



Integrating morphology and DNA barcoding to assess cetacean diversity in Brazil

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Abstract

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

Keywords Whales · Dolphins · Stranding · Morphological · Identification · molecular

Introduction

Brazil has one of the world's most extensive coastlines, spanning almost 8000 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 and 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 and 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).

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Cetaceans (i.e., whales, dolphins and porpoises) comprise one of the taxonomic groups that lack basic information, mainly regarding their ecological function in the aquatic ecosystems, 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, 2011b; 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 (>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 Bressema et al. 2007; Fruet et al. 2012; Lemos et al. 2013; Prado et al. 2016; Dick et al. 2019). 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 and 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 parts, such as the skull or teeth, are lost and the original skin color is no longer present, morphology-based identification is virtually impossible for most species.

This was precisely the case in the first record of the Omura’s whale (*Balaenoptera omurai*) on the coast of Brazil and the Southwestern Atlantic (Cypriano-Souza et al. 2016). The authors were only able to reach unambiguous

identification of the specimen after generating information from three mitochondrial DNA (mtDNA) segments (control region, *cytochrome-b* (*cyt-b*), and *cytochrome oxidase c subunit I* (*coxI*)) and comparing them with sequences of these same segments deposited in molecular databases. Based on these results, it was demonstrated that there is potential cryptic diversity of cetaceans in Brazil, which is “hidden” due to the lack of use of molecular techniques as diagnostic tools for these taxa (Sholl et al. 2008; Cypriano-Souza et al. 2016). A similar situation occurred when Hrbek et al. (2014) found substantial molecular divergence in mtDNA genes supporting the split of the Amazon river dolphin genus *Inia* into two species: *I. geoffrensis* and *I. araguaiaensis*, the latter being the only cetacean species endemic to Brazilian waters. Afterwards, also based on mtDNA control region and *coxI* sequences, Siciliano et al. (2016) detected the presence of the two species of *Inia* and extended the range of the new species *I. araguaiaensis* into the Amazon delta.

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

pacificus) and the newly described minimal-beaked whale (*Berardius minimus*) (e.g., Dalebout et al. 1998, 2002, 2004; Yamada et al. 2019).

To establish the correct identification of these mammals, which are frequently found washed ashore and often in advanced state of decomposition, DNA barcoding becomes a very useful tool (Hebert et al. 2003; Alfonsi et al. 2013). Analysis based on a fragment of the mitochondrial gene *cytochrome c oxidase subunit 1 (coxI)* is a powerful tool to identify individuals at the species level (Hebert et al. 2003). Recently, Falcão et al. (2017) published a DNA barcoding study on marine mammal species from Brazil and Canada, but it covered a small portion of the Northeastern Brazilian coast and only four individuals of four species (*Physeter macrocephalus*, *Peponocephala electra*, *Sotalia guianensis*, and *Tursiops truncatus*).

There are few studies integrating *coxI* and morphology to identify cetacean species (Amaral et al. 2007; Viricel and Rosel 2011; Alfonsi et al. 2013). Until now, there are virtually no cetacean studies including morphological voucher material, such as skulls, to compare with *coxI* results, probably because they need a large number of cetacean species with both DNA samples and bones collected from the same individual.

In the present study, we evaluate the potential of DNA barcoding for the monitoring of cetacean diversity along the coast of Brazil and its inner waters. Based on the establishment of a consortium of 16 institutions from the Brazilian stranding network, included in the project “Tetrapoda DNA Barcodes¹” of the Brazilian Barcode of Life (BrBOL) initiative, tissue samples were collected from stranded cetaceans as well as few biopsies taken from live animals along the Brazilian coast. Most DNA samples were associated with voucher material deposited in scientific collections (e.g., skull and/or skeletons) that could be identified to species level based on morphological characters, which allowed a controlled assessment of the molecular identifications performed with the *coxI* gene. We additionally evaluated the quality and reproducibility of the cetacean taxonomic identification performed by the consortium field researchers, by identifying degraded carcasses, describing intraspecific variation for some dolphin species, and by evaluating the hypothesis that *coxI* can be an efficient molecular marker to identify cetacean species (Hebert et al. 2003; Taylor et al. 2017). Finally, we discuss the results with a focus on method validation and its potential inconsistencies in cases of morphological vs. molecular mismatches (Viricel and Rosel 2011).

Methods

Samples

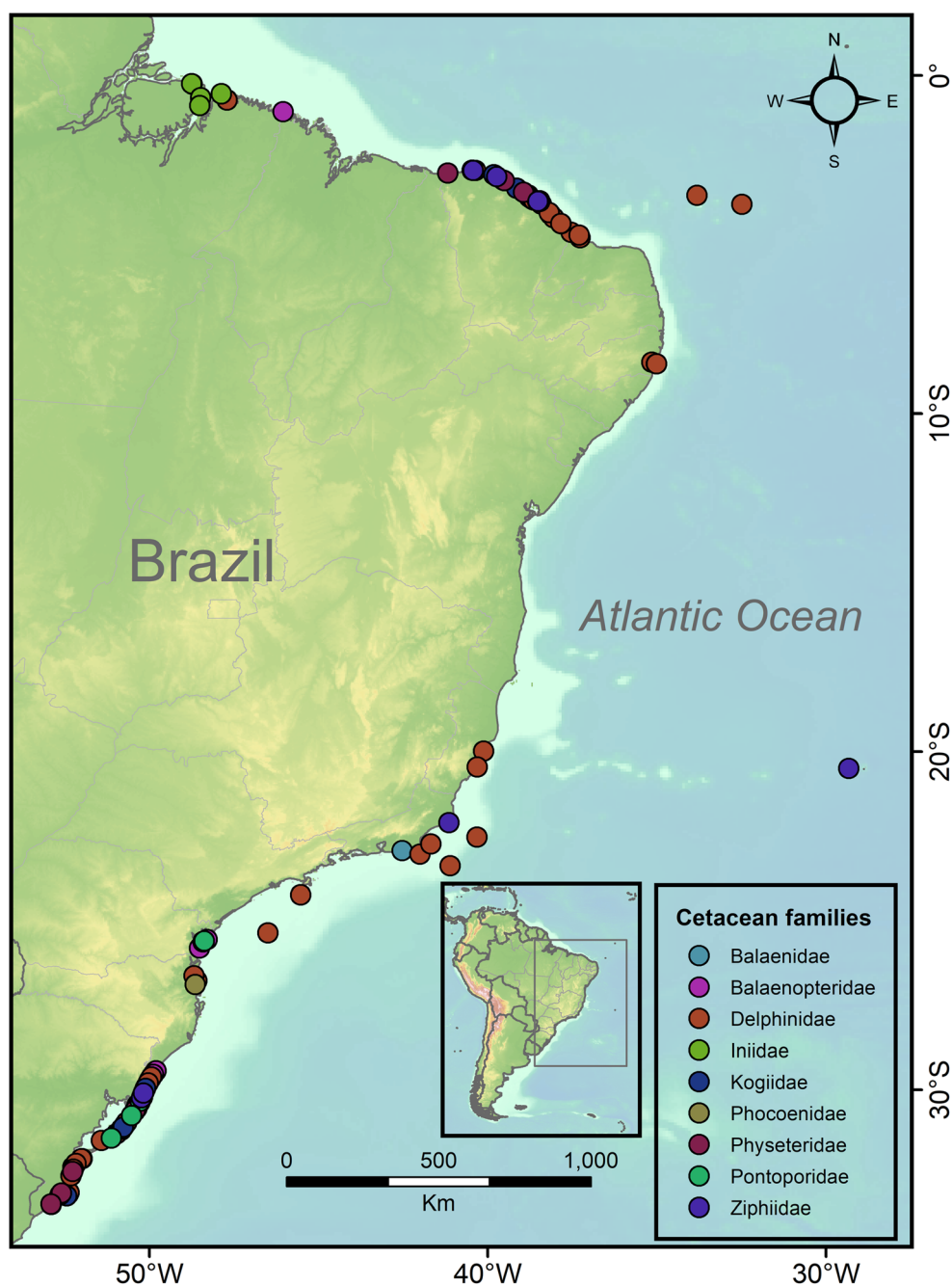
A collaborative stranding network of 16 research institutions investigating aquatic mammal strandings along the Brazilian coast and inner Amazon basin waters obtained tissue samples from 143 cetacean carcasses. The specimens were recovered during regular beach surveys or notified by locals, from 1989 to 2018, including samples from four regions: south, southeast, northeast, and north (see Fig. 1; Table 1). Additionally, we also included seven biopsy samples of cetaceans detected during onboard surveys of oceanic waters. These samples were collected in waters surrounding the São Pedro e São Paulo Archipelago (also known as Saint Paul’s Rocks) (00° 56’ S; 29° 22’ W) and Campos and Santos Basins (from 21° 40’ S to 27° 00’ S). These samples were obtained under *SISBIO* (Brazilian Biodiversity authorization and information system) license numbers 12022-1 and 19665-1.

