Stranded alone: The first reported Peruvian population of Agarophyton chilensis is a single-male's clone

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Abstract:

Gracilariales, a red macroalgal order, is considered one of the top ten most invasive algal taxa. While *Agarophyton vermiculophyllum* is a well know example of an algal invader worldwide, its congeneric species *A. chilensis* has mainly remained within its original distribution range for thousands of years, and was only engaged in a few dispersal journeys to arrive from New Zealand to Chile over 19 000 years ago. Nowadays, *A. chilensis* is intensively farmed along the Chilean coast and the establishment of new populations has mostly been due to intentional/planned cultivation practices. No other source of range expansion has ever been reported, since its first description. However, in 2014 it was sighted for the first time at the docking of a small artisanal port in Peru, which nowadays represents its northernmost population. We genetically assessed 60 specimens taken from Peru in 2015 and 2017, using two different types of molecular markers (the ribosomal Internal Transcribed Spacer 2, ITS2 and six nuclear microsatellites). Altogether, our results suggest the population in Perú to be an unattached, vegetative, single male's clonal population. This population has sustained itself successfully, reproducing asexually through fragmentation, for at least four consecutive years and seems to have expanded in the area. The ITS2 sequences from these individuals corresponds all to one haplotype, the one ubiquitous among populations from Chile, the eastern coast of New Zealand and the Chatham Islands, not allowing to narrow down the potential area of origin of the Peruvian population. Based on first the allelic frequencies of six microsatellite loci estimated for 28 Chilean and five loci for seven New Zealander populations and second the analyses of Bayesian clustering, we propose the natural or unintentional anthropogenic introduction of a single male's thallus from Chile to be the source of the Peruvian population.

Keywords: Agarophyte; Colonization; Microsatellite; Population genetic; Range expansion; Red alga

Introduction

Algal colonizers have previously been reported to successfully expand their original distribution range by spreading their reproductive propagules (spores or zygotes) or thallus fragments, either through: oceanic current systems[1–3], rafting on floating substrata [4–10], attached to ship hulls, in ballast waters, or as a result of other anthropogenic activities such as aquaculture and the aquarium trade[11,12]. It is thought that at least 346 algal taxa have successfully expanded their original biogeographic range and formed new populations worldwide[12]. Amongst these, at least 17 non-buoyant seaweeds have colonized new transoceanic territories across vast distances of open ocean[10,13]. Species of the red macroalgal order Gracilariales are recognized among the top ten invasive algal taxa and *Agarophyton vermiculophyllum* (previously known as *Gracilaria vermiculophylla*[14]) is a well know example of an algal invader worldwide[11]. *Agarophyton chilensis*[15], despite its name, is originally from New Zealand and it is considered most likely that recurrent colonizers naturally expanded the species distribution range to the Chatham Islands and to the southern part of the Pacific coasts of South America dating back at least 19 000 years ago[16,17].

In late February 2014, the presence of *A. chilensis* at the Grau Port in Morro Sama, Tacna, Peru, was reported for the first time. First specimens were

collected in May of the same year and were morphologically and genetically (based on the ribulose 1,5-bisphosphate carboxylase/oxygenase gene, *rbc*L, plastid) coherently identified[18]. *Agarophyton chilensis* is a red alga commonly found and cultivated along the shores of Chile. Here, it is found from Raul Marin, in the South (43°S), all the way up to the Bahia Mejillones (23°S) in northern Chile[15,19,20]. At its currently northernmost distribution, in Coquimbo (29°S) and Caldera (27°S, an introduced population likely originating from Coquimbo), *A. chilensis* is intensively farmed and harvested for the production of agar[15,19]. All northern populations, north of Coquimbo, at 29°S, are populations introduced by fishermen for cultivation purposes and were not part of the historical natural distribution range of the species[15,21]. Some of these northern farms have already disappeared probably due to poor management choices (Wilkomirsky, personal communication). Among these farms, La Herradura Bay, Coquimbo (29°S), remains the oldest reported and established population, dating back to at least the 1950s. In Peru, *A. chilensis* is not farmed, which indicates that the new Peruvian population was not intentionally introduced, and it has been hypothesized to have rather come from a nearby farm in northern Chile. However, since the *rbc*L genetic marker studied in the Morro Sama locality is not variable at the species level (i.e., same sequence across the entire Pacific range[16]) for *A. chilensis,* the origin of the new Peruvian population remained unsolved[18]. Two scenarios could have led to the colonization of the Peruvian coasts: (1) a direct transoceanic colonization from New Zealand to Peru, or (2) a natural or anthropogenic range expansion from Chilean populations to the North.

Agarophyton chilensis, has a complex reproductive life cycle with an alternation between haploid (male and female gametophytes) and diploid (tetrasporophytes) individuals. The species can reproduce sexually through the production of spores or asexually by fragmentation of its thallus. The latter resulting in individuals unattached to the substrata (lacking attachment discs) and forming populations of thalli drifting over, or embedded in sandy/muddy seabeds, which are commonly found in estuaries and bays[19]. Farmers also take advantage of fragmentation to seed their farms with cuttings of thalli and grow new algae[22]. In this process of induced asexual reproduction in farms, a loss in fertility of the farmed unattached population (which also mainly comprises vegetative tetrasporophytes) has further been recorded[19]. In contrast, sexually reproducing natural populations are composed of individuals growing anchored to pebbles, shells or the rocky substratum by attachment discs[19]. Most individuals in natural populations are fertile, and the populations typically comprise a mixture of males, females, and tetrasporophytes[19]. In the case of Peru, the first collected individuals were reported to be infertile and growing buried in sand[18], and thus, so far, rather resembling a farmed Chilean population. Yet, there is little knowledge on the demographics of the Peruvian population since an assessment of population dynamics and genetics is still needed.

This study seeks to comprehensively assess the putative origin as well as the phase and sex ratio of the Peruvian population of *A. chilensis*, combining knowledge on the biology of the species, metadata of the site, and phenotypic and genotypic data. Using population genetics including a large genetic data base

of the ribotype of the Internal Transcribed Spacer 2 (ITS2) and microsatellite nuclear DNA markers of *A. chilensis* specimens collected along its entire distribution range (seven locations from New Zealand, including Chatham Islands, and 28 in Chile[16,19]), we verify whether or not the newly found Peruvian population is representative of another case of transoceanic colonization from New Zealand or rather a range expansion from the Chilean coast. Additionally, Peruvian samples were taken in 2015 and 2017 to assess potential temporally recent genetic and demographic changes within the population of Morro Sama.

Materials and Methods

Sampling location, direct thalli examinations, and DNA extraction

The 12th of September of 2015, 30 samples of *Agarophyton chilensis* were collected under the structure of a small dock for artisanal fishers' boats[18], at the Grau Port, in Morro Sama, Tacna, Peru (17° 59' 39.7" S and 70° 52' 59.1" W; Fig. 1). Another 30 specimens were sampled on the 31st of august, 2017. During both samplings, a brief field evaluation of the *A. chilensis* population was carried out and basic environmental parameters were measured using a field thermometer (for temperature in °C), a WTW Multiparameter (Multi 3630, for pH), and an Atago Master Refractometer (for salinity).

Both collections were evaluated under a stereoscope in search for reproductive structures as well as attachment discs. For further genetic analyses, a fragment

of each of the 60 samples was kept in dry silica gel prior DNA extraction following the protocol in Cohen et al. (2004)[17].

