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SHORT COMMUNICATION

# Remaining genetic diversity in Brazilian Merganser (*Mergus octosetaceus*)

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**Abstract** The Brazilian Merganser is a very rare and threatened species that nowadays inhabits only a few protected areas and their surroundings in the Brazilian territory. In order to estimate the remaining genetic diversity and population structure in this species, two mitochondrial genes were sequenced in 39 individuals belonging to two populations and in one individual collected in Argentina in 1950. We found a highly significant divergence between two major remaining populations of *Mergus octosetaceus*, which suggests a historical population structure in this species. Furthermore, two deeply divergent lineages were found in a single location, which could due to current or historical secondary contact. Based on the available genetic data, we point out future directions which would contribute to design strategies for conservation and management of this threatened species.

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## Introduction

The Brazilian Merganser, *Mergus octosetaceus* (Vieillot, 1817), is a critically endangered (IUCN 2010), fish-eating, riverine duck that inhabits fast-flowing, clear rivers (Antas 1996) and wild population is estimated to be less than 250 individuals (BirdLife_International 2000). Its historical range comprised the tropical and subtropical Atlantic forest and the Cerrado regions of Brazil, Argentina and Paraguay (Hughes et al. 2006). At the present time, only a few protected areas and their surroundings in Brazil still hold populations of the species, namely Jalapão State Park (Tocantins state), the National Parks of Chapada dos Veadeiros and Emas (both Goiás state), and Serra da Canastra (Minas Gerais state), with recent discoveries at other sites in Minas Gerais and Bahia states. It is considered locally extinct in Paraguay and Argentina, although there have been rare sightings in recent decades in Argentina (Hughes et al. 2006). At the Serra da Canastra National Park is found the largest remaining population, with approximately 80 individuals documented (Lamas 2006).

Following the extinction of *Mergus australis* (Auckland Islands Merganser) in the first decades of the twentieth century, *M. octosetaceus* is the only remaining member of the seaduck tribe (Mergini) in the Southern hemisphere (Livezey 1995; Antas 1996). Both Southern hemisphere species presented fragmented distributions and shared unique characteristics as they were the only non-migratory Mergini that also lack sexual dichromatism. However, according to Livezey (1995), each southern species likely

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resulted from independent trans-equatorial colonization from the Northern hemisphere. The Brazilian Merganser is considered the most threatened of all mergansers, being a rare species that naturally occurs in low densities. It is currently endangered by several threats to its original habitat, perhaps the most important of which is the increased sediment input into rivers, resulting in turbidity and sedimentation, which in turn reduces fish populations and merganser hunting success (Hughes et al. 2006).

Considering the current estimates for the total remaining population of the Brazilian Merganser (BirdLife_International 2000), we expect an ongoing erosion of its current genetic diversity. In such a reduced population, it should occur a significant reduction of diversity by genetic drift alone, but inbreeding is also inevitable in each isolated locality (Frankham et al. 2002). It is generally accepted that higher genetic variation increases the ability of a population to adapt and survive environmental changes, therefore a diagnosis of the remaining genetic diversity in threatened species is important in the design of appropriate management programs (Frankham et al. 2002).

Although a few ecological and behavioral studies have been conducted on the Brazilian Merganser, hitherto no studies of its remaining genetic diversity have been published. Aiming to contribute for an ongoing conservation plan, we have quantified the remaining diversity in the Brazilian Merganser by sequencing two mitochondrial genes from two different populations where management is currently in progress.

## Methods

### Sampling and DNA extraction

The samples were collected from 2002 to 2008 at the Serra da Canastra National Park and Chapada dos Veadeiros National Park (Fig. 1). The tissue samples included unhatched eggs with and without embryos ($n = 12$), feathers from abandoned nests ($n = 8$), molted feathers found during fieldwork ($n = 1$) and blood collected during captures ($n = 18$). For molted feathers, the genomic DNA of each feather was extracted separately. For the eggs that contained embryos, we collected the tissue from heart and liver of the formed fetus. For the eggshells and eggs that did not contain embryos, genomic DNA was extracted from $\sim 1$ cm$^2$ of the inner egg membrane.