Voucher specimens (osteological material or photos that unequivocally identify the species) from the carcasses sampled in this study are deposited in their respective scientific collections, except for some stranded baleen whales. This is the first cetacean barcoding study that includes voucher material, allowing reproducibility of species identification, performed by field correspondents or researchers from the collaborating institutions. In the field, specimens were initially identified by experienced researchers or trained assistants, following guidelines suggested by the American Society of Mammalogists in the protocol *Acceptable Field Methods in Mammalogy: Preliminary Guidelines Approved by the American Society of Mammalogists* (ad hoc Committee on Acceptable Field Methods in Mammalogy 1987, http://mammalogy.org/uploads/committee_files/ACUC1987.pdf) and by Geraci and Lounsbury (2005); both protocols were adopted by Brazilian stranding marine mammal networks (IBAMA 2005). The identification of each specimen was performed through a combination of diagnostic characters of body and skull morphology, when necessary. Moreover, information related to total length, sex, and the condition of each carcass, including the state of the decomposition (Geraci and Lounsbury 2005), was also recorded whenever possible. Tissue samples were collected and stored in 70% ethanol or 20% DMSO saturated with NaCl and sent to Laboratory of Genetics and Molecular Biology (LGBM) at the University of Vale dos Rio dos Sinos ($n = 140$). A few samples were also sent to the Laboratory of Genomics and Molecular Biology at the Pontifícia Universidade Católica do Rio Grande do Sul ($n = 10$).

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

¹ Tetrapoda DNA Barcodes: building an integrated network DNA barcoding of amphibians, reptiles, birds and mammals.

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



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

Analysis

We manually selected only high-quality *coxI* sequences, with high and clear peaks for each nucleotide, based on the observation of electropherograms conducted with ChromasPro 2.6.6 (<http://www.technelysium.com.au>). A total of 150 consensus sequences were automatically aligned (with minor manual correction) in ClustalW implemented in MEGA 7 (Kumar et al. 2016), with subsequent edition in BioEdit 5.0.9 (Hall 1999). After the alignment, we compared the *coxI* sequences with those available in GenBank (www.ncbi.nlm.nih.gov).

Table 1. Number of sequences of the gene cytochrome c oxidase subunit 1 (*cox1*) 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 *cox1* currently (April 2020) available in the NCBI and BOLD databases are also indicated. NA = not available

Common name	Species	NCBI	BOLD	Shared between NCBI/BOLD	Number of sequences obtained*
Delphinidae					
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
Kogiidae					
Pygmy sperm whale	<i>Kogia breviceps</i>	4	4	4	2
Dwarf sperm whale	<i>Kogia sima</i>	4	7	4	6
Physeteridae					
Sperm whale	<i>Physeter macrocephalus</i>	88*	71*	70	10
Pontoporiidae					
Franciscana dolphin	<i>Pontoporia blainvillei</i>	3	4	3	11
Phocoenidae					
Spectacled porpoise	<i>Phocoena dioptrica</i>	NA	NA	-	1
Burmeister's porpoise	<i>Phocoena spinipinnis</i>	1	1	1	3
Iniidae					
Araguaian river dolphin	<i>Inia araguaiaensis</i>	47	47	47	3
Amazon river dolphin	<i>Inia geoffrensis</i>	39	39	39	2
Ziphiidae					
Amoux'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
Balaenidae					
Southern right whale	<i>Eubalaena australis</i>	4	4	4	5
Balaenopteridae					
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*.

nih.gov) and BOLD systems (www.boldsystems.org), which are the two main public databases of DNA barcode data for all

taxa (Meiklejohn et al. 2019), using the Basic Local Alignment Search Tool (BLAST) (blast.ncbi.nlm.nih.gov).

The molecular identifications suggested by both GenBank and BOLD were based on the percentage of similarity among sequences. The first criterion we used was based on 3% as a suggested lower limit for genetic divergence (dA) between species (Hebert et al. 2003); values close to this limit were considered to attain the lowest level for cetacean species delimitation based on the *coxI* marker (Siciliano et al. 2016; Taylor et al. 2017). The second criterion was a direct assessment of the level of similarity of each sequence with the suggested by GenBank. Here, we highlight that, for those species with no *coxI* sequence currently available in the databases, the similarity search retrieved the closest taxon or no result was returned. Cases of molecular vs. morphology mismatch (Viricel and Rosel 2011; Alfonsi et al. 2013), due to incongruence between the species identification suggested by *coxI* sequences (from GenBank or BOLD) and the morphological identification informed by collaborating researchers, were further investigated. Whenever possible, a revision of the species identification was conducted by requesting skull or carcass images to the field correspondents. External traits or diagnostic characters of the skull were analyzed to confirm the identification. In cases of uncertainties, additional marine mammal specialists were consulted. This procedure was conducted for species of the polyspecific genera such as *Balaenoptera* and *Stenella*, as well as to the monospecific genera *Orca* and *Pseudorca*. In addition, field notes on the specimens collected were also double-checked in the catalogue books of the scientific collections, mainly regarding the decomposition stage (including images from the sampling), which could explain some of the mismatch results (see Discussion section).

