

## **Integrating morphology and DNA barcoding to assess cetacean diversity in Brazil**

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## Abstract

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4 Stranded cetaceans (whales, dolphins and porpoises) are frequently used to obtain data on species  
5 occurrence and demographic trends. Accurate species-level identification of these individuals is crucial,  
6 but often challenging or impossible when relying solely on morphological features (e.g. for highly decayed  
7 specimens). To aid in the development of a reliable molecular assay for cetacean DNA-based identification,  
8 we tested the efficacy of the standardized DNA barcode segment of the *coxI* gene in identifying cetaceans  
9 occurring off the Brazilian coast and in its continental waters. We generated *coxI* sequences from 150  
10 specimens (collected by 16 Brazilian institutions), most of which included voucher material (skulls,  
11 skeletons and/or images) deposited in scientific collections. This allowed a direct comparison between their  
12 morphological and molecular identification. *CoxI* sequences correctly identified ~93% of the samples,  
13 comprising 33 species (70% of the 47 cetaceans reported for Brazilian waters). Two species (*Berardius*  
14 *arnuxii* and *Phocoena dioptrica*) were sequenced for *coxI* for the first time. For only two dolphins (*Stenella*  
15 *coeruleoalba* and *S. clymene*) and a right whale (*Eubalaena australis*), *coxI* failed to identify the species  
16 due to overlapping distributions of intra- vs. interspecific divergences. Only one right whale species occurs  
17 in the southern hemisphere, facilitating identification in this case. *Stenella* dolphins present extensive  
18 sympatry and potential inter-species hybridization, suggesting that nuclear markers may be required for  
19 their reliable identification. These results indicate that DNA barcoding can reliably identify most stranded  
20 cetaceans, and highlight the importance of voucher materials to validate the construction of a reliable DNA-  
21 based identification system.  
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37 **Keywords:** whales, dolphins, stranding, morphological, identification, molecular  
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## 1. INTRODUCTION

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Brazil has one of the world's most extensive coastlines, spanning almost 8,000 km (Ab'Saber 2001), as well as some of its largest freshwater basins (FAO 2016). The biological diversity of these ecosystems has been substantially impacted by increasing anthropogenic changes in the freshwater, marine and coastal regions, threatening the survival of many species and even entire communities (Amaral & Jablonski 2005; Costa et al. 2005).

Knowledge about the existing diversity in the continental, coastal and oceanic regions of Brazil is essential to understand the functioning of its different ecosystems, as well as to ensure the sustainable use and conservation of their living resources (e.g. Longo & Amado Filho 2014). The current knowledge about the aquatic communities in these regions is still insufficient to guarantee their conservation, especially in view of the growing economic interest in exploring these areas, even with the implementation of important research programs in the Brazilian oceanic regions in the last decades (e.g. REVIZEE, Archipelago Program and Oceanic Islands). Cetaceans (*i.e.* whales, dolphins and porpoises) are one of the taxonomic groups that lack basic information, mainly regarding their ecological function in the aquatic ecosystem, making it difficult to establish effective conservation plans and mitigation strategies in the face of environmental impacts (Zerbini et al. 2004; Ott et al. 2009; Siciliano et al. 2012).

Currently, there are confirmed records of 47 cetacean species in Brazil, out of the 90 that are recognized worldwide (Pinedo et al. 1992, 2002; Zerbini et al. 1997, 2004; ICMBio 2011a, b; Hrbek et al. 2014; Cypriano-Souza et al. 2016; Bastida et al. 2018). Eight of them are classified as threatened, and eight are considered "Data Deficient" (DD) in the Brazilian Red List (ICMBio 2018). Moreover, six species are classified as globally threatened and 12 as "Data Deficient" by the IUCN (2020).

Most information about this remarkable cetacean diversity (*ca.* 50% of the global diversity) is usually based on specimens found dead or stranded along the Brazilian coast and continental waters, mostly related to anthropogenic activities (Greig et al. 2001; Van Bressemer et al. 2007; Fruet et al. 2012; Lemos et al. 2013; Prado et al. 2016; Barreto et al. 2020). In this context, the Brazilian Stranding Network of Aquatic Mammals (REMAB) was created in 2011. This initiative includes four regional networks: the Northern (REMANOR), the Northeastern (REMANE), the Southeastern (REMASE), and the Southern (REMASUL) aquatic mammal networks. REMAB is coordinated by the National Aquatic Mammal Center (*Centro de Mamíferos Aquáticos – CMA/ICMBio/MMA*) and operates throughout the nation. The purpose of these networks is to exchange information and experience among institutions and to support government decisions on aquatic mammal conservation in Brazil.

However, the completeness and reliability of the information surveyed by these networks hinges upon accurate species-level identification of detected cetaceans, which is hampered by two challenges: 1) many individuals observed in-water are difficult to identify by the few exposed parts of the body, especially given the morphological similarity between some species; and 2) the advanced decomposition state frequently observed in stranded carcasses (Meirelles et al. 2009; Sholl et al. 2013). In this context, unambiguous species identification often depends on the analysis of collected osteological material (e.g. Pinedo et al. 2002; Meirelles & Furtado-Neto 2004) or molecular identification (e.g. Sholl et al. 2013; Siciliano et al. 2016; Cypriano-Souza et al. 2016). It is important to highlight that when diagnostic body

1 parts, such as the skull or teeth, are lost and the original skin color is no longer present, morphology-based  
2 identification is virtually impossible for most species.

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4 This was precisely the case in the first record of the Omura's whale (*Balaenoptera omurai*) on  
5 the coast of Brazil and the Southwestern Atlantic (Cypriano-Souza et al. 2016). The authors were only able  
6 to reach unambiguous identification of the specimen after generating information from three mitochondrial  
7 DNA (mtDNA) segments [control region, *cytochrome-b* (cyt-*b*), and *cytochrome oxidase c subunit I* (*coxI*)]  
8 and comparing them with sequences of these same segments deposited in molecular databases. Based on  
9 these results, it was demonstrated that there is potential cryptic diversity of cetaceans in Brazil, which is  
10 "hidden" due to the lack of use of molecular techniques as diagnostic tools for these taxa (Sholl et al. 2008;  
11 Cypriano-Souza et al. 2016). A similar situation occurred when Hrbek et al. (2014) found substantial  
12 molecular divergence in mtDNA genes supporting the split of the Amazon river dolphin genus *Inia* into  
13 two species: *I. geoffrensis* and *I. araguaiaensis*, the latter being the only cetacean species endemic to  
14 Brazilian waters. Afterwards, also based on mtDNA control region and *coxI* sequences, Siciliano et al.  
15 (2016) detected the presence of the two species of *Inia* and extended the range of the new species *I.*  
16 *araguaiaensis* into the Amazon delta.

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18 In some notable cases, such as in the family Ziphiidae (beaked whales), genetic analyses play a  
19 critical role in identifying cryptic diversity (e.g. Dalebout et al. 1998, 2002; Yamada et al. 2019), correcting  
20 previous erroneous identifications (e.g. Yamada et al. 2019), or even validating (or revalidating) taxonomic  
21 propositions (Dalebout et al. 2004; Yamada et al. 2019). The elusive behavior of these cetaceans, with little  
22 exposure on the surface and aversion to vessels, and their common offshore distribution (MacLeod et al.  
23 2006), make information on this family particularly difficult to obtain (Dalebout et al. 1998). Ziphiids are  
24 rarely found washed ashore on the Brazilian coast, even in regions with a long time series of beach surveys  
25 (e.g. Meirelles et al. 2009; Prado et al. 2016; Vianna et al. 2016; Barreto et al. 2020). In general, the  
26 identification of beaked whales is based on the analysis of skull and teeth morphology of stranded  
27 specimens, mainly adult males (e.g. Reyes et al. 1995; Mead, 2008). However, erroneous identifications of  
28 beached specimens are not uncommon, mainly due to carcass decomposition (Dalebout et al. 1998) and  
29 lack of some of diagnostic features used for species recognition (shape and position of erupted mandibular  
30 teeth) in females and juveniles (Reyes et al. 1995). Additionally, the geographic distribution of several  
31 beaked whales is poorly known and their occurrence in some regions can be somewhat unexpected  
32 (Siciliano & Santos 2003; MacLeod et al. 2006). Moreover, some morphologically similar species have  
33 overlapping distributions, making the identification of these elusive whales even more challenging  
34 (Dalebout et al. 1998, 2002). In this context, the inclusion of molecular identification techniques that allow  
35 comparisons with reference databases comprising samples that are validated with voucher materials, is  
36 crucial for the identification of beaked whale specimens, especially in the case of cryptic or poorly sampled  
37 species, such as Perrin's beaked whale (*Mesoplodon perrini*), Longman beaked whale (*Indopacetus*  
38 *pacificus*) and the newly described minimal-beaked whale (*Berardius minimus*) (e.g. Dalebout et al. 1998,  
39 2002, 2004; Yamada et al. 2019).

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41 To establish the correct identification of these mammals, which are frequently found washed  
42 ashore and often in advanced state of decomposition, DNA barcoding becomes a very useful tool (Hebert  
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1 et al. 2003; Alfonsi et al. 2013). Analysis based on a fragment of mitochondrial gene *cytochrome c oxidase*  
2 *subunit 1 (coxI)* is a powerful tool to identify individuals at the species level (Hebert et al. 2003). Recently,  
3 Falcão et al. (2017) published a DNA barcoding study on marine mammal species from Brazil and Canada,  
4 but it covered a small portion of the Northeastern Brazilian coast and only four individuals of four species  
5 (*Physeter macrocephalus*, *Peponocephala electra*, *Sotalia guianensis* and *Tursiops truncatus*).  
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8 There are few studies integrating *coxI* and morphology to identify cetacean species (Amaral et  
9 al. 2007, Viricel & Rosel 2011; Alfonsi et al. 2013). Until now there are virtually no cetacean studies  
10 including morphological voucher material, such as skulls, to compare with *coxI* results, probably because  
11 they need a large number of cetacean species with both DNA samples and bones collected from the same  
12 individual.  
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16 In the present study, we evaluate the potential of DNA barcoding for the monitoring of cetacean  
17 diversity along the coast of Brazil and its inner waters. Based on the establishment of a consortium of 16  
18 institutions from the Brazilian stranding network, included in the project “Tetrapoda DNA Barcodes<sup>1</sup>” of  
19 the Brazilian Barcode of Life (BrBOL) initiative, tissue samples were collected from stranded cetaceans as  
20 well as few biopsies taken from live animals along the Brazilian coast. Most DNA samples were associated  
21 with voucher material deposited in scientific collections (e.g. skull and/or skeletons) that could be identified  
22 to species level based on morphological characters, which allowed a controlled assessment of the molecular  
23 identifications performed with the *coxI* gene. We additionally evaluated the quality and reproducibility of  
24 the cetacean taxonomic identification performed by the consortium field researchers, by identifying  
25 degraded carcasses, describing intraspecific variation for some dolphin species and by evaluating the  
26 hypothesis that *coxI* can be an efficient molecular marker to identify cetacean species (Hebert et al. 2003;  
27 Taylor et al. 2017). Finally, we discuss the results with a focus on method validation and its potential  
28 inconsistencies in cases of morphological vs. molecular mismatches (Viricel & Rosel 2011).  
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## 41 2. METHODS

