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1 **Genetic resources of macroalgae: development of an efficient**
2 **method using microsatellite markers in non-model organisms**

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30 S. Mauger: original concept, laboratory work, bioinformatics analysis, analysis of molecular data, led
31 manuscript drafting and editing; A. Baud: sampling, led manuscript drafting and editing; G. Le
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34 manuscript; M. Valero: drafting and editing manuscript; P. Potin: funding and supervision, drafting
35 and editing manuscript; C. Destombe: funding and supervision, sampling and editing manuscript.

36

37 **ABSTRACT**

38 Red and brown seaweeds are species with high ecological and economic importance. Here we report
39 the feasibility of cost-effective molecular marker development in 6 species from different phyla.
40 Microsatellite markers of two brown seaweed species *Alaria esculenta* and *Pylaiella littoralis*, and of
41 four red seaweed species *Calliblepharis jubata*, *Gracilaria gracilis*, *Gracilaria dura* and *Palmaria*
42 *palmata* were identified and characterized in genomic sequences obtained using Double-Digest
43 Restriction site Associated DNA (ddRAD). A total of 64,623,186 reads were generated from two runs
44 of multiplexed Illumina Miseq sequencing, from which 30,636 reads containing microsatellite motifs
45 including 15,443 where primer pairs could be designed. Five hundred seventy-six primers pairs were
46 selected for amplification trials to determine levels of polymorphism. Of these 576 loci, 338 could be
47 amplified and 142 of the 338 loci were polymorphic. A total of 28 usable polymorphic markers were
48 developed in *A. esculenta*, 18 in *P. littoralis*, 11 in *C. jubata*, 14 in *G. gracilis*, 21 in *G. dura* and 13 in
49 *P. palmata*. The overall number of alleles per locus ranged from 2 to 22. These 105 new microsatellite
50 markers will be useful for further studies of population genetics, breeding programs and conservation
51 genetics of these species. Compared with traditional approaches, our study yielded thousands of
52 microsatellite loci for six different species in a short time and with affordable costs. This study, based
53 on the use of ddRADseq, provided preliminary data about the genetic structure and reproduction mode
54 of these six non-model species based on a small number of individuals from two geographically-
55 distant populations and on the genetic structure and reproduction mode of two non-model species, i.e.

56 detection of clonality for two red algae, *C. jubata* and *G. dura* and detection of highly genetically
57 divergent populations corresponding probably to different cryptic species under the name *P. littoralis*.

58

59 **Keywords:** genetic diversity, microsatellite markers, seaweed, Rhodophyta, Ochrophyta, ddRAD.

60

61 **1. Introduction**

62

63 Evaluation of genetic resources is crucial for the sustainable conservation of economically or
64 ecologically important species. It is also a key step in the domestication process of new species [1],
65 [2]. In the context of global change, most of the seaweed biomass in Europe comes from harvesting
66 wild populations (FAO, 2018), questioning the sustainability of the wild genetic resources [3].
67 Moreover, the development of seaweed aquaculture since the 1950s in China for the Japanese kelp, or
68 more recently in the 1980s in South East Asia and East Africa for tropical red seaweeds, has led to
69 long-distance translocations of a few genotypes. These aquaculture practices are raising concern about
70 their possible detrimental effects on local adaptation of wild populations that may be impacted by
71 human-mediated gene-flow when located in the vicinity of farms [4]. There is therefore, an urgent
72 need for genetic markers to conduct genetic studies on an increasing number of seaweed species that
73 may be negatively impacted by intensive exploitation of their natural populations, the future
74 development of aquaculture or the impact of global change. For example, recent studies reveal that
75 marine heat waves in recent years have played a role in the decline of several macroalgal species on
76 the coast of northern Spain [5], [6]; a possible influence of latitudinal patterns of genetic diversity on
77 the vulnerability of the kelp *Ecklonia radiata* to a marine heatwave on the South-Eastern Australian
78 coast [7]; multi-decadal decline in the cover of the giant kelp *Macrocystis pyrifera* at the southern
79 limit of its Australian range [8]. These studies highlight the need to step up coastal monitoring efforts
80 in order to analyse the impact of extreme temperature events on algal genetic biodiversity.

81 Even if genome-wide studies are more accessible as a result to next generation sequencing (NGS)
82 methods, there are still numerous non-model, but nonetheless valuable, algae species for which

83 genomic data are not available. This restricts the possibility to search for molecular markers *in silico*.
84 Double Digested Restriction site Associated DNA sequencing (ddRADseq) has already been used to
85 efficiently develop molecular markers such as Single Nucleotides Polymorphism (SNPs) [9] and, more
86 recently, Short Sequence Repeats (SSRs) or microsatellite markers for non-model species [10], [11].
87 For over twenty years, microsatellite markers have set the standard in the field of population genetic
88 markers, offering the possibility of population analysis over time and providing useful insights based
89 on the results of the many studies carried out with this type of molecular marker. These markers have
90 the characteristic of being transferable between related species of the same genus, opening the
91 possibility of studying the same locus in different species [12]. They also provide excellent results in
92 paternity analysis, where SNPs have not yielded accurate information [13]. Although SNPs have better
93 genome coverage than microsatellites, the bioinformatic skills required, the computational power and
94 the cost of reagents limit their application to large numbers of individuals. Moreover, development of
95 SNPs, also requires a greater number of sample replications because of the high error rate compared to
96 microsatellite markers. Recent studies have indicated that, when addressing genetic structure or
97 patterns of genetic diversity, a study with a dozen of microsatellite markers gave similar results to
98 those obtained with thousands of SNP markers [13], [14]. Microsatellite markers are therefore better
99 suited (cheaper) for large sample studies and require less bioinformatic skills and computer power than
100 SNPs [12], [15], making them more accessible. The ability of microsatellite markers to uncover
101 genetic variations at the same locus beyond time and species, along with their accessibility, makes
102 them a gold standard in population genetic studies.

103 Since 2010, data on whole genome sequencing have been accumulating for seaweeds facilitating the
104 search for microsatellites (i.e., for *Ectocarpus* species 7, referred to as *Ectocarpus siliculosus* [16]; for
105 *Saccharina japonica* [17]; for *Ecklonia radicata* [18]). However, developing new microsatellite
106 markers for a non-model species can be challenging when there is a lack of genomic data for *in silico*
107 research [see for review: 19] because the traditional microsatellite marker isolation approach, such as
108 generation of an enriched library followed by cloning and Sanger sequencing or NGS pyrosequencing,
109 is a costly, labour-intensive and often inefficient process. Although the use of expressed sequence tag
110 (EST) databases has proved to be a valuable source for the development of SSR markers that are

111 potentially transferable between taxa [20], there are still some taxonomic groups where this type of
112 sequence remains rare. Other Methods to develop microsatellite markers rapidly and cost-effectively
113 are needed for population genetic studies of non-model species. An efficient method was developed
114 during the last ten years for non-model animals and plants [10], [11], based on the generation of
115 double-digest restriction site associated DNA (ddRADseq; [9]) from genomic DNA of a few
116 individuals of the species of interest using Illumina sequencing technology. Microsatellite markers are
117 mined *in silico* using tools that require limited bioinformatic skills and computer power compared to
118 SNP development.

119 Marine macroalgae are a valuable resource with a wide range of applications, from ecosystem
120 services, for example by acting as carbon sinks and fish nurseries, to food and feed production [21],
121 [22]. As the demand for seaweed biomass increases, new tools such as species-specific genetic
122 markers are needed for the sustainable exploitation of wild resources as well as for the implementation
123 of cultivation of those valuable species [4]. Since ddRADseq allows DNA tagging for sample-pooled
124 sequencing, the aim of this study was to extend the method of Mori et al. [11] to multiple species
125 using a maximum of two sequencing runs. In this way, it was possible to increase yields and reduce
126 the cost of microsatellite marker development for economically important non-model species of
127 seaweeds. We focused our study on 6 seaweed species, commonly found along the European coast,
128 which are known for their socio-economic, ecological and scientific values. *Palmaria palmata* and
129 *Alaria esculenta* are used for human consumption, cosmetics and animal feed [23], [24].
130 *Calliblepharis jubata* and *Pylaiella littoralis* are used for cosmetics [25]. *Gracilaria gracilis* and
131 *Gracilaria dura* are known for their high agar content and serves as model species to test hypotheses
132 related to the evolution of sex [26], [27], [28], [29]. Except for *A. esculenta* none of those six North
133 Atlantic species are cultivated at large scales [30] but several cultivation trials have been conducted in
134 the past twenty years for *P. palmata* [31], and more recently for *P. littoralis* and *C. jubata* [32]. The
135 development of microsatellite markers, which was performed for individuals from two geographically-
136 distinct populations, provided a set of new preliminary data on the genetic structure and mode of
137 reproduction of the six non-model species: the two brown seaweeds (Phaeophyceae): *A. esculenta* and
138 *P. littoralis* and the four red algae (Rhodophyta): *P. palmata*, *C. jubata*, *G. gracilis* and *G. dura*.

139

140 **2. Materials and methods**

141

142 *2.1. Sample collection and DNA extraction*

143

144 A total of 275 individuals from six species of seaweed were collected from thirteen sampling locations
145 (Table 1). The ploidy levels were known (either using phenological observations or flow-cytometry
146 analyses) prior to genotyping for all study species except *G. dura* and *C. jubata*. Only later after
147 genotyping, could ploidy levels could be assessed for all individuals. Upon collection, a piece of tissue
148 was cut out from a spot that was free of algal and animal epiphytes and stored in silica gel. No specific
149 treatments were carried out to remove bacteria from the tissue but we estimate the percentage of
150 bacterial DNA after sequencing using the KAIJU software version 1.9.2 developed by Menzel et al.,
151 2016 [33]. Total genomic DNA was extracted from 15 to 20 mg of dry tissue using the Nucleospin 96
152 plant kit (Macherey-Nagel, Germany). The extraction was performed according to the manufacturer's
153 instructions except that samples were left in the PL1 lysis buffer at 65°C for 15 min rather than 30
154 min. The extracted DNA was eluted into 120 µl of the supplied elution buffer.

155

156 *2.2. DNA library preparation, sequencing and assembly*

157

158 Two individuals of each species of seaweed (one per locality, except Quitao for *G. dura*) were used to
159 construct the genomic libraries according to the methods described by Peterson et al [9] with the
160 following modifications (Table 1). Double-digest restriction site associated DNA was generated with
161 *PstI* and *HhaI* (New England Biolabs, USA) restriction enzymes in a total reaction volume of 50µl
162 using 300 ng of high-quality genomic DNA by incubating for 3 hours at 37°C (Fig S1). Adapter
163 ligation was performed following the instructions of Peterson et al [9] with a unique P1 adaptor for
164 each sample (Table 1) and ligation reactions were inactivated using NucleoMag NGS clean-up beads
165 (Macherey-Nagel, Germany) with a final elution into 40µl of water. Individual PCR amplifications

166 were carried out in 40µl reaction volumes containing 10 µl of Adapter-ligated DNA, 170 nM of
167 indexed primer mix at 10 µM, 200 µM of dNTPs (Thermo Fisher Scientific Inc., USA), 1X of Q5®
168 Reaction Buffer, 1X of Q5® High GC Enhancer, 0.8 Unit of Q5® High-fidelity DNA Polymerase
169 (New England Biolabs, USA). DNA amplifications were carried out in a Bio-Rad DNA Engine Peltier
170 Thermal Cycler using the following conditions: initial denaturation phase at 98 °C for 30 sec, followed
171 by 15 cycles of denaturation at 98 °C for 20 s, annealing at 60 °C for 30 sec, extension at 72 °C for 40
172 sec, and a final extension at 72 °C for 10 min. PCR products were purified with NucleoMag NGS
173 clean-up beads (Macherey-Nagel, Germany) with a final elution in 40µl of water. The pooling was
174 done before the size selection to generate two libraries with 6 samples each to obtain about 8 million
175 reads per sample (Illumina performance specifications for Miseq v3 kits is between 22-25 million
176 reads per run), Lib1 (*A. esculenta*, *P. littoralis* and *C. jubata*) and Lib2 (*G. dura*, *P. palmata* and *G.*
177 *gracilis*). The Sage Science Pippin-Prep (sagescience.com) method was used to carry out the size
178 selection to obtain the fragments of interest of between 350 and 550 bp (between 480 and 680 bp with
179 the full library construction fragments). Size selection and quality of both libraries were checked using
180 a Bioanalyzer and High Sensitivity DNA Chips (Agilent Technologies), and the final concentrations
181 were estimated through quantitative PCR (qPCR) using a KAPA Library Quantification Kit (Roche
182 Molecular Systems, Inc) and a LightCycler® 480 System (Roche Molecular Systems, Inc).
183 Sequencing was performed in two runs on a MiSeq sequencer (Illumina, San Diego, CA, USA) using
184 a Miseq Reagent Kit v3 600 cycles (2 x 300 paired-end reads) and libraries at 15pM mixed with 5% of
185 Phix control v3 (Illumina, San Diego, CA, USA).

