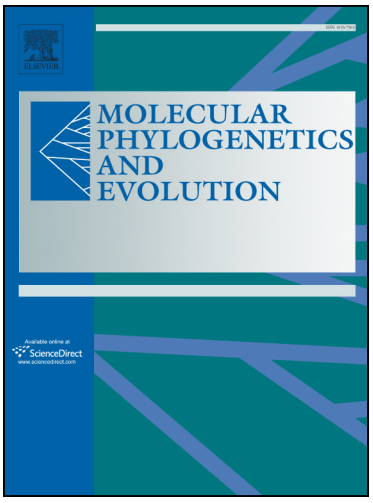

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Cryptic species and independent origins of allochronic populations within a seabird species complex (*Hydrobates* spp.)

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ABSTRACT

Humans are inherently biased towards naming species based on morphological differences, which can lead to reproductively isolated species being mistakenly classified as one if they are morphologically similar. Recognising cryptic diversity is needed to understand drivers of speciation fully, and for accurate estimates of global biodiversity and assessments for conservation. We investigated cryptic species across the range of band-rumped storm-petrels (*Hydrobates* spp.): highly pelagic, nocturnal seabirds that breed on tropical and sub-tropical islands in the Atlantic and Pacific Oceans. In many breeding colonies, band-rumped storm-petrels have sympatric but temporally isolated (allochronic) populations; we sampled all breeding locations and allochronic populations. Using mitochondrial control region sequences from 754 birds, cytochrome *b* sequences from 69 birds, and reduced representation sequencing of the nuclear genomes of 133 birds, we uncovered high levels of genetic structuring. Population genomic analyses revealed up to seven unique clusters, and phylogenomic reconstruction showed that these represent seven monophyletic groups. We uncovered up to six independent breeding season switches across the phylogeny, spanning the continuum from genetically undifferentiated temporal populations to full allochronic species. Thus, band-rumped storm-petrels encompass multiple cryptic species, with non-geographic barriers potentially comprising strong barriers to gene flow.

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1. Introduction

Humans, as predominantly visual species, are inherently biased towards naming taxa based on differences in physical appearance, and most species are thus classified primarily on morphological differences (Bickford et al., 2007). If multiple species are morphologically similar, they may be mistakenly grouped into one species – often referred to as cryptic species (Bickford et al., 2007; Fiser et al., 2018; but see Struck et al., 2018 for a discussion on cryptic species definitions). The presence of undiagnosed cryptic species can lead to underestimates of global biodiversity (Pfenninger and Schwenk, 2007; Trontelj and Fiser, 2009) and regional diversity (Chek et al., 2003), and hinder our understanding of speciation as researchers may overlook drivers involved in the development of reproductive isolation. Mechanisms involved in the formation of cryptic species, as well as whether certain taxonomic groups and geographical areas are more likely to harbour hidden diversity, represent important knowledge gaps (Bickford et al., 2007; Funk et al., 2011).

Recognizing cryptic species also has important implications for conservation, as range sizes, global population sizes and niche width can be vastly over-estimated and habitat specificity underestimated if multiple species are classified as one (Funk et al., 2011; Irwin et al., 2001). Range size and abundance are key criteria used by the International Union for the Conservation of Nature (IUCN) to determine species conservation status (Funk et al., 2011; IUCN, 2017), and so uncovering whether one species actually encompasses multiple cryptic species with more restricted ranges is imperative for accurate assessment of their conservation status (Funk et al., 2011). Further, undiscovered cryptic species can have

consequences for captive breeding and release of endangered species. For example, Chinese giant salamanders (*Andrias davidianus*) comprise multiple genetic lineages in nature, but there is evidence of genetic homogenization due to captive breeding (Yan et al., 2018).

The reasons why species diverge without concomitant morphology change is thus an important area of study both to answer evolutionary questions and for conservation (Fiser et al., 2018). One reason why species may show no or little morphological divergence is that they have speciated recently (Bickford et al. 2007; Fiser et al., 2018); however, this cannot explain all cases of cryptic species. For example, the copepod *Cletocamptus deitersi* appears to represent at least four species that diverged in the Miocene (Bickford et al., 2007; Rocha-Olivares et al., 2001); *Bolitoglossa* salamanders include multiple cryptic species with deep genetic divergences, also dating to the Miocene (Elmer et al., 2013); and a relatively old divergence, potentially around 2.4 million years ago, was found between two cryptic *Phylloscopus* warbler species (Irwin et al., 2001).

Organisms with non-visual reproductive signals, for example calls or odours, are particularly likely to speciate without changes in morphology (Bickford et al., 2007). Differences in chemosensory traits have been implicated in speciation of some taxonomic groups (particularly insects), but are often overlooked in vertebrates (Smadja and Butlin, 2009). Similarly, acoustic signals are routinely used to differentiate some groups, for example anurans (Wang et al., 2017). However, cryptic species of birds were once considered rare due to the perceived importance of vision in the recognition of sexual characteristics (Jones, 1997). Phylogenetic niche conservatism (PNC), otherwise known as morphological constraint, may also mean that organisms diverge without changes in morphology (Fiser et al., 2018). Fiser et al. (2018) suggest three predictions if PNC is the cause of morphological stasis: morphological constraint due to selection; similar ecological niches among the cryptic taxa; and speciation in allopatry.

The band-rumped storm-petrel species complex (genus *Hydrobates*, previously *Oceanodroma* - all species within the Hydrobatinae were recently subsumed within the genus *Hydrobates* by the European Taxonomic Commission - see Wallace et al., 2017 for a phylogeny) presents an excellent opportunity to investigate drivers of cryptic divergence. Band-rumped storm-petrels are small, highly pelagic seabirds with a widespread distribution, breeding on tropical and sub-tropical islands in the Atlantic and Pacific Oceans (Brooke, 2004; Smith et al., 2007; Fig. 1). They also have a series of sympatric populations that breed in distinct seasons, known as allochronic populations (Friesen et al. 2007; Fig. 1). In Japan and Hawaii birds breed only during the hot season (Friesen et al., 2007; Raine et al., 2017; Fig. 1). In Berlengas, the Canary Islands and Vila in the Azores, populations breed only during the cool season. In contrast, sympatric but seasonally separated populations breed in the Galápagos, Praia and Baixo in the Azores, Desertas, Ascension Island and St. Helena Island (Bennett et al., 2009; Friesen et al., 2007; Monteiro and Furness, 1998; Smith et al., 2007; Fig. 1). The length of the breeding seasons is not known exactly for all locations. However, in the Azores, the two seasonal populations are known to be distinct and non-overlapping (Bolton et al., 2008). Individual storm-petrels are unlikely to breed twice within a year due to the energy constraints of breeding and the annual moult cycle (details of moult cycle in Bolton et al., 2008). In Cape Verde birds at different breeding stages are found almost year-round (Friesen et al., 2007). In Selvagem, the birds have been suggested to have two seasons (Faria, 1998; Friesen et al., 2007; Sangster et al., 2012), although they have also been reported as having year-round breeding (Mougin et al., 1990). Further study is needed to be sure of this, but for the purposes of our manuscript we consider them to be seasonal breeders (Fig. 1).

Genetic work using mitochondrial and microsatellite markers found hot season breeders in the Azores to be genetically differentiated from sympatric cool season breeders

(Friesen et al., 2007; Silva et al., 2016: Smith et al., 2007). Hot season breeders in the Azores are also smaller with more deeply forked tails, thinner bills and proportionately longer wings than sympatric cool season breeders (Bolton et al., 2008; Monteiro and Furness, 1998). Importantly, hot season breeders are distinct in their vocalisations and do not respond to vocalizations of cool season breeders implying pre-mating reproductive isolation (Bolton, 2007), and so were consequently named as a separate species, Monteiro's storm-petrel (*H. monteiroi*; Bolton et al., 2008). Likewise, Cape Verde birds were found to be genetically divergent from the other populations (Friesen, 2015; Friesen et al., 2007), and to have distinct vocalizations (Bolton, 2007) suggesting strong premating isolation. They too have been suggested to be a distinct species, the Cape Verde storm-petrel (*H. jabejabe*; Sangster et al., 2012). Seasonal breeding populations in the Galápagos and Desertas were weakly genetically differentiated, with some differentiation in morphology and vocalisations in the Galápagos (Friesen at al., 2007; Smith and Friesen, 2007), possibly representing an earlier stage along the speciation continuum. Seasonal populations in the South Atlantic, on Ascension Island and St. Helena, have not been included in genetic studies until now.

Aside from the hot season breeders in the Azores and the birds in Cape Verde, all other populations are currently classified as *H. castro*. The winter breeders within the North Atlantic were suggested to represent a separate species from the hot season breeders on Selvagem and Desertas, with the winter breeders putatively named Grant's storm-petrel (Robb et al., 2008). Birds breeding on St Helena were previously described as a separate subspecies, *H. c. helena*, based on morphological differences (Matthews, 1934). In the Pacific, previous work indicated genetic differentiation between breeders in the Galápagos versus Japan (Friesen et al., 2007; Smith et al., 2007). Differences in morphology were found between the Galápagos and other populations, leading to the suggestion that they be

recognised as a separate subspecies, *H. c. bangsi*, though this was based on just one bird (Nichols, 1914).

High resolution data are needed to resolve the phylogeny across the range of the band rumped storm-petrel species complex, both for conservation and to fully elucidate drivers of divergence. These previous studies indicate the likelihood of strong genetic structuring within the complex, without strong morphological differentiation, and storm-petrels may demonstrate cryptic diversification for several reasons. They are burrow nesting seabirds that are nocturnal at breeding colonies, and so visual signals are unlikely to be important in courtship (Bretagnolle, 1989). Instead, acoustic signals are used to attract mates and maintain pair bonds (Bretagnolle, 1996); thus differences in vocalisations probably represent a more important indicator of reproductive isolation than do morphological differences (Bolton, 2007; Bretagnolle, 1996).

Many seabirds must fly long distances over large expanses of ocean to find feeding areas, particularly in regions of low productivity such as the tropics (Hertel and Balance, 1999; Spear and Ainley, 1997). Wing shape and size – and other features related to flight – are key adaptations for foraging (Spear and Ainley, 1997), which suggests that band-rumped storm-petrels are morphologically constrained due to selection. Populations of band-rumped storm-petrels share a similar niche (e.g. nocturnal at colonies, breeding in rock crevices on tropical islands; Bolton et al., 2004), and are allopatric either in space or in time (see below; Fig. 1) and so could be a case of PNC as described by Fiser et al. (2018).