### 42 2.1 Samples

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44 A collaborative stranding network of 16 research institutions investigating aquatic mammal  
45 strandings along the Brazilian coast and inner Amazon basin waters obtained tissue samples from 143  
46 cetacean carcasses. The specimens were recovered during regular beach surveys or notified by locals, from  
47 1989 to 2018, including samples from four regions: south, southeast, northeast and north (see Fig. 1 and  
48 Table 1). Additionally, we also included seven biopsy samples of cetaceans observed during onboard  
49 surveys of oceanic waters. These samples were collected in waters surrounding the São Pedro e São Paulo  
50 Archipelago (also known as Saint Paul’s Rocks) (00°56’S; 29°22’W) and Campos and Santos Basins (from  
51 21°40’S to 27°00’S).  
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59 <sup>1</sup> “Tetrapoda DNA Barcodes<sup>1</sup>: building an integrated network DNA barcoding of amphibians, reptiles,  
60 birds and mammals”,  
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1 Voucher specimens (osteological material or photos that unequivocally identify the species)  
2 from the carcasses sampled in this study are deposited in their respective scientific collections, except for  
3 some stranded baleen whales. This is the first cetacean barcoding study that includes voucher material,  
4 allowing reproducibility of species identification, performed by field correspondents or researchers from  
5 the collaborating institutions. In the field, specimens were initially identified by experienced researchers or  
6 trained assistants, following guidelines suggested by the American Society of Mammalogists in the protocol  
7 *Acceptable Field Methods in Mammalogy: Preliminary Guidelines Approved by the American Society of*  
8 *Mammalogists* (ad hoc Committee on Acceptable Field Methods in Mammalogy 1987,  
9 [http://mammalogy.org/uploads/committee\\_files/ACUC1987.pdf](http://mammalogy.org/uploads/committee_files/ACUC1987.pdf)) and by Geraci & Lounsbury (2005); both  
10 protocols were adopted by Brazilian stranding marine mammal networks (IBAMA 2005). The  
11 identification of each specimen was performed through a combination of diagnostic characters of body and  
12 skull morphology, when necessary. Moreover, information related to total length, sex and the condition of  
13 each carcass, including the state of the decomposition (Geraci & Lounsbury 2005) was also recorded  
14 whenever possible. Tissue samples were collected and stored in 70% ethanol or 20% DMSO saturated with  
15 NaCl and sent to Laboratory of Genetics and Molecular Biology (LGBM) at the University of Vale dos Rio  
16 dos Sinos. Few samples were also sent to Laboratory of Genomics and Molecular Biology at the Pontificia  
17 Universidade Católica do Rio Grande do Sul.

26 DNA was extracted using a phenol/chloroform protocol, and the quality and concentration of  
27 DNA were verified in 1% agarose gel electrophoresis. The concentrations of genomic DNA were estimated  
28 with Nanodrop UV spectrophotometry (Thermo Scientific Wilmington, DE). The DNA samples were  
29 diluted in deionized water until reaching a concentration of approximately 100 ng/ul when necessary.

32 We amplified *coxI* fragments with polymerase chain reactions (PCRs) by applying two primer  
33 pairs, VF1d, VF1i, VR1 and VR1d, which targeted approximately 800 base pairs (bp) (see Supplementary  
34 Material 1 for details). PCR results were verified on 1% agarose gels stained with GelRed (Biotium,  
35 Hayward, CA, USA). PCR products were purified using Shrimp Alkaline Phosphatase (SAP) and  
36 exonuclease I (New England Biolabs), following the manufacturer's recommendation. Amplicons were  
37 sequenced in both directions using universal primers (M13-FP and M13R-pUC, see Supplementary  
38 Material 1).

## 43 2.2 Analysis

46 We manually selected only high-quality *coxI* sequences, with high and clear peaks for each  
47 nucleotide, based on the observation of electropherograms with ChromasPro 2.6.6  
48 (<http://www.technelysium.com.au>). Furthermore, the samples contain data regarding the date and place of  
49 collection and primers used in PCR (Hanner 2009).

52 A total of 150 consensus sequences were automatically aligned (with minor manual correction)  
53 in ClustalW implemented in MEGA 7 (Kumar et al. 2016), with subsequent edition in BioEdit 5.0.9 (Hall  
54 1999). After the alignment, we compared the *coxI* sequences with those available in GenBank  
55 ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and BOLD systems ([www.boldsystems.org](http://www.boldsystems.org)), using the Basic Local Alignment  
56 Search Tool (BLAST) ([blast.ncbi.nlm.nih.gov](http://blast.ncbi.nlm.nih.gov)), which are the two main public databases of DNA barcode  
57 data for all taxa (Meiklejohn et al. 2019). The molecular identifications suggested by both GenBank and  
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1 BOLD were based on the percentage of similarity among sequences. Here, we highlight that for those  
2 species with no *coxI* sequence currently available in the databases, the similarity search retrieved the closest  
3 taxon or no result was returned. Cases of molecular vs. morphology mismatch (Viricel & Rosel 2011;  
4 Alfonsi et al. 2013), due to incongruence between the species identification suggested by *coxI* sequences  
5 (from GenBank or BOLD) and the morphological identification informed by collaborating researchers,  
6 were further investigated. Whenever possible, a revision of the species identification was conducted by  
7 requesting skull or carcass images to the field correspondents. External traits or diagnostic characters of the  
8 skull were analyzed to confirm the identification. In cases of uncertainties, additional marine mammal  
9 specialists were consulted. This procedure was conducted for species of the polyspecific genera such as  
10 *Balaenoptera* and *Stenella*, as well as to the monospecific genera *Orca* and *Pseudorca*. Moreover, field  
11 notes on the specimens collected were also double-checked in the catalogue books of the scientific  
12 collections, mainly regarding the decomposition stage (including images from the sampling), which could  
13 explain some of the mismatch results (see Discussion).  
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20 Genetic divergences (dA) (intraspecific and interspecific) were calculated using the K2P model  
21 (Kimura 1980) for those species that did not exhibit a clear-cut barcoding gap to establish the interval of  
22 genetic divergence between them (e.g. some delphinids). According to Hebert et al. (2003), the lower limit  
23 for genetic divergence (dA) between species is around 3%. Values closer to this limit were considered in  
24 the present study as the lower level for cetacean species delimitation using the *coxI* marker (Siciliano et al.  
25 2016; Taylor et al. 2017).  
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30 To test the hypothesis that all *coxI* sequences belonging to the same cetacean species form a  
31 monophyletic cluster, we performed two types of phylogenetic reconstruction: a Neighbor-Joining tree (NJ)  
32 tree using the Kimura 2-parameter (K2P) model implemented in the software MEGA 7 (Kumar et al. 2016);  
33 and a maximum likelihood (ML) tree recovered with the program RAxML 8.2 (Stamatakis 2014). For the  
34 latter, we used GTR+4G as the substitution model, as estimated with jmodeltest2 (Darriba et al. 2012). To  
35 perform these phylogenetic analyses, we assembled and aligned our 150 *coxI* consensus sequences with the  
36 71 sequences available on the BOLD platform, totaling 221 *coxI* sequences representing 33 cetacean taxa.  
37 The species *Hippopotamus amphibius*, available on the BOLD platform (GBMA2411-09), was used as  
38 outgroup.  
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### 44 3. RESULTS

45 We recovered *coxI* sequences spanning 644 bp to 847 bp from 150 samples representing 33  
46 species. The recorded species were distributed in nine cetacean families, including both odontocetes (i.e.  
47 dolphins, porpoises and toothed whales) and mysticetes (i.e. baleen whales) (Table 1). A total of 865 and  
48 857 *coxI* sequence records were identified in the NCBI and BOLD nucleotide databases, respectively,  
49 representing 898 cetacean specimens (Fig. 2A; Table 1). We are adding 150 sequences which will represent  
50 14.4% growth in the number of specimens and 16.8% of all cetacean samples in the databases (Fig. 2A).  
51 The number of individuals per species ranged from one to 11 (mean = 4.6). Two species were sequenced  
52 for *coxI* for the first time (*Berardius arnuxii* and *Phocoena dioptrica*). The molecular identification was in  
53 accordance with the external morphology-based identification in 92.7% of the specimens (Table 2).  
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1 Regarding the 11 mismatches between the molecular and morphological identifications among  
2 150 cetacean carcasses (i.e. ~7% of the sample) (Table 2), we can assign them to four causes: 1) incorrect  
3 morphological identification; 2) recent taxonomic changes (species splitting); 3) incomplete molecular  
4 databases, and 4) absence of barcoding gap between species.  
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### 8 **3.1.1 Incorrect morphological identification**

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10 The first mismatch case between morphological and molecular identifications was found in the  
11 ECOMEGA/FURG 45 specimen. The specimen was in an advanced state of decomposition and was  
12 identified during fieldwork as a killer whale (*Orcinus orca*), but both molecular databases indicated a  
13 complete match (100% identity) with the false killer whale (*Pseudorca crassidens*). Unfortunately, the skull  
14 was missing, but the pictures from the head of the dead specimen were sent to two marine mammal  
15 specialists, who concluded based mainly on external morphology and tooth counts (likely nine) that the  
16 specimen was probably a *P. crassidens* (Fig. 3a). Despite a little overlap between the dental formula of  
17 these two species (typically, 7 to 10 teeth per tooth row in *P. crassidens* and 10 to 12 in *O. orca*), the  
18 number of teeth generally is smaller in *P. crassidens* (Jefferson et al. 1993).  
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25 Two other molecular-morphological mismatches due to incorrect morphological identification  
26 in the field were reported: a) GEMARS 1491, putatively identified during fieldwork as a humpback whale  
27 (*Megaptera novaeangliae*), but with both molecular databases identified as a southern right whale  
28 (*Eubalaena australis*) (NCBI= 99.18; BOLD= 98.38); and b) ECOMEGA/FURG 63, morphologically  
29 identified as a sei whale (*Balaenoptera borealis*) but with both molecular databases identified as a Bryde's  
30 whale (*Balaenoptera brydei*) (NCBI=99.23; BOLD=99.22). In both cases, the specimens were found in an  
31 advanced state of decomposition i.e. code 4 according to the classification determined by Geraci &  
32 Lounsbury (2005). Based on the carcass conditions, we presume that the morphological evaluation of the  
33 species identity was very difficult, leading the collectors to misidentify the two specimens.  
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### 39 **3.1.2 Recent Taxonomic Changes (species splitting)**

