

Sympatric population divergence within a highly pelagic seabird species complex (*Hydrobates* spp.)

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Both physical and non-physical barriers can restrict gene flow among seabird populations. Understanding the relative importance of non-physical barriers, such as breeding phenology, is key to understanding seabird biodiversity. We investigated drivers of diversification in the Leach's storm-petrel species complex (*Hydrobates* spp.) by examining population genetic structure across its range. Variation in the mitochondrial control region and six microsatellite loci was assayed in birds sampled from breeding colonies throughout the North Atlantic and North Pacific (*H. leucorhoa leucorhoa*), as well as from San Benito Islands (*H. l. chapmani*), and two seasonal populations in Guadalupe (summer breeding *H. socorroensis* and winter breeding *H. cheimomnestes*), Mexico. Weak but significant differentiation was found between populations of *H. l. leucorhoa* breeding in the Atlantic versus North Pacific, as well as between *H. l. chapmani* and *H. l. leucorhoa*, and between *H. socorroensis* and *H. cheimomnestes* within Guadalupe. In contrast, strong differentiation in both mitochondrial DNA and microsatellites was found between *H. leucorhoa* and both *H. socorroensis* and *H. cheimomnestes*. Phylogenetic reconstruction suggested the Guadalupe seasonal breeding populations are sister taxa, at least in their mitochondrial DNA. Non-physical barriers to gene flow appear to be more important than physical barriers in driving divergence within the Leach's storm-petrel species complex. In particular, allochronic speciation may have occurred between the seasonal populations within Guadalupe. Further work should include higher resolution sequencing to confirm results, and an increased sampling effort, particularly within the California area, to fully resolve the relationship between *H. l. leucorhoa* and *H. l. chapmani*.

Seabirds are highly mobile organisms, having fewer physical barriers to gene flow than many other taxa (Friesen et al. 2007a, Friesen 2015). Large areas of land or ice can act as physical barriers to gene flow because many seabirds are tied to the ocean for feeding and taking-off for flight (Friesen et al. 2007a). However, land and ice alone cannot explain the diversity of seabirds, and many other factors appear to drive divergence between seabird populations (Friesen et al. 2007a, Friesen 2015). Some of the most important non-physical barriers include philopatry, differences in non-breeding distributions, and ocean regimes (Friesen 2015).

Breeding phenology can also act as a non-physical barrier to gene flow, and in cases of allochronic

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speciation, temporal separation of breeding time drives divergence in sympatry (Alexander and Bigelow 1960, Taylor and Friesen 2017). Allochronic speciation has occurred between two populations of band-rumped storm-petrels (*Hydrobates* spp.), where individuals were found to breed in both the hot and cool seasons in the Azores, even using the same nest sites (Monteiro and Furness 1998). Genetic differentiation between the two seasonal populations, coupled with differences in morphology and vocalisations, led to the hot season population being named as a distinct species, Monteiro's storm-petrel (*H. monteiroi*; Friesen et al. 2007b, Bolton et al. 2008).

Investigation of genetic relationships among conspecific populations can help to elucidate factors driving diversification, as in *H. castro* and *H. monteiroi* (Friesen et al. 2007b, Wallace et al. 2017) and Hawaiian and Galapagos petrels (*Pterodroma sandwichensis* and *P. phaeopygia* respectively; Welch et al. 2011). However, in many species or species complexes detailed genetic study has not been undertaken across the whole range. This limits both our knowledge of these taxa, and identification of factors that may act as strong barriers to gene flow. One such example is the Leach's storm-petrel species complex (*Hydrobates* spp. [*Oceanodroma*]; Procellariiformes: Hydrobatidae), in which phylogenetic relationships among populations across the range remain largely unknown (AOU Classification Committee 2016, but see below).

The Leach's storm-petrel *Hydrobates leucorhoa* (Vieillot 1818) species complex is broadly distributed throughout the North Atlantic and North Pacific Basins. Breeding colonies in the Pacific extend from northern Japan through the Aleutian Archipelago and along western North America from southern Alaska to islands off Baja California. Atlantic breeding colonies are distributed in northern USA and eastern Canada, Iceland, Norway and Scotland (del Hoyo et al. 1992; Huntington et al. 1996, Fig. 1). Wintering Leach's storm-petrels disperse into equatorial waters: non-breeding birds from Atlantic colonies occur as far south as southern Africa, and those from Pacific colonies occur as far south as the equator, though exact distributions are not known for any of the populations. The species is highly pelagic and generally approaches land only for breeding (Huntington et al. 1996).

Over most of its large distribution, the Leach's storm-petrel complex exhibits little morphological variation and differentiation has not been reported among Atlantic colonies. Birds in the North Pacific resemble those in the Atlantic, although they are slightly smaller (Ainley 1980, Power and Ainley 1986).

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Excluding Japan, where morphology has not been extensively studied, the Pacific colonies show clinal variation in morphology: birds from the north have longer wings, tarsi, and bills, and a larger number of white feathers on the rump patch relative to birds from more southern areas (Ainley 1980, Power and Ainley 1986). At the most southern end of the range, on the Coronado and San Benito Islands in northern and central Baja California, birds are smaller with more rounded wings and a deeper tail fork, and have dark rumps (with few light vs. dark upper tail coverts; Ainley 1980, Power and Ainley 1986, Adams et al. 2016).

Birds nesting on Guadalupe and surrounding islets, however, are different from those from other colonies in the Baja region. Here, two seasonal breeding populations occur, one in the winter and one in the summer, as seen in many colonies of band-rumped storm-petrels (Ainley 1980, Power and Ainley 1986, Smith et al. 2007). The winter breeding population is more similar morphologically to the other Pacific colonies, though smaller in size. Most winter breeding individuals have an intermediate coloured rump patch and are lighter brown in fresh plumage than the sympatric summer breeders (Power and Ainley 1986, AOU Classification Committee 2016). The summer breeding birds are the smallest in the species complex, and mostly have white rumps, though dark-rumped individuals also occur (Ainley 1980, AOU Classification Committee 2016). As well as being morphologically distinct from each other and from all other colonies, the summer and winter breeding populations differ in vocalizations (Ainley 1980).

The patterns of morphological differentiation across the range of the Leach's storm-petrel species complex have resulted in a taxonomy that has been characterized as "complex and confused" (del Hoyo et al. 1992). Traditionally, four subspecies were recognized (Power and Ainley 1986). The majority of birds, including those from all colonies in the Atlantic and North Pacific, were included in the subspecies *H. l. leucorhoa*. Another subspecies, *H. l. chapmani*, encompasses the birds breeding on the Coronado Islands south to San Benito Islands. The remaining two subspecies represent the allochronic populations breeding on Guadalupe and nearby islands, with *H. l. socorroensis* breeding in the summer and *H. l. cheimommestes* in the winter (Ainley 1980, Power and Ainley 1986). The Pacific population of *H. l. leucorhoa* has been considered a separate subspecies *beali* because of its smaller size, but that arrangement has not been generally accepted (AOU Classification Committee 2016). In 2016, the AOU Classification Committee (2016) proposed to elevate the allochronic populations as two distinct species, Townsend's storm-petrel *H. socorroensis*

(Townsend 1890) for the summer breeders and Ainley's storm-petrel *H. cheimomnestes* (Ainley 1980) for the winter breeders. The justification for full species status was given as the differences in vocalizations and morphology of these populations, both from each other and from all other colonies. This was soon implemented in the IOC World Bird List (Gill and Donsker 2017).

