Received: 3 August 2020 | Revised: 18 February 2021 | Accepted: 19 February 2021

DOI: 10.1111/mec.15860

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Genomic signatures of clonality in the deep water kelp *Laminaria rodriguezii*

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Funding information
MARFOR-Biodiversa/0004/2015; Clonix2D, Grant/Award Number: ANR-18-CE32-0001

Abstract

The development of population genomic approaches in non-model species allows for renewed studies of the impact of reproductive systems and genetic drift on population diversity. Here, we investigate the genomic signatures of partial clonality in the deep water kelp *Laminaria rodriguezii*, known to reproduce by both sexual and asexual means. We compared these results with the species *Laminaria digitata*, a closely related species that differs by different traits, in particular its reproductive mode (no clonal reproduction). We analysed genome-wide variation with dd-RAD sequencing using 4,077 SNPs in *L. rodriguezii* and 7,364 SNPs in *L. digitata*. As predicted for partially clonal populations, we show that the distribution of F_{IS} within populations of *L. rodriguezii* is shifted toward negative values, with a high number of loci showing heterozygote excess. This finding is the opposite of what we observed within sexual populations of *L. digitata*, characterized by a generalized deficit in heterozygotes. Furthermore, we observed distinct distributions of F_{IS} among populations of *L. rodriguezii*, which is congruent with the predictions of theoretical models for different levels of clonality and genetic drift. These findings highlight that the empirical distribution of F_{IS} is a promising feature for the genomic study of asexuality in natural populations. Our results also show that the populations of *L. rodriguezii* analysed here are genetically differentiated and probably isolated. Our study provides a conceptual framework to investigate partial clonality on the basis of RAD-sequencing SNPs. These results could be obtained without any reference genome, and are therefore of interest for various non-model species.

KEYWORDS
heterozygote excess, kelp, mode of reproduction, partial clonality, population genomics

1 | INTRODUCTION

Partial clonality, defined as a mode of reproduction where species reproduce both sexually and asexually, occurs in a wide variety of organisms across the tree of life, and is particularly widespread in fungi, plants, rotifers, cladocerans and insects (Halkett, Simon, Balloux, 2005). Clonal reproduction can occur through different mechanisms such as fragmentation, vegetative reproduction, mitotic parthenogenesis, or some forms of meiotic parthenogenesis (De Meeûs et al., 2007; Jaron et al., 2020). Partial clonality has attracted considerable

attention among evolutionary biologists due to the potential effects of asexuality on the evolutionary trajectory of species since reproductive modes have a major impact on the level of genome-wide genetic diversity and its distribution within and among populations (Duminil et al., 2007; Halkett et al., 2005). Moreover, reproductive modes profoundly affect other biological traits that play important roles in colonization processes and the spread of populations, such as population growth and dispersal (González de León et al., 2016; Kettenring & Mock, 2012), and in buffering and resilience after catastrophic events (Becheler et al., 2020).

Theoretical models predict that when clonality predominates, it will increase heterozygosity (Balloux et al., 2003; De Meeûs et al., 2006; Marshall & Weir, 1979), which may ultimately lead to higher levels of observed heterozygosity (H_o) than those expected (H_e) under panmixia. This deviation has been observed empirically through negative values of fixation index F_{IS}, reported in various partially clonal populations (in animals Adjeroud et al., 2014; Halkett, et al., 2005; angiosperms Alberto et al., 2005; Arnaud-Haond et al., 2007, and red and brown macroalgae Guillemin et al., 2008; Ardehed et al., 2015; Coleman & Wernberg, 2018, Pardo et al., 2019). Without sex, alleles do not segregate independently, which implies that heterozygosity can be preserved rather than reduced over generations (Judson & Normark, 1996). Increasing clonal reproduction can increase effective population size at the allelic scale but it decreases the genotypic effective population size: as a consequence asexual populations can maintain higher allele diversity but lower genotype diversity compared to sexual ones (Balloux et al., 2003). Asexual reproduction also leads to specific patterns of the distribution of F_{IS} over the genome, characterized by a high occurrence of extreme, mainly negative, values (Reichel et al., 2016; Stoeckel & Masson, 2014). These last theoretical studies reported, among other points, that high rates of asexuality increased the variance of the distribution of F_{IS} compared to that obtained from fully sexual populations (Stoeckel & Masson, 2014) but also as compared to fully clonal populations (De Meeûs et al., 2006). Some variation in F_{IS} among loci can also indicate the occurrence of rare recombination events as in the automictic parthenogenetic species *Artemia parthenogenetica* (Nougué et al., 2015). In addition, if asexuality is stable over generations, the accumulation of mutations in different alleles can increase sequence differences among alleles, which corresponds to the "Meselson effect" (Balloux et al., 2003; Birky, 1996; De Meeûs et al., 2007; Judson & Normark, 1996). This effect has been empirically demonstrated in ancient asexual *Timema* stick-insects (Schwander et al., 2011) and in the human pathogen *Trypanosoma brucei gambiense* (Koffi et al., 2009; Weir et al., 2016) and *Candida albicans* (Nébavi et al., 2006). Conversely the long term persistence without sex of Bdelloid rotifers, referred as asexual scandals, has been challenged by the suggestion of noncanonical sex (Schwander, 2016).

In this article, we investigated how population genomic studies of partially clonal species allow analysis of both the rate of clonality and its consequences for the distribution of F_{IS}. With the development of thousands of single nucleotide polymorphism (SNP), we will test whether the observed empirical distribution of F_{IS} follows the aforementioned predicted variations in F_{IS} among loci. The development of reduced representation sequencing methods (for review see Davey et al., 2011) now allows such investigations in non-model species. These approaches are also useful to more precisely delineate clonal lineages, where microsatellite loci have failed to detect any genetic variation, as in the case of the selfing species *Sargassum muticum* in the introduction range (Le Cam et al., 2019). Among the diversity of reduced representation sequencing methods, restriction site associated DNA sequencing (RAD sequencing; Baird et al., 2008) is useful to efficiently develop enough SNPs to study the genomic distribution of population genetic statistics.

We used the kelp *Laminaria rodriguezii* as a model species for the study of partial clonality. *Laminaria rodriguezii* is a deep water kelp endemic to the Mediterranean Sea, mainly reported from depths below 70 m, rarely found in shallower waters on seamounts or in upwelling systems (Ballesteros, 2006), and with a maximum depth recorded at 260 m in the Adriatic Sea (Ercegović, 1960). The rare populations of this species are exposed to various levels of disturbances, such as direct destruction by fishing gear and a decline in water transparency, which has led to *L. rodriguezii* being classified as endangered (Zuljevic et al., 2016). Besides sexual reproduction, *L. rodriguezii* can reproduce asexually by stoloniferous or rhizoidal growth. This mode of asexual reproduction involves somatic cells and should lead to clonal lineages. Vegetative reproduction is only known in two *Laminaria* species: *L. rodriguezii* (Huvé, 1955) and the northeast Pacific *L. sinclairii* (Demes & Graham, 2011). *Laminaria rodriguezii* can also reproduce sexually with a haplo-diplobiontic life cycle involving the alternation of microscopic dioicous unisexual haploid gametophytes with large diploid sporophytes typically found in kelps (Bringloe et al., 2020). Even if we do not know the importance of self incompatibility in this species, self fertilisation (i.e., fertilisation between male and female gametophytes originating from a single sporophyte parent), was reported to be possible experimentally in most studied species of Laminariales (see the recent review of Goecke et al., 2020). *Laminaria rodriguezii* then presents a mixed reproductive system, for which the genetic consequences have not been studied. This deep water species is also characterized by small and highly fragmented populations (Araújo et al., 2016). Therefore, the populations of *L. rodriguezii* should be impacted by a reduction in genetic polymorphism through drift, a reduction that can be counterbalanced by the aforementioned effects of asexual reproduction on allele diversity (Balloux et al., 2003).

We specifically studied here whether the levels of asexual reproduction have discernable effects on the empirical genomic distribution of diversity and F_{IS} among loci. We included populations of the fully sexual congeneric species, *L. digitata*, with different levels of microsatellite genetic diversity (Liesner et al., 2020) to take into account the consequence of the mode reproduction with the confounding effect of variation in the strength of genetic drift in our interpretations. *Laminaria digitata* is analysed only for comparative purposes, and we did not develop the study of the genetic structure of this species in this article. We also interpret our results in the light

of previous theoretical studies, especially considering the full distribution of F_{IS} among loci (Stoeckel & Masson, 2014). Among other factors, we tested whether the clonal rates in populations of *L. rodriguezii* lead to a shift toward highly negative F_{IS} values among loci, as expected for the highest levels of clonality (Stoeckel & Masson, 2014). We used a large set of SNPs in order to access the most complete distribution of F_{IS} values over populations of genomes. We also evaluated the robustness of our results by considering different cut-offs in the filtering of SNPs. The implications of the inferred genetic diversity and genetic structure for the management and conservation of this rare deep water kelp *L. rodriguezii* are discussed.

