“More people are comfortable with old problems than with new solutions.”

—Charles Brower

Impacts of the Kelp Conveyor Hypothesis on Population Genetics of Subtidal Red Algae (Rhodophyta)

by

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ABSTRACT

Endemic species of Haida Gwaii are commonly attributed to populations persisting in glacial refugia; however, kelp rafting has been recently proposed to explain the disjunct distributions of macroalgal species previously considered endemic to Haida Gwaii. Using mtDNA from five predominantly subtidal species and three predominantly intertidal species of red algae, this study tested the hypothesis that disjunct distributions between Haida Gwaii and California are caused by kelp rafting of subtidal, non-buoyant species. In support of this hypothesis, genetic connectivity of populations in California and Haida Gwaii was greater in subtidal species than intertidal species and allele frequencies suggest direct migration of subtidal species from Haida Gwaii to California. Interestingly, unique Haida Gwaii alleles in subtidal and intertidal species indicate long residence times for some populations, suggesting glacial refugia and kelp rafting may be jointly responsible for disjunct distributions observed at the population level for macroalgae along this coast.

DEDICATION

To my friends, for all the support, laughter, and fond memories you've given me.

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Introduction

Seaweed biogeography is rooted in the concept of *isolation-by-distance* (IBD) (Wright 1943), which describes a positive correlation between genetic and geographic distance. Wright's IBD concept was later applied to the *stepping stone* model (Kimura 1964), which has since been used to explain population structure in numerous macroalgal studies (Coleman and Brawley 2005; Bouza *et al.* 2006; Alberto *et al.* 2011). The model assumes that, within a single generation, populations exchange migrants only with nearby populations. Consequently, gene flow between distant populations occurs in a stepwise process over multiple generations, resulting in a positive correlation between geographic and genetic distance (IBD). This stepping stone pattern is reflected well by typical spore dispersal of seaweeds; spores are short-lived (4 – 11 days) (Santelices 1990), subject to local currents and upwelling (Kumagai *et al.* 2018; De Jode *et al.* 2019) and disperse at most several kilometers before settling (Gaylord *et al.* 2002). Evidently, population structure of macroalgae adheres well to the stepping stone model in theory and practice; in a meta-analysis of IBD in seaweed populations, Durrant *et al.* (2013) found that IBD significantly explained population structure in all 49 studies examined.

While short range spore dispersal is common, buoyant macroalgae are notorious for regularly drifting long distances and causing persistent, high gene flow between geographically distant populations (Van den Hoek 1987; Macaya 2016). The ability for the kelp species *Nereocystis luetkeana* (K.Mertens) Postels & Ruprecht and *Macrocystis pyrifera* (Linnaeus) C.Agardh to form "kelp rafts" is particularly well-studied; *M.*

pyrifera in particular is known to form rafts in excess of 3m in diameter and predicted to drift upwards of 1,000km (Hobday 2000a, Hernández-Carmona 2006). Kelp rafts are responsible not only for long-distance migration in the kelp species themselves but are also known to raft rich communities of marine flora and fauna as "passengers" (Hobday 2000b; Fraser *et al.* 2011). For those passenger species with limited dispersal (e.g. non-buoyant species), kelp rafting can result in unexpected connectivity between geographically distant populations (Nikula *et al.* 2013).

On the archipelago of Haida Gwaii in northern British Columbia (BC), Canada, glacial refugia hypotheses are often invoked to explain the high frequency of terrestrial and marine endemic species (Calder and Taylor 1968; Warner *et al.* 1982). The existence of at least one specific refugium through the height of the Fraser glaciation (19,000 – 17,000 years ago) is supported (Mathewes and Clague 2017) at which time populations were completely isolated from those outside the refugium. However, while genetic patterns of terrestrial species match refugial hypotheses (Calder and Taylor 1968; Ramsey *et al.* 2004), Saunders (2014) demonstrated evidence against the refugial hypothesis in the two macroalgal genera *Callophyllis* Kützing and *Mastocarpus* Kützing. In that study, the author found that haplotypes common to California and northern BC (including Haida Gwaii, Prince Rupert and Tahsis) were more frequent in the predominantly subtidal *Callophyllis* (10%) than the predominantly intertidal *Mastocarpus* (1.8%), despite relatively even sampling of both genera. To explain the disproportionate occurrence of disjunct alleles in the subtidal *Callophyllis,* the *kelp conveyor* hypothesis was proposed, which was supported by other evidence most

notably predominantly disjunct distributions between California and northern British Columbia for 33 seaweed species (Saunders 2014).

The kelp conveyor hypothesis posits that buoyant kelp raft from California and transport non-buoyant, subtidal macroalgae to Haida Gwaii, thus facilitating long-distance gene flow in otherwise poorly dispersing species. Kelp rafts, likely formed of *M. pyrifera* and *N. luetkeana*, are dislodged in large quantities in California as a result of high wave action in winter storms (Ebeling *et al.* 1985; Seymour *et al.* 1989). These winter storms coincide with the nearshore Davidson Current, a northbound system flowing from California to Alaska from November to February (Schwartzlose and Reid 1972). Reaching speeds of 0.5m/s, Merilees (2008) predicted that kelp rafts travelling on the Davidson Current could reach Haida Gwaii from southern Oregon in six to eight weeks—considerably less time than the predicted maximum lifespan of *M. pyrifera* rafts (~14 weeks) (Hobday 2000c). The kelp conveyor is predicted to predominantly affect low intertidal and subtidal species rather than mid- to high-intertidal species owing to the habit of putative rafter species *M. pyrifera* and *N. luetkeana* to attach in the subtidal (Bushing 1994). Should the kelp conveyor hold true, genetic connectivity of populations in Haida Gwaii and California is expected to be significantly higher in subtidal species than intertidal species, but similar between other population pairs not predicted to be affected by the kelp conveyor. Moreover, the direct gene flow from California to Haida Gwaii is expected to increase allelic richness in subtidal Haida Gwaii species relative to intertidal species and result in disjunct alleles (present in California and Haida Gwaii) in subtidal species. As intertidal species are predicted to be largely unaffected by the kelp

conveyor, California populations of intertidal species are expected to be highly differentiated from Haida Gwaii populations, as predicted by the IBD model.

Interestingly, many predictions of the kelp conveyor hypothesis are similar to predictions posed by previous hypotheses of glacial refugia on Haida Gwaii, complicating tests of these two competing hypotheses. Kelp rafting of passenger species with poor dispersal (i.e. non-buoyant species) can lead to highly similar disjunct populations across large geographic ranges (Fraser *et al.* 2013), a phenomenon which is often also attributed to glacial refugia (Hoarau *et al.* 2007, Guillemin *et al.* 2018). Krueger-Hadfield *et al.* (2011) reported *Chondrus crispus* Stackhouse had strong population structure at fine geographic scales (<100m), demonstrating that, for this non-buoyant species, dispersal is limited and IBD predictions hold at small scales. Interestingly however, Provan *et al.* (2013) failed to find evidence of population structure in *C. crispus* at very large geographic scales (>200km), suggesting that forces other than IBD (such as kelp rafting or refugial effects) affect this species' range-wide demographics. Distinguishing between kelp rafting and refugial effects in geographically distant, genetically similar (but not identical) populations requires an examination of how these populations differ; populations separated by glacial refugia will be actively diverging and, as a result, are likely to contain private alleles unique to each population. Conversely, gene flow (mediated by kelp rafting) acts strongly against genetic divergence even at very low rates (Hartl and Clark 1997, p. 195) and if the kelp conveyor hypothesis is correct, the diversity of Haida Gwaii populations will represent a subset of the diversity present in California. Furthermore, while species throughout the subtidal and intertidal zones would persist in refugia, the kelp conveyor is predicted to

predominantly impact low intertidal and subtidal species, which may aid in distinguishing the two hypotheses. If the kelp conveyor is to be considered against withstanding glacial refugia hypotheses of Haida Gwaii, it is necessary to contrast the haplotypic patterns typical of kelp rafting and refugia in marine species.

Recent analyses of population demographics have recognized the weakness inherent in describing complex processes by a single measure (e.g. F_{st}, $G_{st,}$ etc.) and recommend the simultaneous use of multiple measures for more robust hypothesis testing (Chao *et al.* 2015). To this end, Jost (2008) proposed a measure of allelic differentiation, termed Jost's *D,* to be used alongside common fixation measures (such as G_{st}). The popular fixation measure G_{st} (Nei 1973) is a powerful tool for estimating migration between populations (Jost 2009, Ryman and Leimar 2009); however, the maximum value of G_{st} is constrained by unequal diversity between populations (Gerlach *et al.* 2010, Hedrick 2005), while *D* is not (Jost 2008). In the present study, both Jost's *D* and G_{st} were used to jointly infer gene flow between California and Haida Gwaii resulting from the kelp conveyor hypothesis.

In support of the kelp conveyor hypothesis, the results of this study show higher genetic connectivity between California and Haida Gwaii populations of subtidal species than of intertidal species and they highlight the presence of disjunct alleles common to California and Haida Gwaii in subtidal species. Genetic connectivity was inferred by differentiation (*D*) and fixation (G_{st}) calculated from the mitochondrial cytochrome *c* oxidase subunit I (COI-5P) from populations on Haida Gwaii, Vancouver Island, Oregon and California. Permutation tests were applied to pairwise *D* and G_{st} of subtidal and intertidal species to detect the significance of subtidal versus intertidal habitat on

genetic connectivity between California and Haida Gwaii. While private Haida Gwaii alleles were present in most species, further supporting evidence in the literature for marine refugia on the archipelago, high connectivity between Haida Gwaii and California populations was disproportionately found in subtidal species, suggesting co-existence of the kelp conveyor and glacial refugia hypotheses. This study marks the first test of the kelp conveyor hypothesis at the population level and demonstrates support for this hypothesis in species with continuous distributions on the northeast Pacific coast.

Methods

Site & Species Selection

To determine whether the effects of a kelp conveyor are preferentially impacting low intertidal/subtidal species, five predominantly low intertidal/subtidal species of red algae (*Bossiella frondifera* [Manza] P.W.Gabrielson, K.A.Miller, Martone & K.R.Hind; *Calliarthron tuberculosum* [Postels & Ruprecht] E.Y.Dawson; *Callophyllis edentata* Kylin; *Cryptopleura ruprechtiana* [J.Agardh] Kylin; *Erythrophyllum delesserioides* J.Agardh) and three predominantly mid-intertidal species (*Mastocarpus intermedius* S.C.Lindstrom, Hughey & Martone; *Prionitis sternbergii* [C.Agardh] J.Agardh*; Pyropia perforata* [J.Agardh] S.C.Lindstrom) with continuous distributions along the Northeast Pacific coast were selected. As both statistical measures used in this study (*D* and G_{st}) have been shown to be accurate down to sample sizes as low as n=10 (Jost 2008), species were selected for this study based on a minimum of 10 pre-existing records from California, Haida Gwaii and Vancouver Island and supplemented with new collections from central Oregon. All Californian collections were acquired from Monterey Bay, while Haida Gwaii collections spanned six sites across the archipelago. A map of all site locations can be seen in Figure 1.

