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Comparative phylogeography of *Pteropus samoensis* and *P. tonganus* (Pteropodidae: Chiroptera) in the South Pacific

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Pteropids are large, highly mobile bats that are distributed widely across islands of the Pacific and Indian Oceans, southern Asia, and Australia. Dispersal behaviors and colonization patterns of pteropid species among oceanic islands are poorly known. In the southern Pacific, *Pteropus samoensis* and *P. tonganus* have partially overlapping ranges, existing in sympatry on the Samoan and Fijian archipelagos. These species exhibit differences in morphology and roosting behavior, with *P. samoensis* being smaller and tending to roost solitarily or in small groups. Here, we use genetic data to explore whether these species also exhibit differences with regard to patterns of population genetic structure within and between these archipelagos. Phylogenetic analyses of mitochondrial DNA are consistent with earlier morphological recognition of different subspecies of *P. samoensis* on the Samoan vs. Fijian archipelagos. Patterns of mtDNA haplotype sharing suggest that *P. tonganus* experiences restricted gene flow between, but not within archipelagos, while *P. samoensis* shows significant structuring both between and within archipelagos. Species-level differences in patterns of population structure among islands within archipelagos may be due to interspecific differences in morphology, roosting ecology, and/or feeding ecology that can be affected by human influences. Our results directly bear on the conservation of these species, suggesting that (1) populations of both species from the archipelagos of Samoa and Fiji should each be considered as separate conservation units, (2) *P. samoensis* are much less likely than *P. tonganus* to naturally supplement local populations through inter-island dispersal, and (3) *P. tonganus* may experience more severe population bottlenecks during and following cyclones resulting in lower mitochondrial genetic diversity than in *P. samoensis*.

Key words: dispersal, Fiji, phylogeography, population genetics, *Pteropus samoensis*, *P. tonganus*, Samoan archipelago, South Pacific

INTRODUCTION

Old World fruit bats (family Pteropodidae) are highly mobile animals that often occupy large home ranges. Dispersal behaviors and colonization patterns of these bats across island chains in the South Pacific are not well documented, because it is extremely difficult to monitor inter-island movements using traditional tracking methods such as radio telemetry or mark-recapture techniques. *Pteropus vampyrus*, one of the largest pteropid species, is known to travel hundreds of kilometers between roosting sites across southeast Asia within a year (Epstein *et al.*, 2009), with one bat traveling 130 km over a 2-hour period. Less is known about the role of open ocean as a barrier to pteropid dispersal among islands, particularly for smaller species such as *P. samoensis* and *P. tonganus*. Molecular techniques can help us to understand the colonization history of bats among isolated populations, as well as more recent migration patterns (e.g., O'Brien *et al.*, 2009; Almeida *et al.*, 2014). The goal of this study is to investigate and compare patterns of population structure in two sympatric species of Old World fruit bats, *P. samoensis* and *P. tonganus*, on the archipelagos of Fiji and Samoa (here, our use of the terms 'Samoan archipelago' and 'Samoa' are inclusive of the U.S. territory of American Samoa and the Independent State of Samoa). The two species have similar body sizes and wing morphologies (Richmond *et al.*, 1998), and broad overlap has been noted in their plant resource use although *P. tonganus* has been found to feed more often in agroforest and to consume

more species of cultivated plants than *P. samoensis* (Banack, 1998).

Pteropus samoensis is endemic to the Samoan and Fijian archipelagos, with two subspecies currently recognized: *P. s. samoensis* in Samoa and *P. s. nawaiensis* in Fiji (Wiles and Brooke, 2009). This species is unique in that it often is seen flying during the daytime, and it roosts solitarily or in small groups typically comprised of either a female and young of the year, or a mated pair (Brooke, 2001). Roosting bats are generally cryptic, and are not easily disturbed from their roosts (Brooke *et al.*, 2000). Throughout its range, *P. samoensis* is more forest-based in its habitat preferences, and forages less often in agricultural areas and at forest edges (Banack, 1998). Individuals of this species are medium-sized, with an adult body mass of 400–500 g and a forearm length of 130–155 mm (Brooke, 1997). The numbers of *P. s. samoensis* on the Samoan archipelago have been reduced by cyclones and hunting pressures, and the IUCN currently lists this subspecies as 'Near Threatened' (Brooke and Wiles, 2008). *Pteropus s. nawaiensis* was found to be moderately common in some lowland areas of Viti Levu and Vanua Levu, the two largest islands in Fiji, and, while the species also occurs on some medium-sized islands, it usually avoids smaller islands (Palmeirim *et al.*, 2007).

Pteropus tonganus is found throughout many islands of the southwest Pacific Ocean in a variety of habitats (Miller and Wilson, 1997). This species frequents areas close to human habitation in American Samoa where hunting of fruit bats has been banned (RCBU, personal observation). In Fiji and the Independent State of Samoa where such protections are not in place, *P. tonganus* are not commonly observed near human settlements, although they may forage in plantations at night. Adult body mass averages around 600 g, with a forearm length of 120–160 mm (Miller and Wilson, 1997). Several subspecies of *P. tonganus* are currently recognized throughout its range from New Guinea to the Cook Islands, but the bats in the Fijian and Samoan archipelagos are currently considered members of the same subspecies, *P. t. tonganus* (Miller and Wilson, 1997). *Pteropus tonganus* individuals are highly gregarious and their noisy colonies are easy to find, making this species more susceptible to hunting and human disturbance than is *P. samoensis* (Brooke *et al.*, 2000; Utzurrum *et al.*, 2003). Populations on Fiji are documented as widespread and plentiful on both large and small islands, with more than half of the world's population of *P. t. tonganus* likely to occur on Fiji (Palmeirim *et al.*, 2007).

Thus, *P. tonganus* and *P. samoensis* have demonstrated marked differences in their use of the environment and in their rate of encounter with humans. It is not known whether differences between these species also exist in their ability or tendency to move among islands within and between the Samoan and Fijian archipelagos. In this study, we use mitochondrial D-loop sequences and nuclear microsatellite data to investigate genetic structuring of their populations across the sampled range. We investigate genome-wide patterns of structure and assess whether these patterns accurately reflect the current subspecific divisions within *P. samoensis* and *P. tonganus*. Identifying genetically distinct lineages in natural populations is important for determining conservation priorities and can inform management decisions.

Materials and Methods

Study Sites and Sample Collection

Pteropus samoensis were sampled on Viti Levu, Vanua Levu, and Vanua Balavu in Fiji, and on Tutuila, Savai'i, Ofu, and Olosega in the Samoan archipelago (Fig. 1). In addition to these islands, *P. tonganus* were sampled on Ta'u and Upolu in the Samoan archipelago. For the purposes of this study, Ofu and Olosega are considered one sample location because they are geographically adjacent to each other (separated by only 137 m) and sample numbers are low on both islands. A total of 91 *P. tonganus* and 38 *P. samoensis* were sampled (Table 1) between 1998–2003. Specimen details including netting location, age, and sex are provided in Supplementary Table S1. Additionally, two captive *P. rodricensis* individuals from the Lubee Bat Conservancy were sampled as outgroups. Specimen collection protocols were approved by the Institutional Animal Care and Use Committee of the University of Tennessee, Knoxville (protocol #890).

Data Collection

Genomic DNA was isolated from 5 mm wing tissue biopsies (Worthington Wilmer and Barratt, 1996) using phenol-chloroform extraction (Sambrook *et al.*, 1989) or a DNeasy® DNA isolation kit (QIAGEN), with overnight incubation at 55°C. DNA isolates were quantified using a Hoefer DyNA Quant® 200 fluorometer (Amersham Pharmacia), and diluted to a standard concentration of 10 ng/μL for polymerase chain reaction (PCR).

