



RESEARCH ARTICLE

## Conservation genetics of threatened Red-billed Tropicbirds and White-tailed Tropicbirds in the southwestern Atlantic Ocean

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### ABSTRACT

Seabirds are of considerable conservation significance due to their key role in ecosystems and their generally high level of threat, especially from invasive species in breeding sites and as a result of bycatch during commercial fishing activities. We examined the genetic diversity, evidence for a bottleneck, and effective population size ( $N_e$ ) of Red-billed Tropicbirds (*Phaethon aethereus*) from the Abrolhos Archipelago and White-tailed Tropicbirds (*P. lepturus*) from Fernando de Noronha Archipelago, both in the southwestern Atlantic Ocean. In addition, intraspecific genetic distance was assessed between 2 Red-billed Tropicbirds breeding on Fernando de Noronha and the larger population on Abrolhos, and between 5 White-tailed Tropicbird individuals breeding on Abrolhos and the breeding population on Fernando de Noronha, to identify relict populations. From 11 microsatellite loci, observed heterozygosity was 0.471 for White-tailed Tropicbirds and 0.267 for Red-billed Tropicbirds. Additionally,  $N_e$  was estimated to be as low as 100–200 individuals for each species, ~25–50% of recent census estimates for the largest population of each species. No deviation from mutation-drift equilibrium was detected, but both species had  $M$ -ratios indicative of populations that had experienced a bottleneck or been recently established, and seem to have persisted as small populations over the past few centuries on both archipelagos. Small population size coupled with low evolutionary potential make these populations vulnerable to extinction. In our study sites and worldwide, tropicbirds show slow population growth and vulnerability to introduced predators. These species would benefit from both colony-based management and a better understanding of patterns of genetic diversity within and among populations.

**Keywords:** bottleneck, effective population size, genetic diversity, *Phaethon aethereus*, *Phaethon lepturus*, seabirds, microsatellites

### Genética da conservação de *Phaethon aethereus* e *P. lepturus* ameaçados de extinção no Oceano Atlântico sul-ocidental

#### RESUMO

A conservação das aves marinhas é importante devido ao seu papel chave nos ecossistemas e geralmente elevado grau de ameaça de extinção, ocasionado especialmente por espécies invasoras nos locais de reprodução e captura incidental em pescarias comerciais. Neste estudo, duas populações de *Phaethon aethereus* e *P. lepturus* de Abrolhos e Fernando de Noronha, ambas no Oceano Atlântico sudoeste, foram amostradas para investigar a diversidade genética, ocorrência de gargalo populacional, e tamanho efetivo ( $N_e$ ). Também foi medida a distância genética intraespecífica de populações relictuais a partir de 2 *P. aethereus* reproduzindo em Fernando de Noronha e 5 *P. lepturus* reproduzindo em Abrolhos. A partir dos 11 microssatélites analisados, a heterozigosidade observada (*P. lepturus* = 0,471; *P. aethereus* = 0,267) ficou abaixo dos valores reportados para aves classificadas como em perigo ou criticamente em perigo globalmente. Além disso, o  $N_e$  foi estimado em 100–200 indivíduos, cerca de 25–50% dos tamanhos populacionais estimados através de censos recentes das populações maiores de ambas as espécies. Desvios quanto ao equilíbrio entre mutação-deriva não foram detectados, mas ambas as espécies apresentaram razões- $M$  características de populações que enfrentaram gargalo populacional histórico ou que foram fundadas recentemente, e parecem ter tamanhos populacionais pequenos há alguns séculos em ambos os arquipélagos. Tamanhos populacionais pequenos em conjunto com baixo potencial evolutivo tornam estas populações mais suscetíveis à extinção. Nos locais de estudo e também globalmente, *Phaethon* spp. têm demonstrado crescimento populacional lento e vulnerabilidade a predadores introduzidos. Estas espécies serão beneficiadas por

monitoramento das colônias e um melhor entendimento dos padrões de diversidade genética dentro e entre as populações.

*Palavras-chave:* diversidade genética, gargalo de garrafa populacional, tamanho efetivo da população, microssatélites, aves marinhas

## INTRODUCTION

Protecting seabird populations and their breeding sites is critical for ecosystem-based management and for maintaining marine biodiversity (Ronconi et al. 2012). In general, seabirds forage over wide areas around colonies (Ceia et al. 2014, Soanes et al. 2016) and directly influence terrestrial food webs by transporting marine matter to breeding sites (Caut et al. 2012). Intraspecific genetic variation is generally high in seabirds, and even geographically close populations may have distinct gene pools and represent evolutionarily significant units (Friesen et al. 2007, Friesen 2015). Seabirds currently represent the bird group most threatened by extinction, with the highest rates of population declines among all bird taxa (Croxall et al. 2012). Major threats include introduced predators in breeding sites (Fukami et al. 2006) and incidental mortality associated with commercial fishing (Bugoni et al. 2008).