Cryptic species were traditionally thought to be more common in species with low dispersal abilities which may impose limitations to gene flow among populations and promote population differentiation and diversification (Baker et al., 1995; Jörger and Schrödl, 2013), although exceptions exist (e.g. Slapeta et al., 2006). Band-rumped storm-petrels are highly vagile, and so present an interesting case of potential cryptic diversification with very

large range sizes, an exception to cases of cryptic species with low dispersal abilities. Many seabirds exhibit natal philopatry, the tendency for individuals to return to the site where they were born or hatched to breed. Philopatry has been hypothesised to increase the likelihood of cryptic diversification between colonies despite the potential for individuals to travel easily between breeding sites (Brooke, 2004). The extent of natal philopatry in band-rumped storm-petrels is unknown; however adults appear to nest at the same colony every year implying at least breeding philopatry (Bried and Bolton, 2005; Harris, 1969; Monteiro and Furness, 1998). Unrecognised diversity in wide ranging species has additional conservation implications because it may lead to incorrect assumptions about how a species distribution is related to environmental variation (Murray et al., 2008).

In the present study, we used samples from all known breeding colonies of the band-rumped storm-petrel species complex including previously unstudied colonies on Kauai Island (Hawaii), hot and cool season populations on both Ascension and St. Helena Islands, and two new seasonal time points in Cape Verde, enabling a comprehensive phylogeny of the band-rumped storm-petrel species complex for the first time. We used a combination of mitochondrial DNA sequences and high throughput sequencing of nuclear DNA (double-digest restriction site-associated DNA sequencing, ddRADseq; Peterson et al., 2012) to address two hypotheses pertaining to cryptic speciation in these birds.

First, we hypothesised that populations of band-rumped storm-petrels in different archipelagos, including newly sampled populations in Hawaii, Ascension Island (hot and cool populations) and St. Helena (hot and cool populations), will show genetic divergence from one another. Not all seabirds show genetic structuring within, or even between, ocean basins (Friesen, 2015). However, previous studies (mentioned above) indicated that regional populations of band-rumped storm-petrel differ genetically, and so we hypothesise this to be the case globally. Secondly, if populations from different archipelagos are genetically

distinct, indicating that allochronic populations in different regions arose independently, we hypothesise that allochrony acted as a barrier to gene flow between sympatric allochronic populations in all areas, as has occurred in the Azores (Friesen et al. 2007). If so, we expect to find genetic differentiation between sympatric seasonal populations.

Addressing these questions will provide insights into cryptic diversification in the band-rumped storm-petrel in particular and in seabirds generally, and will have strong taxonomic and conservation implications for the species complex. Our results will also clarify the process of divergence by allochrony, an underappreciated mechanism of cryptic divergence found in taxonomic groups as diverse as seabirds, corals (e.g. Rosser, 2015) and insects (e.g. Santos et al., 2007; reviewed in Taylor and Friesen, 2017).

2. Materials and methods

2.1. Sample collection and DNA extraction

Blood was sampled from 754 breeding band-rumped storm-petrels captured across their global range, of which 562 birds were used previously by Friesen et al. (2007; Table 1; Fig. 1). Breeding season was determined by moult stage and brood patch at time of capture. Samples were stored either in lysis buffer (Seutin et al., 1991) or dried on filter paper. Due to the complicated field logistics, samples were collected in different years and so we make the assumption that genetic structure has been stable over time, at least for the last ~15 years (approximately two generations) since we started collecting samples. DNA was extracted by standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation (Maniatis et al., 1982). DNA concentrations were quantified using a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY, USA) and purity was assessed using a Nanodrop ND-

1000 spectrophotomer (Nanodrop Technologies Inc., Wilmington, DE, USA).

2.2. Mitochondrial DNA

Mitochrondrial control region DNA sequences were available from 562 birds (Friesen et al., 2007). These data were augmented with new data obtained using primers OcL61 and H530 (Friesen et al., 2007; Smith et al., 2007; Table 1), following the protocols from Smith et al. (2007). Three individuals per sampling location, for a total of 69 birds, were also sequenced for a 1,045 base pair (bp) fragment of the cytochrome *b* gene to help resolve phylogenetic relationships, using the primers b6 and 23 (Wallace et al., 2017). The PCR cycle consisted of denaturation for 3 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds, 50°C for 45 seconds, and 72°C for 45 seconds, finishing with 72°C for 3 minutes (Patterson et al., 2011; Wallace et al., 2017). PCR products were sequenced by Genome Quebec (McGill University, Montreal) on a 3730xl DNA Analyzer Platform (Applied Biosystems). Sequence quality and variable sites were confirmed from chromatograms by eye and aligned using Geneious v.6.1.5 (Kearse et al., 2012).

2.3. ddRAD Sequencing

Genomic libraries were prepared for 146 birds for ddRADseq by l'Institut de biologie intégrative et des systèmes (IBIS) de l'Université Laval. The restriction enzymes *SbfI* and *MspI* were used in DNA digestion, and barcoded forward adapters were then ligated to the samples. The barcodes ranged from 4bp to 8bp in length and differed by at least 2bp. The reverse adapter was designed as a Y-adapter to prevent the PCR amplification of *MspI-MspI*

fragments (Poland et al., 2012). Size selection was performed using a BluePippin (Sage Science), retaining library molecules between 250 and 500bp, before the final PCR step. The library was sequenced at the Genome Quebec Innovation Centre (McGill University), using single-end 150bp sequencing on four lanes of an Illumina HiSeq (Illumina, San Diego, CA, USA).

2.4. SNP data filtering

Raw reads were de-multiplexed sequentially by barcode length using STACKS 1.45 (Catchen et al., 2013). Low quality reads (with a Phred score below 10), and those with uncalled bases, were filtered out and reads were truncated to 142bp. De-multiplexed reads were aligned to the Leach's storm-petrel genome (unpublished data from SYW Sin et al.) using the 'end-to-end mode' and the 'very_sensitive' settings in Bowtie2 (Langmead and Salzberg, 2012). Alignments were quality filtered in Samtools (Li et al., 2009), only retaining alignments with a mapping quality of at least 10.

Alignment files were run through STACKS, requiring a depth of 10 reads to form a stack (m = 10) and removing loci with a minor allele frequency of less than 0.05, and randomly selecting one SNP per locus. Individuals were grouped in the population map based on sampling site and season, giving 16 populations (Table 2). Three filtering settings were used: to be retained loci had to occur (1) in at least 50% of individuals within every population and all 16 populations (henceforth SNP Dataset 1), (2) in at least 80% of individuals within a population but only 14 out of the 16 populations (henceforth SNP Dataset 2), or (3) in at least 80% of individuals within a population and all 16 populations (henceforth SNP Dataset 3). We used VCFtools to screen for loci that deviated from Hardy-Weinberg expected proportions, and to calculate the average depth of sequencing for each

individual (Danecek et al., 2011). Observed and expected homozygosity, nucleotide diversity and the inbreeding coefficients were calculated in STACKS.

2.5. Tests of assumptions and population genetic analyses

We tested the control region sequences for deviations from selective neutrality in ARLEQUIN 3.5 (Excoffier and Lischer, 2010) using Ewans-Watterson and Chakraborty tests (Chakraborty, 1990; Ewens, 1972; Watterson, 1978), and estimated nucleotide diversity (π, Tajima, 1983) and haplotypic diversity (h, Nei, 1987) for each sampled population.

To investigate genetic differentiation between populations, the SNP datasets were used to assign individuals to genetic clusters using STRUCTURE 2.3.4 (Pritchard et al., 2000; Pritchard et al., 2010). Each run used a burn-in of 50,000 followed by 100,000 MCMC iterations, assuming correlated allele frequencies and using an admixture model without location as prior information. All populations were run for K = 1-10, with 10 runs for each value of K. Due to hierarchical genetic structuring, individuals assigned to each K were analysed in a second STRUCTURE analysis using the same parameters, for K = 1-5 for the North Atlantic populations (excluding Cape Verde and the Azores hot populations) and K = 1-10 for the rest. The subsequent populations were then also analysed separately, for K= 1-4 for the South Atlantic populations, K= 1-4 for the Hawaii and Japan populations (which also included the Azores hot population for SNP Dataset 1), and for K= 1-4 for the Galápagos populations. To assess the potential number of genetic clusters we calculated delta K (ΔK) using STRUCTURE HARVESTER (Earl and vonHoldt, 2012; Evanno et al., 2005), and the posterior probability of each K based on recommendations in the STRUCTURE documentation (Pritchard et al., 2010). STRUCTURE plots were redrawn using Pophelper which includes a CLUMPP function to combine independent runs (Francis, 2016). A

principal component analysis (PCA) of the SNP data was performed and plotted in R software version 3.3.2 (R Development Core Team, 2006) using the packages ADEGENET (Jombart, 2008) and ADE4 (Dray and Dufour, 2007).

We calculated pairwise F_{st} and Φ_{st} between all populations, and between the seven major groups identified in the STRUCTURE and phylogenomic analyses, using the HIERFSTAT package in R (Goudet, 2005) using the Weir and Cockerham calculation (WC84; Weir and Cockerham, 1984) for the SNP datasets, and using ARLEQUIN for the full mitochondrial control region dataset. We calculated Φ_{st} by analysis of molecular variance (AMOVA) using Kimura's two-parameter model of substitution (Kimura, 1980), with a gamma distribution shape parameter (α) of 0.45 as has been used for similar species (Baker and Marshall, 1997). Significance was assessed using 10,100 permutations, and Type 1 statistical errors were addressed using a Benjamini-Yekutieli (B-Y) correction (Benjamini and Yekutieli, 2001; Narum, 2006). We constructed a statistical parsimony haplotype network of the full control region sequence dataset using PopArt (Clement et al., 2000; Leigh and Bryant, 2015; PopArt: http://popart.otago.ac.nz). We also constructed a haplotype network for the cytochrome *b* sequences. Because the program masks missing data we trimmed 109bp from the start and 43bp from the end of the sequences, and removed three individuals with high levels of missing data.

2.6. Phylogenetic reconstruction

We constructed a mitochondrial gene tree using concatenated control region and cytochrome *b* sequences from three individuals per population using BEAST 1.8.4 (Drummond et al., 2012). We used the General Time Reversible substitution model with a gamma distribution and a proportion of invariant sites (GTR+I+G), which was selected as an

appropriate model of sequence evolution using the Akaike Information Criterion (AIC) in jMODELTEST2 (Darriba et al., 2012). We used a strict molecular clock with a uniform distribution, and the Yule species tree prior (Drummond et al., 2006), with individuals not assigned to any populations *a priori*. We ran the analysis for 100,000,000 generations, sampling every 1,000 generations, and discarded the first 10% of trees as burn-in. We assessed convergence by ensuring all effective sample size (ESS) values were over 200 using TRACER 1.5 (Rambaut and Drummond, 2007). The analysis was run three times, the resultant tree files were combined using LogCombiner version 1.8.4, and a consensus tree was generated in TreeAnnotator 1.8.4 (Drummond et al*.,* 2012). The tree was visualized in FIGTREE 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

PGDSpider 2.1.0.2 (Lischer and Excoffier, 2012) was used to convert the VCF file containing the SNP data into a Phylip file. A maximum-likelihood tree was generated in RAxML 8.2.9 (Stamatakis, 2014). We used a General Time Reversible model with a gamma distribution of substitution rates and a proportion of invariant sites (GTRGAMMAI), and used the rapid bootstrap algorithm using 2000 replicates. The Cape Verde population was set as an outgroup for the analysis as it is known to be basal to the rest of the group (Wallace et al., 2017).

