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Review of population structure studies for Sotalia guianensis and a proposal for Management Units

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4	Management Units
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15	Abstract
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17	This paper reviews published and unpublished genetic population structure data
18	for <i>Sotalia guianensis</i> , and presents a proposal of Management Units for the species, to
19	guide future research and conservation recommendations.
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21	Introduction
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23	Sotalia guianensis is a small coastal delphinid that faces human-related threats
24	along most of its distribution, which ranges from Nicaragua to southern Brazil (Flores
25	and Da Silva, 2009). Using the IUCN criteria adapted for regional scale, the species was
26	classified as "Vulnerable" by the Brazilian Ministry of the Environment (MMA, 2014).
27	That assessment was based on data showing a 37% decline in 15 years for the
28	Guanabara Bay population (Azevedo et al. 2017), and the projection that many other
29	populations would also be decreasing or were expected to decrease in the following
30	decade.
31	Delimiting Management Units (MU) is important for the evaluation of abundance
32	and mortality, and thus for the identification of populations at higher risk of local
33	extinction, and also to devise proper conservation and management actions. Genetic
34	data is the gold standard for the definition of MU. The genetic population structure of S.
35	guianensis has been studied along its distribution using the mitochondrial control region
36	("mtDNA") and microsatellites. This paper reviews published and unpublished genetic
37	data and provides a preliminary proposal of MU for the species, and discusses other
38	types of evidence that corroborate the proposed population subdivisions.

40 Results and discussion

Twelve genetic studies analyzed the population structure of *S. guianensis*, five at macro geographic scale, and the remaining at fine scale. Table 1 summarizes results from all those studies and provides the population limits evidenced by them. The ensuing population delimitation is presented in Figure 1.

Macroscale population limits are shown in Figure 1, and served as a basis for a proposal of MU (Figure 2). This scenario should be accepted provisionally, but further studies are needed to confirm the population differentiation indicated with a question mark ("?"). In these cases, genetic data is ambiguous regarding differentiation, but ignoring it would imply a higher risk of type 2 error, which could lead to extinction of a differentiated population (further discussed below and in Table 1).

52 Overall, both mtDNA and microsatellites support marked divergence between 53 *Sotalia guianensis* north and south of the Amazon Estuary (Caballero *et al.* 2018). 54 However, population differentiation was also detected between localities in northern 55 South America (Caballero *et al.* 2018) and along the Brazilian Coast (Cunha *et al.* 56 2005, Cunha 2007, Cunha *et al.*, *in prep.*), and it was this level of genetic differentiation 57 that was considered adequate for the delineation of MU.

58 Cunha et al. (2005) was the first study to present evidence of genetic differentiation for the species. Analyses of mitochondrial control region sequences 59 supported three populations along the Brazilian coast (North, Northeast and 60 South/Southeast). It is worth noting that all samples from Rio de Janeiro southwards 61 present a single haplotype, and therefore mtDNA is unable to detect differentiation in 62 63 this part of the range (Cunha et al. 2005, 2010). Later, microsatellite analyses corroborated the existence of genetic structure in Brazil, indicating the presence of at 64 least four, and up to seven populations, when a larger and geographically more 65 complete dataset of mtDNA sequences was considered (Cunha 2007, Cunha et al., in 66 67 prep.). Caballero et al (2010) presented evidence from mtDNA suggesting strong population structure, with possibly four populations units: Colombian Caribbean + 68 Maracaibo Lake (Venezuela), French Guiana, Amazon Estuary and Southeastern Brazil. 69 Caballero et al. (2018) also detected structure at macroscale, using mtDNA and 70 71 microsatellites, confirming previous findings, including evidence for five populations, 72 four from the Amazon estuary northwards, and one southwards. Also, initial evidence 73 presented suggested that the Orinoco River should be considered as a separate population unit. 74

75 Besides those macroscale studies, recent evidence of micro-geographic structure 76 was also taken into account for the MU proposal. For example, BA, ES and RJ are shown as distinct populations in Figures 1 and 2, because there is evidence of 77 microscale differentiation within each of these regions (Hollatz et al. 2011, Santos F. 78 2015, Santos T. 2015, Reblin 2016, Mendes 2019, Farro et al. in prep.). Therefore, on 79 the macroscale each of these units should be managed independently, but the existence 80 of local differentiated populations within them must not be neglected. Refinement of 81 microscale divisions are being developed and will be incorporated in future updates of 82 this map, once studies currently underway are completed. 83

In addition, besides finer resolution of populations already defined, future genetic studies are required to investigate regions still not analyzed (blank areas in Figures 1 and 2).

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88 Table 1: Summary of genetic findings in population genetic studies of S. guianensis. AP:

89 Amapá, PA: Pará, CE: Ceará, RN: Rio Grande do Norte, SE: Sergipe, BA: Bahia, ES: Espírito

90 Santo, RJ: Rio de Janeiro, SP: São Paulo, PR: Paraná, SC: Santa Catarina. mtDNA:

91 mitochondrial DNA control region, micsats: microsatellites.

