Genetic differentiation and historical demography of wood stork populations in Brazilian wetlands: Implications for the conservation of the species and associated ecosystems.

Running title: Conservation of wood storks in Brazilian wetlands

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ABSTRACT

1. Wetlands are increasingly threatened by anthropogenic actions worldwide. Genetic monitoring of associated wildlife provides valuable data to support their conservation. Waterbirds such as the wood stork (*Mycteria americana*) are good bioindicators of wetlands disturbances and destruction.
2. This study investigates past and contemporary levels of genetic diversity, differentiation and demographic processes in 236 wood storks from two major wetlands in Brazil in which breeding colonies are concentrated, using nine microsatellite loci and a 237-bp untranslated fragment of the mitochondrial Control Region.
3. Amapá populations (northern region) showed slightly higher levels of genetic diversity than Pantanal populations (central western region) and both populations had a low number of effective breeders.
4. Results from assignment tests, *F*-statistics, AMOVA, spatial and non-spatial Bayesian clustering analyses support the hypothesis of ongoing gene flow among colonies within regions, but significant differentiation between regions.
5. The better supported Bayesian coalescent models based on both markers indicated that the northern population exchanged migrants with unsampled populations, and that the central western population was founded by individuals from the north. Mitochondrial estimates revealed that the timing of population divergence broadly overlapped the Last Glacial Maximum (LGM), and that the central western population expanded more recently than the northern population.
6. The results support the hypothesis that the coastal wetlands in northern Brazil remained stable enough to shelter large wood stork populations during the LGM and storks colonized freshwater wetlands in the central western region following deglacial warming.
7. Conservation policies and protective measures should consider Amapá and Pantanal wood stork populations as genetically differentiated units and priority should be given to Amapá populations which represent the source gene pool. Continuous genetic monitoring of wood storks could help detect genetic signs of changes in demographic trends that could reflect alterations or degradation in wetlands.

Keywords: birds, genetics, indicator species, wetlands.

INTRODUCTION

Wetlands constitute a complex interface between aquatic and terrestrial environments (Junk *et al*., 2014; Kingsford, Basset, & Jackson, 2016), and can be either riverine, palustrine, lacustrine, estuarine, groundwater dependent or constructed wetlands (Kingsford *et al*., 2016). These ecosystems currently cover only 1.1% of the Earth's surface (Kingsford *et al*., 2016), and approximately 20% of the surface of South America (Junk, 2013). Recent estimates report that wetlands provide more than 25% of highly valuable and essential ecosystem services (Kingsford *et al*., 2016). Wetlands also provide a habitat for many species of birds, mammals and other vertebrates, which depend on these environments for breeding, nesting, roosting and foraging (Brandolin, Ávalos, & de Angelo, 2013). Despite their value, wetlands are heavily impacted by anthropogenic activities and climate change, and are increasingly modified, reduced, fragmented or destroyed (Brandolin *et al*., 2013; Junk, 2013). Hence, wetlands are declining throughout the world and the rates of loss of these environments in South America are the highest of any continent (Kingsford *et al*., 2016). Past climatic fluctuations in wetlands may as well have exerted an impact on the demographic dynamics of the wildlife dependent on these ecosystems, causing extinction and recolonization events (Gray *et al.*, 2013). In face of current threats to wetlands and associated species, population genetic studies can provide valuable data to assist conservationists and contribute to habitat protection and management policies, thereby supporting ecosystem conservation (Faulks, Kerezsy, Unmack, Johnson, & Hughes, 2016; Willoughby *et al*., 2015).

Waterbirds such as storks, herons, egrets, ibises and spoonbills, are conspicuous inhabitants of wetlands. These birds are very sensitive to environmental changes and alterations in the hydrological cycle of wetlands and are good bioindicators of the status of these ecosystems (Amat & Green, 2010; Del Lama, Dosualdo Rocha, Figueiredo Jardim, Tsai, & Frederick, 2011; Frederick, Gawlik, Ogden, Cook, & Lusk, 2009; Kushlan, 1993; Mistry, Berardi, & Simpson, 2008). Waterbirds are therefore regarded as good models to explore the effects of contemporary environmental changes on population demography (Gray, Hagy, Nyman, & Stafford, 2013). In the wild, waterbirds can be relatively easily accessed at their breeding sites for the purposes of population genetic monitoring. Natural

78 populations of waterbirds can be either panmictic or exhibit genetic structure, which occurs
79 when a population is composed of subpopulations that differ in genetic composition
80 (Chakraborty, 1993). Assessing levels of genetic diversity and the degree of gene exchange
81 (or isolation) between subpopulations can offer clues regarding historical and contemporary
82 events as well as aspects of the ecology and behavior of waterbirds (Friesen, Burg, &
83 McCoy, 2007; Geraci *et al.*, 2012; Reudink *et al*., 2011). Thus, gathering genetic
84 information on waterbirds that breed in threatened wetlands can help identify past and
85 current demographic trends, predict the potential fate of populations, and contribute to
86 defining the conservation status of species.

87 The present study focuses on the wood stork *Mycteria americana* Linnaeus, 1758, a
88 long-legged wading bird that has been used as a model to help monitor and protect some of
89 the ecosystems in which it inhabits (Frederick *et al*., 2009). Native to the Americas, this
90 species breeds in spatially restricted colonies associated with freshwater and estuarine
91 wetlands ranging from the southeastern United States to northern Argentina (BirdLife
92 International, 2012). Wood storks can rapidly abandon traditional breeding sites if changes
93 in water levels preclude efficient foraging to fulfill their energy requirements in the
94 reproductive season (Bryan, Meyer, Tomlinson, Lauritsen, & Brooks, 2012; Frederick &
95 Meyer, 2008). Even minor shifts in the hydrological regime constitute a threat to the fate of
96 populations by imposing serious negative effects on nesting and foraging ecology (Griffin,
97 Morris, Rodgers, & Snyder, 2008) and hindering the ability of parents to raise their young
98 (Bryan *et al*., 2012). Although currently listed as 'least concern', decreasing population
99 trends worldwide highlight the need to reconsider the conservation status of the wood stork
100 in much of its distribution range (BirdLife International, 2012). Populations breeding in the
101 US have recently been re-categorized as 'threatened', mostly due to continual habitat loss,
102 modification and fragmentation (North Florida Ecological Services Field Office, 2014). In
103 Brazil, most wood storks breeding colonies are concentrated in the Pantanal wetland
104 (Antas, 1994; Cardoso, 2011) and the northern coastal region (Miño & Del Lama, 2014).
105 Some isolated colonies are located outside those areas in the states of Minas Gerais and
106 Bahia, but are opportunistically occupied and abandoned after a few breeding cycles (S. N.
107 Del Lama, pers. comm., April 2016). The major wetlands in which wood storks reproduce

in Brazil are differentially affected by anthropogenic impacts: the Brazilian Pantanal in the central western region of the country is highly menaced by land conversion for pastures, agriculture, and dam building, and undergoes heavy pesticide use, as well as other threats (De Pinho & Marini, 2012; Junk *et al*., 2006); the coastal wetlands of Amapá state in the northern region still represent a more or less pristine ecosystem, with more than half legally protected in the form of conservation units (Ministério Público do Estado do Amapá, 2011).