186 To obtain clean, high-quality reads, we discarded low quality raw sequences with adapter
187 contamination or N > 10%. We used STACKS software version 2.52 [34] to demultiplex the pooled
188 samples based on a unique P1 adaptor (Table 1). Overlapping paired-end sequencing reads were
189 assembled using the FLASH v1.2.11 software [35]; the minimum required overlap length between two
190 reads to provide a confident overlap (m) was fifteen and the maximum overlap length expected in
191 approximately 90% of read pairs (M) was three hundred. To construct the reference sequences, the
192 dDocent pipeline version 2.8.7 [36] was run using default parameters and procedures as described in
193 the user manual, except that percent clustering similarity was set at 95% for the reference assembly.

194

195 2.3. *Microsatellite loci screening and primer design*

196

197 Microsatellite motifs were identified using the QDD pipeline program version 3.1.2 [37] and all
198 reference sequences with sizes greater than 80 bp (PIPE1 in QDD) were used for detection. Both
199 perfect and imperfect di-, tri-, tetra-, penta- and hexanucleotide motifs were targeted. Dinucleotide
200 motifs with at least 4 repeats and longer motifs with at least 3 repeats were selected (PIPE1 in QDD).
201 Reads with at least 95% identity were clustered as consensus sequences, while those that could not be
202 grouped were classified as singletons using Blast+ [38] (PIPE2 in QDD). Primers were designed using
203 the PRIMER v3.0 software [39]. Duplicate primers were removed and unique primers of the putative
204 loci were retained. Primers were mainly selected according to the type and size of the microsatellite
205 motif, including also the following criteria: primer length between 18 bp and 27 bp; 50 to 75% GC
206 content; a distance greater than 20 bp between the primer motif and the repeated motif and amplicon
207 sizes between 100 bp and 450 bp (PIPE3 in QDD). In order to test these primers cost-effectively for
208 amplification, we reduced the number of selected microsatellite regions to 96 (the capacity of a
209 microtiter plate) for each species by selecting the ones with the highest number of repeats for each
210 motif category.

211

212 2.4. *Amplification trials and levels of polymorphism of microsatellite loci*

213

214 For each species, positive amplification of 96 primer pairs was tested using DNA from 8 random
215 individuals. Polymorphic level and genetic diversity characterization of the primer pairs that amplified
216 positively were tested using between 35 and 48 individuals per species (Table 1 - Fig 1).
217 Amplifications were carried out in 10 μ L reaction volume with 2 μ L of DNA template diluted 1:100
218 and following the method of Guzinski et al. [40]. The PCR products labeled, with the four different
219 colors, were pooled (24 pools in total) and diluted 1:10 with water. Next, 2 μ L of the diluted PCR
220 product pool was added to 10 μ L of loading buffer made up of 0.5 μ L of the SM594 size standard [41]

221 and 9.5 μ L of Hi-Di formamide, denatured at 95°C for 3 min, and run in an ABI 3130 XL capillary
222 sequencer (Applied Biosystems, USA). Genotypes were scored manually in Genemapper version 4.0
223 (Applied Biosystems).

224

225 2.5. Genetic analyses

226

227 The results obtained for the populations of diploid individuals were analyzed with the following
228 software. Prior to the genetic analysis, we tested for null alleles using MICRO-CHECKER v2.2.3 [42].
229 GENEPOP v4.7.5 [43] was used to test for linkage disequilibrium (global test employing Fisher's
230 method, the following Markov chain parameters were used: 100,000 for dememorization, 1000
231 batches and 50,000 iterations per batch). HIERFSTAT v0.5-10 R package [44] was used to obtain the
232 number of observed alleles per locus (N_a), observed heterozygosity (H_o), expected heterozygosity
233 (H_e), the within-population deviation from Hardy-Weinberg equilibrium (F_{IS}) and its significance per
234 locus and for all loci (tested with a permutation test; based on 10,000 permutations). The Polymorphic
235 information content (PIC) was calculated with CERVUS v3.0.7 [45].

236 A modified analysis was used for the triploid individuals (*G. dura*): AUTOTET v1 software [46], a
237 program for the population analysis of allotetraploid microsatellite data, was used for the genetic
238 analysis and F_{IS} index; its significance per locus and all loci (tested with a permutation test; based on
239 10,000 permutations) was determined using SPAGeDi v1.5d software [47]. The Polymorphic
240 information content (PIC) was calculated using "polysat" v.1.7 R package according to Clark and
241 Jasieniuk [48].

242 The number of distinct Multilocus Genotypes (MLG), the genotypic richness (R) and the Genotype
243 accumulation curves (based on 10,000 permutations) were obtained with the "poppr" v2.9.3 R
244 package [49].

245 Finally, to estimate the level of genetic differentiation between localities, pairwise F_{ST} [50] values
246 were computed in HIERFSTAT v0.5-10 R package [44]. The significance of the comparisons was

247 estimated by performing 10,000 bootstraps over loci, with the comparisons judged significant if the
248 bootstrap-generated confidence intervals did not overlap zero.

249

250 **3. Results**

251

252 *3.1. ddRAD sequencing and assembly*

253

254 The sequencing procedure generated a total of 64.6 million reads (6.3 to 14.4 million per species) for
255 the six studied species [51] (Table S1). The percentage of bacterial DNA in the reads was less than 5%
256 with an average of 2.94% (Table S2). After editing/trimming, 377.7 Mb of high-quality sequences
257 (41.9 to 111.4 Mb per species) were available, which were assembled into 1,351,981 contigs (134,653
258 to 400,288 contigs per species). We obtained an average contig length of 294.0 bp (217.2 to 406.4 bp
259 per species), an average contig N50 size of 279.3 bp (173 to 407 bp per species) and an average GC
260 content of 51.2% (49.9% to 53.8% per species) (Table S1).

261

262 *3.2. Microsatellite loci development and characterization*

263

264 Among the 1,351,981 assembled contigs, a total of 30,636 microsatellite motifs were found in the six
265 species (1,755 to 11,487 per species) using the QDD pipeline (Table S1). The microsatellite sequences
266 contained 14,288 (46.6%) di-, 14,529 (47.4%) tri-, 861 (2.8%) tetra-, 481 (1.6%) penta- and 477
267 (1.6%) hexanucleotide motifs (Table S3). After choosing regions with flanking sequences of sufficient
268 length and requiring a product size of between 100 and 450 bp, we obtained 15,443 primer pairs (752
269 to 6,592 per species – Table S1) corresponding to 7,327 (47.4%) di-, 6,997 (45.3%) tri-, 525 (3.4%)
270 tetra-, 316 (2.0%) penta- and 278 (1.8%) hexanucleotide motifs (Table S3). A total of 576 primer pairs
271 were tested for all species (96 primer pairs each, focusing on the ones with the highest number of
272 repeats), 338 were amplified including 104 (30.7%) di-, 77 (22.7%) tri-, 63 (18.6%) tetra-, 67 (19.8%)
273 penta- and 27 (7.9%) hexanucleotide motifs (Table S3). The 338 loci retained after the amplification

274 tests (see Table S1 for details per species) were subsequently checked for level of polymorphism
275 within the sample of 8 individuals per species. A total of 142 out of these 338 putative loci including
276 31 for *A. esculenta*, 28 for *P. littoralis*, 15 for *C. jubata*, 20 for *G. gracilis*, 28 for *G. dura* and 20 for
277 *P. palmata* produced PCR products of the expected size, were polymorphic (Table S1) and
278 corresponding to 54 (38.0%) di-, 28 (19.7%) tri-, 27 (19.0%) tetra-, 27 (19.0%) penta- and 6 (4.2%)
279 hexanucleotide motifs (Table S2). Therefore, the rate of success for each of the five motif categories in
280 terms of acquisition of polymorphic microsatellite was 33.5% for di-, 19.1% for tri-, 23.5% for tetra-,
281 26.0% for penta- and 12.2% for hexanucleotide repeats (Table S3).

282

283 3.3. Genetic diversity

284

285 All the loci analyzed in this study (142 microsatellites markers for 6 species) were selected for their
286 amplification success and their polymorphism in each species. This allowed us to confirm the ploidy
287 levels for all individuals in the 4 species for which ploidy levels were known prior to genotyping (i.e.,
288 *A. esculenta*, *P. littoralis*, *P. palmata* and *G. gracilis*). For *G. dura* and *C. jubata*, the ploidy level was
289 determined after genotyping and triploid individuals were detected in *G. dura*. Data for the number of
290 alleles, null allele frequency, genetic diversity indices and departure from random mating (F_{IS}) are
291 given in Table S4 for each species, locus and population. However, among the loci that correctly
292 amplified, several markers (3 in *A. esculenta*, 10 in *P. littoralis*, 4 in *C. jubata*, 6 in *G. gracilis*, 7 in *G.*
293 *dura* and 7 in *P. palmata*) were discarded because they appeared to be either monomorphic within
294 populations, or because they showed a significant frequency of null alleles, or an extreme departure
295 from random mating suggesting non-Mendelian inheritance (F_{IS} values > 0.6 or F_{IS} values < -0.6).
296 Overall, from our study, 28 markers appeared to be useful for *A. esculenta*, 18 for *P. littoralis*, 11 for
297 *C. jubata*, 14 for *G. gracilis*, 21 for *G. dura* and 13 for *P. palmata* (corresponds to the markers in bold
298 in the Table S4).

299 The level of diversity was highly variable across species (Table S4). The mean number of alleles (N_a)
300 computed over all loci and populations (i.e. global, Table S4) was higher in the two brown seaweeds

301 *A. esculenta* (6.7) and *P. littoralis* (8.0) compared to *C. jubata* (3.8), *G. gracilis* (3.4), *G. dura* (6.2)
302 and *P. palmata* (5.7). However, this trend was not retrieved for the other estimate of gene diversity
303 (H_e). The highest H_e value was observed in the red alga *G. dura* (0.758) and explained by fixed
304 heterozygosity compared to all other species: the two brown seaweeds *A. esculenta* (0.535) and *P.*
305 *littoralis* (0.496) and the three remaining red algae *C. jubata* (0.344), *G. gracilis* (0.412) and *P.*
306 *palmata* (0.489). Finally, polymorphic information content (PIC) was higher for *P. littoralis* (0.678)
307 and *G. dura* (0.725) compared to *A. esculenta* (0.532), *C. jubata* (0.335), *G. gracilis* (0.421), and *P.*
308 *palmata* (0.478).

309 Differences in the level of clonality were observed among species. No signal of clonality could be
310 detected in *A. esculenta*, *P. littoralis*, *G. gracilis* and *P. palmata* as all multilocus genotypes (MLG)
311 were distinct ($n = MLG$) and genotype richness (R) was maximum and equal to 1 (Table 2). In
312 contrast, the number of multilocus genotypes (MLG) were not distinct in *G. dura* and *C. jubata* ($n >$
313 MLG) and the mean values of genotypic richness (R) over populations were low: 0.378 and 0.848
314 respectively (Table 2). For these two partially clonal species, F_{IS} values were significantly negative,
315 revealing heterozygous excess, while for the four others sexually reproducing species, F_{IS} values were
316 not significantly different from 0, revealing random mating (Table 2). The genotype accumulation
317 curves showed that between five and twelve loci were sufficient to differentiate all non-clonal
318 individuals for each of the studied populations (Fig. 2).

319 Finally, the pattern of genetic differentiation among populations was highly significant ($P < 0.001$) for
320 all studied species (Table 2). However, the magnitude of F_{ST} values were very different among species
321 ranging from 0.118 in *P. palmata* to 0.482 in *P. littoralis* (Table 2).

