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Genetic diversity, population structure, and historical demography of a highly vagile and human-impacted seabird in the Pacific Ocean: The red-tailed tropicbird, *Phaethon rubricauda*

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Abstract

1. Many seabird breeding colonies have recovered from heavy anthropogenic disturbance after conservation actions. The widely distributed red-tailed tropicbird, *Phaethon rubricauda*, was used as a model species to assess potential anthropogenic impacts on the genetic diversity of breeding colonies in the Pacific Ocean.
2. Cytochrome *c* oxidase subunit I and control region sequences analyses were conducted across the range of the species in the Pacific Ocean. The study sites were at islands without human-related disturbance (non-impacted islands) and with human-related disturbance (impacted islands). We hypothesized that (i) breeding colonies of the red-tailed tropicbird on impacted islands have lower genetic diversity compared with colonies on non-impacted islands, and (ii) breeding colonies of the red-tailed tropicbird show significant fine and broad-scale genetic structure across the Pacific Ocean. Bayesian skyline analyses were conducted to infer past changes in population sizes.
3. Genetic diversity was similar between impacted and non-impacted islands. There was significant broad-scale genetic structure among colonies separated by over 6,000 km, but a lack of significant fine-scale genetic structure within Australasia and Hawai'i, although a significant level of differentiation was found within Chile with Φ_{ST} analyses. Skyline analyses showed that effective population sizes

remained relatively constant through time, but experienced either a slight decrease or the end of an expansion event through the last 1,000 years. These changes may be related to the arrival of humans on Pacific islands.
4. Impacted islands may have received immigrants from other relatively close islands, buffering the loss of genetic diversity. However, it is also possible that colonies have retained ancestral variation or that a large effective population size coupled with a long generation time (13 years) has prevented the loss of genetic diversity in human-impacted islands. Future research using higher-resolution markers is needed to resolve the population genetic structure of the red-tailed tropicbird in an ecological time-scale.

KEYWORDS
anthropogenic disturbance, breeding seabirds, conservation actions, dispersal, mtDNA sequences, oceanic islands, population differentiation

1 | INTRODUCTION

Seabird species worldwide have been largely affected by anthropogenic disturbance and are still threatened by human activities such as fisheries by-catch (Zhou, Jiao, & Browder, 2019), manufacturing processes resulting in discharge of marine litter (Battisti et al., 2019), and new ocean infrastructure (Kelsey, Felis, Czapanskiy, Pereksta, & Adams, 2018), among others. The introduction of mammalian predators, including domestic animals and pests, has heavily impacted seabird populations on islands (Bolton, Stanbury, Baylis, & Cuthbert, 2014; Jones et al., 2008; Krajick, 2005). Seabirds are particularly vulnerable to anthropogenic disturbance because they are long-lived and have late sexual maturation, small clutch size, and extended chick-rearing periods (Schreiber & Burger, 2002). They are usually monogamous, have biparental care and exhibit colonial breeding (Schreiber & Burger, 2002).

The conservation of seabirds is important because they are reliable indicators of many aspects of the marine environment, such as food availability (Cairns, 1988). Some species have been considered as ecosystem sentinels based on their capacity to respond to changes in ecosystem structure and function (reviewed by Hazen et al., 2019). Several studies have reported the recovery (i.e. re-colonization and population growth) of heavily-affected seabird populations after the implementation of conservation actions, such as the creation of parks or wildlife refuges, some of which involved the eradication of human-introduced mammalian predators (e.g. Borrelle, Boersch-Supan, Gaskin, & Towns, 2018; Buxton, Anderson, Moller, Jones, & Lyver, 2015; Buxton, Major, Jones, & Williams, 2013; Gaskin, 2011; Hatfield, Reynolds, Seavy, & Krause, 2012; Young, 2010). However, it is likely that the genetic diversity of some recovered populations has been reduced by bottleneck effects resulting from past extirpation or drastic reductions of colonies. In small populations, genetic drift and increased inbreeding can reduce genetic diversity (Allendorf & Luikart, 2007). Therefore, recovered, but isolated populations may be genetically vulnerable to ongoing human-induced impacts and to unpredictable natural disturbance (e.g. El Niño-Southern Oscillation). In this sense, determining genetic diversity and the underlying mechanisms of population differentiation in impacted populations is crucial for informing effective conservation management (Friesen, Burg, & McCoy, 2007).

A model species to assess the potential anthropogenic impacts on the genetic diversity of seabird colonies is the red-tailed tropicbird, *Phaethon rubricauda* (Phaethontiformes). This species is widely distributed throughout tropical regions of the Pacific and Indian Oceans where it breeds on oceanic islands (Fleet, 1974; Schreiber & Burger, 2002). The red-tailed tropicbird is listed as 'least concern' according to IUCN (www.iucn.org) criteria because of its large geographic range and moderately small to large population sizes (BirdLife International, 2019). Hatfield et al. (2012) considered the red-tailed tropicbird and other seabird species on a Hawaiian island to be resilient because populations have grown to large numbers following conservation actions, despite heavy human disturbance in the past. It is worth noting that the red-tailed tropicbird has been reported in recent, historical, and prehistoric records from remote Rapa Nui (Easter Island) while other seabird species were lost following Polynesian colonization (Jaramillo, Johnson, Rothfels, & Johnson, 2008). These reports indicate that red-tailed tropicbird populations were able to persist and recover on highly disturbed islands. The population genetics of the red-tailed tropicbird has not been assessed and the genetic conservation status of this species is unclear.