Little molecular genetic information exists relating to geographic variation in the Leach's storm-petrel species complex. Paterson and Snyder (1999) found weak but significant geographic variation in randomly amplified polymorphic DNA (RAPDs) among Leach's storm-petrels from three colonies in Atlantic Canada ($F_{ST} = 0.04$, $p < 0.05$). More recently, Bicknell et al. (2012) assayed 18 nuclear microsatellite loci and 357 base pairs of the mitochondrial control region in individuals from nine colonies from both the Atlantic and Pacific, all from *H. l. leucorhoa*. They found genetic differentiation between the Atlantic and Pacific colonies in both Φ_{ST} and F_{ST} ($\Phi_{ST} = 0.32$, $F_{ST} = 0.04$, both $p < 0.001$), with results from molecular assignments suggesting two genetic populations. However, there was evidence of some gene flow between the ocean basins.

In the present study, we examine variation in mitochondrial DNA (mtDNA) and six nuclear microsatellite loci among birds from 17 Atlantic and Pacific colonies representing all four former and presently recognized subspecies and species in the Leach's storm-petrel species complex. We address four hypotheses. Firstly, given the effectiveness of land as a barrier to gene flow in seabirds (Friesen 2007a, Friesen 2015), and the finding of differentiation between Atlantic and Pacific colonies reported by Bicknell et al. (2012), we expect to see genetic differentiation between Atlantic and Pacific colonies in our data. Secondly, we expect *H. l. chapmani* to be genetically differentiated from *H. l. leucorhoa*, given differences in morphology. Thirdly, we expect the newly named species *H. socorroensis* and *H. cheimomnestes* to be genetically distinct both from the two subspecies of *H. leucorhoa* and from one another, given differences in vocalizations and morphology. Lastly, we hypothesize that *H. socorroensis* and *H. cheimomnestes* diverged in sympatry via allochrony, as in the Azores races of the band-rumped storm-petrels (Friesen et al. 2007b, Smith et al. 2007, Bolton et al. 2008, Silva et al. 2016), even though Power and Ainley (1986) assumed both Guadalupe seasonal populations diverged separately from *H. l. leucorhoa*. In their phylogenetic reconstruction of the Hydrobatinae Wallace et al. (2017) did not find the two Guadalupe taxa to be

monophyletic sister taxa, however sequences from only two individuals from each were used. With an increased sample size we predicted *H. socorroensis* and *H. cheimomnestes* to be monophyletic sister taxa if they arose through allochryony within Guadalupe; in contrast, if they diverged in two separate vicariance events from *H. l. leucorhoa* then they would not represent sister taxa.

Methods

Sampling and DNA Extraction

Tissues were sampled from 283 Leach's storm-petrels captured at 17 breeding colonies (Table 1, Fig. 1), and stored either in lysis buffer (Seutin et al. 1991) or dried on filter paper. Sampling was conducted across the global range of the species and included all recognized species and subspecies. DNA was prepared using the DNeasy method (Qiagen, Mississauga, Ontario) or by standard proteinase K digestion and phenol/chloroform extraction (Maniatis et al. 1982).

Mitochondrial DNA sequencing

A 333 base pair (bp) fragment of the mitochondrial control region, including parts of domains I and II, was amplified using PCR primers OL-L40 (5'-TTCTCCAAGATCTGTGGCTTG-3') and OL-H530 (5'-GTATGGTCTCTGAAGCTAGTAA-3'; Friesen et al. 2007b). Individual PCR reactions were conducted in volumes of 15 μ L containing 1.5 mM MgCl₂, 10 mM Tris (pH 8.4), 50 mM KCl, 0.1% gelatin, 0.625% bovine serum albumin, 0.4 μ M of each primer, 200 μ M deoxynucleotide triphosphates (dNTPs), and 0.5 units of *Taq* DNA polymerase (Qiagen, Mississauga, Ontario). The temperature profile consisted of 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s. PCR products were sequenced on a 3730xl DNA Analyzer Platform (Applied Biosystems, California) operated by Genome Quebec (McGill University, Montreal). Sequences were confirmed from chromatograms, trimmed and aligned using Geneious ver. 6.1.5 (Kearse et al. 2012).

Microsatellite genotyping

Variation was assayed at six dinucleotide microsatellites using PCR primers and protocols developed for the

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band-rumped storm-petrel (*Hydrobates castro*; Sun et al. 2009). Five loci were amplified in two multiplex reactions (OC49, OC79 and OC84 together; OC63 and OC51 together), while OC87 was amplified individually. Amplifications were generally done in a volume of 10 μ L containing 1 \times Multiplex Mix (Qiagen), 0.15 μ M primers, and 0.5 μ M D4-labelled M13F. After a 15min activation step at 94°C the temperature profile of the initial PCR cycle consisted of 94°C for 45 s, 60°C for 45 s and 72°C for 30 s. The annealing temperature was reduced by 0.5°C in each of the following 16 cycles. The final 25 cycles were run with an annealing temperature of 52°C. PCR products were sized using a Beckman Coulter (California) CEQ 8000 automatic sequencer.

Tests of assumptions and genetic variation

The program Arlequin (ver. 3.5, Excoffier and Lischer 2010) was used to test microsatellite variation for departures from Hardy-Weinberg proportions and linkage equilibrium, and to test control region sequences for deviations from selective neutrality using Ewans-Watterson and Chakraborty tests (Ewens 1972, Watterson 1978, Chakraborty 1990). Haplotypic diversity (h , Nei 1987) and nucleotide diversity (π , Tajima 1983) were calculated for the control region sequences. The program Micro-Checker (Van Oosterhout et al. 2004) was used to check for the presence of null alleles in the microsatellite data.

Population genetic structure

Population genetic structure was indexed using F_{ST} (microsatellites) or Φ_{ST} (mitochondrial control region sequences) calculated by analysis of molecular variance (AMOVA) using Arlequin. Significance of F-statistics was assessed by randomization using 10,100 permutations of the data. F_{ST} and Φ_{ST} were calculated for all pairwise comparisons between breeding colonies (unpooled analysis), and between samples pooled by species (*H. socorroensis* and *H. cheimomnestes*) or subspecies (*H. l. chapmani*) but with the Atlantic and Pacific populations of *H. l. leucorhoa* considered separately, given the results of Bicknell et al. (2012). For mitochondrial control region sequences, we used Kimura's two-parameter model of substitution (Kimura 1980) and defined the gamma distribution shape parameter (α) as 0.45. For the microsatellite data, the weighted averages over loci are reported for F-statistics. Inflated probabilities of Type I statistical errors due to multiple comparisons were addressed by applying a Benjamini-Yekutieli correction (Benjamini and

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Yekutieli 2001, Narum 2006).

