Investigating the origin of vagrant dusky groupers, *Epinephelus marginatus* (Lowe, 1834), in coastal waters of Réunion Island

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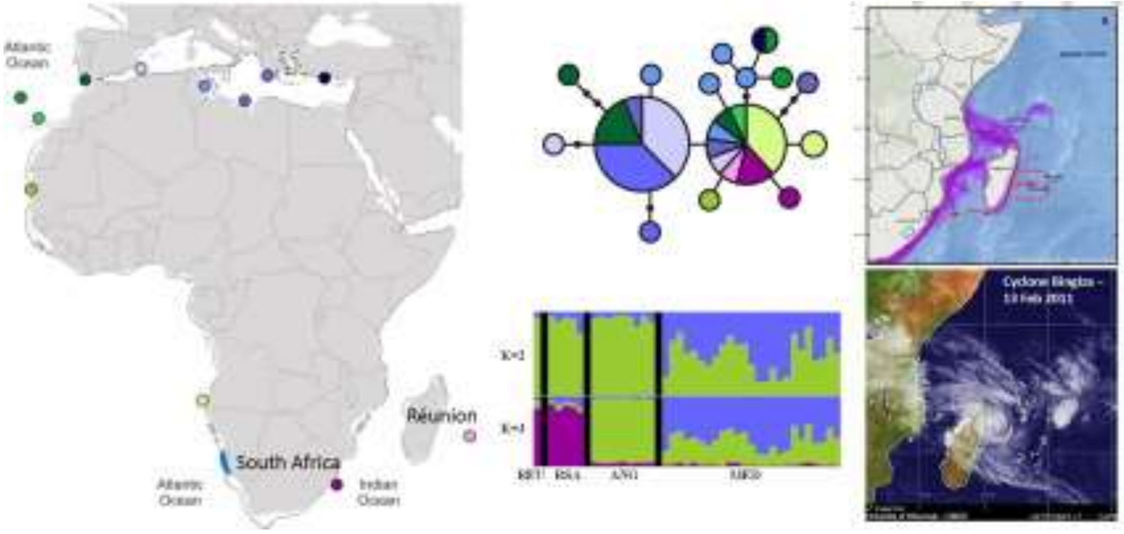
Running title: Long distance dispersal of *E. marginatus*

Highlights

- Sixteen month old Epinephelus marginatus specimen was collected in Réunion, 2500 km from the nearest known population.
- Genetic analyses clustered the specimen with South Africa.
- Hydrodynamic modeling indicated no possible direct connection between South Africa and Réunion during spawning months.
- Long-distance dispersal to Réunion was likely mediated by a cyclone in February 2011, coinciding with hatching.

Graphical abstract



Abstract Due to their geographic isolation, biotas of oceanic islands are likely influenced by episodic long distance dispersal events, but such observations are scarce. In June 2012, fishermen from Réunion Island caught an unknown specimen of grouper, identified as dusky grouper *Epinephelus marginatus* (Lowe, 1834). This was highly unexpected considering the large distance of its closest verified occurrence (South Africa, ~2500 km). To identify the origin of this specimen and the mechanisms driving this potential long distance colonization, we combined genetic analyses and hydrodynamic connectivity modeling approaches. Molecular markers and samples from various locations across the distribution range resulted in the identification of three putative source populations. The Réunion specimen clustered genetically with South Africa. The estimated spawning period in relation to the connectivity modeling of larvae showed no possible direct connection between South Africa and Réunion. However, connectivity was predicted through intermediate stepping stone populations likely located around the southern tip of Madagascar, where the occurrence of the species has yet to be verified. The results further highlight the potential role of the cyclone Bingiza (February 2011) in the connection between Madagascar and Réunion. This shows that cyclones may be an important driver in long distance colonization of oceanic islands.

Keywords: colonization, connectivity, cyclones, microsatellites, mtDNA, range expansion

1. Introduction

Understanding cases of colonization of isolated volcanic islands through long distance dispersal provides insight into founding events and diversification that are fundamental processes underlying oceanic island endemism (Cowie & Holland, 2008; Parent *et al*., 2008). These oceanic islands are often distant from large land masses and the chance of colonization and subsequent divergence of populations is dependent on several biological and physical factors. These factors include the dispersal ability of the organisms, the size and geographic distance of the source population, the number of founders and the frequency of founding events, as well as the availability of suitable habitat post dispersal. How these factors interact leads to the high endemism observed on volcanic islands (Lees & Gilroy, 2013).

Oceanic dispersal potential is often governed by the life history traits of larvae (e.g. duration of larval life), suitable habitat and the features of the transient habitats (e.g. ocean currents; Gosline, 1955; Cowen *et al*., 2000; White *et al*., 2010). This is particularly important in some bentho-pelagic species where the adults are highly sedentary. Oceanographic features such as ocean currents, upwelling zones and eddies maintain or prevent connectivity over periods of time (Cowen *et al*., 2000). However, long distance sporadic dispersal is also possible through various mechanisms in the marine environment. These occur during environmental disturbances, such as cyclones (McKinnon *et al*., 2003) or ENSO events which affect oceanographic features (Hoareau *et al*., 2012) and could lead to dispersal events for species with long pelagic larval duration. In the case of cyclones, they are known to disrupt marine ecosystems and have a major effect on shelf-water where most of the bentho-pelagic species spawn. Such events result in larval displacement and together with associated wind-stress currents, cyclones can transport pelagic larvae to new environments (McKinnon *et al*., 2003). Very few of these events have been described in the literature, but the mechanism is probably important for colonization of remote islands.

