1 **Manatee genomics supports a special conservation area in the Guianas coastline**
2 **under the influence of the Amazon River plume**

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6 Sibelle T. Vilaça^{1,2,6#}, Camilla S. Lima^{3#}, Camila J. Mazzoni^{1,2}, Fabricio R. Santos^{3*},

7 Benoit de Thoisy^{4,5*}

8 1 Leibniz Institute for Zoo and Wildlife Research, Department of Evolutionary Genetics, Berlin,

9 Germany.

10 2 Berlin Center for Genomics in Biodiversity Research, Berlin, Germany.

11 3 Laboratório de Biodiversidade e Evolução Molecular, Departamento de Biologia Geral, ICB,

12 Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, CP 486, 31270-010 Belo

13 Horizonte, MG, Brazil.

14 4 Institut Pasteur de la Guyane, 97300, Cayenne, French Guiana, France.

15 5 Kwata NGO, 97300 Cayenne, French Guiana, France.

16 6 Current address: Trent University, Peterborough, ON, Canada.

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18 **#both authors contributed equally to this work**

19 ***corresponding authors: benoit@kwata.net, fsantos@icb.ufmg.br**

20

21 Abstract

22

23 The West Indian manatee (*Trichechus manatus*) occurs along the Atlantic coastline and
 24 adjacent freshwater systems of South, Central and North America, from Alagoas
 25 (Brazil) to Florida (USA) and Greater Antilles. The Amazonian manatee (*Trichechus*
 26 *inunguis*) is the only Sirenian adapted exclusively to freshwater and endemic to the
 27 Amazon River basin. Previous studies have reported hybrids between *T. inunguis* and *T.*
 28 *manatus* close to the mouth of the Amazon River, composing a likely extensive hybrid
 29 zone along the Guianas coast of South America under the influence of the Amazon
 30 River plume. We have used ddRAD SNP data, and sequences of nuclear and mtDNA
 31 loci to characterize the genomic composition of manatees along the French Guiana
 32 coastline. We found this population to be formed by introgressed or later generation
 33 interspecific hybrids, and also describe the first *T. inunguis* found outside the Amazon
 34 River basin. Our results indicate that *T. inunguis* can survive in the Amazon River
 35 plume and have colonized independent water streams of the Guianas coastline where
 36 they likely hybridize with *T. manatus*. This hypothesis offers a plausible explanation for
 37 the known extension of the hybrid zone between the two species along the Guianas
 38 coastline. It also reinforces the importance of the Amazon plume, which flows
 39 westwards to the Guianas coastline and favors the dispersion of the freshwater species.
 40 This habitat functions as a large estuary-like system that provides an ecological
 41 continuum from Amazon River mouth to the disconnected waterflows of the Guianas,
 42 which deserves a status of special conservation area.

43

44 **Keywords:** *Trichechus*, hybridization, hybrid zone, population genomics, ddRAD,
 45 Amazon River plume

47 **Introduction**

48

49 Manatees (*Trichechus spp.*) are charismatic and vulnerable aquatic species that can be
50 found in the Americas (*T. manatus* and *T. inunguis*) and coastal regions of Africa (*T.*
51 *senegalensis*) (Deutsch, Self-Sullivan, & Mignucci-Giannoni, 2008). The West Indian
52 manatee, *T. manatus*, can live in both salt and freshwater environments. With exception
53 of the population inhabiting the northeastern coast of Brazil (Santos et al., 2016), it is
54 also found in rivers and lake systems along its coastal distribution. Previous
55 observations have recorded *T. manatus* up to 1100 km upstream in the Orinoco River
56 (Castelblanco-Martinez et al., 2003) and up to 650 km in the Magdalena River in
57 Colombia (Montoya-Ospina et al., 2001). Conversely, the Amazonian manatee (*T.*
58 *inunguis*) is highly specialized to freshwater environments and has only been reported
59 in the Amazon River basin so far.

60

61 French Guiana (FG), an overseas department of France in South America, has a stable
62 manatee population, allegedly belonging to the *T. manatus* species, estimated to have
63 between 50 and 250 mature individuals (UICN France et al., 2017)□. The main threats
64 to the FG manatee population are poaching, bycatches and degradation of coastal water
65 quality (UICN France et al., 2017). Despite a significant population decrease during the
66 last century, recolonization of some areas has been observed (de Thoisy et al., 2003).
67 Due to small population sizes, few catches per year are enough to maintain its declining
68 trend and suggest a regional threatened status (UICN France et al., 2017). Sighting
69 records are widespread in FG, both in dense and well-preserved mangroves and on the
70 lower parts of all river systems. Important seasonality in habitat preference has been
71 noticed in relation to strong seasonal fluctuations in the relative contributions of marine

72 and continental waters to the estuarine waters (Castelblanco-Martínez, dos Reis, & de
73 Thoisy, 2017). However, FG manatees also make use of large freshwater streams, with
74 records up to 100 km inland (UICN France et al., 2017).