Decreasing population size over several generations can lead to inbreeding and loss of genetic diversity (Hartl and Clark 2007), with a corresponding loss of evolutionary potential (Frankham et al. 1999). Depressed genetic diversity can also increase the probability of local extinction, such that ignoring genetic aspects can make management actions ineffective (Frankham 2005, Palstra and Ruzzante 2008). Informative molecular markers (e.g., simple sequence repeats) and statistical techniques can be used to identify past population declines based on contemporary genetic diversity, providing managers with a better understanding of seabird conservation status (Taylor and Friesen 2012).

The Brazilian Red List of threatened species includes 17 seabird species, 7 of which nest on Brazilian islands (MMA 2014). This includes 2 pantropical colonial tropicbirds (Order Phaethontiformes), whose only breeding colonies in the southwestern Atlantic Ocean are the Abrolhos and Fernando de Noronha archipelagos. The Red-billed Tropicbird (*Phaethon aethereus*) breeds mainly in burrows on Abrolhos, with a few individuals breeding in cliffs on Fernando de Noronha (Mancini et al. 2016). Conversely, Fernando de Noronha is the main breeding site for the White-tailed Tropicbird (*Phaethon lepturus*), although a few individuals have been recorded to nest in open burrows on Abrolhos (Leal et al. 2016).

Both species are globally classified as Least Concern by the IUCN due to their large geographic ranges and population sizes (>50,000 for White-tailed Tropicbird and 5,000–20,000 for Red-billed Tropicbird; BirdLife

International 2013a, 2013b). However, both species are decreasing globally due to predation by invasive species (BirdLife International 2013a, 2013b). This global trend is repeated on the Abrolhos and Fernando de Noronha archipelagos, which host introduced rats, cats, and lizards. These threats are reducing breeding success and causing populations to decline on these archipelagos, as is also occurring in other tropicbird populations worldwide (Lee and Walsh-McGehee 2000, Catry et al. 2009, Sarmiento et al. 2014, Leal et al. 2016). In addition to invasive species, Abrolhos and Fernando de Noronha have a long history of threats to seabirds from human activities. Indeed, diaries of sailors from the early 16<sup>th</sup> century document the use of seabirds for food (d'Abbeville et al. 1963, Keynes 2001, Markham 2011). Moreover, Fernando de Noronha was a prison camp for more than 200 yr (1737–1942), during which time local vegetation was almost completely suppressed to avoid prisoner escape (Lins-e-Silva 2003).

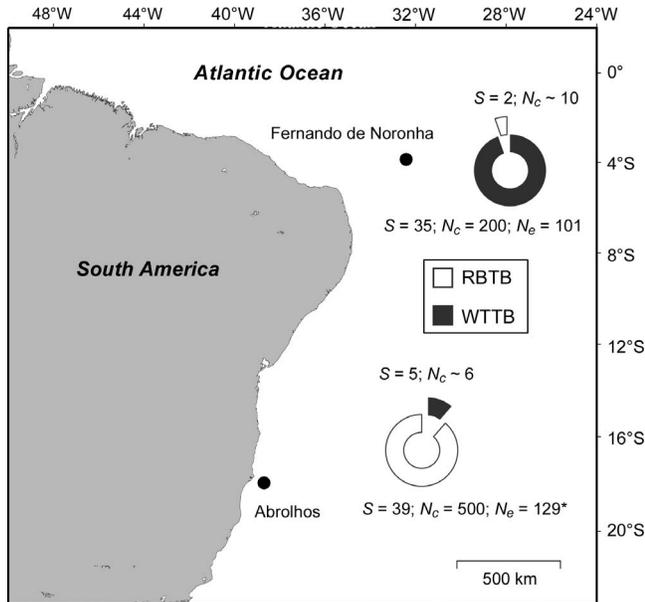
Tropicbirds on these archipelagos likely have a long history of declining populations, but the genetic consequences remain unknown. Maintaining genetic diversity may be especially important for the slow process of population recovery, even with successful management. Once a tropicbird population has declined, quick recovery is unlikely due to their ground-nesting habit, low fecundity, frequent absences from the nest, and lack of parental care after fledging (Orta 1992). In addition, tropicbirds often have low hatching success due to suboptimal environmental conditions or disturbance (Castillo-Guerrero et al. 2011, Sarmiento et al. 2014, Leal et al. 2016).