In an effort to provide data to support the conservation and management of the species and its associated ecosystems in Brazil, the aim of the present study was to investigate the population genetics of wood storks that breed in colonies in the Amapá coastal wetlands and the Pantanal freshwater wetland. Both nuclear and mitochondrial markers were comparatively analyzed to explore hypothesis regarding genetic differentiation and past demographic history of populations established in these important wetlands subjected to different forms of anthropogenic pressure. The main objectives were to: 1) evaluate levels of genetic differentiation in order to define conservation units; and 2) assess past demographic processes in order to identify the source population. The first hypothesis assumes that there is some degree of regional breeding-site phylopatry in this species (Coulter, Rodgers, Ogden, & Depkin, 1999; Frederick & Meyer, 2008; Frederick & Ogden, 1997); thus, the expectation is that populations from different regions will exhibit higher levels of differentiation than populations within regions. Assuming that the Equatorial region of Brazil remained humid enough during the Last Glacial Maximum (LGM: circa 20,000 *yr* BP) to harbor wetlands (Aleixo, 2004), the second hypothesis states that wood stork populations occupied these restricted areas at the time and then expanded their range during interglacial periods; thus, the expectation is that northern populations in the Amapá coastal wetlands (Amazon region) represent source genetic pools. If Pantanal populations derive from Amapá populations, Pantanal colonies are expected to exhibit lower levels of genetic diversity.

METHODS

Study sites and sampling

Wood stork were sampled from three breeding colonies situated in the coastal wetlands of the state of Amapá (northern Brazil) and eight colonies situated in the Pantanal floodplain in the states of Mato Grosso and Mato Grosso do Sul (central western Brazil) (Fig. 1, Table S1 in Supporting Information). Larger colonies are more stable over time (Tsai, Reichert, Frederick, & Meyer, 2016) and were chosen for sampling; the number of breeding pairs in these colonies ranged between 200 and 2000. The Amapá wetlands are environmentally heterogeneous due to the interaction between regular tide pulses and freshwater inflow (Junk *et al*., 2006). This region has a super-humid tropical climate with mean annual temperature of ~26.6 °C and a yearly rainfall regime defining a short dry season (three months) (Sant'anna Neto, Galvani, & Vieira, 2015; Torres & El-Robrini, 2006). Wood stork colonies in Amapá are established during the peak of the dry period, on temporal tidal flood plains, and nests are made in shrubs and trees. The Pantanal is the world's largest tropical wetland, with 90% of its area situated in the central western region of Brazil (138,000 km^2). This alluvial plain has a warm savannah climate, with mean annual temperature of ~25 °C and a seasonal hydrological cycle, with a wet season extending from December to May, followed by a six-month dry season (Junk *et al*., 2014). In the Pantanal, wood storks breed annually during the dry period, nesting on trees and shrubs in riparian forest and open wood savanna habitats. In both Brazilian regions, nests are irregularly distributed in patches of vegetation, reflecting the disposition of shrubs and trees suitable for nesting. Thus, to obtain a representative sample, nestlings were sampled from nests throughout the colony, covering most of its surface. Nests were accessed with climbing equipment or ladders. Blood (0.10 ml) was taken from the brachial vein of nestlings aged two to three weeks (n = 236; one per nest) using sterile disposable syringes with 3% EDTA as anticoagulant, stored in sterile microtubes containing absolute ethanol and kept at -20 °C until processing.

DNA extraction, genotyping and sequencing

Genomic DNA was isolated using proteinase K digestion, followed by a standard phenol-chloroform procedure (Sambrook & Russell, 2001). Samples were amplified by polymerase chain reaction (PCR) at nine species-specific microsatellites (Table S2), following

Tomasulo-Seccomandi *et al.* (2003) and using fluorescent primers (IDT, Iowa, USA). PCRs were conducted in a Veriti® thermal cycler (Applied Biosystems®, Foster City, CA, USA) and genotypic data were collected using a MegaBACE®1000 automatic sequencer, with ET 550-R as the internal size standard (GE Healthcare, Piscataway, NY, USA). Alleles were scored using the MegaBACE Fragment Profiler® v1.2 (GE Healthcare, Piscataway, NY, USA). A subset of samples was amplified at a fragment of the mitochondrial Control Region (mtDNA CR) following Lopes *et al.* (2011). Sequencing was performed in ABI Prism 3700/3730 machines using the Big-Dye Terminator Cycle kit (Perkin Elmer, Walthan, MA, USA). Sequences were aligned and visually verified using CodonCode Aligner v2.0.5 (CodonCode Corporation) and BIOEDIT 7.0.9 (Hall, 1999). Coding fragments were trimmed out from all sequences, and further analyses were carried out with 237-bp fragments of untranslated mtDNA CR, in contrast to a previous study (Lopes *et al.* 2011) that also included translated fragments.

Genetic diversity, number of effective breeders and recent demographic changes

Genotypes were checked for null alleles, stuttering and scoring errors using MICRO-CHECKER v2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004), adjusting allele frequencies with the Brookfield 1 method (Brookfield, 1996) to account for null alleles. The number of alleles per locus, number of private alleles, observed heterozygosity (H_O), unbiased expected heterozygosity (UH_E) and inbreeding coefficients were computed in GENALEX v6.5 (Peakall & Smouse, 2012). Allelic richness was estimated in FSTAT v2.9.3.2 (Goudet, 1995). Linkage between loci and deviations from Hardy-Weinberg equilibrium (*HWE*) were assessed in GENEPOP v4.2 (Rousset, 2008) with 1,000 dememorizations, 1,000 batches and 10,000 iterations. To account for multiple comparisons, significance levels were corrected with the Bonferroni procedure (Rice, 1989). Recent reductions in population size were investigated using two-tailed Wilcoxon tests in BOTTLENECK v1.2.02 (Piry, Luikart, & Cornuet, 1999), assuming a two-phase mutation model (70% single-step mutations and 30% multistep mutations) with 10,000 iterations. The effective number of breeders (N_b) was estimated using the sibship

196 assignment method implemented in COLONY v2.0.6.1 (Jones & Wang, 2010; Wang, 2009,
197 2013), assuming a sibship *prior* of 2 for each parent and jackknifing among loci.