Intraspecific and interspecific genetic divergence (dA) were calculated using the K2P model (Kimura 1980) for those species that did not exhibit a clear-cut barcoding gap to establish the interval of genetic separation between them (e.g., some delphinids). A barcoding gap is a lack of overlap between intraspecific and interspecific nucleotide divergence in the investigated taxa (Viricel and Rosel 2011).

To test the hypothesis that all *coxI* sequences belonging to the same cetacean species form a monophyletic cluster, we performed two types of phylogenetic analyses: a maximum likelihood (ML) tree reconstructed with the program RAxML 8.2 (Stamatakis 2014); and as secondary method, a neighbor-joining tree (NJ) tree using the Kimura 2-parameter (K2P) model implemented in the software MEGA 7 (Kumar et al. 2016). For the ML, we used GTR+4G as the substitution model, as estimated with jmodeltest2 (Darriba et al. 2012). To perform these phylogenetic analyses, we assembled and aligned our 150 *coxI* consensus sequences with the 71 sequences available on the BOLD platform, totaling 221 *coxI* sequences representing 33 cetacean taxa. The species *Hippopotamus amphibius*, available on the BOLD platform (GBMA2411-09), was used as the outgroup.

Results

We recovered *coxI* sequences spanning 644 to 847 bp from 150 samples representing 33 species. The recorded species were distributed in nine cetacean families, including both odontocetes (i.e., dolphins, porpoises and toothed whales) and mysticetes (i.e., baleen whales) (Table 1). A total of 865 and 857 *coxI* sequence records were identified in the NCBI and BOLD nucleotide databases, respectively, representing 898 cetacean specimens (Fig. 2a; Table 1). With this study, we are adding 150 sequences which represent a 14.4% growth in the number of specimens and 16.8% of all cetacean samples in the databases (Fig. 2a). The number of individuals per species ranged from one to 11 (mean = 4.6). Two species were sequenced for *coxI* here for the first time (*Berardius arnuxii* and *Phocoena dioptrica*). The molecular identification was in accordance with the external morphology-based identification in 92.7% of the specimens (Table 2).

Fig. 2 **a** 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

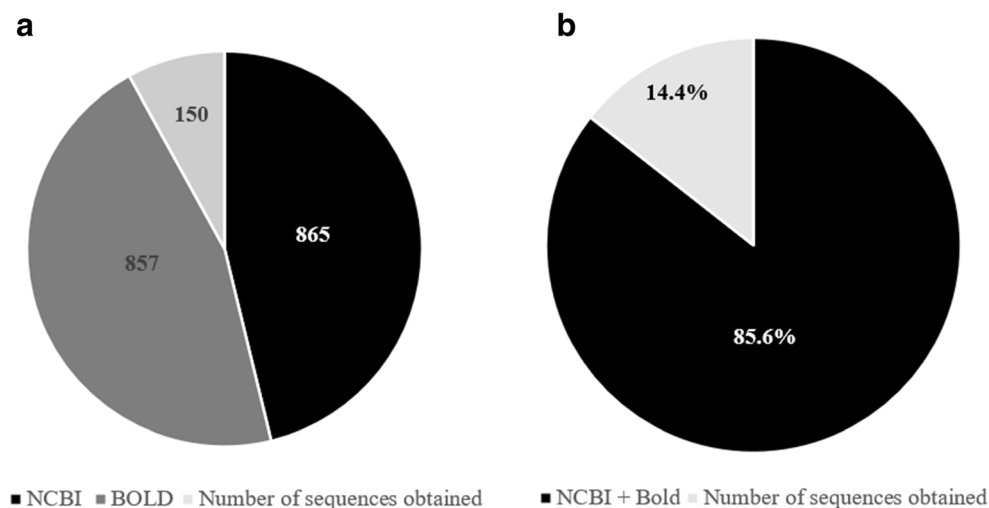


Table 2 Detailed information on each sample examined: specimen and; institution acronym (institution responsible for collecting); GenBank accession number; MID: morphological identification; NCBI ID: molecular identification suggested by GenBank platform; BOLD ID: molecular identification suggested by 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; coordinates of the: sampling site. *Specimens with molecular-morphological mismatch

Specimen	GenBank accession number	MID	NCBI ID	BOLD ID	NCBI(%)	BOLD (%)	Lat	Long
Family Balaenidae								
**GEMM1-Lagos 051	MW4446705	<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
^b GEMARS 1456	MW4446728	<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	MW4446729	<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	MW4446713	<i>Eubalaena australis</i>	<i>Eubalaena australis</i>	<i>Eubalaena glacialis</i>	100	100	22° 26' 00" S	42° 49' 00" W
Family Balaenopteridae								
GEMARS 0469	MW4446668	<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	MW4446681	<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	MW4446719	<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
^d LEC#119	MW4446720	<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	MW4446682	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	100	100	29° 26' 27.10" S	49° 47' 57.80" W
^e EOMEGA/FURG minke	MW4446721	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	<i>Balaenoptera bonaerensis</i>	99.58	99.65	Rio Grande - RS	
*EOMEGA/FURG 63	MW4446679	<i>Balaenoptera borealis</i>	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	99.23	99.22	32° 2' 24.00" S	52° 0' 9.97" W
EOMEGA/FURG 61	MW4446656	<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
EOMEGA/FURG 62	MW4446657	<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
^f MPEG 39691	MW4446661	<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	MW4446669	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	100	100	31° 4' 23.34" S	50° 16' 49.87" W
GEMARS 1425	MW4446727	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	99.71	99.81	30° 22' 36.77" S	50° 16' 49.87" W
GEMARS 1694	MW4446674	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	99.54	99.39	30° 10' 17.99" S	50° 11' 53.69" W
LEC#154	MW4446726	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	<i>Balaenoptera byrdi</i>	100	99.34	25° 33' 39.69" S	48° 17' 27.78" W
02C0100/421 AQUASIS	MW4446678	<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	MW4446725	<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
^g AQUASIS 02C0211/418	MW4446709	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	3° 33' 54.32" S	38° 47' 20.84" W
AQUASIS 02C0212/645	MW4446664	<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	MW4446722	<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	MW4446723	<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	MW4446724	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	99.85	99.85	30° 15' 51.88" S	50° 14' 8.16" W
*GEMARS 1491	MW4446662	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	99.18	98.38	30° 37' 48.80" S	50° 25' 41.20" W
GEMARS 1683	MW4446673	<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	MW4446672	<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	MW4446659	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	<i>Megaptera novaeangliae</i>	100	100	30° 3' 37.84" S	50° 9' 18.99" W
Family Physcteridae								
AQUASIS 02C0410/308	MW4446781	<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
AQUASIS 02C0410/338	MW4446691	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	3° 6' 33.00" S	39° 30' 38.00" W
AQUASIS 02C0411/542	MW4446663	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	3° 43' 3.30" S	38° 31' 20.30" W
AQUASIS 02C0411/809	MW4446652	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	101	3° 27' 19.00" S	38° 56' 12.00" W
AQUASIS 02C0412/792	MW4446667	<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
EOMEGA/FURG 17	MW4446782	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	32° 25' 34.10" S	52° 15' 57.49" W
EOMEGA/FURG 18	MW4446783	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	99	100	33° 3' 39.13" S	52° 35' 55.00" W
EOMEGA/FURG 19	MW4446778	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	33° 7' 7.79" S	52° 38' 2.76" W

