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## Phylogeography of the green turtle, *Chelonia mydas*, in the Southwest Indian Ocean

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### Abstract:

Patterns of mitochondrial DNA (mtDNA) variation were used to analyse the population genetic structure of southwestern Indian Ocean green turtle (*Chelonia mydas*) populations. Analysis of sequence variation over 396 bp of the mtDNA control region revealed seven haplotypes among 288 individuals from 10 nesting sites in the Southwest Indian Ocean. This is the first time that Atlantic Ocean haplotypes have been recorded among any Indo-Pacific nesting populations. Previous studies indicated that the Cape of Good Hope was a major biogeographical barrier between the Atlantic and Indian Oceans because evidence for gene flow in the last 1.5 million years has yet to emerge. This study, by sampling localities adjacent to this barrier, demonstrates that recent gene flow has occurred from the Atlantic Ocean into the Indian Ocean via the Cape of Good Hope. We also found compelling genetic evidence that green turtles nesting at the rookeries of the South Mozambique Channel (SMC) and those nesting in the North Mozambique Channel (NMC) belong to separate genetic stocks. Furthermore, the SMC could be subdivided in two different genetic stocks, one in Europa and the other one in Juan de Nova. We suggest that this particular genetic pattern along the Mozambique Channel is attributable to a recent colonization from the Atlantic Ocean and is maintained by oceanic conditions in the northern and southern Mozambique Channel that influence early stages in the green turtle life cycle.

**Keywords:** *Chelonia mydas*, mitochondrial DNA, control region, phylogeography, Mozambique Channel, Indian Ocean.

58 **Introduction**

59  
60 The green turtle (*Chelonia mydas*) is a large, long lived, herbivorous reptile that grazes on  
61 marine macrophytes in shallow tropical and sub-tropical waters around the world (Limpus *et al.*  
62 1994, Limpus & Chaloupka 1997). Because green turtle hatchlings are rarely seen between the  
63 time they leave their natal beach and when they first appear in shallow water foraging habitats  
64 (Musick & Limpus 1997), Carr (1987) named this interval the *lost year*. Available evidence  
65 now indicates that this *lost year* involves at least several years of drifting in oceanic gyre  
66 systems in a passive migration that may circumnavigate entire ocean basins (Bowen *et al.* 1995;  
67 Lahanas *et al.* 1998; and Bolten *et al.* 1998). Green turtles grow slowly, often taking some 25 to  
68 30 or more years to reach maturity (Limpus and Walter 1980). During this developmental  
69 period, they occupy a series of foraging habitats dispersed over an extensive area. Upon  
70 reaching adulthood, reproductive females typically make long distance migrations between  
71 feeding sites and their natal breeding beaches (Limpus *et al.* 1992). They show great fidelity to  
72 both nesting (Meylan 1982) and feeding grounds (Limpus *et al.* 1992), even though these may  
73 be separated by thousands of kilometers (Mortimer & Carr 1987). They typically lay multiple  
74 clutches within a season (Carr & Ogren 1960), with 1 to 9 or more years separating successive  
75 breeding seasons (Le Gall *et al.* 1985; Limpus *et al.* 1994; Miller 1996; Limpus *et al.* 2001).

76  
77 Attempts have been made to define green turtle population boundaries for this globally  
78 distributed endangered species in order to identify functional units of management. Although  
79 flipper tagging (Le Gall & Hugues 1987), satellite (Pelletier *et al.* 2003) and acoustic telemetry  
80 (Taquet *et al.* 2006) provide useful information about contemporary demography, site fidelity  
81 and migrations of individual animals, the data produced are strongly biased toward females and  
82 intensively surveyed locations, especially nesting beaches. In contrast, genetic studies tend to  
83 focus population rather than individual level and can offer unique perspectives on historical  
84 population dynamics. When complemented by tagging studies, genetic tools can elucidate the  
85 geographical boundaries of breeding populations and provide information about their  
86 migrations through feeding, breeding and developmental ranges (Bowen & Karl 1997).

87  
88 Mitochondrial DNA (mtDNA) has proven particularly effective for detecting population  
89 structure in marine turtles (FitzSimmons *et al.* 1999), and several studies have successfully  
90 used mtDNA variants to resolve population boundaries among breeding sea turtles (Bowen *et al.*  
91 1992; Bowen *et al.* 1994; Broderick *et al.* 1994; Norman *et al.* 1994; Encalada *et al.* 1996;  
92 Bass *et al.* 1996; Bowen *et al.* 1998; Dutton *et al.* 1999). In general, these studies have revealed  
93 a significant level of population subdivision on both regional and global scales and found that  
94 rookeries, often separated by hundreds of kilometers, may form genetically discrete populations  
95 or Management Units (Moritz 1994). The maternal inheritance of mtDNA also tends to  
96 accentuate genetic differences among populations compared to nuclear genes because it has a  
97 smaller effective population size. In many circumstances, female-inherited markers offer a  
98 distinct advantage because they provide perspectives on female reproductive behaviour that are  
99 paramount to species survival (FitzSimmons *et al.* 1999). Nevertheless, mtDNA does not  
100 capture the entire population genetic history of a particular species and inferences of population  
101 connectivity and isolation can be misleading especially if male-mediated gene flow is  
102 substantially different to that of females, as it was shown in some green turtle populations (Karl  
103 *et al.* 1992; FitzSimmons *et al.* 1997a,b; FitzSimmons *et al.* 1999; Roberts *et al.* 2004).

104

105 Among the significant green turtle rookeries that occur in the South West Indian Ocean, some  
106 have been well described. At the French Eparses Islands (Europa, Juan de Nova, Tromelin and  
107 Glorieuses) green turtle populations have been monitored since the 1980's (Le Gall *et al.* 1985;  
108 Le Gall & Hughes 1987; Le Gall 1988). The green turtles of the Seychelles archipelago are  
109 well known (Frazier 1984; Mortimer 1984, Mortimer *et al. in press*), especially those at  
110 Aldabra (Frazier 1971; Mortimer 1988). Other studies include those of green turtles at Mayotte  
111 (Ciccione S unpublished data), Comoros (Frazier 1984; Ciccione S unpublished data), North  
112 East of Madagascar (Bourjea J unpublished data), Kenya (Okemwa *et al.* 2004), and Tanzania  
113 (Muir 2005). These studies have shown that the patterns of movements and behaviour of green  
114 turtles in this region conform to those found elsewhere in the world, but a detailed appraisal of  
115 the entire region has yet to emerge. In fact, information on nesting turtles is either sparse or  
116 lacking in other adjacent countries, especially Mozambique, South of Madagascar and Somalia,  
117 where both nesting and foraging habitat as well as human exploitation of this species occur (Le  
118 Gall & Hughes 1987; Rakotonirina & Cooke 1994).

