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Chapter 14

MOLECULAR ECOLOGY AND SYSTEMATICS OF *SOTALIA* DOLPHINS

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ABSTRACT

Molecular markers have the potential to disclose genetic variation and provide clues on macro and microevolutionary issues. The taxonomic and phylogenetic status of species lie within the realm of macroevolution while intraspecific matters, such as geographic population structure, social organisation and mating system, pertain to microevolution. This chapter describes the findings on the molecular systematics and ecology of Sotalia dolphins, and is divided in two sections, each focusing on one of those topics.The first section shows how molecular markers have helped to settle the issue of species composition within the genus Sotalia – a matter of debate for over 140 years. To explain the controversy, a brief history of taxonomic changes in the genus since the first species descriptions is included. In addition, the section also makes phylogenetic considerations and discusses the timing of the speciation between the two accepted Sotalia species.The second section deals with the molecular ecology of Sotalia, presenting results and prospects of studies on population structure, phylogeography and social structure. Although many studies are still underway, some important findings have already been produced. The section also includes comments on new analytical developments that promise to widen our knowledge on those issues. The two sections close with a discussion of the relevance of results for the conservation and management of Sotalia species. At least two important results stem from molecular systematics and ecology studies of Sotalia dolphins, both with immediate application to their conservation. At the end of the chapter there is a presentation of the prospects for new discoveries in these fields in the near future.

Keywords: *Sotalia*, population structure, phylogeography, social structure, molecular ecology.

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INTRODUCTION

Sotalia dolphins are among the smallest members of the Delphinidae family. These dolphins occur along the Atlantic coast of Central and South America, as well as in the Amazon River basin (Figure 1). Marine *Sotalia* are found from Honduras to the state of Santa Catarina, in southern Brazil (Simões-Lopes, 1987; da Silva and Best, 1996), in a seemingly continuous distribution that might be limited from extending southwards by low water surface temperatures (Borobia et al., 1991). Throughout that range it has many local common names, such as "boto-cinza" (Brazil), "tonina" (Venezuela and Colombia) and "lam" (Nicaragua). The distribution of riverine *Sotalia* comprises most of the Amazon River basin from Brazil as far as Peru, Ecuador and Colombia (da Silva and Best, 1996). Locally, this dolphin is known as "tucuxi" (Brazil), "bufeo-negro" or "bufeo-gris" (Colombia and Peru). There are also records of *Sotalia* dolphins in the Orinoco River, up to 800 km inland, and some disputed reports in the Upper Orinoco (Borobia et al., 1991; Boher et al., 1995). Those sightings may be attributed to marine *Sotalia*, since it inhabits bays and estuaries and is frequently seen entering rivers along the South American coast (da Silva and Best, 1996).

Marine and riverine *Sotalia* are morphologically very alike: both are dark gray in the *dorsum*, and light gray, white or pinkish in the ventral area, with a poorly developed lateral stripe extending from the eye to the pectoral fin. The beak is moderately long and slender, and the melon small and rounded. The dorsal fin is triangular, pectoral fins are large and the body is stocky (Jefferson et al., 1993). The main morphological difference between them is size with a maximum recorded total length for marine Sotalia of 206 cm, in contrast to 152 cm for freshwater *Sotalia* (Barros, 1991; da Silva & Best, 1996). There are also meristic and morphometric differences, but those are modal rather than absolute (Fettuccia, 2006).

Marine and riverine *Sotalia* are different not only in ecology but in life history traits: they use different acoustic signals and have distinct reproductive parameters (such as gestation length and birth seasonality (da Silva & Best, 1996; Rosas & Monteiro-Filho, 2002). Some of those differences may have arisen as adaptations to the different environments they inhabit.

The infrageneric taxonomy of *Sotalia* remained uncertain for over a century, and was solved only recently, when morphological (Monteiro-Filho et al., 2002) and genetic data (Cunha et al., 2005; Caballero et al., 2007) showed that marine and riverine *Sotalia* are different species.

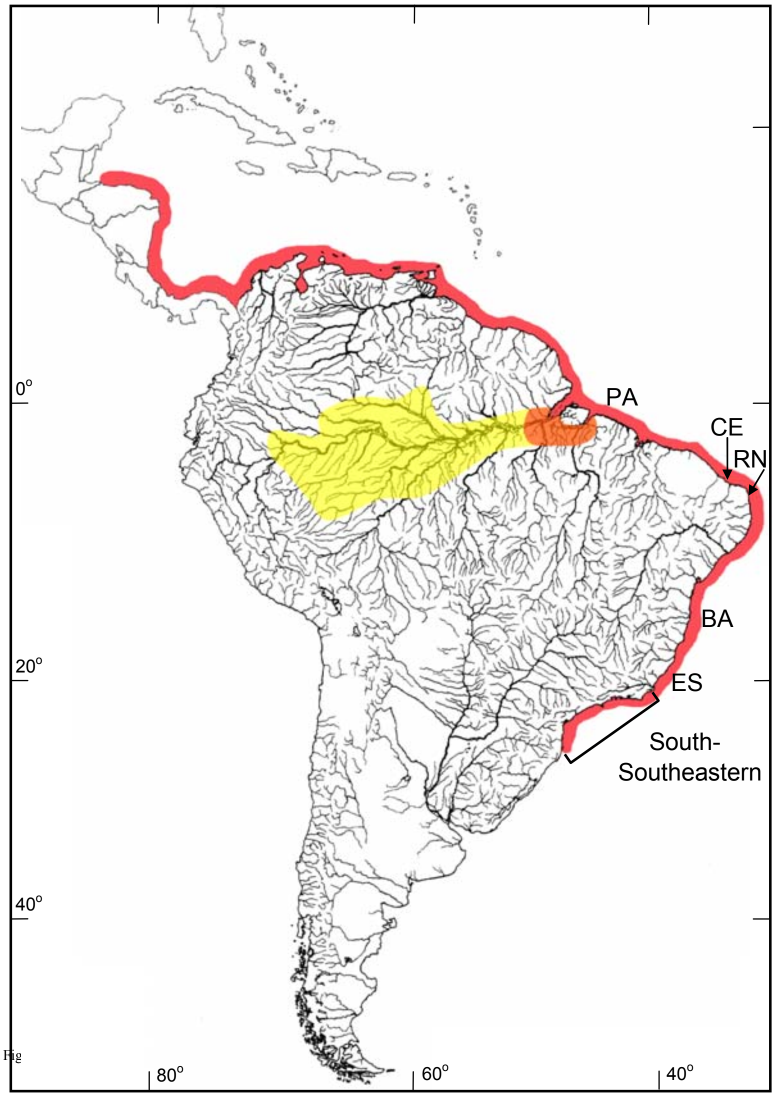

Figure 1. Distribution of *Sotalia fluviatilis* (yellow) and of *S. guianensis* (red), and potential area of sympatry between the two species (orange). Abbreviations correspond to localities cited in the text (PA: Pará, CE: Ceará, RN: Rio Grande do Norte, BA: Bahia, ES: Espírito Santo, South-Southeastern: includes samples from Rio de Janeiro, São Paulo, Paraná and Santa Catarina).

