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ABSTRACT

Delphinid populations show highly variable patterns of genetic diversity and population structure. Previous studies indicate that habitat discontinuities and geographic isolation are major drivers of population division in cetaceans. Spinner dolphins (*Stenella longirostris*) are distributed in all tropical oceans, but they are particularly common around islands and atolls. This species occurs in shallow waters at daytime to rest and socialise, and feeds on offshore mesopelagic prey overnight. Here we investigated the genetic population structure of spinner dolphins in the Southwest (SW) Indian Ocean along a west-east geographical gradient, from eastern Africa to the Mascarene archipelago. We combined analyses of 12 microsatellite loci, mtDNA control region sequences, and sighting data to assess genetic differentiation and characterise habitat preferences of these populations. Significant genetic structure among the three sampled sites (Zanzibar, Mayotte and La Réunion) was observed using both types of molecular markers. Overall, our results indicate that geographic isolation, and potentially other factors such as shallow water habitats to rest and socialise, may be important drivers of the genetic population structure of insular spinner dolphins in this region.

Keywords: spinner dolphin, *Stenella longirostris*, genetic structure, microsatellites, mtDNA, East Africa.

INTRODUCTION

Understanding factors influencing population connectivity has been a central and long-standing research avenue in marine ecology (e.g. Cowen et al. 2000; 2007; Selkoe et al 2008). Past studies have shown that the genetic structure of marine populations is driven by a number of processes, including water currents, sea floor topography, water temperature and life history (e.g., Fullard et al. 2000; Fontaine et al. 2007; Pelc et al. 2009; Ciannelli et al. 2010; Mendez et al. 2011; 2013). In cetaceans, factors that can lead to discontinuous relationships between genetic and geographic distance include habitat characteristics, intra-species niche partitioning (e.g. foraging specialization) and kinship, combined with demographic processes (e.g., Hoelzel 2009; Möller et al. 2007, 2011, Louis et al. 2014a,b; Viricel and Rosel 2014).

Beside its fundamental importance in marine ecology, understanding the spatial structure and genetic connectivity of marine populations is also critical for conservation and management purposes. Indeed, while no marine ecosystem is completely unaffected by human activities, threats to populations of marine organisms vary geographically (Halpern et al. 2008). In the Southwest (SW) Indian Ocean for instance, the importance of marine mammal bycatch is spatially variable and it seems to primarily affect inshore species, including coastal delphinids (Kiszka et al 2008). Thus, delimiting biologically meaningful conservation units (i.e., based on population structure assessments) will be a crucial step toward preserving the marine megafauna of this region.

The spinner dolphin (*Stenella longirostris*) is one of the most abundant and widely distributed tropical delphinids (Perrin 2009). Four subspecies are currently recognised, based on morphological and ecological differences (Perrin and Gilpatrick 1994; Perrin et al. 1999). The Gray's spinner dolphin (*S. longirostris longirostris*), hereafter the spinner dolphin, is primarily an insular subspecies, and its distribution includes the Atlantic, Indian and Pacific Oceans (Perrin and Gilpatrick 1994). In French Polynesia, Hawaii and the Maldives, spinner dolphins enter atolls, sheltered bays and lagoons through reef channels in the morning and leave in the afternoon to feed offshore overnight (Würsig et al. 1994; Anderson 2005; Gannier and Petiau 2006), essentially on mesopelagic prey (Perrin et al. 1973; Dolar et al. 2003). Around the lagoon of Mayotte, in the Mozambique Channel (SW Indian Ocean), spinner dolphins primarily inhabit the outer slope of the barrier reef to rest and socialise, and rarely enter the lagoon (Kiszka et al. 2010a, 2011). During the past 20 years, extensive work has been conducted on the movements, behaviour, social, and genetic population structure of insular spinner dolphins, particularly in the Pacific Ocean (Norris et al. 1994; Karczmarski et al. 2005; Oremus et al. 2007; Andrews et al. 2010). These studies highlight that spinner dolphins may form "fission-fusion" societies, with groups forming and separating over short periods of times, such as around the big island of Hawaii (Karczmarski et al. 2005; Andrews et al. 2010). However, social structure may vary according to habitat characteristics and geographical isolation. Indeed, at the remote Midway atoll (Hawaii), spinner dolphins form stable groups with high level of site fidelity, limited emigration/immigration and strong inter-individual associations (Karczmarski et al. 2005). In this region, gene flow is more restricted among populations showing a fluid social structure (the Kona Coast of the island of Hawaii) than among populations with stable social groups (Midway and Kure Atolls) (Andrews et al. 2010).

In the southwestern Indian Ocean, the spinner dolphin is one of the most common small cetacean species in tropical and subtropical waters, particularly around islands and reef complexes off Zanzibar (Amir et al. 2002), Mayotte (Kiszka et al. 2010a, 2011) and the Comoros archipelago (Kiszka et al. 2010c), La Réunion (Dulau-Drouot et al. 2008, Condet and Dulau-Drouot, 2016), Madagascar (Rosenbaum 2003) and Mauritius (Webster et al. 2015). Spinner dolphins (particularly the *S. l. longirostris* form) are rarely observed in open ocean waters (> 2,000 m), but can occur for short periods of time between islands (particularly in island chains), mostly when undertaking overnight foraging trips (e.g. Kiszka et al. 2011, Mannocci et al. 2013, Thorne et al. 2012). As this species occurs in coastal and reef-associated waters, spinner dolphins are impacted by human activities, including past hunting and bycatch off Zanzibar (Stensland et al. 1998, Amir et al. 2012), direct hunting and bycatch in south-western Madagascar (Razafindrakoto et al. 2008) and disturbance from dolphin-watching activities such as on the west coast of Mauritius (Webster et al. 2015). However, the geographical extent of the influence of such direct and indirect effects on populations is unknown.