119  
120 The South West Indian Ocean, especially the Mozambique Channel, is of particular  
121 biogeographic interest. Suitable green turtle feeding habitat, due to warm water flows, are  
122 found very close to the tip of South Africa while suitable habitat is absent from the west coast  
123 of South Africa due to upwelling and cold water flows. Previous protein and total mtDNA  
124 RFLP genetic studies inferred that cold waters of South Africa have been a major  
125 biogeographic barrier for green turtle dispersal (Bonhomme *et al.* 1987; Bowen *et al.* 1992).  
126 Bowen *et al.* (1992) found no evidence of gene flow occurring between Indian and Atlantic  
127 Oceans over the last 1.5 million years but did not sample rookeries in the Mozambique  
128 Channel. If there is any contact between green turtles in the Indian and Atlantic Oceans, then  
129 the Mozambique Channel is the most likely place for this to occur.

130  
131 The purpose of this study is to survey the patterns of mtDNA control region sequence variation  
132 of nesting green turtles at 10 different rookeries in the South West Indian Ocean, principally  
133 along the Mozambique Channel. The patterns of mtDNA variation will be used to i) define  
134 groups of rookeries that comprise discrete genetic populations, ii) investigate the patterns of  
135 dispersal and subdivision of rookeries in this region and iii) determine if there is any evidence  
136 of contact between green turtles from Indian and Atlantic Oceans.

137  
138  
139 **Material and Methods**

140  
141 *Sampling*

142  
143 A total of 288 nesting females were sampled on different dates from 10 different nesting sites in  
144 the western Indian Ocean (Figure 1A and Table 1), that geographically fall into three groups.  
145 Those from the South Mozambique Channel (called here SMC) include Europa and Juan de  
146 Nova (French Eparses islands); while those from the North Mozambique Channel (called here  
147 NMC) include the Mohéli (Comoros), Mayotte (French territory), Nosy Iranja (Madagascar),  
148 Glorieuses (French Eparses Island), and three sites in the Republic of Seychelles. The last  
149 group, out of the Mozambique Channel, is composed only by Tromelin (French Eparses  
150 Island). In the French Eparses islands, Europa was sampled in 1997 (n=24) and again in 2003  
151 (n=9), for a total of 33 samples; Tromelin (n=44), Juan de Nova (n=20) and Glorieuses (n=39)

152 were sampled respectively in 1997, 1999 and 2004. Mayotte (n=41), Mohéli (n=34), Nosy  
153 Iranja (n=13) were sampled in 2004. In the Republic of Seychelles, Aldabra (n=31), Cosmoledo  
154 (n=26), and Farquhar (n=7) were sampled in 1996.

155  
156 Typically, the source of mtDNA for the majority of turtles was either skin or blood. Blood  
157 samples were taken from the cervical sinus (*after* Owens & Ruiz 1980) and stored in either  
158 lysis buffer or frozen in ACD-B (Becton Dickinson solution). Skin samples were taken from  
159 either the neck or flipper region and stored in 20% DMSO (Dimethyl Sulfoxide) saturated salt  
160 solution (Dutton 1996). All adult turtles encountered in this study were tagged. In some cases  
161 however, mtDNA was obtained from tissues of dead embryos or hatchlings found in the bottom  
162 of hatched-out nests (Mortimer & Day 1999) with only one sample per clutch and per female to  
163 avoid resampling the same matriline.

164  
165  
166 *Mitochondrial DNA control region extraction, amplification and sequencing*

167  
168 DNA was extracted from small amounts of blood (20 µl) or tissue (0.1g) by overnight digestion  
169 at 56°C in a 1x TE buffer, proteinase K (0.5mg/ml) and SDS (0.01%) solution. Digested  
170 proteins and cellular material were salted out by centrifugation (13 000 rpm for 20 min at 4°C)  
171 in the presence of Ammonium acetate. The DNA was subsequently pelleted by adding 1  
172 volume of cold EtOH to the supernatant and further centrifugation (13 000 rpm for 20 min at  
173 4°C). Residual salts were removed by rinsing the DNA pellet twice with 100% and 70% EtOH,  
174 respectively wash. The DNA was resuspended in 1x TE buffer. An alternative rapid protocol  
175 was also used and involves a proteinase K (0.2 mg/ml) digestion in 0.5 ml of 1x TE buffer and  
176 5% Chelex (Biorad) solution for 4 to 12 hours at 55-60°C with frequent vortexing. The  
177 suspension was heated at 95°C for 5 min and then centrifuged for 5 min at 13 000 rpm. The  
178 supernatant was collected and used as template for subsequent PCR amplifications.

179  
180 A portion (~ 396 bp) of the mtDNA control region was amplified by PCR using the TCR-5 (5'-  
181 TTGTACATCTACTTATTACCAC-3') and TRC-6 (5'-GTACGTACAAGTAAAACCTACCGTATGCC-3') primers (Norman  
182 *et al.* 1994). Amplifications were performed in a total volume of 25 µl containing 5-50 ng of  
183 whole DNA, 10 mM of each dNTP, 10 µM of each primer, 0.5 Units of high fidelity Advantage  
184 2 polymerase mix (BD Biosciences) and the corresponding reaction buffer (1x). Cycling  
185 parameters were 93°C for 1 min, followed by 35 cycles at 93°C for 40 sec, 55°C for 50 sec, and  
186 72°C for 40 sec, and a final extension at 72°C for 2 min (Fitzimmons *et al.* 1997a).  
187 Amplification was verified by electrophoresis of 4µl of each reaction in a 1% agarose gel,  
188 together with a 100 bp DNA ladder (New England Biolabs).

