A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem

10 authors, including:

Samuel Starko
University of British Columbia - Vancouver
16 PUBLICATIONS   **24** CITATIONS

SEE PROFILE

Marybel Soto Gomez
University of British Columbia - Vancouver
13 PUBLICATIONS   **99** CITATIONS

SEE PROFILE

Sandra C. Lindstrom
University of British Columbia - Vancouver
87 PUBLICATIONS   **1,708** CITATIONS

SEE PROFILE

Patrick T Martone
University of British Columbia - Vancouver
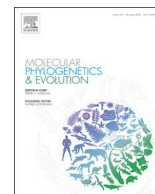**56** PUBLICATIONS   **1,014** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Coastal microbiomes View project

Phylogenomics of kelps View project

A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem

Samuel Starko[a,b,c,*], Marybel Soto Gomez[a], Hayley Darby[a], Kyle W. Demes[d], Hiroshi Kawai[e], Norishige Yotsukura[f], Sandra C. Lindstrom[a], Patrick J. Keeling[a,d], Sean W. Graham[a], Patrick T. Martone[a,b,c]

[a] Department of Botany & Biodiversity Research Centre, The University of British Columbia, 6270 University Blvd., Vancouver V6T 1Z4, Canada
[b] Bamfield Marine Sciences Centre, 100 Pachena Rd., Bamfield V0R 1B0, Canada
[c] Hakai Institute, Heriot Bay, Quadra Island, Canada
[d] Department of Zoology, The University of British Columbia, 6270 University Blvd., Vancouver V6T 1Z4, Canada
[e] Department of Biology, Kobe University, Rokkodaicho 657-8501, Japan
[f] Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0809, Japan

ARTICLE INFO

ABSTRACT

Reconstructing phylogenetic topologies and divergence times is essential for inferring the timing of radiations, the appearance of adaptations, and the historical biogeography of key lineages. In temperate marine ecosystems, kelps (Laminariales) drive productivity and form essential habitat but an incomplete understanding of their phylogeny has limited our ability to infer their evolutionary origins and the spatial and temporal patterns of their diversification. Here, we reconstruct the diversification of habitat-forming kelps using a global genus-level phylogeny inferred primarily from organellar genome datasets, and investigate the timing of kelp radiation. We resolve several important phylogenetic features, including relationships among the morphologically simple kelp families and the broader radiation of complex kelps, demonstrating that the initial radiation of the latter resulted from an increase in speciation rate around the Eocene-Oligocene boundary. This burst in speciation rate is consistent with a possible role of recent climatic cooling in triggering the kelp radiation and pre-dates the origin of benthic-foraging carnivores. Historical biogeographical reconstructions point to a northeast Pacific origin of complex kelps, with subsequent colonization of new habitats likely playing an important role in driving their ecological diversification. We infer that complex morphologies associated with modern kelp forests (e.g. branching, pneumatocysts) evolved several times over the past 15–20 MY, highlighting the importance of morphological convergence in establishing modern upright kelp forests. Our phylogenomic findings provide new insights into the geographical and ecological proliferation of kelps and provide a timeline along which feedbacks between kelps and their food-webs could have shaped the structure of temperate ecosystems.

1. Introduction

Brown algae in the order Laminariales (commonly referred to as 'kelps') are the most productive and one of the most ecologically significant clades of macroalgae in the world (Leigh et al., 1987; Steneck et al., 2002; Teagle et al., 2017). The biomass-rich communities formed by kelps provide a marine analog to terrestrial forests, reaching tens of metres high and producing dynamic and essential habitats along temperate coastlines worldwide (Bolton, 2010; Graham, 2004; Kawai et al., 2016; Steneck et al., 2002). Kelps increase the productivity of nearshore ecosystems both as a source of food (Duggins et al., 1989) and by

providing habitat for a variety of other organisms (Miller et al., 2018; Teagle et al., 2017). Kelps have also long provided ecosystem services to humans both directly as a harvestable resource (e.g., for food and valuable extracts; Bartsch et al., 2008), and indirectly by increasing ecosystem productivity (Kremen, 2005; Smale et al., 2013; Steneck et al., 2002). There is even evidence that kelps facilitated transcontinental travel of people to the Americas (Braje et al., 2017; Erlandson et al., 2007). Despite their global distribution and ubiquitous role as foundation species in nearshore ecosystems, the evolutionary history of the kelps is not entirely understood, and many questions remain about their early evolution and diversification (Bolton, 2010; Kawai et al., 2016, 2013).

Kelps are commonly thought to have originated in the North Pacific (e.g. Estes and Steinberg, 1988), but the timing and geographical paths by which their subsequent diversification occurred has been contested (Bolton, 2010; Domning, 1989; Estes and Steinberg, 1989; Kawai et al., 2016). Two of the nine Laminariales families (Akkesiphycaceae and Pseudochordaceae) are endemic to the coast of North Japan, which has the highest kelp species richness of any coastline in the world (Bolton, 2010). Based on this relative richness, it was hypothesized that kelps originated in the western Pacific and spread globally from there (Bolton, 2010; Lüning and tom Dieck, 1990; Kawai et al., 2016). However, the eastern Pacific has several more genera than the western Pacific, many of which are endemic to the coastline between Alaska and California (Bolton, 2010; Estes and Steinberg, 1988). Rather than basing these arguments on relative taxon richness in different areas, we need to explicitly reconstruct how the spatial distribution of kelps has changed over time using a comprehensive understanding of their phylogenetic history. Kelps are found primarily in temperate regions (Assis et al., 2017; Bolton, 2010; Graham et al., 2007), and their global range is currently decreasing as climate change and extreme marine heat waves drive range contractions around the world (Filbee-Dexter and Wernberg, 2018; Krumhansl et al., 2016; Wernberg et al., 2016, 2012). Yet, for much of the earth's history, the oceans, including the Pacific, were warmer than they are today. Until the Late Cenozoic, much of the current range of kelps (e.g. California) was tropical, and unlikely to support kelp communities in their current form (Estes and Steinberg, 1988), except perhaps in deep water refugia (Graham et al., 2007). Thus, it has been proposed that the radiation of complex kelps, currently distributed globally, occurred only recently (Estes and Steinberg, 1988). This is supported by molecular phylogenetic evidence showing relatively little sequence divergence among divergent kelp species (Saunders and Druehl, 1992). Moreover, kelp-associated species, such as stipe-grazing limpets, which have specific shell morphologies allowing them to graze cylindrical kelp stipes, do not appear in the fossil record until the Pliocene, further suggesting that kelp-dominated ecosystems are not ancient (Estes and Steinberg, 1988). Since the Eocene-Oligocene boundary, global climate has cooled substantially, as evidenced by shifts in the distribution of marine taxa (Ivany et al., 2000; Lindberg and Marincovich, 1988; Vermeij et al., 2018) and changes in polar ice cover (Cramer et al., 2009). It is therefore generally thought that kelps evolved and diversified during periods of global cooling, perhaps taking advantage of newly available mid-latitude habitats (Bolton, 2010; Vermeij et al., 2018). This hypothesis is consistent with early molecular clock analyses that suggested that most kelp diversity evolved within the past 30 million years (Saunders and Druehl, 1992; Silberfeld et al., 2010). However, it has yet to be tested explicitly using a dated phylogeny inferred using a broad taxon sampling of Laminariales.

Although not particularly species-rich (~120 species), the kelps are morphologically highly diverse, varying by several orders of magnitude in mature thallus biomass (Starko and Martone, 2016a) and exhibiting a wide range of ecological strategies, even among closely related species (Lane et al., 2006; Starko et al., 2019; Starko and Martone, 2016b; Steneck et al., 2002). While vicariance and geographical isolation play important roles in speciation of marine taxa (Cánovas et al., 2011; Cowman and Bellwood, 2013; Neiva et al., 2018), adaptive radiation–the diversification of lineages to fill different ecological niches–is also responsible for the production of marine biodiversity (Aguilar-Medrano et al., 2015; Ingram, 2011; Palumbi, 1994; Schluter, 2000) and may explain the wide morphological and ecological diversity of kelps. Grazers (especially sea urchins) and their predators (especially sea otters) control kelp populations and represent one of the most studied examples of trophic cascades (Burt et al., 2018; Byrnes et al., 2006; Estes and Duggins, 1995; Guenther et al., 2012; Steinberg et al., 1995), leading to the hypothesis that top predators created an ecological opportunity for the kelps by providing an environment with unusually low herbivory rates (Estes and Steinberg, 1988). Although

ancestral sea otters did not appear until the Late Miocene (and modern *Enhydra* in the Pliocene), top predators have a long history in the North Pacific (Estes and Steinberg, 1988; Vermeij et al., 2018). In particular, *Kolponomos*, which appeared in the North Pacific in the Late Oligocene (Vermeij et al., 2018), and benthic-foraging odobenids, which radiated in the Miocene (Repenning, 1976) were both likely similar to sea otters, ecologically. This suggests that there has been potential for predatory mammals to promote kelp populations for much longer than the appearance of sea otters would suggest (Vermeij et al., 2018). Yet, the role that top predators may have played in the success and adaptive radiation of kelps has also been contested (Domning, 1989; Vermeij et al., 2018) and molecular clock analyses of some members of Laminariales suggest that kelps might pre-date any of these top predators (Saunders and Druehl, 1992; Silberfeld et al., 2010). An alternative hypothesis is that kelp-dominated ecosystems evolved from the bottom-up: cooling of the North Pacific could have stimulated the adaptive radiation of kelps, fueling coastal productivity and promoting the evolution of complex consumer networks. Thus, there is clear need for a formal analysis of kelp diversification to determine which hypotheses, if any, are consistent with the temporal patterns of kelp evolution.

