

TAXONOMIC STATUS OF THE GENUS *SOTALIA*:  
SPECIES LEVEL RANKING FOR “TUCUXI”  
(*SOTALIA FLUVIATILIS*) AND “COSTERO” (*SOTALIA*  
*GUIANENSIS*) DOLPHINS

S. CABALLERO

Laboratory of Molecular Ecology and Evolution,  
School of Biological Sciences,  
University of Auckland,  
Private Bag 92019, Auckland, New Zealand  
and  
Fundación Omacha, Diagonal 86A #30–38, Bogotá, Colombia

F. TRUJILLO

Fundación Omacha, Diagonal 86A #30–38, Bogotá, Colombia

J. A. VIANNA

Sala L3–244, Departamento de Biologia Geral,  
ICB, Universidad Federal de Minas Gerais,  
Avenida Antonio Carlos, 6627 C. P. 486,  
31270–010 Belo Horizonte, Brazil  
and

Escuela de Medicina Veterinaria,  
Facultad de Ecología y Recursos Naturales,  
Universidad Andres Bello  
Republica 252, Santiago, Chile

H. BARRIOS-GARRIDO

Laboratorio de Sistemática de Invertebrados Acuáticos (LASIA),  
Postgrado en Ciencias Biológicas,  
Facultad Experimental de Ciencias, Universidad del Zulia,  
Avenida Universidad con prolongación Avenida 5 de Julio,  
Sector Grano de Oro, Maracaibo, Venezuela

M. G. MONTIEL

Laboratorio de Ecología y Genética de Poblaciones,  
Centro de Ecología,  
Instituto Venezolano de Investigaciones Científicas (IVIC),  
San Antonio de los Altos, Carretera Panamericana km 11,  
Altos de Pipe, Estado Miranda, Venezuela

S. BELTRÁN-PEDREROS

Laboratorio de Zoológia,  
Coleção Zoológica Paulo Burheim,  
Centro Universitário Luterano de Manaus, Manaus, Brazil

M. MARMONTEL

Sociedade Civil Mamirauá,  
Rua Augusto Correa No.1 Campus do Guamá,  
Setor Profissional, Guamá, C. P. 8600,  
66075-110 Belém, Brazil

M. C. SANTOS

Projeto Atlantis/Instituto de Biologia da Conservação,  
Laboratório de Biologia da Conservação de Cetáceos,  
Departamento de Zoologia, Universidade Estadual Paulista (UNESP),  
Campus Rio Claro, São Paulo, Brazil

M. ROSSI-SANTOS

Instituto Baleia Jubarte-Barão do Rio Branco, 26,  
45900-000 Caravelas, Brazil

F. R. SANTOS

Sala L3-244, Departamento de Biologia Geral,  
ICB, Universidad Federal de Minas Gerais,  
Avenida Antonio Carlos, 6627 C. P. 486,  
31270-010 Belo Horizonte, Brazil

C. S. BAKER

Laboratory of Molecular Ecology and Evolution,  
School of Biological Sciences,  
University of Auckland,  
Private Bag 92019, Auckland, New Zealand  
and  
Marine Mammal Institute,  
Hatfield Marine Science Center,  
Oregon State University,  
2030 SE Marine Science Drive,  
Newport, Oregon 97365, U.S.A.  
E-mail: scott.baker@oregonstate.edu

#### ABSTRACT

Dolphins of the genus *Sotalia* are found along the Caribbean and Atlantic coasts of Central and South America and in the Amazon River and most of its tributaries. At present, the taxonomy of these dolphins remains unresolved. Although five species were described in the late 1800s, only one species is recognized currently (*Sotalia fluviatilis*) with two ecotypes or subspecies, the coastal subspecies (*Sotalia fluviatilis guianensis*) and the riverine subspecies (*Sotalia fluviatilis fluviatilis*). Recent morphometric analyses, as well as mitochondrial DNA analysis, suggested recognition of each subspecies as separate species. Here we review the history of the classification of this genus and present new genetic evidence from ten nuclear and three mitochondrial genes supporting the elevation of each subspecies to the species level under the Genealogical/Lineage Concordance Species Concept and the criterion of irreversible divergence. We also review additional evidence for this taxonomic revision from previously published and unpublished genetic, morphological, and ecological studies. We propose the common name “costero” for the coastal species, *Sotalia guianensis*

(Van Bénédén 1864), and accept the previously proposed “tucuxi” dolphin, *Sotalia fluviatilis* (Gervais, 1853), for the riverine species.

Key words: tucuxi, mtDNA, nuclear DNA, taxonomy, *Sotalia guianensis*, *Sotalia fluviatilis*.

Dolphins of the genus *Sotalia* are endemic to the Caribbean and Atlantic coasts of South America, ranging from Nicaragua to southern Brazil (Borobia *et al.* 1991, Carr and Bonde 2000) and inhabiting the Amazon River and most of its tributaries (Borobia *et al.* 1991, da Silva and Best 1996).

Alexander von Humboldt was probably the first naturalist to document the presence of coastal dolphins that ascended the mouths of rivers in Venezuela. During his travels in northern South America, between 1799 and 1804, he noted the presence of relatively small dolphins with prominent dorsal fins about 130 km up from the mouth of the Orinoco River, in San Fernando de Apure, Venezuela (Hershkovitz 1962). From these first descriptions, it is safe to assume that the dolphins described by Humboldt belonged to the genus later recognized as *Sotalia* (Hershkovitz 1962).

The taxonomy of this genus has been controversial. In the late 1800s, five species were described, three from riverine specimens and two from coastal specimens (Rice 1998). The first riverine species was described by Gervais in 1853 as *Delphinus fluviatilis*, from a specimen collected in the Peruvian Amazon close to Pebas (van Bree 1974, Robineau 1990). Gray placed this species in the genus *Sotalia* in 1866 (Robineau 1990). In 1855 Gervais also described *Delphinus pallidus* from a specimen collected near Nauta in the Peruvian Amazon. However, *Delphinus pallidus* is now considered to be a coloration variant of the original *Delphinus fluviatilis* (Hershkovitz 1966, Robineau 1990). In 1856 Gray described a third riverine species, originally named *Steno tucuxi*, from a specimen collected in the Brazilian Amazon near Santarém (Hershkovitz 1966, da Silva and Best 1994). This species was later classified in the genus *Sotalia* by Flower (1883), but it is now recognized as a synonym of *Sotalia fluviatilis* (da Silva and Best 1994).

Van Bénédén initially described one coastal species, *Delphinus guianensis* (Van Bénédén 1864), based on three specimens collected from the mouth of the Marowijna River in the border between Surinam and French Guiana (Williams 1928, Hershkovitz 1962, Husson 1978). This species was also reclassified by Gray as a member of the genus *Sotalia*. In 1866 (Hershkovitz 1966, da Silva and Best 1994) Van Bénédén described a second coastal species in 1875, from a specimen collected in Rio de Janeiro Bay, Brazil. This species was designated as *Sotalia brasiliensis* and it is now considered a synonym of *Sotalia guianensis* (Hershkovitz 1966, da Silva and Best 1994).

Recent reviews have resulted in a series of revisions (Cabrera 1961), first reducing the number of species to two, one riverine, *Sotalia fluviatilis*, and one coastal, *Sotalia guianensis*, and later to one species, *Sotalia fluviatilis*, with two ecotypes or subspecies, *Sotalia fluviatilis fluviatilis* (riverine subspecies) and *Sotalia fluviatilis guianensis* (coastal subspecies) (Borobia *et al.* 1991, da Silva and Best 1994, Rice 1998). Tridimensional morphometric analysis of skull shape showed significant differences between riverine and coastal specimens suggesting the designation of each subspecies as separate species again (Monteiro-Filho *et al.* 2002). Furthermore, Cunha *et al.* (2005) reported differences between the two subspecies along their Brazilian distribution

based on the phylogenetic analysis of mitochondrial (mt) DNA (control region and cytochrome *b*).

Although cetacean species have generally been recognized under some interpretation of the Biological Species Concept (BSC) (Mayr 1963), the criterion of reproductive isolation is difficult to apply to allopatric forms or populations (Endler 1977, Rice 1998). An alternative is the use of the Genealogical/Lineage Concordance Species Concept (GCC) (Avice and Ball 1990, Avice and Wollenberg 1997, Avice 2000). This approach was used in defining a new species of beaked whale (*Mesoplodon perrini*) (Dalebout *et al.* 2002, 2004) and reviewed by a specialized workshop on cetacean taxonomy (Reeves *et al.* 2004). This concept attempts to reconcile elements from both the BSC and the Phylogenetic Species Concept (PSC), stating that phylogenetic diagnoses should be based on multiple independent genetic traits such as information contained in multiple loci (Avice and Ball 1990, Avice and Wollenberg 1997).

Here we present a formal proposal to recognize each *Sotalia* subspecies as full species under the GCC, based on diagnostic genetic characters consistent with the criterion of "irreversible divergence" (Reeves *et al.* 2004). We include analysis of DNA sequences from ten nuclear introns, three of which have fixed-site differences between coastal and riverine *Sotalia*, and three gene fragments of the mitochondrial genome. Our geographic sampling extends that of Cunha *et al.* (2005), with independent samples from populations along most of the distribution range of the two subspecies, including locations along the Amazon River and some of its tributaries as well as coastal locations in Nicaragua, Colombia, Venezuela, French Guiana, and Brazil. In support of our proposal, we also review unpublished morphometric data (Borobia 1989), as well as published biogeographical data (Borobia *et al.* 1991, da Silva and Best 1996) and ecological information for each *Sotalia* subspecies (Best and da Silva 1984, Borobia and Barrios 1989, da Silva and Best 1996, Santos *et al.* 2001, Rosas and Monteiro-Filho 2002).

## METHODS

### *Sample Collection and DNA Extraction*

A total of seventy-six samples of skin, liver, bone, or teeth samples were obtained from coastal and riverine *Sotalia* in twenty-one locations grouped into nine geographic regions throughout its range (Fig. 1, Table 1, Appendix 1). Tissue samples were obtained from dolphins found stranded dead or dolphins drowned in fishing nets ( $n = 55$ ). Bones and teeth were obtained from skeletal remains found in the field ( $n = 8$ ) and from museum specimens ( $n = 4$ ). Skin samples from the Colombian Caribbean were obtained from captive ( $n = 4$ ) as well as free ranging ( $n = 2$ ) dolphins. Samples from captive dolphins were obtained by removing a small piece of skin from the tail. Skin from free-ranging dolphins was collected using a small biopsy dart deployed from a modified veterinary capture rifle (Krützen *et al.* 2002). Skin and liver samples were stored in 70% ethanol at  $-20^{\circ}\text{C}$ . Bones and teeth samples were stored at room temperature in individual sealed bags. Two DNA samples were obtained from the DNA and Tissue Archive at the Southwest Fisheries Science Center (NOAA, La Jolla, CA). DNA extraction from tissue samples followed the protocol of Sambrook *et al.* (1989), modified for small samples by Baker *et al.* (1994). DNA was extracted from bones following a silica-guanidinium thiocyanate based protocol described by Pichler *et al.* (2001a). Samples collected in Brazil ( $n = 30$ ) were analyzed at Universidade Federal de Minas Gerais (UFMG) in Belo Horizonte, Brazil. Other samples ( $n = 46$ ) were analyzed at the University of Auckland.

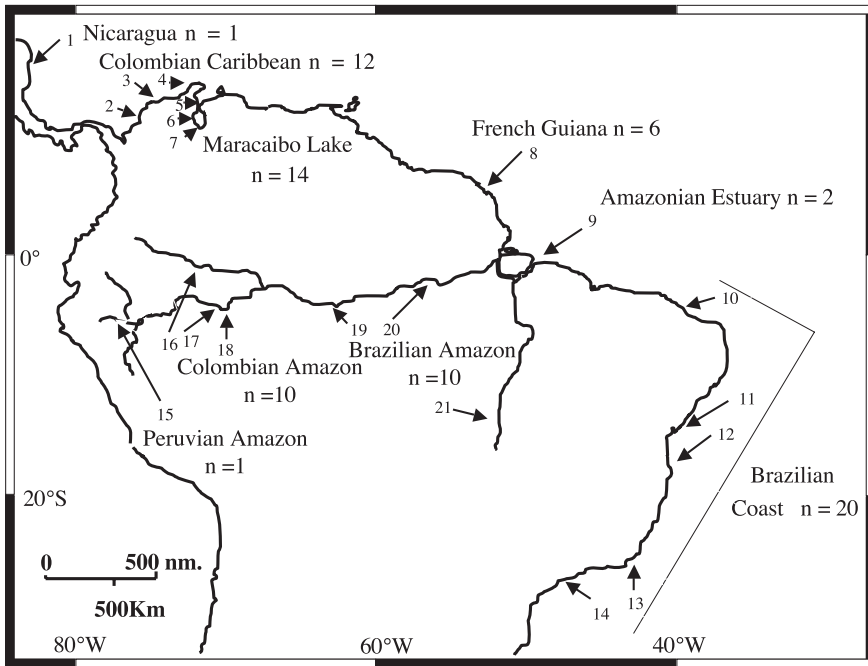


Figure 1. Distribution of coastal and riverine *Sotalia* showing geographic regions, sampling locations (numbers refer to Table 1), and sample sizes included in this study.