Table 2 (continued)

Specimen	GenBank accession number	MID	NCBI ID	BOLD ID	NCBI(%)	BOLD (%)	Lat	Long
ECOMEGA/FURG 20	MW4446779	<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	MW4446780	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	<i>Physeter macrocephalus</i>	100	100	30° 36' 54.90" S	50° 24' 55.80" W
Family Kogiidae								
ECOMEGA/FURG 33	MW4446775	<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	MW4446776	<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
AQUASIS 02C0511/703	MW4446650	<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
AQUASIS 02C0511/726	MW4446666	<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
AQUASIS 02C0512/585	MW4446710	<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	MW4446695	<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	MW4446670	<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	MW4446777	<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
Family Ziphiidae								
*GEMARS 1155	MW4446774	<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	MW4446686	<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
02C0810/683	MW4446665	<i>Ziphius sp.</i>	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	100	100	2° 59' 18.00" S	39° 44' 6.00" W
02C0812/305	MW4446772	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	<i>Ziphius cavirostris</i>	100	100	3° 43' 9.16" S	38° 30' 40.05" W
^b UNIV/ALI AB02	MW4446708	<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	MW4446671	<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
Family Iniidae								
*MPEG 38764	MW4446742	<i>Inia geoffrensis</i>	<i>Inia arguataiensis</i>	<i>Inia arguataiensis</i>	100	99.81	0° 15' 8.58" S	48° 22' 35.91" W
*MPEG 42122	MW4446741	<i>Inia geoffrensis</i>	<i>Inia arguataiensis</i>	<i>Inia arguataiensis</i>	100	99.81	0° 14' 48.81" S	48° 44' 51.40" W
*MPEG 42055	MW4446743	<i>Inia geoffrensis</i>	<i>Inia arguataiensis</i>	<i>Inia arguataiensis</i>	100	99.81	0° 43' 21.28" S	48° 17' 29.22" W
MPEG 42179	MW4446784	<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	MW4446785	<i>Inia geoffrensis</i>	<i>Inia geoffrensis</i>	<i>Inia geoffrensis</i>	100	100	0° 14' 48.81" S	48° 44' 51.40" W
Family Pontoporiidae								
GEMARS 0215	MW4446738	<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	MW4446731	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99	99.84	31° 25' 57.36" S	51° 7' 18.94" W
GEMARS 0530	MW4446732	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99	99.85	29° 51' 5.02" S	50° 3' 36.36" W
GEMARS 0550	MW4446730	<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 0634	MW4446734	<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	MW4446735	<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	MW4446733	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	100	100	RS	
GEMARS 0749	MW4446736	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99.82	100	25° 37' 46.54" S	48° 24' 46.99" W
GEMARS 1487	MW4446737	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	100	99.84	25° 35' 56.15" S	48° 22' 38.95" W
LECH#01	MW4446739	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99.85	100		
LECH#1	MW4446740	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	<i>Pontoporia blainvillei</i>	99.69	99.69		
Family Delphinidae								
GEMM-Lagos # 57	MW4446707	<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
GEMM-Lagos BC04	MW4446712	<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	MW4446680	<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
GEMARS 0419	MW4446693	<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
^p A288 /IO -USP	MW4446687	<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	MW4446752	<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	MW4446753	<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	MW4446751	<i>Delphinus sp.</i>	<i>Delphinus delphis</i>	<i>Delphinus delphis</i>	98.32	98.57	33° 5' 35.30" S	52° 25' 42.43" W
ECOMEGA/FURG 38	MW4446771	<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

Table 2 (continued)

Specimen	GenBank accession number	MID	NCBI ID	BOLD ID	NCBI(%)	BOLD (%)	Lat	Long
AQUASIS 02C1812/588	MW446647	<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	MW446694	<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	MW446685	<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
AQUASIS 02C0212/342	MW446645	<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
AQUASIS 02C2512/389	MW446646	<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	MW446757	<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	MW446773	<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	MW446701	<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	MW446702	<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	MW446696	<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	MW446758	<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	MW446653	<i>Orcinus orca</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	100	32° 21' 3.60" N	52° 14' 34.80" W
AQUASIS 02C1511/783	MW446641	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	100	100	2° 48' 10.10" S	40° 27' 24.80" W
AQUASIS 02C1511/784	MW446642	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	<i>Peponocephala electra</i>	100	100	2° 48' 10.10" S	40° 27' 24.80" W
AQUASIS 02C1512/669	MW446639	<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	MW446770	<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	MW446769	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	99	99.5	31° 55' 00" S	-51° 50' 00" W
ECOMEGA/FURG 02	MW446767	<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	MW446766	<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	MW446768	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	100	31° 26' 7.98" S	51° 7' 6.35" W
GEMARS 1659	MW446697	<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	MW446684	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	<i>Pseudorca crassidens</i>	100	99.53	30° 12' 15.70" S	50° 12' 46.91" W
CEUNES-UFES#1	MW446765	<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
GEMM-Lagos #39	MW446706	<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
AQUASIS 02C1121/614	MW446690	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	100	100	2° 53' 19.40" S	41° 10' 53.80" W
GEMM-Lagos BC02	MW446711	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	99	99.41	21° 40' S	-
GEMM-Lagos BC03	MW446759	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	<i>Stenella attenuata</i>	100	101	25° 21' 54.00" S	46° 29' 45.92" W
AQUASIS 02C1151/531	MW446698	<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
AQUASIS 02C1151/543	MW446699	<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
*AQUASIS 02C1152/333	MW446644	<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
AQUASIS 02C1152/733	MW446651	<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	MW446703	<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	MW446750	<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
AQUASIS 02C1142/295	MW446704	<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	MW446692	<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	MW446643	<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	MW446658	<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	MW446748	<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	MW446749	<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	MW446744	<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	MW446746	<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	MW446745	<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	MW446747	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	99	99.69		
GEMM-Lagos BC009	MW446787	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	<i>Stenella frontalis</i>	100	100	24° 14' 28.32" S	45° 31' 23.16" W
GEMM-Lagos BC 051	MW446660	<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