For the BEAST analysis of the SNP data, we used a subset of three individuals per population, chosen using an online random number generator, to ensure reasonable times for analyses given the large panels of SNPs and requisite computational power. PGDSpider was used to convert the VCF file into a nexus file to input into the SNAPP version 1.3.0 package in BEAST 2.4.2 (Bouckaert et al., 2014; Bryant et al., 2012). Trees were generated with individuals grouped by the major groups identified in the RAxML phylogeny and STRUCTURE, using default parameters and 2,000,000 MCMC iterations, discarding the first 10% of trees as burn-in. We assessed convergence in TRACER version 1.5 (Rambaut and

Drummond, 2007), ensuring the ESS values were all over 200. The consensus tree was generated using TreeAnnotator and the tree was visualized in FIGTREE. All resultant trees (excluding the first 10% as burn-in) were also visualized using DENSITREE 2.2.5 (Bouckaert, 2010). Only Dataset 3 was used to create a BEAST tree due to the computational resources and length of time needed to reach convergence.

3. Results

We used 333bp of mitochondrial control region sequence from 754 birds encompassing 281 unique haplotypes. No deviations from neutral expectations were found using the Ewens-Watterson test, though four populations were significant using the Chakraborty test: Azores cool season birds on the islet of Vila, Selvagem hot season, Cape Verde birds sampled in June and sampled in November on the islet of Raso (Table 1). We also obtained 1,040bp sequence of cytochrome *b* for three birds from each of the populations, resulting in 69 cytochrome *b* sequences with 53 haplotypes.

For the ddRADseq data, we obtained 732.2 million reads from 146 individuals. Due to missing data, this was reduced to 133 individuals for the final dataset (Table 2). SNP Dataset 1, where loci had to occur in at least 50% of individuals within a population and in all 16 populations, contained 3,788 SNPs; SNP Dataset 2, where loci had to occur in at least 80% of individuals within a population but only 14 out of the 16 populations, contained 3,707 SNPs; and SNP Dataset 3, where loci had to occur in at least 80% of individuals within a population and all 16 populations, contained 2,608 SNPs. We present the results from SNP Dataset 3, which had the strictest filtering, as results were similar overall among all three datasets. The results for Datasets 1 and 2 are in the supplementary material (Figs. S1A-S2E, S5C, and S7A and S7B; Tables S6-S15). The levels of missing data were low and had a

relatively even distributed across populations, ranging from 0-7% per individual, with only 1 individual with more than 5% missing data and with 120 out of the 133 individuals having no missing data (Table S1). Due to the filtering settings used, there were no missing SNPs in any of the 16 populations, and no SNPs were found to deviate from Hardy-Weinberg expected proportions. The mean depth of sequencing ranged from 38.3 to 839.2, with an overall mean for all retained individuals of 328.8 times coverage.

3.1. Population genetic analyses

Results from STRUCTURE HARVESTER indicated that the most likely number of genetic populations (K) for the global datasets was two. All samples from the North Atlantic except for the Azores hot season and Cape Verde birds assigned to one genetic population with high probability, whereas the rest assigned with high probability to the other genetic population (Fig. 2A). When the two clusters were analysed separately, the North Atlantic populations split into two groups, with Selvagem June and three birds caught from Selvagem in October clustering separately (Fig. 2B). For the other cluster, STRUCTURE HARVESTER indicated K = 5 was most probable, separating into Cape Verde, South Atlantic populations, Galápagos populations, Hawaii and Japan, and the Azores hot population (Fig. 2C). Japan and Hawaii separated into K = 2 when run separately (Fig. 2D). The South Atlantic populations split into K = 2 when run separately, with the Ascension Island and St Helena samples assigning to different populations (Fig. 2E). No further substructure was found within the Galápagos. The STRUCTURE plots for K = 2-10 from the analysis including all populations, and the delta K outputs and average log likelihood scores for all runs with Dataset 3 are available in the supplementary material (Figs. S3A-S3C and S4A-S4F). Calculating the posterior probability of each K as per the STRUCTURE manual (Pritchard et al., 2010) gave the most likely K to

be six populations for the global dataset, separating samples from the Azores hot population, Cape Verde, the rest of the North Atlantic, Galápagos hot and cool populations, Hawaii and Japan, and the South Atlantic populations.

The PCA indicated distinct clusters corresponding to samples from Cape Verde, Azores hot, the rest of the North Atlantic, South Atlantic, Galápagos, Hawaii, and Japan (Fig. 3). PCA1 explained 25.37% of the variance, and PCA2 explained 10.12% (see Fig. S5A for the PCA with all 16 populations coloured separately). The clusters in our PCA correspond to the clusters found in the STRUCTURE analysis, but with samples from the South Atlantic populations clustering together, and with Selvagem grouping with the rest of the North Atlantic (Figs. 2A-E). Plotting PCA3, which explains 9.06% of the variation, results in the same groupings (Fig. S5B).

Haplotypes from Cape Verde populations were clearly separated from all others on the haplotype network including all mitochondrial control region sequences, although a few haplotypes were shared with other populations (Fig. 4). The Azores hot populations also separated out, sharing only one haplotype with a Cape Verde bird and two haplotypes with birds from the rest of the North Atlantic (Fig. 4). Haplotypes from the South Atlantic and Pacific birds clearly clustered together, although the South Atlantic, Galápagos, Hawaii and Japan populations did not share any haplotypes with each other within this dataset. The cytochrome *b* haplotype network showed a similar pattern (Fig. S6).

The pairwise Φ_{st} values from the mitochondrial control region sequences were generally very high and statistically significant between all 24 populations, with the significant values (after B-Y correction) ranging from 0.05 up to 0.93 (Table S2). Of 276 pairwise comparisons, only 26 were low and non-significant, ranging from 0 to 0.16 (seven of which were significant before B-Y correction; Table S2). Non-significant Φ_{st} values were evident between populations within the North Atlantic excluding Azores hot and Cape Verde

birds, between the two Azores hot populations, within Cape Verde populations, and within South Atlantic populations. Φ_{st} between the two Galápagos populations was not significant after correction (Table S2).

A similar pattern was seen using the SNP dataset. Pairwise F_{st} estimates between the North Atlantic populations, excluding the Azores hot and Cape Verde birds, were generally low ranging between 0 – 0.07. F_{st} estimates were generally low between the South Atlantic populations and between the seasonal populations in the Galápagos ranging between 0.01 - 0.02. All other pairwise comparisons were high, ranging between 0.16 – 0.67 (Table S3).

Estimates of Φ_{ST} from the mitochondrial control region between the seven groups found in the STRUCTURE analysis were high, ranging between 0.52 and 0.86. With the SNP data, F_{st} values were also high, between 0.16 and 0.63 (Table 3).

3.2. Phylogenetic reconstruction

In the gene tree generated using the mitochondrial data (69 birds sequenced for the mitochondrial control region and cytochrome *b*), haplotypes from birds from Cape Verde form a monophyletic group distinct from all other populations (Fig. 5). Haplotypes from the Azores hot population formed a sister group to the rest of the North Atlantic (excluding Cape Verde), which also formed a monophyletic group. Haplotypes from the South Atlantic populations were more closely related to the Pacific populations than to the North Atlantic birds; however relationships within the South Atlantic and Pacific clade did not resolve, having low support for the basal nodes within that group (Fig. 5).

The RAxML tree generated using the SNP data showed a similar topology to the mitochondrial gene tree, with the same seven major groups present, but the Azores hot population was basal to all populations apart from Cape Verde, instead of sister to the North

Atlantic clade as in the mitochondrial tree (Fig. 6). The South Atlantic and Pacific clade resolved better, though one node was still poorly supported (Fig. 6). The South Atlantic populations formed a sister group to all the Pacific birds, Hawaii and Japan were recovered as sister groups, which in turn were sister to the Galápagos populations (Figs. 6). Selvagem hot season breeders form a clade within the North Atlantic group, along with three Selvagem cool season birds (Fig 6). The SNAPP tree gave the same topology as the RAxML trees, but with the Azores hot population as sister to the North Atlantic clade (Fig. 7). The densitree showed little uncertainty between the seven groups (Fig. 7).

4. Discussion

Using a combination of population genomic and phylogenomic analyses we found substantial cryptic diversity in the band-rumped storm-petrel species complex. We uncovered seven genetic clusters that were distinct throughout our analyses of ddRADseq variation and were reciprocally monophyletic for mtDNA variation, consistent with our first hypothesis that populations from different archipelagos are genetically distinct. We also found both hot and cool season breeders within three clades, supporting the assertion that allochronic populations in different regions arose independently. We found allochronic populations to be at different stages along the speciation continuum, from genetically undifferentiated temporal populations to full allochronic species.

4.1. Cryptic species of band-rumped storm-petrel and the process of cryptic divergence

Reciprocal monophyly of genes, preferably from numerous loci, can be used as evidence that populations represent separately evolving metapopulations (De Quiroz, 2007).

Using thousands of genome-wide SNPs we recovered seven monophyletic groups within the band-rumped storm-petrel species complex (Figs. 6 and 7). These results are corroborated by the mitochondrial gene tree (Fig. 5) and our population genomic analyses. As such, band-rumped storm-petrels likely comprise multiple cryptic species. Our findings support suggestions of taxonomic differences between (i) Cape Verde (all seasons), (ii) hot-season birds from Azores, (iii) hot and cool season birds elsewhere in the Atlantic, (iv) South Atlantic, (v) Galápagos birds from both seasons and (vi) Japan and Hawaii populations. Populations in Japan and Hawaii perhaps also represent species, but investigation into responses to their vocalizations using playback would clarify their status given the lower level of genetic differentiation between the two. Our analysis provides no support for genetic or taxonomic separation among other North Atlantic cool season and hot season populations (the former often referred to as 'Grant's storm-petrel').

Although band-rumped storm-petrel populations are not easily distinguishable based on morphology, our results show high levels of genetic differentiation across their range. Five of the genetic groups that we uncovered are currently recognised as a single species, supporting our contention of cryptic diversity. One important question remaining in the study of cryptic diversification is the role of different species concepts in influencing the recognition of cryptic species (Bickford et al., 2007). The recently described unifying species concept defines a species as a 'separately evolving metapopulation lineage' (De Queiroz, 2007). Under this definition, evidence of lineage separation, for example reproductive isolation, ecological separation, diagnosability, and/or reciprocal monophyly, provides support for inferring a species boundary. Not all criteria need to be supported but ideally multiple lines of evidence should be used (De Queiroz, 2007).

In this paper we highlighted an example where testing for reproductive isolation (traditionally thought of as the biological species concept) is problematic, and as Irwin et al.