#### PCR Amplification and Sequencing

Three mitochondrial genetic markers were amplified and sequenced from all available samples (Table 2): a 627 base pair (bp) portion of the mitochondrial DNA control region (CR), a 425 bp fragment of the cytochrome *b* gene (*Cyt-b*), and a 1,044 bp fragment of the NADH dehydrogenase subunit 2 gene (*ND2*).

Ten nuclear introns (4,312 bp in total) were screened for ten *Sotalia* (five coastal and five riverine) as well as two other delphinid species: *Sousa chinensis* and *Steno bredanensis* (Table 2). Seven introns (including all Y chromosome introns) showed no variation between coastal and riverine *Sotalia* samples. The other three, including the first exon and first partial intron of the  $\alpha$ -Lactalbumin gene (*Lac-1*), the first Actin intron (*Act-1*), and the Glucocerebrosidase intron (*GBA*), showed fixed nucleotide differences between the initial ten samples of coastal and riverine *Sotalia* so were sequenced for the remaining eighteen specimens. Thus, a total of 28 *Sotalia* samples with high quality DNA (21 coastal and 7 riverine) from seven geographic locations (Table 1) were sequenced for these three introns.

For samples sequenced at the University of Auckland, free nucleotides and primers were removed from the PCR products using SAP (shrimp alkaline phosphatase) and ExoI (exonuclease I) (USB) and directly sequenced in both directions using the standard protocols of Big Dye terminator sequencing chemistry on an ABI 3100 automated capillary sequencer. Samples sequenced at Universidade Federal de Minas Gerais (UFMG) were amplified following the previously described protocol, cleaned using 20% PEG (Polyethyleneglycol), and sequenced using an ETDye terminator kit on a MegaBACE automated capillary sequencer (Amersham Biosciences, Piscataway, NJ).

Table 1. Summary of sampling locations, tissue type, and number of mitochondrial and nuclear sequences obtained for coastal and riverine *Sotalia* (see Appendix 1). Numbers in parenthesis before each sampling location correspond to the number of this sampling location in Figure 1.

Geographic region	Sampling location	Subspecies	Sample size and type	mtDNA	nuDNA
Nicaragua	(1) Mouth of the Layasiksa River, Waunta Lagoon	Coastal	1 tooth <sup>a</sup>	1	-
Colombian Caribbean	(2) Morrosquillo Gulf (Córdoba province)	Coastal	4 skin 1 tooth	5	4
Maracaibo Lake	(3) Santa Marta (Magdalena province)	Coastal	3 skin	3	1
	(4) La Guajira province	Coastal	4 skin	4	3
	(5) Zapara Island	Coastal	11 skin	14	1
	(6) Barranquitas	Coastal	2 skin		
	(7) Mouth of the Catarumbo River	Coastal	1 bone		
	(8) Cayenne	Coastal	6 skin	6	4
French Guiana	(9) Belém (Pará state)	Coastal	2 skin	2	-
Amazonian Estuary	(10) Ceará state	Coastal	1 liver	1	-
Brazilian Coast	(11) Bahía state	Coastal	2 skin	2	1
	(12) Espírito Santo state	Coastal	2 skin	2	1
Peruvian Amazon	(13) Rio de Janeiro state	Coastal	2 skin 1 DNA <sup>b</sup>	3	-
	(14) Cananéia estuary (São Paulo state)	Coastal	12 skin	12	6
	(15) Curaray River	Riverine	1 skin	1	1
	(16) Caquetá River	Riverine	2 bone	2	-
	(17) Puerto Nariño (Amazonas province)	Riverine	2 skin 4 tooth	6	-
	(18) Leticia (Amazonas province)	Riverine	1 skin 1 tooth	2	1
Brazilian Amazon	Unknown	Riverine	1 DNA <sup>b</sup>	1	1
	(19) Tefé (Amazonas state)	Riverine	7 skin	7	4
	(20) Santarém (Pará state)	Riverine	1 bone	1	-
	(21) Formoso Araguaia River	Riverine	1 bone	1	-

<sup>a</sup> Sample donated by the USNM: United States National Museum Smithsonian Institution, Washington, DC.

<sup>b</sup> Sample donated by the SWFSC: Southwest Fisheries Science Center, La Jolla, CA.

Table 2. Summary of loci and amplification conditions used in study of *Sotalia* and outgroups.

Locus	Primer name	Annealing temperature	Observed product size	Reference
mtDNA				
CR	TRO	52°C	531 bp	R. LeDuc (SWFSC)
	D			
CR	t-Pro-whale M13Dlp1.5	55°C	800 bp	DNA surveillance (www.dna-surveillance.ac.nz)
	Dlp8			
CR	t-Pro-whale M13Dlp1.5	55°C	400 bp	Baker <i>et al.</i> (1998)
	Dlp4			
Cyt- <i>b</i>	Tglu	55°C	464 bp	Palumbi (1996)
	CB2			
ND2	ILP5100R	55°C	1,050 bp	T. Mclenachan (Alan Wilson Centre, Massey University)
	BatH4823			
	tRNA-metF			
ND2	BatL4235	55°C	Sequencing	T. Mclenachan (Alan Wilson Centre, Massey University)
	BatH4461			
nuDNA (autosomal introns)				
Act-1	Act-3	55°C	980 bp	Palumbi and Baker (1994 and Conway (2005)
	Act-1385			
Lac-1	LacIR	54°C	600 bp	Milinkovitch <i>et al.</i> (1998)
	LacIIF			
GBA	GBA-F	55°C <sup>a</sup>	310 bp	Roca <i>et al.</i> (2001)
	GBA-R			
CHRNA1	CHRNA1-F	55°C <sup>a</sup>	360 bp	Roca <i>et al.</i> (2001)
	CHRNA1-R			
CAT	CAT-F	55°C <sup>a</sup>	520 bp	Lyons <i>et al.</i> (1997)
	CAT-R			
IFN	IFN-F	55°C <sup>a</sup>	340 bp	Lyons <i>et al.</i> (1997)
	IFN-R			
nuDNA (Y chromosome introns)				
DBY7	DBY7-F	55°C <sup>a</sup>	400 bp	Hellborg and Ellegren (2003)
	DBY7-R			
DBY8	DBY8-F	55°C <sup>a</sup>	200 bp	Hellborg and Ellegren (2003)
	DBY8-R			
SMCY7	SMCY7-F	55°C <sup>a</sup>	500 bp	Hellborg and Ellegren (2003)
	SMCY7-R			
UBE1Y7	UBE1Y7-F	50°C <sup>a</sup>	500 bp	Hellborg and Ellegren (2003)
	UBE1Y7-R			

<sup>a</sup>Amplification using *Taq*GOLD.

DNA extracted from bones, teeth, or degraded skin proved unsuitable for amplification of nuclear genes. However, most of these samples were suitable for amplification of mtDNA. To protect against contamination, samples were run in at least two separate PCR reactions, including extraction blanks. To improve the confidence and accuracy of our results, PCR products from the two independent amplifications were sequenced in both forward and reverse directions separately and then compared.

### Data Analyses

Sequence quality was evaluated using the program *Pbred* v.020425 (Ewing and Green 1998, Ewing *et al.* 1998). Sequences with *Pbred* scores  $\leq 20$  (a base call having a probability of more than 1/100 of being incorrectly called) were resequenced. Sequences with *Pbred* score values between 20 and 40 (a probability between 1/100 and 1/10,000 of being incorrectly called) were checked by eye to confirm polymorphic sites. A polymorphic site was indicated by a secondary peak with a height  $\geq 30\%$  of the height of the primary peak and by a slight decline in the *Pbred* score. All sequences were edited manually and aligned using Sequencher 4.1 software (Genes Code Corporation, Ann Arbor, MI). For the combined mitochondrial data set (2,096 bp), as well as for the combined nuclear data set (4,312 bp), haplotypes (in the case of mtDNA) or genotypes [in the case of nuclear DNA (nuDNA)] were defined using MacClade (Maddison and Maddison 2000). Sequences were submitted to GenBank as accession numbers EF027006 to EF027092.

Using the program MEGA2 (Kumar *et al.* 2001), we calculated the number of variable and fixed-site differences for all genes, as well as the proportion of synonymous and non synonymous substitutions in the case of the two protein coding genes studied (*Cyt-b* and ND2). The model of nucleotide substitution for the two combined data sets was tested in Modeltest v3.06 (Posada and Crandall 1998), and the settings for this model were used in the Neighbor-Joining and Maximum-Likelihood phylogenetic reconstructions with bootstrap resampling performed in PAUP version 4.0b1 (Swofford 2002). A Maximum Parsimony reconstruction with bootstrap resampling was also performed with PAUP. *Steno bredanensis* (rough-toothed dolphin) and *Sousa chinensis* (Indo-Pacific humpbacked dolphin) were used as outgroups for these analyses. A Partitioning of Homogeneity Test was conducted with PAUP to determine if phylogenies reconstructed from combined mitochondrial genes (mtDNA) and combined nuclear genes (nuDNA) differed significantly from the total combined data set (mtDNA + nuDNA).

To estimate the time of divergence between the coastal and riverine *Sotalia*, we constructed a molecular clock using mtDNA CR sequences from sixteen coastal and thirteen riverine *Sotalia* and one harbor porpoise (*Phocoena phocoena*) sequence (GenBank accession number Y13875). These sequences were compared to estimate the rate of nucleotide substitution per lineage per year ( $\lambda$ ) using the equation  $\lambda = d_1/2T_1$ , where  $d_1$  is the adjusted divergence and  $T_1$  is the time since divergence (Li 1997) between the Phocoenidae and Delphinidae lineages (Harlin *et al.* 2003). This date was assumed to be between 10 and 11 mya based on the fossil record (Barnes 1985) and from molecular data (Waddell *et al.* 2000). Due to software limitations, we used the Tamura–Nei model of nucleotide substitution (Nei and Kumar 2000) in MEGA2 to estimate  $d_1$  with gamma-corrected Tamura–Nei pairwise distance ( $\alpha = 0.5$  from the Modeltest output). Net nucleotide divergence ( $d_2$ ) between and within *Sotalia* subspecies was calculated for the combined mitochondrial data set (2,096 bp) as well as for the initial 450 bp of the mitochondrial CR. We used the Tamura–Nei model (Nei and Kumar 2000) in MEGA2 to estimate  $d_2$  with a pairwise gamma-corrected Tamura–Nei distance ( $\alpha = 0.5$  from the Modeltest output). The previously calculated rate of nucleotide substitution per lineage per year ( $\lambda$ ) was then used to calculate the divergence time between riverine and coastal *Sotalia* ( $T_2$ ) using the equation  $T_2 = d_2/2\lambda$ .

Net nucleotide divergence between coastal and riverine *Sotalia* was also calculated from the total nuDNA combined data set (4,312 bp) using MEGA2 with a



gamma-corrected Tamura–Nei pairwise distance and the parameters from the Modeltest output.

## RESULTS

### *DNA Sequence Data*

*mtDNA*—A total of 2,096 bp of the mitochondrial genome (CR, ND2, and *Cyt-b*) was amplified and sequenced from 51 *Sotalia* specimens representing coastal and riverine subspecies. An additional 25 samples of poorer quality were sequenced for only a short fragment of the mtDNA CR (350–400 bp). A total of 31 different haplotypes were identified within these 76 samples (Table 3), 29 of which were distinguished by substitutions in the CR, and 2 that were distinguished by additional variable sites in the *Cyt-b* gene. A total of 85 variable sites were found along the 2,096 bp of the combined mtDNA genes data set (Table 3). For the CR, 37 variable sites were found, 11 of which represented fixed-site differences between the haplotypes corresponding to coastal and riverine *Sotalia*. For *Cyt-b*, 12 variable sites were found, 5 of which represented fixed-site differences between the 2 subspecies. For the ND2 gene, 36 variable sites were found, 20 of which were fixed between subspecies. Four of these fixed-site differences corresponded to non synonymous substitutions (Table 3).

