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A lack of spatial genetic structure of *Gymnothorax chilospilus* (moray eel) suggests peculiar population functioning

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Moray eels form a speciose lineage that belongs to Elopomorpha, a super-order with a worldwide distribution. As is the case for many anguilliform fish, moray eels are characterized by distinctive life history traits, notably a prolonged larval dispersal phase that determines settlement to distant reef habitats, thus influencing the spatial structuring of their populations. They can be very abundant in coral reef ecosystems and represent major meso-predators, playing a crucial role in food webs. Yet, due to their elusive nature (i.e. cryptic behaviour, nocturnal activity), these organisms are generally difficult to study and collect. To our knowledge, only a few studies performed over large geographical and phylogenetic scales have been conducted. We used a unique and cost-efficient sampling approach, involving forced regurgitation from sea snake predators, to collect large numbers of a widespread moray eel species (*Gymnothorax chilospilus*). When combined with the development of 11 new microsatellite markers, this efficient sampling technique allowed us to examine the genetic structure of *Gymnothorax* populations occurring in the South Lagoon of New Caledonia. Analyses revealed a lack of genetic differentiation among populations. This result echoes the strong genetic homogeneity of populations of their main predator, the sea snake. This convergence might result from a distinctive trait involved in population functioning of both moray eels and sea snakes, where immature individuals emerge from common breeding grounds and disperse over long distances before settlement.

ADDITIONAL KEYWORDS: 454 pyrosequencing – microsatellites – dispersal – larval stage – Muraenidae – New Caledonia.

INTRODUCTION

Moray eels belong to the anguilliform fish order and form a speciose lineage that belongs to the super-order of the Elopomorpha (Santini *et al.*, 2013; Chen *et al.*, 2014; Coluccia *et al.*, 2015). They are widely distributed and display high variation in body size, colour patterns, diet and feeding structures (Mehta & Wainwright, 2007). Despite this diversity, moray eels are characterized by a set of consistent and distinctive life history traits: they are elongated, lack pelvic fins and disperse over vast oceanic distances during a prolonged planktonic leptocephali larval stage, which usually exceeds 100 days (Reece *et al.*, 2010a; Reece & Mehta, 2013). The leptocephali larvae exhibit a unique morphology and ecology (Mochioka & Iwamizu, 1996). Following

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metamorphosis and settlement into benthic habitats, most moray eels shift from a vagrant to a sedentary, highly secretive life style. Their cryptic habits help to explain why their ecological importance has been overlooked until recently (Ineich *et al.*, 2007). In coral reefs, moray eels can be very abundant and are key mesopredators, feeding on a wide diversity of vertebrates (e.g. fish) and invertebrates (e.g. crustaceans, annelids, cephalopods) (Brischoux & Bonnet, 2008; Briand *et al.*, 2016). Additionally, moray eels accumulate traces of heavy metals and organic pollutants and provide essential information regarding contaminations present in their habitats (Bonnet *et al.*, 2014; Briand *et al.*, 2014). However, difficulties involved in observing and collecting free-living individuals have impeded the establishment of mark–recapture studies, thus inhibiting the assessment of population size, population structure, population viability and demographic traits. Recruitment from dispersing planktonic cohorts, which sustain settled populations, cannot be evaluated.

Some of these difficulties can be circumvented with genetic markers. We used molecular microsatellite markers to examine populations of a widespread moray eel, *Gymnothorax chilospilus*. Abundant raw material is needed but moray eels are not easily sampled in the field (Ineich *et al.*, 2007). To get around this issue, we used sea snake sampling, a cost-efficient approach (Bonnet, 2012). Sea kraits (sea snakes) are top predators that feed almost exclusively on anguilliform fish, notably moray eels (Reed *et al.*, 2002; Brischoux *et al.*, 2007). Large numbers of moray eels can be collected across large areas via forced regurgitation of these sea snakes (Bonnet, 2012). Additionally, because most prey items are intact or poorly digested, we are able to collect abundant material for DNA analyses (Brischoux & Bonnet, 2008).