Epinephelus marginatus (Lowe, 1834; Serranidae) is a protogynous hermaphrodite species (Fennessy, 2006) distributed in disjunct habitats throughout the Mediterranean Sea, the Atlantic Ocean (South America, Northeast Atlantic and Angola) and limited ranges from South Africa to southern Mozambique and in Oman (Heemstra & Heemstra, 2004). This species has a pelagic larval duration of approximately 20-30 days (Spedicato *et al*., 1998; Macpherson & Raventos, 2006) which allows dispersal through ocean currents before recruitment (Maggio *et al*., 2006). The adults are considered sedentary and aggregate to spawn but are not known to undertake long spawning migrations. This indicates that larval movement is the most obvious dispersal mechanism in this species as observed in other marine species with a bentho-pelagic life cycle (Kinlan *et al*., 2005).

The Western Indian Ocean (WIO) region has a number of oceanographic features which may enhance or restrict movement of larvae. The region west of Madagascar is strongly influenced by the southward flowing Agulhas Current and a number of eddies forming annually within the Mozambique Channel (Schouten *et al*., 2003). The region east of Madagascar is mainly influenced by the South Equatorial Current flowing east to west and that splits into the East Madagascar Current and the Mozambique Current (Tomczak & Godfrey, 2003). The climate is tropical and is characterized by episodic cyclones generally from November to March annually.

The rare occurrence of *E. marginatus* in the coastal waters of Réunion Island (21°21′34″S 55°46′04″E) enabled the evaluation of connectivity between South Africa and Réunion. The specimen collected in June 2012 and identified based on photographs was compared genetically to other available samples from various locations, including the nearest known population (*i.e.* South Africa). The barcoding gene COI was used to verify the species identity of the specimen captured. Using mitochondrial cytochrome *b* sequences and genotypes at nine microsatellite loci we evaluated the source population of this long distance dispersal event. We tested whether the specimens could originate directly from the southern African coast or from a closer as yet un-described population in the WIO. To provide a broader perspective of the genetic variability within the species we also included specimens collected from Angola in the Atlantic and from the Mediterranean Sea and described the observed population structure.

2. Materials and methods

2.1 Sample collection and ageing of specimen

In addition to the specimen captured in Réunion Island (1), samples were collected from South Africa (6), Angola (11) and Mediterranean Sea (22) and stored in 95% ethanol at -20°C (Fig. 1A & Fig. 1B). The size of the specimen from Réunion (Supplementary Data S1) was 28.4 and 33.7 mm for the standard and the total length respectively. The total length was used to determine possible age of the specimen according to a von Bertalanffy growth model previously obtained for the species in South Africa (Fennessy, 2006). The hatching date of the captured specimen was then back-calculated using the age and the capture date (11 June 2012).

2.2 Molecular data analyses

Cytochrome oxidase I (COI, 617 bp, Reunion individual) was amplified using the primers of Ivanova *et al.* (2007). The cytochrome *b* gene (cyt *b*, 973 bp, 22 individuals) was amplified using universal primers (Hoareau unpublished; Supplementary Data S2) designed according to