Table 2 (continued)

Specimen	GenBank accession number	MID	NCBI ID	BOLD ID	NCBI(%)	BOLD (%)	Lat	Long
AQUASIS 02C1131/226	MW446677	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	3° 43' 38.69" S	38° 27' 29.67" W
AQUASIS 02C1131/672	MW446640	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	3° 32' 35.70" S	33° 48' 38.70" W
AQUASIS 02C1131/681	MW446649	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	4° 43' 39.90" S	37° 17' 48.60" W
AQUASIS 02C1132/403	MW446689	<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	MW446683	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	<i>Stenella longirostris</i>	100	100	29° 48' 9.44" S	50° 2' 2.65" W
AQUASIS 02C1210/601	MW446648	<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	MW446764	<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	MW446638	<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	MW446715	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	<i>Steno bredanensis</i>	99	100	30° 57' 57.13" S	50° 40' 13.69" W
AQUASIS 02C1412/290	MW446761	<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
AQUASIS 02C1412/406	MW446762	<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
AQUASIS 02C1412/508	MW446717	<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
AQUASIS 02C1412/523	MW446763	<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	MW446760	<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	MW446716	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	92	99.69		
PA226 /IO -USP	MW446718	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	<i>Sotalia guianensis</i>	100	100	3° 43' 38.69" S	38° 27' 29.67" W
AQUASIS 02C1312/696	MW446700	<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	MW446714	<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	MW446756	<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	MW446754	<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	MW446755	<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
UNIVALI ii015573	MW446676	<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
UNIVALI ii014622	MW446688	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	100	100	26° 46' 59.05" S	48° 35' 45.49" W
Family Phocoenidae								
*UNIVALI ii 47907	MW446675	<i>Phocoena dioptrica</i>	<i>Phocoena spinipinnis</i>	*	98	*	26° 53' 31.48" S	48° 38' 24.91" W
ECOMEGA/FURG 68	MW446655	<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	MW446654	<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	MW446786	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	<i>Phocoena spinipinnis</i>	98.92	98.91	Rio Grande—	RS

^a GEMM-Lagos: Grupo de Estudos de Mamíferos, Aves e Répteis Marinhos e Costeiros da Região dos Lagos

^b GEMARS: Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul

^c MN: Museu Nacional da Universidade Federal do Rio de Janeiro

^d LEC: Laboratório de Ecologia e Conservação de Mamíferos do Centro de Estudos do Mar, Universidade Federal do Paraná

^e ECOMEGA/FURG: Laboratório de Ecologia e Conservação da Megafauna Marinha da Universidade Federal do Rio Grande

^f MPEG: Museu Paraense Emílio Goeldi/ Grupo de Estudos de Mamíferos Aquáticos da Amazônia

^g AQUASIS: Associação de Pesquisa e Preservação de Ecossistemas Aquáticos

^h UNIVALI: Universidade do Vale do Itajaí

ⁱ Instituto Oceanográfico da Universidade São Paulo

^j Departamento de Ciências Agrárias e Biológicas da Universidade Federal do Espírito Santo

Regarding the 11 mismatches between the molecular and morphological identifications among 150 cetacean carcasses (i.e., ~7% of the sample) (Table 2), we can assign them to four causes: (1) incorrect morphological identification; (2) recent taxonomic changes (species splitting); (3) incomplete molecular databases, and (4) absence of barcoding gap between species. Each of them will be treated separately below.

Incorrect morphological identification

The first mismatch case between morphological and molecular identifications was found in the ECOMEGA/FURG 45 specimen. The specimen was in an advanced state of decomposition and was identified during fieldwork as a killer whale (*Orcinus orca*), but both molecular databases indicated a complete match (100% identity) with the false killer whale (*Pseudorca crassidens*). Unfortunately, the skull was missing, but the pictures from the head of the dead specimen were sent to two marine mammal specialists, who concluded based mainly on external morphology and tooth counts (likely nine) that the specimen was probably a *P. crassidens* (Fig. 3a). Despite a little overlap between the dental formula of these two species (typically, 7 to 10 teeth per tooth row in *P. crassidens* and 10 to 12 in *O. orca*), the number of teeth is generally smaller in *P. crassidens* (Jefferson et al. 1993).

Fig. 3 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



Two other molecular-morphological mismatches due to incorrect morphological identification in the field were reported: (a) GEMARS 1491, putatively identified during fieldwork as a humpback whale (*Megaptera novaeangliae*), but identified with both molecular databases as a southern right whale (*Eubalaena australis*) (NCBI = 99.18% identity; BOLD = 98.38% identity) and (b) ECOMEGA/FURG 63, morphologically identified as a sei whale (*Balaenoptera borealis*) but identified with both molecular databases as a Bryde's whale (*Balaenoptera brydei*) (NCBI = 99.23% identity; BOLD = 99.22% identity). In both cases, the specimens were found in an advanced state of decomposition, indicating code 4 according to the classification determined by Geraci and Lounsbury (2005). Based on the carcass conditions, we presume that the morphological evaluation of the species identity was very difficult, leading the collectors to misidentify the two specimens.

Recent taxonomic changes (species splitting)

Three specimens of river dolphins (MPEG 38764, MPEG 42122 and MPEG 42055) were identified by the field researchers as *Inia geoffrensis*, but the molecular identification with both databases indicated that they should be classified as *I. araguaiaensis* (NCBI=100% identity; BOLD=99.81% identity for all cases). These mismatches are explained by the fact that, at the time the samples, were collected and

deposited in the museum collection (between 2007 and 2012; for details, see Siciliano et al. 2016), *I. araguaiaensis* had not been formally described (Hrbek et al. 2014). Until the formal description of this taxon in 2014 and the deposit of its sequences in the molecular databases, Amazon and Araguaia-Tocantins river dolphins were jointly identified as *I. geoffrensis*.

Incomplete molecular databases

The GEMARS 1155 specimen was morphologically identified as Arnoux's beaked whale (*Berardius arnuxii*), while the molecular identification performed with both databases indicated that it was Baird's beaked whale (*Berardius bairdii*) (NCBI=99.70% identity; BOLD=99.69% identity). These species show very slight morphological differences, and the validity of these species had already been questioned (Balcomb 1989 in Jefferson et al. 1993). However, studies based on the mitochondrial *cytochrome b* gene supported clear-cut molecular differences and recognized them as distinct species (Dalebout et al. 2004). At the same time, it is noteworthy that these two species exhibit antitropical distributions, with *B. bairdii* occurring only in the North Pacific Ocean (Kasuya 2009) and that *B. arnuxii coxI* sequences were not previously represented in these databases. Thus, we conclude that the molecular identification in this case did not match the morphological identification purely because of the lack of *B. arnuxii* reference sequences, making *B. bairdii* the closest available species for similarity-based clustering.