We successfully developed a set of microsatellite markers on *G. chilospilus*, a species largely consumed by sea kraits (Brischoux *et al.*, 2007). We then searched for possible spatial genetic structure (SGS) among moray eel populations sampled in the South Lagoon of New Caledonia. The remarkable breeding system of moray eels, involving a prolonged planktonic larval stage prior to settlement, may favour genetic mixing among populations if leptocephali disperse randomly. However, canalized propagule dispersal and natal homing may result in significant spatial structuring of the populations, as shown in marine organisms where planktonic propagules disperse over great distances (Grosberg & Quinn, 1986; Blanquer *et al.*, 2009). In ascidians, kin recognition among settling larvae leads to the formation of genetically distinct colonies (Grosberg & Quinn, 1986); in sponges, the philopatric dispersal of larvae can promote 'isolation by distance' (Blanquer *et al.*, 2009); and in many anadromous fish and whales, natal homing maintains SGS (Hasler & Scholz, 1983; Baker *et al.*, 2013). Thus, assessing possible existence vs. lack of SGS among moray eel populations is necessary to better understand population functioning of these cryptic and major predators.

MATERIAL AND METHODS

Moray eel sampling

A wide range of moray eel species occur in large numbers in the reef ecosystems of New Caledonia (Ineich *et al.*, 2007). We collected, during a long-term field study, more than 2000 individuals of approximately 50 species of anguilliform fish (Brischoux *et al.*, 2007; Bonnet, 2012; Briand *et al.*, 2014). Sea kraits (sea snakes, genus *Laticauda*) are abundant in the coral reefs of the western Pacific Ocean. They are specialized predators of cryptic anguilliform fish, notably moray eels. Prey aminals were obtained by gentle forced regurgitation and were often intact or only partly digested, as sea kraits swallow their prey whole. After each prey item was cleaned with purified freshwater, a tissue sample of 0.2– cm0.5 cm^3 of dorsal muscle was collected using a sterile scalpel and subsequently preserved in 90% ethanol. Multiple prey species are rarely mixed in the stomach of snakes, thus insuring that items were accurately assigned to a single species.

We used a subsample of 89 *Gymnothorax chilospilus* (Bleeker, 1865), a major prey animal of sea kraits (Brischoux & Bonnet, 2008), collected from nine islets of the South-West Lagoon of New Caledonia (Fig. 1). As sea kraits forage around their home islet, and both sea kraits and their prey are highly sedentary, we assumed that their prey were representative of moray eel populations living within a broad radius of 5–10 km around each sampling site (Brischoux *et al.*, 2007, 2009). Thus, we grouped moray eels from sites in close proximity to one another and obtained four main sampling areas, each of which included at least 17 specimens (Fig. 1, Table 2). Great distances (> 25 km) and various obstacles (e.g. discontinuous reefs, vast unfavourable sandy bottoms, deep floors) separate the four main sampling areas (details in Fig. 1 and Andréfouët *et al.*, 2004). Adult moray eels are sedentary, moving less than 1 km around their home reef, and occupy small home ranges (~20 ha; Pereira *et al.*, 2017). Consequently, we estimated that the moray eels sampled in each of the four areas, spread across more than 350 000 ha (Fig. 1), represented a distinct population.

We also considered *G. eurostus* (N = 3), *G. fimbriatus* (N = 8) and *G. undulatus* (N = 6), three species commonly consumed by sea kraits, to test the applicability