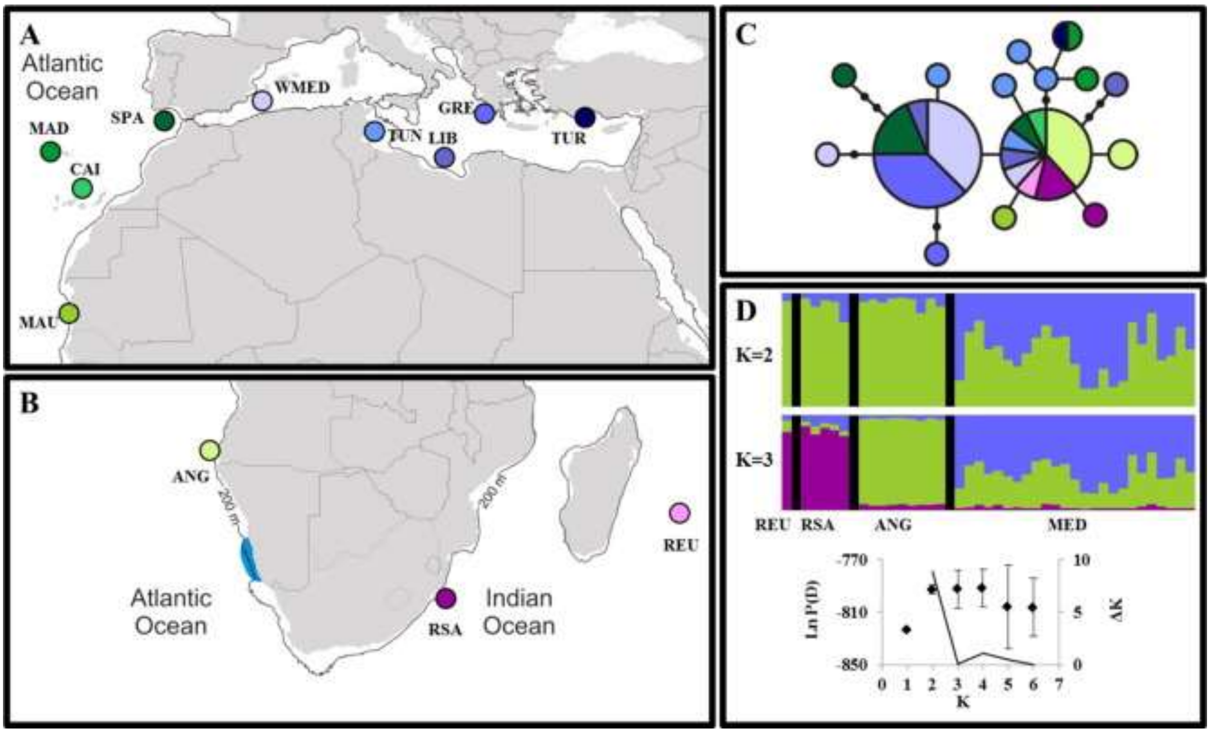


Fig. 1. Locations of specimen collections (newly generated and from GenBank) and genetic results for *Epinephelus marginatus*. (A) Map of locations from the Mediterranean Sea and Northeast Atlantic: western Mediterranean (WMED), Tunisia (TUN), Libya (LIB), Greece (GRE), Turkey (TUR), Spain (SPA), Madeira Archipelago (MAD), Mauritania (MAU), Canary Island (CAI) and (B) map of sampling locations within the Southeast Atlantic and Western Indian Ocean: Angola (ANG), South Africa (RSA) and Réunion Island (REU); the blue shaded area represents the Lüderitz upwelling zone (Namibia). For both maps the 200 m isobath is indicated. (C) Haplotype network for cytochrome *b* throughout the distribution of *E. marginatus* in the East Atlantic and WIO; colors correspond to map A and B. (D) STRUCTURE results for sampling locations in REU, RSA, ANG and MED. Results are shown for two and three populations respectively. The graph indicates the most likely number of clusters based on Ln probability of the data and Δ*K*.

Hoareau & Boissin (2010). These loci were amplified using Polymerase Chain Reaction (PCR) at 55°C and sequenced on an ABI3500xl using a previously described protocol (Reid *et al.,* 2012). Sequence editing, assembly and alignments were done in CLC Workbench 4.1.1 (CLC Bio A/S, Aarhus, Denmark) and MEGA version 5 (Tamura *et al*., 2011), respectively. A BLAST analysis of the COI fragment in NCBI was used to identify the species. Additional cyt *b* sequences (cyt *b*, 22) were downloaded from GenBank and incorporated into the analyses (Supplementary Table S1). Summary statistics and genetic differentiation indices were calculated for cyt *b* in ARLEQUIN version 3.11 (Excoffier *et al*., 2005; Table 1; Table 2). A haplotype network was constructed from 44 cyt *b* sequences (714 bp) in TCS version 1.21 using a 95 % confidence limit (Clement *et al.,* 2000).

Nine microsatellites were amplified in three multiplexes in 40 samples and included: Orla11-71, Orla9-204, Orla3-27, Orla7-167, Orla23-61, Orla18-49, Orla14-42 (Gotoh *et al*., 2013), EPI35 and EPI12 (Muths *et al*., 2011; Table 1). The multiplex PCR conditions and reagents are detailed in Reid *et al*. (2012). These products were screened on an ABI3500xl automated sequencer (Applied Biosystems). GENEMARKER version 1.5 (SoftGenetics, State College, PA, USA) was used to score the data. Summary statistics (Table 1) were calculated in GENETIX version 4.0 (Belkhir et al. 1996-2004). Null allele detection and corrected genetic differentiation using the ENA method were calculated in FREENA (Chapuis & Estoup, 2007). The number of genetic clusters was identified using STRUCTURE version 2.3.2 (Pritchard *et al*. 2000) with a burn-in of 300,000, followed by 300,000 MCMC steps and the locations as a prior (Hubisz *et al*., 2009). The clustering was confirmed using the *ad hoc* statistic *Δ*K (Evanno *et al.,* 2005). Additionally, GENECLASS2 (Piry *et al*., 2004) was used to assign the Réunion specimen

Table 1: Diversity indices based on microsatellite genotypes and cytochrome *b* sequences for *Epinephelus marginatus*.

			Microsatellites											Cytochrome *b*		
			IO (6)			AO (9)			MS (25)					IO (4)	AO (16)	MS (24)
	Dye	***Na***	H_O	H_E	**ENA**	H_O	H_E	**ENA**	H_O	H_E	**ENA**					
Orla11-71^{M1}	6-FAM	4	0.16	0.48	0.21	0.1	0.11	0	0.17	0.30	0.13	H		2	7	10
Orla9-204^{M1}	VIC	5	0.5	0.48	0	0.5	0.37	0	0.52	0.52	0	θ_k		0.87 (0.18, 4.26)	4.18 (1.62, 10.5)	5.91(2.65,12.8)
Orla3-271^{M2}	6-FAM	5	0.16	0.56	0.24	0.22	0.64	0.25	0.28	0.36	0.06	θ_H		0.74 (0.93)	3 (1.73)	1.84 (0.93)
Orla7-167^{M3}	NED	8	0.75	0.83	0	0.66	0.83	0.05	0.17	0.35	0.16	θ_S		0.54 (0.54)	3.31(1.50)	3.48 (0.54)
Orla23-61^{M3}	6-FAM	5	0.16	0.48	0.21	0.5	0.37	0	0.45	0.50	0.02	θ_π		0.50 (0.61)	1.975 (1.31)	1.75 (1.17)
Orla18-49^{M3}	VIC	7	0.5	0.40	0	0.5	0.67	0.08	0.55	0.70	0.05	***Fs***		0.17	-1.75	**-4.53**
Orla14-42^{M3}	PET	14	1	0.76	0	1	0.86	0	0.95	0.80	0	D		-0.61	**-1.52**	**-1.72**
EPI35^{M1}	PET	3	0	0	0	0.14	0.13	0	0.31	0.68	0.21	-		-	-	**-**
EPI12^{M2}	VIC	8	0.66	0.56	0	0.66	0.53	0	0.60	0.59	0	-		-	**-**	**-**

