# Absence of genetic structure among ecologically diverse populations indicate high plasticity in a pantropical seabird

**Conservation Genetics**

**Mariana Scain Mazzochi[1,2*], Vitória Muraro[1,2], Nelson Jurandi Rosa Fagundes[3,4], Leandro Bugoni[1,2]**

[1]Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Rio Grande, Rio Grande do Sul, Brasil
[2]Labortório de Aves Aquáticas e Tartarugas Marinhas, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, Campus Carreiros, Rio Grande, Rio Grande do Sul, Brasil
[3]Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil
[4]Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil
[*]corresponding author: marianasmazzochi@gmail.com

## Supplementary Online Material 1

## Primers and PCR protocols for mtDNA genes

Primers for ATPase 6/8 were ATP6-intH: TGGGATTAGATGTTTTCTTG and LysL: CAGCACTAGCCTTTTAAGCT (Faria et al. 2007) and for ND2, two pairs: H5766s: GATGAGAAGGCYAGGATTTTTCG and Lmet: CCCATACCCCGAAAATGATG (Cibois et al. 2016); and H5766: GATGAGAAGGCTAGGATTTTKCG and MetL: AAGCTATCGGGCCCATACCCG, which amplify the same region. We have used those two primer sets for ND2 because they were already available in the laboratory.

PCR was conducted in the thermocycler with 20 µl reactions containing dNTPs (2 mM), buffer 10×, $MgCl_2$ (50 mM), each primer (5 µM), Platinum™ Taq DNA Polymerase (5 U/µl) and DNA (1.5 ng/µl). For ND2, reaction was performed with a touchdown program of 30 cycles (3 minutes at 95 °C, 10 cycles of 30 s in 95 °C + 40 s at 60–56.5 °C

[-0.5 °C in each cycle] + 40 s at 72 °C, 30 cycles of 30 s at 95 °C + 40 s at 55 °C + 40 s at 72 °C and 7 min at 72 °C for elongation). For ATPase 6/8, reaction was performed with a touchdown program of 30 cycles (3 minutes at 95 °C, 10 cycles of 30 s at 95 °C + 30 s at 55–50.5 °C [-0.5 °C in each cycle] + 45 s at 72 °C, 30 cycles of 30 s at 95 °C + 30 s at 50 °C + 45 s at 72 °C and 10 min at 72 °C for elongation).

## References

Cibois A, Thibault JC, Rocamora G, Pasquet E. 2016 Molecular phylogeny and systematics of blue and grey noddies (Procelsterna). *Ibis* **158**, 433–438. (https://doi.org/10.1111/ibi.12363)

Faria PJ, Baus E. Morgante JS, Bruford MW. 2007 Challenges and prospects of population genetic studies in terns (Charadriiformes, Aves). *Genet. Mol. Biol*. **30**, 681–689. (https://doi.org/10.1590/S1415-47572007000400029)

## Supplementary Online Material 2

## Figures and tables

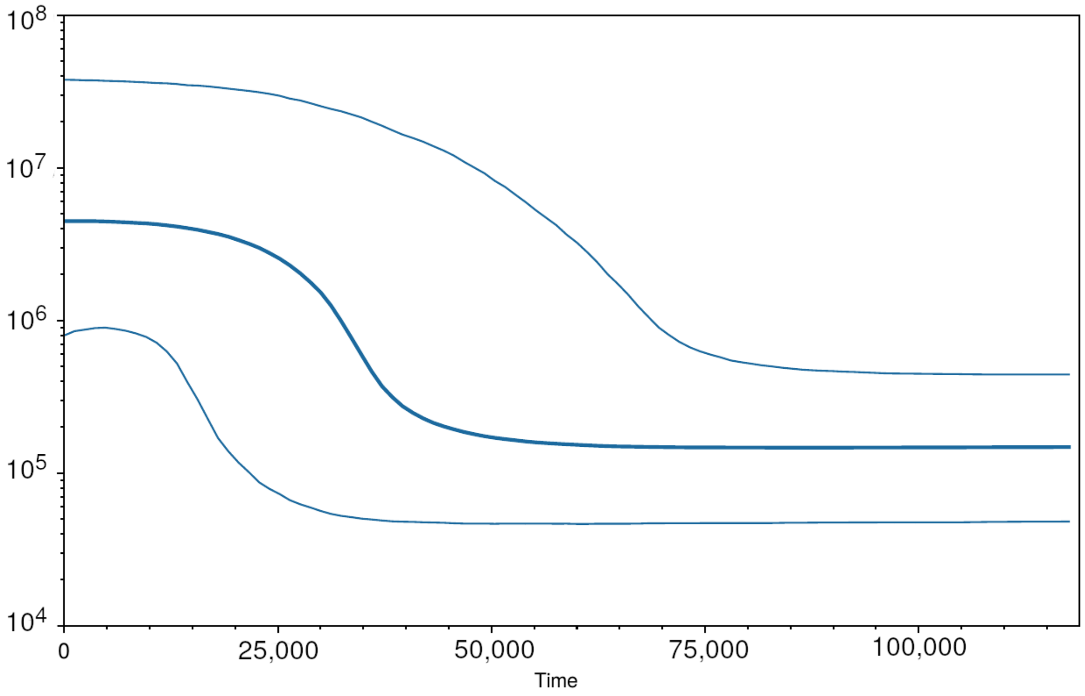

**Figure S1.** Bayesian Skyline plot obtained by two mitochondrial genes of brown noddies from Southwestern Atlantic Ocean populations. Horizontal axis represents time (x = 0: present time) and the vertical axis represents effective population size. Central bold line is the median and external lines represent confidence intervals of 95%.

