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**Introduction**

Taxonomy and systematics play a critical role in the conservation of biodiversity (e.g. Ryder, 1986; Mace, 2004; Aleixo, 2009). Taxonomic names are important for recognition and clear definition of the units to be conserved. Usually, species or subspecies levels are the preferred target of conservation policies and biodiversity monitoring programs (e.g. Karl and Bowen, 1999; Haig *et al.*, 2006; Morrison *et al.*, 2009). In addition, for widely distributed species with considerable fine-scale structuring such as bottlenose dolphins (genus *Tursiops*) (Wells and Scott, 2009), it is important to determine stock units to guide conservation and management procedures at the local level (e.g. Dizon *et al.*, 1992; Moritz, 1994).

In this report, we attempt to compile the current information on morphology, genetics, stable isotopes, acoustics and parasites of bottlenose dolphins along the Southwest Atlantic Ocean (SWAO), which includes waters of Brazil, Uruguay and Argentina. In addition, we briefly review the proposed taxonomy for the genus along the Atlantic coast of South America.

The original source included 11 working papers presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, carried out in Cassino Beach, Rio Grande, Brazil, in 21-23 May 2010. In addition, 47 articles in peer-reviewed journals, two books, six book chapters, four doctoral, four master's and two bachelor theses concerning the bottlenose dolphins in the SWAO complemented this information. Two conference abstracts and personal communications provided by specialists were also used as supplementary data.

**Report of the Working Group on Taxonomy and Stock Identity of bottlenose dolphins in the Southwest Atlantic Ocean**

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## Taxonomy

The taxonomy of the genus *Tursiops* has been debated for a long time. The worldwide distribution and great morphological plasticity of the genus led to a variety of opinions on the status of nominal species (Wells and Scott, 2009). Since the genus *Tursiops* was proposed by Gervais in 1855, more than 20 nominal species have been described in the literature (Hershkovitz, 1966; Rice, 1998). Currently, only two species are recognized by the Society for Marine Mammalogy's Committee on Taxonomy (2015): the common bottlenose dolphin, *T. truncatus* (Montagu, 1821), and the Indo-Pacific bottlenose dolphin, *T. aduncus* (Ehrenberg, 1833). Nevertheless, a third species, the Burrnun dolphin, *T. australis*, was recently described by Charlton-Robb *et al.* (2011).

Historically, the bottlenose dolphins from the Argentinean coast of the La Plata River were described as a distinct species (*T. gephyreus*) by Lahille in 1908, based on two specimens (one male and one female syntypes<sup>1</sup>). True (1914) accepted *T. gephyreus* as a valid species and pointed out that it differs from *T. truncatus* 'mainly in the smaller number of caudal vertebrae, the greater relative length of the beak and, perhaps, the coloration'. Following the validation made by True (1914), this name was applied by almost all researchers to mention the bottlenose dolphins of the SWAO until the 1970s, when only one species (*T. truncatus*), highly polymorphic, was adopted for all the oceans (Rice, 1998). Although *T. truncatus* is currently considered as the only valid name for bottlenose dolphins in the SWAO, many authors after Lahille (1908) have also described marked differences in the animals from southern Brazil, Uruguay and Argentina.

Pilleri and Gihl (1972) analyzed the skull of a bottlenose dolphin captured by fishermen in La Coronilla, department of Rocha, Uruguay and described it as *T. gephyreus* based on morphological skull characteristics. Leboeuf (1981) also proposed, based on the analysis of skulls from southern Brazil (Rio Grande do Sul, n=17) and Argentina (n=20), that bottlenose dolphins from this region should be classified as a distinct taxon and that *T. gephyreus* should be considered a valid species. Barreto (2000) analyzed a larger sample (n=81) of bottlenose dolphins from different regions of the SWAO, including Brazil, Uruguay and Argentina, and found significant differences in skull characters between individuals collected to the north and the south of Santa Catarina State (approx. 27°30'S), southern Brazil. The animals collected in these two regions revealed differences in almost all (*i.e.* 57/59) of the metric variables used. Based on this cranial osteological differentiation, as well as on preliminary genotypic data, the author proposed two different forms of bottlenose dolphins in the region. The specimens collected north of Santa Catarina (called 'northern form') were found to be significantly smaller

than the ones collected south of it ('southern form') in most (*i.e.* 51/58) of the cranial characters analyzed. Considering the morphological differences found between the two forms, Barreto (2000) recommended the use of the subspecies *T. truncatus gephyreus* for the 'southern form'. However, the taxonomic status of the 'northern form' was not defined by the author, since the samples analyzed did not include specimens from other regions of the Atlantic.

The differences between the northern and southern forms of bottlenose dolphins of the SWAO were also corroborated by more recent morphological studies, still using linear measurements and meristic variables<sup>2,3,4</sup> (Wickert, 2010; 2013), as well as geometric morphometrics (Toledo, 2013). All these studies showed significant differences in skull measurements between animals from the two areas. In addition to the metric variables, Barreto *et al.*<sup>3</sup> found that one of the main diagnostic skull characters to differentiate the two morphotypes is the shape of the pterygoids. In the 'northern form', the pterygoid bones are very close to each other, being usually in contact and their posterior borders are almost perpendicular to the longitudinal axis (Figure 1A), whereas in the 'southern form', the pterygoid bones are more separated and the posterior borders are more oblique to the longitudinal axis (Figure 1B). As mentioned by Leboeuf (1981), this character was also used by Lahille (1908) for the definition of *T. gephyreus*.

Barreto *et al.*<sup>3</sup> also pointed out that this cranial osteological differentiation between the northern and southern forms probably results from reproductive isolation, despite the fact that one cannot exclude the influence of an additional clinal variation related to water temperature, as early hypothesized by Barros (1991).

