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Research Article

Genetic variability in mitochondrial and nuclear genes of *Larus dominicanus* (Charadriiformes, Laridae) from the Brazilian coast

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Abstract

Several phylogeographic studies of seabirds have documented low genetic diversity that has been attributed to bottleneck events or individual capacity for dispersal. Few studies have been done in seabirds on the Brazilian coast and all have shown low genetic differentiation on a wide geographic scale. The Kelp Gull is a common species with a wide distribution in the Southern Hemisphere. In this study, we used mitochondrial and nuclear markers to examine the genetic variability of Kelp Gull populations on the Brazilian coast and compared this variability with that of sub-Antarctic island populations of this species. Kelp Gulls showed extremely low genetic variability for mitochondrial markers (cytb and ATPase) and high diversity for a nuclear locus (intron 7 of the β-fibrinogen). The intraspecific evolutionary history of Kelp Gulls showed that the variability found in intron 7 of the β-fibrinogen gene was compatible with the variability expected under neutral evolution but suggested an increase in population size during the last 10,000 years. However, none of the markers revealed evidence of a bottleneck population. These findings indicate that the recent origin of Kelp Gulls is the main explanation for their nuclear diversity, although selective pressure on the mtDNA of this species cannot be discarded.

Key words: cytb, FIB7 intron, phylogeography, recent origin, selective sweep.

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Introduction

The gull genus *Larus*, which consists of 25 species worldwide, has a complex evolutionary history. Twenty-one species of *Larus* occur in the Northern Hemisphere and hybridization among several species in areas of secondary contact has been documented (Liebers *et al.*, 2001; Crochet *et al.*, 2003; Pons *et al.*, 2004). Most studies of the genus *Larus* have addressed the systematics of this group and have reported recent divergence among various species (Crochet *et al.*, 2003; Pons *et al.*, 2005). Some studies have concluded that the last glaciations had a strong influence on the evolutionary pressures acting on this group (Liebers *et al.*, 2001, 2004; Crochet *et al.*, 2003). In contrast to Northern Hemisphere species, little is known about the four species of *Larus* that occur in the Southern Hemisphere. Indeed, the work described in here is the first study to examine the population genetics of this group.

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Larus dominicanus (Kelp Gull) (Charadriiformes: Laridae) is a common species that is widely distributed in the Southern Hemisphere, including South America, southern Africa, Australia, New Zealand, sub-Antarctic islands and the Antarctic Peninsula (Burguer and Gochfeld, 1996). Several studies have documented a large increase in the size of Kelp Gull populations in recent decades (Quintana and Yorio, 1998; Steele and Hockey, 1990). For example, the Kelp Gull population of the Valdez Peninsula, Argentina, reportedly increased from 3,200 to 6,500 breeding pairs in 10 years (Quintana and Yorio, 1998). These increases in population size may reflect the fact that the Kelp Gull is a competitive species that preys on several seabird species and displaces many species from their breeding sites (Quintana and Yorio, 1998). However, the effects of Kelp Gulls on coastal wildlife are not confined to other seabirds. Thomas (1988) and Rowntree *et al.* (1998) observed Kelp Gulls injuring Right Whales (*Eubalaena australis*) by picking off skin and fat when the whales surfaced to breathe. These authors argued that intense harassment by gulls caused Right Whales to abandon breeding areas before their young are sufficiently strong for the open sea.

For several species of seabirds, there are few or no vicariant processes and extrinsic barriers to dispersion (Congdon *et al.*, 2000), leading to the expectation that low levels of population structure should be observed. On the other hand, strong philopatry, a wide geographic distribution or historic separation can create substantial genetic differentiation in some species (Genovart *et al.*, 2003). Several studies had shown low population structure in seabirds (Austin *et al.*, 1994; Friesen *et al.*, 1996). On the Brazilian coast, the few studies that have been done have shown low genetic differentiation on a wide geographic scale. Faria *et al.* (2010) observed low genetic structure in the South American Tern (*Sterna hirundinacea*) on the Brazilian coast. Gonçalves *et al.* (2007) also found low genetic structure in *Calidris pusilla* on the north coast of Brazil. A similar pattern was observed for *Sula dactylacta* and *Sula leucogaster* along the Brazilian coast (Baumgarten MM, 2003, PhD thesis, Universidade de São Paulo, São Paulo, Brazil). In all three cases, low genetic differentiation was attributed to a high capacity for dispersion that homogenized the genetic variability among population or to a population bottleneck that reduced the genetic diversity through the loss of a large number of individuals.

For many years, phylogeographic studies were based on the analysis of a single locus for mitochondrial markers (Austin *et al.*, 1994; Brown *et al.*, 2004; Helbig and Seibold, 1999; Avise *et al.*, 2000). However, more recently, the use of nuclear markers to infer evolutionary history has proven to be highly informative; these markers allow inferences on more remote demographic events that have helped to clarify the evolutionary history of many groups (Hare *et al.*, 2002; Godinho *et al.*, 2006; Melo-Ferreira *et al.*, 2009). Mitochondrial and nuclear markers have different effective population sizes and modes of inheritance such that demographic events shape the variation in these genetic markers in distinct ways (Zink and Barrowclough, 2008). In addition, because mitochondria do not generally undergo recombination any selection events on a mitochondrial gene would tend to decrease the diversity at linked loci through hitch-hiking or background selection (Bazin *et al.*, 2006). Since mitochondria show maternal inheritance the pattern observed for this molecule faithfully represents the history of the population based on female patterns (Ballard and Whitlock, 2004). In contrast, nuclear markers reflect both male and female histories (Zhang and Hewitt, 2003), have deeper coalescence times and experience recombination, thus making distinct unlinked markers independent. Consequently, studies that use both markers should provide a more accurate evolutionary history of the group being investigated.

Into this context, Kelp Gulls are an interesting group in which to study phylogeography patterns because of their wide distribution, fast-growing populations and intense interaction with other species. The use of nuclear and mitochondrial markers should allow us to reconstruct the evolutionary history of this species on the Brazilian coast. The aim of the present study was to estimate the divergence of the Kelp Gull from its sister groups and to describe the genetic variability of this species in populations on the Brazilian coast in comparison with sub-Antarctic islands populations of this species. Two major questions were addressed: 1) Are Brazilian populations genetically different from each other and from non-Brazilian populations? and 2) Is there genetic evidence for a population bottleneck or expansion in the recent evolutionary history of Kelp Gulls?