(2001) stated 'It is ironic that the biological species concept is only directly applicable to sympatric taxa'. This can be extended to include allochronic populations, which, even while sympatric, do not meet in time, and thus cannot be tested directly for evidence of reproductive isolation. Differences in vocalizations could be used as an indication of reproductive isolation, given their importance for attracting mates in storm-petrels (Bretagnolle, 1996). Playback experiments have shown that Azores hot season birds do not respond to calls from either Azores cool season birds or Galápagos hot season birds; the Galápagos hot season birds do not respond to Azores hot or cool season breeding birds; and Cape Verde birds do not respond to Azores hot or cool or Galápagos hot season breeders (Bolton, 2007). Further study into differences in vocalisations and responses across the range is needed; however such studies on remote, spatially and temporally separated band-rumped storm-petrel populations are logistically complex (Bolton, 2007).

Measures of phenotypic variation across populations are often used to define species (i.e. diagnosability, De Queiroz, 2007). Some work has been done on this in band-rumped storm-petrels (see Introduction) but studies are constrained by the same logistical concerns as for the playback experiments. In addition, the seasonal populations studied thus far show slight differences in morphology, potentially reflecting adaptation to different climatic conditions during the breeding seasons (Monteiro and Furness, 1998). For example, the Azores cool season birds have shorter wings and tails than sympatric hot season breeders (Monteiro and Furness, 1998) and the Galápagos cool season breeders have shorter bills, tarsi and wings than the sympatric hot season breeders (Smith and Friesen, 2007). If morphological differences between seasonal populations across the range represent parallel adaptations to the climate during the breeding season, they might confound the use of morphological differences to distinguish species, something that could be true in other species complexes with parallel adaptations. The use of the unifying species concept may

help to resolve the problem of differences between species concepts in identifying cryptic species, as identified by Bickford et al. (2007). This is particularly true in remote regions where applying the biological species concept is difficult, and will aid in the discovery of as yet unrecognized cryptic diversity.

Cryptic diversification remains an understudied process (Bickford et al., 2007), and the discovery of cryptic diversity in band-rumped storm-petrels highlights important unanswered questions. Most examples of cryptic species thus far have come from temperate regions (though not all, for example Funk et al., 2011), and further study is needed to assess whether tropical and marine ecosystems harbour disproportionate cryptic diversity (Bickford et al., 2007). Similarly, prioritizing habitats for conservation relies on accurate estimates of species richness (Bickford et al., 2007). Discovering areas with high levels of endemism, such as Cape Verde or the Galápagos, will be facilitated by the investigation of cryptic diversity, as found in band-rumped storm-petrels.

Uncovering cryptic diversity also gives insight into drivers of diversification, which may be otherwise missed; for example here we show that non-geographic barriers to gene flow promote diversification in band-rumped storm-petrels (discussed below). Non-geographic barriers may also act as important impediments to gene flow in other storm-petrel species, for example the Leach's storm-petrel species complex (Taylor et al., 2018). Allochrony provides a potentially important and underappreciated mechanism that can drive cryptic speciation without any geographical barriers. Many more examples likely remain undiscovered across a wide variety of taxa (Taylor and Friesen, 2017).

4.2. Geographic and non-geographic barriers to gene flow

Both the Azores hot population and the Cape Verde birds were recently named as distinct species (Bolton et al., 2008; Sangster et al., 2012), and our results confirm the genetic distinctness of both. We found further cryptic diversity, with populations in the Pacific genetically differentiated from those in the Atlantic. Therefore, land likely has acted as an historical barrier to gene flow between Atlantic and Pacific populations. However, the South Atlantic birds comprise a lineage that is sister to the Pacific populations, not to the other Atlantic ones (Figs. 5-7), which implies historical migration between the South Atlantic and Pacific.

The Azores hot population may be basal to all other populations except the Cape Verde birds with some gene flow between it and the sympatric Azores cool season breeders, creating the discordance evident in our phylogenies. Conversely, the Azores hot population could be sister to the North Atlantic group but with some gene flow between it and the Hawaii and/or Japan birds. Both scenarios could produce the observed phylogenetic patterns. Long distance dispersal between the Atlantic and Pacific Oceans has been observed in Swinhoe's Storm-petrel (*H. monorhis;* Silva et al., 2016b). The patterns seen with the Azores hot populations, and the sister relationship between the South Atlantic and Pacific populations, may be attributable to similar long-distance dispersal events, which should be explicitly tested in future work. Alternatively, the different topology evident in the RAxML tree could be due to a lack of power for the program, as the topology of the tree was not as well resolved, especially as the number of SNPs used in the analyses was reduced (Figs. 6, S7A and S7B). The BEAST phylogeny, in contrast, was better resolved with stronger support for the nodes and recovered the Azores hot birds as sister to the rest of the North Atlantic (Fig. 7), in agreement with the mitochondrial phylogeny (Fig. 5).

We found strong genetic structuring within both ocean basins, implying that non-geographic barriers to gene flow were also important in diversification in the band-rumped

storm-petrel cryptic species complex. The potential philopatry of storm-petrels, as well as differences in ocean regimes (e.g. differences in sea surface temperature, location of upwellings and prey species), may facilitate differentiation. Cape Verde appears to be a hotspot of endemism for procellariforms with up to four endemic taxa (Cape Verde petrel, *Pterodroma feae;* Cape Verde shearwater*, Calonectris edwardsii;* Boyd's shearwater, *Puffinus lherminieri boydi*; and Cape Verde storm-petrel studied here), which could be associated with adaptation to foraging in the complex ocean currents in the area (Friesen, 2015). Similarly, ocean currents around the Galápagos are associated with genetic divergence in many seabird species (Friesen, 2015). Analysis of the location of genetic groups in relation to differences in ocean regime would enable an explicit test of the importance of adaptation to ocean regimes versus philopatry as drivers of genetic differentiation in band-rumped storm-petrels, as the two scenarios are difficult to distinguish using genomic data alone.

4.3. Independent origins of allochronic populations

Testing for parallel diversification can be difficult, and as such relatively few examples have been proven (Perez-Pereira et al., 2017; Rundel et al., 2000; Schluter and Nagel, 1995). We found three separate monophyletic groups with both hot- and cool-season breeding populations across the range of band-rumped storm-petrels, with the Azores hot birds forming a monophyletic group on their own, and with Selvagem hot season breeders forming a distinct lineage within the North Atlantic group (Figs. 5 and 6). Given that the Azores hot season breeders form their own clade, with the Azores cool season breeders embedded within the rest of the North Atlantic samples, the allochronic populations in the Azores *could* have formed through historical isolation and recolonization of the cool season population from the rest of the North Atlantic. Alternatively, the pattern could also be

explained by ongoing gene flow between the cool season breeders in the Azores and the rest of the North Atlantic cool season breeders (Friesen et al. 2007), especially given the proximity of the islands.

The Selvagem hot season breeders show a low level of genetic differentiation from the rest of the populations within that clade, separating out in STRUCTURE (Figs. 2B). Three Selvagem cool season birds grouped with the Selvagem hot season breeders in STRUCTURE and the RAxML trees, which may indicate that they were hot season breeders caught during their non-breeding season. Our results support the likelihood that two breeding seasons exist in Selvagem. Perhaps the breeding seasons are protracted and overlap, which may have led to the suggestion of year-round breeding (Mougin et al., 1990). The Selvagem hot season breeders appear to be differentiating, and may represent an earlier stage along the speciation continuum than the Azores hot season breeders.

Even using a large SNP dataset, the level of genetic differentiation between many of the seasonal populations is very low, with seasonal breeders not showing signatures of differentiation within Ascension Island, St. Helena Island, the Galápagos, or Desertas. The results raise the interesting question as to whether these populations represent incomplete lineage sorting and recent allochronic divergence in these areas, or plasticity in breeding time and gene flow between seasonal breeding populations. Allochrony can contribute to speciation across taxonomic groups, and factors that might be involved, such as plasticity in breeding time, should be investigated to understand phenological shifts, particularly in light of anthropogenic climate change (Taylor and Friesen, 2017). Few cases of allochronic divergence have been studied in detail, but in some cases plasticity is thought to be involved. For example, Marshall *et al*. (2003; 2011) hypothesized that plasticity played a role in the initial life cycle switches of 13-year and 17-year periodical cicadas (*Magicicada* spp.), driven by climatic processes. Life cycle length may then have become canalized if the extreme

climatic conditions persisted over a number of years (Marshall and Cooley 2000; Marshall et al. 2011). Our finding of low genetic differentiation between seasonal populations may indicate a potential role for plasticity in the formation of seasonal populations of band-rumped storm-petrel.

Our results overall provide evidence for up to six independent formations of sympatric allochronic breeders (or breeding time changes): in the Azores where the hot season breeders have now genetically diverged to species level, on Selvagem where hot season breeders show a lower level of differentiation and still sit within the major North Atlantic clade, on Desertas where hot season breeders sit within the North Atlantic clade with the cool season breeders and are either at an early stage of divergence or are exchanging genes potentially due to plasticity, in the Galápagos where hot and cool birds form a monophyletic clade and are also either at an early stage of divergence or are exchanging genes, on Ascension Island, and on St Helena Island. The latter two could comprise just one breeding time change as they are admixed on the phylogeny; however they separated into two clusters in STRUCTURE: hot and cool populations on Ascension Island, and hot and cool populations on St Helena Island (Fig. 2E). This may indicate that two breeding time changes occurred in the South Atlantic, with seasonal populations arising independently on each island and also either at an early stage along the speciation continuum or still exchanging genes.

A major question arises from our results: what has driven the formation of populations with different breeding seasons multiple times in band-rumped storm-petrels? Monteiro and Furness (1998) postulated that competition for nest sites may underlie the evolution of the allochronic populations. Storm-petrels are predated by larger seabirds, and predation was suggested as another potential (though less likely) explanation for seasonal breeding in the Azores populations of band-rumped storm-petrels (Monteiro & Furness

1998). Competition for food resources may also drive individuals to breed at a new time (Monteiro & Furness 1998), and if not a driver of a new breeding time, available food is likely key to allow the persistence of different breeding season. An interesting avenue for future research would be to test whether the locations of seasonal populations of band-rumped storm-petrels relate to available food resources. Not all global populations have developed two breeding seasons, and an investigation as to whether the location of allochronic populations is linked to areas with multiple seasonal upwellings would be worthwhile. For example, upwelling-favourable winds are strong year-round in the North-West African upwelling system (Meunier *et al*. 2012), which may relate to why birds in Cape Verde demonstrate year-round breeding.

4.4. Conservation implications for the band-rumped storm-petrel species complex

The recognition of cryptic species is key to accurate assessment of conservation status of individual taxa given the use of range size and abundance as key listing criteria by the IUCN (Funk et al., 2011; IUCN, 2017). It is also central to understanding the threats and related conservation actions that may be specific to different geographic areas. Recognising populations representing evolutionary significant units (ESUs, reciprocally monophyletic for mitochondrial DNA and differing in allele frequencies of nuclear loci) is also pivotal to conserving important components of genetic diversity (Moritz, 1994).