A total of 392 pre-existing records of the 5' end of the cytochrome *c* oxidase subunit I gene (COI-5P) spanning 1996-2017 from the Barcode of Life Data Systems (BOLD) were supplemented by new collections in Haida Gwaii, Oregon and Vancouver Island. Collections were made in the intertidal or subtidal using SCUBA. From each collection, a $1cm^2$ section of material was preserved in silica and returned to the Connell Memorial Herbarium (UNB) at the University of New Brunswick. DNA was extracted

with a QIAGEN TissueLyser II and QIAxtractor prior to amplification following protocols outlined in Saunders and McDevit (2012) using the primers M13LF3 and M13Rx. Successful amplicons were sent to Genome Quebec for sequencing using the primers M13F and M13R following Saunders and Moore (2013) prior to editing by eye in Geneious v8.1.9 (Kearse *et al.* 2012). Except for Oregon collections, all data used in this study were collected for previous barcoding studies and sites were delineated *post hoc* by defining a 10km radius around densely-sampled areas. Available collections beyond the radius of these newly defined sample sites were pruned from the dataset. Sample sizes for each site, region and species are detailed in Table 1.

Data Analyses

Minimum-spanning networks were generated using TCS v1.2.1 (Clement 2000) for each population to identify disjunct alleles which may have dispersed by long-distance rafting. Diversity was assessed as allelic richness, calculated with PopGenReport version v3.0.4 (Adamack and Gruber 2014, Gruber and Adamack 2015) in R v3.5.3 (R Core Team 2019). To account for unequal sample sizes within species, sample sizes were rarefied by reducing sample sizes to be equal to the lowest sample size observed across all populations for a species prior to calculating allelic richness. The magnitude of gene flow between populations was jointly inferred using pairwise Jost's D (Jost 2008) and Nei's G_{st} calculated with the mmod v1.3.3 package (Winter 2012) to estimate allelic differentiation and nearness to fixation, respectively.

To account for uneven sampling effort across species and regions, a non-parametric Chao-1 estimator (Chao 1984) was used to estimate expected haplotype

richness per species and region with 9,999 bootstrap replicates using PAST3 v.3.24 software (Hammer *et al.* 2001). The Chao-1 estimator is an abundance-based measure of richness dependent on the frequency of singletons and doubletons observed in a sampled population. Specifically, the estimator assumes no overlooked diversity when all rare alleles have been sampled at least twice.

The effect of habitat on differentiation and fixation was tested using methods modified from Gerlach *et al.* (2010) as follows: for each pair of populations, a test statistic was calculated as the difference between the mean *D* or Gst of intertidal and subtidal species. Thereafter, a null distribution of 10,000 permutations was generated for each pair of populations by randomly shuffling habitat (intertidal and subtidal) labels among all eight species and recalculating the test statistic using the newly shuffled groups. Precise *p*-values were calculated following Manly (1997, p.62), assessed at alpha=0.05 and a Benjamini-Hochberg correction (Benjamini and Hochberg 1995) was applied to account for multiple testing. This process was repeated for both *D* and G_{st} independently and the effect of habitat was found to be significant on gene flow between a given pair of populations only if both differentiation and fixation were significant for that population pair.

Results

Rarefied allelic richnesses (*A*) for all eight species are reported in Table 2. Overall, subtidal species tended to a higher allelic richness than intertidal species and the average allelic richness of all eight species in California (*A*=5.1) was nearly twice that of other regions. Allelic richnesses among subtidal species decrease south to north from California to Vancouver Island. Interestingly, a small increase in mean subtidal richness was observed in Haida Gwaii ($A_{subtidal}$=3.0) relative to Vancouver Island ($A_{subtidal}$=2.8); this pattern was not observed in intertidal species. Interestingly, allelic richness of subtidal species on Haida Gwaii was more than twice that of intertidal species on average, while richness of subtidal and intertidal species in Oregon and Vancouver Island were similar.

For the purposes of identifying undersampled populations, non-rarefied, observed allelic richness versus expected allelic richnesses computed with the Chao-1 estimator are reported in Table 3. Overall, populations in Haida Gwaii, Vancouver Island and Oregon were well-sampled and relatively few haplotypes were likely overlooked. The Chao-1 estimator indicated low sampling effort in California uncovered less than half the expected number of haplotypes in California populations of *Cryptopleura ruprechtiana* and *Erythrophyllum delesserioides* and, for *C. ruprechtiana*, considerable diversity was also overlooked in Haida Gwaii.

Differentiation (*D*) and fixation (G_{st}) for all pairwise comparisons can be seen in Table 4 and the associated *p*-values for these comparisons are shown in Table 5. No significant effect of habitat (i.e. intertidal vs. subtidal) was initially detected for any population pair when considering both *D* and G_{st} simultaneously (Table 5). However,

closer inspection of *C. ruprechtiana* revealed much higher differentiation between California and Haida Gwaii than other subtidal species (Figure 2), possibly owing to a predominantly mid-intertidal habitat in California (see discussion). As this species was also found to be uniquely undersampled in both Haida Gwaii and California (Table 3), further analyses were performed with and without the inclusion of *C. ruprechtiana* among the subtidal group.

Interestingly, subtidal populations of California were genetically more similar to Haida Gwaii (D=0.13, G_{st}=0.055) (~2,000km distant) than the former were to Oregon populations (D=0.42, G_{st}=0.16) (~850km distant), while intertidal species typically maintained similar differentiation for all pairwise comparisons (Figure 2). When considering fixation between California and Vancouver Island, Nei's G_{st} was significantly closer to fixation among intertidal species than subtidal species (Figure 3), however, D did not differ significantly by habitat for this population pair. Neither fixation nor differentiation was significantly affected by habitat for any other population pair. Differentiation of subtidal populations was found to be lower overall (mean subtidal D=0.3, mean intertidal D=0.67).

Minimum-spanning networks and allele distributions are shown in Figures 4 – 11. Two of five subtidal species (*C. ruprechtiana* [Figure 7] and *E. delesserioides* [Figure 8]), exhibited a single, disjunct allele common in California and Haida Gwaii that was rare or absent in Oregon and Vancouver Island. Indeed, the most common allele in the subtidal species *E. delesserioides* dominated Haida Gwaii (81%) and California (50%) but was absent elsewhere. No disjunct alleles were detected in the subtidal species *C. edentata* (Figure 6) or the predominantly intertidal species *M.*

intermedius (Figure 9) and *P. sternbergii* (Figure 10); however, two such alleles were found in *P. perforata* (Figure 11); one disjunct allele of *P. perforata* was common to Haida Gwaii, Vancouver Island and California while the other was common to Oregon and Haida Gwaii and absent elsewhere. Likewise, while one disjunct allele was found in the subtidal species *C. tuberculosum*, it was common to Oregon and Haida Gwaii, but absent in California and Vancouver Island (Figure 5).

Discussion

The present study provides evidence for significantly higher gene flow between California and Haida Gwaii in subtidal species than intertidal species (Figure 2, Figure 3) which, when considered in tandem with disjunct alleles present at these two sites strongly supports the kelp conveyor hypothesis (Figure 8). While patterns of gene flow and allelic richness in subtidal species were generally consistent with predictions set forth by the kelp conveyor hypothesis, population-level patterns of intertidal species were varied and not always consistent with the expected stepping stone migration model, possibly resulting from the co-existence of the kelp conveyor and glacial refugia hypotheses.

Community- and Species-level Impacts of the Kelp Conveyor

Kelp rafting is known to be a strong driver of dispersal and genetic similarity between island populations of the kelp rafts themselves (Guillemin *et al.* 2016) and a variety of marine fauna (Hobday 2000b; Fraser *et al.* 2011), but comparatively little work has investigated the potential impacts of rafting on associated macroalgae. Saunders (2014) reported that 18% of species uncovered during routine DNA barcode surveys in common between Haida Gwaii and California exhibited a disjunct distribution; however, the results of the present study suggest that effects of kelp rafting are more common than previously suspected. Of the eight species selected for this study, three subtidal species exhibit low differentiation ($D \leq 0.2$) and degrees of fixation ($G_{st} \leq 0.1$) between Haida Gwaii and California (*C. tuberculosum, B. frondifera, E. delesserioides*) consistent with possible kelp rafting effects. If this similarity were due to

recent shared ancestry between refugial populations rather than contemporary gene flow caused by kelp rafting, then both subtidal and intertidal species would be expected to have low differentiation and fixation between Haida Gwaii and California. The results presented here indicate a high occurrence of impacted species in the marine subtidal flora of Haida Gwaii and call for further investigation into raft-borne communities.

While the effects of kelp rafting are relatively poorly studied for associated macroalgae, rafts formed of *M. pyrifera* have been associated with more than 800 species of passenger fauna (Foster and Schiel 1985, Bushing 1994) in the Northeast Pacific. Hobday (2000b) investigated 73 associated macrofaunal taxa and found that, in general, the success of these macrofauna on kelp rafts is determined by raft longevity and that the density of passenger species rarely declined with raft age. Indeed, the density of two common passenger species (*Idotea resecata* Stimpson and *Pugettia producta* Randall) significantly increased with raft age. Therefore, if kelp rafts are capable of drifting from California to Haida Gwaii, the macrofauna passengers are likely to survive the journey as well. Moreover, patterns of kelp-rafted macrofauna in this region are already evident in the literature; an investigation of the population structure of 50 marine macroinvertebrates revealed higher connectivity among subtidal species than intertidal species (Kelly and Palumbi 2010) despite the biogeographic barrier at Cape Mendocino. Furthermore, beach-cast red abalone (*Haliotis rufescens* Swainson) have been reported from the southern shores of Haida Gwaii (Merilees 2008, Sloan *et al.* 2010), roughly 800km north its range (California – Oregon) (Hobday and Tegner 2002). In one case the abalone shell was attached to a remnant *Nereocystis luetkeana* holdfast (identified through molecular tools) and the authors concluded that the kelp (and the

attached abalone) likely rafted from the abalone's home range of Oregon (Sloan *et al.* 2010). Considered collectively with the results of the present study, the kelp conveyor appears to be the best explanation for these observations.