Materials and Methods

Phylogenetic analysis

The phylogenetic analyses of the *Larus* group reported by Liebers *et al.* (2004) and Pons *et al.* (2005) located the Kelp Gull in a group consisting of large white-headed gulls. Based on these analyses, we sought to estimate the divergence of the Kelp Gull from its sister groups. For this, we used 105 specimens, *i.e.*, five specimens of *L. dominicanus* and 98 individuals from other taxa of the genus *Larus*; the phylogeny was rooted with the two individuals from genus *Rissa* with 405 bp of cytochrome b, obtained from GenBank (Supplementary material Table S1). The sequences were aligned by eye using Bioedit v. 7.0 (Hall, 2001). The most appropriate model of DNA evolution for the sequences was inferred with MODELTEST (Posada and Cradall, 1998) implemented in PAUP v. 4.0 (Swofford, 2000). Bayesian inferences were done using BEAST v.1.4.8 software (Drummond and Rambaut, 2007). This analysis was based on the following assumptions: a restricted molecular clock, a 2% per million years substitution rate and a 3.3 Mya (Million years ago) divergence between the genera *Rissa* and *Larus* (Paton *et al.*, 2003). We then used a portioned Bayesian Inference search in a Markov-Chain Monte Carlo analysis to run four independent chains, each with 10,000,000 steps and sampled every 1000 steps, excluding a burn-in of 1,000,000 trees. The parameter analysis was visualized by means of Tracer v. 1.4.1 software and the trees were connected in TreeAnotator and visualized in FigTree (Drummond and Rambaut, 2007).

Intraspecific analysis

Population sampling

This study was based on samples from seven islands on the Brazilian coast and two sub-Antarctic islands (Figure 1). Individuals were captured using a hand net and marked with a metal ring provided by CEMAVE/IBAMA (license no. 1060) during the breeding seasons from 2002 to 2005. Blood samples were collected from the brachial vein of all captured individuals and stored in 100% ethanol at room temperature. This blood collection technique did not injure the gulls or result in any deaths. The samples were stored in the Laboratório de Biologia Evolutiva e Conservação de Vertebrados (LABEC/IB-USP). Total genomic

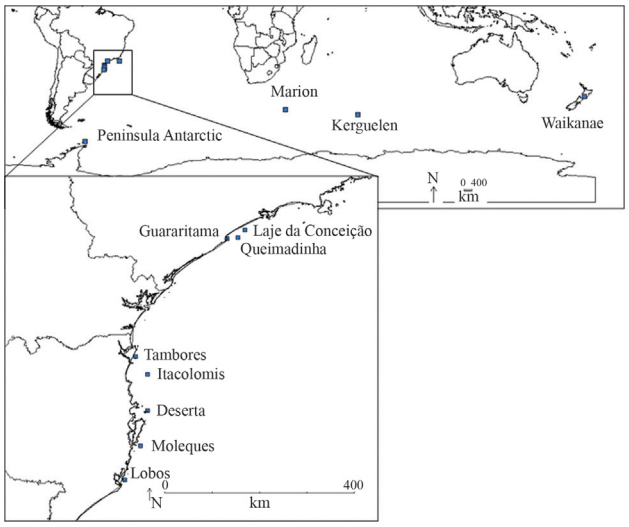

Figure 1 - Locations of the breeding colonies of *L. dominicanus* sampled in the Southern Hemisphere: São Paulo (Laje Conceição, Guaraitama and Queimadinha), Santa Catarina (Tambores, Itacolomis, Deserta, Moleques and Lobos), Antarctic Peninsula, Marion Island and Kerguelen Island.

DNA was isolated from blood samples by a standard phenol/chloroform technique, precipitated with ethanol and resuspended in Tris-EDTA (TE) buffer (Sambrook *et al.*, 2001). In addition, sequences available in GenBank from Kerguelen Island and New Zealand were included in the analysis to compare genetic diversity.

Molecular methods

Two regions of mitochondrial DNA (*ATPase 8* and *6* of ~750 bp and *cytochrome b* (*cytb*) of ~350 bp) and one nuclear (intron 7 of β-*fibrinogen*, ~900 bp) were used in this study. *ATPase 8* is the second most variable region of the mitochondrial genome in birds, but it is short (165-168 pb). The *cytb* gene is a well-conserved region with an estimated substitution rate of ~2%/Myr for the entire avian mitochondrial genome (Shields and Wilson, 1987; Bridge *et al.*, 2005). Intron 7 of β-*fibrinogen* is located on chromosome 4 in chicken (*Gallus gallus*) and has been used in other phylogeographic studies (Bridge *et al.*, 2005; Godinho *et al.*, 2006; Gonçalves *et al.*, 2007); this intron has an evolutionary rate of 0.53%/Myr in the pigeon (Johnson and Clayton, 2000). The *ATPase 8* and *6* fragments were amplified using primers *Lys* (Sorenson *et al.*, 1999) and *int-H* (Faria *et al.*, 2007), *cytb* was amplified using primers *L15008* and *H1532b* (Desjardin and Morais, 1990) and intron 7 of β-*fibrinogen* was amplified using primers FIB7U and Fib7L (Prychitko and Moore, 2000). The reactions for *ATPase 8* and *6* and *cytb* were run in a final volume of 10 μL containing 1X *Taq* platinum polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 2 mM of each dNTP, 10 pmol of primer, 0.5 units of *Taq* polymerase and approximately 20 ng of DNA. The PCR cycling sequence consisted of 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 58 °C for 35 s and 72 °C for 45 s, with a final extension at 72 °C for 10 min. The PCR for β-*fibrinogen* PCR was done in a final volume of 10 μL containing 1X PCR buffer (50 mM Tris-HCl, 50 mM NaCl, pH 8.5), 200 uM of each dNTP, 0.5 units of Go*Taq* DNA polymerase (Promega), 5 pmol of each primer and approximately 50 ng of DNA. The PCR began with an initial denaturation of 5 min at 95 °C followed by 38 cycles of 30 s at 95 °C, 45 s at 60 °C, 1 min at 72 °C and a final extension of 7 min at 72 °C.

The PCR products were purified using EXOI-SAP (1:1). Sequences were obtained using BigDye terminator (version 3.1) (Applied Biosystems) according to the manufacturer's recommendations and the primers used for PCR; the sequences were analyzed on an Applied Biosystems 3100 genetic analyzer. Electropherograms were checked by eye in ChromasLite (www.technelysium.com.au). The alignments were adjusted by eye in Bioedit v.5.06 software (Hall, 2001). A Bayesian approach run with the program PHASE (Stephens *et al.*, 2001) was used to identify haplotypes of heterozygotes in the nuclear intron; this program reconstructs the haplotype as implemented in DNAsp v. 5.10.01 software (Librado and Rozas, 2009). The RDP (Recombination Detection Program) v.3 (Martin *et al.*, 2005) was used to detect recombination in the set of aligned DNA sequences, based on the following methods: the original RDP, bootscanning, GENECONV, Maximum Chi Square, Chimaera, Sister Scanning and 3SEQ. All of these non-parametric recombination detection methods provided a detailed breakdown of the recombination breakpoint locations and of the identities of recombinant and parental sequences.