A similar result was observed with the UNIVALI ii 47907 specimen, which had been morphologically identified as a spectacled porpoise (*Phocoena dioptrica*) based on the unique pigmentation pattern of the species (e.g., double eye patch) and the large and rounded dorsal fin typical of males (Goodall 2009) (Fig. 4). However, the molecular approach identified it as Burmeister's porpoise (*Phocoena spinipinnis*) (NCBI=97.99% identity; BOLD=97.98% identity). This mismatch resulted from the lack of *coxI* sequences of *P. dioptrica* in both GenBank and BOLD databases

Absence of barcoding gap between species

The sample AQUASIS 02C1152/333 was morphologically identified as a Clymene dolphin (*Stenella clymene*). This identification was confirmed by GenBank (NCBI=100% identity), but it was ambiguous in the BOLD database, which reported 100% similarity with both *Stenella frontalis* and *Stenella clymene*. This specimen was very weak when it was rescued, according to the Marine Mammal Rehabilitation Center (CRMM), and it died a few hours after arrival. The fresh conditions of the carcass allowed the precise observation of a typical *S. clymene* coloration pattern, including the three-part color of the body, the dark mark on the upper side of the beak ("moustache") and the distinct eye-stripe, some of the most distinctive features of the species (Perrin 2009; Jefferson et al. 1993) (Fig. 5).

Likewise, the molecular results suggested that the GEMARS 1240 specimen was a short-beaked common dolphin (*Delphinus delphis*) with both databases (NCBI = 100% identity; BOLD = 99.85% identity), but the specimen was morphologically identified during fieldwork and also after the skull examination by a marine mammal expert as a striped dolphin (*Stenella coeruleoalba*). Taking into account the morphological diagnosis of deep palatal grooves in the *D. delphis* skull and the fact that in the GEMARS 1240 specimen this trait was absent, we are confident in the morphological identification as *S. coeruleoalba* (Fig. 6a and b). In addition to these dolphin species, we found two cases that seem to reflect the inexistence of a barcoding gap among right whale species. According to the external morphology, the samples MN60458 and GEMM-Lagos 051 were identified as southern right whales (*Eubalaena australis*), and these identifications were supported by a BLAST comparison against GenBank (MN60458, NCBI=100% identity; GEMM-Lagos 051, NCBI=99% identity). However, the BOLD analysis identified both samples as *E. glacialis* (MN60458, BOLD=100% identity; GEMM-Lagos 051, BOLD=99.69% identity), a species that only occurs in the North Atlantic (Rosenbaum et al. 2000). Although these findings could also be related to an erroneous deposit of sequences in the BOLD platform, we believe that the results are more likely derived from a weak or absent barcoding

Fig. 4. UNIVALI 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>





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

gap between these species, as noticed by Viricel and Rosel (2011).

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 intraspecific 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 neighbor-joining tree correctly distinguished all the analyzed cetaceans (Fig. 7), except the species of Delphinidae, which presented a small inter-specific genetic

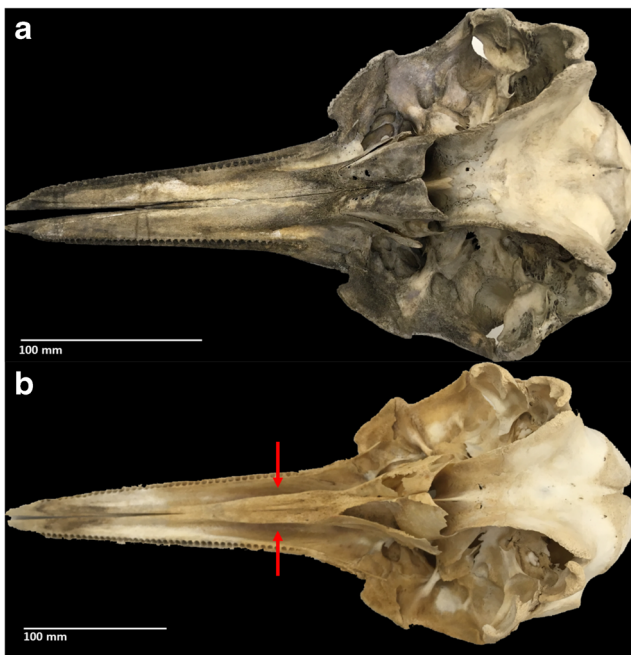


Fig. 6. Ventral view of skulls: **a** Striped dolphin (*Stenella coeruleoalba*) (GEMARS 1240). **b** common dolphin (*Delphinus delphis*), highlighting the prominence of the palatal grooves (pointed out by the arrows)

Table 3 The inter- and intra-specific divergences (K2P pairwise distances) between cetacean species of the subfamily Delphininae of the present study that the *coxI* marker was not efficient to identify at species level

Species	Genetic divergences (%)				
	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