This study aims to characterise genetic diversity and population structure of spinner dolphins in the SW Indian Ocean, particularly from Zanzibar (Tanzania), Mayotte (Comoros archipelago) and La Réunion (Mascarene archipelago) (Fig. 1). These islands were selected because they are located along a west-east gradient, from continental waters of Africa (Zanzibar) to the most isolated and remote oceanic islands (La Réunion). We also characterise depth preferences of spinner dolphins at these sites to evaluate their reliance on shallow-water habitat and to estimate habitat size around the two oceanic islands. Resting habitat availability has been suggested to influence population size and dispersal in other island-associated spinner dolphin populations (Andrews et al. 2010). We evaluate the relationship between our estimations of genetic diversity

and habitat size: islands with more suitable habitats are expected to sustain greater population sizes, which would maintain greater genetic diversity. Geographic distances separating the coasts of the three sites are greater than 900 km. Considering previous knowledge gathered in the Pacific (Andrews et al. 2006; Oremus et al. 2007; Andrews et al. 2010), we hypothesised that geographic isolation is a major driver of the genetic population structure of spinner dolphins in this region, and that sampled islands should contain genetically distinct populations.

MATERIAL AND METHODS

Sample collection and DNA extraction

In total, tissue samples collected from 69 individual spinner dolphins off Zanzibar (n=21), Mayotte (n=29) and La Réunion (n=19) were available for this study (Fig. 1). For Zanzibar, muscle tissues were collected from bycaught animals in drift gillnets between 2000 and 2004 and were stored frozen at -20°C. Samples from Mayotte and La Réunion were collected during dedicated biopsy surveys undertaken in territorial waters from 2006 to 2011 from small boats. Biopsy attempts were made opportunistically, when groups and individuals were easily approachable and when conditions were optimal (Beaufort < 2, dolphins closely approaching the boat). Optimal weather conditions allowed stability of the research boat and better chances to sample the animals successfully and safely (Kiszka et al. 2010b). Blubber and skin biopsies were collected using a crossbow (BARNETT Veloci-Speed® Class, 68-kg draw weight and BARNETT Panzer V Class, 68-kg draw weight) with Finn Larsen (Ceta-Dart, Copenhagen, Denmark) bolts and tips (dart 20-mm long, 7-mm-diameter). Biopsy samples were preserved individually in 90% ethanol before shipping and subsequent analysis. Biopsy sampling was

conducted under French scientific permit #78/DAF/2004 (September 10, 2004) and permit #032/DAF/SEF/2008 (May 16, 2008) for Mayotte and MC/2009/336 for La Réunion. Genomic DNA was extracted from ~ 25 mg of tissue (muscle or skin) using a Nucleospin Tissue kit (Macherey-Nagel) following the manufacturer's protocol.

Microsatellite genotyping and mitochondrial DNA (mtDNA) sequencing

Twelve microsatellite loci previously optimized for *S. longirostris* were genotyped (Table 1). PCR reactions included ~20 ng of genomic DNA, 0.5 U Taq polymerase, 0.25 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR Buffer, 0.125 μM of each primer in a 20 μL final volume. PCR profiles were as follows: initial 5 min denaturation step at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at a specific annealing temperature (see Table 1), 45 s at 72°C, and by a final 7-min extension step at 72°C. All PCRs were conducted in a Techne TC-5000 thermocycler. PCR products were visualized using polyacrylamide gels on the LICOR 4300 DNA Analyser. Allele sizes were determined by eye using a size standard, and by two different researchers to ensure consistency in scoring.

A portion of the mtDNA control region was amplified using primers Dlp-1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') (Baker et al. 1998) and Dlp-8G (5'-GGAGTACTATGTCCTGTAACCA-3') (Dalebout et al. 2005). PCR reactions included 0.5 U Taq polymerase, 0.25 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR Buffer, 0.125 μM of each primer and ~50 ng of genomic DNA in a 50 μL reaction volume. PCRs were conducted in a Techne TC-5000 thermocycler using the following profile: initial 5 min denaturation step at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C, 45 s at 72°C, and by a final 7-min extension step at

72°C. PCR products were purified and sequenced by Genoscreen (Lille, France). Sequences were edited in Chromas sequence viewer v. 2.33 (Chromas Technelysium) and were aligned using BioEdit v.5.0.6 (Hall 1999).

Microsatellite analyses

We tested for departures from Hardy-Weinberg or linkage equilibrium within each sampled site using Genepop v. 4.2 (Raymond and Rousset 1995) with 10,000 dememorizations, 1,000 batches and 10,000 iterations per batch. The sequential Bonferroni technique (Holm 1979) was applied to correct for multiple tests. The presence of null alleles and scoring errors was assessed using Micro-checker v. 2.2.3 (van Oosterhoot et al. 2004) within each site. We searched for potential duplicates within biopsied animals by comparing their multi-locus genotypes (i.e., searching for identical genotypes in the dataset, and for genotypes with less than three different alleles overall) using Genalex v. 6.41 (Peakall and Smouse 2006). The mitochondrial haplotypes of samples with matching microsatellite genotypes were compared to confirm they were duplicates of the same individual. We investigated whether related individuals were included in the dataset by calculating maximum-likelihood estimates of pairwise relatedness using ML-Relate (Kalinowski et al. 2006). To avoid biases in population inferences that could result from family structure (Anderson and Dunham 2008), we removed one individual from each pair of potential relatives, i.e., individuals showing a pairwise relatedness value greater than 0.45 (as in Viricel and Rosel 2014). Allele richness, observed and expected heterozygosity were calculated using FSTAT v. 2.9.3.2 (Goudet 1995) and Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010), respectively. These molecular diversity indices were calculated for the whole dataset and for each site separately.