Comparison of mtDNA versus nDNA diversity

For intraspecific analyses, a fragment of *cytb* (312 bp) was sequenced in 83 individuals from the Brazilian coast, seven individuals from the Antarctic Peninsula, and five individuals from Marion Island. These sequences were compared with those available in GenBank from New Zealand (NC006007, AY293619 and AF268497) and Kerguelen Island (AF444259). The coding region of *ATPase 6* and *8* was sequenced for 58 *L. dominicanus* individuals from the Brazilian coast. Intron 7 of the β-*fibrinogen* gene (858 bp) was sequenced in 66 individuals from the Brazilian coast, seven individuals from the Antarctic Peninsula and three individuals from Marion Island.

Descriptive analyses including haplotype diversity (h), nucleotide diversity (π) and Theta per gene (θ) were calculated for all loci using DNAsp v.5.10.01 (Librado and Rozas, 2009). Tajima's D value (Tajima, 1989) and Fu and Li's D* and F* values (Fu and Li, 1993) and the significance of these neutrality test statistics were calculated using DNAsp v.5.10.01 (Librado and Rozas, 2009). DNAsp v.5.10.01 was also used to calculate parameters for the demographic history of the population based on mismatch distributions; unimodal curves are expected in populations

that have undergone rapid population expansion (Rogers and Harpending, 1992). The Raggedness statistic and Ramos-Onsins and Rozas R^2 statistics (Ramos-Onsins and Rozas, 2002) were used to test for deviations between the observed and expected on mismatch distribution. Haplotype networks were constructed using the median-joining method (MJN) (Bandelt *et al.*, 1999) as implemented in the NETWORK v.4.1.0.8 software

We used coalescent simulations to generate the predicted levels of genetic diversity based on *a priori* specified demography history, thus allowing us to contrast nuclear and mitochondrial markers based on the following assumptions or hypothesis: 1) If a demographic event affected the gull populations it would leave signatures in the entire mitochondrial and nuclear genomes; on the other hand, selective events would leave signatures only at individual loci and unlinked loci, 2) Liebers *et al.* (2001) stated that the last glaciation affected the distribution of these gulls; consequently, if the last glaciation reduced the population size then the ecological expansion observed today is a consequence of expansion after this period, and 3) if this species has a recent origin, as described for other seabirds (Crochet *et al.*, 2003; Pons *et al.* (2005), then the diversity observed in mitochondrial DNA and nuclear DNA is the result of ancestral polymorphism, with or without expansion of the species.

The simulations based on these three scenarios were run in SIMCOAL2 (Laval and Excoffier, 2004): Scenario 1 – the *L. dominicanus* population has experienced continuous expansion from the time of its divergence from other *Larus* species to the present, Scenario 2 – the population passed through a bottleneck that reduced 90% its original population size 10,000 years ago followed by subsequent expansion and Scenario 3 – the population has a recent origin with regard to the time of divergence from *Larus fuscus* complex to form *L. dominicanus* as estimated here and has not experienced expansion or a bottleneck. This approach allowed us to compare the results for all Brazilian populations with simulated data generated under each model, with the following additional assumptions: a substitution rate of 2%/Myr for *cytochrome b* (Shields and Wilson, 1986) and 0.53%/Myr for β-*fibrinogen* (Johnson and Clayton, 2000). The initial population sizes were calculated for each population on the Brazilian coast based on the variability of the β-*fibrinogen* locus (Table 1). The generation time used in this simulation for *L. dominicanus* was 10 years based on that estimated for *L. michaelis* from field data (Crochet *et al.*, 2003). The estimated divergence time between *L. dominicanus* and the *L. fuscus* complex in this work was used to calculate the number of generations from the divergence up to the present day. For each scenario, 10,000 simulations were run and the SIMCOAL2 results were then analyzed using ARLEQUIN v.3.01 (Excoffier *et al.*, 1992) to estimate Tajima's D values for each simulation. The values obtained during each run were used to construct the distribution of Tajima's D values for each *L. dominicanus* population on the Brazilian coast. Finally, the observed Tajima's D values for each Brazilian population were compared with the distribution generated by the simulations.

Population analysis

Population analyses were done only for intron 7 of β-*fibrinogen* since the mitochondrial locus showed insufficient variation. Each island was considered as a single population. We estimated the haplotype diversity (h), nucleotide diversity (π) and Theta (θ) per gene for each population using DNAsp v. 5.10.01. In addition, Tajima's D value, Fu and Li's D* and F* values and Ramos-Onsins and Rozas R^2 statistics were calculated for all populations and the signifi-

Table 1 - Analysis of the genetic diversity of intron 7 of β-*fibrinogen* in various *L. dominicanus* populations, showing the number of polymorphic sites (S), haplotype number (NH), haplotype diversity (H), nucleotide diversity (π) and theta (θ) per gene. The parameters used in the neutrality test of intron 7 of the β-*fibrinogen* gene are also shown.

Localities		N	S	NH	H	π	Θ	Tajima's D	Fu and Li's D*	Fu and Li's F*
Guararitama	SP	36	18	23	0.933	0.00528	3.973	0.205	0.545	0.512
Queimadinha	SP	24	11	16	0.960	0.00442	3.181	0.287	0.355	0.384
Laje Conceição	SP	10	9	7	0.933	0.00433	3.181	1.488	0.904	1.197
Moleques	SC	20	16	13	0.905	0.00428	4.408	-0.511	1.518**	1.055
Tambores	SC	12	4	5	0.667	0.00150	1.325	-0.016	1.195	1.004
Deserta	SC	22	13	15	0.905	0.00526	3.566	1.256	1.038	1.282
Lobos	SC	14	13	10	0.945	0.00563	4.088	0.900	1.102	1.998
Antarctica	IA	14	6	4	0.571	0.00232	1.887	0.699	0.505	0.634
Marion	IA	6	0	1	0	0	0	0	0	0
Total		152	33	66	0.906	0.00478	5.895	-0.628	0.840	0.297

N – number of individuals. SP – islands in São Paulo state, Brazil; SC – islands in Santa Catarina state, Brazil; IA – islands in the Antarctic region. **$p < 0.02$.

cance of these test statistics was assessed using DNAsp v. 5.10.01 (Librado and Rozas, 2009).

The overall differentiation of intron 7 of β-*fibrinogen* among the sampled populations was quantified using pairwise F-statistics. Empirical F_{ST} values were compared with a null distribution of no difference between the populations to test for significance. The analyses were implemented in ARLEQUIN with 10,000 permutations. Molecular distances were estimated by the method of Tamura (1992) which allows for unequal nucleotide frequencies. The transition-to-transversion ratios, as well as the overall nucleotide frequencies, were computed from the original data. The partitioning of genetic differences among populations was assessed using hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992), with 10,000 permutations and estimates of molecular distance (Tamura, 1992).

The Mantel test was used to assess the association between geographic distance and genetic distance for all population pairs. The test was initially included all islands (Brazilian and sub-Antarctic ones) and was then run by considering the Brazilian islands and the Antarctic Peninsula without Marion Island since the latter locality had only one haplotype.