While there has been substantial work on kelp phylogenetics (Boo et al., 1999; Jackson et al., 2017; Kawai et al., 2017, 2013; Lane et al., 2006), we still lack a single analysis that robustly resolves the relationships across a broad enough sampling of Laminariales to explicitly and reliably test hypotheses about the spatial and temporal patterns of kelp origins and radiation. The first extensive molecular kelp phylogeny (Lane et al., 2006) stimulated numerous ecological hypotheses and revolutionized our understanding of the lineage (Bolton, 2010). Although it did not firmly resolve relationships among several key clades, it demonstrated that many important morphological traits, such as branching or production of pneumatocysts (buoyant floats), are distributed throughout kelp phylogeny, suggesting multiple origins of these traits. Jackson et al (2017) added to this work by using a transcriptome dataset to robustly resolve many of the family-level relationships within Laminariales. However, several important kelps were not investigated in that study, directly limiting our ability to make inferences about the evolution of important ecological characters and biogeographical events (Kawai et al., 2016). In particular, previous work has not resolved the relationships between three morphologically distinct kelp families (Akkesiphycaceae, Chordaceae, and Pseudochordaceae) and the other kelp families (Alariaceae, Agaraceae, Arthrothamnaceae, Aureophycaceae, Laminariaceae, and Lessoniaceae), which are often collectively referred to as "ancestral" and "derived" kelps, respectively. The latter terminology is misleading, as it tends to confuse individual traits with the taxa that possess them (Crisp and Cook, 2005); in contrast, a key approach in modern comparative biology is to infer whether individual traits are ancestral or derived. Akkesiphycaceae, Chordaceae and Pseudochordaceae are distinct from other kelps in that they have simple thallus structures and eyespots on their zoospores. Akkesiphycaceae also has anisogamous gametes and sexually monomorphic gametophytes (Kawai et al., 2013). All other members of Laminariales produce complex, modular thalli (with clear distinction between stipe and blade), lack eyespots on zoospores and have sexually dimorphic gametophytes with oogamous reproduction (Kawai et al., 2013) (hereafter "complex kelps"). It has been hypothesized that major aspects of the morphology and reproductive biology of Akkesiphycaceae, Chordaceae and Pseudochordaceae are ancestral, but characteristics of these taxa are distinct from those in closely related orders (Kawai et al., 2013; Silberfeld et al., 2010). Thus, the validity of these trait-focused hypotheses concerning ancestral vs. derived traits should be tested using well-supported phylogenetic inferences based on a broadly representative taxon sampling, which remains to be accomplished for kelps (Kawai et al., 2016, 2013).

Here we present the results of a broadly-sampled phylogenomic analysis that uses gene sets assembled from organellar genome data (and published transcriptome and organellar genome data sets) and

also includes the nuclear rDNA genes (18S and 28S). We sample most extant kelp genera (22 of 29 genera sampled, representing all nine kelp families), and explore the implications of inferred trees for understanding kelp evolution. We date the kelp phylogeny using fossil and biogeographical calibrations to reconstruct the timing of radiations, the dispersal history of key lineages and the appearance of adaptations that are crucial to the structuring of nearshore ecosystems. Specifically, we ask: What are the relationships among extant kelp genera (including *Akkesiphycus*, *Pseudochorda* and *Chorda*)? Have speciation rates changed through time or across kelp phylogeny and are shift(s) consistent with known patterns of ancient climate and trophic structure? Where did kelps originate and over what spatial scales have they diversified? And when did kelp forest-forming morphologies first appear?

2. Materials & methods

2.1. Taxon and gene sampling

We included 40 species of kelp and eight outgroups in our phylogenomic analyses; 34 kelps and six outgroups were included for the plastid data-matrix, and 40 kelps and eight outgroups were included in the mitochondrial and combined data-matrices. New sequence data was acquired for 25 kelp species and three outgroups (*Sphacelaria* sp., *Desmarestia aculeata* and *Analipus japonicus*). Tissues were collected from around the world (Tables S1-S3), dried and stored in silica gel for all taxa except for *Chorda asiatica, Pseudochorda nagaii* and *Akkesiphycus lubricus*, which were cultured from accessioned collections. Previously published annotated plastomes (n = 4), mitomes (n = 14) and transcriptomes (n = 8) were downloaded from GenBank and incorporated into analyses (Tables S4, S5).

2.2. Library preparation and sequencing

We used next generation sequencing to produce phylogenomic datasets for 28 species of brown algae. DNA extractions were performed using one of three methods (Table S2): (a) Qiagen DNeasy Plant Extraction Mini Kit as per the manufacturer's instructions, (b) brown algal extraction buffer (Saunders and McDevit, 2012) followed by the Qiagen Wizard DNA Clean-Up Kit (Qiagen, Hilden, Germany), or most often (c) a modified CTAB protocol (Doyle, 1987; Rai et al., 2003). DNA libraries were prepared using Bioo NEXTflex Rapid DNA Library Preparation Kit (Bioo Scientific Corporation, Austin, TX, USA), New England Biolab Ultra II DNA Library Preparation Kit (New England Biolabs, Ipswich, MA, USA) or NuGEN Ovation Ultralow Library Preparation Kit (NuGEN Technologies, Inc, San Carlos, CA, USA), following manufacturer instructions (Table S2). Total DNA samples with concentrations between 10 and 1000 ng/uL were sheared (to 400-bp fragments) using a Covaris s220 sonicator (Covaris Inc, Woburn, MA, USA) and size-selected (to 550-650-bp fragments) using magnetic beads (Agencourt AMPure XP Magnetic beads: Beckman Coulter Genomics, Brea CA; NucleoMag NGS Size-selection and Clean-up beads: Macherey-Nagel, Bethlehem, PA, USA). For quality control, we quantified all libraries using Qubit fluorometry (Thermofisher Scientific, Waltham, MA, USA) to ensure a minimum DNA mass of 200 ng, verified fragment size profiles using BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) and measured concentrations of adaptor-ligated fragments using the Kapa Illumina GA library quantification kit (Roche Diagnostics, Basel, Switzerland). Most libraries were sequenced as 125-bp paired-end reads on an Illumina HiSeq 2500 multiplexed with a maximum of 32 samples per lane. However, one sample (*Egregia menziesii*) was sequenced on an Illumina HiSeq 2000 as 100-bp paired-end reads and multiplexed with 28 other samples. Barcoding sequences (CO1-5P, ITS, or *rbcL*) were used to check species identities using BLAST (Altschul et al., 1990) (see Table S2).

2.3. Assembly, alignment and phylogenetic inference

We used a reference-based assembly approach to retrieve individual genes for assembling organellar gene sets. The focus was on protein-coding genes and the large and small ribosomal DNA genes from both organellar genomes. Paired-end reads were combined and mapped to mitochondrial and plastid genome references of *Costaria costata* (GenBank accessions NC_023506 and NC_028502, respectively) using CLC Genomics workbench v. 6 and 7 (CLC Bio, Aarhus, Denmark). The consensus sequences were extracted using an average minimum read depth of 10x. Organellar data from previously published transcriptomes (Jackson et al., 2017) were also extracted by mapping contigs to the same references. We then aligned each gene individually using MAFFT (Katoh et al., 2002), and manually checked and edited sequences in Geneious R 7.1.9 (http://www.geneious.com; Kearse et al., 2012). We compiled three different concatenated matrices: one from plastid data, a second from mitochondrial data, and a third from combined plastid, mitochondrial, and nuclear ribosomal genes (28S rDNA, 18S rDNA). We partitioned each data-matrix both by gene and by codon position (first, second and third position for the protein-coding genes, leaving the rDNA genes as individual partitions). We used PartitionFinder2 (Lanfear et al., 2017) with the random clustering algorithm and AICc to find optimal partitioning schemes and models that we used in subsequent phylogenetic analyses. There were 268 initial partitions for the plastid alignment, 80 for the mitochondrial alignment and 350 for the combined data-matrix, resulting in an optimal partitioning scheme of 114, 55 and 156 partitions, respectively. Maximum likelihood phylogenetic inference was conducted using RAxML 8.2.10 (Stamatakis, 2014), with 20 independent search replicates for the best tree and 1000 bootstrap replicates to assess branch support. Bayesian analyses were conducted in MrBayes (Ronquist et al., 2012) using 5,000,000 Markov Chain Monte Carlo (MCMC) iterations. ESS values were > 200 for individual parameters, which we visualized using Tracer (Rambaut et al., 2014). Gaps were treated as missing data. Phylogenetic analyses were performed using the CIPRES Science Data Portal (Miller et al., 2011).

We performed a Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) to determine whether inferred relationships between the Akkesiphycaceae-Pseudochordaceae-Chordaceae and complex kelp clades were significantly better than alternative hypotheses for these relationships. We produced likelihood trees of all three possible topologies by inputting constraint trees into RAxML. We then tested for differences in the likelihood of these topologies using CONSEL (Shimodaira and Hasegawa, 2001).

To characterize the phylogenetic placement of five *Saccharina* species not included in the main phylogenomic dataset, we produced alignments of the 5′-end of *cox1* and *nad6* using data from this study and previously published sequences (Table S6). Sequences were aligned using MAFFT and edited manually in Geneious. Gene trees were inferred using the GTR + G model in RAxML 8.2.10 (Stamatakis, 2014). These trees allowed us to place all but six species that have yet to be sequenced, into appropriate genera that we redefined here (see Table S6).