322

323 **4. Discussion**

324

325 The development of NGS has improved our capacity to identify and develop microsatellite markers
326 (for review see [52]). In particular, polymorphic loci in model and non-model, plant or animal, species
327 can now be identified rapidly using ddRAD sequence data [10], [11], [53], [54]. The ddRADseq

328 method [9] was largely developed during the last 10 years for population genetics studies in many
329 organisms to search for SNPs and to decrease the cost of whole genome sequencing for genotyping. It
330 is still the object of continuous innovations [55] like those we have developed for multiple seaweed
331 species. However, even if several studies have developed ddRADseq methods for SNP discovery in
332 seaweeds in recent years (firstly in the brown algal model *Ectocarpus* [56] and mostly in the kelp or
333 large brown algal species *Undaria pinnatifida* [14]; *Saccharina latissima* [40]; *Ecklonia radiata* [57];
334 *Sargassum thunbergii* [58] and *Durvillaea antarctica* [59]), none of them was exploited to discover
335 microsatellites because markers were already available.

336 In this study, implementing the protocol developed by Mori et al. [11] with only two multiplexed
337 libraries for the 6 species, we successfully developed around 20 microsatellite markers per species in
338 different brown and red seaweeds of economic and ecological interest. This highly efficient method
339 allows rapid identification of microsatellite motifs distributed throughout the genome, ultimately
340 revealing polymorphic markers in the target species with an efficiency of about 25%. Interestingly, in
341 *G. gracilis*, more than 50% of the microsatellite loci already identified using classical library cloning
342 methods [60], [61] were found in this study confirming good coverage of the genome.

343

344 4.1. *Characteristics of microsatellites between species*

345

346 Our data yielded ten times more microsatellite motifs in brown than in red seaweeds but this
347 difference could not be clearly linked to their genome size (Table S1). However, for *Gracilaria* and
348 *Calliblepharis*, it may be important to consider their small genome size for future development of
349 microsatellite markers by, for example, adapting the coverage (e.g., sequencing 2 species per run
350 instead of 3 or more).

351 The occurrence and the frequency of different types of microsatellite motifs seem to be characteristic
352 of a species, which can be distant or closely related [12], [62], [63]. In our study, the distribution of
353 microsatellite marker repeats nucleotide types (di, tri, tetra, penta and hexanucleotide) was similar to
354 that obtained for the red alga *G. lichenoides* [64]. Dinucleotide and trinucleotides repeats were found

355 to be the most abundant motif, on average 46.6% and 47,4% respectively (Table S3), probably
356 because short repeats are the starting point for subsequent extensions of Short Tandem Repeats (STRs)
357 [65] which are the most variable type of DNA sequence in the genome [66]. The most common class
358 of polymorphic marker contained dinucleotide motifs (35% - 40% of the polymorphic markers) for
359 each species except for *A. esculenta* and *P. littoralis* for which polymorphic markers with
360 tetranucleotide and pentanucleotide motifs were the most abundant, respectively (32.3% and 35.7% of
361 the polymorphic markers respectively). In contrast to what was found for plants by Zhu *et al.* [63],
362 hexanucleotide repeats in our study were the least abundant type of polymorphic markers. This could
363 be due either to the phylogenetic distance between algae and land plants [63] or to an artefact related
364 to the size of the fragments present in the ddRADseq libraries (partial genome vs complete genome) or
365 to the types of restriction enzymes used for library construction.

366 The consequent yields and largely sufficient genome coverage imply that the method used in this
367 study could be extended to more than 6 species. Future studies should be conducted to find the
368 adequate balance between numbers of species and genome coverage, accounting for genome structure
369 and length.

370

371 4.2. *New perspectives of using next-generation sequencing for population-level studies*

372

373 Several methods have been developed in many organisms to lower the cost and/or streamline next-
374 generation sequencing for population-level studies [55]. Future developments for seaweeds will
375 depend of many contingencies. With the need to develop knowledge on new species of seaweed to
376 domesticate or to conserve in natural populations, only a few laboratories are developing approaches,
377 depending on the means available for these studies. Large whole-genome sequencing efforts such as
378 the Darwin tree of life in the UK, Atlasea in France and the ERGA (European Reference Genome
379 Atlas) project at the EU level will offer new opportunities to enlarge the search for genetic markers for
380 novel species. For the development of multiple PCR, synthesizing unique barcodes for each sample or
381 even a subgroup of samples is expensive. Moreover, multi-, or single-level multiplexing and

382 demultiplexing of samples can be cumbersome, time consuming, and costly. Therefore, approaches for
383 targeted sequencing-based genetic screening such as the use of high multiplex PCR coupled to NGS
384 (Hi-Plex, [67]) can be developed for only a few species of seaweeds and mainly models of economic
385 importance [68].

386

387 4.3. *Limited population genetics data could provide useful information on genetic diversity*
388 *and reproductive systems*

389

390 The preliminary results of the population genetic analysis, even though they were carried out on a few
391 populations, provided interesting information about the reproductive systems and diversity of the
392 study species. Indeed, two of the four red algal species, *C. jubata* and *G. dura*, showed strong
393 signatures of asexual reproduction (heterozygous excess, low genotypic richness, repeated multilocus
394 genotypes). It is known that most Rhodophyta species are able to reproduce vegetatively either by
395 thallus fragmentation or propagule dispersal [69], [70], [71], however, the significance of asexual
396 reproduction in the field remains poorly known except for *Phymatolithon calcareum* (maerl beds)
397 [72], cultivated *G. chilensis* in farms [73] and the introduced species *G. vermiculophylla* [74]. Our
398 results for *C. jubata* suggest that this species partially undergoes natural fragmentation. Indeed, the
399 rhizoids could play a central role in vegetative propagation [71], by detaching from the parent thallus
400 to give a new individual. However, no field observations had been made until now. To our knowledge,
401 our results confirm this hypothesis for the first time. In contrast, in the genus *Gracilaria*, such
402 specialized organs have never been observed and vegetative multiplication occurs by spontaneous
403 fragmentation of the thallus. Cuttings are routinely used to reproduce macroalgae in aquaculture [75].
404 However, cuttings are not generally capable of giving rise to a new perennial individual [27]. In both
405 *G. chilensis* and *G. vermiculophylla* species for which there is strong evidence for asexual
406 reproduction [73], [74], it was shown that vegetative fragmentation could be facilitated by farming
407 practices (in *G. chilensis*) and explained rapid colonization during biological invasion (in *G.*
408 *vermiculophylla*). In the case of *G. dura*, as the individuals are attached to rocks, the fragmentation

409 hypothesis can be rejected. Instead, sexual reproduction could occur either by apomixis or apogamy,
410 as observed in some triploid individuals of the red alga *Caloglossa leprieurii* [76]. Further
411 investigation is therefore needed to better understand how and by what means this species reproduces
412 asexually. The results obtained for *G. gracilis* and *P. littoralis*, confirmed that individuals reproduced
413 sexually by outcrossing [77], [78]. Finally, our results suggested the occurrence of random mating
414 systems in natural populations of *A. esculenta* and *P. palmata*.

415 The genetic diversity observed in this study was of the same order of magnitude as that observed
416 previously for the same or closely related species. Overall, genetic diversity tended to be higher in
417 brown than in red algae, probably due to differences in their effective population sizes. For example,
418 the genetic diversity indices found in *A. esculenta* ($He = 0.505$ and $He = 0.566$) was close to that
419 recorded for other kelp beds from the same region *Saccharina latissima* ($He = 0.549$), *Laminaria*
420 *digitata* ($He = 0.534$) and *Laminaria hyperborea* ($He = 0.591$) [79], [80]. In the filamentous alga *P.*
421 *littoralis*, genetic diversity ($He = 0.556$) was slightly reduced compared with that found in a previous
422 study ($He = 0.608$) maybe because we used cross-amplification of microsatellite markers from
423 *Ectocarpus siliculosus* [78]. In *G. gracilis*, we studied the same population using 14 newly developed
424 markers (GN2, located at Cape Gris Nez) that was genotyped 20 years ago using seven microsatellite
425 markers. Lower genetic diversity values were observed in our study ($He = 0.514$ in 2001, [77] vs $He =$
426 0.464 in 2021, this study) suggesting that temporal changes could have happened in this population.
427 Finally, these markers were used to estimate differentiation between populations (F_{ST}). Even though
428 the scale of distance between populations varied greatly between species (from 50 to 2200 km), it was
429 possible to detect significant genetic differentiation even between populations separated by distances
430 of 50 km. Finally, in *Pylaiella* where the occurrence of two cryptic species along the French coast has
431 been reported by Geoffroy et al. [78], the high values of genetic differentiation (F_{ST} close to 0.5)
432 observed between the two studied populations suggest that they belong to different cryptic species.

433

434 **5. Conclusion**

435

436 We were able to rapidly develop species-specific molecular markers for six phylogenetically distantly-
437 related species of red and brown seaweeds with relatively low cost (in time and money). These
438 markers are suitable for studying the diversity and genetic structure, and can also be used to detect and
439 decipher clonality processes involved in the reproduction of these species. This work, which uses this
440 rapid and inexpensive method, could facilitate evolutionary analyses in a wide variety of non-model
441 taxa. The applications of these new species-specific genetic markers are numerous, such as breeding,
442 selection and conservation programs, genetic resources studies, management of harvested stocks and
443 monitoring of the origin of processed products. In addition, the use of ddRAD sequencing in the
444 microsatellite development process still allows for the detection of SNPs with the generated data.

445

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457

458 **Ethical consent**

459 This article does not contain any studies with human participants or animals performed by any of the
460 authors.

461

462 **Consent for publication**

463 All authors read and approved the manuscript for the publication in *Algal Research*.

464

465 **Declaration of competing interest**

466 The authors declare that they have no conflicts of interest concerning the present article.

467

468 **Availability of data**

469 All data generated during this study are openly available in a Dryad Digital Repository

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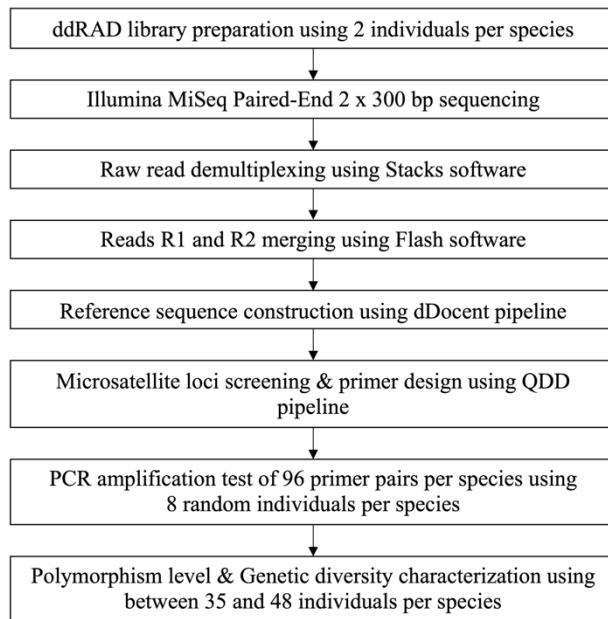


Fig. 1 Workflow and experimental set-up used for microsatellite markers development using ddRADseq