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41 Three specimens of river dolphins (MPEG 38764, MPEG 42122 and MPEG 42055) were  
42 identified by the field researchers as *Inia geoffrensis*, but the molecular identification with both databases  
43 indicated that they should be classified as *I. araguaiaensis* (NCBI=100; BOLD=99.81 for all cases). These  
44 mismatches are explained by the fact that, at the time the samples were collected and deposited in the  
45 museum collection (between 2007 and 2012, for details see Siciliano et al. 2016), *I. araguaiaensis* had not  
46 been formally described (Hrbek et al. 2014). Until the formal description in 2014 of this taxon and the  
47 deposit of its sequences in the molecular databases, Amazon and Araguaia-Tocantins river dolphins were  
48 jointly identified as *I. geoffrensis*.  
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### 54 **3.1.3 Incomplete molecular databases**

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56 The GEMARS 1155 specimen was morphologically identified as Arnoux's beaked whale  
57 (*Berardius arnuxii*), while the molecular identification performed with both databases indicated that it was  
58 Baird's beaked whale (*Berardius bairdii*) (NCBI=99.70; BOLD=99.69). These species show very slight  
59 morphological differences, and the validity of these species had already been questioned (Balcomb 1989 in  
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1 Jefferson et al. 1993). However, studies based on the mitochondrial *cytochrome b* gene supported clear-cut  
2 molecular differences and recognized them as distinct species (Dalebout et al. 2004). At the same time, it  
3 is noteworthy that these two species exhibit antitropical distributions, with *B. bairdii* occurring only in the  
4 North Pacific Ocean (Kasuya 2009), and that *B. arnuxii coxI* sequences were not previously represented in  
5 these databases. Thus, we conclude that the molecular identification in this case did not match the  
6 morphological identification purely because of the lack of *B. arnuxii* reference sequences, making *B. bairdii*  
7 the closest available species for similarity-based clustering.  
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10 A similar result was observed with the II47907 specimen, which had been morphologically  
11 identified as a spectacled porpoise (*Phocoena dioptrica*) based on the unusual pigmentation pattern of the  
12 species (e.g. double eye patch) and the large and rounded dorsal fin typical of males (Goodall 2009) (Fig.  
13 4). However, the molecular approach identified it as Burmeister's porpoise (*Phocoena spinipinnis*)  
14 (NCBI=97.99; BOLD=97.98). This mismatch resulted from the lack of *coxI* sequences of *P. dioptrica* in  
15 both GenBank and BOLD databases  
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#### 20 **3.1.4 Absence of barcoding gap between species**

21 The sample AQUASIS 02C1152/333 was morphologically identified as a Clymene dolphin  
22 (*Stenella clymene*). This identification was confirmed by GenBank (NCBI=100), but it was ambiguous in  
23 the BOLD database, which reported 100% similarity with both *Stenella frontalis* and *Stenella clymene*. This  
24 specimen was very weak when rescued, according to the Marine Mammal Rehabilitation Center (CRMM),  
25 and it died a few hours after arrival. The fresh conditions of the carcass allowed the precise observation of  
26 a typical *S. clymene* coloration pattern, including the three-part color of the body, the dark mark on the  
27 upper side of the beak ('moustache') and the distinct eye-stripe, some of the most distinctive features of the  
28 species (Perrin 2009; Jefferson et al. 1993) (Fig. 5).  
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35 Likewise, the molecular results suggested that the GEMARS 1240 specimen was a short-beaked  
36 common dolphin (*Delphinus delphis*) with both databases (NCBI=100%; BOLD=99.85%), but the  
37 specimen was morphologically identified during fieldwork and also after the skull examination by a marine  
38 mammal expert as a striped dolphin (*Stenella coeruleoalba*). Taking into account the morphological  
39 diagnosis of deep palatal grooves in the *D. delphis* skull and the fact that in the GEMARS 1240 specimen  
40 this trait was absent, we are confident in the morphological identification as *S. coeruleoalba* (Figs. 6a and  
41 6b). In addition to these dolphin species, we found two cases that seem to reflect the inexistence of a  
42 barcoding gap among right whale species. According to the external morphology, the samples MN60458  
43 and GEMM 0051 were identified as southern right whales (*Eubalaena australis*), and these identifications  
44 were supported by a BLAST comparison against GenBank (MN60458, NCBI=100; GEMM 0051,  
45 NCBI=99). However, the BOLD analysis identified both samples as *E. glacialis* (MN60458, BOLD=100;  
46 GEMM 0051, BOLD=99.69), a species that only occurs in the North Atlantic (Rosenbaum et al. 2000).  
47 Although these findings could also be related to an erroneous deposit of sequences in the BOLD platform,  
48 we believe that the results are more likely derived from a weak or absent barcoding gap between these  
49 species, as noticed by Viricel and Rosel (2011).  
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### 3.2 Inter and Intraspecific distances of *coxI*

Intra- and interspecific genetic divergences for *Delphinus delphis*, *Stenella clymene*, *Stenella coeruleoalba*, *Stenella frontalis* and *Tursiops truncatus* of the family Delphinidae are detailed in Table 3. Measurements of intra-specific variation ranged from 0 to 0.56% while interspecific variation ranged from 0.38% to 2.56%, with a mean divergence of 1.5%. The neighbour-joining tree correctly distinguished all the analyzed cetaceans (Fig. 7), except the species of Delphinidae, which presented a small intrer-specified genetic divergence. However, some species of this family formed clades with high bootstrap support values (>90%): *Sotalia guianensis*, *Steno bredanensis*, *Grampus griseus*, *Stenella attenuata* and *Globicephala melas*.

The same comparative scenario for *coxI* intra- and inter-specific genetic divergences for the three species of *Eubalaena* (*E. australis*, *E. glacialis* and *E. japonica*) is presented in Table 4, based on the analysis of only 11 sequences deposited in both GenBank and BOLD databases. The measurements of inter-specific variation for *coxI* marker of the three species of *Eubalaena* were very small, less than 1%. Moreover, when the standard deviations are taken into account, the limits of divergence among the three species did not support three groups, suggesting the inexistence of a gap among right whales. However, these results must be interpreted with caution, because of the small sample size available for this analysis. Although *coxI* was able to correct the misidentification of the specimen GEMARS 1491 (*Megaptera novaeangliae* cf.) to a right whale, the highest score (98.38) of the BLAST search of this sequence in the BOLD platform (i.e. the most similar sequences to the query) was shared among four sequences, two *E. australis* and two *E. glacialis*. Moreover, this same searching tool of the Bold platform identified two other southern right whale samples (MN60458 and GEMM 051) as *E. glacialis* and even in the cases that the Brazilian samples were correctly identified as *E. australis* (GEMARS 1456, and GEMARS 1467) the first five results of the target sequences also include *E. glacialis* and *E. japonica*.

### 3.3 Phylogenetic reconstruction through Maximum-likelihood

Although there were problems with determining inter-specific limits for some species of Delphinidae, the maximum likelihood (ML) tree reconstructed most species in well-defined clades (Fig. 8), supporting the use of *coxI* as a useful marker for species identification in cetaceans, except for *Delphinus delphis*, the only species that did not form a monophyletic group.

## 4. DISCUSSION

This study generated 150 sequences from the *coxI* region of 33 cetacean species, which represent 70% of the Brazilian diversity of this taxon. The molecular identification was in accordance with external morphology-based identification in ~93% of the specimens. We detected 11 cases of molecular-morphological mismatched identifications; all were solved in favor of the molecular identification, except three cases in which we observed an absence of barcoding gap between delphinid species (genera *Stenella* and *Delphinus*) and probably two cases among the right whales (*Eubalaena* spp.). Overall, these results demonstrate that DNA barcoding data are highly efficient as a tool for taxonomic identification of cetacean species along the Brazilian coastal and continental waters.

1 Alfonsi et al. (2013) were able to amplify *coxI* sequences of good quality from 150 highly  
2 degraded carcasses of marine mammals found along the Brittany coast in France. They correctly identified  
3 all specimens, that represent around 16% of the specimens recovered every year along the coast of France  
4 and concluded that DNA barcoding, even with certain constraints, is very useful for the French stranding  
5 network. In face of their findings, Alfonsi et al. (2013) suggested that DNA barcoding could be useful for  
6 the monitoring of marine mammal strandings at three levels: i) by providing a confirmation or an additional  
7 degree of taxonomic determination of rare species identified by field researchers, mainly in uncommon  
8 stranding events of rare or deep-living species (Thompson et al. 2012); ii) by helping the identifications at  
9 species level when it is not possible to identify the animal by the external morphology due to highly  
10 degraded carcasses or even when morphology-based identification only reaches the genus or family levels,  
11 due to incomplete skeleton or skull, and iii) by offering intraspecific genetic variability, which allows  
12 genetic structure analysis, and eventually monitoring population movements (Pauls et al. 2012).  
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18 In general, most species identified here with the molecular approach were very common in the  
19 coastal region, with no challenging identification, such as franciscana dolphins (*Pontoporia blainvillei*) and  
20 the common bottlenose dolphin (*Tursiops truncatus*). There were also records of rarely found stranded  
21 specimens belonging to Ziphiidae and Phocoenidae and oceanic and deep-diving species such as pygmy,  
22 dwarf and sperm whales (Pinedo et al. 2002; Prado et al. 2016), and the only endemic cetacean species for  
23 Brazil, the recently described Araguaian River dolphin. It is worth mentioning that the present study is one  
24 of the few involving DNA barcoding sequences of samples associated with voucher materials deposited in  
25 scientific collections, enabling morphological checking whenever necessary, and thus providing greater  
26 reliability of the use of the molecular marker. According to Hanner (2009), as part of the BOLD quality  
27 control, DNA barcodes must be associated with specimen records linked to institutional (e.g. museum)  
28 material making them the most valuable as reference accessions. This is particularly important in cases of  
29 rare species, which usually have no sequences deposited in molecular platforms. The accuracy of DNA  
30 barcoding relies upon the level of taxonomic representation for each group and the amount of intraspecific  
31 genetic diversity represented in the databases (Gaubert et al. 2015).  
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40 It is important to mention that the present study contributed with the inclusion of the first *coxI*  
41 sequences of the spectacled porpoise (*Phocoena dioptrica*) and Arnoux's beaked whale (*Berardius*  
42 *arnuxii*), in both GenBank and Bold databases. The spectacled porpoise is a small cetacean with  
43 circumpolar distribution in Antarctic and subantarctic waters, with only one previous record published for  
44 the Brazilian coast (Pinedo et al. 2002). There was another unpublished record in August 2016 for Cassino  
45 beach (ca. 32°11'S; 52°09'W, in Rio Grande do Sul State - Ecomega unpubl. data), southern Brazil. The  
46 specimen analyzed in the present study was collected at Navegantes Beach, Santa Catarina State  
47 (26°53'40"S; 48°38'32"W) in July 2017, and represents the northernmost record of this species in the  
48 Atlantic Ocean (Barreto unpubl. data).  
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54 The Arnoux's beaked whale was first reported in Brazilian waters based on the collection of a  
55 floating dead specimen close to the coast of São Sebastião, São Paulo State, in southeastern Brazil (Siciliano  
56 & Santos 2003). The specimen sequenced in the present study (GEMARS 1155) stranded in the  
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1 municipality of Balneário Pinhal, Rio Grande do Sul State (30°14'29"S; 50°13'37"W), in January 2004,  
2 representing the second confirmed record of the species in Brazilian waters (Ott et al. 2013).

