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ORIGINAL ARTICLE

One species for one island? Unexpected diversity and weak connectivity in a widely distributed tropical hydrozoan

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Isolation by distance (IBD) is one of the main modes of differentiation in marine species, above all in species presenting low dispersal capacities. This study reports the genetic structuring in the tropical hydrozoan *Lytocarpia brevirostris* α (*sensu* Postaire *et al*, 2016b), a brooding species, from 13 populations in the Western Indian Ocean (WIO) and one from New Caledonia (Tropical Southwestern Pacific). At the local scale, populations rely on asexual propagation at short distance, which was not found at larger scales; identical genotypes were restricted to single populations. After the removal of repeated genotypes, all populations presented significant positive F_{IS} values (between 0.094*** and 0.335***). Gene flow was extremely low at all spatial scales, between sites within islands (< 10 km distance) and among islands (100 to > 11 000 km distance), with significant pairwise F_{ST} values (between 0.012*** and 0.560***). A general pattern of IBD was found at the Indo-Pacific scale, but also within sampled ecoregions of the WIO province. Clustering analyses identified each sampled island as an independent population, whereas analysis of molecular variance indicated that population genetic differentiation was significant at small (within island) and intermediate (among islands within province) spatial scales. The high population differentiation might reflect the life cycle of this brooding hydrozoan, possibly preventing regular dispersal at distances more than a few kilometres and probably leading to high cryptic diversity, each island housing an independent evolutionary lineage.
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INTRODUCTION

Seascape connectivity, the process linking habitat patches and populations through the exchange of organisms across the marine environment, is a key driver of population dynamics, genetic structuring and diversification processes of marine organisms (Palumbi, 1992; Paulay and Meyer, 2002; Cowen *et al.*, 2007; Boissin *et al.*, 2011; Bowen *et al.*, 2013). Knowledge of seascape and population connectivity ideally forms the basis for the definition of management and conservation units (Cowen *et al.*, 2007; Christie *et al.*, 2010; White *et al.*, 2010; Thomas *et al.*, 2012). Indeed, a high proportion of marine organisms rely on the dispersal of their larval stage by ocean currents for population maintenance over time and colonization of new habitats. Stretches of open ocean are thus often regarded as environmental barriers reducing the dispersal (that is, including the transport and recruitment phases) of propagules (gametes and larvae) over large geographic scales for organisms presenting short pelagic larval duration, direct development, brooding and/or holobenthic life cycles (for example, Mokhtar-Jamaï *et al.*, 2011). Hence, marine species with low dispersal capacities are expected to have narrow distribution ranges and strong population differentiation with geographic distance, that is, an isolation by distance (IBD) pattern (Slatkin, 1993). This pattern is thought to increase the number of speciation opportunities, mainly allopatric, by favouring vicariant events and the formation of independent evolutionary lineages over time (Paulay and Meyer, 2002; Malay and Paulay, 2010) leading *in fine* to speciation (De Queiroz, 1998).

The relative importance of environmental barriers opposing the dispersal of organisms is often estimated via the assessment of population connectivity across geographic distances. This connectivity represents the genetic linking of local populations through the effective dispersal (that is, transport, recruitment and reproduction) of individuals (larvae, juveniles or adults) among them (Sale *et al.*, 2005). It represents a continuum, from an absence of connectivity (closed populations, mainly self-recruiting) to high connectivity (open populations, most of the recruitment happens via migration of individuals), in which the life cycle and reproductive strategy are important traits that shape population differentiation and connectivity. Nevertheless, Shanks (2009) observed that the relationship between direct or indirect development, pelagic larval duration, swimming capacity and dispersal capacity is not always straightforward, as a large body of literature exists, detailing examples of lecithotrophs, brooders and direct developers, with biogeographic ranges spanning oceans (Ayre and Hughes, 2000; Kyle and Boulding, 2000; Boissin *et al.*, 2008). Thus, other aspects, for example, larval behaviour and species ecology, must be considered to explain the patterns of marine population connectivity (Shanks, 2009), as well as oceanic circulation and historical sea-level variations (Treml *et al.*, 2007; Ayre *et al.*, 2009; Schiavina *et al.*, 2014). In order to achieve a more comprehensive understanding of the maintenance of natural populations over time, it is necessary to multiply the number of biological models studied.

Hydrozoans are ubiquitous in all marine ecosystems with species often presenting broad biogeographic distributions and a variety of life

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cycles and reproductive features (Bouillon *et al.*, 2006). The Aglaopheniidae family (Marktanner-Turneretscher, 1890) represents one of the largest, with over 250 valid species (Bouillon *et al.*, 2006) and is particularly diversified in tropical marine ecosystems. The diversity of this family is still under assessment, as recent publications highlighted that morphological characters provide little clues to the evolutionary history and species richness of this taxon, presenting extensive cases of morphological convergence and low anagenesis (Leclère *et al.*, 2007; Moura *et al.*, 2012; Postaire *et al.*, 2016a, b). Aglaopheniidae species generally do not have a medusa stage but brood their larvae in dedicated structures of the colony (named gonothecae; Millard, 1975) and planulae are released only when mature (Boero *et al.*, 1992). Hydrozoan planulae usually crawl rather than swim and settle in less than 24 h, suggesting low dispersal capacities (see Gili and Hughes (1995) for a review of hydrozoan ecology). Intuitively, this feature contradicts the extensive geographic ranges spanning several major biogeographic provinces of these species. *Lytocarpia brevirostris* (Busk, 1852) is a typical tropical Aglaopheniidae brooding species, found on coral reefs throughout the Indo-Pacific region (Millard, 1975; Gravier-Bonnet, 2006; Gravier-Bonnet and Bourmaud, 2006, 2004, 2012; Di Camillo *et al.*, 2011). This orange feather-like colonial hydrozoan is composed of two sympatric cryptic species, which can be identified using molecular markers (either typical barcoding markers or microsatellites; Postaire *et al.*, 2016b). In this study, we focus on one of these cryptic species, named *L. brevirostris* α in Postaire *et al.* (2016b). Hydrozoans are among the least studied cnidarians for population genetics, but the few studies available to date (Darling and Folino-Rorem, 2009; Schuchert, 2014) highlighted the high genetic differentiation among individuals sampled from different regions. Considering these previous results, one could argue that each population restricted to a small geographic region potentially represents an independent species (*sensu* Samadi and Barberousse, 2006). Indeed, the classification of organisms into species may be hampered by variation of genetic and morphological characters within and among populations. Furthermore, gene flows across ocean basins are known to vary in intensity and direction according to oceanic circulation (Dawson, 2001; Hohenlohe, 2004; Cowen *et al.*, 2007; Weersing and Toonen, 2009), influencing both population differentiation and speciation processes. Thus, the wide distributions of marine brooding hydrozoans could be (1) a taxonomic artefact and species represent complexes of sibling species with limited dispersal abilities or (2) a single species with an extensive population subdivision (Schuchert, 2014). One of the solutions to start answering the problem is to conduct an analysis of population structuring based on an extensive hierarchical sampling of populations at large geographic scales.

