Population genetics of *Narella versluysi* (Octocorallia: Alcyonacea, Primnoidae) in the Bay of Biscay (NE Atlantic)

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Abstract

Octocoral species are globally distributed in all oceans and may form dense communities known as vulnerable marine ecosystems. Despite their importance as deep-water habitats , the underlying genetic structure and gene-flow patterns of most deep-water populations remains largely unknown. Here, we evaluated genetic connectivity of the primnoid octocoral *Narella versluysi* across the continental shelf of Bay of Biscay, spanning 360 km (95 samples from submarine canyons, ranging from 709–1247 m depths). We report 12 novel microsatellite markers which were used to genotype 83 samples from six populations. Sixteen samples were sequenced for three mitochondrial DNA regions (Folmer region of COI with an adjacent intergenic region igr1, MT-ND2 gene, and mtMutS homolog 1 region). All sequence haplotypes and genetic clusters were found in multiple sites spanning more than 200 km. Overall, our analyses suggest there is high gene flow between colonies of *N. versluysi* among all study sites. There is no significant geographic

structure and no pattern of isolation by distance or depth. Connectivity is facilitated by the prevailing current which runs along the shelf break, and could be a mechanism to connect all of the sampled locations. The high connectivity over large geographic distance is a positive sign for a potentially vulnerable organism and may provide some resilience to disturbance. This information is crucial for a better understanding of how this fragile benthic fauna may respond to climatic and anthropogenic disturbances, which is a cornerstone for effective habitat management.

Keywords

Cold water corals; microsatellites; population genetics; submarine canyons; gorgonians; deep-sea;

Introduction

Members of the subclass Octocorallia are an important part of benthic ecosystems (Roberts et al. 2009), with over 3000 extant species of soft corals, gorgonians and sea pens (Cairns, 2007; Daly et al. 2007). They have a worldwide distribution and may occur in a significant portion of deep oceans (Yesson et al. 2012). In fact, around 74% of the octocoral global diversity is found at depths greater than 50 m (reviewed in Roberts and Cairns, 2014). In cold or deep water environments, they can create dense patches or fields of biological, conservation and socio-economic value (Foley and Armstrong, 2010; Braga-Henriques, 2014) therefore recognized as (1) Vulnerable Marine Ecosystems (VMEs) under the Food and Agriculture Organization of the United Nations (FAO), (2) priority marine habitats through the Oslo and Paris Conventions (OSPAR), and (3) components of Ecologically or Biologically Significant Marine Areas (EBSAs) under the CBD scientific criteria. Hence, when taking into account their remoteness and our limited overall knowledge, it becomes greatly challenging to protect those ecosystems from increasing anthropogenic disturbances such as industrial fishing (Wheeler et al. 2005, Clark et al. 2016), litter (van den Beld et al. 2017a), mining (Larsson et al. 2013), pollution (White et al. 2012), ocean warming (Barnett et al. 2005) and acidification (Guinotte et al. 2006).

Evidence of declines in deep-sea populations around fishing areas have prompted

international efforts to prioritise research on connectivity (Clark et al. 2012), including on corals that are slow-growing organisms with low recovery potential (Althaus et al. 2009). Besides increased risk of biomass loss, octocoral habitats are also particularly vulnerable to physical damage due to the three-dimensional or whip-like morphologies of most common foundation species (Braga-Henriques et al. 2013). A reduced resilience and low genetic diversity is thus highly expected in exploited grounds. Consequently, an improved understanding of population connectivity, and therefore potential dispersal and recovery, is imperative to guide and support further policy development on sustainable management and conservation of cold water octocoral habitats (Jones et al. 2007; Mengerink et al. 2014; Baco et al. 2016).

While our knowledge of population genetics using microsatellites in reef-building scleractinians has increased considerably over the last decade, both in tropical (e.g. *Acropora*: Baums et al 2005, Wang et al. 2009) and deep, cold-water environments (e.g. *Lophelia pertusa*: Morrison et al. 2008, Dahl et al. 2012; Becheler et al. 2017; *Solenosmilia variabilis* and *Desmophyllum dianthus*: Miller & Gunasekera, 2017), less attention has been given to the use of these markers in octocorals inhabiting deep waters. This is partly due to the logistic constraints of sampling non-reef-building corals at depth. Many of those species are seldom found in numbers conducive to sampling for population genetics studies, and alternative approaches have been used to examine, for example, the connectivity of wide-ranging species over regional or global scales using traditional sequencing (Baco and Cairns, 2012; Herrera et al. 2012). However, a few relatively small scale genetic studies of octocorals have been reported using microsatellite markers. For instance, five microsatellite markers were developed for four populations and 128 samples of the Mediterranean gorgonian *Eunicella singularis* (Alcyonacea: Gorgoniidae) from depths of 15–35m to examine the profile of host and symbiont populations in relation to thermal tolerances (Pey et al. 2013). Six loci were used on 104 colonies and 385 larvae of the gorgonian *Paramuricea clavata* (Alcyonacea: Plexauridae) from southern France to investigate reproductive success in a shallow (20m) population (Mokhtar-Jamaï et al. 2013). Also, four populations of the tall sea pen *Funiculina quadrangularis* (Pennatulacea: Funiculinidae) were genetically profiled using 10 microsatellite markers for 176 specimens in Scottish sea lochs at depths of 18–35m (Wright et al. 2014). Finally, ten loci were used to examine connectivity for the gorgonian *Callogorgia delta* (Alcyonacea: Primnoidae) in the Gulf of Mexico (Quattrini et al. 2015).