3 Another record of a poorly known cetacean for which we provide new *coxI* sequences is Fraser's  
4 dolphin (*Lagenodelphis hosei*). There was a mass stranding event of 10 dolphins along 156 km of sandy  
5 beaches in the Rio Grande do Sul State coast, between September and November 1997 (Pinedo et al. 2001;  
6 Moreno et al. 2003), and four of these specimens were analyzed in this study. This stranding was not an  
7 isolated event; other stranded animals were reported in Uruguay as well as in Rio de Janeiro state coast. As  
8 a final counting, around 100 specimens were reported for the Southwestern Atlantic coast in 1997 (for a  
9 review see Moreno et al. 2003).

10 According to Galimberti et al. (2015), it is there is a hidden biodiversity within the mammal  
11 record. The BOLD System had barcoded by the end of May 2015 about 2,850 mammal species, at least  
12 300 unnamed clusters (i.e. not assigned taxonomic rank). Currently, there are approximately 3,587 species  
13 with barcodes recognized in the MammaliaBoL in 2020, 75 of which are cetaceans. Taking into account  
14 the requirement of *coxI* sequences associated to voucher material, Galimberti et al. (2015) emphasized that  
15 the standardized molecular reexamination of museum-deposited voucher specimens and the comparison  
16 with other reference information allow the fast detection of misidentification or uncertainties that typically  
17 occurs in the field.

18 This was particularly true for the case of specimen GEMARS 1491 found on the coast of Rio  
19 Grande do Sul, putatively identified in the field as a humpback whale (*Megaptera novaeangliae* cf.), but  
20 genetically as a southern right whale (*Eubalaena australis*). As mentioned earlier, when we examined the  
21 field notes presented in the catalogue book of the scientific collection, we found that the specimen was in  
22 an advanced state of decomposition, almost buried in sand, and that there was a highlighted note in the  
23 labels saying cf. (*confero*, in Latin), which means "need to confirm" or "need to compare with" (Sigovini  
24 et al. 2016), which supports the care referred to by Galimberti et al. (2015). Moreover, according to the  
25 field notes, the specimen had some sessile whale barnacles still attached to its exposed skin, which led  
26 researchers to believe it was a humpback whale or a southern right whale. Considering that there was no  
27 clear clue in the notes in favor of humpback whale identity, we conclude that the molecular identification  
28 is correct.

29 Francis et al. (2010) commented that field identification for many mammals is difficult because  
30 it is based on the analysis of internal structures such as skull or dentition, which is particularly true for  
31 cetaceans. In these cases, the existence of voucher material as well as DNA samples in scientific collections  
32 becomes crucial to confirm the identification of specimens through complementing approaches (i.e. DNA  
33 barcodes and voucher material). For cetacean taxonomy, one of the main constraints relies on the small  
34 number of reference collections, which are in general dispersed among several museums. The present study  
35 had the privilege to include 16 institutions in Brazil with vast scientific collections, which allowed us to  
36 detect cases of doubtful or incorrect morphological identification in the field, as was the case of  
37 ECOMEGA/FURG 45. This specimen was identified during fieldwork as a killer whale (*Orcinus orca*),  
38 but both molecular databases indicated greater similarity with the false killer whale (*Pseudorca crassidens*).  
39 Due to this incongruity, the available voucher material was reexamined and, taking into account the number  
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1 of tooth pairs visible on the photographs of the stranded animal and the range of tooth pairs described for  
2 both species, the specimen was considered to have a higher probability of being *P. crassidens*, in agreement  
3 with the molecular identifications. Although these two species have completely different external  
4 appearance, skull morphology is quite similar (Heyning and Dahlheim 1988; Baird 2009). Thus, in the  
5 field, when some of the main external morphological traits are missing or are not clearly visible, as in the  
6 case of this specimen, misidentification can occur, highlighting the importance of molecular analyses.  
7 However, two other cases involving the combination of field identification, barcoding information and the  
8 reexamination of the skull morphology from two dolphin specimens revealed unsolved morphological-  
9 molecular mismatches. In the case of GEMARS 1240, both molecular databases suggested that the  
10 specimen was a short-beaked common dolphin (*Delphinus delphis*), but the absence of a prominent  
11 trapezoid-shaped palatal ridge and the deep palatal grooves on the skull of the voucher specimen  
12 corroborated the morphological identification as *S. coeruleoalba* (Jefferson et al. 1993). In the case of the  
13 02C1152/333, the specimen showed a typical coloration pattern of *S. clymene* and this identification was  
14 corroborated by one of the molecular databases (NCBI). Nevertheless, the search in the BOLD System  
15 database resulted in an ambiguous identification with equal probability for *Stenella frontalis* and *Stenella*  
16 *clymene*. It is worth to mention that both cases above involve member of the subfamily Delphininae, which  
17 show an overlap between intra- and inter-specific *coxI* genetic variation, suggesting that *coxI* is an imperfect  
18 barcode for these species.  
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Nevertheless, we cannot rule out that the morphological and molecular mismatch of these specimens represent cases of hybridization between *S. coeruleoalba* and *D. delphis* (GEMARS1240) and between *S. clymene* and *S. frontalis* (02C1152/333). The introgressive hybridization between *S. coeruleoalba* and *D. delphis*, where males of *D. delphis* mate and produce fertile hybrids with females of *S. coeruleoalba*, has been recently reported in the Mediterranean (Antoniou et al. 2018). Moreover, the existence of other presumed interspecific hybrids in the genus *Stenella* (*S. clymene* x *S. longirostris* and *S. attenuata* x *S. longirostris*) has been reported in Brazilian waters (Silva Jr. et al. 2015).

Despite this hypothesis of natural hybridization, two unsolved mismatches (AQUASIS 02C1152/333 e GEMARS 1240) between the morphological and molecular identifications found in this study involved delphinids (*i.e.* Delphinidae family species), as already reported in other studies (e.g. Amaral et al. 2007; Viricel and Rosel 2011; Alfonsi et al. 2013), corroborating the limited efficiency of this marker in identifying these species, mainly within the subfamily Delphininae. Moreover, the neighbor-joining analysis showed that *D. delphis*, *S. frontalis* and *T. truncatus* species do not form monophyletic groups, probably due to introgression processes, as reported for *D. delphis* and *S. coeruleoalba* (Kessler 2019) or due to insufficient time of divergence of some species within the taxa (Zhou et al. 2011). Conversely, through methods that use an evolutionary model, such as maximum likelihood, it was possible to recover a greater number of monophyletic groups that corresponded to the sequences identified at species level for all cetaceans in this study, except for *D. delphis*. Moreover, *D. delphis*, *T. truncatus*, *S. coeruleoalba*, *S. frontalis* and *S. clymene* presented very low interspecific *coxI* distances, which ranged from 2.56% (*S. clymene* vs. *S. coeruleoalba*) to 0.38% (*D. delphis* vs. *S. frontalis*). Due to the difficulty of species delimitation, the Delphinidae has been the target of studies that seek to solve evolutionary relationships among its members using information regarding morphology, genetics and, recently, acoustics and

1 historical biogeography (Amaral et al. 2007; Vollmer et al. 2019). Additional work is required to clarify  
2 species boundaries in this group, thus allowing a more direct assessment of the power of DNA barcoding  
3 for their accurate identification.

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5 In addition to the members of the subfamily Delphininae, our results also indicated a poor  
6 resolution of *coxI* to discriminate among right whales species (*Eubalaena* spp.). Although *coxI* was able to  
7 correct the misidentification of the specimen GEMARS 1491 (*Megaptera novaeangliae* cf.) to a right  
8 whale, the difficulty to discriminate among right whale species using another mtDNA region (*cytochrome*  
9 *b*) was previously mentioned by Viricel and Rosel (2011), but the efficiency of the *coxI* to discriminate in  
10 particular for these taxa was not detailed by those authors. Taking into account the recent divergent time of  
11 these species (Rosenbaum et al. 2000), our results are not surprising. However, since only *E. australis* is  
12 distributed in the southern hemisphere and all three extant species have an antitropical distribution  
13 (Rosenbaum et al. 2000), this limitation of *coxI* would not be a problem to discriminate the southern right  
14 whale from other large baleen whales in Brazilian waters.

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16 In summary, the main concerns regarding the identification of cetaceans using the *coxI* gene are  
17 related to: i) cetaceans that seem not to have the “barcoding gap”, which is a lack of overlap between  
18 intraspecific and interspecific nucleotide divergence in the investigated taxa (Viricel & Rosel 2011); ii)  
19 potential hybrids, which would require the use of biparentally inherited nuclear genes to establish the  
20 identification of the species; iii) taxonomic updates that have not been updated in specimen identifications.  
21 All these concerns are relevant but, according to Galimberti et al. (2015) “...reference sequences constitute  
22 the main core of the DNA barcoding initiative and their absence or the lack of control of the correct  
23 identification of the source specimens by expert taxonomists, can irremediably affect the assignment of  
24 newly generated query sequences”. This is why the existence of voucher material related to every *coxI*  
25 sequence is important.

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27 Although DNA barcoding still generates controversies, when it is considered as a “taxonomic  
28 service” it becomes a very interesting tool, able to contribute to the knowledge of mammal diversity,  
29 providing information on the biology, distribution and conservation of mammals mainly on rare or poorly  
30 investigated taxa (Galimberti et al. 2015). A hidden biodiversity is also observed in large whales, which  
31 had their last species described in 2003 as *Balaenoptera omurai*, based on comparisons of external  
32 morphology, osteology and mitochondrial DNA data (Wada et al. 2003). The species distribution was  
33 recently expanded to Brazilian waters based on a stranded specimen identified by *cytochrome b* and *coxI*  
34 sequences (Cypriano-Souza et al. 2016), since identification through its external morphology had been  
35 compromised due to the decomposition process. Moreover, DNA barcoding proved to be more effective in  
36 discriminating cryptic or morphologically similar species, such as the species of genus *Inia*. The DNA  
37 barcoding approach allowed the researchers to recognize the existence of a distinct lineage confined to the  
38 Araguaia-Tocantins basin (Hrbek et al. 2014) as well as Marajó Bay (Siciliano et al. 2016), in northern  
39 Brazil.