189  
190 Products were purified with the SEQueasy Kleen Kit (Biorad) and run through a 3730XL  
191 sequencing analyser (Applied Biosystems). The sequencing reactions (forward and reverse)  
192 were performed with dye terminators (Bigdye 3.1, Applied Biosystems) on a Primus 96  
193 thermocycler (MWG Biotech).

194  
195  
196 *Data Analysis*

198 Sequence alignments were performed with the software Clustal W (Thompson *et al.* 1994).  
199 Neighbor-joining analysis (Saitou & Nei 1987) was implemented with the NEIGHBOR  
200 procedure of the program Phylip 3.5 (Felsenstein 1993). Bootstrap analysis was computed  
201 using of the SEQBOOT (500 replicates) and CONSENSE procedures from the Phylip package.  
202 The neighbor-joining tree was drawn with the software Tree View 1.5 (Page 1996).

203  
204 Differentiation between populations was assessed with Wright's fixation index  $F_{st}$  (10000  
205 replicates; Wright 1951), estimated by  $\theta$  (Weir & Cockerham 1984) with the Genetix 4.02  
206 software package (Belkhir *et al.* 2001). This software was also used to estimate the number of  
207 migrants per generation ( $Nm$ ). AMOVA (analysis of molecular variance approach, Excoffier *et*  
208 *al.* 1992) was performed using Arlequin, v. 2.0 (Markov chain length: 10 000; Schneider *et al.*  
209 2000) to examine genetic structuring among rookeries and among different groups of regional  
210 rookeries.

211  
212 Correlation between genetic (measured as  $F_{st}/(1-F_{st})$  following Rousset 1997) and geographic  
213 distance matrices was tested with a Mantel non parametric permutation test (Mantel 1967) as  
214 implemented in Genetix 4.02. The geographic distances between the different nesting sites  
215 corresponded to the shortest sea distance between rookeries.

216  
217

## 218 **Results**

219  
220 *Mitochondrial DNA polymorphism*

221  
222 A total of 40 polymorphic sites were found (Table 2) corresponding to 39 substitutions, one  
223 insertion and one deletion. Seven mtDNA haplotypes were observed among the 288 green  
224 turtles sampled from 10 rookeries in the south-western Indian Ocean (Table 1 and Figure 1A).  
225 Six of the 7 haplotypes described here have been found elsewhere: CM8 (GenBank accession  
226 nos. Z50130) occurs in South Atlantic and West African Rookeries (Encalada *et al.* 1996) and  
227 is the first time this variant has been found in the Indian Ocean. Haplotypes C3, D2, A1 and  
228 A2 are known to occur in several other rookeries throughout the Indo-Pacific (Dethmers *et al.*  
229 Submitted; GenBank accession nos. AY955204, AY955205, AY955215 and AY955219,  
230 respectively). May23 haplotype was found in the Comoros (Formia 2002) and registered in  
231 GenBank as accession nos. AF529030. A new haplotype is described here for the first time:  
232 Glo33 (GenBank accession nos. DQ256086).

233  
234 The observed 7 haplotypes differed by between 1 and 25 substitutions, corresponding to 0.3% –  
235 6.5% (mean = 4.2%) estimated sequence divergence. The neighbour-joining tree of the 7  
236 haplotypes (Figure 2) identified three distinct clades of haplotypes: clade 1 (CM8 alone), clade  
237 2 (including A1 and A2) and clade 3 (including C3 and the rare haplotypes May23, D2 and  
238 Glo33). The new haplotype Glo33 forms a clade with common C3 haplotype and differs by  
239 only two substitutions.

240  
241

242 *Within population diversity*

243

244 Within population diversity range from 1 haplotype at Nosy Iranja (n=13) to 6 (haplotype  
245 diversity:  $h = 0.3708$ ; Table 1) at Mohéli (n=34; Table 1 and Figure 1A). The northern (NMC-  
246 Tromelin) regional set of rookeries has higher levels of haplotypic heterogeneity (mean 3.3  
247 haplotypes,  $h = 0.3723$ ) compared to those from the south (SMC, mean 2.5 haplotypes,  $h =$   
248  $0.3425$ ). All 7 haplotypes were found in the NMC rookeries, with C3 at high frequencies, A2 at  
249 intermediate frequencies and several rarer haplotypes (CM8, May23, D2, A1 and Glo33). In  
250 contrast for the SMC only 3 haplotypes were found in Juan de Nova ( $h = 0.5632$ ; CM8 at high  
251 frequency, C3 at intermediate frequency and a single occurrence of haplotype A2; Table 1) and  
252 only 2 haplotypes were found in Europa ( $h = 0.1174$ ; CM8 in high frequency and C3 in low  
253 frequency). Nucleotide diversities on the other hand were similar in both the NMC and  
254 Tromelin ( $\pi = 0.0184$ ) and SMC ( $\pi = 0.0221$ ) because most rookeries are comprised of a  
255 mixture of divergent haplotypes.

256  
257

#### 258 *Differentiation among nesting sites population structure*

259

260 Tests for population differentiation were estimated using Wright's fixation index ( $F_{st}$ ) based on  
261 haplotype frequency. Results are presented in Table 3. Comparisons between SMC rookeries  
262 (Europa and Juan de Nova) and all other rookeries were highly significant ( $F_{st} = [0.307-0.912]$ ;  
263  $P < 0.001$ ). There is also a significant differentiation inside SMC between Europa and Juan de  
264 Nova populations ( $F_{st} = 0.303$ ;  $P < 0.05$ ). Farquhar has a small sample size but it is also slightly  
265 but significantly differentiated from most other NMC rookeries ( $F_{st} = [0.147-0.501]$ ;  $P < 0.05$ )  
266 with the exception of Glorieuses, Cosmoledo and Aldabra ( $F_{st} = [0.160 - 0.012]$ ;  $P = [0.066$ ;  
267  $0.340]$ ). But all comparisons among the NMC rookeries excluding Farquhar were not significant  
268 ( $F_{st} < 0.17 - P = [0.056; 0.610]$ ). Comparisons between pooled NMC rookeries and Tromelin  
269 were also statistically insignificant ( $F_{st} < 0.0466 - P = [0.081; 0.558]$ ). We therefore recognise  
270 two genetic stocks in SMC (Europa and Juan de Nova) and a single genetic stock in the NMC  
271 comprising Aldabra, Cosmoledo, Glorieuses, Nosy Iranja, Mohéli, Mayotte, Farquhar and  
272 Tromelin.