Here, we use microsatellites to assess genetic diversity and identify bottleneck events in the Red-billed Tropicbird population of Abrolhos and in the White-tailed Tropicbird population of Fernando de Noronha. We also estimate the effective population sizes of the same populations and characterize the genetic identity of the tiny populations of White-tailed Tropicbird from Abrolhos and Red-billed Tropicbird from Fernando de Noronha.

## MATERIALS AND METHODS

### Study Area and Sampling

The Fernando de Noronha Archipelago covers 21 km<sup>2</sup> and is comprised of 21 islands of volcanic origin, located 345 km east of mainland Brazil (Almeida 1955). The smaller Abrolhos Archipelago, ~1700 km southwest of Fernando de Noronha and 70 km from the Brazilian coast, consists



**FIGURE 1.** Fernando de Noronha and Abrolhos archipelagos in the southwest Atlantic Ocean, where we studied the conservation genetics of Red-billed Tropicbirds (*Phaethon aethereus*; RBTB) and White-tailed Tropicbirds (*P. lepturus*; WTTB) in 2010–2013. Sample sizes ( $S$ ) are numerically and graphically represented, while census-based population sizes ( $N_c$ ) and effective population sizes ( $N_e$ ) are numerically presented.  $N_c$  was obtained from Mancini et al. (2016). \*  $N_e$  for Red-billed Tropicbirds from Abrolhos was estimated using 0.01 as the minimum allele frequency.

of 5 volcanic islands arranged circularly and totaling 0.16 km<sup>2</sup> (IBAMA/FUNATURA 1991; Figure 1). Abrolhos has a population of ~500 Red-billed Tropicbirds, but just 10 White-tailed Tropicbirds. The relative abundance of the 2 species is reversed on Fernando de Noronha, with ~200 White-tailed Tropicbirds and only 6 Red-billed Tropicbirds (Leal et al. 2016, Mancini et al. 2016).

Sampling was carried out in August and November, 2010, July and August, 2011, and March, 2013. Breeding birds were captured at their nests and blood samples were collected with a syringe and needle and stored on sterile filter paper. Every sampled tropicbird was banded to avoid resampling. Hereafter, population size from census techniques will be referred as ' $N_c$ ' and effective population size as ' $N_e$ '.

### DNA Extraction and Genotyping

DNA was extracted from 39 samples from Red-billed Tropicbirds and 40 samples from White-tailed Tropicbirds following a cetyltrimethylammonium bromide (CTAB) protocol (Boyce et al. 1989). From the total genomic DNA, 11 microsatellite loci (P3A3, P3D7, P3C1, P4F2, P3F3, P3F5, P3A4, P3G12, P3F7, P4G1, and P3H10) designed from White-tailed Tropicbird samples were

amplified through polymerase chain reaction (PCR), with conditions and sequences as described by the Molecular Ecology Resources Primer Development Consortium (2011). Fluorophores (HEX or FAM) were incorporated into the PCR products by M13(-21) tail (Schuelke 2000). PCR products were analyzed in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Fragment sizing was performed using PEAK SCANNER 1.0 (Applied Biosystems). To avoid biased reading between genotyping rounds, ~5–10% of already genotyped samples were reassayed in the subsequent genotyping.

### Data Analysis

**Genetic diversity.** Potential scoring errors, such as presence of null alleles, stuttering, and large allelic dropout, were checked with MICRO-CHECKER 2.2.3 using 10,000 iterations and Bonferroni correction (van Oosterhout et al. 2004). Nonpolymorphic loci were removed from the datasets. For Red-billed Tropicbirds from Abrolhos and White-tailed Tropicbirds from Fernando de Noronha, the following calculations were performed considering 1,000,000 Markov chain steps and 100,000 dememorisation steps: number of alleles per locus ( $A$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity from Hardy-Weinberg proportions ( $H_E$ ; Nei 1978), average heterozygosity, and deviations from Hardy-Weinberg equilibrium (HWE; Guo and Thompson 1992) after Bonferroni correction for multiple tests (Rice 1989). These analyses, as well as linkage disequilibrium tests considering 10,000 iterations with Bonferroni correction, were performed in ARLEQUIN 3.1 (Excoffier et al. 2005b). One-locus and global estimates of the inbreeding coefficient,  $F_{IS}$  (Weir and Cockerham 1984), were performed in GENEPOP 4.2.2 (Rousset 2008).