Red-tailed tropicbirds are highly mobile, and capable of long-distance movements. For example, a banded adult travelled 4,344 km in a 3-year period (Jenkins & Robertson, 1969), a banded chick was recovered as an adult 6,000 km away from its banding site (Le Corre, Salamolard, & Portier, 2003), and another chick banded at Johnston Island was recovered as an adult breeding 1,330 km away, at O'ahu Island (Eric A VanderWerf, unpublished data). However, certain 'mechanisms' including large expanses of low-productivity ocean, high natal philopatry, and population-specific non-breeding areas, may promote and maintain genetic structure in many seabird species

(Friesen et al., 2007). In fact, the red-tailed tropicbird has been considered to be a philopatric species (Schreiber & Schreiber, 1993). Friesen et al. (2007) stated that the red-tailed tropicbird probably shows population genetic structure and suggested that this prediction should be investigated to help define adequate management units. In this regard, morphological differences have been reported among red-tailed tropicbird populations from different regions, even leading to the naming of subspecies (Peters, 1931). Later, Tarburtonv (1989) detected differences in the intensity of the pink suffusion in the streamer, egg size, culmen length, and wing length among breeding populations throughout the species' geographic range, but discarded the existence of subspecies and attributed the differences to possible genetic divergence among populations. Similarly, Ismar et al. (2011) suggested that the morphological distinction of the breeding colony at Kermadec Islands (New Zealand) is the consequence of genetic isolation from other populations. Therefore, given all the above, levels of gene flow among breeding colonies of the red-tailed tropicbird may be low.

The aim of this study was to determine levels of genetic diversity and differentiation among colonies of the red-tailed tropicbird. Mitochondrial DNA sequences were used for a population-level genetic study throughout the range of the species in the Pacific Ocean. The study sites were on islands either never inhabited by humans and free from introduced predators (hereafter non-impacted islands): Salas & Gómez Island (Chile) and Meyer/Herald Islands (Kermadec, New Zealand); inhabited by humans with presence of introduced predators (hereafter impacted islands): Rapa Nui (Chile), Kaua'i and O'ahu (Hawai'i); or never inhabited by humans, but heavily disturbed by introduced predators in the past (hereafter impacted island): Phillip Island, off Norfolk Island (Australia). Among the Chilean islands, the closest to Rapa Nui is Salas & Gómez Island (separated by ca. 370 km). Although Salas & Gómez Island receives large amounts of anthropogenic litter (Luna-Jorquera, Thiel, Portflitt-Toro, & Dewitte, 2019; Miranda-Urbina, Thiel, & Luna-Jorquera, 2015), there are no known direct anthropogenic impacts at Salas & Gómez Island because it has never been occupied by humans, it is difficult to access, and, in contrast to Rapa Nui, is free of introduced mammals. Similarly, Meyer/Herald Islands, located a few kilometres to the northeast of Raoul Island (Kermadec, New Zealand), have never been inhabited by humans and are free of introduced predators (Gaskin, 2011). The Islands of Kaua'i and O'ahu are part of the south-eastern Hawaiian Islands where red-tailed tropicbirds are restricted to breeding on steep coastal cliffs, small islets, and within fenced or otherwise protected areas due to the presence of introduced mammals (VanderWerf & Young, 2014). Finally, Phillip Island, currently part of an Australian National Park, was ecologically devastated by the introduction of mammals, but these were all eradicated by 1988 (Coyne, 2010; Priddel, Carlile, Evans, Evans, & McCoy, 2010).

The hypotheses to be tested in this study were: (i) breeding colonies of the red-tailed tropicbird on impacted islands have lower genetic diversity compared with colonies on non-impacted islands; and (ii) breeding colonies of the red-tailed tropicbird show significant fine- and broad-scale genetic structure across the Pacific Ocean.

2 | METHODS

2.1 | Sampling and DNA extractions

Blood samples were obtained from 149 specimens from six islands in the Pacific Ocean, hereafter grouped in three regions: Chile, Australasia, and Hawai'i (Table 1, Figure 1). In Phillip, Meyer, and Salas & Gómez islands (Figure 1), birds were sampled across the island where they were accessible. In Kaua'i and O'ahu (Figure 1), birds were sampled at Kilauea Point National Wildlife Refuge, Lehua Islet, and Halona Point. Samples from Rapa Nui (Figure 1) were obtained from the colony at the Rano Raraku volcano, on the south-east side of the Island. Adult birds were captured by hand at their nests and their head was covered with a cotton bag to reduce their vision and therefore reduce manipulation stress. A small blood sample was taken from the brachial or tarsal vein with a hypodermic syringe or by puncture and collection in a capillary tube then spread on an FTA card (Whatman paper, GE Healthcare Life Sciences). The blood was air-dried, and cards were stored at ambient temperature. Only samples from New Zealand were preserved in Seutin buffer (Seutin, White, & Boag, 1991). Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen).