Genomic DNA was also extracted from feathers and toepad tissue of four skins collected from 1950 to 1954 in the region of Arroyo Urugua-í from Misiones, Argentina (Fig. 1), and held at the Museo Argentino de Ciencias Naturales of Buenos Aires.

For the nests that contained both eggshells and feathers, each sample was considered a different source of DNA, i.e., feathers and eggshells were separately extracted. In this case, we have considered for the analysis only the eggshells or the feathers, to avoid sample redundancy. For one nest we obtained samples for more than one breeding season and each sample was extracted separately in order to control if the same pair or female (in cases where only nest remains were available) occupied the same nest for more than one season.

Genomic DNA was extracted using standard phenol–chloroform protocol after digestion with proteinase K (Vilaça and Santos 2010). For feathers, eggshells and museum samples, DTT was added in the proteinase K step.

### DNA amplification and sequencing

For blood or other good quality tissue samples, 1200 bp of the mtDNA Cytochrome b (Cyt-B) were amplified with the primers L14841 and H16065 (Sorenson et al. 1999). Amplification and sequencing was performed as described in Vilaça and Santos (2010). For samples which only feathers, eggshells or toepad tissue were available, a smaller fragment (350 bp) was amplified and sequenced with the primers L14841 and H15149 (Sorenson et al. 1999).

A 700 bp segment of the mtDNA control region (CR) was amplified and sequenced with the primers Duck. L81 and Duck. H768 (Munoz-Fuentes et al. 2005) using previously described protocols.

### Data generation and analysis

High quality sequence assembly and alignment were performed as previously described (Vilaça and Santos 2010). Summary statistics (nucleotide diversity, gene diversity, mean number of nucleotide differences) were estimated using the program DnaSP version 5 (Librado and Rozas 2009). To estimate the amount of population differentiation we performed an Analysis of Molecular Variance (AMOVA) with the software Arlequin 3.5 (Excoffier and Lischer 2010). We used the Median-Joining network analysis (Bandelt et al. 1999) to depict the relationships among haplotypes as implemented in Network 4 software (http://www.fluxus-engineering.com). For assessing any departure from neutrality we used an online platform (http://wwwabi.snv.jussieu.fr/achaz/neutralitytst.html) to calculate the normalized Tajima's D (Tajima 1989), and Fay and Wu's H (Fay and Wu 2000). The Fu's Fs test (Fu 1997) was calculated with Arlequin. For the tests that require an outgroup, a *Mergus merganser* sequence retrieved from Genbank (EU585654) was used. Significant negative values of the D and Fs statistics reflect a population expansion or genetic


**Fig. 1** Map with the current distribution of *Mergus octosetaceus* populations (*grey*) (Ridgely et al. 2007), including the sampled sites (*stars*): National Parks of Serra da Canastra (SC) and Chapada dos Veadeiros (CV), and Misiones (MA)

hitchhiking (evidenced by an excess on number of alleles), while the H statistic is more sensitive to positive selection evidenced by high-frequency-derived variants (Przeworski 2002). The combination of the three statistics enables to discriminate demographic effects from natural selection.

To access the confidence on clade separation we performed a phylogenetic analysis using the software BEAST v. 1.6.1 (Drummond and Rambaut 2007). As outgroups we used a 398 bp fragment of the CR from *Mergini* species published by Solovyeva and Pearce (2011), which include sequences of *M. merganser* ($n = 4$), *Mergus squamatus* ($n = 4$), *M. serrator* ($n = 4$), *Bucephala islandica* ($n = 1$), *B. clangula* ($n = 1$) and *B. albeola* ($n = 1$). The analysis was run for 10,000,000 generations, with a Yule prior and convergence was checked on Tracer (Rambaut and Drummond 2007) to assure that all statistics and estimated parameters had a Effective Sample Size (ESS) of at least 200 (which indicate that the MCMC chain ran long enough to get a valid estimate of the parameter). For accessing the relationships under the Mergini tribe, we only used the CR, which has been suggested as a better marker than Cytochrome B to investigate phylogenetic relationships among the Anatinae (Donne-Gousse et al. 2002).

