Chapter 2

HOW ARE AMAZON AND ORINOCO RIVERS RELATED? PRELIMINARY RESULTS ON THE COMPARATIVE HISTORY, STRUCTURE AND DYNAMICS OF GIANT OTTERS, *PTERONURA BRASILIENSIS*, FROM WESTERN AMAZONIA

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ABSTRACT

This is a preliminary study on the possible genetic structure and phylogeography of the Giant otter (*Pteronura brasiliensis*) in the upper Amazon basin (Colombia, Peru, Bolivia and western Brazilian Amazon) and in the Orinoco basin (Colombia) by means of sequences of the *mtCyt-b* gene. The animals from the upper Amazon and those from the Orinoco basin were not isolated and no significant heterogeneity was detected. Our results showed that the animals clustered in two main clades, one only integrated by upper Amazon individuals, with moderate haplotype diversity, and the second by other upper Amazon animals as well as the Orinoco animals and one sampled in the Bolivian Amazon with a higher haplotype diversity. No population expansion processes were detected with different procedures and the Bayesian analyses determined that the two main clades diverged around 37,000 years ago, while within each one of the clades, the divergences times among haplotypes were around 10,000-20,000 years ago. These time splits were correlated with climatic changes in the fourth glacial period of the Pleistocene and during the Holocene.

Keywords: *Pteronura brasilensis*, Giant otter, *mt* Cytochrome gene, genetic heterogeneity, Upper Amazon, climatic changes, Pleistocene, Holocene

INTRODUCTION

An acute debate in Neotropical biogeography concerns the importance of Pleistocene climatic and geological events in generating species richness and organizing current population structure (Hewitt, 2000; Bennett, 2004). Some works suggested a scenario where most of the observed divergences and speciation events are associated with paleogeographic events of the late Miocene (Moritz et al., 2000; Marroig and Cerqueira, 1997). Most of the studies, however, support a more significant role to the climatic cycles of the Pleistocene, associated with recent and rapid divergence processes in several mammal clades (Patton and Silva, 2001; de Thoisy et al., 2010; Lavergne et al., 2010; Ruiz-García et al., 2010a,b).

The Pleistocene period was marked by significant changes in environmental conditions, notably with the uplift of the Panama Isthmus, and a tendency for aridity reflected by expansion of savannas in replacement of forests (Van der Hammen and Hooghiemstra, 2000). Fauna communities consequently reorganized, with biotic exchange between the Americas (Webb, 2006), and a trend towards large-sized mammalian groups typical of open habitats, notably in Western Amazonia (Rossetti et al., 2004). In addition, there was an environmental change from the large middle Miocene lake to a fluvial system during the Plio-Pleistocene (Shepard et al., 2010) which may have expanded the distribution of terrestrial species, while at the same time limiting the aquatic species to restricted fluvial areas.

In this chapter we expect to contribute to this debate, providing preliminary explorations of the population history of the largest fresh water Amazonian carnivore mammal, the Giant otter *Pteronura brasiliensis*.

Diversification of most of the 13 current living otter species occurred during the Pliocene, with the noticeable exception of the Giant otters that diverged from a Eurasian ancestor in the late Miocene, 7.7 to 7.4 MYA (Koepfli et al., 2008). The species colonized South American during the Great American Interchange and consequently has a South American history confined to the Pleistocene. The Giant otter is endemic to the rainforests and wetlands of South America, the northern extent of its range occurs near the Caribbean Sea, and the southern range limit reaches Northern Argentina, although Argentine and Uruguayan populations are considered extinct. The species is now found in Orinoco, Amazon, and La Plata River, Parana and Paraguay systems, and in the unrelated freshwater systems of the Guiana shield. The genetic structure of the Giant otter population has been investigated in the southern part of its range, and has shown complex population structuring, only partly explained by river drainages (Garcia et al., 2007).

The current work aims to complement those first investigations on Giant otter phylogeography, focusing now in the western Amazon, with animals from the major freshwater drainages of the upper Amazon and the Orinoco. Specifically, we explore the evolutionary history, population genetic structure and dynamics of Giant otters, and attempt to provide data relevant for the conservation of this increasingly threatened species.

MATERIAL AND METHODS

We used 21 skin samples from Colombia, Peru, Brazil and Bolivia. Eighteen of the samples were related to the upper Amazon or tributaries, and three to the Orinoco basin. Samples were collected as follows: eight from the Peruvian Amazon [one from Urarinas, Puhinauva channel at the Ucayali River, four from Nanay and Pintayacu rivers (tributaries of the Amazon River), one from San Rafael at the Curaray River (tributary of the Napo River), one from the Mazan River (tributary of Napo River) and one from Sandoval Lake at the Tambopata River (affluent of the Madre de Dios River)], 10 from Colombia (one from the Bita River [tributary affluent of the Orinoco River] and another from the Orinoco River both in the Vichada Department, two from the Putumayo River, three from the Amacayacu River [tributary affluent of the Amazon River], one from “El Paujil” in the Inirida-Orinoco Rivers in the Guainia Department and two from the Caqueta River]), one from Bolivia (Alejandría, Mamoré River) and one from Brazil (Atalaia do Norte, Yavari River).