2.2 | Polymerase chain reaction amplification, sequencing, and alignment

Polymerase chain reaction (PCR) primers to amplify a segment of the cytochrome *c* oxidase subunit I (COI) gene and the control region were designed using Primer3 (Untergasser et al., 2012) and the complete mitochondrial sequences of *P. rubricauda* (GenBank® Accession Number: AP009043.1) and *Phaethon lepturus* (GenBank® Accession Number: KR349465.1). The COI primers used were PrCOI_F1-5´-GAT CTG TAC TCA TCA CCG CC-3´ and PrCOI_R1-5´-GAT GCA GTG TGT ACC CTG AG-3´, and the control region primers were PrCR_F1-5´-ACA GCA AAT TAG ACC TCC CC-3´ and PrCR_R1-5´-CCT GAA GCT AGT AAC GCA GG-3´. A 600-bp fragment of the COI gene was amplified in 149 individuals (Table 1) and a 260-bp fragment of the control region was amplified in 143 individuals (Table 1). Although both genes are physically linked as they are both part of the mitochondrial genome, analyses were done separately as the COI gene is a protein-coding region evolving slower than the non-coding control region.

Reactions of 30 μL total volume for partial amplification of both genes consisted of ~300 ng of DNA, 1× PCR buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl, 0.1% stabilizer), 2 mM $MgCl_2$, 0.6 μM of each primer, 0.25 mM of each dNTPs, 3 U of Taq polymerase, and 0.4 mg mL^{-1} of bovine serum albumin. PCR cycles for both genes were performed on an Agilent SureCycler 8800 Thermal Cycler, as follows: 95°C for 2 min, followed by 35 cycles of 94°C for 40 s, 50°C for 40 s, 72°C for 40 s, and a final extension at 72°C for 10 min. PCR amplification was verified using 1% agarose gels. PCR products were purified and sequenced by Macrogen Inc (Seoul, South Korea). Sequences were aligned using the software Geneious R10 (https://www.geneious.com).

TABLE 1 Sampling sites by region, codes and diversity indices for cytochrome *c* oxidase subunit I (COI) and control region (CR) sequences of the red-tailed tropicbird, *Phaethon rubricauda*

Gene	Region	Sampling site	Code	Impacted islands	*n*	*S*	*Nh*	*h*	π
COI	Chile	Rapa Nui	RN	Yes	44	13	8	0.854	0.0061
		Salas & Gomez Island	SG	No	32	7	5	0.712	0.0026
	Australasia	North Meyer Islet, New Zealand	Mey	No	11	5	3	0.582	0.0027
		Phillip Island, Australia	Phi	Yes	25	6	4	0.547	0.0036
	Hawai'i	Kaua'i Island	Kau	Yes	24	12	5	0.540	0.0059
		O'ahu Island	Oah	Yes	13	11	4	0.603	0.0064
		Total			149	18	13	0.822	0.0074
CR	Chile	Rapa Nui	RN	Yes	39	13	9	0.803	0.0119
		Salas & Gomez Island	SG	No	33	11	12	0.769	0.0089
	Australasia	North Meyer Islet, New Zealand	Mey	No	12	11	6	0.848	0.0129
		Phillip Island, Australia	Phi	Yes	25	12	10	0.890	0.0166
	Hawai'i	Kaua'i Island	Kau	Yes	22	13	11	0.857	0.0126
		O'ahu Island	Oah	Yes	12	8	5	0.803	0.0106
		Total			143	20	30	0.907	0.0165

Impacted islands are those that have suffered anthropogenic impacts, as stated in the introduction.
Abbreviations: *n*, number of samples; *S*, number of segregating sites; *Nh*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity

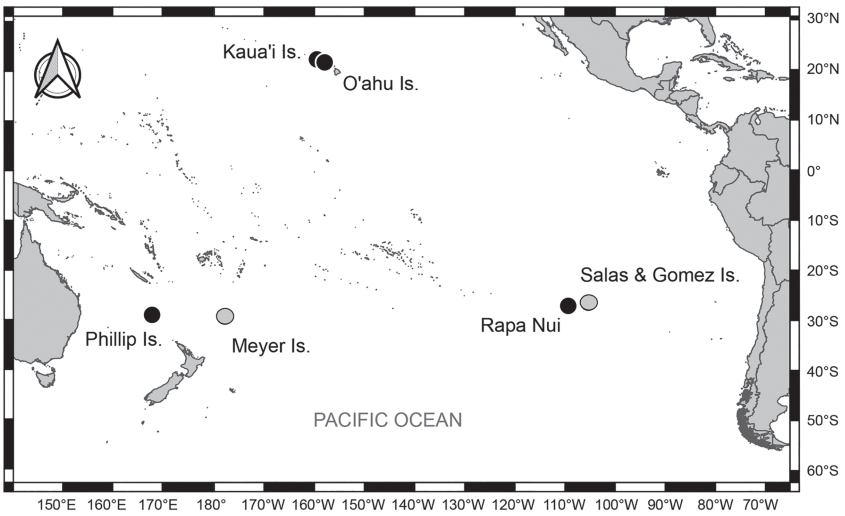

FIGURE 1 Map showing the sampling sites in Chile, Australasia, and Hawai'i for the red-tailed tropicbird, *Phaethon rubricauda*. Black-filled circles indicate impacted islands (islands that have suffered direct anthropogenic impacts), and grey-filled circles indicate non-impacted islands (never inhabited by humans and free from introduced predators)

2.3 | Data analyses

Genetic diversity indices, the number of segregating sites (*S*), the number of haplotypes (*Nh*), haplotype diversity (*h*), and nucleotide diversity (π) were calculated in DnaSP 6.11.01 (Rozas et al., 2017). Pairwise genetic differentiation using the index Φ_{ST} between sampling sites were estimated using the software ARLEQUIN 3.5.2.2 (Excoffier, Laval, & Schneider, 2005) with pairwise difference as the distance method and 20,000 permutations. ARLEQUIN was also used to perform a Mantel test to evaluate a pattern of isolation by distance. The Mantel test was performed with 10,000 permutations using linearized Φ_{ST} values and the shortest marine distance (ln m) between sites as estimated using Google Earth Pro 7.3.2.