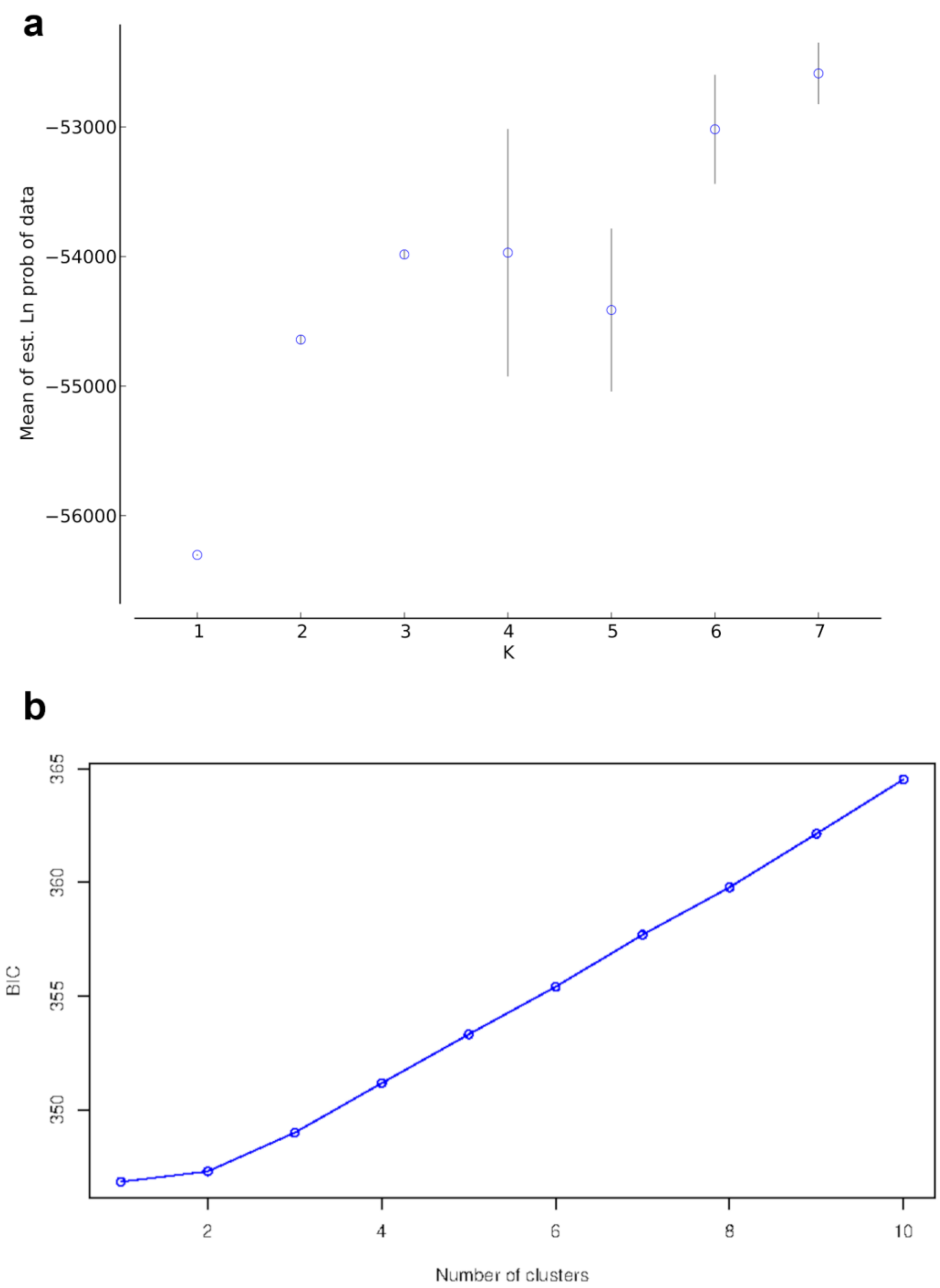

**Figure S2.** Number of genetic groups estimated by two methods from 2062 SNPs belonging to UCEs loci from six brown noddies *Anous stolidus* populations. *a* - Values of log-normal probability of genetic groups generated in STRUCTURE software. The white circles are the mean values for each *K*, and the lines are the standard deviation. *b* - Bayesian Information Criterion for each *K* calculated in DAPC package in RStudio.