The Azores hot population of band-rumped storm-petrels is already recognised as a distinct species, *H. monteiroi* (Bolton et al., 2008), and is listed as Vulnerable on the IUCN Red List due to its small population size and restricted range (IUCN, 2017). All other populations have currently been assessed by the IUCN under *H. castro* (or *O. castro*), and are listed as Least Concern because the species ‘has an extremely large range’ and ‘the

population size is very large' (IUCN, 2017). Given the restricted ranges of the well-delineated genetic clusters uncovered in our study, re-assessment will likely be needed for a more accurate assessment of the levels of threat impinging on the various components of the band-rumped storm-petrel species complex. Some populations of this species are already considered threatened in parts of their range, such as in Hawaii, where the population was recently up-listed to Endangered by the US Fish & Wildlife Service (USFWS, 2016). Under-estimation of threat level is a key problem with cryptic species, for example in *Engystomops* toadlets and *Hypsiboas* treefrogs (Funk et al., 2011). Uncovering the need for different conservation strategies also requires the recognition of cryptic species, such as in *Pipistrellus pipistrellus* and *P. pygmaeus* bats (Davidson-Watts et al., 2006).

5. Conclusion

We assessed patterns of genetic differentiation across the entire range of the band-rumped storm-petrel species complex. We uncovered high levels of cryptic diversity in these nocturnal seabirds, suggesting that band-rumped storm-petrels likely represent multiple species. We also found that non-geographic barriers may be stronger impediments to gene flow than geographic land barriers. Finally, we also report the independent formation of multiple allochronic populations spanning the speciation continuum in band-rumped storm-petrels. Further research is needed to understand whether the lack of genetic differentiation between some sympatric seasonal populations represents ongoing gene flow and plasticity in breeding time, or incomplete lineage sorting due to recent breeding time switches. Answering such questions will give important insight into the processes underlying allochronic speciation, an under-studied mechanism that can lead to cryptic divergence in many taxa (Taylor and Friesen, 2017).

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Competing Interests Statement

The authors have no conflicts of interest to declare.

Author Contributions

RST conducted field work, lab work, analysed the data and wrote the manuscript; MB, AB, PD-C, AFR and JG-S helped conceive the study and conducted field work, TB and PD-C conducted lab work, SCL aided with the analysis of data and VLF conceived the study

and helped write the manuscript. RST, MB, AB AFR, JG-S, AR and VLF helped generate

funding.

Appendix A. Supplementary material

Supplementary data to this article can be found online

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Table 1

Sample sites with their abbreviations, and descriptive statistics for mitochondrial control region results. N = sample size, h = haplotype diversity, π = nucleotide diversity as a percent. The Ewens-Watterson values show the observed and expected F values, none of which were significant. The Chakraborty values are probabilities for deviation from neutrality.

Country	Geographic Region	Sampling site	Abbreviation	Breeding Season	N	Number of haplotypes	h	π	Ewans-Watterson	Ch
Portugal	North Atlantic Ocean	Berlengas September	BLS	Cool	19	9	0.91±0.04	1.21±0.71	0.14/0.18	0.8
	North Atlantic Ocean	Azores, Praia September	PRS	Cool	40	10	0.78±0.05	0.66±0.42	0.24/0.21	0.2
	North Atlantic Ocean	Azores, Praia June	PRJ	Hot	45	21	0.94±0.02	1.72±0.94	0.08/0.08	0.5
	North Atlantic Ocean	Azores, Baixo September	BXS	Cool	13	5	0.81±0.08	0.43±0.32	0.25/0.31	0.7
	North Atlantic Ocean	Azores, Baixo June	BXJ	Hot	47	19	0.99±0.02	1.95±1.05	0.09/0.10	0.6
	North Atlantic Ocean	Azores, Vila September	VLS	Cool	49	20	0.87±0.03	1.22±0.70	0.15/0.09	0.0
	North Atlantic Ocean	Madeira, Desertas November	DSN	Cool	26	10	0.80±0.07	0.66±0.43	0.23/0.18	0.1
	North Atlantic Ocean	Madeira, Desertas August	DSA	Hot	32	15	0.89±0.04	1.62±0.90	0.14/0.11	0.1
	North Atlantic Ocean	Madeira, Selvagem October	SVO	Cool	39	20	0.91±0.03	0.95±0.56	0.11/0.08	0.0
	North Atlantic Ocean	Madeira, Selvagem June	SVJ	Hot	32	12	0.79±0.06	0.57±0.37	0.23/0.15	0.0
Spain	North Atlantic Ocean	Canary Islands December	CID	Cool	5	5	1.00±0.13	1.21±0.85		
Cape Verde	North Atlantic Ocean, West Africa	Branco June	BRJ	Hot	26	18	0.94±0.03	2.17±1.18	0.09/0.07	0.2
	North Atlantic Ocean, West Africa	Raso January[1]	RJA	Year-round	12	9	0.91±0.08	4.81±2.61	0.17/0.14	0.3
	North Atlantic Ocean, West Africa	Raso April	RA	Year-round	25	13	0.92±0.03	0.83±0.51	0.12/0.12	0.5
	North Atlantic Ocean, West Africa	Raso June	RJU	Year-round	50	24	0.88±0.04	0.92±0.55	0.14/0.07	0.0
	North Atlantic Ocean, West Africa	Raso November[1]	RN	Year-round	84	28	0.84±0.04	0.91±0.53	0.17/0.07	0.0
United Kingdom	South Atlantic Ocean	Ascension Island June[1]	AIJ	Cool	23	14	0.96±0.02	1.54±0.87	0.09/0.10	0.8
	South Atlantic Ocean	Ascension Island November[1]	AIN	Hot	28	17	0.95±0.02	1.57±0.87	0.08/0.08	0.5
	South Atlantic Ocean	St Helena June[1]	SHJ	Cool	18	15	0.98±0.02	1.17±0.69	0.07/0.08	0.7
	South Atlantic Ocean	St Helena November[1]	SHN	Hot	35	20	0.95±0.02	0.97±0.58	0.07/0.08	0.6
Galápagos	East Pacific Ocean	Plaza Norte May[2]	PNM	Cool	34	26	0.98±0.01	1.27±0.73	0.05/0.05	0.6
	East Pacific Ocean	Plaza Norte December[3]	PND	Hot	30	25	0.98±0.01	1.93±1.05	0.05/0.05	0.5
Hawaii	Central Pacific Ocean	Kaua'i September[1]	KS	Hot	11	9	0.96±0.05	1.69±1.00	0.12/0.13	0.7
Japan	West Pacific Ocean	Hidejima August	HA	Hot	31	12	0.85±0.05	2.08±1.12	0.18/0.15	0.2

[1]New population sampled in this study

[2]Sometimes referred to as wet season (Smith and Friesen, 2007)

[3]Sometimes referred to as dry season (Smith and Friesen, 2007)

Table 2

Sampling sites and number of sampled individuals from each location and time point for ddRADseq. H_o = observed heterozygosity, H_e = expected heterozygosity, π = nucleotide diversity as a percent, F_{is} = inbreeding coefficient.

Country	Geographic Region	Sampling Site	Breeding Season	N	H_o	H_e	π	F_{is}
Portugal	North Atlantic Ocean	Berlengas September	Cool	8	0.0011	0.0010	0.11	0.0001
	North Atlantic Ocean	Azores, Praia September	Cool	6	0.0011	0.0010	0.11	0.0001
	North Atlantic Ocean	Azores, Praia June	Hot	6	0.0012	0.0012	0.13	0.0003
	North Atlantic Ocean	Madeira, Desertas November	Cool	8	0.0011	0.0011	0.11	0.0001
	North Atlantic Ocean	Madeira, Desertas August	Hot	8	0.0011	0.0011	0.12	0.0001
	North Atlantic Ocean	Madeira, Selvagem October	Cool	8	0.0011	0.0011	0.12	0.0002
	North Atlantic Ocean	Madeira, Selvagem June	Hot	7	0.0010	0.0010	0.11	0.0001
Cape Verde	North Atlantic Ocean, West Africa	Raso June	Year-round	7	0.0005	0.0005	0.06	0.0002
United Kingdom	South Atlantic Ocean	Ascension Island June	Cool	13	0.0014	0.0014	0.15	0.0003
	South Atlantic Ocean	Ascension Island November	Hot	10	0.0014	0.0014	0.15	0.0002
	South Atlantic Ocean	St Helena June	Cool	11	0.0013	0.0014	0.14	0.0003
	South Atlantic Ocean	St Helena November	Hot	12	0.0013	0.0014	0.14	0.0003
Galápagos	East Pacific Ocean	Plaza Norte May	Cool	7	0.0010	0.0010	0.11	0.0001
	East Pacific Ocean	Plaza Norte December	Hot	8	0.0010	0.0010	0.11	0.0001
Hawaii	Central Pacific Ocean	Kaua'i September	Hot	6	0.0012	0.0012	0.13	0.0002
Japan	West Pacific Ocean	Hidejima August	Hot	8	0.0011	0.0011	0.12	0.0002

Table 3
Estimates of Φ_{st} from the mitochondrial control region (above diagonal, all values $P < 0.05$) and F_{st} from the SNP data (below diagonal) for pairwise comparisons of the seven major population groups found in STRUCTURE.

		Azores Hot	North Atlantic	Cape Verde	South Atlantic	Galápagos	Hawaii	Japan
	n	92	281	171	104	64	11	31
Azores Hot	6		0.52	0.83	0.76	0.81	0.77	0.66
North Atlantic	45	0.32		0.78	0.72	0.74	0.72	0.56
Cape Verde	7	0.54	0.60		0.84	0.86	0.84	0.82
South Atlantic	46	0.23	0.40	0.50		0.81	0.77	0.69
Galápagos	15	0.34	0.48	0.63	0.33		0.73	0.73
Hawaii	6	0.21	0.41	0.59	0.22	0.28		0.66
Japan	8	0.23	0.41	0.60	0.25	0.31	0.16	

Fig. 1. Breeding areas of the band-rumped storm-petrel species complex. Red circles indicate hot season breeding, blue circles indicate cool season breading, and purple circles indicate year-round breeding. Circles with both red and blue halves indicate areas separate both hot and cool season breeding populations. Current taxonomy indicates Azores hot season breeders as *Hydrobates Monteiroi*, Cape Verde birds as *Hydrobates jabejabe*, and all others as *Hydrobates castro*. South Atlantic populations have been considered a subspecies, *H. c. Helena*, as have the Galápagos populations, *H. c. bangsi*. Inset painting shows birds from the Azores, with the cool season breeding morphology demonstrated on the left and hot season morphology on the right.

Fig. 2. Molecular assignments of band-rumped storm-petrels based on analysis of SNPs using STRUCTURE showing the plots with the most likely number of clusters indicated by STRUCTURE HARVESTER. A) The plot for K= 2 for all populations. B) The plot for K = 2, when including the North Atlantic group only. C) The plot for K = 5 when removing the North Atlantic birds. D) The plot for K = 2 when including the Hawaii and Japan populations only. E) The plot for K = 2 when including the South Atlantic populations only.