Bayesian Skyline plots were constructed with BEAST v. 1.4.6 (Drummond and Rambaut, 2007) to estimate historical changes in population size over time. Since this method for estimating historical demography assumes that sequences are sampled from a single panmitic population the analysis considered all of the populations as a single group. This method uses Markov-Chain Monte Carlo sampling techniques to estimate the posterior distribution of effective population size given a set of aligned DNA sequences and a model of molecular evolution and takes into consideration uncertainty in the genealogical process (Drummond *et al.*, 2005). The best-fit model of molecular evolution selected based on the criteria of Posada and Cradall (1998) was implemented in PAUP v.4.0 (Swofford, 2000). The skyline plot was run for 20,000,000 steps with parameters logged every 2,000 steps and a burn-in of 1,000,000 trees, under a strict molecular clock based on a substitution rate of 0.53%/Myr for β-fibrinogen of *G. gallus* (Jonhson and Clayton, 2000). Skyline plots were constructed using TRACER v.1.4.1 (Drummond and Rambaut, 2007).

Results

Phylogenetic analyses

Phylogenetic Bayesian analysis based on *cytochrome b* was done using the HKY+G model. The inferred topology showed short branch lengths within gull species (Figure 2). *Larus dominicanus* appeared as a monophyletic clade and the sister clade consisted of six species from the *fuscus* complex: *L. taymyrensis*, *L. glaucoides*, *L. glaucescens*, *L. smithsonianns*, *L. marinus* and *L. shiasagus*

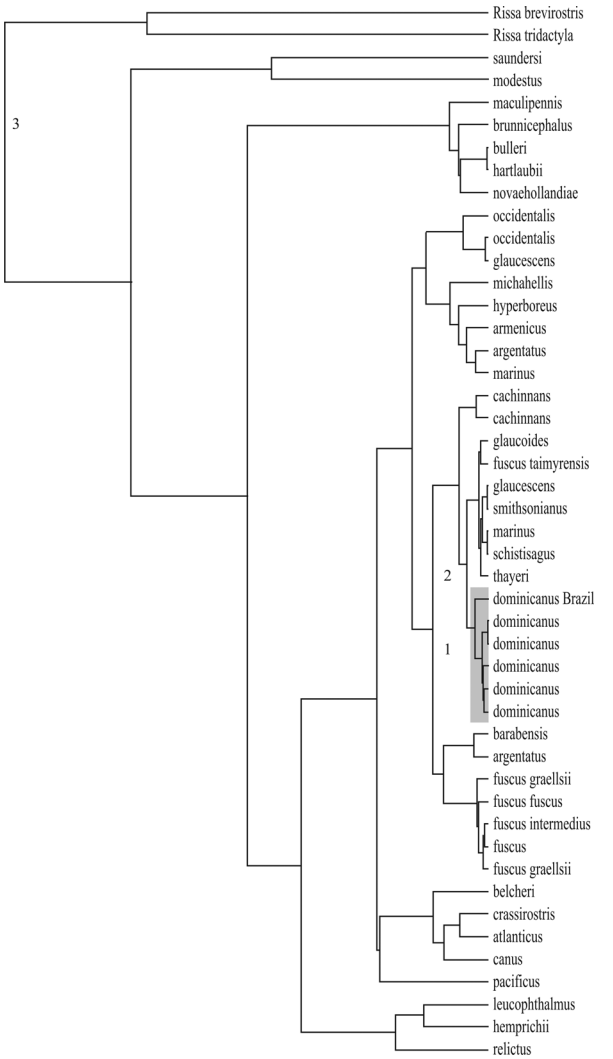

Figure 2 - Bayesian tree based on mtDNA (a fragment of *cytochrome b*) depicting the phylogenetic relationships of gull species. The numbers indicate the date of the corresponding clade (1 = 153,184 years ago, 2 = 241,202 years ago, 3= 3,300,00 years ago) and the light grey shading indicates the *dominicanus* clade.

(Figure 2). The estimated divergence time of *L. dominicanus* from the *fuscus* complex was ~241,200 years ago. The time to the most recent common ancestor of *L. dominicanus* was ~153,184 years ago.

Intraspecific analyses

Comparison of mtDNA and nDNA diversity

Larus dominicanus from the Brazilian coast showed only one haplotype for *cytochrome b* while the sequences from the Antarctic Peninsula, Marion Island, Kerguelen Island and New Zealand represented another haplotype that differed by a single substitution (Table 2). The coding region of *ATPase 6* and *8* contained three haplotypes with two polymorphic sites (Table 2). The most common haplotype found on the Brazilian coast (HBRA01) was identical to the sequences found in New Zealand (NC006007, AY293619) (Figure 3). On the other hand, intron 7 of

Table 2 - Genes analyzed in *L. dominicanus* showing the number of polymorphic sites (SNP), haplotype diversity (h), nucleotide diversity (π), theta per gene (θ), Tajima's D value and Fu and Li's D* and F* values among loci.

Gene	N	Size	SNP	H	Pi (π)	θ	Tajima's D (ci)	P	Fu and Li's F* (ci)	P	Fu and Li's D* (ci)	P
cytochrome b	95	315	1	0.273	0.00083	0.00064	0.460 (-1.30; 1.82)	0.74	0.565 (-2.00; 1.24)	0.75	0.496 (-2.01; 0.94)	0.48
ATPase 8 and *6*	58	583	2	0.358	0.00063	0.00074	-0.323 (-1.44; 1.81)	0.39	0.513 (-2.01; 1.28)	0.67	0.723 (-1.89; 0.98)	0.88
Intron 7 of β-*fibrinogen*	152	858	28	0.896	0.00465	1.053	0.0517 (-1.65; 1.95)	0.61	0.13 (-2.21; 1.70)	0.12	1.74 (-2.14; 1.57)	0.02

ci – confidence interval.

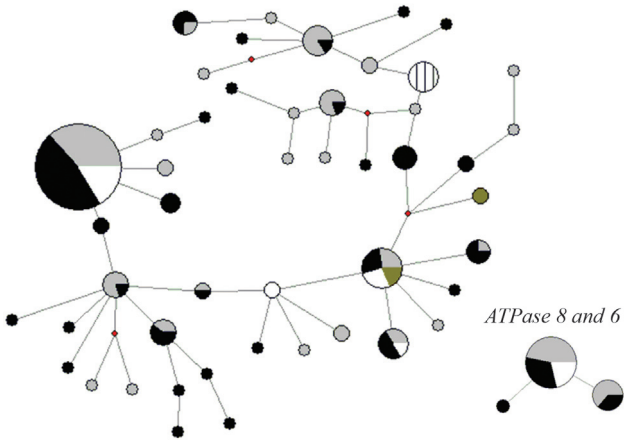

Figure 3 - Median joining networks based on *ATPase 6* and *8* mtDNA and intron 7 of β-*fibrinogen* (Fib7) nuclear DNA from *L. dominicanus* in the Southern Hemisphere. São Paulo (grey), Santa Catarina (black), Antarctic (white), Marion Island (vertical lines) and outgroup (green).