198 The number of polymorphic sites (*PS*), average number of pairwise nucleotide
199 differences (*k*), haplotype diversity (*h*) and nucleotide diversity (π) (Nei, 1987) were
200 computed using DNASP v5.10.01 (Librado & Rozas, 2009). Relationships among
201 haplotypes were assessed using a TCS network model (Clement, Posada, & Crandall, 2000)
202 visualized in POPART (Leigh & Bryant, 2015). Standard tests of selective neutrality, R_2
203 (Ramos-Onsins & Rozas, 2002), *D* (Tajima, 1989) and F_S (Fu, 1997), and associated 95%
204 confidence intervals, were computed in DNASP with 1,000 simulations and a neutral
205 infinite-sites model assuming a large constant population size (Hudson, 1990). Selective
206 neutrality was rejected when significant small R_2 ($P < 0.05$) and significant negative
207 F_S ($P < 0.02$) values were obtained.

208

209 **Genetic structure**

210 The distribution of nuclear and mitochondrial variation was inspected on different
211 hierarchical levels (within colonies, among colonies and between regions) using analysis of
212 molecular variance (AMOVA) (Excoffier, Smouse, & Quattro, 1992) in GENALEX V6.5
213 and estimating pairwise Φ_{ST} between colonies and regions in ARLEQUIN v3.5 (Excoffier &
214 Lischer, 2010). Genetic differentiation at nuclear markers was investigated by conducting
215 frequency-based assignment tests (Paetkau, Slade, Burden, & Estoup, 2004) in GENALEX
216 v6.5 and at mtDNA by computing θ (Weir & Cockerham, 1984) in FSTAT v2.9.3.2 (Goudet,
217 1995). Significance levels were adjusted using the Bonferroni procedure (Rice, 1989).
218 Genetic differentiation was also inspected through microsatellites-based Bayesian
219 clustering analyses, in STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000), using a
220 dataset with separate samples ($K = 1$ to 15) as well as pooling colonies into regions (north
221 and central west, $K = 1$ to 3). The running parameters were no *a priori* information on
222 sampling location, admixture model, correlated allele frequencies, degree of admixture
223 inferred from the data, λ of one, 10^6 runs of the Markov Chain Monte Carlo process, burn-
224 in of 10^4 and five independent runs with 20 iterations for each *K*. The most likely number
225 of differentiated populations (clusters) was determined applying the method proposed by

Evanno, Regnaut, and Goudet (2005) to STRUCTURE outputs using STRUCTURE HARVESTER v0.6.1 (Earl & vonHoldt, 2012). Complementary Bayesian spatial clustering analyses were performed in TESS v3 (Caye, Deist, Martins, Michel, & François, 2016), incorporating linear global trend surfaces in the *prior* distribution and considering both CAR and BYM models, which address spatial autocorrelation and complex spatiotemporal processes (e.g., massive migration and local mating), respectively (Durand, Jay, Gaggiotti, & François, 2009). Ten exploratory runs of TESS were conducted to determine the maximum number of clusters (*K* max.), setting 10,000 sweeps, a burn-in of 5,000 and starting with a neighbor joining tree. Thirty runs were then conducted for each *K*, setting 50,000 sweeps and a burn-in of 10,000. The model with the lowest value for the deviance information criterion (*DIC*) and stabilizing at the lowest number of clusters was chosen as the one that best explained the genetic variation in the data. The results of all cluster analyses were visualized graphically using POPHELPER V.1 (Francis, 2017).

Historical demography

Demographic processes were inferred by mismatch analyses carried out by comparing the distribution of observed pairwise nucleotide differences among mtDNA haplotypes *versus* the expected distribution under a model of sudden population expansion (Rogers & Harpending, 1992; Schneider & Excoffier, 1999). Analyses were run in ARLEQUIN V3.5, computing the sum of squared deviations (SSD) and the raggedness index (*r*) (Harpending, 1994) and their respective confidence intervals, using 10,000 replicates of the parametric bootstrap procedure. Time since population expansion (*t*) in mutational units was estimated using the formula $t = \tau /2u$ (Rogers & Harpending, 1992), in which u is the mutation rate of the assayed fragment and *tau* (τ) is the mode of the mismatch distribution obtained for each sample. Divergence rates were set at 2%, 6% and 10% (Div/MY) to account for uncertainties in the mutation rate of the assayed fragment (Lopes, Brito, Henrique-Silva, & Del Lama, 2006). To express time since expansion in years before present (*YBP*), a generation time of four years was used based on the age of the wood stork at first breeding (Coulter *et al*., 1999; Lopes *et al*., 2006).

Main past demographic scenarios were modeled for populations using an approximate Bayesian computation (ABC) framework based on information gathered from the literature and previous analyses. Initially, five demographic scenarios were simulated, with different combinations of colonization events followed by either population stability or exponential population growth, and different migration parameters, totaling 14 candidate models. Data simulations (100,000 for each model) were performed with the scripts given in Perez, Bonatelli, Moraes, and Carstens (2016), using empirical sample sizes. *Priors* for the parameters were drawn from simulated uniform distributions: divergence time (τ) was drawn from a distribution of 11,000 to 25,000 years and the effective population size of the ancient population (N_e) was drawn from a distribution of 50 to 10,000 individuals. The mutation rate of microsatellites was set at $1.1x10^{-8}$, as determined for *Gallus gallus* (Hillel *et al*. 2003), and transformed into theta values (θ). *Priors* related to specific models were also sampled from uniform distributions: the migration rate (M) was drawn from a distribution of 0.1 to 10 individuals per generation; for models simulating long dispersal, population contraction rates during colonization (θrF-A) were computed as the ratio between the θ in the ancient population (drawn from 0.001 to 0.1) and the magnitude of population expansion after colonization (θrC-A), calculated as the ratio between the current θ and that of the ancient population (sampled from 0.01 to 1). After the first run with 14 initial models, a second ABC run was performed, following a hierarchical procedure (Fagundes *et al.*, 2007), by choosing the five best performing models (ranked by PP) tested in the first run. This procedure is adequate for determining the behavior of the best model in relation to different sets of models (Pelletier & Carstens, 2014). The model that yielded the highest PP and Bayes' factor of relative support (BF) values in the second run was chosen as the one better that best fit the genetic data.