2 | MATERIALS AND METHODS

2.1 | Sampling, DNA extraction

Individuals of *L. rodriguezii* were sampled from four sites in the Mediterranean, three in Eastern Provence (Banc Magaud_1, Banc Magaud_2, Cap Camarat) and one in Southern Corsica (Bonifacio), between 65 to 76 m depth (Figure 1). Sampling was done by scuba divers between June and August 2018. Sampling for DNA collection was performed by collecting a small piece of tissue from the blade of sporophytes. More precisely, a total of 47 samples were collected from sporophytes not connected to each other by a stolon, and with a minimum distance of 2 m between sampled blades. This strategy aimed to promote the sampling of distinct genets. For one of the two Banc du Magaud sites, namely Banc Magaud_2, we used a different sampling design: the spatial distance between the sampled sporophytes was recorded along a regular transect of 20 m to study the spatial genetic structure inside the population. Regarding *L. digitata*, we used samples from five northeastern Atlantic populations of *L. digitata* collected in 2018 (Table S1, Supporting Information). Genomic DNA was extracted using the Nucleospin 96 plant kit (Macherey-Nagel), according to the manufacturer's protocol.

2.2 | ddRAD-sequencing

Two double-digest RAD-sequencing libraries (ddRAD-seq) with 47 individuals of *L. rodriguezii* and 125 individuals of *L. digitata* were prepared according to Peterson et al., (2012). To minimize bias in library

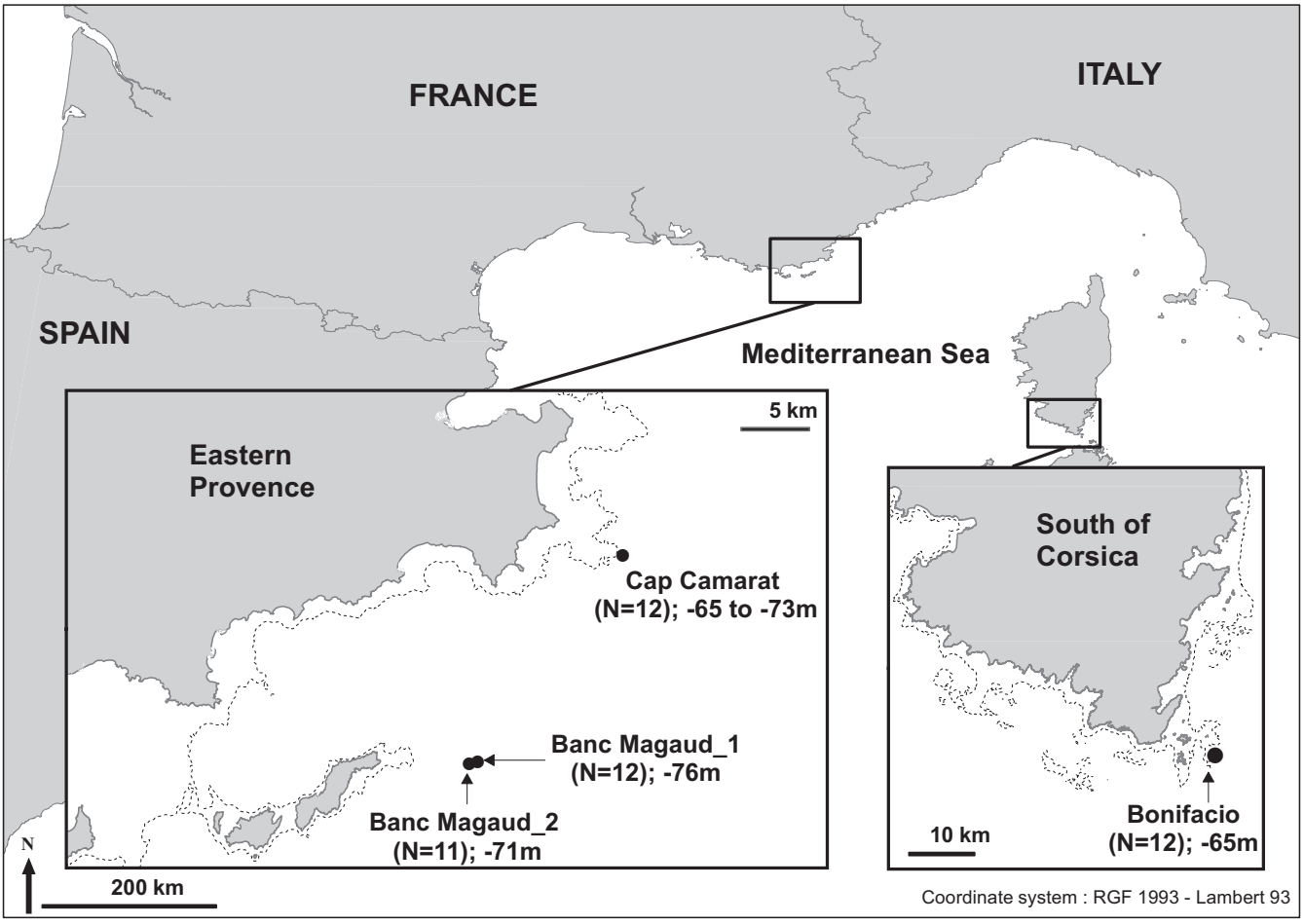

FIGURE 1 Sampling map of *Laminaria rodriguezii* in Eastern Provence and Southern Corsica, reporting the depth in meters of sampling sites and the number of individuals sampled per site (N)

preparation, all DNA samples were randomized across libraries, and two and five replicates of *L. rodriguezii* and *L. digitata*, respectively, were included. DNA concentrations were estimated with PicoGreen (Invitrogen), and 100 ng of genomic DNA was double digested using PstI and HhaI (NEB) for 12 h at 37°C. The purification of digested DNA was conducted by the NucleoMag NGS clean-up and size select kit (Macherey-Nagel) according to the manufacturer's protocol. When these steps were completed, the ligation of sequencing adapters to restriction digested DNA was performed as follows: P1 adapters (overhang PstI), including unique barcodes (6–12 bp) and P2 universal adaptor (overhang HhaI) were ligated to DNA using 0.5 µl (400 U/µl) of T4 DNA ligase (NEB), 6 µl of 10x T4 ligase buffer (NEB) and incubated for 12 h at room temperature. Before PCR amplification, an additional purification step was done with the NucleoMag NGS clean-up and size select kit. Amplification by PCR was restricted to 15 cycles and performed with the Q5 hot Start High-Fidelity DNA polymerase kit (NEB). Samples were then multiplexed, and automated size selected for a range between 400 and 900 bp using a Pippin Prep (Sage Science). Finally, the libraries were sequenced with the paired-end method (2 × 150 bp) on an Illumina Hiseq 4000 platform (Génome Québec Innovation Centre).

2.3 | Genotyping and SNP filtering

Raw quality reads were checked using FastQC v0.11.7 (Andrews, 2010) and trimmed to 137 bp after the removal of adapters sequencing by Trimmomatic (Bolger et al., 2014). Then, paired-reads were demultiplexed by both index and barcode using Stacks's process_radtags (Catchen et al., 2011). Thanks to the phylogenetic proximity between *L. rodriguezii* and *L. digitata* (Žuljević et al., 2016), we were able to use the draft genome of *L. digitata* (M. Cock, unpublished data) to align our reads with the BWA-mem algorithm in BWA v0.7.17 (Li & Durbin, 2009). Aligned reads were then assembled into loci and finally, we called single nucleotide polymorphisms (SNPs) with the Stacks v2.4 pipeline (Rochette et al., 2019). Aligned reads of mapping quality below 30 (which means a probability of incorrect alignment below 0.001) were excluded to minimize errors resulting from the erroneous assembly of paralogous loci as a single locus. To avoid a strong linkage between SNPs, we kept only the first SNP of each RAD locus. Further filtering was performed on the basis of minor allele frequency, missing data rates for both individuals (i.e., the percentage of missing genotypes per individual) and loci (the percentage of nongenotyped individuals per locus), using VCFtools v0.1.17 (Danecek et al., 2011) (Table 1). We aimed to identify the signatures of clonality from putatively neutral loci. To this end, we excluded outlier loci identified as involved under divergent selection with *pcadapt* v3.5 (Luu et al., 2017). Pcadapt starts by analysing population structure with a PCA. Then candidate outliers are identified as those which are excessively correlated with population structure (Luu et al., 2017). The outlier identified with pcadapt should then correspond to inflated signals of genetic differentiation.