**Effective population size.** Contemporary  $N_e$  estimates were obtained for Red-billed Tropicbirds from Abrolhos and White-tailed Tropicbirds from Fernando de Noronha using a single-sample estimator based on the linkage-disequilibrium model implemented in NeEstimator v2 (Do et al. 2014). Two allele frequencies were adopted as cutoffs: 0.02, which is recommended for sample sizes of >25 individuals (Waples and Do 2010); and 0.01, as both datasets presented low allelic richness (see Results). The mating system was defined as monogamous, as is typical for tropicbirds (Nelson 2005, Leal et al. 2016) and for seabirds as a whole (Schreiber and Burger 2001). Estimates of 95% confidence intervals (CI) were calculated by jackknifing over loci for each estimate.

**Population bottlenecks.** Although only contemporary samples were available, 3 complementary approaches were used to identify population bottlenecks (Kim and Sappington 2013). The first was a graphical method, which illustrates the frequency of rare alleles in the population;

an L-shaped graph indicates that the population is naturally in mutation-drift equilibrium, whereas other patterns are indicative of a population that has experienced a recent bottleneck (Luikart et al. 1998).

The second approach was to test for heterozygosity excess, assuming mutation-drift equilibrium as the null hypothesis. This analysis was performed in BOTTLENECK 1.2 (Cornuet and Luikart 1996), based on the Infinite Allele Model (IAM), which is more powerful when heterozygosity is between 0.3 and 0.8 and there are reduced sample sizes and numbers of loci (Cornuet and Luikart 1996), and on the 2-phase mutation model (TPM), which considers microsatellite mutation rates as intermediate between the IAM and stepwise mutation model (Di Rienzo et al. 1994). TPM computations were performed assuming variance among multiple steps = 12 and proportion of multi-step mutations = 0.22, following the recommendations of Peery et al. (2012). Statistical significance was computed both for the TPM and IAM using a 1-tailed Wilcoxon's signed ranked test to compare observed and expected heterozygosities based on 10,000 replications (Cornuet and Luikart 1996).

The third approach was to calculate the  $M$ -ratio of Garza and Williamson (2001), with the adjustment proposed by Excoffier et al. (2005a). The ratio consists of dividing the number of alleles by the range in allele size + 1 for each locus, indicating the number of unoccupied potential allelic states (Garza and Williamson 2001). The  $M$ -ratio was shown to be small when a severe population decline had occurred, and assumes that a population has gone through a bottleneck when  $M < 0.68$  in a dataset with  $\geq 7$  loci (Garza and Williamson 2001). The test for heterozygosity excess and the  $M$ -ratio are the most effective approaches to minimize Type I error (detecting a bottleneck that has not occurred) and Type II error (not detecting a bottleneck that has occurred), and a comparison between them can provide information on the timing of a bottleneck (Williamson-Natesan 2005).

**Intraspecific genetic distance.** Genetic distance between sampling sites was assessed using a standardized covariance matrix for each species and analyzed in a bidimensional plot generated by Principal Coordinate Analysis (PCoA). PCoA was used to estimate the genetic distance between populations from both islands. Due to the small sample sizes of Red-billed Tropicbirds from Fernando de Noronha and White-tailed Tropicbirds from Abrolhos, PCoA was run with a correction for distinct sample sizes. The standardization option divides the covariance inputs by the square root of  $N - 1$ , thus correcting values accounting for sample size (Orlóci 1978). These analyses were performed with GENALEX 6.5 (Peakall and Smouse 2012). Controlling for genotyping errors was not possible due to the small sample size of

White-tailed Tropicbirds from Abrolhos and Red-billed Tropicbirds from Fernando de Noronha.

## RESULTS

### Genetic Diversity

A total of 35 White-tailed Tropicbirds were sampled from Fernando de Noronha. Locus P4G1 did not amplify and polymorphism was not detected in P3G12. There was no evidence for genotyping errors in the dataset. A total of 60 alleles were recorded (6.0 alleles per locus), with an average observed heterozygosity of 0.471 (Table 1). Linkage disequilibrium was not detected, but a deviation from the expected genotypic proportions of HWE was observed in locus P3D7. Locus P3D7 was removed from subsequent analyses, which were thus run with 8 loci. Global  $F_{IS}$  was estimated at  $-0.006$ .