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41 Twelve cetaceans recorded for Brazil are classified by IUCN as “Data Deficient”, mainly due to  
42 the lack of taxonomic or ecological information about these animals (ICMBio/MMA 2018; Hrbek et al.  
43 2014; Cypriano-Souza et al. 2016; IUCN 2020). This scarcity of data and the accelerated process of  
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1 degradation and pollution of the marine and freshwater environments occupied by these species reinforce  
2 the need for studies that can help and optimize the production of knowledge about this group, enabling the  
3 elaboration of conservation plans (MMA 2014).  
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5 Considering this scenario, stranded cetacean carcasses can provide valuable information about  
6 the richness and patterns of occurrence of this group in Brazilian waters (Sholl et al. 2008; Meirelles et al.  
7 2009; Prado et al. 2016; Barreto et al. 2020; Milmann et al. 2020), once correctly identified. Therefore,  
8 despite some recognized limitations (Galimberti et al. 2015), our results reinforce that DNA barcodes, when  
9 properly used, can be a valuable tool for the scientific community involved in the stranding networks and  
10 to support decision-makers and conservation policies.  
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## 14 5. ACKNOWLEDGEMENTS

15 The authors would like to thank all members of 16 institutions involved due to their field work  
16 and sampling activity *Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS)*,  
17 *Ecosistemas Aquáticos (Aquasis)*, *Laboratório de Biologia Molecular da Universidade do Vale dos Sinos*,  
18 *Fundação Osvaldo Cruz (FIOCRUZ)*, *Universidade Federal do Espírito Santo (UFES)*, *Universidade de*  
19 *São Paulo (USP)*, *Universidade Federal de Rio Grande (FURG)*, *Universidade Federal do Paraná*  
20 *(UFPR)*, *Grupo de Estudos de Mamíferos Aquáticos da Amazônia (GEMMAM)*, *Universidade do Vale do*  
21 *Itajaí (UNIVALI)*, *Fundação Mamíferos Aquáticos (FMA)*, *Grupo de Estudos de Mamíferos, Aves e*  
22 *Répteis Marinhos e Costeiros da Região dos Lagos (GEMM/Lagos)*, *Organização Consciência Ambiental*  
23 *(ORCA/ES)*, *Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)*, *Instituto de*  
24 *Desenvolvimento Sustentável Mamirauá (IDSM)*. We also thank the curators MSc. Mauricio Tavares from  
25 the *Museu de Ciências e História Natural (MUCIN)* of the *Universidade Federal do Rio Grande do Sul*  
26 *(UFRGS)*, Dr. João Oliveira from the *Museu Nacional do Rio de Janeiro (MNRJ)*, Dr. Marcos César de  
27 Oliveira Santos from the *Instituto Oceanográfico da Universidade de São Paulo (IO-USP)*, Dr. Silvana  
28 Botta, and the lab. assistants Lilia Fidélis and Wagner Vaz of the *Laboratório de Ecologia e Conservação*  
29 *da Megafauna Marinha (Ecomega-FURG)*, the staff of the *Centro de Biodiversidade Subtropical (CBS-*  
30 *FURG)* as well as a lot of students who have been working with all the cited institutions for the collection  
31 and maintenance of part of the specimens analyzed in this study.  
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43 Finally, the authors would like to thank the organizations supporting this project: Coordenação  
44 e Aperfeiçoamento de Pessoal de Nível Superior (CAPES-PROSUC) (CAPES) (Finance Code 001) for the  
45 provision of the master's degree to VSS and Conselho Nacional de Pesquisa e Desenvolvimento Científico  
46 e Tecnológico (CNPq), which provided research fellowship to LRO (CNPq 303813/2011-3, 308650/2014-  
47 0 and 310621/2017-8), ERS (PQ 310597/2018-8), SS (CNPq 306076/2019-5) and EE (PQ 305040/2008-  
48 1, 311327/2011-7, 310803/2015-2 and 309068/2019-3). The research groups Ecologia e Conservação da  
49 Megafauna Marinha (Ecomega-FURG/CNPq) and Observa Litoral (Uergs/CNPq) contributed to this study.  
50 This study was financed in part by the project “Tetrapoda DNA Barcodes: construction of a DNA barcoding  
51 database for amphibians, reptiles, birds and mammals”, part of the Brazilian Barcode of Life (BrBOL)  
52 initiative (MCT/CNPq/FNDCT N°. 50/2010). S. Siciliano is supported by Program INOVA Fiocruz.  
53 Samples collected by Aquasis (Projeto Manatí) were sponsored by Petrobras through Petrobras  
54 Socioambiental Program. Samples ii015573, ii014622, and ii047907 were collected under permit ABIO n°  
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640/2015, issued by IBAMA. Long-term beach surveys performed by Eomega-FURG has been sponsored by Yaqu Pacha (Germany).

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## Captions

**Figure 1.** Sampling sites of 150 cetacean specimens collected along the Brazilian continental, coastal and oceanic areas. The specimens were grouping by family

**Figure 2A.** Number of sequences of the cytochrome c oxidase subunit 1 gene (*coxI*) obtained in this study and currently available for cetaceans in NCBI and BOLD databases. **B.** Growth of *coxI* records in the aggregated databases as a result of this study.

**Figure 3.A.** ECOMEGA/FURG 45 specimen: a *Pseudorca crassidens* found stranded in advanced state of decomposing. The species identification was based on teeth counting as well as DNA barcoding analysis. Photo: ECOMEGA/FURG. **B.** GEMARS 1491 specimen found on the coast of Rio Grande do Sul and identified as *Megaptera novaeangliae* cf., but genetically it was *Eubalaena australis*. Photo: image bank of GEMARS.

**Figure 4.** II47907 specimen of spectacled porpoise (*Phocoena dioptrica*). Photo: PMP-BS (2020) Ocorrência de Fauna Alvo Individual - ii 047907. Available at <https://simba.petrobras.com.br/simba/web/sistema/pmp/1/individualfaunaoccurrence/33573>.

**Figure 5.** AQUASIS 02C1152/333 specimen of Clymene dolphin (*Stenella clymene*) still alive during its treatment. Photo: Aquasis photo bank.

**Figure 6.** Ventral view of skulls: **A.** Striped dolphin (*Stenella coeruleoalba*) (GEMARS 1240). **B.** common dolphin (*Delphinus delphis*), highlighting the prominence of the palatal grooves (pointed out by the arrows).

**Figure 7.** Neighbour-joining tree generated from pre-existing sequences in BoldSystem and the 150 sequences generated in this study. The colors indicate different families of cetaceans. The colors indicate different families of cetaceans: dark green: Iniidae, brown: Balaenidae, purple: Pontoporiidae, blue: Physeteridae, light green: Kogiidae, pink: Ziphiidae, light blue: Balaenopteridae, coral: Phocoenidae and yellow: Delphinidae.

**Figure 8.** Maximum-likelihood tree generated from pre-existing sequences in BoldSystem and the 150 sequences generated in this study. The colors indicate different families of cetaceans: dark green: Iniidae, brown: Balaenidae, purple: Pontoporiidae, blue: Physeteridae, light green: Kogiidae, pink: Ziphiidae, light blue: Balaenopteridae, coral: Phocoenidae and yellow: Delphinidae.

**Table 1.** Number of sequences of the gene cytochrome c oxidase subunit 1 (*coxI*) obtained from 33 cetaceans species along the Brazilian coast and continental waters by a collaborative stranding network of 16 research institutions. The number of sequences of *coxI* currently (April 2020) available in the NCBI and BOLD databases are also indicated. NA = not available.

**Table 2.** Detailed information on each sample examined: specimen; institution (acronym of the institution responsible for collecting); MID: Morphological identification; NCBI ID: molecular identification suggested by GenBank platform; BOLD ID: molecular identification suggested by BOLD SYSTEM platform; NCBI%: percentage of similarity with the cetacean species deposited in NCBI; BOLD%: percentage of similarity with the cetacean species deposited in BOLD platform; Locality: sampling site. \*Specimens with molecular-morphological mismatch.

**Table 3.** Cetacean species of the subfamily Delphininae of the present study whose *coxI* marker was not efficient to identify at species level taking into account pairwise inter- and intra-specific distances.

**Table 4.** *CoxI* pairwise inter- and intra-specific distances among species of *Eubalaena*, including the two specimens of the present study and sequences deposited in both GenBank and BOLD System databases.

## Declarations

### Conflicts of interest/Competing interests (include appropriate disclosures)

All authors (Vanessa S. Silva - VSS, Natália Skueresky, Fernando Lopes, Tabata K. Koch, Paulo Henrique Ott, Salvatore Siciliano, André S. Barreto, Eduardo R. Secchi, Ana Carolina O. de Meirelles, Vitor Luz Carvalho, João C. G. Borges, Daniel Danilewicz, Ana Paula C. Farro, Lupércio A. Barbosa, José Martins S. Jr., Camila Domit, Inês Serrano, Tiago Silva, Cristine Trinca, Miriam Marmontel, Neusa Renata Emin-Lima, Victor Hugo Valiati, Eduardo Eizirik, Larissa Rosa de Oliveira) declare they had no conflicts of interest whatsoever.

### Availability of data and material (data transparency)

All barcode sequences and their respective accession numbers will be available in GenBank or Bold System when the manuscript will be published.

**Code availability:** Not applicable

### Authors' contributions

L.R.O. and E.E. developed the conceptualization of the study. L.R.O., E.E. and VHV designed the experiments. P.H.O., L.R.O., D.D., R.M. A.B., E.R.S., A.C.O.M., V.L.C., J.C.G.B., L.A.B., J.M.S.Jr., C.D., I.S., T.S., C.T., M.M. and N.R.E. conducted the sampling activity. V.S.S., N.S. A.P.F. and T.K.K., conducted mtDNA sequences generation in the laboratory and V.H.V., V.S.S. and F.L. performed the bioinformatics analysis. L.R.O., V.H.V., E.E. and P.H.O. conducted the validation of the data. L.R.O. and E.E. were responsible for funding acquisition and project administration. V.S.S., L.R.O., V.H.V., S.S., E.E., P.H.O., F.L., D.D., R.M. A.B., E.R.S., A.C.O.M., V.L.C., J.C.G.B., L.A.B., J.M.S.Jr., C.D., I.S., T.S., C.T., M.M. and N.R.E. wrote the original draft. L.R.O. and E.E. and P.H.O. reviewed and edited the final version of the manuscript.

### Consent to participate

All authors (Vanessa S. Silva, Natália Skueresky, Fernando Lopes, Tabata K. Koch, Paulo Henrique Ott, Salvatore Siciliano, André S. Barreto, Eduardo R. Secchi, Ana Carolina O. de Meirelles, Vitor Luz Carvalho, João C. G. Borges, Daniel Danilewicz, Ana Paula C. Farro, Lupércio A. Barbosa, José Martins S. Jr., Camila Domit, Inês Serrano, Tiago Silva, Cristine Trinca, Miriam Marmontel, Neusa Renata Emin-Lima, Victor Hugo Valiati, Eduardo Eizirik, Larissa Rosa de Oliveira) declare that they consent to participate in the manuscript.