In recent years, an increased sampling effort across the continental shelf of the Bay of Biscay has revealed a hidden coral diversity and abundance at the submarine canyon areas, including octocoral taxa such as *Narella spp.* (van den Beld et al. 2017b). The latter is the most species-rich genus within the “quintessential” deep-water octocoral family Primnoidae (Cairns and Bayer, 2009). It comprises 46 species distributed globally at depths ranging from 129 m down to a deepest observation at 4594 m (Cairns and Bayer, 2003; Taylor and Rogers, 2017). As recently as 2007, five new species were reported from deep seamounts in the Gulf of Alaska, and it is predicted there are more species yet to be discovered in this group (Cairns and Baco, 2007). Indeed, the systematic account of *Narella* species continues to increase with new descriptions from a variety of ocean basins, i.e. 14 new species in eleven years: six from Hawaiian Islands and adjacent seamounts (Cairns and Bayer, 2008), five from New Zealand (Cairns, 2012), and three from SW Indian ocean ridge (Taylor and Rogers, 2017). Two species have been so far reported to the NE Atlantic, *N*. *bellissima* (Kükenthal, 1915) and *N*. v*ersluysi* (Hickson, 1909), both having amphi-Atlantic distributions. Despite our growing knowledge of distributional ranges (spatial and in deep) and habitat preferences (Braga-Henriques et al. 2011, 2013; van den Beld et al. 2017b), baseline information such as dispersal strategy (e.g. type of larvae, larval lifespan, dispersal distance), reproductive mode (broadcast spawner or brooder) and population genetics, is still lacking for those species. Nonetheless, genetic research using barcoding regions of the mitochondrial genome has been employed at the generic level, revealing significant variation in populations from the Eastern Pacific (Baco and Cairns, 2012).

N. versluysi is found across the central North Atlantic including obseravations from Bermuda, straits of Florida, Azores, W Ireland and Bay of Biscay at depths of 550-3100m (Cairns and Bayer, 2003). It is an unbranching *Narella* species, reaching heights up to 78cm (Cairns and Bayer, 2003), and is associated with *Lophelia/Madrepora* reefs and mixed coral habitats in Bay of Biscay (van den Beld et al. 2017b), and associated with *Pheronema carpenteri* sponges in Azores (Braga-Henriques 2014). It is reported in Bay of Biscay at depths of 678-1734m where it is the most abundant gorgonian cold water coral (van den Beld et al. 2017b), making it suitable for population genetic studies. Here we assess genetic diversity and connectivity patterns among populations of *N*. *versluysi*at the spatial scale of the Bay of Biscay, covering depths of 700–1250 m and spanning more than 360 km. Combined analysis of mitochondrial and nuclear DNA data was carried out to

infer population structure and dispersal capacity. Novel microsatellite markers for the studied species are herein reported.

Methods

Study sites and sampling

The Bay of Biscay is home to a series of submarine canyons that connect the continental shelf and rise (Bourillet et al. 2003). Significant stands of cold-water coral reefs formed by *L*. *pertusa* and *Madrepora oculata* are typically found occurring on those areas at depths between 600–900m (Arnaud-Haond et al. 2017). The BobEco cruise aboard the IFREMER research vessel "*Pourquois Pas*?" (September 09 to October 11, 2011) surveyed eight of those canyons located on the edge of the continental shelf of the Bay of Biscay with the remotely operated vehicle (ROV) Victor 6000 (IFREMER, France). Ninety-five colonies of *Narella* from six locations, in the bathymetric range of 709–1247 m, were collected with the manipulator arm of the ROV and placed into a series of labelled boxes for transport to the surface (see Becheler et al. 2017 for details of sampling equipment). Once on deck, specimens were photographed, labelled, sub-sampled for taxonomic identification and genetic analysis and preserved in ethanol (70% and 100%, respectively). The remainder of each sample was frozen at - 80°C for long term storage and reference. Species-level identification was obtained to all samples by ABH using morphological distinctive characters, which includes polyp size, number of pairs of adaxial buccal scales, number of polyps per whorl, nature of adaxial buccal scales, among others (Grasshoff, 1982; Cairns and Bayer, 2003). Of the 95 collected samples, 83 were haphazardly selected to perform the genetic analysis (Fig.1, Table 1). CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) does not apply to octocorals.

Molecular Analysis

Total genomic DNA extraction was performed on 1 cm sections of sample using the DNeasy Tissue kit (QIAGEN Ltd. West Sussex, UK), following the manufacturer's instructions and adapted for a digestion time of 1–2 days. Fifteen samples covering all study sites were selected for microsatellite development. Microsatellite markers (from the nuclear genome) are typically more variable than sequence regions of mitochondrial DNA

(mtDNA) and thus can offer resolution to the population level. This approach was only possible due to the large sample size as a result of a greater sampling effort and research investment (FP7/EU CoralFISH). Samples were sent to Ecogenics GmbH (http://www.ecogenics.ch; Zurich, Switzerland) for enriched genomic library construction and microsatellite primer development. Twelve primer pair combinations were developed producing variable fragment lengths. Eighty-three samples were genotyped for these regions by Ecogenics. Results from sequencing were examined using the software GENEMAPPER v 4.1 (Applied Biosystems Inc.). Chromatogram peaks were automatically scored based on fragment size and verified by manual inspection. A matrix of sample and allele pairs was produced for each locus.

In order to estimate genetic connectivity and population genetic differentiation, a subset of samples (N=16) were selected for mitochondrial DNA (mtDNA) sequencing. These markers were used to avoid cryptic variation, confounding effects of phenotypic variation, and convergent evolution of morphological characters. Three mtDNA regions were amplified: (1) Folmer region of COI with an adjacent intergenic region igr1, (2) subunit of NADH dehydrogenase MT-ND2 gene, and (3) mtMutS homolog 1 region (aka msh1). PCRs were performed on successful extractions using 15µl reaction volumes consisting of: 10µl PCR mastermix (Qiagen ltd), 1µl forward and reverse primers (0.2 pm concentration), 1-2µl whole genomic DNA, 1-2µl H_2O. Primer sets used in the amplification were ND42599F/MUT3458R for mtMutS (McFadden et al. 2011), COII8068xF/COIOCTR for the Folmer region of COI with an adjacent intergenic region igr1 (McFadden et al. 2011), and 16S647F/ND21418R for the ND2 (McFadden et al. 2004). Thermal cycling conditions follow the original recommendations (McFadden et al. 2004; McFadden et al. 2011). PCR products were resolved on a 1–1.5% agarose gel stained with ethidium bromide (10 mg/mL). Successful amplifications were sent to Macrogen Inc (Seoul, South Korea http://dna.macrogen.com) for purification and sequencing. Forward and reverse reads were aligned into contigs with manual inspection of all base calls. All sequences were aligned and missing data for PCR products were completed with consensus sequences. Resulting sequences were deposited in GenBank accession numbers MH660458-MH660522.