273

274 The screening of mtDNA variation shows a frequency shift of haplotypes from Europa to  
275 Tromelin Atolls. The CM8 haplotype is the most common in the SMC (Europa and Juan de  
276 Nova) whereas the C3 haplotype is most frequent in the NMC (Seychelles, Nosy Iranja,  
277 Mohéli, Mayotte and Glorieuses) and in Tromelin. The change in frequency of the CM8  
278 haplotype from south to north Mozambique Channel is particularly informative. It is nearly  
279 fixed at Europa (94%), dominant at Juan de Nova (55%), present at Mayotte (12%), rare at  
280 Mohéli (3%) and is absent from the other NMC rookeries surveyed (Figure 1A).

281

282 Estimates of gene flow (Table 3) show that there is little exchange between SMC and NMC  
283 rookeries ( $Nm < 1$ ) compared to exchange among rookeries within each of these regions  
284 (typically  $Nm > 1$ ). There was some evidence for restricted gene flow between Farquhar and  
285 some of the more distant rookeries within the NMC rookeries ( $Nm = [0.34 - 1.65]$ ) compared to  
286 the closest rookery Cosmoledo ( $Nm = 19.98$ ).

287

288 AMOVA was used to compare four hypotheses about hierarchical structuring among South  
289 West Indian Ocean rookeries (Table 4). The first model (GP1) had two groups, all the NMC  
290 rookeries and all the SMC rookeries. The second model (GP2) had three groups, Farquhar, the

291 remainder of the NMC rookeries and SMC rookeries. The third model (GP3) had three groups,  
292 Europa, Juan de Nova and all the NMC rookeries. The fourth model (GP4) had four groups  
293 Europa, Juan de Nova, Farquhar and the remainder of the NMC rookeries. According to  
294 among-group variance ( $F_{CT}$ ) component test results, all four models were statistically  
295 significant but the GP3 model explained the highest among group variance ( $F_{CT}$ ) and is  
296 consistent with our earlier identification of just three genetic stocks within this region.  
297

298 We used a Mantel test to determine if the observed patterns of population genetic structure  
299 were consistent with a one-dimension isolation-by-distance model (Figure 3) and found a  
300 significant correlation ( $P < 0.001$ ,  $R^2 = 0.3565$ ; slope = 0.002) between genetic and geographic  
301 pairwise distance measures. Concerned that the divergent SMC rookeries might be driving this  
302 pattern we ran the same model without Europa and Juan de Nova and found no correlation  
303 between the genetic and geographic distance measures ( $P = 0.147$ ;  $R^2 = 0.018$ ; slope = 0.00004).  
304

## 305 **Discussion**

### 306 *Evidence for gene flow around the Cape of Good Hope*

307  
308  
309 Most of the haplotypes identified in this study conform to expectations and occur elsewhere in  
310 Indo-Pacific Oceans rookeries (Dethmers *et al.* Submitted) or are novel and occur in low  
311 frequency. The remarkable discovery of an Atlantic Ocean haplotype (CM8, Encalada *et al.*  
312 1996) represents the first time that any Atlantic Ocean haplotype has been recorded among any  
313 Indo-Pacific nesting populations. The observation of this Atlantic variant mixed with Indo-  
314 Pacific haplotypes in a same rookery (Table 1) reinforces the fact that Atlantic and Indo-Pacific  
315 lineages are not cryptic species. Until now, several green turtle genetic studies have shown that  
316 there is a fundamental phylogenetic split distinguishing all green turtles in Atlantic Ocean and  
317 the Mediterranean Sea from those in Indian and Pacific Oceans (Bonhomme *et al.* 1987; Avise  
318 *et al.* 1992; Bowen *et al.* 1992). Because of prevailing cold water conditions, the Cape of Good  
319 Hope has been commonly assumed to be an absolute barrier to the mixing of Atlantic and Indo-  
320 Pacific populations of green turtles but it has not been an impermeable barrier to all tropical  
321 species (Briggs 1974).  
322

323 Had Bowen's *et al.* (1992) total mtDNA study surveyed populations from the southwest Indian  
324 Ocean, they would have found the same remarkable pattern despite the present studies  
325 enhanced power using mtDNA sequence data. Using microsatellite data Roberts *et al.* (2004)  
326 demonstrated recent or ongoing male-mediated gene flow among populations within Indian and  
327 Atlantic Ocean Basins. Although their study did not include samples from the southwest Indian  
328 Ocean it did provide compelling evidence that at least the occasional male was capable of  
329 rounding the Cape of Good Hope. Our study of southwest Indian Ocean rookeries demonstrates  
330 for the first time a recent matrilineal link between Atlantic and Indian Ocean green turtle  
331 populations. The observation that an Atlantic mtDNA haplotype occurs in adjacent Indian  
332 Ocean waters and not *vice versa* is a significant observation as it indicates that the direction of  
333 matrilineal gene flow is likely to be from the Atlantic to the Indian Ocean. Likewise, the  
334 observation that only a single Atlantic haplotype has been observed and that it occurs in high  
335 frequency among SMC rookeries suggests that gene flow is not ongoing. If the Indian and  
336 Atlantic Oceans were connected by substantial amounts of contemporary gene flow then we  
337

338 would expect to detect additional Atlantic haplotypes in the SMC. If the colonization event was  
339 more ancient then we would expect to have detected novel variants of the CM8 haplotype with  
340 our intensive sampling of the SMC region.

341  
342 A growing number of studies document an Indian and East Atlantic phylogeographic  
343 connection in different marine species, like bigeye tuna (Chow *et al.* 2000; Durand *et al.* 2005),  
344 hammerhead sharks (Duncan *et al.* 2006), trumpetfishes (Bowen *et al.* 2001) or the urchin  
345 diadema (Lessios *et al.* 2001). Almost all cases of marine dispersal in this region are from the  
346 Indian to the Atlantic Ocean, usually attributed to passive drift by larvae in the Agulhas current.  
347 However, in a recent study on hammerhead shark (*Sphyrna lewini*), Duncan *et al.* (2006)  
348 showed a connection between these two oceans. The authors strongly support that the Indo-  
349 West Pacific hammerhead shark haplotypes most closely related to the Atlantic lineage are the  
350 product of a recent dispersal from the Atlantic into the Indo-Pacific, and that gene flow in this  
351 opposite direction is possible because this species is an active swimmer at every life stage  
352 (Duncan *et al.* 2006). Green turtles are also active swimmers at every life stage and may present  
353 the second example of active dispersal from the Atlantic into the Indian Ocean.