From the empirical and simulated mtDNA sequence data, a set of summary statistics (*SuSts*) was computed: the proportion of polymorphic sites (π), the number of segregating sites (S), Tajima's D and θ_H statistic (Fay & Wu, 2000) using a custom PERL script written by N. Takebayashi (available at: http://raven.iab.alaska.edu/~ntakebay/teaching/programming/coalsim/scripts/msSS.pl). The simulated nuclear data were converted into microsatellite alleles using 'microsat' (available

285 at: http://massey.genomicus.com/software.html#microsat) and further formatted using a
286 script from Perez *et al.* (2016) to compute the number of alleles (*A*), expected
287 heterozygozity (H_E) and a migration parameter (*nM*) (Excoffier, Laval, & Schneider, 2007;
288 Garza & Williamson, 2001) in ARLEQUIN (Excoffier & Lischer, 2010). Pairwise F_{ST} values
289 were computed between populations for both the simulated and empirical data. The
290 empirical *SuSts* were employed for running the ABC modeling with the best method
291 (chosen as described above) using the R package abc v1.4 (Csilléry, François, & Blum,
292 2012) with a threshold level of 0.005, resulting in 14,000 simulations retained in the
293 posterior probabilities.
294 Population genetic parameters were estimated using neural networks, transforming
295 parameters with a *logit* correction and setting the *priors* as interval boundaries to ensure
296 that the estimates would lie within these boundaries. For τ and *Ne*, present in all models,
297 estimation was conditional on the overall posterior probabilities. Specific parameters of the
298 best model were estimated using only the simulations for that model. The performance of
299 the ABC procedure was assessed by posterior predictive checks.
300

301 **RESULTS**

302 **Nuclear diversity, effective number of breeders, recent demographic processes and**
303 **structure**

304 Populations in both regions exhibited moderate microsatellite diversity; Amapá exhibited
305 slightly higher levels of UH_E (Table 1, Table S2 in Supporting Information). There was no
306 evidence of linkage between any loci. Significant deviation from *HWE* was detected at
307 locus *WSμ 20* and *WSμ 24* in four Pantanal colonies and at loci *WSμ 08*, *WSμ 09* and *WSμ*
308 *20* in all Amapá colonies (Table S2 in Supporting Information). Only locus *WSμ 20*
309 evidenced null alleles in most samples (mean null frequency = 0.21), but removing this
310 locus from the dataset did not significantly change the results of the subsequent population
311 genetic analyses. Thus, the final analyses involved a dataset with all nine loci using
312 adjusted allele frequencies at *WSμ 20*.
313 The N_b was 25 (95% CI: 15 to 44) for Amapá colonies and 22 (95% CI: 13 to 40)
314 for Pantanal colonies. There was no evidence of a recent reduction in population size that

could have affected genetic diversity in any population (all $Ps > 0.1$) (Table S3 in Supporting Information). When colonies were set as the potential source in the assignment tests, on average, only 8.05% of the storks were assigned to their colonies of origin (Fig. S1 in Supporting Information). However, when regions were set as the source, on average, 63.13% of storks were correctly assigned to their regions of origin (Fig. S1 in Supporting Information). AMOVA using the entire dataset showed that most nuclear diversity was distributed within (86.00%, $P = 0.001$) and among individuals (13.00%, $P = 0.001$). However, when pooling individuals from colonies within regions, genetic diversity was significantly partitioned between regions (1.00%, $P = 0.001$). None of the pairwise F_{ST} values between colonies within regions were significant (Table S4 in Supporting Information), indicating a lack of a strong nuclear structure. However, significant differentiation between regions was found when pooling genotypes from colonies into regions ($F_{ST} = 0.006$, $P = 0.01$).

The STRUCTURE results revealed that, when colonies were analyzed separately, the highest Delta K (1.73) occurred at $K = 10$ (Table S5 in Supporting Information), with additional peaks at $K = 8$ and $K = 3$ (Fig. 2A). However, when pooling genotypes from colonies into regions, the proportion of individuals strongly assigned to clusters was maximized at $K = 2$ [$LnP(K) = -2990.98$] and the highest Delta K (3.86) was obtained for two population clusters (Fig. S2 in Supporting Information). Likewise, TESS results supported two main genetic components (Fig. 2B) – one from each region –, with genetic contributions from unsampled populations, as indicated by the model with the highest likelihood, as well as the lowest and most stable *DIC* values ($K = 10$) (Fig. S3 in Supporting Information).

Mitochondrial diversity, demographic processes and genetic structure

Fourteen polymorphic sites defined 13 haplotypes in the overall sample. The Amapá and Pantanal populations had ten and seven haplotypes, respectively (Table 2). The most frequent and widespread haplotype (A023) occurred in 19 individuals from Amapá and 11 individuals from Pantanal (Fig. S4 in Supporting Information). Colonies from both regions shared most haplotypes, five haplotypes were private to Amapá and three other haplotypes

were unique to Pantanal. Amapá had slightly higher haplotype and nucleotide diversities than Pantanal (Table 2). Haplotypes from both regions occupy both interior and terminal positions of the network (Fig. S4 in Supporting Information). There was low but significant differentiation between Amapá and Pantanal ($\Phi_{ST} = 0.18$, $P < 0.001$), and AMOVA revealed that 8.11% of the variation was significantly distributed between regions ($P < 0.001$). Similarly, θ was low (0.005) and significant ($P = 0.05$).

Historical demography

Assuming neutrality of the assayed untranslated mtDNA sequence, demographic stability could not be rejected due to non-significant *Fs*, *D* and R2 values (Table 2). However, the negative Fu's F_S and the unimodal pattern of the mismatch distribution curve suggest population expansion in Amapá (Fig. S5 in Supporting Information). Mitochondrial estimates revealed that the Pantanal population expanded more recently (Table 2).