The program Structure (ver. 2.3.4, Pritchard et al. 2000, Pritchard et al. 2010) was used to determine the number of genetically distinct populations (K) within the samples using the microsatellite data. The program was run for values of K from one to five for 10 replicate runs, with the ‘admixture’ model and assuming correlated allele frequencies. A burn in of 10,000 and total running length of 100,000 iterations were used, and the program was run both with and without sampling location included as prior information. Delta K (ΔK) was calculated using Structure Harvester to give an indication of the appropriate number of clusters (Evanno et al. 2005, Earl and vonHoldt 2012). Structure plots were redrawn using Pophelper (Francis 2016). A principal component analysis of the microsatellite data was then performed and plotted in R software ver. 3.3.2 (R Development Core Team 2006) using the packages adegenet (Jombart 2008) and ade4 (Dray and Dufour 2007). A statistical parsimony haplotype network of the control region sequences was constructed using PopArt (Clement et al. 2000; <http://popart.otago.ac.nz>). The program masks any sites with a gap or missing data, and thus 12 individuals were removed from the dataset to construct the network.

Phylogenetic reconstruction

A species tree was constructed for the control region sequences using the *Beast algorithm (Heled and Drummond 2010) implemented in Beast ver. 1.8.4 (Drummond et al. 2012). Individuals were grouped by species (for the Guadalupe races *H. socorroensis* and *H. cheimomnestes*) or subspecies (for the San Benito population *H. l. chapmani*), with the exception of the Atlantic and Pacific populations of the subspecies *H. l. leucorhoa*, which were separated as above. Seven sequences from *H. castro* (all North Atlantic cool season breeders) were used as an outgroup (Taylor et al. unpublished data). The model of sequence evolution was selected using the Bayesian Information Criterion (BIC) in jModelTest2 (Darriba et al. 2012). The Hasegawa-Kishino-Yano substitution model was selected, with a proportion of invariant sites (HKY + I). We used a strict molecular clock, and the Yule species tree prior (Drummond et al. 2006). The *BEAST analysis was run for 10^9 generations, sampling every 10^5 generations, and discarding the first 10% as burn-in. Convergence was assessed using the effective sample size (ESS) values in Tracer ver. 1.5 (Rambaut and Drummond 2007), ensuring all ESS values were over 200. The analysis was run three times, with the resultant tree files

combined using LogCombiner ver. 1.8.4, and a consensus tree was generated in TreeAnnotator ver. 1.8.4 (Drummond et al. 2012). The tree was visualized in FigTree ver. 1.4.3 (Rambaut 2012).

Results

Test of assumptions and genetic variation

Eighty-three control region haplotypes were found in 278 individuals from 17 breeding colonies across the range of the Leach's storm-petrel species complex (Table 1, Fig. 2). The haplotype network separated most of the Guadalupe birds from the rest and placed the San Benito haplotypes closer to the Atlantic and other Pacific *H. leucorhoa* haplotypes than to the Guadalupe ones (Fig. 2). Variation was high and similar among sites; no deviations from neutral expectations were found, except for the San Benito population (Chakraborty test, $p = 0.05$). However, San Benito samples did not show a significant deviation from neutrality using the Ewans-Watterson test (Table 1). Fifty-eight variable sites were found, and while no fixed differences occurred between any subspecies, some variants were found in only one or two species or subspecies. In particular, a substitution at position 213 occurred in all birds from Guadalupe with the exception of two summer breeding individuals, and a substitution at position 196 was found in most Guadalupe summer breeders and only six Guadalupe winter breeders. Similarly, position 66 had a single nucleotide polymorphism that was found in most San Benito birds but no other populations.

All microsatellite loci were variable, although only two alleles were observed for OC79 (Supplementary material Appendix 1, Table A1). Some weak departures from Hardy-Weinberg proportions were found; these were usually due to homozygote excess and may indicate the presence of null alleles, however no locus was consistently out of Hardy-Weinberg across populations (Supplementary material Appendix 1, Table A1). The Guadalupe summer breeding population showed significant departures from Hardy-Weinberg proportions in four of the six loci due to homozygote excess suggesting the possibility of non-random breeding in this population (Supplementary material Appendix 1, Table A1). Four of the 17 populations showed evidence for linkage between OC63 and OC51. All analyses were therefore re-run excluding OC51 and results were consistent with those using all six loci, so results with all six loci are presented below. Micro-Checker indicated the possible presence of null alleles at microsatellite loci OC63

and OC49, so all analyses were re-run excluding these loci. Results were also consistent with those using all loci, except some pairwise F_{ST} estimates lost significance. However, these results were from comparisons between populations with small sample sizes, and thus power was low.

Population genetic structure

The global estimate of Φ_{ST} from mitochondrial variation was large and highly significant both when Arlequin was run with no grouping of the breeding colonies (unpooled dataset), and when the subspecies and species were pooled as stated above (0.40 and 0.47 respectively, for both $p < 0.001$). The global F_{ST} values for the microsatellite data were also significant for both the unpooled and pooled datasets (0.16 for the unpooled and 0.17 for the pooled dataset, for both $p < 0.001$). Most differentiation appeared to be due to the Guadalupe populations (Supplementary material Appendix 1, Table A2): pairwise estimates of Φ_{ST} between *H. socorroensis* and all other populations except *H. cheimomnestes* in the unpooled dataset were high and significant, ranging from 0.35 to 0.61 ($p < 0.001$ for all; Supplementary material Appendix 1, Table A2). Estimates of F_{ST} from microsatellites based on the unpooled dataset showed a similar trend, ranging from 0.04 to 0.25 ($p < 0.001$ for all but two comparisons, which could be due to the smaller sample size for the Central Aleutian and Semidi Islands, Supplementary material Appendix 1, Table A1). Similarly, estimates of Φ_{ST} and F_{ST} between *H. cheimomnestes* and all other populations excluding *H. socorroensis* were all high and significant, ranging from 0.57 to 0.72 for Φ_{ST} and 0.17 to 0.39 for F_{ST} ($p < 0.001$ for all; Supplementary material Appendix 1, Table A2). *H. socorroensis* and *H. cheimomnestes* also differed significantly from each other, though not as much as for other colonies ($\Phi_{ST} = 0.27$ and $F_{ST} = 0.06$, $p < 0.001$ for both; Supplementary material Appendix 1, Table A2).

The San Benito subspecies (*H. l. chapmani*) was highly differentiated from all other populations in the mitochondrial data, with Φ_{ST} estimates ranging from 0.48 to 0.72 ($p < 0.001$ for all), and was also differentiated from most other sites in the microsatellite data, with F_{ST} estimates ranging from 0.03 to 0.35 ($p < 0.001$ for all but four comparisons; Supplementary material Appendix 1, Table A2).

With the pooled data (Atlantic *H. l. leucorhoa*, Pacific *H. l. leucorhoa*, *H. socorroensis*, *H. cheimomnestes* and *H. l. chapmani*) all pairwise Φ_{ST} estimates were high and significant, ranging from 0.19

to 0.72 ($p < 0.001$ for all; Table 2). Pairwise F_{ST} estimates were also all significant, ranging from 0.01 to 0.38 ($p < 0.001$ for all; Table 2). Within *H. l. leucorhoa*, the global estimate of Φ_{ST} was considerably lower, though still significant overall (0.12, $p < 0.001$). Similarly, the global estimate of F_{ST} was low, though still significant (0.02, $p < 0.05$).