The mitochondrial D-loop region was amplified with primers RodmtU and RodmtL (Brown *et al.*, 2011). For both species, all samples from Fiji were sequenced and at least six individuals of each species from each of the Samoan islands were sequenced, when possible, for a total of 71 *P. tonganus* (54 from Samoa, 17 from Fiji) and 36 *P. samoensis* (25 from Samoa, 11 from Fiji). PCRs were performed in 12 μL volumes containing

1 U Taq DNA polymerase (Promega), 1X PCR buffer, 1.04 mM $MgCl_2$, 0.1 mM dNTPs (Promega), 10 ng genomic DNA, and 10 pmol of each primer (Integrated DNA Technologies). PCR cycling parameters were as follows: denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 52.5°C for 1 min, and elongation at 72°C for 1.5 min. PCR products were purified using a MinElute Gel Extraction Kit (Qiagen), and sequenced using RodmtU on an ABI 3100 automated sequencer (Applied Biosystems; UT Genomics Core). Sequences were edited using Sequencher v.4.5 (Gene Codes Corporation), and deposited into GenBank (accession numbers JQ012845–JQ012894, KT192553–KT192554).

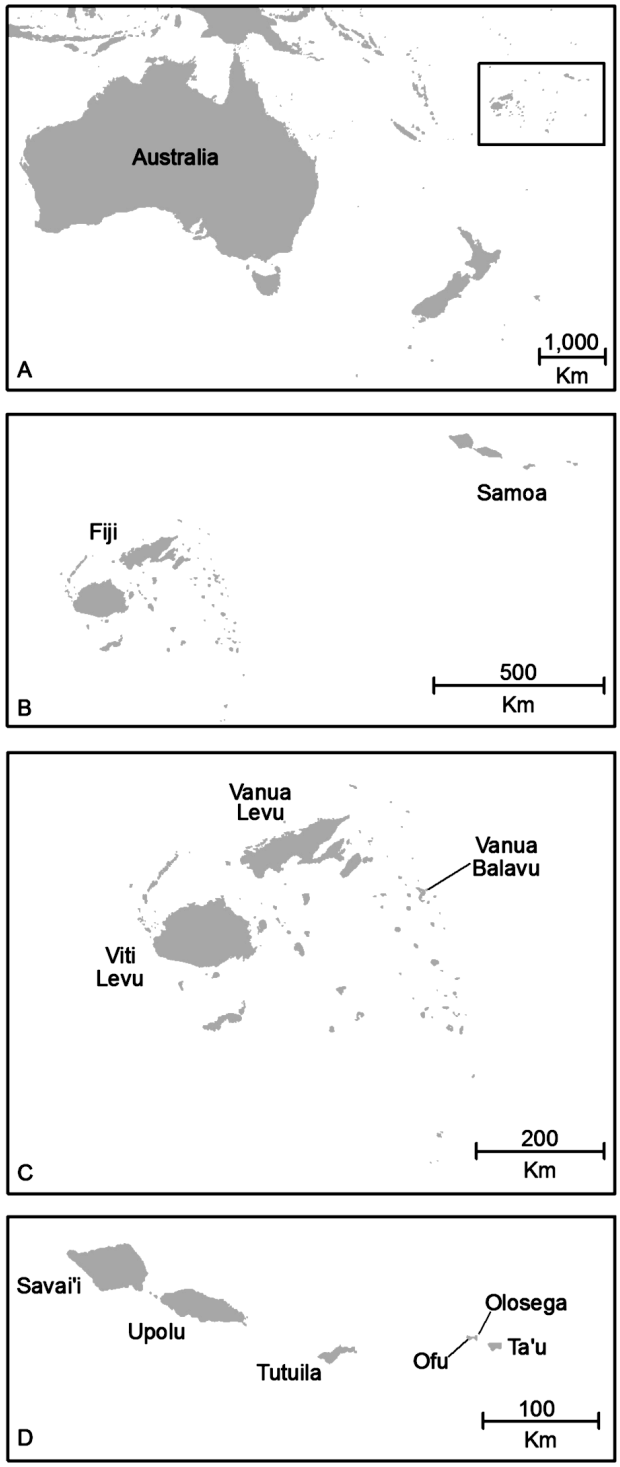

FIG. 1. Map of sampled islands. A — Map of major islands in the South Pacific. The box encloses the archipelagos of Fiji and Samoa; B — An exploded view of the Fijian and Samoan archipelagos. Note that the Samoan archipelago (here labeled 'Samoa') includes the political units of American Samoa and the Independent State of Samoa; C — The islands of Fiji, with sampled islands labeled; D — Islands of the Samoan archipelago, with sampled islands labeled

Microsatellite genotypes were determined at 6 loci using primer pairs described by Brown *et al.* (2011) and O'Brien *et al.* (2007). PCRs were carried out as described above with locus-specific PCR information provided in Supplementary Table S2. BSA (10 ng; Promega) was included in the reaction for locus Ph4 to improve amplification. The reverse primer of each pair was labeled with HEX, 6-FAM, or TET fluorescent dye (Integrated DNA Technologies; Supplementary Table S2). PCR cycling parameters were as follows: denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing for 1 min (annealing temperatures given in Supplementary Table S2), and elongation at 72°C for 1 min, with the exception of primer Ph4, which consisted of 26 cycles with annealing and elongation steps for 30 s. A final elongation step of 30 minutes at 72°C was added for locus A3 to reduce stuttering. Differently labeled and/or -sized loci were pooled for genotyping with 0.75 mL of TAMRA size standard (Applied Biosystems), using 1–1.5 μL of each PCR product, and then brought to 15 μL with Hi-Di Formamide (Applied Biosystems). PCR fragments were denatured at 95°C for 10 min, put immediately on ice, and separated by capillary electrophoresis in an ABI PRISM 3100 genetic analyzer (UT Genomics Core). Raw allelic peak data were sized and binned using Genescan v.3.1 software (PerkinElmer). Previously genotyped samples were included in each run to ensure consistent allele sizing.

Data Analysis

We estimated a phylogeny from our mitochondrial sequence data using MrBayes v.3.0b4 (Huelsenbeck and Ronquist, 2001). Based on the results of a ModelTest v.3.06 (Posada and Crandall, 1998) analysis, we specified the HKY+I+Γ model with four categories for the Γ distribution. Four MCMC chains were run in MrBayes for 1 million steps with a sampling frequency of 100. The first 10% of sampled trees were discarded as a burn-in, and a 50% majority consensus tree was constructed from the remaining sampled genealogies.

Measures of diversity were estimated from mtDNA sequence data and nuclear microsatellite data for each island sampled. Diversity statistics for DNA sequence data were estimated using DnaSP v.5.10.1 (Librado and Rozas, 2009), and included the number of haplotypes (n_h), haplotype diversity (Hd), nucleotide diversity (π), and the number of segregating sites (S). Mismatch distribution analyses were conducted in DnaSP as a rough test for population expansion. Diversity statistics for nuclear microsatellite loci were estimated using GenePop v.4.2 (Raymond and Rousset, 1995; Rousset, 2008), and included the number of alleles (n_A), observed heterozygosity (H_O), and expected heterozygosity (H_E).