## Results and discussion

For the longer Cytochrome B (Cyt-B) sequences, we obtained 1028 bp for 10 individuals (eight from Serra da Canastra and two from Veadeiros), comprising five haplotypes displaying 53 polymorphisms. For the remaining 29 individuals, we obtained smaller Cyt-B sequences that allowed evaluating 323 bp haplotypes. In all analysis we

have used the 323 bp Cyt-B sequences to allow genetic comparisons among all 39 individuals. In the 39 samples, for which we considered 323 bp sequences, we identified seven haplotypes with 26 variable sites. No frameshift or stop codons were observed for any Cyt-B sequences. The summary statistics for Cyt-B data is shown in Table 1.

The Cyt-B median-joining network showed two haplotype clusters (Fig. 2) with an average nucleotide difference between them of 8.1%. These divergent clusters were separated by 20 mutations in the network. Individuals from cluster 2 ($n = 18$) occurred in the populations of both Serra da Canastra and Chapada dos Veadeiros, and although we observed cluster 1 ($n = 21$) only in individuals from Serra da Canastra, we cannot exclude its occurrence in Chapada dos Veadeiros due to the small sample size.

For the CR, we obtained 623 bp sequences for 17 individuals, identifying only two divergent haplotypes that differed on 24 nucleotide sites. Despite fewer individuals were sequenced for the CR likely due to the low quality DNA samples, we detected two highly differentiated CR haplotypes corresponding to the same pattern of two highly divergent clades found for Cyt-B (Fig. 2). When compared with another *Mergini* species, levels of divergence within each lineage are similar to another endangered species, *M. squamatus*, in which four haplotypes differed by a single nucleotide changes on the mtDNA CR for samples from Russia and China (Solovyeva and Pearce 2011). Divergence between the two *M. octosetaceus* lineages was comparable to the differentiation on the CR observed between subspecies of *M. merganser* (*M. merganser merganser* × *M. m. americanus*) which diverged at a mean of 28 nucleotide differences in 888 bp of the mtDNA CR (Hefti-Gautschi et al. 2009). Although no description of previous *M. octosetaceus* subspecies has been published, it may be a possible that discontinuous populations in South America accumulated divergence in the past resulting in both clades. There is no evidence of reproductive isolation between *M. octosetaceus* populations or lineages in this case, as no reproductive barrier has been recorded among the three *M. merganser* subspecies, despite high differentiation was observed between two subspecies located in distant geographic areas (Hefti-Gautschi et al. 2009). The occurrence of both divergent lineages in a single population of *M. octosetaceus* occupying a relatively small area is a strong indication that those mtDNA divergent lineages found in simpatry are likely due to secondary contact, either historical or recent.

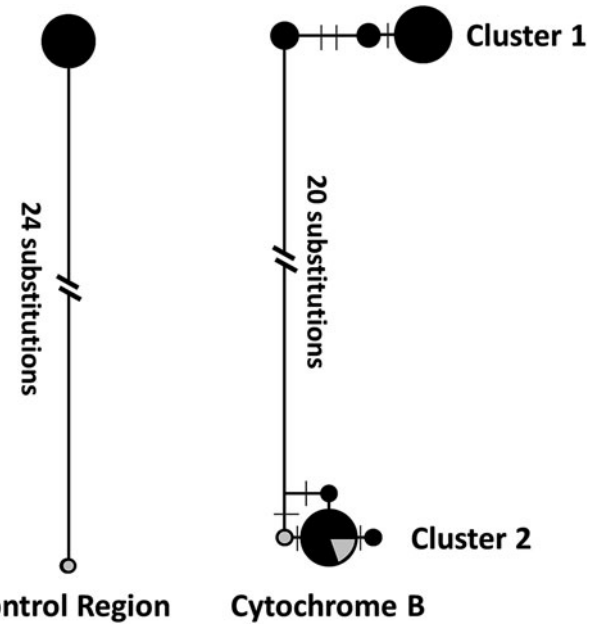


**Fig. 2** Phylogeographic network describing the relationship among haplotypes for 323 bp Cyt-B sequences (on the *right*) and for 623 bp CR sequences (on the *left*). *Black* haplotypes found in the Serra da Canastra National Park. *Gray* haplotypes found in Chapada dos Veadeiros National Park. Numbers of substitutions are assigned in the branches, either by mutation number or transversal bars