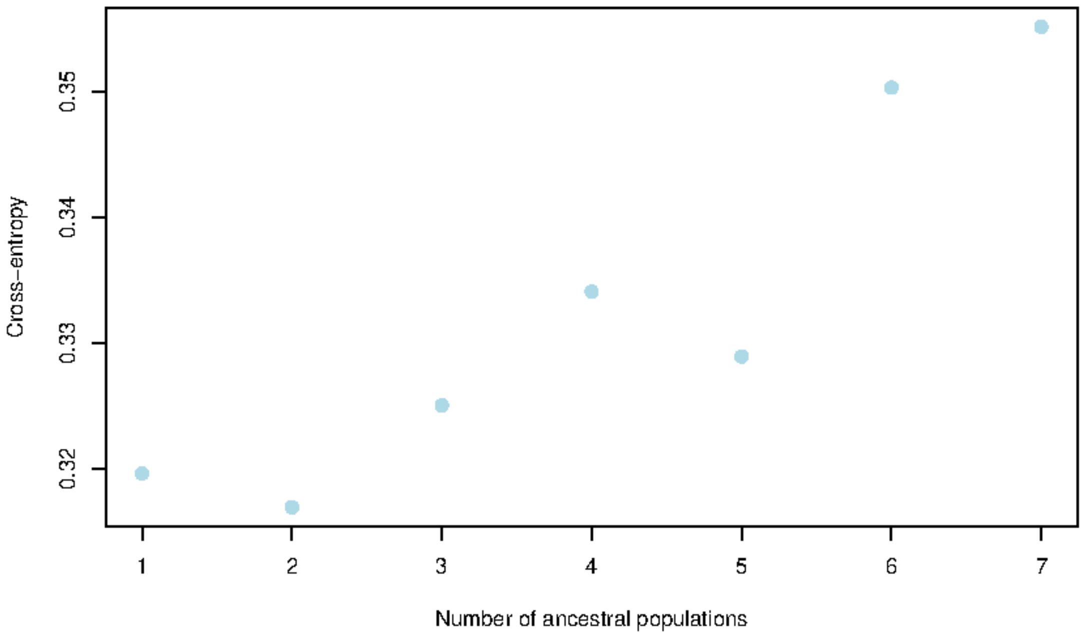

**Figure S3.** Number of genetic groups for populations of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean estimated by the cross-entropy criterion in sNMF package (2062 SNPs belonging to UCEs loci).

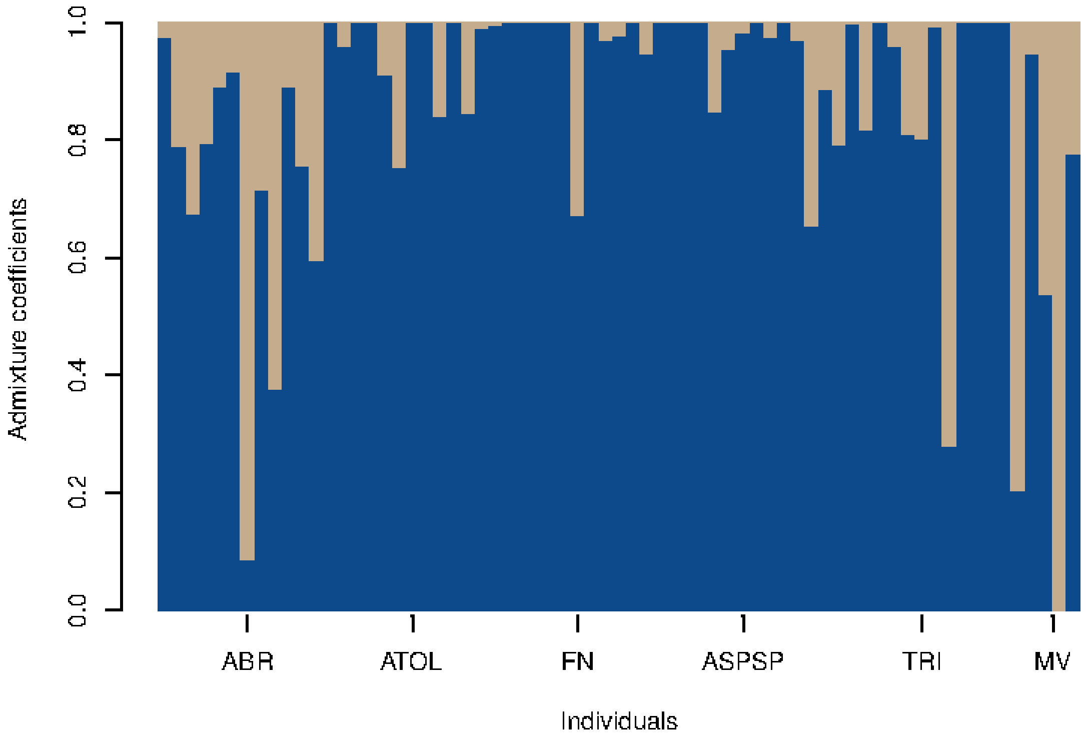

**Figure S4.** sNMF plot for $K = 2$ generated through 2062 SNPs belonging to UCEs loci of six populations of brown noddies *Anous stolidus* in Southwestern Atlantic Ocean. ABR - Arquipélago de Abrolhos; ATOL – Rocas Atoll; FN - Fernando de Noronha Archipelago; SPSPA - São Pedro e São Paulo Archipelago; TRI - Trindade; MV - Martin Vaz.