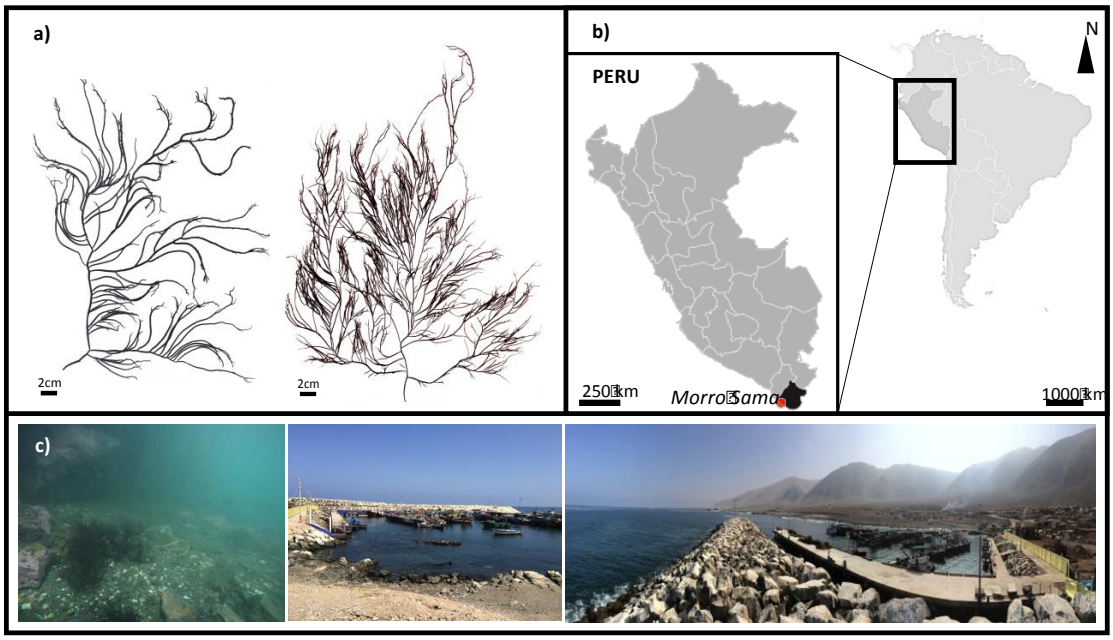


Fig. 1: Description of the Peruvian population of *Agarophyton chilensis*, Morro Sama, Tacna, Peru. Panel (a) are photographs of two samples taken in September 2015, by N. Arakaki. Panel (b) indicates the exact sampling location of Puerto Grau, in Morro Sama, Tacna Region, Perú. Panel (c) are three photographs: The *A. chilensis* population *in situ* (underwater); the shoreline and access road to the Port; and the structure of the docking site (from left to right).

Genetic Data acquisition

The ploidy level and sex of the vegetative Peruvian individuals were determined using the genetic sex markers published by Guillemin et al. (2012)[23]. Ploidy level given by genetic sex markers was cross-checked with microsatellite

155 genotypes (i.e., haploid gametophytes should not present more than one allele
156 for each microsatellite locus).

157 Microsatellite data were generated according to Guillemin et al. (2005; with the
158 following codes for each of the six microsatellite loci: Grc-8B2, Grc-7F12, Grc-
159 2B2, Grc-6C7, Grc-7D3, and Grc-AC/CT23)[24] on an ABI 3100 sequencing
160 platform (Applied Biosystem, Foster City, CA). Genotypes from Morro Sama were
161 added to previously published genetic data sets composed of a total of 2112
162 individuals from seven locations in New Zealand (including the Chatham Islands)
163 and 28 locations in Chile (for more details on the sample locations and site
164 descriptions of published samples see Guillemin et al. 2008, 2014[16,19], and the
165 suppl. Table S1). All 182 individuals from New Zealand were diploids and
166 individuals from Chile were composed of 1394 diploids and 536 haploids. Allele
167 sizes among the different data sets were calibrated by including individuals of
168 known genotypes from Chile in each separate fragment analysis run. A total of
169 six microsatellite loci were scored in South America and two localities in New
170 Zealand (Scorching Bay (P-SCB) and Pautahanui Inlet (P-PIW) in the south of the
171 northern island; locality codes, as in Guillemin et al. (2008, 2014) [16,19]; suppl.
172 Table 1). Locus Grc-AC/CT23[24], was missing for the rest of the New Zealand
173 samples, for which only five microsatellites were scored. Please note that the
174 DRYAD data set for microsatellites genotypes used in Guillemin et al. (2014)[16]
175 is available at http://hdl.handle.net/10255/dryad.73932.

176 Additionally, the Internal Transcribed Spacer 2 (ITS2, operon of nuclear
177 ribosomal genes) was sequenced for a subset of the Peruvian samples collected
178 in 2015. The ITS2 PCR reactions and sequencing followed the protocol described

179 in Cohen et al. (2004)[17] and were performed on an ABI 3100 Sequencer
180 (Applied Biosystem, Foster City, CA). Sequences were edited using
181 CHROMAS[25] and multiple sequence alignments were constructed with
182 BIOEDIT[26].

183

184 *Microsatellites and ITS data analyses*

185 First, allelic frequencies and probabilities to generate the Peruvian genotypes
186 under the null hypothesis of free recombination [27] were estimated in various
187 populations and regions. Allele frequencies were estimated using GenoDive
188 v.2.0b27[28], for each microsatellite locus, for every single sampling location in
189 Chile and New Zealand (i.e., 35 locations in total), or among regions (after
190 grouping locations following Guillemin et al. 2008, 2014)[16,19]. Regions in New
191 Zealand were assigned as in Guillemin et al. (2014)[16]: West New Zealand, East
192 New Zealand, and Chatham Islands (suppl. Table 1). Eight regions were defined
193 in Chile (i.e., Caldera, Coquimbo, Concepción, Valdivia, Seno Reloncavi, Channel
194 of Chacao, Chiloé Island, and Continental Chiloé; Guillemin et al. 2008[19], suppl.
195 Table 1). Within each location or region, the likelihood of producing a Peruvian
196 genotype was estimated using the product of the frequency of each allele
197 characterizing the genotype (i.e., two per locus for diploid genotypes, one per
198 locus for haploid genotypes). Since only five microsatellites were available for
199 most populations from New Zealand (including Chatham Islands) these
200 calculations were done (1) using five microsatellite loci among samples from all
201 available locations (in South America and New Zealand), and (2) using six
202 microsatellite loci among samples from South America.

Second, using allele frequencies estimated above, genic differentiation between the population of Morro Sama and all the other populations sampled was calculated using the Fisher's exact probability test and 1,000 permutations, in GENEPOP v.4.2[29,30]. Genic differentiation was estimated using the five microsatellite loci available in all populations.

Third, Bayesian clustering assuming genetic admixture and correlated allele frequencies was performed in STRUCTURE v.2.3.4[31,32] with a burn-in of 50,000 and a run-length of 100,000 iterations, making 10 runs for each K from 1 to 15 and without any prior population assignment, to evaluate with which individuals of the 35 total sampling locations, the Peruvian samples were more likely to cluster with. In order to achieve this, two different datasets were consecutively run: (1) using all sampling sites and all available microsatellite markers (six loci among South American samples and two sites in New Zealand, and five loci for the remaining sites in New Zealand and Chatham Island, with the sixth locus coded as missing data), (2) using only those sampling sites with which the Peruvian samples clustered within the first data set and all six available microsatellite markers. For STRUCTURE analyses, haploids were artificially diploidized as homozygotes at every locus. The most likely number of K was determined using the Evanno method implemented in STRUCTURE HARVESTER v.0.6.94[33].