In this study, we used microsatellites to assess the population structuring of *L. brevirostris* α. We sampled populations in two biogeographic provinces as defined by Spalding *et al.* (2007): the Western Indian Ocean (WIO: Reunion Island, Rodrigues, Madagascar and Scattered Islands) and the Tropical Southwestern Pacific (TSP: New Caledonia). We evaluated patterns of connectivity across three spatial scales: within sampling site (short distance connectivity), among islands within a biogeographic province (intra-regional comparisons) and between biogeographic provinces (inter-regional connectivity). To our knowledge, this is the first study on genetic connectivity in marine hydrozoans.

METHOD

Sample collection and DNA extraction

Each sampling site was explored randomly using SCUBA diving. *L. brevirostris* individuals (defined as a plume), which present a scarce and patchy distribution on outer reef slopes, often found in shaded caverns or on vertical cliffs, were

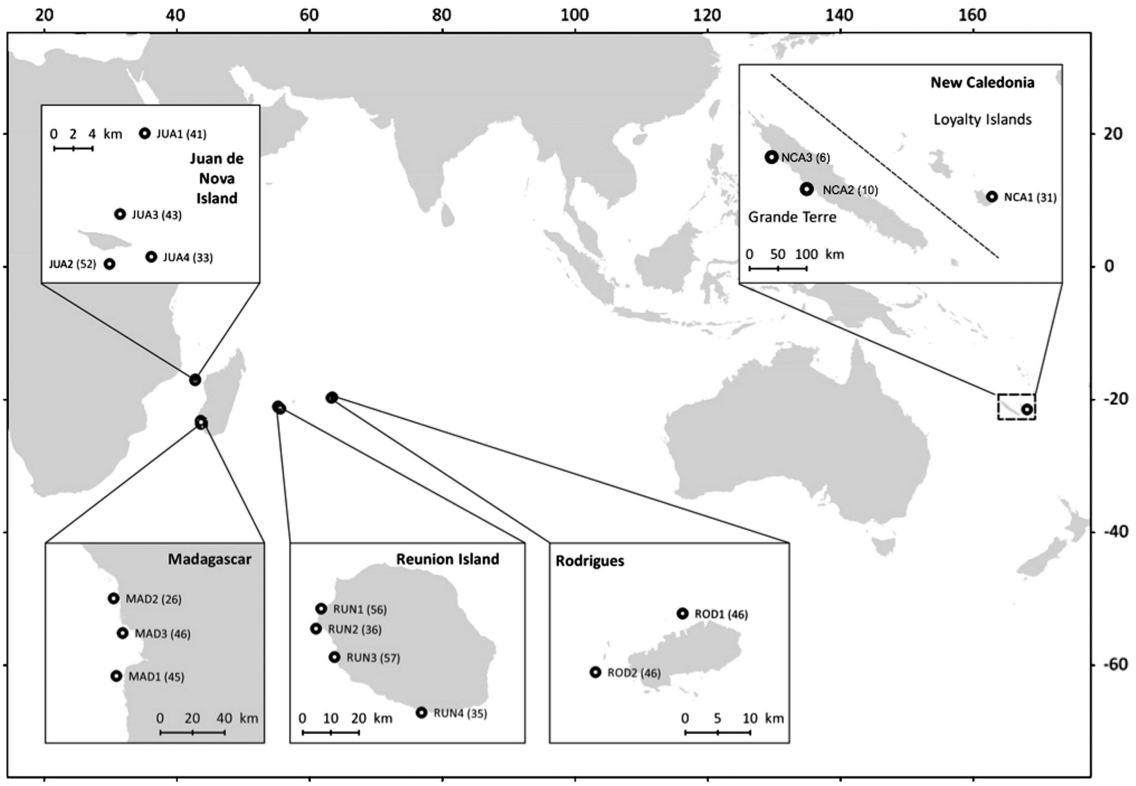

Figure 1 Sampling locations of *L. brevirostris* α in the WIO and the TSP with population names and the number of individuals sampled (in parentheses).

Table 1 ***L. brevirostris*** **α samples (*N* = 593) used in this study**

Marine Province	*Ecoregion*	*Island*	*Site name*	*Population*	*Latitude*	*Longitude*	N	N_{MLG}	R	H_O	H_E	F_{IS}	*Ar(26)*	*Ap(26)*
WIO	Mascarene Islands	Reunion Island	Cap La Houssaye	RUN1	−21.01766	55.23836	56	36	0.636	0.374	0.416	0.104*	2.919 ±0.142	0.009 ±0.009
			Passe de l'Ermitage	RUN2	−21.08659	55.22065	36	13	0.342	0.344	0.447	0.238**	2.753 ±0.231	0.148 ±0.085
			Saint Leu	RUN3	−21.18419	55.28418	57	42	0.732	0.438	0.488	0.104**	3.322 ±0.277	0.011 ±0.007
			Manapany	RUN4	−21.37500	55.58358	35	23	0.647	0.354	0.477	0.263*	3.073 ±0.463	0.121 ±0.112
		Rodrigues	Petits Patés	ROD1	−19.65386	63.41501	46	13	0.266	0.452	0.475	0.051***	3.086 ±0.602	0.237 ±0.130
			Ilot Cocos	ROD2	−19.74016	63.28660	46	11	0.222	0.331	0.433	0.238***	2.933 ±0.611	0.161 ±0.102
	Western/Northern Madagascar	Juan de Nova Island	Juan 2	JUA1	−16.95337	42.76011	41	34	0.825	0.489	0.636	0.235***	4.472 ±0.565	0.200 ±0.102
			Juan 6	JUA2	−17.08177	42.72536	52	39	0.745	0.545	0.661	0.177***	5.051 ±0.638	0.144 ±0.083
			P7	JUA3	−17.03280	42.73580	43	36	0.833	0.462	0.647	0.290***	5.292 ±0.567	0.313 ±0.139
			Biodiv 7	JUA4	−17.07470	42.76650	33	25	0.750	0.572	0.707	0.194***	5.600 ±0.510	0.220 ±0.105
		Madagascar	Anakao	MAD1	−23.66586	43.59985	45	27	0.590	0.395	0.472	0.166***	3.978 ±0.631	0.129 ±0.055
			Ifaty	MAD2	−23.19096	43.58291	26	18	0.680	0.350	0.526	0.341***	4.087 ±0.681	0.068 ±0.054
			Tuléar	MAD3	−23.40370	43.63818	46	24	0.511	0.363	0.512	0.294***	3.723 ±0.555	0.189 ±0.109
TSP	New Caledonia	Maré	Sud Cap Coster	NCA1	−21.48673	168.11940	31	31	1	0.454	0.592	0.239***	4.291 ±0.632	1.691 ±0.393
		Grande Terre	Népoui	NCA2	−21.41855	164.96713	10	10	1	0.433	0.592	0.177***		
			Kouma	NCA3	−20.81090	164.30625	6	6	1	0.509	0.608	0.280***		