213 Population structure was assessed using a Bayesian approach implemented with Structure v. 2.3.4
214 (Pritchard et al. 2000), which infers the number of populations (*K*) present in a dataset based on
215 assumptions of Hardy-Weinberg and linkage equilibria within populations. Analyses were
216 conducted using the admixture and correlated allele frequencies models, with and without prior
217 information on individual location (option "LOCPRIOR"). Including prior information on sample
218 locations can improve population inferences, particularly when the level of population
219 differentiation is weak or recent (Hubisz et al. 2009). To verify that using prior information did
220 not artificially result in distinct clusters, we conducted additional Structure runs with the
221 LOCPRIOR option after randomizing the sample location information in the input file. Three
222 randomized input files were created. All Structure runs included 300,000 Markov chain Monte
223 Carlo iterations and a 50,000 step burn in. Ten replicate runs were performed for *K* values
224 between 1 and 5. Convergence was assessed by examining *alpha* and likelihood plots, and by
225 comparing individual membership probabilities across replicate runs. The best *K* was chosen by
226 comparing mean log probabilities among *K* values, and when $K = 1$ was ruled out, by applying
227 Evanno's method using ΔK (Evanno et al. 2005).
228
229 To assess genotypic variation among individuals and among the three locations, we applied a
230 principal component analysis (PCA) to the microsatellite data using the package adegenet
231 (Jombart 2008) in R v. 3.1.2 (R Core Team, 2015). In the PCA, allele frequencies were scaled
232 using the centring option. An analysis of molecular variance (AMOVA, Excoffier et al. 1992)
233 was conducted in Arlequin v. 3.5.1.2. (Excoffier and Lischer 2010) to estimate genetic
234 differentiation among the three islands. Pairwise F_{ST} estimates were calculated and significance
235 was assessed using 10,000 permutations. We tested for isolation by distance (IBD) by conducting

a Mantel test comparing pairwise genetic distances ($F_{ST}/(1-F_{ST})$, Rousset 1997) with log-transformed (base 10) geographic distances among sampling locations. Geographic distances between population pairs were calculated as the Euclidean distance between the approximate centres of the areas where samples were collected. The Mantel test was performed using IBDWS v. 3.23 (Jensen et al. 2005) with 10,000 randomizations. Finally, we investigated the occurrence of private alleles in each population identified using Genalex v. 6.41.

MtDNA sequence analyses

Diversity indices (haplotype and nucleotide diversities) were calculated for each site using Arlequin. We used JModeltest v. 0.1.1 (Guindon and Gascuel 2003; Posada 2008) and the Akaike Criterion to determine the most appropriate model of substitution given our sequence alignment. AMOVAs comparing the populations identified using Structure were performed in Arlequin. Genetic differentiation was measured using both F_{ST} and Φ_{ST}. For Φ_{ST}, distances between haplotypes were calculated using the model of substitution selected with JModeltest. Significance was assessed using 10,000 permutations. We evaluated IBD as described above for microsatellite data. A median-joining network was constructed using Network v.4.6.1.2 (Bandelt et al. 1999) with default parameters to represent relationships among haplotypes.

Habitat size

In order to relate patterns of genetic structure and availability of habitat used by spinner dolphins, we created spinner dolphin habitat maps based on their depth preferences around Zanzibar, Mayotte and La Réunion. From 2004 to 2008, sighting data were collected around Mayotte (n = 168 sightings in 224 days of survey) and La Réunion (n = 51 sightings in 278 days of survey)

during small-boat dedicated surveys (see Dulau-Drouot et al. 2008; Kiszka et al. 2011 for sampling protocols). For surveys undertaken around Mayotte and La Réunion, the sampling effort did not follow predefined transects and was not homogeneous. However, both surveys covered shallow inshore waters, the outer slope of the reef and deep oceanic habitats (depth > 500 m around Mayotte: Kiszka et al. 2011 and depth > 1,000 m around La Réunion: Dulau-Drouot et al. 2008). For Zanzibar, as sighting data were unavailable, we used geographic locations of bycatch events that were recorded during a bycatch monitoring program coordinated by the Institute for Marine Sciences, University of Dar es Salaam, based on Zanzibar between 2000 and 2007 (n = 27 records). These data were used to determine habitat preferences of spinner dolphins using depth as the main variable. We chose to focus habitat analyses on depth as it was previously identified as one of the main explanatory variables explaining spinner dolphin distribution patterns in this region (Kiszka et al. 2011, Condet & Dulau-Drouot 2016) and in other parts of the world (e.g. Thorne et al. 2012). Depth data were extracted from the GEBCO_2014 Gridded bathymetric dataset (30 arcsecond resolution) hosted on the British Oceanographic Data Center (http://www.bodc.ac.uk). We considered that the preferred habitat corresponded to the depth range where 95% of spinner dolphin observations were made. Thus, we excluded 2.5% of the deepest and 2.5% of the shallowest observations to determine i) the preferred depth range for Mayotte and La Réunion separately (we did not include Zanzibar in this comparison as observations from Zanzibar come from bycatch events and are thus not directly comparable to sighting data from the other two islands), ii) the overall preferred depth range for the three islands together. For the second calculation, group size information available for sightings around Mayotte was taken into account. Our reasoning was that using group size information (when available) better reflects the preferred habitat of these populations (i.e. observing large groups in an area carries more weight than observing a single individual). We

283 computed the total area available within the depth range limits obtained for the three islands
284 together, and mapped these areas for Mayotte and La Réunion. It was not computed for Zanzibar
285 since available habitat within this depth-range is virtually infinite along the East African
286 continental shelf relative to the two islands. The retrieval of individual depths, the computation of
287 projected surfaces and the mapping were all performed with the marmap package v0.9.2 (Pante
288 and Simon-Bouhet 2013) in R v3.1.2.
289
290
291 **RESULTS**
292
293 Microsatellite data
294 Each sample was genotyped at 8 to 12 loci. No significant departure from Hardy-Weinberg or
295 linkage equilibrium was observed (after sequential Bonferroni correction) in the three sampled
296 sites. Furthermore, no scoring errors were identified using Micro-checker. Possible null alleles
297 were detected for loci EV94 and 415/416 in Mayotte; however, since this issue was restricted to
298 one site, we kept these two loci in all analyses. A duplicate sample was identified (100%
299 matching genotypes and haplotype) in La Réunion, where the same individual was biopsied
300 twice. Five pairs of potential relatives were detected using ML-Relate: two pairs in La Réunion,
301 one pair in Zanzibar, and two pairs in Mayotte. Only one individual from each pair was kept in
302 population analyses conducted with both types of markers (mtDNA and microsatellites) to avoid
303 the potential bias of including relatives. The final microsatellite dataset included 63 individuals
304 (Table 2). The number of alleles per locus ranged from six to 25 (Table 1). Observed
305 heterozygosity and mean allele richness were similar among the three sites (Table 2).
306