To assess ribotype diversity present in Peru, the ITS2 sequences obtained were compared to the sequences available from Chile and New Zealand (108 sequences from Chile and 93 from New Zealand, Guillemin et al. 2014[16]) using MEGA7[34].

Results

Characteristics of the Morro Sama Agarophyton chilensis population

In 2015 the Peruvian *A. chilensis* population covered only an area of about 18 m in diameter. During the field evaluation in 2017, the site was described as sheltered, without currents, turbid with 10 m visibility, depths ranging from 1-4 m, sea surface temperature of 16-17°C, pH of 7.65, 34.7 salinity, and a total *A. chilensis* population of about 100 m x 25 m consisting of isolated patches separated by approximately 1 meter between patches. The substratum was described as mostly muddy-sandy, with a couple of rocks and some boulders. The evaluation of the population further reported estimates of a 1:9 ratio of fixed to buried or entangled (loose) specimens or fragments.

None of the specimens collected in 2015 or 2017 showed presence of reproductive structures and they were categorized as vegetative samples. No fixation discs were observed, suggesting that the entire population was composed of loose thalli.

Among all 60 samples, the sex marker only amplified PCR products representative to the male marker[23]. The results of the sex markers' amplifications were coherent with the single, unique genotype obtained in Peru (see below; with no more than one allele at each microsatellite locus).

From Peru, 29 individuals collected in 2015 and 28 in 2017 were successfully genotyped at all six microsatellite loci. All 57 Peruvian samples corresponded to one single genotype: 357bp for locus Grc-8B2, 192bp for locus Grc-7F12, 318bp

for locus Grc-2B2; 201bp for locus Grc-6C7; 297bp for locus Grc-7D3 and 220bp for locus Grc-AC/CT23 (the genotype is given as allele lengths of each of the six microsatellite loci, in base-pairs (bp) following Guillemin et al. 2005)[24]. All allele lengths found in Peru had already been recorded in Chile[19,24] (Guillemin et al. 2005, 2008). Further, we found one single ribotype among all Peruvian sequenced ITS2 fragments (480 bp) generated for 22 samples collected in 2015 (corresponding to "r1", after Guillemin et al. 2014[16]).

Altogether, our results suggest the population in Peru to be an unattached, vegetative, single male's clonal population.

Possible origins of Morro Sama's male clone

The ribotype r1 corresponds to the one ubiquitous among Chilean populations and to the most common ribotype on the eastern coast of New Zealand and the Chatham Islands[16]. Therefore, based on this genetic marker it is only possible to discard the western coast of New Zealand as possible origin for the Peruvian population.

For the data set of five microsatellites, the likelihood to produce the Peruvian genotype was 0.00 in all populations and regions of New Zealand, 0.03 in the region of Coquimbo (north Chile), and 0.23 in the region of the Channel of Chacao (south Chile; see suppl. Table S1 for further details). For the complete data set of six microsatellites, the likelihood to produce de Peruvian genotype was the highest in the region of the Channel of Chacao followed by the region of Coquimbo (0.14 and 0.02, respectively; suppl. Table S1). Among the 28 Chilean

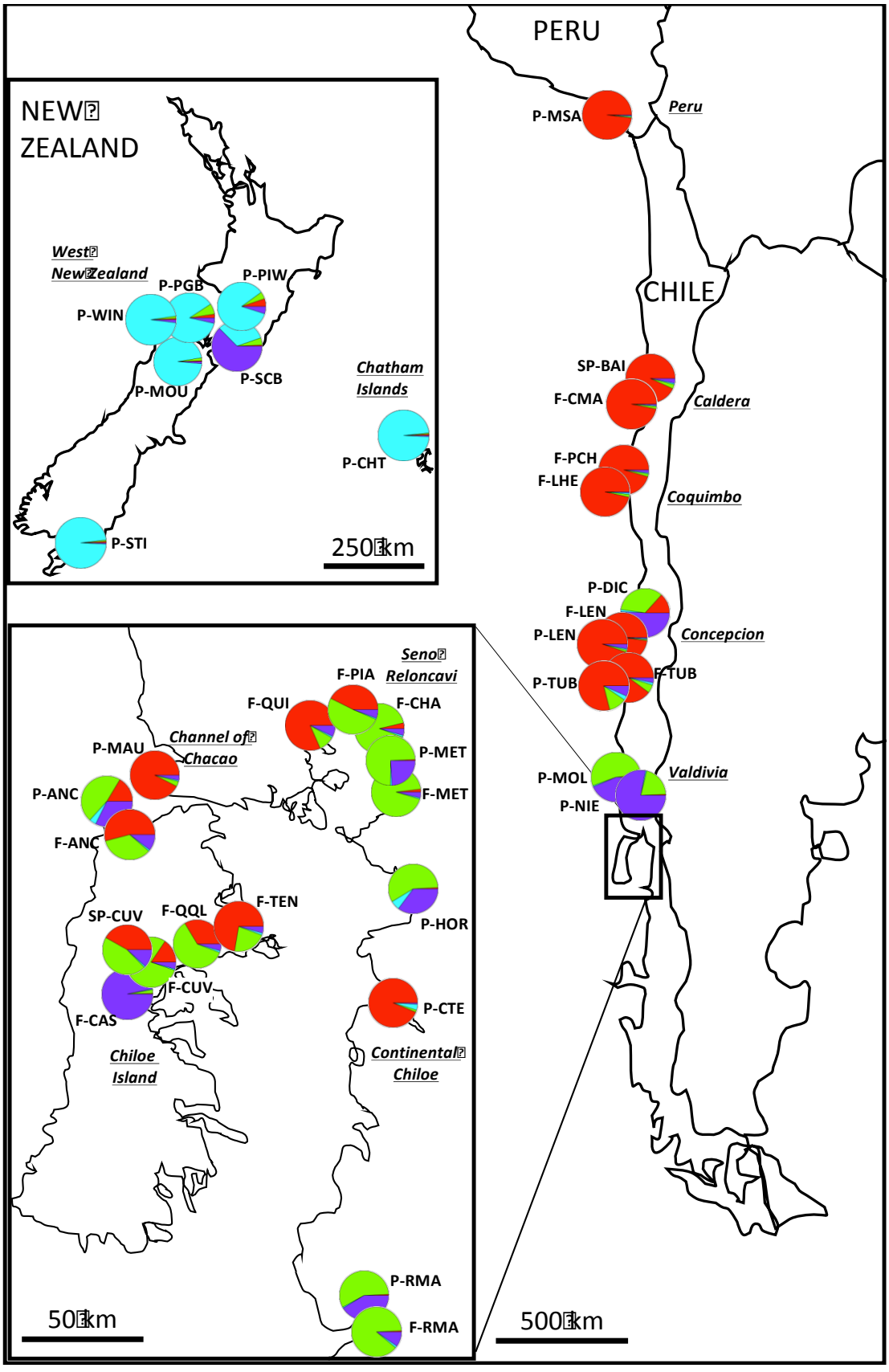
sampling locations (no grouping into regions), the most likely to produce the Peruvian genotype was the location of Chaitén (in the continental Chiloé region, p = 0.10), followed by the location of Maullín (in the Channel of Chacao region, p = 0.05). Amongst the northern locations, the most likely source of the Peruvian genotype was Playa Changas (in the region of Coquimbo, p = 0.01). Please note that our values of likelihood to produce the Peruvian genotype within a Chilean population are comparable to ones already estimated for *A. chilensis* in Chile using the same molecular markers. Indeed, the probability of a given multilocus genotype to be observed in the N samples genotyped from its own sampling site as a consequence of different events of sexual reproduction was estimated to be in between 3.3E-06 and 0.125 for diploid samples and between 4.5E-04 and 0.586 for haploid samples within our Chilean data set (see Supplementary Online Material from Guillemin et al. 2008[19]).