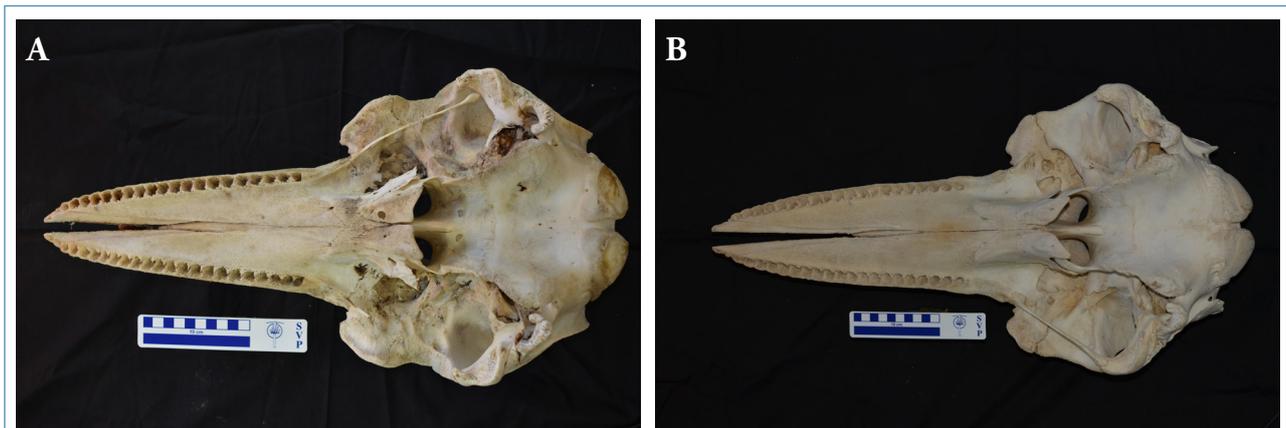
In view of these findings, Barreto *et al.*<sup>3</sup> and Wickert (2010) proposed that the subspecies *T. truncatus gephyreus* should in fact be elevated to the species category. This idea was further supported by a recent study based on both morphological and morphometric analysis of 192 skulls of bottlenose dolphins from Brazil, Uruguay and Argentina (Wickert, 2013). Nevertheless, a formal validation of this proposal, with the redescription of *T. gephyreus* Lahille, 1908, is still pending. Moreover, Toledo (2013) analyzing 424 specimens from the northwest and southwest Atlantic found about the same cranial

<sup>1</sup>Since the male syntype of *T. gephyreus* was lost, the female specimen was recently designed as the lectotype of the species (Varela *et al.*, 2010).

<sup>2</sup>Wickert, J.C., Moreno, I.B., Oliveira, L.R., Ott, P.H., Danilewicz, D. and Machado, R. (2008). *Tursiops gephyreus* Lahille 1908, no Rio Grande do Sul, evidência da existência de duas espécies no Brasil. Page 211 in Abstracts, 12<sup>a</sup> Reunión de Trabajo de Especialistas en Mamíferos Acuáticos de América del Sur, 13-17 October 2008, Montevideo, Uruguay.

<sup>3</sup>Barreto, A.S., Moreno, I.B. and Miranda, C.M. (2010) Geographic variation of the genus *Tursiops* in Southwestern Atlantic. Working Paper 46 presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, Cassino, Brazil, 21-23 May 2010.

<sup>4</sup>Santos, M.C.O., Rosso, S. and Ramos, R.M.A. (2010) Bottlenose dolphins (genus *Tursiops*) in southeastern Brazil: insights on geographic variation based on skull morphology. Working Paper 51 presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, Cassino, Brazil, 21-23 May 2010.



**Figure 1.** Ventral view of skulls of the two forms of bottlenose dolphins from the Southwest Atlantic (*sensu* Barreto, 2000). A – Specimen from the ‘northern form’ (GEMARS 1495). B – Specimen from the ‘southern form’ (GEMARS 0333). Note the differences on the shape of the pterygoid bones (Photos by Janaína Wickert and Ignacio Moreno).

differences, but instead of a division in ‘northern’ and ‘southern’ forms proposed a separation of nearshore and offshore groups. These groups would have a parapatric distribution with an overlapping stranding area from the coast of São Paulo, in Brazil, to Argentina.

In addition to the results from osteological analyses, genotypic data (Moreno *et al.*, 2009; Ott *et al.*, 2009; Fruet *et al.*, 2014; Costa *et al.*, 2015), as well as information on color patterns and external morphology of bottlenose dolphins in southern Brazil (*e.g.* Cremer *et al.*, 2009) and Argentina (Vermeulen and Cammareri, 2009; Goodall *et al.*, 2011), also revealed that the genus *Tursiops* presents a more complex variation than a simple latitudinal differentiation in the SWAO.

Therefore, the separation of *Tursiops* in a northern and a southern form, regardless of its taxonomic status, does not seem to be the only effective conservation strategy to apply for the bottlenose dolphins in the SWAO as further discussed in this report. A comprehensive understanding of the taxonomic status and population structure of bottlenose dolphins in this region depends on a more detailed analysis of individuals from coastal and oceanic zones along different latitudes. In addition, for a complete understanding of taxonomy and stock discreteness of bottlenose dolphins in the southernmost areas of Argentina (between 53°S and nearly 55°S), future genetic studies on both ocean basins off South America will be necessary, since a connection between Atlantic and Pacific animals is possible via the southern fjords and channels of South America (Olavarria *et al.*, 2010; Goodall *et al.*, 2011).