Genus *Sotalia* Gray 1866

Sotalia was described to accommodate a marine species from the South American continent, originally attributed to *Delphinus* (*Delphinus guianensis*). In the same year, Gray proposed the *Sotalia* sub-genus *Tucuxa* for a riverine species from the Amazon (*Steno tucuxi*): that species was later relocated to *Sotalia* by Flower (1883). Also in 1866, Gray described the sub-genus *Sousa,* using *Steno lentiginosus* (later synonymized with *Sousa chinensis*), from India, as its type species. Interestingly, many species originally described as *Delphinus* and *Steno* from the Old World were assigned to *Sotalia*, before being finally placed in *Sousa* (Iredale & Troughton, 1934; Fraser & Purves, 1960), almost 100 years after the description of that genus.

Among the South American species reclassified in *Sotalia*, three were riverine dolphins collected in Peru and Brazil (*Delphinus fluviatilis*, *D. pallidus* and *Steno tucuxi*) and the other species was estuarine, described based on three dolphins collected at the mouth of the Marowijne River, in the border between Suriname and the French Guiana (*Delphinus guianensis*). In 1875 a fifth species was added to the genus *Sotalia* (the marine *S. brasiliensis*, whose type locality was Guanabara Bay, Brazil).

All those species were described based on few individuals from single location, at a time when barely anything was known about their ranges, so their diagnoses were incomplete and full of inconsistencies. As more specimens were examined and more data on their distribution were gathered, the three freshwater species were lumped into *Sotalia fluviatilis*, and the two marine were grouped as *Sotalia guianensis* (True, 1889; Cabrera, 1961; Carvalho, 1963).

Later, some authors argued that the differences between *S. fluviatilis* and *S. guianensis* were too subtle and attributable to phenotypic variability, and that *Sotalia* should be regarded as monotypic (Mitchell, 1975; Leatherwood & Reeves, 1983). This proposal was reinforced by a morphometric study that concluded that differences between marine and riverine *Sotalia* were mainly a consequence of size variation, and concluded that they should be considered a single species, without subspecific differentiation (Borobia, 1989). Since then, most authors adopted the binomial *S. fluviatilis*, regarding *S. guianensis* as a synonym, but acknowledging marine and riverine populations as different ecotypes (Borobia et al., 1991; Jefferson et al., 1993; da Silva & Best, 1996; Rice, 1998; Flores, 2002). Other researchers preferred to distinguish the two *Sotalia* forms using the subspecific denomination *S. fluviatilis fluviatilis* and *S. fluviatilis guianensis*. A summary of the taxonomic changes in the genus *Sotalia* and the type localities of the species described for this genus in South America are displayed in Figures 2 and 3 respectively.

The first indication that the lumping of *Sotalia* species should be reassessed was given by Furtado-Neto (1998). A phylogenetic analysis of mitochondrial cytochrome b sequences showed that marine and riverine *Sotalia* were different, but that result needed further confirmation, since only a single riverine sample was analyzed.

The second indication was provided by geometric morphometrics: Monteiro-Filho and co-workers (2002) found significant differences in shape and size between marine and riverine *Sotalia* skulls, suggesting that they belonged to different species. The main difference was in the alignment of the rostrum and occipital condyle: in marine animals, the location of the *foramen magnum* is posterior, indicating that the cranium would be in line with the vertebral column. In freshwater specimens, the *foramen magnum* is located more ventrally, so the cranium would point downwards (Monteiro-Filho et al., 2002).