TABLE 1 Comparison of population genetic summary statistics (mean and range of values) in *Laminaria rodriguezii* and *Laminaria digitata*

	Laminaria rodriguezii			*Laminaria digitata*		
Filtering thresholds	**SNP counts**	**Missingness**	**Coverage**	**SNP counts**	**Missingness**	**Coverage**
Locus genotyped	70,026	13.97%	11.29	609,699	12.26%	14.11
> 75% of the samples						
> 75% of the populations						
SNP kept						
observed heterozygosity <80%						
Linkage disequilibrium	27,769	13.18%	11.88	58,830	13.45%	14.36
single SNP per locus						
Minor allele frequency (MAF)	16,865	13.51%	12.18	21,090	15.01%	14.43
MAF >2%						
Missingness	13,235	4.46%	13.91	15,150	4.73%	17.24
per individual <30%						
per SNP <20%						
Coverage	4,366	2.27%	22.17	7,538	3.40%	21.63
mean read depth >15 X						
mean read depth <100 X						
Outliers detection	4,077	2.29%	22.04	7,364	3.39%	21.64
putatively neutral						

%P, average percentage of polymorphic loci within populations rarified at n = 10 by random resampling (see methods for details), H_o, observed heterozygosity, H_e, expected heterozygosity, F_{IS}, fixation index

2.4 | Impact of SNP filtering on population genetic summary statistics

RAD-seq is subject to different sources of genotyping errors (Mastretta-Yanes et al., 2015) that may generate spurious inferences in surveys of genetic variation. However, the noise of artefactual SNPs can be efficiently overcome by applying a rigorous filtering strategy of raw RAD-seq data sets (e.g., McKinney et al., 2017; O'Leary et al., 2018) and measuring its effects on population genetic summary statistics (Shafer et al., 2017). Care was taken in this study to minimize the impact of artifactual SNPs on the true biological signal of the data sets (see Supporting Information "Materials S1"). To achieve this, we studied the impact of different filtering cutoffs on several parameters of genetic diversity and departure from panmixia: (i) the mean F_{IS} and standard deviation (SD) among loci, (ii) the overall F_{ST} and SD among loci, and (iii) the SNP error rate, defined as the proportion of SNP mismatches between replicate pairs (Mastretta-Yanes et al., 2015). We specifically explored the effect of the maximum rate of missing data allowed per SNP (max-missing), the minor allele frequency (MAF), and the mean read depth per locus across all individuals (meanDP), or by considering only loci for which genotypes were strictly identical between replicates. Further details are given in Supporting Information Materials S1.

2.5 | Clonality in *Laminaria rodriguezii*

Because of somatic mutations and sequencing errors, we do not expect to get two strictly identical genotypes over thousands of loci even if they belong to the same clonal lineage. Consequently, we considered that genotypes with very low divergence corresponded to the same clonal lineage. We chose the maximum distance between replicates (d = 0.022 for *L. rodriguezii*, d = 0.020 for *L. digitata*, see Results) as a threshold to identify repeated genotypes. Distances were computed as the ratio of the number of allelic differences between two individuals (the Hamming distance, implemented in the function *diss.dist* of the Poppr v2.8.2 R package). Samples of *L. rodriguezii* separated by a distance lower than the aforementioned threshold were clustered into clonal lineages (referred here as MLLs Multi-Locus Lineages, see Arnaud-Haond, Migliaccio, et al., 2007) using the UPGMA clustering algorithm (implemented in Poppr v2.8.2 R packages: Kamvar et al., 2014, 2015). The matrix of genetic distances was additionally used for the construction of a network with the neighbour-net method implemented in SplitsTree, which was used to visualize genetic proximity of samples from the same clonal lineages, and also the differences among populations (Huson, 1998). We computed the genotypic richness (R) index within populations (Dorken & Eckert, 2001), as follows:$R = \frac{G-1}{N-1}$, where G is the number of distinct genotypes detected in the population and N the total number of individuals sampled. We thus considered each cluster of clonal individuals as a single genotype to calculate G. The distribution of clonal membership was analysed with the Pareto β index as described in Arnaud-Haond, Duarte, et al., (2007) using the Poppr v2.8.2 R package. Finally, the average values of genetic diversity and population differentiation of *L. rodriguezii* were estimated in two complementary ways: firstly, including all genotyped individuals, secondly by keeping only one representative of each clonal lineage.

2.6 | Genetic diversity

The within-population genetic diversity was assessed with the data sets of *L. rodriguezii* and *L. digitata*, after the removal of potentially selected loci. Given that the percentage of polymorphic loci (%P) may be affected by the variations in sample sizes, we used a random sampling approach implemented in a custom bash script (available at https://github.com/rebecca-cj/revegetation). The percentage of polymorphic loci was calculated from 100 random resamplings (with replacement between samples) for each sample size at the population level. In addition, estimates of observed heterozygosity (H_o), expected heterozygosity (H_e), and F_{IS} (Weir & Cockerham, 1984) were calculated with the GENEPOP v1.0.5 R package (Rousset, 2008). The same data sets were also analysed with the HierFstat R package (Goudet, 2005) to compute the distribution of F_{IS} values across loci. We used a two-sample Kolmogorov-Smirnov (KS) test to test for differences in the distribution of F_{IS} between populations. We used the KS D statistic as the maximum distance between cumulative distributions to assess the magnitude of these differences. The *p*-value of the D statistic was computed using a bootstrapping method (10^6 bootstraps), with the Matching v4.9.7 R package (Sekhon, 2008). The distributions were divided into 10 discrete classes of F_{IS} for which the frequency of occurrence in populations was shown as histograms. The Global Hardy-Weinberg test (Score [U] test) (Raymond & Rousset, 1995), implemented in GENEPOP, was used to test departure from panmixia across all loci for the different populations. The test was performed twice: firstly by considering the alternative hypothesis of heterozygote excess and secondly by considering heterozygote deficiency. The *p*-value of each test was approximated by Markov chain (MCMC) algorithm with the following settings; dememorization: 10 000, batch: 100, iterations per batch: 10 000. Then, a multisample score test (Raymond & Rousset, 1995) was performed to obtain a global *p*-value per population. Finally, the two-sided *p*-value of the Global Hardy-Weinberg test (Score [U] test) was obtained by computing the following formula: Pmin + (1 - Pmax) where Pmin and Pmax are the smaller and the higher probabilities of unilateral tests, respectively (De Meeûs, 2012). Linkage disequilibrium (LD) among loci was analysed with the standardized index of multilocus association $\overline{r_d}$ (Agapow & Burt, 2001) in Poppr v2.8.2. The $\overline{r_d}$ index is a summary statistic of linkage disequilibrium based on the variance of pairwise distances among individuals (see details in Agapow & Burt, 2001). This index is particularly useful for data sets with a high number of loci for which pairwise approaches are difficult to apply. To avoid the consequences of a Wahlund effect, we computed the $\overline{r_d}$ index separately for each sampling site. Genotypes were permuted 1,000 times among individuals inside populations to test for linkage disequilibrium.

2.7 | Population differentiation in *Laminaria rodriguezii*

The overall level of genetic differentiation (overall F_{ST}) was estimated in Genepop v4.2.2. We performed a G-based test of genotypic differentiation across loci (Goudet et al., 1996) with the same software. The *p*-value for each locus was computed using the Markov chain method with default parameters (500,000 iterations). The significance of the test was evaluated by the method of the harmonic mean *p*-values (HMP) (Wilson, 2019), implemented in the harmonicmeanp v3 R package. The test compares P_R, the HMP of a set of *p*-values *R*, to α_L, the significance threshold for a false positive rate weighted by ω_R, the total number of individual *p*-values (see Table 1 in Wilson, 2019). The HMP *p*-value is significant when $P_R \leq \alpha_L \omega_R$. We used a false positive rate of 0.05 and an equal weight for each *p*-value, considered as similar to Bonferroni correction.

3 | RESULTS

3.1 | Sequencing and SNPs filtering

Approximately 1.2 billion (1,154,828,457) raw reads were produced from 49 samples of *L. rodriguezii* (i.e. 47 individuals and two replicates) and 130 samples of *L. digitata* (i.e., 125 individuals and five replicates). After quality filtering and read trimming, an average of 2.7 million (± 1.5 million SE) and 2.3 million (± 2.1 million SE) high-quality reads per individual were retained for *L. rodriguezii* and *L. digitata*, respectively. The percentage of reads mapped to the *L. digitata* genome, ranged from an average of 85.4% (min: 53.9%; max: 92.2%) for *L. rodriguezii*, to 94.2% (min: 67.9%; max: 97.6%) for *L. digitata*. The initial filtering of loci, which retained those shared by a high proportion of individuals, resulted in catalogs of 44,716 loci and 60,942 loci for *L. rodriguezii* and *L. digitata*, respectively. At this stage, the number of SNPs was more than eight times higher for *L. digitata* than for *L. rodriguezii* (Table 1). Additional quality filtering steps followed by outlier exclusion resulted in the selection of 4,077 putatively neutral SNPs among 43 individuals of *L. rodriguezii* and 7,364 putatively neutral SNPs among 116 individuals of *L. digitata* (Table 1). Specifically, 289 SNPs (6.62%) and 174 SNPs (2.31%) were identified as outliers for *L. rodriguezii* and *L. digitata*, respectively. The rate of missing data was slightly higher in *L. digitata* (i.e., 3.36%) than in *L. rodriguezii* (i.e., 2.24%). The mean SNP error rate across replicates was estimated to be 1.06% in *L. digitata* and 3.89% in *L. rodriguezii* data sets.