Results from Structure and Structure Harvester, both with and without sampling location as a prior, indicated that genetic variation in the Leach's storm-petrel species complex is partitioned into two genetic populations ($K=2$, Fig. 3), which include 1) *H. l. leucorhoa* from throughout the North Pacific and Atlantic, and *H. l. chapmani*, and 2) samples from Guadalupe, including both *H. socorroensis* and *H. cheimomnestes*. Assignment probabilities suggest that three birds may be migrants, as they did not cluster according to their sampling sites (with Q values over 0.9), including one individual sampled on Daikoku Island, Japan, one individual sampled on Cleland Island, Canada, and one Guadalupe summer breeding bird (*H. socorroensis*) (Fig. 3). This same individual (Specimen 2A) did not group with the other Guadalupe individuals in the phylogeny generated by Wallace et al. (2017), and is potentially a migrant from *H. l. leucorhoa*. Additionally, four birds had Q values around 0.5 from Structure, suggesting they may be hybrids (one from the Western Aleutian Islands, one from the Semidi Islands, U.S.A., and two from St. Lazaria Island, U.S.A.). Results of the PCA were consistent with Structure: the first two principal components gave two major clusters corresponding to the Structure groupings, with one Guadalupe summer breeding individual (2A) that did not group with the Guadalupe cluster (Fig. 4). However, the first two principal components only explained 13.1% of the variation. Specimen 2A from Guadalupe also shared a haplotype with Pacific birds in the haplotype network (Fig. 2).

Phylogenetic reconstruction

The phylogeny generated in Beast, which was made after removal of specimen 2A from the dataset, grouped the Guadalupe seasonal colonies as sister populations with strong support (Fig. 5). A sister group relationship between *H. l. chapmani* and *H. l. leucorhoa* had high support, agreeing with the relationship recovered by Wallace et al. (2017) using cytochrome b.

Discussion

Using six nuclear microsatellites and 333bp of the mitochondrial control region, we found some differentiation between Leach's storm-petrels in different ocean basins. However a key result was that breeding colonies within the Pacific are strongly differentiated, particularly in the southernmost part, where no obvious physical barriers to gene flow occur. Further, phylogenetic reconstruction indicated that the two Guadalupe populations may be sister taxa and so could have diverged via allochronic speciation (Fig. 5).

Differentiation between Atlantic and Pacific colonies of *H. l. leucorhoa*

Genetic differentiation between ocean basins in *H. l. leucorhoa* is not surprising, and is consistent with patterns of genetic differentiation in other Holarctic seabird species, for example Atlantic vs. Pacific populations of common murre *Uria aalge* (reviewed by Friesen et al. 2007, Friesen 2015). However, the degree of inter-ocean variation we report in the Leach's storm-petrels is smaller than in most other species, suggesting that some gene flow occurs between oceans. Bicknell et al. (2012) found stronger differentiation between the ocean basins ($\Phi_{ST} = 0.32$, $F_{ST} = 0.04$, both $p < 0.001$), with Structure dividing Atlantic and Pacific colonies of *H. l. leucorhoa* into two genetic clusters (Bicknell et al. 2012). Even after removing the Guadalupe birds from our data set, Structure did not divide the populations any further. This is probably a limitation of using just six microsatellites, compared to the 18 loci used by Bicknell et al. (2012). However, Bicknell et al. (2012) also found evidence for gene flow between the ocean basins, which may limit genetic differentiation and is consistent with very slight morphological differences between Atlantic and Pacific colonies (Ainley 1980, Power and Ainley 1986).

Differentiation between *H. l. leucorhoa* and *H. l. chapmani*

Although no obvious barrier to gene flow exists between the Pacific *H. l. leucorhoa* and *H. l. chapmani* colonies, the two subspecies show differences in morphology (Ainley 1980, Power and Ainley 1986). The genetic data reported here, particularly the mitochondrial sequences, are consistent, with the evidence for morphological differentiation. Lower differentiation in the pooled nuclear data indicates either some on-going gene flow, or recent divergence between the subspecies. However, to truly resolve this relationship samples need to be included from colonies situated between British Columbia and the San Benito Islands, particularly around California, an area representing a gap in this study (Fig. 1). Effects of incomplete lineage sorting also

need to be excluded. The cline in morphology from the Aleutian Islands to the San Benito Islands is thought to have a major transition within the California Channel Islands (Ainley 1980, Adams et al. 2016). In the Farallon Islands in northern California, individuals are mostly white-rumped, with only 6% of birds having an almost or completely dark rump (Adams et al. 2016). Within the northern part of the California Channel Islands the proportion rises to 28% dark-rumped individuals, however the proportion of dark-rumped individuals in the southern part of the California Channel Islands is less clear (Adams et al. 2016). *H. l. chapmani* individuals breed as far north as the Coronado Islands, and are mostly dark-rumped as within the San Benito Islands (Ainley 1980, Power and Ainley 1986, Adams et al. 2016). More research is needed to fully understand the transition in morphology in the California area, perhaps with special emphasis on morphology related to differences in feeding. Distributions at sea may be one of the drivers of diversification, and additional data will allow testing of how these birds relate genetically to both the *H. l. leucorhoa* colonies further north and the San Benito *H. l. chapmani* birds. An isolation by distance genetic pattern between *H. l. leucorhoa* and *H. l. chapmani* may be observed when including the missing region.

Differentiation and evolutionary history of *H. socorroensis* and *H. cheimomnestes*

Wallace et al. (2017) found the Leach's storm-petrel species complex to be monophyletic, but could not resolve the relationship between *H. socorroensis* and *H. cheimomnestes*. They sequenced two individuals each for *H. socorroensis* and *H. cheimomnestes*, and found one of the summer breeders did not group with the other Guadalupe individuals (Wallace et al. 2017). Using 25 summer breeders and 28 winter breeders in our data set, Structure identified this same individual (specimen 2A) as one of the possible migrants or vagrants (Fig. 3). Using mitochondrial DNA, our increased sample size supports the monophyly of *H. socorroensis* and *H. cheimomnestes*, supporting the hypothesis of allochronic divergence in sympatry (Fig. 5). Higher resolution data (for example ddRADseq) is needed for a more rigorous test of this hypothesis, and to confirm the monophyly of *H. socorroensis* and *H. cheimomnestes* in their nuclear DNA. It is also possible that summer and winter breeders diverged in allopatry, perhaps on different islets around Guadalupe, and then came into sympatry after divergence. This possibility would be difficult to refute but is less likely since it would require three evolutionary events: 1) evolution of reproductive isolation in allopatry, 2) change in distribution of one or both species into Guadalupe, and 3) extirpation of one or both species from the rest of

the ancestral range. Similarly, the reason why *H. socorroensis* birds show deviations from Hardy-Weinberg proportions at four out of six loci is unclear (Table 2). Even after the removal of specimen 2A, three out of the four loci still showed significant deviation. Perhaps the summer breeding population is small, or undergoing inbreeding, but further investigation using a large number of loci is needed to test this result and confirm it is not due to the presence of null alleles. Deviations from Hardy-Weinberg proportions could also be due to sampling summer individuals that breed on different islets that differ genetically, and so further investigation to assess levels of genetic structure between Guadalupe islets is also needed.