Abbreviations: MLG, multi-locus genotype; N_{MLG}, number of unique MLG; NS, non-significant; TSP, Tropical Southwestern Pacific; WIO, Western Indian Ocean.
For each population are given: total sample size (N), N_{MLG}, clonal richness $R = [(N_{MLG} - 1)/(N - 1)]$, observed ($H_O$) and expected ($H_E$) heterozygosities, inbreeding coefficient (F_{IS}), allelic richness *Ar*(26) ± s.e. and private allelic richness *Ap*(26) ± s.e.
GPS coordinates of sampling sites are in decimal degrees. H_O, H_E and F_{IS} were calculated keeping one representative per MLG per population. With the F_{IS}, is indicated the test significance for deviation to Hardy–Weinberg equilibrium: $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

sampled between 3 and 25 m depth. When encountered, individuals were collected and placed individually in sequentially numbered plastic bags, to approximate distances between individuals (the closeness in number reflects the proximity of individuals). *Lytocarpia brevirostris* grows by asexual propagation and individual plumes are sometimes linked by a stolon, thus forming colonies (defined hereafter as the aggregation of individuals forming a genet). Consequently, to minimize the probability of sampling members from the same genet, we collected, where possible, individuals at least few centimetres to several decimetres apart. Larger individuals (3–10 cm high) with visible reproductive structures were preferentially collected to avoid misidentification with other species from the genus *Lytocarpia*. A total of 593 collected *L. brevirostris* α individuals (Figure 1 and Table 1) were primarily identified using morphological characters (Millard, 1975) and verified using microsatellite data (Postaire *et al.*, 2016b). *L. brevirostris* was relatively rare in New Caledonia compared with the WIO and only 3 sites presented more than 5 individuals (Figure 1 and Table 1) among 18 explored sites all around the island.

Specimens were fixed and preserved in 90% ethanol for later DNA extraction. Before DNA extraction, all reproductive structures were removed from the individuals to avoid genotyping progeny issued from sexual reproduction and thus distinct individuals. DNA was extracted from one or two primary branches of the hydrocaulus per individual using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

Microsatellite genotyping

We used 16 microsatellite loci specific for the *L. brevirostris* species complex (Postaire *et al.*, 2015a). Amplifications, thermocycling and genotyping conditions were the same as in Postaire *et al.* (2015b). Identical multi-locus genotypes (MLGs) were identified with GENCLONE v. 2.0 (Arnaud-Haond and Belkhir, 2007) using the maximum set of loci for each sampling site and keeping only the individuals without missing data. For subsequent analyses, were kept only the loci presenting <10% of missing data for the entire data set (that is, 10 loci: Lb01, Lb02, Lb03, Lb05, Lb06, Lb07, Lb08, Lb10, Lb11 and Lb16; see Results) and only one representative per MLG (that is, one individual per colony; Table 1).

All tests in this study were corrected for false discovery rate in multiple tests (Benjamini and Hochberg, 1995). We used MICROCHECKER v. 2.3 (van Oosterhout *et al.*, 2004) to check for scoring errors and to estimate null allele frequencies. Linkage disequilibrium (LD) was tested using Arlequin v. 3.5 (Excoffier and Lischer, 2010) among all pairs of loci in each population with a permutation test ($n = 10^3$). Observed (H_O) and expected (H_E) heterozygosities, F_{IS} indices and tests for Hardy–Weinberg equilibrium were computed using the software Arlequin v. 3.5 (Excoffier and Lischer, 2010) for all populations and over all loci. Mean allelic richness [*Ar*(g)] and mean private allelic richness [*Ap*(g)] were calculated. We applied a rarefaction method to obtain estimates of both values independently of sample size using ADZE v. 1.0 (Szpiech *et al.*, 2008). We chose to compare both values to a common sample size of $g = 26$ as it was the minimum number of individuals found per site, excluding NCA2 and NCA3 as these populations were too small (⩽10 individuals; Table 1).

Clustering individuals and populations, analyses of population differentiation and gene flow

We investigated the population structure using different approaches: measures of differentiation and a Bayesian clustering method. First, the geographic origin of samples (that is, site) was treated as an *a priori* defined population. Owing to low sampling size, NCA2 and NCA3 were pruned from this set of analyses. Population-level pairwise F_{ST} comparisons and Fisher's exact tests of population differentiation were performed in Arlequin v. 3.5 (Excoffier and Lischer, 2010). The significance of the observed F_{ST} statistics was tested using the null distribution generated from 5×10^3 non-parametric random permutations. To infer mechanisms that may be responsible for the observed patterns of population structure, we compared estimates of genetic differentiation to geographic distances among sites of the WIO and the TSP. Euclidean distances between sampling locations were measured with Google Earth v. 7.1 (http://earth.google.fr/) using site coordinates (Table 3) and taking into account the regional pattern of oceanic currents (Schott *et al.*, 2009). We used a Mantel test (Mantel, 1967) to evaluate the correlation between linearized genetic differentiation (Slatkin's distance $= F_{ST}/(1 - F_{ST})$) and the $\log_{10}$ of the geographic distance between sites (Table 3). This relationship is

expected to be positive and linear in the context of a two-dimensional IBD model (Rousset, 1997). All Mantel tests were performed using the programme GENODIVE (Meirmans and van Tienderen, 2004) with 10^4 random permutations to assess significance. Population differentiation was also assessed without *a priori* stratification of samples.