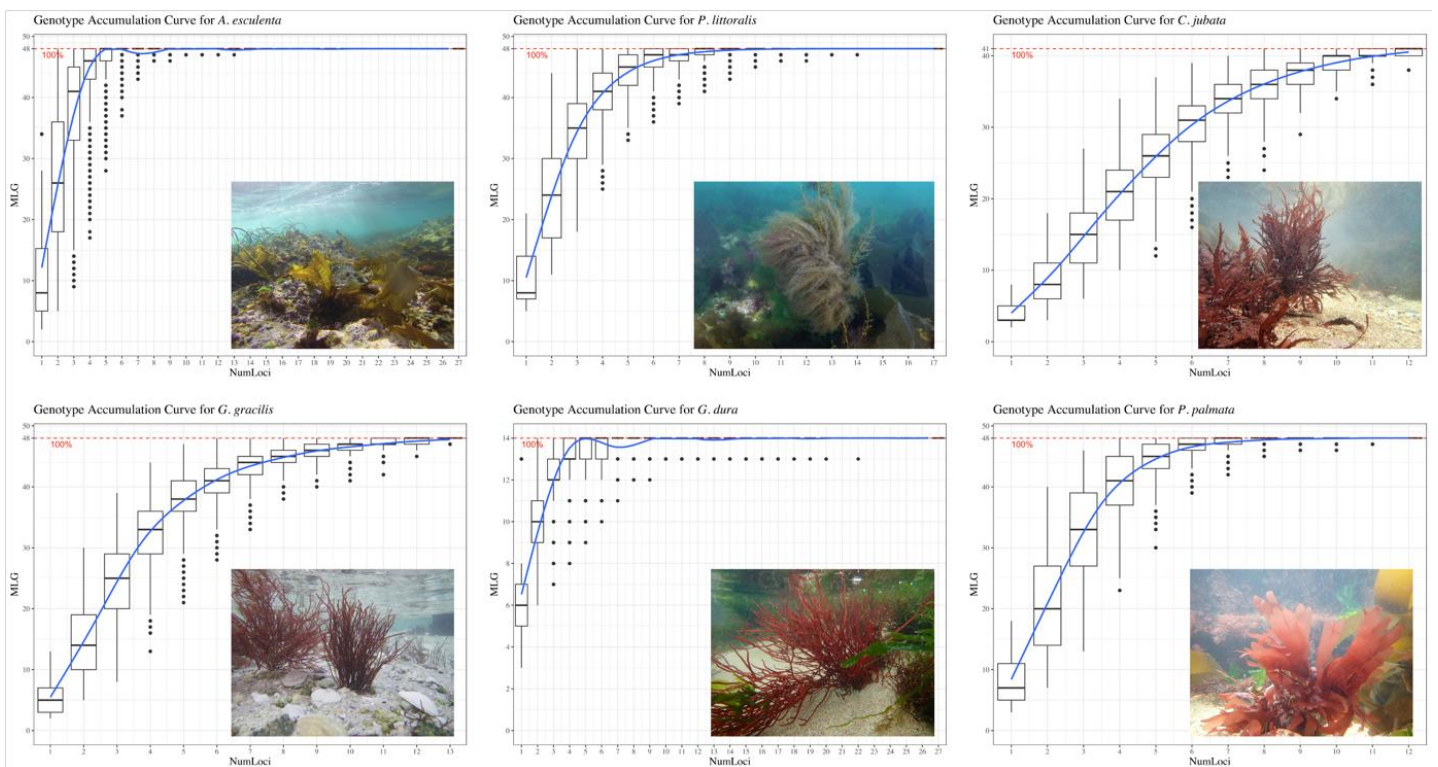


Fig. 2 Accumulation curves of the genotypes of the 6 species based on 10,000 permutations with “poppr” v2.9.3 R package. Photo credits: © Destombe C. for *G. gracilis* and *G. dura*; © Baud A. for *A. esculenta* and *P. palmata*; © Thomas W. for *P. littoralis*.

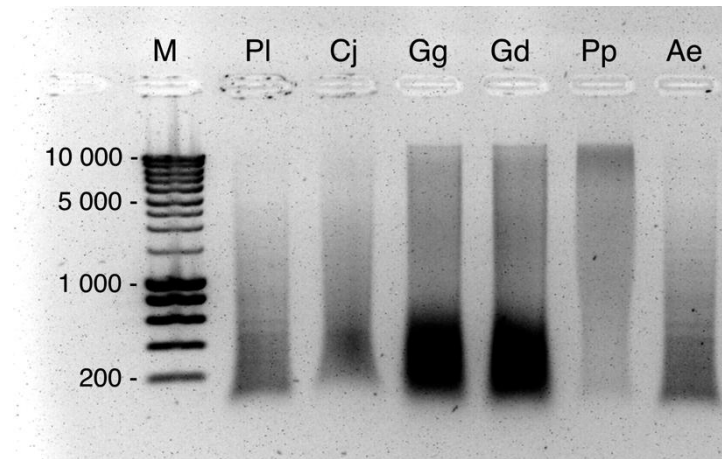


Fig. S1 Agarose gel electrophoresis (1% w/v) of *PstI/HhaI* restriction enzyme-digested algal DNA. Lane PI, *Pylaella Littoralis*. Line Cj, *Calliblepharis jubata*. Line Gg, *Gracilaria gracilis*. Line Gd, *Gracilaria dura*. Line Pp, *Palmaria palmata*. Line Ae, *Alaria esculenta*. Line M, SmartLadder 200bp-10kb (Eurogentec, Seraing, Belgium).

Table 1 Geographical location of the populations used in the study, sample size (n), sexual phenotypes and barcodes used for ddRADseq.

Species	Populations	n	Phase (ploidy level)	Latitude (N)	Longitude (W)	P1 adaptor Sequence 3'-5'
<i>Alaria esculenta</i>	Roscoff, France	24	sporophyte (2n)	48.7316	-3.9907	CTGGTT
	Plouguerneau, France	24	sporophyte (2n)	48.6365	-4.5674	AAGATA
<i>Pylaiella littoralis</i>	Wissant, France	24	sporophyte (2n)	50.9045	1.6326	ACTTCC
	Ile de Aransa, Portugal	24	sporophyte (2n)	41.1571	-8.6892	TTACGG
<i>Calliblepharis jubata</i>	Roscoff, France	24	tetrasporophyte (2n)	48.4344	-3.5920	AAGGAA
	Le Havre, France	24	tetrasporophyte (2n)	49.7105	-1.8719	ATTCAT
<i>Gracilaria gracilis</i>	Gris-Nez, France	24	tetrasporophyte (2n)	50.8727	1.5924	CTGGTT
	Porto, Portugal	24	tetrasporophyte (2n)	41.1532	-8.6804	AAGATA
<i>Gracilaria dura</i>	Locquirec, France	12	mixed phase (3n)	48.6954	-3.6528	AACGAA
	Coroso, Spain	15	mixed phase (3n)	42.5691	-8.9778	ATTCAT
	Quitao, Portugal	8	mixed phase (3n)	41.1945	-8.7090	Not used for sequencing
<i>Palmaria palmata</i>	Roscoff, France	24	tetrasporophyte (2n)	48.7261	-3.9685	ACTTCC
	Belle-Ile, France	24	tetrasporophyte (2n)	47.3781	-3.2252	TTACGG

Table 2 Mean genetic diversity among loci for the populations sampled for each the 6 species.

Species	Populations	<i>n</i>	<i>nLoc</i>	<i>Na</i>	<i>A</i>	<i>MLG</i>	<i>R</i>	<i>He</i>	<i>Fis</i>	<i>Loc dist</i>	<i>Fst</i>
<i>Alaria esculenta</i>	Roscoff, France	24	28	4.1	4.24 ± 0.15	24	1	0.505	-0.031	50	0.136*
	Plouguerneau, France	24	28	5.8	3.32 ± 0.10	24	1	0.566	-0.006		
<i>Pylaiella littoralis</i>	Wissant, France	24	18	5.3	4.09 ± 0.14	24	1	0.556	-0.083	2200	0.482*
	Ile de Aransa, Portugal	24	18	3.7	2.97 ± 0.20	24	1	0.436	-0.008		
<i>Calliblepharis jubata</i>	Roscoff, France	24	13	2.9	2.42 ± 0.17	20	0.826	0.346	-0.131\$	510	0.132*
	Le Havre, France	24	13	2.5	2.29 ± 0.12	21	0.870	0.394	-0.285\$		
<i>Gracilaria gracilis</i>	Gris-Nez, France	24	14	2.9	2.65 ± 0.10	24	1	0.464	-0.024	2150	0.262*
	Porto, Portugal	24	14	2.8	2.28 ± 0.17	24	1	0.362	-0.075		
<i>Gracilaria dura</i>	Locquirec, France (a)	12	28	3.8	3.43 ± 0.36	7	0.636	0.655	-0.186\$	1470 (a-b)	0.263* (a-b)
	Coroso, Spain (b)	15	28	3.2	2.86 ± 0.31	4	0.214	0.674	-0.390\$	1600 (a-c)	0.299* (a-c)
	Quitao, Portugal (c)	8	28	2.5	2.54	3	0.286	0.617	-0.350\$	130 (b-c)	0.279* (b-c)
<i>Palmaria palmata</i>	Roscoff, France	24	13	5.1	3.51 ± 0.22	24	1	0.514	0.023	300	0.118*
	Belle-Ile, France	24	13	3.9	3.12 ± 0.19	24	1	0.465	-0.014		

n Number of genotyped individuals.

nLOC Number of loci retained as suitable polymorphic markers (i.e., polymorphic, no-significant *Nf*, no-significant *Fis* for no clonal species).

Na Means of number of alleles per locus.

A Standardized allelic richness for a common sample size of 8 individuals.

MLG Number of distinct Multilocus Genotypes.

R Genotypic richness $(G-1) / (N-1)$ (Dorken and Eckert, 2001).

He Expected heterozygosity.

Fis Inbreeding coefficient as defined by Weir and Cockerham (1984) calculated overall polymorphic loci, *Fis* values significantly different from zero are shown with \$.

Loc Dist distance between two localities in km.

Fst Pairwise estimates of genetic differentiation obtained with HIERFSTAT (Goudet 2014) *, $P < 0.001$.

Table S1 Steps involved in the definition of microsatellite loci for each of the species studied.

	<i>Alaria esculenta</i>	<i>Pylaiella littoralis</i>	<i>Calliblepharis jubata</i>	<i>Gracilaria gracilis</i>	<i>Gracilaria dura</i>	<i>Palmaria palmata</i>	Total	Mean
Genome size estimation (Mb)	381.5	322.5	156.0	72.5	72.5	782.0		
Number of raw reads	13,059,425	6,283,637	7,161,739	12,544,477	14,367,115	11,206,793	64,623,186	10,770,531
Number of unique cleaned sequences	222,863	134,653	172,899	221,730	199,548	400,288	1,351,981	225,33
n bases (Mb)	52.2	54.7	64.0	53.5	41.9	111.4	377.7	62.9
Min length (bp)	80	80	80	80	80	80	-	80
Max length (bp)	572	549	577	552	577	578	-	567.5
Average length (bp)	242.2	406.4	371.0	246.0	217.2	281.4	-	294.0
N50 (bp)	202	407	383	204	173	307	-	279.3
(G+C) _s	51.1%	53.8%	49.9%	50.1%	51.1%	51.4%	-	51.2%
Number of sequences with microsatellite	10,965	11,487	1,888	2,016	1,755	2,625	30,636	5,122.7
Number of sequences with primers	5,095	6,592	752	1,169	805	1,030	15,443	2,419.3
Number of primer pairs tested	96	96	96	96	96	96	576	96
Number of positives amplifications	61	54	67	52	36	68	338	56.3
Number of polymorphic markers	31	28	15	20	28	20	142	23.6
Number of polymorphic and usable markers	28	18	11	14	21	13	105	17.5

Table S2 Percentage of bacterial DNA and algae DNA in ddRADseq reads.

Species	Algae DNA	Bacterial DNA
<i>Alaria esculenta</i>	98.18%	1.82%
<i>Pylaiella littoralis</i>	95.72%	4.28%
<i>Calliblepharis jubata</i>	98.87%	1.13%
<i>Gracilaria gracilis</i>	96.36%	3.64%
<i>Gracilaria dura</i>	97.16%	2.84%
<i>Palmaria palmata</i>	96.06%	3.94%
Mean	97.06%	2.94%

Estimation of the percentage of bacterial DNA contamination in ddRADseq reads using the KAIJU software version 1.9.2 (Menzel et al., 2016).

Table S3 Steps involved in the selection of microsatellite markers, with the percentage of loci with specific motif types (Di-, Tri-, Tetra-, Penta- and Hexanucleotide) associated to each step.