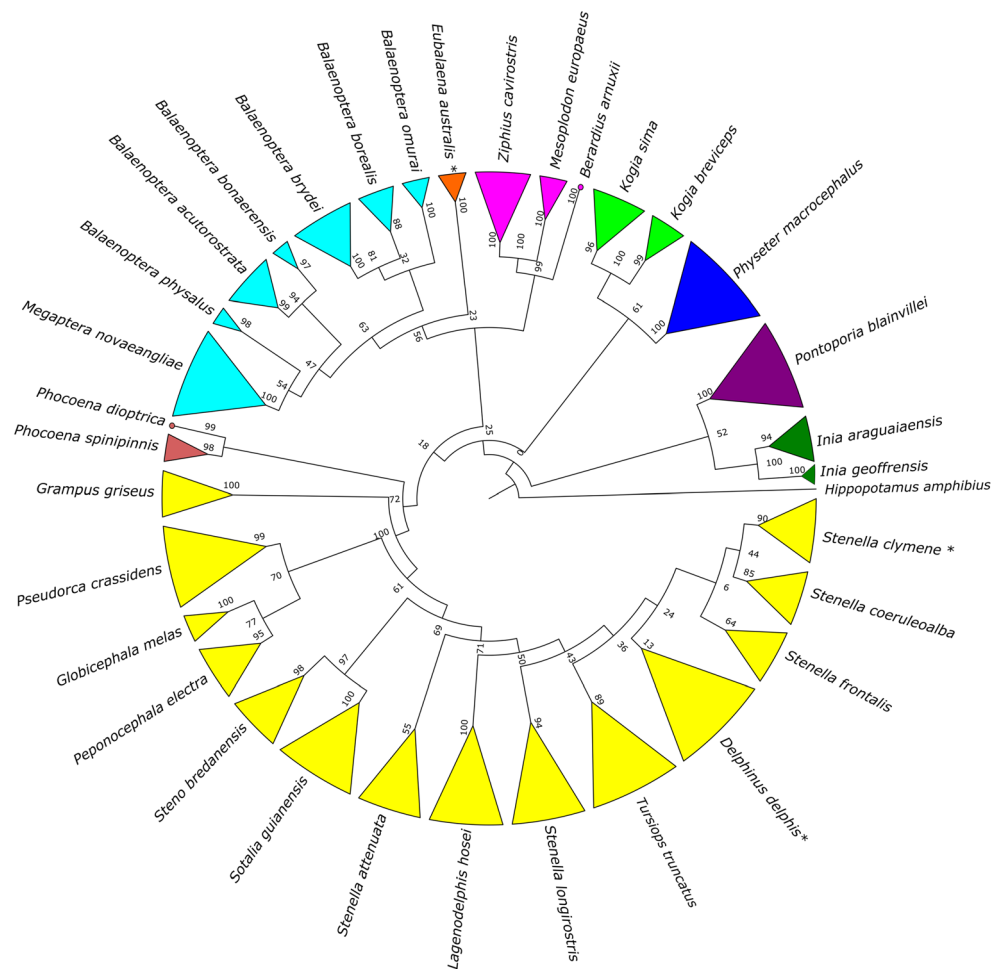
divergence. However, some species of this family formed clades with high bootstrap support values (>90%): *Sotalia guianensis*, *Steno bredanensis*, *Grampus griseus*, *Stenella attenuata*, and *Globicephala melas*.

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 the GenBank and BOLD databases. The measurements of inter-specific variation for the *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 lack 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 identity score (98.38%) of the BLAST search for 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 (MN 60458 and GEMM-Lagos 051) as *E. glacialis* and even in the cases that the Brazilian samples were correctly identified as *E. australis* (GEMARS 1456 and GEMARS 1467), and the first five results of the target sequences also included *E. glacialis* and *E. japonica*.

Phylogenetic reconstruction using maximum-likelihood

Although there were problems with determining inter-specific limits for some species of Delphinidae, both phylogenetic analyses had similar results, but especially in the maximum likelihood (ML) tree reconstructed most species as well-defined clades (Fig. 8), supporting the use of *coxI* as a useful marker for species identification in cetaceans, except for

Fig. 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: Physteridae, light green: Kogiidae, pink: Ziphiidae, light blue: Balaenopteridae, coral: Phocoenidae and yellow: Delphinidae



- (i) by providing a confirmation or an additional degree of taxonomic determination of rare species identified by field researchers, mainly in uncommon stranding events of rare or deep-living species (Thompson et al. 2012);
- (ii) by helping the identification at species level when it is not possible to identify the animal by the external morphology due to highly degraded carcasses or even when morphology-based identification only reaches the genus or family levels, due to an incomplete skeleton or skull
- (iii) by potentially allowing an assessment of intraspecific genetic variability, which enables genetic structure analysis and possibly monitoring population movements (Pauls et al. 2012).

In general, most species identified here with the molecular approach were very common in the coastal region, with no challenging identification, such as franciscana dolphins (*Pontoporia blainvillei*) and the common bottlenose dolphin (*Tursiops truncatus*). There were also records of rarely found stranded specimens belonging to Ziphiidae and Phocoenidae and oceanic and deep-diving species, such as pygmy, dwarf, and sperm whales (Pinedo et al. 2002; Prado et al. 2016), and the only

endemic cetacean species for Brazil, the recently described Araguaian River dolphin. It is worth mentioning that the present study is one of the few involving DNA barcoding sequences of samples associated with voucher materials deposited in scientific collections, enabling morphological checking whenever necessary and thus providing greater reliability of the use of the molecular marker. According to Hanner (2009), as part of the BOLD quality control, DNA barcodes must be associated with specimen records linked to institutional (e.g., museum) material making them the most valuable as reference accessions. This is particularly important in cases of rare species, which usually have no sequences deposited in molecular platforms. The accuracy of DNA barcoding relies upon the level of taxonomic representation for each group and the amount of intraspecific genetic diversity represented in the databases (Gaubert et al. 2015).

It is important to mention that the present study contributed with the inclusion of the first *cox1* sequences of the spectacled porpoise (*Phocoena dioptrica*) and Arnoux's beaked whale (*Berardius arnuxii*), in both GenBank and Bold databases. The spectacled porpoise is a small cetacean with circumpolar distribution in Antarctic and subantarctic waters, with only one previous record published for the Brazilian coast (Pinedo et al. 2002).

There was another unpublished record in August 2016 for Cassino beach (ca. 32° 11' S; 52° 09' W, in Rio Grande do Sul State—Eomega, unpubl. data), southern Brazil. The specimen analyzed in the present study was collected at Navegantes Beach, Santa Catarina State (26° 53' 40" S; 48° 38' 32" W) in July 2017, and represents the northernmost record of this species in the Atlantic Ocean (Barreto, unpubl. data).

Amoux's beaked whale was first reported in Brazilian waters based on the collection of a floating dead specimen close to the coast of São Sebastião, São Paulo State, in southeastern Brazil (Siciliano and Santos 2003). The specimen sequenced in the present study (GEMARS 1155) stranded in the municipality of Balneário Pinhal, Rio Grande do Sul State (30° 14' 29" S; 50° 13' 37" W), in January 2004, representing the second confirmed record of the species in Brazilian waters (Ott et al. 2013).

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

According to Galimberti et al. (2015), there is a hidden diversity within the mammal record. The BOLD System had barcoded about 2850 mammal species by the end of May 2015, with at least 300 unnamed clusters (i.e., not assigned to a species). Currently, there are approximately 3587 species with recognized barcodes in the MammaliaBoL, 75 of which are cetaceans. Taking into account the requirement of *coxI* sequences associated to voucher material, Galimberti et al. (2015) emphasized that the standardized molecular reexamination of museum-deposited voucher specimens and the comparison with other reference information would allow the fast detection of misidentification or uncertainties that typically occur in the field.