Cytochrome b was used as a target marker because data resulting from genetic variability of mitochondrial DNA are commonly used to study species’ natural history and dispersal patterns, and are adequate markers to investigate Evolutionarily Significant Units and Management Units (Moritz, 1994). Primers were designed specifically for the work and are available upon request, and allowed to obtain a fragment sequence of 689 bp.

Population structure was investigated according to two hypothesized scenarios: populations were defined (i) according to the two main river basins, Orinoco and Amazon; and (ii) according to strongly supported clades identified with gene tree reconstructions.

DNASP 5.1 (Rozas et al., 2003) and ARLEQUIN 3.1 (Excoffier et al., 2005) were used to examine nucleotide site polymorphism, haplotypic diversity (H_d), and nucleotide diversity (π) of each group of samples. Gene trees were constructed using both the Maximum Likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969) conducted in Mega 5.0 (Tamura et al., 2011), and the Median Joining haplotype Network (Bandelt et al., 1999). Genetic variation was partitioned according to the above mentioned hypotheses. Fixation indices were estimated using an analysis of molecular variance (AMOVA), as implemented in ARLEQUIN. Pairwise F_{CT} significance tests and number of migrants among clades were also calculated using ARLEQUIN.

Historical patterns of demographic history were investigated with two strategies. Under the assumption of neutrality, deviations in Tajima’s D (Tajima, 1989), Fu’s Fs (Fu, 1997), and Harpending raggedness index (Harpending, 1994) were used to test for a recent population expansion or bottleneck. Second, Bayesian skyline plots (BSP) constructed in BEAST 1.4.7 (Drummond et al., 2005) were used to describe evolution of efficient population size over time, assuming a mutation rate of 0.039 / site / MY (Nabholz et al., 2008). Divergence clades and time of the most recent common ancestors (tMRCA) for the different clades were inferred with BEAST 1.4.7 using an approach based on the mitochondrial DNA mutation rate defined for *Pteronura brasiliensis* (0.039 mutation / site / MY, Nabholz et al., 2008). No outgroup was used, in order to accommodate recently debated issues about molecular dating of recent phylogenetic splits (Ho et al., 2008).

RESULTS AND DISCUSSION

The phylogeography and population history of Neotropical mammals have been investigated in several large terrestrial vertebrates, including primates (Cortes-Ortiz et al., 2003; Lavergne et al. 2010; Ruiz-García et al., 2006a, 2010a,b, 2011a,b), terrestrial carnivores (Tchaika et al 2007; Eizirik et al. 2001; Ruiz-Garcia et al., 2006b, 2007a, 2009a), and ungulates (Ruiz-García et al., 2007b, 2009b; de Thoisy et al., 2010). Those works suggest recent and rapid diversification stages, often altering with secondary admixtures. Among studies of large aquatic vertebrates, fresh water turtles (Escalona, 2009; Pearse et al., 2006), Neotropical otters (Trinca et al., 2012), the Giant otter in the southern part of its range (Garcia et al., 2007), caimans (Vasconcelos et al., 2008), capybaras (Maldonado et al., 2011) and river dolphins (Banguera-Hinestroza et al. 2002, Ruiz-García et al., 2008; Ruiz-García, 2010c,d; Caballero et al., 2010) revealed complex histories and important levels of genetic structure.