3.2 | Clonal structure of *Laminaria rodriguezii*

In *L. rodriguezii*, the distribution of pairwise genetic distances was discontinuous with a gap from the threshold d = 0.022 to the lowest distance reported above this threshold (d = 0.042) (Figure 2a). This threshold corresponded to the maximum distance observed among replicates. The use of this threshold allowed us to identify four different clonal lineages with several members: namely MLL-A, MLL-B, MLL-C and MLL-D, composed of three, three, seven, two samples respectively (see the network, Figure 3). By selecting only one sample for each clonal lineage, we finally retained 32 different samples in our data analyses from the 43 samples initially present in the whole data set. At Bonifacio (Southern Corsica), we reported a relatively strong occurrence of clonal lineages (R = 0.20), with the dominance of a few large clones (β = 0.56), which contrasted with the low occurrence of clonal lineages (R = 0.90) of small size (β = 2.66) in Eastern Provence (Table S2, Supporting Information). For instance, the maximum clonal size reported was seven at Bonifacio against three in Eastern Provence. The analysis of the spatial genetic structure along the transect at Banc Magaud_2 (Figure S1, Supporting Information) revealed that: (i) individuals belonging to the same clonal lineage were relatively close to one another (less than 1 m), and (ii) the genetic distance between individuals increased with spatial distance (Mantel test, R = 0.40, $p < 0.001$). For the other sites, the sampling protocol (with a minimum spacing of 2 m), did not allow a similar investigation of the spatial genetic structure. Contrary to *L. rodriguezii*, no pairwise distance among distinct individuals of *L. digitata* was below the distance threshold defined by replicates (Figure 2b), indicating that there was no cluster of genotypes grouped in clonal lineages. The lowest divergence among individuals in *L. digitata* was around d = 0.035, and corresponded to intrapopulation differences within the Helgoland population.

Besides the detection of clonal lineages, we observed in *L. rodriguezii* three individuals, with distinct long branches in the phylogenetic networks (indicated as outliers in Figure 3). These three outlier individuals shared a common characteristic: the highest proportion of heterozygous genotypes among loci per individual, with an average of 33% against 16% in other individuals (Figure S2, Supporting Information). These outlier individuals also had the highest percentage of private alleles among all individuals (average percentage of private alleles at the individual level (Pr) =20.3% for outlier individuals and Pr =1.0% among the other 40 genotyped individuals, see Figure S2, Supporting Information). By definition, these private alleles were different among the three individuals.

3.3 | Genetic diversity

Laminaria rodriguezii and *Laminaria digitata* displayed similar expected heterozygosity (H_e), with an average H_e within populations of 0.14 and 0.15, respectively (Table 2). The proportion of polymorphic loci (P) within populations (estimated with the minimum sampling size *n* = 10 and 100 random resamples) was higher in *L. rodriguezii* (average *p* = 54.42%) than in *L. digitata* (average *p* = 39.39%) (see Table 2, Figure S3, Supporting Information). We observed a departure from panmixia in both species, with an excess of heterozygotes in *L. rodriguezii*, and a deficit of heterozygotes in *L. digitata* (see Table 3 for *L. rodriguezii* and Table S1, Supporting Information for *L. digitata*). The heterozygotes excess was statistically significant (Score [U] test, $p < 0.001$) within populations for *L. rodriguezii*, except

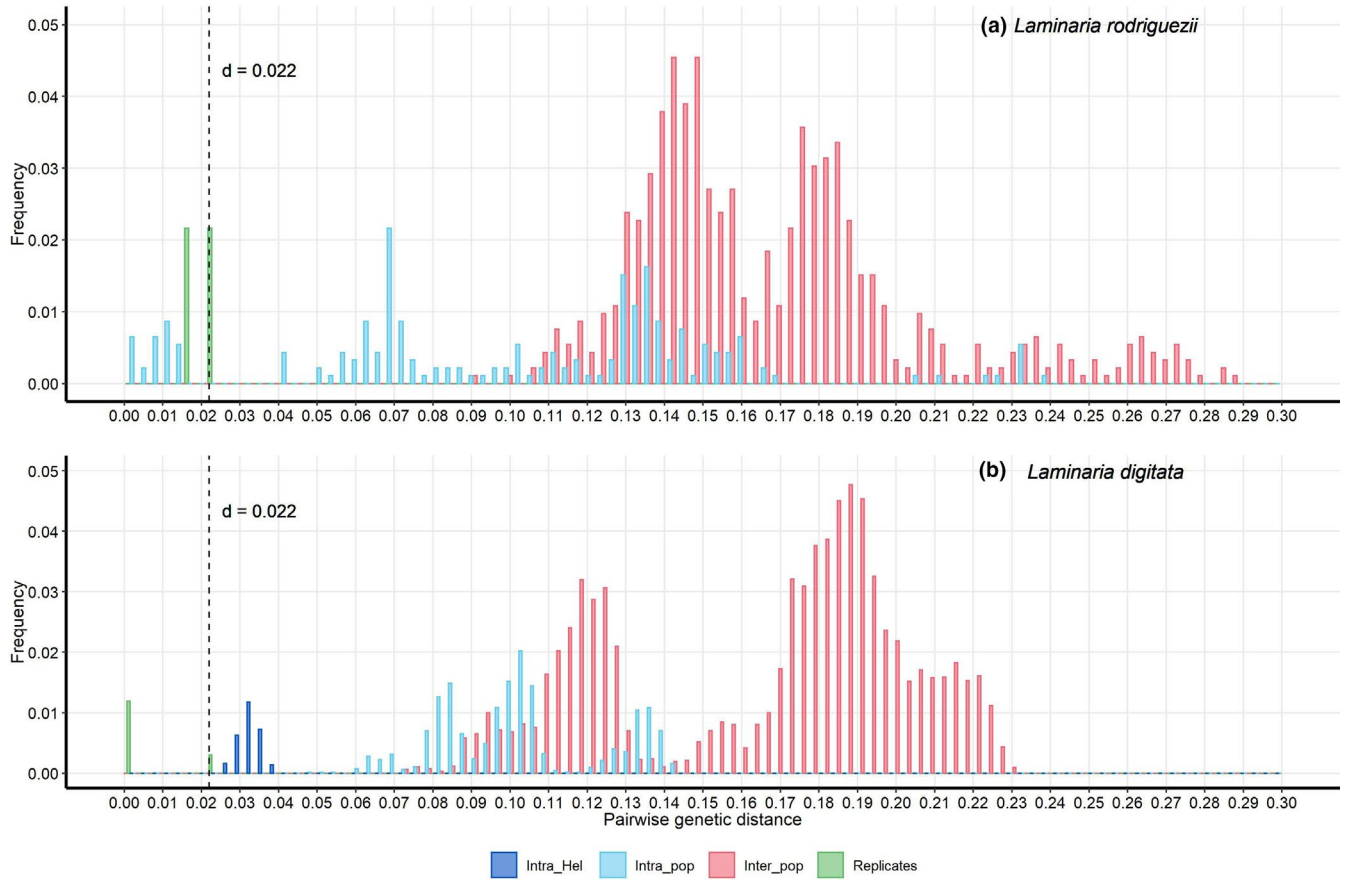

FIGURE 2 Frequency of pairwise genetic distances between individuals of (a) *Laminaria rodriguezii* and (b) *Laminaria digitata*. Intrapopulational (Intra-pop), interpopulational (Inter-pop), within Helgoland (Intra-Hel) and replicates (Replicates) comparisons are coloured according to the legend. The frequency of pairwise genetic distances across replicates was multiplied by 40 to create an optimum bin size. The vertical dotted line shows the distance threshold (d = 0.022) as reported as the maximum value of replicate pairs [Colour figure can be viewed at wileyonlinelibrary.com]

for Banc Magaud_1. The heterozygotes deficit was significant (Score [U] test, $p < 0.001$) within populations of *L. digitata*.

The distributions of F_{IS} were significantly different for all intra- and interspecific comparisons of *L. rodriguezii* and *L. digitata* populations (KS tests, $p < 0.001$, D statistic: 0.07–0.53). At the intraspecific level, the most striking difference in the distributions of F_{IS} (KS tests, D statistic: 0.26–0.27) was detected for *L. rodriguezii* between Eastern Provence (Banc Magaud_1, Banc_Magaud_2 and Cap Camarat) and Bonifacio (Figure 4a). The distributions of F_{IS} in Eastern Provence were moderately skewed to negative values with a long tail to positive values (skewness =0.69; kurtosis =4.07). The distribution of F_{IS} in Bonifacio was highly skewed toward negative values (skewness = −0.95; kurtosis =3.14) and showed a much higher proportion of extreme negative values as above to −0.8 (13.6%) in comparison to those measured in Eastern Provence (average, 0.6%). Similarly, the variance in F_{IS} among loci was higher at Bonifacio (var =0.13) than in Eastern Provence (var =0.08, 0.07 and 0.06 for Banc Magaud_1, Banc Magaud_2, and Cap Camarat, respectively).

In contrast to these results, F_{IS} values were mainly positive in *L. digitata*, whatever the population considered even if the distribution mode of F_{IS} is always observed between −0.1 and 0 (Figure 4b). Extreme negative F_{IS} (above −0.8) and even moderate to high negative values (those between −0.5 and −0.3) were almost absent in *L. digitata* with frequencies of 0.03% and 0.06%, respectively. The distribution mode of F_{IS} was observed for slightly negative values between −0.1 and 0 whatever the population considered (Figure 4b). The asymmetry of the distributions, as revealed by the positive skewness (skewness =0.98; kurtosis =3.32) is indicative of a long tail toward high and extreme positive values (0.5 to 1). This class of extreme positive F_{IS} corresponded to 28% of all loci in Helgoland. At the intraspecific level, the second major difference in the shape of the distribution of F_{IS} was reported between Helgoland and all other populations of *L. digitata* (KS tests, D statistic 0.11–0.23).