The results of cross-validation tests of demographic coalescent models showed that seven PCA axes explained 95% of the variation contained in the *SuSt* and accurately retrieved the best simulated model among the 14 candidates (data not shown). The models assuming panmixia were not supported (Table 3). The best scenario (Model 11) assumes that the Amapá population exchanged genes with unsampled populations and that the Pantanal population was founded by genes from the Amapá population (Table 3, Fig. S6 in Supporting Information). This scenario yielded the highest posterior probabilities and very high values for Bayes' Factor of relative support, both considering the full set of candidate models as well as when compared to the reduced set of models (Table 3). Posterior predictive checks showed that nearly all *SuSts* yielded simulated datasets containing the empirical values, which indicates a good fit between both datasets (Table S6). Past demographic parameter estimates showed that the Amapá and Pantanal populations diverged during the LGM (parameter τ, Table 4) from a large source population, as shown by the *Ne* in the upper range of the simulated values (Table 4). During the founder event, the Pantanal population was reduced to less than 10% (parameter $\theta rF\text{-}A\ P$, Table 4) and then underwent moderate exponential growth (parameter $\theta rC\text{-}A\ P$). The Amapá population also experienced exponential growth, and is currently larger than the Pantanal population

375 (parameter *θrC-A AP*, Table 4). Coalescent simulations also showed that gene migration of
376 moderate magnitude likely existed between the Amapá population and unsampled
377 populations (parameter *nM*, Table 4).
378
379 **DISCUSSION**
380 Using molecular markers with different modes of inheritance, the present study revealed
381 significant genetic differentiation among wood stork populations settled in two major
382 wetlands located in northern and central western Brazil. The coalescent analyses support
383 genetic exchange between the Amapá population and unsampled populations, as well as
384 demographic expansion and changes in population size that broadly overlapped the LGM.
385 These results have implications for the conservation of wood stork populations and wetland
386 management policies in Brazil.
387
388 **Genetic diversity, effective population size and recent demography**
389 Wood storks breeding colonies in the Amapá and Pantanal wetlands have moderate
390 microsatellite diversity (Table 1), within the range observed in jabiru storks (*Jabiru*
391 *mycteria*) in the Brazilian Pantanal (Lopes *et al*., 2011), Oriental storks (*Ciconia boyciana*)
392 in China (Huang & Zhou, 2011) and white storks (*Ciconia ciconia*) in Europe (Shephard,
393 Galbusera, Hellemans, Jusic, & Akhandaf, 2009; Shephard, Ogden, Tryjanowski, Olsson,
394 & Galbusera, 2013). Storks therefore appear to conform to the trend of low-moderate
395 nuclear genetic diversity described for waterbirds (mean *He*: 0.44 to 0.83; see Table S1 in
396 Eo, Doyle, & DeWoody, 2011). The smaller effective population sizes, lower annual
397 reproductive rates and longer life expectancies of these birds, reported to live up to 18 years
398 in nature (Animal Diversity Web, 2016), may explain the low diversity pattern observed
399 (Eo *et al*., 2011). Wood stork populations from Amapá and Pantanal showed low levels of
400 mtDNA diversity (Table 2) and lower haplotype diversity than jabiru storks from Pantanal
401 (Lopes *et al*., 2011), white storks from Europe (Shephard *et al*., 2013) and Oriental storks
402 from China (Zan *et al*., 2008). According to the mitochondrial theory of ageing, species
403 with a long lifespan would have lower mutation rates due to reduced oxidative damage in
404 the mitochondria, which would translate to low levels of mtDNA diversity (Nabholz,

405 Glémin, & Galtier, 2009). The Amapá wood stork population harbors slightly higher levels
406 of nuclear and mtDNA diversity than the Pantanal population, as expected if the former
407 was source. Coalescent modeling also showed that the Pantanal population was reduced to
408 less than 10% of its current size during past colonization events.

409 Neither marker showed signs of a recent bottleneck in the populations examined.
410 Thus, it seems that the ongoing threats that increasingly affect Brazilian wetlands may not
411 have yet significantly reduced the genetic variation of wood stork colonies. However,
412 estimates of the effective number of breeders in Amapá and Pantanal populations were
413 rather low, suggesting that only a few reproductive wood storks may be contributing to the
414 gene pools. Thus, these colonies may be prone to genetic depletion, which could hinder
415 population survival in the long term (Willoughby *et al*., 2015).

416

417 **Genetic structure**

418 Previous studies based only on one type of molecular marker found low levels of genetic
419 structure between wood stork breeding colonies located in the Brazilian Pantanal (Del
420 Lama, Lopes, & Del Lama, 2002; Lopes, Rocha, & Del Lama, 2004; Lopes *et al.*, 2006;
421 Lopes *et al*., 2011; Rocha, Del Lama, & Regitano, 2004). In agreement, the results of
422 genetic estimation, assignment tests, Bayesian clustering and coalescent models performed
423 in the present study support ongoing gene flow between wood stork colonies within the
424 Pantanal as well as among those settled within the Amapá wetlands. ABC coalescent
425 analyses also showed that Amapá population may exchange migrants with unsampled
426 populations (Table 3). In northern South America, breeding colonies of wood storks can be
427 found in Guyana (Mistry *et al*., 2008), Suriname (Spaans & de Jong, 1982), Colombia
428 (Arango, 2014), the Llanos grassland of Venezuela (Vilella & Baldassarre, 2010) and
429 Ecuador (Coulter *et al*., 1999; Santander, Muñoz, & Lara, 2006). Further genetic studies
430 including samples from northern South America are needed to broaden knowledge on the
431 effective dispersal of wood storks.

432 Despite ongoing gene flow among colonies within regions and no apparent barriers
433 to gene exchange, data from the present study also show that wood stork populations from
434 different Brazilian regions are significantly structured and do not constitute a panmictic

435 unit. The concurrent results of the assignment tests, AMOVA, significant F_{ST} (Table S4 in
436 Supporting Information) and Φ_{ST} values and Bayesian clustering analyses (Fig. 2; Fig. S2
437 in Supporting Information; Fig. S3 in Supporting Information) suggest the existence of two
438 main genetic components: one comprising storks breeding in the Amapá coastal wetlands in
439 northern Brazil, and another comprising those breeding in the Pantanal freshwater wetland
440 in central western Brazil. Furthermore, the ABC analyses did not support any of the models
441 which assumed a single panmictic population (Table 3; Fig. S5 in Supporting Information),
442 reinforcing the existence of genetic differentiation. Collectively, these results support the
443 hypothesis of regional breeding-site phylopatry (Coulter *et al*., 1999; Frederick & Meyer,
444 2008; Frederick & Ogden, 1997). Significant population structure in the absence of clear
445 barriers to gene flow has also been found in the roseate spoonbill (*Platalea ajaja*) (Miño &
446 Del Lama, 2014) and the great egret (*Ardea alba egretta*) (Corrêa, Del Lama, Rossi de
447 Souza, & Miño, 2016) inhabiting the same breeding colonies in both regions. Such a
448 pattern common to three highly dispersive waterbirds suggests that some unidentified factor
449 may be limiting genetic exchange between individuals inhabiting the two Brazilian regions
450 analyzed.
451
452 **Demographic history**