No population subdivision was found when Structure was run without prior information (Fig. 2a). Conversely, genetic structure was detected when individual location was used as prior information. The most likely number of populations present in the dataset was three based on comparison of the mean log probabilities and ΔK (Fig. 2b), and corresponded to the three sampled sites (Fig. 2c): within each site, all individuals were assigned to the same cluster with high ancestry proportions ($q > 0.8$, mean $q = 0.95$). Randomizing sample locations in the Structure input file and applying the LOCPRIOR option resulted in no structure being detected by the program (most likely K = 1; see Appendix 1).

The first principal component of the PCA separated individuals from Mayotte from the other two locations, while the third principal component showed two separate clusters for individuals from La Réunion and Zanzibar (Appendix 2). Some overlap in the PC space was observed among the three clusters. Together, the first three principal components explained 12.7% of the total genotypic variation. The AMOVA conducted among the three populations revealed relatively weak, but significant genetic differentiation (Overall $F_{ST} = 0.020$ $p < 0.0001$; Table 3). The Mantel test conducted to examine IBD was not significant ($r = 0.21$, $R^2 = 0.04$, $p = 0.51$). Private alleles were detected within each of the three sites (Mayotte: 31; La Reunion: 9; Zanzibar: 11).

MtDNA data

The final control region sequence alignment was 720 bp-long and included 28 unique haplotypes (Genbank accession # XXXXXXXX-XX). The sequence alignment included 42 substitutions and no indels. The model selected using JModeltest was Tamura-Nei (Tamura and Nei 1993) with a gamma correction ($\alpha = 0.726$). Analyses of mitochondrial sequences supported the population

structure detected using microsatellite loci: significant differences in mitochondrial haplotype frequencies (after sequential Bonferroni correction) were observed among all sites using F_{ST} (Overall $F_{ST} = 0.084$ $p < 0.0001$; Table 4). However, none of the pairwise comparisons were significant when distances among haplotypes were incorporated in the AMOVA (i.e., using Φ_{ST}, Table 4). The test for IBD was not significant ($r = 0.96$, $R^2 = 0.93$, $p = 0.17$). There was one shared haplotype between La Réunion and Zanzibar, and three shared haplotypes between Mayotte and Zanzibar. Mayotte and La Réunion had no haplotypes in common (Fig. 3). No obvious phylogeographic structure was observed on the haplotype network.

Habitat size

Comparing the depth distribution data of the two oceanic islands revealed that the preferred depth range of spinner dolphins around La Réunion (16 to 935 m) was larger than the one calculated for Mayotte (5 to 175 m). When all observation data are combined, the locations used by 95% of the individuals sighted around Zanzibar, Mayotte and La Réunion have a depth range of 9 to 162 m. The total surface available around Mayotte and La Réunion within this depth-range (9 to 162 m) was 1,036 and 327 km², respectively (Figure 4). The habitat surface corresponding to spinner dolphins' depth preferences is thus three times larger in Mayotte than in La Réunion.

DISCUSSION

Marine organisms with high dispersal capacities can show weak genetic structure across large geographic distances. For instance, the short-beaked common dolphin (*Delphinus delphis*) and

353 the Portuguese dogfish (*Centroscymnus coelolepis*) each form a single panmictic population
354 across the eastern Atlantic (Veríssimo et al. 2011; Moura et al. 2013). However, even low levels
355 of genetic differentiation can correspond to restricted levels of dispersal in a demographic sense
356 and can be associated with adaptive divergence (e.g., Knutsen et al. 2011; Aykanat et al. 2015),
357 and therefore represent important findings in terms of conservation and management.
358 In highly dispersive marine organisms, incorporating spatial information as prior information in
359 genetic analyses can help reveal genetic differentiation (Selkoe et al. 2008), as illustrated in the
360 present study. Indeed, we did not detect any structure when using a Bayesian approach to detect
361 the number of genetically distinct populations based on microsatellite data alone. Including prior
362 information about sample location in the Bayesian analysis allowed retrieving three populations,
363 corresponding to the distinct islands that were sampled. Our results are in accordance with
364 expectations of Latch et al. (2006) and Hubisz et al. (2009), as the F_{ST} estimates we obtained
365 from analysis of microsatellite data fell right in the range where genetic structure can remain
366 undetected without prior information ($0.01 < F_{ST} < 0.03$). This approach (Structure with
367 LOCPRIOR: Hubisz et al. 2009) does not seem to falsely inflate genetic structure as shown in
368 previous studies (e.g. Christie et al. 2010; Russello et al. 2012; Viricel 2012). Results from the
369 three randomizations we conducted indicate the genetic clusters we observed using prior
370 information are biologically significant (Appendix 1). Furthermore, the Mantel test comparing
371 pairwise genetic and geographic distances was not significant, suggesting IBD did not confound
372 our Structure results. The three distinct populations inferred from Structure and observed in the
373 PCA based on nuclear data were supported by the significant genetic differentiation estimated
374 with mitochondrial DNA sequences using F_{ST}. Possible explanations for the lack of significant
375 differentiation observed using Φ_{ST} are that the observed genetic differentiation is recent and that

not enough time has passed for new mutations to accumulate within populations; or that migration rates among these populations are greater than the mutation rate of the mitochondrial DNA control region. In these cases, incorporating distances among haplotypes in the AMOVA can increase noise in the analysis (Bird et al. 2011), rendering Φ_{ST} less informative than F_{ST}.