Then, the geographic origin of samples was no more considered, allowing us to use samples from NCA2 and NCA3. We performed a discriminant analysis of principal components (DAPC) in the R (R Development Core Team, 2004) package *adegenet* (Jombart, 2008; Jombart *et al.*, 2010). DAPC is a non-model-based method that maximizes the differences between groups, while minimizing variation within groups without prior information on individuals' origin. This method does not assume Hardy–Weinberg equilibrium or the absence of LD. We used the function find.clusters() to assess the optimal number of groups with the Bayesian information criterion (BIC) method (that is, K with the lowest BIC value is ideally the optimal number of clusters). We tested values of K ranging from 1 to 30, but BIC values may keep decreasing after the true K value in case of genetic clines and hierarchical structure (Jombart *et al.*, 2010). Therefore, the rate of decrease in BIC values was visually examined to identify values of K beyond which BIC values decreased only slightly (Jombart *et al.*, 2010). The dapc() function was then executed using the best grouping, retaining axes of PCA sufficient to explain $\geqslant 90\%$ of total variance of data. Afterwards, Bayesian clustering analyses were performed to estimate the most probable number of populations (K) given the data, as implemented in the programme STRUCTURE v. 2.3.2 (Pritchard *et al.*, 2000), using the admixture model with correlated allele frequencies (Falush and Pritchard, 2003). This analysis assumes that the data set is composed of K populations and individuals are assigned to each putative population under Hardy–Weinberg equilibrium and minimized LD. We studied the assignment of samples again using a hierarchical approach. Four independent runs were conducted for each value of K from 1 to 10 with a burn-in period of 5×10^4 steps followed by 5×10^5 Markov chain Monte Carlo iterations. We used the statistic proposed by Evanno *et al.* (2005) to estimate the number of clusters K implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). The STRUCTURE outputs of the best number of K were summarized with CLUMPP v. 1.0 (Jakobssen and Rosenberg, 2007) and formatted with DISTRUCT v. 1.1 (Rosenberg, 2004). Finally, Arlequin v. 3.5 (Excoffier and Lischer, 2010) was used to perform hierarchical analysis of molecular variance using clusters identified by STRUCTURE as groups. A hierarchical analysis of molecular variance (Excoffier *et al.*, 1992), using provinces as groups and islands as populations was finally performed.

RESULTS

Multi-locus genotyping and potential importance of asexual reproduction

Over the 16 available loci for *L. brevirostris* α, 15 loci amplified correctly (that is, presented $<10\%$ of missing data) on samples from Reunion Island (all except Lb13), 12 loci (all except Lb04, Lb09 and Lb12, Lb13) on samples from Juan de Nova Island, Madagascar and Rodrigues (WIO except Reunion Island) and 10 loci (all except Lb04, Lb09, Lb12, Lb13, Lb14 and Lb15) on samples from New Caledonia (TSP). Our analysis of 609 individuals yielded 470 MLGs, indicating that asexual propagation occurred in the sampled populations. The individuals sharing the same MLG were found close together (that is, small difference in field numbers; Figure 2). Moreover, none of the MLGs were shared by different populations. In other words, clones were confined to their sites. Consequently, only one representative of each MLG was used for further analyses.

Genetic variability

Global significant LD among loci was detected ($P<0.05$, 68 significant tests over 720 after false discovery rate correction, that is, 9.4%) in the global dataset. However, more than half of the positive tests (38 out of 68) occurred in two populations with low clonal richness (ROD1 and ROD2) and might just represent their low genetic diversity. All loci were polymorphic, with a total number of alleles ranging from eight (Lb01, Lb07) to 23 (Lb02) (mean $\pm$ s.e. $= 14.4 \pm 1.8$). Some loci were monomorphic in several populations: Lb02 in population NCA1, Lb01 and Lb06 in ROD1 and ROD2, Lb05 in RUN2 and Lb10 in RUN4. Observed heterozygosities ranged from 0.331 to 0.572 (mean $\pm$ s.e. $= 0.429 \pm 0.019$) for ROD2 and JUA4, respectively, and unbiased expected heterozygosities from 0.416 to 0.707 (mean $\pm$ s.e. $= 0.543 \pm 0.023$) for RUN1 and JUA4, respectively. Mean allelic richness per locus ranged from 2.919 ± 0.142 (s.e.) in RUN1 to 5.600 ± 0.510 (s.e.) in JUA4 and mean number of private alleles per locus ranged from 0.009 ± 0.009 (s.e.) in RUN1 to 1.691 ± 0.393 (s.e.) in NCA1. Multi-locus F_{IS} values were all significantly positive ($P<0.05$; Table 1) and ranged between 0.051*** for ROD1 and 0.341*** for MAD3. Over all loci, significant heterozygote deficiencies were found in all populations (after false discovery rate correction). For each locus in each population, the presence of null alleles was checked. Null alleles were detected for several loci in several populations. However, as (1) LD was not constant among loci, (2) not a single locus was monomorphic over all populations, (3) the number of loci with null alleles was not constant between populations and (4) in several cases, the F_{IS} value was significantly positive without evidence of null alleles, we decided to keep the ten loci presenting $<10\%$ of missing data for further analyses.

Genetic clusters

Population structuring was evidenced by results of DAPC and STRUCTURE analyses that showed genotypes clustering according to their geographic origin. STRUCTURE outputs revealed that the plot of LnP(D) as a function of K showed a clear plateau starting at $K=5$ (Figure 3a), the most probable number of clusters. Each cluster corresponded to a sampled island, except for MLGs from New Caledonia (Figure 3b). When analysing the genetic clustering of these

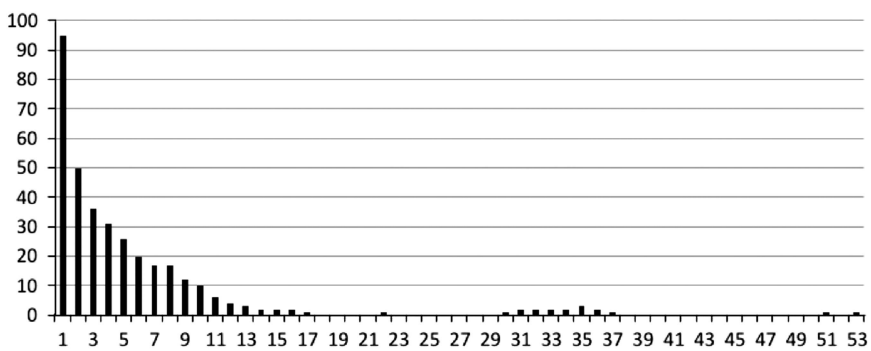

Figure 2 Distribution of the difference in field numbers (sequentially numbered sampling bags) between pairs of individuals presenting the same MLG.

