

<http://dx.doi.org/10.5597/lajam00150>

REPORT OF THE WORKING GROUP ON TAXONOMY AND GENETICS

ANTONIO M. SOLÉ-CAVA^{1,*}, SUSANA CABALLERO², CIBELE R. BONVICINO³,
THAIS G. C. SHOLL^{4,5}, IGNACIO B. MORENO⁶, PAULO A. C. FLORES⁷

Introduction

Genetic data are extremely useful for the study of natural populations, particularly of endangered species (*e.g.* Avise and Hamrick, 1996; Solé-Cava, 2001; Avise, 2004). Genetic data have been applied in recent years to the study of *Sotalia* dolphins, where they have been helpful in clarifying an old controversy in the taxonomy of this genus by indicating the existence of differentiated evolutionary units along the South and Central American coast. These studies have also helped in sexing samples from biopsies or highly degraded tissues and in identifying body parts illegally commercialized in the Amazon Region. This report presents a summary of the discussions of the Taxonomy and Genetics working group undertaken during the 'Workshop on Research and Conservation of the genus *Sotalia*' held in Armação dos Búzios, Rio de Janeiro, Brazil, 19-23 June 2006. We present a brief report on the state of the art of genetic studies on *Sotalia*, as well as suggestions for future research. We hope that the use of genetic markers, together with sound analytical approaches, will help improving our knowledge on many aspects of the ecology and evolutionary biology of *Sotalia*.

Taxonomy and systematics

The taxonomic status of the genus *Sotalia* was discussed in the workshop based on recent independent analyses using molecular markers (Cunha *et al.*, 2005; Caballero *et al.*, 2007; Cunha and Solé-Cava, 2010), as well as morphological (Borobia, 1989; Monteiro Filho *et al.*, 2002) and ecological data (Best and da Silva, 1984; Rosas and Monteiro Filho, 2002). After considering the number of species that historically have described or assigned to the genus *Sotalia* (see the Introduction to this volume), consensus was reached that coastal and riverine *Sotalia* are true separate species.

The scientific name for the riverine species should be *Sotalia fluviatilis* (Gervais and Deville *in* Gervais, 1853) (see the Introduction to this volume; Robineau, 1990) and for the coastal species *Sotalia guianensis* (Van Bénédén, 1864). The working group agreed on the use of 'tucuxi' as the common name, in any language, for *S. fluviatilis*. Possible common names for *Sotalia guianensis* were discussed; however, no consensus on the common name for *S. guianensis* was achieved during the workshop. This was mainly because of the different common names used for this species in various regions and languages, including 'boto-cinza' (Brazil), 'delfin gris' or 'bufeo gris' (Colombia), 'tonina' (Venezuela) or 'lam' (Nicaragua). A common name, Guiana dolphin, was proposed for formal use in English publications; however, it was agreed that local common names should be accepted as well in a regional context (see Flores *et al.* 2010 this volume).

Phylogeography and population units

Sotalia guianensis

Based on the discussion of two working papers on phylogeography and population structure of *S. guianensis* (Cunha and Solé-Cava, 2006⁸; Caballero *et al.*, 2010a this volume), which showed high differentiation among *S. guianensis* populations, we preliminarily suggest the recognition of eight population units for conservation and management purposes (Figure 1).

Sotalia fluviatilis

Preliminary data presented here were obtained from samples collected in the main course of the Amazon River and a couple of tributaries. The limited available data do not indicate the same level of structuring found in *S. guianensis*, suggesting some level of connectivity among

¹ Laboratório de Biodiversidade Molecular, Dept. de Genética, Inst. Biologia, Ilha do Fundão, Sl. A2-098, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ Brasil

² Laboratorio de Ecología Molecular de Vertebrados Acuáticos, Departamento de Ciencias Biológicas, Universidad de los Andes, Carrera 1 No. 18A - 10, Bogotá, Colombia