354

355

#### 356 *Regional differentiation*

357

358 The analysis of the genetic variability of nesting turtles in the South West Indian Ocean shows  
359 a significant population differentiation between those in the SMC including Europa and Juan de  
360 Nova, and the remaining nesting sites that were sampled in the NMC including Mohéli,  
361 Mayotte, Glorieuses, Nosy Iranja, Seychelles and Tromelin (Figure 1A, Table 3). For example,  
362 there is a high genetic differentiation ( $F_{st}=0.646$ , Table 3) between Europa and Mayotte  
363 although the two populations are less than 1200 kilometers apart. Inside SMC, there is a  
364 significant population differentiation between Europa and Juan de nova. Our data also show  
365 that Farquhar may be differentiated from both rookeries in the NMC (excluding Cosmoledo)  
366 and Tromelin (Table 3). This result must be taken with caution as the sample size of Farquhar is  
367 small ( $n=7$ ) due to the limited number of nesting females present at this remote island when the  
368 survey was conducted. However, more intensive sampling may not necessarily lead to the  
369 identification of further population genetic structuring here as the well sampled and more  
370 distant comparisons of Tromelin and pooled SMC rookeries were also insignificant.

371

372 It is rare to see such clear patterns of isolation by distance (IBD) in marine turtles even though  
373 it is expected in a species that has natal homing. Our results showed a pattern of IBD (Figure 3)  
374 when run on the entire data set. However there was no relationship between genetic and  
375 geographic distance for comparisons among rookeries in the NMC and Tromelin. The  
376 decreasing frequency of the CM8 variant from SMC rookeries to NMC rookeries points to IBD  
377 operating within the Mozambique Channel but not among rookeries in the rest of the southwest  
378 Indian Ocean. This pattern is consistent with a colonization process whereby rookeries closest  
379 to the Atlantic Ocean source populations (eg Europa) receive more immigrants than those more  
380 distant (eg Juan de Nova). In subsequent generations migration and possible selection could act  
381 to further disperse the CM8 lineage throughout the Mozambique Channel beyond the initial  
382 founder populations.

383



384 Data from turtle tagging studies in the Mozambique Channel (Hughes 1982; Le Gall & Hughes  
385 1987) are consistent with the general observation that most nesting turtles migrate less than  
386 1000 km between breeding and foraging habitat; although distances greater than 2600km have  
387 been recorded for sea turtles (Miller 1997). These observations indicate that the length of the  
388 Mozambique Channel is not a biological barrier during the migration of adult turtles. As  
389 highlighted by Pelletier *et al.* (2003) we suggest that the unique and unusual oceanography in  
390 the Mozambique Channel may contribute to the green turtle population structure observed in  
391 the Mozambique Channel, influencing particularly the early stages in the life cycle of green  
392 turtles.

393

394

#### 395 *Oceanography in the Mozambique Channel*

396

397 At the seabird nesting islands in the Mozambique Channel, studies have shown that subspecies  
398 of *Phaethon lepturus* (Le Corre & Jouventin 1999), *Puffinus lherminieri* (Le Corre 2000b) and  
399 *Sula sula* (Le Corre 1999), nesting in Europa (South Mozambique Channel), have phenotypic  
400 patterns that differ from the equivalent species nesting in other islands of the Indian Ocean. Le  
401 Corre (1999; 2000a,b) suggested that few successful exchanges of individuals occur between  
402 the North and South Mozambique Channel and that Europa seabird populations are isolated  
403 from the other nesting colonies of the Indian Ocean. This biogeographic pattern may be linked  
404 to oceanic conditions in the Mozambique Channel particularly at the south end where there is a  
405 peculiar pattern of sea-surface temperatures (Le Corre 2000b).

406

407 Several authors have already emphasized the unusual oceanic conditions that occur in the  
408 southern Mozambique Channel, where there is an increase of sea-surface temperature (Piton *et*  
409 *al* 1981), the occurrence of meanders (Ludjeharms *et al* 1981; Donguy & Piton 1991) and a  
410 convergence zone between different currents (Piton & Magnier 1976; Piton & Laroche 1993).  
411 Recent studies in the Mozambique Channel showed that the average drift in the southern part is  
412 a dynamic area swept by an intermittent train of large anticyclonic eddies (~200 km in  
413 diameter) leading to a southward transport along the African coast (De Ruijter *et al.* 2002;  
414 Schouten *et al.* 2003; Quartly & Srokosz 2004; Lutjeharms *et al.* 2000; Figure 1B). These  
415 currents are likely to play a role in hatchling dispersal as they spend the first few years of their  
416 life in oceanic waters (Carr 1987). Hatchlings emerging from nests south of the Mozambique  
417 Channel should drift southward. On the western side of the Mozambique Channel, oceanic  
418 movement consists of strong anti-clockwise eddies (De Ruijter *et al.* 2002), whereas on the  
419 eastern side the flow is weak and variable. In the northern part of the Mozambique Channel, the  
420 flow seems to be quite variable, but on average may consist of an anti-clockwise gyre in the  
421 Comoro Basin (Lutjeharms 2005). The South Equatorial Current carries water westward in  
422 North of the Comoros, but part of this will go south into the Mozambique Channel, part  
423 northward as the East African Coastal Current (Figure 1B; Schouten *et al.* 2003). As Girard *et*  
424 *al.* (2005) have showed that currents around Europa act as a constraint for adult green turtles,  
425 one theory would be that juveniles from the NMC do move part northward and part southward,  
426 but are mostly retained in this way in the intense western Mozambique Channel eddies. This  
427 would mean that they would only occasionally pass close to Juan de Nova and on the whole  
428 would not reach Europa Island. A test for this theory would come from the haplotypes found at  
429 the Mozambique and south west Madagascar coasts: if these have Indo-Pacific genetic  
430 characteristics, the unusual characteristics at Europa Island would be a localised exception.