2.4. Divergence time estimation

Molecular clock analyses were performed in BEAST 1.8.4 using a 9561 bp alignment of plastid (*psa*A, *psb*A, *rbc*L) and mitochondrial (*atp*6, *cox*1, *cox*3, *nad*6) protein-coding genes, and one ribosomal locus (ITS). To maintain the well resolved tree topology from phylogenomic analyses, we used a constraint tree and unselected the tree estimation operators when creating the xml file in BEAUTI to restrict MCMC exploration to estimating parameters, rather than tree topology (Drummond et al., 2012). *Pseudolessonia laminarioides*, which has previously been resolved as the sister group of the giant kelp clade (*Postelsia, Nereocystis, Macrocystis* and *Pelagophycus*; Cho et al., 2006) was included in this analysis by using three previously published sequences

(*rbc*L, *psa*A, ITS). We used the plastid-inferred topology for Agaraceae; note that preliminary analyses using the mitochondrion-inferred topology for the family yielded similar results for this and subsequent analyses (not shown here). We converted the starting tree into a chronogram (with millions of years as the branch length units) based on the reduced seven-gene matrix using the penalized likelihood method implemented in the R package "ape" with the function "chronos" (Paradis et al., 2004). We used a birth-death model and three calibrations, two were fossil-based calibrations and one was biogeographic. BEAST analyses were run for 10,000,000 generations, sampled every 1000 and three independent runs were combined to achieve convergence and ESS values > 200 for all parameters. The only reliable fossil of a kelp is *Julescrania* from the Monterey Bay Miocene deposits (Bolton, 2010; Parker and Dawson, 1965; Silberfeld et al., 2010). This was used to set a minimum age of 13 MY for the stem-node of the giant kelps (*Nereocystis, Postelsia, Pelagophycus* and *Macrocystis*). The Monterey deposit also included a few species of Cystoseiraceae brown algae (Parker and Dawson, 1965), and so the crown node of the Fucales outgroups (*Fucus vesiculosus* and *Sargassum horneri*) was also given a minimum age of 13 MY. In both cases, a log-normal distribution, with a SD of 2MY was used for fossil calibration. We also assigned a uniform prior with a maximum age of 5.3 MY and a minimum age at present to the crown node of Atlantic *Laminaria* species (*L. digitata* and *L. hyperborea*), as they are known to have speciated in the Atlantic, after the opening of the Bering Strait (Rothman et al., 2017).

2.5. Diversification analyses

To determine whether diversification rates shifted through time in response to changes in ancient climate or biotic conditions such as the appearance of sea otters, we performed two variant analyses. First, we tested for shifts in speciation and extinction through time using BAMM (Bayesian Analysis of Macroevolutionary Mixtures) (Rabosky, 2014). We used this program to fit diversification-through-time models in a Bayesian framework, while accounting for missing taxa. We used priors generated from our time tree (see previous section) in the R package "bammtools" and used 100,000,000 MCMC iterations to obtain ESS values > 200. We also assessed lineage-through-time plots using "phytools" to provide an additional line of evidence for temporal patterns of diversification (Revell, 2012). To account for missing taxa in our time tree, we used addTaxa to randomly resolve polytomies within genera that were not completely sampled in our phylogenomic the analysis. We produced a distribution of 100 possible trees that contained these missing species and were therefore nearly complete, missing only six monospecific genera from the Far East Russia whose taxonomic classification is yet to be resolved (i.e., *Costularia, Tauya, Undariella, Feditia, Phyllaria*, and *Streptophyllopsis*). We included *Eualaria fistulosa* as a species on the *Alaria* stem branch based on morphology and previous molecular analyses (Lane et al., 2007).

2.6. Historical biogeography

Ancestral range reconstruction was performed using a likelihood framework in the R-package BioGeoBears (Matzke, 2013). BioGeoBears is unique in its use of a "J" parameter (jump parameter; founder-event speciation) allowing for simultaneous dispersal and cladogenesis. We ran analyses of dispersal-extinction-cladogenesis models both with (DEC + J) and without (DEC) this parameter. We then used AIC criteria and a likelihood test to determine the model of best fit. Biogeographical areas were chosen to reflect the makeup of regional floras, consistent with the analysis of Bolton (Bolton, 2010). Including a large number of areas can be problematic for ancestral range reconstructions (Landis et al., 2013). Therefore, we simplified Bolton's scheme by grouping all southern hemisphere areas together as Southern Oceans, and also grouped Atlantic and Arctic regions to reflect dispersal from the Pacific to the Atlantic occurring through the Arctic via the Bering Strait

(Cánovas et al., 2011; McDevit and Saunders, 2010). Modern geographical distributions of species were taken from the extensive online taxonomic database, AlgaeBase (Guiry and Guiry, accessed March 2018). Although the modern distribution of some species includes multiple areas, the highest likelihood ranges for all ancestors had two or fewer areas. Thus, to minimize issues associated with a large number of area combinations, we assessed the relative probability of ranges containing a maximum of two areas.

2.7. Morphological ancestral state reconstruction

We aimed to reconstruct the evolution of key traits associated with morphological complexity and the formation of upright kelp forests. To look at morphological complexity, we considered two types of branching that we define here in the context of kelp evolution: split branching and true branching. Split branching involves programed cell death along the longitudinal axis of a blade, forming slits. In most cases, slits extend into the stipe and cause bifurcation. However, in some species, these slits do not extend into the stipe and instead dissect only the blade (e.g. *Laminaria digitata*). Environmentally-driven blade tearing or ripping (as in *Saccharina nigripes*) was not considered to be split branching. True branching occurs in kelps that have secondary meristems, and is often associated with reproductive blades (i.e., sporophylls). True branching can occur on the blade (e.g. *Ecklonia*), on the stipe (e.g. *Alaria, Pterygophora*) or both (e.g. *Egregia*). The forked stipe of *Ecklonia arborea* was considered to be a special case of true branching in the blade, in which the base of the blade (which already possesses sporophylls through true branching) branches early in development and then later becomes part of the stipe (Setchell and Gardner, 1925). We performed ancestral state reconstruction of split branching using three possible character states, relating to whether the splitting is absent, occurs in the blade only or extends into the stipe, causing branching. Reconstruction of true branching was performed using four possible states: unbranched, true branching in the stipe (e.g. sporphylls of Alariaceae), true branching of the blade (e.g. sporophylls of *Ecklonia*) or both (e.g. *Egregia*). For upright traits, we scored species on whether they have tissue cavitation (i.e., air bladders) that serve to float kelp fronds towards the surface and/or stiff stipes that hold the fronds up without buoyancy. We defined tissue cavitation as any air-filled bladder, including pneumatocysts and hollow stipes. Thus, species with stiff, hollow stipes were scored as having both characters present. We performed reconstructions using two states for each character (present or absent) and assigned a character to be present in species that only exhibit the character sometimes (e.g. *Ecklonia arborea, Saccharina longissima*; Matson and Edwards, 2006; Rothman et al., 2017). Morphological characterization of species was based on descriptions provided by Setchell & Gardner (1925), Fritsch (1935), Druehl & Clarkston (2016) and other relevant references (e.g. Matson and Edwards, 2006; Rothman et al., 2017). We also performed ancestral state reconstruction on all kelps (including Chordales) for eight additional morphological and life history traits (Figs. S1-S4) using Ectocarpales (putative sister clade to the kelps; Silberfeld et al., 2010) as an outgroup. We performed all ancestral state reconstruction in the R package "ape" using equal rate ("ER") and multiple rate ("ARD" and "SYM") transition models and compared reconstruction fits using AIC. In all cases, equal rate models out-performed more complex transition matrices. To compare phlorotannin content of different kelp species, data were taken from the literature (Dubois and Iken, 2012; Steinberg, 1985; Van Alstyne et al., 1999).

3. Results and discussion

3.1. Data characteristics

We assembled three data matrices: (a) a 71,153 bp plastid gene alignment that included 88 protein-coding genes shared by all included

species (of 127 genes in the plastome of *Costaria costata* for reference; Zhang et al., 2015) and two plastid rDNA genes (rns and rnl); (b) a 24,490 bp mitochondrial gene alignment that included 26 protein-coding (of 63 genes in the mitome of *Laminaria digitata* for reference; Oudot-Le Secq et al., 2002) and two mitochondrial rDNA genes (rns and rnl); and (c) a 98,840 bp combined alignment for 40 kelps and eight outgroups that also included genes for the nuclear SSU and LSU ribosomal RNAs (i.e., 18S and 28S rDNA genes), the largest data matrix compiled for kelps to date. The plastid alignment comprised 34 kelp species and six outgroup taxa, and the mitochondrial and combined datasets included 40 kelp species and eight outgroups. Sequence data sets were newly recovered for 28 species here (Tables S1-S3), with additional data from publicly available genome or transcriptome data sets (Tables S4-S5). All alignments include species from 22 currently recognized kelp genera from all nine families, and outgroups from five brown algal orders (Desmarestiales, Ectocarpales, Fucales, Ralfsiales, Sphacelariales). Most of the gene sets are complete or nearly complete, although partial gene sets were recovered for a few taxa (e.g., 44% and 81% recovery here of plastid and mitochondrial data for *Aureophycus aleuticus*, Tables S2; lower recovery or missing organellar gene sets for several previously published taxa, Tables S5). Concatenated alignments and trees generated for this study are available at http://purl.org/phylo/treebase/phylows/study/TB2:S23066 and at http://github.com/sstarko/KelpPhylogenomics.