β-*fibrinogen* had a comparatively higher genetic diversity with 55 haplotypes, 28 polymorphic sites, three singletons and 25 parsimonius sites (Figure 3 and Table 2). All of these sequences are available through GenBank accession numbers FJ668863-FJ668936. None of the methods implemented in RDP3 software provided any evidence of recombination in intron 7 of β-*fibrinogen*, a finding that allowed the use of all haplotypes in subsequent analyses.

Tajima's D value and Fu and Li's D* and F* values for *cytochrome b*, *ATPase 6* and *8* and intron 7 of β-*fibrinogen* revealed no significant deviation from neutrality and the expected equilibrium (Table 2), except for Fu and Li's D* value for intron 7 of β-*fibrinogen*. The mismatch distribution of intron 7 of β-*fibrinogen* showed a wave signal (multiple peaks) consistent with a stable population (Raggedness index = 0.0110 and Ramos-Onsins and Rozas $r^2 = 0.0723$, p = 0.60; intial theta = 1.89 and tau = 2.19) (Figure 4). However, the mismatch distribution for loci from mtDNA did not show the expected bias for *cytochrome b* (Raggedness statistic r = 0.280 and Ramos-Onsins and Rozas $r^2 = 0.1367$; estimated initial theta = 0.00 and tau = 0.273) and *ATPase 6 and 8* (Raggedness statistic r = 0.199 and Ramos-Onsins and Rozas $r^2 = 0.0925$; estimated initial theta = 0.000 and tau = 0.370).

The best scenario from simulations that could explain the variation found in nuclear intron 7 of β-*fibrinogen* was the hypothesis of a recent origin and neutral evolution, without marked expansion. None of the models used in the simulations were compatible with the diversity found at the *cytochrome b* locus. We therefore chose to show only the outcomes of the simulations for this scenario (Figure 5) because other simulations showed distributions that did not include the values observed in the populations.

Population analysis

When Tajima's D value and Fu and Li's D* and F* values for intron 7 of β-*fibrinogen* were analyzed for each

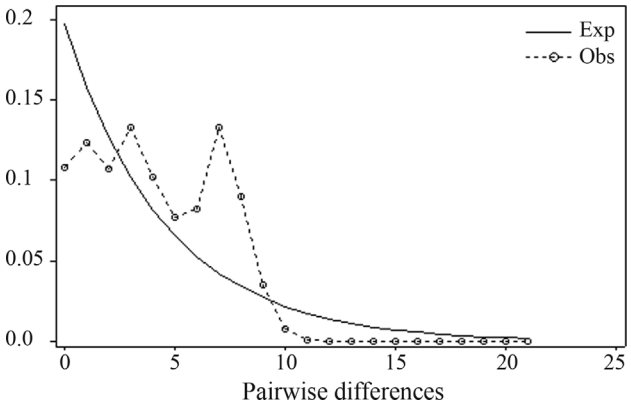

Figure 4 - Mismatch distribution of the fragment of intron 7 of β-*fibrinogen* nuclear DNA from *L. dominicanus* in the Southern Hemisphere. Exp – expected, Obs – observed.

population separately they revealed no significant deviation from neutrality and the expected equilibrium, except for the Moleques population in Santa Catarina that deviated significantly from Fu and Li's D test (Table 1).

AMOVA of intron 7 of the β-fibrinogen revealed significant differentiation among populations, but a large part of the total variation was found within the population (92%; Table 3). The Marion Island population was the most differentiated population of *L. dominicanus* because of a single exclusive haplotype (Table 4). There was also differentiation between the Antarctic and São Paulo islands (Guararitama, Queimadinha, Laje da Conceição) but there was no marked differentiation between Santa Catarina islands (Moleques, Tambores, Itacolomis) and the Antarctic. This weak structure was corroborated by the locus network (Figure 3) which detected no exclusive haplotypes for any region, except for Marion Island.

The Mantel test detected a correlation between distance and genetic differentiation when all populations were used in the test (r = 0.826) but this was not significant (test $t = 2.5752$; p = 0.9950). The correlation found using that the Mantel test without Marion Island was r = 0.324 (test $t = 1.2651$; p = 0.8971). The Bayesian Skyline analysis indicated rapid growth that started ~10,000 years ago and has continued up to the present time, with no sign of retraction during the evolutionary history of these lineages of intron 7 of the β-fibrinogen gene (Figure 6).

Discussion

Phylogenetic analysis

The phylogenetic analysis indicated that the *Larus* group has a recent origin: *L. dominicanus* diverged from the *fuscus* complex 241,201 years ago and the time to the most recent common ancestor was estimated at 153,184 years ago (Figure 2). Other researchers have also shown that the *Larus* group has a recent origin. The *argentatus-fuscus* complex diverged between 100,000 and 170,000 years ago (Crochet and Desmarais, 2000); the species into this com-

Table 3 - Analysis of molecular variance (AMOVA) for intron 7 of the β-*fibrinogen* gene of *L. dominicanus* populations.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among population	8	7.903	0.03565 Va	7.8
Within population	139	58.374	0.41996 Vb	92.2
Total	147	66.277	0.45560	100.00

Fst = 0.074, p = 0.00. Va: covariance component due to differences among populations within populations. Vb: covariance component among individuals within populations.

Table 4 - Pairwise F_{ST} values for intron 7 of β-*fibrinogen* (below diagonal) among populations of *L. dominicanus.* Significant values (p < 0.05) are indicated in bold.

	Laje	Gua	Quei	Mole	Tam	Des	Lob	Ant	Marion
Laje	-								
Gua	-0.225	-							
Quei	-0.119	0.000	-						
Mole	0.025	0.038	**0.112**	-					
Tam	-0.041	-0.015	0.044	0.026	-				
Des	-0.009	-0.011	0.191	0.037	-0.004	-			
Lob	**0.161**	**0.120**	**0.270**	0.033	**0.107**	**0.147**	-		
Ant	**0.114**	**0.088**	**0.242**	0.003	0.066	0.067	0.082	-	
Marion	**0.539**	**0.407**	**0.434**	**0.625**	**0.548**	**0.424**	**0.844**	**0.780**	-