Genic differentiation assessed between the Peruvian population and the rest of the populations sampled were in all case highly significant (all $p<0.0001$)

Using the Evanno method in the Bayesian clustering analysis of STRUCTURE, K=2 and K=4 were suggested as the most likely total number of genetic groups in our data set, differing slightly from the results in Guillemin et al. (2014)[16] where K=5 was estimated for the whole *A. chilensis* distribution area. Results of K=2 can be found in Figure S1 and clustering results for K=4 are given for each population in Figure 2. For K = 2, the Peruvian genetic cluster (P-MSA, in red, Figure S1) also included 12 Chilean populations (SP-BAI, F-CMA, F-PCH, F-LHE, P-LEN, F-LEN, P-TUB, F-TUB, P-MAU, F-QUI, P-CTE, F-TEN). For K = 4, one group was clustering all sites from New Zealand and Chatham Islands, except P-SCB

297 (light blue in Figure 2). P-SCB shows a mixture of light blue and purple genetic
298 group. Both light green and purple genetic groups were mostly present in
299 populations located in Southern Chile (from the region of Valdivia to the region
300 of Chiloé Continental) and in P-DIC (region of Concepción). The Peruvian genetic
301 cluster (P-MSA, in red, Fig. 2) also included another 12 Chilean populations with
302 over 70% assignment to that cluster (i.e.: SP-BAI, F-CMA, F-PCH, F-LHE, F-LEN, P-
303 LEN, F-TUB, P-TUB, F-QUI, P-MAU, P-CTE, F-TEN). Among these 13 South
304 American populations (i.e., P-MSA and the 12 populations listed above), another
305 STRUCTURE analysis was performed to narrow down which populations were
306 closest to the Peruvian genotype. The Evanno method suggested 4 genetic
307 clusters among these 13 populations. Some Chilean populations highly clustered
308 with P-MSA with: 77% of the population of Chaitén (P-CTE), 38% of the
309 population of Maullín (P-MAU), and 31% of the population of Playa Changa (F-
310 PCH) assigned to the Peruvian cluster. All other populations were rather
311 assigned to one of the other 3 genetic clusters.

312


PERU
NEW ZEALAND
West New Zealand
P-PGB
P-PIW
P-WIN
P-MOU
P-SCB
Chatham Islands
P-CHT
P-STI
250 km
P-MSA
Peru
CHILE
SP-BAI
F-CMA
Caldera
F-PCH
F-LHE
Coquimbo
P-DIC
F-LEN
P-LEN
Concepcion
P-TUB
F-TUB
P-MOL
P-NIE
Valdivia
Seno Reloncavi
F-PIA
F-QUI
F-CHA
P-MET
F-MET
Channel of Chacao
P-MAU
P-ANC
F-ANC
P-HOR
SP-CUV
F-QQL
F-TEN
F-CUV
F-CAS
P-CTE
Chiloe Island
Continental Chiloe
P-RMA
F-RMA
50 km
500 km

313

Fig. 2: Genetic clustering of *Agarophyton chilensis* using six microsatellite loci. Sampling sites are coded with P and F (for natural vs. farmed populations, respectively) and a three-letter code for the population's name (following Guillemin et al. 2008, 2014, and as in suppl. Table S1). The assigned regions are given in underlined bold letters (see also suppl. Table S1). Pie charts indicate the proportion of the population's individuals belonging to a total of four (K=4) possible genetic clusters as assigned by STRUCTURE v.2.3.4[31,32]. Each of these K-clusters is coded by a different color.

Discussion

This study infers the putative origin of the newly found Peruvian population of *Agarophyton chilensis,* and describes the phase and sex ratios of this population using genetic data available for specimens across the species' entire, currently known distribution[16,18,19]. In brief, the Peruvian population was found to comprise clones of one single vegetative male of *A. chilensis*, which seemingly has been reproducing asexually through fragmentation of its thallus, forming a small, patchy population. This population has sustained itself successfully for at least four consecutive years and appears to have grown in area (2014[18]; and from this study for 2015 and 2017). While the ITS2 nuclear DNA marker could not discard New Zealand as a possible transoceanic source, based on allele frequencies of microsatellite markers, our study strongly suggests Chile as possible source of the Peruvian male, whereas all New Zealand locations were discarded. Limited dispersion of Gracilariales spores[19] suggests that the Peruvian population rather originated from a single stranded fragment

of the thallus of a male specimen than from one haploid spore. Further, all the samples analyzed during both sampling periods were vegetative indicating that the population has not been reproducing sexually in recent years. The reasons for this remain undetermined but may be due to the recent (and singular) arrival of the species to Peru or the lack of favorable environmental conditions. Similarly, in a study on *A. vermiculophyllum*, newly arrived thalli seemed to rather reproduce vegetatively through fragmentation, forming a vegetative unattached population[35].

To date, invasion and expansion of geographic ranges among *Agarophyton* have been proposed to rely on the arrival of one or a few floating tetrasporophytes, which increase the initial population density and algal biomass via vegetative reproduction through fragmentation, after which the production of a few reproductive structures by these long-standing unattached populations may lead to the production of haploid spores and the fixation of male and female gametophytes on nearby hard substrata, finally recovering the sexual life cycle of the species[16,19,35,36]. In the case of the Morro Sama population, even if the male clone developed functional reproductive structures (i.e., male gametangia), due to the absence of close-by reproductive females, the sexual life cycle cannot be restored. Indeed, in Gracilariales, the male gametes are short-lived (<6h)[37] and fecundation occurs at a few meters at most[38,39]. The nearest known locality where *A. chilensis* females have been reported is located in the region of Caldera, almost 1000 km away from Morro Sama (Guillemin et al. 2008). Additionally, most surveyed farms and/or sandy unattached populations have been described to be highly dominated by

362 vegetative tetrasporophytes (in *A. chilensis*[16,19] and in *A. vermiculophyllum*
363 [36,40]). In Chile, only one *A. chilensis* farm mostly contained female
364 gametophytes, the rest being only or mostly tetrasporophytes[19], with
365 commonly complete absence of male gametophytes. Ecological differences
366 between phases may be one explanation for the dominance of vegetative
367 tetrasporophytes in unattached populations[41–43]. For instance, in *A. chilensis*,
368 vegetative tetrasporophytes have enhanced growth when compared to
369 gametophytes, with the male's growth rate being slowest in this species[41,42].
370 Altogether, Morro Sama is a unique case among unattached, recently described
371 *Agarophyton* populations, and the first asexually reproducing population
372 dominated by male thalli ever reported.