431  
432 Those oceanic elements may contribute to the green turtle genetic structuring in the  
433 Mozambique Channel, slowing down the exchanges between these two opposite zones. Further  
434 studies are needed to fully elucidate the genetic structure of green turtles nesting along the  
435 Mozambique Channel and to distinguish the relative importance of ongoing oceanographic  
436 processes from historical patterns of colonization. An expanded study incorporating rookeries  
437 from the East African coast, and eastern and southwestern coasts of Madagascar will help us to  
438 better understand the mechanisms responsible for structuring among NMC-Tromelin and the  
439 SMC green turtle populations. Of particular interest would be the relationships between genetic  
440 characteristics of the nesting green turtles, oceanography and seasonality of nesting. For  
441 instance, do nesting green turtles in Mozambique coast, at the same latitude of Europa  
442 (22°21'S), have the same mtDNA genetic structure as those nesting at Europa?

443  
444  
445 *Green turtle Management Units*

446  
447 Several rookeries of the South West Indian Ocean are important nesting sites for green turtles  
448 (Frazier 1984; Mortimer 1984; Le Gall 1988; Mortimer 1988; Van Buskirk & Crowder 1994;  
449 Mortimer & Day. 1999). Genetic analysis of sea turtle population structure can provide an  
450 essential management tool to identify genetically distinct Management Units (MUs) within a  
451 region (Dizon *et al.* 1992; Moritz 1994). Our genetic data suggest that rookeries of green turtles  
452 in Europa, Juan de Nova and the NMC-Tromelin belong to three separate genetic populations  
453 and should be considered as independent MUs. Our inability to differentiate Tromelin from  
454 other NMC rookeries most likely reflects the limitations of a single locus marker and a recent  
455 shared history rather than ongoing gene flow.

456  
457 The genetic markers we have characterised for each Management Unit are suitable for  
458 assessing stock composition in regional harvested and resident populations of green turtle. The  
459 assessment of multiple harvests and feeding assemblages throughout this region will help to  
460 define the geographic extent of migration and threatening processes that impact on green turtle  
461 populations. The delineation of Management Areas for each Management Unit relies on a  
462 combination of tag returns, satellite tracking and genetic analysis of foraging and harvested  
463 populations all of which are currently being evaluated for this region.

464  
465  
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769  
770

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785  
786

## 787 **Figure Legends:**

788  
789 **Figure 1: A.** Geographical locations of the ten green turtle nesting sites sampled in the South  
790 West Indian Ocean. The pie-chart shows the frequencies of the haplotypes per nesting site. **B.**  
791 Main oceanic movements in the South West Indian Ocean and nesting green turtle population  
792 boundaries inferred from mtDNA data. The following abbreviations were used; SEC: South  
793 Equatorial Current; SEMC: South East Madagascar Current; EACC: East African Coastal  
794 Current; AC: Agulhas Current; CB: Comoro Basin. The numbers (1, 2, 3, and 4) in red show  
795 the different nesting green turtle genetic stocks proposed in this study.

796

797 **Figure 2:** Neighbour-joining tree based on the mtDNA control region sequences. Bootstrap  
798 values (500 replicates) are indicated on the branches. Three clades of haplotypes were  
799 identified, called respectively 1, 2 and 3. Haplotype Cm8 is nested in the Atlantic Ocean clade  
800 B of Encalada *et al.* (1996). Haplotypes A1 & A2 and haplotypes C3 & D2 are nested in the  
801 Indo-Pacific Ocean clades V and I respectively of Dethmers *et al.* (submitted).

802  
803 **Figure 3:** Regression of genetic distances,  $F_{st}/(1-F_{st})$ , versus geographic distances (km) in the  
804 ten green turtle nesting sites sampled for mitochondrial DNA data. Regressions were performed  
805 with (x) and without (o) Europa and Juan de Nova.

806 **Tables and Figures:**

807  
808 **Table 1:** Mitochondrial DNA variants detected among green turtle population nesting in ten  
809 different sites in the South West Indian Ocean. Haplotype ( $h$ ) and nucleotide diversity ( $\pi$ ) for  
810 the ten populations in the North Mozambique Channel (NMC) and South Mozambique Channel  
811 (SMC).  
812

		Date of sampling	CM8	C3	May23	D2	Glo33	A1	A2	total	Haplotype diversity ( $h$ )	Nucleotide diversity ( $\pi$ )
SMC	Europa	1997/2003 1999	31	2						33	0.1174	0.0076
	Juan de Nova		11	8					1	20	0.5632	0.0360
	Total SMC		42	10					1	53	0.3425	0.0221
NMC	Nosy Iranja	2004		13						13	0	0
	Mayotte	2004	5	30	2			1	3	41	0.4524	0.0231
	Mohéli	2004	1	27	2	1		1	2	34	0.3708	0.0133
	Glorieuses	2004		31			1		7	39	0.3441	0.0168
	Cosmoledo	1996		24				3	4	31	0.3871	0.0210
	Aldabra	1996		18				1	7	26	0.4646	0.0249
	Farquhar	1996		3				1	3	7	0.7143	0.0342
	Total NMC		6	146	4	1	1	7	26	191	0.3964	0.01962
	Tromelin	1997		38					6	44	0.2410	0.0132
	Total		48	194	4	1	1	7	33	288	0.5063	0.0289

813

814 **Table 2:** Polymorphic sites corresponding to the 7 green turtle haplotypes detected in the South West Indian Ocean from a 396bp  
 815 fragment of mtDNA control region sequence.  
 816