The relationships between COI and control region haplotypes were assessed with haplotype networks using the TCS method (Clement, Posada, & Crandall, 2000) as implemented in PopArt (http://popart.otago.ac.nz). The software BAPS 6 was used to perform a Bayesian analysis of population structure under a population mixture analysis with the spatial clustering of individuals model (Corander, Siren, & Arjas, 2008) and using all the sequences obtained for both genes (i.e. not only haplotypes). The upper bound to the number of populations was set to six.

Tajima's (*D*) (Tajima, 1989) and Fu's (*Fs*) (Fu, 1997) tests were conducted in DnaSP to assess neutrality and as a first estimate of potential population sizes changes in the past. Bayesian skyline analyses (Drummond, Rambaut, Shapiro, & Pybus, 2005) were implemented in the software BEAST 1.10.4 (Suchard et al., 2018) to further infer potential historical fluctuations in effective population size (*Ne*). Analyses were performed for each region considering the results of the genetic structure analyses. All analyses were run under

the Jukes–Cantor model for COI and control region sequences, which was the best nucleotide substitution model determined with jModelTest 2.1.10 (Darriba, Taboada, Doallo, & Posada, 2012) for both genes. The mutation rate used was 1.8%/My for the COI gene (Lavinia, Kerr, Tubaro, Hebert, & Lijtmaer, 2016) and 20.8%/My for the control region (Quinn, 1992; Ritchie, Millar, Gibb, Baroni, & Lambert, 2004) under the strict clock model. The analyses were run for 100,000,000 iterations sampled every 10,000 iterations with a burn-in of 10%. All operators were optimized automatically. Adequate mixing of the Markov chain Monte Carlo was examined using TRACER 1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) inspecting that effective sample sizes were >200. To ensure convergence, two independent runs were performed for each dataset and the results combined using LogCombiner 1.10.4 (Suchard et al., 2018). The skyline plots where obtained using TRACER.

3 | RESULTS

COI and control region sequences were obtained for 149 and 143 individuals, respectively. As expected, given the higher mutation rate of the control region, diversity indices were higher for the control region compared to the COI gene (Table 1). The analyses of COI sequences resulted in 18 segregating sites and 13 haplotypes. Haplotype and nucleotide diversities ranged from 0.540 to 0.854 and from 0.0026 to 0.0064, respectively (Table 1). The analyses of control region sequences resulted in 20 segregating sites and 30 haplotypes. Haplotype and nucleotide diversities ranged from 0.769 to 0.890 and from 0.0089 to 0.0166, respectively (Table 1). Diversity indices for both genes were similar between impacted and non-impacted islands (Table 1). Haplotypes sequences were deposited in GenBank® (Accession numbers MN198934–MN198946 for the COI gene and MN198947–MN198976 for the control region).Pairwise Φ_{ST} values for both markers revealed significant genetic differentiation among sampling sites from the three regions and between Rapa Nui and Salas & Gómez Island (within Chile), although the Φ_{ST} value was lower compared with the inter-region comparisons (Tables 2 and 3). There was a lack of significant genetic differentiation within the other two regions, Australasia (between Phillip and Meyer islands) and Hawai'i (between Kaua'i and O'ahu islands) (Tables 2 and 3). These results coincide with a pattern of isolation by distance detected with mantel tests (COI: $r = 0.80$, $P = 0.038$; control region: $r = 0.79$, $P = 0.036$). In the COI network (Figure 2), the haplotype with highest frequency was found in individuals from all six islands. However, there was a slight differentiation of haplogroups by region, which was also detected in the control region network (Figure 3). A Bayesian analysis of population structure corroborated the regional differentiation detected by the Φ_{ST} analyses. Both COI and control region sequences revealed the existence of three genetic clusters, each representing one of the regions (Figure 4, only the plot for the COI gene is presented as the plot for the control region showed the same results).

Tajima's (*D*) and Fu's (*Fs*) analyses indicated that sequences of both genes did not deviate from neutrality and did not indicate expansion or reduction events for any of the three regions (Table 4). Only one value was significant, the Fu's (*Fs*) obtained with the analysis of control region sequences for Australasia. Regarding the Bayesian skyline analyses, the two genes gave different signals about changes in population sizes through time for two of the regions. For Chile and Hawai'i, the COI gene showed that population sizes were relatively constant through time up to around 5,000–10,000 years ago, then the effective population sizes dropped, with a more pronounced decrease in Chile compared to Hawai'i (Figure 5a, c). Interestingly, the control region showed a population expansion event starting around 2,500 and 7,000 years ago for Chile and Hawai'i, respectively. These expansion events reached a plateau through the present for both regions (Figure 5 d, f). By contrast, the skyline analyses for Australasia showed that effective population sizes were relatively constant through time and experienced a slight reduction around 1,000 years ago through to the present for both genes (Figure 5 b, e).