### Consent for publication

All authors (Vanessa S. Silva, Natália Skueresky, Fernando Lopes, Tabata K. Koch, Paulo Henrique Ott, Salvatore Siciliano, André S. Barreto, Eduardo R. Secchi, Ana Carolina O. de Meirelles, Vitor Luz Carvalho, João C. G. Borges, Daniel Danilewicz, Ana Paula C. Farro, Lupércio A. Barbosa, José Martins S. Jr., Camila Domit, Inês Serrano, Tiago Silva, Cristine Trinca, Miriam Marmontel, Neusa Renata Emin-Lima, Victor Hugo Valiati, Eduardo Eizirik, Larissa Rosa de Oliveira) declare that they consent for publication of the manuscript.

## **Ethical approval**

The study was based on voucher specimens deposited in scientific collections. All specimens in the scientific collections were found dead, stranded along the coast. No animal was intentionally caught or killed during the summarized research. Consequently, no submission to the institutional ethics or committee on the use of animals is required in Brazil.

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**Table 1.** Number of sequences of the gene cytochrome c oxidase subunit 1 (*coxI*) obtained from 33 cetaceans species along the Brazilian coast and continental waters by a collaborative stranding network of 16 research institutions. The number of sequences of *coxI* currently (April 2020) available in the NCBI and BOLD databases are also indicated. NA = not available. \* all accession numbers of the obtained sequences will be mentioned in the published version of the manuscript.

Common name	Species	NCBI	BOLD	shared between NCBI/ BOLD	Number of sequences obtained*
<b>Delphinidae</b>					
common dolphin	<i>Delphinus delphis</i>	29	19	16	8
common bottlenose dolphin	<i>Tursiops truncatus</i>	122	125	122	7
rough-toothed dolphin	<i>Steno bredanensis</i>	4	4	4	4
Atlantic spotted dolphin	<i>Stenella frontalis</i>	9	10	9	6
striped dolphin	<i>Stenella coeruleoalba</i>	11	13	11	5
Clymene dolphin	<i>Stenella clymene</i>	13	13	13	6
spinner dolphin	<i>Stenella longirostris</i>	117	117	117	5
pantropical spotted dolphins	<i>Stenella attenuata</i>	97	97	97	4
Fraser's dolphin	<i>Lagenodelphis hosei</i>	4	2	2	8
Guiana dolphin	<i>Sotalia guianensis</i>	4	5	2	7
long-finned pilot whale	<i>Globicephala melas</i>	10	11	9	1
false killer whale	<i>Pseudorca crassidens</i>	11	8	8	8
Risso's dolphin	<i>Grampus griseus</i>	8	11	6	3
melon-head whale	<i>Peponocephala electra</i>	7	8	8	4
<b>Kogiidae</b>					
pygmy sperm whale	<i>Kogia breviceps</i>	4	4	4	2
dwarf sperm whale	<i>Kogia sima</i>	4	7	4	6
<b>Physeteridae</b>					
sperm whale	<i>Physeter macrocephalus</i>	88*	71*	70	10
<b>Pontoporiidae</b>					
franciscana dolphin	<i>Pontoporia blainvillei</i>	3	4	3	11
<b>Phocoenidae</b>					
spectacled porpoise	<i>Phocoena dioptrica</i>	NA	NA	- 1	1
Burmeister's porpoise	<i>Phocoena spinipinnis</i>	1	1		3
<b>Iniidae</b>					
Araguaian River dolphin	<i>Inia araguaiaensis</i>	47	47	47	3
Amazon River dolphin	<i>Inia geoffrensis</i>	39	39	39	2
<b>Ziphiidae</b>					
Arnoux's beaked whale	<i>Berardius arnuxii</i>	NA	NA	-	1
Gervais' beaked whale	<i>Mesoplodon europaeus</i>	10	10	10	1
Cuvier's beaked whale	<i>Ziphius cavirostris</i>	26	29	26	4
<b>Balaenidae</b>					
southern right whale	<i>Eubalaena australis</i>	4	4	4	5
<b>Balaenopteridae</b>					

humpback whale	<i>Megaptera novaeangliae</i>	10	12	10	9
common minke whale	<i>Balaenoptera acutorostrata</i>	5	6	5	4
Antarctic minke whale	<i>Balaenoptera bonaerensis</i>	3	3	3	2
sei whale	<i>Balaenoptera borealis</i>	5	5	5	3
Bryde's whale	<i>Balaenoptera brydei</i>	4	3	3	5
fin whale	<i>Balaenoptera physalus</i>	158	161	158	1
Omura's whale	<i>Balaenoptera omurai</i>	8	8	8	1

\* Some sequences attributed to the known synonym *Physeter catodon*.

**Table 2.** Detailed information on each sample examined: specimen; institution (acronym of the institution responsible for collecting); MID: Morphological identification; NCBI ID: molecular identification suggested by GenBank platform; BOLD ID: molecular identification suggested by BOLD SYSTEM platform; NCBI%: percentage of similarity with the cetacean species deposited in NCBI; BOLD%: percentage of similarity with the cetacean species deposited in BOLD platform; Locality: sampling site. \*Specimens with molecular-morphological mismatch.

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
<b>Family Balaenidae</b>								
*GEMM 051	GEMM-Lagos	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	<i>Eubalaena glacialis</i>	99	99.69	22°55'57.86"S	42°31'25.21"W
GEMARS 1456	GEMARS	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	100	100	29°43'49.35"S	49°59'44.92" W
GEMARS 1467	GEMARS	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	100	100	29°26'53.80"S	49°48'32.68" W
*MN 60458	MN/UFRJ	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	<i>Eubalaena glacialis</i>	100	100	22° 26'00"S	42°49'00"W
<b>Family Balaenopteridae</b>								
GEMARS 469	GEMARS	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	99.05	99.04	31° 1'30.00"S	50°42'45.00" W
GEMARS 1042	GEMARS	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	99.08	99.08	30°37'54.50"S	50°25'44.80" W
GEMARS 1468	GEMARS	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	99.15	99.13	30° 9'13.60"S	50°11'35.30" W
LEC#119	UFPR	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	<i>Balaenoptera acutorostrata</i>	98.90	98.9	25°48'42.85"S	48°30'51.49" W
GEMARS 1269	GEMARS	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	100	100	29°26'27.10"S	49°47'57.80" W
ECOMEGA/FURG minke	ECOMEGA/FURG	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	99.58	99.65	Rio Grande - RS	
*ECOMEGA/FURG 63	ECOMEGA/FURG	<i>Balaenoptera borealis</i>	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	99.23	99.22	32° 2'24.00"S	52° 0'9.97" W
ECOMEGA/FURG 61	ECOMEGA/FURG	<i>Balaenoptera borealis</i>	<i>Balaenoptera borealis</i>	<i>Balaenoptera borealis</i>	100	99.35	31°26'16.91"S	55° 0'0.00" W
ECOMEGA/FURG 62	ECOMEGA/FURG	<i>Balaenoptera borealis</i>	<i>Balaenoptera borealis</i>	<i>Balaenoptera borealis</i>	99.70	99.7	32°17'31.52"S	52°15'41.58" W
MPEG 39691	MPEG	<i>Balaenoptera borealis</i>	<i>Balaenoptera borealis</i>	<i>Balaenoptera borealis</i>	100	100	01° 3'52.34"S	46° 2'31.21" W
GEMARS 1406	GEMARS	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	100	100	31° 4'23.34"S	50°16'49.87" W
GEMARS 1425	GEMARS	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	99.71	99.81	30°22'36.77"S	50°16'49.87" W
GEMARS 1694	GEMARS	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	99.54	99.39	30°10'17.99"S	50°11'53.69" W
LEC#154	UFPR	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	<i>Balaenoptera brydei</i>	100	99.34	25°33'39.69"S	48°17'27.78" W
02C0100/421	AQUASIS	<i>Balaenoptera omurai</i>	<i>Balaenoptera omurai</i>	<i>Balaenoptera omurai</i>	100	100	3°32'11.60"S	38°47'51.80" W
GEMARS 0826	GEMARS	<i>Balaenoptera physalus</i>	<i>Balaenoptera physalus</i>	<i>Balaenoptera physalus</i>	97.81	97.79	29°55'14.60"S	50° 5'36.80" W
02C0211/418	AQUASIS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	3°33'54.32"S	38°47'20.84" W
02C0212/645	AQUASIS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	3°36'41.30"S	38°45'19.10" W
GEMARS 0597	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	99.84	99.84	30° 4'4.51"S	50° 9'32.01" W
GEMARS 1409	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	99.84	99.84	29°39'54.40"S	49°57'31.08" W
GEMARS 1451	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	99.85	30°15'51.88"S	50°14'8.16" W
*GEMARS 1491	GEMARS	<i>Megaptera novaeangliae</i>	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	99.18	98.38	30°37'48.80"S	50°25'41.20" W
GEMARS 1683	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	30° 8'32.24"S	50°11'19.50" W
GEMARS 1684	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	29°32'44.89"S	49°52'44.12" W
GEMARS 1685	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	30° 3'37.84"S	50° 9'18.99" W

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
GEMARS 1685	GEMARS	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	30° 3'37.84"S	50° 9'18.99" W
<b>Family Physeteridae</b>								
02C0410/308	AQUASIS	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	99.84	100	3°42'27.93"S	38°27'51.71" W
02C0410/338	AQUASIS	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	3° 6'33.00"S	39°30'38.00" W
02C0411/542	AQUASIS	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	3°43'3.30"S	38°31'20.30" W
02C0411/809	AQUASIS	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	101	3°27'19.00"S	38°56'12.00" W
02C0412/792	AQUASIS	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	99.85	99.85	2°53'16.50"S	41°10'38.90" W
ECOMEGA/FURG 17	ECOMEGA/FURG	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	32°25'34.10"S	52°15'57.49" W
ECOMEGA/FURG 18	ECOMEGA/FURG	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	99	100	33° 3'39.13"S	52°35'55.00" W
ECOMEGA/FURG 19	ECOMEGA/FURG	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	33° 7'7.79"S	52°38'2.76" W
ECOMEGA/FURG 20	ECOMEGA/FURG	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	98	99.7	33°23'11.00"S	52°54'16.49" W
GEMARS 0941	GEMARS	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	30°36'54.90"S	50°24'55.80" W
<b>Family Kogiidae</b>								
ECOMEGA/FURG 33	ECOMEGA/FURG	<i>Kogia breviceps</i>	<i>Kogia breviceps</i>	<i>Kogia breviceps</i>	100	99.37	33° 8'46.03"S	52°26'33.72" W
GEMARS 1496	GEMARS	<i>Kogia breviceps</i>	<i>Kogia breviceps</i>	<i>Kogia breviceps</i>	99.68	99.68	31° 6'40.82"S	50°46'10.74" W
02C0511/703	AQUASIS	<i>Kogia sima</i>	<i>Kogia sima</i>	<i>Kogia sima</i>	100	99.83	3°19'55.83"S	39° 8'25.13" W
02C0511/726	AQUASIS	<i>Kogia sima</i>	<i>Kogia sima</i>	<i>Kogia sima</i>	99.33	99.33	2°56'7.00"S	39°48'59.00" W
02C0512/585	AQUASIS	<i>Kogia sima</i>	<i>Kogia sima</i>	<i>Kogia sima</i>	99.33	99.32	3°19'55.83"S	39° 8'25.13" W
GEMARS 1311	GEMARS	<i>Kogia sima</i>	<i>Kogia sima</i>	<i>Kogia sima</i>	100	99.85	29°57'32.00"S	50° 6'40.10" W
GEMARS 1407	GEMARS	<i>Kogia sima</i>	<i>Kogia sima</i>	<i>Kogia sima</i>	99.06	99.84	30°11'59.35"S	50°12'39.92" W
GEMARS 1421	GEMARS	<i>Kogia sima</i>	<i>Kogia sima</i>	<i>Kogia sima</i>	100	99.85	30°33'25.13"S	50°22'16.14" W
<b>Family Ziphiidae</b>								
*GEMARS 1155	GEMARS	<i>Berardius arnuxii</i>	<i>Berardius bairdii</i>	<i>Berardius bairdii</i>	99.70	99.69	30°14'29.07"S	50°13'37.48" W
MN 84736	MN/UFRJ	<i>Mesoplodon europaeus</i>	<i>Mesoplodon europaeus</i>	<i>Mesoplodon europaeus</i>	99	99.41	22° 6'1.16"S	41° 8'27.35" W