<b>Base positions</b>	32	45	71	82	87	88	89	92	93	95	108	109	110	111	112	135	136	146	147	149
<b>Haplotypes</b>																				
Glo33	T	C	A	G	T	A	C	T	C	G	A	A	T	A	C	G	G	C	T	T
May23	T	C	A	A	T	A	C	T	T	G	A	A	G	A	C	G	G	C	T	T
D2	T	C	A	G	T	A	C	T	T	G	A	A	G	A	C	G	G	C	T	T
CM8	T	C	G	A	T	G	C	C	T	G	A	A	G	C	T	A	A	C	C	C
A2	C	C	A	A	C	G	T	T	T	A	G	G	A	A	C	G	A	C	C	C
A1	C	-	A	A	C	G	T	T	T	A	A	G	A	A	C	A	A	T	C	C
C3	T	C	A	G	T	A	C	T	T	G	A	A	G	A	C	G	G	C	T	T
<b>Base positions</b>	151	153	155	163	222	226	236	248	290	307	328	329	336	343	344	345	347	353	359	360
<b>Haplotypes</b>																				
Glo33	A	C	A	C	C	A	A	G	A	T	A	T	A	T	G	G	T	A	C	-
May23	A	C	A	C	C	A	A	G	A	T	A	T	A	T	G	G	T	A	C	-
D2	A	C	A	C	C	A	A	G	A	T	A	C	A	T	G	G	T	A	C	-
CM8	G	T	G	T	T	G	C	G	G	C	G	T	A	T	A	A	T	G	T	T
A2	A	T	G	T	T	A	A	A	A	T	A	T	G	C	A	A	T	A	C	-
A1	A	T	G	T	T	A	A	A	A	T	A	T	G	T	A	A	C	A	C	-
C3	A	C	A	C	C	A	A	G	A	T	A	T	A	T	G	G	T	A	C	-

817

818 **Table 3:** Genetic differentiation ( $F_{st}$ ) between the 10 locations sampled in the South West Indian Ocean (above diagonal) and  
 819 estimation of the number of migrant per generation ( $Nm$ ; below diagonal). The significance of permutation test (10 000 permutations)  
 820 are shown for  $P < 0.05$  (\*) and  $P < 0.001$  (\*\*\*)  
 821

<i>Nm</i>	<i>Fst</i>	Europa	Juan de Nova	Nosy Iranja	Mayotte	Mohéli	Glorieuses	Cosmoledo	Aldabra	Farquhar	Tromelin
Europa			0.3030 *	0.9113 ***	0.6465 ***	0.7343 ***	0.7497 ***	0.7125 ***	0.7388 ***	0.7368 ***	0.8031***
Juan de Nova	1.22			0.5831 ***	0.3151 ***	0.4160 ***	0.4502 ***	0.5280***	0.3757 ***	0.4189 ***	0.5280***
Nosy Iranja	0.03	0.19			0.0793	0.0406	0.0842	0.1742	0.078	0.5011*	0.0466
Mayotte	0.13	0.49	4.46			-0.0106	-0.017	0.0304	0.004	0.1473*	0.0326
Mohéli	0.09	0.32	14.97	∞			-0.0023	0.0374	-0.0111	0.2027*	0.0023
Glorieuses	0.08	0.27	5.07	14.97	∞			0.0035	-0.0112	0.1604	-0.0118
Cosmoledo	0.1	0.39	1.52	7.03	6.43	70.41			-0.0001	0.0124	0.0425
Aldabra	0.09	0.32	4.6	89.66	∞	∞	∞			0.1317	0.0014
Farquhar	0.09	0.54	0.34	1.39	0.98	1.09	19.98	1.65			0.2911*
Tromelin	0.06	0.2	10.17	7.12	106.43	∞	5.63	173.86	0.61		

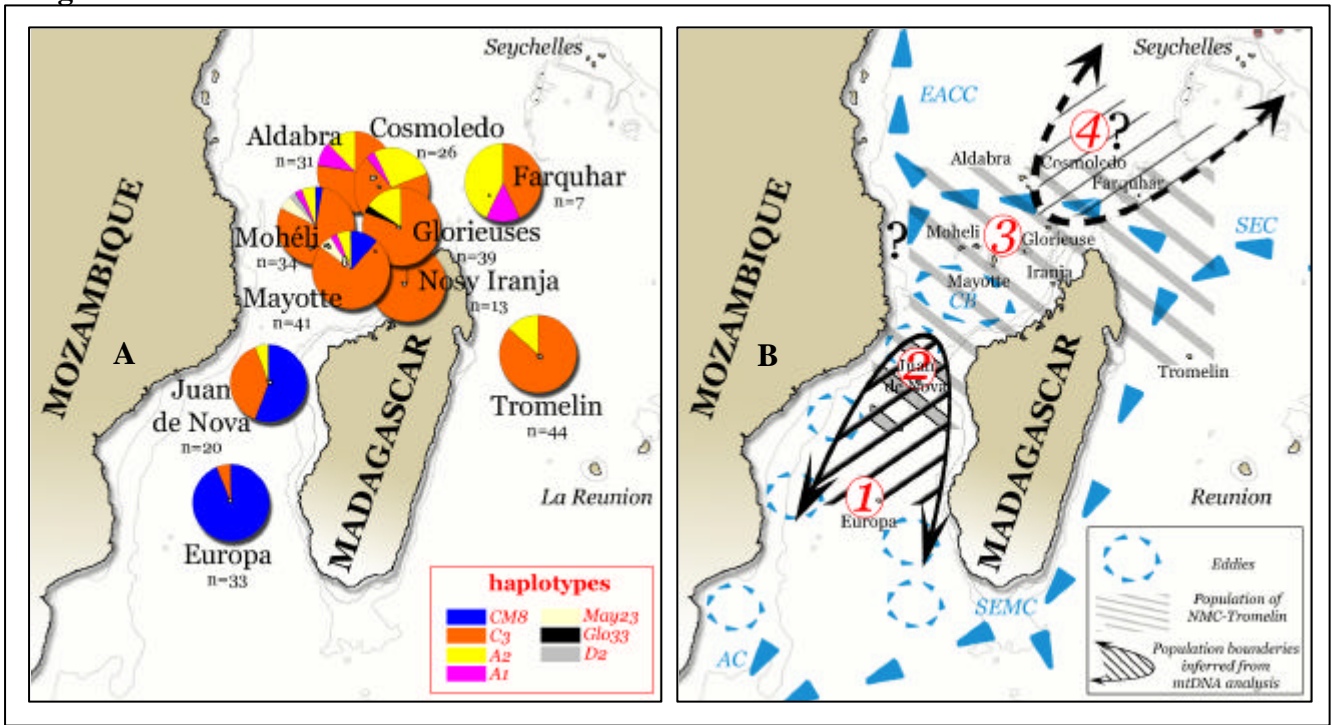
822

823 **Table 4:** Analysis of variance (AMOVA) results for the South West Indian Ocean groups of  
 824 green turtle nesting sites. AG is the among-groups component variance; AP/WG is the among-  
 825 populations/within-group component of variance; WP is the within-population component of  
 826 variance. The significance of permutation test (10 000 permutations) are shown for  $P < 0.05$  (\*),  
 827  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*).  
 828