Interestingly, *D* and G_{st} values for *Cryptopleura ruprechtiana* were generally more similar to intertidal species than other subtidal species (Figure 2, Figure 3), suggesting the habitat of *C. ruprechtiana* may be more similar to intertidal species than subtidal species. While the collections of *C. ruprechtiana* used in this study are predominantly subtidal (~70%) in both Haida Gwaii and California populations, *C. ruprechtiana* is reported to be predominantly intertidal in California and Oregon (Augyte 2011). A reduced occurrence of *C. ruprechtiana* individuals growing subtidally in California where buoyant kelp species attach may explain the higher differentiation and fixation of *C. ruprechtiana* populations. Conversely, the predominantly mid- to high- intertidal species *Pyropia perforata* (J.Agardh) S.C.Lindstrom exhibited unexpected connectivity between Haida Gwaii and Oregon suggestive of kelp rafting effects (Figure 2, Figure 3). One plausible explanation for this result lies in the heteromorphic life history of *Pyropia* species. Members of this group have a large, leafy gametophytic stage that is abundant and obvious in the mid-intertidal zone, while the sporophyte stage (*conchocelis*) is microscopic and grows endolithically in stones, as well as in shells and barnacles (Drew 1949; Martinez 1990), which are themselves known associates of kelp rafts (Macaya *et al.* 2005; Fraser *et al.* 2011; Wichmann 2012). Relatively little is known about the *conchocelis* phase of *P. perforata,* however the *conchocelis* stage of some members of this group are reported to dwell almost exclusively in the subtidal zone (Tribollet *et al.* 2018). Thus, it remains possible that the

conchocelis stage of *P. perforata* is responsible for a disjunct distribution by way of kelp rafting.

The kelp conveyor hypothesis predicts a unidirectionally northward migration, however, the results presented here are ambiguous with respect to directionality. Roman (2006) found that populations receiving gene flow from multiple sources (e.g. simultaneous stepping stone and kelp-rafted inputs) may have relatively high diversity; it would follow that under the northward predictions of the kelp conveyor hypothesis, Haida Gwaii would have higher diversity than nearby populations (i.e. Vancouver Island). Interestingly, while allelic richness of subtidal populations is higher in Haida Gwaii ($A_{subtidal}$=3.0) than in Vancouver Island ($A_{subtidal}$=2.8), subtidal populations of California ($A_{subtidal}$= 5.8) are also more diverse than Oregon ($A_{subtidal}$=2.8) (Table 2). Given these data, a northward, southward or bidirectional kelp conveyor is plausible. However, while a northward migration remains plausible on the nearshore Davidson current, a southward migration lacks an appropriate mechanism. The California current flows up to 1,000km offshore and, importantly, travels along the northeast Pacific coast only from Oregon to California. At present, the kelp conveyor hypothesis of strict northward movement is the only explanation with a plausible mechanism that fits the data presented here.

Barriers to Gene Flow

Differentiation and fixation between California and Oregon were consistently higher than Oregon and Vancouver Island across both intertidal and subtidal species,

despite similar geographic distances between sites (~700km). A genetic break between California and Oregon populations is consistent with previous reports of a biogeographic barrier near Cape Blanco, Oregon, resulting from a combination of physical structure and coastal upwelling (Magnell *et al.* 1990, Barth *et al.* 2000). Evidence of gene flow across such a barrier has been demonstrated for several macrofauna passengers of *Durvillea antarctica* (Chamisso) Hariot kelp rafts as well as passengers of *Macrocystis* rafts crossing the sub-tropical (Nikula *et al.* 2013) and Antarctic polar fronts (Leese *et al.* 2010, Fraser *et al.* 2016), respectively. Thus, the gene flow in subtidal species across the Cape Blanco barrier apparent in this study further suggests gene flow between California and Haida Gwaii crosses the Cape Blanco barrier by way of kelp rafting, rather than stepwise, short-distance spore dispersal. Interestingly, disjunct alleles observed in the subtidal *C. tuberculosum* and intertidal *P. perforata* (although see comments above regarding the conchocelis stage) are consistent with long-range dispersal and suggest kelp rafting may also occur from Oregon to Haida Gwaii using the same mechanisms described in the original kelp conveyor hypothesis.

The subtidal species *Erythrophyllum delesserioides* J.Agardh appears to have highly limited dispersal leading to strong population structure among nearby regions. Uniquely high differentiation and fixation was detected between Vancouver Island and Haida Gwaii populations of *E. delesserioides* (Figure 2, Figure 3). A stepping stone migration pathway would predict a recolonization of Haida Gwaii's eastern shore from British Columbia's (BC) mainland following glacial periods; however, despite intensive sampling of the eastern coastline, *E. delesserioides* is rare and restricted to southern regions on the eastern coast (Figure 11), suggesting this coast harbours few habitable

zones for *E. delesserioides* and limited dispersal may be responsible for the strong population structure between mainland BC populations and Haida Gwaii populations. Despite this strong population structure at relatively small scales, in addition to the Cape Blanco barrier described above, differentiation between Haida Gwaii and California remains lower in *E. delesserioides* than intertidal species (D=0.20 and D=0.83, respectively). This result coupled with known kelp raft associations (Devinny and Kirkwood 1974) largely suggests this species colonized Haida Gwaii by some vector aside from the classic stepping stone model.

Glacial Refugia

The presence of glacial refugia on Haida Gwaii has long been hypothesized to explain high rates of endemism on the archipelago (Warner *et al.* 1982; Heusser 1989; Reimchen and Byun 2005; Shafer *et al.* 2010), however, a review of Pacific refugia shows that macroalgal endemism in this region is poorly studied (Shafer *et al.* 2010). Saunders (2014) identified 33 genetic species occurring predominantly in northern BC (including Haida Gwaii, Prince Rupert and Vancouver Island north of Tahsis) and California, but not encountered in southern BC, and he reported a higher proportion of disjunct alleles in subtidal species versus intertidal species, a result inconsistent with refugial hypotheses for subtidal species. The results of the present study further suggest that glacial refugia alone are not responsible for what initially appeared to be endemism in the marine flora of Haida Gwaii.

Herbert *et al.* (2001) suggest dominant currents in the northeast Pacific collapse in advance of glaciations and reorganize post-glaciation when surface sea temperatures

rise. Given the private alleles observed in Haida Gwaii for five of eight species (Figures 3, 4, 6, 7 and 8) separated from non-private alleles by up to three base pair substitutions, and a red algal COI-5P mutation rate of 0.007 substitutions site^{-1} per million years (Bringloe and Saunders 2018), it seems likely that these populations existed on the archipelago prior to the last glacial maximum (LGM). Thus, it seems most likely that both the kelp conveyor hypothesis and previous glacial refugia hypotheses exist in tandem. Kelp rafting mediates gene flow from the California region to Haida Gwaii during interglacial periods and desists when the currents collapse pre-glaciation.

During glacial periods, small populations of macroalgae likely persist in the Haida Gwaii refugia, resulting in bottlenecks and the appearance of private alleles over evolutionary time. The presence of one or more private alleles in all but two species (*C. edentata* and *P. sternbergii*) suggests refugial populations existed for subtidal and intertidal species examined in this study. While subtidal species appear to be rafting on the kelp conveyor, it remains unclear whether this kelp conveyor is a recent phenomenon (occurring following the end of the LGM) or a recurring phenomenon that occurs regularly during interglacial periods and collapses with the California current system during glacial periods.

Low Sample Size and Unequal Effort

Considering the high allelic richness in California, combined with relatively low sampling effort in this region, it is unsurprising to find considerable overlooked diversity in this region for *C. ruprechtiana* and *E. delesserioides* (Table 4). However, as *E. delesserioides* was well sampled in Haida Gwaii and Vancouver Island, it is unlikely

that other alleles relevant to the kelp conveyor or glacial refugia hypotheses were overlooked. Conversely, observed haplotype richness of *C. ruprechtiana* was less than half the expected richness in both Haida Gwaii and California, suggesting the possibility of disjunct alleles present in California and Haida Gwaii that may have been overlooked in this study. Remaining Haida Gwaii and California populations of intertidal and subtidal species were determined to be well-sampled by the Chao-1 estimator, suggesting the conclusions drawn herein are based on sufficient sampling sizes.

Conclusions

Studies of biogeography require strong foundational knowledge regarding sources of biodiversity and factors affecting speciation. This study provides the first support of the kelp conveyor hypothesis at the population level and adds to the growing body of work on the diverse marine flora of Haida Gwaii. Considering this work, it seems clear that both kelp rafting and glacial refugia are jointly responsible for the disjunct distribution of alleles in many marine species on the archipelago. Moving forward, using multiple loci to uncover historic bottlenecks (as in Heled and Drummond 2008) in population histories of Haida Gwaii species might reveal the kelp conveyor to be a recent phenomenon (occurring only since the LGM) or an ancient one that has been affecting interglacial populations on Haida Gwaii for many glacial cycles, further elucidating changing population histories of marine species around the archipelago.

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Table 1 Sample sizes for each site and species used in the present study. MA: Masset, RS: Rennell Sound, SC: Skidegate Channel, KU: Kunxalas Heritage Site, LY: Lyell Island, BU: Burnaby Island, KG: Kunghit Island, NK: Nootka, VA: Vancouver, VC: Victoria, BF: Bamfield, AR: Cape Arago, SR: Seal Rock, MO: Monterey Bay. Site locations are shown in Figure 1.

		Haida Gwaii							Vancouver Island				Oregon		California
Species	Total Collections	MA	RS	SC	KU	LY	BU	KG	NK	VA	VC	BF	AR	SR	MO
Bossiella frondifera	34		7				9					7			11
Calliarthron tuberculosum	196					9	28	12	34			54	27		32
Callophyllis edentata	178					5	40	5		5	8	44	17	12	5
Cryptopleura ruprechtiana	169			40	21	5	10	7				20	26	10	30
Erythrophyllum delesserioides	76		12	20								12	19	5	8
Mastocarpus intermedius	77					10	20					17	7		23
Prionitis sternbergii	103						9			17		32	23	14	8
Pyropia perforata	122	7				1	4	5		13		46	24	10	12

Table 2 Allelic richness (*A*) of all populations. To account for variable sample sizes within a species, rarefaction of sample sizes was performed for each population by reducing the sample size to equal the lowest sample size across all populations for a species prior to calculating allelic richness. The rarefied sample size (n_r) is also shown. Site codes are: CA=California, HG=Haida Gwaii, VI=Vancouver Island, OR=Oregon.