*nuDNA*—Initial screening showed one fixed-site difference between coastal and riverine *Sotalia* in each of three introns, a 592 bp fragment of Lac-1, 950 bp of Act-1, and 308 bp of GBA. The presence of the three fixed sites was confirmed in an additional sample of coastal ( $n = 16$ ) and riverine ( $n = 2$ ) *Sotalia* for a total of twenty-eight specimens compared. All were transitions (A to G or G to A, Fig. 2). One variable site (position 32) was found in Act-1 in one individual from the Colombian Caribbean (a heterozygote) as evidenced by double peaks. Otherwise, no variable sites or heterozygotes were found in the screening.

Across the total of ten introns used in the initial screening, only one fixed-site difference in the Y chromosome intron UBE1Y7 was found to be diagnostic for *Sotalia* when compared to *Sousa chinensis*. Four additional fixed-site differences in the Y chromosome intron DBY8 and in the autosomal introns GBA, CAT, and Lac-1 were shared between *Sotalia* and *Sousa chinensis* relative to *Steno bredanensis* (Fig. 2).

### *Phylogenetic Analyses*

For the three mitochondrial genes, Modeltest indicated the best-fit model of substitution to be the HKY + I + G. The Partition of Homogeneity Test found no conflict in phylogenies ( $P = 0.97$ ) for the individual and combined mitochondrial data set. Phylogenetic reconstructions by Maximum Parsimony, Maximum Likelihood, and Neighbor Joining showed clear reciprocal monophyly for individual and combined genes between haplotypes of the two *Sotalia* subspecies (data for the Maximum Parsimony reconstruction based on the combined mitochondrial data set shown only, Fig. 2).

For the three nuclear loci, the best-fit model of nucleotide substitution was the HKY. A Partitioning of Homogeneity Test showed no conflicting phylogenies ( $P = 0.96$ ) for the combined nuDNA data set or the total combined data set (mtDNA + nuDNA,  $P = 0.99$ ). Phylogenetic reconstructions by Maximum Parsimony, Neighbor



Table 3. Continued.

Haplotype	Variable sites			Cytochrome b (425 bp)
	Control region (627 bp)	ND2 (1044 bp)		
M	.....TC	.....	.....?	.....
N	.....T	.....	.....?	.....
O	A...T.....C..T	.....	.....	.....
P	.....T.C.....C..?	.....	.....	.....
Q	.....T.C.....C..ACT	.....	.....	.....
R	.....T.C.....C..CT	.....	.....	.....
S	TTGTCG..GGGT.CC..T...A.....CG.AATG	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT?	.T.TA...G.C.
T	TT.TCG..GGG..CC..T...A.....CG.AATG	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT?	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT?	.T.TA...G.C.
U	TTGTCG..GGG..CC.T			
V	TTGTCG..GGGT.CC.T			
W	TTGTCG..GGG..CC..T...A.....CGCAATG	??TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	??TCGCCATG..GG.ATTTTG..CTCCG.TATAGT?	.T.TA...G.C.
X	TTGTCG..GGG..CC..T...A.....CG.AATG	..TCGCCATGC.GG.ATTTTG..CTCCG.TATAG??	..TCGCCATGC.GG.ATTTTG..CTCCG.TATAG??	.T.TA...G.C.
Y	TTGTCG..GGG..CC..T...A.....C???????	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	.T.TA...G.C.
Z	TTGTCG..GGGT.CC..T...A.....CG.AATG	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	.T.TA...G.C.
AA	TTGTCG..GGG..C...			
BB	TT.TCG..GGG.C...T...A...A.			
CC	TTGTCG..GGG..CC..T...A.....CG.AATG	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	..TCGCCATG..GG.ATTTTG..CTCCG.TATAGT.	.T.TA...G.C.
DD	TT.TCGC..GGG..C...TT.C.A...CG.AATG	..TCGCCA.G.CG..ATTTTTG..CTCCG.TATA.??	..TCGCCA.G.CG..ATTTTTG..CTCCG.TATA.??	.T.TA..TG.C.
EE	TT.TCGCTGGG..C...TT.C.A...C???????	..TCGCCA.G.CG..ATTTTTG..CTCCG.TATA.T.	..TCGCCA.G.CG..ATTTTTG..CTCCG.TATA.T.	.T.TA...G.C.

Bone samples

Bone samples

Bone samples

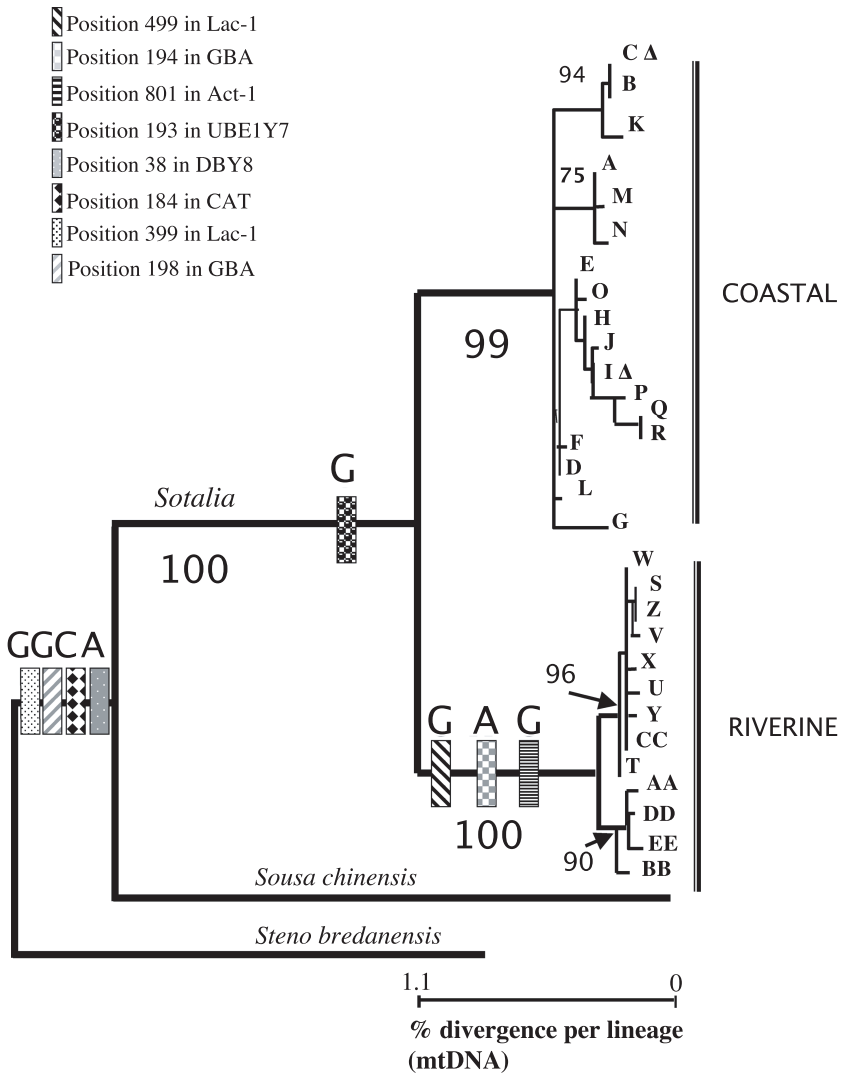


Figure 2. Maximum Parsimony phylogenetic reconstruction of the combined mitochondrial haplotypes (2,096 bp) of *Sotalia* and outgroups with bootstrap values from 1,000 replicates. Letters at the end of the branches represent haplotype codes. ( $\Delta$ ) indicates haplotypes distinguished on the basis of the *Cyt-b* gene. The percent divergence was calculated in MEGA2 using the Tamura–Nei distance option and the settings for the  $\alpha$  and  $T_i/T_v$  output from Modeltest. Vertical bars on tree represent nuclear fixed-site differences between coastal and riverine *Sotalia* as cladistic characters, showing their derived state in riverine *Sotalia*, and fixed differences shared between *Sotalia* and *Sousa chinensis* relative to *Steno bredanensis*.

Joining, and Maximum Likelihood showed clear reciprocal monophyly for individual and combined gene fragments (including the combined mtDNA + nuDNA) between haplotypes and genotypes of the two subspecies with high bootstrap support (data not shown). The small number of fixed-site differences found between both subspecies

at the nuclear level (synapomorphies) were mapped as cladistic characters onto the phylogenetic reconstruction for the combined mitochondrial haplotypes (Fig. 2).

#### *Genetic Divergence Within and Between Coastal and Riverine Sotalia*

After controlling for within-species differences, net nucleotide divergence between the coastal and riverine subspecies was 2.2% for the combined mtDNA data set, 2.5% when considering only 450 bp of the CR, and 1.1% considering only 425 bp of *Cty-b*. Average difference within the riverine subspecies was estimated as 0.2% for the combined mtDNA gene fragment, 0.6% for the CR only, and 0.1% for *Cty-b*, compared to 0.4%, 1%, and 0.1% respectively for the coastal subspecies. For comparison, we calculated the net divergence between coastal *Sotalia* and *Steno bredanensis* to be 10.6% and between *Sotalia* and *Sousa chinensis* to be 10.9% for the CR only. Net divergence between coastal and riverine *Sotalia* at the nuclear level was 0.07% calculated from the 4,312 bp of the total data set of ten introns, and less than 0.001% within each subspecies. For comparison, net divergence at the nuclear level was 0.09% between *Sotalia* and *Sousa chinensis* and 0.16% between *Sotalia* and *Steno bredanensis*.

#### *Divergence Time Between Coastal and Riverine Sotalia*

Given the assumed divergence time of 10–11 mya and the net divergence of 24% between *Sotalia* and *Phocoena phocoena* (harbor porpoise) calculated for the mtDNA CR with the Tamura–Nei model, the rate of nucleotide substitution was estimated to range from  $1.11 \times 10^{-8}$  to  $1.22 \times 10^{-8}$  bp<sup>-1</sup> yr<sup>-1</sup>. Given the net divergence of 2.5% for the CR, this suggests that coastal and riverine *Sotalia* diverged approximately 1–1.2 mya.

### DESCRIPTION

Our genetic evidence supports recognition of the two *Sotalia* subspecies as full species based on the four criteria of the GCC (Avice and Ball 1990, Avice and Wollenberg 1997, Avice 2000).

(1) *Concordance across sequence characters within genetic locus leading to conclusive exclusion*—In the combined mitochondrial data set, thirty-six fixed-site differences were found between coastal and riverine *Sotalia*. Four of these fixed-site differences corresponded to non synonymous substitutions in the mitochondrial gene ND2, suggesting some functional as well as neutral divergence. An additional three fixed-site differences were found among the combined nuclear gene fragments. We consider that these sites represent the primary molecular diagnostic characters distinguishing the two species. The low number of fixed-site differences at the nuclear level was expected, due to the slower rates of evolution of the nuclear genome (Hare *et al.* 2002).

(2) *Concordance in genealogical patterns across multiple loci, both mitochondrial and nuclear*—Phylogenies constructed from mtDNA lineages (*i.e.*, haplotypes) as well as from nuclear intron sequences showed a pattern of reciprocal monophyly and fixed characters satisfying the Exclusivity Criterion and Cladistic Haplotype Aggregation method of species delimitation for the GCC (Sites and Marshall 2003). For this method, a population is considered a species if the haplotypes of all its members

are joined in a contiguous section of an unrooted parsimony cladogram, forming monophyletic groups that are separated and distinct from other such clades by a single branch that contains character-state changes leading to fixed differences (Sites and Marshall 2003).