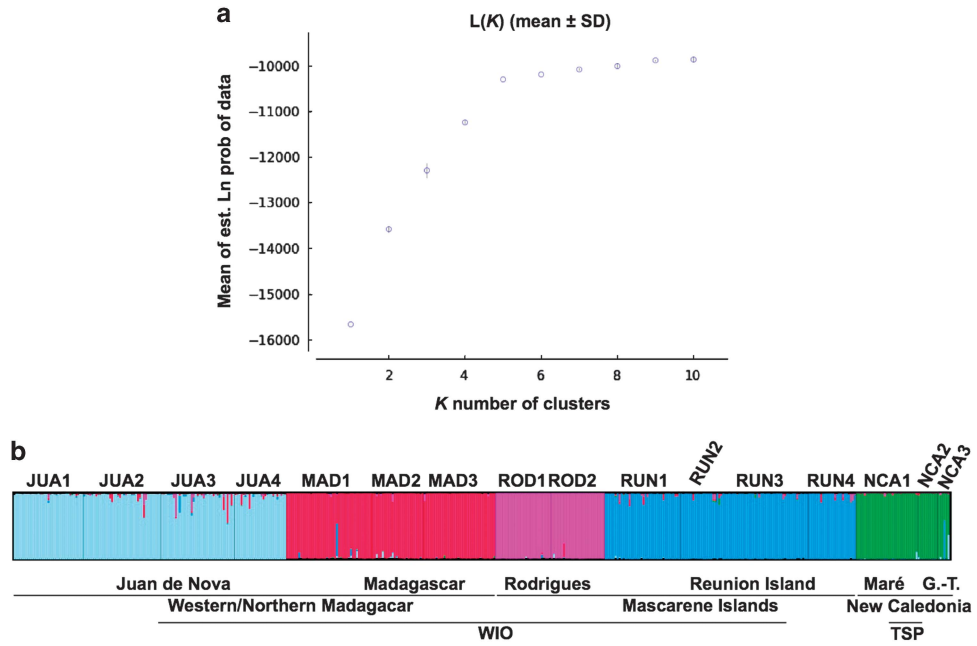

Figure 3 *L. brevirostris* α. Assignment probabilities of MLGs to putative clusters using an admixture model. (**a**) Mean LnP(*K*) values. (**b**) Average probability of membership (*y* axis) of MLGs ($N=470$, *x* axis) in $K=5$ clusters as identified by STRUCTURE. G.-T., Grande-Terre.

MLGs alone (data not shown), they clustered according to their island of origin, that is, Maré for NCA1 and west coast of Grande-Terre for NCA2 and NCA3.

DAPC also identified clusters corresponding to individuals sampled from the same island. In the successive values of *K* (number of cluster tested), the initial decline in BIC values slowed at $K=5$ (Figure 4a). As the three first axes explained > 95% of the variance, we decided to present the DAPC results in two ordination plots: (1) first and second axes, and (2) second and third axes. DAPC separated the WIO from the TSP along the first PCA axis (75.5% of variance; eigenvalue = 19656.61). The second axis explained less variability (11.2% of variance; eigenvalue = 19656.61), slightly separating WIO colonies from the TSP and plotting colonies from Reunion Island distant from those of other populations in the WIO (Figure 4b). The third axis (8.62% of variance; eigenvalue = 2244.09) separated colonies sampled in Rodrigues from those originating from Juan de Nova and Madagascar (Figure 4c). As in STRUCTURE, when analysing with DAPC the clustering scheme of MLGs only from New Caledonia, they clustered according to their island of origin.

Using provinces as groups and islands as populations, analysis of molecular variance revealed a highly significant genetic structuring among islands within provinces (WIO and TSP) and within islands ($P<0.001$; Table 2), but not between provinces. The genetic variation explained by differences among islands within provinces was higher than the genetic variation explained by differences among provinces (26.31%*** and 11.22%^{NS}, respectively) while the highest amount of genetic variation was found within islands (62.47%***).

Assessment of connectivity over different geographic scales and IBD

All pairwise differentiation tests were significant but none of the exact Fisher's tests (after false discovery rate correction). Pairwise F_{ST} values indicated a high and significant differentiation between populations (Table 3), ranging from 0.012*** to 0.560***. Concerning Reunion Island populations, the highest differentiation was found between RUN2 and RUN4 populations ($F_{ST}=0.203$***). In the WIO, the lowest differentiation occurred between populations from the same island (minimum $F_{ST}=0.013$*, between JUA1 and JUA2) and the maximum F_{ST} values occurred between populations ROD2 (Rodrigues) and RUN4 (Reunion Island) ($F_{ST}=0.560$***). Populations ROD1 and ROD2 were highly differentiated from all other populations of the WIO (F_{ST} ranged from 0.249*** to 0.560***). When comparing populations from the WIO with the population from New Caledonia, F_{ST} values were also high and highly significant, ranging from 0.297*** to 0.475*** (Table 3).

In general, population differentiation in *L. brevirostris* α was low between populations from the same island and high at every other scale. Mantel tests revealed a significant positive correlation between transformed F_{ST} values and the $\log_{10}$ of the geographic distances among sites both within the WIO province ($n=78$, $r=0.624$***, $R^2=0.390$) and within each of the two ecoregions, Western/Northern Madagascar and the Mascarene Islands ($n=21$, $r=0.977$***, $R^2=0.955$ and $n=15$, $r=0.957$***, $R^2=0.917$, respectively). Similarly, a strong pattern of IBD was evidenced at the Indo-Pacific scale ($n=91$, $r=0.639$***, $R^2=0.408$; Figure 5).