Reduction step		Di	Tri	Tetra	Penta	Hexa	Total
QDD output							
	<i>Alaria esculenta</i>	3,644 (33.5%)	6,406 (59.0%)	394 (3.6%)	256 (2.4%)	165 (1.5%)	10,865
	<i>Pylaiella littoralis</i>	5,397 (47.0%)	5,436 (47.3%)	334 (2.9%)	162 (1.4%)	158 (1.4%)	11,487
	<i>Calliblepharis jubata</i>	1,397 (74%)	425 (22.5%)	44 (2.3%)	7 (0.4%)	15 (0.8%)	1,888
	<i>Gracilaria gracilis</i>	1,223 (60.7%)	699 (34.7%)	37 (1.8%)	22 (1.1%)	35 (1.7%)	2,016
	<i>Gracilaria dura</i>	1,037 (59.1%)	662 (37.7%)	15 (0.9%)	12 (0.7%)	29 (1.7%)	1,755
	<i>Palmaria palmata</i>	1,590 (60.6%)	901 (34.3%)	37 (1.4%)	22 (0.8%)	75 (2.9%)	2,625
	Total	14,288 (46.6%)	14,529 (47.4%)	861 (2.8%)	481 (1.6%)	477 (1.6%)	30,636
Primer3 output							
	<i>Alaria esculenta</i>	1,862 (36.5%)	2,771 (54.4%)	220 (4.3%)	152 (3.0%)	90 (1.8%)	5,095
	<i>Pylaiella littoralis</i>	3,132 (47.5%)	2,983 (45.3%)	231 (3.5%)	124 (1.9%)	122 (1.9%)	6,592
	<i>Calliblepharis jubata</i>	505 (67.2%)	218 (29.0%)	22 (2.9%)	6 (0.8%)	1 (0.1%)	752
	<i>Gracilaria gracilis</i>	686 (58.7%)	428 (36.6%)	23 (2.0%)	16 (1.4%)	16 (1.4%)	1,169
	<i>Gracilaria dura</i>	514 (63.9%)	264 (32.8%)	9 (1.1%)	8 (1.0%)	10 (1.2%)	805
	<i>Palmaria palmata</i>	628 (61.0%)	333 (32.3%)	20 (1.9%)	10 (1.0%)	39 (3.8%)	1,030
	Total	7,327 (47.4%)	6,997 (45.3%)	525 (3.4%)	316 (2.0%)	278 (1.8%)	15,443
Selection (highest number of repeats)							
	<i>Alaria esculenta</i>	24 (25.0%)	24 (25.0%)	24 (25.0%)	24 (25.0%)	0 (0%)	96
	<i>Pylaiella littoralis</i>	24 (25.0%)	24 (25.0%)	24 (25.0%)	24 (25.0%)	0 (0%)	96
	<i>Calliblepharis jubata</i>	24 (25.0%)	24 (25.0%)	24 (25.0%)	24 (25.0%)	0 (0%)	96
	<i>Gracilaria gracilis</i>	28 (29.2%)	15 (15.6%)	22 (22.9%)	15 (15.6%)	16 (16.7%)	96
	<i>Gracilaria dura</i>	36 (37.5%)	36 (37.5%)	6 (6.3%)	8 (8.3%)	10 (10.4%)	96
	<i>Palmaria palmata</i>	25 (26.0%)	24 (25.0%)	15 (15.6%)	9 (9.4%)	23 (24.0%)	96
	Total	161 (28.0%)	147 (25.5%)	115 (20.0%)	104 (18.1%)	49 (8.5%)	576
Positive amplifications							
	<i>Alaria esculenta</i>	16 (26.2%)	12 (19.6%)	15 (24.5%)	18 (29.5%)	0 (0%)	61
	<i>Pylaiella littoralis</i>	14 (25.9%)	10 (18.5%)	13 (24%)	17 (31.4%)	0 (0%)	54
	<i>Calliblepharis jubata</i>	21 (31.3%)	12 (17.9%)	14 (20.8%)	20 (29.8%)	0 (0%)	67
	<i>Gracilaria gracilis</i>	12 (23%)	12 (23%)	10 (19.2%)	5 (9.6%)	13 (25%)	52
	<i>Gracilaria dura</i>	22 (61.1%)	11 (30.5%)	0 (0%)	1 (2.7%)	2 (5.5%)	36
	<i>Palmaria palmata</i>	19 (27.9%)	20 (29.4%)	11 (16.1%)	6 (8.8%)	12 (17.6%)	68
	Total	104 (30.7%)	77 (22.7%)	63 (18.6%)	67 (19.8%)	27 (7.9%)	338
Rate of success to obtain positive amplification		104/161 (64.5%)	77/147 (52.3%)	63/115 (54.7%)	67/104 (64.4%)	27/49 (55.1%)	338/576 (58.7%)
PCR product of the expected size and polymorphic							
	<i>Alaria esculenta</i>	9 (29.0%)	3 (9.7%)	10 (32.3%)	9 (29.0%)	0 (0%)	31
	<i>Pylaiella littoralis</i>	5 (17.9%)	7 (25.0%)	6 (21.4%)	10 (35.7%)	0 (0%)	28
	<i>Calliblepharis jubata</i>	6 (40.0%)	0 (0%)	4 (26.7%)	5 (33.3%)	0 (0%)	15
	<i>Gracilaria gracilis</i>	8 (40.0%)	6 (30.0%)	4 (20.0%)	0 (0%)	2 (10.0%)	20
	<i>Gracilaria dura</i>	19 (67.9%)	9 (32.1%)	0 (0%)	0 (0%)	0 (0%)	28
	<i>Palmaria palmata</i>	7 (35.0%)	3 (15.0%)	3 (15.0%)	3 (15.0%)	4 (20.0%)	20
	Total	54 (38.0%)	28 (19.7%)	27 (19.0%)	27 (19.0%)	6 (4.2%)	142
Rate of success to obtain polymorphic marker		54/161 (33.5%)	28/147 (19.1%)	27/115 (23.5%)	27/104 (26.0%)	6/49 (12.2%)	142/576 (24.7%)

Table S4 Technical information (primer sequence, dye) characteristics (repeat motif, observed size range) and polymorphism estimates (*Nf*, *Na*, *Ho*, *He*, *F_{IS}*, *PIC*) for microsatellites selected for this study for each of the six species.

Alaria esculenta

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Dye	Roscoff							Plouguerneau							Global					
					<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>F_{IS}</i>	<i>PIC</i>	<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>F_{IS}</i>	<i>PIC</i>	<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>PIC</i>
Aesc_01	F: GCATTTTCGGTCTGTTCGGT R: GGTTTCGGTTTCGGTTATGTT	(GGTTC)14	230-370	NED	24	0.021	11	0.750	0.815	0.080	0.771	24	0.048	19	0.833	0.926	0.100	0.899	48	0.063	22	0.791	0.870	0.894
Aesc_02	F: GTCATCGGTTGGTTTGTGAG R: GTGTGAGGGAATGTCAAGGAA	(GGTTT)18	227-381	FAM	24	0	8	0.916	0.850	-0.077	0.813	24	0	10	0.833	0.840	0.008	0.803	48	0.005	15	0.875	0.845	0.875
Aesc_03	F: AAAGCAACAACATCAACA R: CCCGTCAACCTTATCCTT	(ACA)41	200-274	PET	24	0	8	0.833	0.779	-0.068	0.731	24	0.052	14	0.791	0.896	0.117	0.866	48	0.022	17	0.812	0.838	0.833
Aesc_04	F: GCTGCTATCCTGAACATAAA R: GACACCACCAACCCATAAC	(GT)22	153-197	FAM	24	0	6	0.666	0.641	-0.039	0.592	24	0.007	12	0.791	0.825	0.040	0.787	48	0.053	13	0.729	0.733	0.782
Aesc_05	F: ACCGCACCAAATACGAGAAA R: CAGCAGCAGGTAGTGAAGAATG	(TG)18	197-213	NED	24	0.014	6	0.750	0.785	0.045	0.731	24	0.008	6	0.750	0.769	0.024	0.717	48	0.031	6	0.750	0.777	0.769
Aesc_06	F: CGTACTGCTGCCACTACTCT R: AATACTGATTTGGAGGGATG	(CTA)48	318-399	FAM	24	0.003	6	0.708	0.731	0.031	0.670	24	0	8	0.791	0.703	-0.124	0.669	48	0.022	9	0.750	0.717	0.750
Aesc_07	F: TGCTGGATGTGGATTGTTAGA R: AACTCCTTCCGTTACACTGCT	(AC)22	102-128	FAM	24	0	3	0.666	0.612	-0.088	0.516	24	0	9	0.916	0.859	-0.066	0.825	48	0	9	0.791	0.736	0.726
Aesc_08	F: CGAATCCCTTGCCTGTG R: GCGTCCGTCITTTATGGTTG	(CCAT)23	214-312	NED	24	0.065	4	0.583	0.682	0.144	0.607	24	0	4	0.666	0.680	0.020	0.608	48	0.062	5	0.625	0.681	0.684
Aesc_09	F: ATCAAGCAGCAGAGCAAGAAG R: GCAACAGAAACCGAACAGAAA	(GCCT)18	163-215	HEX	24	0	4	0.833	0.712	-0.169	0.652	24	0	4	0.666	0.606	-0.098	0.536	48	0	5	0.750	0.659	0.648
Aesc_10	F: TCAAGAATAGATGCCGAAAGAA R: ATGAAATCCCAACTCC	(AG)31	225-237	FAM	24	0	4	0.750	0.683	-0.096	0.609	24	0.053	6	0.625	0.694	0.100	0.633	48	0	6	0.687	0.689	0.636
Aesc_11	F: CGTATCACCCAGACTCTTC R: GCCTGTGTTCCGCTGTTC	(GACA)18	269-309	HEX	24	0	3	0.708	0.597	-0.184	0.507	24	0	7	0.833	0.752	-0.107	0.704	48	0	7	0.770	0.675	0.633
Aesc_12	F: GCAGCCCGTAGCCGATGA R: TAACCAAGCAGTCAATCCACC	(TGAT)41	237-365	FAM	24	0	3	0.333	0.296	-0.125	0.270	24	0.087	7	0.666	0.823	0.190	0.774	48	0.012	8	0.500	0.559	0.632
Aesc_13	F: GCATTTATTCTTCATCTTCTG R: GCTGTGCTGCCGAAACTAC	(ACACG)10	249-319	PET	24	0	5	0.750	0.699	-0.072	0.628	24	0.177*	7	0.333	0.523	0.363\$	0.491	48	0.101*	9	0.541	0.611	0.627
Aesc_14	F: CGCAAGTGAGCAAGCAAA R: ATCTGGAGGAGGGTTGGG	(AAAGC)13	135-225	HEX	24	0.190*	5	0.375	0.636	0.411	0.555	24	0.292*	5	0.250	0.713	0.649\$	0.633	48	0.245*	6	0.312	0.675	0.603
Aesc_15	F: CCGTCAAGGAACGAAGGTG R: AACCGTAACCGAACAGTAA	(AT)18	355-363	NED	24	0	4	0.666	0.644	-0.033	0.561	24	0	3	0.625	0.556	-0.123	0.479	48	0	4	0.645	0.600	0.572
Aesc_16	F: GCAGAGCAAGAACACACCCA R: AATGAGTAATGAACGGTATTT	(AC)17	296-306	HEX	24	0	4	0.500	0.423	-0.182	0.391	24	0.026	4	0.500	0.538	0.070	0.426	48	0.060	6	0.500	0.480	0.529
Aesc_17	F: GCACTTCCCATCTGACCT R: ACTGCCTGGTATTTGTGG	(TCTGA)11	164-199	HEX	24	0	3	0.375	0.369	-0.014	0.310	24	0	4	0.750	0.640	-0.171	0.564	48	0	5	0.562	0.505	0.493
Aesc_18	F: AGGCATTGGCAGGACAC R: AAACCATCTCAAATCAAATCC	(AC)17	263-273	HEX	24	0	2	0.458	0.467	0.019	0.353	24	0.151*	6	0.375	0.538	0.304	0.496	48	0.098*	6	0.416	0.503	0.489
Aesc_19	F: CGTCCGATGATGTTCTCTG R: TCAGTCCAGAGGTGCTTATTT	(GGTC)15	120-152	PET	24	0.073	4	0.541	0.615	0.120	0.560	24	0	3	0.416	0.354	-0.176	0.316	48	0.035	5	0.479	0.485	0.466
Aesc_20	F: TAGTAGTGGCAGTGTGCGGAG R: CGAGTTGTTTCGTGTGAG	(TG)16	270-290	FAM	24	0	2	0.500	0.478	-0.045	0.359	24	0	3	0.333	0.296	-0.125	0.270	48	0.086	3	0.416	0.387	0.425
Aesc_21	F: GGTAGCACTTTGGTGCTGA R: GCTTTAGACCTCTCTCTCT	(GGAT)15	221-253	PET	24	0.087	3	0.458	0.570	0.196	0.457	24	0	3	0.375	0.376	0.004	0.325	48	0.095	4	0.416	0.473	0.424
Aesc_22	F: ATTGGGCAGTTTCTCGGTC R: GTTGGTTACGCTACGACGGTT	(CATAC)17	159-209	NED	24	0	4	0.291	0.268	-0.087	0.252	24	0	6	0.625	0.599	-0.042	0.537	48	0	6	0.458	0.433	0.422

