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WHOLE-GENOME SEQUENCING REVEALS FORGOTTEN LINEAGES AND RECURRENT HYBRIDIZATIONS WITHIN THE KELP GENUS *ALARIA* (PHAEOPHYCEAE)[1]

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The genomic era continues to revolutionize our understanding of the evolution of biodiversity. In phycology, emphasis remains on assembling nuclear and organellar genomes, leaving the full potential of genomic datasets to answer long-standing questions about the evolution of biodiversity largely unexplored. Here, we used whole-genome sequencing (WGS) datasets to survey species diversity in the kelp genus *Alaria*, compare phylogenetic signals across organellar and nuclear genomes, and specifically test whether phylogenies behave like trees or networks. Genomes were sequenced from across the global distribution of *Alaria* (including *Alaria crassifolia*, *A. praelonga*, *A. crispa*, *A. marginata*, and *A. esculenta*), representing over 550 GB of data and over 2.2 billion paired reads. Genomic datasets retrieved 3,814 and 4,536 single-nucleotide polymorphisms (SNPs) for mitochondrial and chloroplast genomes, respectively, and upwards of 148,542 high-quality nuclear SNPs. WGS revealed an Arctic lineage of *Alaria*, which we hypothesize represents the synonymized taxon *A. grandifolia*. The SNP datasets also revealed inconsistent topologies across genomic compartments, and hybridization (i.e., phylogenetic networks) between Pacific *A. praelonga*, *A. crispa*, and putative *A. grandifolia*, and between some lineages of the *A. marginata* complex. Our analysis demonstrates the potential for WGS data to advance our understanding of evolution and biodiversity beyond amplicon sequencing, and that hybridization is potentially an important mechanism contributing to novel lineages within *Alaria*. We also emphasize the importance of surveying phylogenetic signals across organellar and nuclear genomes, such that models of mixed ancestry become integrated into our evolutionary and taxonomic understanding.

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Abbreviations: **Kbp, thousand base pairs; LD, linkage disequilibrium; Mbp, million base pairs; ML, maximum-likelihood; SNP, single-nucleotide polymorphism; WGS, whole-genome sequencing**

Genomic datasets are ushering in a new era of evolutionary analyses for phycologists (Oliveira et al. 2018). These datasets promise to reveal exceptional insights into the distribution of biodiversity across marine environments, the ways species and populations are related, and functional genomic aspects underpinning evolutionary processes ranging from major transitions (e.g., multicellularity, endosymbiosis; Cock et al. 2010) to the capacity for adaptation under advanced climate change (Wood et al. 2021). Estimates of biodiversity and phylogenetic relationships among algal taxa are now commonly validated through amplicon sequencing (e.g., DNA barcoding; Saunders 2005), which serves as a proxy for genomic evolution. Several options, however, now exist to move past these proxies. Microsatellites are commonly employed in tandem with DNA barcoding of organellar markers as a means to fetch genetic information from the nuclear genome (e.g., Neiva et al. 2018, Grant and Bringloe 2020). Restriction-site–associated DNA sequencing (RADseq) has proven a significant step forward in improving the resolution of genetic datasets (i.e., 1,000s of single-nucleotide polymorphisms, SNPs), particularly in non-model organisms for which reference genomes remain unavailable (e.g., Kobayashi et al. 2018, Guzinski et al. 2020, Le Cam et al. 2020, Mao et al. 2020, Reynes et al. 2021). Showcasing the power of genome scale genotyping, Flanagan et al. (2021) used >62K SNPs to pinpoint the source of global introductions of *Agarophyton vermiculophyllum* to a ˜50 km section of coastline in Japan.

Whole-genome sequencing (WGS), the comprehensive analysis of all genomic information in a given sample, is quickly emerging as the next logical step for genetic analyses in the field of phycology (Bringloe et al. 2020a). Emphasis of WGS, however, has remained on sequencing and functional analysis of genomes (e.g., Cock et al. 2010, Ye et al. 2015, Lipinska et al. 2019, Shan et al. 2020), with few studies only recently moving beyond this aim. For instance, Jenkins et al. (2021) applied WGS to the study of genetic diversity in Northeast Atlantic maerl-bed species, which was used to debunk putative introgression between species and showcase substantial genetic differentiation even among adjacent populations. Graf et al. (2021) also used a whole-genome approach in the kelp *Undaria pinnatifida* to distinguish cultivated, natural, and globally introduced populations. Bringloe et al. (2021) used WGS to survey epi-endobiont diversity of kelp and infer the discovery of a parasitic brown alga. The versatility of WGS datasets to answer a wide variety of phycological questions, ranging from the assembly of genomes to population genomics to biodiversity surveys, further justifies this approach as the future standard for genetic analyses.

Alaria is a genus of kelp (Laminariales, Phaeophyceae) found in Arctic to temperate waters of the Northern Hemisphere, with its greatest species diversity in the Northwest Pacific. Its life history alternates between haploid microscopic gametophytes and diploid macroscopic sporophytes, the latter of which forms crucial marine habitat (Bringloe et al. 2020a). *Alaria* is also a genus of interest for aquaculture, with *Alaria esculenta* cultivated in the Atlantic, and *A. marginata* now being cultivated in the Northeast Pacific (Stekoll 2019, Kraan 2020). *Alaria* is the second largest genus in the Laminariales and is included in one of four families (Alariaceae) in this order. More than 108 species names have been proposed to classify diversity within the genus, reflecting a wide range of morphologies, plastic features, and ultimately taxonomic confusion (Widdowson 1971, Kraan 2020). Adding to taxonomic complications, inbreeding experiments have established successful crosses between *Alaria* and sometimes distantly related, even intrafamilial species, indicating reproductive barriers are permeable (Kraan and Guiry 2000, Liptack and Druehl 2000). Hybridization studies, in concert with DNA barcoding efforts, have thus reduced the number of proposed *Alaria* species to eight, of which *A. angusta* and *A. ochotensis* have yet to be verified with molecular data (Fig. 1; Lane et al. 2007, Kraan 2020).

Confusion surrounding diversity within *Alaria* has persisted despite extensive efforts to DNA barcode species. The first comprehensive DNA barcoding survey of the genus was conducted by Lane et al. (2007), who employed the partial mitochondrial gene cytochrome *c* oxidase I (*cox*I-5P), the nuclear internal transcribed spacer (ITS) of the ribosomal cistron, and the chloroplast RuBisCO operon spacer (*rbc*Sp) to assess species' delineations recognized on the basis of morphology. Given morphological species intermixed throughout genetic groups, the now synonymized species *Alaria nana*, *A. taeniata*, and *A. tenuifolia* were folded into *A. marginata* (Lane et al. 2007). Lane et al. (2007) hypothesized genetic patterns in the Northeast Pacific were driven by past isolation during Pleistocene glaciation events, allowing nuclear and mitochondrial genomes to diverge before recombining during the current day interglacial period, a hypothesis further validated with larger surveys of organellar and microsatellite DNA in the Gulf of Alaska (Grant and Bringloe 2020). On the basis of ITS region data, Lane et al. (2007) further proposed an "*A. esculenta*" clade that included *A. esculenta*, *A. crispa*, *A. praelonga*, and *A. crassifolia*; however, species statuses remained in