Statistical analysis of microsatellite data

A test of linkage disequilibrium was performed between all pairs of loci over all sites using FSTAT 2.9.3.2 (Goudet 2001). Observed and expected heterozygosity levels were calculated using the R package adegenet (Jombart and Ahmed, 2011) and tested for signifcant deviation from Hardy Weinberg equilibrium using the hw.test function in the R package pegas (Paradis, 2010). Between population Fst values were calculated with the function pairwise.neifst and significance was tested using 1000 bootstrap replicates with the function boot.ppfst in the R package hierfstat (Goudet and Jombart, 2015). Geographic and genetic (Fst) distances between sites were compared using a mantel randomisation test using the mantel.randtest function in the R package adegenet (Jombart and Ahmed, 2011). A k-means clustering procedure was performed based on a principal components analysis of microsatellite data (Jombart et al. 2010). This process divides samples into a small number of groups with similar genetic profiles. The genetic structure of biological populations was investigated using a discriminant analysis of principal components (DAPC) process (Jombart et al. 2010).

An analysis was performed to test the power of datasets of similar size to produce significant results. Effective population size (Ne) was estimated using NeEstimator V2 (Do et al. 2014). The program PowSim (Ryman and Palm, 2006) was used with population size (Ne=20), 100 simulations and 25 generations of drift. PowSim checks for significant Fst results based on simulated datasets (of a given size, i.e. equal to the observed). If simulations produce significant results then we can assume that datasets of equivalent size are sufficient to produce significant results (Ryman and Palm, 2006).

All statistical assessment of population connectivity was performed on the microsatellite data. DNA sequence data were examined purely descriptively and no formal statistical tests were performed on this dataset.

Results

Mitochondrial DNA sequences were obtained for 16 samples (genbank accession numbers MH660458-MH660522). Sequence variation was very low between samples, with only the MutS region showing any variability. The COI sequences were 785 base pairs (bp) with 0 variable sites, the ND2 region was 788 bp with 0 variable sites, the MutS region was

866 bp with 1 variable site creating 2 haplotypes (note only 15 samples were sequenced for the MutS region and the combined analysis was performed with missing data for the one sample without a sequence). These haplotypes were spread over the study area with both haplotypes seen in 3 of 6 sites, geographically spanning >200 km (Fig. 2). Both haplotypes span the full depth range from ca. 700-1200 m (Fig. 3).

Canyon	Longitude	Latitude	Depth (m)	Samples	Genotyped	Sequenced
Croisic	46° 22.90'N	4° 40.71'W	837-848	6	4	3
Crozon	47° 22.67'N	6° 37.53'W	1136-1247	21	20	4
Guilvinec	46° 56.10'N	5° 21.65'W	811-849	9	7	3
Lampaul	47° 37.73'N	7° 32.06'W	729-1138	21	19	2
Morgat-Douarnenez	47° 19.45'N	6° 21.06'W	709-823	22	17	3
Petite Sole	48° 07.34'N	8° 48.84'W	919-931	16	16	1
Total			709-1247	95	83	16

***Table** 1 Number and location of samples collected in this study*

Analysis of the microsatellite data provides evidence that populations of *N. versluysi* are not genetically differentiated at the study area (Fig. 2, 4), i.e. across the six canyons of the Bay of Biscay spanning a distance of hundreds of kilometres (>360 km). Twelve microsatellite loci were genotyped for this species (see Table 2 for details) and the majority of these were significantly linked (linkage test, $p<0.05$). The four unlinked markers yielding the most complete dataset selected for analysis (Narver_02442, Narver_03299, Narver_04747, Narver_17311). This created a dataset of 45 alleles for 4 markers, and all subsequent analysis will be based on these markers. There were 66 unique genotypes across 83 samples with the most populus genotype shared by 7 samples. The majority of markers showed significant deviation from Hardy Weinburg equilibrium (Table 3), although all markers fitting Hardy Weinburg are in the unlinked set of markers. The power analysis indicates that these four markers are sufficient to detect significant population structure in datasets of this size (all replication runs produced significant results at $p<0.05$).

There is no observable geographic pattern in the data (Table 4). Genetic distances (Fst) between populations are all close to zero (Table 4). There is no pattern of isolation by distance ($p=0.41$) or depth ($p=0.73$).

242

243

Locus	Forward Primer (5'-3')	Reverse Primer (5'-3')	Motif	Alleles	Size bp
Narver_01031	GCTGCCGTATATTCGTAGCG	AAGAAGGCATTGTGGTTGCC	(CA)	20	122-165
Narver_02199	TTTGTATTTACACAGGCAGGC	TCCCAGTTGGTTTGAAGTTGC	(AGAC)	19	142-223
Narver_02442	GTGCTTACAGACACACACGC	GCCAGGAGGTCATGTTTGC	(CA)	10	64-87
Narver_03299	CACTGCTGAGGGGGTAATAAG	TGTCTGTCTGTCCAGTGGTG	(CAC)	24	192-310
Narver_03969	GCCCATTTACTCACTCGTCC	GTGCTTGGGTGGATGGATAG	(CATT)	14	179-287
Narver_04747	TAAGGGCAACCTATCCCACG	GTTGCGATATTAGCGATCCCG	(ATTG)	6	110-130
Narver_07831	GTTGGTGCTGGTGGTGATTG	CAACGACGAGGAGAACATGC	(GTT)	11	179-235
Narver_10479	ATGTGGATCTCTGAGTAGCAG	TCAAACATCGCCGAGTAACG	(AC)	8	183-196
Narver_11984	GGAATGACAGGGAGGCAAAC	AGGGAATAAGACACACAACAATGAG	(GACA)	24	110-210
Narver_12411	TTGTTGTCCTTGCGGTTGTC	TCAGCAGCTTGCTCGAATAC	(TGT)	12	94-134
Narver_14768	CGTTGGTCTCCGTTTAGCTG	TGAACGGCAATTACCACAGG	(ATC)	14	151-201
Narver_17311	TGGTGGTGGATTTGGACGAG	CTTAAGATGGCGGCGTACC	(GGA)	5	103-119