³ Depto. de Medicina Tropical, Instituto Oswaldo Cruz/FIOCRUZ, Divisão de Genética, Rua Prefeito João Felipe, 310, Santa Teresa, Rio de Janeiro, RJ, 20251-140 Brasil

⁴ Projeto de Monitoramento de Aves e Mamíferos Marinhos na Bacia de Campos, CENPES/PETROBRAS, Rio de Janeiro, RJ, 21041-210 Brasil

⁵ ENSP/FIOCRUZ, Bolsista PIBIC/CNPq, Rua Leopoldo Bulhões, 1480-térreo Manguinhos, Rio de Janeiro, RJ, 21041-210 Brasil

⁶ Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul, Rua Felipe Néri, 382 conj. 203, Porto Alegre, RS, 90440-150 Brasil

⁷ Instituto de Pesquisa e Conservação de Golfinhos e Coordenador, Núcleo de Unidades de Conservação IBAMA, Gerex Manaus, R. 'Ministro João Gonçalves de Souza', s/n, Distrito Industrial, Manaus, AM, 69075-830 Brasil

*Corresponding author, e-mail: sole@biologia.ufrj.br

⁸ CUNHA, H.A. AND SOLÉ-CAVA, A.M. (2006). Phylogeography of *Sotalia guianensis* along the Brazilian coast. Page 22 *in* Siciliano, S., Borobia, M., Barros, N.B., Marques, F., Trujillo, F. and Flores, P.A.C. (Eds), Book of Abstracts, *Workshop on Research and Conservation of the genus Sotalia*, 19-23 June 2006, Armação dos Búzios, Rio de Janeiro, Brazil. *Latin American Journal of Aquatic Mammals* 8(1-2) (supplement). <http://dx.doi.org/10.5597/lajam00147.a012>