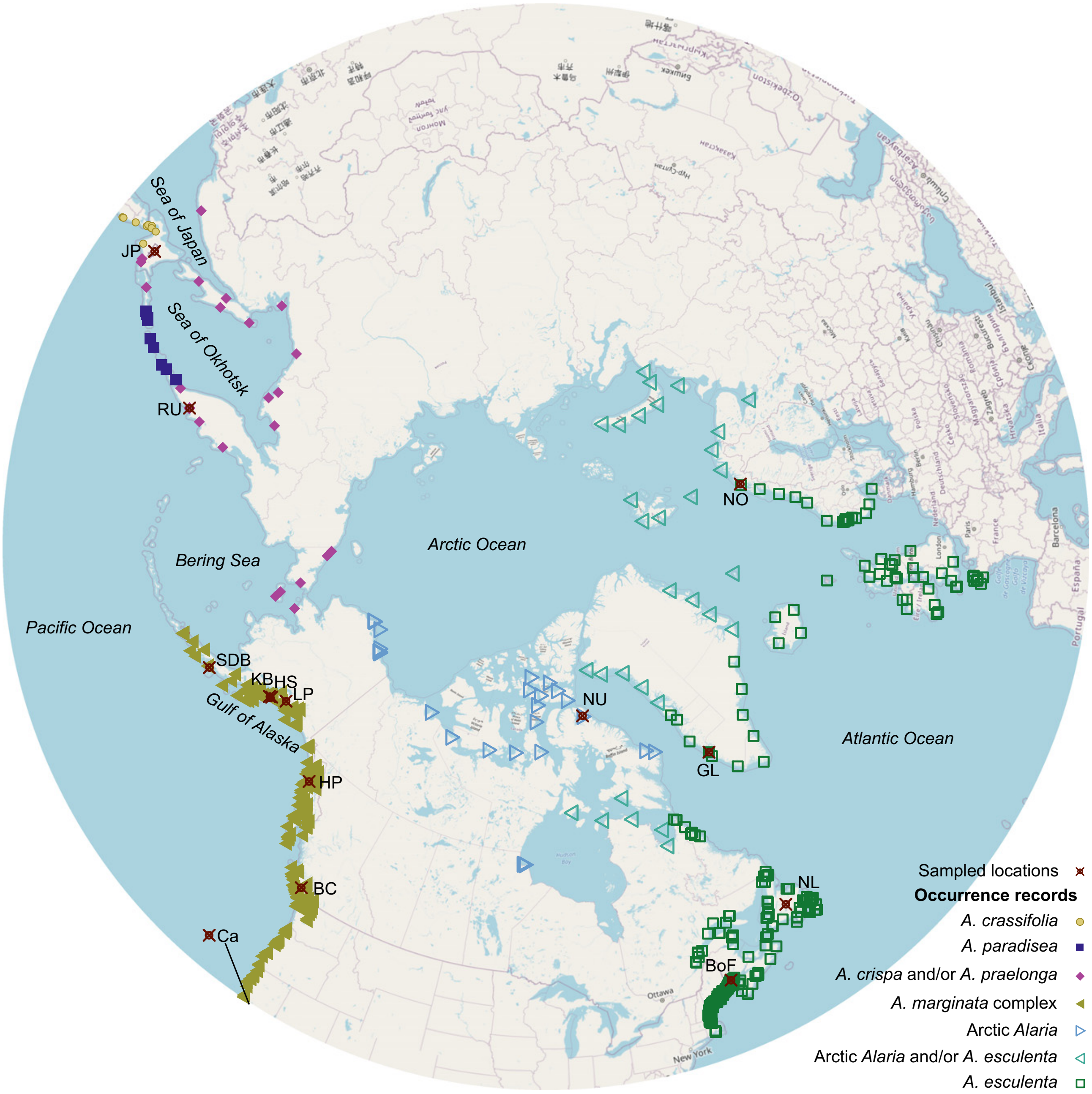

FIG. 1. Distribution map of *Alaria* species occurrence records, and locations sampled (brown dots) for the current study. Distributions are curated based on previous molecular studies (viz. Lane et al. 2007, Klimova et al. 2018a,b, Bringloe and Saunders 2019, Klochkova et al. 2019, Bringloe et al. 2020b, Grant and Bringloe 2020), and the current study. Note, not depicted here are *Alaria angusta* and *A. ochotensis* purportedly from the sea of Okhotsk (species thus far not supported with molecular data); see also the distribution map of Kraan (2020). Occurrence data derived from the Lüning (1990), the Macroalgal Portal, Barcode of Life Data System, and the above studies (data available on Figshare: https://doi.org/10.6084/m9.figshare.14740959.v1). JP = Japan; RU = Kamchatka, Russia; SDB = Sand Dollar Beach (Sand Point); KB = Kayak Beach (Kachemak Bay); HS = Homer Spit (Kachemak Bay); LP = Lowell Point (Seward); HP = Halibut Point (Sitka); BC = British Columbia (Vancouver Island); NU = Nunavut, Canada; GL = Greenland; NO = Norway; NL = NewFoundland, Canada; BoF = Bay of Fundy, Canada. [Color figure can be viewed at wileyonlinelibrary.com]

effect given support from organellar markers. The taxonomic standing of *A. crispa* and *A. esculenta* as separate species remains supported by morphological and molecular investigations, but has been debated in recent years (Klimova et al. 2018a, Bringloe and Saunders 2019).

Here, we take a genomic approach to understanding species diversity in the genus *Alaria*. Our main objective was to clarify the nature of phylogenetic relationships within *Alaria* using WGS data. Specifically, we: (i) investigated evolutionary scenarios within the genus by comparing phylogenetic results across genomic compartments (i.e., mitochondrial, chloroplast, and nuclear SNP datasets), testing the assumption that species relationships behave in a tree-like manner, and generating time-calibrated organellar phylogenies, and (ii) considered whether phylogenetic results based on standard DNA barcode markers faithfully reproduce patterns detected in fully resolved genomes. Though our objectives have implications for systematics, we did not formally pursue taxonomic revisions. Rather, we sought to provide a new framework for understanding diversity and phylogenetic relationships in *Alaria* by enhancing the resolution of genetic datasets, thus clarifying some of the confusion that has persisted despite decades of experimental and molecular research within the genus.

MATERIALS AND METHODS

Whole-genome sequencing and SNP-calling. Sporophyte tissue of *Alaria* was collected from Kamchatka (Russia), the Gulf of Alaska (Sand Dollar Beach, Sand Point [SDB]; Kayak Beach, Kachemak Bay [KB]; Homer Spit, Kachemak Bay [HS]; Lowell Point, Seward [LP]; Halibut Point, Sitka [HP], British Columbia (Vancouver Island, Canada [BC]), the eastern Canadian Arctic (Eclipse Sound, Nunavut), Southwest Greenland, the Bay of Fundy (Atlantic Canada), and northern Norway (Troms; Table 1, Fig. 1). Gametophyte samples were additionally sourced from the Kobe University Macroalgal Culture Collection (KU-MACC). Total genomic DNA was extracted using either a modified CTAB protocol (Cremen et al. 2016) or a QIAGEN DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Extracted DNA was sent to either GENEWIZ (Suzhou, China), where libraries were generated using the Illumina VAHTS Universal DNA Library prep kit and protocols, and sequenced on the NovaSeq System (paired-end, 150 bp reads, ~15–30 Gb of data/specimen), or BGI (Hong Kong), where libraries were generated using standard BGI protocols, and sequenced on the DNBSEQ-G400 platform (paired-end, 150 bp reads, ~20 Gb of data/specimen, except in gametophyte culture samples [KU-791, KU-793, KU-1164, KU-3288] wherein 50 GB of data · specimen^{-1} were generated). In total, five putative species of *Alaria* were sequenced, including *A. crassifolia* ($n = 1$), *A. praelonga* ($n = 1$), *A. crispa* ($n = 4$; considered *A. esculenta* sensu lato by some authors; Klochkova et al. 2019), *A. marginata* ($n = 7$), Arctic *Alaria* ($n = 5$), and *A. esculenta* ($n = 4$; Table 1), representing over 550 GB of data and over 2.2 billion paired reads. Note, specimens initially identified as *A. esculenta* collected from Baffin Island, Nunavut, Canada, are referred to as Arctic *Alaria* throughout the text. Prior to analysis, all reads were trimmed using Trimmomatic v.11.0.2 (Bolger et al. 2014), with a hard trim of the first 15 bp, trimming bases with a quality score below 10 from the 3′ end, and keeping reads with an average read quality score of 20 and minimum length of 75 bp.

Organellar genomes were de novo assembled for all samples using default settings in NOVOPlasty v.4.2 (Dierckxsens et al. 2017). Read coverage typically exceeded 1,000× for the organelles. SNPs for the organellar genomes were called by aligning organellar genomes using the MAUVE alignment function (Darling et al. 2004) in Geneious Prime v.2021.1.1 (Kearse et al. 2012).

Reference nuclear scaffolds for read mapping were generated from *Alaria esculenta* (KU-791) using SPAdes v.3.13.0 with specified k-mer values of 21, 33, 55, 77 (Nurk et al. 2013). The assembly contained 438.3 Mbp in 508,190 scaffolds, with a maximum scaffold size of 1.28 Mbp, N50 of 5,122 bp, L50 of 20,285, and peak read coverage of ~60× (Fig. S1 in the Supporting Information). The genome size of *A. esculenta* is reported to be 612.5 Mbp (Kapraun 2005), a typical size reported in other kelp (e.g., Ye et al. 2015, Shan et al. 2020). Note also that repeat regions are abundant in brown algae, accounting for 54% of the whole genome in *Undaria pinnatifida*, which is of the same family as *Alaria* (Alariaceae). Given the limitation of short-read data, these portions of the *A. esculenta* genome cannot be accurately represented in our draft assembly. Scaffolds <1,000 bp were removed from the assembly, after which 79,128 remained. Kraken2 v.2.0.8 (Wood and Salzberg 2014) was used to classify and remove bacterial and human scaffolds using the standard Kraken2 database (i.e., unclassified-out flag was used), which includes complete genomes for bacteria, archaea, viruses, and humans, along with known vectors (built March 18, 2019), resulting in another 49,514 scaffolds removed. Finally, 2,152 scaffolds with >60 kmer coverage (~140× read coverage) were removed to ensure organellar sequences, and high copy regions were not present in the reference nuclear scaffolds. The final reference contained 83.5 Mbp in 27,462 scaffolds, with a maximum scaffold size of 56,189 bp, N50 of 3,854 bp, and L50 of 5,988. Note, our strategy was to conservatively retain high confidence reference scaffolds for read mapping, rather than assemble a relatively contiguous draft genome of *A. esculenta.* The final scaffolds used for read mapping can be assessed via Figshare (https://doi.org/10.6084/m9.figshare.14740959.v1).

To call nuclear SNPs, reads for each specimen were mapped to the reference nuclear scaffolds for KU-791 described above. This was done using Bowtie2 v.2.3.4 (Langmead and Salzberg 2012), and using a 10% divergence threshold for mapping high-quality reads. SAM files were then converted to BAM format and sorted using SAMtools v.1.9 (Li et al. 2009). BCFtools v.1.9 (Danacek et al. 2021) was used to compile all the sorted BAM files into VCF format, call SNPs, and filter according to the following criteria: heterozygous SNPs with allelic balance >5 or <0.2 were discarded; SNPs with a quality score of ≥30 (i.e., 1/1,000 chance of a calling error) were kept; SNPs with a minimal read depth of 15 and a maximal read depth of 100 were kept (roughly twice the mean coverage in our gametophyte samples for which more sequencing was conducted); SNP sites passing the filtering criteria in all specimens were kept (i.e., no missingness). A combination of BCFtools and VCFtools v.0.1.16 (Danacek et al. 2011) were used to filter the SNPs. First, the +setGT plugin in BCFtools was used to change SNP calls to missing according to the allelic balance and read depth criteria. Filtering commands in VCFtools were then used to remove sites with low-quality scores and missing data. Indels were not kept for analysis. The filtering parameters kept 148,542 SNPs. The nuclear SNPs were then pruned for loci in linkage disequilibrium (LD) using PLINK v.1.9 (Purcell et al. 2007), such that sites with an r^2 value exceeding 0.1 were removed using a 50 variant count sliding window. Our intent was to thin sites as a means to minimize LD, compensating for the fragmented nature of the nuclear scaffolds, which prevented analysis across large genetic distances. The nuclear dataset used for ADMIXTURE analysis (Alexander and Lange 2011) consisted of 24,242 SNPs. In order to produce a rooted nuclear SNP tree, a SNP dataset including *Undaria pinnatifida* was also generated. This

TABLE 1. *Alaria* and *Undaria* specimen list and accession information. Short read data generated for this project fall under BioProject PRJNA675898.