A total of 37 Red-billed Tropicbirds were sampled from Abrolhos. A total of 21 alleles were observed (2.3 per locus), with an average observed heterozygosity of 0.267 (Table 1). There was no evidence for linkage disequilibrium. Locus P3F3 did not amplify, and evidence for null alleles was identified in P3A4. Among the 9 remaining loci, P3D7, P3A3, and P3F7 showed no polymorphism, and P3C1 deviated from HWE. After removing these loci, 5 loci remained for subsequent analyses. Global  $F_{IS}$  was estimated at 0.016.

### Effective Population Size

Estimates for White-tailed Tropicbirds from Fernando de Noronha were  $N_e = 101$  (CI = 54–331) and  $N_e = 143$  (CI = 73–704), using allele frequency cutoffs of 0.02 and 0.01, respectively. For Red-billed Tropicbirds from Abrolhos, the effective population size estimate had a negative value ( $N_e = \text{infinite}$ ) when using an allele frequency cutoff of 0.02, and  $N_e = 129$  (CI = 19–infinite) with a cutoff of 0.01.

### Population Bottlenecks

There were a large number of rare alleles in both species, with a greater number in White-tailed Tropicbirds; however, both species were characterized by an L-shaped allele frequency distribution curve (Figure 2). Both populations were in mutation-drift equilibrium, based on Wilcoxon signed rank tests in the TPM and IAM. The  $M$ -ratio for Red-billed Tropicbirds was  $M = 0.55$  and for White-tailed Tropicbirds was  $M = 0.63$ . Thus, the L-shaped allele frequency distribution graphs and heterozygosity excess analyses were not consistent with recent bottleneck events, but the  $M$ -ratio for both tropicbirds was below the cutoff proposed by Garza and Williamson (2001), suggesting that these populations have been small for a few centuries.

**TABLE 1.** Microsatellite diversity of 35 White-tailed Tropicbirds (*Phaethon lepturus*) sampled from Fernando de Noronha Archipelago and of 37 Red-billed Tropicbirds (*P. aethereus*) sampled from Abrolhos Archipelago (see Figure 1 for archipelago locations). Shown are the number of alleles per locus ( $A$ ), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ; Nei 1978), inbreeding coefficient values per locus ( $F_{IS}$ ; Weir and Cockerham 1984), average heterozygosity ( $H$ ), and the average numbers of alleles per locus and global  $F_{IS}$  ( $\bar{x}$ ).

Locus	White-tailed Tropicbird				Red-billed Tropicbird			
	$A$	$H_O$	$H_E$	$F_{IS}$	$A$	$H_O$	$H_E$	$F_{IS}$
P3G12	1	0.000	0.000	—	2	0.027	0.027	0.000
P3D7	5	0.514 *	0.564	—	1	0.000	0.000	—
P3A3	3	0.121	0.172	0.298	1	0.000	0.000	—
P4F2	22	0.848	0.935	0.094	2	0.200	0.182	-0.096
P3C1	4	0.200	0.187	-0.069	3	0.351 *	0.470	—
P3F7	11	0.788	0.854	0.078	1	0.000	0.000	—
P4G1 <sup>a</sup>	—	—	—	—	4	0.457	0.494	0.075
P3A4 <sup>b</sup>	5	0.323	0.339	0.048	—	—	—	—
P3F3 <sup>a</sup>	2	0.451	0.389	-0.163	—	—	—	—
P3H10	3	0.676	0.544	-0.248	3	0.114	0.110	-0.034
P3F5	4	0.314	0.282	-0.116	4	0.457	0.462	0.010
$H$	—	0.471	0.474	—	—	0.267	0.291	—
$\bar{x}$	6.0	—	—	-0.006	2.3	—	—	0.016

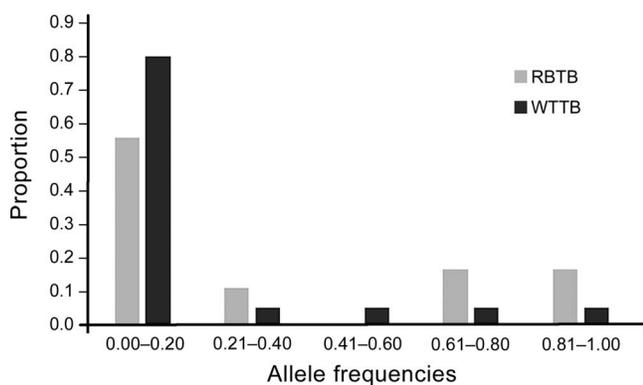
<sup>a</sup> Locus did not amplify.