### Review of the available information

#### *Phenotypic data*

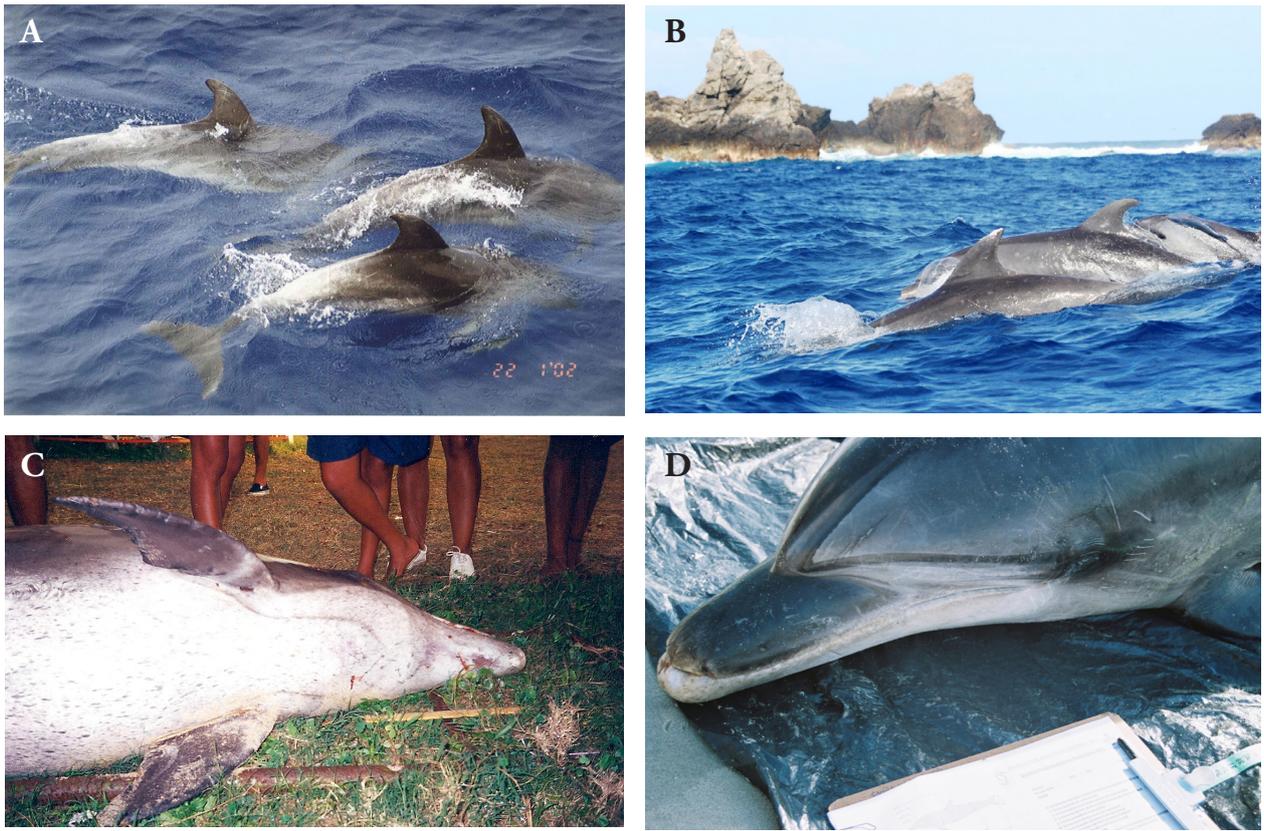
#### *Color pattern and external morphology*

Morphological differences between nearshore and offshore populations of bottlenose dolphin have been described in many geographic locations around the world (*e.g.* Van Waerebeek *et al.*, 1990; Mead and Potter, 1995; Sanino and

Yáñez, 2001; Perrin *et al.*, 2011). In the SWAO, however, this information is incipient and the patterns are still poorly understood. Although considerable variation exists, the pigmentation of bottlenose dolphin in the SWAO resembles the general color pattern described for *T. truncatus* in other regions (Wells and Scott, 1999). The animals have a dark to medium gray dorsal cape, presenting sometimes a light spinal blaze (*sensu* Mitchell, 1970; see an example in Simões-Lopes and Daura-Jorge, 2008). A paler gray covers the sides, grading to white, sometimes with a pinkish hue, on the ventral region.

The existence of color pattern differences in offshore bottlenose dolphins in Brazilian waters was first proposed by Simões-Lopes<sup>5</sup> and it was also described in Simões-Lopes and Daura-Jorge (2008). According to these authors, the offshore bottlenose dolphins in southern Brazil are darker in color with a taller and more falcate dorsal fin compared to the inshore animals. The offshore dolphins also present the anterior edge of the dorsal fin and the dorsal surface of the peduncle with a distinctive lighter coloration. An intense depigmentation in the caudal peduncle area was also reported by Cremer *et al.* (2009) for bottlenose dolphins sighted on the border of the continental slope in southern Brazil (26°46’S, 46°47’W) (Figure 2A). This depigmented area contained overlapping bite marks and tooth rakes and thus it is possible that this color pattern could be either the result of social interactions or at least reinforced by those (A.S. Barreto, pers. obs.). Nevertheless, this pattern was not observed in individuals of the resident offshore population of *T. truncatus* in São Pedro and São Paulo Archipelago (SPSPA, offshore area of northeastern Brazil, 00°55’N, 29°20’W) (P.H. Ott, pers. obs.) (Figure 2B).

<sup>5</sup>Simões-Lopes, P.C. (1996) Offshore and coastal bottlenose dolphins in southern Brazil: Preliminary comparisons on coloration. Page 84 in Abstracts, 7<sup>a</sup> Reunión de Trabajo de Especialistas en Mamíferos Acuáticos de América del Sur, 22–25 October 1996, Viña del Mar, Chile.