The preferred depth range of spinner dolphins we inferred from sighting and bycatch data (i.e., for the three locations analysed together) confirmed that the species is associated with relatively shallow-water habitat at these locations, which corresponds to their resting grounds. The wider depth range observed for the population of La Réunion (also described in Dulau-Drouot et al. 2008) may reflect the very narrow continental shelf of the island, with depth increasing rapidly from the shore, compared to Mayotte. In fact, a recent habitat modelling study showed that despite the wide depth range of spinner dolphin observations aroung La Réunion, most sightings occur between 51 and 63 m of depth, within a "core habitat" also characterised by flat and light-colored seabeds (Condet & Dulau-Drouot 2016). Geographic isolation and the reliance of spinner dolphins on appropriate shallow-water resting habitat during daytime are likely factors causing and/or maintaining divergence between populations occupying Mayotte and La Réunion. Although we cannot tease apart the relative role of each of these two factors, hypotheses can be made based on what has been observed in other small delphinid species. Indeed, large geographic distance from continental waters does not seem to be a sufficient driver to cause restricted gene flow in pelagic dolphins found around oceanic islands as illustrated by population structure studies on Atlantic spotted dolphin (*Stenella frontalis*) and common bottlenose dolphin (*T. truncatus*) populations from the Azores: the pelagic morphotypes within these two species form panmictic populations over large distances, from the Azores to offshore waters of the

399 northwestern Atlantic at least 4500 km away (Quérouil et al. 2007; Viricel & Rosel 2014). On the
400 other hand, some pelagic island-associated delphinids show restricted gene flow across short
401 distances within an archipelago such as common bottlenose dolphin populations around Hawaii
402 (Martien et al. 2012), or the rough-toothed dolphin (*Steno bredanensis*), which displays long-
403 term site fidelity around the Society Islands (French Polynesia), and fine-scale genetic
404 differentiation between two islands only 170 km apart (Oremus et al. 2012). Thus, daily reliance
405 on near-shore, insular habitats may be a predominant driver of population structure in pelagic
406 delphinids, even more so than geographic isolation.
407
408 The genetic subdivision we observed is consistent with other studies conducted on *S. longirostris*
409 in the Pacific Ocean, which indicated that insular populations are generally discrete. Levels of
410 genetic differentiation were similar to those observed among Society (French Polynesia) and
411 Hawaii archipelago (Oremus et al., 2007; Andrews et al. 2010). Spinner dolphins found around
412 islands of the Society Archipelago (French Polynesia) form relatively closed communities
413 showing strong island fidelity (Oremus et al. 2007). Gene flow among these communities is
414 restricted, despite the short geographic distances separating some of these islands (i.e. tens of
415 kilometers). Oremus et al. (2007) suggest that these communities are characterised by a
416 metapopulation dynamics, which would explain the high genetic diversity and large island
417 effective population sizes estimated from their molecular data. In the Hawaiian archipelago, fine-
418 scale genetic structure is also observed, but patterns of gene flow vary according to social
419 structure and habitat availability (Andrews et al. 2010). Overall, both the genetic and social
420 structure of this species seems to be influenced by the availability and extent of resting areas
421 (Karczmarski et al. 2005; Andrews et al. 2010). In Hawaiian populations, genetic differentiation
422 increased with geographic distance among islands. In the present study, the tests for IBD we

conducted for both types of markers were not significant. We have to note, however, that these tests had low statistical power since only three populations were sampled. Thus, future studies analyzing samples from additional islands in the SW Indian Ocean would better allow testing for IBD. Additionally, photo-identification data would complement present findings, as site fidelity may constitute another factor driving population structure in spinner dolphins from the SW Indian Ocean.

The genetic diversity we measured (Table 2) was similar to what has been reported for this subspecies in French Polynesia (Oremus et al. 2007), and was greater than the diversity observed in Hawaii (Andrews et al. 2010). Despite the genetic differentiation of spinner dolphins from La Réunion and Mayotte, the genetic diversity of these two populations is similar to the diversity of spinner dolphins from the coast of Zanzibar. Factors influencing their population genetic diversity include effective population size (linked to drift) and immigration rates. Given the differences in habitat size among the three sites we compared, the respective local population size of the populations occupying these sites may differ. Thus, we hypothesize that the similar levels of genetic diversity we observe in these populations likely reflect low but recurrent gene flow, which may be sufficient to maintain genetic diversity within island communities. Spinner dolphins from the SW Indian Ocean could thus be under a metapopulation dynamics, similarly to what findings from Oremus et al. (2007) indicate for populations in the Society Archipelago. Alternatively, the genetic divergence we measured could be recent, and these populations may have so far retained ancestral polymorphisms.

The present study constitutes the first population structure assessment for the spinner dolphin in the SW Indian Ocean and our findings have important conservation implications. Indeed, the

habitat preferences and patterns of restricted gene flow we identified suggest spinner dolphin populations found off Mayotte and La Réunion are demographically independent from each other. Their differentiation makes them potentially vulnerable if directly impacted by human activities, and spinner dolphins found off these two islands should therefore be treated as two distinct conservation units at the national level. Analysing samples from other islands (e.g. Madagascar, Mauritius) within this region would allow further evaluating the genetic isolation of these populations. Bycatch levels of spinner dolphins in gillnets off Zanzibar (Amir et al. 2002) should be considered as a cause of concern, and this issue highlights the need of a population structure assessment along the east coast of Africa. Protecting important resting habitat is an important step toward the conservation of insular spinner dolphin populations. Dedicated surveys help assessing whether current marine protected areas encompass such habitats (e.g. in la Réunion: Dulau-Drouot et al. 2008) and habitat modelling studies (e.g. Thorne et al. 2012, Condet & Dulau-Drouot 2016) allow identifying key areas where new conservation efforts should be focused.