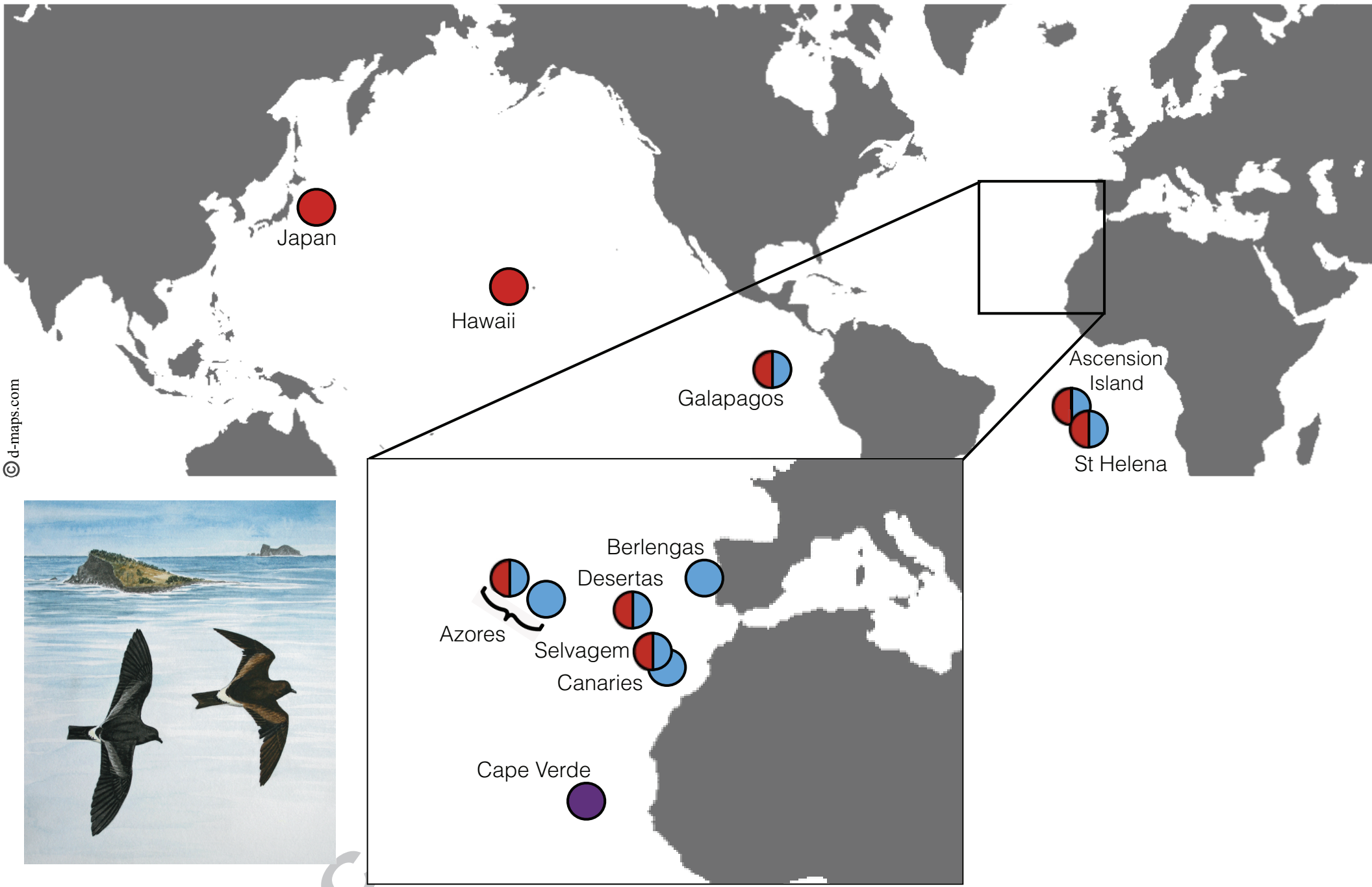
Fig. 3. Results of a principal component analysis of the band-rumped storm-petrel species complex seven genetic groups. Ellipses and colours have been chosen to label the six resultant non-overlapping clusters.

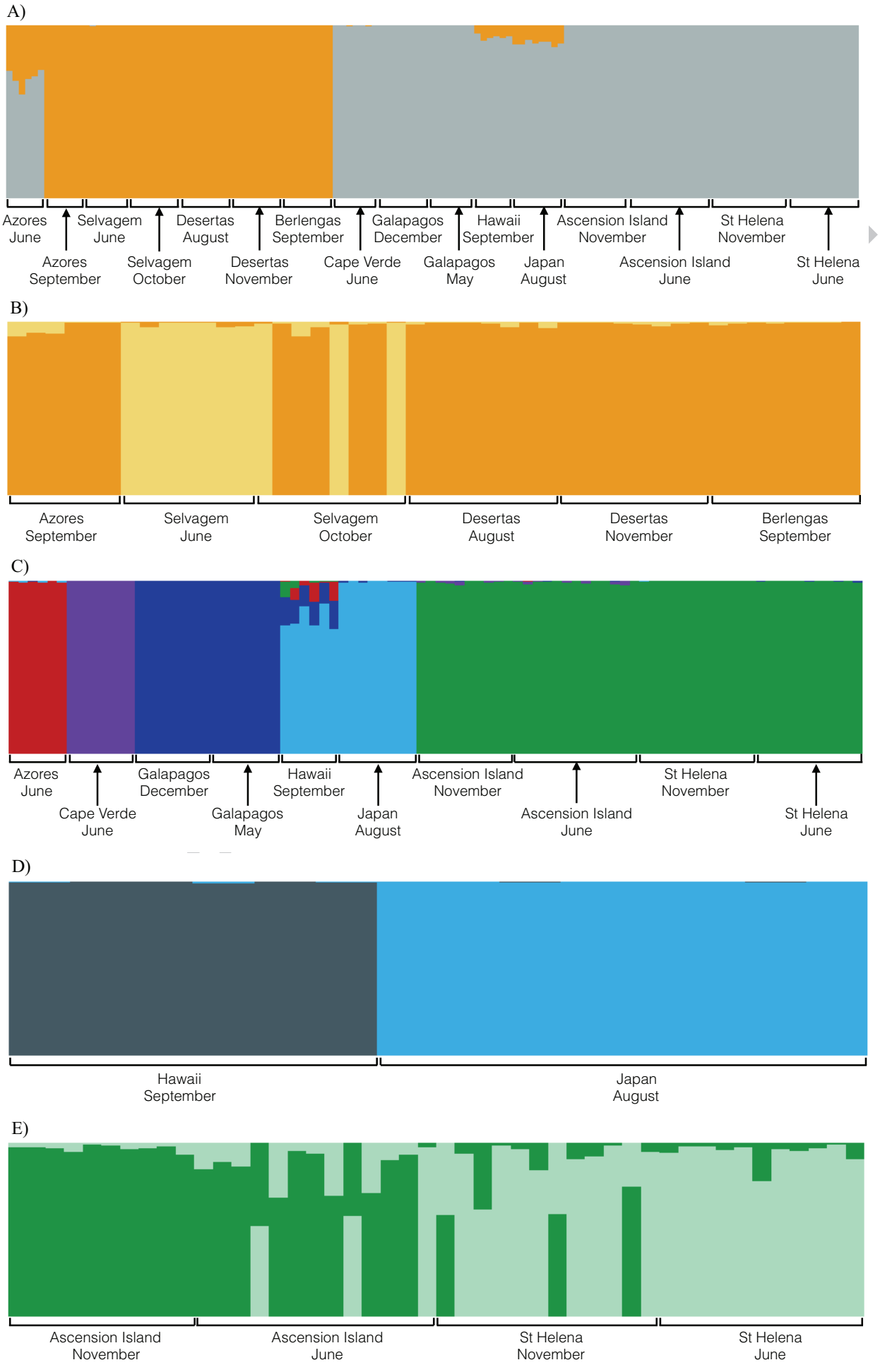
Fig. 4. Statistical parsimony network of 333bp of the mitochondrial control region of 754 band-rumped storm-petrels. Circle size relates to the number of samples with each haplotype. Black circles represent unsampled or now-extinct haplotypes.

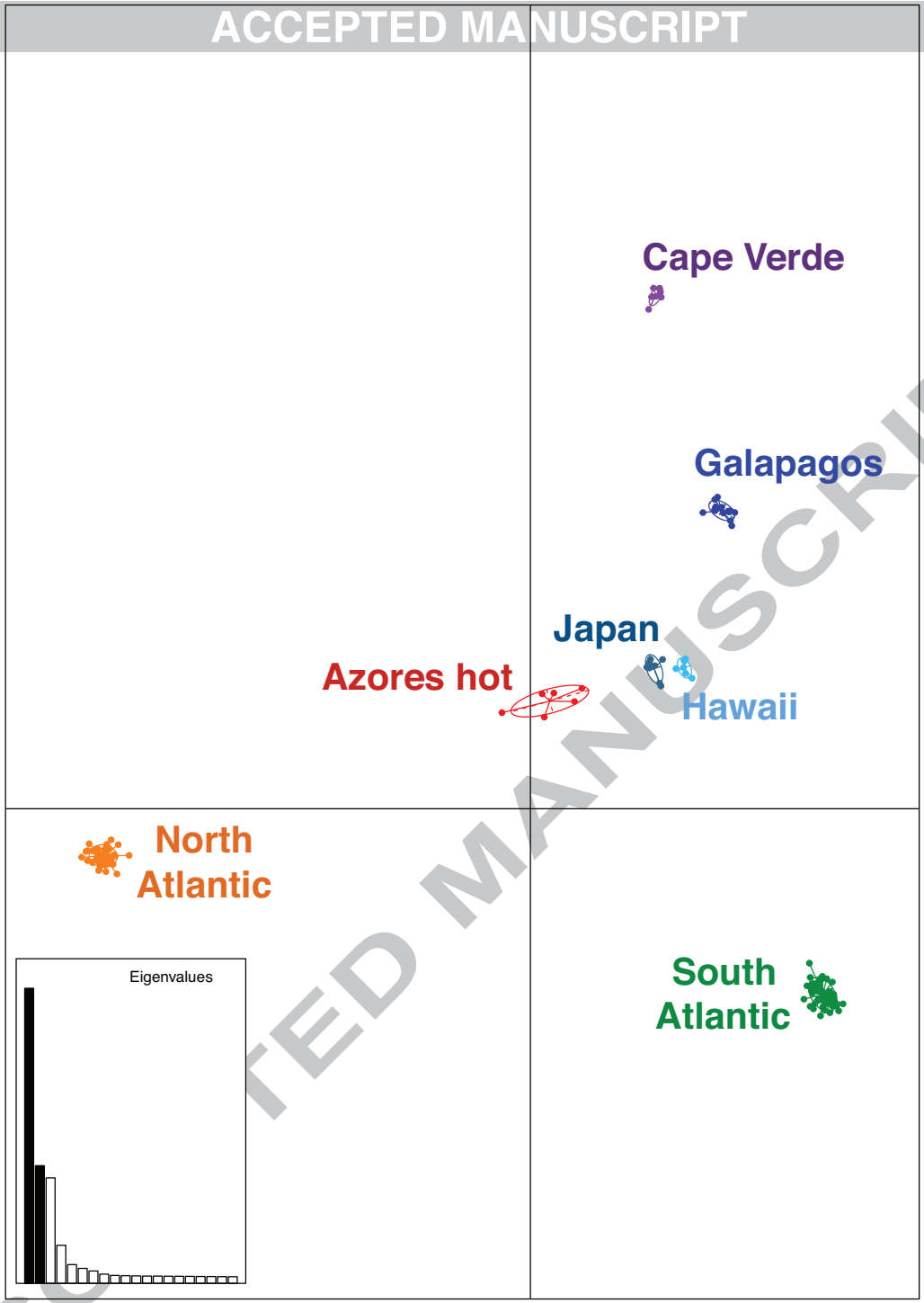
Fig. 5. Bayesian phylogenetic reconstruction of concatenated cytochrome *b* and control region mitochondrial DNA sequences of the band-rumped storm-petrel species complex, generated in BEAST. Posterior probability values are given above nodes to show support for the seven groups uncovered in our analyses.