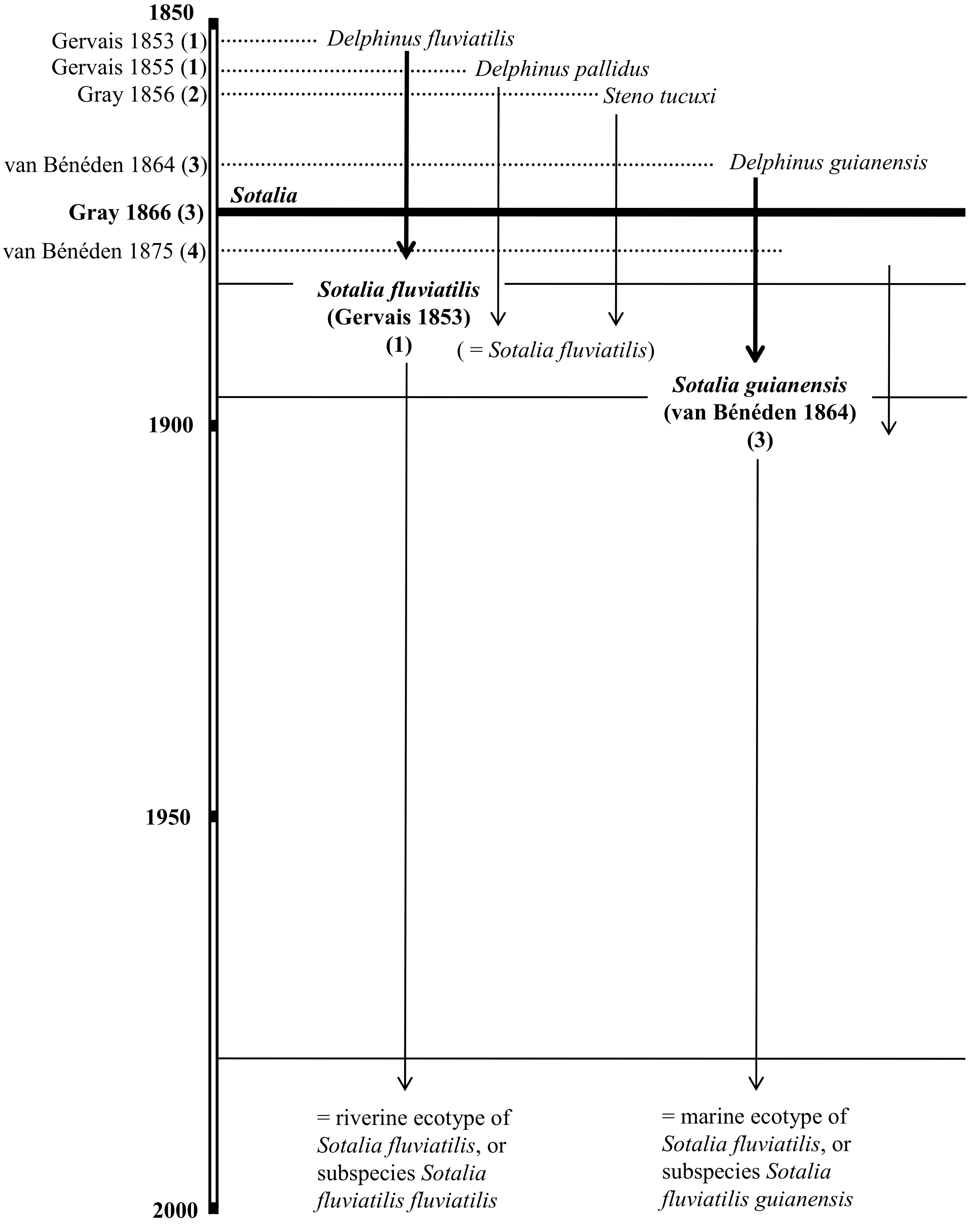

Figure 2. Time-line of descriptions of *Sotalia* species from South America and summary of subsequent nomenclature changes. (····) species description; (▬) genus description; (—) synonymization. Numbers in parentheses refer to type localities, shown in Figure 3.

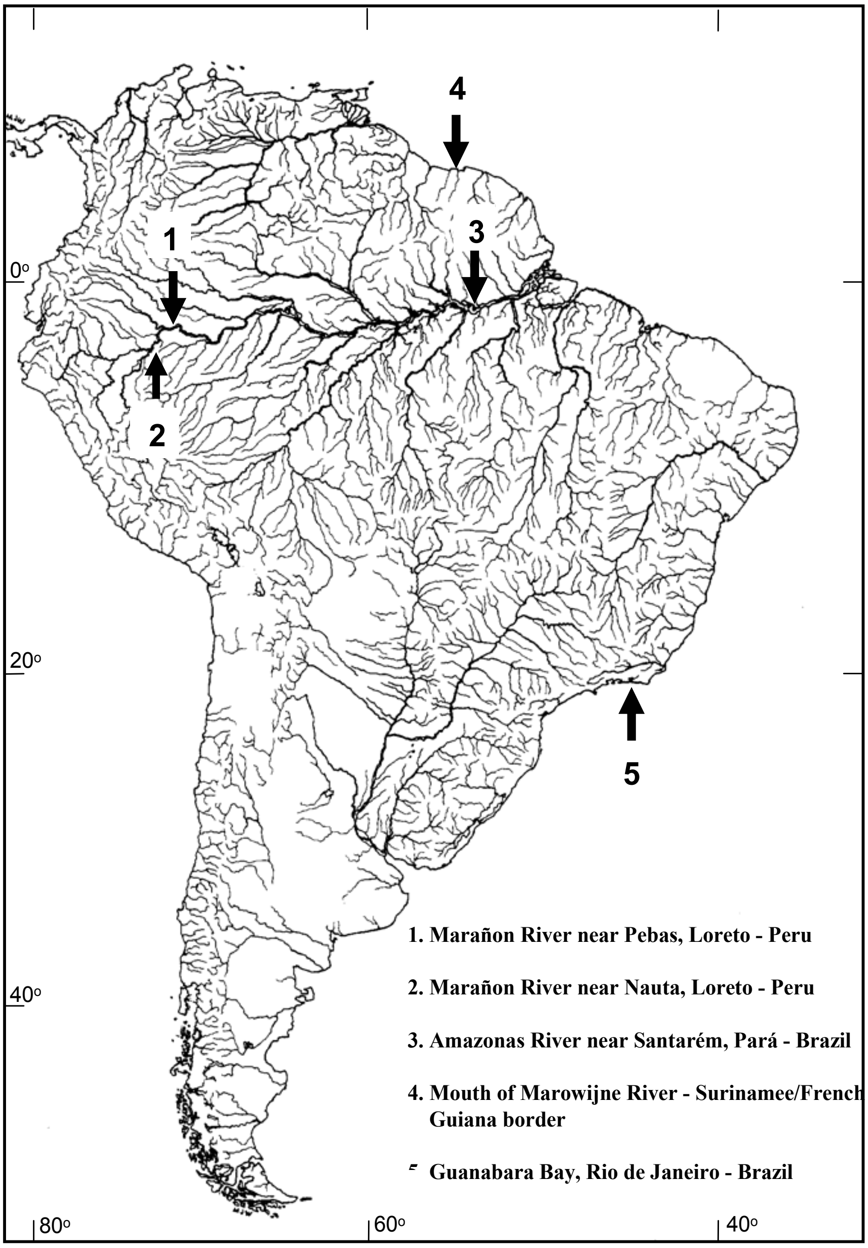

Figure 3. Type localities of *Sotalia* species from South America. Numbers correspond to those depicted between parentheses in Figure 2.

Molecular Systematics

Taxonomy

As morphological analyses revealed significant differences between marine and riverine *Sotalia* (Monteiro-Filho et al., 2002), genetic analyses were essential to settle the issue of specific differentiation. This is because morphological differences might arise in response to different selection regimes and might not reflect reproductive isolation. Additionally, Monteiro-Filho et al. (2002) did not examine any skull from the Amazon Estuary, so the possibility that marine and riverine *Sotalia* formed extremes of a cline could not be ruled out. Significant differences in the skull had been previously reported by Borobia (1989), but a conservative conclusion supporting a single species was reached, among other reasons, due to the lack of samples from the Amazon Estuary, which could represent a transitional zone.

The use of molecular data in taxonomy and phylogeny has intensified over the last decades. Molecular systematics has benefits and disadvantages over traditional, morphology-based systematics (Hillis, 1987). Molecular markers are useful because they reveal a larger amount of variation, due to the large number of characters available in comparison with morphological analyses. Besides, genetic differences usually accumulate faster than phenotypic differences and, when genotypes are analyzed, environmental effects such as plasticity or convergence do not confound the analyses (Mayr, 1963; Avise, 2004). Those are invaluable features, especially in the delimitation of species. The detection of reproductive isolation and of monophyletism, which are pre-requisites of many species concepts, is also straightforward when genotypes are analyzed (Mayr, 1963; Hillis, 1987; Knowlton, 2000; Avise, 2004). On the other hand, molecular analyses demand expensive equipment and samples preserved in a way not to destroy DNA. Hence, integrating molecular and morphological approaches maximizes the likelihood of understanding true evolutionary relationships (Hillis, 1987; Knowlton, 2000; Avise, 2004).