**Figure 2.** Examples of the color variation in bottlenose dolphins (genus *Tursiops*) in Brazilian waters. A – Bottlenose dolphins observed on the border of the continental slope, with 200m in depth, (26°46'S, 46°47'W) in Santa Catarina, southern Brazil (Photo by Marta Cremer, UNIVILLE). B – Bottlenose dolphins in the vicinities of São Pedro and São Paulo Archipelago (00°56'N, 29°22'W), distant *ca.*1010km northeast of the coast of Rio Grande do Norte, Brazil (Photo by Lucas Milmann, UESC/GEMARS). C - An adult female (280.0cm length, GEMM 017) incidentally caught in Barra de São João (22°35'S, 41°59'W), Rio de Janeiro, southeastern Brazil, showed several small brown spots in the ventral surface (Photo by Salvatore Siciliano, GEMM-Lagos/Fiocruz). D - A 253.0cm male (LEC/CEM#02) stranded alive in Pontal do Sul (25°31'S, 48°21'W), Paraná, southern Brazil, presenting dark stripes extending from the apex of the melon to the blowhole and to the dark patches around the eyes (Photo by Camila Domit, LEC/UFPR).

A few bottlenose dolphins sighted in coastal waters of Arraial do Cabo (22°57'S, 42°00'W), southeastern Brazil, also showed a distinctive lighter coloration on the peduncle (S. Siciliano, pers. obs.). In addition, an adult female (280.0cm length) incidentally caught in Barra de São João (22°35'S, 41°59'W), Rio de Janeiro, showed several small brown spots in the ventral surface (Figure 2C) (S. Siciliano, unpub. data). Another example of the great color variation existing within this species in the SWAO was observed in an individual from southern Brazil. A 253.0cm male that stranded alive in Pontal do Sul (25°31'S, 48°21'W), Paraná, presented dark stripes extending from the apex of the melon to the blowhole and to the dark patches around the eyes (C. Domit, unpub. data) (Figure 2D).

Along the Argentinean coast, two distinct morphotypes have been described among the bottlenose dolphins that year-round inhabit the shallow waters of San Antonio Bay, in the northern region of San Matías Gulf (40°50'S, 64°50'W), Rio Negro Province. In this area, a few individuals can be easily

distinguishable by a darker coloration, a notably shorter beak and a more falcate dorsal fin (Vermeulen and Cammareri, 2009). Considering that individuals of both forms are residents of the same coastal area, the reported variation is clearly more complex than an 'inshore' *versus* 'offshore' differentiation, as pointed out by the authors.

The available information on external morphology of stranded bottlenose dolphins in this region is even scarcer (*e.g.* Schmiegelow, 1990). To date, the total length is the only external measurement reported for the species in different areas along the SWAO (see below).

#### *Total length (TL) and Condylbasal Length (CBL)*

In many parts of the world, the body size of bottlenose dolphins seems to increase towards higher latitudes (Wells and Scott, 2009). In addition, offshore specimens of *T. truncatus* in the western North Atlantic seem to be larger than those found along the coast (*e.g.* Hohn, 1980; Turner *et al.*, 2006).

**Table 1.** Total body length (TL) of bottlenose dolphins (genus *Tursiops*) along the Southwest Atlantic Ocean. Sample size (N). Note that immature and mature individuals of both sexes are reported.

#	Region	N	TL (cm)	Study
1	Northeastern Brazil	48	138.0 – 321.0	Meirelles <i>et al.</i> (2016 this volume)
2	Southeastern Brazil (Espírito Santo)	1	280.0	Siciliano <i>et al.</i> (2007)
3	Southeastern Brazil (Rio de Janeiro)	11	162.0 – 300.0	Siciliano <i>et al.</i> (2007)
4	Southeastern Brazil (Rio de Janeiro)	13	160.0 – 335.0	Moura <i>et al.</i> (2016 this volume)
5	Southeastern Brazil (Rio de Janeiro)	15	87.0 – 300.0	Lodi (2016 this volume)
6	Southeastern Brazil (São Paulo)	1	240.0	Schmiegelow (1990)
7	Southeastern Brazil (São Paulo)	8	193.0 – 315.0	Siciliano <i>et al.</i> (2007)
8	Southeastern Brazil (São Paulo)	40	150.0 – 330.0	Santos <i>et al.</i> (2010)
9	Southeastern Brazil (São Paulo)	7	252.0 – 312.0	Santos (1999); Santos <i>et al.</i> <sup>4</sup>
10	Southern Brazil (Paraná)	6	205.0 – 294.0	Siciliano <i>et al.</i> (2007)
11	Southern Brazil (Paraná)	10	204.0 – 290.0	Domit <i>et al.</i> <sup>7</sup>
12	Southern Brazil (Santa Catarina)	5	204.0 – 301.0	Simões-Lopes and Ximenez (1993)
13	Southern Brazil (Santa Catarina)	1	320.0	Simões-Lopes <i>et al.</i> (1993)
14	Southern Brazil (Northern Rio Grande do Sul)	43	120.0 – 346.0	P.H. Ott (unpub. data)
15	Southern Brazil (Southern Rio Grande do Sul)	4	231.0 – 337.0	Barros (1991)
16	Southern Brazil (Southern Rio Grande do Sul)	147	124.5 – 384.0	Fruet <i>et al.</i> (2012)
17	Uruguay	8	178.0 – 371.0	P. Laporta (unpub. data)
18	Argentina (La Plata River)	1	276.0	Lahille (1908)*
19	Argentina (Samborombón Bay / La Plata River)	1	320.0	Mermoz (1977)
20	Argentina (San Antonio Bay)	1	310.0	García <sup>8</sup>
21	Argentina (Tierra del Fuego)	6	277.0 – 310.0	Marchesi <i>et al.</i> <sup>9</sup> , Goodall <i>et al.</i> (2011)

\* Holotype (male) of *Tursiops geophysus*.

Herein, we compile the information of total body length (TL) and condylobasal length (CBL) measurements of bottlenose dolphins from different geographic locations along the SWAO (Tables 1 and 2). Nevertheless, these data should be viewed with caution because the sample includes immature and mature individuals of both sexes. In addition, for some areas such as the northern Brazilian coast, the records of bottlenose dolphin are based only in skulls and partial skeletons. Therefore, no information on TL of the specimens is available<sup>6</sup> (Siciliano *et al.*, 2008).