DISCUSSION

We investigated the genetic structure and connectivity among populations of a widely distributed hydrozoan species, *L. brevirostris* α (*sensu* Postaire *et al.*, 2016b) across three spatial scales in the Indo-Pacific region using a set of 16 microsatellite loci. The study revealed that populations of this brooding hydrozoan were characterized by low connectivity even at the smallest spatial scale (a few kilometres), presented an IBD pattern, and that the detected genetic clusters correspond to the sampled islands. Our results are congruent with those of the only other molecular study on marine hydrozoans, based on two markers, where high genetic differentiation was observed among populations (Schuchert, 2014, but see also Schuchert, 2005). Such pattern of low population connectivity may be typical for other marine species with similar life cycles.

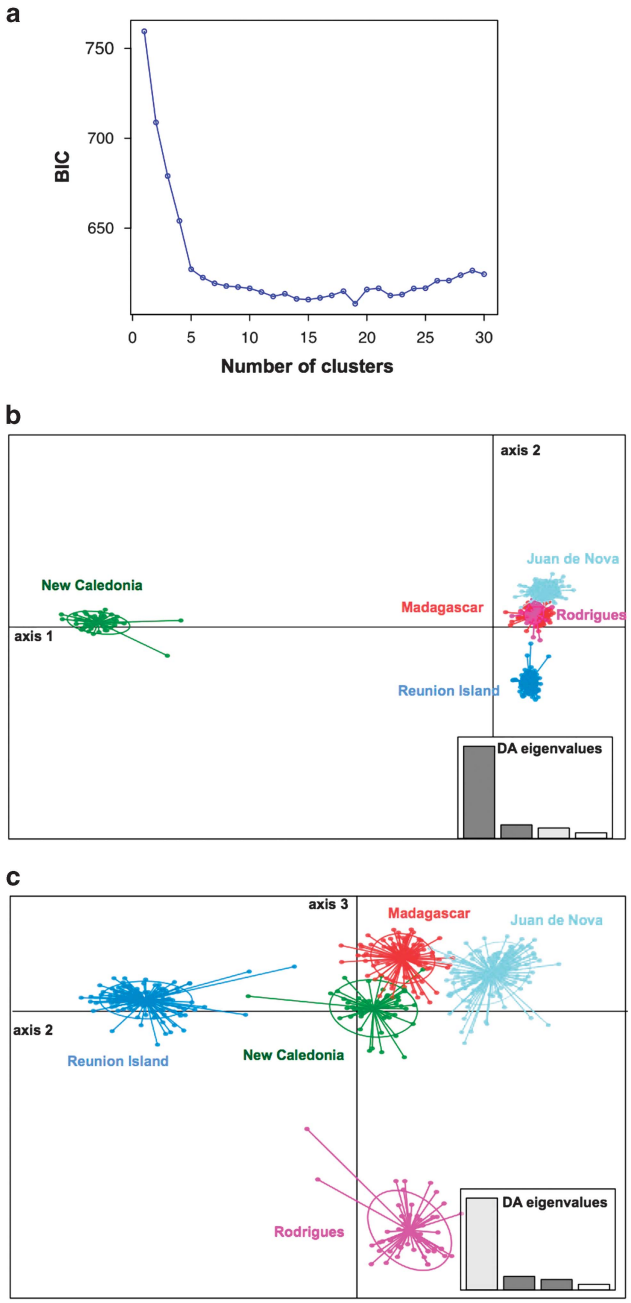

Figure 4 *L. brevirostris* α. DAPCs of MLGs sampled in the WIO and the TSP. (**a**) The BIC. (**b**) Scatter plots of the MLGs using the first and second components and (**c**) the second and third components.

Table 2 Analysis of molecular variance of *L. brevirostris* α samples grouped according to STRUCTURE clusters

Source of variation	*d.f.*	*% of variance*	P-*value*
Structure clusters $K=5$			
Among groups	4	29.85	<0.0001
Among populations within groups	9	4.34	<0.0001
Within populations	892	65.81	<0.0001

Populations grouped according to structure clusters.

High genetic differentiation of *L. brevirostris* α populations

Pairwise F_{ST} values (Table 3) revealed the high isolation of all sampled populations and highlighted the extreme differentiation of populations from Rodrigues and New Caledonia from all the others, underscoring the isolated position of Rodrigues in the WIO. Using microsatellites also allowed the identification of strong and significant genetic structuring at smaller geographic scales, indicating that gene flow is low even at distances less than 10 km (for example, between JUA2 and JUA4 populations from Juan de Nova Island). Private alleles were found in several populations, with the population from New Caledonia presenting the highest mean number of private alleles. The finding that individuals from several populations were difficult to amplify for certain loci (probably because of existing null alleles) supports the inference of high divergence among populations from the WIO and the TSP. Bayesian clustering and PCA analyses further confirmed the high isolation of *L. brevirostris* α populations across the Indo-Pacific as they identified sampled colonies from each island as putative populations, whereas the analysis of molecular variance showed that a high and significant proportion of the global differentiation occurred among islands. Furthermore, Mantel tests over several geographic scales revealed that population differentiation is related to geographic distance: the IBD pattern is detected at scales ranging from 100 to >1 000 km, but absent at the local scale. Supporting this general pattern of population isolation, genetic indices of diversity (mean allelic richness and heterozygosity) were slightly different between marine ecoregions: overall, populations from the Mascarene Islands present a slightly lower allelic richness and lower heterozygosity (Table 1). These dissimilarities might reflect differences in current selective pressures among islands, but might also attest to past climatic and geological events (sea level change) that modified the dynamics of the sampled populations, for example, population bottlenecks. This aspect merits further study.

Extremely high genetic differentiation between populations of a single species is unusual, although similar levels have been documented over large geographic scales in sponges (Chaves-Fonnegra *et al.*, 2015), coastal sharks (Ashe *et al.*, 2015) and marine mammals (Fruet *et al.*, 2014), but also at distances <100 km in terrestrial animals (Sethuraman *et al.*, 2013) or freshwater diatoms (Vanormelingen *et al.*, 2015). Our results indicate that the populations of *L. brevirostris* α present clear geographic boundaries and are consistent with two of the characteristics of a metapopulation model (Grimm *et al.*, 2003): (1) local populations have their own dynamics and (2) they are connected by limited dispersal.