The average multilocus estimates of F_{IS} within populations ranged from 0.12 to 0.43 for *L. digitata* (Table S1, Supporting Information) whereas mean values were mainly negative within populations in *L. rodriguezii*, with a range from −0.47 in Bonifacio to −0.02 at Banc Magaud_1 (Table 3A). After applying the correction for repeated genotypes, the average multilocus estimates of F_{IS} increased to a range from F_{IS} = −0.26 at Bonifacio to F_{IS} = −0.01 at Banc Magaud_2 (Table 3B). After the correction for repeated genotypes, the heterozygosity excess only remained significant at Bonifacio (Score [U] test, $p < 0.001$).

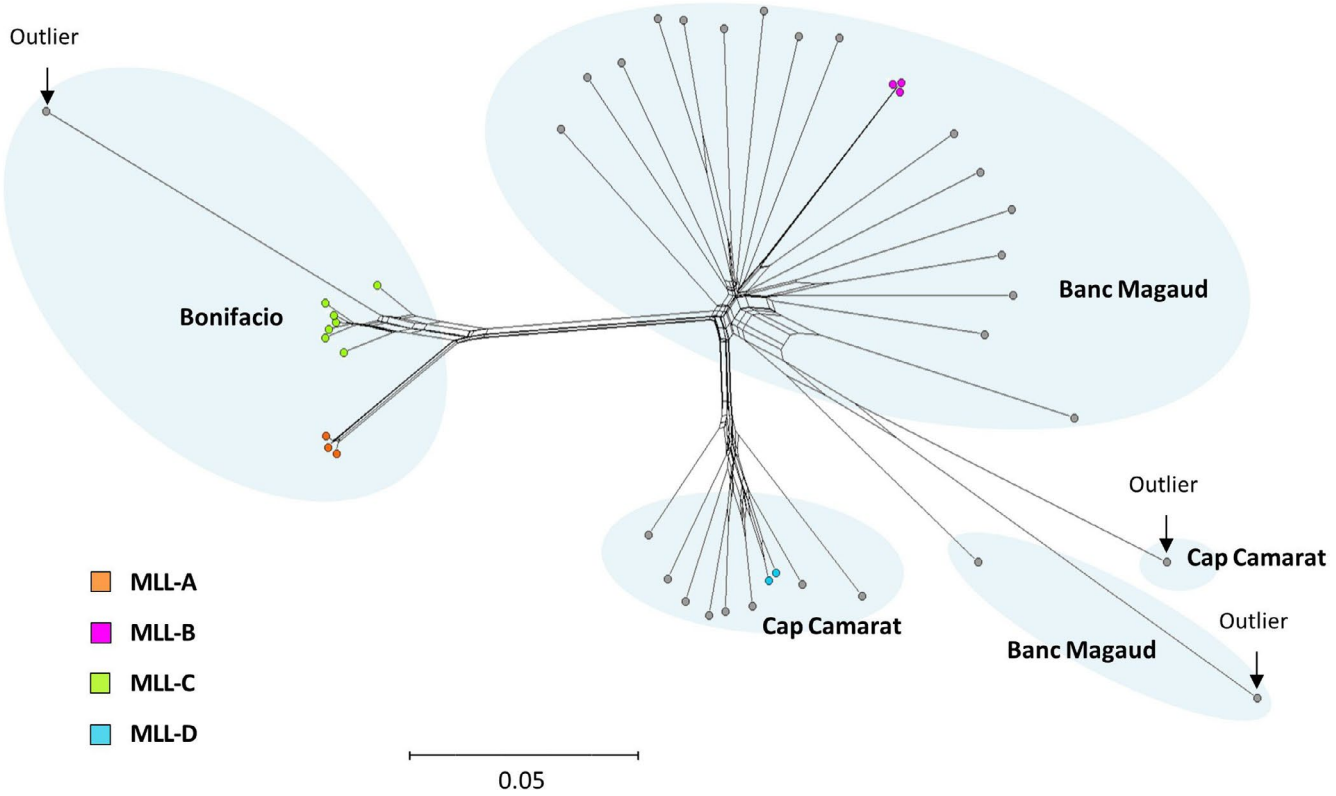

FIGURE 3 Phylogenetic networks of *Laminaria rodriguezii* represented with the neighbor-net method. Each dot represents an individual, Individuals belonging to the same clone (MLLs, multilocus lineages) are coloured according to the legend. Individuals characterized by the highest proportion of heterozygous genotypes and private alleles are indicated as outliers [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Quality filters used to build the final RAD-seq data sets of *Laminaria rodriguezii* and *Laminaria digitata*

	%P	H_o	H_e	F_{IS}
L. rodriguezii				
Average	54.42	0.16	0.14	−0.14
Range	40.96– 72.14	0.11– 0.19	0.11– 0.18	−0.47 to −0.02
L. digitata				
Average	39.39	0.11	0.15	0.26
Range	21.44–54.89	0.09–0.14	0.12– 0.19	0.12– 0.43

For each filtering step, the number of SNP passing the filter (SNPs count), as well as the mean missing data rate per individual (missingness) and the mean sequencing depth per individual (coverage) are reported. Loci were initially built in both species from the draft genome of *L. digitata* using the Stacks v.2.4 bioinformatics pipeline. The threshold of missing genotypes per individual <30% has deleted four and nine individuals in *L. rodriguezii* and *L. digitata* data sets respectively. See Methods for details

The distribution of the index of multilocus association (Figure 5) was nearly identical whatever the number of SNPs subsampled (i.e., 1,000; 2,000 and 3,000 random SNPs). The mean $\overline{r_d}$ was statistically significant ($p < 0.001$) within all populations of both species. For *L. rodriguezii*, the $\overline{r_d}$ index reached high levels within all populations (mean $\overline{r_d}$ =0.08 to 0.27), except at Banc Magaud_1 (mean $\overline{r_d}$ =0.01) for which there was no repeated sampling of a clonal lineage (Table 3). The values of $\overline{r_d}$ decreased slightly by keeping only one representative of each clonal lineage (Table 3, Figure 5). For *L. digitata*, the mean $\overline{r_d}$ ranged between 0.01 to 0.03 within all populations (Table S1, Figure 5), except for Helgoland that displayed a remarkably high level of linkage disequilibrium similar to values observed in *L. rodriguezii* (mean $\overline{r_d}$ =0.20).

3.4 | Population differentiation in *Laminaria rodriguezii*

Laminaria rodriguezii exhibited high levels of population structure with a global F_{ST} of 0.28 when considering all individuals (n = 43).

TABLE 3 Genetic variation within populations of *Laminaria rodriguezii* (a) before and (b) after applying the clonal correction

Population	Label	*N*	*Ho*	*He*	F_{IS}	$\overline{r_d}$
(a)						
Banc Magaud_1	BM_1	10	0.18	0.17	−0.02	**0.01**
Banc Magaud _2	BM_2	11	0.19	0.18	**−0.04**	**0.08**
Cap Camarat	CAM	11	0.11	0.11	**−0.05**	**0.10**
Bonifacio	BON	11	0.16	0.11	**−0.47**	**0.27**
(b)						
Banc Magaud_1	BM_1	10	0.18	0.17	−0.02	**0.01**
Banc Magaud _2	BM_2	9	0.19	0.19	−0.01	**0.05**
Cap Camarat	CAM	10	0.11	0.11	−0.02	**0.10**
Bonifacio	BON	3	0.20	0.16	**−0.26**	**0.20**

Clonal correction consists of keeping only one representative of clonal lineages. N, number of individuals successfully genotyped, H_o, observed heterozygosity, H_e, expected heterozygosity, F_{IS}, fixation index, $\overline{r_d}$, average multilocus linkage disequilibrium. F_{IS} and $\overline{r_d}$ values highlighted in bold indicate significant heterozygote excess within populations ($p < 0.001$) and significant linkage disequilibrium ($p < 0.001$), respectively

After applying the clone correction (i.e., one sample per clonal lineage), the estimate of genetic differentiation decreased (global F_{ST} =0.18). After correcting for multiple testing, genotypic differentiation among populations of *L. rodriguezii* was significant (HMP significance threshold,$\alpha_L\omega_R = 0.032$; HMP *p*-value *PR =0*), with and without the clone correction.

3.5 | Negative F_{IS} and high F_{ST} in *L. rodriguezii* over SNP filtering methods

For *L. rodriguezii*, the mean F_{IS} and overall F_{ST} varied between the reference data set and the filtered data sets (Table 4). However, the mean F_{IS} was always negative, with high variance among loci, whatever the levels of stringency. In the same way, the overall F_{ST} stayed high and it was never below 0.27. The maximum rate of missing data allowed per SNP (max-missing) had a very limited effect on mean F_{IS} and overall F_{ST}, which ranged from −0.06 to −0.07 and from 0.27 to 0.30, respectively. The highest variance was associated with MAF cutoff as the filter excluded the lowest polymorphic loci, and subsequently increased the level of genetic variation in the data set. Therefore, we choose to keep the MAF cutoff at 2% so as not to inflate the levels of genetic variation in *L. rodriguezii*. The SNP error rate decreased from 5.22% in the reference data set to 2.74% in the data set which was filtered by excluding loci with low coverage (i.e., meanDP >15x). Genotyping errors were probably nonrandomly distributed and occurred mostly in loci with low coverage, so we excluded them by applying meanDP >15x.