373 *Agarophyton chilensis* is negatively buoyant, however, fragments of thalli could
374 either be transported (1) entangled on marine vessels[44,45], (2) on other
375 positively buoyant rafts like tree trunks or other large floating
376 seaweeds[10,46,47], (3) carried by birds (e.g., in *A. vermiculophyllum* by either
377 migrating birds[48], or as nesting material of local birds,[49]), or (4) attached to
378 other imported marine goods such as oysters, shells, or clams (e.g., in *A.*
379 *vermiculophyllum*[50–52]). In terms of geographic distance and likelihood of
380 drift through natural currents or anthropogenically induced transport, northern
381 Chilean populations could be regarded as the more likely source for the arrival of
382 male propagules in southern Peru. Morro Sama is located directly on the path of
383 the Humboldt Current that flows north from 42°S (i.e., North of the Chiloé
384 Island)[9] and is found only 600 km away from Bahia Mejillones, the northern-
385 most point where *A. chilensis* was transported to be cultivated in Chile. The farm

of Bahia Mejillones seems to have disappeared nowadays due to poor management choices (Wilkomirsky, personal communication) but Morro Sama is still located only 980 km north of the region of Caldera (27°S) and 1,170 km north of the region of Coquimbo (29°S), the oldest established farm in northern Chile[15]. However, at its northern distribution in Chile, *A. chilensis* populations are farms mostly or even entirely composed of vegetative tetrasporophytes[19] and our genetic investigation did not refer to a northern population as the most likely source of the Peruvian male. Nonetheless, even if barely reproductive, farmed tetrasporophytes along Chile's northern coasts are still able to produce haploid gametophytes via sexual reproduction every once in a while and these spores can attach and grow on pebbles and shells scattered close to the farms (few fixed males recorded up to 27°S, SP-BAI, region of Caldera[19]). Thus, it is still possible that the male thallus fragment from Morro Sama originated from a rare male specimen in northern Chile in the Coquimbo or the Caldera region. Our genetic data, however, suggest the region of the Channel of Chacao to be the most likely source of the Peruvian genotype (see our results from STRUCTURE; Channel of Chacao is located more than 2,600km south of Morro Sama). In the region of the Channel of Chacao and around the Island of Chiloé, large natural populations of *A. chilensis* are established at the opening of estuaries and rivers growing from spores and fixed on small pebbles, shells, or in rock pools. *Agarophyton* thalli seem highly resistant and resilient to physiological stress and are able to survive long trips while drifting[16,40,50]. Supporting this idea, ecophysiological studies have demonstrated that *Agarophyton* thalli have the ability to withstand a wide range of physico-chemical conditions (in *A. chilensis*[53]; in *A. vermiculophyllum*[48,54]). Furthermore, it has been shown

411 that rapid evolution can occur during range expansion by sieving advantageous
412 genotypes during transport, which for example allows for successful
413 establishment in new environments[55]. In *A. vermiculophyllum*, adaptation to
414 new environmental conditions during colonization has been proposed due to
415 higher tolerance to extreme heat, cold, or low-salinity[56] observed in non-
416 native populations compared to native ones. Hence, the mere distance between
417 Channel of Chacao and Morro Sama, and their contrasting latitudinal position
418 (i.e., the two locations are separated by 25°) does not represent a sufficient
419 impediment as to reject South Chile as a possible source of the Peruvian clone.
420 Indeed, *A. chilensis* has already undertaken and survived the much longer
421 journey from New Zealand to Chile and was able to colonize most of Chile's
422 coast[16] and long-distance dispersal has been suggested as an important factor
423 for the distribution of marine macrophytes in the southern Pacific Ocean[13].

424 Regardless of the exact Chilean source population, natural colonization by rafting
425 thalli was possibly fostered by the strong northward-flowing Humboldt
426 Current[9,10]. Yet introduction linked to anthropogenic activities is not to be
427 discarded, especially since the traffic of boats from Morro Sama has been
428 reported in the northern parts of Chile[57–59]; and the Peruvian population of *A.*
429 *chilensis* is actually found right at the landing pier for artisanal fisheries
430 (Desembarcadero de Pesca Artesanal, DPA) of the Grau Port (Sama District,
431 Tacna Province), a very small port built in 1995, with the capacity of mooring up
432 to 15 artisanal fishing boats, which daily provide a wide variety of marine
433 products.

Despite the high numbers of samples and locations included in this study, the source of the Peruvian male thallus still remains elusive, especially because of the anthropogenic farming activities in Chile[19]. Indeed, fresh thalli are commonly exchanged between fishermen's and southern populations were used to seed the artificially introduced farms in the North[19], potentially blurring accurate genetic signature for single geographic sites/regions._*Agarophyton chilensis*, because of its economic interest, is relatively well known in Chile where most of the natural and farmed populations have been mapped. However, some recognized populations (unattached in the seawater lagoon of Puerto Saavedra, region of Araucanía, 38°S; attached on rocks at Punta Curaumilla or buried in sand in Quintero, region of Valparaíso, 33°S and 32°S, respectively) have not yet been genotyped. It could be interesting to add these populations to the Chilean data set in order to better infer the origin of the Moro Sama population. Potentially, the utilization of next generation sequencing techniques and the acquirement of much higher numbers of genetic markers, such as SNPs, for population genetics may give a better resolution of the colonization pathways and sources of *Agarophyton* populations. Until then, the Peruvian *A. chilensis* population does not seem to be an ecological threat nor is the species categorized as invasive but it will be interesting to keep on monitoring the further development and proliferation of this isolated and unique clonal male.

References

[1] E. Rothaeusler, I. Gomez, I.A. Hinojosa, U. Karsten, L. Miranda, F. Tala, M. Thiel, Kelp rafts in the Humboldt Current: Interplay of abiotic and biotic

458 factors limit their floating persistence and dispersal potential, Limnol.
459 Oceanogr. 56 (2011) 1751–1763. doi:10.4319/lo.2011.56.5.1751.

460 [2] T.A. Norton, Dispersal by macroalgae, Br. Phycol. J. 27 (1992) 293–301.
461 doi:10.1080/00071619200650271.

462 [3] B. Gaylord, D.C. Reed, P.T. Raimondi, L. Washburn, S.R. McLean, A
463 physically based model of macroalgal spore dispersal in the wave and
464 current-dominated nearshore, Ecology 83 (2002) 1239–1251.
465 doi:10.2307/3071939.

466 [4] A. Ingólfsson, Floating clumps of seaweed around Iceland: natural
467 microcosms and a means of dispersal for shore fauna, Mar. Biol. 122
468 (1995) 13–21. doi:10.1007/BF00349273.

469 [5] J.P. Wares, C.W. Cunningham, Phylogeography and historical ecology of the
470 North Atlantic intertidal, Evolution 55 (2001) 2455–2469.
471 www.sci.qut.edu.au/NRS/mantel.htm.

472 [6] M. Thiel, L. Gutow, The ecology of rafting in the marine environment: II.
473 The rafting organisms and community, Oceanogr. Mar. Biol. 43 (2005)
474 279–418. doi:10.1201/9781420037449.ch7.

475 [7] J.F. Muhlin, C.R. Engel, R. Stessel, R.A. Weatherbee, S.H. Brawley, The
476 influence of coastal topography, circulation patterns, and rafting in
477 structuring populations of an intertidal alga, Mol. Ecol. 17 (2008) 1198–
478 1210. doi:10.1111/j.1365-294X.2007.03624.x.

479 [8] C.I. Fraser, G.C. Zuccarello, H.G. Spencer, L.C. Salvatore, G.R. Garcia, J.M.

Waters, Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the southern hemisphere, PLoS One 8 (2013) 1–12. doi:10.1371/journal.pone.0069138.

[9] M. Thiel, E. Macaya, E. Acuña, The Humboldt Current system of northern-central Chile: oceanographic processes, ecological interactions and socioeconomic feedback, Oceanogr. Mar. Biol. 45 (2007) 195–344. doi:Book_Doi 10.1201/9781420050943.

[10] E.C. Macaya, B. López, F. Tala, F. Tellier, M. Thiel, Float and raft: role of buoyant seaweeds in the phylogeography and genetic structure of non-buoyant associated flora, in: Z. Hu, C. Fraser (Eds.), Seaweed Phylogeography, Springer Netherlands, Dordrecht, 2016: pp. 97–130. doi:10.1007/978-94-017-7534-2.