Scale	Detected divisions	Data	Reference	Observations
Macro	3: Brazil - North (PA), Northeast (RN) and South/Southeast (RJ+SP+PR+SC)	mtDNA	Cunha <i>et al.</i> 2005	
Macro	4: Brazil - AP+PA, CE+RN, SE+BA+ES, RJ+SP+PR+SC	mtDNA, micsats	Cunha <i>et al., in</i> <i>prep</i> ; Cunha 2007	At least 4, but some analyses support further differentiation between CE and RN; and SE, BA and ES
Macro	4: Colombian Caribbean + Maracaibo Lake (Venezuela), French Guiana, Amazon Estuary and Southeastern Brazil	mtDNA	Caballero <i>et al.</i> 2010	
Macro	6: Colombian Caribbean (CC), Maracaibo Lake (ML), Orinoco River (OR), French Guiana (FG), Amazon Estuary, Brazil	mtDNA, micsats	Caballero <i>et al.</i> 2018	Differentiation between French Guiana and Brazil/PA supported by mtDNA but not microsatellites, meaning potential male mediated gene flow occurs. Strong differentiation between group North and South of the mouth of the Amazon. Orinoco Rive defined as a population Unit.
Micro	2: RJ	Micsats	Hollatz <i>et al.</i> 2011	Genetic differentiation between two bays
Micro	3: RJ	Micsats	Santos F. 2015, Santos T. 2015	Genetic differentiation between three bays and adjacent coastal area
Micro	2: ES	mtDNA	Reblin 2016	
Micro	ES?	Micsats	Ywasaki-Lima <i>et</i> <i>al.</i> 2017	Claimed differentiation between north and south ES not supported by data (F_{ST} , p > 0.05; Structure, K=1)
Micro	3: BA and ES	mtDNA	Mendes 2019	
Micro	3: ES	mtDNA	Farro <i>et al., in</i> prep.	





98 Table 2: Ongoing population genetic structure studies that will improve population delimitation.

Abbreviations as in Table 1.

	Geographic			Estimated	
Scale	coverage	Data	Reference	conclusion	Observations
Macro	Brazilian coast	mtDNA, micsats	Luana Barbosa, PhD Thesis	2021	Refinement of macroscale population structure along the Brazilian coast by increased sampling including previously unsampled regions (specially in NE Brazil)
Micro	SP, SC	micsats	Luana Barbosa, PhD Thesis	2021	
Micro	RJ	micsats	Haydée Cunha	2021	Long term monitoring - project includes genetic analyses of RJ populations
Micro	ES	mtDNA, micsats	Ana Farro	2021	Long term monitoring - project includes genetic analyses of ES populations



Figure 2: Proposal of MU delimitation based on genetic evidence (see Table 1 and text for details). Colored polygons show sampling area for each MU. CCOL: Colombian Caribbean; VEML: Maracaibo Lake, Venezuela; VEOR: Orinoco River, Venezuela; FRGU: French Guiana; BRNO: Brazil North; BRNE1: Brazil Northeast 1; BRNE2: Brazil Northeast 2; BRNE32: Brazil Northeast 3; BRNE4: Brazil Northeast 4; BRSE1: Brazil Southeast 1; BRSE2: Brazil Southeast 2; BRS/SE: Brazil South/Southeast. Note that due to vast unsampled regions in north South America limits between each MU were not drawn.

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129 At least some of the MU proposed here are also supported by other lines of 130 evidence, as summarized below.

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132 <u>Morphology:</u>

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Ramos *et al.* (2010) presented morphologic and morphometric evidence of differentiation between animals from Rio de Janeiro and Espírito Santo and those from São Paulo. Body size was smaller for specimens from São Paulo. Also, cranial metric characters differentiated the specimens studied in four groups, corresponding to Northern RJ, Southern RJ, São Paulo and Espírito Santo.

Fettuccia (2010) investigated geographic variation in cranial and post-cranial characters along the species distribution, and found clinal differences in the shape of the vomer. Many specimens from the North of South America (Colombia, Amapá and Pará) had a wider vomer, which is less frequent in Northeastern Brazil, rare in Southern Brazil, and absent in Southern Brazil.

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- Pollutant accumulation:

Lailson-Brito *et al.* (2010) presented results on bioaccumulation patterns of DDT, PCBs and HCB for Guiana dolphins in three bays in Southeastern Brazil, Ilha Grande and Sepetiba Bay, Guanabara Bay and Paranaguá Bay. These patterns were distinct for each bay, with higher concentration of DDT found in samples from Paranaguá, while Guanabara Bay showed higher mean values of PCBs. The highest mean values of HCB was also found in samples from Guanabara Bay.

- 154 <u>Stable Isotopes</u>:
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156 Carbon and nitrogen stable isotope analyses indicated the existence of at least
157 four ecological populations in the Brazilian coast: Amazon Estuary+Ceará, Espírito
158 Santo, Northern Rio de Janeiro and Santa Catarina (Botta 2011).

In addition C and N stable isotope of *Sotalia guianensis* from Rio de Janeiro State evidenced differences in feeding habitat utilization between populations from Ilha Grande and Sepetiba bays, suggesting high site fidelity of these populations and slight niche differentiation (Bisi *et al.* 2013).

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