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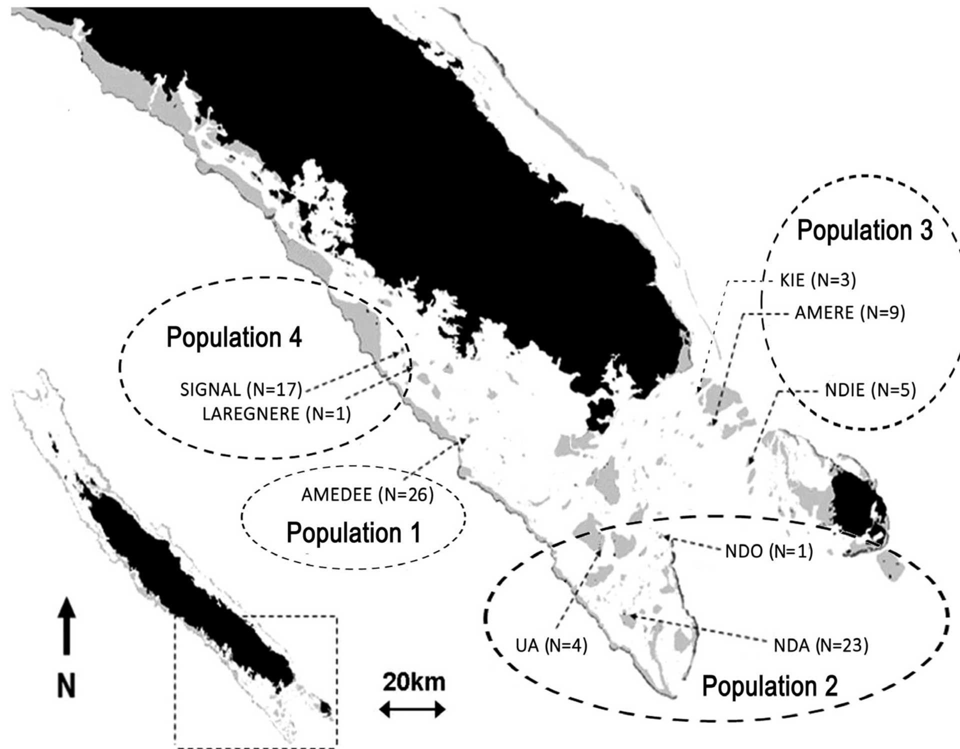

Figure 1. Map of South Lagoon of New Caledonia with the nine sampled sites, the number of moray eels (*Gymnothorax chilospilus*) collected at each site, and the four corresponding populations. The land areas are represented in black, coral reefs in light grey and the limit of the lagoon in grey. Three populations were represented by more than one sampling islet (populations 2, 3 and 4). However, in population 1, numerous small islets (not easily visible at this scale) are spread among the three islets considered without major obstacles between them (e.g. deep channel) and thus they were considered relatively interconnected (Andréfouët *et al.*, 2004). Similarly, the three islets of population 3 belong to a continuous reef superstructure. However, populations 2 and 3 are separated by deep water with little favourable habitat for *Gymnothorax* species (hard reefs) and the barrier reef is interrupted. The same rationale applies for the four populations (e.g. presence of a deep channel between populations 1 and 4).

of the microsatellite markers across *Gymnothorax* species (Brischoux *et al.*, 2007).

Microsatellite isolation and amplification

We isolated microsatellite markers using 454 pyrosequencing. For genomic library construction, we extracted DNA in *G. chilospilus* from 89 individuals using a desalted-out protocol (Miller *et al.*, 1988). We then measured DNA concentration using a Nanodrop ND-1000 device, and we sent the samples to Genoscreen (Lille, France; www.genoscreen.fr) to construct a microsatellite-enriched genomic library; eight of these extraction products displayed in an equimolar amount. To maximize the genomic diversity detected by our study and to encompass the geographical sites sampled, we randomly selected two individuals collected from each of the four sampled populations. Using samples from these individuals, Genoscreen constructed a microsatellite-enriched genomic library through a partial 454 GS FLX sequencer run with titanium chemistry, as previously described by Malausa *et al.* (2011). Briefly, total DNA was mechanically fragmented and enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT and ATCT repeat motifs. The resulting reads were analysed with the QDD software (Meglécz *et al.*, 2010) to identify reads containing microsatellite motifs, as well as to design primers for PCR amplification.

Among the 220 reads containing microsatellite motifs, Genoscreen designed and selected a panel of 47 primer pairs, mainly according to size (i.e. higher than 100 bp) and the number of repeated microsatellite motifs of amplified fragments (Supporting Information, File S1). Each PCR contained 0.5 U Taq polymerase (Fast-Start Taq kit, Roche, Basel, Switzerland), 6 pmol dNTP (FastStart Taq kit), 37.5 pmol $MgCl_2$, 10 pmol each of primers, and 1 μL of extracted DNA. Cycling conditions consisted of an initial denaturation of 10 min at 95 °C, then 40 cycles

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Table 1. Names and sequences of primers, number of repetitions and motif of the microsatellite molecular markers, size range of amplified fragments, label dyes and associated poolplex of different molecular markers