4 | DISCUSSION

We used RAD-sequencing to analyse the genome-wide diversity of the rare *Laminaria rodriguezii*. We found that intraindividual genomic diversity is a powerful tool to identify clonal lineages. We also proposed that the observed F_{IS} distribution and linkage disequilibrium at the genome scale can be compared with theoretical models to search for a discernable signal of clonality, even in this partially clonal reproducing species. Finally, we will discuss here the findings of high heterozygosity within some individuals that raise questions about the mechanisms allowing the persistence of such genomic features.

4.1 | RAD-seq as a powerful tool for detection of clonal lineages

The identification of clonal lineages in natural populations was addressed by assessing the probability that identical or slightly distinct genotypes belong to the same clonal lineage. That can be achieved through the development of standardized methods allowing for investigation of the influence of somatic mutations and scoring errors (Arnaud-Haond et al., 2007a). However, while this methodological framework is particularly robust for a small number of loci, RAD-seq and the amount of data generated by next generation sequencing (NGS) brings new challenges. The main concern is that increasing the number of loci is equivalent to increasing the number of missing data and genotyping errors that can subsequently affect the detection of clonal lineages. This is especially the case for reduced representation sequencing (e.g., RAD-seq) sensitive to missing data and genotyping error, thus necessitating appropriate filtering strategy (e.g., Mastretta-Yanes et al., 2015; Shafer et al., 2017; Andrews et al., 2018; Boscari et al., 2019). How then can we find an adequate distance threshold for collapsing slightly distinct genotypes into clonal lineages? For that purpose, we can define a threshold using the pairwise genetic distances, a threshold below which genotypes are assumed to belong to the same clonal lineage. Some studies have fixed a cutoff based on the gap in pairwise genetic distances (e.g., Shrestha et al., 2014), while others have defined an arbitrary 95% genotype similarity threshold (e.g., Locatelli & Drew, 2019). Nevertheless, such

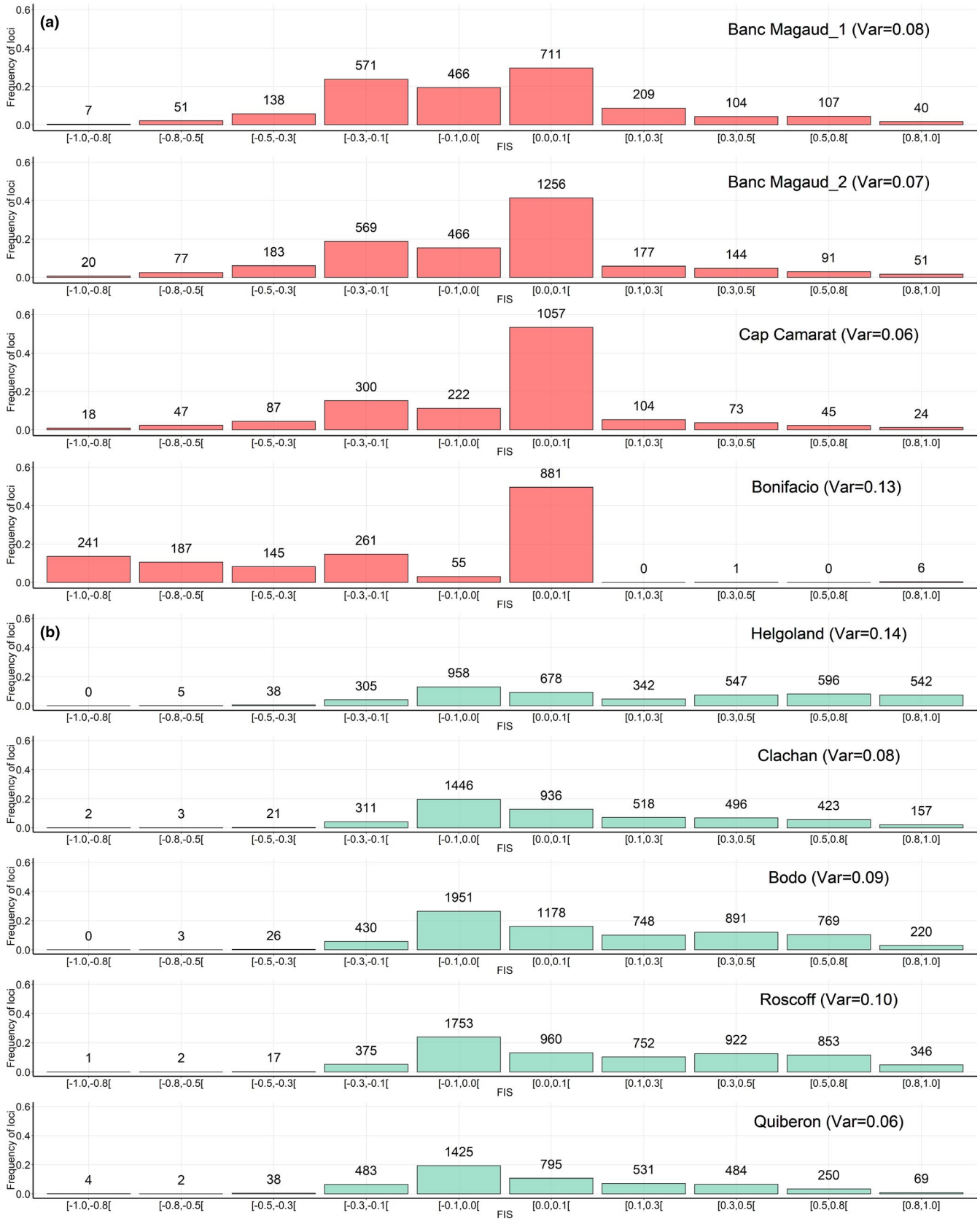

FIGURE 4 Distributions of the fixation index F_{IS} among loci within populations of (a) *Laminaria rodriguezii*, and (b) *Laminaria digitata* computed across 4,077 and 7,364 putatively neutral SNPs, respectively. The F_{IS} values were distributed into 10 discrete classes and the number of occurrences for each class is indicated on top of histogram bars. The variance of F_{IS} among loci (Var) is indicated in brackets next to population labels [Colour figure can be viewed at wileyonlinelibrary.com]