### 3.2. Phylogenetic relationships among kelps

Using a combined plastid, mitochondrial and nuclear data set comprising up to 120 genes from 40 kelps, we resolved relationships among most extant kelp genera with strong support (Fig. 1). For the portion of diversity that was previously analyzed by Jackson et al

(2017), we inferred a largely congruent tree. However, our results improve on their analyses by sampling many more taxa within this group and by including three additional families of key interest to the evolution of the kelps. Jackson et al (2017) resolved relationships among five kelp families using a 16-taxon, 152-gene alignment based on transcriptome data. Our results are consistent with these family-level relationships and differ only by the positions of *Ecklonia* and *Cymathaere*, which are strongly supported in both the likelihood and Bayesian analyses presented here. Our analysis swapped the relative positions of these taxa compared to Jackson et al (2017) who found only moderate support for this node. Family-level circumscriptions inferred here (and in Jackson et al., 2017) also correspond broadly to the clade circumscriptions inferred in earlier five- and eight-gene analyses (Kawai et al., 2013; Lane et al., 2006), although relationships among and within the family-level clades have often been poorly supported or variable in previous studies, and the position of some species in some genera (e.g. *Egregia*, *Ecklonia*) drifted depending on sampling scheme (Kawai et al., 2017, 2013; Lane et al., 2006) and phylogenetic inference approaches (Kawai et al., 2013). Thus, the well-supported and consistent (across genomic compartments and inference methods) phylogenetic relationships that we infer here significantly advance our understanding of relationships within the Laminariales.

Jackson et al. (2017) also placed *Aureophycus* (Aureophycaceae) as the sister group of Alariaceae in a separate 24-taxon, eight-gene analysis, with moderate likelihood bootstrap support. This placement of *Aureophycus* was also moderately supported by a recent study that used an eleven-gene alignment (Kawai et al., 2017). We confirm this relationship here, but with maximum support (Fig. 1). We also resolve most intrafamilial relationships at current taxon sampling with strong support (Fig. 1). Minor exceptions include the precise placement of *Undaria* within Alariaceae (weakly supported by bootstrap analysis,
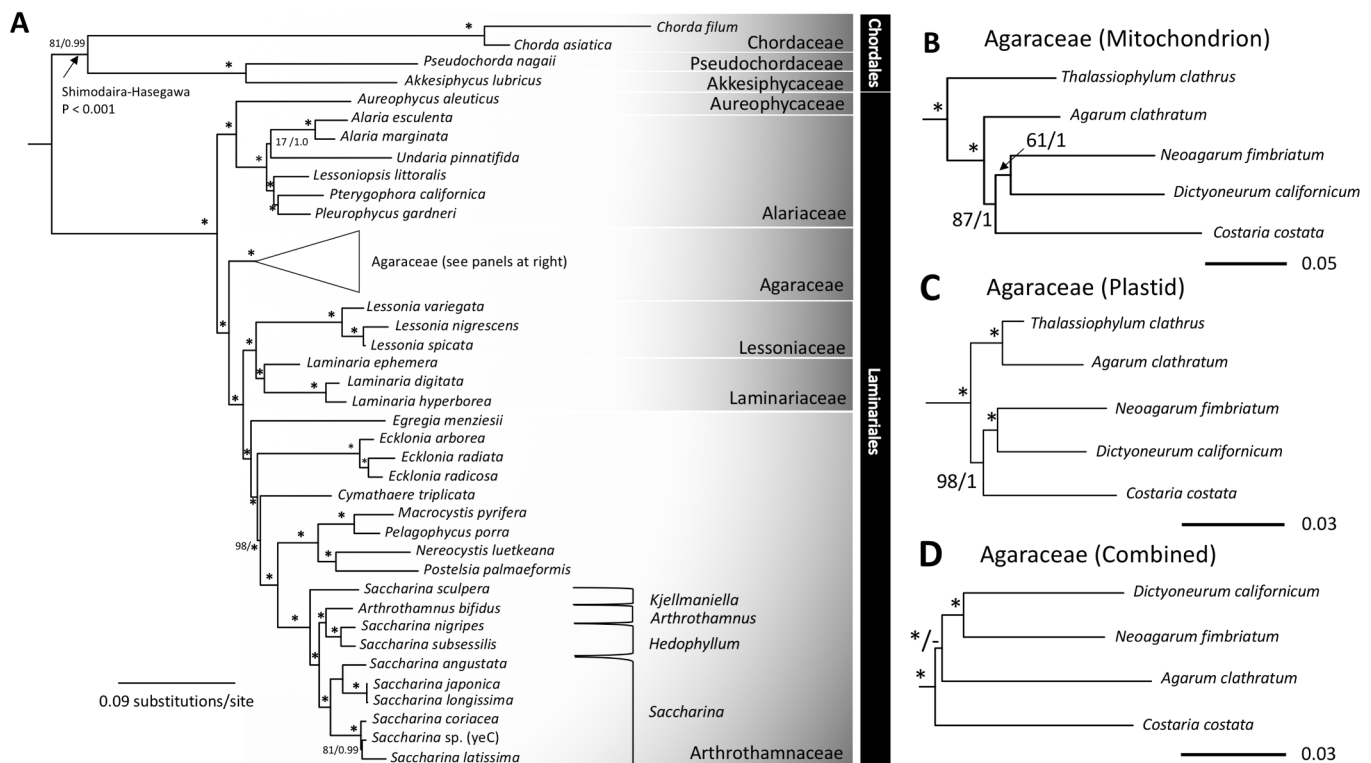
Fig. 1. (A) Phylogenetic reconstruction of the kelps based on partitioned maximum likelihood analyses of combined plastid, mitochondrial and ribosomal genes. Support values are shown for both ML bootstrapping and Bayesian posterior probability. Asterisks (*) indicate full support using both methods; other support values are shown beside branches (bootstrap /posterior probability). *Thalassiophyllum* was not included in analyses of the combined matrix, but its conflicting placements within Agaraceae based on mitochondrial and plastid data are shown in panels (B) and (C), respectively. The topology of Agaraceae from the concatenated alignment excluding *Thalassiophylum* is shown in panel (D). The hyphen (-) indicates no support (i.e., a conflict between ML and Bayesian inference methods. Scale bars in all panels represent substitutions per site. Our proposed taxonomic scheme is also shown with orders in black boxes and families in grey boxes (see Section 3.5).
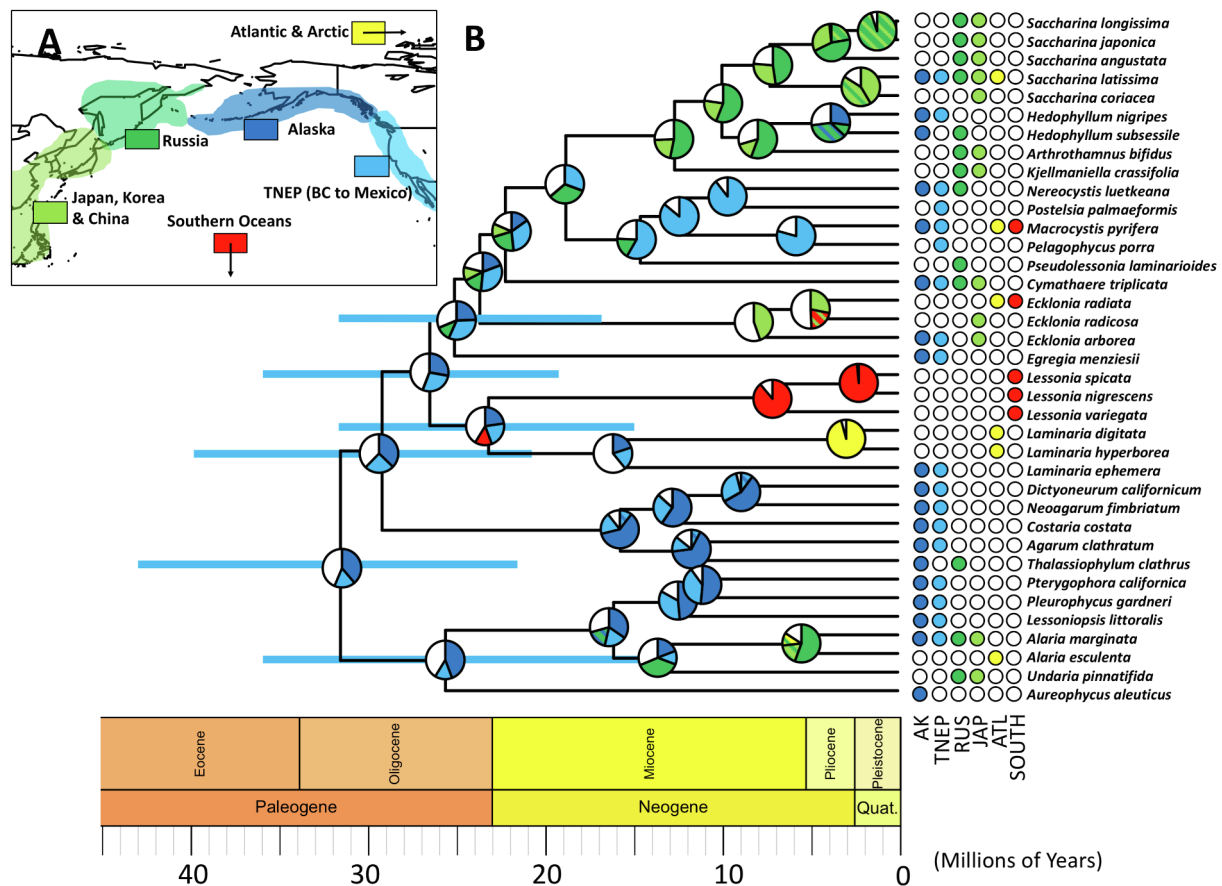
Fig. 2. Historical biogeography of the complex kelps. A time-adjusted phylogeny generated in BEAST 1.8.4 using two fossil and one biogeographical time calibration and a subset of genetic data (see text). Pie charts on nodes indicate the most likely biogeographical distribution of that ancestor, as predicted by DEC + J model in BioGeoBears. White colour on the pie chart indicates the combined relative likelihood of ranges that were less than 10%. Node bars near the base of the tree indicate uncertainty in divergence time (95% confidence intervals). Other node bars were removed for clarity but are available in Fig. S9.