75

76 Previous studies involving *T. manatus* populations (Garcia-Rodriguez et al., 1998;
77 Vianna et al., 2006; Santos et al., 2016) have identified three deeply divergent
78 mitochondrial DNA (mtDNA) clusters composing two Evolutionary Significant Units
79 (ESUs) that occupy two major geographic regions: Caribbean coast of Antilles, South
80 and Central America, Mexico Gulf coast and Florida (USA) (clusters I and II); and the
81 northeastern Atlantic coast of South America (Brazil and the Guianas), where cluster III
82 was exclusively found (Vianna et al., 2006). However, seven manatees found along the
83 Guianas coastline (corresponding to Guyana, French Guiana and the state of Amapá,
84 Brazil) and one manatee found in the Amazon River close to the mouth were reported to
85 be hybrids with mixed morphology and unexpected mtDNA lineages for their habitat
86 (Garcia-Rodriguez et al., 1998; Vianna et al., 2006)□.

87

88 Out of the eight samples, three manatees from FG were morphologically classified as *T.*
89 *manatus* (presence of nails, absence of white patch on the chest), but presented mtDNA
90 haplotypes typically found in *T. inunguis* (haplotypes T2 and R1). Furthermore,
91 microsatellite data confirmed the hybrid status of four of the eight manatees (two from
92 FG and two from Brazil), and a karyotype of a single individual from Amapá (Brazil)
93 indicated that it was a likely second-generation hybrid, a male offspring between a
94 female F1 hybrid and a male *T. manatus* (Vianna et al., 2006). Due to the supposedly
95 high prevalence of hybrids along the Guianas coast, this region located west of the
96 Amazon River mouth was suggested to be a hybrid zone where *T. manatus* and *T.*

97 *inunguis* individuals may contact and produce hybrids, further isolating the two *T.*
 98 *manatus* ESUs (Santos et al., 2016).

99

100 Hybridization has been recognized as a widespread phenomenon within animals, and it
 101 plays an important role in species evolution (Mallet, 2005). In endangered species,
 102 hybridization is often a consequence from declining population numbers due to
 103 anthropogenic activities (Fitzpatrick et al., 2015). When populations of closely related
 104 species – that have close or overlapping distribution and can still interbreed – decreases
 105 in size and consequently mating partners are rare, species hybridization is a likely
 106 outcome. Hybridization and introgression can have negative effects for a population
 107 (e.g. outbreeding depression), and the rise of hybrid individuals can further complicate
 108 the endangerment status of a species. If hybrids frequency is shown to be increased due
 109 to anthropogenic population decline of parental species, other alternatives should be
 110 discussed to avoid further decline of rare parental species and populations. However, in
 111 populations where hybridization is a natural phenomenon and does not pose a threat to
 112 the non-hybrid populations, improved management strategies can be developed to
 113 ensure the protection of hybrids. However, hybrids are usually not taken into
 114 consideration in conservation regulation and plans (Stronen & Paquet, 2013), and
 115 therefore the specimens and their habitat may be not eligible to be protected (but see
 116 Allendorf et al. 2001). Therefore, understanding hybridization events (i.e. their
 117 extension in space and time, ecological factors, interbreed, survival rate etc) and their
 118 causes (i.e. anthropogenic *versus* natural) is fundamental so that hybrids can be
 119 appreciated in conservation efforts.

120

121 In this study, we used a genomic approach to (i) investigate a previously recognized
122 hybrid zone between manatee species, in order to (ii) understand the extent of
123 hybridization, and (iii) to discuss ecological and evolutionary hypothesis for their
124 occurrence and conservation implications.

125

126 **Methods**

127

128 *Sampling*

129 Tissue material from eight samples from French Guiana were collected from stranded
130 animals, found dead on beaches and/or in mangroves. If the carcasses were not too
131 degraded, phenotypical information was collected, such as presence of nails, skin color
132 and extant of white patches on the chest and belly, expected to differentiate West Indian
133 and Amazonian manatees. Kidney and liver samples were obtained from a dead *T.*
134 *manatus* individual from the Tierpark Berlin Zoo, a born in captivity descendant of
135 maternal grandparents from Georgetown, Guyana, and unknown father (Bell, 2001).
136 Two *T. inunguis*, two *T. manatus* and a likely F2 hybrid from Amapá, Brazil (Vianna et
137 al., 2006) were also included.