<sup>b</sup> Genotyping errors observed.

\* Locus with significant deviation from the proportions expected according to Hardy-Weinberg equilibrium.

### Intraspecific Genetic Distance

The PCoA for White-tailed Tropicbirds was performed with 8 loci (P3G12, P3A3, P3C1, P3F7, P3A4, P3F3, P3H10, and P3F5). In the 5 White-tailed Tropicbirds from Abrolhos, 8 exclusive alleles were found, including 1 private allele at locus P3G12 for which the population from Fernando de Noronha had no polymorphism. The 2 major coordinates explained 43% of the total variance, although coordinate 1 was sufficient to differentiate the 5 White-tailed Tropicbirds sampled from Abrolhos from the population from Fernando de Noronha (Figure 3A).



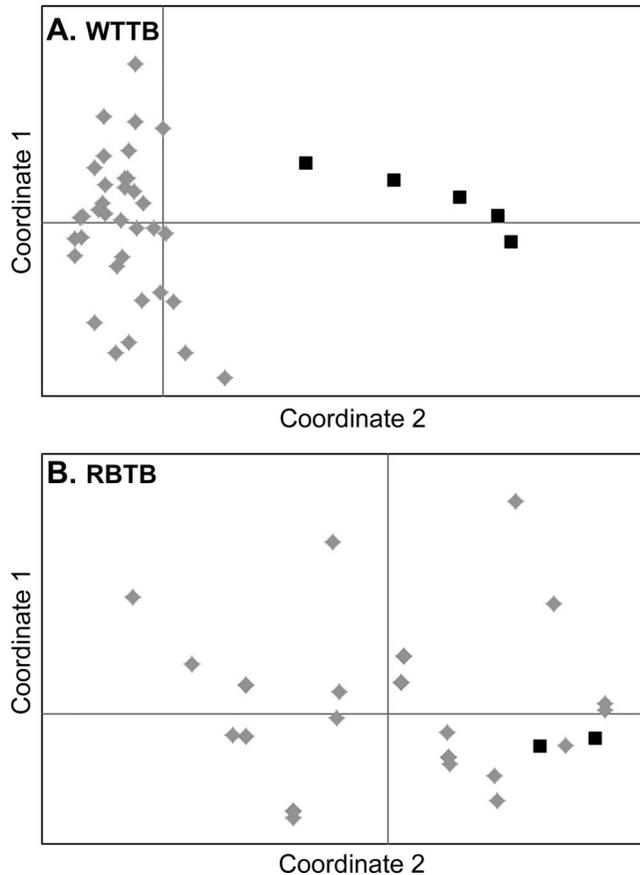
**FIGURE 2.** Allele frequency distributions of 37 Red-billed Tropicbirds (*Phaethon aethereus*; RBTB) sampled from Abrolhos Archipelago, and 35 White-tailed Tropicbirds (*P. lepturus*; WTTB) sampled from Fernando de Noronha Archipelago, off the coast of Brazil.

For Red-billed Tropicbirds, the PCoA was performed with 7 loci (P3G12, P3D7, P3A3, P4F2, P4G1, P3H10, and P3F5). The 2 Red-billed Tropicbirds sampled from Fernando de Noronha had 4 private alleles, but were grouped with the population from Abrolhos when looking at coordinates 1 and 2, which explained 45% of the total variance (Figure 3B).

## DISCUSSION

### Genetic Diversity

To our knowledge, this is the first conservation genetics study of Phaethontiformes. Despite their smaller population, White-tailed Tropicbirds from Fernando de Noronha had higher genetic diversity than Red-billed Tropicbirds from Abrolhos, even when comparing only the 4 loci that successfully amplified for both species. Furthermore, Red-billed Tropicbirds had 3 nonpolymorphic loci, the same loci in which White-tailed Tropicbirds from Fernando de Noronha had 19 alleles. Additionally,  $N_e$  estimates for both populations were lower than documented values for other seabird species (Valle 1995, Morris-Pocock et al. 2012, Ramírez et al. 2013) and below the level considered necessary to maintain the evolutionary potential of a population, i.e.  $N_e = 500-5,000$  (Franklin and Frankham 1998, Lynch and Lande 1998), although the 95% confidence intervals for both species' estimates encompassed the lower limit of this proposed range at the 0.01 allele frequency cutoff. Census-based population size estimates for both tropicbird populations reinforce the idea that they