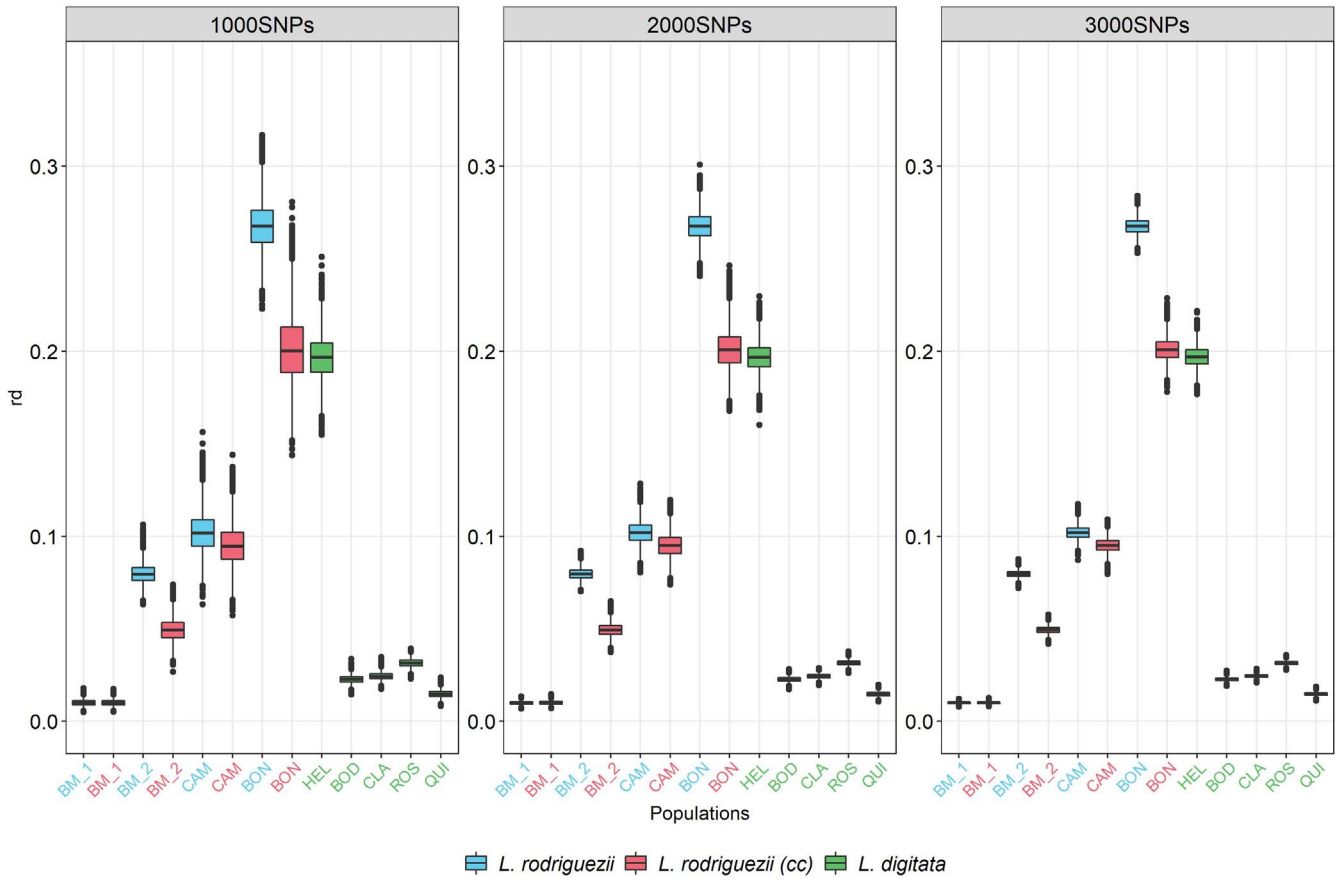

FIGURE 5 Distributions of the standardized index of multilocus association $\overline{r_d}$ within populations of *Laminaria rodriguezii* before (highlighted in blue) and after (highlighted in red) clonal correction (cc). Comparison of the index value was done with five populations of *Laminaria digitata* (highlighted in green). The $\overline{r_d}$ values were estimated using 10,000 iterations by random subsampling of 1,000; 2,000 and 3,000 SNPs. The abbreviations used for populations are those reported in Table 3 for *L. rodriguezii* and Table S1 for *L. digitata* [Colour figure can be viewed at wileyonlinelibrary.com]

approaches can be inaccurate, particularly when different sources of noise (i.e., genotyping error, missing data, somatic mutation) can accentuate differences between clonal genotypes above the detection threshold. This also depends on the genetic diversity of the populations: clonal lineages are more easily identified in populations where the diversity among sexually produced individuals is high, i.e., in partially clonal species. In this study, we proposed defining a threshold on the basis of the pairwise genetic distance across replicates. While this approach seems to be useful in evaluating the genetic distance expected as a result of genotyping errors and somatic mutations, it has rarely been reported, except in *Leptopsammia pruvoti* (Boscari et al., 2019). For this organism genotyped with 1386 SNPs (with the 2b-RAD protocol), slightly distinct genotypes below a distance threshold set at d = 0.043 were collapsed into clonal lineages. For *L. rodriguezii*, this cutoff was lower (d = 0.022), even if of a similar order as in *L. pruvoti*. This difference highlights the importance of not considering a common threshold but rather of adjusting it using genotype mismatch across replicates. Note that using the same method on both species, the threshold was very similar between *L. rodriguezii* and *L. digitata*.

4.2 | The genomic patterns of clonality

Our results show how the distribution of F_{IS} among loci can be used to study species and populations with various signals of clonality including partial clonality. Signatures of clonality in natural populations are usually inferred from repeated genotypes, along with mean negative values of F_{IS}, and linkage disequilibrium across loci (Balloux et al., 2003; Halkett, et al., 2005). Nevertheless, recent studies reported that one of these signatures, genotypic richness, had poor relevance for measuring the relative importance of sexual versus clonal reproduction (Arnaud-Haond et al., 2020), even using relatively large sample sizes (from 100 to 500 individuals) (Stoeckel et al., 2020). This is because the estimates of genotypic richness are strongly dependent on sampling strategy and density, basically decreasing with increasing sampling effort (Arnaud-Haond, Duarte, et al., 2007; Becheler et al., 2017; Gorospe et al., 2015). In our study, by looking at the F_{IS} distribution in the genome, we propose a new analysis which seems useful to detect partial clonal reproduction. Our approach confirmed the prediction that inter-locus variance of F_{IS} (rather than an average value) increased as

TABLE 4 The effect of SNP filtering cutoffs on the mean (± SD) F_{IS} over loci, overall (± SD) F_{ST} over loci and the SNP error rate in *Laminaria rodriguezii* [Colour table can be viewed at wileyonlinelibrary.com]

Quality filter	Cutoff	SNPs count	Missingness (%)	Coverage	Mean (± SD) F_{IS}	Overall (± SD) F_{ST}	SNP error rate (%)
Reference data set		21 357	4.11	13.00	−0.07 (0.20)	0.27 (0.18)	5.22
Max-missing (%)	< 15	21 114	3.97	13.08	−0.07 (0.20)	0.27 (0.18)	5.17
	< 10	19 468	3.33	13.52	−0.07 (0.20)	0.27 (0.18)	4.94
	< 5	14 480	1.95	14.88	−0.07 (0.19)	0.28 (0.18)	4.30
	0	5 479	0.00	18.12	−0.06 (0.18)	0.30 (0.19)	2.90
MAF (%)	> 2	12 735	4.28	13.31	−0.08 (0.26)	0.30 (0.22)	7.07
	> 5	5 094	4.54	16.18	−0.12 (0.34)	0.37 (0.26)	4.92
	> 10	3 428	4.56	17.29	−0.14 (0.37)	0.42 (0.26)	3.86
MeanDP (X)	> 5	19 245	3.59	13.71	−0.08 (0.20)	0.28 (0.18)	4.88
	> 10	11 773	2.52	17.41	−0.11 (0.22)	0.31 (0.21)	3.92
	> 15	6 163	1.90	21.85	−0.13 (0.23)	0.33 (0.22)	2.74
Max-missing cutoff for fixed MAF (2%) and meanDP (>15)	< 20	3 911	2.14	21.83	−0.15 (0.29)	0.34 (0.25)	3.71
	< 10	3 850	1.99	21.81	−0.14 (0.28)	0.35 (0.25)	3.70
	< 5	3 464	1.41	21.90	−0.12 (0.28)	0.35 (0.25)	3.53
	0	1 813	0.00	22.71	−0.10 (0.27)	0.37 (0.26)	2.68
Matching genotypes		3 638	2.14	22.07	−0.15 (0.29)	0.35 (0.25)	0.00

As thresholds, we considered the maximum rate of missing data allowed per SNP (max-missing), the minor allele frequency (MAF), the mean read depth per locus across all individuals (meanDP). For each cutoff value we reported the number of SNP passing the filter (SNPs count), the mean missing data rate per individual (missingness), and the mean read depth per individual (coverage). We considered an additional set of SNPs (matching genotypes) for which replicates have exactly the same multilocus genotypes (SNP error rate =0)

clonal rates increased (Balloux et al., 2003; Halkett, et al., 2005; Stoeckel & Masson, 2014; Reichel et al., 2016; Stoeckel et al., 2020). We also reported (i) contrasted distribution of F_{IS} between species, with F_{IS} shifted towards negative values in the partially clonal species *L. rodriguezii*, and towards positive values in the fully sexually reproducing species *L. digitata*, and (ii) striking differences in distributions of F_{IS} among populations of *L. rodriguezii*, with higher interlocus variance in F_{IS} at Bonifacio (Corsica) than in the three localities of Eastern Provence. Remarkably, the empirical distributions of F_{IS} in Eastern Provence matched well with the simulation results of Stoeckel and Masson (2014) which predicted that most F_{IS} values are concentrated between −0.3 and 0.1 below intermediate levels of clonality.

Genetic drift is another possible explanation for negative F_{IS} values. In a finite population with random mating, genetic drift due to sampling effect indeed tends to create a slightly negative mean F_{IS}, but the effect is small, of order 1 / (2 N) where N is the size of the population (see Figures 3 and 4 in Stoeckel & Masson, 2014). For the fully sexual dioicous species *L. digitata*, this drift effect could explain why the distribution mode of F_{IS} is between −0.1 and 0 whatever the population considered. However, the probability of highly negative values increases when genetic drift is combined with intermediate rates of clonality (Stoeckel & Masson, 2014). Nevertheless, Stoeckel and Masson (2014) showed that values below −0.3 remain rare if the clonality rate is below 0.9. This is indeed what is observed for all *L. rodriguezii* populations, suggesting that their clonality rates are intermediate, except at Bonifacio, the Corsican population for which we observed the highest proportion of F_{IS} below −0.3 and even below −0.8. In addition, the estimates of genotypic richness (R), Pareto (β), and linkage disequilibrium ($\overline{r_d}$) emphasized the degree of distinction between the Eastern Provence and Bonifacio populations, thus strengthening our hypothesis of a higher level of clonality in Corsica than in Eastern Provence. A more in-depth investigation of the combined effects of drift and clonal reproduction on the distribution of F_{IS} would be useful in this context. Parameters other than reproductive mode differ between the two species compared here, such as the levels of genetic drift, the investigated geographical range, and potentially their demographic history. The population of Helgoland for the fully sexually reproducing species, *L. digitata*, reached a high level of linkage disequilibrium comparable to the Bonifacio population of *L. rodriguezii* for which the signature of clonality was clearly discernible. Nevertheless, Helgoland had a distribution of F_{IS} markedly different from *L. rodriguezii*, with a majority of positive values. Helgoland is a rocky island surrounded by several hundreds of kilometers of sandy seafloor. This population shows the highest levels of positive F_{IS}, the lowest values of pairwise genetic distances between individuals, and the highest value of linkage genetic disequilibrium observed for *L. digitata*. This is most probably explained by the effect of low effective size and maybe of a bottleneck. This population also showed the lowest level of genetic diversity observed for *L. digitata* when genotyped with the microsatellite locus in the study of Liesner et al., (2020). There is a lack of

case studies to conclude more precisely on the role of drift and of demographic history in the distribution of inter locus F_{IS} variance. However, the theoretical results of Stoeckel and Masson (2014) and Stoeckel et al. (2020) agree well with our experimental results. This paves the way toward a better assessment of the importance of clonality through genomic studies, but it remains the case that the estimation of clonality rates is still only possible using temporal sampling (Ali et al., 2016; Becheler et al., 2017).