	Species	A_{CA}	A_{OR}	A_{VI}	A_{HG}	n_r
Subtidal	*B. frondifera*	3.3	NA	4.0	2.9	7
	C. tuberculosum	9.4	7.0	3.7	4.0	27
	C. edentata	2.0	1.0	1.2	1.0	5
	C. ruprechtiana	8.5	3.6	3.0	3.4	20
	E. delesserioides	6.0	2.4	2.0	3.5	8
	Subtidal Mean	5.8	2.8	2.8	3.0	NA
Intertidal	*M. intermedius*	3.7	3.0	3.2	1.4	7
	P. sternbergii	6.0	3.2	1.4	1.0	9
	P. perforata	1.9	1.7	2.1	1.9	9
	Intertidal Mean	3.9	2.6	2.2	1.4	NA
	Overall Mean	5.1	3.1	2.6	2.5	NA

Table 3. Observed (left) and expected (right) number of haplotypes. Expected allele counts were calculated with a Chao1 indicator and 9,999 bootstrap replicates as implemented in PAST3. Similarity between observed and expected numbers of haplotypes indicates sufficient sampling effort in Haida Gwaii, Oregon and Vancouver Island, however a large amount of overlooked diversity likely exists in California populations for *C. ruprechtiana* and *E. delesserioides*. Observed haplotype numbers less than half the expected value are indicated by bold text.

	Species	California	Haida Gwaii	Oregon	Vancouver Island
Subtidal	*B. frondifera*	4/5	3/4	NA	3/3
	C. tuberculosum	4/5	5/6	3/3	6/7.5
	C. edentata	2/2	1/1	1/1	2/2
	C. ruprechtiana	**9/30**	**7/17**	4/5	3/3
	E. delesserioides	**3/11**	2/3	3/3	2/2
Intertidal	*M. intermedius*	6/7.5	2/2	3/4	4/4
	P. sternbergii	6/6.75	1/1	**7/16**	3/4
	P. perforata	4/7	5/8	3/3	14/16

Table 4. Pairwise differentiation (*D*) and fixation (G_{st}) for all species. Differentiation and fixation were used to jointly infer migration between population pairs. Significant effect of habitat on both differentiation and fixation is denoted by bold text. An asterisk (*) denotes a significant effect of habitat on G_{st} but not *D* observed between California and Vancouver Island populations. *C. ruprechtiana* values are reported here, however, due to the unusually high *D* and G_{st} of this species, *C. ruprechtiana* was not included among subtidal species for these analyses. Population codes are: CA=California, HG=Haida Gwaii, OR=Oregon, VI=Vancouver Island.

	Species	CA – HG	CA – OR	CA – VI	HG – OR	HG – VI	VI – OR
	C. ruprechtiana	0.85/0.25	0.97/0.29	0.93/0.2	0.017/0.015	0.19/0.1	0.2/0.11
Subtidal	*B. frondifera*	0.19/0.065	NA	0.23/0.052	NA	0.14/0.26	NA
	C. tuberculosum	0.078/0.023	0.21/0.053	0.029/0.0079	0.055/0.022	0.023/0.002	0.16/0.06
	C. edentata	0.053/0.1	0.052/0.22	0.049/0.15	0.0/0.076	0.00013/0.004	0.0045/0.028
	E. delesserioides	0.20/0.032	1/0.21	1/0.21	0.95/0.22	0.98/0.26	0.56/0.24
	Subtidal Mean	**0.13/0.055**	0.42/0.16	0.33/0.11*	0.41/0.11	0.29/0.13	0.24/0.11
Intertidal	*M. intermedius*	1/0.44	1/0.27	1/0.2	1/0.54	0.54/0.29	1/0.26
	P. sternbergii	0.61/0.29	0.52/0.13	0.6/0.26	0.032/0.055	0/0	0.28/0.04
	P. perforata	0.88/0.23	1/0.32	0.88/0.23	0.90/0.24	0.067/0.019	1/0.27
	Intertidal Mean	**0.83/0.32**	0.84/0.24	0.83/0.23*	0.64/0.28	0.21/0.10	0.68/0.19

Table 5. Pairwise p-values calculated from comparisons of habitat (intertidal versus subtidal) on differentiation (D) and fixation (G_{st}). Benjamini-Hochberg correction has been applied to all *p*-values to account for multiple testing. Columns *D and G_{st}* are *p*-values for pairwise comparisons after removing *C. ruprechtiana* from analyses. Significant values are indicated by bold text.**

Species	*D*	G_{st}	*D**	G_{st} *
California – Haida Gwaii	0.194	0.0942	**0.0006**	**0.0003**
California – Oregon	0.384	**0.0006**	0.252	**0.0003**
California – Vancouver Island	0.384	0.448	0.252	0.208
Haida Gwaii – Oregon	0.384	0.494	0.302	0.601
Haida Gwaii – Vancouver Island	1.0	1.0	1.0	1.0
Vancouver Island - Oregon	0.419	0.494	0.609	0.732

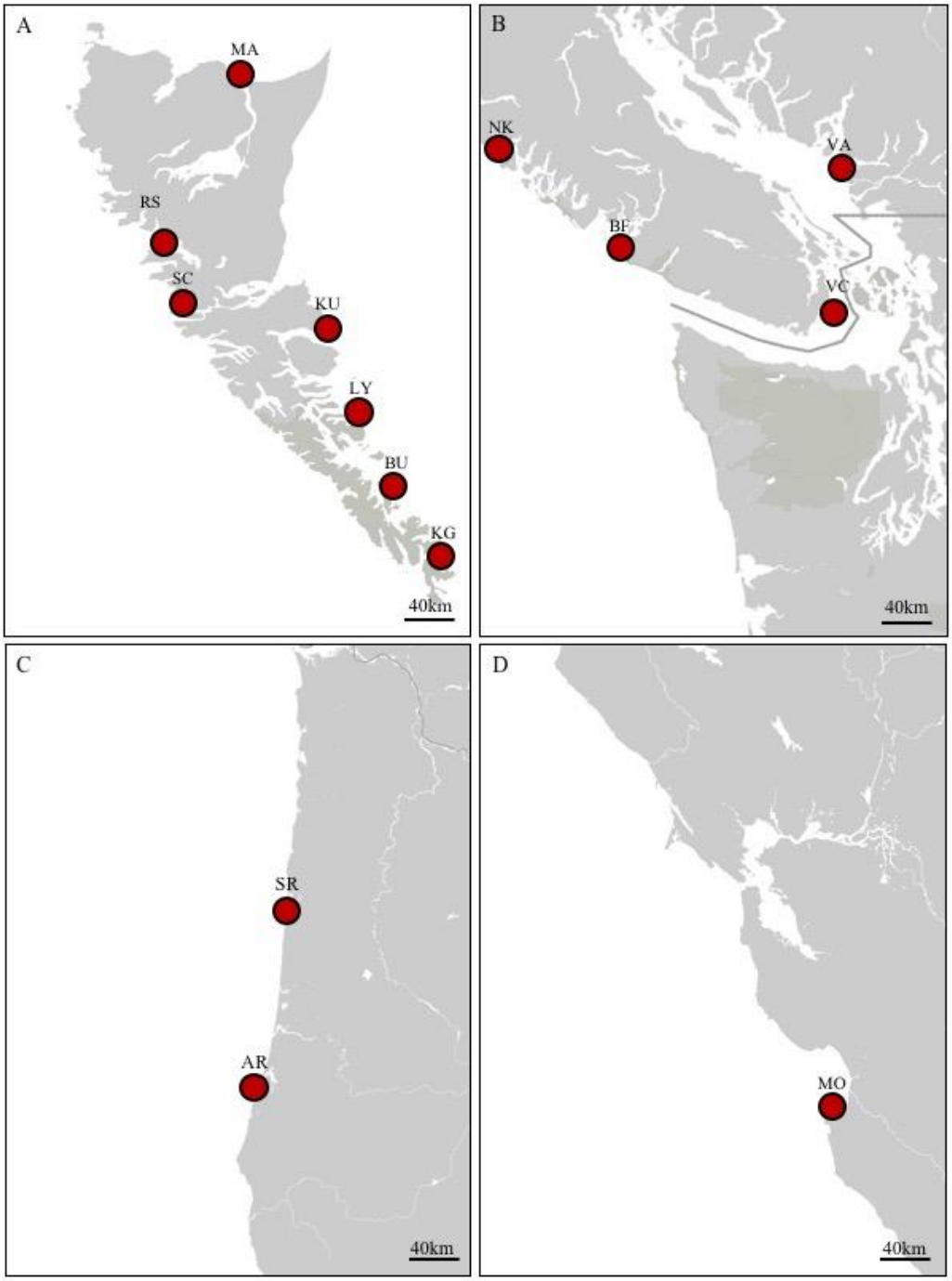

Figure 1. All sampling sites used in this study. A: Haida Gwaii, B: Vancouver Island, C: Oregon, D: California. All sites span a 10km radius and, excepting Oregon, were selected post-hoc and assigned a radius of 10km. For site codes see Table 1. Adjacent regions are separated by 600-800km.