The CR phylogeny (Fig. 3) showed a monophyletic *M. octosetaceus* (posterior probability = 1.0), divided in two genetic lineages corresponding to the two haplotypes. Although Livezey (1995) places the Brazilian Merganser as a basal species within the *Mergus* genus, our results showed *M. serrator* as the most basal *Mergus*.

When taking into account sampling localities (Serra da Canastra × Chapada dos Veadeiros), the AMOVA also showed a significant degree of population divergence ($\Phi st = 0.37$, $P = 0.04$) due to limited gene flow. The

**Table 1** Summary of Cytochrome B data for 323 bp sequences

| | Serra da Canastra | Chapada dos Veadeiros | Total |
|---|---|---|---|
| *n* | 35 | 4 | 39 |
| Ht | 6 | 2 | 7 |
| $\pi$ | 0.038 | 0.001 | 0.039 |
| Gene diversity | 0.721 | 0.500 | 0.723 |
| k | 12.437 | 0.500 | 12.744 |
| Tajima's D | 2.89 ($P < 0.001$) | −0.61 ($P = 0.39$) | – |
| Fu's Fs | 12.84 ($P < 0.01$) | 0.17 ($P = 0.35$) | – |
| Fay and Wu H | −0.46 ($P = 0.19$) | – | – |

*n* Sample size, *Ht* number of haplotypes, $\pi$ nucleotide diversity, *k* average number of nucleotide differences

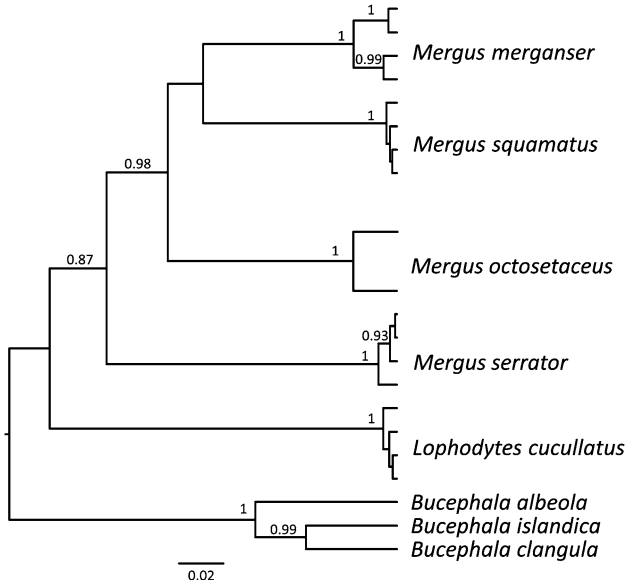


**Fig. 3** Bayesian tree describing the relationship among mtDNA Control Region haplotypes (398 bp) from several *Mergini* species. Posterior probabilities >0.5 are shown

neutrality tests were shown significant for the Serra da Canastra population, where positive values of Fs and D indicated a signal of bottleneck or selection. Since Fu's Fs test is sensitive to demographic effects (Fu 1997) and the H test is sensitive to positive selection but not to demographic changes (Fay and Wu 2000), the combination of the three statistics indicate a population size reduction as the main cause of departure from neutrality for the Serra da Canastra population, and not positive selection. The neutrality test results should be taken with care in Chapada dos Veadeiros because of the reduced sample size.