***Table** 2 Microsatellite markers developed for Narella versluysi with reference to primer sequences, motifs, number of alleles and fragment size range.*

	All regions			Guilvinec			Croisic			Petite Sole			Lampaul			Crozon			Morgat-Dourarnenez	
Loci	**N(a)**	**Ho**	**He**	**N(a)**	**Ho**	**He**	**N(a)**	**Ho**	**He**	**N(a)**	**Ho**	**He**	**N(a)**	**Ho**	**He**	**N(a)**	**Ho**	**He**	**N(a)**	**Ho**	**He**
Narver_01031	20	0.890*	0.902	8	0.714	0.847	5	0.750	0.750	10	1.000**	0.787	12	0.944*	0.870	14	0.850*	0.896	13	0.882	0.894
Narver_02199	19	0.143***	0.917	4	0.000**	0.667	5	0.250**	0.781	6	0.154***	0.592	10	0.231***	0.802	11	0.071***	0.895	12	0.154***	0.899
Narver_02442	10	0.476	0.549	5	0.714	0.551	2	0.000	0.375	5	0.813	0.586	5	0.389*	0.623	5	0.450	0.420	6	0.294**	0.481
Narver_03299	24	0.783**	0.864	6	0.429**	0.776	6	1.000	0.813	8	1.000**	0.770	10	0.737**	0.832	16	0.800	0.890	14	0.706	0.848
Narver_03969	14	0.831***	0.797	5	0.857	0.714	4	1.000	0.719	4	0.750	0.527	8	0.737***	0.814	7	0.850	0.753	10	0.941	0.853
Narver_04747	6	0.329	0.386	2	0.429	0.337	2	0.000	0.375	4	0.250	0.229	3	0.263	0.234	5	0.450	0.440	4	0.375	0.580
Narver_07831	11	0.667***	0.836	7	1.000	0.833	3	0.333	0.611	5	0.688*	0.703	7	0.588**	0.815	9	0.650*	0.839	9	0.688	0.848
Narver_10479	8	0.474***	0.777	4	0.429	0.724	3	0.750	0.656	5	0.786**	0.732	6	0.375***	0.738	6	0.350**	0.635	6	0.412**	0.775
Narver_11984	24	0.904***	0.941	9	1.000*	0.867	6	1.000	0.781	13	1.000***	0.871	14	0.789***	0.910	16	0.900	0.914	16	0.882	0.915
Narver_12411	12	0.976*	0.893	7	1.000	0.827	7	1.000	0.844	10	1.000***	0.842	10	1.000*	0.874	10	1.000	0.863	11	0.882	0.869
Narver_14768	14	0.854***	0.859	7	0.857	0.796	5	0.750	0.688	8	0.875***	0.838	11	0.944*	0.864	7	0.750	0.796	9	0.882	0.822
Narver_17311	5	0.366	0.364	3	0.571	0.439	2	0.000	0.375	3	0.438	0.354	2	0.222	0.198	3	0.400	0.471	2	0.412	0.327

***Table** 3 Heterozygosity statistics for all microsatellite markers over all stations. N(a) = Number of alleles, Ho = Observed heterozygosity and He = Expected heterozygosity. Asterisks indicate significance levels (* p<0.05, ** p<0.01, *** p<0.001).*

244

Site	**Croisic**	**Petite Sole**	**Lampaul**	**Crozon**	**Morgat-Douarnenez**
Guilvinec	-0.083	0.010	-0.010	-0.015	-0.022
Croisic		-0.019	-0.038	-0.063	-0.070
Petite Sole			0.024	0.040	0.037
Lampaul				0.046	0.027
Crozon					-0.004

***Table** 4 Pairwise genetic distance (Fst) values using θ estimator for all sites (after 10000 permutations). Slightly negative numbers should be treated as not difference from zero. No significant Fst values were found (p>>0.05).*

245

Discussion

There is strong connectivity between populations of the primnoid gorgonian *N*. *versluysi* in the Bay of Biscay, which suggests potential genetic cohesion over this region. The population structure shows no geographic patterning, and every sequence haplotype and microsatellite cluster is seen in at least four sites spanning more than 200 km.

These findings agree with the lack of genetic structure found across the same locations for the scleractinian *L*. *pertusa* (Becheler et al 2017). Considering that both species are affected by the same currents and hydrographic regime, and were surveyed under randomly sampling schemes, it is therefore feasible to assume that similar reproductive or dispersal strategies are underlying the observed connectivity pattern on this scale. However, these hypotheses are difficult to ascertain as little is known about reproductive biology and early life-history stages of *Narella* species.