^{M} indicates the multiplex. The statistics are indicated by the following abbreviations: number of alleles (N_A), observed (H_O) and expected (H_E), frequency of null alleles (ENA), estimates of θ based on average number of nucleotide differences (θ_k), haplotype diversity (θ_h), number of segregating sites (θ_S), mean pairwise differences (θ_π) and neutrality tests (Fu's Fs and Tajima's D). The values in parentheses indicate the standard deviations and values in bold are significant ($P<0.05$). IO, AO and MS indicate the Indian Ocean, Atlantic Ocean and Mediterranean Sea respectively, the values in brackets indicate the sample sizes per region.

Table 2: Genetic differentiation based on microsatellite genotypes (below diagonal) and cytochrome *b* sequences (above diagonal) for *Epinephelus marginatus*.

	Mediterranean	Atlantic Ocean	Indian Ocean
Mediterranean	*	**0.067**	**0.166**
Atlantic Ocean	**0.119 (0.116)**	*	-0.040
Indian Ocean	**0.140 (0.160)**	**0.120 (0.123)**	*

Values in bold are significant at $P<0.05$. Values in brackets indicate uncorrected pairwise F_{ST} values to the other populations using the Bayesian method described by Rannala and Mountain (1997) and the probability computation described by Paetkau *et al*. (2004) using all microsatellite loci.

2. 3 Hydrodynamic modeling of larval connectivity

To better understand the possible dispersal patterns of the *E. marginatus* larvae in the region, we modeled larval movement around South Africa, Madagascar and the Mascarene Archipelago (Fig. 1B). We used the modeling framework of Treml *et al*. (2008). This framework uses the Marine Geospatial Ecology Tools software (Roberts *et al*., 2010) by implementing the Coral Reef Connectivity Simulation tool. This framework uses a two-dimensional Eulerian advection–diffusion algorithm to simulate the movement of propagules by dynamic ocean currents and turbulent diffusion.

To assess the larval transport, we used shoreline data from the global self-consistent, hierarchical, high-resolution shoreline database (Wessel and Smith, 1996), suitable habitat locations from FishBase (www.fishbase.com) and daily-averaged surface geostrophic current velocities (AVISO (http://www.aviso.oceanobs.com/) using 1/4° resolution "Global DT all sat" products) for the months of the spawning period. All the geographic products were organized as rasters and placed into the World Mercator coordinate system with the WGS84 datum at a 25 km cell size.

Specific criteria used were pelagic larval durations (PLDs) of 20, 30 and 40 days respectively across the most likely period of spawning of the individual identified in Réunion. Larvae were released from 444 sites within the WIO region with an initial larval density of 10 000 larvae per kilometer squared (default value). The release time was back-calculated for each new moon corresponding to "new moon date - PLD" and running until the PLD elapsed. The diffusivity coefficient was also set by default (25 $m^2.s^{-1}$). The connectivity results were reported for each month that corresponded to plausible hatch dates for the specimen captured in Réunion.

3. Results

Both the color patterns (see photographs in the supplementary Data S1) and the BLAST analysis results of the COI sequence (GenBank accession: KX242297) confirmed that the specimen was a dusky grouper. Based on the total body length (33.7 cm), the specimen was identified to be approximately 16 months old when captured on the 11th June 2012. Therefore, the hatching month was back-calculated to January 2011, which coincided with summer months and the main spawning period in South Africa (Fennessy, 2006). This provides a likely range for the spawning period of the specimen from October 2010 to March 2011.

The 22 cyt *b* sequences generated in this study were deposited in GenBank (accession numbers: KX242275-KX242296). Two high frequency haplotypes were detected using the cyt *b* sequences (Fig. 1B). One haplotype is more specific to the Mediterranean (16 individuals), while the other included the samples collected in Angola, South Africa and Réunion (28; Φ_{ST} between regions of 0.09, p-value =0.00195 ± 0.0014, Fig. 1C, Table 2). The levels of null alleles detected at microsatellite loci in some locations (Table 1) were sufficiently low to not have an influence on the genetic differentiation estimates (Table 1, Table 2). The microsatellite data (Supplementary Data S3) indicated genetic subdivision between the Mediterranean Sea and the rest of the locations ($\Delta K = 2$, Fig. 1D). Further sub-structuring was observed between the Southeast Atlantic (Angola) and the Indian Ocean (South Africa; Fig. 1, Table 2). The specimen from Réunion Island was genetically very close to South African samples as these locations consistently clustered together based on STRUCTURE results, and as already suggested by the