The maximum total length reported for males and females of the species in the SWAO was 384.0 and 340.0cm, respectively, for specimens stranded at the southern portion

of Rio Grande do Sul, southern Brazil (Fruet *et al.*, 2012). The total length of the male syntype of *T. geophysus* from the La Plata River was 276.0cm (Lahille, 1908). Although some variation was detected among the regions (Table 1), the largest animals reported in northeastern Brazil (Meirelles *et al.*, 2016) were smaller than those recorded along Rio Grande do Sul and Uruguay (Fruet *et al.*, 2012; J. Wickert, unpub. data; P. Laporta, unpub. data). However this pattern is not

<sup>6</sup>Sousa, M.E.M., Arcoverde, D.L., Costa, A.F., Emin-Lima, N.R., Santos, G.M.A., Martins, B.M., Rodrigues, A.L.F., Siciliano, S. e Silva Júnior, J.S. (2010) O golfinho-nariz-de-garrafa (*Tursiops truncatus*) na Costa Norte do Brasil. Working Paper 13 presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, Cassino, Brazil, 21-23 May 2010.

<sup>7</sup>Domit C., Weber Rosas, F.C., Rosso-Londoño, M.C., Ougo, G., Bracarense, A.P.F.R., Domiciano, I.G., Beloto, N. e Monteiro-Filho, E.L.A. Ocorrência de *Tursiops truncatus* (Montagu, 1821) no litoral do estado do Paraná, no período de 1997/1999 e 2007/2009. Working Paper 15 presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, 21-23 May 2010, Rio Grande, Brazil.

<sup>8</sup>N. Garcia, pers. comm., 10 May 2010.

<sup>9</sup>Marchesi M.C., Torres, M., Bayer, S., García Peredo, M., Benegas, L.G. and Goodall, R.N.P. (2010) The southernmost records of *Tursiops* sp. Working Paper 06 presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, 21-23 May 2010, Rio Grande, Brazil.

**Table 2.** Condylobasal length (CBL) of the skull of bottlenose dolphins (genus *Tursiops*) along the Southwest Atlantic Ocean. Sample size (N). Note that immature and mature individuals of both sexes are included.

#	Region	N	CBL (mm)	Study
1	Northern Brazil	3	486.5 - 606.5	Sousa <i>et al.</i> <sup>6</sup>
2	Northeastern Brazil	5	501.0 - 521.0	Barreto (2000)
3	Southeastern Brazil	5	502.0 - 528.0	Barreto (2000)
4	Southeastern Brazil (São Paulo)	9	494.0 - 547.0	Santos (1999), Santos <i>et al.</i> <sup>4</sup>
5	Southeastern Brazil (São Paulo)	2	468.0 - 542.0	Schmiegelow (1990)
6	Southern Brazil (Paraná)	5	470.0 - 572.0	Domit <i>et al.</i> <sup>7</sup>
7	Southern Brazil (Paraná)	9	494.0 - 541.0	Barreto (2000)
8	Southern Brazil (Santa Catarina, north to 27°30'S)	5	497.0 - 541.0	Barreto (2000)
9	Southern Brazil (Santa Catarina, south to 27°30'S)	7	558.0 - 580.0	Barreto (2000)
10	Southern Brazil (northern Rio Grande do Sul)	25	496.4 - 621.0	Wickert <i>et al.</i> <sup>2</sup>
11	Southern Brazil (southern Rio Grande do Sul)	38	520.0 - 606.0	Barreto (2000)
12	Southern Brazil (southern Rio Grande do Sul)	17	528.3-600.0	Leboeuf (1981)
12	Uruguay	6	564.0 - 607.0	Barreto (2000)
13	Uruguay	11	532.0 - 583.0	P. Laporta (pers. obs.)
14	Uruguay	7	565.0 - 607.0	Barreto (2000)
15	Argentina (Samborombón Bay / La Plata River)	1	540.0	Mermoz (1977)
16	Argentina	20	516.0-614.0	Leboeuf (1981)
17	Argentina (Chubut)	2	564.0 - 584.0	Marchesi <i>et al.</i> <sup>9</sup> , Goodall <i>et al.</i> (2011)
18	Argentina (Tierra del Fuego)	5	509.0 - 524.0	Marchesi <i>et al.</i> <sup>9</sup> , Goodall <i>et al.</i> (2011)

a simple clinal gradient, since animals from northeastern Brazil are larger than the largest reported from Paraná or Santa Catarina, further south (Table 1).

The asymptotic body length of the species in southeastern Brazil was estimated at 301.3cm based on the analysis of body size maturity of 22 individuals, using the *Gompertz* model (Siciliano *et al.*, 2007), at an estimated age of about 20 years.

The CBL of physically mature animals varies greatly along the SWAO (Table 2), although this variation does not seem to be clinal (Barreto, 2000). The largest skull reported (621.0mm) is from a specimen from Rio Grande do Sul<sup>2</sup>. The animals included in the 'southern form' group seem to have larger skulls than their northern counterpart. Barreto (2000) found that all adult bottlenose dolphins, except three specimens, collected south of Santa Catarina (27°30'S), Brazil, have CBL larger than 553mm (n=53), while all specimens to the north of this locality have CBL equal or smaller than 541mm (n=14). Nevertheless, Sousa *et al.*<sup>6</sup> reported a CBL of 606.5mm for a specimen stranded in Pará State (*ca.* 00°35'S), northern coast of Brazil. This is almost the same size of the largest skulls reported from

the coasts of Uruguay (564.0 to 607.0mm, n=6) and Buenos Aires Province, Argentina (565.0 to 607.0mm, n=8) (Barreto, 2000). In addition, the CBL of the animals from the provinces of Chubut and Tierra del Fuego (Argentina), in the southernmost areas, varied from 564.0 to 584.0mm (n=2) and from 509.0 to 524.0mm (n=5), respectively<sup>9</sup> (Goodall *et al.*, 2011).