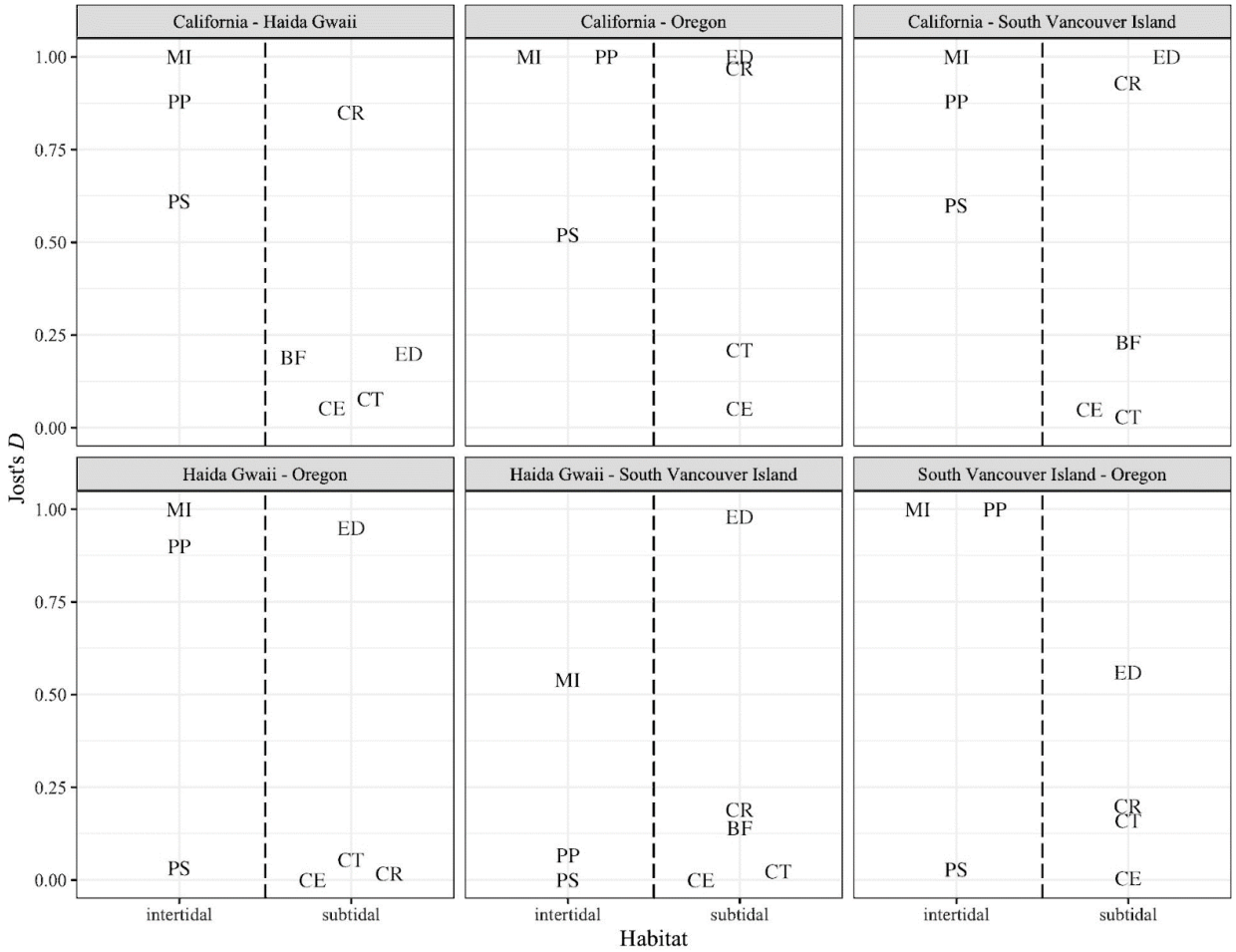

Figure 2. Estimates of differentiation (*D*) for all species and population pairs. Species are sorted according to predominant habitats. Horizontal variation between species within the figure is for visualization purposes only. Species codes for subtidal species are: BF: *Bossiella frondifera,* CT: *Calliarthron tuberculosum,* CE: *Callophyllis edentata*, CR: *Cryptopleura ruprechtiana,* ED: *Erythrophyllum delesserioides* and for intertidal species are MI: *Mastocarpus intermedius*, PS: *Prionitis sternbergii,* PP: *Pyropia perforata*.

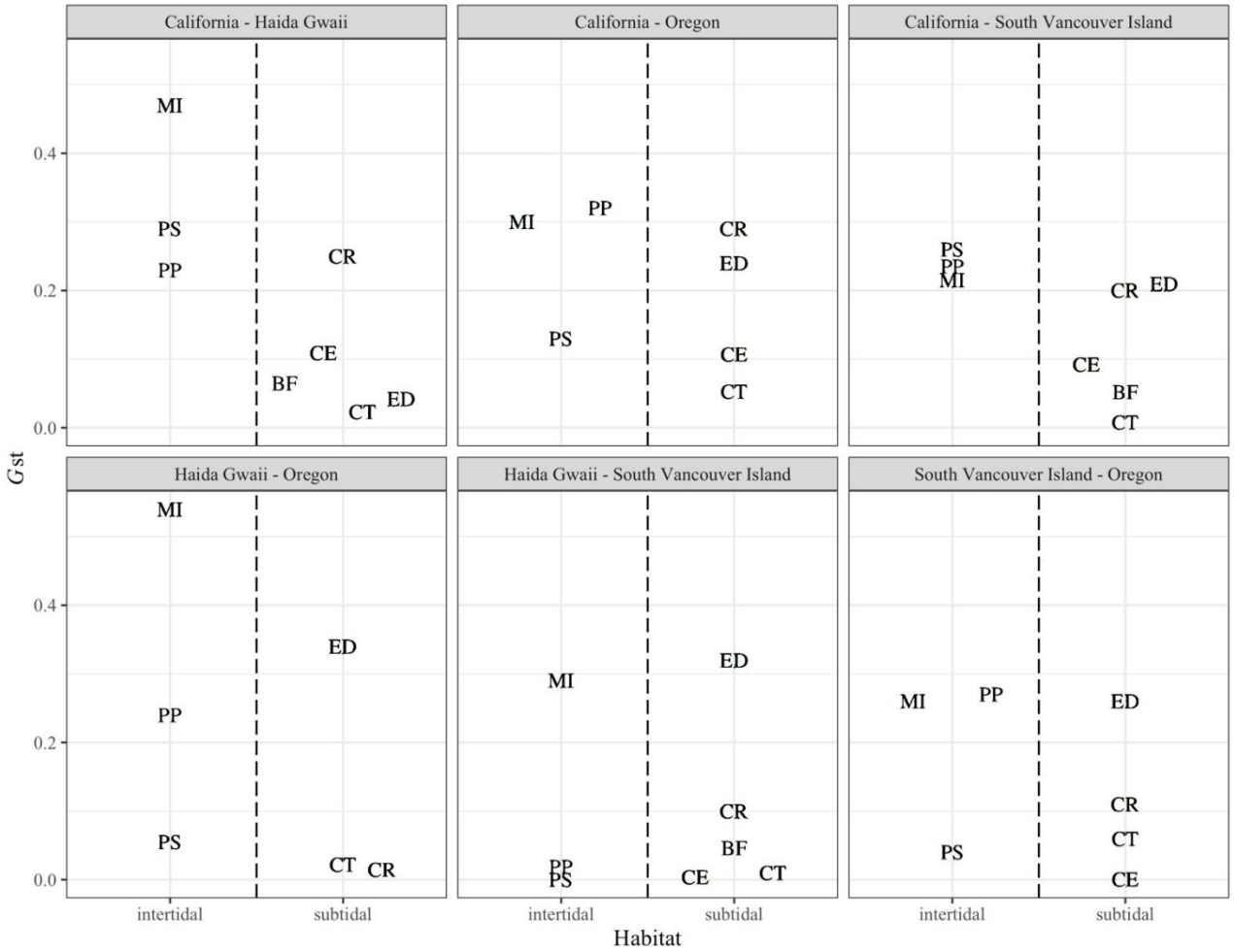

Figure 3. Estimates of fixation (G_{st}) for all species and population pairs. Species are sorted according to predominant habitats. Horizontal variation between species within the figure is for visualization purposes only. Species codes for subtidal species are: BF: *Bossiella frondifera,* CT: *Calliarthron tuberculosum,* CE: *Callophyllis edentata*, CR: *Cryptopleura ruprechtiana,* ED: *Erythrophyllum delesserioides* and for intertidal species are MI: *Mastocarpus intermedius*, PS: *Prionitis sternbergii,* PP: *Pyropia perforata*.

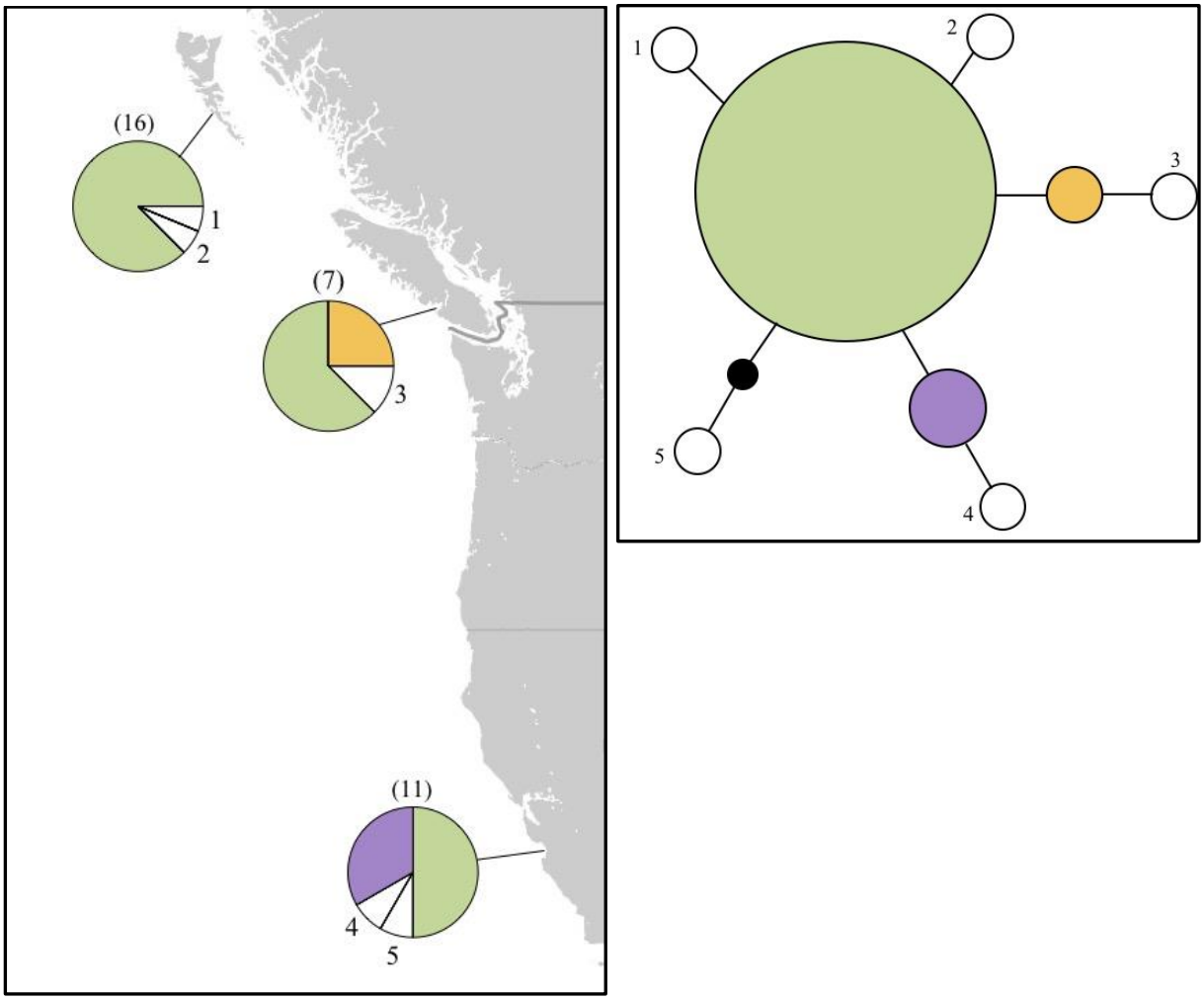

Figure 4. Allelic distribution and minimum-spanning network for the subtidal species *Bossiella frondifera*. Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles.