Fig. 1), and local relationships among a few members of *Saccharina* (i.e., *S. coriacea*, *S. latissima*, and an undescribed species which may all be populations of *S. latissima*; Neiva et al., 2018) (Fig. 1). Our results confirm that the widespread genus *Laminaria* and the only genus found exclusively in the southern hemisphere, *Lessonia*, are sister clades (as reported by Jackson et al., 2017; Kawai et al., 2017), although there are substantial differences in the estimated crown ages of these sister families (Fig. 2). This is especially evident when considering that our dataset is likely to cover the entire phylogenetic variation within *Lessonia* (Martin and Zuccarello, 2012) but not *Laminaria* (Rothman et al., 2017). Our analyses also uncovered that *Arthrothamnus bifidus* is nested within the *Saccharina* clade, a finding with taxonomic implications (see Section 3.5).

Our study included three additional kelp families, the morphologically simple Akkesiphycaceae, Chordaceae and Pseudochordaceae, that were excluded (Jackson et al., 2017; Lane et al., 2006) or whose placements were not well resolved (Kawai et al., 2017, 2013) in past phylogenetic studies. Our combined analyses resolve these three families as a clade (the "ACP clade," which we also define as a new order, see Section 3.5) that is sister to the larger radiation of complex kelps. We also recovered Akkesiphycaceae and Pseudochordaceae as sister taxa with maximum support (Fig. 1). Although support for a broader three-family ACP clade was moderate (82% bootstrap support and 1.0 posterior probability in ML and Bayesian partitioned analyses, respectively), a Shimodaira-Hasegawa test in CONSEL (Shimodaira and Hasegawa, 2001, 1999) found significantly higher likelihood for this arrangement than alternative hypotheses of ACP paraphyly (LH difference = 8.91, P < 0.001).

The existence of the ACP clade has important implications for our understanding of kelp morphological evolution. Species in this clade were previously thought to possess ancestral characteristics (or were viewed as ancestors of other modern lineages). Their collective placement here as a clade comprising three families, sister to the complex kelps, underscores the need to revisit hypotheses of ancestral morphology and historical biogeography in a modern phylogenetic framework. Ancestral state reconstruction of a range of morphological and reproductive characters suggest that many traits that are unique to members of the ACP clade are not ancestral to all of the kelps (Figs. S1-S4). For example, sexual monomorphism of gametophytes and multicellular paraphyses are more likely to be derived characteristics of certain species than ancestral traits of the kelps. In contrast, the absence of an eyespot on the zoospore (which is present in members of the ACP clade as well as the sister order Ectocarpales) is a shared, derived trait of the complex kelps only. Our results also suggest that the ancestor of the complex kelps was perennial and that obligately annual life histories have evolved several times from ancestors that our ancestral state reconstruction suggest are perennial (Fig. S4). This has important implications because phylogenetically disparate members of this clade exhibit similar patterns of seasonal growth including endogenous growth rhythms (Bartsch et al., 2008; Lüning, 1991; Schaffelke and Lüning, 1994). Reconstructions also indicate that discoid holdfasts, which have been hypothesized to be ancestral traits (Kawai et al., 2016, 2013), may be ancestral traits of the ACP families but not of the complex kelps (Fig. S4).

Separate plastid and mitochondrial analyses were done before combining genes from these different genomic compartments (summarized in

Figs. S5-S8). These separate analyses are generally highly congruent with the tree inferred when these data sets are all combined (Figs. S9-S10), typically with strong support for individual relationships in each case. Some minor differences that are not strongly supported in individual analyses are highlighted in each supplementary figure. A single strong conflict within ingroup taxa concerns relationships in Agaraceae (Fig. 1B–D). In the plastid analysis, *Thalassiophyllum clathrus* is well supported as the sister of *Agarum clathratum* (Fig. 1B), but in the mitochondrial analysis it is instead strongly supported as the sister group of the remaining Agaraceae (Fig. 1C). This discrepancy was also documented by Kawai et al. (2017), who used a subset of genes from each organelle. Because of these conflicting signals and the possibility of differences in plastid and mitochondrial evolutionary history, we did not include *T. clathrus* in our combined-matrix analyses, leading to yet a third topology within Agaraceae in likelihood analyses (Fig. 1D, Fig. S9); Bayesian analysis of the combined alignment inferred relationships within the Agaraceae that were consistent with plastid-based analyses (Fig. S10). Conflicting evolutionary histories of plastids and mitochondria may arise through a variety of evolutionary processes, such as rapid radiation and incomplete lineage sorting, or hybrid speciation (Baack and Rieseberg, 2007; Joly et al., 2009; Maddison et al., 2006); Agaraceae may therefore provide an opportunity to explore these processes in kelps.

3.3. Temporal and spatial patterns

The highly supported and broadly sampled analysis of kelp phylogeny described above allowed us to reliably explore the spatial and temporal diversification of kelps. In order to explore the temporal patterns of kelp evolution, we used three time calibrations to date divergence events: two fossils from the Monterey Bay Miocene deposits (c. 13 MYA) and one trans-arctic biogeographical calibration in the genus *Laminaria* (see Methods). Time-calibrated molecular clock analyses suggest that while the entire kelp lineage (crown age) is approximately 73 million years old (49 – 120 MY based on 95% highest posterior densities, HPD; Table S7; Fig. S11), complex kelps – which comprise most of the diversity of modern kelps - diversified recently, with a crown age around 31.5 (21 – 43) MYA, near the Eocene-Oligocene (EO) boundary (Fig. S11). Our clade age estimates are generally consistent with previously published time-calibrated trees where these overlap, perhaps largely due to use of similar time calibration points. For example, Silberfeld et al. (2010) examined the entire brown algal radiation based on a subset of kelps, and found divergence times that are consistent with our estimates for the complex kelps and the ACP clade, and also dated the radiation of the complex kelps to c. 30 MYA. Rothman et al. (2017) dated the split between *Laminaria* and Arthrothamnaceae to approximately 25 MYA, matching a nearly identical estimate here (Fig. 2; Fig. S11). A molecular clock analysis with small subunit rRNA (SSU) is also more or less consistent with our results (but did not rely on similar calibration points), with 0.66% sequence divergence between *Alaria* and *Nereocystis*, possibly equivalent to c. 16 – 30 MY divergence time (Saunders and Druehl, 1992).

The diversification of complex kelps was associated with an increase in speciation rate (Fig. 3), the timing of which is consistent with large changes in climatic, oceanographic and biotic conditions. During the EO boundary, waters of the North Pacific saw a significant drop in temperature (as demonstrated by ∂O^{18} in Fig. 3), which may have provided cool water habitat that was ideal for an initial kelp radiation. Moreover, the EO boundary was associated with a marine mass extinction event that saw a substantial loss of taxonomic diversity in the world's oceans (Ivany et al., 2000). Although detrimental to some groups, mass extinctions are hypothesized to provide novel ecological opportunities for other lineages (Erwin, 1998; Mahler et al., 2010), and this phenomenon may have contributed to the proliferation of kelps in the North Pacific.

Historical biogeographical analysis suggests that much of the ACP and complex kelp clades diversified on opposite sides of the Pacific,
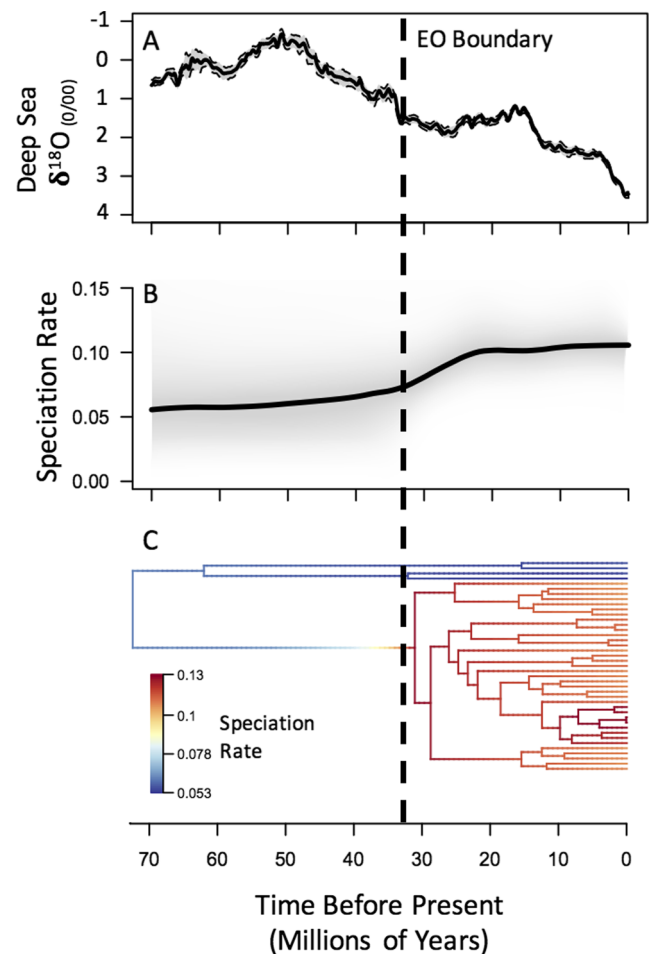