Aesc_23	F: GATAACGAAACGAAACAGACC R: CCTGGCTGGGTGTATGAAA	(AAACC)15	290-300	NED	24	0.036	3	0.375	0.376	0.004	0.325	24	0.105	2	0.250	0.338	0.262	0.275	48	0.186	3	0.312	0.357	0.405
Aesc_24	F: AAATGAAACGAACTGAACC R: CTCGGGTAAGGAGGCGTG	(AAACG)10	220-245	PET	24	0	4	0.500	0.490	-0.018	0.436	24	0	5	0.333	0.302	-0.101	0.285	48	0	6	0.416	0.396	0.383
Aesc_25	F: AGGGAGGGAGAAGGAGAAAGA R: GTTGTGATTGGTCGGTGGTT	(AAAC)25	193-225	NED	24	0	2	0.625	0.500	-0.250	0.371	24	0	2	0.416	0.422	0.012	0.328	48	0	2	0.520	0.461	0.356
Aesc_26	F: TCGGACACATCCCTTACCC R: CCCTGTAGTTAGTTTCTGTGTT	(TTTG)20	190-217	HEX	24	0	3	0.250	0.230	-0.086	0.212	24	0.035	4	0.458	0.525	0.127	0.454	48	0.028	4	0.354	0.377	0.353
Aesc_27	F: CGATACGACAGGCTACTTTCA R: AAGGACAAACATACCGACC	(GATA)14	284-304	PET	24	0	2	0.166	0.155	-0.069	0.141	24	0	4	0.500	0.499	-0.001	0.429	48	0.009	5	0.333	0.327	0.332
Aesc_28	F: ATCCGACCCGTGAGGTTT R: CGATGATTTATCCAGGGCTT	(ACCCG)12	223-258	HEX	24	0.075	3	0.208	0.266	0.217	0.244	24	0	6	0.458	0.428	-0.069	0.400	48	0.010	6	0.333	0.347	0.326
Aesc_29	F: GATAGCAAACGAACCGTAGCA R: TATGCCGAGGAAGGCTGA	(TGTC)20	223-271	NED	24	0	3	0.208	0.193	-0.074	0.178	24	0	3	0.416	0.413	-0.006	0.369	48	0	3	0.312	0.303	0.286
Aesc_30	F: AGGTTTGTTCAGTTGTCGGTGA R: GTCTGGGTGGAGCCTGTGTAG	(CT)18	375-391	NED	24	0	2	0.333	0.337	0.010	0.275	24	0	2	0.083	0.081	-0.022	0.077	48	0.022	2	0.208	0.209	0.195
Aesc_31	F: TCCAGAAATCCGCTTATCAA R: AGGGAAATGGGCAAACTAA	(GAT)43	218-224	PET	24	0	2	0.041	0.041	0	0.040	24	0	2	0.083	0.081	-0.022	0.077	48	0	2	0.062	0.061	0.059
Mean					24	0.013	4.1	0.519	0.505	-0.031	0.449	24	0.015	5.8	0.564	0.566	-0.006	0.515	48	0.029	6.7	0.541	0.535	0.532

Pylaiella littoralis

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Dye	Wissant						Ile de Aransa						Global							
					n	Nf	Na	Ho	He	Fis	PIC	n	Nf	Na	Ho	He	Fis	PIC	n	Nf	Na	Ho	He	PIC
Plit_01	F: CACCACTATCATTACAGTCAGGG R: GAAGCAGCACTCCAGCGA	(ACA)30	183-450	PET	24	0.195*	17	0.541	0.913	0.407	0.880	24	0.191*	17	0.541	0.933	0.420	0.900	48	0.205*	28	0.541	0.923	0.929
Plit_02	F: ACCGACAGCAGCAATCAG R: GCACCTAGAACACGCTACCC	(GTA)58	251-362	FAM	23	0.039	19	0.826	0.920	0.103	0.891	24	0.166*	13	0.583	0.923	0.368	0.889	47	0.119*	22	0.704	0.922	0.920
Plit_03	F: AAATGCGAACAACCTCACTT R: CGACAGACACGCTGAAACC	(TC)29	218-272	PET	24	0.078*	13	0.750	0.916	0.181	0.885	24	0.163*	7	0.500	0.797	0.372	0.743	48	0.148*	18	0.625	0.856	0.890
Plit_04	F: CGTTCCTTGTTGTGCTTAC R: GTAGATGCTTGCTGACCCAGA	(CT)33	138-170	NED	24	0	9	0.875	0.820	-0.066	0.781	24	0.180*	5	0.458	0.750	0.388	0.683	48	0.118*	11	0.666	0.785	0.855
Plit_05	F: AGTCATCCCTGCTCTGCC R: ATGGGCTCGTCTCTGGTG	(ACAT)42	242-374	NED	24	0.051	20	0.833	0.946	0.119	0.920	24	0	2	0.458	0.467	0.019	0.353	48	0.125	22	0.645	0.707	0.834
Plit_06	F: CGACTCCCTGGTTCTCTTCA R: CGTTTGTCCACGCATTACA	(TTG)44	350-401	HEX	24	0	7	0.791	0.731	-0.081	0.676	24	0	5	0.791	0.676	-0.170	0.605	48	0.029	12	0.791	0.704	0.828
Plit_07	F: GCGAAAGACAACAACAACACTAC R: CGACACACGAGAAGGACAGA	(GTAT)36	194-301	HEX	24	0	6	0.833	0.762	-0.092	0.713	24	0.061	6	0.500	0.567	0.118	0.529	48	0.106	12	0.666	0.664	0.808
Plit_08	F: CTTTCTACCGCATCCACCAG R: TTCTTCTCAACTAAACGCCA	(AACAC)11	238-313	HEX	24	0	9	0.958	0.817	-0.172	0.781	24	0	3	0.541	0.530	-0.020	0.435	48	0.050	11	0.750	0.673	0.799
Plit_09	F: CACAATGGAAATCAGGTCA R: TTCGTGTCGTTCTAAGCC	(ACA)39	250-301	NED	24	0.047	7	0.583	0.631	0.076	0.574	24	0.235*	8	0.416	0.809	0.485	0.760	48	0.193*	10	0.500	0.720	0.792
Plit_10	F: TGTCTTACGCCTGTTTCGTTT R: GAGTGTCCGTGGGAAGGTC	(TGT)37	240-309	NED	24	0.030	9	0.750	0.837	0.103	0.793	24	0.122*	7	0.583	0.798	0.269	0.746	48	0.081*	11	0.666	0.817	0.791
Plit_11	F: CCTGCTCGCTGAAGATTGT R: TGTATGAAATGGAGTTGAGGAAA	(AATGC)15	162-212	FAM	24	0.068	8	0.708	0.836	0.152	0.794	24	0.070	8	0.666	0.772	0.137	0.726	48	0.082	10	0.687	0.804	0.787
Plit_12	F: CATTATCTCCGCGCAA R: GCCAGGTCTAAGTTTCACA	(AAGGC)10	153-213	HEX	24	0.077	3	0.500	0.615	0.188	0.533	24	0.002	7	0.541	0.559	0.032	0.518	48	0.168	8	0.520	0.587	0.748
Plit_13	F: GTAGACGGCACAGCACACAA R: TGTTCAGCAGCGTAGTCCC	(GGCAC)15	212-277	FAM	24	0	9	0.625	0.564	-0.107	0.529	24	0.195*	6	0.416	0.744	0.440	0.691	48	0.166*	10	0.520	0.654	0.748
Plit_14	F: CTGCGAGAAACAGAACCGA R: GAGAGGTGTGAATGGAGCGT	(AAAC)13	157-211	PET	24	0.051	6	0.375	0.399	0.061	0.372	24	0.121*	4	0.416	0.613	0.320	0.529	48	0.233*	9	0.395	0.506	0.707

Plit_15	F: TTTGGAGTTGTTCTGTGTTTGA R: GCGTTTCACTTGTATCCCACC	(TGAT)14	269-289	PET	24	0.020	3	0.416	0.465	0.105	0.410	24	0	3	0.583	0.527	-0.106	0.420	48	0.164	6	0.500	0.496	0.701
Plit_16	F: CACCGTATGCTTGAATGCTC R: GAACCTCAGATGGGAAACCCT	(CTT)36	251-308	NED	24	0.126	5	0.500	0.635	0.212	0.576	24	0.093	2	0.166	0.224	0.258	0.195	48	0.268	7	0.333	0.429	0.668
Plit_17	F: ACTACTGGAACCCGCATCGT R: CTAECTCGGAACACACAGCCC	(ATC)40	252-315	FAM	24	0	4	0.416	0.361	-0.152	0.332	24	0	2	0.500	0.496	-0.007	0.368	48	0.180	6	0.458	0.428	0.660
Plit_18	F: TCAGTCAGTCAGTCAATCAATCA R: AGGTTTCGTGTCAAAGCAGAA	(CAAT)29	250-306	FAM	24	0	3	0.958	0.603	-0.588	0.514	24	0	2	0.208	0.190	-0.095	0.169	48	0.099	5	0.583	0.396	0.649
Plit_19	F: GCACCTGAGACAAGAGACGG R: ACAGCAACATCAACAGCAACA	(TGTGA)21	260-295	FAM	24	0	3	0.458	0.385	-0.187	0.348	24	0	5	0.625	0.555	-0.125	0.513	48	0.117	6	0.541	0.470	0.638
Plit_20	F: CATAGGGTGTCTTGGCATT R: CGCACGACTTCTGTCTTACC	(GCTCA)17	351-396	FAM	24	0	2	0.208	0.190	-0.095	0.169	24	0.014	5	0.666	0.680	0.020	0.620	48	0.193	6	0.437	0.435	0.638
Plit_21	F: AAGGAAGGACAGCACAATGAA R: AGGTCGCACAACAATAACTGG	(CAAAA)10	285-360	PET	24	0	3	0.416	0.381	-0.092	0.336	24	0	2	0.791	0.503	-0.571	0.375	48	0.055	4	0.604	0.442	0.612
Plit_22	F: TTGATTGGTCTCTCCAGGTGT R: ATAGGTGCGTGTGTAGCCGT	(TC)38	219-303	HEX	24	0.124	5	0.541	0.719	0.246	0.648	24	0.169*	4	0.291	0.509	0.427	0.462	48	0.183*	5	0.416	0.614	0.605
Plit_23	F: AGGAGCAGGGTGAGAGAAGAG R: TAGGAAAGTGAGGCTGGACAA	(GA)34	286-336	NED	24	0	6	0.750	0.625	-0.200	0.583	24	0	4	0.208	0.197	-0.055	0.187	48	0.143	8	0.479	0.411	0.597
Plit_24	F: GTGTGTTCTTGGTGCCTGTC R: CGCATAGAGAGCGAGAGATT	(CT)29	196-228	PET	24	0	2	0.375	0.309	-0.210	0.258	24	0.179	4	0.208	0.331	0.371	0.298	48	0.273	6	0.291	0.320	0.593
Plit_25	F: CCTTCGCTTTGCCTCGTT R: AAAGTCCATTCTTTCGGT	(TCTGC)13	274-324	NED	24	0.144*	5	0.500	0.725	0.310	0.661	24	0	2	0.458	0.402	-0.139	0.317	48	0.105*	6	0.479	0.563	0.584
Plit_26	F: GCAGGAATGGCAGAGAGG R: CGACAGAGAACTGAAAGGTGG	(CGGCA)11	173-213	HEX	24	0	4	0.458	0.445	-0.028	0.402	24	0	2	0.125	0.119	-0.045	0.110	48	0.268	6	0.291	0.282	0.574
Plit_27	F: CGTTGCGAGGTGATGATG R: TCAAGGTGATTTGGGCTTT	(ACAG)13	224-248	PET	24	0	5	0.375	0.334	-0.122	0.313	24	0	2	0.166	0.155	-0.069	0.141	48	0.272	6	0.270	0.245	0.535
Plit_28	F: TACCACCAAGAAGAGTAGGG R: CAGAAACAACACAAATCGG	(GGTGT)15	264-289	HEX	24	0	3	0.708	0.565	-0.253	0.496	24	0.051	3	0.250	0.295	0.153	0.264	48	0.111	3	0.479	0.430	0.533
<i>Mean</i>					<i>24</i>	<i>0.019</i>	<i>5.3</i>	<i>0.592</i>	<i>0.556</i>	<i>-0.083</i>	<i>0.509</i>	<i>24</i>	<i>0.026</i>	<i>3.7</i>	<i>0.444</i>	<i>0.436</i>	<i>-0.008</i>	<i>0.379</i>	<i>48</i>	<i>0.150</i>	<i>8.0</i>	<i>0.518</i>	<i>0.496</i>	<i>0.678</i>