Cunha et al. (2005) clarified the taxonomic status of *Sotalia* dolphins using sequences of the mitochondrial control region and the cytochrome b of 56 samples (12 riverine and 44 marine). This was the first study to include samples of the Amazon Estuary in analyses of differentiation between *Sotalia* ecotypes. Three phylogenetic approaches were used, and all of them recovered the same topology, displaying marine and freshwater *Sotalia* as reciprocally monophyletic groups (Figure 4). This result was corroborated by a Nested Clade Analysis (NCA; Templeton, 1998) of the same data. Notwithstanding some of its limitations in analyses of recently diverged lineages, NCA is a powerful tool that quantitatively and qualitatively investigates population structure and evolutionary history, including speciation (Templeton, 1998, 2001; Sites and Marshall, 2003). The NCA of *Sotalia* samples indicated a relatively old allopatric fragmentation event, which separated marine and riverine populations (Figure 5). Fragmentation events are evidence of speciation, especially if they: (a) are in higher level (older) clades; (b) reflect the separation of two clusters by several mutational steps and (c) coincide with independent evidence from other type of data (Templeton, 2001). The fragmentation observed between the two *Sotalia* ecotypes meets all three conditions. Interestingly, dolphins from Pará, at the mouth of the Amazon River, were genetically much closer to dolphins from Santa Catarina (4,700 km southwards, along the coast) than to the geographically closer (2,000 km) riverine dolphins (Cunha et al., 2005).

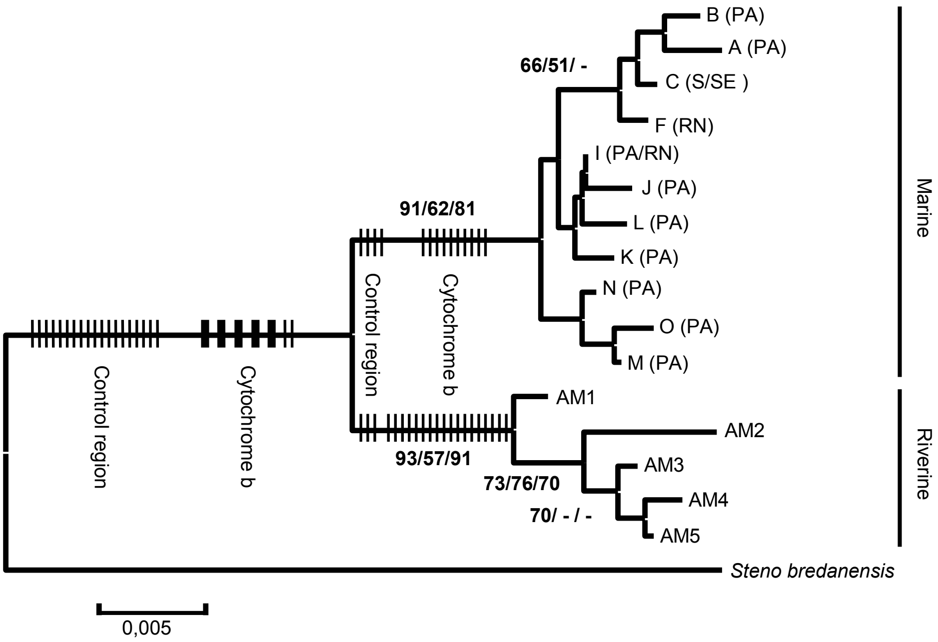

Figure 4. Neighbor-Joining (NJ) phylogenetic tree (p distances) of *Sotalia* spp. control region haplotypes. Maximum-Likelihood (ML) and Maximum Parsimony (MP) retrieved the same topology. Bootstrap values (NJ/ML/P) higher than 50% are shown. Hypothetical synapomorphies of control region and cytochrome b haplotypes from marine and riverine species are indicated by vertical bars. Thicker bar corresponds to 10 synapomorphies. Adapted from Cunha et al. (2005).

Therefore, both the phylogenetic and NCA approaches supported the same conclusion: riverine and marine populations of *Sotalia* are deeply divergent. This result, along with distinct ecological and geographical distributions and the morphometric differentiation observed between them (Monteiro-Filho et al., 2002), led Cunha et al. (2005) to conclude that marine and riverine *Sotalia* belonged to different species. At least three criteria for the recognition of *taxa* as distinct species were fulfilled by those data (morphological and molecular population aggregation analysis, cladistic haplotype aggregation and Templeton's test of cohesion - Sites & Marshall, 2003).

In 2003, an international workshop on the molecular systematics of Cetaceans recognized that there was, in the field, a "traditional tendency to err in the direction of avoiding designating too many *taxa* rather than making sure that all potentially recognized *taxa* have been designated'' (Reeves et al., 2004). As a consequence, guidelines for the recognition of full species were established. According to the Workshop's guidelines, an argument for species status should be accepted only when there were at least two independent primary lines of evidence for its existence, such as morphology and genetics (Reeves et al., 2004). Therefore, together, the results presented by Monteiro-Filho et al. (2002) and Cunha et al. (2005) fulfilled those guidelines. Marine and riverine species of *Sotalia* could be separated not only on the basis of two primary types of evidence (morphology and genetics, respectively), but also of secondary ones (i.e., distribution and ecology). Based on priority criteria, the revalidation of *Sotalia guianensis* (van Bénéden 1864) was recommended for the marine ecotype, while the riverine form holds the binomial *Sotalia fluviatilis* (Gervais 1853, van Bree, 1974).