4 | DISCUSSION

The analyses of COI and control region sequences of the red-tailed tropicbird from Chile, Australasia, and Hawai'i showed that breeding colonies on impacted islands did not have a lower genetic diversity compared with colonies on non-impacted islands, which is contrary to our first hypothesis. Regarding our second hypothesis, we found significant broad-scale genetic structure across the Pacific Ocean (i.e. among the regions), but not at a fine-scale (i.e. within the regions), although a significant level of differentiation was found within Chile with Φ_{ST} analyses.

4.1 | Fine-scale assessment

The lack of evidence for anthropogenic impact on the genetic diversity of red-tailed tropicbird colonies at impacted islands (in this study, Rapa Nui, Phillip, Kaua'i, and Oha'u islands) may indicate that at least limited gene flow exists within the regions. It is possible that impacted islands have received immigrants from other relatively close, non-impacted islands, thereby buffering the loss of genetic diversity (e.g. Lombal et al., 2020; Ramírez et al., 2013). Nevertheless, these results should be viewed with caution when considering management actions because it is possible that red-tailed tropicbird breeding colonies within the regions have retained ancestral variation and experienced a recent process of divergence that was undetected using mitochondrial DNA sequences (see Friesen et al., 2007). Moreover, mitochondrial DNA sequence variation reflects only female-mediated gene flow. It is also possible that a large effective population size coupled with the long generation time of this species (13 years, Birdlife International, 2019) has prevented the loss of genetic diversity in human-impacted islands (e.g. Lombal et al., 2020; Welch et al., 2012). Given all the above, we will investigate levels of genetic diversity and population differentiation using genome-wide single nucleotide polymorphisms (SNPs), across the same breeding colonies surveyed here.

TABLE 2 Pairwise Φ_{ST} values for the cytochrome *c* oxidase subunit I sequences between sampling sites (below diagonal) and *P* values (above diagonal)

	RP	SGI	Mey	Phi	Kau	Oah
RP		0.007	<0.0001	<0.0001	<0.0001	<0.0001
SGI	**0.06***		<0.0001	<0.0001	<0.0001	<0.0001
Mey	**0.34**	**0.59**		0.26	<0.0001	0.0001
Phi	**0.23**	**0.41**	0.01		<0.0001	<0.0001
Kau	**0.38**	**0.61**	**0.61**	**0.59**		0.89
Oah	**0.36**	**0.63**	**0.60**	**0.58**	0	

Note: Codes as in Table 1. Significant Φ_{ST} values are shown in bold. The significance was tested using 20,000 permutations.
*Significant differentiation after sequential Bonferroni correction ($P < 0.016$).

TABLE 3 Pairwise Φ_{ST} values for the control region sequences between sampling sites (below diagonal) and *P* values (above diagonal)

	RP	SGI	Mey	Phi	Kau	Oah
RP		0.015	0.00005	0.0002	<0.0001	0.00005
SGI	**0.06***		<0.0001	<0.0001	<0.0001	<0.0001
Mey	**0.41**	**0.53**		0.23	<0.0001	<0.0001
Phi	**0.20**	**0.30**	0.02		<0.0001	<0.0001
Kau	**0.24**	**0.43**	**0.45**	**0.33**		0.79
Oah	**0.30**	**0.49**	**0.50**	**0.36**	0	

Note: Codes as in Table 1. Significant ΦST values are shown in bold. The significance was tested using 20,000 permutations.
*Significant differentiation after sequential Bonferroni correction ($P < 0.016$).

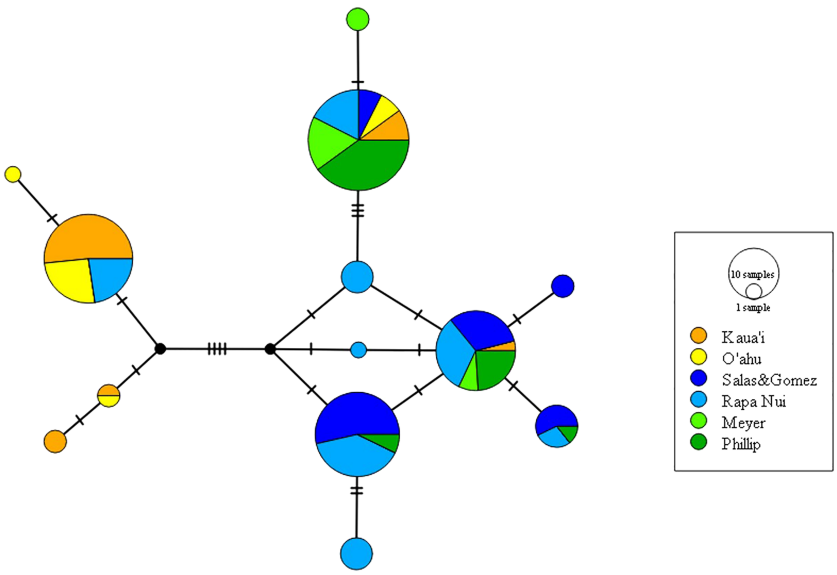

FIGURE 2 Cytochrome *c* oxidase subunit I haplotype network of the red-tailed tropicbird, *Phaethon rubricauda*. The circles represent the haplotypes and the colours the sampling locations as shown in the legend. The scale on the right side of the figure indicates the relationship between the size of the circles and the frequency of the haplotypes. Lines on connecting branches represent mutations and black dots represent inferred intermediate steps