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
02C0810/683	AQUASIS	<i>Ziphius</i> sp.	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	100	100	2°59'18.00"S	39°44'6.00" W
02C0812/305	AQUASIS	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	100	100	3°43'9.16"S	38°30'40.05" W
AB02	UNIVALI	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	100	100	20°29'43.01"S	29°19'41.02" W
GEMARS 1484	GEMARS	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	99.85	99.85	30° 6'0.78"S	50°10'20.82" W
<b>Family Iniidae</b>								
*MPEG 38764	MPEG	<i>Inia geoffrensis</i>	<i>Inia araguaiaensis</i>	<i>Inia araguaiaensis</i>	100	99.81	0°15'8.58"S	48°22'35.91" W
*MPEG 42122	MPEG	<i>Inia geoffrensis</i>	<i>Inia araguaiaensis</i>	<i>Inia araguaiaensis</i>	100	99.81	0°14'48.81"S	48°44'51.40" W
*MPEG 42055	MPEG	<i>Inia geoffrensis</i>	<i>Inia araguaiaensis</i>	<i>Inia araguaiaensis</i>	100	99.81	0°43'21.28"S	48°17'29.22" W
MPEG 42179	MPEG	<i>Inia geoffrensis</i>	<i>Inia geoffrensis</i>	<i>Inia geoffrensis</i>	100	100	0°15'3.22"S	48°44'11.75" W
MPEG 42180	MPEG	<i>Inia geoffrensis</i>	<i>Inia geoffrensis</i>	<i>Inia geoffrensis</i>	100	100	0°14'48.81"S	48°44'51.40" W
<b>Family Pontoporiidae</b>								
GEMARS 0215	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	98	99.85	30°20'29.87"S	50°16'3.18" W
GEMARS 0424	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99	99.84		
GEMARS 0530	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99	99.85	31°25'57.36"S	51° 7'18.94" W
GEMARS 0550	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	100	100		
GEMARS 0634	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99	100	29°51'5.02"S	50° 3'36.36" W
GEMARS 0745	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	100	100	29°59'3.08"S	50° 6'53.64" W
GEMARS 0748	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	100	100		
GEMARS 0749	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99.82	100		
GEMARS 1487	GEMARS	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	100	99.84	RS	
LEC#01	UFPR	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99.85	100	25°37'46.54"S	48°24'46.99" W
LEC#71	UFPR	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99.69	99.69	25°35'56.15"S	48°22'38.95" W
<b>Family Delphinidae</b>								
# 57	GEMM-Lagos	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	99.56	99.69	23° 2'14.21"S	42° 0'10.87" W
BC04	GEMM-Lagos	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	99.68	99.67	22°44'0.13"S	41°40'39.61" W
GEMARS 0221	GEMARS	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	99.85	99.85	31°18'30.00"S	50°58'0.00" W

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
GEMARS 0419	GEMARS	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	99.68	99.68	29°57'52.00"S	50° 6'51.00" W
PA288	IO –USP	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	100	99.84	24° 21' 00" S	46°40' 00"W
ECOMEGA/FURG 7	ECOMEGA/FURG	<i>Delphinus sp.</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	100	99.68	33° 3'35.30"S	52°23'52.40" W
ECOMEGA/FURG 8	ECOMEGA/FURG	<i>Delphinus sp.</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	99	99.23	32°12'8.46"S	52°10'32.70" W
ECOMEGA/FURG 6	ECOMEGA/FURG	<i>Delphinus sp</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	98.32	98.57	33° 5'35.30"S	52°23'42.43" W
ECOMEGA/FURG 38	ECOMEGA/FURG	<i>Globicephala melas</i>	<i>Globicephala melas</i>	<i>Globicephala melas</i>	100	99.68	33° 8'8.48"S	52°25'58.58" W
02C1812/588	AQUASIS	<i>Grampus griseus</i>	<i>Grampus griseus</i>	<i>Grampus griseus</i>	99.38	99.37	4°38'39.80"S	37°32'15.40" W
GEMARS 1236	GEMARS	<i>Grampus griseus</i>	<i>Grampus griseus</i>	<i>Grampus griseus</i>	99.22	99.52	29°41'47.94"S	49°58'36.30" W
MPEG 38480	MPEG	<i>Grampus griseus</i>	<i>Grampus griseus</i>	<i>Grampus griseus</i>	100	99.83	0°43'13.14"S	47°42'13.64" W
02C0212/342	AQUASIS	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	100	99.83	4° 7'3.00"S	38° 8'16.00" W
02C2512/389	AQUASIS	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	99.69	99.84	3° 5'36.00"S	39°31'56.00" W
ECOMEGA/FURG 22	ECOMEGA/FURG	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	99.84	99.84	32° 2'45.13"S	52° 0'30.53" W
ECOMEGA/FURG 23	ECOMEGA/FURG	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	100	98.89	30°58'39.88"S	50°22'53.38" W
GEMARS 0467	GEMARS	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	100	99.85	31° 4'15.00"S	50°44'26.00" W
GEMARS 0488	GEMARS	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	100	99.85	30°57'44.40"S	50°40'4.00" W
GEMARS 1453	GEMARS	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	100	99.84	29°58'36.00"S	50° 7'22.54" W
GEMARS 0435	GEMARS	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	<i>Lagenodelphis hosei</i>	100	100	30° 8'54.88"S	50°11'28.92" W
* ECOMEGA/FURG 45	ECOMEGA/FURG	<i>Orcinus orca</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	100	32°21'3.60"N	52°14'34.80" W
02C1511/783	AQUASIS	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	100	100	2°48'10.10"S	40°27'24.80" W
02C1511/784	AQUASIS	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	100	100	2°48'10.10"S	40°27'24.80" W
02C1512/669	AQUASIS	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	100	100	4°12'59.50"S	38° 2'47.10" W
CEUNES-UFES#6	UFES	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	100	100	20°27'23.13"S	40°18'23.52" W
ECOMEGA/FURG 01	ECOMEGA/FURG	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	99.5	31°55' 00" S	-51°50' 00" W
ECOMEGA/FURG 02	ECOMEGA/FURG	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	99	99.4	32° 22' 00" S	52° 18' 00" W
ECOMEGA/FURG 04	ECOMEGA/FURG	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	98	99.83	32°11'5.68"S	52° 9'49.46" W
ECOMEGA/FURG 05	ECOMEGA/FURG	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	100	31°26'7.98"S	51° 7'6.35" W

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
GEMARS 1659	GEMARS	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	99.85	29°37'19.88"S	49°55'58.48" W
GEMARS 1665	GEMARS	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	99.53	99.53	30°12'15.70"S	50°12'46.91" W
CEUNES-UFES#1	UFES	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	99.87	99.87	19°59'2.64"S	40° 6'53.07" W
#39	GEMM-Lagos	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	99.56	99.55	22°32'3.26"S	40°18'45.79" W
02C1121/614	AQUASIS	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	100	100	2°53'19.40"S	41°10'53.80" W
BC02	GEMM-Lagos	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	99	99.41	21°40' S	-
BC03	GEMM-Lagos	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	100	101	25°21'54.00"S	46°29'45.92" W
02C1151/531	AQUASIS	<i>Stenella clymene</i>	<i>Stenella clymene</i>	<i>Stenella clymene</i>	99.35	99.83	4°23'28.70"S	37°49'44.50" W
02C1151/543	AQUASIS	<i>Stenella clymene</i>	<i>Stenella clymene</i>	<i>Stenella clymene</i>	100	99.83	2°48'35.00"S	40°21'34.00" W
*02C1152/333	AQUASIS	<i>Stenella clymene</i>	<i>Stenella clymene</i>	<i>Stenella frontalis/ S. clymene</i>	100	100	2°56'44.34"S	39°47'58.58" W
02C1152/733	AQUASIS	<i>Stenella clymene</i>	<i>Stenella clymene</i>	<i>Stenella clymene</i>	100	99.85	4° 3'31.30"S	38°10'50.50" W
GEMARS 0795	GEMARS	<i>Stenella clymene</i>	<i>Stenella clymene</i>	<i>Stenella clymene</i>	100	99.71	30° 0'15.40"S	50° 7'49.80" W
CEUNES-UFES 01C1152/99	UFES	<i>Stenella clymene</i>	<i>Stenella clymene</i>	<i>Stenella clymene</i>	99.69	100	3°48'47.85"S	32°29'0.36" W
02C1142/295	AQUASIS	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	100	100	4°48'6.60"S	37°16'0.80" W
GEMARS 0047	GEMARS	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	100	100	30°15'12.32"S	50°13'54.79" W
*GEMARS 1240	GEMARS	<i>Stenella coeruleoalba</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	100	99.85	31°10'19.20"S	50°49'30.00" W
GEMARS 1346	GEMARS	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	99.7	99.69	30° 5'46.80"S	50°10'14.90" W
GEMARS 1416	GEMARS	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	100	100	30°27'9.36"S	50°21'26.46" W
GEMARS 1478	GEMARS	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	<i>Stenella coeruleoalba</i>	100	100	30°13'25.50"S	50°12'8.89" W
ECOMEGA/FURG 29	ECOMEGA/FURG	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	100	100	32°21'19.51"S	52°14'49.24" W
ECOMEGA/FURG 30	ECOMEGA/FURG	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	99	100	31°30'11.77"S	51°24'45.18" W
GEMARS 1174	GEMARS	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	99.69	99.68	30°32'26.63"S	50°21'32.81" W
GEMARS 1488	GEMARS	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	99	99.69		
BC 009	GEMM-Lagos	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	100	100	24°14'28.32"S	45°31'23.16" W
BC 051	GEMM-Lagos	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	99.85	99.84	23°22'57.32"S	41° 6'28.98" W
02C1131/226	AQUASIS	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	3°43'38.69"S	38°27'29.67" W