Fig. 3. Patterns of ocean climate and kelp diversification through deep time. (A) Pacific deep sea ∂^{18}O (a proxy for ocean temperature) over the past 70 million years (data from Cramer et al 2009) (Cramer et al., 2009). Speciation rate plotted through time (B), and across the kelp phylogeny (C) as estimated using BAMM.

although uncertainty remains about the predicted ancestral distributions of several deeper nodes in the kelp phylogeny (Fig. 2). The geographic origin of the common ancestor to the kelps (including the ACP families) remains unresolved (Fig. S12). Ancestral nodes in the ACP clade most likely arose in the northwestern Pacific waters, but much of the early diversification of complex kelps likely occurred on the eastern side of the Pacific Ocean, especially Alaska (Figs. 2, S12). Although the complex kelps likely initially diversified in the northeast Pacific, transcontinental, trans-equatorial and polar dispersal have all occurred repeatedly across the tree (Figs. 2, S12). For example, the most recent common ancestor of the Arthrothamnaceae is predicted to have an eastern Pacific origin, yet *Saccharina* and allied clades –nested within Arthrothamnaceae- were likely to have originated and largely diversified in Asia (Figs. 3, S10). *Saccharina* sensu lato species have subsequently recolonized the eastern Pacific and other parts of the globe several times (Figs. 3, S10). Our analyses also suggest that lineages have crossed the equator at least four times independently (*Macrocystis, Laminaria, Ecklonia, Lessonia*) or separately invaded the North Atlantic through the arctic at least four times (*Alaria, Saccharina s.s, Saccharina s.l., Laminaria*). Including additional species from diverse genera may improve the resolution at which we understand the historical pathways of kelp dispersal and may reveal even more dispersal events. For example, *Laminaria* spp. may have crossed the equator twice (Rothman et al., 2017) and the dispersal pathways of some widespread genera still remain unclear (e.g. *Ecklonia*). Future work could also incorporate

growing knowledge of how geographic features and environmental conditions have changed through deep time (e.g., Cramer et al., 2009; Shackleton and Boersma, 1981; Vermeij et al., 2018; Zachos et al., 2001). For example, warmer conditions may have limited the historical ranges of kelps to the far North Pacific and made the Bering Sea suitable habitat for a wider range of kelp species, perhaps increasing connectivity between opposite sides of the Pacific Ocean.

These results follow previous work demonstrating the importance of long distance dispersal events in establishing the contemporary distribution of modern kelp taxa (Bolton, 2010). The DEC + J biographical model, which allows for species to disperse to a new environment concurrently with speciation events, outperformed the more restrictive DEC model (Δ AIC = 11.6296, p < 0.001) but our conclusions were independent of the model of dispersal and speciation used in BioGeoBears (Matzke, 2013). Across the phylogeny, our analysis reveals that many kelp taxa are endemic to areas that were likely not inhabited by their recent ancestors. Thus, geographic isolation may have played a fundamental role in the speciation of kelps. This is consistent with previous work on species-rich kelp genera that exist along latitudinal (López-Cristoffanini et al., 2013) or longitudinal clines (Starko et al., 2018).

A general caveat of these analyses is that we did not include seven monospecific genera due to difficulty in obtaining suitable material (*Eualaria, Costularia, Tauya, Undariella, Feditia, Phyllaria*, and *Streptophyllopsis*). With the exception of *Eualaria*, these taxa are all restricted in distribution to Far East Russia (Bolton, 2010). Recent molecular evidence suggests that *Tauya* is nested within Arthrothamnaceae (Klochkova et al., 2017); thus, dispersal to Russia from Alaska could explain its distribution. *Eualaria* is a member of Alariaceae (Lane et al., 2007) and is found exclusively in Alaska and Far East Russia (Bolton, 2010; Lane et al., 2007), and so the exclusion of *Eualaria* and *Tauya* is unlikely to have influenced our general conclusions. It is more difficult to predict how inclusion of the remaining five genera would affect tree topology or downstream analyses. None of these taxa have been sequenced (they have only rarely been collected or examined). We speculate that their inclusion might increase the probabilities of Russian origins for some clades, which would support even more back-and-forth dispersal across the Pacific. Inclusion of these species into the phylogeny is a priority, and the essential next step in reconstructing the evolutionary history of Laminariales.

Although geographic isolation and allopatric speciation doubtlessly played an important role in the evolutionary history of kelps, sympatric speciation has also likely occurred, as several kelp subclades appear to have diversified over relatively small spatial ranges (Fig. 2, Fig. S12). For example, the *Saccharina s.l.* clade likely originated in the western Pacific before spreading globally (Figs. 2, S10). Adaptive radiation is an essential process linking ecology and evolution and may be promoted when resources are underutilized due to mass extinction, during the invasion of new habitats, or because key innovations allow a lineage to exploit a new set of resources (Givnish, 2015; Schluter, 2000, 1996). Kelp species are known to be functionally diverse, and play many different ecological roles, even among sympatric members of the same family (Bolton, 2010; Starko and Martone, 2016b; Steneck et al., 2002). For example, *Postelsia palmaeformis* invests nearly half of its dry biomass in attachment to the substratum, allowing it to live in the high intertidal of extremely wave-exposed sites. In contrast, its sister species, *Nereocystis luetkeana*, invests less than 10% of its biomass into attachment, and lives in calmer subtidal environments (Starko and Martone, 2016a). These closely related species differ drastically in habitat and morphology, but both co-occur in the northeast Pacific. Our phylogenetic reconstruction (Fig. 1) suggests that this is not an anomaly. Instead, closely related species often differ in morphology (Fig. 4) and life history (Fig. S4). Phenotype-environment correlations are well documented in the literature (Druehl and Elliot, 1996; Estes and Steinberg, 1988; Starko and Martone, 2016a; Augyte et al., 2018) and studies on survival and growth of genetically distinct intraspecific kelp

populations (Blanchette et al., 2002; Kawamata, 2001; Roberson and Coyer, 2004) directly demonstrate that trait differences can influence fitness. Taken together, this collective evidence suggests that ecologically-driven divergent selection may have promoted kelp diversification and adaptive radiation (Lago-Lestón et al., 2010; Palumbi, 1994; Roberson and Coyer, 2004).

In many clades, bursts of radiation are followed by a slowing in speciation rate as niche space is filled by newly evolved species (i.e. diversity-dependent speciation; Phillimore and Price, 2008; Rabosky, 2014, 2013). However, our data suggest that kelps have radiated at a constant rate since the initial burst, with possible evidence of an additional rate increase in the clade containing *Saccharina* and allies (Fig. 3). This largely constant speciation rate suggests that diversification did not slow in recent periods as kelps were diversifying ecologically. Therefore, there may be remaining unoccupied niche-space across which kelps will continue to radiate. This hypothesis that kelps have yet to saturate their ecological niche-space in marine environments is consistent with the ubiquity of high intraspecific differentiation (Lane et al., 2007; McDevit and Saunders, 2010), local adaptation (Assis et al., 2017; Neiva et al., 2018) and species complexes (Saunders and McDevit, 2014; Starko et al., 2018) in modern kelp floras.

3.4. Evolution and assembly of kelp forest ecosystems

Although our time-calibrated analysis points to a more ancient origin of the Laminariales sensu lato than previously proposed by some authors (Bolton, 2010; Estes and Steinberg, 1988), it still suggests that much of the diversity that we associate with modern kelp communities evolved relatively recently. Our analyses suggest that since the initial burst (c. 31 MYA) near the crown node of the complex kelps, clade-wide speciation rates have not changed through time (Fig. 3). A constant speciation greater than extinction would lead to exponential growth in the number of lineages through time (Stadler et al., 2014). Indeed, the number of kelp species has approximately doubled in the past 5 million years and increased approximately six-fold in the last 15 million years (Fig. 5). Upright growth forms and branching are two major morphological characteristics associated with complex three-dimensional kelp forest habitats that also appear to have evolved independently and multiple times relatively recently (Fig. 4). Our analyses suggest that stiff stipes evolved from flexible ones at least five separate times, and tissue cavitation evolved four times across the sampled taxa. Increased taxonomic sampling, especially within the most morphologically variable genera (e.g. *Laminaria, Ecklonia*) that contain species both with and without these complex morphological features, may alter some of these conclusions, for example by revealing additional independent origins of these characters. Tissue cavitation, in the form of stipe hollowing has also been documented in *Laminaria pallida* and *Ecklonia maxima* that likely evolved from ancestors without hollow stipes (Rothman et al., 2017, 2015). Moreover, *Eualaria fistulosa* has airbladders in its midrib that act as buoyant floats and is the only species in the Alariaceae to do so (Setchell and Gardner, 1925), suggesting that tissue cavitation evolved independently in this species. Branching (through splitting and secondary meristems) has also appeared repeatedly across the kelp tree over the past 15–20 MY and was likely lost in some taxa (e.g. *Pleurophycus*, Fig. 4). Evidence that these ecologically-relevant traits evolved in parallel several times across kelps provides additional support for the hypothesis that kelp diversification was driven by the process of adaptive radiation.