453 Evidence from the present study supports the hypothesis that wood stork populations in
454 northern Brazil established earlier and remained stable during the climatic changes of the
455 LGM. The Amapá population exhibited a more ancient pattern of expansion (18.73 kyr BP;
456 Table 2) and diverged from the Pantanal population between 11 and 23 kyr BP (Table 4),
457 overlapping the period after the LGM. The relatively large mean simulated coalescent
458 estimates of ancient population size may reflect the historical presence of a large population
459 in the equatorial region of Brazil (Table 4). Taken together, these findings suggest that the
460 Amapá wetland remained stable enough for many years, sheltering healthy wood stork
461 breeding populations. Although the effects of Quaternary climatic and hydrological
462 changes on different South American biomes are a matter of continuous research and
463 debate, most paleoclimatic reconstructions of the Neotropics currently accept that
464 seasonally dry forests, open woodlands and savannas once dominated the landscape

(Sant'anna Neto *et al*., 2015). Highly concordant paleopalynological and paleo-sedimental records suggest that a more humid climate contributed to the persistence of wetlands in the equatorial regions of South America during the LGM (Sant'anna Neto *et al*., 2015). At the time, savannas would have expanded and dominated the northern portion of the country in the Brazilian Amazon, where the state of Amapá is located (see Fig. 4.1 in Sant'anna Neto *et al*., 2015). In the central western region of Brazil, deglacial warming began immediately following the LGM and was marked by the onset of a wetter climate (Sant'anna Neto *et al*., 2015). A sharp shift to wetter and humid conditions occurred between 12.8 and 12.2 kyr BP, when the Pantanal wetland was a shallow body of water (Whitney *et al*., 2011). Mitochondrial estimates in wood storks indicate a more recent demographic expansion in the Pantanal sample (Table 2) and ABC analyses strongly support the founding of the Pantanal population by individuals from Amapá (Table 3). These results broadly agree with the timing of past events in both Brazilian regions examined, including the geological formation of the Pantanal floodplain (Assine & Soares, 2004; Junk *et al*., 2014). Collectively, therefore, the results suggest that wood storks could have continuously occupied the coastal wetlands of Amapá during the LGM, and migrated southward when deglacial warming began, colonizing the Pantanal freshwater wetland. A similar pattern of more ancient demographic changes in Amapá and a more recent expansion in the Pantanal has been found for roseate spoonbills (Miño & Del Lama, 2014) and great egrets (Corrêa *et al*., 2016). Such findings of a similar historical demography in species with foraging patterns and colonial reproduction comparable to the wood stork suggest that these organisms may respond similarly to future climate changes.

Conservation implications

Genetic monitoring of waterbird populations can support conservation of Brazilian wetlands. Following recent guidelines for the use of genetic monitoring to rank conservation priorities (Willoughby *et al*., 2015), this study contributes novel genetic data to assist in the conservation of wood storks breeding in such ecosystems. The populations studied here breed in Brazilian wetlands which are important biodiversity reservoirs as well as stopover and breeding sites for a variety of birds (Junk *et al*., 2014). However, these

wetlands are subjected to different types and levels of anthropogenic pressure. The Brazilian portion of the Pantanal is highly threatened: only 5% of its surface is currently protected by the Ramsar Convention (Ramsar Sites, 2017). This wetland is mainly managed by private land owners and is strongly impacted by habitat conversion and deforestation for cattle grazing, agriculture, hydroelectric projects, and water pollution (De Pinho & Marini, 2012; Junk *et al*., 2006). In contrast, the Amapá coastal wetlands are legally protected in the form of conservation units, which cover approximately 62% of the state, including a recently declared Ramsar site (Ministério Público do Estado do Amapá, 2011). However, deterioration stemming from dam building, urban development and global climate change also menace the Amapá wetlands (Junk *et al*., 2014). Moreover, recent changes in the Brazilian "forest code" implemented in 2012 significantly reduced the surface of the mandatory permanent protection zones of wetlands (Wittmann *et al*., 2015). Hence, it is expected that the coastal wetlands of Amapá will be impacted by the concurrent effects of deforestation, accelerating climate change and other anthropogenic disturbances that are taking place in the Amazon basin and surrounding areas, which will bring drier conditions to the region (Swann, Longo, Knox, Lee, & Moorcroft, 2015). Increasing habitat destruction, modification and pollution in wetlands (Junk *et al*., 2014) can reduce population size and further cause genetic deterioration of associated wildlife (Willoughby *et al*., 2015). Thus, the continuity of wood stork populations in the Amapá and Pantanal wetlands is of considerable concern.

The present study identified different genetic pools of wood storks breeding in Brazil: solid evidence indicates that colonies established in the Amapá coastal wetlands and those in the Pantanal freshwater wetland should be regarded as separate groups of interbreeding individuals. This pattern of genetic differentiation could be partially explained by past population movements in response to palaeoclimatic changes in wetlands. Thus, the novel information contributed here can help predict responses to future environmental changes as well as estimate probabilities of population survival. A pattern similar to that observed in this study has been previously reported for other waterbirds inhabiting the same wetlands (Corrêa *et al*., 2016; Miño & Del Lama, 2014). This multi-

524 species genetic pattern should be taken into account to support future protection measures,
525 management strategies and conservation policies directed at species and wetlands.

526 Previous studies on wood stork breeding colonies settled in the Pantanal have
527 resulted in changes in management practices, mainly in the state of Mato Grosso. For
528 example, eco-touristic practices involving visitation to colonies have changed after studies
529 involving wood storks (Bouton, 1999; Bouton, Frederick, Rocha, Barbosa Dos Santos, &
530 Bouton, 2005). Such studies also influenced the design of the 25th Article of State Law
531 9,096 of 2009, which prohibits catching small fishes up to 1,000 meters from breeding
532 colonies, in order to preserve the feeding items of foraging adult waterbirds and their
533 nestlings. In addition, the state government of Mato Grosso supported research aimed at
534 locating new breeding colonies of waterbirds (Cardoso, 2011). The exchange of
535 information between scientists and local authorities has provided good results regarding the
536 conservation of this region of the Pantanal. This scheme should also be implemented in the
537 state of Mato Grosso do Sul. It is highly likely that the Brazilian environmental authorities
538 will be sympathetic to this approach since it is within the scope of the National Strategic
539 Plan for Protected Areas (*PNAP*) (República Federativa do Brasil, Decreto N° 5.758, 2006)
540 and has proven to be very effective in the Pantanal area.