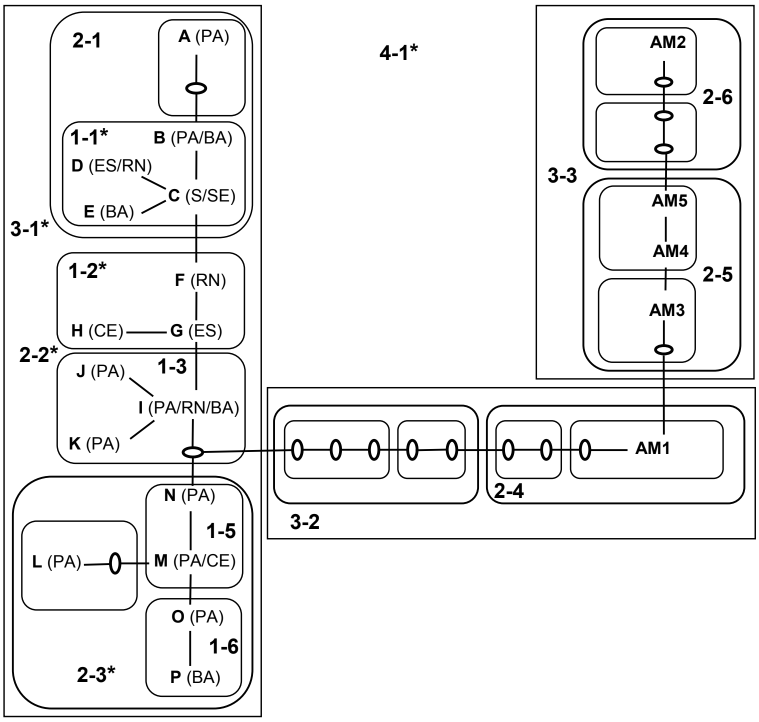

Figure 5. Parsimony network of haplotypes from the Brazilian coast, with nested clade design. Ovals represent missing intermediaries. Clades with significant associations ($P < 0.05$) are marked with an asterisk. Hierarchical level is denoted as 1-x for first level, 2-x for second, etc, where x identifies each clade. AM: Amazonas (*S. fluviatilis*). *S. guianensis* - PA: Pará; CE: Ceará; RN: Rio Grande do Norte; BA: Bahia; ES: Espírito Santo; S/SE: South-Southeastern (Rio de Janeiro, São Paulo, Paraná and Santa Catarina). Adapted from Cunha (2007).

Another important finding was the presence of *S. guianensis* at the mouth of the Amazon River. The freshwater load of the Amazon River reaches hundreds of kilometers into the sea (Muller-Karger et al., 1988), so the animals sampled in Pará were actually living in freshwater. It would be interesting to analyze samples from intermediate locations along the Amazon River, to detect how far upriver *S. guianensis* occurs, and verify if there is sympatry in any region with *S. fluviatilis*. For that, a joint analysis of mitochondrial sequences and microsatellites would be crucial, since it would allow not only the detection of any possible hybridization in the area but also its polarity.

Recently, Caballero et al. (2007) analyzed sequences from introns of three nuclear genes (lactalbumin, actin and glucocerebrosidase) and another mitochondrial marker (ND2) including South America and Caribbean samples. Their study, based on a larger dataset both in genes analysed and geographical breadth gave support to the conclusion of Cunha et al. (2005), confirming the specific status of *S. guianensis* and *S. fluviatilis*.

Two issues related to genus *Sotalia* remain unclear: the range and species identity of *Sotalia* dolphins in the Orinoco River, and the taxonomic status of *Sotalia* dolphins from southern Maracaibo Lake. In the Orinoco River, there are frequent records of *Sotalia* dolphins at Ciudad Bolívar, some 300 km from the river's mouth, but those may correspond to *S. guianensis*, which can reach several kilometers upriver (da Silva and Best, 1996; Mead & Koehnken, 1991; Flores & da Silva, 2008). Boher et al. (1995) reported a sighting in the Middle Orinoco, 800 km inland. In addition, there are disputed reports of *Sotalia* dolphins in the Upper Orinoco, and even in the Apure River (Hershkovitz, 1963; Borobia et al., 1991; Boher et al. 1995). However, *Sotalia* dolphins were not recorded in the Upper Orinoco and Apure Rivers, nor in the lower reaches of most of the major tributaries of the Orinoco, during a long term study conducted between 1983 and 1990 (Mead & Koehnken, 1991). It is believed that *Sotalia* dolphins cannot traverse the rapids at the Casiquiare channel, which connects the Orinoco and Amazon River basins (da Silva and Best, 1996). This barrier has existed since the uplift of the Mérida Cordillera (10 mya; Lundberg et al., 1998), which predates the split between *Sotalia* species (see next section). Thus, *Sotalia* dolphins in the Middle Orinoco are likely to be an isolated population of *S. guianensis*.

Another interesting issue concerns *Sotalia* dolphins found in the southern, freshwater, portion of the Maracaibo Lake. That population is morphologically different from the marine *Sotalia* that inhabit the northern portion of the Lake, where it opens to the Gulf of Venezuela. Dolphins from southern Maracaibo are smaller than marine *Sotalia*, and about the same size as *S. fluviatilis* (Casinos et al., 1981; da Silva & Best, 1996; León, 2005). However, there is no connection between the Maracaibo Lake and the present day known range of riverine *Sotalia*, and the Maracaibo Lake has been isolated from the Amazon basin for the last 8-10 million years (Hoorn et al., 1995; Días de Gamero, 1996). The morphological distinctiveness of the southern Maracaibo Lake population could result from true phenotypic plasticity, unlike that found between *S. guianensis* and *S. fluviatilis*. However it may also indicate a lack of gene flow with the marine *Sotalia* from the mouth of the lake and the Gulf of Venezuela. Indeed, genetic differentiation between those areas was reported by Caballero et al. (2006). Those authors observed some exclusive haplotypes in samples from the lake, but did not attribute the variation to specific differentiation.

Timing of Speciation

The divergence between *S. fluviatilis* and *S. guianensis* observed by Cunha et al. (2005) was 2.5%, for both the control region and the cytochrome b. The evolutionary rates of those markers have been estimated at between 0,5% and 1% per million years (My) for the control region of cetaceans (Hoelzel et al., 1991) and 1%/My for the cytochrome b (Irwin et al., 1991). Hence, the speciation event that separated both lineages probably happened between 5 and 2.5 mya, during the Pliocene. At that time, the Amazon River was already flowing along its present course, with its outlet to the Atlantic (Hoorn et al., 1995; Lundberg et al., 1998). For the last 4 my, several sea level oscillations occurred, as a consequence of glacial and interglacial periods. During the periods of sea level rise, river discharge was prevented, and freshwater inflow into the Amazon basin increased, causing the inundation of the Amazon craton (Lundberg et al., 1998). The highest marine transgression happened around 2.5 mya (Klammer, 1984). It is possible that *Sotalia* colonized the Amazon basin during one of those transgression/inundation events. Regardless of the putative timings of speciation, dolphins that colonized the Amazon River system probably had an Atlantic origin, because the alternative explanation (entrance from the Caribbean via present day Maracaibo Lake and Paleo-Orinoco system) would require a much older divergence (>10 mya).

Caballero et al. (2007) calibrated a molecular clock for the control region using the estimated divergence between *Sotalia* and *Phocoena phocoena* based on the fossil record (10-11 my). Therefore, they arrived at a faster substitution rate, and dated the divergence between *S. fluviatilis* and *S. guianensis* at 1 to 1.2 mya, during the Pleistocene. This dating is also compatible with environmental oscillations in the Amazon basin (Caballero et al., 2007). Due to the lack of *Sotalia* fossils, it is not possible yet to decide which of the two scenarios is more likely.

Evolutionary Relationships

Sotalia is one of the several Delphinidae genera. The Delphinidae family is regarded as a taxonomic “trash basket”, because its members are very diverse in shape and size, and share no exclusive characteristics. Some of the characteristics of delphinids are a marine distribution, presence of beak, presence of a falcate dorsal fin and presence of conical teeth. However, there are exceptions to each of those features (Jefferson et al., 1993).