The summer and winter breeding races on Guadalupe were both recently elevated to species status (*H. socorroensis*, and *H. cheimomnestes* respectively; Gill and Donsker 2017). However, this taxonomic change was done without information on their genetic relationships with either the other Leach's storm-petrel species complex colonies, or with each other (AOU Classification Committee 2016). Genetic data presented here show the Guadalupe birds to be highly genetically differentiated from all other locations, and to have lower but significant differentiation between the summer and winter breeders. Thus, the genetic data are consistent with morphological and behavioural observations (Ainley 1980, Jehl and Everett 1985, Power and Ainley 1986).

Drivers of diversification in Guadalupe and nearby islands

While land is often a barrier to gene flow in seabirds (Friesen et al. 2007a), clearly it is not always the most important. Genetic differentiation over small spatial scales between populations without land barriers has been found in other seabird species, for example in shy albatrosses (*Thalassarche cauta*; Abbott and Double 2003), Gentoo penguins (*Pygoscelis papua*; Levy et al. 2016), and a number of Southern Ocean species (reviewed in Munro and Burg 2017). Here we found some genetic differentiation between birds in different ocean basins, but much stronger differentiation among breeding colonies within the Pacific where no obvious physical barriers to gene flow occur. Further, most of this differentiation is confined to the southernmost part of the distribution. A similar genetic pattern is found in some other seabird species along the Pacific Coast of North America, including Cassin's Auklets (*Ptychoramphus aleuticus*, Wallace et al. 2015) and the Guadalupe murrelet (***Synthliboramphus hypoleucus***), a species endemic to Guadalupe recently elevated from subspecies status based on genetic distinctiveness (Birt et al. 2012; Chesser et al. 2012). With *H.*

socorroensis, *H. cheimomnestes* and (the probably now extinct) *H. macrodactyla*, Guadalupe has three endemic storm-petrel species (Aguirre-Muñoz et al. 2011, AOU Classification Committee 2016).

What factors may have driven divergence of the Guadalupe populations from other populations of the Leach's storm-petrel species complex are not clear. Similarly, that San Benito birds are more similar genetically to *H. l. leucorhoa* than to the Guadalupe populations may seem surprising given San Benito is further south than Guadalupe (Fig. 1). Guadalupe has (or had) a high number of endemic species across taxonomic groups including birds, mammals, invertebrates, plants and lichens, and this high level of endemism may be driven by its isolation, 260 km west of the North American coast (Garcillán et al. 2008). Isolation may seem an unlikely explanation for highly pelagic seabirds such as storm-petrels, especially given the low level of genetic differentiation seen across the vast range of *H. leucorhoa*. Alternatively, differences in foraging behavior during the breeding season has putatively lead to genetic differentiation in species such as black-browed albatrosses (*Thalassarche melanophris*; Burg and Croxall 2001). Similarly, foraging ranges of Guadalupe murrelets during the breeding season (offshore habitats) differ from their sister species, the Scripps's murrelet (*S. scrippsi*; coastal southern California and Baja California; Birt et al. 2012). Perhaps *H. socorroensis* and *H. cheimomnestes* are adapted to forage in offshore areas, whereas *H. leucorhoa* forage in the coastal upwellings. Differences in non-breeding distributions can also contribute to divergence (Friesen 2015), such as in Cape and Australasian gannets (*Sula capensis* and *S. serrator*; Ismar et al. 2011; Patterson et al. 2011).

Adaptation to different ocean regimes is another possible driver of genetic divergence in the absence of geographical barriers in seabirds (Friesen 2015). The Guadalupe and San Benito populations sit at the end of the California Eastern Boundary Upwelling Ecosystem (EBUE), an area of high marine productivity (Fréon et al. 2009) which may support a high level of seabird diversity. Seasonality occurs in the California Current System (CCS) around Baja California, whereby the region is uniform and cold in the winter, but subdivided into two oceanographic sub regions in the summer (Durazo 2015). Differences in cold-water upwellings used for feeding during the breeding season may have contributed to differences in breeding time and genetic differentiation in Cassin's auklets in Baja California in comparison to populations breeding further north (Wallace et al. 2014), and to earlier egg laying in the San Benito population of Xantus's murrelet (Wolf et al.

2005). Perhaps genetic differentiation between *H. l. chapmani* and *H. l. leucorhoa* is driven by the differences in the coastal cold water upwellings. Cassin's auklets in the California Channel Islands are morphologically intermediate between those in Baja California and the more northern birds, and were found to be genetically more similar to the Baja California populations (Wallace et al. 2014). Leach's storm-petrels in the California Channel Islands appear also to be morphologically intermediate (Adams et al. 2016) and so including samples from the area is an important next step to fully resolve the genetic relationship between *H. l. chapmani* and *H. l. leucorhoa*.

Allochrony, or differences in breeding time, may be an important driver of speciation in a diversity of taxonomic groups, including arthropods and several classes of vertebrates (reviewed by Taylor and Friesen 2017). Allochronic speciation has perhaps driven the divergence between seasonal populations within Guadalupe, as seen in seasonal populations of the band-rumped storm-petrel (Friesen et al. 2007b). In fact, two seasonal breeding populations may be more common than we think within many storm-petrel species (Spear and Ainley 2007). Further study within these cryptic seabirds is warranted to understand the drivers behind the change in breeding time. For example, change may be facilitated by ecological opportunity with the high availability of food resources promoting two breeding seasons, and/or perhaps driven by competition for nest sites (Taylor and Friesen 2017).

Management implications

Moritz (1994) recommended separate management of “evolutionary significant units” (ESUs): populations that are “reciprocally monophyletic for mtDNA alleles and also differ significantly for the frequency of alleles at nuclear loci”. Such populations are likely to be both demographically and genetically independent, and are essentially equivalent to “distinct population segments” under the US Endangered Species Act, and “designatable units” under the Canadian Species at Risk Act. Moritz also recommended recognition of “management units” (MUs) - populations with “significant differences in allele frequencies, regardless of the phylogeny of alleles” - the idea being that populations that exchange so few alleles as to be genetically different will also be demographically independent. Variation in both mitochondrial and nuclear DNA suggests that the *H. leucorhoa* species complex constitutes at least two ESUs: (1) *H. l. leucorhoa* and *H. l. chapmani*, and (2) the two Guadalupe species. Results of the present study also suggest that Atlantic versus

Pacific populations of *H. l. leucorhoa*, and *H. l. chapmani* should be treated as separate management units within *H. leucorhoa*, and that *H. socorroensis* and *H. cheimomnestes* should be treated as separate management units within Guadalupe. However, given the use of only six microsatellites here our results may be underestimating the number of ESUs, particularly as Bicknell et al. (2012) found stronger differences between Atlantic and Pacific *H. l. leucorhoa* populations using a larger number of microsatellites. An increased number of genetic markers is needed to determine whether stronger genetic structuring is present.