[11] S.L. Williams, J.E. Smith, A global review of the distribution, taxonomy, and impacts of introduced seaweeds, Annu. Rev. Ecol. Evol. Syst. 38 (2007) 327–359. doi:10.1146/annurev.ecolsys.38.091206.095543.

[12] J. Neiva, E.T.A. Serrão, J. Assis, G.A. Pearson, J.A. Coyer, J.L. Olsen, G. Hoarau, M. Valero, Climate oscillations, range shifts and phylogeographic patterns of North Atlantic Fucaceae, in: Z.M. Hu, C.I. Fraser (Eds.), Seaweed Phylogeography, Springer Netherlands, Dordrecht, 2016: pp. 279–308. doi:10.1007/978-94-017-7534-2.

[13] T.M. Smith, P.H. York, B.R. Broitman, M. Thiel, G.C. Hays, E. Van Sebille, N.F. Putman, P.I. Macreadie, C.D.H. Sherman, Rare long-distance dispersal of a marine angiosperm across the Pacific Ocean, Glob. Ecol. Biogeogr. (2018)

487–496. doi:10.1111/geb.12713.

[14] C.F.D. Gurgel, J.N. Norris, W.E. Schmidt, H.N. Le, S. Fredericq, Systematics of the Gracilariales (Rhodophyta) including new subfamilies, tribes, subgenera, and two new genera, *Agarophyton gen. nov.* and *Crassa gen. nov.*, Phytotaxa 374 (2018) 1–23.

[15] C.J. Bird, J. McLachlan, E.C. de Oliveira, *Gracilaria chilensis* sp. nov. (Rhodophyta, Gigartinales), from the Pacific South America, Can. J. Bot. 64 (1986) 2928–2934. doi:10.1139/b86-387.

[16] M.L. Guillemin, M. Valero, S. Faugeron, W. Nelson, C. Destombe, Tracing the trans-pacific evolutionary history of a domesticated seaweed (*Gracilaria chilensis*) with archaeological and genetic data, PLoS One 9 (2014) 1–17. doi:10.1371/journal.pone.0114039.

[17] S. Cohen, S. Faugeron, E.A. Martínez, J.A. Correa, F. Viard, C. Destombe, M. Valero, Molecular identification of two sibling species under the name *Gracilaria chilensis* (Rhodophyta, Gracilariales), J. Phycol. 40 (2004) 742–747. doi:10.1111/j.1529-8817.2004.03203.x.

[18] N. Arakaki, W.E. Schmidt, P. Carbajal, S. Fredericq, First occurrence of *Gracilaria chilensis*, and distribution of *Gracilariopsis lemaneiformis (Gracilariaceae, Gracilariales)* in Peru on the basis of *rbc*L sequence analysis, Phytotaxa 208 (2015) 175–181.

[19] M.L. Guillemin, S. Faugeron, C. Destombe, F. Viard, J.A. Correa, M. Valero, Genetic variation in wild and cultivated populations of the haploid-diploid red alga Gracilaria chilensis: how farming practices favor asexual

526 reproduction and heterozygosity, Evolution 62 (2008) 1500–1519.
527 doi:10.1111/j.1558-5646.2008.00373.x.

528 [20] B. Santelices, R. Ugarte, Ecological differences among Chilean populations
529 of commercial *Gracilaria*, J. Appl. Phycol. 2 (1990) 17–26.
530 doi:10.1007/BF02179765.

531 [21] A.H. Buschmann, M.D.C. Hernández-González, G. Aroca, A. Gutierrez,
532 Seaweed farming in Chile: a review, Aquaculture 194.3-4 (2001) 203-220.

533 [22] A.H. Buschmann, F. Briganti, C.A. Retamales, Intertidal cultivation of
534 *Gracilaria chilensis* (Rhodophyta) in southern Chile: long term invertebrate
535 abundance patterns, Aquaculture 156 (1997) 269–278.
536 doi:10.1016/S0044-8486(97)00132-4.

537 [23] M.L. Guillemin, O.R. Huanel, E.A. Martínez, Characterization of genetic
538 markers linked to sex determination in the haploid-diploid red alga
539 *Gracilaria chilensis*, J. Phycol. 48 (2012) 365–372. doi:10.1111/j.1529-
540 8817.2012.01116.x.

541 [24] M.L. Guillemin, C. Destombe, S. Faugeron, J.A. Correa, M. Valero,
542 Development of microsatellites DNA markers in the cultivated seaweed,
543 *Gracilaria chilensis* (Gracilariales, Rhodophyta), Mol. Ecol. Notes. 5 (2005)
544 155–157. doi:10.1111/j.1471-8286.2005.00867.x.

545 [25] C. McCarthy, Chromas, Sch. Biomol. Biomed. Sci. Fac. Sci. Technol. Griffith
546 Univesity Brisbane, Technelysium Pty Ltd, Aust. (1997).
547 http://trishul.sci.gu.edu.au/~conor/chromas.htm/.

548 [26] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and
549 analysis program for Windows 95/98/NT, Nucleic Acids Symp. Ser. 41
550 (1999) 95–98. doi:citeulike-article-id:691774.

551 [27] M. Tibayrenc, F. Kjellberg, F.J. Ayala, A clonal theory of parasitic protozoa:
552 The population structures of *Entamoeba, Giardia, Leishmania, Naegleria,*
553 *Trichomonas,* and *Trypanosoma* and their medical and taxonomical
554 consequences, Proc. Natl. Acad. Sci. 87 (1990) 2414–2418.

555 [28] P.G. Meirmans, P.H. Van Tienderen, GENOTYPE and GENODIVE: Two
556 programs for the analysis of genetic diversity of asexual organisms, Mol.
557 Ecol. Notes. 4 (2004) 792–794. doi:10.1111/j.1471-8286.2004.00770.x.

558 [29] M. Raymond, F. Rousset, GENEPOP (version 1.2): population genetics
559 software for exact tests and ecumenicism, J. Hered. 86 (1995) 248–249.
560 http://jhered.oxfordjournals.org/content/86/3/248.short (accessed
561 August 10, 2014).

562 [30] F. Rousset, GENEPOP'007: A complete re-implementation of the GENEPOP
563 software for Windows and Linux, Mol. Ecol. Resour. 8 (2008) 103–106.
564 doi:10.1111/j.1471-8286.2007.01931.x.

565 [31] J.K. Pritchard, M. Stephens, P. Donnelly, Inference of population structure
566 using multilocus genotype data, Genetics 155 (2000) 945–959.

567 [32] D. Falush, M. Stephens, J.K. Pritchard, Inference of population structure
568 using multilocus genotype data: linked loci and correlated allele
569 frequencies, Genetics 164 (2003) 1567–1587.

570 [33] D.A. Earl, B.M. VonHoldt, STRUCTURE HARVESTER : a website and
571 program for visualizing STRUCTURE output and implementing the Evanno
572 method, Conserv. Genet. Resour. 4 (2012) 359–361. doi:10.1007/s12686-
573 011-9548-7.

574 [34] S. Kumar, G. Stecher, K. Tamura, MEGA7: Molecular Evolutionary Genetics
575 Analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–
576 1874. doi:10.1093/molbev/msw054.