(3) *Concordance with biogeographical patterns*—The distribution of *Sotalia* showed complete concordance with the phylogenetic patterns observed in our analysis. Coastal and riverine populations occur in physical isolation (allopatry) with little overlap possible only at the mouth of the Amazon River and the Amazonian Estuary (Borobia *et al.* 1991, da Silva and Best 1996). With the number of samples analyzed to date, we cannot exclude the possibility of some hybridization of *Sotalia* in this area of overlap, as observed between the Antillean manatee (*Trichechus manatus*) and the Amazonian manatee (*T. inunguis*) (Vianna *et al.* 2006). Outside this area, however, we have found no evidence of either nuclear or mitochondrial gene flow or introgression between the two proposed species (Caballero *et al.* 2003, Cunha *et al.* 2005).

(4) *Concordance with morphological characters*—Coastal and riverine *Sotalia* differ in various morphological characteristics including slight differences in body coloration, dimensions of the orbital region, and number of teeth. However, these are average differences and not discontinuities. Nevertheless, considered together, these average differences suggest some “morphological divergence.” Borobia (1989) recorded and compared using paired *t*-tests a total of 37 cranial character measurements from 58 *Sotalia* skulls including 21 riverine and 38 coastal specimens. Out of the 37 cranial characters compared, 29 were significantly different with a *P* value <0.001. These characters included the length of the rostrum, the number of teeth in the upper maxilla, the internal length of the braincase, and the length of the left tympanic cavity. Characters such as the preorbital width, supra and postorbital widths, as well as the condylobasal length were found to provide best discrimination between riverine and coastal specimens (Borobia 1989). Overall, coastal specimens tended to have larger skulls than riverine specimens, as well as larger body sizes.

Monteiro-Filho *et al.* (2002) conducted tridimensional morphometric analysis of 22 landmarks from skulls of 92 coastal and 13 riverine specimens of unknown age. They found significant shape differences between coastal and riverine *Sotalia*. In riverine specimens, the rostrum and the occipital condyle pointed downwards, relative to the anteroposterior axis of the skull, and in coastal specimens these were aligned along this axis.

#### TAXONOMIC TREATMENT

Order Cetacea (Brisson, 1762)  
Family Delphinidae (Gray, 1821)  
Genus *Sotalia* (Gray, 1866)  
*Sotalia fluviatilis* (Gervais, 1853)

#### *Holotype and Type Locality*

Rostrum and mandibles held at the Laboratory of Comparative Anatomy at the Natural History Museum (Laboratoire d'Anatomie Comparée du Muséum d'Histoire Naturelle), Paris, France. Collection number JAC: 1880–550 (Robineau 1990). Collected in Pebas (3°19'8"S, 71°49'02"W), Marañon River, upper Amazon, Peru.

### Synonyms

*Sotalia pallidus* (Gervais, 1855) and *Sotalia tucuxi* (Gray, 1856).

### Specimens Examined and Referred Specimen

DNA was examined from twenty-one specimens (Appendix 1). No analysis of skull morphology or external morphometrics of these specimens has been included in this study. As we have not examined the DNA of the holotype, we refer to specimen BA02 as representative of the genetic characters described for *Sotalia fluviatilis* (see Appendix 1). The skull of this specimen (Fig. 4A, C, E, G) is accessioned at Sociedade Civil Mamirauá (Belém, Brazilian Amazon) and a skin sample and DNA are accessioned at Universidade Federal de Minas Gerais (Belo Horizonte, Brazil) for future reference.

### Morphological Description

*Sotalia fluviatilis* is a small delphinid with a moderately long, slender beak (Fig. 3A). The dorsal fin is triangular, short, and high, sometimes hooked at the peak (da Silva and Best 1996). Coloration is dark gray on the dorsum and rosy pink to white or light gray on the ventral side. A lateral area of light gray occurs behind the pectoral fin and another extends from mid body to the level of the anus (da Silva and Best 1996). Pectoral fins and flukes are dark gray underneath (da Silva and Best 1996). Mean body length is 1.4 m, with the largest recorded adults being a 1.49-m male and a 1.52-m female ( $n = 17$ , in Best and da Silva 1984; da Silva and Best 1994, 1996). Mean body measurements are from tip of jaw to the blowhole, 26.4 cm; from tip of jaw to the insertion of flippers, 39.0 cm; from tip of jaw to angle of gape of mouth, 22.7 cm; maximum length of flippers, 24.8 cm; and length of flukes tip to tip, 39.4 cm ( $n = 8$ , in da Silva and Best 1996). The number of upper teeth ranges from 28 to 35 ( $n = 38$ , in Borobia 1989). Mean cranial measurements are condylobasal length = 334.3 mm (range 288–369); greatest preorbital width = 126.9 mm (range 112.3–135.1); greatest postorbital width = 139.4 mm (range 126–148); least supraorbital width = 125.4 mm (range 110.9–133.8) ( $n = 21$ , in Borobia 1989).

### Distribution

*Sotalia fluviatilis* is found throughout the Amazon River drainage, including some of its most important tributaries, like the Putumayo and Caquetá rivers (Colombia) (Trujillo *et al.* 2000), the Ucayali and Marañón rivers (Peru), Negro, Madeira, and Tapajos rivers (Brazil), and the Napo and Cuyabeno rivers (Ecuador) (Borobia *et al.* 1991; da Silva and Best 1994, 1996). Although the distribution of *Sotalia fluviatilis* seems to be continuous along the Amazon River and most of its tributaries, the number of distinct populations that might exist is unknown (Caballero 2006).

### Etymology

We accept the previously proposed “tucuxi,” “tucuchi-una” from the Tupi language of the Mayana indigenous group in the Amazon Region (da Silva and Best 1996) as the common name for the riverine species *Sotalia fluviatilis*.

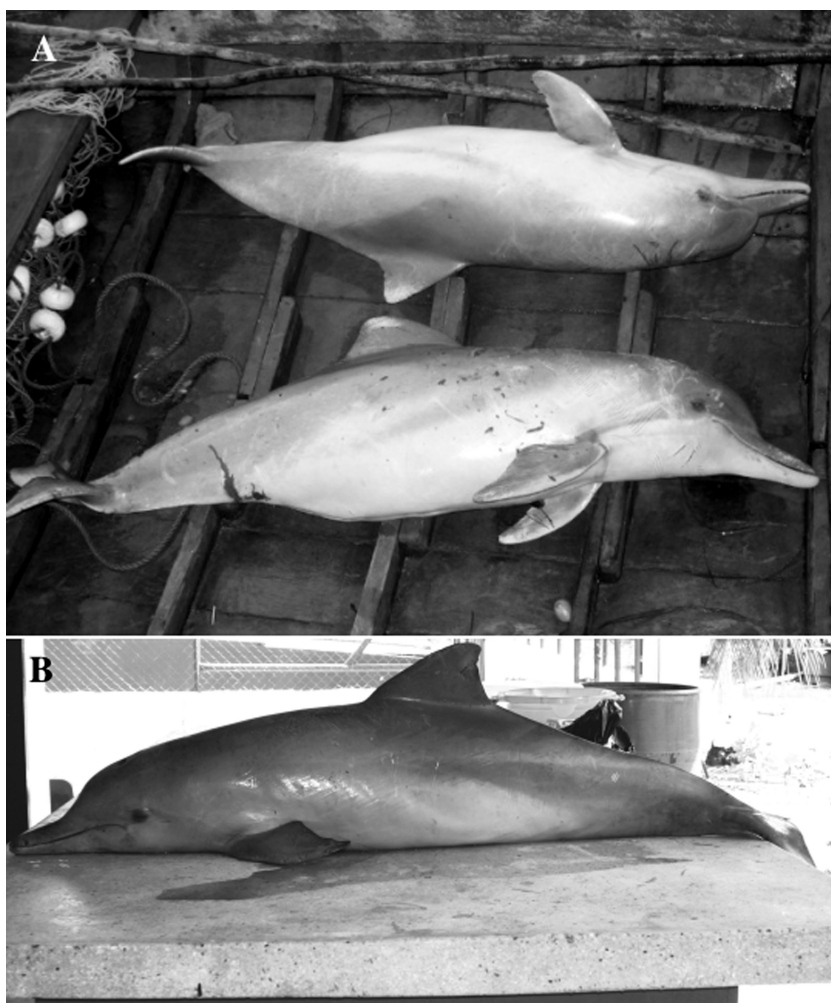


Figure 3. External morphology and coloration. (A) *Sotalia fluviatilis* (two stranded individuals, Lago Tefé, Brazilian Amazon [Photo: Miriam Marmontel, SCM]); (B) *Sotalia guianensis* (stranded individual, Cispatá Bay, Colombian Caribbean. [Photo: Salomé Dussan, CVS]).

#### TAXONOMIC TREATMENT

Order Cetacea (Brisson, 1762)  
Family Delphinidae (Gray, 1821)  
Genus *Sotalia* (Gray, 1866)  
*Sotalia guianensis* (Van Bénédén, 1864) (survive)

#### *Holotype and Type Locality*

Skeleton and skull, 152 cm total length, held at the Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium (Van Bénédén 1864, Williams 1928, Husson



1978). (Previously held at the Stuttgart Museum.) Collection number IRSNB 1516 (Borobia *et al.* 1991). Collected at the mouth of the Maroni River (5°30'00"N, 54°01'98"W), on the border between French Guiana and Surinam. Husson (1978) refers to the type locality as Marowijne River. Considering the largely (if not completely) allopatric distribution of *Sotalia fluviatilis* and *Sotalia guianensis*, we assume this to be the correct holotype for *Sotalia guianensis*.

#### Paratypes

Two additional specimens were obtained with the holotype (co-types). One of these is damaged, lacking the rostrum (Husson 1978). They are held at the collection of Louvain University, Belgium (Husson 1978). No further information on the collection number of these specimens is available at present.

#### Synonyms

*Sotalia brasiliensis* (Van Bénédén, 1875)

#### Specimens Examined and Referred Specimen

DNA was examined from fifty-five specimens (Appendix 1). No analysis of skull morphology or external morphometrics of these specimens has been included in this study. As we have not examined the DNA of the holotype, we refer to specimen CCBC 0103 as representative of the genetic characters described for *Sotalia guianensis* (see Appendix 1). The skull of this specimen (Fig. 4B, D, F, H) is accessioned at Corporación Autónoma Regional de los Valles del Rio Sinú y del Rio San Jorge (Montería, Colombia), and a skin sample and DNA are accessioned at the University of Auckland Molecular Archive (Auckland, New Zealand) for future reference.

#### Diagnosis

*Molecular*—Based on 2,096 bp segment of the MtDNA genome and 1,850 bp segment of nuDNA (three introns), there are 39 fixed-site differences (diagnostic sites) that distinguish *Sotalia guianensis* from *Sotalia fluviatilis*.

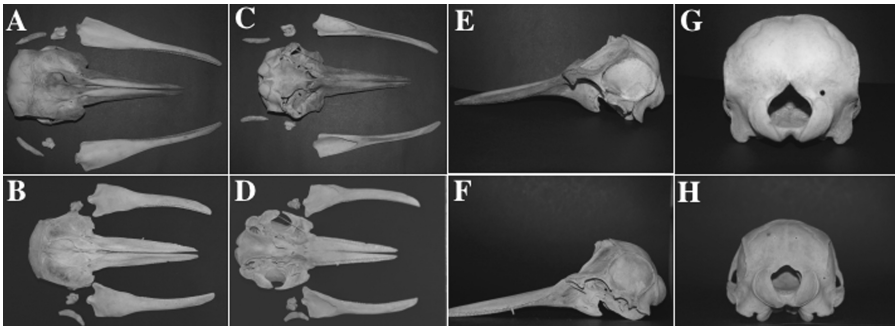


Figure 4. Views of the skull of *Sotalia fluviatilis* (referred specimen BA 02, above) and *Sotalia guianensis* (referred specimen CCBC 0103, below). Dorsal (A, B); ventral (C, D); lateral (left, E, F) and posterior (G, H).

*Morphological*—Although a large number of average morphological differences (not discontinuities) have been found between *Sotalia fluviatilis* and *Sotalia guianensis* (Borobia 1989), the best separation of the two species is based on four cranial measurements: (1) condylobasal length, (2) greatest preorbital width, (3) greatest postorbital width, and (4) least supraorbital width (Borobia 1989). In all cases, averages and ranges of these measures were significantly greater for *Sotalia guianensis*. Mean body length is also significantly greater for *Sotalia guianensis* (da Silva and Best 1996).