Patterns of population genetic structure were investigated using several methods. Mitochondrial haplotype sharing within and between archipelagos were depicted graphically using Circos (Krzywinski *et al.*, 2009) and by constructing haplotype

networks based on pairwise differences in TCS (Clement *et al.*, 2000). Supplementary Table S1 provides details regarding haplotype sharing by individuals. Differentiation among island samples was explored using pairwise F_{ST} values calculated separately for mtDNA sequence data and microsatellite data. Mitochondrial genetic structure between and within archipelagos also was investigated using an analysis of molecular variance (AMOVA); significance of these results was assessed using 16,000 permutations of the data. Mantel tests were used to explore the impact of geographic distance (ln-transformed great circle distances between the midpoints of islands; Supplementary Table S3) on mitochondrial genetic distance (Slatkin's linearized F_{ST}; Rousset 1997), with significance determined using 16,000 permutations of the data. Both pairwise F_{ST} and AMOVA analyses were conducted using Arlequin v.3.5 (Excoffier and Lischer, 2010), with statistical significance determined by permutation tests (16,000 permutations). Population structure for microsatellite variation was further examined using STRUCTURE v.2.1 (Pritchard *et al.*, 2000) to estimate the number of genetic clusters (K) that best explain the data and to assign individuals to subpopulations based on their genotypes without regard to sampling locations. Ten replicates of each run for K = 1–10 for *P. tonganus* and K = 1–7 for *P. samoensis* (based on number of islands sampled) were performed using the admixture model with a burn-in period of 100,000 and 1,000,000 MCMC steps after burn-in. The number of clusters that best represents the data was determined as proposed by Evanno *et al.* (2005).

Results

Genetic Diversity in P. samoensis

We sequenced an average of six *P. samoensis* individuals per island from Samoa and Fiji (Table 1), although this average is inflated by an overrepresentation of samples from Tutuila. Two to eight unique haplotypes were detected per island, corresponding to a haplotype diversity (Hd) of 0.5–1.0. Except for Vanua Balavu in Fiji, per-island measures of sequence diversity including nucleotide diversity, haplotype diversity, and number of segregating sites were relatively high for this species (Table 1). We conducted mismatch distribution analyses on better-sampled islands, including Savai'i (n = 6) and Tutuila (n = 17). The null model of population expansion was supported for both populations based on Harpending *et al.*'s (1998) raggedness index (Savai'i: r = 0.1556, P = 0.55; Tutuila: r = 0.1205, P = 0.947).

Thirty-eight individuals were genotyped at six microsatellite loci, including 27 individuals from Samoa and 11 individuals from Fiji. Per-island diversity was low, with an average of 3.72 alleles per locus in Samoa and 2.78 alleles per locus in Fiji. Observed heterozygosity was also low, but not significantly different than expected under Hardy-Weinberg equilibrium (Table 1).

Genetic diversity in P. tonganus

We sequenced an average of 8.9 individuals per location from the islands of Ofu, Olosega, Savai'i, Ta'u, Tutuila, and Upolu in Samoa, and Vanua Balavu, Vanua Levu, and Viti Levu in Fiji. Despite having higher overall sample sizes for this species compared to *P. samoensis*, we observed lower

Table 1. Sample numbers and locations, and basic diversity measures. Calculations for the mitochondrial sequence dataset include sample size (n_1), the number of unique haplotypes (n_h), haplotype diversity (Hd), nucleotide diversity (π), and the number of segregating sites (S). Calculations for the nuclear microsatellite data include sample size (n_2), average number of alleles (n_A) per locus, average observed heterozygosity per locus (H_O), and average expected heterozygosity per locus (H_E)

Island/Archipelago	Mitochondrial D-loop				Microsatellite loci				
	n_1	n_h	Hd	π	S	n_2	n_A	H_O	H_E
Pteropus samoensis									
Olosega/Ofu, Samoa	2	2	1.000	0.036	15	2	1.50	0.250	0.250
Savai'i, Samoa	6	5	0.933	0.040	36	7	4.83	0.571	0.588
Tutuila, Samoa	17	8	0.882	0.039	36	18	4.83	0.380	0.432
Vanua Balavu, Fiji	4	2	0.500	0.001	1	4	2.33	0.375	0.446
Vanua Levu, Fiji	3	3	1.000	0.040	25	3	2.83	0.556	0.544
Viti Levu, Fiji	4	4	1.000	0.057	41	4	3.17	0.542	0.501
Pteropus tonganus									
Olosega/Ofu, Samoa	12	5	0.667	0.008	12	15	4.83	0.600	0.606
Savai'i, Samoa	6	4	0.867	0.016	13	7	4.33	0.571	0.559
Ta'u, Samoa	10	2	0.200	0.005	10	15	5.33	0.600	0.611
Tutuila, Samoa	18	7	0.693	0.013	19	27	5.83	0.519	0.598
Upolu, Samoa	8	5	0.857	0.016	13	10	5.83	0.667	0.684
Vanua Balavu, Fiji	5	5	1.000	0.023	21	5	4.17	0.567	0.633
Vanua Levu, Fiji	3	3	1.000	0.030	19	3	3.50	0.611	0.744
Viti Levu, Fiji	9	8	0.972	0.028	46	9	6.33	0.704	0.773

mitochondrial sequence diversity (e.g., $\pi = 0.008$ for *P. tonganus* in Ofu and Olosega ($n = 12$), versus $\pi = 0.036$ for *P. samoensis* in Ofu and Olosega ($n = 2$ — Table 1). Mismatch distribution analyses on samples pooled by archipelago were consistent with a model of population expansion (Samoa: $r = 0.147$, $P = 0.87$; Fiji: $r = 0.023$, $P = 0.16$).

Ninety-one individuals were genotyped at six loci from eight islands in Samoa and Fiji, with an average sample size of 11.4 individuals per island. Per-island diversity was slightly higher in *P. tonganus* than in *P. samoensis*, with an average of 5.23 alleles per locus in Samoa and 4.67 alleles per locus in Fiji. Observed heterozygosity was also higher in *P. tonganus* than *P. samoensis*, and islands where $n \geq 5$ fit Hardy-Weinberg expectations.

Population Genetic Structure in P. samoensis

Multiple analyses of genetic structure in *P. samoensis* demonstrate significant differentiation of island populations at the mitochondrial D-loop. Pairwise F_{ST} analyses indicate the presence of significant structure in comparisons of Savai'i and Tutuila with most other islands (Table 2). Even where these analyses are not statistically significant, calculated F_{ST} values are quite high between almost all pairs of islands. Similarly, AMOVA results show significant structure both between archipelagos ($\phi_{CT} = 0.564$, $P < 0.01$) and among islands within archipelagos ($\phi_{SC} = 0.327$, $P < 0.01$). This pattern is further supported by a lack of shared haplotypes between islands (Fig. 2A, Supplementary Fig. S1), although it should be noted that sample sizes in this species are generally very low. Mantel tests indicate that these results cannot be explained by simple isolation-by-distance ($r = 0.612$, $P = 0.093$); however, this test may suffer from low sample sizes. In a phylogenetic analysis, samples from Samoa and Fiji represent monophyletic clades within *P. samoensis* (Fig. 3), and a mitochondrial haplotype network shows the two archipelagos separating into two haplogroups distinguished by at least 29 mutations (Supplementary Fig. S1).

Patterns of genetic structure at the nuclear microsatellite loci were less consistent across analyses. Pairwise F_{ST} values (Table 2) indicate some differentiation among islands, particularly in comparisons including better-sampled islands (Savai'i, $n = 7$; Tutuila, $n = 18$). However, clustering analyses using STRUCTURE revealed the strongest support for $K = 1$, and thus could not reject a model of panmixia for this species.