Populations in Guadalupe in particular warrant special conservation attention given their restricted breeding ranges. The birds are largely restricted to breeding on islets and not on the main island, likely due to predation by introduced cats which are thought to have led to the extinction of the Guadalupe storm-petrel (*H. macrodactyla*; Aguirre-Muñoz et al. 2011) and several terrestrial bird taxa (Barton et al. 2004, Barton et al. 2005). An oil spill near this island or introduction of invasive species (in addition to the cats already present) could seriously threaten the survival of these populations (Croxall et al. 2012, Doherty et al. 2016). The feral goat population was eradicated between 2003-2006 (Aguirre-Muñoz et al. 2011); a program to control feral cats has been carried out since 2003 (Hernández-Montoya et al. 2014). Given the importance of Guadalupe for storm-petrels, it is key to continue with such long-term conservation efforts.

Conclusions and future work

For the first time, the genetic relationships between all recognized species and subspecies within the Leach's storm-petrel species complex were investigated. We show that genetic differentiation has largely been driven by non-physical barriers at a small geographic scale, and we report a possible case of allochronic speciation. The likely recent divergence of the three groups breeding in southern California and Mexico suggest that strong breeding philopatry and assortative mating in connection with feeding conditions at sea, leading to spatial segregation during the wintering period, might explain the observed pattern. Higher resolution data, such as the use of high throughput sequencing, is needed to confirm the sister relationship between the Guadalupe populations. Distributions at sea for small seabirds such as Leach's storm-petrels are virtually unknown and difficult to obtain using current tracking technology. Analyses of diet for the various populations, for example using stable isotopes, might help resolve the mechanisms behind the observed differentiation (Gladbach et al. 2007). Investigation into the possible ecological significance of the differences

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in rump colour, for example to discover whether it has a role in species recognition, would also be useful. In addition, filling sampling gaps, particularly within the California region, is a key next step to fully understand the observed cline in morphology along the Pacific coast of North America. Increased sampling of the Atlantic colonies, coupled with higher resolution data, may also help resolve the weak genetic structuring between the ocean basins.

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FIGURE LEGENDS

Figure 1: The breeding distribution of the Leach's storm-petrel is shown with black lines. Arrows indicate locations of colonies sampled for genetic analysis of Leach's storm-petrels. The circle encompasses three of the four species and subspecies, GS is *H. socorroensis*, GW is *H. cheimomnestes*, SB is *H. l. chapmani*, the rest are all *H. l. leucorhoa*. See Table 1 for sample site abbreviations.

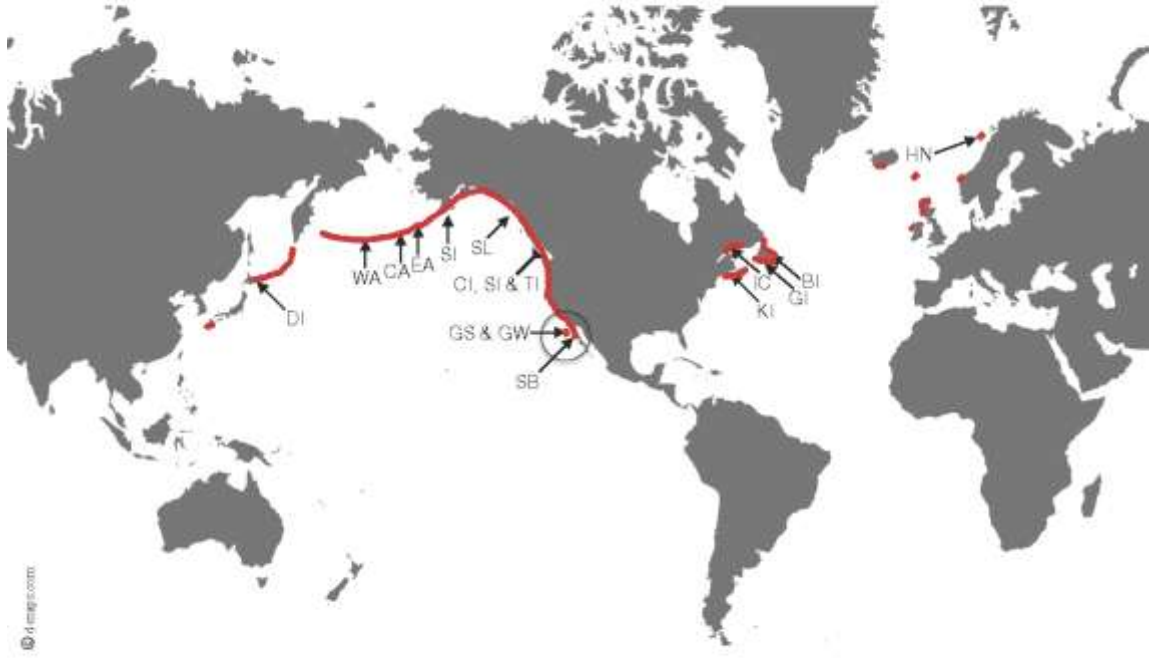


Figure 2: Statistical parsimony haplotype network for Leach's storm-petrels based on a 333 base pair region of the mitochondrial control region. Circle size relates to the number of samples with each haplotype. Black circles represent hypothetical haplotypes not found. Twelve individuals with missing data or gaps were removed from the dataset, resulting in 71 haplotypes being displayed in the network.

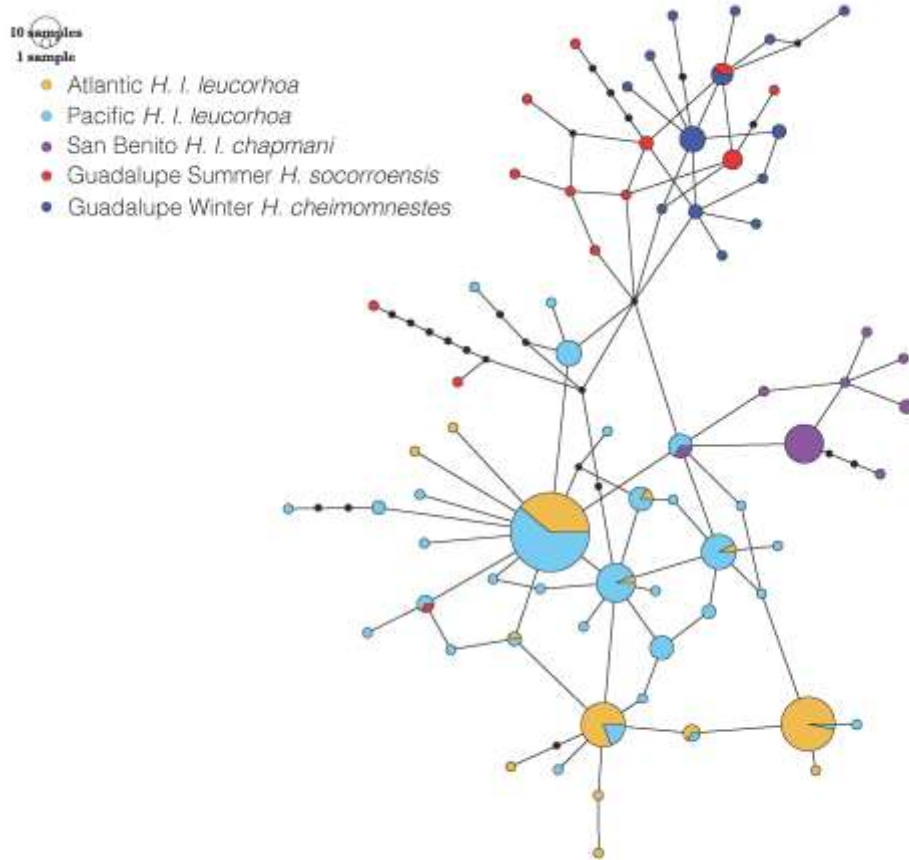


Figure 3: Probabilities of assignment of individuals to two genetic populations based on microsatellite variation for Leach's storm-petrels (blue and purple), generated by Structure without location as prior information. Each vertical bar represents one individual. The arrow indicates a Guadalupe summer individual with a high probability of being an immigrant (Specimen 2A). One Daikoku bird and one Cleland bird also had high probabilities of being immigrants from Guadalupe.