4.3 | Extreme levels of individual genomic heterozygosity

We reported an intriguing signal of genome-wide heterozygosity in three of the 43 individuals in *L. rodriguezii*. These outlier individuals were characterized by an exceptionally high proportion of heterozygous genotypes compared to other individuals. Given the relatively low sample sizes, we do not really know how these individuals depart from the population distribution in individual heterozygosity, but we can discuss several hypotheses regarding their origin. High heterozygosity at particular loci can correspond to paralogs assembled in the same RAD locus depending on assembly parameters (McKinney et al., 2017; Nadukkalam Ravindran et al., 2018; Verdu et al., 2016). However, for the following reasons, we would argue that polymorphisms detected within these outlier individuals do not correspond to artefactual loci: firstly, sequencing reads were mapped to the reference genome of a closely related species, followed by stringent filter criteria, including for maximum heterozygosity. Secondly, paired-end sequencing protocols provide additional information on correct read placement since RAD loci are retained when both reads are properly mapped (according to the insert length between forward and reverse reads). Thirdly, if paralogs were responsible for high levels of heterozygosity, we would not expect to observe this in only three individuals. On the other hand, recent studies have revealed that both cross-contamination among samples and environmental contamination, especially during library preparation and/or sequencing, can lead to artifactual results, with the sequencing of loci of different origins in the same sample (Ballenghien et al., 2017; Laurin-Lemay et al., 2012). Here, the three outlier individuals were also characterised by the highest proportion of private alleles at the individual level, and they were not grouped on the network. Such private alleles can then not be explained by cross-contamination among samples of the same species. Rather they could be explained by the accumulation of mutations along with clonal reproduction, thus increasing the divergence among loci in the same individuals, a potential signal of the Meselson effect (Birky, 1996; Welsh & Meselson, 2000; De Meeûs et al., 2007). These outlier individuals could then belong to a relatively ancient asexual lineage. However, we lack temporal sampling to get more precise estimates of the rates of clonal reproduction, and therefore, the hypothesis of clonality to explain these outlier individuals remains highly speculative. A high frequency of heterozygous loci could also be observed following hybridization among well differentiated genetic lineages. Hybridization has indeed been proposed as the main driver of high heterozygosity in parthenogenetic animals (Jaron et al., 2020). Here, the hybrid hypothesis should involve several hybridizations from three different sources to explain the observed pattern of private alleles, as well as the genetic distance among the three outliers. It would therefore be interesting to analyse the genomic diversity of other *L. rodriguezii* populations in the Mediterranean in order to go further on this topic. Nevertheless, the populations of *L. rodriguezii* are scarce, and there are no other congeneric species in this area.

4.4 | Implications for conservation

Our results emphasise that *L. rodriguezii* is spatially structured into genetically distinct populations. At Banc Magaud_2 we also evidenced a significant spatial genetic structure: regarding the low occurrence of clonality in this population, this structure is better explained by reduced dispersal through haploid spores. Although high levels of population genetic structure are not uncommon in kelps (e.gBillot et al., 2003; Coleman et al., 2009; Durrant et al., 2018), our results contrast with the lack of genetic differentiation among populations of *L. digitata* spatially distant by up to 10 km (Billot et al., 2003; Robuchon et al., 2014). In this respect, the spatial scale of dispersal of *L. rodriguezii* is presumably smaller than that reported in other species of the genus *Laminaria* (Santelices, 1990). The patchy distribution and small population sizes of *L. rodriguezii* can probably explain this pattern of high and local genetic differentiation through strong local genetic drift and limited gene flow among populations. The identification of such a local genetic structure has important implications for management and conservation. The high genetic drift and limited gene flow can explain the lower genetic diversity expected in *L. rodriguezii* compared to *L. digitata*, a species with a wider geographical distribution, exhibiting semi-continuous and dense populations (see Frankham, 1996; Hamrick & Godt, 1990; Levy et al., 2016). Furthermore, the range of *L. rodriguezii* has been drastically reduced during the last half-century, especially in the Adriatic Sea, where the species has suffered losses of 85% from its historical range (Žuljević et al., 2016). However, the observed heterozygosity and the percentage of polymorphic loci were higher in *L. rodriguezii* when compared to *L. digitata*. This can be explained by the conservation of heterozygous genotypes with low recombination rates (Judson & Normark, 1996; Balloux et al., 2003; see Meloni et al., 2013 for an empirical example), as genetic drift under clonal reproduction acts on genotype frequencies rather than allele frequencies (Reichel et al., 2016; Stoeckel & Masson, 2014). Further studies would be necessary to test with genomic data whether the current fragmented range and small populations of *L. rodriguezii* correspond to a demographic reduction from a larger ancestral population. The extensive cartography and monitoring of the biocenoses down to 100 m along French coasts do point to the rarity of this species. The conservation of these last isolated populations should become a high priority for stakeholders.

5 | CONCLUSION

Our study shows that despite limitations on the detection of clonal lineages, SNPs derived from RAD sequencing are interesting markers for population genomic studies of partially clonal species. The interaction between demographic history and reproductive mode shaped the distribution of F_{IS} and linkage disequilibrium in *L. rodriguezii*. Interestingly, even if our loci were mapped to a reference genome for assembly, we did not use the physical location of loci in our analyses, which indicates that similar approaches could be envisioned with de novo assembly. In order to decipher the respective impact of demography and reproduction in our results, one next step could be to use summary statistics linked with these distributions (such as higher moments) for evolutionary inferences, using for example Approximate Bayesian Computation (Csilléry et al., 2010).

ACKNOWLEDGEMENTS

This project was funded, by the MARFOR Biodiversa/0004/2015 project (http://marfor.eu/), and by the Agence Nationale de la Recherche, Grant/ Award Number: ANR-18-CE32-0001 (Clonix2D). Lauric Reynes also received support from the GDR Génomique Environnementale (GDR3692). The project leading to this publication has received funding from the European FEDER Fund under project 1166-39417. This work was also funded by a Ph.D. Fellowship from Région PACA and the Calanques National Park. The authors would like to thank Julie Deter, in charge of the scientific coordination during sampling, the team of research divers of Andromède Océanologie for the prowess in sampling *Laminaria rodriguezii* at 76 meters depth, the Biogenouest genomics core facility (Genomer Plateforme génomique at the Station Biologique de Roscoff) for their technical support, Claire Daguin-Thiébaut and Quentin Langevin for useful discussions regarding the optimization processes in purpose-extracted high-quality DNA in brown macroalgae, Marck Cock providing the draft genome of *Laminaria digitata*, Thierry Gosselin for very useful discussions on the quality control of RADseq data, and finally Solenn Stoeckel for his comments on the manuscript. We deeply thank Thierry De Meeûs, three anonymous reviewers and the corresponding editor, Tatiana Giraud, for their comments that enabled us to greatly improve the current version of this manuscript. Finally, we would like to thank Michael Paul for English language proofreading.

AUTHOR CONTRIBUTIONS

Thierry Thibaut and Didier Aurelle devised the project and were responsible for the main conceptual ideas. Florian Holon performed the sampling of *Laminaria rodriguezii*. Aurélie Blanfuné contributed to sample preparation. Stéphane Mauger and Lauric Reynes performed the RAD-sequencing experiment and analysed sequencing reads. Corinne Cruaud and Arnaud Couloux provided the draft genome of *Laminaria digitata*. Lauric Reynes performed research, analysed data, and wrote the manuscript in close collaboration with Didier Aurelle and Myriam Valero. All authors provided critical feedback and helped to shape the research, analysis, and manuscript.

DATA AVAILABILITY STATEMENT

Individual high-quality reads (dd-RADseq) that support the findings of this study are openly available in a Dryad Digital Repository (https://doi.org/10.5061/dryad.hmgqnk9dq). The home-made scripts used to generate the distribution of F_{IS} values into classes, the histograms of pairwise genetic distances, and the graphs representing the percentage of polymorphic loci are available in the public repository: https://github.com/LauricReynes

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Reynes L, Thibaut T, Mauger S, et al. Genomic signatures of clonality in the deep water kelp *Laminaria rodriguezii*. *Mol Ecol*. 2021;30:1806–1822. https://doi.org/10.1111/mec.15860