Species	Sample ID	Date collected	Collector	Lat.	Long.	SRA	Mito	Chloroplast
A. crassifolia	KU-1164	NA	NA	NA	NA	SAMN20286326	MZ488957	MZ504650
A. crispa	A1	9-Jul-2018	A. Klimova	53.259438	159.777157	SAMN20286318	MZ488949	MZ504642
A. crispa	A5	19-Jul-2018	A. Klimova	52.637206	158.407990	SAMN20286319	MZ488950	MZ504643
A. crispa	A6	24-Oct-2018	A. Klimova	52.912625	158.636948	SAMN20286320	MZ488951	MZ504644
A. crispa	A8	21-Aug-2018	A. Klimova	52.913000	158.637000	SAMN20286339	MT767059	MT767060
A. esculenta	AT001	06-Jun-2019	G. Saunders	45.044000	−66.809000	SAMN20286338	MT767061	MT767062
A. esculenta	TTB000076	23-Aug-2019	D. Krause-Jensen, B. Olesen	64.079000	−51.467000	SAMN20286336	MZ488967	MZ504660
A. esculenta	TTB0000137	20-May-2019	K. Filbee-Dexter	69.652460	17.887450	SAMN20286337	MZ488968	MZ504661
Arctic *Alaria*	PI001	29-Aug-2019	K. Filbee-Dexter	72.753500	−77.622150	SAMN20286329	MZ488960	MZ504653
Arctic *Alaria*	PI20	29-Aug-2019	K. Filbee-Dexter	72.753500	−77.622150	SAMN20286330	MZ488961	MZ504654
Arctic *Alaria*	TTB000023	29-Aug-2019	K. Filbee-Dexter	72.753500	−77.622150	SAMN20286333	MZ488964	MZ504657
Arctic *Alaria*	TTB000026	29-Aug-2019	K. Filbee-Dexter	72.753500	−77.622150	SAMN20286334	MZ488965	MZ504658
Arctic *Alaria*	TTB000053	29-Aug-2019	K. Filbee-Dexter	72.753500	−77.622150	SAMN20286335	MZ488966	MZ504659
A. esculenta	KU-791	NA	NA	NA	NA	SAMN20286324	MZ488955	MZ504648
A. marginata	KU-793	NA	NA	NA	NA	SAMN20286325	MZ488956	MZ504649
A. marginata	LP-3	18-May-2019	S. Grant	60.349970	−149.26320	SAMN20286328	MZ488959	MZ504652
A. marginata	HP-4	19-Mar-2019	S. Grant	57.432900	−135.22310	SAMN20286321	MZ488952	MZ504645
A. marginata	HS-2	19-May-2019	S. Grant	59.363300	−151.25350	SAMN20286322	MZ488953	MZ504646
A. marginata	KB-10	20-May-2019	S. Grant	59.212000	−151.55570	SAMN20286323	MZ488954	MZ504647
A. marginata	SDB-15	29-Aug-2019	S. Grant	55.202160	−160.25610	SAMN20286332	MZ488963	MZ504656
A. marginata	SAM001	04-Aug-2020	S. Starko	48.411780	−123.37870	SAMN20286331	MZ488962	MZ504655
A. marginata	A93071	NA	NA	NA[a]	NA	SRS4572731	MN395660	MZ156044
A. praelonga	KU-3288	NA	NA	NA	NA	SAMN20286327	MZ488958	MZ504651
U. pinnatifida	M23	NA	NA	NA	NA	SRR10224200	NC_023354	NC_028503

Samples collected were wild sporophytes, with the exception of gametophyte cultures (KU-XXX) of Kobe University Macro-Algal Culture Collection and *Undaria* (Shan et al. 2020). NA = Not available; SRA = Short read archive.

[a] Collected from Santa Cruz, California, USA.

dataset was generated using the same methods described above, except using a threshold of 20% for read mapping to the *U. pinnatifida* genome, filtering to remove SNPs from low complexity and repeat regions, and filtering for a minor allele frequency of 0.03 to retain phylogenetically informative SNPs. The final dataset consisted of 21,614 SNPs, of which 2,255 were phylogenetically informative (i.e., variable) within *Alaria* and used for phylogenetic analysis. All the commands described above are provided in the supplemental material (Appendix S1 in the Supporting Information). All SNP datasets, raw and filtered and in VCF and fasta formats, are available on Figshare (https://doi.org/10.6084/m9.figshare.14740959.v1).

Phylogenetic analysis and hybridization analyses. Maximum-likelihood (ML) phylogenetic trees were built for each organellar genome, using *Undaria pinnatifida* as an outgroup in order to root the trees. Mitochondrial *cox*1 and chloroplast *rbc*L trees were additionally generated, as these represent standard DNA barcodes (i.e., amplicon level resolution). RAxML trees of the organellar markers/genomes were produced in Geneious Prime v.2021.1.1 using a GTR GAMMA substitution model (Stamatakis 2014); gene trees were partitioned according to codon position. The nuclear SNP dataset (without *Undaria*, and prior to LD pruning) was visualized as an uncorrected distance phylogenetic network. A network approach had the advantage of not forcing tree-like topologies and additionally helped to visualize any uncertainty or shared genetic information among species (i.e., spread out edges in the networks). In order to visualize nuclear SNPs as a network, the VCF file was converted to fasta format using

PGDSpider v.2.1.1.5 (Lischer and Excoffier 2012) before importing into SplitsTree v.5.2.24 (Huson and Bryant 2006). A ML tree of the nuclear SNPs was also produced using a GTR substitution model in Geneious, both for the pruned dataset to accompany the ADMIXTURE analysis (24,242 SNPs), the SNPs dataset containing *U. pinnatifida* (2,255 SNPs), and the full SNP dataset of just *Alaria* (148,542 SNPs). Results from the analysis with *Undaria* were used to root the ML tree of 148,542 SNPs. A phylogenetic network was also generated from the dataset after pruning for LD (Fig. S2 in the Supporting Information).

To test for signatures of mixed ancestry within species, the pruned dataset, consisting of 24,242 SNPs, was analyzed using ADMIXTURE at *k* values 2–6. Loglikelihood values and 5-fold cross-validation error at the various values of *k* are provided in Figure S3 in the Supporting Information. The output was visualized as barplots in R (R Core Team 2021). A PCA of the pruned SNP dataset was also generated in R using the output from PLINK in order to inform outgroup positions in the nuclear phylogeny (Fig. S4 in the Supporting Information). The pruned nuclear SNP dataset was further assessed for signs of hybridization among species using Dsuite v.0.4 (Malinsky et al. 2021). Specifically, D (ABBA-BABA) and f_4-ratios were calculated for trios of *Alaria*. The D statistic measures the number of shared alleles between the specified trios, and investigates departures of shared allele frequencies expected solely from incomplete lineage sorting; put differently, D measures the "tree-likeness" of a phylogeny. Given species P1, P2, and P3, with known relationships ((P1, P2) P3), P3 is expected to randomly sort with P1 and P2 at the same frequency (i.e., incomplete lineage sorting). Departures in the sorting frequencies therefore indicate migration or hybridization of genetic information (depending on whether the test is conducted at the population or species level). Departures from $D = 0$ are assessed by jackknifing the dataset into 20 blocks; the range in D values obtained is used to calculate its standard error, which is divided into the overall D statistic to produce the *Z*-score (*Z*-cores > 2–3 are typically considered significant).

Two hybridization analyses were conducted, one to assess hybridization in the "*Alaria esculenta* complex" and one to assess in the "*Alaria marginata* complex," as per Lane et al. (2007). The *A. esculenta* complex comprised *A. praelonga*, *A. crispa*, Arctic *Alaria*, and *A. esculenta*. Based on the nuclear SNP results, three *A. marginata* lineages were identified for the Dsuite analysis: the specimen from British Columbia (Canada); two specimens midway between British Columbia and the Eastern Aleutian Islands, comprising HP and LP; and three specimens farther along the Gulf of Alaska, and into the Aleutian Islands, comprising HS, KB, and SDB (Fig. 1). The p-value threshold for the analysis of the *A. esculenta* complex was corrected for the four tests performed, setting alpha to 0.0125. Note that, in the analysis of the *A. esculenta* complex, data for *A. praelonga* was from a haploid specimen, which had the potential to bias allele frequencies in this species. Though an important caveat, we expected the impact on allele frequencies to be minimal given the analysis was performed at the species level (i.e., most variants were likely to represent fixed differences, as in homozygous alleles).