#### *Morphological Description*

The coloration of *Sotalia guianensis* is gray on the dorsum and rosy pink light gray on the ventral side (da Silva and Best 1996) (Fig. 3B). The pectoral fins and flukes are gray underneath (da Silva and Best 1996). In some individuals, there is a light streak of gray that slopes anteriorly and ventrally from the upper edge of the caudal peduncle for 10–15 cm (da Silva and Best 1996). Mean body length is 1.7 m ( $n = 17$ , in Husson 1978) with the largest recorded adults being a 1.87-m male and a 2.06-m female (Barros 1991). Mean body measurements are from tip of jaw to the blowhole, 25.9 cm; from tip of jaw to the insertion of flippers, 41.2 cm; from tip of jaw to angle of gape of mouth, 22.9 cm; maximum length of flippers, 29.2 cm; and length of flukes tip to tip, 42.2 cm ( $n = 4$  or 5, in da Silva and Best 1996). The number of upper teeth ranges from 30 to 36 ( $n = 38$ , in Borobia 1989). Mean cranial measurements are condylobasal length = 375.2 mm (range 337–400); greatest preorbital width = 142.6 cm (range 130.1–152.2); greatest postorbital width = 159.1 mm (range 135.7–173); least supraorbital width = 139.8 mm (range 124.4–158) ( $n = 38$ , in Borobia 1989).

#### *Distribution*

*Sotalia guianensis* is distributed along the Caribbean and Atlantic coasts of South America, from Nicaragua (13°N) (Carr and Bonde 2000) and possibly Honduras (up to 15°N) (Edwards and Schnell 2001) to Florianopolis in southern Brazil (27°S) (Geise and Borobia 1987, Borobia *et al.* 1991). It has also been reported in some Caribbean islands including Trinidad and Tobago (da Silva and Best 1996) and the Abrolhos Archipelago of Brazil (Borobia *et al.* 1991). One population has also been described in Maracaibo Lake (Hershkovitz 1962), a large estuarine system located in northwestern Venezuela. Individuals from this population seem to have a smaller body size than individuals from other coastal populations (Casinos *et al.* 1981, Rice 1998), as well as some differences in the measures of a few cranial features (Casinos *et al.* 1981).

*Sotalia* dolphins reported in the lower Orinoco River, 300 km up river, close to Ciudad Bolívar (Venezuela), appear to be *Sotalia guianensis* (Borobia *et al.* 1991, Boher *et al.* 1995, Trujillo *et al.* 2000), but additional data are required for confirmation. Evidence for overlap in the distribution (sympatry) of *Sotalia fluviatilis* and *Sotalia guianensis* in the mouth of the Amazon River is uncertain (da Silva and Martin 2000). Surveys and additional collection of genetic samples are needed in order to clarify boundaries in the distribution of each species in this region. Although the distribution of *Sotalia guianensis* seems to be continuous along the coast, the number of distinct populations that might exist is unknown (Caballero 2006).

### Etymology

We propose the common name “costero” (Spanish for coastal) for the coastal species *Sotalia guianensis*. The common name “estuarine dolphin” has been proposed previously (Rosas and Monteiro-Filho 2002), but we consider that this name is misleading, as the distribution of these dolphins is not restricted to estuaries and other dolphins are also estuarine (e.g., *Orcaella*). These dolphins are known locally with a variety of names, such as tonina (Venezuela), lam (Nicaragua), Guyanese dolphin or Surinam dolphin (French Guiana and Surinam), and boto or boto-cinza in Brazil (da Silva and Best 1996). The proposed “costero” is applicable across the entire range, as this species is rarely, if ever, sighted in pelagic waters.

## DISCUSSION

### Evidence of “Irreversible Divergence”

“Irreversible divergence” was considered important for delimiting cetacean species by a recent workshop on cetacean taxonomy (Reeves *et al.* 2004). The workshop considered that the criterion of irreversible divergence required at least two lines of evidence. Multiple morphological characters were considered likely to be correlated and, thus, to represent only a single line of evidence. However, genetic characters from unlinked loci were considered to represent multiple lines of evidence. Based on these guidelines, the proposed species-level ranking of coastal and riverine *Sotalia* is supported by at least three lines of evidence: morphology, as reviewed here; mtDNA, as presented here and by Cunha *et al.* (2005); and single-copy nuclear DNA, as represented by three introns. No evidence presented or reviewed was in conflict with the proposal (*i.e.*, all genetic loci were either in agreement or were non informative).

Although the workshop did not offer guidelines on the degree of genetic divergence required to recognize species, we employed a comparative approach, using the divergence between other accepted cetacean sister-taxa (assuming similar rates of molecular evolution in other cetacean species), to evaluate the proposed species of *Sotalia* (Table 4). For the mtDNA CR, this comparison showed that nucleotide divergence of the two *Sotalia* was within the range of other accepted species. The net divergence of 2.5% for *Sotalia guianensis*/*Sotalia fluviatilis* was identical to that of the Chilean and Commerson’s dolphins (*Cephalorynchus commersoni* and *C. eutropia*) (Pichler *et al.* 2001b), greater than that of the shortbeaked and longbeaked common dolphins (*Delphinus delphis* and *D. capensis*) (Rosel *et al.* 1994) and the dusky and Pacific white-sided dolphins (*Laagenorhynchus obscurus* and *L. obliquidens*) (Hare *et al.* 2002), but less than that of Indian Ocean bottlenose and bottlenose dolphins (*Tursiops aduncus* and *T. truncatus*) as reported by Wang *et al.* (1999) and that of the recently proposed snubfin and Irrawaddy dolphins (*Orcaella heinsobni* and *O. brevirostris*) (Beasley *et al.* 2005).

For nuclear introns, net divergence between *Sotalia guianensis* and *Sotalia fluviatilis* was consistent with divergence between dusky and Pacific white-sided dolphins (*Laagenorhynchus obscurus* and *L. obliquidens*) as reported by Hare *et al.* (2002). Although no value for divergence was calculated for the Southern right and North Atlantic right whales (*Eubalaena australis* and *E. glacialis*) (Gaines *et al.* 2005), the proportion of fixed sites observed in nuclear introns between these whale species was similar to the proportion of fixed sites in nuclear introns of coastal and riverine *Sotalia* (Table 4).

Table 4. Summary of comparative nucleotide divergence (%) and fixed-site differences in mtDNA and nuDNA for selected sister species of cetaceans.

Species and sample size	Number of base pairs (bp) analyzed		% Divergence		Fixed differences		Reference
	mtDNA	nuDNA	mtDNA	nuDNA	mtDNA	nuDNA	
Tucuxi dolphin ( <i>S. flaviatilis</i> ) ( <i>n</i> = 21)	450 bp (CR only) or 425 bp (Cyt- <i>b</i> only)	4,312 bp (introns)	2.5 % (450 bp CR only) 1.1 % (425 bp Cyt- <i>b</i> only)	0.07% (net)	36 (CR, Cyt- <i>b</i> , ND2) 11 (CR only)	3 (≅1/1,400 bp)	This study
<i>vs.</i> "costero" dolphin ( <i>S. guianensis</i> ) ( <i>n</i> = 55)							
Commerson's dolphin ( <i>C. commersonii</i> ) ( <i>n</i> = 47) <i>vs.</i> Chilean dolphin ( <i>C. eutropia</i> ) ( <i>n</i> = 20)	485 bp (CR)	n.r.	2.5 %	n.r.	3	n.r.	Pichler <i>et al.</i> (2001 <i>b</i> )
Shortbeaked common dolphin ( <i>D. delphis</i> ) ( <i>n</i> = 11) <i>vs.</i> longbeaked common dolphin ( <i>D. capensis</i> ) ( <i>n</i> = 18)	404 bp (CR)	n.r.	%	n.r.	1 4 <sup>a</sup>	n.r.	Rosel <i>et al.</i> (1994)
Bottlenose dolphin ( <i>T. truncatus</i> ) ( <i>n</i> = 21) <i>vs.</i> Indian Ocean bottlenose dolphin ( <i>T. aduncus</i> ) ( <i>n</i> = 19)	397 bp (CR)	n.r.	4.4 %	n.r.	7	n.r.	Wang <i>et al.</i> (1999)

Table 4. Continued.

Species and sample size	Number of base pairs (bp) analyzed		% Divergence		Fixed differences		Reference
	mtDNA	nuDNA	mtDNA	nuDNA	mtDNA	nuDNA	
Irrawaddy dolphin ( <i>O. brevirostris</i> ) ( <i>n</i> = 24) vs. Snubfin dolphin ( <i>O. heinsobhani</i> ) ( <i>n</i> = 4)	403 bp (CR)	n.r.	5.9 %	n.r.	16	n.r.	Beasley <i>et al.</i> (2005)
Southern right whale ( <i>E. australis</i> ) ( <i>n</i> = 45) vs. North Atlantic right whale ( <i>E. glacialis</i> ) ( <i>n</i> = 2)	289 bp (CR)	n.r.	3%–7%	n.r.	n.r.	n.r.	Baker <i>et al.</i> (1999)
Southern right whale ( <i>E. australis</i> ) ( <i>n</i> = 11) vs. North Atlantic right whale ( <i>E. glacialis</i> ) ( <i>n</i> = 8)	292 bp (CR)	14,823 bp (introns)	n.r.	n.r.	3	14 (≅1/1,000 bp) (Monophyly)	Gaines <i>et al.</i> (2005)
Dusky dolphin ( <i>L. obscurus</i> ) ( <i>n</i> = 5) vs. Pacific whitesided dolphin ( <i>L. obliquidens</i> ) ( <i>n</i> = 6)	496 bp (Cyt- <i>b</i> )	7,118 bp (introns)	1.22 %	0.03%–0.21% (Net) (Average: 0.086%)	5	1 <sup>a</sup>	Hare <i>et al.</i> (2002)

n.r.: not reported.

<sup>a</sup>Close to fixation.

We further considered that ecological adaptation provides evidence of irreversible divergence. This ecological adaptation would be directed by divergent natural selection, which would be expected when considering the distinct environments where the two proposed sister species are found (Schluter 2001). The pronounced differences in salinity between the riverine and coastal-estuarine waters, as well as the changes in the water level at different times of the year, represent different selective pressures. Salinity ranges from less than 0.05 ppm (parts per million) in the Amazon River to over 35 ppm on the Atlantic coast of Brazil with a steep cline in the last 200 km of the mouth of the river (Tundisi *et al.* 1999). For example, although both coastal and riverine *Sotalia* prey mostly on pelagic fish from the same families (*i.e.*, *Sciaenidae* and *Clupeidae*), they prefer different species found exclusively either in freshwater or saltwater, and no overlap in prey species has been observed in diet studies (Best 1984, Borobia and Barrios 1989, Santos *et al.* 2002).

Borobia (1989) proposed that the differences found in the skull measurements around the eye orbit between riverine and coastal *Sotalia* could be related to the relative importance of vision in coastal and freshwater environments. Monteiro-Filho *et al.* (2002) proposed that differences in cranial shape between coastal and riverine *Sotalia* could reflect functional distinctions between both subspecies and, ultimately, adaptation to two very different environments and selective pressures (Schluter 2001). Dissimilarities in echolocation clicks have also been attributed to ecological differences (Kamminga *et al.* 1993). The dominant echolocation frequency of coastal *Sotalia* (55–65 kHz) is higher than that of riverine *Sotalia* (40–45 kHz), and the signal of the latter is more similar to that of the sympatric Amazon River dolphin (*Inia geoffrensis*) (36–46 kHz, Kamminga *et al.* 1993).

Finally, adaptation to seasonal fluctuation in the water levels of the Amazon River and its tributaries has influenced the seasonality of reproduction in riverine *Sotalia*, perhaps contributing to an incipient prezygotic isolating mechanism. A high proportion of births in riverine *Sotalia* occur during the dry season (October–November) when water levels are low (Best and da Silva 1984). Births in coastal *Sotalia* occur year round (Santos *et al.* 2001, Rosas and Monteiro-Filho 2002) with a peak in the rainy season (May–November) in some localities (Bössenecker 1978). Duration of gestation is also longer in coastal individuals, 11.6–11.7 mo (Rosas and Monteiro-Filho 2002), compared to 10.0–10.3 mo in riverine *Sotalia* (Best and da Silva 1984). Other differences in reproductive parameters include ovarian activity restricted to the left ovary in riverine *Sotalia* and activity in both ovaries in coastal *Sotalia* (Rosas and Monteiro-Filho 2002). Furthermore, seasonal testicular activity has been suggested in riverine *Sotalia* (Best and da Silva 1984), whereas it has not been detected in coastal *Sotalia* (Rosas and Monteiro-Filho 2002).