There are few population genetic studies within the regions considered here for highly vagile seabird species. Within the Australasia region as defined here, there are several seabird species that breed at both Norfolk islands (which include Phillip Island) and Kermadec Islands (which include Meyer/Herald islands), including the only two populations in the world of the white-necked petrel, *Pterodroma cervicalis* (Priddel et al., 2010). To the best of our knowledge, there are no published studies assessing population differentiation in seabirds between these two island groups. Studies within Australasia involving other islands are scarce. For example, a population genetic study using mtDNA sequences, nuclear introns, and microsatellite loci found that the small population of the providence petrel, *Pterodroma solandri* at Phillip Island is at a low genetic risk because it is genetically connected to the founder and larger population located ca. 900 km southwest, at Lord Howe Island (Lombal et al., 2017). By contrast, significant genetic structure was found in the flesh-footed shearwater, *Ardenna carneipes*

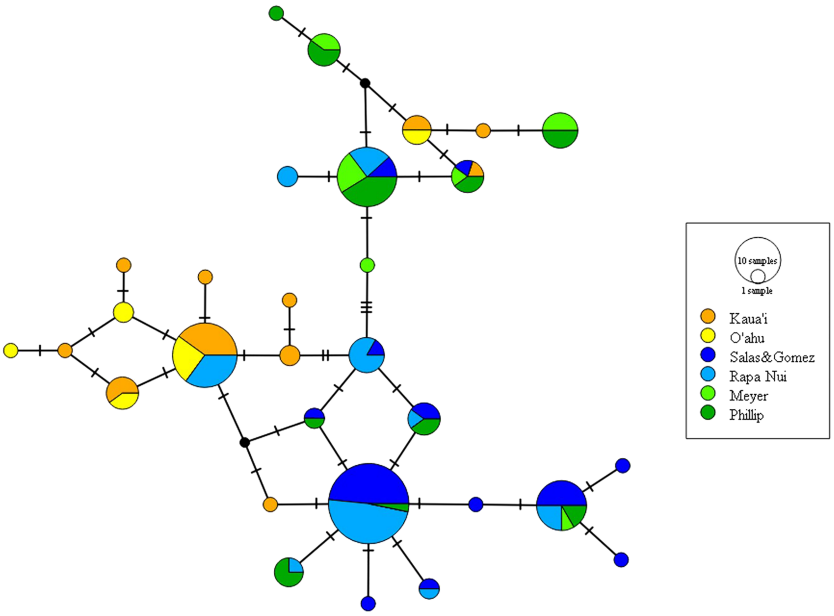

FIGURE 3 Control region haplotype network of the red-tailed tropicbird, *Phaethon rubricauda*. The circles represent the haplotypes and the colours the sampling locations as shown in the legend. The scale on the right side of the figure indicates the relationship between the size of the circles and the frequency of the haplotypes. Lines on connecting branches represent mutations and black dots represent inferred intermediate steps

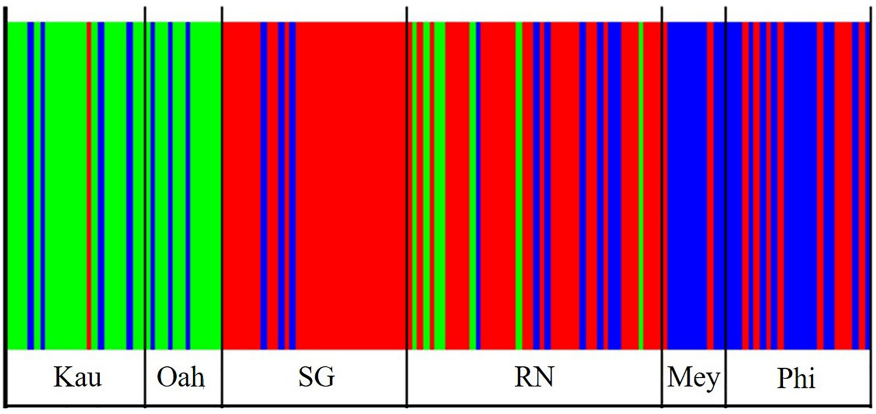

FIGURE 4 Population assignment of individuals (vertical lines) to putative population clusters based on Bayesian analysis of population structure (BAPS software) for cytochrome *c* oxidase subunit I sequences of the red-tailed tropicbird, *Phaethon rubricauda*. The plot was obtained using mixture analysis and the spatial clustering of individuals model. The resulting clusters are represented by colours and coincide with the three regions: green for Hawai'i, red for Chile and blue for Australasia. Codes as in Table 1

TABLE 4 Values and probabilities of Tajima's (*D*) and Fu's (*Fs*) tests of the cytochrome *c* oxidase subunit I (COI) and control region (CR) sequences of the red-tailed tropicbird, *P. rubricauda* in each region

Gene	Region	Tajima's (*D*)	*P*	Fu's (*Fs*)	*P*
COI	Chile	0.02	>0.10	1.20	>0.10
	Australasia	0.55	>0.10	0.63	>0.10
	Hawai'i	0.44	>0.10	0.62	>0.10
CR	Chile	−0.22	>0.10	0.35	>0.10
	Australasia	1.28	>0.10	1.66	<0.05
	Hawai'i	−0.37	>0.10	−0.99	>0.10

between Lord Howe Island and New Zealand (Lombal et al., 2018). Differences in foraging area during the breeding season was considered as the main factor influencing the divergence between those two populations rather than the geographic distance between them (Lombal et al., 2018).