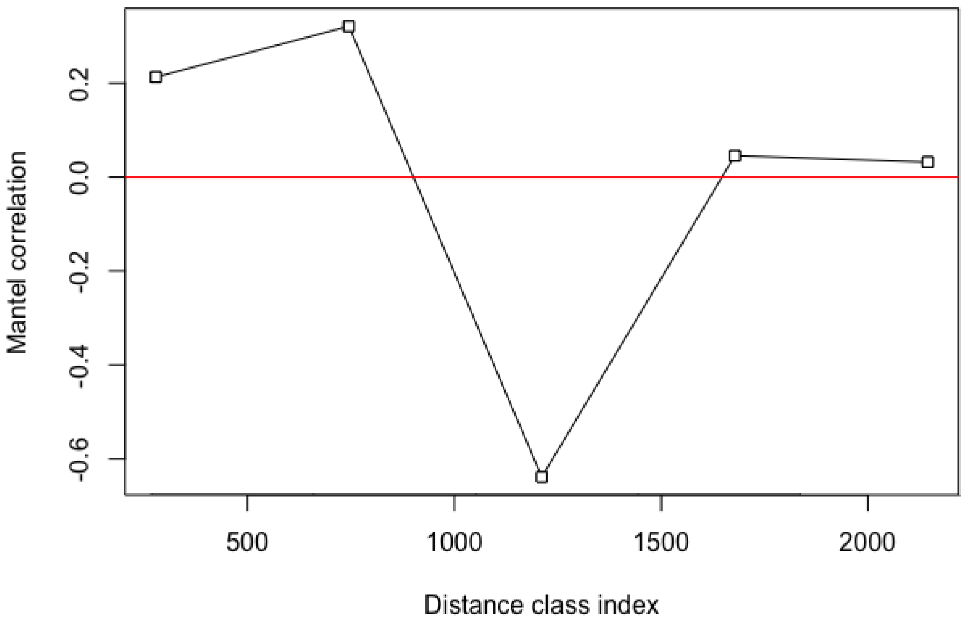

**Figure S5.** Mantel's correlogram of geographic and genetic distances (mitochondrial genes) between populations of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean. X-axis presents the indexes (medians) of the four geographical distance (km) classes, and Y-axis presents Pearson's correlation for each class. White boxes indicate no statistically significant correlations.

a

| | GenDist | SST | Chlα | Sal | C | N | DistCoast | DistGeo |
|---|---|---|---|---|---|---|---|---|
| GenDist | 1 | -0.04 | 0.06 | -0.15 | 0.23 | 0.41 | 0.54* | 0.05 |
| SST | | 1 | 0.15 | 0.93* | -0.19 | 0.42 | 0.15 | 0.9* |
| Chlα | | | 1 | 0.14 | -0.14 | 0.44 | 0.14 | 0.31 |
| Sal | | | | 1 | -0.2 | 0.21 | -0.02 | 0.96* |
| C | | | | | 1 | -0.06 | -0.07 | -0.2 |
| N | | | | | | 1 | 0.11 | 0.04 |
| DistCoast | | | | | | | 1 | 0.11 |
| DistGeo | | | | | | | | 1 |

b

| | SST | Sal | Chlα | C | N | DistCoast |
|---|---|---|---|---|---|---|
| SST | 1 | -0.97* | 0.48 | 0.32 | 0.58 | -0.51 |
| Sal | | 1 | -0.49 | -0.33 | -0.46 | 0.29 |
| Chlα | | | 1 | -0.43 | 0.8 | -0.48 |
| C | | | | 1 | -0.18 | 0.18 |
| N | | | | | 1 | -0.57 |
| DistCoast | | | | | | 1 |

**Figure S6.** Mantel's tests between genetic (mitochondrial genes), geographic and environmental distances between populations of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean. *a* - Pearson's correlations between distance matrices calculated through Mantel's tests. *b* - Pearson's correlations between centralized values of environmental variables. GenDist - pairwise genetic distance calculated through two mitochondrial genes ($F_{ST}$); SST - sea surface temperature; Sal - sea surface salinity; C - $\delta^{13}$C; N - $\delta^{15}$N; DistCoast - minimal distance from each island or archipelago to the South American continent; DistGeo - geographical distance between each pair of islands or archipelagos; * - statistically significant values.