Acknowledgements

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Compliance with ethical standards

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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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696

Tables

Table 1. Twelve microsatellite loci genotyped in this study. PCR annealing temperature (Ta), reference, number of alleles, observed (*Ho*) and expected (*He*) heterozygosity are given for each locus.

Locus	Ta (°C)	Reference	Number of alleles	*Ho*	*He*
415/416	45	Amos et al. 1993	12	0.772	0.889
GT575	60	Bérubé et al. 2000	10	0.679	0.778
GT6	60	Caldwell et al. 2002	6	0.635	0.676
AAT44	55	Caldwell et al. 2002	10	0.770	0.710
KWM12a	46	Hoelzel et al. 1998	10	0.889	0.844
MK5	50	Krützen et al. 2001	13	0.786	0.912
MK6	50	Krützen et al. 2001	18	0.879	0.931
GATA98	54	Palsbøll et al. 1997	9	0.655	0.804
PPHO142	50	Rosel et al. 1999	9	0.804	0.810
PPHO131	57	Rosel et al. 1999	12	0.755	0.834
EV1	47	Valsecchi and Amos 1996	16	0.868	0.902
EV94	54	Valsecchi and Amos 1996	25	0.772	0.925

Table 2. Mitochondrial DNA (mtDNA) and microsatellite diversity indices for *Stenella longirostris* from each site: N, sample size; No. haplo, number of haplotypes; π, nucleotide diversity; *h*, haplotype diversity; AR, allele richness; H_o, observed heterozygosity; H_e, expected heterozygosity

	mtDNA				Microsatellites			
	N	No. haplo	π	*h*	N	Mean AR	*Ho*	*He*
Zanzibar	20	9	0.013	0.826	20	7.1	0.747	0.790
Mayotte	19	14	0.015	0.965	27	8.2	0.773	0.835
La Réunion	16	9	0.012	0.900	16	7.5	0.798	0.818

706 Table 3. AMOVA results obtained from analysis of microsatellite data from 63 *S. longirostris*
707 individuals. Pairwise F_{ST} values are shown below diagonal and corresponding p-values
708 above diagonal.
709

	Zanzibar	Mayotte	La Réunion
Zanzibar	NA	<0.001*	<0.001*
Mayotte	0.024	NA	0.009*
La Réunion	0.025	0.013	NA

710 *significant p-value after sequential Bonferroni correction
711
712 Table 4. AMOVA results for mitochondrial DNA sequences. Pairwise F_{ST} and Φ_{ST} values are
713 shown below and above diagonal, respectively.
714

	Zanzibar	Mayotte	La Réunion
Zanzibar	NA	0.050	0.014
Mayotte	0.058*	NA	0.066
La Réunion	0.130*	0.067*	NA

715 *significant p-value after sequential Bonferroni correction
716
717

718 **Figure Legends**

719 **Figure 1.** Study area and sample locations. Sample sizes are indicated for each site. The 200 m
720 and 1,000 m isobaths are represented by darker lines.

721 **Figure 2.** Bayesian clustering analysis (Structure) results obtained from analysis of 12
722 microsatellite loci (a) without any prior information and (b) using prior information about
723 sample location ("LOCPRIOR" option). The mean log probability (LnP(*K*)) is given for each
724 *K* tested and the Δ*K* from Evanno's method is shown between successive *K* values. (c) The
725 barplot represents individual ancestry proportions for the three populations obtained using
726 the "LOCPRIOR" option.

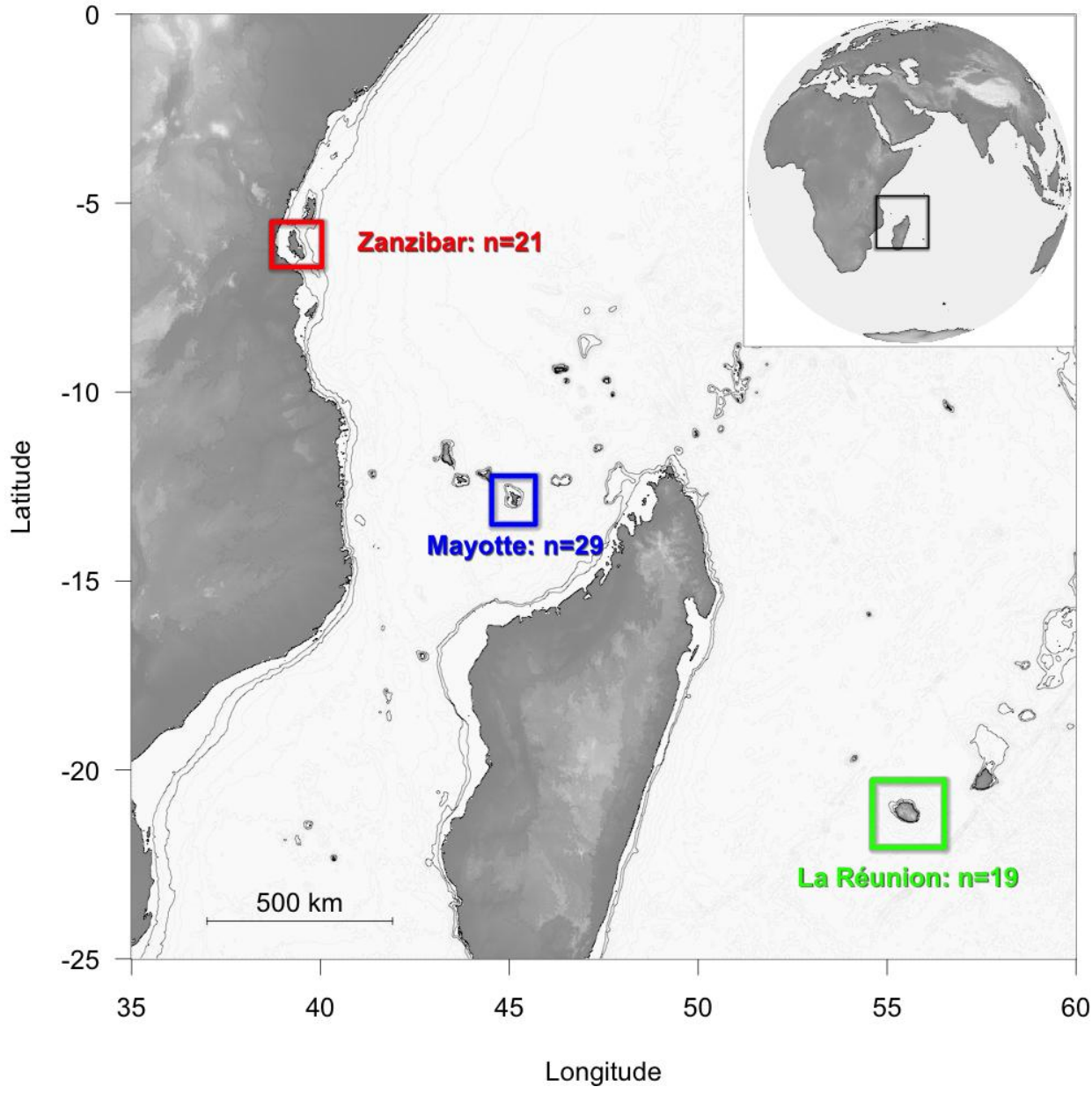
727 **Figure 3.** Median-joining network of 28 mitochondrial control region haplotypes observed in 63
728 *S. longirostris* individuals. Filled circles represent haplotypes and their size is proportional to
729 their frequency in the dataset. Circles are shaded in colours proportionally to the number of
730 individuals from each population (Mayotte: blue; La Reunion: green; Zanzibar: red).
731 Unsampled or extinct intermediate haplotypes are shown as black dots. Each line
732 corresponds to one mutational step, except when a number of mutations is adjacent to it.