Seabird population genetic studies considering Hawaiian Islands have reported high levels of gene flow within the north-western Hawaiian Islands in the black-footed albatross, *Phoebastria nigripes*, using microsatellite loci (Ando et al., 2014) and SNPs (Dierickx, Shultz, Sato, Hiraoka, & Edwards, 2015). In contrast, using mitochondrial DNA sequences, nuclear introns, and microsatellite loci, significant genetic differentiation was reported within south-eastern Hawaiian Islands (also called Main Hawaiian Islands) in the endemic Hawaiian petrel, *Pterodroma sandwichensis* (Welch et al., 2012). Population-specific non-breeding distributions or strong natal philopatry were considered the most likely mechanisms for genetic isolation among populations even at very short geographic distances (i.e. 75 km) (Welch, Fleischer, et al., 2012). The breeding colonies of the red-tailed tropicbird assessed in this study at Kaua'i and O'ahu (impacted islands) are part of the Main Hawaiian Islands. It is possible that the colonies at Kaua'i and O'ahu receive immigrants from the relatively close breeding colonies found in the north-western Hawaiian Islands, which have been the focus of conservation efforts in recent decades (Hatfield et al., 2012). This has been reported for another vagile seabird species, the Laysan albatross, *Phoebastria immutabilis* (Young, 2010). Using control region sequences, microsatellite DNA loci, and banding records, Young (2010) found Laysan albatross from

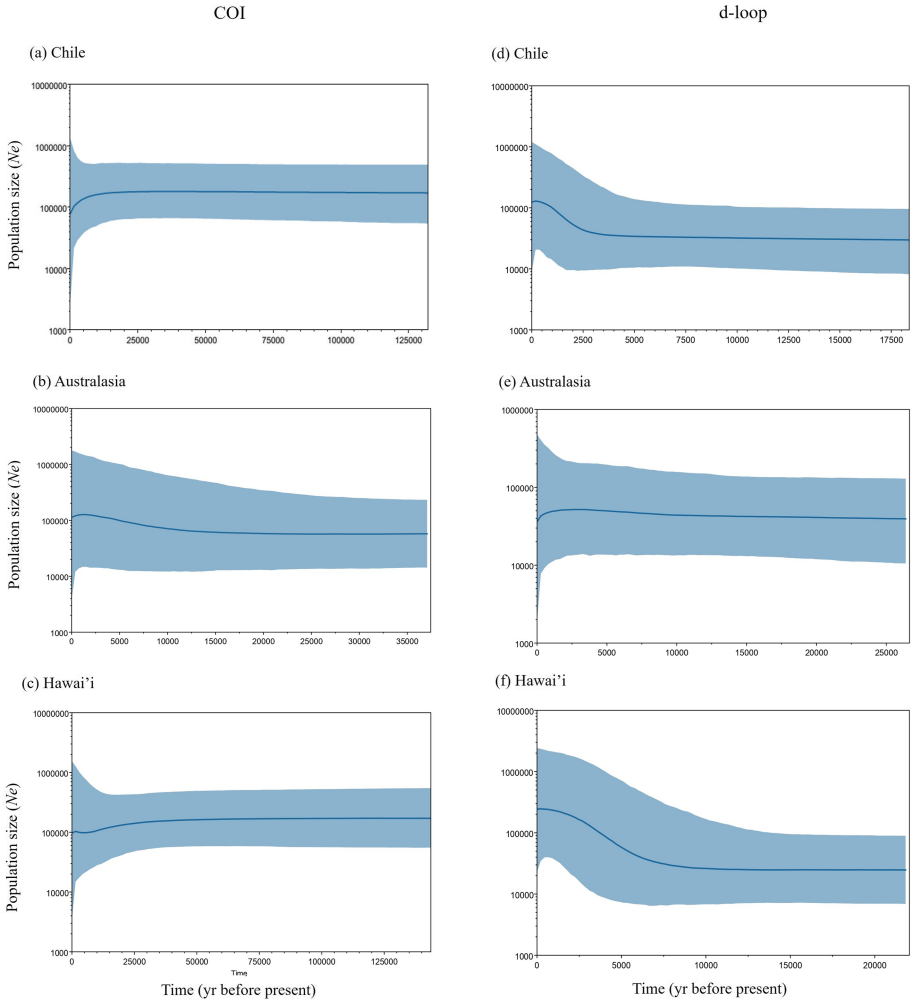

FIGURE 5 Bayesian skyline plots showing changes in effective population size (*Ne*) through time for each region using a mutation rate of 1.8%/My for the cytochrome *c* oxidase subunit I (COI) sequences (a–c), and a mutation rate of 20%/My for the control region sequences (d–f). The line indicates the median estimate and the shadow area represents the 95% upper and lower confidence limits