TABLE 2. Pairwise F_{ST} for *P. samoensis* and *P. tonganus*. Estimates for the mtDNA data are shown below the diagonal; those for microsatellite data are shown above diagonal. Asterisks indicate significant values at $P < 0.01$. Samoa includes OO, Sav, Tau, Tut, Up; Fiji includes VB, VL, VT

Location	*P. samoensis*						*P. tonganus*						
	OO	Sav	Tut	VB	VL	VT	OO	Sav	Tau	Tut	Up	VB	VL	VT
Ofu/Olosega (OO)	–	0.117	0.166	0.250	0.111	0.158	–	-0.012	-0.005	0.001	0.007	0.151*	0.191*	0.068*
Savai'i (Sav)	0.279	–	0.112*	0.086*	0.004	0.032	0.012	–	0.010	0.006	0.012	0.249*	0.281*	0.135*
Ta'u (Tau)							-0.102	-0.049	–	0.003	-0.007	0.109*	0.174*	0.085*
Tutuila (Tut)	-0.175	0.285*	–	0.142*	0.167*	0.078	-0.023	-0.043	-0.024	–	0.015	0.177*	0.201	0.087*
Upolu (Up)							0.081	-0.123	0.074	-0.008	–	0.105*	0.125*	0.052
Vanua Balavu (VB)	0.928	0.832*	0.777*	–	0.077	0.054	0.502*	0.327*	0.551*	0.357*	0.300	–	0.056	0.022
Vanua Levu (VL)	0.616	0.701	0.671*	0.637	–	-0.070	0.387*	0.164	0.458*	0.230	0.167	0.080	–	0.011
Viti Levu (VT)	0.484	0.664*	0.643*	0.592	0.110	–	0.331*	0.183*	0.333*	0.229*	0.163	-0.010	-0.022	–

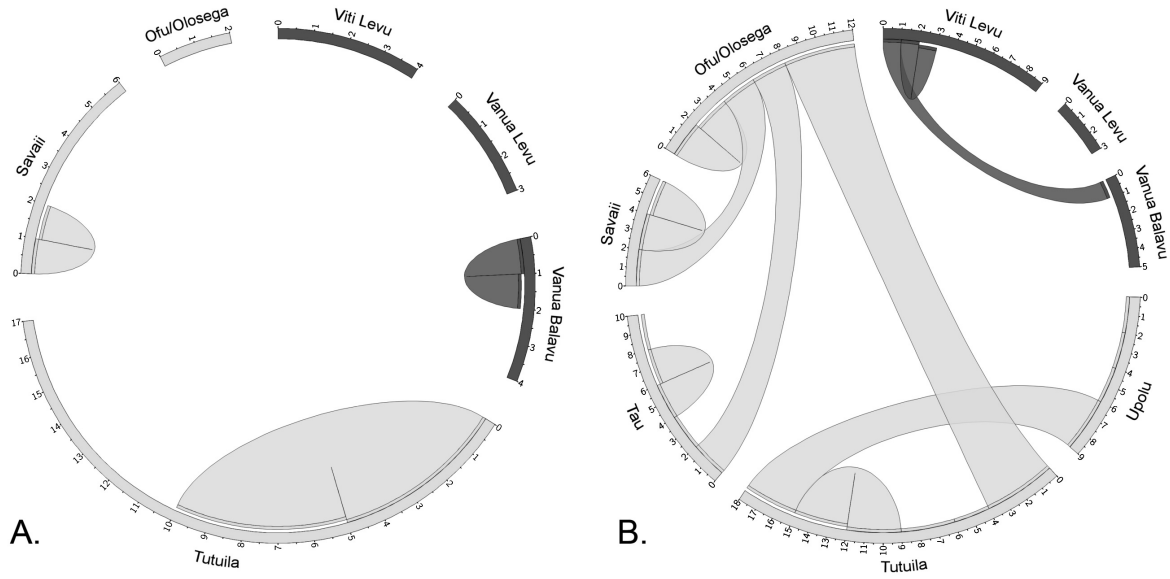

FIG. 2. Circos diagrams showing shared haplotypes within and among archipelagos for A — *P. samoensis*; B — *P. tonganus*. For each island, numbered segments depict sampled individuals. Light grey, Samoan islands; dark grey, Fijian islands

Population Genetic Structure in P. tonganus

Analyses of the mitochondrial D-loop sequences consistently showed a pattern of genetic structuring between, but not within, archipelagos. Pairwise F_{ST}'s were significant only between islands from different archipelagos; not all calculations between islands from different archipelagos were significant, but all were markedly higher ($F_{ST} \geq 0.164$) than those between islands in the same archipelago ($F_{ST} \leq 0.081$ — Table 2). AMOVA results show significant structure between archipelagos ($\phi_{CT} = 0.316$, $P < 0.01$), but not among islands within archipelagos ($\phi_{SC} = 0.006$, $P = 0.54$). There is evidence of haplotype sharing within, but not between, archipelagos (Fig. 2B and Supplementary Fig. S2), and Mantel tests reveal significant isolation-by-distance across the entire dataset ($r = 0.483$, $P = 0.011$) but not within archipelagos (Samoa: $r = -0.254$, $P = 0.85$; Fiji: $r = -0.230$, $P = 0.50$). However, samples from Samoa and Fiji do not constitute reciprocally monophyletic clades in phylogenetic analyses (Fig. 3), and the haplotype network for this species similarly fails to indicate clearly distinguishable haplogroups (Supplementary Fig. S2).

Nuclear microsatellite loci are largely consistent with a model of genetic structuring between archipelagos, but not among islands within archipelagos. Pairwise F_{ST} analyses show significant differentiation only between islands of different archipelagos (Table 2). STRUCTURE analyses support $K = 2$ populations, with the clusters largely distinguishing Samoan from Fijian islands (Fig. 4).

DISCUSSION

Our results provide comparative analysis of genetic diversity and population structuring in *Pteropus samoensis* and *P. tonganus*, with the two species sampled mostly from the same islands in Samoa and Fiji. Patterns of genetic diversity described here differ between the species, particularly at the mitochondrial D-loop, which is significantly more diverse in *P. samoensis* than in *P. tonganus*, despite our lower sample sizes for the former. Both species exhibit significant genetic differentiation between the archipelagos. In *P. samoensis*, multiple analyses of mtDNA sequence data support this pattern and are consistent with described morphological distinctions between the subspecies *P. s. nawaiensis* in Fiji and *P. s. samoensis* in Samoa (Andersen, 1912; Banack, 2001). Although *P. tonganus* is not recognized as different subspecies in Samoa and Fiji (Miller and Wilson, 1997), our analyses of both mitochondrial and nuclear data indicate a significant restriction of gene flow between the archipelagos for this species. A comparison of the haplotype network (Supplementary Fig. S2) with the phylogeny (Fig. 3) shows that, while *P. tonganus* haplotypes are not shared between archipelagos,

the haplotypes do not segregate into clearly distinguishable haplogroups, which is also reflected in the lack of cladistic distinction between archipelagos in the phylogeny (Fig. 3). We reflect on reasons for this below.

We detected interspecific differences in patterns of population structure among islands within archipelagos, with restricted gene flow among islands for *P. samoensis* and a lack of genetic structure among islands for *P. tonganus*. We propose three

FIG. 3. Bayesian phylogeny for *P. samoensis* and *P. tonganus*. The tree is rooted on homologous sequences from *P. rodricensis*. * indicates Bayesian posterior probability ≥ 95%. Taxonomically informative nodes are labeled as such. Samoa includes Ofu, Olosega, Savai'i, Ta'u, Tutuila, and Upolu; Fiji includes Vanua Balavu, Vanua Levu, and Viti Levu

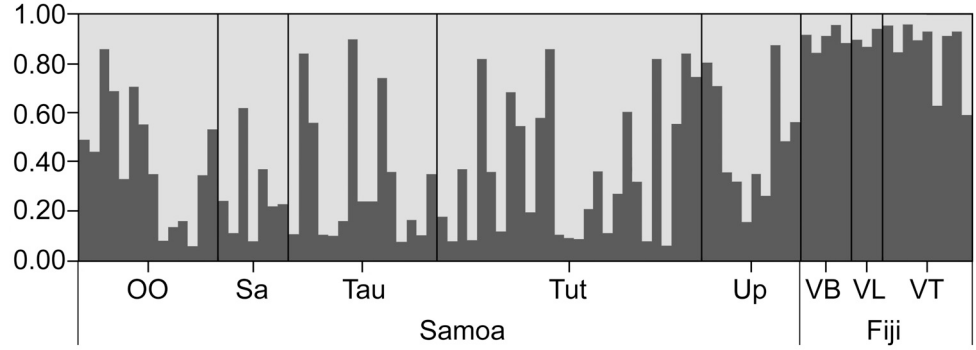

FIG. 4. STRUCTURE clustering output for *P. tonganus* for $K = 2$. Samoa includes OO, Sav, Tau, Tut, Up; Fiji includes VB, VL, VT

explanations to address these differences: morphology, roosting ecology, and feeding ecology, and encourage further behavioral and/or genetic studies to test the validity of these ideas.