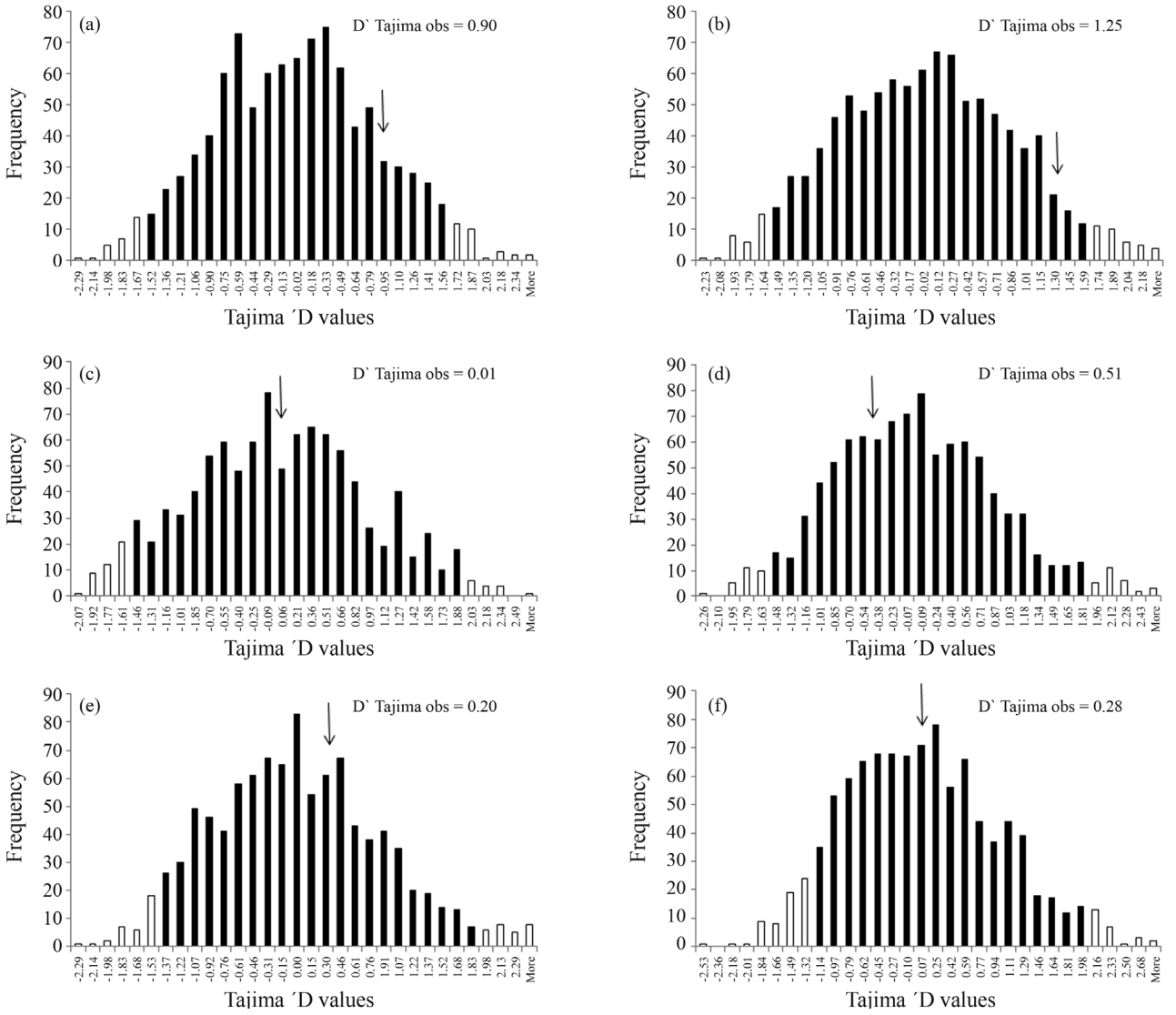

Figure 5 - Histogram of the simulation for intron 7 of β-*fibrinogen* from *L. dominicanus* populations along the Brazilian coast. Recent origin scenario: (a) Lobos Island – RS, (b) Deserta Island – SC, (c) Tambores Island – SC, (d) Moleques do Sul Island – SC, (e) Queimadinha Island – SP and (f) Guararitama Island – SP. The arrows indicate the position of our data in the histogram.

plex share several haplotypes and hybridization is observed in their contact zone in the Northern Hemisphere. Given *et al.* (2005) also reported a recent origin for masked gulls since they estimated that nine species in the Southern Hemisphere diverged from a common ancestor ~0.5 Mya. Overall, there is a consensus regarding the recent divergence of the genus *Larus* and the cause of extensive hybridization among species in the Northern Hemisphere (Liebers *et al.*, 2001, 2004; Crochet *et al.,* 2003; Given *et al.,* 2005).

Comparison of mtDNA and nDNA diversity

The mtDNA results for Kelp Gulls were lower than those found in another groups of birds in which the same *cytochrome b* region was analyzed. Brooke and Rowe (1996) reported a haplotype diversity of 0.68 and nucleotide diversity of 0.0013 for *Pterodroma magenta* whereas the corresponding values for *Brichyranphys perdix* were 0.70 and 0.004, respectively (Friesen *et al.*, 1996). Proudfoot *et al.* (2006) observed a nucleotide diversity of 0.013 for *Glaucidium brasilianum* and Mundy *et al.* (1997) reported a corresponding value of 0.0043 for *Lanius ludovicianus*. All of these indicators of diversity in seabirds or other groups were considerably higher than in Kelp Gulls, for which the haplotype diversity was 0.273 and the nucleotide diversity 0.00083. On the other hand, the haplotype diversity (0.89) of intron 7 of β-*fibrinogen* was similar to that of seabirds such as *Calidris pussilla* on the northern coast of Brazil (Gonçalves *et al.*, 2007).

The low genetic diversity observed for *cytochrome b* and *ATPase 8* and *6* was compatible with both demographic and selective processes. Demographic factors include bottlenecks and founder effects, both of which reduce popula-

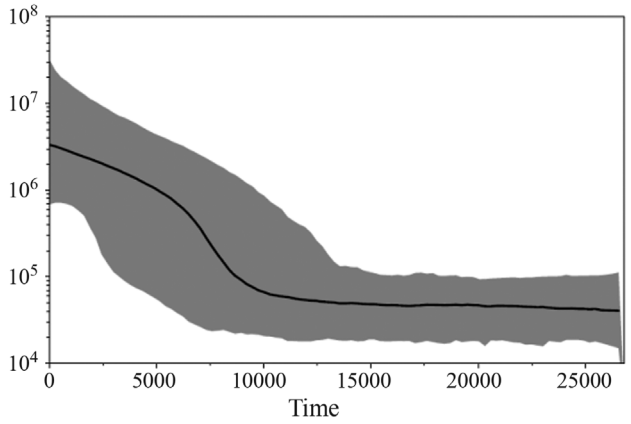

Figure 6 - Bayesian skyline plot for intron 7 sequences of β-*fibrinogen* with a log-normal restricted clock and a generation time of 10 years. The y-axis shows the effective number of individuals. The thick solid line is the estimated median and the gray shaded area shows the 95% highest density limits. The x-axis is scaled as thousand years ago (Ya).

tion size temporally and result in an increased rate of genetic drift (Galtier *et al.*, 2000). On the other hand, the rapid fixation of a new, favorable allele through directional selection (selective sweep) can also reduce genetic variability at a locus under selection and at linked loci (Galtier *et al.*, 2000). A reduction in genetic variability can transform an abundant species into a species with a high probability of extinction because of stochastic events such as infection by new pathogens. Nevertheless, the β-fibrinogen gene showed high diversity, indicating that the genetic variability of Kelp Gulls is not at a critical level (Table 2).