The handful of studies to date about sexual reproductive processes in primnoid octocorals (less than 4.5% of the global diversity, see Kahng et al. 2011) indicate that they can either be broadcast spawners (*Primnoa resedaeformis*: Mercier and Hamel, 2011; *Primnoa pacifica:* Waller et al. 2014) or brooders with both internal and external fertilization (e.g. *Thouarella* spp: Brito et al. 1997; *Fannyella* spp.: Orejas et al. 2007) and that these possibly exhibit seasonal reproductive cycles. Although the broadcasting mode has been described for *L*. *pertusa* (Waller and Tyler, 2005), a precautionary approach is advocated when taking into account the contrasting patterns of connectivity between this species and *M*. *oculata* over the same area (Becheler et al. 2017), both keystone reef-builder scleractinians co-occurring in Bay of Biscay (Arnaud-Haond et al. 2017) and broadcast spawners (Waller and Taylor, 2005). While *L*. *pertusa* is panmictic at this regional scale, *M*. *oculata* shows significant genetic structure across a distance of ca. 500 km (Becheler et al. 2017). Miller et al. (2010) found, however, no genetic differentiation in populations of *M*. *oculata* and *Solenosmilia variabilis* (also co-distributed reef-builder scleractinian species) across a large area from the southern Pacific Ocean (Miller et al. 2010). Conversely, a genetic structuring pattern was detected in taxonomically unrelated coral species (a solitary scleractinian and two antipatharians) at the same spatial scale (Miller et al. 2010), suggesting geographical barriers to dispersal. These add to the mounting evidence that unravelling connectivity and population structure in deep sea fauna, though crucial for a sustainable ocean, is a challenging exercise due to the lack of generalised connectivity

patterns (Hilário et al. 2015; Baco et al. 2016).

Additionally, the role of asexual reproduction mechanisms such as fragmentation (Highsmith, 1982; Lasker, 1984), fission (Benayahu and Loya, 1985), polyp detachment (Rosen and Taylor, 1969; Sammarco, 1982) and clonal planulae produced via parthenogenesis (Brazeau and Lasker, 1989; Hartnoll, 1975; Yeoh and Dai, 2010) cannot be ruled out and this reproductive mode might also contribute to the observed connectivity pattern amongst populations of *N*. *verslyusi*. However, its effectiveness in the deep sea has been mostly assessed in scleractinian corals (e.g. Waller et al. 2002; Le Goff-Vitry et al. 2004; Le Goff-Vitry and Rogers, 2005; Dahl et al. 2012; Miller and Gunasekera, 2017).

It is during the planktonic phase that dispersal occurs for most benthic invertebrate species and therefore the maintenance of connectivity between populations (e.g. Kinlan and Gaines 2003; Bradbury et al. 2008; Treml et al. 2008; Cowen and Sponaugle, 2009; Faurby and Barber, 2012), and habitat resilience to anthropogenic stressors (Jones et al. 2009; Lett et al. 2010), greatly depend on intrinsic biological drivers (e.g. planktonic larval type and duration, swimming ability and other pre-settlement traits, recruit mortality, reproductive output) and how these interact with the surrounding environment (Ayata et al. 2010; Etter et al. 2015; Cardona et al. 2016). The location of sites selected for this study aligns with the strong north-westward current that follows the contours of the shelf (Koutsikopoulos and Le Cann, 1996) and this physical process might indeed facilitate the transport of *N*. *versluysi* larvae and promote enhanced food supply to the new recruits throughout the study area (Thiem et al. 2006; Van Rooij et al. 2010; Soetaert et al. 2016). Furthermore, early post-settlement survivorship of these might be boosted by its attachment onto elevated and structurally complex habitats, i.e. *L*. *pertusa* and *M*. *oculata* reefs (van den Beld, 2017b), avoiding the accumulation of fine-grained sediments due to swifter currents (Genin, 1986; Lacharité and Metaxas, 2013). In adult colonies access to food and resilience to sediment exposure are likely enhanced by its morphology. This species, although flagelliform (apart from a few reports on poorly branched colonies), can attain heights of up to 1 m from the reefs exhibiting large and strongly calcified polyps (Cairns and Bayer, 2003 and ABH pers. observations).

Moreover, we see no pattern of isolation by depth, as has been reported for the primnoid *C. delta*, in Gulf of Mexico, over a slightly shallower (400–914m) depth range (Quattrini et al. 2015), and other *Narella* species in the Hawaiian archipelago (Baco and Cairns, 2012). The pattern for Hawaiian *Narella* was mixed, with depth related isolation reported for some

311 species (*N. alaskensis*) and not others (*N. macrocalyx/arbuscula/sp. 2* Baco and Cairns,
312 2012). Nevertheless, *N*. *versluysi* has a wider bathymetric distribution (600–3000 m depths)
313 than sampled in this study (709–1247 m), so depth-related trends could be detected by
314 sampling from deeper populations or at coarser scales (Zardus et al. 2006; Rex and Etter
315 2010; Baco and Cairns, 2012). For example, genetic differentiation with depth has been
316 detected in the solitary scleractinian *Desmophyllum dianthus* across large geographic
317 scales and that was consistent with the stratification of water masses, which could be
318 indicative of larval retention in certain layers (Miller et al. 2011). Patterns of genetic
319 structuring along depth gradients have been also identified in the precious octocoral
320 *Corallium rubrum* (Constantini et al. 2011) and stylasterids (Lindner et al. 2008).

321 Our samples span a geographic distance of 300km, which fits a pattern of genetic
322 connectivity in deep-water species over hundreds of kilometers (Taylor and Roterman,
323 2017). However, *N. versluysi* is reported as far across the North Atlantic as the coast of
324 Florida, almost 7,000km distant. It seems unlikely that the level of genetic cohesion
325 reported in this study will be apparent over the full range. For example *L. pertusa*, which
326 shows high connectivity in Bay of Biscay (Becheler et al. 2017), has significant
327 differentiation between regions of the NE Atlantic, and strong differences on the
328 transatlantic scale (Morrison et al. 2011). This fits with a pattern of basin-scale genetic
329 variability for cold water corals, rather than local variation or differentiation with depth
330 (Herrera et al. 2012). However, patterns of connectivity in the deep-sea vary between
331 species, with some species reportedly maintaining genetic homogeneity over hundreds of
332 kilometres (Miller et al. 2010).