#### *Timeframe for Speciation of Sotalia fluviatilis*

The proposed divergence time of 1.0–1.2 mya for coastal and riverine *Sotalia* is consistent with the values reported by Avise *et al.* (1998) for time of initial divergence between sister species in many mammalian groups. These results are also consistent with the prominent role of Pleistocene events in the differentiation of extant mammalian sister species (Avise *et al.* 1998, Knowlton 2000). By comparison Cunha *et al.* (2005) proposed an older time of divergence between coastal and riverine *Sotalia*

(between 2.5 and 5.0 mya) based on a slower molecular clock calculated by Hoelzel *et al.* (1991). Further confirmation of the proposed dates is currently not possible given the lack of fossil record for *Sotalia* (da Silva and Best 1994).

The time frame of 1.0–1.2 mya proposed in our study coincides with a series of marine transgressions and regressions that occurred in the Amazon basin at the end of the Pliocene and during the Pleistocene (Plio-Pleistocene 3.4 mya–125,000 B.P.; Marroig and Cerqueira 1997, Gorrington *et al.* 2003). Based on geological evidence (Marroig and Cerqueira 1997), it has been estimated that about 2.5 mya, the ocean level was 180 m above the present and since then a general trend of descending sea level has occurred. From 2.5 mya to the present, it is thought that the entire Amazon basin underwent alternating periods of ponding with sedimentation as well as regional erosion (Putzer 1984, Müller *et al.* 1995). This series of ponding events, accompanied by tectonic activity in the western Amazon, has been proposed as the “Amazon Lagoon” (Lago Amazonas) hypothesis (Frailey *et al.* 1988, Campbell 1990). According to this hypothesis, the Amazonian lowland could have been covered by water until approximately 750,000 B.P. (Marroig and Cerqueira 1997). Tectonic events, climatic change (cooling) and environmental change have been proposed as the cause for the transition of the Amazon Basin from a large lake to a braided fluvial system in the early Pleistocene (Brooks *et al.* 1981, Räsänen *et al.* 1987, Rossetti *et al.* 2005). We suggest that these transitional events isolated the riverine *Sotalia* from the coastal populations, maintaining a possible connection only in the mouth of the Amazon River, and promoting the divergence of these two species closer to the date of 750,000 B.P.

The relatively recent divergence of *Sotalia* is also suggested by comparison with the wider distribution of *Inia*. Although the family Iniidae is ancient, having occupied the Amazon drainage for around 15 million yr (Hamilton *et al.* 2001), divergence of the Bolivian Amazon subspecies (*Inia geoffrensis boliviensis*) is more recent, having occurred between 5 and 6 mya (Banguera-Hinestroza *et al.* 2002) by the formation of the Madeira-Mamoré rapids. As *Sotalia* is not found in the Bolivian Amazon above the rapids, this seems to suggest an upper time limit of 5 mya for its occurrence in the Amazon.

A similar upper bound is suggested by the divergence of the Amazon and Orinoco subspecies (*Inia geoffrensis geoffrensis* and *Inia geoffrensis humboldtiana*). Connectivity between the Amazon and Orinoco *Inia* subspecies is suspected through the Casiquiari Channel, which connects the Upper Orinoco and the upper Rio Negro, one of the main tributaries of the Amazon River (Banguera-Hinestroza *et al.* 2002). This is, at present, the only possible point of contact between the Amazon and Orinoco river basins after isolation of the Orinoco drainage from the main Amazon drainage initiated with the uplift of the Eastern Andean Cordillera in the Late Middle Miocene (*ca.* 12 mya) and continued with the uplift of the Mérida Cordillera in the Late Pliocene (5–3.4 mya) (Díaz de Gamero 1996, Audemard and Audemard 2002). If *Sotalia* entered the Amazon and expanded toward other tributaries, and the divergence of the coastal and riverine proposed sister species occurred between 5.0 and 2.5 mya as suggested by Cunha *et al.* (2005), we would expect *Sotalia* to be found in the upper Orinoco, with a distribution similar to that of *I. g. humboldtiana* and similar connectivity between Orinocoan and Amazonian *Sotalia* populations. To date, presence of *Sotalia* in this region has not been confirmed and sightings of *Sotalia* (presumably coastal transients) are restricted to the lower Orinoco and its mouth (Borobia *et al.* 1991, Boher *et al.* 1995, Rice 1998).

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#### SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article online:  
Supplementary Appendix 1

Appendix I. List of *Sotalia* specimens examined in genetic analyses (this study) and specimens reported in other studies that have not been used in genetic analyses to date but can be assigned to *Sotalia guianensis* or *Sotalia fluviatilis* based solely on partial allopatry. One asterisk (\*) indicates specimens used only for mtDNA analyses and two asterisks (\*\*) indicate specimens used for both mtDNA and nuDNA analyses. Specimens referred for further genetic analyses are denoted in bold.

Identification number	Location/Region	Country	Sample collector/Collection/ Depository of genetic material	Date	Sex	Nature of specimen	Genetic analyses	Reference
<b><i>Sotalia guianensis</i></b>								
USNM571558 /SWFCS z 44079*	Mouth of the Layasiksa River, Waunta Lagoon	Nicaragua	T. Carr /USMN/SWFSC, UAMA	1992	Male	Complete skeleton, DNA	Yes	Carr and Bonde (2000)
<b>CCB C0103**</b>	<b>Cispatá Bay, Colombian Caribbean</b>	<b>Colombia</b>	<b>S. Dussan/CVS/UAMA</b>	<b>2003</b>	<b>Female</b>	<b>Skull Skin DNA</b>	<b>Yes</b>	<b>This study</b>
CCBC 0104**	Cispatá Bay, Colombian Caribbean	Colombia	S. Dussan/CVS/UAMA	2004	Male	Skull Skin, DNA	Yes	This study
CCIR 0103*	Cispatá Bay, Colombian Caribbean	Colombia	CEINER/UAMA	1986	Unknown	Skull, DNA	Yes	This study, Borobia <i>et al.</i> (1991)
CCMOR 0103**	Morrosquillo Gulf, Colombian Caribbean	Colombia	S. Caballero/UAMA	2003	Male	Skin, DNA	Yes	This study
CCMOR 0203**	Morrosquillo Gulf, Colombian Caribbean	Colombia	S. Caballero/UAMA	2003	Female	Skin, DNA	Yes	This study
CCSM 0199*	Ciénaga, Colombian Caribbean	Colombia	S. Caballero/UAMA	1999	Female	Skin, DNA	Yes	This study
CCSM 0103**	Santa Marta, Colombian Caribbean	Colombia	N. Jimenez, M. C. Rosso/UJTL/UAMA	2003	Male	Skin, DNA	Yes	This study
CCSM 0203**	Santa Marta, Colombian Caribbean	Colombia	N. Jimenez, M. C. Rosso/ UJTL/UAMA	2003	Female	Skin, DNA	Yes	This study
CCGU 0101**	Guajira province, Colombian Caribbean	Colombia	S. Caballero/captive animal, ASM/UAMA	2001	Male	Skin, DNA	Yes	This study
CCGU 0201**	Guajira province, Colombian Caribbean	Colombia	S. Caballero/captive animal, ASM/UAMA	2001	Male	Skin, DNA	Yes	This study
CCGU 0301**	Guajira province, Colombian Caribbean	Colombia	S. Caballero/captive animal, ASM/UAMA	2001	Female	Skin, DNA	Yes	This study
CCGU 0401**	Guajira province, Colombian Caribbean	Colombia	S. Caballero/captive animal, ASM/UAMA	2001	Male	Skin, DNA	Yes	This study
ZMA 19784	San Antero, Colombian Caribbean	Colombia	P.J. Bössenecker/ZMA	1977	Unknown	Complete skeleton	No	Bössenecker (1978) Borobia <i>et al.</i> (1991)
ZMA 19775	San Antero, Colombian Caribbean	Colombia	P.J. Bössenecker/ZMA	1977	Unknown	Complete skeleton	No	Bössenecker (1978) Borobia <i>et al.</i> (1991)
ZMA 19780	San Antero, Colombian Caribbean	Colombia	P.J. Bössenecker/ZMA	1977	Unknown	Complete skeleton	No	Bössenecker (1978) Borobia <i>et al.</i> (1991)
ZMA 19776	San Antero, Colombian	Colombia	P.J. Bössenecker/ZMA	1978	Unknown	Complete	No	Bössenecker (1978)

	Caribbean					skeleton		Borobia <i>et al.</i> (1991)
ML 0197*	Mouth of the Catatumbo River, Maracaibo Lake	Venezuela	Unknown/UZV	1997	Unknown	Partial skeleton, DNA	Yes	This study
ML 0199*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	1999	Unknown	Skin, DNA	Yes	This study
ML 0100*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	1999	Unknown	Skin, DNA	Yes	This study
ML 0700*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2000	Male	Skin, DNA	Yes	This study
ML 2100*	Barranquitas, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2000	Unknown	Skin, DNA	Yes	This study
ML 2200*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2000	Unknown	Skin, DNA	Yes	This study
ML 2300*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2000	Female	Skin, DNA	Yes	This study
ML 0101*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2001	Female	Skin, DNA	Yes	This study
ML 0102*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2002	Female	Skin, DNA	Yes	This study
ML 0202**	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2002	Male	Skin, DNA	Yes	This study
ML 0302*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2002	Unknown	Skin, DNA	Yes	This study
ML 0402*	Barranquitas, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2002	Female	Skin, DNA	Yes	This study
ML 0502*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2002	Unknown	Skin, DNA	Yes	This study
ML 0602*	Zapara Island, Maracaibo Lake	Venezuela	H. Barrios-Garrido, M. G. Montiel/UZV	2002	Unknown	Skin, DNA	Yes	This study
FMNH 18801	Maracaibo Lake	Venezuela	W. H. Osgood/FMNH	1911	Unknown	Rostrum	No	Borobia <i>et al.</i> (1991)
FMNH 18802	Maracaibo Lake	Venezuela	W. H. Osgood/FMNH	1911	Unknown	Rostrum	No	Borobia <i>et al.</i> (1991)
FMNH 34906	Maracaibo Lake	Venezuela	D. L. Bitancourt-Suere/FMNH	1912	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
FMNH 34907	Maracaibo Lake	Venezuela	D. L. Bitancourt-Suere/FMNH	1912	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
EBRG 2748	Maracaibo Lake	Venezuela	A. Casinos/EBRG	1981	Unknown	Skull	No	Casinos <i>et al.</i> (1981) Borobia <i>et al.</i> (1991)
EBRG (no number)	Maracaibo Lake	Venezuela	A. Casinos/EBRG	1981	Unknown	Skull	No	Casinos <i>et al.</i> (1981) Borobia <i>et al.</i> (1991)
MBUCV 2819	Maracaibo Lake	Venezuela	A. Casinos/MBUCV	1981	Unknown	Skull	No	Casinos <i>et al.</i> (1981) Borobia <i>et al.</i> (1991)
USNM 571461 /SWFSC z 44078	Saint John Parish, Tobago	Trinidad and Tobago	E. D. Caesar/USNM/SWFSC	1987	Unknown	Skull, DNA	No	Borobia <i>et al.</i> (1991)
USNM 253476 /SWFSC z 44077	Junction of the Mazaruni and Cuyuni Rivers	British Guiana	S. H. Williams/USNM/SWFSC	1925	Male	Complete skeleton DNA	No	Borobia <i>et al.</i> (1991)
CM 60941	Cuyuni River, near	British	S. H. Williams/CM	1925	Unknown	Soft tissue in	No	Williams (1928)