the north-western Hawaiian Islands recolonized Kaua'i, O'ahu, and Lehua islands, where colonies were extirpated in the past (before 1970s) due to high human disturbance. However, a potential genetic connectivity between red-tailed tropicbird colonies at Kaua'i and O'ahu remains to be investigated.The absence of anthropogenic impact on the genetic diversity of the breeding colony at Rapa Nui may not be explained by a pattern of genetic connectivity between Rapa Nui (impacted island) and Salas & Gómez (non-impacted island). Although the Bayesian analysis of population structure showed that one genetic cluster was composed mainly of the individuals from Rapa Nui and Salas & Gómez, the Φ_{ST} analyses between these islands showed significant genetic differentiation. However, the Φ_{ST} value (0.06 for COI and control region) was considerably lower compared to the Φ_{ST} values between islands from the different regions (0.23 to 0.63 for COI and 0.20 to 0.53 for control region). Nevertheless, there may be mechanisms that limit the dispersal of the red-tailed tropicbird between Rapa Nui and Salas & Gómez. One possible explanation for the significant differentiation between the breeding colonies at these islands may be differences in foraging grounds during the breeding or non-breeding seasons, as inferred for other seabird species (e.g. Lombal et al., 2018; Welch, Fleischer, et al., 2012). However, there are no studies comparing foraging strategies among these two red-tailed tropicbird colonies. The absence of anthropogenic impact on the genetic diversity of Rapa Nui may have any of four explanations: (i) recent divergence and retained ancestral variation; (ii) a large effective population size coupled with a long generation time has prevented the loss of genetic diversity; (iii) some level of male-mediated immigration from Salas & Gómez island sufficient to buffer the loss of genetic diversity; or (iv) Rapa Nui has been receiving immigrants from other, more distant breeding colonies that were not included in this study. Red-tailed tropicbirds in the southern Pacific Ocean also breed at Desventuradas Islands (Aguirre, Johow, Seeger, Johow, & Rubio, 2009) and Pitcairn islands (de Brooke, 1995) separated from Rapa Nui by ca. 2,900 km to the west and 1,500 km to the east, respectively.

4.2 | Broad-scale assessment

Although the haplotypes with higher frequencies (probably ancestral haplotypes) were found in individuals from the different regions, pairwise genetic differentiation and Bayesian analyses of population structure significantly differentiated the three regions. This broad population structure may be related to the detected pattern of

isolation by distance. However, geographic distance alone may not be the factor limiting the dispersal of red-tailed tropicbirds. The regions considered in this study are separated by 6,000–8,000 km and there are records of individuals dispersing over 4,000 km using band-recovery data (Jenkins & Robertson, 1969; Le Corre et al., 2003). Tracking records of red-tailed tropicbirds using global location sensing loggers in the Indian Ocean, showed that individuals marked on Europa Island and Nosy Be, Madagascar, mostly stay around Madagascar during the breeding season and disperse all through the Indian Ocean (up to ca. 6,000–7,000 km) during the non-breeding period (data owner: Matthieu Le Corre, Birdlife International Seabird Tracking Database, 2019). Therefore, although the red-tailed tropicbird can disperse widely, there may be mechanisms other than the geographic distance alone that have historically limited gene flow among the regions. It is possible that large expanses of low-productivity ocean together with other mechanisms such as non-breeding distribution promote genetic differentiation among the regions in the red-tailed tropicbird in the Pacific Ocean. Both factors have been reported to limit gene flow in seabirds (Friesen et al., 2007). However, the distribution of red-tailed tropicbirds during breeding and non-breeding periods across the Pacific Ocean is largely unknown (but see Adams, Felis, & Czapanskiy, 2020). The significant genetic differentiation among the regions found in this study is consistent with previous studies that reported morphological differences among red-tailed tropicbird populations from different regions (Ismar et al., 2011; Peters, 1931; Tarburton, 1989). This broad-scale population structure will be further assessed using SNPs loci as this higher-resolution molecular marker will allow to assess if levels of population differentiation have changed in a more recent (ecological) time scale.

4.3 | Past changes in population sizes

Overall, demographic analyses showed that red-tailed tropicbird population sizes in the three regions have been relatively constant through time. However, skyline analyses showed either, a slight decrease in effective population size or the end of a population expansion event through the last 1,000 years. These changes in effective population sizes may be related to the high anthropogenic disturbance that Pacific oceanic islands experienced after human arrival which started about 3,500 years ago in west Polynesia and Micronesia reaching almost all of the Pacific islands by about 1,000 years ago, causing extinctions, extirpations, and reductions of bird's populations (Steadman, 1995).

4.4 | Conservation implications

Although there was a lack of evidence for anthropogenic impact on the genetic diversity of red-tailed tropicbird colonies at highly human-disturbed islands, we suggest that each island should be considered as a unique evolutionary unit for conservation plans and management of red-tailed tropicbird colonies, at least until further research using higher resolution markers confirm a lack of population differentiation among islands in an ecological time-scale. This would be the best precautionary approach considering that red-tailed tropicbird colonies have been shown to be vulnerable to anthropogenic disturbance and populations have been extirpated or highly reduced in the past (e.g. Gaskin, 2011; Hatfield et al., 2012; Jaramillo et al., 2008; VanderWerf & Young, 2014). In this sense, we highlight the importance to preserve the red-tailed tropicbird colonies at Rapa Nui. This island is one of the few breeding sites of this species in Chile and is threatened by introduced species (Flores, Lazo, Campbell, & Simeone, 2017; Luna, Brokordt, & Luna-Jorquera, 2018; Varela, Luna, & Luna-Jorquera,- 2018). This study showed a lack of female-mediated gene flow with the closest breeding colony found at Salas & Gómez island. Potential genetic connectivity with the other two relatively close breeding areas (Desventuradas and Pitcairn islands) is unknown.

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