138

139 *DNA extraction and Sanger sequencing*

140 DNA extractions were performed using the Dneasy Blood and Tissue kit (Qiagen). The
141 mtDNA D-loop region was amplified using the primers L15926 and H16498 (Kocher et
142 al., 1989). The amplification cycle followed Vianna et al. (2006)□, with annealing
143 temperature of 55 °C. Nuclear genes (Apolipoprotein B - APOB, Amyloid precursor
144 protein - APP, BMI1 proto-oncogene – BMI1, and CAMP Responsive element
145 modulator – CREM), were amplified following Murphy et al. (2001) (Table S1). New

146 primers for the APOB gene were designed using as reference the *T. manatus* GenBank
 147 sequence JN413954 (Meredith et al., 2011). For all loci, the PCR mix was prepared
 148 with final volume of 25 μ L, containing 1X Taq reaction buffer; 1.5 mM of $MgCl_2$; 200
 149 μ M of dNTPs; 0.5 μ M of each primer; 0.5 U of Platinum Taq DNA Polymerase
 150 (Thermo Fisher Scientific). PCR products were purified by the polyethylene glycol
 151 (PEG) method (20% PEG 8000, 2.5M NaCl) (Sambrook, Fritsch, & Maniatis, 1989)
 152 and sequenced on the ABI 3130xl Genetic Analysis (Applied Biosystems) using the
 153 BigDye Termination v3.1 Cycle Sequencing kit. The same primers used for
 154 amplification were used for DNA sequencing. Chromatograms were analyzed in
 155 SeqScape v2.6 and consensus sequences were aligned using the ClustalX (Larkin et al.,
 156 2007) algorithm in the software MEGA 6 (Tamura et al., 2013). The relationships
 157 between haplotypes were inferred with the software NETWORK (Fluxus-engineering)
 158 using the median-joining algorithm (Bandelt, Forster, & Röhl, 1999). Summary
 159 statistics were calculated in DNAsp v5 (Rozas et al., 2003).

160

161 *Double-digest RADseq*

162 Double-digest RAD (ddRAD) libraries were prepared following a previous protocol
 163 (Peterson et al., 2012) with adapter modifications (Meyer & Kircher, 2010), including
 164 two *T. inunguis* from Brazil, five manatees from French Guiana, and a *T. manatus* from
 165 Tierpark Zoo (Germany). In brief, 1 μ g of genomic DNA was digested with the
 166 enzymes *MseI* and *EcoRI* at 37 °C for at least two hours in a reaction volume
 167 proportional of DNA. The ligation of adapters was performed immediately after the
 168 digestion. Inline barcodes of varying sizes (5 to 9 bp) were added to the P5 adapter. The
 169 adapters and overhangs matching the enzymes were added. A sixteen-cycle indexing
 170 PCR was then performed in which one of the 50 barcodes described by Meyer and

171 Kircher (2010) was added to the P7 adapter, was then cleaned with 1.5X AMPure XP
 172 magnetic beads and quantify in the Qubit 2.0 using the dsDNA HS assay (Life
 173 Technologies) and checked in the Agilent 2100 Bioanalyzer High Sensitivity DNA
 174 (Agilent Technologies). The indexed samples were then equimolarly pooled in two
 175 libraries and selected by size in BluePippin using the 1.5% cassette with R2 marker
 176 (Sage Science). We select the sequences in a range between 350-400 bp and checked
 177 with the Agilent Bioanalyzer 2100 High Sensitivity DNA chip. No PCR was performed
 178 after the samples were pooled to avoid chimera formation (DaCosta & Sorenson, 2014).
 179 Then the libraries were characterized using the KAPA SYBR® FAST (Kapa
 180 Biosystems) kit and run in the in-house Illumina NextSeq 500 using a 300-cycle mid
 181 output v2 kit or in the in-house MiSeq with a 300-cycle kit.