Although the two species are similar in morphology, *P. tonganus* has larger body size and larger wings than *P. samoensis*. For *P. tonganus*, the aspect ratio (the square of wingspan divided by wing area) is 8.11 and wing loading (weight divided by wing area) is 34.1–37.0 Newtons/m^2, whereas the same measurements for *P. samoensis* are 6.74–6.86 and 28.2–29.6 N/m^2, respectively (Norberg *et al.*, 2000). Higher wing loading is associated with faster, more efficient flight, whereas lower wing loading is associated more with soaring rather than flapping flight. Bats with higher aspect ratios are less maneuverable but fly faster over longer distances (Norberg and Rayner, 1987). In a meta-analysis of factors influencing population structure in bats, Olival (2012) found that lower wing aspect ratio and, to a lesser extent, lower wing loading were predictive of population structuring. Our results are consistent with Olival (2012), suggesting that, compared to *P. samoensis*, its flight morphology may allow *P. tonganus* to fly further (dispersal distances of at least 325 km, inferred from our data) and more effectively travel between islands within an archipelago. The fact that *P. tonganus* is distributed throughout much of the south Pacific from the Cook Islands to New Guinea (Miller and Wilson, 1997) is further support for the greater capacity of this species to disperse long distances. However, the suggestion that *P. tonganus* has a higher tendency towards inter-island dispersal than *P. samoensis* implies that, all else being equal, the former species would have a larger effective population size and thus more genetic diversity, which is not what we observe in our mitochondrial data.

The roosting ecology of these species might also contribute to the different patterns of population structure. *Pteropus tonganus* roosts in large colonies in coastal and upland forest, hillsides, and exposed points protruding out into the ocean, whereas *P. samoensis* roosts singly or in small family units in more sheltered forest (Cox, 1983; Brooke *et al.*, 2000). These more concentrated and exposed roosting habits of *P. tonganus* make this species likely to suffer more from chance catastrophes (such as cyclones) than *P. samoensis*, which is more dispersed across the landscape. Indeed, Pierson *et al.* (1996) documented periodic bottlenecks in *P. tonganus* due to cyclones. These differences could also make *P. tonganus* more prone to inter-island dispersal and storm-assisted transport, thus facilitating gene flow in this species by individuals who survive such experiences. In addition, these roosting habits may make *P. tonganus* an easier target for hunting. Whether due to hunting pressures or to storms, these differences in roosting ecology mean that *P. tonganus* would likely experience more severe bottlenecks than *P. samoensis*, which might explain the lower observed genetic diversity in the former species. Our mismatch distribution analyses support the idea that both species have experienced relatively recent population bottlenecks, but our data are insufficient to quantify the date or magnitude of these demographic events. We anticipate that larger genetic datasets with more loci will be able to test the idea that *P. tonganus* has experienced more intense bottlenecks than *P. samoensis*.

Differences in feeding ecology might further contribute to the different patterns of genetic diversity observed between these species. *Pteropus tonganus* utilizes more agricultural food resources such as breadfruit and coconut and banana flowers, while *P. samoensis* relies more on forest resources including leaves of trees and epiphytes (Banack, 1998). Following cyclones, forest vegetation recovers much faster (within a week) than agricultural vegetation (six months or more). With more concentrated

populations competing for a more restricted food base, we expect that *P. tonganus* would be more prone to storm-induced population crashes. Because their resources are close to human habitations, *P. tonganus* might also experience higher rates of predation from humans, dogs, and cats. These interspecific differences in feeding ecology would result in a more severe population bottleneck and subsequent loss of genetic diversity from *P. tonganus* than from *P. samoensis*, as we observe in our data.

Studies of gene flow among isolated Pacific island populations recently have provided insight into the dispersal patterns of several species of pteropids. Brown *et al*. (2011) found a pattern of movement for *P. mariannus* within the Mariana Islands and Palau that is similar to that found here for *P. tonganus,* with *P. mariannus* showing dispersal within each archipelago but significant structure between the two geographically isolated groups of islands. Similarly, Chan *et al.* (2011) found little genetic structure among samples of *P. seychellensis comorensis* from the Comoro Islands. Okada *et al*. (2014) discovered a lack of inter-island movement for *P. pselaphon* in the Ogasawara Islands, a pattern similar to that documented here for *P. samoensis*. Almeida *et al*. (2014) noted that multiple colonizations of isolated islands by distantly related pteropid species has occurred several times, and tends to be successful when there are ecological differences between the sympatric species, as there appear to be between *P. samoensis* and *P. tonganus*. Our study provides the first population level molecular data for these two partially co-distributed species, and, while some islands have limited sample sizes, these results provide new insights into patterns of gene flow in these species.

Small sample sizes are an issue in some of our analyses, especially for *P. samoensis*. For example, small sample size prevents us from concluding whether the low mitochondrial diversity detected in *P. samoensis* in Vanua Balavu results from constraints on population size on this small island or chance sampling of similar haplotypes. In this species, sampling is strongly biased towards Tutuila in Samoa. To some extent, this limits our power to detect statistically significant genetic structuring, particularly with pairwise F_{ST} analyses. The small number and low diversity within microsatellite loci in this study also present a limitation for the STRUCTURE analyses (Puechmaille, 2016), although reanalyses of datasets subsampled to alleviate issues of low and uneven sampling did not yield different results for either species (analyses not shown). We suspect that the lack of genetic structure detected using this approach in *P. samoensis* may be a result of low variation at the loci used (average n_A = 2.7–3.7), and encourage future research to investigate this question using larger datasets.

In conclusion, our analyses demonstrate that the open ocean between the Samoan and Fijian archipelagos represents a significant barrier to gene flow for both *P. samoensis* and *P. tonganus*. Within archipelagos, *P. tonganus* appears to be much more prone to dispersing among islands, while *P. samoensis* experiences considerably less inter-island gene flow. These interspecific differences in patterns of genetic structuring may be due to differences in morphology, roosting ecology, feeding ecology, or any combination thereof. Our results have direct conservation implications for these species, suggesting that populations of both species from Samoan and Fijian islands should each be treated as separate conservation units. In addition, future conservation efforts should consider that *P. samoensis* appear to be much less likely than *P. tonganus* to naturally supplement local populations through inter-island dispersal. Thus, managers may need to consider more intensive management actions such as assisted dispersal for *P. samoensis* if this species becomes locally extirpated from an island.