The evolutionary relationships among delphinids are far from understood, so at present it is difficult to ascertain the phylogenetic position of *Sotalia*. Traditionally, *Sotalia* has been grouped with *Sousa* and *Steno* based on morphology. In fact, *Sousa* dolphins were originally assigned to *Sotalia*. The grouping with *Steno* might have resulted from the use of primitive morphological features in pre-cladistic analyses, but has endured to the latest classifications (reviewed in LeDuc et al., 1999). The most accepted morphological classification was proposed by Perrin (1989). This classification maintains *Sotalia*, *Sousa* and *Steno* as closely related (Subfamily Stenoninae). *Sousa* is a genus with two recognized species: *S. teuszii* from the Eastern Atlantic, and *S. chinensis* from the Indo-Pacific. A third species, *S. plumbea*, occurring in the Western Indian Ocean, is regarded by most authors as a synonym of *S. chinensis*. *Sousa* dolphins are morphologically similar to *Sotalia*. *Steno* is a monotypic genus comprised of *S. bredanensis*, a larger dolphin found around the globe in tropical and subtropical waters (Jefferson et al., 1993).

Molecular markers have also been used to investigate delphinid evolution. LeDuc et al. (1999) reassessed the phylogenetic relationships within Delphinidae using full cytochrome b sequences (about 1.2 kilobases) of 33 species. Among several interesting findings, their analysis placed *Sousa* outside Stenoninae, which comprised *Steno* and *Sotalia*. Stenoninae, however, had low bootstrap support. According to their results, *Sousa* belongs to Subfamily Delphininae.

The most recent analyses used a less complete *taxon* sampling (17 species) but a larger number of sequences (5.2 kilobases, including two mitochondrial and ten nuclear markers; Caballero et al., 2008). Differently from the work by LeDuc et al (1999), Caballero et al. showed *Sousa* and *Sotalia as* sister taxa within Delphininae, separated from *Steno*. The combined phylogeny grouped *Sousa* with the Delphininae species in the analyses, and both

Sotalia species as a monophyletic clade branching from this grouping. *Steno* is placed with Globicephalinae, *Orcaella* and *Grampus*.

The phylogenetic position of *Sotalia* will probably remain unsettled until the taxonomy of *Steno* and *Sousa* is resolved. None of the above mentioned studies included *S. teuszii*, which is the *Sousa* species geographically closer to *Sotalia*, or *Sousa* dolphins from Australia, which may belong to a third species according to mitochondrial control region sequences (Frère et al., 2008). The existence of other species of *Steno* is also still an open issue, since very little is known about those dolphins (Jefferson, 2002).

Conservation Aspects

The uncertainty about the taxonomic situation of *Sotalia* dolphins hindered the evaluation of their conservation status, and combined with the lack of information on their biology and ecology, determined their classification as "data deficient" by the International Union for the Conservation of Nature (IUCN; 2008) and the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, 2001). The clarification of the specific status of both *Sotalia* species was an important first step toward the proper assessment of their conservation status.

One of the consequences of the recognition that the two ecotypes of *Sotalia* constitute different species is that *Sotalia fluviatilis* becomes the only exclusively freshwater delphinid in the world (Cunha et al., 2005). To date, there are only three other living species of cetaceans known to exist exclusively in freshwater, two of them belonging to the Platanistidae (*Platanista gangetica* and *P. minor*) family, and the other to the Iniidae family (*Inia geoffrensis*, which probably includes a fourth species, *Inia boliviensis*, Banguera-Hinestroza et al. 2002). The baiji (*Lipotes vexillifer*, Family Lipotidae) was another river dolphin, endemic to the Yangtze River, but is now believed extinct in the wild (Turvey et al., 2007).

At least four other dolphin species can be found both at sea and in rivers: three are delphinids (*Sousa chinensis*, *S. teuszii* and *Orcaella brevirostris*), and the other is a phocoenid (*Neophocaena phocaenoides*). However, there is no agreement about the degree of differentiation between their marine and riverine populations, except for *Orcaella brevirostris*. Beasley et al. (2005) demonstrated, using molecular analyses, that there are two *Orcaella* species (*O. brevirostris* and *O. heinsohni*), and that *Orcaella brevirostris* has both coastal and riverine populations. Therefore, *Sotalia fluviatilis* is the first delphinid living exclusively in freshwater.

S. fluviatilis is endemic of the Amazon River and its main tributaries, from Brazil to Colombia, Ecuador and Peru (da Silva & Best, 1996; Flores, 2002). The Amazon River basin has been experiencing a steep increase in human activities in the last decades, most of them potentially harmful to the Amazon river dolphins. Several anthropogenic threats have been identified (ex. direct and indirect catch, building of dams, habitat loss and degradation, heavy-metal contamination - Best and da Silva, 1989), but their effects on *S. fluviatilis* populations remain unknown (IBAMA, 2001; Reeves et al., 2003). Those potential threats, combined with the newly found endemism of *S. fluviatilis*, may jeopardize its persistence.

River dolphins are the most endangered cetaceans, because they share their endemic, restricted habitat with increasing human populations and are therefore exposed to several direct and indirect human-related threats (Reeves et al., 2003). For that reason, they have been granted special conservation status. The newly found endemism of *S. fluviatilis* implies that its conservation status should be reassessed, and it also should be included in the river dolphin category for conservation purposes.

The molecular identification of *Sotalia* species also led to an important discovery: dolphin-derived products, illegally sold in the Brazilian Amazon as love charms, do not belong to the red boto (*Inia geoffrensis*), as advertised by sellers. Instead, all samples that had actually been obtained from dolphins belonged to the marine *S. guianensis* (Cunha & Solé-Cava, 2007; Gravena et al., 2008; Sholl et al., 2008). *S. guianensis* amulets were detected not only in Belém (Pará state, at the Amazon estuary) but in Manaus and Porto Velho, despite the availability of botos and of *S. fluviatilis* in those areas. In one market (Ver-o-peso of Porto Velho, Rondônia), 90% of the eyeballs sold were in fact from pig or sheep (Gravena et al., 2008). The assessment of the impact of this illegal activity depended on the identification of the targeted species. Now that *S. guianensis* has been recognized as possibly the only species currently used, authorities can act on the sources of charms, which are likely to be the Amazon estuary and adjacent Pará and Amapá coasts. *S. guianensis* has been intentionally caught in those areas to be used as shark bait (Pinedo, 1985) - a single boat had 83 specimens on board (footage done by IBAMA and broadcasted by a Brazilian television network on 07/16/2007). Dolphin charms may originate both from by-catch from legal fisheries, and as a second commodity of the illegal bait catch.