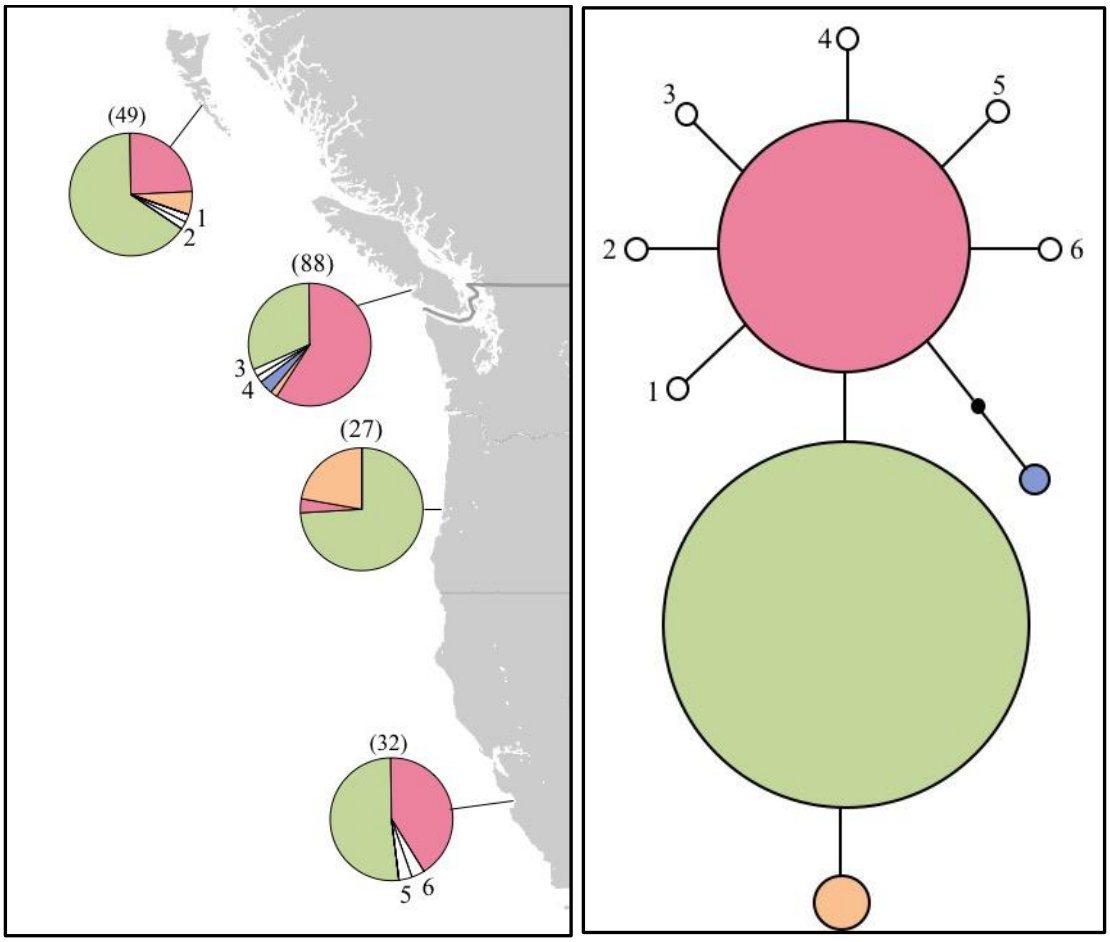

Figure 5. Allelic distribution and minimum-spanning network for the subtidal species *Calliarthron tuberculosum*. Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles.

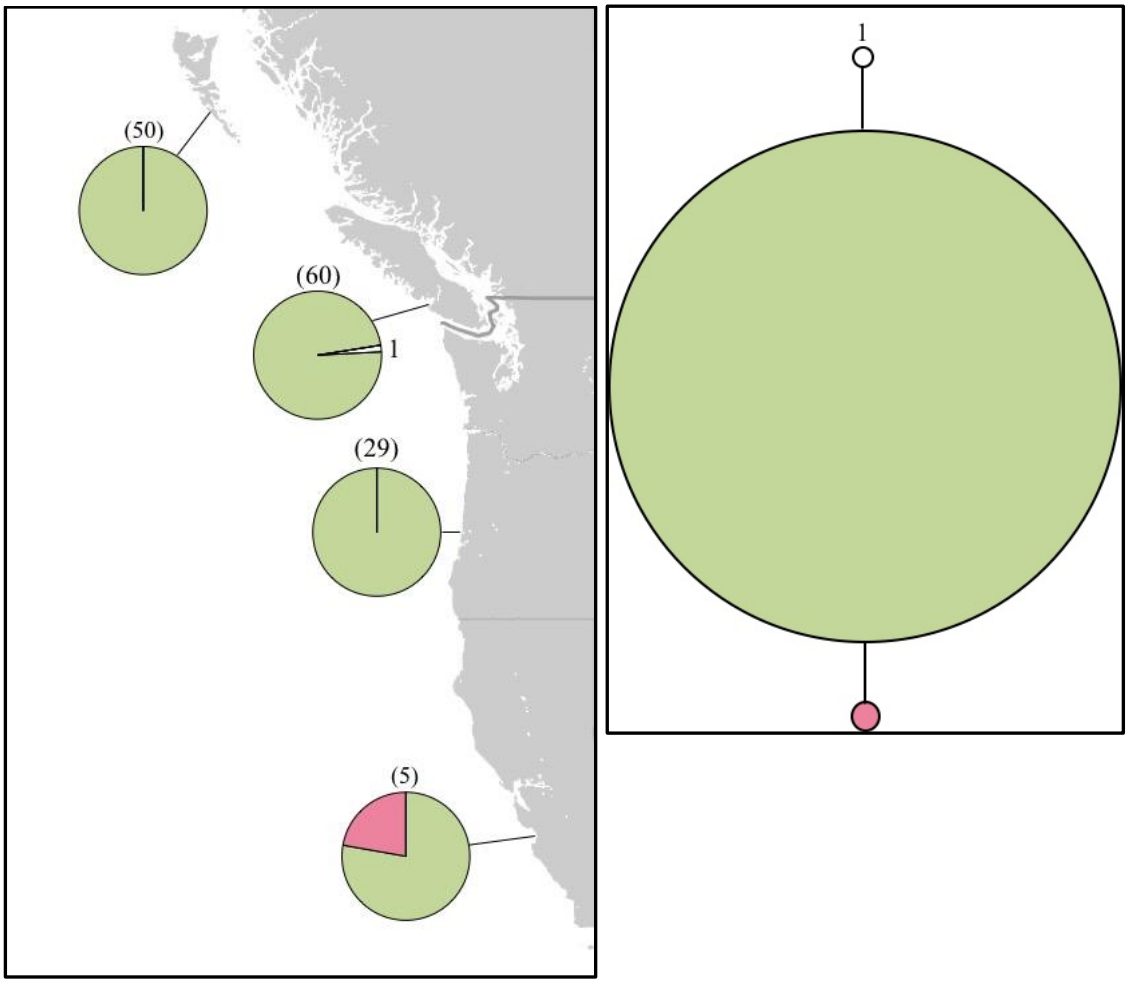

Figure 6. Allelic distribution and minimum-spanning network for the subtidal species *Callophyllis edentata.* Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles.

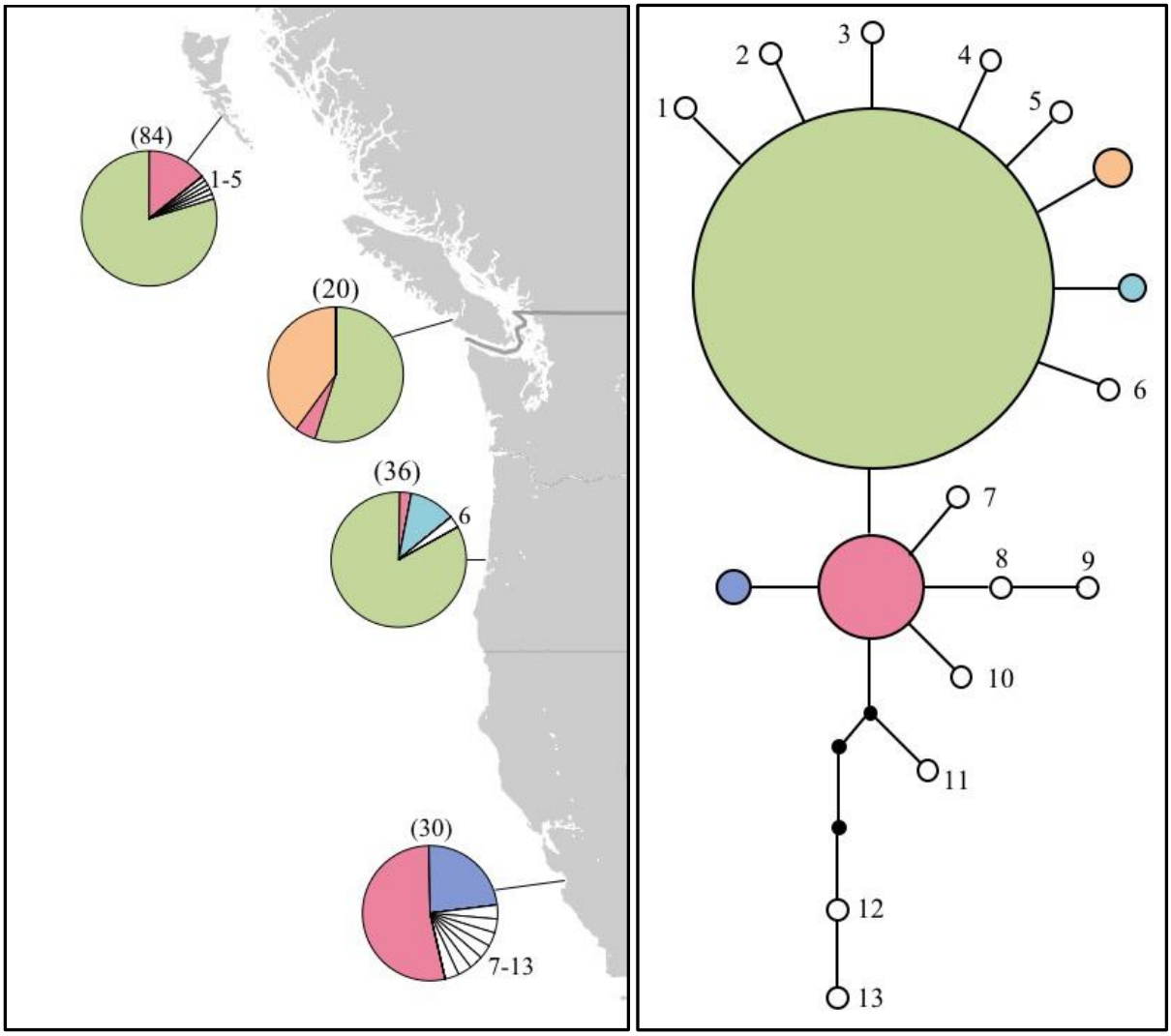

Figure 7. Allelic distribution and minimum-spanning network for the subtidal species *Cryptopleura ruprechtiana*. Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles.