541 Results from the present study also revealed that Amapá wood stork populations
542 belong to the source gene pool and should therefore be given high conservation priority.
543 This novel information can be used by the Brazilian National Wetland Committee (*Comitê*
544 *Nacional de Zonas Úmidas*, *CNZU*) to help formulate conservation strategies and
545 determine areas that may potentially become new Ramsar sites. A prerequisite of the *CNZU*
546 is that Ramsar sites lie only within protected areas (Ramsar Sites, 2017; Witmann *et al*.,
547 2015). Thus, expanding the national network of Ramsar sites in the Amapá wetlands is a
548 realistic proposal and would be an efficient measure to preserve the source gene pool of the
549 species. Furthermore, this study revealed that Amapá populations may be exchanging
550 migrants with other unsampled populations, which underscores their utmost importance for
551 enabling continuous gene flow between breeding colonies in neighboring areas of northern
552 South America. By preserving the processes that generate and shape genetic diversity in
553 Ramsar sites, the Brazilian government would be complying with its commitments of

554 evaluating and monitoring protected areas, which is among the goals of the *PNAP*. Findings
555 from the present study indicate that such a task can be effectively supported by the genetic
556 monitoring of waterbird populations.

557

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568

569 **SUPPORTING INFORMATION**

570 Additional supporting information may be found online in the supporting information tab
571 for this article**.**

572 **Supporting Tables**

573 **Table S1.** Sampling information on wood stork populations.

574 **Table S2**. Statistics of diversity at nine microsatellites per *locus* and colony.

575 **Table S3.** Results of BOTTLENECK tests in wood stork populations.

576 **Table S4.** Pairwise F_{ST} values and corresponding probabilities observed between colonies
577 within regions and between regions.

578 **Table S5**. Raw results of application of the method described by Evanno *et al.* (2005) to
579 the outputs of STRUCTURE analyses.

580 **Table S6.** Posterior Predictive Checks (*PPC*) for ABC simulated summary statistics (*SuSts*)
581 of genetic diversity.

582

583 **Supporting Figures**

584 **Figure S1**. Histograms showing results of assignment tests.

585 **Figure S2.** Plot of Delta *K* vs. *K* obtained from STRUCTURE results.

586 **Figure S3.** Plot of Deviance Information Criterion (*DIC*) vs. *K* max resulting from spatial
587 Bayesian clustering analyses in TESS.

588 **Figure S4.** TCS network of mitochondrial haplotypes.

589 **Figure S5**. Graphics showing results of mismatch analyses.

590 **Figure S6**. Graphic representation of ABC models of past demographic changes.

591

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TABLES

Table 1. Mean estimates of diversity (± standard error) based on nine microsatellites for wood storks from Pantanal freshwater wetland and Amapá coastal wetlands, Brazil. *n*: number of samples genotyped, *Na*: number of different alleles, *A*: allelic richness, *Ho*: observed heterozygosity, *UHe*: unbiased expected heterozygosity, F_{IS}: inbreeding coefficient (Weir & Cockerham 1984), *PA*: number of private alleles.

Sample (Region)	Colony	*n*	*Na*	*A*	*Ho*	*UHe*	F_{IS}	*PA*
Pantanal	Mimoso	6.78 ± 0.15	2.00 ± 0.17	1.98	0.36 ± 0.084	0.37 ± 0.07	-0.08 ± 0.12	0
(center-west)	Fazenda Ipiranga	7.00 ± 0.00	2.00 ± 0.29	1.97	0.35 ± 0.089	0.33 ± 0.08	-0.14 ± 0.08	0
	Porto da Fazenda	24.78 ± 0.15	2.67 ± 0.24	2.25	0.34 ± 0.075	0.41 ± 0.06	0.18 ± 0.09	1
	Baia de Gaiva	12.00 ± 0.00	2.44 ± 0.24	2.28	0.33 ± 0.072	0.41 ± 0.07	0.15 ± 0.08	0
	Baia Bonita	10.00 ± 0.00	2.44 ± 0.50	2.26	0.33 ± 0.101	0.37 ± 0.09	0.12 ± 0.12	0
	Fazenda Retirinho	15.00 ± 0.00	2.78 ± 0.28	2.37	0.34 ± 0.066	0.44 ± 0.06	0.14 ± 0.13	0
	Rio Vermelho	9.00 ± 0.00	2.67 ± 0.33	2.42	0.42 ± 0.103	0.41 ± 0.06	0.03 ± 0.06	0
	Porto da Fazenda 2000	10.00 ± 0.00	2.22 ± 0.22	2.11	0.34 ± 0.091	0.38 ± 0.08	0.13 ± 0.15	1
	Tucum 2000	13.00 ± 0.00	2.44 ± 0.29	2.24	0.36 ± 0.091	0.38 ± 0.08	0.03 ± 015	1
	Fazenda Ipiranga 2000	9.89 ± 0 .11	2.44 ± 0.38	2.23	0.36 ± 0.008	0.38 ± 0.07	0.04 ± 0.10	1
	Entire region	117.44 ± 0.242	3.89 ± 0.48	3.76	0.35 ± 0.073	0.40 ± 0.07	0.14 ± 0.09	4
Amapá	Se Cria	26.00 ± 0.33	2.78 ± 0.28	2.39	0.38 ± 0.071	0.43 ± 0.70	0.14 ± 0.10	0
(north)	Fazenda Zelândia	21.78 ± 0.46	2.67 ± 0.24	2.32	0.45 ± 0.092	0.41 ± 0.07	-0.08 ± 0.07	0
	Macacoari	34.78 ± 0.86	2.78 ± 0.28	2.32	0.39 ± 0.085	0.40 ± 0.07	0.02 ± 0.08	1
	Se Cria 2007	23.67 ± 0.47	3.00 ± 0.29	2.37	0.48 ± 0.081	0.43 ± 0.06	-0.10 ± 0.06	0
	Fazenda Zelândia 2007	6.78 ± 0.15	2.33 ± 0.29	2.28	0.35 ± 0.092	0.38 ± 0.07	0.04 ± 0.17	0
	Entire region	113.00 ± 1.81	3.33 ± 0.24	3.30	0.42 ± 0.078	0.42 ± 0.07	0.04 ± 0.07	1