182

183 Illumina reads were first demultiplexed according to P7 indexes. The software ipyrad
 184 0.7.19 (Eaton, 2014) was used to demultiplex the P5 inline barcodes, trim the adapters
 185 and quality trimming. Since samples from FG were in an advanced state of
 186 decomposition, we first estimated the contamination level in each sample. A subsample
 187 of 20,000 merged sequences was randomly extracted and blasted against the NCBI nt
 188 database. BLAST results were visualized in MEGAN6 (Huson et al., 2007) and levels
 189 of bacterial contamination were evaluated. Samples that remained in the analysis after
 190 evaluating the levels of contamination were submitted to a stepwise filtering process.
 191 First, all reads were blasted (blastn) against the Florida manatee genome (GenBank
 192 Accession Number GCA000243295) and to the African elephant genome
 193 (GCA000001905). Sequences with blast hits below an e-value threshold of 10e-50 were
 194 retained. Subsequently, reads with no hits to either genome was blasted against a
 195 *Clostridium noyi* genome (see Results for reasoning) with a similar threshold. The

196 remaining reads were finally blasted against a database of 8344 bacterial genomes and
197 all reads presenting hits below the threshold were excluded from the clustering step.
198 Clustering and SNP calling were performed in ipyrad. We used the following
199 parameters: minimum coverage for a cluster: 8; clustering threshold: 0.90; data type:
200 pairedddrad; minimum samples in a final locus: 6. The remaining parameters were left as
201 default.

202

203 Supposedly hybrid individuals used for ddRAD were originally from the Guianas region
204 where the hybrid zone was reported (Vianna et al., 2006). As controls we used two
205 Amazon manatees (*T. inunguis*) from Brazil and a West Indian manatee (*T. manatus*)
206 represented by a Florida manatee (*T. manatus latirostris*) genome (Foote et al., 2015).
207 The consensus sequences of each RAD locus were mapped to the Florida manatee
208 genome using bowtie2 (Langmead & Salzberg, 2012) in order to identify the
209 homologous regions. In parallel, all raw reads available in GenBank were mapped back
210 to the genome contig sequences, also using bowtie2. Resulting SAM files were
211 analyzed using customized python scripts for the identification of alleles at the target
212 loci. The Florida manatee genotypic information was then added to the ddRAD results
213 for subsequent analyses.

214

215 The software STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) was used to
216 identify hybrids and possible different genetic groups. A total of twenty replicates were
217 performed for each value of K from 1 to 5 (burn-in period: 20,000, 2×10^6 iterations)
218 under the admixture model and the assumption of correlated allele frequencies among
219 populations. The USEPOPINFO model was used to specify the two parental species
220 (POPFLAG=1) by assigning the two *T. inunguis* samples from Brazil and the *T.*

221 *manatus* from Florida, USA as learning samples, while ancestry was estimated for all
222 FG samples. The software CLUMPAK (Kopelman et al., 2015) was used to summary
223 and visualize the results. Samples identified as pure individuals were subsequently
224 assigned as learning samples. The observed heterozygosity (Hobs) was estimated using
225 the R package adegenet 2.1.0 (Jombart & Ahmed, 2011). The number of differences
226 between samples were estimated in the software Arlequin 3.5 (Excoffier & Lischer,
227 2010).

228

229 Hybridization simulations, using species-specific SNPs between the two-parental
230 species, were performed using the command *hybridize* in adegenet (Fig. S1).

231

232 **Results**

233

234 *Sanger sequencing*

235 A total of 410 bp of mtDNA D-loop and 1,919 bp of nuclear DNA were sequenced,
236 although four samples (M348, M827, M2108 and M2772) were partially sequenced for
237 some nuclear genes (Table 1). Alleles were considered as species-specific based on
238 population studies using larger samples of both species (Vianna et al. 2006; Santos et al.
239 2016, and unpublished data). Summary statistics and diagnostic polymorphisms for all
240 sequenced loci can be seen in Tables S2 and S3.

241

242 **Table 1.** Sampling location and main results obtained for each sample. MtDNA D-loop
243 haplotypes and nuclear haplotypes belonging to each species (*T. manatus*/*T. inunguis*,
244 respectively) are shown in parenthesis.