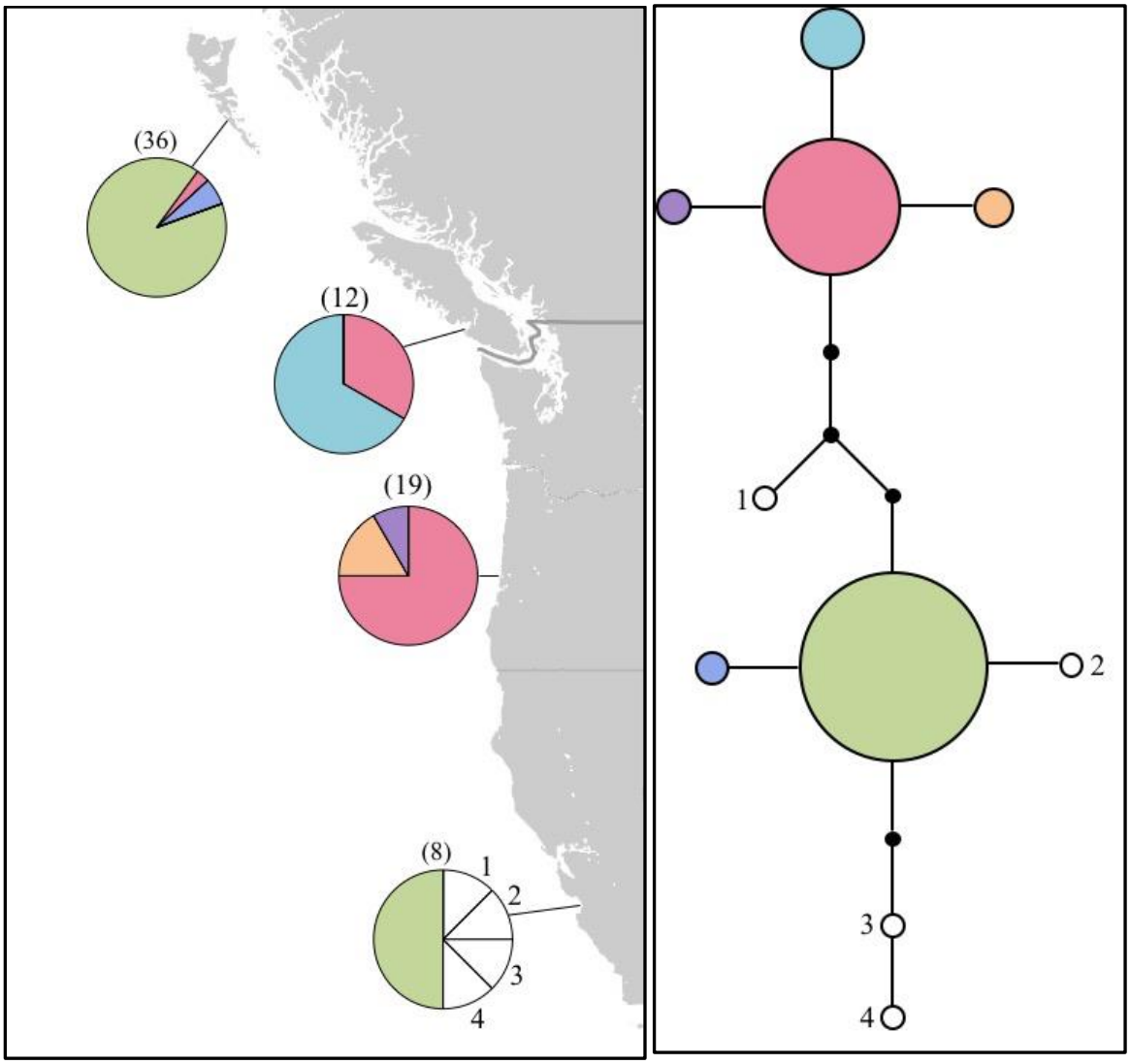

Figure 8. Allelic distribution and minimum-spanning network for the subtidal species *Erythrophyllum delesserioides.* Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles.

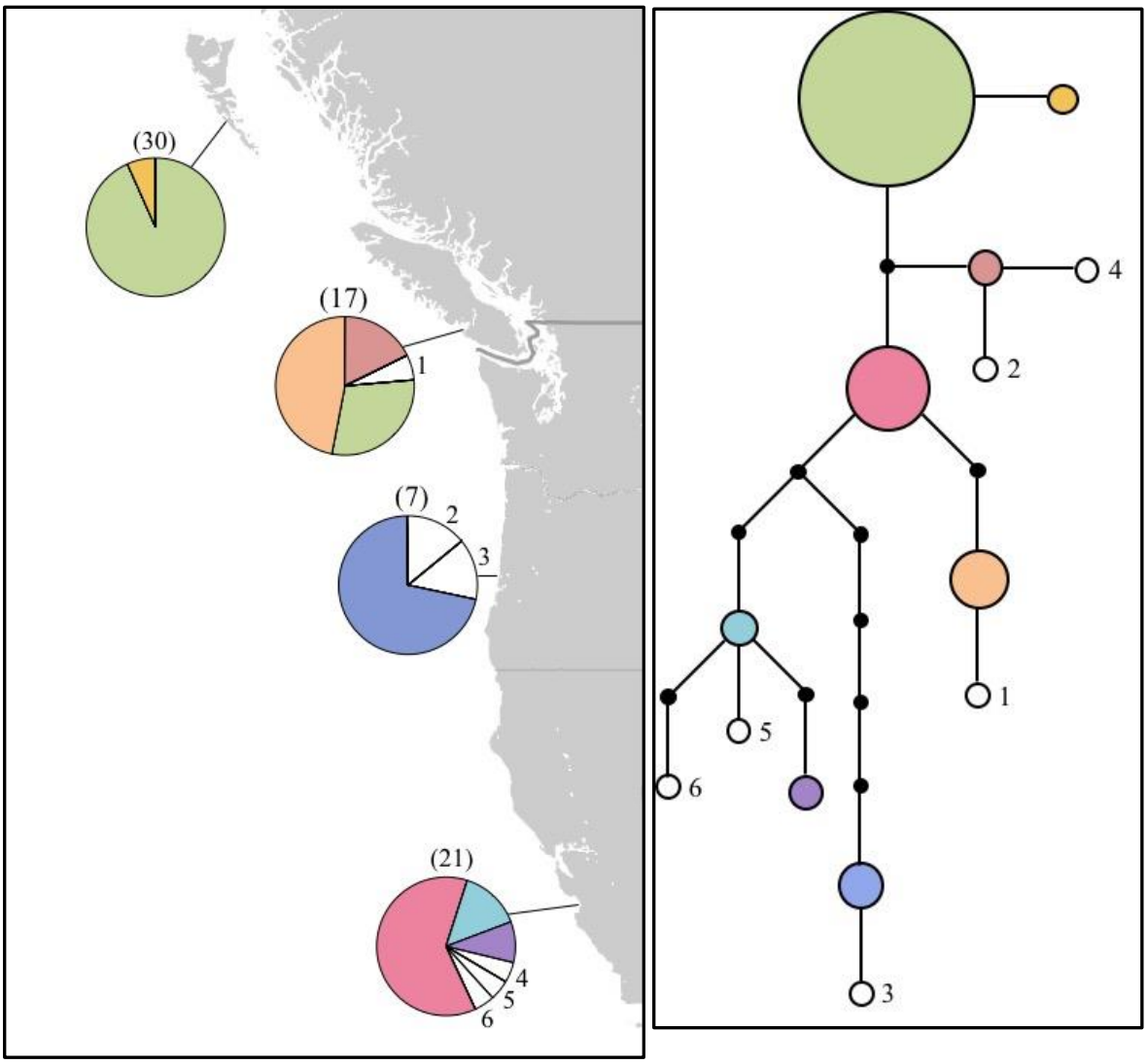

Figure 9. Allelic distribution and minimum-spanning network for the intertidal species *Mastocarpus intermedius.* Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles

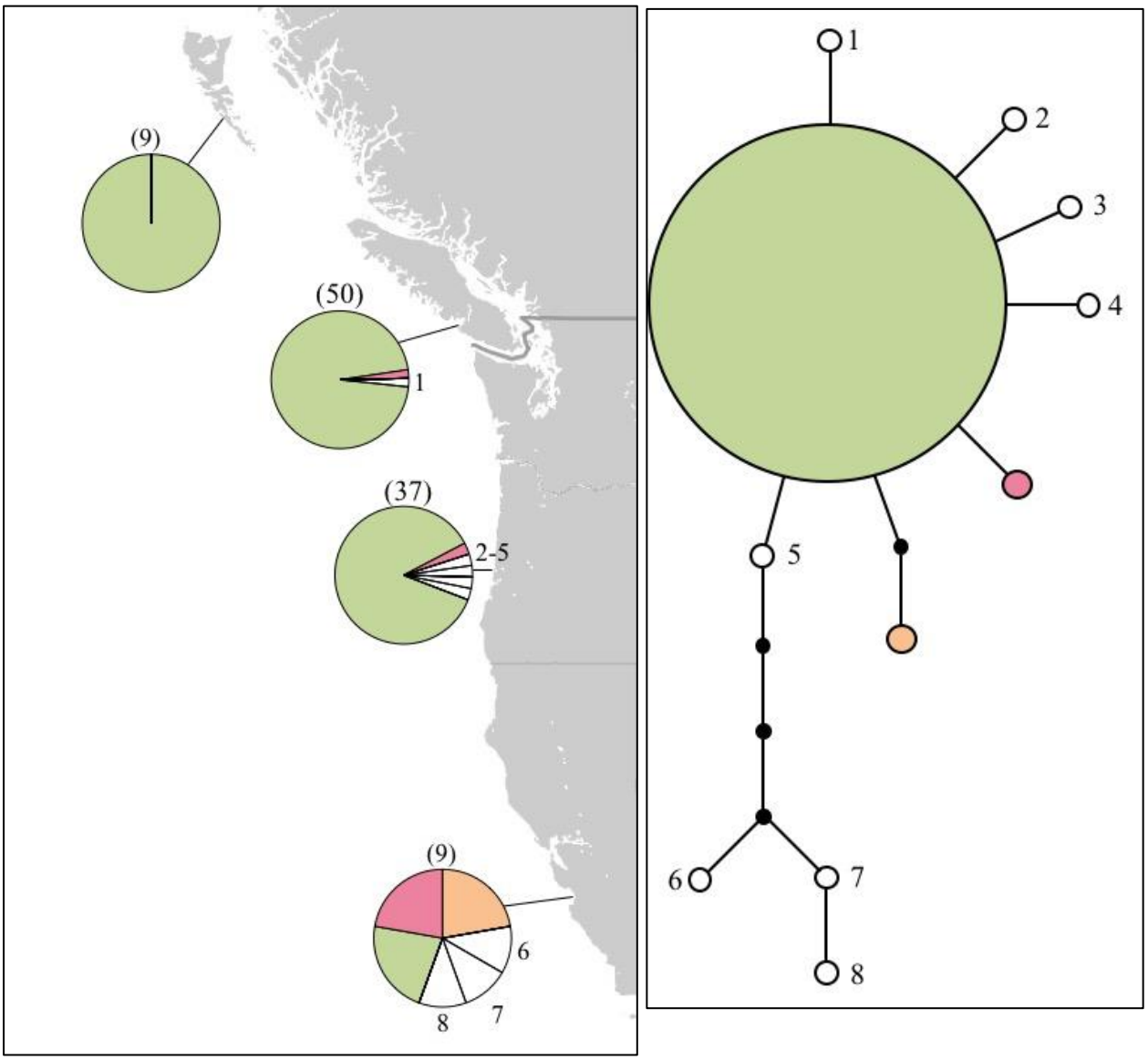

Figure 10. Allelic distribution and minimum-spanning network for the intertidal species *Prionitis sternbergii.* Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles

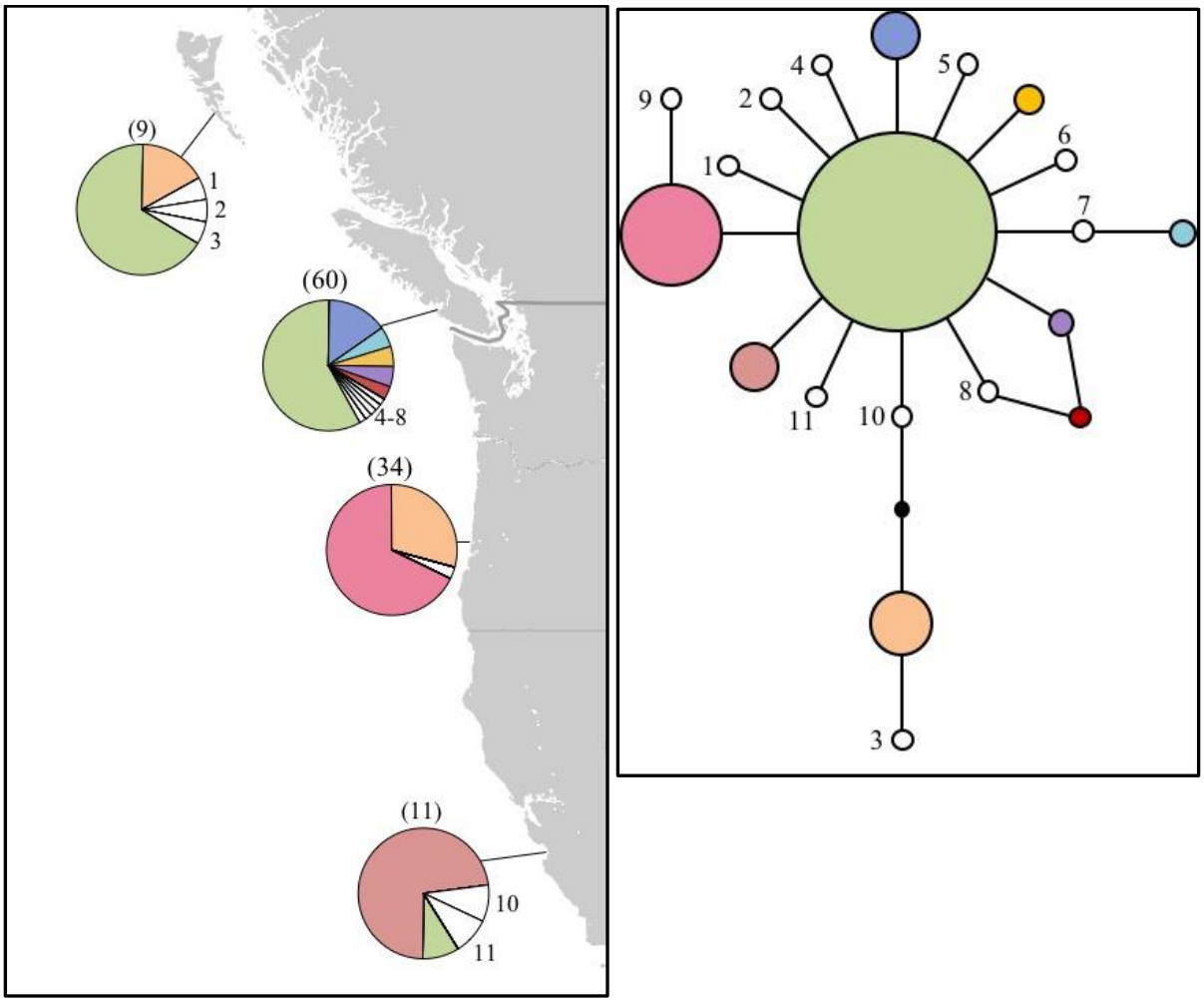

Figure 11. Allelic distribution and minimum-spanning network for the intertidal species *Pyropia perforata.* Numbers in parentheses indicate sample sizes. Numbers adjacent to white alleles indicate singletons. Unsampled haplotypes are denoted by black circles

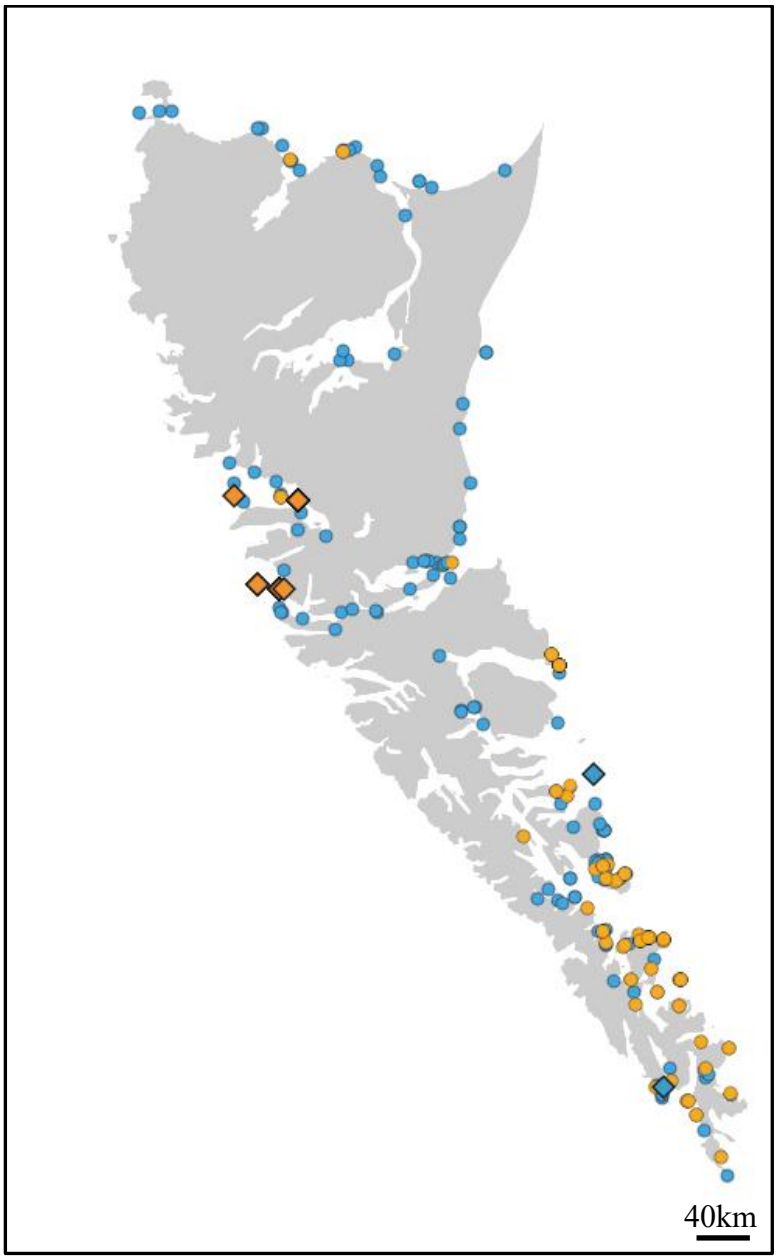

Figure 12. Sampling sites on Haida Gwaii. All sites sampled over 16 years of ongoing barcoding surveys (blue and orange) including all Haida Gwaii sites used in this study (orange). Despite heavy sampling of the eastern coastline, *Erythrophyllum delesserioides* (diamonds) is found more commonly on the exposed, western coast of the archipelago.

Curriculum Vitae

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Publications:

Saunders, G.W., **Brooks, C.M.** & Scott, S. 2019. Preliminary DNA barcode report on the marine red algae (Rhodophyta) from the British Overseas Territory of Tristan da Cunha. Cryptogamie Algol. 40(10): 105-117.

Conference Presentations:

Acadian Entomological Society & Entomological Society of Canada Canadian Society for Ecology and Evolution Joint Meeting, Fredericton, NB. 2019. The Kelp Conveyor Hypothesis: evidence of long-distance migration in the Northeast Pacific. **Brooks C**., Saunders G.W. (oral)

58th Northeast Algal Symposium, Salem, MA. 2019. The Kelp Conveyor Hypothesis: Long Distance Gene Flow in the Northeast Pacific. **Brooks C**., Saunders G.W. (oral)

Phycological Society of America, Vancouver, BC. 2018. The Kelp Conveyor Hypothesis: Exploring Long-Distance Genetic Connectivity of Red Algae in the Northeast Pacific. **Brooks C**., Saunders G.W. (oral)

57th Northeast Algal Symposium, New Haven, CT. 2018. The Kelp Conveyor Hypothesis: Exploring Long Distance Genetic Connectivity of Red Algae in the Northeast. **Brooks C**., Saunders G.W. (oral).

55th Northeast Algal Symposium, Westfield, MA. 2016. Long-Distance Kelp Rafting of Red Algae in the Northeast Pacific. **Brooks C**., Saunders G.W. (oral).