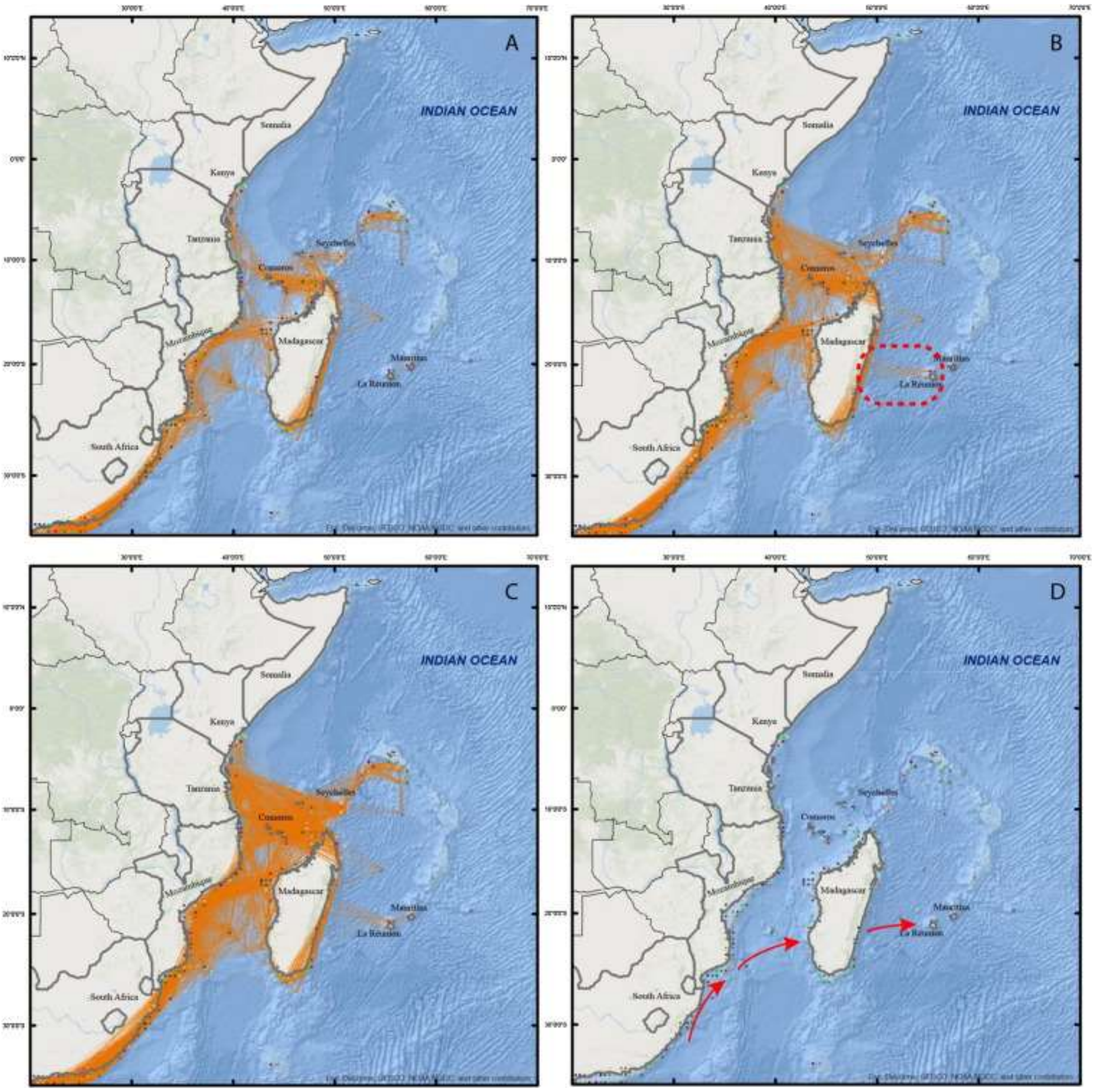


Fig. 2. Connectivity patterns of *Epinephelus marginatus* larvae between sites of suitable habitat for February 2011. Each simulation is based on varying pelagic larval durations. (A) 20 day, (B) 30 days, with the red circle indicating the connection between Madagascar and Réunion Island, (C) 40 days, (D) the region with the red arrows indicates the most likely route of colonization. Dots indicate spawning areas from where larvae were released within suitable habitat and lines indicate connections between sites.

cyt *b* (Fig. 1B). This was also supported by the GENECLASS2 result which assigned the Réunion sample to the South African population with a probability of 99 %.

The modeling results indicated that movement of *E. marginatus* larvae between South Africa and Madagascar was possible during all months modeled (Supplementary Fig.S1, Fig. 2) and for all PLDs (20 to 40 days). Dispersal was investigated over the different plausible months of the hatching event, from November 2010 to March 2011. The modeling indicated a unique possibility for the dispersal event between Madagascar and Réunion Island in February 2011, which coincides with the cyclone Bingiza (Fig. 2A). No direct connection could be identified between South Africa and Réunion Island.

4. Discussion

4.1 Genetic inference for the origin of *Epinephelus marginatus* in Réunion

In June 2012 two specimens, approximately of the same size, of an unknown grouper were observed by local fishermen in waters off Réunion Island at Port de Saint Philippe. One of the specimens was caught and photographed by fishermen and they kept a fin clip in 95% ethanol. Mitochondrial sequences (COI and cyt *b*) confirmed the specimen to be a dusky grouper (*E. marginatus*). The cyt *b* haplotype of this individual clustered with a haplogroup that is dominant in the Southeast Atlantic and WIO off South Africa (Fig. 1C). Microsatellite data further clustered the individual with others from South Africa (Fig. 1D). This specimen therefore likely originated from the WIO region however, uncertainty remained to the exact location of origin. Two hypotheses of origin were tested through larval modeling. The two observed specimens colonized Réunion by a unique long distance dispersal event, either directly from

South Africa or from an as yet unrecorded population within the WIO region located closer to Réunion Island, but genetically similar to South Africa.