Calliblepharis jubata

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Dye	Roscoff						Le Havre						Global							
					<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>PIC</i>	<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>PIC</i>	<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>PIC</i>
Cjub_01	F: GAAGGAAACGGAGCAGCA R: AATGTGGTGACGAGCGATTT	(CAAA)6	247-281	NED	24	0	4	0.583	0.515	-0.131	0.461	24	0.121	4	0.458	0.554	0.173	0.505	48	0.167	8	0.52	0.534	0.728
Cjub_02	F: GTATCCTGTCTGTGCCGCCT R: CCATCAATCACCGTCTACTC	(GA)20	247-261	HEX	24	0	4	0.625	0.535	-0.167	0.477	24	0	4	0.875	0.581	-0.504	0.485	48	0	6	0.75	0.558	0.519
Cjub_03	F: ATTATCTCCAGCATCGTT R: CGGTTTGGTCTGCGAAAGT	(AT)14	274-298	FAM	24	0.233*	5	0.291	0.573	0.491	0.513	24	0.133	3	0.208	0.32	0.35	0.274	48	0.271*	5	0.25	0.447	0.495
Cjub_04	F: CCACAGAAAGCCAGGTAAA R: AACACCCGCTGCTACGAC	(GCACC)4	140-265	NED	24	0.018	3	0.416	0.458	0.090	0.397	24	0	2	0.75	0.472	-0.586	0.359	48	0	3	0.583	0.465	0.435
Cjub_05	F: AACTCTTCTCTCGCTTCGTG R: GATTAGATGGTCTGTGAGGTGG	(CT)9	344-401	PET	24	0	4	0.833	0.562	-0.481	0.459	24	0	2	0.833	0.489	-0.703	0.368	48	0	4	0.833	0.525	0.419
Cjub_06	F: ATAAGAAGCGACAGCAATCCA R: AACAGGTGCGCGGAAG	(TGTT)5	205-261	HEX	24	0	2	0.958	0.500	-0.916	0.375	24	0	2	0.916	0.498	-0.840	0.373	48	0	2	0.937	0.499	0.374
Cjub_07	F: AGAGTGAAGAGACGACGA R: CCTCACCAGTAAACCTACCA	(GA)15	157-169	FAM	24	0	4	0.458	0.438	-0.045	0.388	24	0	2	0.583	0.418	-0.393	0.328	48	0	4	0.52	0.428	0.365

Cjub_08	F: GGCACCACTCCCATTGTATC R: TTACCAACGCACGCTCCT	(GTGTA)4	206-211	PET	24	0	2	0.625	0.507	-0.232	0.375	24	0	2	0.458	0.358	-0.277	0.291	48	0	2	0.541	0.433	0.353
Cjub_09	F: AGCGGTGTGTGTGACC R: ACCAGTGCTTCATTAGTAA	(AC)14	133-137	FAM	24	0.029	3	0.291	0.329	0.115	0.298	24	0	3	0.416	0.414	-0.004	0.371	48	0	3	0.354	0.372	0.336
Cjub_10	F: CCCTGTGTCTAATTTCATCT R: CCTTCCTGCTACTGTGGTTCA	(TGTT)11	366-390	FAM	24	0	2	0.041	0.041	0	0.040	24	0.02	4	0.416	0.414	-0.004	0.371	48	0.064	5	0.229	0.228	0.233
Cjub_11	F: CCCGTTTGTGGGAATG R: GACCTTGCTCTACACCTCTTCT	(GA)9	161-169	PET	24	0	3	0.208	0.193	-0.074	0.178	24	0	2	0.375	0.309	-0.21	0.258	48	0	3	0.291	0.251	0.225
Cjub_12	F: AAGAAAGGAGGAACCCGAAA R: ACTGGCTTGTGGAAACGG	(TGTTG)4	110-115	HEX	24	0.093	2	0.166	0.224	0.258	0.195	24	0.266*	2	0.041	0.259	0.839	0.218	48	0.190*	2	0.104	0.241	0.207
Cjub_13	F: ACAAGCAAGCAAACAAGCAAG R: ATGTGAGGAAACGGAGGAGAG	(AAACA)5	115-130	NED	24	0.049	3	0.250	0.295	0.153	0.264	24	0	2	0.166	0.155	-0.069	0.141	48	0.023	3	0.208	0.225	0.206
Cjub_14	F: TGACAAAGATGGCAAACAACC R: ACGGGAGCGCGGAAGAAA	(GTTT)9	146-162	HEX	24	0	2	0.083	0.081	-0.022	0.077	24	0	2	0.416	0.335	-0.243	0.275	48	0	3	0.25	0.208	0.206
Cjub_15	F: CATCCCTTCCATCTCTTTCT R: GCTGAGTGCGTAAAGTTGTGA	(ACAAA)10	276-281	FAM	24	0	2	0.041	0.041	0	0.040	24	0	2	0.125	0.119	-0.045	0.110	48	0	2	0.083	0.08	0.077
<i>Mean</i>					24	0.009	2.9	0.329	0.312	-0.028	0.272	24	0.013	2.6	0.458	0.375	-0.197	0.318	48	0.023	3.8	0.393	0.344	0.335

Gracilaria gracilis

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Dye	Gris-Nez						Porto						Global							
					<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>PIC</i>	<i>N</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>PIC</i>	<i>N</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>PIC</i>
Ggrac_01	F: TCATAATCGGGCAGCCATC R: CAGCTGAGCGAGCCGTTTC	(CCG)6	161-200	PET	24	0.307*	6	0.458	0.792	0.421	0.731	24	0.247*	6	0.375	0.766	0.510	0.704	48	0.237*	9	0.416	0.779	0.808
Ggrac_02	F: CGCGTCTGAAGAATCGAAGA R: GCAGCATGCAACGAATTAACC	(AC)14	122-150	FAM	24	0.084	7	0.583	0.695	0.161	0.641	24	0.129*	4	0.541	0.762	0.289	0.694	48	0.155*	9	0.562	0.729	0.807
Ggrac_03	F: TCGTAATTAGGCTTCTGCGCT R: ATGCCGGTGTCTTTGAGTCC	(AG)22	114-130	FAM	24	0	7	0.791	0.762	-0.038	0.712	24	0.209*	3	0.333	0.600	0.444	0.514	48	0.156*	9	0.562	0.681	0.784
Ggrac_04	F: CGTCCATCGCTTCTTGTTTC R: GACGCGCGGAGGAGTATAAG	(AGC)5	245-269	FAM	24	0.167*	6	0.500	0.740	0.324	0.676	24	0.033	5	0.666	0.765	0.129	0.708	48	0.129*	8	0.583	0.752	0.778
Ggrac_05	F: GGATGACGAAATGAAGCCCG R: TCCAGAATACCACAAGTTGCA	(AAG)5	212-230	NED	24	0.018	5	0.708	0.749	0.054	0.685	24	0.058	5	0.500	0.582	0.141	0.536	48	0.094	6	0.604	0.665	0.708
Ggrac_06	F: TTTCCACATCGCCACTTCGA R: AGCGACAACATCTAGAGCGG	(AATGCG)7	227-245	FAM	24	0.186	4	0.333	0.491	0.322	0.431	24	0	3	0.750	0.501	-0.494	0.408	48	0.105	4	0.541	0.496	0.633
Ggrac_07	F: CTCTCCACTTCCGGCTTCTG R: TCTCACCTCACTGCACAACG	(AG)9	132-142	HEX	24	0	4	0.791	0.757	-0.045	0.694	24	0	3	0.750	0.526	-0.425	0.405	48	0	5	0.770	0.641	0.631
Ggrac_08	F: AGTATGGCTAACACACGCA R: GTACAAACGCCAAGAGACACG	(AC)12	172-182	HEX	24	0.130*	4	0.500	0.690	0.275	0.621	24	0.193*	5	0.250	0.433	0.423	0.402	48	0.224*	5	0.375	0.562	0.626
Ggrac_09	F: CCTTCCTCATCGCTTCGTC R: CGCATCGCTCTATTCCACCA	(AAGACG)7	104-130	NED	24	0.148	3	0.375	0.516	0.273	0.396	24	0	2	0.416	0.335	-0.243	0.275	48	0.119	3	0.395	0.425	0.562
Ggrac_10	F: CGTCACTATCCACACGCTGA R: CAACTGCCAAGAACAACACTCT	(GT)16	133-141	FAM	24	0	3	0.750	0.635	-0.181	0.549	24	0.043	3	0.375	0.440	0.148	0.388	48	0.032	4	0.562	0.537	0.557
Ggrac_11	F: CCGTGTCTACCGTTGGTACC R: TGAGCTGGTGAACGAGCTTC	(AGCC)8	212-232	HEX	24	0	3	0.625	0.606	-0.031	0.523	24	0	4	0.541	0.493	-0.097	0.402	48	0.009	4	0.583	0.549	0.502
Ggrac_12	F: CGCACTCGCTGACGAAGG R: GCGCTCAGAATGGTGGAGAT	(AGC)5	217-244	FAM	24	0.177	2	0.333	0.481	0.308	0.359	24	0	3	0.541	0.512	-0.056	0.396	48	0.051	3	0.437	0.497	0.378
Ggrac_13	F: TGCAGTGTACCTGTGAGT	(AC)6	189-191	HEX	24	0.029	2	0.500	0.496	-0.007	0.368	24	0.243*	2	0.250	0.501	0.501	0.368	48	0.118*	2	0.375	0.499	0.375

Ggrac_14	R: TTTCGCTGTCCGCTCATICT	(AAGG)6	136-168	PET	24	0	2	0.375	0.309	-0.210	0.258	24	0	2	0.041	0.041	0	0.040	48	0.051	2	0.208	0.175	0.368
	F: CTGTCAAGTGCACGCCGAG																							
Ggrac_15	F: CCTTTAACAAAGCGCGCAA	(AGC)14	100-125	HEX	24	0	5	0.708	0.539	-0.312	0.489	24	0	3	0.208	0.193	-0.074	0.178	48	0	5	0.458	0.366	0.367
	R: CGCAGCGGTGAAGTCATTAG																							
Ggrac_16	F: CGCCATCAAGAGTGAAGTAGC	(AC)7	110-114	HEX	24	0	2	0.458	0.467	0.019	0.353	24	0.117	2	0.291	0.405	0.281	0.317	48	0.06	2	0.375	0.436	0.337
	R: AGACGGCCACAGTGAACCC																							
Ggrac_17	F: CAGCAATCGTAGTCAGCAAGA	(TGT)13	145-178	NED	24	0	2	0.333	0.282	-0.179	0.239	24	0	2	0.541	0.465	-0.163	0.353	48	0	2	0.437	0.374	0.311
	R: GGCGTGGGTGTGGTGT																							
Ggrac_18	F: TCCTGAAGATAGATGATACGA	(CT)11	190-200	HEX	24	0	2	0.291	0.253	-0.150	0.218	24	0.020	2	0.291	0.311	0.064	0.258	48	0	2	0.291	0.282	0.239
	R: AAACGAGAGCAGAAACCCAAG																							
Ggrac_19	F: CCCTGACTCTCTCAGCTGAC	(ACGC)7	127-131	FAM	24	0	2	0.250	0.222	-0.122	0.195	24	0	2	0.125	0.119	-0.045	0.110	48	0	2	0.187	0.171	0.155
	R: GAGCAGCCTGACAACGATGA																							
Ggrac_20	F: GATGAACGGCAGCAGAGAGA	(ACAG)5	285-297	PET	24	0	2	0.208	0.190	-0.095	0.169	24	0	3	0.125	0.121	-0.029	0.115	48	0	3	0.166	0.155	0.145
	R: CCTGACTGAGGCCATAGGTG																							
<i>Mean</i>					<i>24</i>	<i>0.038</i>	<i>2.9</i>	<i>0.467</i>	<i>0.464</i>	<i>-0.024</i>	<i>0.397</i>	<i>24</i>	<i>0.017</i>	<i>2.8</i>	<i>0.392</i>	<i>0.36</i>	<i>-0.071</i>	<i>0.299</i>	<i>48</i>	<i>0.056</i>	<i>3.4</i>	<i>0.43</i>	<i>0.412</i>	<i>0.421</i>