Supplementary Information

Contents: Table S1. Specimen information. Sex is given as M = male, F = female. Subadult, young adult, and adult age classes were determined from a combination of morphological characters including the degree of calcification of the metacarpal-phalangeal joints, pelage characteristics, and nipple size (Utzurrum, 1999); Table S2. PCR conditions for microsatellite loci. Species-specific conditions include the magnesium concentration in mM, annealing temperature (TA), number of alleles observed in total (NA), and the size range of the PCR amplicon. Locus Ph4 is from O'Brien *et al.* (2009); all others are from Brown et al. (2011). The dye added to the reverse primer is labeled as follows: * — 6-FAM, ^ — HEX, and # — TET. Table S3. Geographic distances (km) between the midpoints of each island; Fig. S1. Minimum spanning network for *Pteropus samoensis*. Circles represent sampled haplotypes, with the size of each circle reflecting its relative frequency in the sample. The number at each edge designates the number of substitutions differentiating neighboring haplotypes; Fig. S2. Minimum spanning network for *P. tonganus*. Circles represent sampled haplotypes, with the size of each circle reflecting its relative frequency in the sample. Haplotypes that were shared among islands are represented as pie charts showing the relative frequency on each island where they were detected. The number at each edge designates the number of substitutions differentiating neighboring haplotypes. Supplementary Information is available exclusively on BioOne.

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SUPPLEMENTARY INFORMATION

TABLE S1. Specimen information. Sex is given as M = male, F = female. Subadult, young adult, and adult age classes were determined from a combination of morphological characters including the degree of calcification of the metacarpal-phalangeal joints, pelage characteristics, and nipple size (Utzurrum, 1999)

Individual	Archipelago	Island	Netting location	Latitude	Longitude	Sex	Age	Haplotype label	Identical Sequences	GenBank laccession no.
Pteropus samoensis										
OF62	Samoa	Ofu	Mt. Tumu	-14.175964	-169.660316	F	Adult	OF62	–	JQ012872
OL197	Samoa	Olosega	Unknown	–	–	Unknown	Unknown	OL197	–	JQ012871
SA9	Samoa	Savai'i	Tafua Tai crater	-13.786176	-172.252024	M	Adult	SA9	SA9, SA73	JQ012873
SA10	Samoa	Savai'i	Tafua village road	-13.784987	-172.258066	F	Adult	not sequenced	–	–
SA63	Samoa	Savai'i	Salelologa	-13.775659	-172.230371	F	Young adult	SA63	–	JQ012874
SA64	Samoa	Savai'i	Salelologa	-13.775659	-172.230371	F	Sub-adult	SA64	–	JQ012875
SA68	Samoa	Savai'i	Salelologa	-13.775659	-172.230371	M	Young adult	SA68	–	JQ012876
SA72	Samoa	Savai'i	Tafua	-13.789808	-172.256733	M	Adult	SA72	–	JQ012877
SA73	Samoa	Savai'i	Salelologa	-13.775659	-172.230371	F	Adult	SA9	SA9, SA73	JQ012873
TU5	Samoa	Tutuila	Olomoana, Aoa	-14.26209	-170.577434	F	Adult	TU5	TU5, TU198	JQ012878
TU23	Samoa	Tutuila	Fagasa	-14.286809	-170.718145	M	Unknown	not sequenced	–	–
TU24	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Sub-adult	TU24	TU24, TU25, TU86, TU123, TU167	JQ012879
TU25	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Young adult	TU24	TU24, TU25, TU86, TU123, TU167	JQ012879
TU33	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Sub-adult	TU33	–	JQ012880

TU42	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Adult	not sequenced	–	–
TU43	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Adult	TU82	TU82, TU43	JQ012882
TU44	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Young adult	TU44	TU44, TU83, TU87	JQ012883
TU76	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Adult	TU76	TU76, TU138	JQ012881
TU82	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Adult	TU82	TU82, TU43	JQ012882
TU83	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Adult	TU44	TU44, TU83, TU87	JQ012883
TU86	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Adult	TU24	TU24, TU25, TU86, TU123, TU167	JQ012879
TU87	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Adult	TU44	TU44, TU83, TU87	JQ012883
TU123	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Young adult	TU24	TU24, TU25, TU86, TU123, TU167	JQ012879
TU138	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Sub-adult	TU76	TU76, TU138	JQ012881
TU167	Samoa	Tutuila	Unknown	–	–	Unknown	Unknown	TU24	TU24, TU25, TU86, TU123, TU167	JQ012879
TU178	Samoa	Tutuila	Unknown	–	–	Unknown	Unknown	TU178	–	JQ012884
TU193	Samoa	Tutuila	Unknown	–	–	Unknown	Unknown	TU193	–	JQ012885
TU198	Samoa	Tutuila	Unknown	–	–	Unknown	Unknown	TU5	TU5, TU198	JQ012878
VB92	Fiji	Vanua Balavu	Lomaloma	-17.2928	-178.987	F	Adult	VB92	–	JQ012886
VB96	Fiji	Vanua Balavu	Daliconi	-17.227685	-178.948463	M	Adult	VB96	VB96, VB97, VB98	JQ012887
VB97	Fiji	Vanua Balavu	Daliconi	-17.227685	-178.948463	F	Adult	VB96	VB96, VB97, VB98	JQ012887
VB98	Fiji	Vanua Balavu	Daliconi	-17.227685	-178.948463	F	Young adult	VB96	VB96, VB97, VB98	JQ012887
VL208	Fiji	Vanua Levu	Unknown	–	–	Unknown	Unknown	VL208	–	JQ012888
VL212	Fiji	Vanua Levu	Unknown	–	–	Unknown	Unknown	VL212	–	JQ012889
VL213	Fiji	Vanua Levu	Unknown	–	–	Unknown	Unknown	VL213	–	JQ012890
VT102	Fiji	Viti Levu	Unknown	–	–	Unknown	Unknown	VT102	–	JQ012891
VT232	Fiji	Viti Levu	Unknown	–	–	Unknown	Unknown	VT232	–	JQ012892
VT233	Fiji	Viti Levu	Unknown	–	–	Unknown	Unknown	VT233	–	JQ012893
VTSF4	Fiji	Viti Levu	Suva	-18.028202	-178.384919	Unknown	Unknown	VTSF4	–	JQ012894
Pteropus tonganus										
OF105	Samoa	Ofu	Mt. Tumu	-14.175964	-169.660316	M	Adult	OF105	OF105, TU78, UP19	JQ012845

OF106	Samoa	Ofu	Mt. Tumu	-14.175964	-169.660316	F	Young adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
OL3	Samoa	Olosega	Mt. Piumafua	-14.173414	-169.619462	M	Sub-adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
OL4	Samoa	Olosega	Sili village	-14.165216	-169.620212	M	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
OL56	Samoa	Olosega	Sili village	-14.165216	-169.620212	M	Adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
OL57	Samoa	Olosega	Sili village	-14.165216	-169.620212	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
OL58	Samoa	Olosega	Sili village	-14.165216	-169.620212	M	Adult	OL58	OL58, TU27	JQ012848
OL59	Samoa	Olosega	Sili village	-14.165216	-169.620212	F	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
OL60	Samoa	Olosega	Sili village	-14.165216	-169.620212	M	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
OL61	Samoa	Olosega	Sili village	-14.165216	-169.620212	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6,	JQ012846

									TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	
OL117	Samoa	Olosega	Sili village	-14.165216	-169.620212	F	Young adult	OL117	–	JQ012849
OL140	Samoa	Olosega	Sili village	-14.165216	-169.620212	M	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
SA11	Samoa	Savai'i	Tafua village road	-13.784987	-172.258066	F	Sub-adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
SA65	Samoa	Savai'i	Tafua	-13.789808	-172.256733	M	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
SA66	Samoa	Savai'i	Salelologa	-13.775659	-172.230371	M	Sub-adult	SA66	–	JQ012850
SA67	Samoa	Savai'i	Tafua	-13.789808	-172.256733	F	Young adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
SA69	Samoa	Savai'i	Tafua	-13.789808	-172.256733	F	Sub-adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
SA70	Samoa	Savai'i	Tafua	-13.789808	-172.256733	F	Sub-adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
TA1	Samoa	Ta'u	Lua-Iti crater	-14.224108	-169.4358	F	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TA48	Samoa	Ta'u	Lua-Iti crater	-14.224108	-169.4358	M	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TA49	Samoa	Ta'u	Lua-Iti crater	-14.224108	-169.4358	M	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65,	JQ012846

									TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	
TA50	Samoa	Ta'u	Lua-Iti crater	-14.224108	-169.4358	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TA53	Samoa	Ta'u	Lua-Iti crater	-14.224108	-169.4358	F	Young adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
TA107	Samoa	Ta'u	Faleasao	-14.219695	-169.503609	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TA108	Samoa	Ta'u	Faleasao	-14.219695	-169.503609	F	Adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
TA109	Samoa	Ta'u	Faleasao	-14.219695	-169.503609	M	Young adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TA110	Samoa	Ta'u	Faleasao	-14.219695	-169.503609	F	Young adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
TA113	Samoa	Ta'u	Faleasao	-14.219695	-169.503609	M	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU6	Samoa	Tutuila	Olomoana, Aoa	-14.26209	-170.577434	M	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30,	JQ012846

									TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	
TU7	Samoa	Tutuila	Olomoana, Aoa	-14.26209	-170.577434	M	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU8	Samoa	Tutuila	Olomoana, Aoa	-14.26209	-170.577434	F	Young adult	OL3	OL3, OL56, SA11, SA70, TA53, TA110, TU8	JQ012847
TU27	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Sub-adult	OL58	OL58, TU27	JQ012848
TU28	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Young adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU29	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Young adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU30	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Young adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU32	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Sub-adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU38	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Sub-adult	TU38	TU38, TU75	JQ012852
TU40	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Sub-adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
TU45	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Young	OF106	OF106, OL4, OL57, OL59,	JQ012846

							adult		OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	
TU46	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU55	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU74	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Young adult	TU74	–	JQ012853
TU75	Samoa	Tutuila	Amalau	-14.255055	-170.660097	F	Sub-adult	TU38	TU38, TU75	JQ012852
TU78	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Young adult	OF105	OF105, TU78, UP19	JQ012845
TU81	Samoa	Tutuila	Amalau	-14.255055	-170.660097	M	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
TU84	Samoa	Tutuila	Fagatele	-14.361084	-170.761656	M	Young adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
UP13	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	M	Sub-adult	UP13	–	JQ012854
UP14	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	F	Adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
UP16	Samoa	Upolu	Lalomanu	-14.023391	-171.461026	M	Young	OF106	OF106, OL4, OL57, OL59,	JQ012846

			crater				adult		OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	
UP17	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	F	Young adult	OF106	OF106, OL4, OL57, OL59, OL60 OL61, OL140, SA65, TA1, TA48, TA49, TA50, TA107, TA109, TA113, TU6, TU7, TU28, TU29, TU30, TU32, TU45, TU46, TU55, TU81, UP14, UP16, UP17	JQ012846
UP18	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	F	Sub-adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
UP19	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	F	Sub-adult	OF105	OF105, TU78, UP19	JQ012845
UP20	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	M	Young adult	SA67	SA67, SA69, TA108, TU40, TU84, UP18, UP20	JQ012851
UP21	Samoa	Upolu	Lalomanu crater	-14.023391	-171.461026	M	Sub-adult	UP21	–	JQ012855
VB90	Fiji	Vanua Balavu	Lomaloma	-17.2928	-178.987	M	Adult	VB90	–	JQ012856
VB91	Fiji	Vanua Balavu	Lomaloma	-17.2928	-178.987	F	Adult	VB91	–	JQ012857
VB93	Fiji	Vanua Balavu	Lomaloma	-17.2928	-178.987	M	Adult	VB93	VB93, VT101, VTSF1	JQ012858
VB94	Fiji	Vanua Balavu	Lomaloma	-17.2928	-178.987	M	Adult	VB94	–	JQ012859
VB95	Fiji	Vanua Balavu	Lomaloma	-17.2928	-178.987	M	Adult	VB95	–	JQ012860
VL88	Fiji	Vanua Levu	Natewa village	-16.602298	179.735621	M	Adult	VL88	–	JQ012861
VL89	Fiji	Vanua Levu	Natewa village	-16.602298	179.735621	M	Adult	VL89	–	JQ012862
VL231	Fiji	Vanua Levu	Unknown	–	–	Unknown	Unknown	VL231	–	JQ012863
VT99	Fiji	Viti Levu	Suva	-18.028202	-178.384919	F	Young adult	VT99	–	JQ012864
VT100	Fiji	Viti Levu	Suva	-18.028202	-178.384919	M	Adult	VT100	–	JQ012865
VT101	Fiji	Viti Levu	Suva	-18.028202	-178.384919	M	Adult	VB93	VB93, VT101, VTSF1	JQ012858
VT103	Fiji	Viti	Sigatoka	-18.142498	177.514467	M	Adult	VT103	–	JQ012866

		Levu								
VT104	Fiji	Viti Levu	Sigatoka	-18.142498	177.514467	M	Adult	VT104	–	JQ012867
VT207	Fiji	Viti Levu	Unknown	–	–	Unknown	Unknown	VT207	–	JQ012868
VTSF1	Fiji	Viti Levu	Suva	-18.028202	-178.384919	Unknown	Unknown	VB93	VB93, VT101, VTSF1	JQ012858
VTSF2	Fiji	Viti Levu	Suva	-18.028202	-178.384919	Unknown	Unknown	VTSF2	–	JQ012869
VTSF3	Fiji	Viti Levu	Suva	-18.028202	-178.384919	Unknown	Unknown	VTSF3	–	JQ012870

TABLE S2. PCR conditions for microsatellite loci. Species-specific conditions include the magnesium concentration in mM, annealing temperature (T_A), number of alleles observed in total (N_A), and the size range of the PCR amplicon. Locus Ph4 is from O'Brien *et al.* (2009); all others are from Brown *et al.* (2011). The dye added to the reverse primer is labeled as follows: * 6-FAM, ^ HEX, and # TET

Locus	GenBank accession no.	*P. samoensis*				*P. tonganus*			
		Mg (mM)	T_A (°C)	N_A	Size range (bp)	Mg (mM)	T_A (°C)	N_A	Size range (bp)
A2*	DQ157421	1.04	51.6	9	159–187	1.04	51.6	5	163–171
A3^	DQ157422	1.04	54	12	328–352	1.04	54	10	332–354
C6^	DQ157426	1.04	58	5	286–322	1.04	58	5	283–295
D1#	DQ157425	1.04	59	13	371–469	1.04	59	21	379–475
GA2#	DQ157417	1.15	60.4	5	187–195	1.04	59.2	4	189–197
Ph4*	DQ157418	0.94	55	2	258–262	0.90	54	6	259–280

TABLE S3. Geographic distances (km) between the midpoints of each island

Island	Ofu	Olosega	Sav	Tau	Tut	Up	VB	VL	VT
Ofu	–								
Olosega	6	–							
Savai'i	301	307	–						
Ta'u	19	14	320	–					
Tutuila	108	114	200	126	–				
Upolu	223	228	79	242	121	–			
Vanua Balavu	1063	1068	829	1077	960	1068	–		
Vanua Levu	1214	1220	950	1232	1108	1014	228	–	
Viti Levu	1382	1387	1133	1397	1277	1192	325	208	–