577 [35] S.A. Krueger-Hadfield, G.H. Carmona, R. Terada, J.M. López-Vivas, R.
578 Riosmena-Rodríguez, New record of the non-native seaweed *Gracilaria*
579 *parvispora* in Baja California - A note on Vergara-Rodarte *et al.* (2016),
580 Cryptogam. Algol. 37 (2016) 257–263.
581 doi:10.7872/crya/v37.iss4.2016.257.

582 [36] S.A. Krueger-Hadfield, C.L. Magill, F.S.P.D. Bunker, N. Mieszkowska, E.E.
583 Sotka, C.A. Maggs, When invaders go unnoticed: the case of *Gracilaria*
584 *vermiculophylla* in the British Isles, Cryptogam. Algol. 38 (2017) 379–400.
585 doi:10.7872/crya/v38.iss4.2017.379.

586 [37] C. Destombe, J. Godin, J.M. Remy, Viability and dissemination of spermatia
587 of *Gracilaria verrucosa* (Gracilariales, Rhodophyta), Hydrobiologia
588 204/205 (1990) 219–223.

589 [38] C.R. Engel, M. Valero, Y. Lagadeuc, C. Destombe, Non-random mating in
590 controlled multiple-donor crosses in *Gracilaria gracilis* (Gracilariaceae,
591 Rhodophyta), Eur. J. Phycol. 37 (2002) 179–190.
592 doi:10.1017/S096702620200358X.

593 [39] C.R. Engel, C. Destombe, M. Valero, Mating system and gene flow in the red
594 seaweed Gracilaria gracilis: Effect of haploid-diploid life history and
595 intertidal rocky shore landscape on fine-scale genetic structure, Heredity
596 92 (2004) 289–298. doi:10.1038/sj.hdy.6800407.

597 [40] S.A. Krueger-Hadfield, N.M. Kollars, J.E. Byers, T.W. Greig, M. Hammann,
598 D.C. Murray, C.J. Murren, A.E. Strand, R. Terada, F. Weinberger, E.E. Sotka,
599 Invasion of novel habitats uncouples haplo-diplontic life cycles, Mol. Ecol.
600 25 (2016) 3801–3816. doi:10.1111/mec.13718.

601 [41] M.L. Guillemin, R.D. Sepúlveda, J.A. Correa, C. Destombe, Differential
602 ecological responses to environmental stress in the life history phases of
603 the isomorphic red alga *Gracilaria chilensis* (Rhodophyta), J. Appl. Phycol.
604 25 (2013) 215–224. doi:10.1007/s10811-012-9855-8.

605 [42] M.L. Guillemin, P. Valenzuela, J.D. Gaitán-Espitia, C. Destombe, Evidence of
606 reproductive cost in the triphasic life history of the red alga *Gracilaria*
607 *chilensis* (Gracilariales, Rhodophyta), J. Appl. Phycol. 26 (2014) 569–575.
608 doi:10.1007/s10811-013-0072-x.

609 [43] L.E. Lees, S.A. Krueger-Hadfield, A.J. Clark, E.A. Duermit, E.E. Sotka, C.J.
610 Murren, Nonnative *Gracilaria vermiculophylla* tetrasporophytes are more
611 difficult to debranch and are less nutritious than gametophytes, J. Phycol.
612 54 (2018) 471–482. doi:10.1111/jpy.12746.

613 [44] M.S. Thomsen, T. Wernberg, P. Stæhr, D. Krause-Jensen, N. Risgaard-
614 Petersen, B.R. Silliman, Alien macroalgae in Denmark – a broad-scale
615 national perspective, Mar. Biol. Res. 3 (2007) 61–72.

616 doi:10.1080/17451000701213413.

617 [45] J.M. Ruiz, L. Marín-Guirao, J. Bernardeau-Esteller, A. Ramos-Segura, R.
618 García-Muñoz, J.M. Sandoval-Gil, Spread of the invasive alga *Caulerpa*
619 *racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) along the
620 Mediterranean coast of the Murcia region (SE Spain), Anim. Biodivers.
621 Conserv. 34 (2011) 73–82.

622 [46] C.I. Fraser, R. Nikula, J.M. Waters, Oceanic rafting by a coastal community,
623 Proc. R. Soc. B Biol. Sci. 278 (2011) 649–655. doi:10.1098/rspb.2010.1117.

624 [47] C.I. Fraser, J.M. Waters, Algal parasite *Herpodiscus durvillaeae*
625 (Phaeophyceae: Sphacelariales) inferred to have traversed the Pacific
626 Ocean with its buoyant host, J. Phycol. 49 (2013) 202–206.
627 doi:10.1111/jpy.12017.

628 [48] C.D. Nyberg, I. Wallentinus, Long-term survival of an introduced red alga in
629 adverse conditions, Mar. Biol. Res. 5 (2009) 304–308.
630 doi:10.1080/17451000802428159.

631 [49] A.F. Arnold, The sea-beach at ebb tide: A guide to the study of the
632 seaweeds and the lower animal life found between tide-marks, The
633 Century Co., New York, 1901. doi:https://doi.org/10.5962/bhl.title.46210.

634 [50] S.A. Krueger-Hadfield, N.M. Kollars, A.E. Strand, J.E. Byers, S.J. Shainker, R.
635 Terada, T.W. Greig, M. Hammann, D.C. Murray, F. Weinberger, E.E. Sotka,
636 Genetic identification of source and likely vector of a widespread marine
637 invader, Ecol. Evol. 7 (2017) 4432–4447. doi:10.1002/ece3.3001.

638 [51] A. Sfriso, S. Maistro, C. Andreoli, I. Moro, First record of *Gracilaria*
639 *vermiculophylla* (Gracilariales, Rhodophyta) in the Po Delta lagoons,
640 Mediterranean Sea (Italy), J. Phycol. 46 (2010) 1024–1027.
641 doi:10.1111/j.1529-8817.2010.00893.x.

642 [52] M.S. Thomsen, C.F.D. Gurgel, S. Fredericq, K.J. McGlathery, *Gracilaria*
643 *vermiculophylla* (Rhodophyta, Gracilariales) in Hog Island Bay, Virginia: A
644 cryptic alien and invasive macroalga and taxonomic correction, J. Phycol.
645 42 (2006) 139–141. doi:10.1111/j.1529-8817.2006.00160.x.

646 [53] I. Gómez, F.L. Figueroa, P. Huovinen, N. Ulloa, V. Morales, Photosynthesis of
647 the red alga *Gracilaria chilensis* under natural solar radiation in an estuary
648 in southern Chile, Aquaculture 244 (2005) 369–382.
649 doi:10.1016/j.aquaculture.2004.11.037.

650 [54] D.A. Lyons, R.E. Scheibling, Range expansion by invasive marine algae:
651 rates and patterns of spread at a regional scale, Divers. Distrib. 15 (2009)
652 762–775. doi:10.1111/j.1472-4642.2009.00580.x.

653 [55] C.E. Lee, Evolutionary genetics of invasive species, Trends Ecol. Evol. 17
654 (2002) 386–391. doi:https://doi.org/10.1016/S0169-5347(02)02554-5.

655 [56] E.E. Sotka, A.W. Baumgardner, P.M. Bippus, C. Destombe, E.A. Duermit, H.
656 Endo, B.A. Flanagan, M. Kamiya, L.E. Lees, C.J. Murren, M. Nakaoka, S.J.
657 Shainker, A.E. Strand, R. Terada, M. Valero, F. Weinberger, S.A. Krueger-
658 Hadfield, Combining niche shift and population genetic analyses predicts
659 rapid phenotypic evolution during invasion, Evol. Appl. 11 (2018) 781–
660 793. doi:10.1111/eva.12592.