245

Sample	Sampling location	D-loop	Nuclear	ddRAD
M113	Paraná do Castanho, AM, Brazil	<i>T. inunguis</i> (T)	<i>T. inunguis</i> (0/8)	<i>T. inunguis</i>
M117	Tefé, AM, Brazil	<i>T. inunguis</i> (Q2)	<i>T. inunguis</i> (0/8)	<i>T. inunguis</i>
M22 JAG	Awala, French Guiana	<i>T. manatus</i> (J2)	Introgressed (7/1)	Introgressed
M297 JAG	Awala, French Guiana	<i>T. inunguis</i> (T2)	<i>T. manatus</i> (8/0)	Introgressed
M348 JAG	Awala, French Guiana	<i>T. inunguis</i> (T2)	-	-
M423 JAG	Mana, French Guiana	<i>T. manatus</i> (J2)	Introgressed (7/1)	Introgressed
M524 JAG	Kourou, French Guiana	<i>T. inunguis</i> (T2)	<i>T. manatus</i> (8/0)	Introgressed
M827 JAG	Riviere des Cascades, French Guiana	<i>T. inunguis</i> (T2)	<i>T. inunguis</i> (0/2)	<i>T. inunguis</i>
M2108 JAG	Marina de Dégrad des Cannes, French Guiana	<i>T. inunguis</i> (T2)	-	-
M2772 JAG	Kaw River, French Guiana	<i>T. inunguis</i> (T2)	<i>T. manatus</i> (6/0)	-
Tierpark	Tierpark zoo, Berlin, Germany	<i>T. manatus</i> (M)	Introgressed (7/1)	<i>T. manatus</i>
M035	Oiapoque, AP, Brasil	<i>T. Inunguis</i> (T)	Introgressed (6/2)	-
M042	Baía Formosa, RN, Brazil	<i>T. manatus</i> (M)	<i>T. manatus</i> (8/0)	-
M047	Beberibe, CE, Brazil	<i>T. manatus</i> (M)	<i>T. manatus</i> (8/0)	-

246

247

248 *Trichechus manatus* and *T. inunguis* samples from Brazil had both the mitochondrial
249 and nuclear DNA consistent with their morphological classification and habitat, except
250 for sample M035 (Table 1) from Amapá state (close to the frontier with French Guiana),
251 which is a likely second-generation hybrid previously characterized by mtDNA,
252 microsatellite and karyotype data (Santos et al., 2016). Most of the samples from FG
253 had mixed nuclear and mtDNA from both species (Table 1). Surprisingly, a new D-loop
254 mtDNA haplotype (J2), related to the *T. manatus* cluster II (Fig. S2), was found in two
255 FG samples (M22 and M423). All five FG samples with *T. inunguis* mtDNA had the
256 haplotype T2, previously described in FG samples as hybrids (Vianna et al., 2006).
257 Remarkably, a FG manatee (M827) found in a tributary (Riviere des Cascades, 40 km
258 from the river mouth at sea) of the Cayenne River had both *T. inunguis* mtDNA and
259 nuclear genes (Table 1), which suggests a non-hybrid individual. The analysis of
260 nuclear genes, also indicates that the *T. manatus* from Tierpark Zoo, which is

261 maternally descendant from manatees from Suriname (in the Guianas coastline),
262 presents 1 of 8 haplotypes derived from *T. inunguis*, thus a likely later generation hybrid
263 born in captivity.

264

265 Most FG manatees were found dead in an advanced state of decomposition, but
266 phenotypic information was obtained for two individuals. The individual M423 had a
267 large white patch on its breast (Fig. S3) and no nails on its flippers, both characteristic
268 of *T. inunguis*. The sample M524 had no nails but did not exhibit a white patch. Thus,
269 both individuals presented phenotypes indicative of *T. inunguis* ancestry.

270

271 *ddRAD*

272 A total of 30,486,215 paired sequences were obtained for eleven *Trichechus* samples.
273 Eight samples presented similarities with the bacterial genomes (<1%), indicating a
274 negligible contamination by decomposer organisms. On the other hand, three samples
275 (M2772, M2108, M348) were mostly composed of bacterial sequences, primarily from
276 the genus *Clostridium* and were excluded from further analysis. One sample (M827)
277 had a high presence of bacterial reads from the genus *Clostridium* (Fig. S4), but due to
278 the large number of reads, we were able to filter out bacterial sequences and include this
279 sample in further analysis. Due to the elevated number of reads compared to other
280 samples, a subsample of 2 million reads was used for sample M524.

281

282 After filtering for bacterial reads, samples had an average of 1,379,455 paired reads,
283 although three samples had significant numbers of small fragment carry-over (DaCosta
284 & Sorenson, 2014). All samples had overlapping fragments between 210-260 bp, and
285 therefore all analyses were conducted within this range. Coverage depth varied greatly

286 between clusters (Table S4), with many loci sequenced at low depth. Despite passing
 287 the paralog filter in ipyrad, five loci blasted in two separate regions of the *T. m.*
 288 *latirostris* genome and were excluded. The final dataset consisted of 947 clusters and
 289 2287 SNPs. Genotypic data from the *T. m. latirostris* genome was obtained for 900
 290 RAD loci and a total of 118 SNPs was unique to this.