Potential barriers to gene flow and limited dispersal

Our results clearly indicate that expanses of deep ocean waters represent a barrier to dispersal for *L. brevirostris* α, similar to some other coastal organisms with low dispersal capacities (for example, Ragionieri *et al.*, 2010; Aurelle *et al.*, 2011). First of all, each MLG was restricted to a single population. Moreover, within sampled populations, individuals close to each other (that is, presenting small difference in field numbers) presented a higher probability to share the same MLG, forming a colony (or genet). As colonies of *L. brevirostris* α can grow through stolonial expansion, sampled individuals sharing the same MLG were either connected through their stolon or represent distinct fragments of an ancient extended colony. Thus, colonies are spatially restricted, spanning a few centimetres or decimetres. This clonal range is quite narrow compared with some other clonal marine species, such as scleractinian corals (several kilometres (Baums *et al.*, 2006; Pinzón *et al.*, 2012; Japaud *et al.*, 2015)). Nevertheless, when restricting the data to one individual per

Table 3 ***L. brevirostris*** **α pairwise F_{ST} values (below diagonal) and geographic distance (in kilometres: above diagonal) for all pairs of populations**

Population	*JUA1*	*JUA2*	*JUA3*	*JUA4*	*MAD1*	*MAD2*	*MAD3*	*RUN1*	*RUN2*	*RUN3*	*RUN4*	*ROD1*	*ROD2*	*NCA1*
JUA1		15	9	13	748	696	719	1 388	1 389	1 398	1 434	2 202	2 190	12 704
JUA2	*0.013*		6	4	734	682	706	1 386	1 387	1 397	1 433	2 204	2 191	12 699
JUA3	0.019	0.025		6	470	688	712	1 388	1 388	1 397	1 433	2 204	2 191	12 701
JUA4	*0.020*	*0.014*	0.039		735	683	707	1 383	1 383	1 393	1 429	2 199	2 187	12 696
MAD1	0.216	0.202	0.218	0.203		53	29	1 234	1 230	1 234	1 258	2 097	2 081	12 208
MAD2	0.226	0.215	0.222	0.200	0.122		24	1 226	1 223	1 227	1 253	2 091	2 076	12 240
MAD3	0.238	0.212	0.226	0.215	0.039	0.075		1 225	1 221	1 225	1 250	2 089	2 073	12 221
RUN1	0.341	0.328	0.323	0.300	0.336	0.316	0.337		8	19	53	867	852	11 349
RUN2	0.300	0.288	0.291	0.265	0.328	0.327	0.332	0.073		13	49	870	855	11 347
RUN3	0.338	0.326	0.321	0.293	0.330	0.305	0.335	*0.016*	0.102		38	865	850	11 336
RUN4	0.361	0.343	0.343	0.322	0.337	0.322	0.344	0.112	0.203	0.117		839	823	11 298
ROD1	0.303	0.277	0.270	0.249	0.464	0.431	0.443	0.489	0.508	0.467	0.540		17	10 657
ROD2	0.322	0.296	0.298	0.272	0.486	0.450	0.482	0.516	0.537	0.489	0.560	0.162		10 665
NCA1	0.337	0.331	0.338	0.298	0.426	0.400	0.421	0.417	0.410	0.404	0.452	0.447	0.475	

All values were highly significantly different from 0 ($P<0.001$) after false discovery rate correction, except for four values (in italics) with $0.001<P<0.05$.

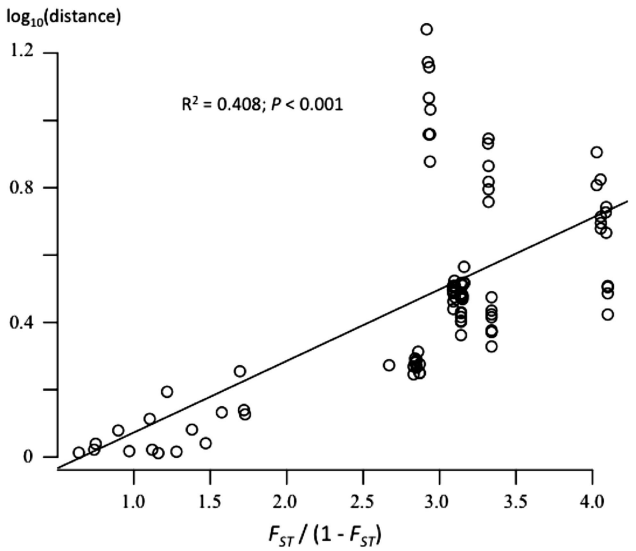

Figure 5 *L. brevirostris* α. Correlation between genetic distances computed as $F_{ST}/(1-F_{ST})$ and the $\log_{10}$ of geographical distances (in kilometres) between site pairs at the in the WIO and the TSP.

colony, all populations showed a significant deviation from Hardy–Weinberg equilibrium due to heterozygote deficiency. Heterozygote deficiency is relatively common in marine colonial organisms, such as scleractinian corals (Ayre and Hughes, 2000; Baums *et al.*, 2005; Underwood *et al.*, 2007; Ridgway *et al.*, 2008), but the exact mechanism driving this effect cannot always be determined (inbreeding or spatial/temporal Wahlund effect). Considering the reproductive strategy of *L. brevirostris* α (absence of medusa stage, internal fertilization of eggs, larviparity), this pattern might be explained by restricted dispersal of gametes and/or larvae. Indeed, observations of larval behaviour in hydrozoans suggest that dispersal after planulae release is low: they tend to settle nearby their mother colony (Sommer, 1990), thus favouring fertilization between related individuals. Furthermore, observations on hydrozoans in aquaria indicate that the life span of male gametes in the water column is only a few hours (Yund, 1990), limiting long distance dispersal and thus gene flow among distant colonies.