The low genetic variability at mtDNA loci and a comparatively higher level of variation at intron 7 of β-*fibrinogen* allowed us to formulate three hypotheses: Hypothesis 1 – demographic events reduced the genetic diversity of both mitochondrial and nuclear genes, but the difference in effective size between these molecules preserved greater diversity at the nuclear gene, Hypothesis 2 – the recent demographic expansion of *L. dominicanus* can explain the low genetic diversity in mtDNA while the high diversity in intron 7 of β-*fibrinogen* reflects ancestral polymorphism; these findings reflect differences in the effective population size for these markers and the divergence time of the species, and Hypothesis 3 – mitochondrial DNA from *L. dominicanus* experienced a selective sweep that reduced its variability.

Hypothesis 1 could be the outcome of founder events or bottleneck events on the Brazilian coast. Recent colonization can lead to reduced genetic diversity since all individuals are descendants of a small founder group (Hartl and Clarck, 1989). If populations on the Brazilian coast are the result of recent colonization then this may have involved specimens from the Pacific coast of South America, the southeast Atlantic Ocean, South Africa or Australia. Regardless of the origin of these populations, this species would use the sub-Antarctic islands and Antarctic Peninsula as a route to migrate to the Brazilian coast. Considering this route of migration, the colonies on the Atlantic coast of South America would be expected to show differentiation in a south-north direction. However, as shown here, there was low genetic differentiation in mitochondrial markers such as *cytochrome b* when compared with populations from Australia, New Zealand and Kerguelen Island. Overall, the low genetic variability of the Brazilian populations compared with other colonies in the Southern Hemisphere was not consistent with the hypothesis that the colonization of this region involved founder events. On the other hand, intron 7 of β-*fibrinogen* showed a cline of differentiation between the Antarctic and Brazilian coast (Table 4), indicating that this hypothesis cannot be discarded; however, its acceptance would require an analysis of samples from the Pacific and other sub-Antarctic islands.

A second possible demographic event that may have occurred in *L. dominicanus* is the loss of genetic diversity as a result of bottlenecks in the last glaciations. Pleistocene glaciers promoted changes in sea temperature, currents and other physiochemical characteristics that may have affected the population history of tropical birds (Peck and Congdon, 2004). Glacial cycles in the Pleistocene that were associated with ecological changes affected the dynamics of gull populations in the Northern Hemisphere (Liebers *et al.*, 2001). Oscillations in sea levels and changes in ecological factors in response to glacial cycles may have reduced the size of the Kelp Gull population. This reduction may have led to the loss of genetic variability through successive reduction and expansion of the population. Sea levels are known to have oscillated during the Quaternary glaciations (Bigarela, 1965). On the Brazilian coast, the sea level was up to 10 meters above its current level (Suguio, 2004) and there is evidence that regressions of approximately 100 meters below the current level occurred in the Pleistocene (Bigarela, 1965). Such fluctuations may have altered the breeding sites for this species. However, the influence of sea level fluctuations on the genetic diversity of seabirds with a wide distribution and high capacity for dispersion remains unclear.

As shown here, Tajima's D value and Fu and Li's D* and F* values and the R2 neutrality tests for intron 7 of β-*fibrinogen* showed no signs of the population expansion that would be expected in the case of a recent bottleneck followed by expansion. Fu and Li's D* and F* values and the R^2 statistic are more sensitive indicators of range expansion when compared to Tajima's D value (Ramos-Onsins and Rozas, 2002). R^2 is more effective when testing small sample sizes, but these tests did not detect any sign of expansion in Kelp Gull. These results were corroborated by mismatch distribution that showed no sign of population expansion, with a multimodal distribution consistent with a population in demographic equilibrium (Excoffier *et al.*, 1992). However, ecological data suggest that Kelp Gulls have increased at a rate of 50% per year (Dantas and

Morgante, 2010) and the Bayesian Skyline plot clearly showed the expansion of *L. dominicanus* from 10,000 years ago up to the present-day, but no sign of bottleneck events (Figure 6). The conditions that prevailed after the last glaciations probably favored the expansion of *L. dominicanus* on the Brazilian coast. Although the summary statistics of the data for intron 7 of β-*fibrinogen* were consistent with a population in equilibrium, the ecological data and Bayesian analysis indicated expansion of the population. Gonçalves *et al.* (2007) also observed marked genetic diversity in *C. pusilla* on the northern coast of Brazil based on an analysis of intron 7 of β-*fibrinogen*; these authors suggested that this diversity was the result of recent growth in population size and that the populations had not experienced recent bottleneck events.

Based on a generation time of 10 years for Kelp Gulls and their recent origin dated at 241,201 years ago (which correspond to 24,120 generations) it seems reasonable to question whether this length of time is compatible with the absence of variation in the mtDNA locus but insufficient for splitting of the nuclear genome. The simulations showed that divergence time did not explain the low genetic variability observed in mtDNA whereas a recent origin explained the genetic diversity seen in β-*fibrinogen* (Figure 5). This simple demographic scenario therefore cannot explain the low genetic diversity in mtDNA and the hypothesis of a selective sweep in mtDNA cannot be discarded. In addition, Bazin *et al.* (2006) have shown that natural selection acting on mtDNA contributes to the homogenization of diversity. These authors affirmed that mtDNA frequently undergoes adaptive evolution involving direct selection that targets the respiratory machinery, *i.e.*, a form of nucleo-cytoplasmic coadaptation. An investigation of other mtDNA markers is needed to confirm this hypothesis.

Population diversity

Intron 7 of the β-*fibrinogen* gene of the Kelp Gull showed similar variation that found in other Charadriiform seabirds. Gonçalves *et al.* (2007) reported a nucleotide diversity of 0.0048 and haplotype diversity of 0.97 for this species whereas we estimated the corresponding values to be 0.0046 and 0.89, respectively. Our results were consistent with previous genetic studies of seabirds on the Brazilian coast (Gonçalves *et al.*, 2007; Faria *et al.*, 2010) in that we observed a low genetic population structure among Kelp Gull populations (F_{ST} = 0.074) and little differentiation when compared with sub-Antarctic islands (Marion and King George) (Table 4). This outcome was corroborated by network analysis that showed identified shared haplotypes among regions, and by the Mantel test that detected no significant correlation between geographic distance and genetic diversity.

The low genetic structure in the Kelp Gull could reflect the current high levels of dispersal since this species can fly hundreds of kilometers on foraging trips and the Brazilian coast has no apparent barriers to seabird dispersal. Marine currents are believed to play an important role in species distribution and dispersion. The Brazilian coast is influenced by a warm, nutrient-poor current that flows from north to south. In contrast, the Falklands current is cold, rich in nutrients and flows from the south up to Rio de Janeiro in the north. Consequently, all of the Kelp Gull colonies examined were influenced by the same currents. The Kelp Gull is a poorly studied species for which important ecological data and information on the migratory routes are still lacking. To understand the population dynamics of this species it will be necessary to gather ecological and demographic information throughout the species' distribution.