Time calibrated organellar phylogenies. To infer the timing of diversification and putative hybridization events, we produced two separate time-calibrated phylogenies based on mitochondrial and chloroplast gene sets, respectively ($n = 8$ genes per organelle; Mitochondrion: *atp*6, *atp*8, *atp*9, *cox*2, *cox*3, *nad*2, *nad*3, *rpl*2, *rps*2; Chloroplast: *atp*A, *atp*B, *dna*B, *pet*A, *psa*A, *psa*B, *psb*A, *rbc*L). We included species from across the Laminariales-Chordales clade for which organellar genomes were available (or sequenced in the present study) totaling to 40 taxa for the mitochondrial analysis and 33 taxa for the chloroplast analysis. To preserve well-described relationships inferred from genome-level datasets of each organelle (Starko et al. 2019), we conducted phylogenetic reconstruction in RaxML v.8.2.12 using a constraint tree at the level of genus. Support values for intrageneric relationships was calculated during the RaxML analysis by conducting 500 bootstrap replicates. We conducted an initial time calibration using penalized-likelihood implemented in the "chronos" package in R (Vrahatis et al. 2016). The resulting tree was then used as a constraint tree for molecular clock analysis conducted in BEAST v.1.10.4 by de-selecting the tree topology operators in BEAUti v.1.10.4 (Drummond et al. 2012). BEAST analyses were run for 5,000,000 iterations and all ESS values were >200, indicating convergence. A single, log-normal time-calibration (minimal age 13 million years ago) was used based on the Monterey Formation Miocene Deposits that includes the only reliable kelp fossil dated to 13 million years ago and believed to be an ancestor to the "giant kelp" lineage (*Macrocystis, Nereocystis, Pelagophycus, Postelsia*; Parker and Dawson 1965).

RESULTS

Whole-genome sequencing datasets were applied to the kelp *Alaria* in order to make evolutionary inferences and better understand the nature of phylogenetic relationships within the genus. Across species of *Alaria,* the number of SNPs in fully resolved organellar genomes was two orders of magnitude greater than the number of SNPs resolved in standard DNA barcode markers. The 658 bp fragment of *cox*I-5P featured 47 SNPs, with a maximal divergence between sequences of 4.41%, while the fully resolved mitochondrial genomes featured 3,814 SNPs in *ca.* 39 Kbp and a maximal divergence between genomes of 4.84% (Fig. 2A). The topology of the *cox*I-5P phylogenetic tree faithfully reflected the topology of the full mitochondrial genomes, with larger bootstrap values in the full mitochondrial tree (Fig. 2A). Meanwhile, *rbc*L featured 22 SNPs in 1,467 bp and maximal divergence between sequences of 0.87%, whereas the fully resolved chloroplast genomes featured 4,536 SNPs in *ca.* 130 Kbp and maximal divergence between genomes of 2.02% (Fig. 2B). The *rbc*L tree, however, failed to meaningfully produce relationships reflected in the full chloroplast tree, and even placed Arctic and Atlantic *Alaria* as non-sister taxa (Fig. 2B). The *rbc*L tree topology featured low bootstrap values, in contrast to the full chloroplast genome tree, which consistently displayed full node support (Fig. 2B). The ML tree of nuclear SNPs similarly displayed nearly perfect bootstrap support, with the exception of a few intraspecific nodes (Fig. 3B). Conflicting topologies, however, were captured in the network of nuclear SNPs, as evidenced by edges representing shared genetic information across lineages (Fig. 3C).

The full organellar phylogenies and nuclear SNP network revealed lineages not present in the DNA barcode data, most notably an Arctic lineage of *Alaria* collected from Nunavut, Canada, previously collected as *A. esculenta* (but referred to as Arctic *Alaria*

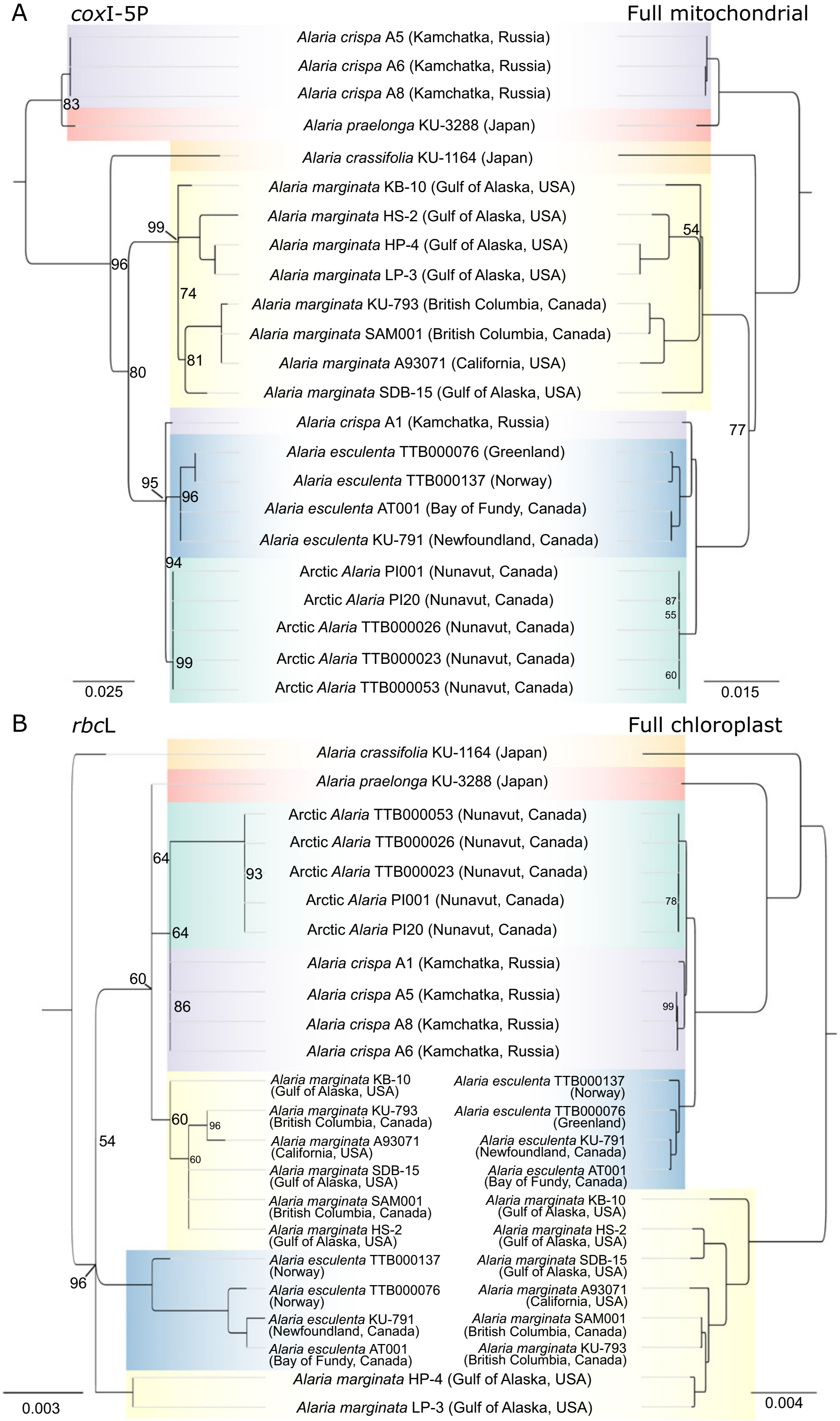

FIG. 2. Maximum-likelihood (ML) organellar phylogenies in *Alaria*. The root node *Undaria pinnatifida* has been trimmed from the trees; the following SNP counts did not include *Undaria* in the alignments. A. *cox*I-5P ML tree, based on 47 SNPs in 658 bp and maximal divergence between sequences of 4.41%. B. Full mitochondrial genome ML tree based on 3,814 SNPs in ca. 39k bp and maximal divergence between genomes of 4.84%. C. *rbc*L ML tree, based on 22 SNPs in 1,467 bp and maximal divergence between sequences of 0.87%. D. Full chloroplast genome ML tree, based on 4,536 SNPs in ca. 130k bp and maximal divergence between genomes of 2.02%. Bootstrap values of 100 are not indicated, and intraspecific nodes are indicated with smaller font size. [Color figure can be viewed at wileyonlinelibrary.com]