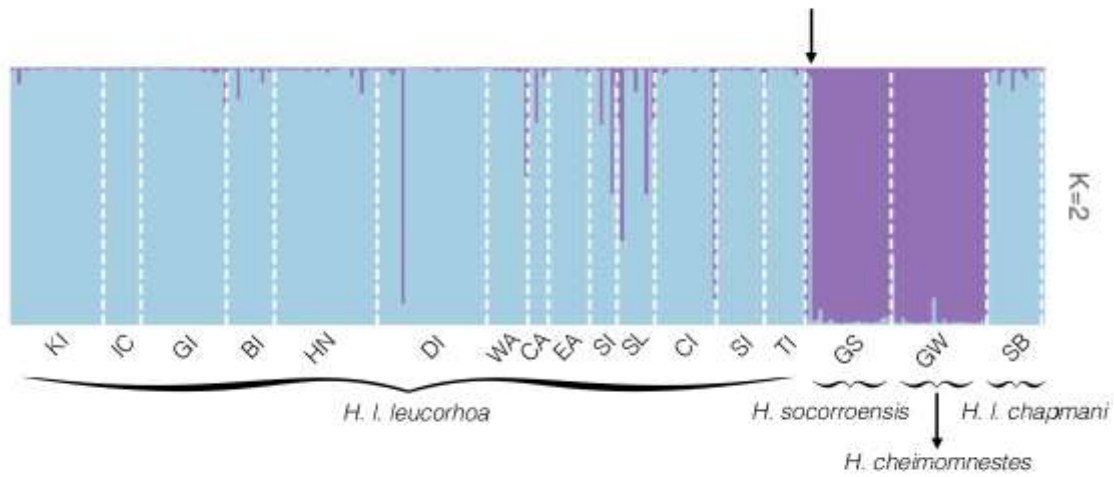


Figure 4: Results of a principal component analysis based on microsatellite variation for Leach's storm-petrels. Blue indicates Atlantic *H. l. leucorhoa* samples, purple indicates Pacific *H. l. leucorhoa*, brown indicates San Benito *H. l. chapmani*, green indicates Guadalupe summer breeders *H. socorroensis*, and orange indicates Guadalupe winter breeders *H. cheimomnestes*.

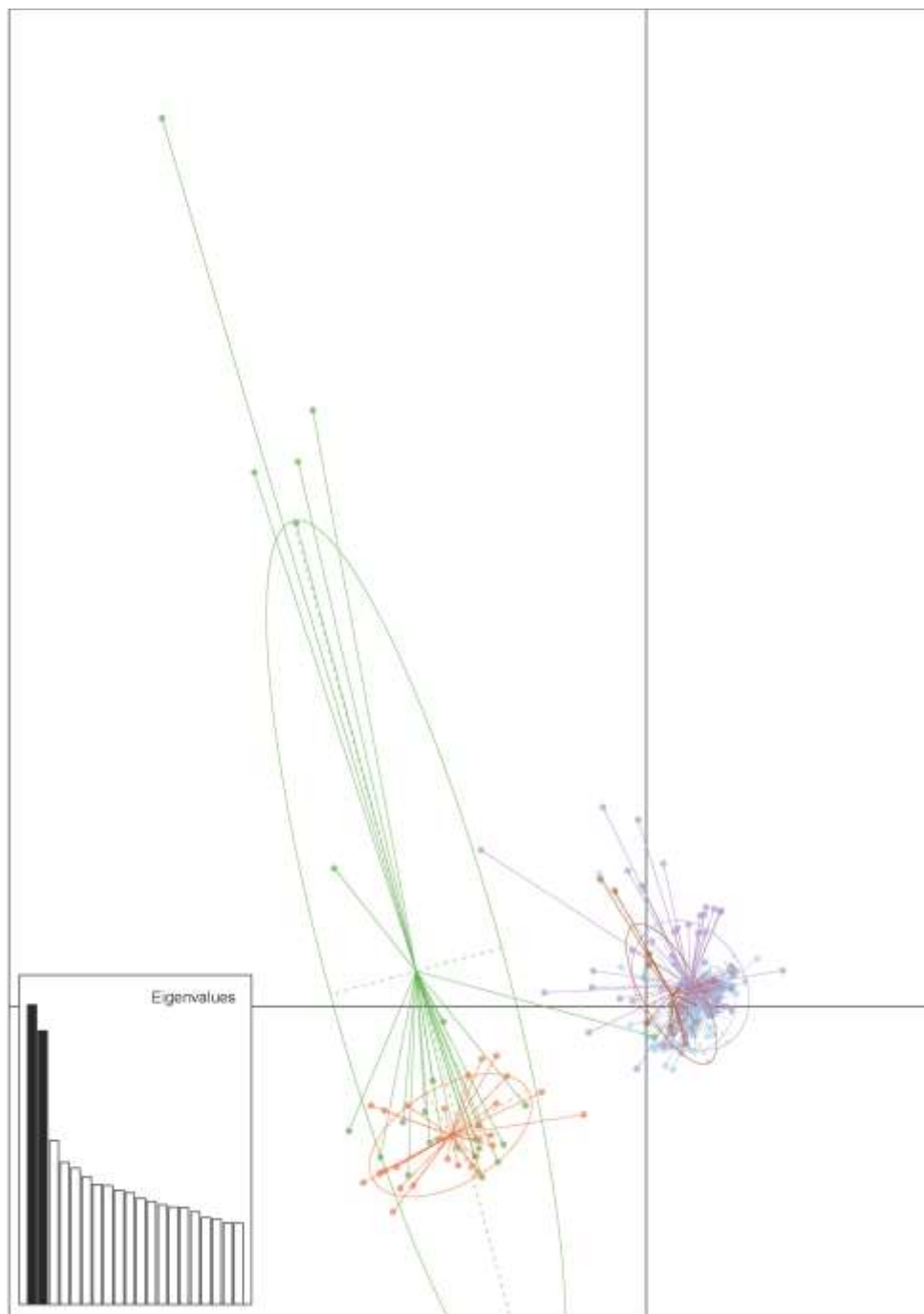


Figure 5: Phylogenetic reconstruction of the Leach's storm-petrel species complex constructed from mitochondrial control region sequence variation using *Beast. *H. castro* is used as an outgroup. Posterior probabilities are shown at each node.