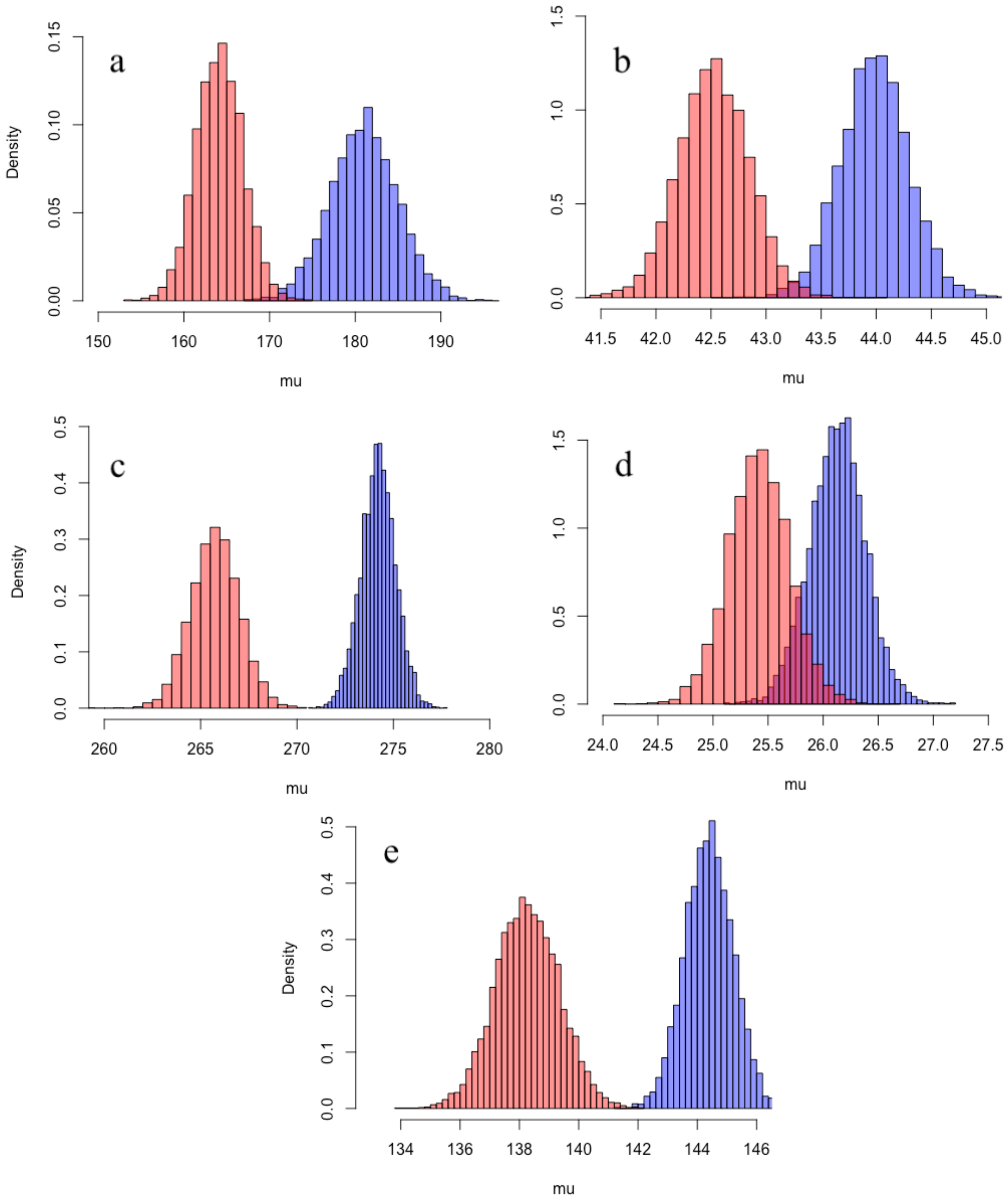

**Figure S7.** *A posteriori* distributions of mean biometric measures of males and females ($\mu$) of brown noddies *Anous stolidus* metapopulation in the Southwestern Atlantic Ocean, simulated by Monte Carlo via Markov Chains. Pink histograms represent females and blue, the males. The three first and the last compared distributions provide decisive evidence and last but one provides strong evidence (Bayes Factor) that males are larger than females. *a* - mass (g); *b* - culmen length (mm); *c* - wing length (mm); *d* - tarsus length (mm); *e* - tail length (mm).