Modern kelp forest architecture is globally variable and dependent on the species that make up regional pools, but there are general features shared by many kelp forest ecosystems (Teagle et al., 2017). Buoyant kelps produce canopies that reach the surface of the water in many regions around the world. For example, *Macrocystis pyrifera*, the most widespread canopy species, produces the dominant canopy in the northeast Pacific and in much of the Southern Hemisphere (Teagle et al., 2017). Stiff-stiped species are also globally distributed, with

Upright growth forms

Branching
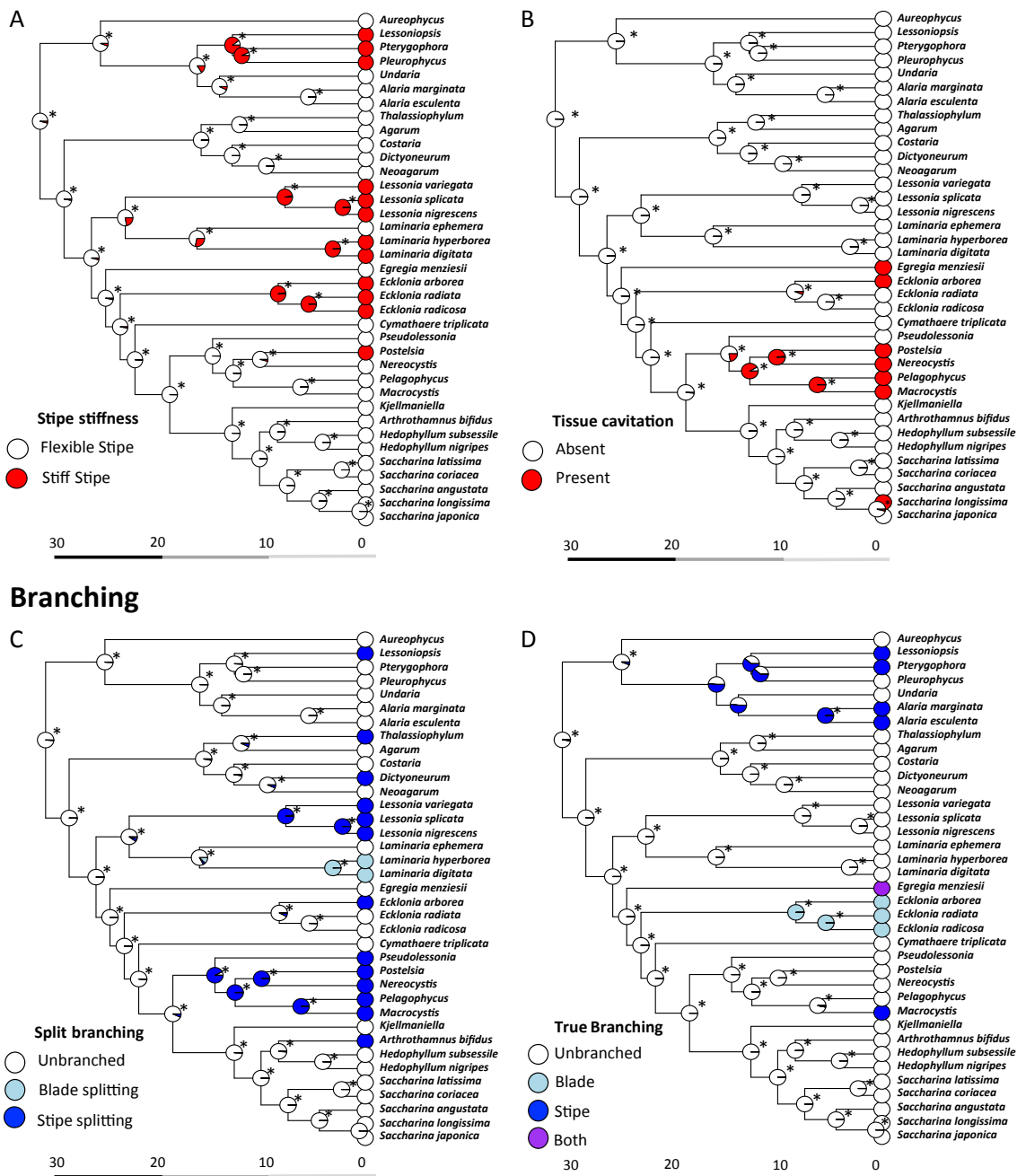
Fig. 4. Likelihood reconstructions of upright traits and morphological complexity in the kelps. Circles at leafs indicate the morphology of species. Pie charts on nodes indicate the relative likelihood (as computed in "ape") of that ancestor possessing a specific morphology. Panel (A) shows the evolution of stipe stiffness. Panel (B) shows the evolution of tissue cavitation (i.e., pneumatocyst, hollow stipe). Panel (C) shows the evolution of split branching (blade splitting that forms multiple connected blades or stipe splitting, in which a blade split progresses into the stipe, causing the branch to form a dichotomy). Panel (D) shows the evolution of true branching (i.e., branching from a true secondary meristem) of the blade and stipe. Both panels include time scales in millions of years. Asterisks (*) indicate significantly higher likelihood of the most likely state than other possible states (LR > 2.0).

species of *Ecklonia*, *Laminaria* or *Lessonia* present in virtually every kelp assemblage around the world (Bolton, 2010). Thus, modern kelp forests almost universally contain species that reach above the benthos. In many subtidal forests, the canopy species, stiff-stiped species and species without upright traits create multiple layers of highly productive habitat. Traits associated with upright growth appeared much more recently than the initial diversification of kelps (Figs. 2, S12). Thus, animal species that are adapted to modern kelp forests likely diverged recently from animals that utilized different habitats. This hypothesis is

consistent with the temporal disconnect between the appearance of stipe-dwelling limpets (~3 MYA) (Estes and Steinberg, 1988) and the more ancient origin of complex kelps (~30 MYA; Figs. 2, S12).

Our results also refute the hypothesis that sea otters or earlier marine mammals stimulated the early diversification of complex kelps (Estes and Steinberg, 1988; Vermeij et al., 2018). Although the kelps represent a radiation in the North Pacific, where there has historically been a longer marine food chain than in other regions (Estes and Steinberg, 1988; Vermeij et al., 2018), we date the kelp radiation to
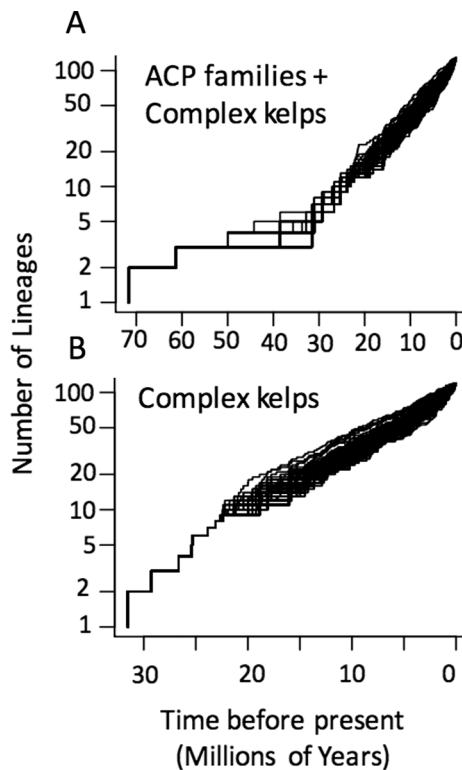
A

Fig. 5. Lineage through time plots for (A) all kelps and (B) complex kelps (excluding ACP clade). Each line represents one of 100 possible, near-complete phylogenies generated using the R package, "addTaxa".

more than 30 million years ago (Figs. 3, S10), pre-dating the appearance of both sea otters and earlier benthic feeding mammals (Estes and Steinberg, 1988; Vermeij et al., 2018), as well as strongylocentrotid sea urchins, which radiated in the Miocene (Kober and Bernardi, 2013; Vermeij et al., 2018). Instead, our results are consistent with the hypothesis that kelp diversification and proliferation provided resources that allowed for the diversification of herbivorous invertebrates and benthic-foraging mammalian carnivores. Kelp presence and abundance increases the productivity of marine animals by providing habitat and increasing food availability for herbivores, detritivores and filter feeders (Duggins et al., 1989). Subtidal invertebrates -such as herbivores and suspension feeders- along coastal shores enriched by kelp diversification may have provided an unexploited resource and encouraged the evolution of a longer food chain. Our timeline also suggests that kelps may have been food for North Pacific sirenians (e.g. Steller's sea cow) since their initial appearance c. 20 MYA (as proposed in Domning, 1976) and herbivorous desmostylians that lived in the North Pacific from the early Oligocene to the late Miocene and may have fed on coastal algae (Domning, 1989; Vermeij et al., 2018). The intercalary meristem of kelps and their rapid growth rate may have made them ideal sources of food for herbivorous mammals that could have eaten large amounts of kelp tissue without damaging the tissues necessary for growth (Vermeij et al., 2018).