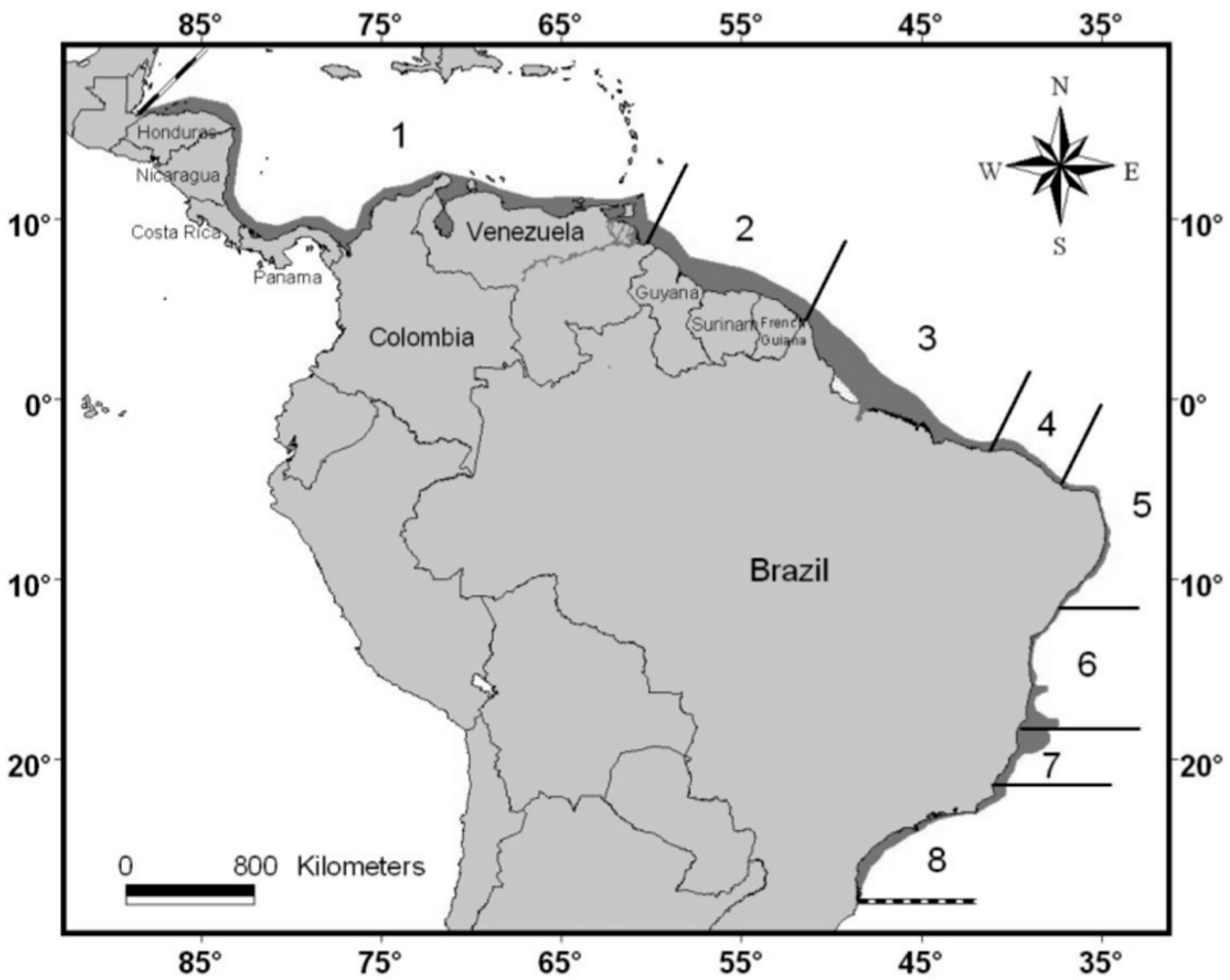


Figure 1. Proposed population units for *Sotalia guianensis*. Numbers correspond to: 1) Caribbean 2) Guianas (following Caballero *et al.*, 2010a this volume); 3) Amazonian Estuary and adjacent regions, 4) Ceará, 5) Rio Grande do Norte/Sergipe, 6) Bahia, 7) Espírito Santo and 8) South/Southeastern Brazil (following Cunha and Solé-Cava, 2006⁸; Cunha, 2007).

Amazonian populations and high genetic diversity in these groups (Caballero *et al.*, 2010b; Caballero *et al.*, 2010a this volume). In order to clearly establish population units for *S. fluviatilis*, sampling is needed in most tributaries of the Amazon River, including Negro, Branco, Xingu, Tapajós, Madeira, Caquetá, Putumayo, Napo, Cuyabeno and Ucayali rivers.

Use of molecular techniques for forensic research in *Sotalia*

Molecular techniques have been successfully used for identifying dolphin market samples in Amazonia, at the Mercado Municipal (Manaus) and Mercado Ver-o-peso

(Belém). All samples have been identified as *S. guianensis* even if they were sold as *Inia geoffrensis* (Gravena *et al.*, 2008; Sholl *et al.*, 2006a⁹; Cunha and Solé-Cava, 2007), suggesting movement of market samples between Belém and Manaus (Gravena *et al.*, 2008; Sholl *et al.*, 2006a⁹).

Use of molecular techniques for sexing biopsies and highly degraded samples

Sex identification for *Sotalia* individuals is difficult given that no obvious sexual dimorphism exists and direct observation of the genital area is usually not possible in the field. Sex identification can be performed on carcasses by direct observation; however, it can be problematic in