Palmaria palmata

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Dye	Roscoff						Belle-Ile						Global							
					<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>PIC</i>	<i>n</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>PIC</i>	<i>N</i>	<i>Nf</i>	<i>Na</i>	<i>Ho</i>	<i>He</i>	<i>PIC</i>
Ppalm_01	F: AGTGTGGAGAGAGCTTTGGC R: CTGCCAAGTCCTTATCAAGC	(AAGATC)14	144-324	HEX	24	0.060	15	0.791	0.919	0.138	0.889	24	0.178*	9	0.541	0.872	0.379	0.830	48	0.127*	17	0.666	0.895	0.886
Ppalm_02	F: ACTATGGTCAACCGTGTGG R: AACCGCCAGATCCTCGATA	(ACGCC)10	247-313	HEX	24	0.060	8	0.625	0.702	0.109	0.652	24	0.064	10	0.750	0.875	0.143	0.839	48	0.077	10	0.687	0.788	0.787
Ppalm_03	F: CTCAAGCGTCTTACATTACA R: CAAGGGAGGCGGACAGTAG	(TA)12	194-233	FAM	24	0.063	9	0.708	0.824	0.140	0.779	24	0.180*	5	0.458	0.735	0.376	0.679	48	0.144*	9	0.583	0.779	0.782
Ppalm_04	F: AGGAGGCCATCAATTCTACA R: TGCCTTTGAGGAGCATAGGT	(ACCGT)5	206-286	NED	24	0.113	7	0.541	0.683	0.207	0.622	24	0.129	5	0.416	0.616	0.324	0.535	48	0.202	9	0.479	0.650	0.780
Ppalm_05	F: GAGGTCGAGAAGCCACCAC R: ATCAGCAGGTTCCGGTTCATG	(AAC)12	317-380	HEX	24	0.062	11	0.666	0.764	0.128	0.710	24	0.195	6	0.458	0.795	0.423	0.736	48	0.147*	13	0.562	0.779	0.771
Ppalm_06	F: TGCAGATGGTGGTTTCGACA R: TACACGTGTGTGCTGAGCAT	(AACAC)7	375-405	NED	24	0.258*	7	0.375	0.804	0.533	0.751	24	0.303*	4	0.250	0.682	0.633	0.603	48	0.285*	7	0.312	0.743	0.699
Ppalm_07	F: CGTGTCTGAGGAGAGCTGG R: CGCTCCGTATCGCGATTT	(AAGG)7	112-142	NED	24	0	5	0.833	0.671	-0.241	0.606	24	0.023	4	0.625	0.686	0.089	0.602	48	0.001	6	0.729	0.678	0.694
Ppalm_08	F: TGGAGGCTACAACCTTTGG R: CGAAGAGACTGCGGGACAAA	(AG)7	116-124	FAM	24	0	5	0.791	0.655	-0.207	0.584	24	0	4	0.958	0.677	-0.414	0.617	48	0	5	0.875	0.666	0.610
Ppalm_09	F: AAACCGTCGCTGTCTTGGC R: CTACACGAACACCTCCCTTGT	(ATC)9	121-160	HEX	24	0	9	0.833	0.714	-0.166	0.663	24	0	3	0.791	0.541	-0.461	0.430	48	0	9	0.812	0.628	0.565
Ppalm_10	F: GCAGAAGATGTTTCAGTGTGG R: GGCTGTAAGTGCTATCTGCT	(AGCC)8	138-186	NED	24	0.116	6	0.500	0.686	0.271	0.608	24	0.095	3	0.166	0.231	0.281	0.212	48	0.206	7	0.333	0.459	0.529
Ppalm_11	F: AACTCTAAGCGTCTCGAA R: TGCAGAAATCCAGGCTGAG	(AT)6	117-123	HEX	24	0	4	0.541	0.516	-0.049	0.441	24	0	2	0.875	0.501	-0.743	0.375	48	0	4	0.708	0.509	0.521
Ppalm_12	F: TGTGTGCAATAGAGAGAGGA R: CTCCAATCTCCGGAGCTCG	(AGCCC)5	111-141	NED	24	0.102	3	0.458	0.573	0.200	0.488	24	0.180*	3	0.125	0.266	0.530	0.239	48	0.232*	5	0.291	0.419	0.518

Ppalm_13	F: AAAGAAAGGCGACACCGACA R: CCACCTCCCATAGCCCAATT	(ACTAGC)6	113-161	HEX	24	0.085	8	0.500	0.652	0.233	0.604	24	0.111	4	0.333	0.392	0.150	0.355	48	0.104	8	0.416	0.522	0.500
Ppalm_14	F: AAGAGCCGACCAAGAAGACG R: AGGGATGTTCCGCCGGCAG	(AGC)13	152-227	PET	24	0	2	0.625	0.500	-0.250	0.371	24	0.090	4	0.416	0.552	0.245	0.481	48	0.015	4	0.520	0.526	0.455
Ppalm_15	F: TAGCCAGAAACCCACAAGC R: CCGCGTGTGCTGAATATAGC	(AG)10	145-153	FAM	24	0.205*	5	0.291	0.505	0.422	0.432	24	0	2	0.208	0.190	-0.095	0.169	48	0.238*	5	0.250	0.347	0.424
Ppalm_16	F: TGCATATTGCCTAATAAAGCACC R: GCTCGAAAACAATGGAATGTCC	(AT)6	99-125	FAM	24	0	4	0.375	0.388	0.035	0.350	24	0	4	0.291	0.266	-0.095	0.248	48	0	4	0.333	0.327	0.306
Ppalm_17	F: CGTCCCACTATTCCCGGG R: TCGTTCTCGCACAAGTCGTT	(AACAGG)6	91-103	FAM	24	0.072	3	0.208	0.228	0.087	0.206	24	0	3	0.375	0.385	0.028	0.344	48	0.028	3	0.291	0.307	0.280
Ppalm_18	F: CGTGAAGGCTAACCTAGAGAGC R: GGCACCAAAATCCTCGAAAGC	(AG)6	348-444	FAM	24	0	3	0.375	0.317	-0.182	0.274	24	0	2	0.375	0.309	-0.210	0.258	48	0	3	0.375	0.313	0.267
Ppalm_19	F: TGTCACCTTGATCGTACCGC R: CGAAGGCCTCAGCTGTGTAT	(AAAT)6	138-146	NED	24	0.170	2	0.208	0.364	0.427	0.291	24	0	2	0.250	0.222	-0.122	0.195	48	0.091	2	0.229	0.293	0.249
Ppalm_20	F: ACCCGCTCGGCATTACAAAT R: AGGAGTGCGAAAGGCTTTGA	(AT)6	142-152	FAM	24	0	4	0.125	0.122	-0.022	0.118	24	0	3	0.333	0.288	-0.153	0.254	48	0	4	0.229	0.205	0.197
<i>Mean</i>					24	0.047	5.1	0.503	0.514	0.023	0.458	24	0.039	3.9	0.467	0.465	-0.014	0.413	48	0.056	5.7	0.485	0.489	0.478

Gracilaria dura

Locus	Primer sequences (5'-3')	Repeat	Size	Dye	Locquirec						Coroso						Quitao						Global				
					motif	range (bp)	n	Na	Ho	He	Fis	PIC	n	Na	Ho	He	Fis	PIC	n	Na	Ho	He	Fis	PIC	n	Na	Ho
Gdura_01	F: GCAGCATGCAACGAATTTAACCC R: CGCCGTCTGAAGAATCGAAGA	(AC)17	103-135	FAM	12	8	0.736	0.840	0.124	0.683	15	5	0.859	0.780	-0.101	0.700	7	5	1.000	0.752	-0.329	0.690	34	11	0.844	0.890	0.839
Gdura_02	F: TACCGGTCGTGGGAATATC R: ACGGTACGGGCAAAAGTACAA	(AAG)14	212-278	NED	12	4	0.667	0.676	0.014	0.619	15	4	0.722	0.575	-0.257	0.462	8	3	0.833	0.598	-0.393	0.606	35	7	0.729	0.836	0.793
Gdura_03	F: GTCGGAGCGTGAGGACGG R: CGCATCGCTCTATCCACCA	(ACG)7	166-184	PET	11	4	0.700	0.738	0.052	0.626	15	4	0.964	0.747	-0.291	0.578	8	3	0.952	0.662	-0.439	0.638	34	5	0.876	0.789	0.785
Gdura_04	F: AGGTTCAATGCCTCCTGACC R: GTACGCTTCGTTGCCGATTC	(AAG)11	183-210	NED	12	4	0.764	0.748	-0.021	0.733	15	6	0.956	0.808	-0.183	0.633	8	3	0.792	0.556	-0.423	0.687	35	6	0.852	0.766	0.782
Gdura_05	F: CATGCGACCCAGATGGTGAA R: GCGTTATGGTCCACTGCTCT	(AG)15	225-331	PET	12	6	0.667	0.618	-0.079	0.661	15	6	1.000	0.791	-0.264	0.816	8	8	0.979	0.829	-0.181	0.609	35	9	0.880	0.814	0.782
Gdura_06	F: TTGTGCGCTGTGAGTAGCAA R: AGCTCGTGAACCTCCGAAAT	(AC)10	335-369	NED	12	5	0.681	0.676	-0.006	0.611	15	4	0.974	0.740	-0.317	0.499	8	3	0.833	0.592	-0.408	0.613	35	6	0.833	0.807	0.777
Gdura_07	F: GCAGAACTGTACGTCGCTCT R: TGCCCTCCTACCGTATGG	(AG)12	277-309	FAM	12	6	0.778	0.719	-0.082	0.627	15	4	0.956	0.732	-0.306	0.560	8	3	0.896	0.660	-0.358	0.646	35	8	0.881	0.826	0.778
Gdura_08	F: TGTTTGATCCTGATCCTACCCT R: CGTACGCGTTTCATAAATGACA	(AG)12	278-312	NED	12	6	0.639	0.726	0.120	0.627	15	4	0.910	0.664	-0.371	0.693	8	5	0.979	0.751	-0.303	0.624	35	7	0.828	0.817	0.773
Gdura_09	F: ACGGCTCGTCATGTTTCTCA R: AGCTACGTTGGTACAGCTCG	(AG)15	102-120	NED	12	5	0.879	0.731	-0.203	0.668	15	6	0.988	0.786	-0.257	0.409	8	2	0.833	0.444	-0.875	0.679	35	7	0.914	0.819	0.765
Gdura_10	F: TAACCGCACTACTTTCGCCA R: AATGTTCTTCGCGTGTGGG	(AGC)7	114-132	PET	12	4	0.903	0.692	-0.304	0.720	15	4	0.881	0.726	-0.214	0.555	8	3	0.875	0.639	-0.369	0.690	35	5	0.887	0.765	0.745
Gdura_11	F: GTGCGTTGGTTTCGGTGATT R: TTACCCGTCGTTTCTGCCTC	(AGC)5	219-234	PET	12	4	0.861	0.673	-0.280	0.737	15	5	0.889	0.774	-0.148	0.560	8	3	0.896	0.642	-0.395	0.518	35	5	0.881	0.768	0.746
Gdura_12	F: CGTTCGCGTTACTGACCAT	(ACT)7	115-151	PET	12	8	0.792	0.842	0.060	0.614	15	4	0.845	0.650	-0.300	0.565	8	3	0.937	0.654	-0.434	0.729	35	9	0.848	0.824	0.755