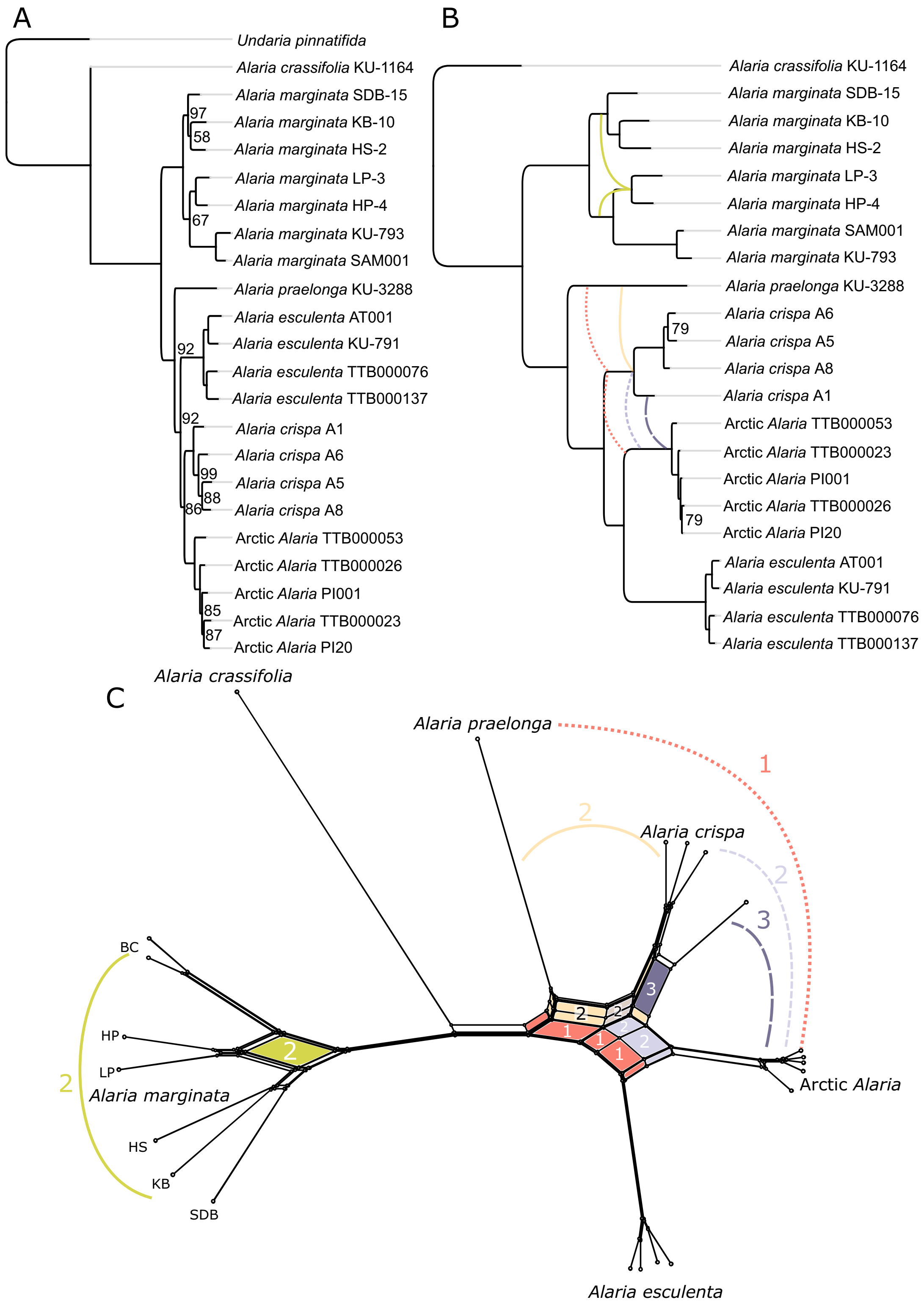

FIG. 3. Phylogenetic relationships in nuclear SNPs among species of *Alaria*. A. Maximum-likelihood (ML) phylogeny of 2,255 SNPs rooted with *Undaria pinnatifida*. B. ML phylogeny of 148,542 nuclear SNPs, rooted according to relationships inferred with previous tree using *Undaria*; added reticulations represent hypothesized hybridizations also depicted in the nuclear SNP network. C. Phylogenetic network of 148,542 nuclear SNPs, with hypothesized hybridizations depicted from early (1) to latest (3); the same edges tested for significant signs of hybridization using ABBA-BABA tests (Table 2). Bootstrap values in the ML trees are 100% unless otherwise indicated. [Color figure can be viewed at wileyonlinelibrary.com]

in this study; Figs. 1, 2, 3). Several *A. marginata* lineages were also confirmed, in particular, mitochondrial genomes differentiated British Columbia (Canada) and more southern California (USA) specimens, and full chloroplast genomes further reflected lineages not present in the *rbc*L data (Fig. 2).

Topologies of the phylogenetic trees differed according to the genome being considered. In particular, *Alaria crispa* was either closely related to *A. praelonga* or *A. esculenta* in the mitochondrial phylogeny, but always closely related to Arctic *Alaria* in the chloroplast phylogeny (Fig. 2). Lineages in the *A. marginata* complex of the mitochondrial genome tree were inconsistent with the lineages in the chloroplast genome tree. In particular, specimens from HP and LP (midway through the Gulf of Alaska; Fig. 1) formed a clade with the more western HS specimen in the mitochondrial tree, but formed a clade with southern specimens BC and Ca in the chloroplast tree (Fig. 2). Specimens from SDB and HS (Aleutian Islands and Gulf of Alaska), also formed separate lineages in the mitochondrial tree, but were closely related in the chloroplast tree. Phylogenetic placement of earlier diverging species also differed across genomic compartments; while *A. praelonga*/*A.crispa* and *A. marginata* diverged from other species in the mitochondrial and chloroplast trees, respectively, the nuclear SNP analysis with *Undaria pinnatifida* as the root taxa supported *A. crassifolia* as the earliest diverging species (Fig. 3A,B; also supported by PCA, Fig. S4). In the phylogenetic network, *A. crassifolia*, *A. praelonga*, *A. crispa*, Arctic *Alaria*, and *A. esculenta* variously shared edges (Fig. 3C). Note that a nearly identical network was produced from the LD pruned SNP dataset (Fig. S2). The *A. marginata* complex similarly displayed edges shared among specimens sampled from midway through the Gulf of Alaska and specimens sampled farther west and into the Eastern Aleutian Islands (SDB, HS, KB) and British Columbia (BC; Fig. 3C).

ADMIXTURE results indicated various levels of shared ancestry at all the k values tested (Fig. 4). In particular, mixed ancestry in *Alaria crispa* and *A. praelonga* was evident when considering two ancestral populations ($k = 2$). One specimen of *A. crispa* showed mixed ancestry across all values of k, with some ancestry derived from Arctic *Alaria* (Fig. 4). Cross-validation error showed strongest support for four ancestral populations ($k = 4$), while the loglikelihood increased consistently with larger values of k (Fig. S3). Dsuite confirmed significant hybridization (large D values) across *A. esculenta*, Arctic *Alaria*, *A. crispa*, and *A. praelonga*, and admixture proportions (f_4-ratios) as large as 0.56 between Arctic *Alaria* and *A. crispa* (Table 2). The *A. marginata* complex similarly displayed significantly elevated hybridization values across three putative lineages in the nuclear network (Fig. 3C). Admixture proportions (f_4-ratio) were high (0.474) between western specimens (SDB, HS, KB) and populations located midway in the Gulf of Alaska (HP and LP; Fig. 1).

Molecular clock analyses conducted separately on each organelle ($n = 8$ genes per compartment) provided similar timelines for the diversification and hybridization within *Alaria*. Both chloroplast and mitochondrial trees (Fig. 5) suggest an initial diversification of the genus sometime between ~3.5–6 million years ago. Incongruencies between tree topologies appear to have manifested as early as this initial diversification. Other discrepancies between trees, however, appeared to correspond to more recent timeframes during the Pleistocene (2.6 million years ago and onwards).

DISCUSSION

Our main objective was to apply WGS datasets to the kelp genus *Alaria* to clarify the nature of phylogenetic relationships and infer evolutionary events, and on this basis showcase the strength of a WGS approach. We show that WGS vastly improved phylogenetic resolution compared to amplicon data, that an Arctic lineage had been overlooked by DNA barcoding efforts, and that hybridization is an important mechanism contributing to novel lineages across the genus. Thus, the paradigm that speciation is tree-like within the genus, and indeed potentially other kelp, must be replaced with a model that recognizes the fundamental importance of mixed ancestry. As far as we know, this is the first genus-wide assessment of diversity based on WGS datasets in a macroalga, and the first to resolve organellar and nuclear SNP variants at this scale (>148K SNPs) in a genus of kelp.

WGS reveals unique Arctic lineage. Among the surprising findings revealed by the phylogenies based on WGS was a unique Arctic lineage of *Alaria* (i.e., Arctic *Alaria*; Figs. 2, 3). This lineage was admittedly present in DNA barcode data (Bringloe et al. 2020b). Being only a couple of *cox*I-5P mutational steps from *A. esculenta*, however, it was previously assumed Arctic populations represented conspecifics (Fig. 1). A similar pattern of closely related *cox*I-5P phylogroups has also been reported in *Saccharina* (Neiva et al. 2018). We hypothesize the lineage corresponding to Arctic *Alaria* represents *Alaria grandifolia*, a synonymized taxon originally characterized by a notably long stipe and sporophylls, large blades (length and width), and an affinity to grow in deep, cold waters, sometimes in locations where *A. esculenta* is present at shallower depth (Edelstein et al. 1967). Though debate persisted whether to consider *A. grandifolia* a subspecies or a larger, deep-water ecotype of *A. esculenta*, Widdowson (1971) and Edelstein et al. (1967) concluded they were separate species. These debates continued until Kraan et al. (2001) demonstrated that purported specimens of