Marker name	Forward primer	Reverse primer	Number of repetitions	Repetition motif	Size range (bp)	Label dyes	Poolplex
GYMC02	AAAGAAGCCCAAAGTCGTGT	GAGGACCCACATACGAAGGA	5	tct	153–191	6FAM	2
GYMC04	TGTCATTCTGTGTCTTGGGG	CAAACGGCCAATCAGTGACA	14	tc	217–291	NED	3
GYMC11	ACTCCTCCCCCGTCTCCTC	ATCTGAAGGTTTACGTGGCG	7	tcc	165–201	6FAM	3
GYMC17	TGTAAACAGTGCGAGCCTTG	TCTCCGTGAGTCAGCTTGAA	16	ac	129–167	PET	3
GYMC19	ACTATTCCGGAGAGCATGCT	TGTGTGCAGCAACCAAAGC	5	tct	121–169	PET	2
GYMC24	TGCATCCCTTGATCATCCATC	TACAAGCCGCGAGAGTTTTG	11	atct	94–158	PET	4
GYMC30	TTTGTCTTGCAGCCCTGGTT	ACAGGGCTGCAATACTGACT	6	cag	109–124	VIC	1
GYMC32	CTTCTGTCCTCGGGTCTCTG	TCCTTGCTTTCCTGACTCGT	14	tc	81–171	VIC	2
GYMC33	GGAGACAATCAGATATTCCGCC	TGCGCCAGTATGAAGGATCT	6	tta	199–238	NED	4
GYMC39	CTGTTCTTCCGCAGTCTGTG	ACAAACAGGCAGAAAACCCC	5	caa	256–289	6FAM	1
GYMC43	AAGATCACGACGAGGGCTTC	ATGAAGACGTGACAGAGGCA	5	gag	206–223	NED	2
GYMC47	GCCCTGCATTGTGTTGTAC	GCAAAAGTGAGGCAAGGTGA	5	ttg	111–127	VIC	3
GYMC48	GTTATGGGGACTGGACAGGA	TCTGTAGCCGACCCTATGCT	8	tga	246–294	VIC	4

of 30 s at 95 °C, 30 s at the annealing temperature of 55 °C, followed by elongation for 1 min at 72 °C and a final elongation step of 10 min at 72 °C. Amplified products were then resolved on electrophoresis gel (2% agarose). For microsatellite markers that produced an amplification band with a sufficient amplification success rate and which seemed to be polymorphic, we ordered forward primers with labelled dyes (6_FAM, HEX, PET, VIC or NED).

We then tested the DNA amplification from these selected labelled primer pairs by resolving the resulting amplicons by electrophoresis on an ABI PRISM 3130 Genetic analyser. PCRs were performed, in simplex, using 0.625 U Taq polymerase (G2, Hot Start, Promega, France), 400 µM of each dNTP (Euromedex), 3.5 µM $MgCl_2$, 0.5 µM primers and 1.5 µL of extracted DNA. We added 2 µL of a 1:100 dilution of PCR product to 9 µL formamide and 0.5 µL 600 LIZ standard (Life Technologies) and visualized it by electrophoresis on an ABI PRISM 3130 Genetic analyser. We determined amplification product size using Genemapper software (Applied Biosystems), followed by visual verification. We then selected microsatellite markers yielding a specific amplification, a sufficient amplification success rate and a correct polymorphism. To maximize efficiency and minimize cost, we pooled amplification products in four poolplexes according to their amplified fragment sizes and dyes (File S1).

Genetic polymorphism

We investigated the genetic polymorphism of the four populations of *G. chilospilus* using the microsatellite marker panel. We used Microchecker v.2.2.3 (Oosterhout *et al.*, 2004) to detect signs of null alleles or scoring errors due to stuttering. Departure from Hardy–Weinberg expectations and linkage disequilibrium were assessed for each selected microsatellite marker using exact tests (1200 permutations), as implemented in FSTAT v.2.9.3.2 (Goudet, 2001). We used standard Bonferroni corrections to adjust analyses involving multiple tests (Rice, 1989). Then, genetic variability and transferability of microsatellite markers were further assessed by their allele number (N_a), observed and expected heterozygosity (H_o and H_e), and F_{is}, all of which were computed using FSTAT v.2.9.3.2 (Goudet, 2001).