333 In this study we see relatively few haplotypes in the sequence data (2 haplotypes over 16
334 samples), but there is only notable intra-specific variation for MutS region (0-0.1%). These
335 are within the maximum infra-specific variations for *Narella* reported for the Eastern Pacific
336 (Baco and Cairns, 2012, ND2: 0-0.13%; COI: 0-0.24%; MutS: 0-0.47%), and well within
337 the maximum inter-specific variation values reported therein. Sequence identity is
338 maintained over distances of more than 200 km, but it is difficult to comment on sequence
339 diversity based on these data as some mitochondrial regions may not be sufficient to
340 reveal *generic* level differences in Primnoidae (Baco and Cairns, 2012), for example
341 maximum infra-specific variation reported in this study exceeds inter-genera variation of
342 *Narella* and other Primnoids (Baco and Cairns, 2012; France and Hoover, 2002).

343

Future effort

The large number of linked markers may be due to the high variability in these markers, in extremis we have 2 markers showing 24 alleles in 83 individuals. The Linkage Disequilibrium test is based on a contingency table comparing the presence/absence of single alleles, but this test may be less reliable in cases of low allele frequency (Delvin and Risch, 1995). When comparing two loci with high allele counts relative to the population sampled, it is highly likely that pairs of co-varying alleles can be found. The markers found to be linked in this study should be tested on larger populations, where they may show independent variation.

The congeneric species *Narella bellissima* was found in several locations alongside *N. versluysi*, but not in sufficient numbers to perform an analysis. The microsatellite markers developed in this study were tested on 5 samples of *N. bellissima*, all markers worked and showed variation, and it is hoped that these markers may be applied to other *Narella* species in future studies.

Conclusion

This is the first study to report population genetics of primnoid populations in the Northeast Atlantic, and the first to date addressing *N*. *verslyusi*. The markers developed herein proved effective for assessing genetic population structure in this species and may be applicable to other members of the genus *Narella*. There is high connectivity between populations over large geographic distances, and no evidence for isolation by distance or depth. The connectivity patterns observed may be facilitated by the predominant northwesterly current that follows the steep shelf-edge topography of the Bay of Biscay. High gene flow between populations indicates a large dispersal potential, suggesting that *N*. *versluysi* is likely to recover after low to moderate disturbance. This information is vital in establishing baseline data and assess the impacts of potential anthropogenic disturbances in this important habitat forming group from deep-water ecosystems, whose diversity and distributional range is commonly updated after new seabed surveys.

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Figure Captions

Fig. *1* Specimen of *Narella versluysi* being collected during the BobEco cruise (© IFREMER 2011). Inset shows the full specimen on deck (right – rightmost specimen) and a close up of downward orientation in polyp whorls (left).

Fig. *2* Geographic distribution of *N. versluysi* samples (crosses). Pies below crosses show genetic clusters based on microsatellite data (see Fig. 4). Pies above crosses show sequence haplotypes (MutS data). Pies are sized proportional to sample numbers, with segments representing individual genetic clusters and haplotypes. Arrows show relative speed of seabed currents.

Fig. *3* Beanplots showing depth profiles of each sequence haplotype (left) and genetic cluster (right). Widths are based on sample density. Thin horizontal lines show individual samples, thick line shows group mean. Dashed line = overall mean.

Fig. 4 Plot of first two principal components based on the discriminant analysis of principal components (DAPC) analysis of microsatellite data. Ellipses show 75% CI around each genetic cluster.

Compliance with Ethical Standards

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Conflict of Interest: All authors declare that they have no conflict of interest.

Ethical approval : All applicable international, national, and/or institutional guidelines for the sampling, care and use of organisms were followed. Approval for this work was obtained from the institutions of all participants.