This reexamination of voucher specimens and their information was particularly true for the case of specimen GEMARS 1491 found on the coast of Rio Grande do Sul, putatively identified in the field as a humpback whale (*Megaptera novaeangliae* cf.), but genetically as a southern right whale (*Eubalaena australis*). As mentioned earlier, when we examined the field notes presented in the catalogue book of the scientific collection, we found that the specimen was in an advanced state of decomposition, almost buried in sand, and that there was a highlighted note in the labels saying cf. (*confero*, in Latin), which means “needs to be confirmed” or “needs to be compared with” (Sigovini et al. 2016), which

supports the care referred to by Galimberti et al. (2015). Moreover, according to the field notes, the specimen had some sessile whale barnacles still attached to its exposed skin, which led researchers to believe it was a humpback whale or a southern right whale. Considering that there was no clear clue in the notes in favor of humpback whale identity, we conclude that the molecular identification is correct.

Francis et al. (2010) commented that field identification for many mammals is difficult because it is based on the analysis of internal structures such as skull or dentition, which is particularly true for cetaceans. In these cases, the existence of voucher material as well as DNA samples in scientific collections becomes crucial to confirm the identification of specimens through complementary approaches (i.e., DNA barcodes and voucher material). For cetacean taxonomy, one of the main constraints lies in the small number of reference collections, which are in general spread among several museums. The present study had the privilege of including 16 institutions in Brazil with vast scientific collections, which allowed us to detect cases of doubtful or incorrect morphological identification in the field, as was the case of ECOMEGA/FURG 45. This specimen was identified during fieldwork as a killer whale (*Orcinus orca*), but both molecular databases indicated greater similarity to the false killer whale (*Pseudorca crassidens*). Due to this incongruence, the available voucher material was reexamined, and taking into account the number of tooth pairs visible on the photographs of the stranded animal and the range of tooth pairs described for both species, the specimen was considered to have a higher probability of being *P. crassidens*, in agreement with the molecular identifications. Although these two species have completely different external appearance, skull morphology is quite similar (Heyning and Dahlheim 1988; Baird 2009). Thus, in the field, when some of the main external morphological traits are missing or are not clearly visible, as in the case of this specimen, misidentification can occur, highlighting the importance of molecular analyses. However, two other cases involving the combination of field identification, barcoding information and the reexamination of the skull morphology from two dolphin specimens revealed unsolved morphological-molecular mismatches. In the case of GEMARS 1240, both molecular databases suggested that the specimen was a short-beaked common dolphin (*Delphinus delphis*), but the absence of a prominent trapezoid-shaped palatal ridge and the deep palatal grooves on the skull of the voucher specimen supported the morphological identification as *S. coeruleoalba* (Jefferson et al. 1993). In the case of the AQUASIS 02C1152/333 specimen, it showed a typical coloration pattern of *S. clymene*, and this identification was corroborated by one of the molecular databases (NCBI). Nevertheless, the search in the BOLD System database resulted in an ambiguous identification with equal support for *Stenella frontalis* and *Stenella clymene*. It is worth mentioning that both cases above involve member of the subfamily

Delphininae, which show an overlap between intra- and inter-specific *coxI* genetic variations, suggesting that *coxI* is an imperfect barcode for these species. Therefore, for these taxa, additional markers must be developed for accurate species-level molecular identification, including complementary mitochondrial segments and likely biparentally inherited nuclear markers as well.

In this context, 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* (AQUASIS 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 et al. 2005). To investigate this possibility, informative nuclear markers must be developed, since the uniparentally-inherited mtDNA is insufficient for such questions. This development is an important avenue to pursue in the field of cetacean genetics and molecular identification.

Regardless of putative hybridization as a potential cause, 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), 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, especially within the subfamily Delphininae. Moreover, the neighbor-joining analysis showed that *D. delphis*, *S. frontalis*, and *T. truncatus* species do not form monophyletic groups, possibly 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 taxon (Zhou et al. 2011). On the other hand, through phylogenetic reconstruction with 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 resolve evolutionary relationships among its members using information regarding morphology, genetics, and, recently, acoustics and historical biogeography (Amaral et al. 2007; Vollmer et al. 2019). Additional work is required to clarify species boundaries in this group, thus allowing a more direct assessment of the power of DNA barcoding for their accurate identification.

In addition to the members of the subfamily Delphininae, our results also indicated a poor performance of *coxI* for discriminating among right whales species (*Eubalaena* spp.). Although *coxI* was able to correct the misidentification of the specimen GEMARS 1491 (*Megaptera novaeangliae* cf.) to a right whale, it was unable to conclusively discriminate among right whale species, as previously observed with another mtDNA region (*cytochrome b*) by Viricel and Rosel (2011). Taking into account the recent divergence of these species (Rosenbaum et al. 2000), our results are not surprising. However, since only *E. australis* is distributed in the southern hemisphere and all three extant species have an antitropical distribution (Rosenbaum et al. 2000), this limitation of *coxI* would not be a problem for discriminating the southern right whale from other large baleen whales in Brazilian waters.

In summary, the main concerns regarding the identification of cetaceans using the *coxI* gene are related to (i) cetaceans that seem not to have a “barcoding gap”; (ii) potential hybrids, which would require the use of biparentally inherited nuclear genes to establish the identification of the species; (iii) taxonomic updates that have not been incorporated in specimen identifications. All these concerns are relevant but, according to Galimberti et al. (2015), “...reference sequences constitute the main core of the DNA barcoding initiative and their absence or the lack of control of the correct identification of the source specimens by expert taxonomists, can irremediably affect the assignment of newly generated query sequences.” This is why the existence of voucher material related to every *coxI* sequence is important.

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

Twelve cetaceans recorded for Brazil are classified by IUCN as “Data Deficient”, mainly due to the lack of taxonomic or ecological information about these animals (ICMbio

2018; Hrbek et al. 2014; Cypriano-Souza et al. 2016; IUCN 2020). This scarcity of data and the accelerated process of degradation and pollution of the marine and freshwater environments occupied by these species highlight the need for studies that can help enhance the knowledge about this group, enabling the elaboration of effective conservation plans.

Considering this scenario, stranded cetacean carcasses can provide valuable information about the richness and patterns of occurrence of this group in Brazilian waters (Sholl et al. 2008; Meirelles et al. 2009; Prado et al. 2016; Dick et al. 2019; Milmann et al. 2020), once correctly identified. Therefore, despite some recognized limitations (Galimberti et al. 2015), our results reinforce that DNA barcodes, when properly used, can be a valuable tool for the scientific community involved in the stranding networks and to support decision-makers and conservation policies.

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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., E.E., and P.H.O. reviewed and edited the final version of the manuscript.

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Data availability All barcode sequences and their respective accession numbers are available in GenBank.

Compliance with ethical standards

Ethics approval and consent to participate The study was based on voucher specimens deposited in scientific collections. All DNA samples used for this research derived from animals stranded and naturally dead, or from biopsied animals. No animal was intentionally caught or killed during the summarized research. The sampling permits (see methods) issued by local authorities in Brazil (*SISBIO* - Brazilian Biodiversity authorization and information system) attest that the authors did not violate any ethical rule for collecting the animal samples. 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.

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