Spatial genetic structure

We removed molecular markers showing both departures from Hardy–Weinberg expectations and null alleles, and estimated pairwise F_{st} values between populations according to Weir & Cockerham (1984) using FSTAT v.2.9.3.2 (Goudet, 2001). Their associated

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Table 2. Genetic diversity parameters for each locus

		Pop 1	Pop 2	Pop 3	Pop 4	All
Geographical coordinates	X (wgs84-utm58S)	651047	693700	709669	650925	–
	Y (wgs84-utm58S)	7513600	7483339	7513383	7526341	–
Loci names	N	26	28	17	18	89
GYMC02	N All	1	2	1	2	2
	H_e	0	0.036	0	0.269	0.076
	H_o	NA	0.036	NA	0.307	0.082
	F_{is}	NA	0	NA	–0.143	–0.072
	Null alleles	no	no	no	no	–
GYMC4	N All	14	16	14	12	20
	H_e	0.936	0.925	0.937	0.924	0.931
	H_o	0.792	0.927	0.857	0.833	0.853
	F_{is}	0.154	–0.002	0.085	0.098	0.084
	Null alleles	no	no	no	no	–
GYMC11	N All	6	5	5	5	6
	H_e	0.585	0.587	0.712	0.648	0.633
	H_o	0.480	0.500	0.500	0.500	0.498
	F_{is}	0.179	0.148	0.298	0.228	0.213
	Null alleles	no	no	no	no	–
GYMC17	N All	12	12	11	10	14
	H_e	0.917	0.91	0.918	0.869	0.904
	H_o	0.790	0.962	0.857	0.750	0.839
	F_{is}	0.139	–0.057	0.066	*0.137*	0.071
	Null alleles	no	no	no	no	–
GYMC19	N All	4	6	5	6	8
	H_e	0.235	0.376	0.255	0.732	0.40
	H_o	0.167	0.269	0.200	0.273	0.258
	F_{is}	*0.29*	0.284	0.215	*0.627*	0.35
	Null alleles	no	no	no	**yes**	–
GYMC24	N All	5	6	5	6	7
	H_e	0.686	0.605	0.656	0.849	0.699
	H_o	0.167	0.269	0.500	0.143	0.283
	F_{is}	*0.757*	0.555	0.238	*0.832*	0.596
	Null alleles	**yes**	**yes**	no	**yes**	–
GYMC30	N All	3	4	6	7	7
	H_e	0.251	0.202	0.614	0.604	0.418
	H_o	0.038	0.215	0.235	0.437	0.243
	F_{is}	*0.847*	–0.062	*0.617*	0.276	0.420
	Null alleles	**yes**	no	**yes**	no	–
GYMC33	N All	11	11	9	11	12
	H_e	0.891	0.883	0.89	0.939	0.901
	H_o	0.454	0.584	0.600	0.385	0.508
	F_{is}	0.49	0.339	0.326	0.59	0.436
	Null alleles	**yes**	**yes**	**yes**	**yes**	–
GYMC39	N All	4	5	4	3	5
	H_e	0.363	0.596	0.481	0.55	0.498
	H_o	0.192	0.482	0.375	0.312	0.334
	F_{is}	*0.47*	0.192	0.221	0.432	0.329
	Null alleles	**yes**	no	no	**yes**	–
GYMC43	N All	6	5	5	4	6
	H_e	0.609	0.456	0.779	0.662	0.627
	H_o	0.520	0.464	0.312	0.334	0.435
	F_{is}	0.146	–0.017	*0.599*	0.496	0.306
	Null alleles	no	no	**yes**	**yes**	–

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Table 2. (Continued)