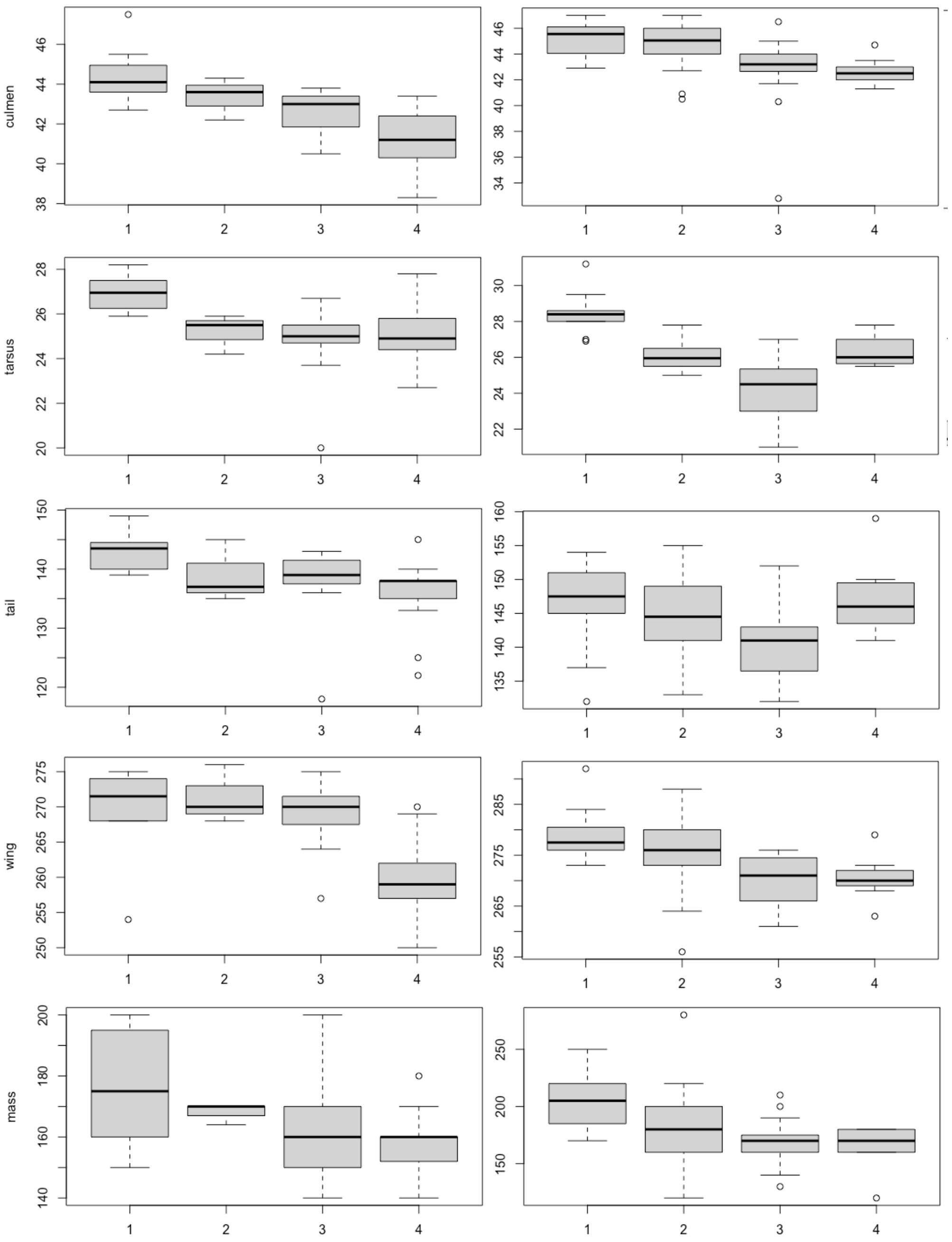

**Figure S8.** Boxplots for five biometrical measures of females (left) and males (right) of brown noddies *Anous stolidus* in four populations in the Southwestern Atlantic Ocean: São Pedro e São Paulo Archipelago (1), Fernando de Noronha Archipelago (2), Rocas Atoll (3) and Abrolhos Archipelago (4). From top to bottom: culmen length, tarsus length, wing length, tail length (mm) and mass (g). Dark lines represent median; bottom and top of boxes represent first and third quartiles, respectively; inferior and superior limits represent minimum and maximum values; and circles are the outliers.

**Table S1.** Genetic diversity values and indexes of each population of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean estimated from ND2 gene. Diversity values and Watterson's Theta (S) are presented in mean ± standard deviation. Neutrality indexes Tajima's *D* and Fu's *Fs* are the test results and their respective *p*-values between brackets. SPSPA - São Pedro e São Paulo Archipelago; FN - Fernando de Noronha Archipelago.

| Population | Haplotype number | Haplotypic diversity | Nucleotide diversity | Watterson's Theta (Theta S) | Tajima's D | Fu's Fs |
|---|---|---|---|---|---|---|
| Metapopulation (n = 109) | 11 | 0.63 ± 0.03 | 0.001 ± 0.001 | 2.08 ± 0.78 | -1.47 (0.049) | -5.65 (0.008) |
| FN (n = 24) | 5 | 0.49 ± 0.11 | 0.001 ± 0.001 | 1.07 ± 0.61 | -1.02 (0.17) | -1.93 (0.051) |
| Abrolhos (n = 21) | 2 | 0.38 ± 0.10 | 0.0007 ± 0.0008 | 0.27 ± 0.27 | 0.65 (0.82) | 0.94 (0.55) |
| Rocas (n = 24) | 4 | 0.59 ± 0.05 | 0.001 ± 0.001 | 0.80 ± 0.50 | -0.40 (0.39) | -0.72 (0.22) |
| SPSPA (n = 19) | 5 | 0.52 ± 0.12 | 0.001 ± 0.001 | 1.14 ± 0.66 | -1.40 (0.08) | -2.55 (0.01) |
| Trindade (n = 16) | 5 | 0.82 ± 0.05 | 0.003 ± 0.002 | 1.50 ± 0.82 | 0.55 (0.72) | -0.01 (0.48) |
| Martin Vaz (n = 5) | 3 | 0.70 ± 0.21 | 0.002 ± 0.002 | 1.44 ± 1.01 | -1.04 (0.08) | -0.18 (0.25) |

**Table S2.** Diversity values and indexes of each population of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean estimated from ATPase 6/8 gene. Diversity values and Theta are presented in mean ± standard deviation. Neutrality indexes D and Fs are the test results and their respective p-values between brackets. SPSPA - São Pedro e São Paulo Archipelago; FN - Fernando de Noronha Archipelago; Rocas - Rocas Atoll.

| Population | Haplotype number | Haplotypic diversity | Nucleotide diversity | Watterson's Theta (Theta S) | Tajima's D | Fu's Fs |
|---|---|---|---|---|---|---|
| Metapopulation (n = 65) | 9 | 0.55 ± 0.05 | 0.001 ± 0.001 | 1.68 ± 0.72 | -0.83 (0.24) | -3.01 (0.06) |
| FN (n = 13) | 4 | 0.67 ± 0.08 | 0.001 ± 0.001 | 1.28 ± 0.76 | 0.25 (0.60) | 0.19 (0.55) |
| Abrolhos (n = 15) | 1 | 0 | 0 | 0 | - | - |
| Rocas (n = 13) | 2 | 0.15 ± 0.12 | 0.0003 ± 0.0004 | 0.64 ± 0.48 | -1.46 (0.06) | 0.36 (0.34) |
| SPSPA (n = 9) | 4 | 0.75 ± 0.11 | 0.001 ± 0.001 | 1.10 ± 0.73 | 0.79 (0.77) | -0.44 (0.25) |
| Trindade (n = 10) | 4 | 0.77 ± 0.09 | 0.002 ± 0.001 | 1.41 ± 0.86 | 0.68 (0.76) | 0.19 (0.49) |
| Martin Vaz (n = 5) | 4 | 0.90 ± 0.16 | 0.002 ± 0.001 | 1.92 ± 1.26 | -0.41 (0.38) | -1.19 (0.09) |