878 **Table 2.** Diversity estimates (± standard error), neutrality tests and time since expansion based on mtDNA Control Region haplotypes
879 found in wood storks from Pantanal and Amapá wetlands. *n*: number of individuals. *Fs*, *D* and R_2 statistics are shown with
880 corresponding probability values (*P*) and confidence intervals (95% CI). The mode of the unimodal mismatch distribution (*Tau,* τ) is
881 also shown. To account for uncertainties in the mutation rate of the fragment analyzed, time since population expansion (YBP: years
882 before present) was estimated using 2%, 6% and 10% divergence rates.
883

	Sample (Region)	
Statistic	**Pantanal (center-west)**	**Amapá (north)**
No. of polymorphic sites, *PS (n)*	7 (44)	10 (39)
Mean no. of nucleotide differences, *k*	1.425	1.973
Haplotypic diversity, $h \pm SD$	0.628 ± 0.000	0.773 ± 0.000
Nucleotide diversity, $\pi \pm SD$	0.006 ± 0.000	0.008 ± 0.000
Fs (95% CI)	0.139 ($P = 0.22$) (-2.42-1.74)	-2.170 ($P = 0.06$) (-4.25-5.69)
D (95% CI)	-0.683 ($P > 0.10$) (-1.69-2.00)	-0.385 ($P > 0.10$) (-1.64-1.85)
R_2 (95% CI)	0.115 ($P = 0.31$) (0.07-0.22)	0.096 ($P = 0.40$) (0.05-0.18)
Tau (τ) (95% CI)	2.051 (0 – 5.27)	2.22 (0.12 – 3.33)
SSD (P)	0.023 (0.40)	0.002 (0.80)
Raggedness (P)	0.306 (0.81)	0.342 (0.90)
Time since expansion (YBP)		
Divergence rate 10%	17,308.01	18,734.16
Divergence rate 6%	28,846.68	31,223.60
Divergence rate 2%	86,540.08	93,670.88

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885
886

887 **Table 3.** List of past demographic coalescent models tested for wood stork breeding colonies from the Pantanal (denoted as P) and
888 Amapá (denoted as AP) wetlands, Brazil. Posterior probabilities (*PP*) and Bayes' Factor of relative support (BF) compared to the best
889 model (highest *PP*, shown in bold) for the first ABC run; Subset posterior probabilities (*SSPP*) and Bayes' Factor of relative support
890 (*SSBF*) for the second ABC run, performed only with the best candidate models for each scenario, marked with an asterisk.

Scenario	**Model**	**Description**	***First run***		***Second run***	
			PP	*BF*	*SSPP*	*SSBF*
1	1*	Single population with constant size.	0.0000	0.0000	0.0000	0.0000
1	2	Single population expanding.	0.0000	0.0000		
1	3	Single bottlenecked population.	0.0000	0.0000		
1	4	Single population expanding after a bottleneck.	0.0000	0.0000		
2	5	P is founded by AP, ongoing bidirectional gene migration.	0.0029	0.0030		
2	6	P is founded by AP; AP is expanding; ongoing bidirectional gene migration.	0.0040	0.0043		
2	7*	P is founded by AP; gene migration from AP to P.	0.0067	0.0070	0.0001	0.0001
3	8	AP is founded by P, ongoing bidirectional gene migration.	0.0002	0.0004		
3	9	AP is founded by P; Pop P is expanding, ongoing bidirectional gene migration.	0.0001	0.0001		
3	10*	AP is founded by P; gene migration from P to AP.	0.0012	0.0012	0.0006	0.0006
4	**11***	**AP exchanges genes with unsampled populations and is expanding; P is founded by AP.**	**0.9487**	**5.3059**	**0.9645**	**27.7816**
4	12	AP derives from unsampled populations; P is founded by AP.	0.0179	0.0188		
4	13	AP derives from unsampled populations and is expanding; P is founded by AP, ongoing bidirectional gene migration.	0.0177	0.0186		

5	14*	P exchange genes with unsampled populations and is expanding, AP is founded by P.	0.0007	0.0007	0.0347	0.0360

Table 4. Demographic parameters and confidence intervals (CI) estimated under neural network regression for wood stork populations in the Amapá (AP) and Pantanal (P) wetlands, Brazil. Population size, *Ne*, is given in no. of individuals and divergence time (τ) is given in years before present and were estimated based on simulations of all models.

Parameters	Median	95% CI
Ne	5,577.91	3,449.29-8,906.61
τ	14,997.65	11,123.63-24,316.30
θ*rF-A P*	0.072	0.030-0.094
θ*rC-A AP*	0.517	0.092-0.891
θ*rC-A P*	0.145	0.030-0.693
nM	0.104	0.100-0.125

π: Proportion of polymorphic sites, *S*: number of segregating sites, Tajima's D, θ*rC-A AP* and θ*rC-A P* is the ratio between the current size of population AP and P, respectively, and the ancient population size; θr*F-A*: ratio between the size of population P and the source population during the founder event; *nM*: Garza and Williamson's (2001) modified index.

FIGURE CAPTIONS

Figure 1. Map showing wood stork sampling sites. Grey-shaded areas in lower left inset indicate location of regions sampled within Brazil. Breeding colonies (black dots) are shown in enlarged sub-figures: three in Amapá coastal wetlands (A) and eight in Pantanal freshwater wetland (B). Full names of colonies are given in Table S1.

Figure 2. Evidence of genetic differentiation between wood stork populations from the Amapá coastal wetlands and Pantanal freshwater wetlands in Brazil. (A) Membership plots showing genetic ancestry of each of 236 individuals resulting from Bayesian clustering analyses in STRUCTURE (Pritchard *et al.,* 2000) for $K = 2$ and 10; (B) Polygons showing membership proportions to populations, and membership plots for $K = 10$ resulting from spatial Bayesian analyses in TESS (Caye *et al.,* 2016). In the membership plots, each individual (y-axis) is represented by a single vertical line broken into segments proportional to its membership coefficients for each cluster. Individuals were grouped into colonies, separated with white dashed line, coded and arranged as in Table S1.