One important result of this study was that the Bayesian skyline plot detected signs of a change in population size, with a clear expansion after the last glacial maximum and no retraction during the glacial period. This outcome is consistent with the population expansion detected based on ecological data from several sites around the world (Quintana and Yorio, 1998; Steele and Hockey, 1990).

Conclusions

L. dominicanus has shown population growth in recent decades, with skyline plots showing a rapid increase during the last 10,000 years. Neutrality tests based on summary statistics showed no deviation from a neutral equilibrium model and there was no sign of bottleneck events. A simple demographic scenario based on the estimated time of divergence between *L. fuscus* and *L. dominicanus* did not explain the low genetic diversity found in mtDNA. In addition, the genetic diversity found in the nuclear gene agreed with the expected neutrality, especially considering that the divergence between the *L. fuscus* complex and *L. dominicanus* occurred 241,201 years ago. Based on these findings, we cannot discard a selective sweep hypothesis for mtDNA, although a recent origin followed by rapid expansion of this species after the last glacial period is the most likely scenario.

Acknowledgments

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Internet Resources

NETWORK ver. 4.1.0.8, http://www.fluxus-engineering.com/sharepub.htm#a1 (December 26, 2011).

Mantel test, http://life.bio.sunysb.edu/morph/soft-mult.html (December 26, 2011).

Supplementary Material

The following online material is available for this article:

Table S1 - List of the 105 taxa of the genus *Larus* used in the phylogenetic analysis. The genus *Rissa* was used to root the tree. The accession numbers are from GenBank.

This material is available as part of the online article from http://www.scielo.br/gmb.

Associate Editor: Fabricio Santos

Table S1 - List of the 105 taxa of the genus *Larus* used in the phylogenetic analysis. The genus *Rissa* was used to root the tree.
The accession numbers are from GenBank.

AB208758.1| *Larus canus*

AB208757.1| *Larus canus*

AB208756.1| *Larus canus*

AB208754.1| *Larus crassirostris*

AB208753.1| *Larus crassirostris*

EF513630.1| *Larus argentatus*

EF513629.1| *Larus argentatus*

EF513628.1| *Larus argentatus*

EF513627.1| *Larus argentatus*

EF513626.1| *Larus cachinnans*

EF513625.1| *Larus argentatus*

EF513624.1| *Larus cachinnan*

EF513623.1| *Larus cachinnans*

EF373138.1| *Larus marinus*

AY964952.1| *Larus hemprichii*

AY964949.1| *Larus relictus*

AY964948.1| *Larus saundersi*

AY964947.1| *Larus maculipennis*

AY964946.1| *Larus bulleri*

AY964945.1| *Larus novaehollandiae*

AY964944.1| *Larus hartlaubii*

AY964943.1| *Larus brunnicephalus*

AY964942.1| *Larus armenicus*

AY964941.1| *Larus cachinnan*

AY964940.1| *Larus glaucescens*

AY964939.1| *Larus leucophthalmus*

AY964938.1| *Larus modestus*

AY964937.1| *Larus crassirostris*

AY964936.1| *Larus atlanticus*

AY964935.1| *Larus belcheri*

AY964934.1| *Larus pacificus*

AY615706.1| *Larus thayeri*

AY615705.1| *Larus thayeri*

AY615704.1| *Larus thayeri*

AY615703.1| *Larus glaucescens*

AY615702.1| *Larus glaucescens*

AY615701.1| *Larus glaucescens*

AY615700.1| *Larus glaucescens*

AY615699.1| *Larus occidentalis*
AY615698.1| *Larus occidentalis wymani*
AY615697.1| *Larus occidentalis wymani*
AY615696.1| *Larus occidentalis occidentalis*
AY615695.1| *Larus occidentalis occidentalis*
AY615694.1| *Larus occidentalis occidentalis*
AJ508148.1| *Larus schistisagus*
AJ508147.1| *Larus occidentalis*
AJ508145.1| *Larus michahellis*
AJ508144.1| *Larus michahellis*
AJ508091.1| *Larus argentatus*
AJ508092.1| *Larus argentatus argenteus*
AJ508094.1| *Larus argentatus argenteus*
AJ508095.1| *Larus argentatus argenteus*
AJ508096.1| *Larus argentatus argenteus*
AJ508097.1| *Larus argentatus argenteus*
AJ508098.1| *Larus argentatus argenteus*
AJ508099.1| *Larus argentatus argenteus*
AJ508100.1| *Larus argentatus argenteus*
AJ508103.1| *Larus argentatus smithsonianus*
AJ508104.1| *Larus argentatus smithsonianus*
AJ508122.1| *Larus fuscus fuscus*
AJ508105.1| *Larus argentatus smithsonianus*
AJ508106.1| *Larus argentatus smithsonianus*
AJ508107.1| *Larus argentatus smithsonianus*
AJ508108.1| *Larus argentatus smithsonianus*
AJ508109.1| *Larus argentatus smithsonianus*
AJ508110.1| *Larus armenicus*
AJ508111.1| *Larus cachinnans barabensis*
AJ508112.1| *Larus cachinnans barabensis*
AJ508113.1| *Larus cachinnans barabensis*
AJ508114.1| *Larus cachinnans*
AJ508115.1| *Larus cachinnans*
AJ508116.1| *Larus cachinnans*
AJ508117.1| *Larus cachinnans*
AJ508118.1| *Larus cachinnans*
AJ508119.1| *Larus cachinnans*
AJ508120.1| *Larus cachinnans*
AJ508121.1| *Larus dominicanus*
AJ508143.1| *Larus michahellis*

AJ508142.1| *Larus michahellis*

AJ508141.1| *Larus michahellis atlantis*

AJ508140.1| *Larus marinus*

AJ508138.1| *Larus hyperboreus*

AJ508123.1| *Larus fuscus fuscus*

AJ508124.1| *Larus fuscus fuscus*

AJ508125.1| *Larus fuscus graellsii*

AJ508126.1| *Larus fuscus graellsii*

AJ508128.1| *Larus fuscus graellsii*

AJ508129.1| *Larus fuscus graellsii*

AJ508130.1| *Larus fuscus heuglini*

AJ508131.1| *Larus fuscus intermedius*

AJ508139.1| *Larus hyperboreus*

AJ508136.1| *Larus hyperboreus*

AJ508135.1| *Larus hyperboreus*

AJ508137.1| *Larus hyperboreus*

AJ508134.1| *Larus glaucoides*

AJ508133.1| *Larus glaucoide*

AJ508132.1| *Larus fuscus taimyrensis*

AJ508127.1| *Larus fuscus graellsii*

Nc006007 | *Larus dominicanus*

AY293619| *Larus dominicanus*

AJ508121| *Larus dominicanus*

AF268497| *Larus dominicanus*

AF 444259| *Larus dominicanus*

DQ385229| *Rissa tridactyla*

AF268523| *Rissa brevirostris*