		Pop 1	Pop 2	Pop 3	Pop 4	All
GYMC48	*N* All	11	10	8	8	12
	H_e	0.904	0.873	0.792	0.781	0.838
	H_o	0.583	0.454	0.584	0.600	0.559
	F_{is}	***0.355***	***0.48***	0.263	0.232	0.333
	Null alleles	**yes**	**yes**	no	no	–
GYMC47	*N* All	5	5	4	4	5
	H_e	0.768	0.786	0.712	0.533	0.700
	H_o	0.200	0.107	0.385	0.176	0.222
	F_{is}	***0.74***	***0.864***	0.459	***0.669***	0.683
	Null alleles	**yes**	**yes**	**yes**	**yes**	–
GYMC32	*N* All	17	15	12	14	20
	H_e	0.948	0.928	0.937	0.94	0.938
	H_o	0.435	0.440	0.428	0.500	0.451
	F_{is}	***0.541***	***0.526***	0.543	0.468	0.520
	Null alleles	**yes**	**yes**	**yes**	**yes**	–
ALL	*N* All	7.62	7.85	6.85	7.08	7.35
	H_e	0.623	0.628	0.668	0.715	0.658
	H_o	0.370	0.439	0.449	0.427	0.422
	F_{is}	0.405	0.301	0.328	0.403	0.359

N: number of analysed individuals per population, H_e: expected heterozygosity, N All: number of alleles and F_{is} have been computed for each population and each loci using FSTAT v.2.9.3.2 software (Goudet 2001). In italic and bold: F_{is} values showing significant departures from Hardy–Weinberg expectations (i.e. significantly different from 0; $P < 0.0009$ after Bonferroni adjustment). We used Micro-checker v.2.2.3 (Oosterhout *et al.*, 2004) to detect signs of null alleles. In grey, both markers removed for genetic structure analyses.

Table 3. Pairwise F_{st} values for each population comparison (below diagonal) and associated *P*-values (above diagonal) computed using FSTAT v.2.9.3.2 software (Goudet, 2001); no genetic differentiation was significant [indicative adjusted nominal level (5%) for multiple comparisons is: 0.008333]

	Pop 1	Pop 2	Pop 3	Pop 4
Pop 1	–	0.3917	0.2750	0.0250
Pop 2	0.0105	–	0.4917	0.0500
Pop 3	0.0043	0.0209	–	0.0667
Pop 4	0.0687	0.0735	0.0405	–

significances were computed and tested using global tests implemented in FSTAT v.2.9.3.2 (Goudet, 2001), with standard Bonferroni corrections applied to cases involving multiple tests. We also accessed the genetic structure using the individual-based approach implemented in the STRUCTURE software (Pritchard *et al.*, 2000). This Bayesian clustering approach estimated both the number *K* of genetic cluster(s) and the admixture coefficient of individuals to be assigned to each inferred cluster. We selected the admixture model and the option of correlated allele frequencies among populations. As recommended by Evanno *et al.* (2005), we replicated 20 independent runs for each value of *K* (with *K* varying from 1 to 10) with a total number of 1 000 000 iterations and a burn-in of 10 000. To determine the number of genetic clusters from STRUCTURE analyses, we used the program STRUCTURE HARVESTER (Earl & VonHoldt, 2011) to compare the mean likelihood and variance per *K* values computed from the 20 independent runs.

RESULTS

Microsatellites

From the 47 primer pairs tested by Genoscreen, 38 were successfully amplified. Electrophoresis (ABI PRISM 3130 Genetic analyser) revealed that ten primer pairs had amplified an unspecified product, 11 showed poor amplification success (i.e. < 50%) and four were monomorphic (File S1). Thus, we first retained 13 polymorphic microsatellite markers (Table 1). The number of alleles per locus ranged from two to 20 and gene diversity (i.e. H_e) ranged from 0 to 0.948 (Table 2). No linkage disequilibrium was detected (adjusted *P*-value threshold = 0.0005), but two loci (i.e. GYMC32 and GYMC47) showed evidence of both null alleles and deviations from Hardy–Weinberg expectations (adjusted *P*-value threshold = 0.0008). These two were discarded. In the remaining loci, we detected sporadic allele nulls not associated with genotyping errors or

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deviations from Hardy–Weinberg expectations, suggesting that these characteristics probably resulted from a homozygosity excess inherent to the analysed populations. Thus, we selected these remaining 11 microsatellite loci.

Spatial genetic structure

We found no evidence for genetic structure, as low F_{st} values revealed no significant genetic differentiation among populations of *G. chilospilus* (Table 3). STRUCTURE and STRUCTURE HARVESTER analyses inferred the highest ΔK value for $K = 3$. However, ΔK was very low and inferences of the individual admixture coefficients suggested similar *q* values for two of three inferred genetic clusters. The ΔK method often fails to find the best K if $K = 1$. Thus, the resulting patterns did not converge toward any significant genetic structure, in agreement with the low F_{st} results.