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
02C1131/672	AQUASIS	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	3°32'35.70"S	33°48'38.70" W
02C1131/681	AQUASIS	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	4°43'39.90"S	37°17'48.60" W
02C1132/403	AQUASIS	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	3°48'28.60"S	38°24'40.40" W
GEMARS 1317	GEMARS	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	29°48'9.44"S	50° 2'2.65" W
02C1210/601	AQUASIS	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	100	99.85	3°35'52.20"S	38°46'12.00" W
ECOMEGA/FURG 11	ECOMEGA/FURG	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	100	100	33° 3'2.99"S	52°22'2.06" W
GEMARS 0512	GEMARS	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	96	99.56	31°33'0.68"S	51°13'47.37" W
GEMARS 1621	GEMARS	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	99	100	30°57'57.13"S	50°40'13.69" W
02C1412/290	AQUASIS	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	100	99.69	4° 3'20.76"S	38°10'55.39" W
02C1412/406	AQUASIS	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	92	99.82	3°39'18.00"S	38°41'9.00" W
02C1412/508	AQUASIS	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	99.26	99.55	3°41'10.63"S	38°38'14.15" W
02C1412/523	AQUASIS	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	98	100	3°43'26.80"S	38°30'7.70" W
LEC#92	UFPR	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	99.54	99.82	25° 36.973'S	48°24'4.41" W
PA186	IO –USP	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	92	99.69		
PA226	IO –USP	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	100	100	3°43'38.69"S	38°27'29.67" W
02C1312/696	AQUASIS	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	100	100	3°48'16.00"S	38°24'49.00" W
GEMARS 1485	GEMARS	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	98.96	99.54	30°10'37.81"S	50°12'8.89" W
GEMARS_ ASPSP_C	GEMARS	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	97.22	97.21	8°30'38.27"S	34°52'41.16" W
GEMARS_ ASPSP_E	GEMARS	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	97.06	96.92	8°32'32.83"S	34°52'4.10" W
GEMARS_ ASPSP_N	GEMARS	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	100	99.85	8°32'34.68"S	34°51'12.00" W
ii015573	UNIVALI	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	100	99.85	26°38'2.83"S	48°40'50.81" W
ii014622	UNIVALI	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	100	100	26°46'59.05"S	48°35'45.49" W

SPECIMEN	INSTITUTION	MID	NCBI ID	BOLD ID	NCBI %	BOLD %	Lat	Long
<b>Family Phocoenidae</b>								
*ii 47907	UNIVALI	<i>Phocoena dioptrica</i>	<i>Phocoena spinipinnis</i>	*	98	*	26°53'31.48"S	48°38'24.91" W
ECOMEGA/FURG 68	ECOMEGA/FURG	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	100	98.99	32°33'17.89"S	52°18'53.28" W
ECOMEGA/FURG 69	ECOMEGA/FURG	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	100	100	33°21'3.02"S	53° 5'27.71" W
ECOMEGA/FURG 70	ECOMEGA/FURG	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	98.92	98.91	Rio Grande - RS	



**Table 3.** Cetacean species of the subfamily Delphininae of the present study whose *coxI* marker was not efficient to identify at species level taking into account pairwise inter- and intra-specific distances.

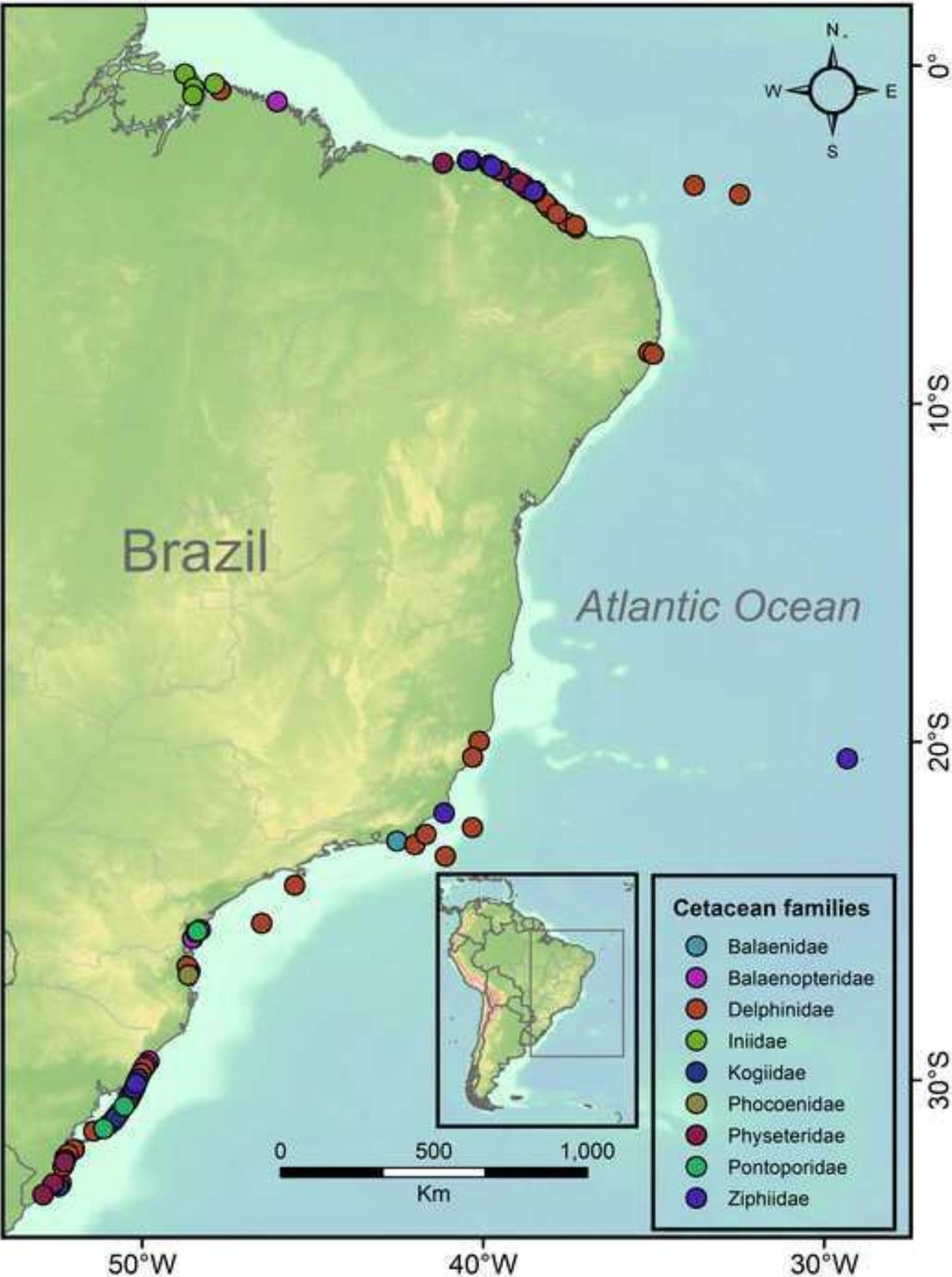
Genetic divergences (%)					
Species	Between species				Within species
	1	2	3	4	5
1 <i>Delphinus delphis</i>					0.56
2 <i>Stenella clymene</i>	1.56				0.00
3 <i>Stenella coeruleoalba</i>	1.73	2.56			0.27
4 <i>Stenella frontalis</i>	0.38	1.16	1.33		0.00
5 <i>Tursiops truncatus</i>	1.03	1.97	2.15	0.70	0.28

**Table 4.** *CoxI* pairwise inter- and intra-specific distances among species of *Eubalaena*, including the two specimens of the present study and sequences deposited in both GenBank and BOLD System databases.

<b>Genetic divergence (%)</b>				
<b>Species</b>		<b>Between species</b>		<b>Within species</b>
		1	2	
1	<i>E. australis</i>			$0.42 \pm 0,2$
2	<i>E. glacialis</i>	$0.56 \pm 0,2$		$0.55 \pm 0,2$
3	<i>E. japonica</i>	$0.82 \pm 0,3$	$0.72 \pm 0,3$	$0.00 \pm 0,0$

Figure 1

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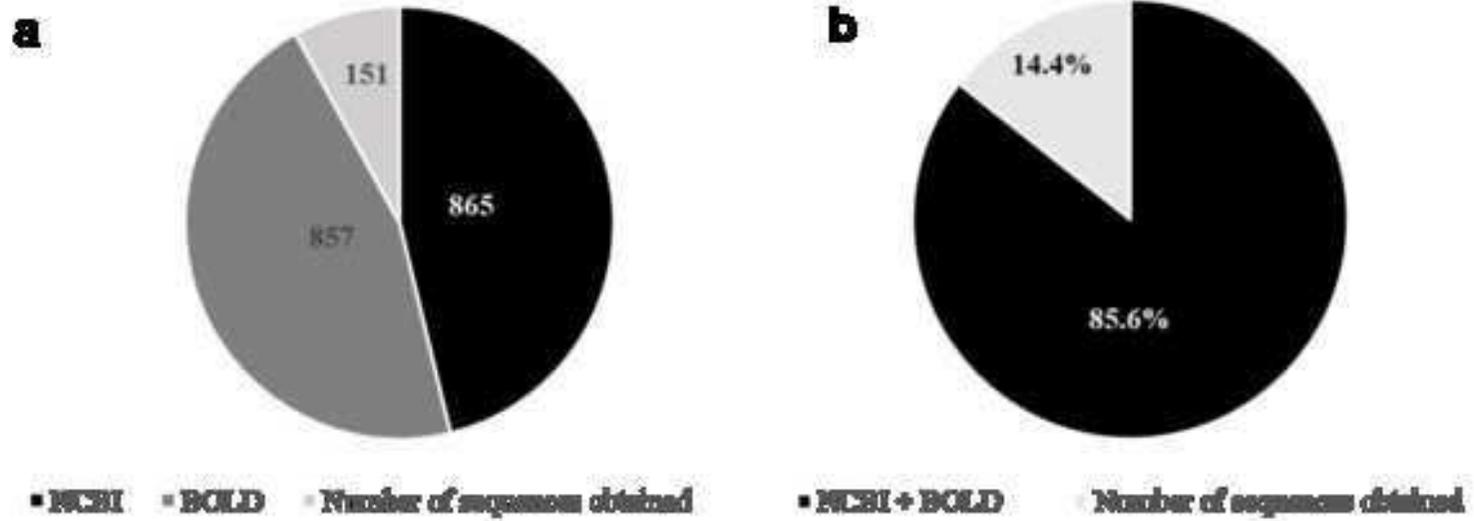










Figure 6

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