In southern Brazil, the physical maturity of the skull of bottlenose dolphins was estimated to occur at five years (Barreto, 1995; 2016).

#### *Genotypic Data*

The first molecular information about the bottlenose dolphins along the Brazilian coast was provided by Barreto (2000). The analysis of 338 base pairs (bp) of the mitochondrial control region (mtDNA) of 16 bottlenose dolphins stranded between Bahia (northeastern Brazil) and Argentina, revealed 11 different haplotypes (*i.e.* unique sequences). A relatively high genetic variability (haplotype diversity [h]=0.882±0.072) was detected for the species in this area (Table 3).

In this same study, a cladistic analysis of the geographical distribution of mtDNA haplotypes revealed that the two

**Table 3.** Genetic diversity of mitochondrial DNA control region of bottlenose dolphins (genus *Tursiops*) along the Southwest Atlantic Ocean. Sample size (N), Sequence length (SL) (in base pairs), Number of haplotypes (H), Haplotype diversity ( $h$ ), Nucleotide diversity ( $\pi$ ).

#	Location	N	SL (bp)	H	$h$	$\pi$	Study
1	Brazil/Argentina	16	338	11	0.882±0.072	-	Barreto (2000)
2	São Pedro and São Paulo Archipelago, offshore Brazilian waters (Equatorial region)	19	316	2	0.105±0.092	0.001±0.001	Ott <i>et al.</i> (2009)
3	Campos Basin-Santos Basin, southeastern Brazil	20	316	6	0.847±0.037	0.017±0.010	Ott <i>et al.</i> (2009)
4	Rio Grande do Sul (northern portion), southern Brazil	20	316	7	0.832±0.057	0.021±0.012	Ott <i>et al.</i> (2009)
5	Florianópolis, Santa Catarina, southern Brazil	8	457	3	0.759±0.096	0.004±0.003	Fruet <i>et al.</i> (2014)
6	Laguna, Santa Catarina, southern Brazil	10	457	1	0.000±0.000	0.000±0.000	Fruet <i>et al.</i> (2014)
7	North of Patos Lagoon Estuary, southern portion of Rio Grande do Sul, southern Brazil	19	457	5	0.542±0.123	0.007±0.004	Fruet <i>et al.</i> (2014)
8	Laguna, Santa Catarina, and Rio Grande do Sul (northern portion), southern Brazil	40	316	8	0.715±0.065	0.017±0.002	Costa <i>et al.</i> (2015)
9	Patos Lagoon estuary, southern portion of Rio Grande do Sul, southern Brazil	63	457	3	0.480±0.062	0.007±0.004	Fruet <i>et al.</i> (2014)
10	South of Patos Lagoon (southern portion of Rio Grande do Sul)/Uruguay	12	457	3	0.648±0.116	0.007±0.004	Fruet <i>et al.</i> (2014)
11	San Antonio Bay, province of Río Negro, Argentina	12	457	1	0.000±0.000	0.000±0.000	Fruet <i>et al.</i> (2014)

samples from Bahia and Rio de Janeiro (northeastern and southeastern Brazil, respectively) were grouped in a separate clade with the only sample from Rio Grande do Sul identified as the 'northern form', by craniometrical features. The remaining 13 samples from southern Brazil and Argentina were grouped in two other clades, which could represent individuals from the estuary and adjacent areas. Nevertheless, since the samples were collected from stranded animals, this hypothesis could not be confirmed. The analysis of molecular variance (AMOVA) among the northern (n=3) and southern (n=13) forms also indicated significant differences between these two groups (Barreto, 2000).

A broader geographical study of genetic variation of mtDNA control region (316bp) in bottlenose dolphins off Brazilian waters, including Rio Grande do Sul (n=20), Campos Basin/Santos Basin (southeastern Brazil, n=20), and SPSPA (n=19) revealed 13 haplotypes for the entire sample (Ott *et al.*, 2009). The haplotype and nucleotide diversities observed for the species were 0.872±0.028 and 0.018±0.0010,

respectively. However, the bottlenose dolphins from SPSPA presented an extremely low genetic diversity ( $h=0.105±0.092$  and  $\pi=0.001±0.001$ ), when compared to Campos Basin/Santos Basin ( $h=0.847±0.037$  and  $\pi=0.017±0.001$ ) and Rio Grande do Sul ( $h=0.832±0.057$  and  $\pi=0.021±0.012$ ) (Table 3). The AMOVA based on mtDNA showed that *T. truncatus* has a high degree of population subdivision along the Brazilian waters and suggests that bottlenose dolphins from SPSPA are genetically isolated from coastal populations. In this sense, no haplotypes were shared between SPSPA and other studied populations (Moreno *et al.*, 2009; Ott *et al.*, 2009).

The bottlenose dolphins from SPSPA were also genetically examined in relation to their effective population size ( $N_e$ ) (Meirelles *et al.*, 2016 this volume). The  $N_e$  was estimated by the direct counting of the reproductive adults sexed by DNA, by the amplification of introns from ZFX and ZFY genes. The sex ratio of this population was 1.11 male to 1 female and the  $N_e$  was estimated in only 12 individuals. However,

the effective population size based on the genetic diversity of 19 sequences of the mtDNA control region (316bp) resulted in a female effective population size of 223 individuals, and the total long-term effective size of about 470 individuals (Oliveira *et al.*, 2016 this volume). The small  $N_e$  from SPSPA, as well as its low levels of mtDNA genetic variability (Table 3) and apparent geographic isolation (Moreno *et al.*, 2009; Ott *et al.*, 2009) indicate that a precautionary approach should be applied to the management of this population.