DISCUSSION

Previous studies assessed phylogenetic and phylogeographic questions involving moray eel populations at broad spatial and time scales using mitochondrial cytochrome *b* genes and nuclear genomic sequences of *RAG-1* and *RAG-2* genes (Reece et al., 2010a, b, 2011; Du *et al.*, 2016). In this study, for the first time, the genetic structure of four populations of *Gymnothorax chilospilus* was examined at a relatively fine spatial scale thanks to a cost-efficient and spatially precise sampling technique, as well as a panel of polymorphic molecular markers. These markers were sufficiently polymorphic and the sample size in each of the main areas was appropriate to establish a lack of population genetic structure.

Among the molecular microsatellite markers selected for *G. chilospilus*, nine were successfully cross amplified in the three other species tested, namely *G. eurostus*, *G. fimbriatus* and *G. undulatus* (File S2). The genus *Gymnothorax* includes most moray eel species (Tang & Fielitz, 2013). In many of the coral reefs of the western Pacific Ocean, over 30 species of these fish are heavily consumed by large numbers of sea kraits. Thus, sea snake sampling is an efficient technique to collect large samples of such fish (Brischoux *et al.*, 2007). This method of forced regurgitation has the potential to provide novel insights into the functioning of populations of a particularly elusive, albeit widespread and abundant, group of predatory fish. Understanding these populations is particularly important because *Gymnothorax* eels are themselves major mesopredators, and they play a central role in coral reef food webs (Briand *et al.*, 2016).

Despite widespread sampling within the lagoon, analyses indicated that the moray eel populations involved in this study were genetically homogeneous in structure, with no significant genetic differentiation between pairwise comparisons. This lack of genetic structure was supported by the Bayesian clustering analysis and thus was probably robust. However, three alternative, non-exclusive explanations should be considered: (1) microsatellite marker resolution, (2) geographical scale of the study area and (3) life history traits of moray eels.

(1) The panel of 11 microsatellite markers was specifically developed for this study, and thus we did not have the benefit of hindsight. The heterozygosity deficit (most F_{is} values were positive) of several molecular markers was compensated for by the high number of alleles found in most of them, ranging from one to 20, and that was probably sufficient to detect genetic structure. However, a high number of alleles combined with a strong heterozygosity deficit may result in a Walhund effect. In such a case, we would not have to reconsider the efficiency of the microsatellite markers but rather the populations' delineation resulting from limited gene flow.
(2) Permanent barriers preventing the movement of individuals between populations can result in spatial genetic structure. Gene flow can only occur if individuals are able to move throughout their respective landscapes. Moray eels navigate within coralline matrixes, avoid open water and remain close to their home reef (Pereira *et al.*, 2017). The bottom of the lagoons that they inhabit are heterogenous and highly structured (Andréfouët *et al.*, 2004). Hard coral reefs suitable for many species of moray eels are patchy habitats spread throughout vast sandy areas or interrupted by deep channels or open water. These obstacles significantly limit the distance of moray eel movements. Yet, the homogeneous genetic structure we found suggests that the landscape of the South Lagoon of New Caledonia exerts a low resistance to moray eel movement. Although adults are sedentary bottom dwellers, attached to heterogeneous structures, larval stages (leptocephalus) are planktonic and thus can easily avoid seafloor obstacles. During their long planktonic stage, moray eels carried by marine currents have ample opportunities for population mixing within the lagoon and across large oceanic areas (Reece *et al.*, 2010a).