Fig. 6. Maximum likelihood phylogenetic reconstruction of relationships among the band-rumped storm-petrels generated in RAxML. Bootstrap support is given for all nodes with a value above 50. Branches are coloured by the breeding time of the individual bird: red for a bird sampled during the hot season, blue for a bird sampled during the cool season, and purple for a bird sampled from a colony with year-round breeding.

Fig. 7. Phylogenomic reconstruction of the seven genetic groups of band-rumped storm-petrels generated in the SNAPP package in BEAST. The left-hand side shows the densitree and the right-hand side shows the consensus tree with posterior probabilities labelled on the nodes.

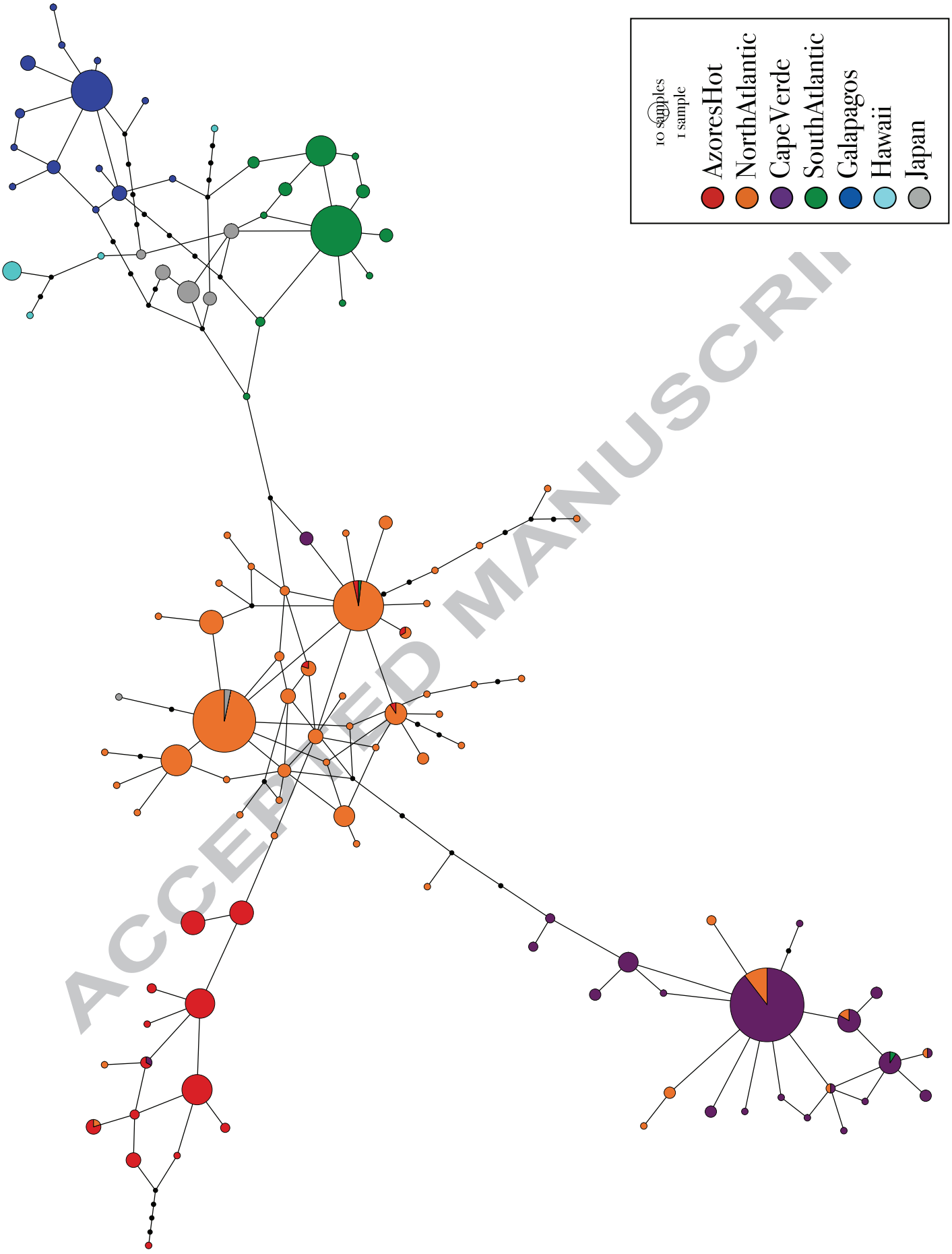


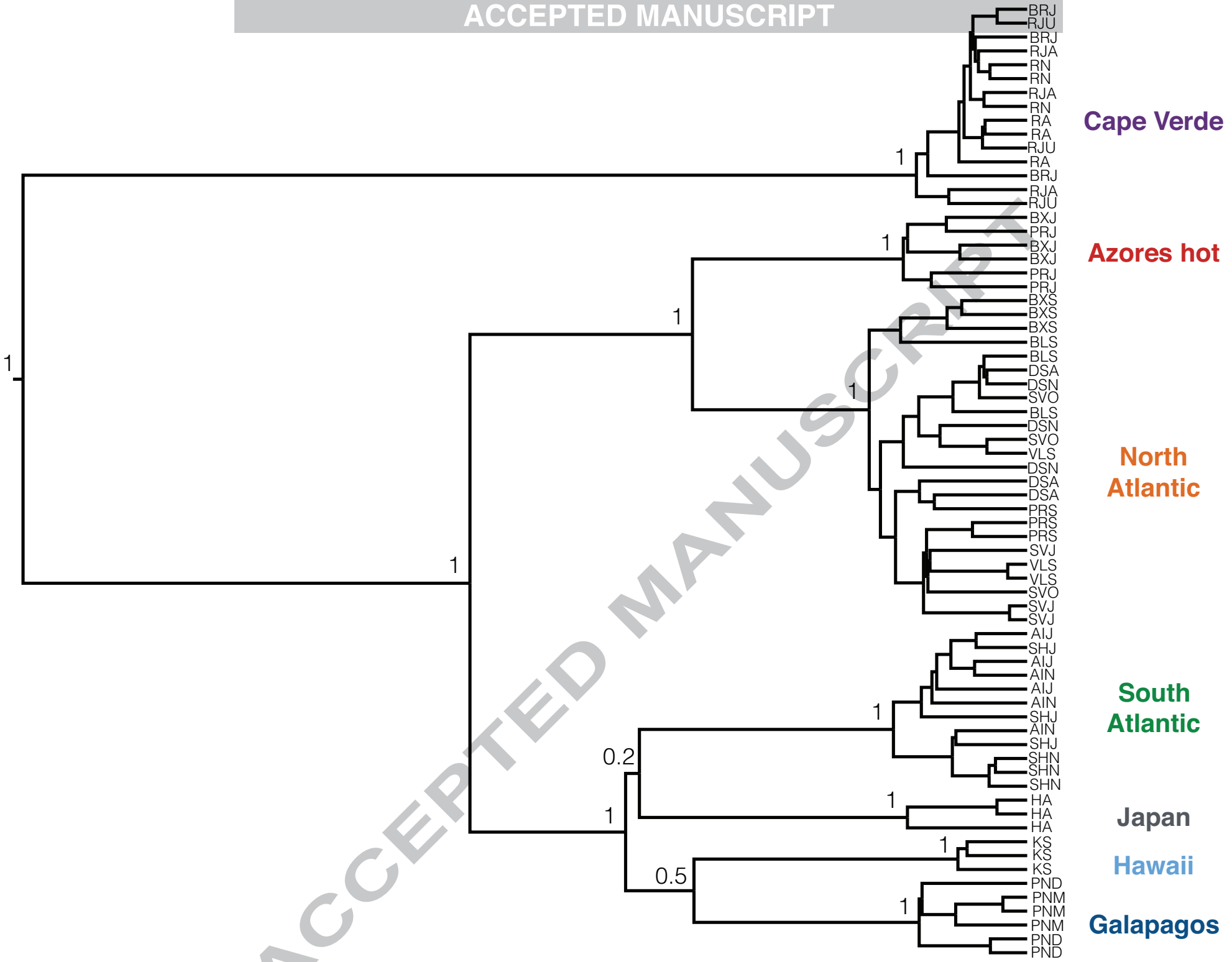