The presence of bottlenose dolphins has also been reported for other oceanic regions in Brazilian waters, including the biological reserve of Rocas Atoll (03°50'S, 33°49'W) (Baracho *et al.*, 2007) and the Trindade Island (20°30'S, 29°20'W) (Carvalho and Rossi-Santos, 2011) (for further records of *T. truncatus* in offshore areas see Lodi *et al.*, 2016 Report on Distribution, this volume). However, to date, there is no genetic information about the relationships among these offshore populations.

On the other hand, the genetic diversity, structure and connectivity among six coastal populations of bottlenose dolphins from southern Brazil, Uruguay and central Argentina were recently investigated based on the analyses of microsatellite (16 loci) and mtDNA control region sequences (457bp) (Fruet *et al.*, 2014; Table 3). Levels of nuclear genetic diversity were remarkably low (mean values of allelic diversity and heterozygosity across all loci were 3.6 and 0.21, respectively). Thirteen polymorphic sites revealed nine distinct mtDNA haplotypes. The number of haplotypes detected in each sampled location varied from one to five, and haplotype diversity ranged from 0 to 0.75. Overall, nucleotide diversity among all individuals was low ( $\pi=0.009$ ), and haplotype diversity moderate ( $h=0.712$ ), although values varied among populations. A fixed unique haplotype was found for San Antonio Bay population, which differed from the most common haplotype by only one mutational step.

In addition, the authors reported a moderate to strong level of population differentiation using both mtDNA and nuclear markers. On a broad geographical scale, strong and significant genetic differentiation was found between bottlenose dolphins from southern Brazil-Uruguay and San Antonio Bay (AMOVA mtDNA  $\Phi_{ST}=0.43$ ; nuclear  $F_{ST}=0.46$ ), with negligible contemporary gene flow detected based on Bayesian estimates. On a finer scale, moderate but significant differentiation (AMOVA mtDNA  $\Phi_{ST}=0.29$ ; nuclear  $F_{ST}=0.13$ ) and asymmetric gene flow were detected between five neighboring populations in southern Brazil-Uruguay. Based on this pattern of genetic differentiation and the negligible migration rates detected, the authors suggested two distinct lineages, or Evolutionarily Significant Units, one placed in Argentina and the other in southern Brazil-Uruguay. In addition, five distinct populations, or Management Units, characterized by low to moderate asymmetrical gene flow were identified in southern Brazil-Uruguay, which are consistent with a metapopulation model (Fruet *et al.*, 2014).

The coastal bottlenose dolphins from southern Brazil (Santo Antônio dos Anjos Lagoon, Mampituba and Tramandaí estuaries) were also studied using both mtDNA control region sequences (316bp; Table 3) and six nuclear microsatellite loci (Costa *et al.*, 2015). The genetic data from 41 free-ranging and stranded specimens revealed the presence of at least three clusters along this portion of the Brazilian coast, with a lower genetic variability detected in the coastal communities. Moreover, the coastal specimens appear to be only neighbors (with no gene flow among them) of a larger offshore population that eventually strands along the coast. The results reinforced the importance of the establishment of management and conservation measures for the species at a local scale (Costa *et al.*, 2015).

#### Stable isotopes

Botta *et al.*<sup>10</sup> evaluated for the first time the regional variation in carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ) stable isotope ratios in *T. truncatus* along the Brazilian coast. The stable isotope signatures in teeth of stranded bottlenose dolphins from São Paulo/Paraná, southeastern/southern Brazil ( $\delta^{13}\text{C}=-13.1\text{‰}$  and  $\delta^{15}\text{N}=15.7\text{‰}$ ) ( $n=5$ ), were significantly more depleted than in the animals from the southern coast of Rio Grande do Sul State, southern tip of Brazil ( $\delta^{13}\text{C}=-10.6\text{‰}$  and  $\delta^{15}\text{N}=17.8\text{‰}$ ) ( $n=24$ ). More recently, Bisi *et al.* (2013) reported muscle isotopic values from seven bottlenose dolphins found stranded along the coast of Rio de Janeiro ( $\delta^{13}\text{C}=-15.5\text{‰}$  and  $\delta^{15}\text{N}=14.9\text{‰}$ ). After accounting for a tissue-dependent  $\Delta^{13}\text{C}$  collagen-muscle ( $\sim 4\text{‰}$ ) (Koch, 2007), carbon isotope values ( $\delta^{13}\text{C}=-11.5\text{‰}$ ) were intermediate between those found in individuals from São Paulo/Paraná and Rio Grande do Sul.

Differences in prey composition of bottlenose dolphins among areas (see Laporta *et al.*, 2016 Report on Biology and Ecology, this volume) should be accounting for some of the observed variation in mean  $\delta^{15}\text{N}$  values found along the Brazilian coast, although local food web enrichment in  $^{15}\text{N}$  due to environmental factors should not be discarded (Botta *et al.*, 2012). Moreover, two groups differing in their  $\delta^{15}\text{N}$  were identified, which were interpreted as a consequence of resource partitioning that may be occurring among bottlenose dolphins from southern Brazil (Botta *et al.*, 2012).

Nearshore, benthos-linked food webs are more  $^{13}\text{C}$  enriched compared to more offshore, pelagic food webs (France, 1995). Following this rationale, differences in carbon isotope values found among bottlenose dolphins could be interpreted as a more inshore and estuarine feeding habitat in southern Rio Grande do Sul and a more offshore foraging in São Paulo/Paraná and Rio de Janeiro regions. However, as in the case of  $\delta^{15}\text{N}$ , these variations could be just

<sup>10</sup>Botta, S., Hohn, A.A., Macko, S., Santos, M.C.O. and Secchi, E.R. (2010) Regional and local variation in carbon and nitrogen stable isotopes ratios in *Tursiops truncatus* from south/southeastern Brazil. Working Paper 58 presented during the *First Workshop on the Research and Conservation of Tursiops truncatus: Integrating knowledge about the species in the Southwest Atlantic Ocean*, 21-23 May 2010, Rio Grande, Brazil.

reflecting differences in local basal isotope values instead of a longitudinal trend in foraging habitats.