**Table S3.** Diversity values and indexes for each population of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean estimated from ATPase 6/8 and ND2 genes. Diversity values are presented by mean ± standard deviation, and D and Fs indexes are the test values and their respective p-values between brackets. SPSPA - São Pedro e São Paulo Archipelago; FN - Fernando de Noronha Archipelago; Rocas - Rocas Atoll.

| Population | Haplotype number | Haplotype diversity | Nucleotide diversity | Watterson's Theta(Theta S) | Tajima's D | Fu's Fs |
|---|---|---|---|---|---|---|
| Metapopulation (n = 59) | 16 | 0.80 ± 0.03 | 0.0016 ± 0.001 | 4.08 ± 1.41 | -1.44 (0.04) | -6.9 (0.005) |
| FN (n = 13) | 7 | 0.84 ± 0.08 | 0.0016 ± 0.0011 | 2.25 ± 1.15 | -0.30 (0.38) | -2.17 (0.07) |
| Abrolhos (n = 12) | 2 | 0.40 ± 0.13 | 0.0003 ± 0.0003 | 0.33 ± 0.33 | 0.54 (0.80) | 0.73 (0.49) |
| Rocas (n = 13) | 4 | 0.65 ± 0.10 | 0.0008 ± 0.0006 | 1.61 ± 0.89 | -1.18 (0.10) | -0.35 (0.34) |
| SPSPA (n = 7) | 5 | 0.90 ± 0.10 | 0.001 ± 0.001 | 2.04 ± 1.22 | 0.36 (0.63) | -1.35 (0.11) |
| Trindade (n = 9) | 5 | 0.86 ± 0.08 | 0.002 ± 0.001 | 3.31 ± 1.69 | 0.33 (0.65) | 0.42 (0.57) |
| Martin Vaz (n = 5) | 4 | 0.90 ± 0.16 | 0.002 ± 0.001 | 3.84 ± 2.24 | -0.80 (0.33) | -0.12 (0.38) |

**Table S4.** Genetic indexes estimated from 2062 SNPs belonging to UCEs loci of six populations of brown noddies *Anous stolidus* in Southwestern Atlantic Ocean. $N_{e\ (es)}$ - effective population size estimated with NeEstimator; $N_{e\ (mi)}$ - mean values of modes of effective population size (4 subreplicates) estimated with Migrate; θ - mean theta estimated with Migrate (4 subreplicates); $F_{IS}$ - inbreeding coefficient; Ho = observed heterozygosity; He - expected heterozygosity; Bartlett's K-squared and respective *p*-values for homogeneity of variances between the values of expected and observed heterozygosity. Bold values are results with $p < 0.05$. SPSPA - São Pedro e São Paulo Archipelago; FN - Fernando de Noronha Archipelago; Rocas - Rocas Atoll; MV - Martin Vaz.

| Statistics | Metapop | Abrolhos | SPSPA | FN | Rocas | Trindade | MV |
|---|---|---|---|---|---|---|---|
| $N_{e\ (es)}$ | 80.3 | 42.1 | 72.7 | 30.7 | 69.6 | 41.5 | inf |
| $N_{e\ (mi)}$ | - | 390 | 390 | 390 | 390 | 1604 | 390 |
| θ | - | $3x10^{-5}$ | $3x10^{-5}$ | $3x10^{-5}$ | $3x10^{-5}$ | $1.2x10^{-4}$ | $3x10^{-5}$ |
| $F_{IS}$ | 0.41 | 0.44 | 0.42 | 0.39 | 0.43 | 0.43 | 0.39 |
| Ho | 0.063 | 0.067 | 0.057 | 0.062 | 0.051 | 0.062 | 0.096 |
| He | 0.112 | 0.115 | 0.094 | 0.097 | 0.086 | 0.105 | 0.138 |
| Bartlett's K-squared | **83.72 ($0.2x10^{-15}$)** | **38.42 ($0.5x10^{-9}$)** | **24.85 ($0.6x10^{-6}$)** | **28.44 ($0.9x10^{-7}$)** | **60.2 ($0.8x10^{-15}$)** | **48.45 ($0.3x10^{-11}$)** | 0.02 (0.88) |

**Table S5.** Median of migration rates between populations of the brown noddy *Anous stolidus* in Southwestern Atlantic Ocean estimated with BayesAss-SNPs. Values between brackets are 95% highest density intervals. Columns are destination populations and rows are origin populations of migrants. SPSPA - São Pedro e São Paulo Archipelago; FN - Fernando de Noronha Archipelago; Rocas - Rocas Atoll.