4.2 Origin of the Reunion specimen using modeling

The larval modeling did not support a direct colonization from South Africa to Réunion Island over the hatching period of the specimen likely ranging from October 2010 and March 2011. This is in agreement with the general oceanographic features in the region characterized by the South Equatorial Current circulating in the opposite direction (Woodberry *et al*., 1989). However, high connectivity was predicted across the Mozambique Channel between suitable habitats off South Africa and Madagascar through all spawning months and PLDs. This result suggests that an established population of dusky grouper occurs along the southern coasts of Madagascar.

A long distance dispersal event was identified from Madagascar to Réunion, and this occurred in February 2011 coinciding with cyclone Bingiza (Fig. 2, Supplementary Fig. S1). Long distance dispersal events have been described during environmental disturbances such as cyclones (McKinnon *et al*., 2003) which are frequent in the WIO during summer months. Cyclone Bingiza, moreover, had a particular position between Madagascar and Réunion at this time, which could have favored long distance dispersal events of larvae between the two islands. Further biodiversity surveys in the SWIO region should provide insight into this rare colonization event, as other species not normally occurring in the SWIO are also likely to be transported through such events. Realized colonization and establishment of new populations would then be dependent on the number of founders, their biology (such as successful breeding) and suitable habitat (Lees & Gilroy, 2013).

4.3 Population structure of *Epinephelus marginatus*

Our study confirmed population structure and showed an additional divergence of the Southeast Atlantic and Indian Ocean (Fig. 1D). Our data indicated colonization into the Southeast Atlantic and then a likely subsequent colonization into South Africa (Fig. 1D). This pattern was also supported by the cyt *b* data which showed that Angola, South Africa and Réunion Island shared a single haplotype (Fig. 1B). Divergence between the Northeast Atlantic and Mediterranean populations of *E. marginatus* has previously been described (Maggio *et al*., 2006; Schunter *et al*., 2011), as well as subtle substructure within the Mediterranean Sea (Schunter *et al*., 2011).

4.4 Conclusions

Studies focusing on the identification of colonization events as reported here provide insight into mechanisms that may lead to founding events in remote regions previously deemed to be unsuitable for particular species. More studies should focus on these types of processes to elucidate the mechanisms underlying the origin of oceanic island endemism. Also, our study indicates a likely additional population or extended range of the described South African population to Madagascar; this species has previously been sighted there although this record was deemed a likely error in the IUCN report on *E. marginatus* (www.IUCNredlist.org).

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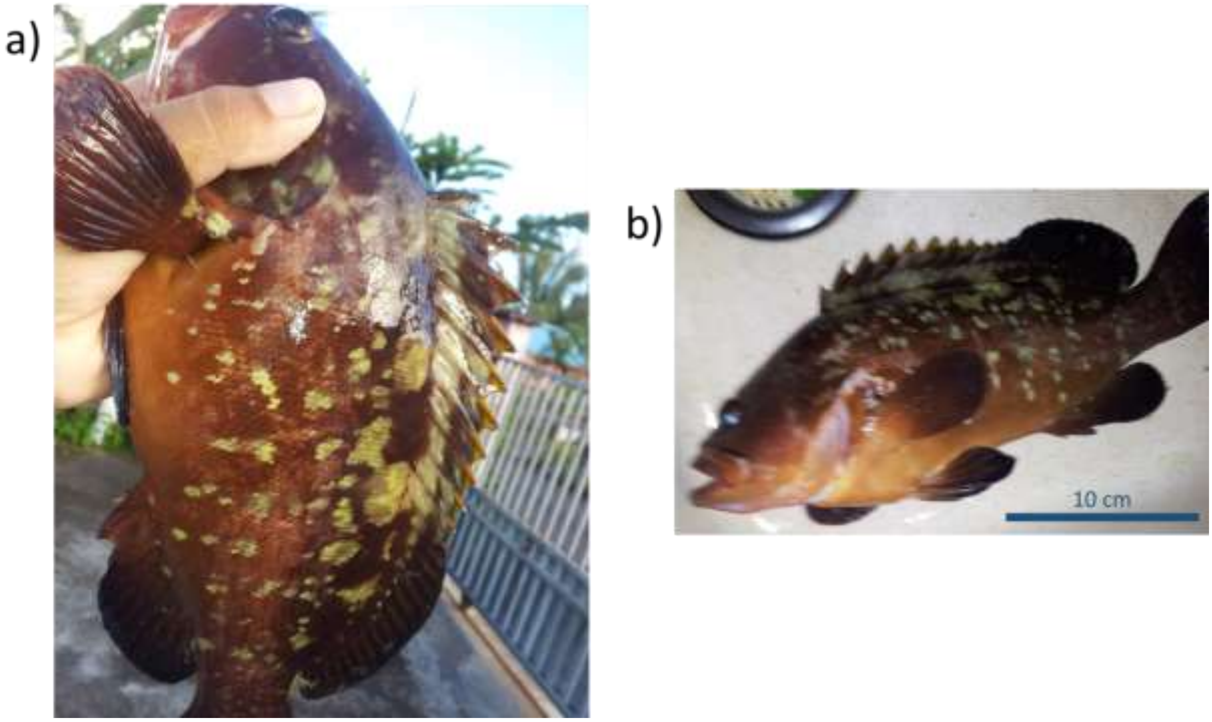
Supplementary information for the dusky grouper sampled at Réunion

Supplementary data S1A and S1B: Photos of the specimen of dusky grouper collected in port de Saint Philippe (Réunion Island) in June 2012 by Mr Florent Collet. The color pattern is characteristic of the species .