Nevertheless, carbon isotope values found in bottlenose dolphins from Rio Grande do Sul correspond to values found in the sympatric franciscana dolphin, *Pontoporia blainvillei*, ( $\delta^{13}\text{C} = -10.7\text{‰}$ ; Botta, 2011), which is known to occupy coastal waters (Danilewicz *et al.*, 2009). On the other hand, bottlenose dolphins from São Paulo and Paraná presented lower  $\delta^{13}\text{C}$  than franciscanas ( $\delta^{13}\text{C} = -12\text{‰}$ ; Botta, 2011). The same pattern was reported for Rio de Janeiro bottlenose dolphins, where the species was classified as a continental shelf species (Bisi *et al.*, 2013). Nevertheless, considering that the common bottlenose dolphins are also sighted in shallow coastal waters off Rio de Janeiro (*e.g.* Lodi and Neto, 2012) and São Paulo (M.C.O. Santos, pers. obs.), further studies are necessary to elucidate these distinguished patterns shown by stable isotope analyses.

Regardless of the roles of different factors that might be influencing the isotopic patterns described, this approach could be a powerful tool for stock identification of the species in the region<sup>10</sup>.

#### Vocalization

Azevedo *et al.* (2007) characterized the whistles from resident bottlenose dolphins from the Patos Lagoon Estuary, southern Brazil. The mean number of whistles emitted per minute per animal was 0.8. Whistles recorded had a wide frequency range, between 1.2 and 22.3kHz. Whistle duration was  $553.3 \pm 393.9\text{ms}$  and 66.6% of the whistles lasted less than 800ms. Differences in the mean values of the whistles' characters were found between this study and other values previously reported for *T. truncatus*, including the bottlenose dolphin population from San José Gulf, Argentina, reported by Ding *et al.* (1995).

Hoffmann (2004) described the whistles from resident bottlenose dolphins from the Tramandaí Estuary, southern Brazil. Whistle frequency and duration ranged between 1.4 and 17.1kHz and from 41 to 1935ms ( $x = 400\text{ms}$ ), respectively. Hoffmann *et al.* (2012) also described the geographic variability of bottlenose dolphin whistle repertoires between the Tramandaí estuary and the SPSPA. For all whistle parameters, except for the final frequency, whistles between the two groups were statistically different. According to the authors, these differences probably resulted from the geographic isolation of bottlenose dolphins from SPSPA, as well as from the ecological and oceanographic characteristics of each area (*i.e.* estuary and oceanic island). For further details on bioacoustic parameters of the species in the SWAO see the Report on Behavioral Ecology (Domit *et al.*, 2016 this volume).

#### Parasites

Skull bone lesions caused by nematodes (*Crassicauda* sp.) have been proposed as a useful tool to distinguish coastal and oceanic common bottlenose dolphins off the Atlantic coast of North America (Mead and Potter, 1995), as well as off the



**Figure 3.** Ventral view of a skull of bottlenose dolphin from southern Brazil, with typical *Crassicauda* lesions (Photo by Janaína Wickert).

Pacific coast of South America (Van Waerebeek *et al.*, 1990).

Barreto (2000) investigated the possible use of these bony lesions to assign the origin (*i.e.* coastal *versus* oceanic) of the specimens in the SWAO. The author compared animals only within the 'southern form', including specimens from Brazil, Uruguay and Argentina. However, significant differences were found in only 7 of 69 metric and meristic variables among the animals with ( $n=21$ ) and without ( $n=28$ ) bone erosions caused by the parasites. Therefore, multivariate analyses did not identify significant differences between the two groups.

The presence of typical *Crassicauda* lesions in the skull of bottlenose dolphins was also noticed in specimens from other localities of Brazil (*e.g.* Rio de Janeiro - Van Bresse *et al.*, 2007; São Paulo, Paraná, Santa Catarina and Rio Grande do Sul - J. Wickert, pers. obs.) (Figure 3), Uruguay and Argentina (A. Barreto and J. Wickert, pers. obs.), but its potential as a biological marker needs further investigations.

In addition to *Crassicauda*, a few other parasites have been reported for bottlenose dolphins in Brazilian waters (Carvalho *et al.*, 2010; Luque *et al.*, 2010; see also Laporta *et al.*, 2016 Report on Biology and Ecology, this volume), although they were not tested for stock identification purposes.

#### Recommendations

To improve our understanding on the taxonomic status and stock identity of bottlenose dolphins in the SWAO, the following actions are recommended:

1. Increase and refine the studies related to taxonomy and stock discreteness using different methodologies (*e.g.* genetics, morphology, parasites, contaminant loads, stable isotopes) along the entire range of the genus *Tursiops* in the SWAO, including specimens from oceanic waters;
2. Conduct collaborative studies on the coloration patterns of bottlenose dolphins based on the analysis of pictures taken from sighted, stranded, or incidentally caught animals in different areas of the SWAO;

3. Estimate population parameters of bottlenose dolphin along its entire distribution in the SWAO.

In relation to the current taxonomy of the bottlenose dolphins in the SWAO, in spite of the morphological differences reported by many authors for the specimens of the 'southern form' (*sensu* Barreto, 2000), we understand that a revised diagnosis and formal redescription of *T. gephyreus* Lahille, 1908, is necessary before the recognition of its validity. In addition, in order to clarify the taxonomic status of the bottlenose dolphins of the 'northern form' (*sensu* Barreto, 2000), we recommend the examination of the type specimens of *T. truncatus* (Montagu, 1821) and *T. compressicauda* (Lesson, 1828).

Finally, for conservation and management of the bottlenose dolphins in the region, we also recommend that the southern and northern forms of *T. truncatus* in the SWAO (*sensu* Barreto, 2000) should be considered as distinct units for management purposes. This distinctiveness should be taken into account in the future evaluations of the threatened status of the bottlenose dolphins in Brazilian waters.

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