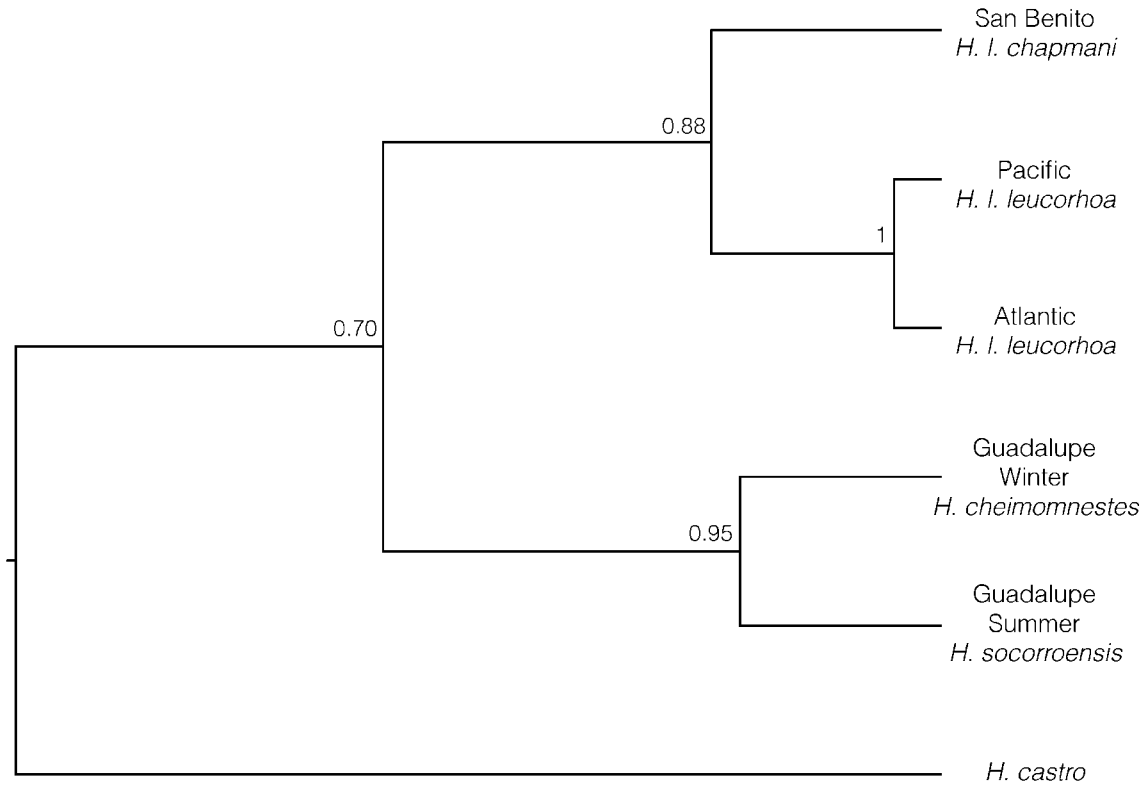


TABLE LEGENDS

Table 1: Descriptive statistics for Leach’s storm-petrel control region variation; N = sample size, h = haplotype diversity, π = nucleotide diversity as a percent. Entries for Ewans-Watterson are observed and expected F values (none significant) and Chakraborty’s tests are probabilities for deviation from neutrality.

Species	Sampling Abbreviation	site	N	Number of haplotype s	h	π	Ewans- Watterso n	Chakrabort y
<i>Hydrobates leucorhoa leucorhoa</i>	Kent Island, New Brunswick, Canada	KI	2 6	4	0.67±0.0 5	0.52±0.3 5	0.35/0.47	0.81
	Ile Corrosol, Quebec, Canada	IC	10	4	0.71±0.1 2	0.64±0.4 4	0.36/0.37	0.61
	Green Island Newfoundland , Canada	GI	2 5	5	0.66±0.0 6	0.66±0.4 2	0.37/0.38	0.53
	Baccalieu Island, Newfoundland , Canada	BI	1 3	6	0.78±0.1 0	0.56±0.3 8	0.28/0.25	0.44
	Hernyken, Norway	HN	1 7	14	0.97±0.0 3	1.21±0.7 1	0.09/0.08	0.59
	Daikoku Island, Japan	DI	3 2	14	0.88±0.0 4	0.51±0.3 4	0.15/0.12	0.19
	Western Aleutian Islands	W A	1 2	9	0.91±0.0 8	0.77±0.5 0	0.22/0.16	0.21
	Central Aleutian Islands	CA	5	4	0.90±0.1 6	0.54±0.4 4	0.28/0.28	0.77
	Eastern Aleutian Islands	EA	1 2	9	0.94±0.0 6	0.52±0.3 7	0.15/0.16	0.72
	Semidi Islands, Alaska, U.S.A	SI	8	7	0.96±0.0 8	0.69±0.4 8	0.22/0.20	0.56
	St. Lazaria Island, Alaska, U.S.A.	SL	9	7	0.94±0.0 7	1.00±0.6 4	0.16/0.17	0.77
	Cleland Island, British Columbia, Canada	CI	2 1	10	0.84±0.0 7	0.50±0.3 4	0.20/0.16	0.22

	Storm Island, British Columbia, Canada	SI	1 6	6	0.62±0.1 3	0.40±0.2 9	0.42/0.27	0.11
	Thomas Island, British Columbia, Canada	TI	1 1	6	0.85±0.0 9	0.47±0.3 4	0.22/0.23	0.65
<i>Hydrobates socorroensis</i>	Isla Guadalupe Summer, Mexico	GS	1 8	13	0.95±0.0 4	1.23±0.7 2	0.10/0.10	0.50
<i>Hydrobates cheimomnestes</i>	Isla Guadalupe Winter, Mexico	G W	2 4	14	0.91±0.0 5	0.57±0.3 7	0.13/0.10	0.20
<i>Hydrobates leucorhoa chapmani</i>	Islas Benito, Mexico	San SB	2 4	9	0.66±0.1 1	0.37±0.2 7	0.37/0.22	0.05

Table 2: Estimates of F_{ST} from microsatellites (below diagonal) and Φ_{ST} from mitochondrial control region sequences (above diagonal) for pairwise comparisons of Leach's storm-petrel species and subspecies, with Atlantic and Pacific *H. l. leucorhoa* colonies grouped separately.

		Atlantic <i>H. l.</i> <i>leucorhoa</i>	Pacific <i>H.</i> <i>leucorhoa</i>	Guadalupe Summer <i>H. socorroensis</i>	Guadalupe Winter <i>H.</i> <i>cheimomnestes</i>	San Benito <i>H. l.</i> <i>chapmani</i>
	n	91	126	18	24	24
Atlantic <i>H. l. leucorhoa</i>	107		0.19**	0.57**	0.65**	0.51**
Pacific <i>H. l. leucorhoa</i>	125	0.01**		0.55**	0.65**	0.49**
Guadalupe Summer <i>H. socorroensis</i>	25	0.27**	0.28**		0.27**	0.61**
Guadalupe Winter <i>H. cheimomnestes</i>	28	0.36**	0.38**	0.06**		0.72**
San Benito <i>H. l. chapmani</i>	17	0.09**	0.06**	0.24**	0.35**	

** significant at $p < 0.01$