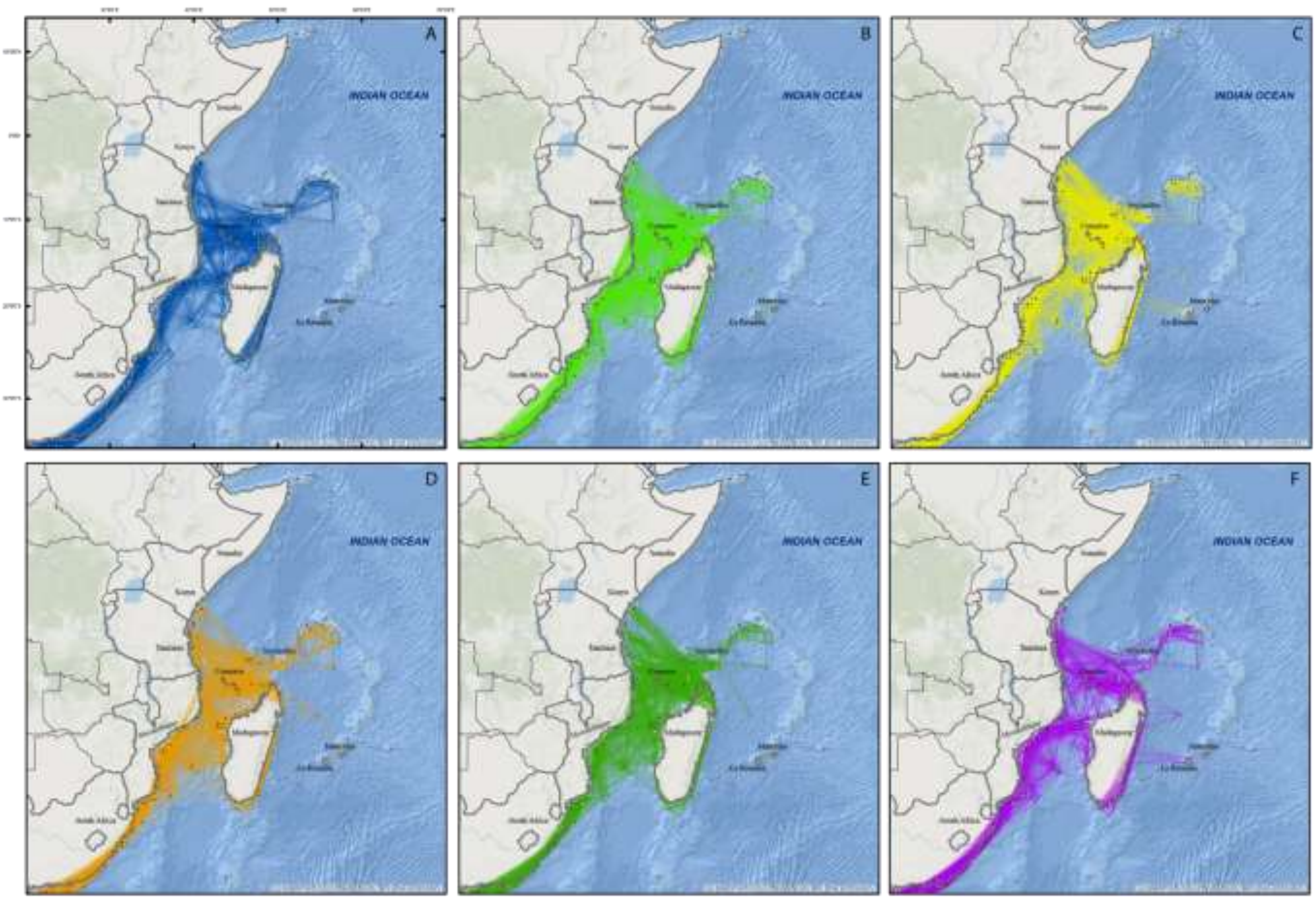


Supplementary Data S2:

Cytochrome *b* primer for teleosts:
F: 5’ –ATGGCAAGCCTACGCAAAACC -3’
R: 5’ –GCAGAGAATAGTTTAAATGCA -3’

Supplementary Table S1: GenBank accession information and locations of *Epinephelus marginatus* cytochrome *b* data incorporated in this study

	CYTB GenBank no	Location	New code
1	AB179763	Spain	SPA1
2	AB179762	Spain	SPA2
3	AB179761	Spain	SPA3
4	AB179760	Spain	SPA4
5	AB179759	Spain	SPA5
6	DQ197952	Canary Island	CA1
7	EF439529	Western Mediterranean	WESM1
8	EF439528	Western Mediterranean	WESM2
9	EF439205	Madeira_Archipelago	MADA1
10	EF439204	Madeira_Archipelago	MADA2
11	EF456002	Mauritania	MAU1
12	EU264009	Greece	GREG1
13	EU264008	Greece	GREG2
14	KC533574	Tunisia	TUN1
15	KC533573	Tunisia	TUN2
16	KC533572	Tunisia	TUN3
17	KC533571	Tunisia	TUN4
18	KC533570	Tunisia	TUN5
19	KC533569	Libya	LIB1
20	KC533568	Libya	LIB2
21	KC533567	Libya	LIB3
22	GU199326	Turkey	TUR1