**FIGURE 3.** Bidimensional plots generated from Principal Coordinate Analyses (PCoA) based on standardized covariance matrices of intraspecific genetic distances. **(A)** Individual genetic distances between White-tailed Tropicbirds (*Phaethon lepturus*; WTTB) from Fernando de Noronha (black squares) and Abrolhos (gray diamonds) archipelagos, and **(B)** Genetic distances between Red-billed Tropicbirds (*P. aethereus*; RBTB) from Fernando de Noronha (black squares) and Abrolhos (gray diamonds) archipelagos.

are at risk, as there are currently only a few hundred tropicbirds breeding on Fernando de Noronha and Abrolhos (Mancini et al. 2016; Figure 1).

Comparing genetic diversity between species using distinct molecular markers, or even distinct loci, should be carried out cautiously, although some broad patterns of genetic diversity have been observed within Aves. There is a trend of decreasing heterozygosity the more threatened a bird species is, and, despite the lower genetic diversity of Red-billed Tropicbirds compared with White-tailed Tropicbirds, both species had mean heterozygosities lower than values observed for bird species classified as globally endangered and critically endangered by the IUCN (Evans and Sheldon 2008). The tropicbird heterozygosities were also lower than values observed for seabird species that have experienced severe population declines (Friesen et al. 2006, Bouzat et al. 2009, Morris-Pocock et al. 2012).

Furthermore, allelic richness and observed heterozygosity for Red-billed Tropicbirds on Abrolhos and White-tailed Tropicbirds on Fernando de Noronha were lower than values observed for White-tailed Tropicbirds on Réunion Island, Indian Ocean (Molecular Ecology Resources Primer Development Consortium 2011), although the loci used were not exactly the same.

The observed between-species differences in this study may reflect marked genetic differences, but may also be a consequence of using cross-species microsatellite amplification, as the primers used in this study were developed from White-tailed Tropicbird samples. Microsatellites are less abundant in avian genomes than in those of other vertebrate groups (Primmer et al. 1997) leading to the widespread use of heterologous primers (i.e. primers developed for closely related species). Cross-species microsatellite amplification has been shown to be successful in species with long generation times (Barbará et al. 2007), including several bird families (e.g., Castro e Souza et al. 2012, Presti et al. 2015). Nonetheless, within-genus phylogeny suggests that White-tailed Tropicbirds and Red-billed Tropicbirds are the most distantly related species within the clade (Kennedy and Spencer 2004). This phylogenetic separation could have affected the results for Red-billed Tropicbirds based on White-tailed Tropicbird primers.

### Population Bottlenecks

No deficiency of rare alleles or heterozygosity excess were observed in either population. Red-billed Tropicbirds from Abrolhos and White-tailed Tropicbirds from Fernando de Noronha had  $M$ -ratios below the critical value proposed by Garza and Williamson (2001). After population decline, heterozygosity tends to be high in populations that have undergone a bottleneck, but this effect only lasts for a few dozen generations (Luikart et al. 1998). The  $M$ -ratio, which is defined by the number of alleles ( $k$ ) and the range of allele size ( $r$ ), tends to decrease after bottleneck events, mainly because the number of alleles is reduced proportionally more than the range of allele size (Peery et al. 2012). However, in contrast to heterozygosity excess, a low  $M$ -ratio can persist for more than 100 generations because not all mutations will increase the  $M$ -ratio and genetic drift removes rare alleles in small populations (Garza and Williamson 2001). Therefore, more distant bottleneck events can be detected by the  $M$ -ratio, even if the population is already in mutation-drift equilibrium (Peery et al. 2012).

Simulations suggest that values of  $M \approx 0.6$  (as found here) are associated with bottleneck events  $\sim 30$ – $50$  generations ago (Garza and Williamson 2001). The generation time for tropicbirds is not accurately known, but it can be estimated with the formula  $(1/m) + b$ , where  $m$  = mean annual mortality and  $b$  = age at first breeding, as

proposed by BirdLife International (available at <http://www.birdlife.org/datazone/info/spcpop>). Using 10% as the mean annual mortality (Nelson 2005) and 5 yr as the age of first breeding in tropicbirds (Hamer et al. 2001, Schreiber and Schreiber 2009, Lowrie et al. 2012), generation time for tropicbirds would be 15 yr, and 30–50 generations would thus correspond to 450–750 yr ago. This broadly corresponds to the period when seabirds were exploited by sailors, as documented in the diaries of early explorers of the region (e.g., d'Abbeville et al. 1963, Markham 2011). Alternatively, low  $M$ -ratios could reflect a founding event, such that Fernando de Noronha and Abrolhos could have been colonized by tropicbird populations from elsewhere in the Atlantic Ocean, such as from Ascension Island or the Lesser Antilles.