291

292 A total of 720 unlinked SNPs was used in the STRUCTURE analysis. The preferred K
 293 was 2 (Fig. 1A). The results are in accordance with the four sequenced autosomal loci,
 294 the FG sample M827 is a non-hybrid *T. inunguis*, the sample from Tierpark is likely a
 295 non-hybrid (or highly-introgressed) *T. manatus* and all samples FG samples showed
 296 admixture between the two parental species (Fig. 1A and 1B). Furthermore, the
 297 assignment of M827 and Tierpark as training samples (added separately or combined)
 298 did not change the results in respect to hybrid admixture proportions (Fig. 1C).

299

301 **Figure 1.** STRUCTURE results for unlinked SNPs. A and B, Admixture results using *T.*
 302 *m. latirostris* and *T. inunguis* from Brazil (M113 and M117) as training samples. C,
 303 Admixture results adding M827 and Tierpark as training samples.

304

305 The differences within samples (Table 2 and Table S5) showed a lower diversity in *T.*
 306 *inunguis* and *T. manatus* (Florida) when compared to *T. manatus* (Tierpark Zoo).
 307 Hybrid samples from FG were more similar to *T. manatus* from Tierpark (with maternal
 308 ancestors from Suriname, Guianas coastline) than from Florida (*T. m. latirostris*). The
 309 newly identified *T. inunguis* from FG (M827) was more closely related to *T. inunguis*
 310 from Brazil than to other FG samples, although it presents the typical mtDNA haplotype
 311 found in FG hybrids (Table 1).

312

313 **Table 2.** Pairwise differences between manatee samples considering the 720 unlinked
 314 SNPs. Both *T. inunguis* samples from Brazil were considered as one group. Upper
 315 diagonal: Corrected pairwise differences between samples; Diagonal: Number of
 316 polymorphic sites within samples; Lower diagonal: Pairwise divergence between
 317 samples.

318

	M827	M423	M297	M22	M524	<i>T. manatus</i> (Tierpark)	<i>T. inunguis</i>	<i>T. manatus</i> (Florida)
M827	24	73	77	74	83	86	0	134
M423	0.62	64	0	0	0	13	101	35
M297	0.67	0.00	52	0	1	17	102	36
M22	0.61	0.00	0.00	70	0	10	95	29
M524	0.61	0.00	0.01	0.00	84	8	109	37
<i>T. manatus</i> (Tierpark)	0.70	0.19	0.25	0.14	0.10	49	108	41
<i>T. inunguis</i>	0.00	0.77	0.79	0.75	0.76	0.80	21	157
<i>T. manatus</i> (Florida)	0.90	0.50	0.55	0.43	0.45	0.60	0.90	6

319

320

321 The simulations of hybridization scenarios (Fig. S1) and the STRUCTURE results
322 showed a similar level of admixture between hybrids individuals (>F2).

323

324

325 Discussion

326

327 This paper explores the genetic diversity of manatees at the frontier region of the
328 distribution areas of two species, along the Guianas coastline with some characteristics
329 of a large estuarine environment close to the Amazon River mouth. Here we (i) validate
330 a pipeline to extract genotypic information from the raw reads of full genomes,
331 overcoming samples limitations for ddRAD analysis, (ii) describe an incongruence
332 between mitochondrial and nuclear DNA in FG manatees, supporting the extent of the
333 hybridization in this population, (iii) report the first *T. inunguis* outside the Amazon
334 Basin, in a French Guiana river at least 40 km away from the sea and (iii) discuss the
335 implications for conservation and management of this hybrid population under
336 influence of the Amazon River plume.

337

338 *Low-quality DNA sample quality for population genomics*

339 The combination of high-coverage sequencing and bioinformatic filtering allowed the
340 use of low-quality samples from decomposing animals found in FG rivers. The high
341 number of bacterial reads was an indicative of the low sample quality. The presence of
342 *Clostridium*, *Lactobacillus* and other Enterobacteriaceae sequences was previously
343 associated with decomposing bodies (Hyde et al., 2013). The samples composed mostly
344 of bacterial reads showed a higher bacterial diversity than the other samples, which
345 could indicate a more advanced of decomposition than the others. Nevertheless, ddRAD

346 data can be used to assess sample quality, local bacterial diversity, and bacterial
347 composition of decaying samples. The use of decomposing animals, can be the only
348 biological material usable to assess the genetic of small populations or that have
349 difficulties in sampling. In this study, they have proven to be a valuable tool to identify
350 SNPs to investigate the hybrid swarming phenomenon occurring along the Guianas
351 coastline (Santos et al., 2016).