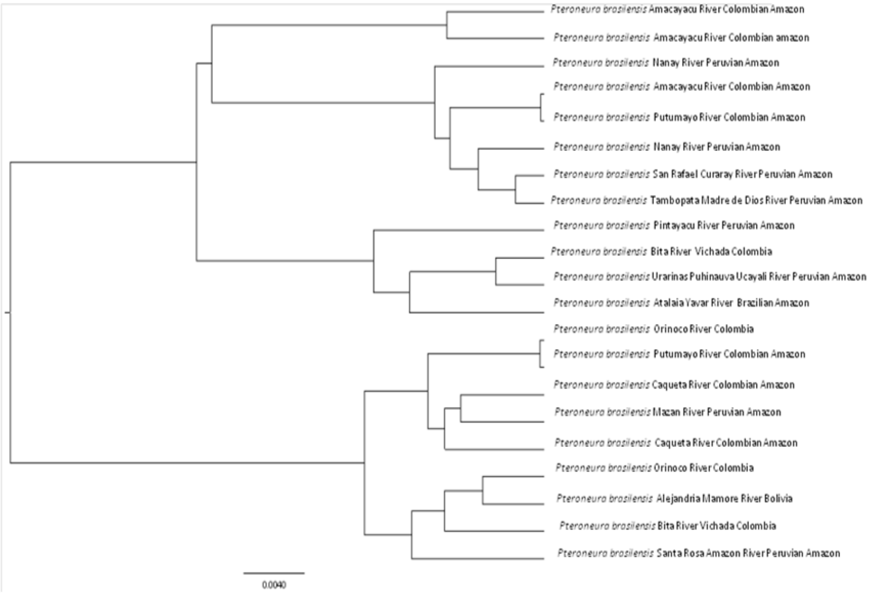

Figure 1. Maximum likelihood tree for the 21 Giant otters analyzed for the *mt Cytb* gene sequences studied.

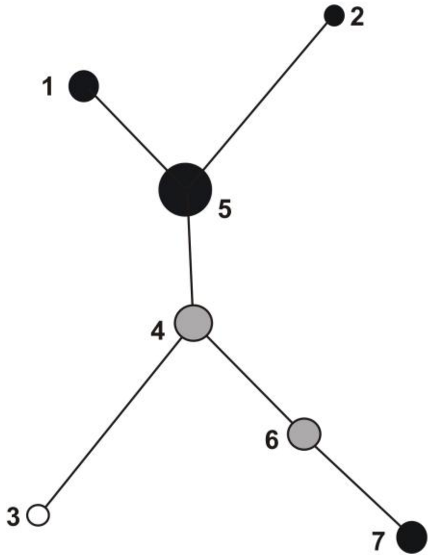

Figure 2. Haplotype network for the Giant Otter from Western Amazonia. Black haplotypes (1,2,5,7): upper Amazonia. Grey haplotypes (4,6) are shared by animals from the Orinoco and the Amazon. The white haplotype (3) is from Rio Madeira, Bolivia.

The present work on Giant otters adds some new features to our knowledge of large fauna inhabiting Neotropical waterways.

Giant otters from the western Amazonian region showed high levels of diversity (haplotypic diversity $H_d = 0.819$). Animals of the two basins did not show significant structural differences ($F_{st} = 0.2$, $p = 0.08$). A phylogenetic tree was constructed using the HKI model and the Maximum Likelihood method. It identified (but did not propose as the most relevant) a cladogram organized on the geographic origin of animals. Two clades were clearly identifiable, one including animals from the Upper Amazon only, and the other one grouping animals from the Orinoco River, Madeira river (Bolivia), and the Upper Amazon (Figure 1). These two clades are well supported and show significant structure (Amova: 59.4% of the genetic variability is observed between clades, $F_{ST} = 0.59$, $p < 0.001$), with only a limited number of migrants (Nm = 0.17). The clade clustering animals from the upper Amazon has a lower haplotypic diversity ($H_d = 0.53$) than the clade that groups animals from different river basins ($H_d=0.80$). The haplotype network also identified these two groups (Figure 2), with two parallel central haplotypes, one from the Upper Amazon, and one shared with the Orinoco and Amazon basins. A starlike structure from these two haplotypes likely suggests either primary (Amazon) or secondary (Orinoco) dispersals from those two regions. Neither Fu's, Tajima's and Harpending's tests nor the Bayesian Skyline Plots showed evidence of demographic expansion, no matter what clade was considered.