⁹ SHOLL, T.G., NASCIMENTO, F.F., LEONCINI, O., BONVICINO, C.R. AND SICILIANO, S. (2006a) Identificação taxonômica de amostras de botos comercializadas na região amazônica através de técnicas moleculares. Page 24 in Siciliano, S., Borobia, M., Barros, N.B., Marques, F., Trujillo, F. and Flores, P.A.C. (Eds), Book of Abstracts, *Workshop on Research and Conservation of the genus Sotalia*, 19-23 June 2006, Armação dos Búzios, Rio de Janeiro, Brazil. *Latin American Journal of Aquatic Mammals* 8(1-2) (supplement). <http://dx.doi.org/10.5597/lajam00147.a014>

the case of highly degraded stranded animals. For example, in a study of vertebrate lesions of stranded animals, only about 60% of the studied individual dolphins could be sexed (Mendonça de Souza *et al.*, 2006¹⁰), which partially hindered the cross-tabulation by sex of the different lesions observed. Molecular markers specific of the X (X-specific Zinc-finger protein) and Y (Y specific Zinc-finger protein and the Sex-determining region) chromosomes have been successfully used to sex *Sotalia* samples (Cunha and Solé-Cava, 2004¹¹; Cunha and Solé-Cava, 2007; S. Caballero, unpub. data). These markers allowed the sex identification of highly degraded samples (codes 2-5 of Geraci and Lounsbury, 1993), and are simple to set up and use in samples associated with ecological studies.

Recommendations and further studies

SYMPATRY BETWEEN *SOTALIA* SPECIES

Further research is needed in order to establish the distribution limits of *S. fluviatilis* and *S. guianensis* in the mouth of the Amazon River and the Amazonian Estuary. This seems to be the most likely geographic area where hybridization between these species could be happening. A study using both nuclear (*i.e.* introns, microsatellites) and mitochondrial DNA markers would allow identification of possible hybrids as well as the direction of hybridization (Caballero and Baker, 2010).

ORINOCO RIVER BASIN: *S. GUIANENSIS* OR *S. FLUVIATILIS*?

The species identity of *Sotalia* in the Orinoco River remains unclear. Sampling in the mouth of the Orinoco and along the river is crucial in order to clarify the taxonomic identity and the limits of the distribution of *Sotalia* in this river as well as their genetic relationship with both coastal and Amazonian groups. It is important to determine whether they are actually vagrants from the coast or if there are established resident populations in this river that could be genetically differentiated from both coastal and Amazonian groups.

GENETIC VARIATION AND POPULATION STRUCTURE

At the population level, additional studies are required to identify the population structure at a fine-scale in *S.*

guianensis and to clarify the existence and number of *S. fluviatilis* population units. Additional 'population markers' (acoustics, stable isotopes, particular parasite species and parasites loads, and morphometrics, among others) would allow further confirmation of population units. 'Population markers' have proven useful for identification and confirmation of population units in other cetaceans, including the franciscana, *Pontoporia blainvillei* (Bordino *et al.*, 2002; Marigo *et al.*, 2002).

Conflicting data exist on levels of cytochrome *b* variation in *S. guianensis*, with some estimates indicating very low intraspecific variation (Cunha *et al.*, 2005; Caballero *et al.*, 2010a this volume), whereas others suggest at least four different haplotypes for the Brazilian coast (Sholl *et al.*, 2006b¹²). More analyses for this marker are needed to try and understand the origins of this discrepancy.

Confirmation of the low genetic diversity in the South/Southeastern Brazil *S. guianensis* population unit (Cunha *et al.*, 2005; Cunha and Solé-Cava, 2006⁸; Flores *et al.*, 2006¹³; Caballero *et al.*, 2010a this volume) based on mitochondrial DNA is needed, including nuclear markers (*i.e.* microsatellites). Male migration between this and neighboring population units requires investigation, as it would complement the knowledge of the level of isolation of this particular population unit. Biparentally inherited molecular markers could be useful for this purpose, as has been shown for bottlenose dolphins in South Australia (*Tursiops aduncus*; Möller and Beheregaray, 2004) and Dall's porpoise (*Phocoenoides dalli*; Escorza-Treviño and Dizon, 2000).

The population structure of *S. guianensis* in the northern limit of the species distribution needs to be investigated, in order to determine genetic diversity in these groups and levels of gene flow between them and the Caribbean population unit.