352

353 *Manatees in French Guiana: a hybrid population*

354 The presence of a *T. manatus* mtDNA clade II haplotype in FG, combined with the
355 presence of *T. inunguis* individuals, is an indication that this population has a more
356 complex dynamic than previously thought. Previous studies have identified that the
357 distribution limit between *T. manatus* clades II and III was between Venezuela and
358 Guyana (Vianna et al., 2006), where a possible historical barrier to gene flow was
359 hypothesized. The presence of clade II in FG highlights the gene flow of western
360 manatees to the hybrid zone, where they meet eastern manatees (from Brazil), as shown
361 by the presence of clade III mtDNA lineages (L, M, N, O) in Guyana (Garcia-Rodriguez
362 et al., 1998), the westernmost country of the Guianas coastline. Thus, this *T. manatus* vs
363 *T. inunguis* hybrid zone is also a secondary contact zone between both *T. manatus* ESUs
364 (Vianna et al., 2006; Santos et al., 2016).

365

366 The extent of the hybrid zone between *T. manatus* and *T. inunguis* as suggested earlier
367 (Vianna et al., 2006; Santos et al., 2016), and the unusual high rate of hybrids supports a
368 possible ecological and biological advantage of hybrids in this peculiar environment.
369 The identification of later generation hybrid manatees indicates that this region may
370 have an old history of hybridization, and hybrids may present higher fitness in the local

ecological conditions. This remarkable finding of an extended distribution of a large aquatic mammal throughout riverine systems separated by hundreds of kilometers of sea implies the unique influence of the largest river basin in the world. The Amazon River discharge at the Atlantic Ocean shapes the distribution of many taxa of the marine biota (Spalding et al., 2007), sometimes promoting vicariance, as it can be seen for the disjoint distribution of West Indian manatees (Santos et al., 2016; Barros et al., 2017), with eastern and western ESUs separated by the hybrid zone acting as a barrier to gene flow. However, the presence of the Amazon River plume seems also to promote the connection of freshwater biota from separate river basins, like catfishes (Barthem et al., 2017), as we have also observed for the Amazonia manatee found in a river in French Guiana.

382

383 *The Amazonian manatee outside the Amazon basin*

The presence of *T. inunguis* reported here for the first time outside the Amazon basin indicates a possible connection between freshwater systems along the Guianas coastline and the Amazon River, in a region under influence of the Amazon plume that runs westwards because of the North Brazil Current (Muller-Karger, McClain, & Richardson, 1988). Indeed, the identification of a large Amazon River mammal in a French Guiana freshwater system supports the idea of a broad and unique low-salinity estuarine-like habitat along the Guianas coastline that connects freshwater biodiversity of further apart river basins under the influence of the Amazon River plume (Muller-Karger, McClain, & Richardson, 1988; Artigas et al., 2003; Anthony et al., 2013; Gensac et al., 2016; Barthem et al., 2017). From the Amazon River mouth to the west of the Guianas coast, the suitable habitats for *T. manatus* are narrow, due to the quite restricted alluvial coastal plain. The fluvial sediment supplies of the Amazon River

396 shaped the coastal landscape during the Quaternary (Anthony et al., 2013; Gensac et al.,
397 2016) and resulted in a continuous mangrove-dominated ecosystem along the Guianas
398 coastline from Amapá (Brazil) to Suriname, with rather close salinity and turbidity traits
399 brought by the Amazon plume. This coastal geomorphology associated with the
400 estuary-like characteristics may thus provide an ecological continuum (Artigas et al.,
401 2003), from the currently accepted distribution of *T. inunguis* in the Amazon Basin
402 towards the area of occurrence of *T. manatus* along the Guianas coastline. Furthermore,
403 the North Brazil Current and the Amazon River plume (Froidefond et al., 2002;
404 Fratantoni & Richardson, 2006) likely promote a westward movement of animals from
405 the Amazon River mouth.

406

407 The newly reported *T. inunguis* in this study has the same mtDNA haplotype as the
408 hybrids and has only been described in FG (Vianna et al., 2006), which implies this
409 hybridization may be a local phenomenon, involving both species. The mtDNA
410 haplotypes and the observed heterozygosity from simulated hybrids indicates that the
411 hybridization phenomenon has been occurring for several generations and that later-
412 generation hybrids in FG tend to mate with each other, thus initial hybrids (F1 and F2)
413 are at least partially fertile, particularly the females, as occurs in other mammals
414 (Allendorf et al., 2001; Stronen & Paquet, 2013; Fitzpatrick et al., 2015). Future
415 sampling efforts at larger geographic extent could help understanding the ratio of
416 hybrids to “pure” manatees in the Guianas coastline, and if hybrids from different
417 generations (F1, F2, etc) are also present in the population.