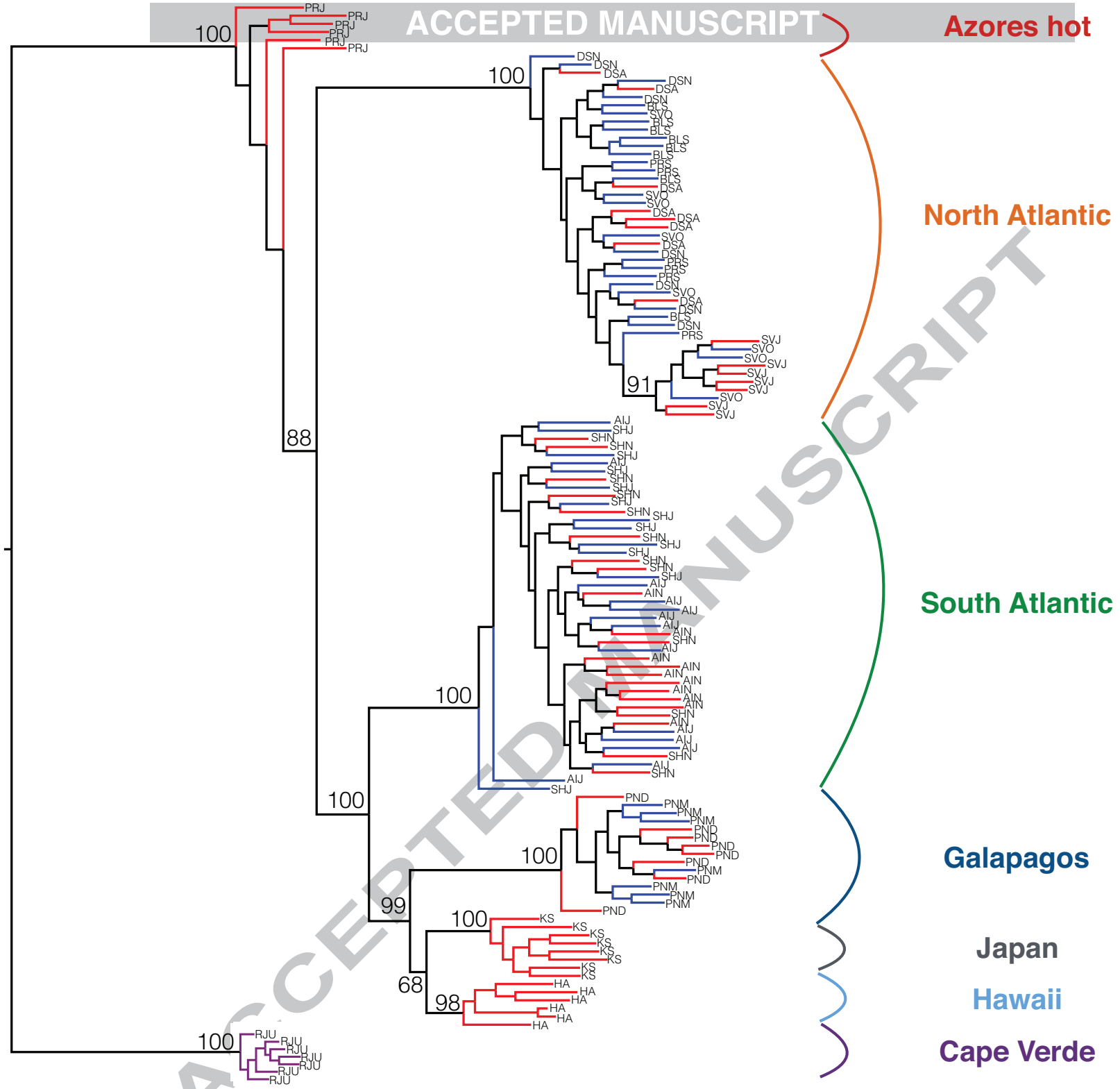


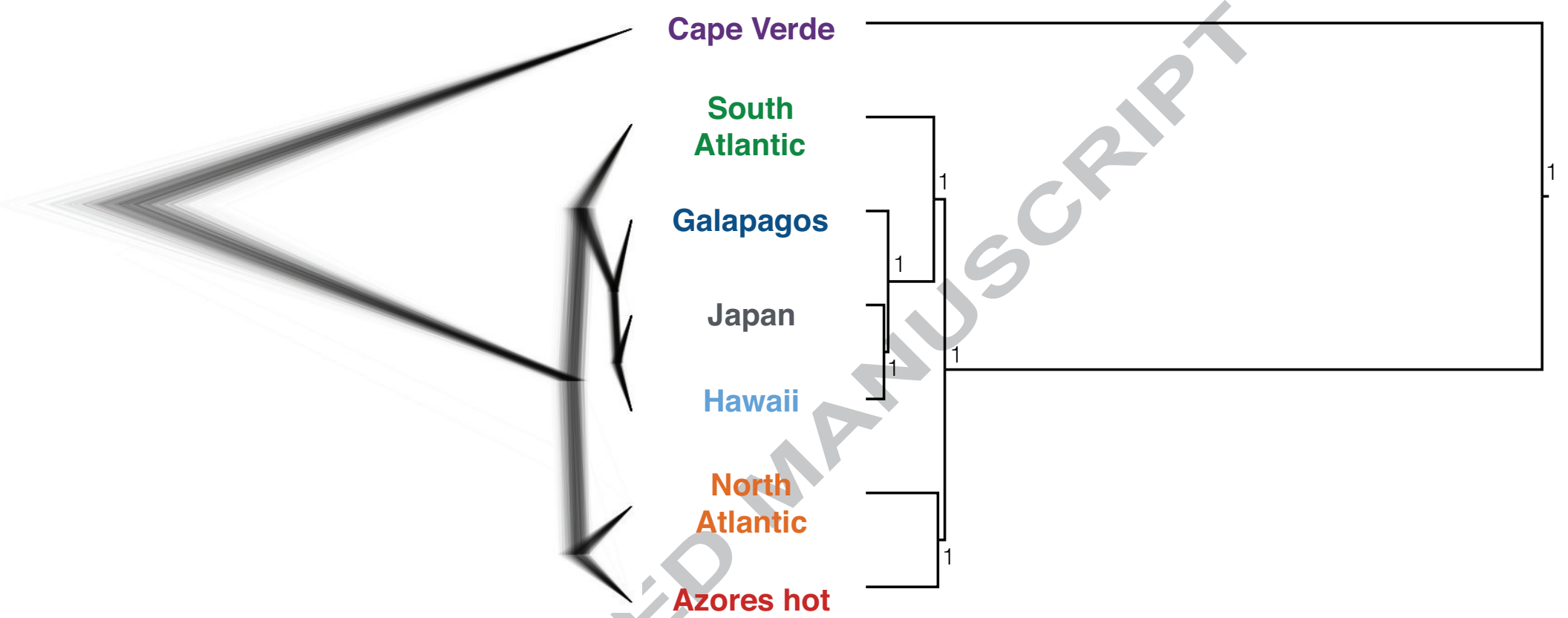
PCA Axis 2 (10.12% Variance)

PCA Axis 1 (25.37% Variance)



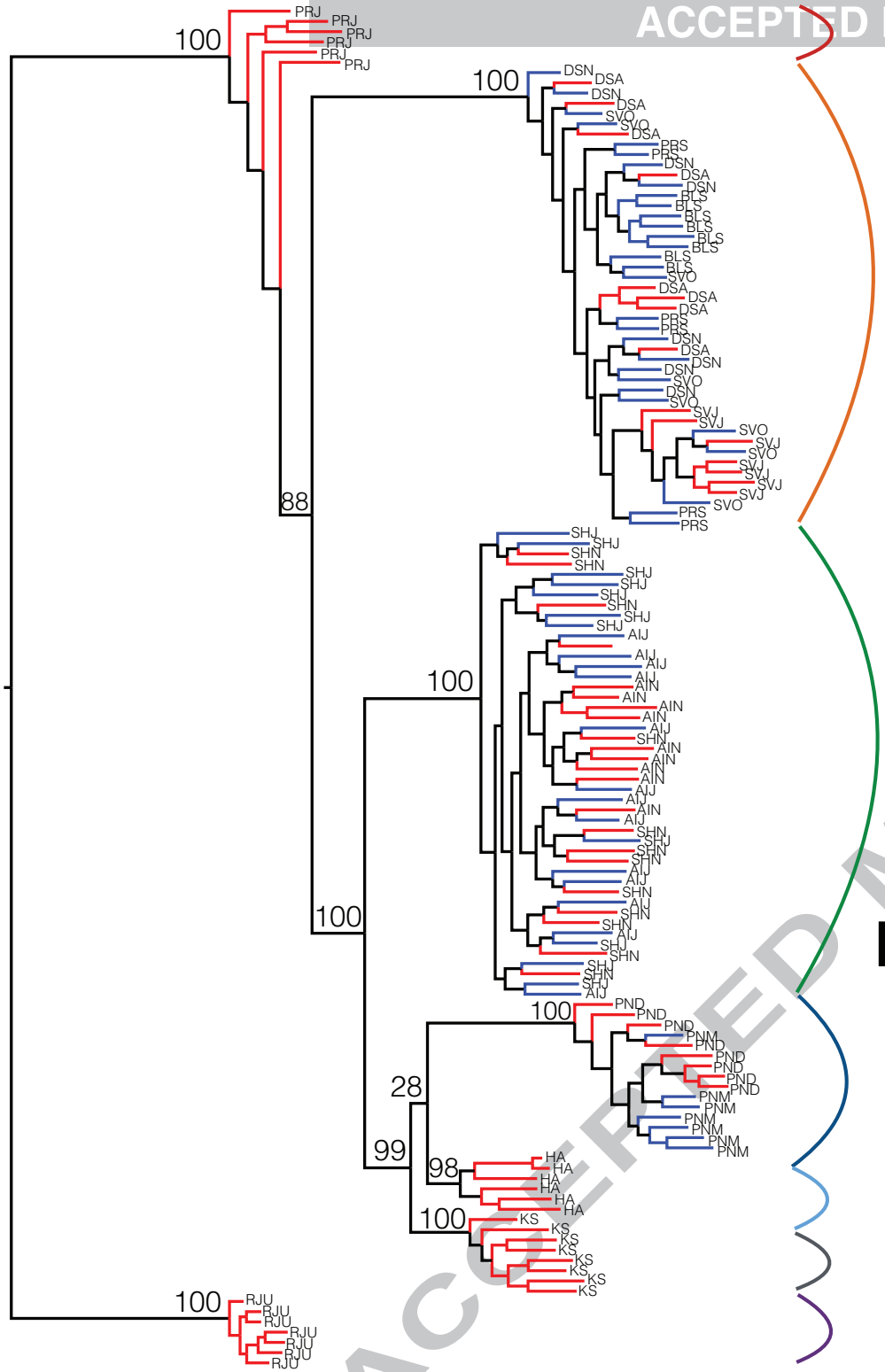






Highlights

- We uncover seven reciprocally monophyletic clades of band-rumped storm-petrel.
- They likely comprise multiple cryptic species needing assessment for conservation.
- Non-geographic barriers were possibly key in driving divergence.
- Allochronic populations are in multiple clades, indicating independent evolution.
- Allochronic populations are at different stages of differentiation.



Azores hot

North Atlantic

South Atlantic

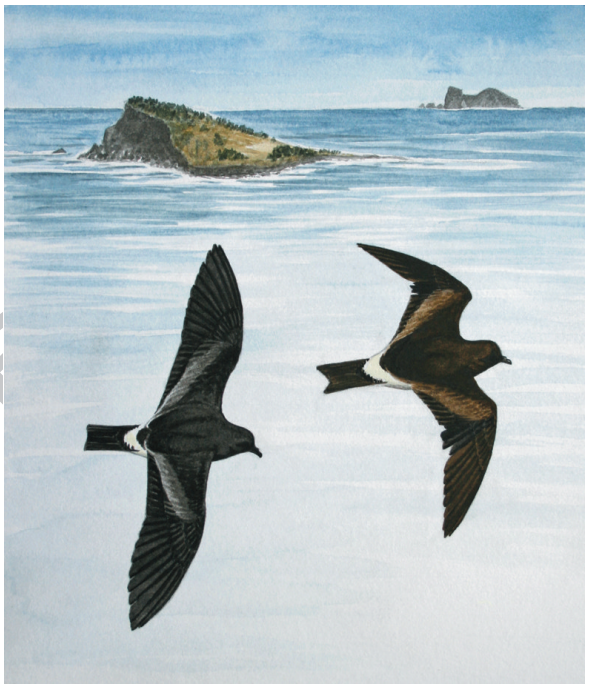
Galapagos

Hawaii

Japan

Cape Verde

Morphology of band-rumped storm-petrels, example from the Azores



Cool season breeder

Hot season breeder