[57] S. Emol, Detenidas 6 naves peruanas en aguas chilenas, El Mercur. Online. (2000). http://www.emol.com/noticias/nacional/2000/06/25/24486/detenidas-6-naves-peruanas-en-aguas-chilenas.html (accessed September 5, 2018).

[58] M. Redacción, Ahora son cinco las embarcaciones retenidas por la armada chilena, D. Correo. (2015). https://diariocorreo.pe/edicion/tacna/ahora-son-cinco-las-embarcaciones-retenidas-por-la-armada-chilena-560190/ (accessed September 5, 2018).

[59] Aqua, Armada: Crece ingreso ilegal de embarcaciones pesqueras peruanas, AQUA - Acuacultura y Pesca (2012). http://www.aqua.cl/2012/04/03/armada-crece-ingreso-ilegal-de-embarcaciones-pesqueras-peruanas/ (accessed September 5, 2018).

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Author Contributions Statement

V.R. did data analysis, prepared the figure and table; V.R. and M-L.G. wrote the main manuscript text; S.M. generated molecular data; J.C.Z.R and N.A. collected and analyzed samples as well as site descriptive information. All authors critically reviewed and approved the manuscript's final version prior submission.

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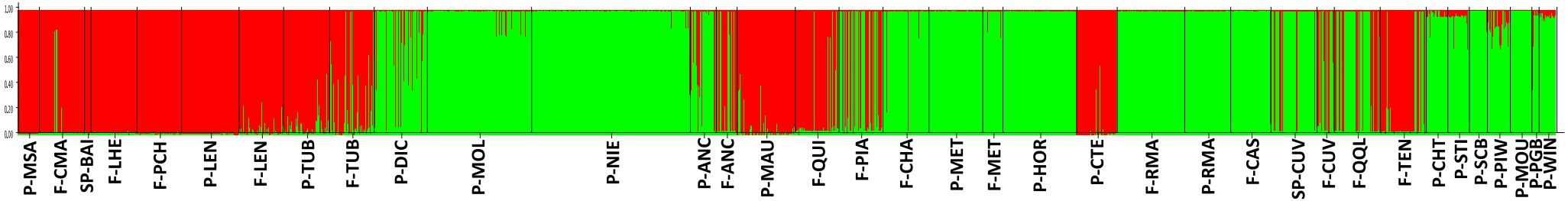
Supplementary Information

Suppl. Table S1: Details on the sampled populations (Natural or Spontaneous vs. Farms (P, SP, or F, respectively)) and probabilities P to generate the Peruvian genotype among each population based on allele frequency analyses of microsatellite data (six vs. five microsatellites (MSATS)). Probabilities to generate the Peruvian genotype among each region are given in bold between brackets. N is the Number of genotyped individuals.

Country	Region	Name	Population	Geoposition	N	P [6 MSATS]	P [5 MSATS]
Perú	Perú	*Morro Sama*	P-MSA	17° 59' S, 70° 52' W	29	1.00 ***(1.00)***	1.00 ***(1.00)***
Chile	Caldera	*Bahía Inglesa*	SP-BAI	27° 09′ S, 70° 53′ W	9	0.003 ***(0.00)***	0.003 ***(0.00)***
		Cultivos Marinos	F-CMA	27° 04′ S, 70° 50′ W	63	0.000	0.000
	Coquimbo	*Playa Changa*	F-PCH	29° 57′ S, 71° 20′ W	62	0.011 ***(0.002)***	0.016 ***(0.003)***
		La Herradura	F-LHE	29° 58′ S, 71° 21′ W	64	0.000	0.000
	Concepción	*Dichato*	P-DIC	36° 32′ S, 72° 56′ W	74	0.000 ***(0.002)***	0.000 ***(0.002)***
		Lenga	P-LEN	36° 45′ S, 73° 11′ W	80	0.002	0.002
			F-LEN	36° 45′ S, 73° 11′ W	62	0.000	0.000
		Tubul	P-TUB	37° 15′ S, 73° 26′ W	64	0.007	0.008
			F-TUB	37° 15′ S, 73° 26′ W	62	0.002	0.002
	Valdivia	*Los Molinos*	P-MOL	39° 50′ S, 73° 23′ W	145	0.000 ***(0.000)***	0.000 ***(0.000)***
		Niebla	P-NIE	39° 52′ S, 73° 23′ W	221	0.000	0.000
	Channel of Chacao	*Maullín*	P-MAU	41° 37′ S, 73° 35′ W	81	0.053 ***(0.014)***	0.064 ***(0.023)***
		Ancud	P-ANC	41° 52′ S, 73° 48′ W	36	0.000	0.000
			F-ANC	41° 52′ S, 73° 48′ W	29	0.004	0.007
	Seno Reloncavi	*Quillape*	F-QUI	41° 32′ S, 72° 44′ W	61	0.013 ***(0.000)***	0.017 ***(0.000)***
		Piedra Azul	F-PIA	41° 30′ S, 72° 47′ W	61	0.000	0.000
		Chaica	F-CHA	41° 38′ S, 72° 39′ W	64	0.000	0.000
		Metri	P-MET	41° 36′ S, 72° 42′ W	75	0.000	0.000
			F-MET	41° 36′ S, 72° 42′ W	28	0.000	0.000

	Chiloé Island	*Tenaún*	F-TEN	42° 20′ S, 73° 23′ W	64	0.009 ***(0.000)***	0.020 ***(0.001)***
		Quiquel	F-QQL	42° 21′ S, 73° 35′ W	64	0.001	0.001
		Curaco de Veléz	SP-CUV	42° 26′ S, 73° 36′ W	24	0.001	0.002
			F-CUV	42° 26′ S, 73° 36′ W	64	0.000	0.000
		Castro	F-CAS	42° 30′ S, 73° 47′ W	56	0.000	0.000
	Continental Chiloé	*Hornopiren*	P-HOR	41° 58′ S, 72° 28′ W	103	0.000 ***(0.000)***	0.000 ***(0.000)***
		Chaitén	P-CTE	42° 55′ S, 72° 42′ W	56	0.102	0.104
		Raul Marín	P-RMA	43° 46′ S, 72° 57′ W	94	0.000	0.000
			F-RMA	43° 46′ S, 72° 57′ W	64	0.000	0.000
New Zealand	Chatham Islands	*Chatham Islands*	P-CHT	43° 52′ S, 176° 33′ W	30	_ ***(_)***	0.000 ***(0.000)***
	East New Zealand	*Stewart Islands*	P-STI	46° 54′ S, 168° 07′ E	30	_ ***(0.000)***	0.000 ***(0.000)***
		Pautahanui Inlet	P-PIW	41° 06′ S, 174° 49′ E	32	0.000	0.000
		Scorching Bay	P-SCB	41° 18′ S, 174° 48′ E	25	0.000	0.000
	West New Zealand	*Moutere Inlet*	P-MOU	40° 59′ S, 173° 01′ E	30	_ ***(_)***	0.000 ***(0.000)***
		Whanganui Inlet	P-WIN	40° 34′ S, 172° 32′ E	25	_	0.000
		Golden Bay	P-PGB	40° 48′ S, 172° 48′ E	10	_	0.000



Suppl. Figure S1: STRUCTURE bar plot from 36 collection sites of Agarophyton chilensis (complete trans-Pacific distribution) based on five polymorphic microsatellite markers. Posterior probability of assignment of A. chilensis samples to one of two (K = 2) genotype clusters are shown in the bar plot by the colors green and red, generated using a Bayesian clustering analysis. Black lines divide the collection sites, which are coded as in Suppl. Table S1.