733 **Figure 4.** Maps showing the surface available for daytime resting for spinner dolphins around
734 Mayotte (top) and La Réunion (bottom). These surfaces were estimated based on the
735 preferred depth-range of spinner dolphins in the SW Indian Ocean. Maps include all
736 sightings, including outlier observations that were not used to determine the preferred depth
737 range (see Material and Methods).

738

739 **Figures**

740 Figure 1.



741

742 Figure 2

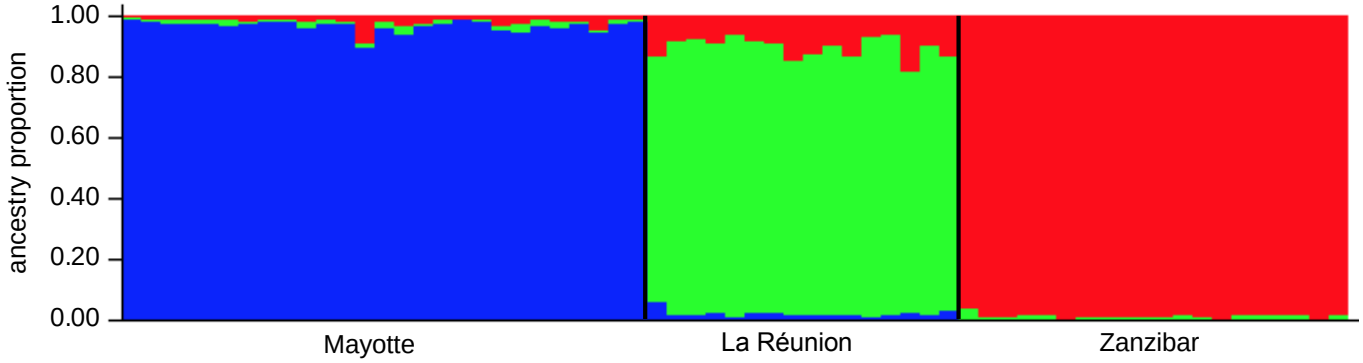
a)

K	LnP(*K*)	Δ*K*
1	**-2885**	**na**
2	-3579	3.4
3	-3078	2.2
4	-3007	5.7
5	-3086	na

b)