Our results involving genetic structure could also suggest that the lagoon area we studied (the populations examined stretched along > 100 km, Fig. 1) merely represents a fragment of a larger population spread over the

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lagoon perimeter. New Caledonia is bordered in the east by the Loyalty Islands, Vanuatu and the Fiji Islands. Each of these systems harbours moray eel populations (Reed *et al.*, 2002). The main marine currents and dominant winds are orientated from these archipelagos toward New Caledonia, and they may well carry the planktonic larval stages of moray eels long distances over periods ranging from several weeks to months (Vega *et al.*, 2006; Reece *et al.*, 2010a). Additionally, large whirlpool systems in the west and south of New Caledonia may carry eels from as far off as Ile des Pins and the Chesterfield Islands (Vega *et al.*, 2006). Thus, moray eel populations settled in New Caledonia may be influenced by large spatial mixing processes. This hypothesis is supported by the findings of Reece *et al.* (2010a) who showed that leptocephalus stages can persist up to 2 years in the pelagic environment, a finding associated with a lack of genetic structure in *Gymnothorax undulatus* populations sampled across 2000 km in the Indo Pacific Ocean. It would be useful to sample remote areas (e.g. Loyalty, Vanuatu and Fiji archipelagos) using sea snake sampling to compare their respective genetic signatures with the results obtained in New Caledonia. Local population mixing within New Caledonia is not excluded from a regional mixing process operating at a larger scale across Western Pacific coral reefs (among distant archipelagos).

(3) Spatial heterogeneity and dispersing individuals can drive gene flow, and they are often considered the source of spatial genetic structures. However, inferring genetic structure from these types of present-day processes (i.e. gene flow or individuals' behaviour) assumes that the genetic signature of past historical events has been erased by a combination of more recent events such as migration and/or drift (Grosberg & Cunningham, 2001). Recent and historical processes can generate genetic patterns at different spatial and temporal scales (Grosberg & Cunningham, 2001). Can evolutionary history explain the lack of SGS observed? The low sea levels that occurred during the Last Glacial Maximum may have influenced the distribution and demographic history of sea snakes (Lane & Shine, 2011; Bech *et al.*, 2016). For example, amphibious Laticaudine sea snakes benefited from low sea levels that provided abundant emergent lands, creating new routes across oceanic spaces and facilitating the colonization of new areas (Lane & Shine, 2011). Based on genetic data, Lane & Shine (2011) inferred that *Laticaudata frontalis*, an endemic species of the Vanuatu Islands, probably derived from small founding populations of New Caledonian *L. saintgironsi*, which had probably derived from northern populations of *L. colubrina* (Lane & Shine, 2011; Bech *et al.*, 2016). Different ecological requirements are necessary for sea snakes and moray eels, but both strongly depend on coral reefs. Their distribution was driven by oceanic and climatic events that occurred in the South Pacific during the Pleistocene. These processes probably influenced the genetic signature of current populations. In agreement with this, using mitochondrial data, Reece *et al.* (2010a) suggested that *G. flavimarginatus* and *G. undulatus* recently experienced a range expansion (during the Pleistocene, approximately 600 000 years ago) followed by episodic fragmentation of populations during oceanic regressions. Thus, it is possible that the lack of genetic structure recorded in *G. chilospilus* populations results from successive historical events during the Pleistocene that confined populations into refuges localized in the lagoons scattered in the southern Pacific Ocean. Geographiccal and ecological constraints could thus explain the high F_{is} values, suggesting potential inbreeding depression in the studied populations. Under this scenario, moray eels of the South Lagoon of New Caledonia experienced a historical bottleneck event, and we are now witnessing the genetic consequences.

CONCLUSION

We found a lack of SGS in moray eel populations in the South Lagoon of New Caledonia. This result echoes the lack of SGS of their main predator sampled in the same area, the sea kraits (Bech *et al.*, 2016). In both cases, the homogeneous genetic structure could be explained by a long dispersal stage of fish larvae and juvenile snakes (Bonnet *et al.*, 2015) and/or by historical events experienced by these organisms during the Pleistocene. Thus, shared larvae/juvenile dispersal traits, combined with a common history, may have shaped the genetic structure patterns of both prey and predator populations in the South Lagoon of New Caledonia. Alternatively, the long planktonic larval stage of moray eels may favour dispersal among distant coral reefs found throughout the western Pacific Ocean. The genetic tools we developed will allow future investigation of the potential role of oceanic currents in influencing the biogeographical patterns of moray eels across large geographical areas, where these fish and their specialized predators occur.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

File S1: Raw data from 454 pyrosequencing of a repeat-enriched genomic library, including names and sequences of primers; number of repetitions and motif of the microsatellite molecular markers; different steps of marker selection and for selected markers; and label dyes and associated poolplex.
File S2: Study of the transferability of the microsatellite markers.

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