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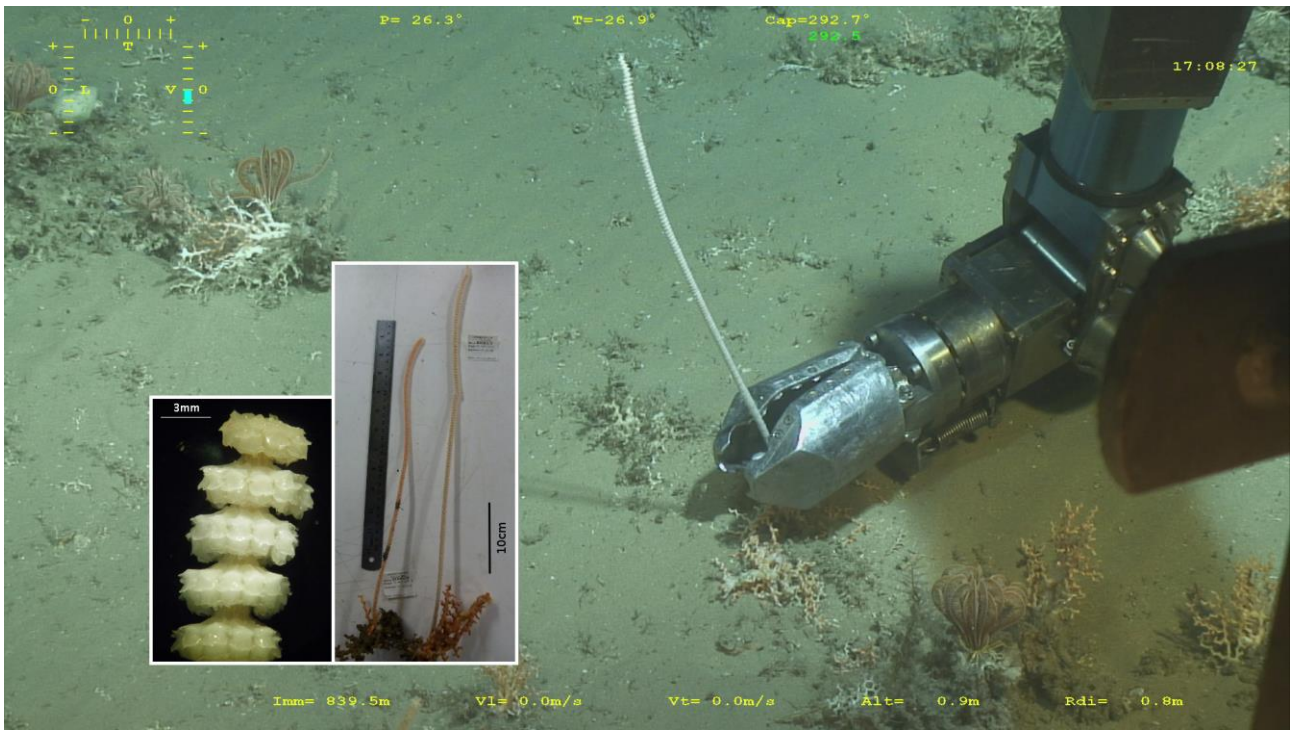
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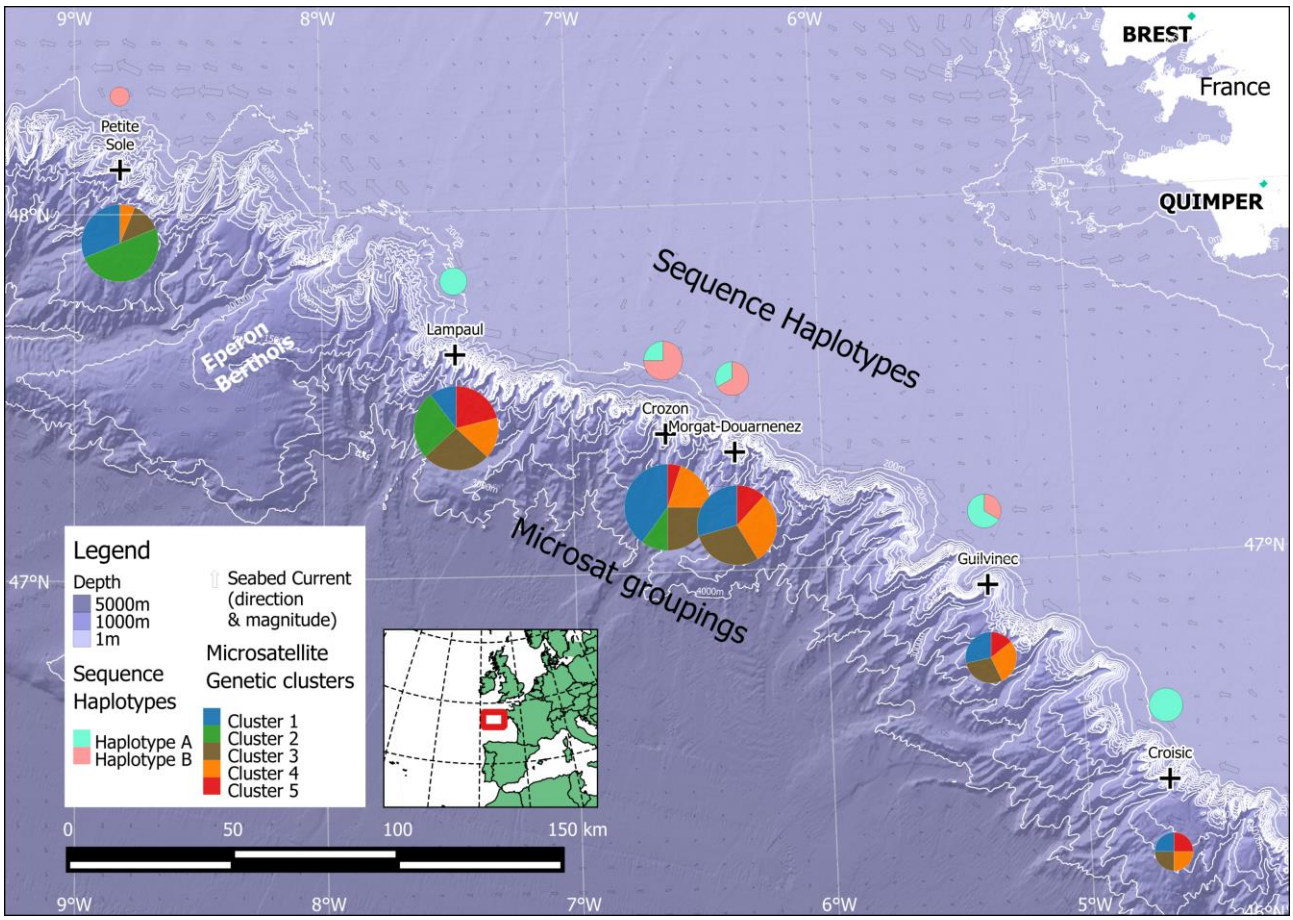
Fig 1