The Maracaibo Lake population was considered part of the Caribbean population unit. However, some mtDNA haplotypes were unique to Maracaibo Lake and genetic diversity was high. Differentiation at the haplotypic level was detected between the Maracaibo Lake groups and the Colombian Caribbean (Caballero *et al.* 2010a this volume). Due to the fact that the Maracaibo Lake groups are under threat due to oil exploitation activities in the

¹⁰ MENDONÇA DE SOUZA, S.M.F., LAETA, M. AND SICILIANO, S. (2006). Lesões ósseas em colunas vertebrais de golfinhos do gênero *Sotalia*, provenientes do litoral norte do Rio de Janeiro, Brasil. Page 32 in Siciliano, S., Borobia, M., Barros, N.B., Marques, F., Trujillo, F. and Flores, P.A.C. (Eds), Book of Abstracts, *Workshop on Research and Conservation of the genus Sotalia*, 19-23 June 2006, Armação dos Búzios, Rio de Janeiro, Brazil. *Latin American Journal of Aquatic Mammals* 8(1-2) (supplement). <http://dx.doi.org/10.5597/lajam00147.a022>

¹¹ CUNHA, H.A. AND SOLÉ-CAVA, A.M. (2004). Marcadores moleculares para sexagem de amostras de *Sotalia fluviatilis* (Cetacea: Delphinidae) provenientes de biópsias e encalhes. 50^o Congresso Nacional de Genética, Florianópolis-SC, Brazil.

¹² SHOLL, T.G., NASCIMENTO, F.F., SICILIANO, S. AND BONVICINO, C.R. (2006b) Diversidade genética em *Sotalia* no Brasil. Page 23 in Siciliano, S., Borobia, M., Barros, N.B., Marques, F., Trujillo, F. and Flores, P.A.C. (Eds), Book of Abstracts, *Workshop on Research and Conservation of the genus Sotalia*, 19-23 June 2006, Armação dos Búzios, Rio de Janeiro, Brazil. *Latin American Journal of Aquatic Mammals* 8(1-2) (supplement). <http://dx.doi.org/10.5597/lajam00147.a013>

¹³ FLORES, P.A.C., DUFFIELD, D.A., SIMÕES-LOPES, P.C. AND WELLS, R.S. (2006). Lack of genetic variability in a small population of marine tucuxi? Page 27 in Siciliano, S., Borobia, M., Barros, N.B., Marques, F., Trujillo, F. and Flores, P.A.C. (Eds), Book of Abstracts, *Workshop on Research and Conservation of the genus Sotalia*, 19-23 June 2006, Armação dos Búzios, Rio de Janeiro, Brazil. *Latin American Journal of Aquatic Mammals* 8(1-2) (supplement). <http://dx.doi.org/10.5597/lajam00147.a017>

area, further studies are required to evaluate population health. This can be achieved by monitoring the population using a combined approach, analyzing genes related to immune function such as the major histocompatibility complex (MHC) (Murray *et al.*, 1999; Caballero *et al.*, 2010c this volume), developing a comprehensive toxicological study of polycyclic aromatic hydrocarbons (PAHs) levels in tissue samples and, ideally, using 'biomarkers' that can indicate alterations in the metabolic paths, reproduction, and in the survival of these dolphins, alterations that could be related with possible detrimental effects of toxic compounds (Downs *et al.*, 2000; Wetzel and Reynolds, 2006¹⁴).

Conclusions

Genetic data can be extremely important for conservation studies when used jointly with ecological, morphological and biological data. This can be clearly seen from the results discussed during the workshop that show how genetic analyses helped testing different hypotheses. We believe a joint and collaborative effort of ecologists, population geneticists and managers will be very fruitful for drawing guidelines for the conservation of *S. guianensis* and *S. fluviatilis*.

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Received 30 November 2009. Accepted 8 November 2010.
Managed by Carlos Olavarría.