| | Abrolhos | Rocas | FN | SPSPA | Trindade | MV |
|---|---|---|---|---|---|---|
| Abrolhos | 0.679 | 0.013 | 0.013 | 0.013 | 0.012 | 0.021 |
| | (0.666–0.719) | (9.845x10$^{-7}$–0.055) | (9.250x10$^{-8}$–0.054) | (1.164x10$^{-7}$0.054) | (9.888x10$^{-7}$–0.049) | (3.953x10$^{-8}$–0.085) |
| Rocas | 0.243 | 0.910 | 0.243 | 0.243 | 0.252 | 0.182 |
| | (0.175–0.305) | (0.840–0.969) | (0.171–0.303) | (0.171–0.303) | (0.190–0.309) | (0.086–0.270) |
| FN | 0.013 | 0.013 | 0.679 | 0.013 | 0.011 | 0.022 |
| | (1.176x10$^{-7}$–0.054) | (1.015x10$^{-6}$–0.054) | (0.666–0.720) | (1.455x10$^{-6}$0.054) | (5.834x10$^{-7}$–0.049) | (4.657x10$^{-7}$–0.086) |
| SPSPA | 0.013 | 0.013 | 0.013 | 0.68 | 0.011 | 0.022 |
| | (2.923x10$^{-6}$–0.054) | (5.565x10$^{-7}$–0.053) | (1.866x10$^{-6}$–0.053) | (0.666–0.720) | (4.255x10$^{-6}$–0.048) | (8.222x10$^{-6}$–0.085) |
| Trindade | 0.013 | 0.013 | 0.013 | 0.013 | 0.678 | 0.022 |
| | (1.105x10$^{-7}$–0.053) | (2.020x10$^{-6}$–0.052) | (5.185x10$^{-6}$–0.054) | (2.002x10$^{-8}$0.053) | (0.666–0.716) | (9.963x10$^{-7}$–0.087) |
| MV | 0.013 | 0.013 | 0.013 | 0.013 | 0.011 | 0.688 |
| | (1.176x10$^{-7}$–0.054) | (2.434x10$^{-6}$–0.054) | (1.205x10$^{-6}$–0.055) | (6.056x10$^{-7}$–0.054) | (1.172x10$^{-6}$–0.048) | (0.666–0.752) |

**Table S6.** Distance classes from Mantel's correlogram (mitochondrial genes) and brown noddies populations included in each one of them. Distance values presented between brackets at the second column are approximated. Third column contains the corrected p-values between brackets.

| Distance classes (km) | Population pairs | Mantel's correlation |
|---|---|---|
| 49-515.6 | Trindade Island and Martin Vaz (49 km), Fernando de Noronha and Rocas Atoll (152 km) | 0.21 (0.19) |
| 515.7-982.2 | Fernando de Noronha and São Pedro e São Paulo Archipelago (631 km), Rocas Atoll and São Pedro e São Paulo Archipelago (726 km) | 0.32 (0.31) |
| 982.3-1448.8 | Abrolhos Archipelago and Trindade Island (1023 km), Abrolhos Archipelago and Martin Vaz (1070 km) | -0.63 (0.1) |
| 1448.9-1915.4 | Abrolhos Archipelago and Rocas Atoll (1656 km), Fernando de Noronha and Abrolhos Archipelago (1714 km), Fernando de Noronha and Trindade Island (1882 km), Fernando de Noronha and Martin Vaz (1888 km), Rocas Atoll and Trindade Island (1913 km), Rocas Atoll and Martin Vaz (1923 km) | 0.04 (0.46) |
| 1915.5-2382 | Abrolhos Archipelago and São Pedro e São Paulo Archipelago (2336 km), São Pedro e São Paulo Archipelago and Trindade Island (2382 km), São Pedro e São Paulo Archipelago and Martin Vaz (2379 km) | 0.03 (0.62) |

**Table S7.** Molecular variance analysis for six populations of the brown noddy *Anous stolidus* in the Southwestern Atlantic Ocean, grouped in four different ways, considering their different breeding phenologies. Populations between brackets connected by a plus sign (+) are grouped. Asterisks (*) beside $F_{ST}$ values indicate statistically significant results. SPSPA - São Pedro e São Paulo Archipelago; FN - Fernando de Noronha Archipelago; Rocas - Rocas Atoll.

| Grouping | Percentage of variation | | $F_{st}$ |
|---|---|---|---|
| | Between populations | In populations | |
| (FN + Rocas Atoll), Martin Vaz, Trindade, SPSPA, Abrolhos | 15.50 | 84.50 | 0.15* |
| (FN + Rocas Atoll), Martin Vaz, Trindade, (SPSPA + Abrolhos) | 12.43 | 87.57 | 0.12* |
| (FN + Rocas Atoll), (Martin Vaz + Trindade), SPSPA, Abrolhos | 16.26 | 83.74 | 0.16* |
| (FN + Rocas Atoll), (Martin Vaz + Trindade), (SPSPA + Abrolhos) | 13.22 | 86.78 | 0.13* |

**Table S8.** Mean and standard deviation of biometrical measures of brown noddies *Anous stolidus* in the Southwestern Atlantic Ocean, *a posteriori* distributions simulated by Monte Carlo via Markov chains and their 95% highest density intervals (HDI).

| Biometrical measure | Mean and standard deviation | | *A posteriori* distribution and HDI | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Mass (g) | 181.76 ± 28.97 | 164.17 ± 15.69 | 181.76 ± 3.81<br>(174.31–189.35) | 164.19 ± 2.68<br>(158.66–169.37) |
| Culmen length (mm) | 43.94 ± 2.27 | 42.52 ± 1.87 | 43.93 ± 0.29<br>(43.37–44.52) | 42.52 ± 0.32<br>(41.9–43.19) |
| Wing length (mm) | 274.53 ± 6.57 | 265.8 ± 7.18 | 274.54 ± 0.87<br>(272.85–276.31) | 265.78 ± 1.26<br>(263.42–268.27) |
| Tarsus length (mm) | 26.15 ± 1.8 | 25.42 ± 1.59 | 26.16 ± 0.23<br>(25.67–26.6) | 25.42 ± 0.27<br>(24.86–25.96) |
| Tail length (mm) | 144.39 ± 5.97 | 138.25 ± 6.26 | 144.38 ± 0.82<br>(142.81–146.01) | 138.26 ± 1.1<br>(136.08–140.38) |