Supplementary Figure S1: Connectivity patterns of *Epinephelus marginatus* larvae between sites of suitable habitat in the SWIO region, based on a PLD of 30 days. Release dates are indicated in brackets: A) September 2010 (07/09), B) October 2010 (07/10), C) November 2010 (05/11), C) December 2010 (05/12), E) January 2011 (04/01), F) February 2011 (02/02).

Supplementary Data S3:

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All together
npops = 4
nloci = 9
 O11-71  O9-204  EPI35  O3-271  EPI12  O7-167  O23-61  O18-49  O14-42
pop = RSA
397          378 378 267 269 151 151 403 403 143 159 167 179 179 179 193 193 351 359
74341        376 376 267 267 151 151 401 401 155 159 171 173 171 171 193 193 355 359
EM2011       378 378 267 269 151 151 403 403 143 143 175 177 171 171 193 201 351 357
GG07A034 376 376 267 267  ?    ?    403 403 143 159 169 171 171 171 193 201 355 359
HM09070   376 378 269 269  ?    ?    401 401 159 159 169 169 171 179 193 197 357 359
pop = Reunion
MEROU     376 376 267 269 151 151 401 407 143 159 173 177 179 179 193 193 347 351
pop = Angola
G97            376 376 267 267 151 151 401 401 159 163 177 177 169 171 193 197 353 355
G149          376 378 267 267 151 159 402 402 159 163 171 171 171 171 197 197 361 365
T1               376 376 267 269 151 151 401 401 159 163 173 175 169 171 195 195 353 365
T173          376 376 267 267 151 151 401 403 159 167  ?    ?    171 171 195 195 365 373
T218           ?     ?     ?     ?     ?     ?    403 403 163 163 175 177 169 171 193 195 355 369
T222          376 376 267 269 151 151 402 404 159 159 171 175 169 171 197 199 343 361
T223          376 376 267 269 ?     ?    401 401 159 163 169 175 171 171 193 195 343 353
T233          376 376 267 267 151 151 402 402 159 163 ?     ?    ?     ?     ?     ?     ?     ?
T234          376 376 267 269 151 151 401 401 159 159 ?     ?    171 171 197 197 359 363
pop = MED
EM15         376 378 269 269 139 139 401 401 159 159  ?    ?     171 171  ?     ?     ?    ?
EM17         376 386 265 277 139 139  ?     ?     ?     ?     ?    ?     165 171  ?     ?     ?    ?
EM4           378 378 267 269 151 151 401 401 127 139 ?     ?     173 173  ?     ?     ?    ?
EM56         376 376 261 277 139 139  ?     ?    ?     ?     ?     ?     171 171  ?     ?     ?    ?
EN2           376 376 267 269 139 147 401 403 143 159 169 171 171 173 193 197 353 365
EN3           376 376 269 269 ?     ?    401 401 159 163 171 173 171 171 197 199 353 357
EN6           376 378 267 269 147 147 401 401 159 159 ?     ?     ?     ?     ?     ?     ?     ?
EN7           376 376 269 269 139 139 401 403 159 163 171 171 171 173 195 203 353 371
EN8           376 376 267 269 139 139 401 401 159 163 175 175 169 171 195 197 353 361
EN9           376 376 267 267 ?     ?    401 403 151 159 171 171 171 171 179 195 351 355
NAL1020    376 376 267 269 151 159 401 403 143 151 171 171 171 171 193 193 353 363
NAL1021    376 376 269 269 151 151 ?     ?    159 163 171 171 173 173 195 199 353 361
NAL1024    376 376 269 269 147 151 ?     ?    151 159 171 171 171 171 193 195 357 369
Nal1025      ?    ?     269 269 139 139 401 401 159 159 171 171 171 171 193 195 353 357
Nal1026      ?    ?     269 269 139 139 401 401 151 159 171 171 171 171 193 193 353 357
NAL1028    376 378 269 269 147 147 401 401 159 159 171 171 169 171 193 197 343 357
NAL1029    376 376 269 269 147 147 401 401 159 159  ?     ?     171 173 193 193 353 357
NE6           376 376 267 269 ?     ?    401 401 159 159  ?     ?     171 171 193 193 353 357
NE7           376 376 267 269 151 159 401 401 159 163 171 175 171 173 197 197 361 373
GRE10       376 376 267 269 139 151 401 401 127 139 171 171 171 171 193 193 349 359
GRE1         376 376 267 269 151 151 401 402 143 159 ?     ?     165 173 197 203 355 359
GRE2         376 376 267 267 139 151 401 401 159 159 171 171 171 173 193 193 357 359
GRE3         376 376 269 269 139 139 401 401 127 139 187 187 171 171 197 197 353 353
GRE4         376 376 267 269 151 159 402 404 159 159 171 171 165 171 197 197 353 355
GRE9         374 374 267 269 147 147 402 402 159 159 171 171 169 171 193 197 353 357
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