Among the six abandoned nests that were sampled (from feathers and eggs), five were sampled for only breeding season, and one of the nests, we sampled along three consecutive years. For the nest where samples were collected for three breeding seasons, we were able to verify that all samples possessed the same mtDNA haplotype. These results help to understand the barely known Brazilian Merganser behavior, since mtDNA data suggest that the same (or related) female (and perhaps the same breeding pair) may occupy the same nest along different breeding seasons.

Only one sequence was obtained from the Argentinean museum samples, due to low quality of preserved tissues. For one individual classified as female and collected in the year of 1950 (reference MACN-Or 32368), 192 bp of the Cyt-B was sequenced. This sequence groups with cluster 1, although it is a different haplotype with two autapomorphic mutations (Supplementary Material). The population in Argentina may now be extinct, but the sequence from a single individual collected half a century ago may represent an exclusive lineage previously found in the Argentinean region of Misiones, since the haplotype was not found in any other location. In addition, as it is more related to the *M. octosetaceus* lineage 1, which has a more southern distribution, it may be a southern remaining of an ancient lineage separation.

The Action Plan for the Conservation of the Brazilian Merganser (Hughes et al. 2006) claims that the current species distribution, like the one expected during the Pliocene/Pleistocene, consists of relatively small and isolated subpopulations. This metapopulation pattern should reflect in a low genetic variability expected within each local population (Hughes et al. 2006). The Action Plan also added that if low genetic diversity and inbreeding were limiting factors, *M. octosetaceus* would probably already be extinct. Our results agree with their claim of historical and current distribution composed of small isolated populations, since low genetic variability was found if we consider each of the divergent clades separately. However, relative isolation and genetic drift can also explain the presence of highly divergent mtDNA lineages in the same location due to the occurrence of secondary contact (see below), either recent, or in historical times before anthropogenic environment degradation.

Current data suggests that the Brazilian Merganser is a sedentary and territorial species that usually occupies the same territory through its entire adult life, but little is known about its dispersal behavior. Our data show two highly divergent clades occupying the Serra da Canastra National Park region. One hypothesis to consider is whether this pattern could be generated by a secondary contact between populations that were pushed into sympatry after being isolated for a long period of time during which they evolved allopatrically. Previous studies (Ramos et al. 2007; Cabanne et al. 2008) suggested that the last Pleistocene glaciations affected the Minas Gerais region (from where Serra da Canastra samples were collected), an area known to be a contact zone for several species of animals and plants. Although there is no suggestion of a subspecies within the Brazilian Merganser, if these two lineages evolved allopatrically, the two gene pools were admixed in more recent times, when the species returned as a single mating gene pool, at least in Serra da Canastra. Considering *Anatidae* species, reproductive barriers are not strong, since several interspecific hybrids in the genus *Mergus* were reported, an also many species belonging to different genus are known to hybridize frequently (Tubaro and Lijtmaer 2002; MacCarthy 2006). Therefore, the two lineages of Brazilian Merganser may have never shown a true reproductive incompatibility (in terms of morphologic/behavioral mechanisms that could lead to speciation), but the isolation of individuals in different regions could have generated the divergence of clades.

Due to the small sample size analyzed, we cannot discard the possibility of presence of clade 1 on Chapada dos Veadeiros. A historical occurrence of both clades in the sampled regions should be also considered, where one clade could have been lost (lineage sorting) due to genetic drift, enhanced by the small population size. Nevertheless, the divergence of clades observed in *M. octosetaceus* is comparable with other intraspecific clades observed in *M. merganser* (Hefti-Gautschi et al. 2009), which displays no evident inter-subspecies reproductive barrier. It supports the idea of an ancient geographic structuration in the Brazilian Merganser, which has been partially erased by secondary contact.

Our results show that recent bottleneck may be reducing the genetic diversity in the species, but a significant population structure still remains along the *M. octosetaceus* distribution, which indicate that restrictions of gene flow are maintained in natural populations. Although this is a preliminary study, care should be taken in any eventual captive breeding plan to ensure effective management of the genetic diversity of the species. Nuclear markers and more populations should also be analyzed in order to build a more complete scenario of the current genetic diversity of the Brazilian Merganser.

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