Molecular Ecology

Molecular markers have been successfully employed to investigate other aspects of the biology of *Sotalia*, especially their population structure and social behaviour. Although studies on *Sotalia* dolphins are still in course, they promise to reveal important data for the conservation of those species.

Population Structure and Phylogeography

Phylogeography is a field of research concerned with the evolutionary and demographic processes that shaped the genealogical lineages within or between closely related species (Avise, 2004). Phylogeographic analyses focus on the species' past, but provide important insights on its present-day population structure. Most endangered species are highly structured, because reductions in abundance contribute to the isolation of populations (O'Brien, 1994; Frankham, 1996; Avise, 2004) and small population sizes increase genetic drift, which accelerates population differentiation. As a result, endangered species are often subdivided in demographically independent units, each with a population size more affected by local birth and death rates than by migration rates. Hence, the persistence of each unit is linked to the evolutionary and demographic processes acting upon it (Moritz, 1994; Avise, 2004; Palsbøll et al., 2007). Population units that should be considered independently for evolutionary biology purposes have been named "Evolutionarily Significant Units" (ESU) (Ryder, 1986). Later, Moritz (1994) proposed the term "Management Units" (MU) to designate units for conservation purposes. MUs are different from ESUs because they are less restrictive and closer to the demographic present of species.

The population structure and phylogeography of *S. guianensis* along the Brazilian coast was investigated by Cunha (2007), using mtDNA control region sequences. Analysis of molecular variance (AMOVA; Excoffier et al. 1992), spatial analysis of molecular variance (SAMOVA; Dupanloup et al 2002) and Nested Clade Analysis (NCA; Templeton 1998, 2001) showed evidence for at least six MUs in Brazil: Pará, Ceará, Rio Grande do Norte, Bahia, Espírito Santo and the South-Southeastern area (from Rio de Janeiro to Santa Catarina states, Figure 1). Those MUs were highly differentiated (Φ_{CT} = 0,485, $P < 10^{-5}$), indicating severe restrictions to gene flow among them. An interesting finding was a lack of variation in the control region of dolphins from South-Southeastern Brazil (between parallels 22º and 25ºS, extending 900 km). NCA and genetic diversity patterns suggest that this homogeneity might have been caused by a recent colonization of the Brazilian coast through a range extension from north to south, which could be linked to a warming up of the Western Atlantic during the Holocene. Thus, the observed homogeneity is probably not due to gene flow within the region, but a consequence of recent foundation (Cunha & Solé-Cava, 2006; Cunha, 2007).

Populations of *S. guianensis* from the northern part of South America and the Caribbean were analyzed by Caballero et al. (2006), who proposed two MU for that area: one for Central America, Colombia and Venezuela, and another for Guyana, Surinamee and French Guiana. The authors advised that dolphins from the Maracaibo Lake, despite being included in the first MU, had some unique haplotypes and their genetic distinctiveness should be further investigated. However, only three individuals from southern Maracaibo were analyzed: the others were from the northern portion of the lake, where it opens to the Gulf of Venezuela. Clearly, further analyses of samples from the Maracaibo must be analyzed to verify their possible genetic distinctiveness.

To date, there is no information on the population structure of *S. fluviatilis*. The only data available suggest that the species has moderate to high genetic diversity, since 12 individuals from the same location in the Central Brazilian Amazon had five different control region haplotypes (Cunha et al., 2005), and 21 dolphins from the Peruvian, Colombian and Brazilian Amazon had 13 haplotypes (combining the control region and ND2, Caballero et al., 2007). Microsatellite variation was also larger in *S. fluviatilis* (H = 0.531) than in *S. guianensis* (H = 0.364; Cunha and Watts, 2007). The reason for a higher level of gene variation in *S. fluviatilis*, in spite of its probably smaller population size, remains to be determined.

Social Structure

Undoubtedly, the newly developed microsatellite markers will be invaluable also for the investigation of the social structure of *Sotalia* dolphins. Besides being highly polymorphic, microsatellites are useful for that purpose because they are bi-parentally inherited.

During the last decade, many interesting results have been found concerning the social behaviour of *S. guianensis*, especially through long-term photo-identification studies. Three local populations in Brazil showed strong residency (North Bay, Santa Catarina - Flores 1999; Cananéia Estuary, São Paulo - Santos et al., 2001; Guanabara Bay, Rio de Janeiro - Azevedo et al., 2004), and that pattern may prove to be a feature of that species throughout its distribution.

In spite of the vast database on social associations built during the long-term monitoring of some *Sotalia guianensis* populations, studies on social structure have been hampered by the absence of easily observable sexual dimorphism. Sex determination of free-ranging *Sotalia* relies on the observation of the animal's ventral area, which is a rare event in the field. Therefore, sexing is only achieved for reproducing females, on the basis of their close, lasting and recurring association with calves. That approach demands a long-term monitoring of the population, and does not allow the detection of males and non-reproductive individuals. Fortunately, remote biopsy darting has been safely and successfully applied to *Sotalia* dolphins, providing samples that can be sexed molecularly. Two genetic systems are usually applied for sex determination in cetaceans: the ZFX/ZFY (Bérubé and Palsbøll, 1996) and the SRY (Palsbøll et al., 1992). Both systems have been tested and optimized for *Sotalia* species, and have been successfully used for sexing biopsy samples (Cunha and Solé-Cava, 2007) (Figure 6). Additionally, those molecular techniques allow the sex determination of carcasses in advanced decaying, when sexing cannot be done by the examination of the genital opening.

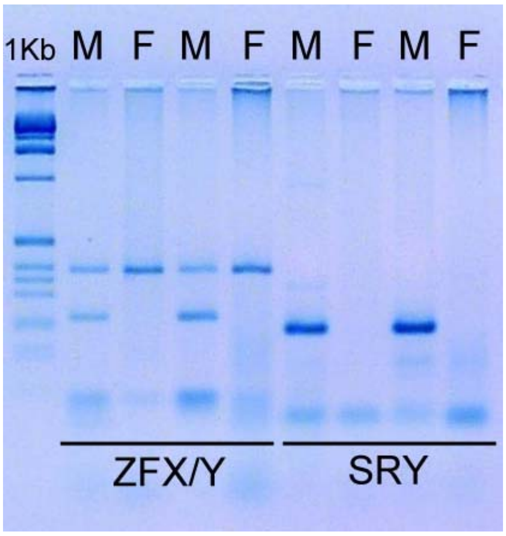

Figure 6. Sex determination patterns of *Sotalia* samples using the ZFX/ZFY and SRY systems. M: male, F: female, 1Kb: DNA size ladder.