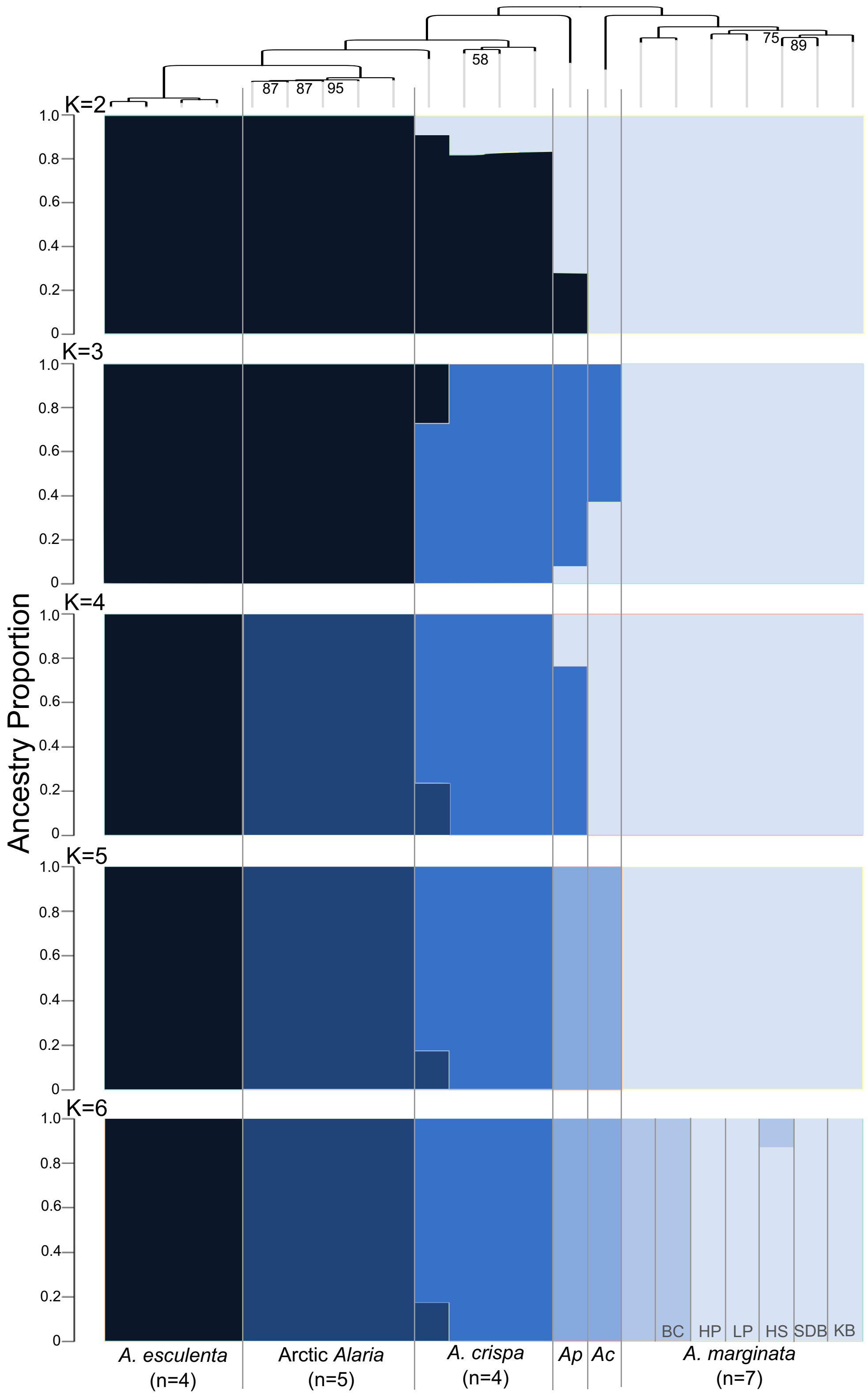

FIG. 4. ADMIXTURE plots for species of *Alaria* depicting mixed ancestry at five values of *k*, based on 24,242 unlinked nuclear SNPs. A maximum-likelihood tree is depicted at the top, wherein bootstrap support is 100% unless otherwise indicated. Ap = *Alaria praelonga*; Ac = *Alaria crassifolia*; *Alaria marginata* complex: SDB = Sand Dollar Beach (Sand Point); KB = Kayak Beach (Kachemak Bay); HS = Homer Spit (Kachemak Bay); LP = Lowell Point (Seward); HP = Halibut Point (Sitka); BC = British Columbia (Vancouver Island). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. DSuite ABBA-BABA results testing for patterns of hybridization (or tree-likeness) among species of *Alaria* based on 24,424 unlinked nuclear SNPs.

(((P1	P2) ↔ P3)O)		D	Z-score	P	f_4-ratio
Alaria esculenta complex						
A. esculenta	*A. crispa*	*A. praelonga*	0.3037	13.67	<0.001	0.1264
A. esculenta	Arctic *Alaria*	*A. crispa*	0.3676	20.35	<0.001	0.5642
Arctic *Alaria*	*A. crispa*	*A. praelonga*	0.0610	3.19	<0.001	0.0202
A. esculenta	Arctic *Alaria*	*A. praelonga*	0.2997	13.37	<0.001	0.1082
Alaria marginata complex						
BC	LP+HP	KB+HS+SDB	0.3262	13.77	<0.001	0.4741

In each test, P3 is expected to sort at equal frequencies with P1 and P2 under incomplete lineage sorting (D = 0). Departures from D = 0 are corrected for multiple tests, such that α = 0.0125 for the *A. esculenta* complex. The tested tree topology is indicated in brackets (O = *Alaria marginata* for *Alaria esculenta* complex, and Arctic *Alaria* for *A. marginata* complex); significant results indicate excess sharing of alleles between P2 and P3. Sample sizes are: *A. esculenta*, n = 3; Arctic *Alaria*, n = 5; *A. crispa*, n = 4; *A. praelonga*, n = 1; *A. marginata*, n = 6. SDB = Sand Dollar Beach (Sand Point); KB = Kayak Beach (Kachemak Bay); HS = Homer Spit (Kachemak Bay); LP = Lowell Point (Seward); HP = Halibut Point (Sitka); BC = British Columbia (Vancouver Island).

A. grandifolia collected from Spitsbergen (its type locality) readily interbred with *A. esculenta* collected from Ireland (interestingly, the broad form was always produced from these crosses). Furthermore, since *rbc*L, RuBisCo spacer, and *rbc*S amplicons did not differentiate specimens of *A. grandifolia* from *A. esculenta* from Canada, *A. grandifolia* was folded into *A. esculenta* (Kraan et al. 2001).

Our results resurrect the debate on whether *Alaria grandifolia* should be reinstated. First hybridization among species, as discussed here and in other work (Kraan and Guiry 2000), indicates reproductive isolation is not a consistent proxy for species boundaries in *Alaria*. Second, though organellar genomes were modestly differentiated in the specimens of Arctic *Alaria*, nuclear SNPs showcased deep divergence between Atlantic and Arctic specimens of *Alaria* (Fig. 3B), as might be expected from selective forces driving a deep-water, Arctic lineage. The morphology and life history of *A. grandifolia* are well-suited for the Arctic environment; in particular, broad blades capture limited sunlight in deep, often turbid, waters during a short growing season, and growth at depth avoids ice scour in the Arctic. Though beyond the scope of the current study, we hypothesize that these features are reflected in the

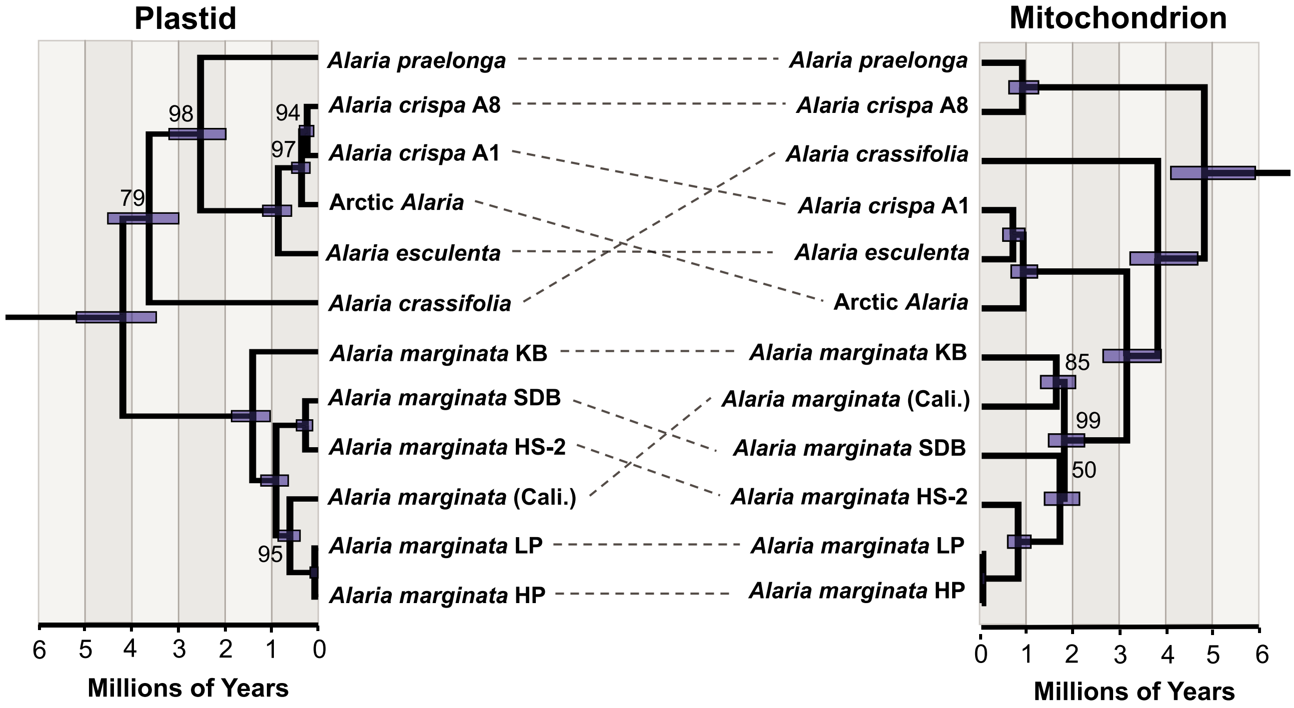

FIG. 5. Time calibrated mitochondrial and chloroplast phylogenies based on gene sets (n = 8 genes) from each compartment. Branch lengths are in millions of years and node bars represent 95% highest posterior densities. SDB = Sand Dollar Beach (Sand Point); KB = Kayak Beach (Kachemak Bay); HS = Homer Spit (Kachemak Bay); LP = Lowell Point (Seward); HP = Halibut Point (Sitka); Cali = Santa Cruz (California). Support values are bootstraps support from ML reconstruction and values of 100% are excluded. [Color figure can be viewed at wileyonlinelibrary.com]

underlying genome of Arctic *Alaria*. An interesting analogue system is the endolithic green alga *Ostreobium*, whose genome clearly exhibits an expansion of light-harvesting proteins and loss of photoprotective and photoreceptor genes, presumed adaptations to low and variable light environments (Iha et al. 2021).