### Intraspecific Genetic Distance

Analysis of genetic distance showed contrasting patterns between the 2 species: White-tailed Tropicbirds from Abrolhos were strongly isolated from the population on Fernando de Noronha, while Red-billed Tropicbirds from Fernando de Noronha were grouped with the Abrolhos population, despite 4 private alleles from birds on Fernando de Noronha. We could not control for genotyping errors due to small sample sizes of White-tailed Tropicbirds from Abrolhos and Red-billed Tropicbirds from Fernando de Noronha, which could have contributed to the high number of private alleles in both groups. Despite the small sample sizes, some precautions were taken for these small populations, such as sampling almost all breeding individuals and randomly reassaying already genotyped individuals for genotyping repetition. Therefore, our findings may suggest strong differentiation between White-tailed Tropicbirds from Fernando de Noronha and Abrolhos.

White-tailed Tropicbirds have bred on Abrolhos in small but relatively stable numbers since at least 1992 (Alves et al. 2004), and, similarly, there are reports of Red-billed Tropicbirds breeding in small numbers on Fernando de Noronha since 1982 (Oren 1982). Records of breeding activities for decades on both archipelagos and site fidelity in tropicbirds based on banding (Leal et al. 2016, M. A. Efe personal observation) suggest that these small populations have been consistently present, at least in recent times. These small populations could be founder populations in the settlement process, or relict populations remaining after a bottleneck event. Other tropicbird colonies in the Atlantic Ocean could be potential sources of immigrants to colonies in the southwestern Atlantic Ocean, even though the few Red-billed Tropicbirds breeding on Fernando de Noronha may have come from Abrolhos, given the small genetic distance between these populations.

Interestingly, there are 2 White-tailed Tropicbird subspecies in the Atlantic Ocean, *P. l. ascensionis* and *P. l. catesbyi* (Orta 1992), with wing length decreasing from the northwest (*catesbyi* from Bermuda) to the southeast (*ascensionis* from São Tomé). Birds in between, from Antilles (*catesbyi*), Fernando de Noronha, and Ascension Islands (*ascensionis*), have intermediate wing length (Le Corre and Cebe 1999). Similarly, it has been suggested that there are 2 Red-billed Tropicbird subspecies in the Atlantic Ocean: *P. a. aethereus* in the southern Atlantic Ocean, and *P. a. mesonauta* in the eastern Atlantic Ocean and Caribbean (Orta 1992, Nelson 2005). The phylogenetic position of tropicbirds from Abrolhos and Fernando de Noronha has not been addressed with modern methods.

### Conservation Implications

Both tropicbird species in this study consisted of 1 population of a few hundred individuals and 1 population of 6–10 individuals. These populations are threatened by introduced rats (*Rattus* spp.), cats (*Felis catus*), and Argentine black and white tegus (*Salvator merianae*).  $N_e$  estimates and low  $M$ -ratios indicate that these populations have been small for a few dozen generations. Although seabird populations can persist with low genetic diversity (Milot et al. 2007), egg, nestling, and adult predation by exotic species represents an even more severe threat when populations have low evolutionary potential, as predation can result in population decreases and, consequently, removal of rare alleles as the population is reduced (Garza and Williamson 2001, Peery et al. 2012). White-tailed Tropicbirds from the 2 archipelagos appear to be genetically distinct, elevating the importance of protecting the small population of White-tailed Tropicbirds on Abrolhos. The White-tailed Tropicbird population on Abrolhos seems to have persisted with almost no growth for a few decades, corresponding to the low breeding success observed in this species worldwide (Schaffner 1991, Ramos et al. 2005, Leal et al. 2016). A global overview of genetic diversity and structure could facilitate the conservation of tropicbirds by revealing their biological diversity, colonization processes, and persistence within and between ocean basins. In parallel with colony-based assessments of evolutionary potential and threats, this could guide global and local actions toward the conservation of tropicbird populations.

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