The residency of local populations of *S. guianensis* could reflect the phylopatry of one of the sexes. In most mammals, females are the phylopatric sex while males disperse (Dobson, 1982). This pattern has been observed in almost all small cetacean species studied so far (e.g. *Tursiops truncatus* - Scott et al., 1990; Duffield Wells 1991; *Delphinapterus leucas* - O'Corry-Crowe & Lowry, 1997; *Phocoena phocoena* - Rosel et al., 1999; *Phocoenoides dalli* - Escorza-Treviño & Dizon, 2000; *Cephalorhynchus hectori* - Pichler and Baker, 2000; *Tursiops aduncus* - Möller & Beheregaray, 2004). It is possible that *S. guianensis* shares the same sex bias in dispersal, but until now that could not be evaluated due to the impossibility of visually sexing the resident animals. The hypothesis of female phylopatry can be tested with the comparison of maternally inherited mitochondrial DNA with bi-parentally transmitted markers such as microsatellites, as well as through studies of social structure coupling photo-identification and biopsy sampling.

The genetic analysis of biopsies from photo-identified dolphins will also provide a finer-scale picture of the social structure of *S. guianensis*, by seeking correlations between kinship and social affiliations, as has been done with other delphinids recently (e.g. Möller et al., 2001, 2006; Krützen et al., 2004).

The above mentioned methods can also help to unveil the social structure of *S. fluviatilis*. The only available information on the social organization of this species are from mark and recapture data, suggesting that *S. fluviatilis* in the Central Amazon is not territorial, but shows strong site fidelity (spending up to 9 years in the same area). Group structure seems to be socially organized by fusion-fission strategies, and some animals have been sighted together 8.5 years after marking (da Silva & Martin, unpublished data).

Another interesting prospect is the investigation of the mating system of *Sotalia* dolphins. Until now, the only hypothesis advanced was of polyandry of both *Sotalia* species, based on their large testis sizes (an indication of sperm competition) (da Silva & Best, 1996; Rosas & Monteiro-Filho, 2002). Mating system can be studied using microsatellites because they have the ability to ascertain paternity. That is useful when different mother-calf pairs from the same group are biopsied, and also when known siblings are sampled (for instance as calves from the same female), since the genotype of the father can be reconstructed from the calf's genotype if the mother's genotype is known. Hence, it is possible to check how many calves from the same cohort are fathered by the same male, and if calves of the same female born in different years are full siblings.

Conservation Implications

Studies on the population structure, phylogeography and social structure of *Sotalia* species will certainly help in the evaluation of their conservation status, and contribute to the design of effective measures for their conservation.

A proper evaluation of the impact of non-natural mortality on populations can only be achieved when their geographical boundaries are known. Additionally, population delimitation is fundamental for the design of effective conservation measures (O'Brien, 1994; Avise, 1997). The goal of any conservation plan should be to preserve the target species both in time and space. That means the entire range of the species should be maintained, which is an obvious challenge because there is hardly any species charismatic enough to stop human plans of growth and development in face of the low ecological responsibility of our species. When there is enough gene flow across the species range, individuals removed by human-related factors are replaced from other areas. But when a species is split into different and isolated populations (i.e. MU), each one evolves independently, since they are not connected (and replenished) through migration. Besides, independent units harbour exclusive genetic variation (locally originated or maintained, and not spread to other units due to restricted gene flow), and it is reasonable to assume that some of that variation may encompass local adaptations. It is crucial to ensure that genetic diversity is preserved, because it constitutes the evolutionary potential of the species. Inappropriate management of units may result in the loss of adaptations, which may jeopardize the short-term viability of some populations, or even the species as a whole (Frankham, 1996; Solé-Cava, 2000; Crandall et al., 2000). Therefore, knowledge on the population structure is of paramount importance, as it enhances the probability of success of management and conservation actions (O'Brien, 1994).

Understanding the social structure of *Sotalia* dolphins may also help in their conservation. For instance, if females of either species prove phylopatric, management must be based on mitochondrial data, even if there is evidence of gene flow with nuclear markers (Avise, 1995; Dizon et al., 1997). Mitochondrial DNA is maternally inherited, so it depicts the history and structuring of female lineages. If only males disperse, populations are unlikely to be recolonized after local extinction, and the most conservative strategy would be to ensure the persistence of each population detected with mitochondrial data. In addition, if mortality rates are higher in areas between populations (which has been demonstrated for some species), that mortality would translate into a higher loss of males compared to females, causing unequal sex-ratio and reduction of the effective population size and genetic variability of the species.

The studies cited above provide the first, and most reliable, data for the establishment of MU for *S. guianensis*. Before their publication, there was no information on genetics, demography, morphology, behaviour, bioacoustics, parasites, ecology or contaminants that could argue for any delimitation of MU for the species, even provisional. That is the present situation for *S. fluviatilis*, but it will change in the near future, as the investigation of its population structure using molecular markers is currently underway.

Many threats to the persistence of both *Sotalia* species have been identified. However, the paucity of information on the taxonomy and biology of *Sotalia* dolphins hindered the evaluation of their conservation status; hence they are considered “data deficient” by the Brazilian environmental agency IBAMA (2001) and by IUCN (2008). Some countries took a precautionary approach and decided to give *Sotalia* a conservation status: in Colombia and Venezuela, both species are regarded as “vulnerable” (Rodríguez-Mahecha et al., 2006; Bolaños-Jímenez et al., 2008), and in Ecuador, *S. fluviatilis* is listed as “endangered” (Tirira, 2001). With the data now available, environmental agencies need to reassess the conservation status of both species, especially in Brazil, because that country encompasses over half of the range of *S. guianensis*, and most of the distribution of *S. fluviatilis.*

CONCLUSIONS AND PROSPECTS

This chapter reviewed the latest results on the molecular systematics and ecology of *Sotalia* dolphins. Some of the issues still require investigation, but several important results have been obtained in those fields during the last few years.

Unquestionably, the most remarkable finding to date was the elucidation that riverine and marine ecotypes of *Sotalia* are different species. Molecular markers were fundamental to settle the issue of specific differentiation between *S. fluviatilis* and *S. guianensis*. The impact of that discovery can be appreciated by considering that all articles published since the work of Cunha and co-workers (2005) accepted the revalidation of *S. guianensis* (22 articles – Web of Knowledge search on October, 2008). A major consequence of the split of *Sotalia* species is the need for reassessment of their conservation status, in recognition of the different conservation requirements of both species. The discovery of an exclusively freshwater habit for *S. fluviatilis* indicates that it should have its conservation priority raised. Secondly, the impact of non-natural mortality need to be re-evaluated for each MU of *S. guianensis* across its entire range, and conservation plans must be devised for those MU that show signs of endangerment.

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