Divergence times among (median = 37,000 years ago, YA) and within clades (medians of well supported nodes ranging from 10,000 to 20,000 YA) are very recent. Although Giant otters can be found in swamp areas, they show a marked preference for watercourses with overhanging vegetation. Population dispersals may have been concomitant with forest expansion in western Amazonia during the Holocene (Thompson et al., 2000). In fact, the temporal splits between the two main clades, and within them, agree quite well with some cold and dry periods during the fourth glacial period and during the Holocene. The middle Pleniglatial which occurred 60,000-26,000 YA was characterized by very cold temperatures although in general this epoch was not especially dry (Van der Hammen, 1992). However, a very dry period began 39,000 YA, which could coincide with the split between the two main clades determined in this study. This dry period continued up to 30,000-26,000 YA in the Bogota savannah causing the disappearance of the Bogota lagoon and the apparition of dry vegetation species, such as *Symplocos*, *Myrica, Myrsine* and *Alnus*. Meanwhile, in the Amazon, *Quercus* also appeared (Van der Hammen, 1980). This period was followed by the most intense cold and dry period of the fourth glaciation, which ranged from 26,000 to 14,000 YA. This period was related to the maximum extension of the glaciers in the northern hemisphere and the total disappearance of the Fuquene lagoon near Bogota (Van Geel and Van der Hammen, 1973). This period could be the most important cause of haplotype diversification within the two main clades. From 14,000 YA to 10,000 YA (Tardiglacial) the climate became hotter and wetter than what it had been previously, but there were some periods that were cold and dry. In the Fuquene lagoon, there were two cold phases, one between the Susacá and the Guantiva periods (12,000-13,000 YA) and the second, the very cold stadial of El Abra (10,000-11,000 YA) (Van der Hammen, 1992). This coincided with the last glacial advance in the Andes (11,800-9,500 YA; Dryas III; Clapperton, 1993). This could have be the last moment of haplotype differentiation in the upper Amazon for *Pteronura brasilensis*. However, keep in mind that during the last 10,000 YA, there were also alternative periods of dry versus wet climate. For instance, in the Agua Sucia Lagoon at the Ariari River, south of San Martín in the Meta Department within the Eastern Colombian Llanos, there were dry periods 6,000-5,000 YA, 4,000 YA and 2,200 YA. Dry periods also occurred in the Amazon 4,000 YA, 2,700-2,000 YA, 1,500-1,200 YA, 700 YA and 400 YA, while in the Magdalena, Cauca and San Jorge river basins, dry periods occurred 7,000-5,500 YA, 4,700 YA, 4,100-3,800 YA, 2,600-2,000 YA, 1,470-1,230 YA, 720 YA and 480 YA (Van der Hammen, 1992). Therefore, these last Holocene dry periods could also have some impact on the genetic haplotype distribution of the Giant otter.

Although considered as a limited cause of speciation (Haffer, 2008; see the case of the jaguar in this book, Ruiz-García et al., 2012), major river systems may represent important biogeographic barriers, widely applied to explain the distribution of several vertebrate groups (Ayres and Clutton-Brock, 1992; Capparella, 1988). This also applies to aquatic species because of varying water types (eg, in the black caiman, de Thoisy et al., 2006) and depth along watercourses (eg, in the pink dolphin, Hollatz et al., 2011). In large vertebrates, a gradient of structure related to the Amazon *versus* Orinoco basins among taxa can be observed, according to their dispersal capabilities. In fresh water turtles, *Podocnemis unifilis* shows signs of structuration (Escolana et al., 2009), but the larger *P. expansa* only has a limited structure (Pearse et al., 2006). Among mammals, the pink dolphin did not show strong genetic differentiation between the two basins (Banguera-Hinestroza et al,. 2002; Ruiz-García, 2010d). Consequently, the absence of structuration in a large Neotropical vertebrate such as the Giant otter, was not unexpected, and may be explained by the existence of the Cassiquiaré channel connecting watersheds (Meade and Koehnken, 1991).

Populations of Giant Otters in Western Amazonia are nevertheless significantly structured. In the southern part of the Giant otter range, populations were also found to be structured, but they did not corroborate with the existence of two evolutionarily separate clades occurring either in the Amazon or Pantanal. The genetic structure was more complex than what was expected considering watersheds only (Garcia et al., 2007). Similar complex structures are observed in different part of the species' range. Those structures may be related to bound tectonic compartments with distinctive sedimentary units commonly bound in major Amazon River systems (Rosseti et al., 2005). Therefore, the cause of vicariance could actually be directly related to differences in landscape evolution of large-scale tectonic compartments: species and/or population differentiation would not be controlled by river location only, but by geomorphological, sedimentological, pedological characteristics inherent to those compartments (Rossetti et al., 2005; Tuomisto et al., 2003). Secondary admixtures, suggested by Orinoco-related haplotypes of animals from the Upper Amazon region, may then be favored by highly dynamic rivers in the region: repeated formations of new major floodplains and loss of other ones led to continuous secondary contacts of formerly separated river systems (Salo et al., 1986), likely favoring allopatric divergence and admixture of associated animal populations.

Considering the relatively small sample size, high population differentiation data and the great number of observed haplotypes indicate that expansion of sampling areas will be necessary for a better characterization of those highly heterogeneous populations, as well as to provide efficient conservation strategies. Some recommendations can nevertheless be pointed out and discussed.

First, management of Giant otter populations cannot rely on a basin-river approach. As previously shown with other taxa (de Thoisy et al., 2010), South American eco-regions, either terrestrial (Dinerstein et al., 1995) or aquatic (Olson et al., 1998), defined on the basis of species richness, beta-diversity, and endemism, failed to reflect adequate management units for large vertebrates. Maintenance of connectivity between watercourses, through lowland and wetland areas preservation, may allow a maintenance of gene flow and population dynamic according to source–sink metapopulation models.

Second, as stated for the Pantanal and South Amazonia regions (Garcia et al., 2007), complex geographic structure underlines the importance of preserving all local populations, which do not cluster in major geographic groups but retain a large and unique genetic diversity.

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