	Kartabo	Guiana				alcohol		Borobia <i>et al.</i> (1991)
CM 60942	Cuyuni River, near Kartabo	British Guiana	S. H. Williams/CM	1967	Unknown	Skull	No	Williams (1928) Borobia <i>et al.</i> (1991)
ZMA 15515	Mouth of Coppename River	Surinam	P. J. H. Van Bree/ZMA	1972	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
ZMA 15571	Mouth of Coppename River	Surinam	J. J. van Dijk/ZMA	1972	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
RNHL 18165	Mouth of the Suriname River	Surinam	Unknown/RNHL	1963	Female	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 18166	Mouth of the Suriname River	Surinam	Unknown/RNHL	1963	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 18167	Mouth of the Suriname River	Surinam	Unknown/RNHL	1963	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 18168	Mouth of the Suriname River	Surinam	Unknown/RNHL	1963	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 21755	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Female	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 21756	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Female	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 22256	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 22257	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 22258	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 22259	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
RNHL 22260	Mouth of the Suriname River	Surinam	Unknown/RHNL	1971	Male	Complete skeleton	No	Husson (1978) Borobia <i>et al.</i> (1991)
ZMA 14641	Mouth of the Suriname River	Surinam	H Lionarons and D. Dekker/ZMA	1971	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
ZMA 15527	Mouth of the Suriname River	Surinam	P. J. van Bree/ZMA	1972	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
IRSNB 1516 (Holotype)	Mouth of the Maroni/Marowijne River	French Guiana	A. Kappler/IRSNB	1861	Unknown	Complete skeleton	No	P. J. Van Bénédén, (1864) Borobia <i>et al.</i> (1991)
SMNH 26376	Mouth of the Maroni/Marowijne River	French Guiana	A. Kappler/SMNH	1865	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
SMNH 26394	Mouth of the Maroni/Marowijne River	French Guiana	A. Kappler/SMNH	1865	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
FG 0101*	Near Cayenne	French Guiana	B. de Toisy/KWATA/UAMA	2001	Female	Skin, DNA	Yes	This study
FG 0201*	Near Cayenne	French Guiana	B. de Toisy/KWATA/AUMA	2001	Unknown	Skin, DNA	Yes	This study
FG 0301**	Near Cayenne	French Guiana	S. Beltrán-Pedrerros/CULM	2001	Male	Skin, DNA	Yes	This study
FG 0401**	Near Cayenne	French Guiana	S. Beltrán-Pedrerros/CULM	2001	Female	Skin, DNA	Yes	This study



FG 0501**	Near Cayenne	French Guiana	S. Beltrán-Pedrerros/CULM	2001	Male	Skin, DNA	Yes	This study
FG 0601**	Near Cayenne	French Guiana	S. Beltrán-Pedrerros/CULM	2001	Female	Skin, DNA	Yes	This study
AE 07*	Belém, PA (Amazonian Estuary)	Brazil	F. R. Santos/UFGM	2000	Female	Skin, DNA	Yes	This study
AE 08*	Belém, PA (Amazonian Estuary)	Brazil	F. R. Santos/UFGM	2000	Female	Skin, DNA	Yes	This study
MZUSP 26867	Vieira, Ilha de São Luis, MA	Brazil	Unknown/MZUSP	1989	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
UFPB 547	Formosa Bay, RN	Brazil	C. Bonvicino/UFPB	1986	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
UFPB 544	Barra de Mamanguape, PB	Brazil	Unknown/UFPB	1986	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
UFPB 548	João Pessoa, PB	Brazil	Unknown/UFPB	1981	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
UFPB 794	João Pessoa, PB	Brazil	Unknown/UFPB	1987	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 23814	Pirambú, SE	Brazil	Unknown/MZUSP	1986	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
BCCE 01*	Fortaleza, CE	Brazil	A. C. O de Meirelles/AQUASIS/UFGM	2004	Unknown	Liver, DNA	No	This study
BCBA 032**	Alcobaça, BA	Brazil	M. Rossi-Santos/IBJ/UFGM	2003	Male	Skin, DNA	Yes	This study
BCBA 038*	Santa Cruz Cabralia, BA	Brazil	M. Rossi-Santos/IBJ/UFGM	2004	Male	Skin, DNA	Yes	This study
MZUSP 23802	Praia do Forte, BA	Brazil	Unknown/MZUSP	1986	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 26873	Ponta de Corumbao, Itamarajú, BA	Brazil	Unknown/MZUSP	1989	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 25430	Barra de Caravelas, BA	Brazil	Unknown/MZUSP	1988	Unknown	Partial skull	No	Borobia <i>et al.</i> (1991)
MZUSP 23801	Praia do Grauca, Caravelas, BA	Brazil	Unknown/MZUSP	1987	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 26866	Abrolhos Archipelago, BA	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 26868	Praia do Pontal, Nova Viçosa, BA	Brazil	Unknown/MZUSP	1989	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
BCES 039**	Regência, ES	Brazil	M. Rossi-Santos/IBJ/UFGM	2004	Male	Skin, DNA	Yes	This study
BCES 040*	Regência, ES	Brazil	M. Rossi-Santos/IBJ/UFGM	2004	Unknown	Skin, DNA	Yes	This study
MZUSP 26870	Guriri, São Mateo, ES	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 26871	Pontal do Ipiranga, Linhares, ES	Brazil	Unknown/MZUSP	1988	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 19365	Regência, ES	Brazil	Unknown/MZUSP	1984	Unknown	Partial skeleton	No	Geise and Borobia (1987)
MZUSP 19366	Regência, ES	Brazil	Unknown/MZUSP	1984	Unknown	Partial skeleton	No	Geise and Borobia (1987)
MZUSP 23809	Regência, ES	Brazil	Unknown/MZUSP	1984	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 26865	Regência, ES	Brazil	Unknown/MZUSP	1987	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
RG 926	Vila Velha, ES	Brazil	Unknown/RG	1983	Unknown	Skull	No	Borobia and Barrios

								(1989)
BCRJ 03*	Praia da Ponte, Ibicui, RJ	Brazil	L. Flach/PUC-MG/UFMG	2003	Male	Skin, DNA	Yes	This study
BCRJ 04*	Rio de Janeiro Bay, RJ	Brazil	L. Flach/PUC-MG/UFMG	2003	Male	Skin, DNA	Yes	This study
USNM STR 02655/z 534*	Unknown, RJ	Brazil	USNM/SWFSC	1970	Male	Skin, DNA	Yes	This study
MZUSP 23813	Atafona, RJ	Brazil	Unknown/MZUSP	1985	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 25631	Atafona, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
UFRJ-AC01	Ilha da Convivência, RJ	Brazil	Unknown/MZUSP	1983	Unknown	Skull	No	Geise and Borobia (1987)
MZUSP 25612	Açu, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25616	Açu, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25606	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP25606	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25607	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25608	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25609	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25619	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25622	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Lodi and Capistrano (1990)
MZUSP 25623	Farol de São Tomé, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Skull	No	Lodi and Capistrano (1990)
MZUSP 25624	Farolzinho, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25625	Farolzinho, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25611	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25613	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25617	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Lodi and Capistrano (1990)
MZUSP 25618	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25620	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25734	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25628	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Lodi and Capistrano (1990)

MZUSP 25629	Praia da Flexeira, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Lodi and Capistrano (1990)
MZUSP 25610	Quinçamá, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Partial skeleton	No	Lodi and Capistrano (1990)
MZUSP 25614	Quinçamá, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25615	Quinçamá, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25621	Quinçamá, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25626	Quinçamá, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25627	Quinçamá, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Lodi and Capistrano (1990)
MZUSP 25630	Quinçamá, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Partial skeleton	No	Lodi and Capistrano (1990)
UFRJ-AC 03	Praia de Manguinhos, Búzios, RJ	Brazil	H. de. G. Bergallo/UFRJ	1984	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 23810	Barra de Maricá, RJ	Brazil	Unknown/MZUSP	1985	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 19541	Niterói, RJ	Brazil	Unknown/MZUSP	1984	Unknown	Skull	No	Borobia and Barros (1989)
MZUSP 23812	Niterói, RJ	Brazil	Unknown/MZUSP	1985	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 24811	Niterói, RJ	Brazil	Unknown/MZUSP	1987	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MNHN-1888-791	Rio de Janeiro, RJ	Brazil	E. Van Bénédén/MNHN	1888	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MNHN-1888-793	Rio de Janeiro, RJ	Brazil	E. Van Bénédén/MNHN	1888	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MNRJ 123	Guanabara Bay, Rio de Janeiro, RJ	Brazil	Unknown/MNRJ	1933	Unknown	Skull	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MNRJ 124	Guanabara Bay, Rio de Janeiro, RJ	Brazil	Unknown/MNRJ	1933	Unknown	Skull	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MZUSP 23811	Praia de São Conrado, Rio de Janeiro, RJ	Brazil	Unknown/MZUSP	1985	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 24812	Praia de São Conrado, Rio de Janeiro, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 26876	Praia de São Conrado, Rio de Janeiro, RJ	Brazil	Unknown/MZUSP	1988	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
FEEMA 706	Ilha Grande, RJ	Brazil	N. C. Maciel/FEEMA	1986	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 9417	Santos, SP	Brazil	Unknown/MZUSP	1961	Unknown	Complete skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MZUSP 9611	Santos, SP	Brazil	Unknown/MZUSP	1961	Unknown	Complete skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
BCPA 03*	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFGM	2003	Male	Skin, DNA	Yes	This study
BCPA 13**	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFGM	2003	Male	Skin, DNA	Yes	This study
BCPA 15**	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFGM	2003	Female	Skin, DNA	Yes	This study
BCPA 19*	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFGM	2004	Unknown	Skin, DNA	Yes	This study

BCPA 21*	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Female	Skin, DNA	Yes	This study
BCPA 53**	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Female	Skin, DNA	Yes	This study
BCPA 57*	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Male	Skin, DNA	Yes	This study
BCPA 59*	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Unknown	Skin, DNA	Yes	This study
BCPA 80*	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Female	Skin, DNA	Yes	This study
BCPA 95**	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Male	Skin, DNA	Yes	This study
BCPA 102**	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Male	Skin, DNA	Yes	This study
BCPA 131**	Cananéia Estuary, SP	Brazil	M. C. Santos/UNESP/UFMG	2004	Male	Skin, DNA	Yes	This study
MZUSP 9821	Cananéia, SP	Brazil	Unknown/MZUSP	1962	Unknown	Complete skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MZUSP 18943	Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 18944	Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 18874	Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1965	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 10230	Praia do Nóbrega, Ilha Comprimida, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 10231	Praia do Nóbrega, Ilha Comprimida, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 10232	Capivari River, Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 10402	Mouth of Bagaçu River, Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 10403	Mouth of Bagaçu River, Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 18923	Pereirinha, Ilha do Cardoso, SP	Brazil	Unknown/MZUSP	1984	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 9605	Praia da Trincheira, Cananéia, SP	Brazil	Unknown/MZUSP	1961	Unknown	Complete skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MZUSP 9606	Praia da Trincheira, Cananéia, SP	Brazil	Unknown/MZUSP	1961	Unknown	Partial skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MZUSP 10228	Praia da Trincheira, Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Partial skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
MZUSP 10227	Praia da Trincheira, Cananéia, SP	Brazil	C. T. de Carvalho/MZUSP	1964	Unknown	Partial skeleton	No	de Carvalho (1963) Borobia <i>et al.</i> (1991)
UFSC 1010	Praia do Tamanco, Florianópolis, SC	Brazil	Unknown/UFSC	1985	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
UFSC 1073	Praia da Daniela, Norte bay, Ilha de Santa Catarina, SC	Brazil	Unknown/UFSC	1989	Unknown	Partial skeleton	No	Simões-Lopes and Ximenez (1990) Borobia <i>et al.</i> (1991)
UFSC 1074	Praia de Canasvieiras, Ilha de Santa Catarina, SC	Brazil	Unknown/UFSC	1989	Unknown	Complete skeleton	No	Simões-Lopes and Ximenez (1990) Borobia <i>et al.</i> (1991)
<b><i>Sotalia fluviatilis</i></b>								
MNHN JAC 1880-550 (Holotype)	Pebas, Marañon River, Peruvian Amazon	Peru	Castelnau and Deville/MNHM	1844?	Unknown	Rostrum and mandibles	No	Robineau (1990)
MNHN JAC 1880-549	Nauta, Marañon River, Peruvian Amazon	Peru	Castelnau and Deville/MNHM	1844?	Unknown	Partial skeleton	No	Robineau (1990)
UF 18785	Pucallpa, Peruvian Amazon	Peru	J. Schimke/UF	1965	Unknow	Skull	No	Borobia <i>et al.</i> (1991)