418

419 *Implications for conservation*

420 The need for conservation of hybrids is a controversial and challenging issue. Within
 421 species, Conservation Units are independent population units that are used to help guide
 422 management and conservation initiative, at the more relevant spatial extent (Funk et al.,
 423 2012). Other commonly discussed conservation units correspond to evolutionarily
 424 significant units (ESUs) and management units (MUs). An ESU can be defined as a
 425 population exhibiting high genetic and ecological distinctiveness (Crandall et al., 2000)
 426 as suggested for *T. manatus* (Vianna et al., 2006; Santos et al., 2016), while MUs are
 427 expected to be demographically independent (Palsbøll, Bérubé, & Allendorf, 2007).

428
 429 Distinctive genetic signatures of the Guianan manatees and their likely adaptive
 430 characters to the ecological conditions of this peculiar coastline and drainages may
 431 allow considering them as a special Conservation Unit, as a distinct MU, if not even an
 432 ESU. In this case, this population would require a particular conservation effort. Even
 433 though global IUCN do not consider hybrids as relevant taxa to be evaluated (IUCN
 434 Standards and Petitions Subcommittee, 2017), regional Red List assessment in French
 435 Guiana (UICN France et al., 2017) concluded it to be in a stable or decreasing trend,
 436 with a small population size due to restricted habitats, and ongoing threats such as
 437 incidental poaching, and to be included in an “endangered” conservation status.

438
 439 Thus, our study shows that French Guiana (and from other neighboring Guianan coasts)
 440 manatees are hybrids between *Trichechus manatus*, the coastal (and associated
 441 freshwater environment) species and *T. inunguis*, the freshwater adapted species from
 442 the Amazon River basin. Because genomic contribution to the local hybrids mostly
 443 comes from *T. manatus*, we conclude this hybridization is occurring for many
 444 generations with the input of *T. inunguis* individuals surviving along the Amazon River

445 plume. The extent of the hybrid zone within the distribution area of *T. manatus* raises
 446 the question of the likely ecological advantages of this introgression of genes from a
 447 freshwater adapted genome. The understanding of associated genetic process cause
 448 could be investigated by searching for natural selection signs throughout the genome of
 449 both species and hybrids, which could also give clues for the putative dominance of
 450 characters and directionality in interbreeding.

451

452 If this particular aquatic ecosystem promoted by the Amazon plume indeed influences
 453 the distribution and population dynamics of many other species along this coastal area,
 454 a regional conservation program should be devised, with an emphasis to preserve this
 455 unique aquatic environment of the Guiana shield coastline

456

457 **Acknowledgments**

458

459 We thank Susan Mbedi (BeGenDiv) and Hannah Ebbinghaus for their technical support
 460 in next-generation sequencing and Max Driller for bioinformatic support. Claudia
 461 Szentkis (IZW) for providing the sample of the captive manatee. STV was supported
 462 by an Alexander von Humboldt Foundation fellowship, CSL by a CAPES (Brazil)
 463 fellowship, and FRS by a CNPq (Brazil) research fellowship. This project was
 464 performed under ICMBio/SISBIO permit 45028 and received funding support from
 465 FAPEMIG, CNPq and Fundação o Boticário from Brazil. Samples from Brazil are kept
 466 in the collection of the Laboratório de Biodiversidade e Evolução Molecular in UFMG,
 467 Belo Horizonte, with a collaboration with ICMBio and Instituto de Desenvolvimento
 468 Sustentável Mamirauá, as part of the National Action Plan for Conservation of Sirenians

469 of Brazil. The use of genetic resources for this study is declared in the SisGen platform
470 according to Brazilian law 13123/2015.

471 Samples from French Guiana are kept in the databank JAGUARS, at Cayenne, French
472 Guiana. The JAGUARS tissue collection is supported by the INDIGEN project, funded
473 by European Community (ERDF funds), the Collectivité Territoriale de Guyane, and
474 the DEAL Guyane.

475 The use of the genetic resources was declared to the French Ministry of Environment
476 under the reference TSP 48704, in compliance with the Access and Benefit Sharing
477 procedure implemented by the Loi pour la Reconquête de la Biodiversité.

478

479

480

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