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658 Fig 2

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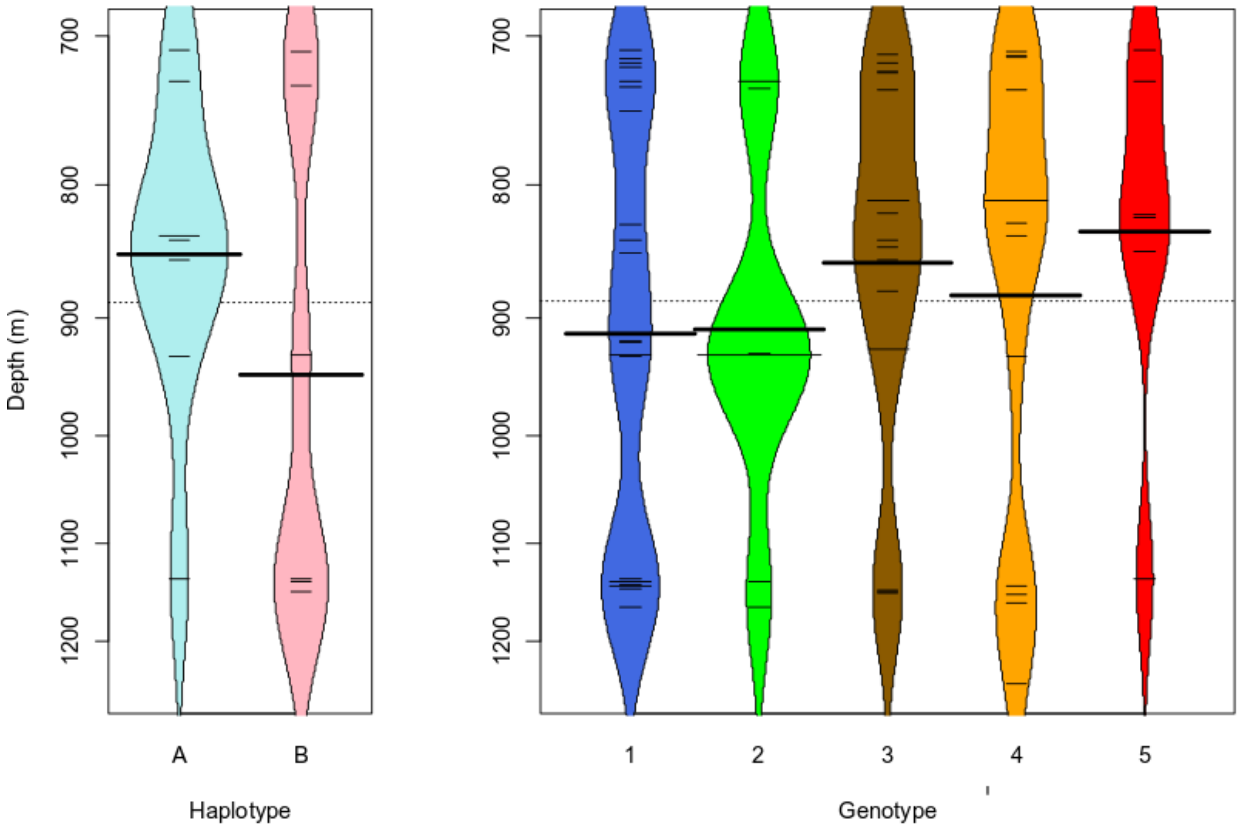

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661 Fig 3

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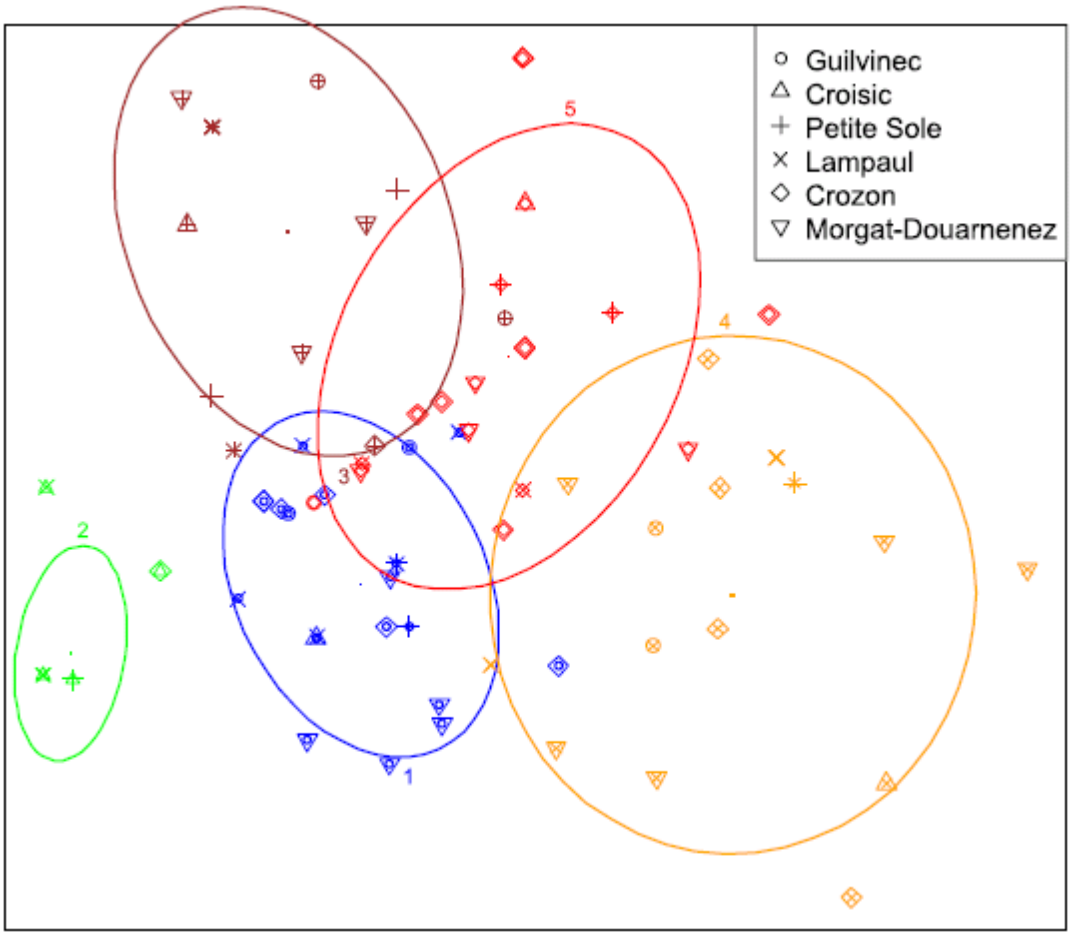
Guilvinec
Croisic
Petite Sole
Lampaul
Crozon
Morgat-Douarnenez
1
2
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663

664 Fig 4