Linking the Arctic lineage of *Alaria* to *A. grandifolia* can be established by generating WGS data from Spitsbergen (Svalbard), the type locality of *A. grandifolia*. A transition zone between Arctic and temperate *Alaria* is expected, where *A. esculenta* is likely phased out at high latitudes due to ice scour. As such, future efforts should collect across the extremes of depths where *Alaria* is expected to occur, to confirm whether both putative *A. grandifolia* and *A. esculenta* are present. Note that *A. grandifolia* is also not likely confined to the Arctic. Edelstein et al. (1967) report a deep-water population in Nova Scotia (Canada), which the authors associated with characteristic Arctic flora at that location (including *Agarum*, *Laminaria* spp., *Desmarestia* spp., *Ptilota*, and *Phycodrys*; see also Wilce 2016, and Bringloe et al. 2020b for characteristic Arctic flora). Crucially, we note that Edelstein et al. (1967) indicated the lower depth limit of *A. esculenta* was 8 m, whereas specimens of *A. grandifolia* were collected from 12 to 19 m. Kraan et al. (2001) collected specimens on Spitsbergen from 5 to 10 m, possibly missing *A. grandifolia* (instead collecting *A. esculenta*). Interestingly, Lüning (1990) recognized *Alaria pylaiei* (Bory) Greville, another traditionally recognized Atlantic species, and *A. grandifolia* as northern forms of *A. esculenta*, while Widdowson (1971) suggested that *A. grandifolia* was a large Arctic form of *A. esculenta*. Note that *A. pylaiei* (type locality in Newfoundland, Canada) outranks *A. grandifolia* for taxonomic priority if both names can be linked to the Arctic lineage. In sum, WGS evidence points to *A. grandifolia* as a distinct species, adapted to deep, cold water environments throughout the Arctic and North Atlantic. Unfortunately, some populations from the southern range of purported *A. grandifolia* may already be extirpated, as Arctic conditions contract northward (Assis et al. 2018).

Recurrent hybridizations in Alaria. Signatures of hybridization were present throughout species of *Alaria*. The most compelling case was *A. crispa*, wherein its positions in organellar genome phylogenies were incongruent with nuclear genomic phylogenies (Figs. 2, 3). In particular, *A. crispa* featured two distinct mitochondrial genomes in the four specimens sequenced, one closely related to *A. praelonga*, the other closely related to *A. esculenta* (Fig. 2A). Meanwhile, the chloroplast genome was a consistent close match to Arctic *Alaria* (Fig. 2B). Altogether, these results suggest organellar capture occurred repeatedly in *A. crispa*, a conclusion further supported by the nuclear network and ADMIXTURE results that showed *A. crispa* shares phylogenetic signal with *A. praelonga* and Arctic *Alaria*. (Figs. 3C, 4). Dsuite results ruled out incomplete lineage sorting (Table 2), suggesting vertical evolution followed by horizontal transfers (i.e., hybridizations) led to sharing of nuclear genomic information.

Hybridizations early in the evolution of *Alaria* were potentially formative events leading to novel lineages. The nuclear network showcased edges representing shared nuclear SNPs between *A. praelonga*, *A. crispa*, and Arctic *Alaria*, and ADMIXTURE results indicated mixing in *A. crispa* and *A. praelonga* when considering two ancestral populations ($k = 2$; Fig. 4). Earlier hybridization and organellar capture events may also have led to different node topology deeper in the respective trees. While mitochondrial and chloroplast genomes supported *A. praelonga/crispa* and *A. marginata* as the earliest diverging lineages, respectively, the nuclear SNP analysis confidently placed *A. crassifolia* as the earliest diverging species (Figs. 2, 3A). We hypothesize that hybridization occurred early in the evolution of *Alaria*, potentially between Arctic/Atlantic and Pacific lineages, leading to a mixed ancestry lineage that would eventually differentiate into *A. praelonga*, *A. crispa*, and Arctic *Alaria*, followed by hybridization and organellar capture during the early Pleistocene among these later evolving species (Fig. 3C). Specimen A1 (collected from Kamchatka, Russia), in particular, appeared to be the result of recent mixing between *A. crispa* and Arctic *Alaria*, with backcrossing with nearby *A. crispa* populations likely washing out some of the genetic signature originally derived from Arctic *Alaria* (Figs. 2, 3). It is worth noting the contemporary distributions of each species (Fig. 1) match the patterns of hybridization described here, in that *A. crispa* is distributed midway between temperate Pacific *A. praelonga* and Arctic *Alaria*. The potential contributions of unsampled species/populations, including those now extinct, however, should not be overlooked. Nonetheless, these results shed new light on previous morphological and molecular work that often conflated these species (Lane et al. 2007, Klimova et al. 2018a, Bringloe and Saunders 2019).

Similar results were also observed in *Alaria marginata*. In particular, HP and LP specimens, sampled midway through the Gulf of Alaska (Fig. 1), were sister to northern specimen HS in the mitochondrial genome tree, but sister to southerly derived specimens (BC and Ca) in the chloroplast genome tree (Fig. 2). Meanwhile, the nuclear network indicated shared edges in HP and LP with two seemingly distinct lineages, SDB, HS, and KB in the north, and BC and Ca in the south (Figs. 1, 3C). No strategy was employed during collections to link the putative lineages to previously recognized morphological species *A. nana*, *A. taeniata*, and *A. tenuifolia*, though the substantial number of fixed

SNP differences between lineages (for instance, compared to populations of *A. esculenta* in the North Atlantic; Fig. 3B,C) suggest our results could potentially be linked to some of these previously recognized species. It is worth noting that all the *cox*I-5P lineages reported by Grant and Bringloe (2020) were recovered here, indicating most of the major genetic lineages of *A. marginata* ought to be represented here in our WGS datasets.

Our results suggest that north (Eastern Aleutian Islands) and south (California, USA; British Columbia, Canada) lineages have come into secondary contact midway through the Gulf of Alaska, as evidenced by organellar capture (Fig. 2), shared edges in LP and HP specimens (Fig. 3C), and significant *D* results (Table 2). Hybridizations between northern and southern populations took place in the early origins of the lineages in the *Alaria marginata* complex and have subsequently been affected by several glacial cycles in the Pleistocene (Fig. 5). These hybridizations between divergent northern and southern populations likely indicate the appearance of a geographic barrier to migration in the early Pleistocene. Contemporary genetic divergences between groups of coastal fishes (Withler et al. 2001), invertebrate (Sunday et al. 2014, Xuereb et al. 2018), and algal (Lindstrom 2006, 2009) populations in the northeastern Pacific have been attributed to a dispersal barrier produced by the North Pacific Current bifurcation into a north-flowing Alaska Coastal Current and south-flowing California Current (Cumins and Freeland 2007). Little is known, however, of the history of the bifurcation, its location, or the strength of diverging current systems over the Pleistocene. Nevertheless, southeast Alaska and northern British Columbia mark a biogeographic transition between the Gulf of Alaska ecoregion and the North American Pacific Fjordland (Spalding et al. 2007). Sequencing of more specimens and population genomic analyses are needed to further explore the geographic extent, timings of hybridizations, and level of reproductive isolation among *A. marginata* lineages.

A key question remains of how *Alaria* genomes hybridize. Chromosome numbers in *Alaria* suggest aneuploidy may be a significant barrier to hybridization. Species in the Northeast Pacific have a haploid chromosome number of 14, as does *A. crispa* (Klimova et al. 2017), whereas *A. praelonga* and *A. crassifolia* have a haploid number of 22 (reviewed by Kraan 2020). Robinson and Cole (1971) curiously report a haploid number of 24 for specimens they identified as *A. grandifolia* from Coronation Island (southern tip of the Gulf of Alaska, USA, a location the authors noted did not match the known distribution of *A. grandifolia* at the time; see also Robinson 1967). Meanwhile, *A. esculenta* is reported to have a haploid number of 28. Feller-Demalsy and Demalsy (1974), however, reported a haploid number of 14 in specimens from Atlantic Canada (St.-Laurent Estuary) and hypothesized Great Britain populations represented polyploids. Despite such differences in chromosome numbers, Kraan and Guiry (2000) made reciprocal crosses between *A. esculenta* populations and produced healthy sporophytes with sporophylls between reciprocal crosses of *A. praelonga* and *A. esculenta* (Irish populations), and between female *A. tenuifolia* and male *A. esculenta* (parthogenophytes were ruled out). Reciprocal crosses between *A. marginata* and *A. nana*, however, did not produce sporophytes. In sum, reproductive barriers appear to exist between some closely related species of *Alaria*, while these barriers are weakened between more distantly related species. Allopolyploidy followed by genome reductions may explain incompatible chromosome numbers among *Alaria* species. Interrogating the genomic data for signatures of gene duplications may shed light on the role of polyploidy in facilitating hybridizations and whether other mechanisms to achieve euploidy are at play.