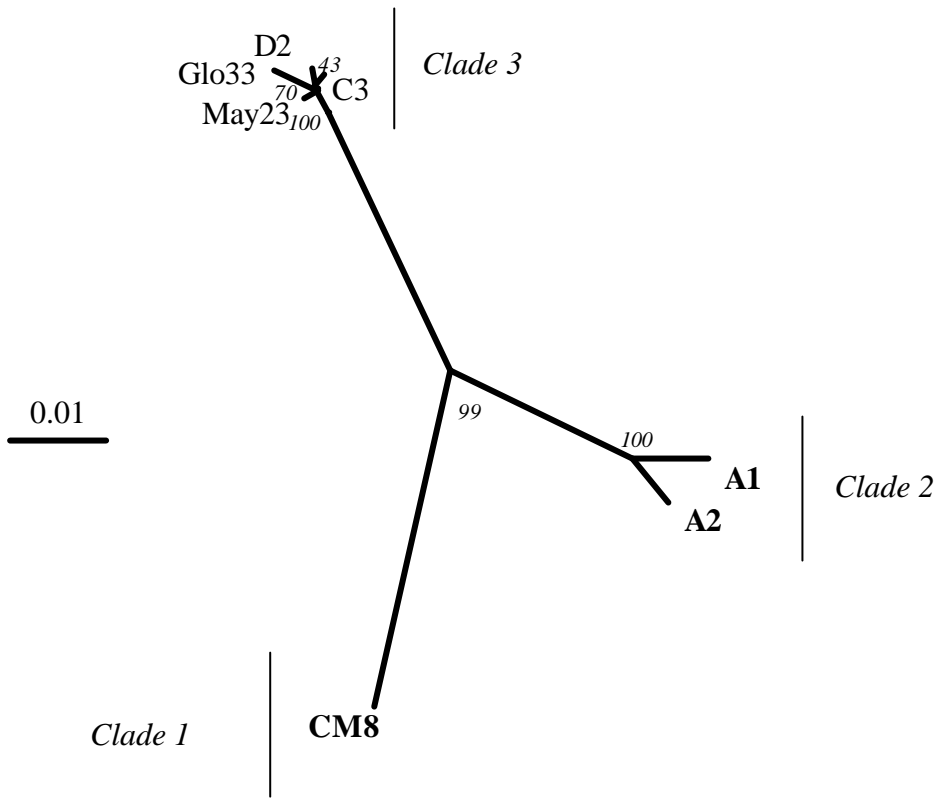
Name	Grouping scheme	Variance component	% of variance	F - statistics	
GP1	Group 1	Europa - Juan de nova	AG	55.84	F <sub>CT</sub> = 0.55835*
	Group 2	Other islands	AP/WG	2.9	F <sub>SC</sub> = 0.06562*
			WP	41.27	F <sub>ST</sub> = 0.58733***
GP2	Group 1	Europa - Juan de nova	AG	53.96	F <sub>CT</sub> = 0.53959*
	Group 2	Farquhar	AP/WG	2.43	F <sub>SC</sub> = 0.05272*
	Group 3	Other islands	WP	43.61	F <sub>ST</sub> = 0.56388***
GP3	Group 1	Europa	AG	57.18	F <sub>CT</sub> = 0.57178*
	Group 2	Juan de nova	AP/WG	1.46	F <sub>SC</sub> = 0.03413*
	Group 3	Other islands	WP	41.36	F <sub>ST</sub> = 0.58640***
GP4	Group 1	Europa	AG	55.65	F <sub>CT</sub> = 0.55653**
	Group 2	Juan de nova	AP/WG	0.76	F <sub>SC</sub> = 0.01720
	Group 3	Other islands	WP	43.58	F <sub>ST</sub> = 0.56416***
	Group 4	Farquhar			

829

830 **Figure 1**



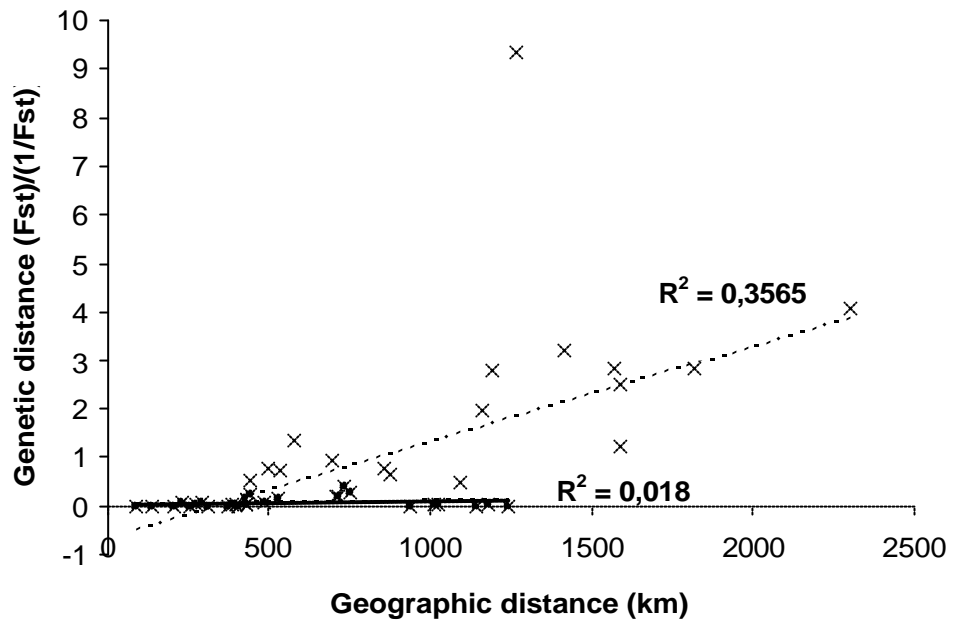
851 **Figure 2**  
852





853 **Figure 3**

855



× All data • without Europa and Juan de Nova