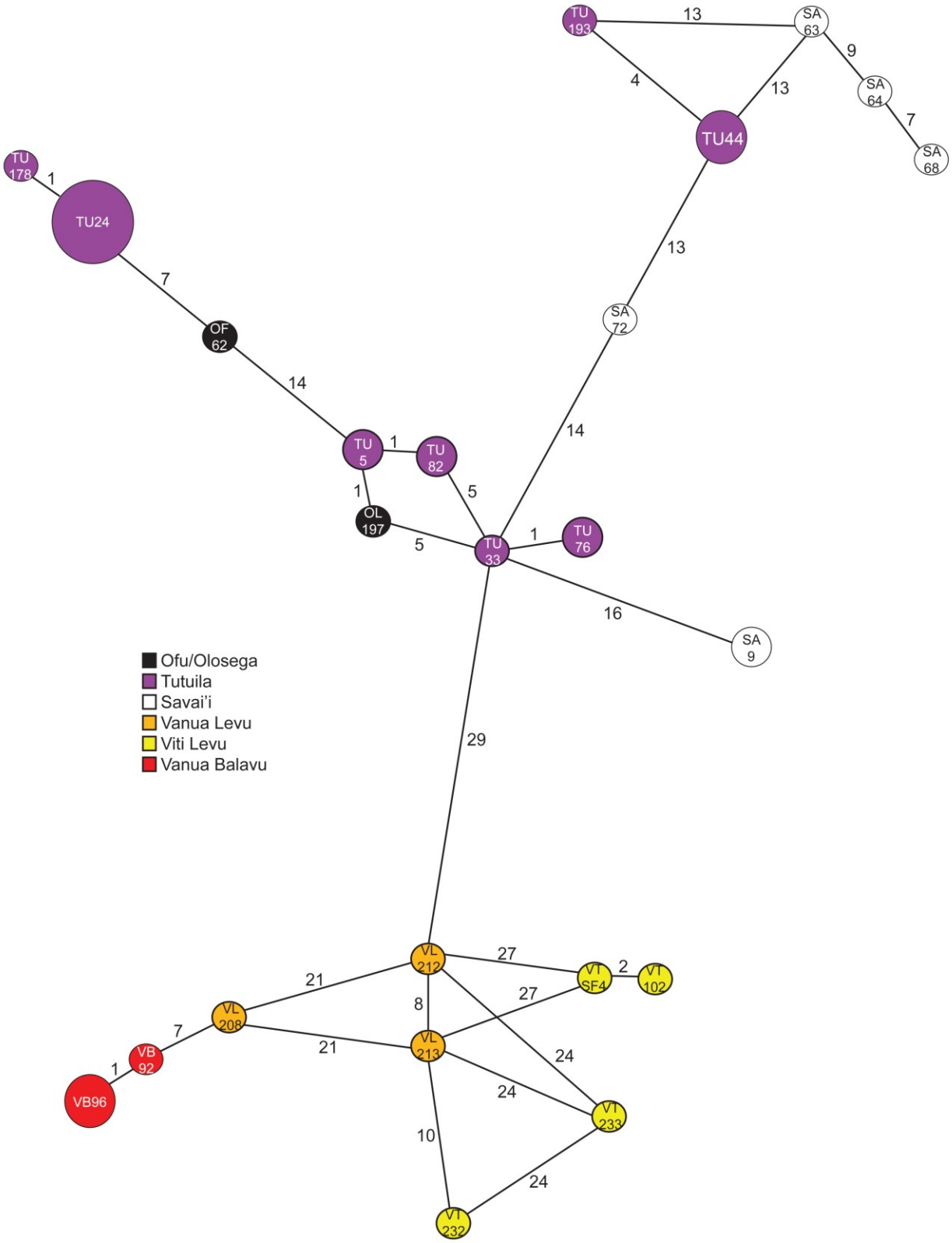

FIG. S1. Minimum spanning network for *Pteropus samoensis*. Circles represent sampled haplotypes, with the size of each circle reflecting its relative frequency in the sample. The number at each edge designates the number of substitutions differentiating neighboring haplotypes

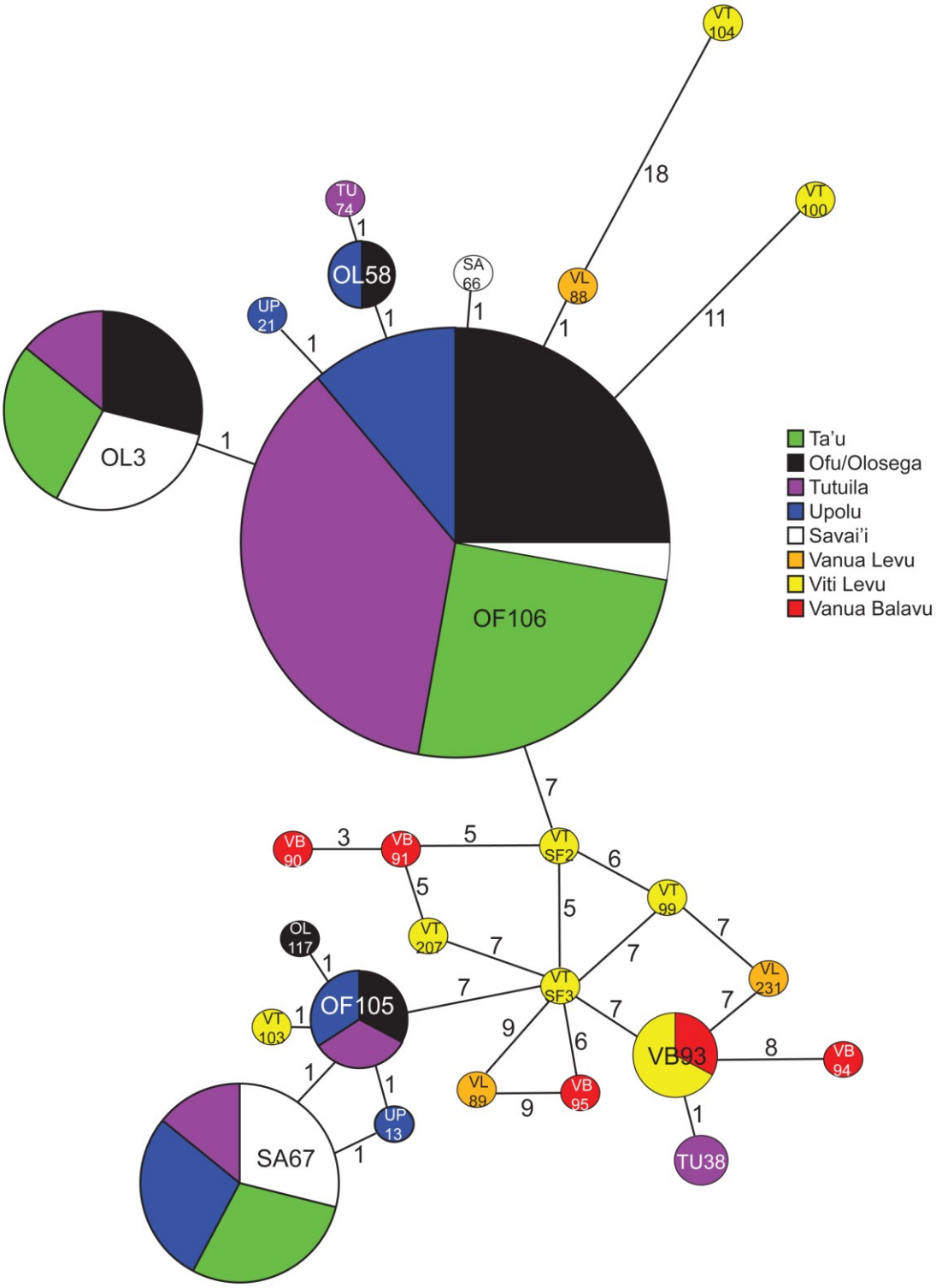

FIG. S2. Minimum spanning network for *P. tonganus*. Circles represent sampled haplotypes, with the size of each circle reflecting its relative frequency in the sample. Haplotypes that were shared among islands are represented as pie charts showing the relative frequency on each island where they were detected. The number at each edge designates the number of substitutions differentiating neighboring haplotypes