Hybridization between species of kelp has long been the topic of phycological investigation, with intergeneric hybridizations even considered by Lewis and Neushul (1995). Druehl et al. (2005) demonstrated that molecular confirmation of parental genomes is necessary for putative hybrids as abnormal morphotypes proved an unreliable proxy, and that many of the crossings originally hypothesized in wild populations could not be confirmed. Hybridization is nonetheless expected to be common among species of kelp. For example, lamoxirene is a female pheromone used in kelps that indiscriminately induces sperm release and attraction to eggs (Maier and Müller 1986). As such, post-zygotic barriers to reproduction are expected to play a dominant role in maintaining kelp species boundaries. As well, Laminariales is a relatively young lineage, radiating within the past 30 Ma (Starko et al. 2019). In concert with potentially slow rates of evolution, reproductive barriers may be slow to develop. Hybridization has been recently confirmed using molecular analyses between wild populations of kelps, in particular, the interfamilial crossing of *Macrocystis pyrifera* and *Lessonia spicata* (Murúa et al. 2020), and intra- and intergeneric *Ecklonia-Eisenia* hybrids (Akita et al. 2021). Our results in *Alaria* add to the growing list of wild kelp populations featuring hybrid species, pointing to a possible re-emerging trend in the literature.

Similar hybridization dynamics may be widespread in brown algae. Hybridizations are especially notable among species of *Fucus* (Coyer et al. 2002, 2007, Moalic et al. 2011) and other fucoids (Hodge et al. 2010; see Bringloe et al. 2020a for examples in other taxa). Secondary contact and hybridizations may also be common in the Arctic environment, as suggested by our results with *Alaria*. Indeed, DNA barcoding efforts in numerous lineages of macroalgae mirror the patterns originally detected in *Alaria* (i.e., unique Arctic haplotypes; Bringloe et al. 2020b), suggesting substantial diversity and hybridizations remain undetected by amplicon sequencing at high latitudes. Microsatellite data in *Saccharina latissima* already point to hybridization in the Arctic (Neiva et al. 2018), a hypothesis also forwarded by McDevit and Saunders (2010). Similar evolutionary scenarios could therefore be explored in other macroalgae residing in the Arctic.

Do evolutionary inferences scale up from amplicon data to full genomes?. A key question remains of whether amplicon data faithfully reflect patterns in full genomes. In *Alaria*, phylogenetic analyses based on fully resolved organellar genomes generally lacked ambiguity, as evidenced by 100% bootstrap support for nearly all nodes (Figs. 2, 3B). The upgrade in resolution was particularly stark in the comparison of *rbc*L and chloroplast genome trees (Fig. 2B). The *cox*I-5P tree, on the contrary, was generally consistent with the full mitochondrial genome tree (Fig. 2A), a consolation for the numerous DNA barcode studies of macroalgae based on this marker. A couple of nodes remained poorly supported in the mitochondrial genome tree; deep partition in phylogenies based on chloroplast genomes are potentially better resolved given their rate of evolution is more conserved within brown algae (Starko et al. 2021). Our tree based on nuclear SNPs was also resolved with nearly 100% confidence (Fig. 3B), though topologies depended on whether the dataset was pruned for linkage disequilibrium (Fig. 4; specimen A1). Importantly, the topologies of the nuclear dataset differed depending on the region targeted (Fig. 3C). Further investigation with a less fragmented reference assembly is needed to rigorously assess heterogeneity in phylogenetic signal across the nuclear genomes of *Alaria*. Due to the high degree of confidence afforded by the genomic datasets, conflicts across genomic compartments were revealed that otherwise could have been incorrectly interpreted as different species or cast as uncertainties due to the limited windows provided by single genes, most notably the conflicting phylogenetic signal in the mitochondrial and chloroplast genomes of *A. crispa* and the *A. marginata* complex (Fig. 1), and differences in deeper node topology of trees (Figs. 2, 3A and B). Furthermore, these incongruences were readily explained based on insights regarding hybridizations gained from the nuclear genome.

Integrating high-resolution genetic information across genomic compartments, with comprehensive sampling across nuclear genomes, should be a high priority for phylogenetic investigations. Phylogenetic signal, to a large extent but with less confidence, does scale up from amplicon data to the genomic level in *Alaria*. Where amplicon data remain severely limited, however, is in providing one angle on a network of evolutionary hypotheses realized at the genomic level. Rather, the evolution of lineages in *Alaria* is a collection of isolation and secondary contact events over millions of years (Fig. 5), where lineages merge and peel away to various degrees. As such, the evolution of species in *Alaria* cannot possibly be captured in 2–3 markers, and species cannot be defined using a strict tree-like model of isolation and differentiation. Rather, a global (i.e., genomic) phylogenetic overview is needed to reflect the multifaceted, network-like nature of species relationships driven by ancestral mixing. We therefore expect WGS to become the gold standard for phylogenetic analysis in the near future, with less cost-prohibitive approaches (e.g., RADseq) continuing to ease the transition from DNA barcodes.

A cost-benefit analysis of WGS versus traditional sanger sequencing warrants consideration. The gross cost of WGS remains high relative to sanger sequencing, but the cost per unit of information is smaller in WGS. Assuming a bidirectional sanger sequencing cost of 10 USD/specimen, and a cost for WGS of 160 USD/specimen for 20GB of 150bp paired-end read data (the cost for the current project for ˜20× coverage on average for *Alaria*), we estimate the cost per SNP and per specimen of sanger sequencing is (10 + 10)/(47 + 22) = ˜0.29 USD, whereas the cost per SNP for WGS is 160/(3,814 + 4,536 + 148,542) = ˜0.00102 USD, 0.35% the cost of the sanger approach. Add to this the potential to further use the WGS data for functional genomic analysis, build reference databases, develop SNP arrays to fine-tune the accuracy of DNA barcodes, and characterize the holobiont, and the cost per unit of information is further diminished. Whether a sanger sequencing approach remains adequate for a given study objective or taxonomic group will remain uncertain until underlying assumptions regarding phylogenetic signal from single genes, particularly in organelles, are validated using WGS.

Conclusions. We have demonstrated the ability of WGS datasets to further uncover overlooked biodiversity, clarify phylogenetic relationships, and provide clear evidence of hybridizations. Nonetheless, several caveats to our analysis warrant discussion. Several species of *Alaria* were not included in our study. In particular, *A. paradisea*, an endemic species to the Kurile Islands, is well supported with molecular evidence (Klimova et al. 2018b). *Alaria angusta* and *A. ochotensis*, both native to the Russian Bering Sea and Sea of Okhotsk, were not included, nor are we aware of any molecular evidence to substantiate the existence of these species. The inclusion of any one of these lineages would have the potential to refine the conclusions presented here. Taxonomic names are also problematic. Here, *A. crispa* was applied to Kamchatka specimens; however, molecular investigations from Bering Island (type locality of *A. angusta*, *A. taeniata*, *A. lanceolata*, *A. laticosta*, and *A. praelonga*) could reveal names that have taxonomic priority, or forgotten species in need of resurrection, changing the designations reported here. Population-level analyses would reveal if the hybridization patterns reported here are fixed at the species level, or if events are limited to certain populations. Bolstering the number of specimens analyzed, particularly in *A. crassifolia* and *A. praelonga* where $n = 1$ in our study, should also be prioritized. Allele phasing should also be explored to tease apart parental lineages in putative hybrids, and adjustments to the SNP filtering criteria could be explored to maximize the amount of biological signal retrieved from WGS datasets. On a final note, our analysis was also limited to SNPs, without any exploration of functional genomic aspects unique to each species. As alluded to with *A. grandifolia*, this avenue promises to yield rich insight on evolution within this genus, and, in concert with transcriptomic datasets, would offer insight on niche occupation in the environment.

Much like the transition from morphological to molecular-based assessments of species diversity and relationships, we foresee a turnover in molecular approaches in phycology, one that pushes past the reliance on DNA barcodes and toward genomic insight. We emphasize the need for phylogenetic research to survey information across organellar and nuclear genomes, and for nuclear genomes to be comprehensively interrogated. Moreover, if shared genomic information proves to be a widely held feature among species, we will have to move past the paradigm of discrete genetic units, and rather view species as the fluid phenomena we understand them to be. Our work here on *Alaria* stands among the first crucial steps of applying WGS datasets to the study of macroalgal diversity and suggests we need to revisit phylogenetic assumptions firmly entrenched in the phycological community. We hope other research groups will rise to the challenge, ushering in exciting new developments in the coming years and decades.

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AUTHOR CONTRIBUTIONS

T.T. Bringloe: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). **D. Zaparenkov:** Conceptualization (equal); Formal analysis (supporting); Methodology (supporting); Writing-original draft (supporting). **S. Starko:** Formal analysis (supporting); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **W.S. Grant:** Conceptualization (equal); Project administration (supporting); Resources (supporting); Writing-review & editing (supporting). **C. Vieira:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **H. Kawai:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **T. Hanyuda:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **K. Filbee-Dexter:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **A. Klimova:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **T.A. Klochkova:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **D. Krause-Jensen:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **B. Olesen:** Methodology (supporting); Resources (supporting); Writing-review & editing (supporting). **H. Verbruggen:** Funding acquisition (supporting); Methodology (supporting); Project administration (supporting); Resources (supporting); Supervision (supporting); Writing-review & editing (supporting).

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DATA AVAILABILITY STATEMENT

All the short-read data can be accessed via the Short Read Archive using the accessions in Table 1. Annotated organellar genomes can similarly be accessed via GenBank using the Table 1 accessions. Final datasets used for analysis in fasta and vcf formats (filtered and un-filtered) can be accessed on FigShare (https://doi.org/10.6084/m9.figshare.14740959.v1). Command-line arguments for all analyses are provided in the supplemental material.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Coverage distribution for assembled reference nuclear scaffolds in *Alaria esculenta* specimen KU-791.

Figure S2. *Alaria* phylogenetic network of 24,242 nuclear SNPs, pruned for linkage disequilibrium.

Figure S3. Cross-validation error and log likelihood values at different values of k used in the ADMIXTURE analysis.

Figure S4. PCA plot of variation in 24,242 nuclear unlinked SNPs in species of *Alaria*.

Appendix S1. Command lines used for bioinformatic analyses