PA 0104**	Curaray River, Peruvian Amazon	Peru	M. Ruíz- García/PUJ/UAMA	2004	Male	Skin, DNA	Yes	This study
CACAQ 0196*	Caquetá River, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	1996	Unknown	Skull, DNA	Yes	This study
CACAQ 0296*	Caquetá River, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	1996	Unknown	Skull, DNA	Yes	This study
CA 0192*	Puerto Nariño, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	1992	Unknown	Skull, DNA	Yes	This study
CA 0193*	Puerto Nariño, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	1993	Unknown	Skull, DNA	Yes	This study
CA 0100*	Puerto Nariño, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	2000	Male	Skin, DNA	Yes	This study
CA 0200*	Puerto Nariño, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	2000	Unknown	Skull, DNA	Yes	This study
CA 0300*	Puerto Nariño, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	2000	Unknown	Skull, DNA	Yes	This study
CA 0204*	Puerto Nariño, Colombian Amazon	Colombia	F. Trujillo/FO/UAMA	2000	Female	Skin, DNA	Yes	This study
CA 0102*	Leticia, Colombian Amazon	Colombia	M. Ruíz- García/PUJ/UAMA	2002	Unknown	Teeth, DNA	Yes	This study
CA 0104**	Leticia, Colombian Amazon	Colombia	M. Ruíz- García/PUJ/UAMA	2004	Male	Skin, DNA	Yes	This study
UF 1215	Leticia, Colombian Amazon	Colombia	Unknown/UF	1956	Unknown	Skull	No	Layne (1958) Borobia <i>et al.</i> (1991)
MCZ 52235	Leticia, Colombian Amazon	Colombia	Tarpon Zoo, Florida/MCZ	1963	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
LACM 19594	Leticia, Colombian Amazon	Colombia	Tarpon Zoo, Florida/LACM	1964	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
UF 17546	Leticia, Colombian Amazon	Colombia	Tarpon Zoo, Florida/UF	1966	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
UF 18782	Leticia, Colombian Amazon	Colombia	Tarpon Zoo, Florida/UF	1966	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
INPA-MA 38	Juruá River, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 39	Juruá River, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 15	Lago Amanã, AM	Brazil	Unknown/INPA	1979	Female	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 16	Lago Amanã, AM	Brazil	Unknown/INPA	1979	Male	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 24	Lago Amanã, AM	Brazil	Unknown/INPA	1979	Male	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 25	Lago Amanã, AM	Brazil	Unknown/INPA	1979	Male	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 26	Lago Amanã, AM	Brazil	Unknown/INPA	1979	Female	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 27	Lago Amanã, AM	Brazil	Unknown/INPA	1979	Female	Partial skeleton	No	Best and da Silva (1984)

INPA-MA 31	Lago Amanã, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 32	Lago Amanã, AM	Brazil	Unknown/INPA	1980	Female	Partial skeleton	No	Best and da Silva (1984)
BA 01*	Amazonas River, AM	Brazil	M. Marmontel/SCM/UFMG	1997	Female	Skin, DNA	Yes	This study
<b>BA 02*</b>	<b>Lago Tefé, AM</b>	<b>Brazil</b>	<b>M. Marmontel/SCM/UFMG</b>	<b>1997</b>	<b>Female</b>	<b>Skull, Skin, DNA</b>	<b>Yes</b>	<b>This study</b>
BA 14**	Lago Tefé, AM	Brazil	M. Marmontel/SCM/UFMG	2004	Male	Skin, DNA	Yes	This study
BA 16*	Lago Tefé, AM	Brazil	M. Marmontel/SCM/UFMG	2004	Male	Skin, DNA	Yes	This study
INPA-MA 05	Lago Tefé, AM	Brazil	Unknown/INPA	1979	Female	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 06	Lago Tefé, AM	Brazil	Unknown/INPA	1979	Female	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 40	Lago Tefé, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)
BA 06**	Solimões River, AM	Brazil	M. Marmontel/SCM/UFMG	2004	Male	Skin, DNA	Yes	This study
INPA-MA 51	Solimões River, AM	Brazil	Unknown/INPA	1981	Male	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 52	Solimões River, AM	Brazil	Unknown/INPA	1981	Male	Partial skeleton	No	Best and da Silva (1984)
BA 05**	Lago Urini, AM	Brazil	M. Marmontel/SCM/UFMG	2003	Male	Skin, DNA	Yes	This study
BA 15**	Paraná do Aranapu, AM	Brazil	M. Marmontel/SCM/UFMG	2004	Female	Skin, DNA	Yes	This study
MZUSP 18948	Lago Boiaçú, RO	Brazil	Expedição Permanente da Amazônia/MZUSP	1968	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 18949	Lago Boiaçú, RO	Brazil	Expedição Permanente da Amazônia/MZUSP	1968	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
INPA-MA 28	Negro River, AM	Brazil	Unknown/INPA	1980	Male	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 36	Negro River, AM	Brazil	Unknown/INPA	1980	Male	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 41	Negro River, AM	Brazil	Unknown/INPA	1980	Female	Partial skeleton	No	Best and da Silva (1984)
CAS 13947	Cuieiras River, AM	Brazil	E. S. Herald/CAS	1965	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
CAS 16658	Cuieiras River, AM	Brazil	R. Brownell Jr. and E. S. Herald/CAS	1965	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
LACM 49708	Cuieiras River, AM	Brazil	Unknown/LACM	1965	Unknown	Partial skeleton	No	Harrison and Brownell (1971) Borobia <i>et al.</i> (1991)
LACM 49709	Cuieiras River, AM	Brazil	Unknown/LACM	1965	Unknown	Partial skeleton	No	Harrison and Brownell (1971) Borobia <i>et al.</i> (1991)
AMNH 92203	Manaus, AM	Brazil	Ollala Brothers/AMNH	1930	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
INPA-MA 42	Manaus, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 43	Manaus, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)

INPA-MA 44	Manaus, AM	Brazil	Unknown/INPA	1980	Male	Partial skeleton	No	Best and da Silva (1984)
INPA-MA 45	Manaus, AM	Brazil	Unknown/INPA	1980	Female	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 46	Manaus, AM	Brazil	Unknown/INPA	1980	Male	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 47	Manaus, AM	Brazil	Unknown/INPA	1980	Female	Complete skeleton	No	Best and da Silva (1984)
INPA-MA 48	Manaus, AM	Brazil	Unknown/INPA	1980	Female	Partial skeleton	No	Best and da Silva (1984)
LACM 30790	100 miles from the junction of Negro and Amazon Rivers, AM	Brazil	A. E. Colburn/LACM	1932	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
INPA-MA 50	Madeira River, AM	Brazil	Unknown/INPA	1981	Unknown	Complete skeleton	No	Best and da Silva (1984)
MNRJ 6030	Lago Serpa, AM	Brazil	Unknown/MNRJ	1937	Unknown	Skull	No	de Carvalho (1963)
AMNH 94169	Lago de Faro, AM	Brazil	Ollala Brothers/AMNH	1931	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 18924	Oriximiná, PA	Brazil	Expedição Permanente da Amazônia/MZUSP	1968	Unknown	Partial skeleton	No	Borobia <i>et al.</i> (1991)
MZUSP 18946	Trombetas River, PA	Brazil	Expedição Permanente da Amazônia/MZUSP	1969	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
MZUSP 19913	Trombetas River, PA	Brazil	Expedição Permanente da Amazônia/MZUSP	1969	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)
BMNH 1856.8.2.3	Santarém, PA	Brazil	H. W. Bates/BMNH	1856	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
BMNH 1856.8.2.3	Santarém, PA	Brazil	H. W. Bates/BMNH	1856	Unknown	Skull	No	Borobia <i>et al.</i> (1991)
BA 10*	Santarém, PA	Brazil	Unknown/UFGM	?	Unknown	Partial skeleton	Yes	This study
USNM 504316 /SWFCS z 455**	Unknown, Brazilian Amazon	Brazil	Unknown /USMN/SWFCS, UAMA	?	Female	Carcass frozen, skin, DNA	Yes	This study
BA 11*	Formoso River, Araguaia, TO	Brazil	Unknown/UFGM	?	Unknown	Partial skeleton	Yes	This study
<b><i>Sotalia</i> sp. (area of possible sympatry)</b>								
MPEG 10945	Marajó Bay, PA	Brazil	R. Barthem/MPEG	1982	Unknown	Complete skeleton	No	Borobia <i>et al.</i> (1991)

Abbreviations of Brazilian states are as follows: AM, Amazonas; BA, Bahia; CE, Ceará; ES, Espírito Santo; MA, Maranhão; MG, Minas Gerais; PA, Pará; PB, Paraíba; PR, Paraná;

RJ, Rio de Janeiro; RN, Rio Grande do Norte; RO, Roraima; SC, Santa Catarina; SE, Sergipe; SP, São Paulo;

Appendix II. Names of institutions (with abbreviations) and their locations.

Institution	Abbreviation	Location
American Museum of Natural History	AMNH	New York, NY (USA)
Associação de Pesquisa e Preservação de Ecossistemas Aquáticos	AQUASIS	Fortaleza, CE (Brazil)
Acuario de Santa Marta	ASM	Santa Marta, Magdalena (Colombia)
British Museum (Natural History)	BMNH	London (England)
California Academy of Sciences	CAS	San Francisco, CA (USA)
Oceanario Islas del Rosario	CEINER	Isla San Martín de Pajarales, Parque Corales del Rosario (Colombia)
Carnegie Museum of Natural History	CM	Pittsburg, PA (USA)
Centro Universitario Luterano de Manaus	CULM	Manaus, AM (Brazil)
Estación Biológica Rancho Grande	EBRG	Maracay, Aragua (Venezuela)
Corporación Autónoma Regional de los Valles del Río Sinú y del Río San Jorge	CVS	Montería, Córdoba (Colombia)
Fundação Estadual de Engenharia do Meio Ambiente	FEEMA	Rio de Janeiro, RJ (Brazil)
Field Museum of Natural History	FMNH	Chicago, IL (USA)
Fundación Omacha	FO	Bogotá (Colombia)
Instituto Baleia Jubarte	IBJ	Praia do Kitongo, Caravelas, BA (Brazil)
Instituto Nacional de Pesquisas da Amazônia	INPA	Manaus, AM (Brazil)
Institut Royal des Sciences Naturelles de Belgique	IRSNB	Brussels (Belgium)
Association pour l'Etude et Conservation de la Faune Sauvage de Guyane-KWATA	KWATA	Cayenne (French Guiana)
Los Angeles County Museum	LACM	Los Angeles, CA (USA)
Museo de Biología de la Universidad Central de Venezuela	MBUCV	Caracas (Venezuela)
Museum of Comparative Zoology, Harvard University	MCZ	Cambridge, MA (USA)
Muséum National d'Histoire Naturelle	MNHN	Paris (France)
Museu Nacional do Rio de Janeiro	MNRJ	Rio de Janeiro, RJ (Brazil)
Museu Paraense Emílio Goeldi	MPEG	Belém, PA (Brazil)
Museu de Zoologia da Universidade de São Paulo	MZUSP	São Paulo, SP, Brazil
Pontifícia Universidade Católica de Minas Gerais	PUC-MG	Belo Horizonte, MG (Brazil)
Pontificia Universidad Javeriana	PUJ	Bogotá (Colombia)
Museu de História Natural de Curitiba	RG	Curitiba, PR (Brazil)
Rijksmuseum van Natuurlijke Historie	RNHL	Leiden (The Netherlands)
Sociedade Civil Mamiará	SCM	Belém, PA (Brazil)
Staatliches Museum für Naturkunde	SMNH	Stuttgart (Germany)
Southwest Fisheries Science Center	SWFSC	La Jolla, CA (USA)
Molecular Archive, The University of Auckland	UAMA	Auckland (New Zealand)
The Florida State Museum of Natural History	UF	Gainesville, FL (USA)
Universidade Federal de Minas Gerais	UFMG	Belo Horizonte, MG (Brazil)
Universidade Federal da Paraíba	UFPB	João Pessoa, PB (Brazil)
Universidade Federal do Rio de Janeiro	UFRJ	Rio de Janeiro, RJ (Brazil)
Universidade Federal de Santa Catarina	UFSC	Florianópolis, SC (Brazil)
Universidad Jorge Tadeo Lozano	UJTL	Bogotá (Colombia)
Universidade Estadual Paulista	UNESP	Rio Claro, SP (Brazil)
United States National Museum Smithsonian Institution	USNM	Washington, D. C. (USA)
Universidad del Zulia	UZV	Maracaibo, Zulia (Venezuela)
Instituut voor Taxonomische Zoölogie, Universitet van Amsterdam	ZMA	Amsterdam (The Netherlands)