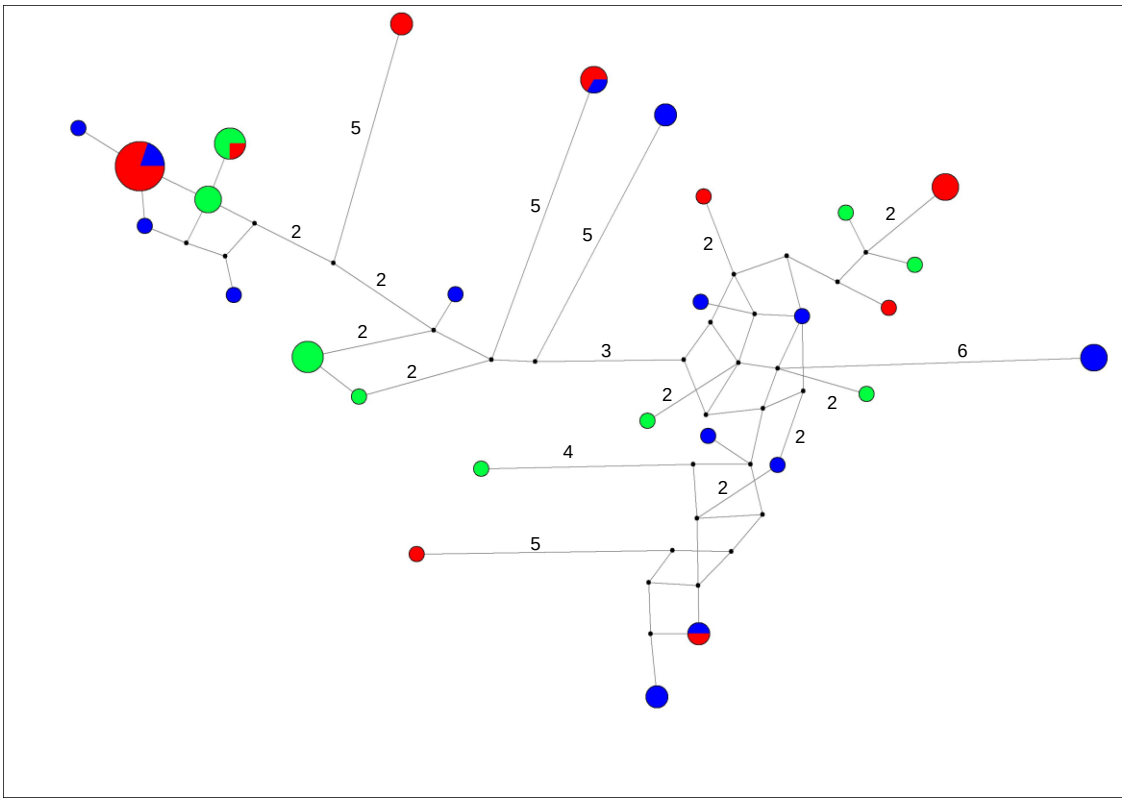
K	LnP(*K*)	Δ*K*
1	-2885	na
2	-2856	3.0
3	**-2808**	**19.4**
4	-2841	0.2
5	-2877	na

c)



743

744 Figure 3



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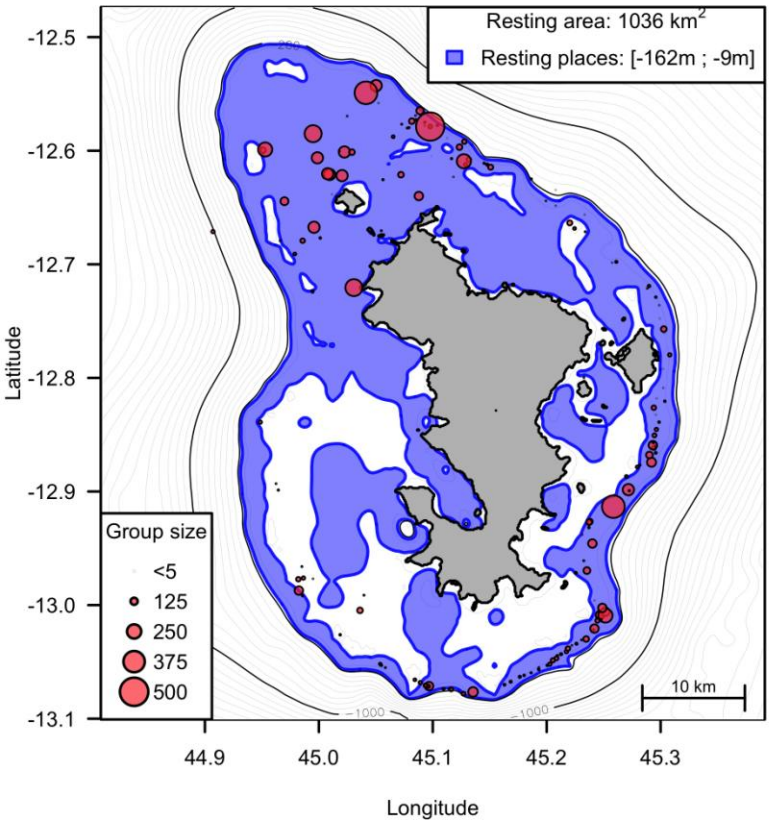
746 Figure 4



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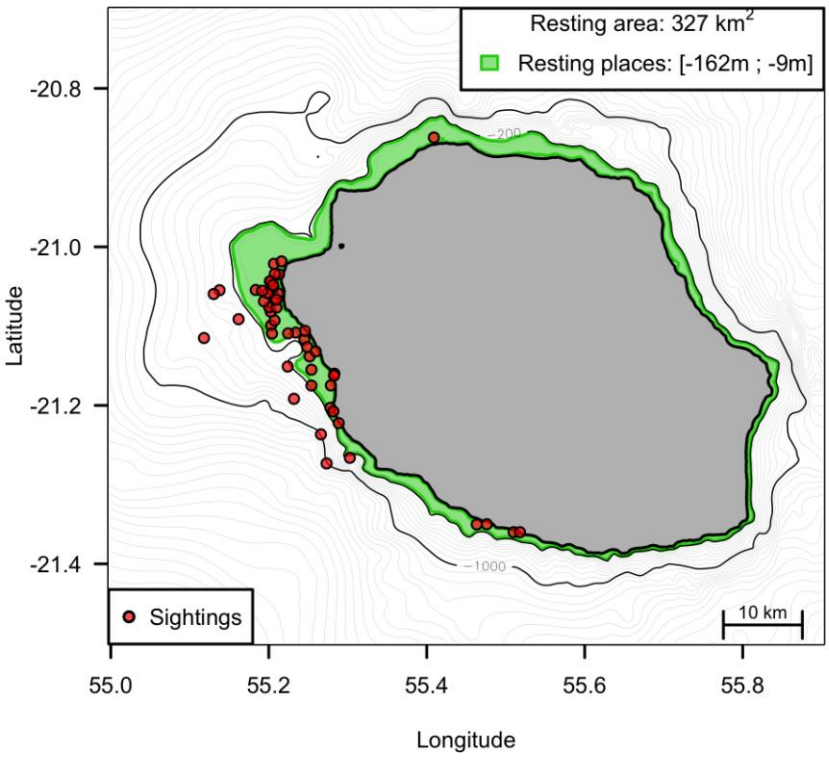

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