The isolation between WIO and TSP populations was quite expected as these two regions are >10 000 km distant (Figure 1). Indeed, several studies found such an Indian Ocean-Western Pacific disjunction (Kochzius and Nuryanto, 2008; Yasuda *et al.*, 2009; Richards *et al.*, 2016). For westward migration, *L. brevirostris* α propagules originating from New Caledonia would have to survive in the plankton for several months to disperse into the WIO. In the opposite direction, propagules from the WIO should be able to survive a pelagic environment a long time but also withstand unfavourable oceanic conditions, as they would migrate along the temperate southern coast of Australia to reach New Caledonia (Schott *et al.*, 2009). Given the reproductive mode of *L. brevirostris* α, such migrations would be extremely rare events. At smaller geographic scales, within the WIO, previous population genetics studies revealed that oceanic gyres isolate Juan de Nova Island from the Southern part of the Mozambique Channel (Bourjea *et al.*, 2006; Krishna *et al.*, 2006; Muths *et al.*, 2011); our results support these findings. In the Mascarene Islands, the Southeast Madagascar current (Schott *et al.*, 2009) could allow connectivity between Rodrigues and Reunion Island populations, but our results indicate that such gene flow is absent or extremely low. Marine circulation models at even smaller scales (that is, around islands and coastal areas) are still under development for most of the sampled islands. Nevertheless, a model of oceanic circulation around Reunion Island indicates high heterogeneity in the direction of currents across seasons and years (Pous *et al.*, 2014), partly explaining the absence of IBD at this geographic scale, because reproduction has been observed throughout the year (BP, HM and CAF Bourmaud, personal observation). To our knowledge, no detailed population genetic study of a marine hydrozoan species presenting biphasic life cycle (that is, medusa and fixed colonial stages) using microsatellites exists yet to compare our findings. However Schuchert (2005), using a mitochondrial marker, found that the genetic diversity of a hydrozoan species with a medusa stage (*Coryne eximia*; Allman, 1859) was modest over wide geographic distances (several oceans) when compared with monophasic species of the same genus (*Coryne muscoides* (Linnaeus, 1761) and *Coryne pintneri* (Schneider, 1898)), supporting the importance of a long-lived planktonic medusa stage in the dispersal capacities of hydrozoans. This aspect needs to be further explored by modelling gene flows in correlation with ocean circulation models, that is, seascape genetics, an approach that has already shown

merit to explain patterns of connectivity in marine organisms, such as bivalves, crustaceans and scleractinians (Treml *et al.*, 2007; Ragionieri *et al.*, 2010; Thomas *et al.*, 2012).

Our results suggest that ocean circulation plays a minor role in determining spatial patterns of genetic differentiation in *L. brevirostris* α populations of the WIO and the TSP. Instead, short-distance exchange of gametes, larval brooding and restricted movements of larvae seem to be mostly responsible of the observed pattern, as they tend to favour small-scale genetic differentiation (within populations or islands). The wide Indo-Pacific distribution of *L. brevirostris* α is better explained by rafting of adult colonies fixed on floating objects. According to Thiel and Gutow (2005), Aglaopheniidae present several life traits enhancing their capacity of travelling through rafting: they can cling on various substrata and notably on natural or artificial floating items (BP and HM, personal observation) and adult individuals feed on plankton, a common pelagic resource. In addition, their capacity of clonal growth facilitates the colonization of new suitable habitats. Thus, even if rafting events are rare, they might occur at a sufficient rate to colonize new islands and explain the repartition of the clade formed by *L. brevirostris* α. This assumption is supported by the presence of both cryptic species of *L. brevirostris* α and β in the WIO and the TSP (Postaire *et al.*, 2016b). The importance of rafting in the marine environment, above all in species presenting direct development, is increasingly recognized (DeVantier, 1992; Johnson *et al.*, 2001; Thiel and Gutow, 2005; Thiel and Haye, 2006; Rocha *et al.*, 2006).

Implications for hydrozoan taxonomy and marine conservation

Our study also provides some taxonomic clues. Indeed, the extensive population subdivision in *L. brevirostris* α is concordant with the phylogeny based on mitochondrial and nuclear markers of this species (Postaire *et al.*, 2016b) and reveals that populations inhabiting the same marine ecoregions (as defined by Spalding *et al.*, 2007) represent independent evolutionary units or even species when considering the genealogical species concept (Baum and Shaw, 1995). As they match several metapopulation characteristics, these groups of populations might actually represent lineages engaged in a speciation process but situated in the 'grey zone' (De Queiroz, 1998, 2005), that is, the evolutionary time during which two lineages are definitively diverging but where criteria commonly used for identifying divergence might not be applicable or in agreement (Pante *et al.*, 2015). As both *L. brevirostris* cryptic species (α and β) present an Indo-Pacific distribution (Millard, 1975; Postaire *et al.*, 2016b), the observed extensive population differentiation within *L. brevirostris* α may reflect very limited gene flow between groups of populations but sufficient to prevent allopatric speciation. On the contrary, the extensive geographic distribution might be the testimony of the antiquity of these clades, each being composed of several species (Le Gac *et al.*, 2004). Indeed, almost all clusters found in this study correspond to monophyletic groups (Postaire *et al.*, 2016b), underlining the low dispersal of *L. brevirostris* α. Controlled crosses are necessary to asses whether these clusters correspond to biological species or interfertile groups with disjunct distribution ranges leading to effective absence of gene flow. Thus, given the high population differentiation and the importance of IBD over several geographic scales in *L. brevirostris* α, a taxonomic revision of *L. brevirostris* might consider individuals from each sampled island as a potential new species (Pante *et al.*, 2014, 2015). The apparent absence of morphological clues, a criterion already known to poorly describe the diversity of Aglaopheniidae (Leclère *et al.*, 2007; Moura *et al.*, 2012; Postaire *et al.*, 2016a; Postaire *et al.*, 2016b), can be explained by the maintenance of similar selective pressures and ecologies (Le Gac *et al.*, 2004).

CONCLUSIONS

This study is one the few presenting data from such an extended geographic scale, notably including the eastern margin of the WIO province (Rodrigues). Our study particularly underlines the population isolation in this brooding species, each island potentially representing an independent metapopulation with high dependence on local recruitment for their maintenance. We believe that our approach provides valuable information for the management and creation of marine protected areas in these particular regions. Indeed, the design of marine reserve networks requires an understanding of effective dispersal (that is, transport, recruitment and reproduction) over several scales to apprehend whether populations in reserves are open or self-recruiting and whether reserve networks can exchange recruits (Briggs, 2005; Jones *et al.*, 2009; Christie *et al.*, 2010). Considering our results, connectivity of populations between islands or even between reefs on the same island might be extremely low for species without long planktonic life phase, underlining the need for multiple protected areas to preserve evolutionary dynamics in these species (Briggs, 2003, 2005; Obura, 2012a, b). In this view, the conservation of the coral reefs of Rodrigues seems particularly important in view of their genetic isolation from other populations of the WIO and the TSP. Future studies on other key benthic marine species, such as scleractinians, molluscs or echinoderms may add support to these findings from hydrozoans.

DATA ARCHIVING

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.s866f.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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