The relative importance of top-down and bottom-up forcing on establishing ecosystem structure is a fundamental debate in ecology and evolution (Estes et al., 2011; Hunter and Price, 1992; Power, 1992) and kelp-dominated ecosystems have set the stage for many well-known studies of these phenomena (Estes et al., 2011; e.g. Estes and Duggins, 1995; Pfister et al., 2017; Reed et al., 2011). Although our results demonstrate that otters and earlier carnivores likely did not stimulate the initial diversification of kelps, top predators clearly exert controls on the structure of coastal ecosystems (Estes et al., 2016; Estes and Duggins, 1995; Estes and Steinberg, 1988; Steinberg et al., 1995; Vermeij, 2012). The initial argument that kelp diversification may have

been stimulated by food chain length was predicated on observations of modern ecology (Estes and Steinberg, 1988). For example, the observation that shallow kelps in the North Pacific are poorly defended against herbivores suggests that kelps in this region may be adapted to longer food-chains (Estes and Steinberg, 1988; Steinberg et al., 1995). Re-evaluating the phylogenetic distributions of chemical defenses, however, suggests that defence compound production (phlorotannins) is highly labile –very different percentages found in closely related species (Fig. S13). For example, within the genus *Laminaria*, species differ by more than an order of magnitude in phlorotannin content covering nearly the whole range of this value demonstrated by any kelp (Van Alstyne et al., 1999; data not shown in Fig. S13). Labile phlorotannin content and perhaps other traits (e.g. Fig. 4, Starko and Martone, 2016b) suggest that food-chain length may have influenced trait evolution, but without influencing the rates of kelp speciation or extinction. Thus the effects of top-down forcing on kelp traits can be understood without invoking a hypothesis that involves the entire duration of kelp evolution. We instead hypothesize that the initial diversification of kelps was promoted by cooling of North Pacific waters and stimulated the lengthening of temperate marine food chains by enhancing coastal productivity (Vermeij et al., 2018). Following this initial burst of diversification, feedbacks between kelps and newly evolving herbivores and carnivores may have shaped the selective landscape of rocky coastlines, influencing trait evolution of kelps and other taxa. For example, despite the finding that the complex kelps radiated ~30 MYA (and diverged from the ACP clade much earlier), the timing of the evolution of many of the traits associated with modern, upright forests is more recent and could have occurred in the presence of a mammalian top predator. In this way, we hypothesize that bottom-up and top-down forcing have both played fundamental roles in the evolutionary assembly of modern temperate ecosystems.

3.5. Taxonomic implications

Our results suggest that taxonomic changes should be made to make kelp classification more reflective of lineages' evolutionary histories and trajectories. The ACP clade is sufficiently distinct (in morphology and phylogenetic distance) from the complex kelps that we describe it here as a new order, Chordales, restricting Laminariales sensu stricto to the complex kelps (Fig. 1). This proposed taxonomic change would also exclude the ACP families from being called "kelps", when restricting usage of the term to the order Laminariales.

Chordales Starko, H.Kawai, S.C.Lindstrom & Martone

Description & Diagnosis: Heteromorphic life history; gametophyte microscopic and filamentous; sporophyte macroscopic with inconspicuous discoid holdfast. Several plastids per cell with no pyrenoid. Oogamous or anisogamous reproduction. Differentiated from the Laminariales by the lack of differentiation between stipe and holdfast and the presence of an eyespot on the zoospore. Constituent families: Akkesiphycaceae, Chordaceae, Pseudochordaceae. Type species: *Chorda filum* (Linnaeus) Stackhouse.

Within *Arthrothamnaceae*, our analyses also provide robust support for the placement of *Arthrothamnus bifidus*, a branched kelp from the western Pacific, as nested within the *Saccharina* clade, forming a sister-group relationship to a clade that contains the generitype of the former genus *Hedophyllum* (*S. subsessilis*) (Fig. 1). Phylogenetic divergence within *Saccharina* is substantial compared to other genera (with the exception of *Laminaria*) with divergence time estimates similar in age to the crown node of the giant kelps (*Macrocystis, Pelagophycus, Nereocystis* and *Postelsia*; Fig. 2). *Hedophyllum*, along with *Kjellmaniella* (now *S. sculpera*), were sunk into *Saccharina* when monophyly of these genera was first reported (Lane et al., 2006). However, analysis of a small number of genes failed to capture the full phylogenetic diversity of this

clade. Thus, in order to be internally consistent with the taxonomic framework used on other members of the Arthrothamnaceae, we support the reinstatement of *Hedophyllum* 1901 Setchell and *Kjellmaniella* 1902 Miyabe, two genera that are morphologically distinct from other *Saccharina* species, and were recognized for over 100 years. This breaks the *Saccharina* clade into four genera that are well supported as clades in our analyses (*Arthrothamnus*, *Hedophyllum*, *Kjellmaniella* and *Saccharina*). The two resurrected genera were also strongly supported as clades that are distinct from *Saccharina* sensu stricto in a recent population-level analysis with extensive intraspecific sampling (Neiva et al., 2018). We analysed several additional single-gene datasets aimed at including species (n = 5) with less available data (Figs. S14-S15, Table S6). These analyses suggest that *Kjellmaniella* is monospecific (*Saccharina gyrata*, formerly *Kjellmaniella gyrata*, is very likely a *Saccharina* species) and *Hedophyllum* contains six species (formerly *S. bongardiana, S. dentigera, S. druehlii, S. nigripes, S. sessilis, S. subsessilis*). A generitype has not been assigned for *Kjellmaniella*. Thus, we designate *K. crassifolia* (also known as *S. sculpera*) as the generitype.

Kjellmaniella Miyabe
Monotypic. Lectotype species: *Kjellmaniella crassifolia* Miyabe 1902: 134
Homotypic synonym: *Saccharina sculpera* C.E.Lane, C.Mayes, Druehl, & G.W.Saunders 2006: 962

Hedophyllum Setchell emend. Starko, S.C.Lindstrom & Martone
Description and diagnosis: Young plant with distinct stipe, blade frequently bullate. Mature plants without stipe, haptera developing from basal margin of blade, or plants maintaining a distinct stipe, usually cylindrical near haptera and flattening toward the base of the blade, which often becomes cordate. Mature blades smooth, frequently lacerated into two or more segments. Haptera stout. Lectotype species: *Hedophyllum subsessile* (Areschoug) Setchell 1901: 122. Designated by Saunders (1901), who attributed the type designation to Setchell.

Hedophyllum bongardianum (Postels & Ruprecht) Yendo
Basionym: *Laminaria bongardiana* Postels & Ruprecht in *Illustr. Alg.*, 1840: 10, pls. XIII, XIV.
Hedophyllum dentigerum (Kjellman) Starko, S.C.Lindstrom & Martone
Basionym: *Laminaria dentigera* Kjellman *Om Beringhafyets Algflora* 1889: 45.
Hedophyllum druehlii (Saunders & McDevit) Starko, S.C.Lindstrom & Martone
Basionym: *Saccharina druehlii* in Saunders & McDevit, *Botany*. 2014. 92: 824..
Hedophyllum nigripes (Rosenvige) Starko, S.C.Lindstrom & Martone
Basionym: *Laminaria groenlandica* Rosenvinge *Meddelelser om Grønland.* 1893: 847
Hedophyllum sessile (C. Agardh) Setchell
Basionym: *Laminaria sessile* Agardh. *Systema Algarum* 1824: 270.
Hedophyllum subsessile (Areschoug) Setchell
Basionym: *Hafgygia bongardiana* f. *subsessilis* Areschoug in *Observ. Phycol.* I, 1883: 5.

4. Conclusions

Our phylogenomic study, the largest compiled to date for kelps, allowed us to identify the spatial and temporal patterns underlying their evolutionary diversification. The ACP families, which had previously been described as having ancestral characters, were shown to comprise a sister clade to the complex kelps and are recognized herein as the new and distinct order Chordales. Complex kelps speciated at a substantially increased rate relative to the ACP families, and likely began diversifying during or after the EO boundary. Together, cooling

of the ocean possibly combined with the opening of niche space from a mass extinction may have allowed for the rapid radiation and ecological diversification of complex kelps independent of invertebrate or marine mammal evolution. Historical biogeographical reconstruction suggests that the early diversification of complex kelps occurred in the northeast Pacific, but dispersal to other regions has been a common occurrence, with substantial diversification occurring more recently in Asia. Branched and upright growth forms evolved multiple times independently within complex kelps but did so recently in all cases. Thus, the three-dimensional kelp forest habitats that are widespread along modern temperate coastlines are likely a near-contemporary occurrence. These results provide new insights into niche construction in temperate waters worldwide and lend insight into the timing of major radiations, dispersal events and the origins of ecologically important characters.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.04.012.

References

Augyte, S., Lewis, L., Lin, S., Neefus, C.D., Yarish, C., 2018. Speciation in the exposed intertidal zone: the case of Saccharina angustissima comb. nov. & stat. nov. (Laminariales, Phaeophyceae). Phycologia 57 (1), 100–112.

Aguilar-Medrano, R., Reyes-Bonilla, H., Polly, P.D., 2015. Adaptive radiation of damselfishes (Perciformes, Pomacentridae) in the eastern Pacific. Mar. Biol. 162, 2291–2303. https://doi.org/10.1007/s00227-015-2759-9.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Assis, J., Araújo, Miguel B., Serrão, Ester A., 2017. Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. Glob. Change Biol. 24, e55–e66. https://doi.org/10.1111/gcb.13818.

Baack, E.J., Rieseberg, L.H., 2007. A genomic view of introgression and hybrid speciation. Curr. Opin. Genet. Dev Genomes Evol. 17, 513–518. https://doi.org/10.1016/j.gde.2007.09.001.

Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C.M., Buck, B.H., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, M.Y., Schubert, H., Schumann, R., Valentin, K., Weinberger, F., Wiese, J., 2008. The genus Laminaria sensu lato : recent insights and developments. Eur. J. Phycol. 43, 1–86. https://doi.org/10.1080/09670260701711376.

Blanchette, C.A., Miner, B.G., Gaines, S.D., 2002. Geographic variability in form, size and survival of Egregia menziesii around Point Conception. California. Mar. Ecol. Prog. Ser. 239, 69–82.

Bolton, J.J., 2010. The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics. Helgol. Mar. Res. 64, 263–279. https://doi.org/10.1007/s10152-010-0211-6.

Boo, S.M., Lee, W.J., Yoon, H.S., Kato, A., Kawai, H., 1999. Molecular phylogeny of Laminariales (Phaeophyceae) inferred from small subunit ribosomal DNA sequences. Phycol. Res. 47, 109–114. https://doi.org/10.1046/j.1440-1835.1999.47220159.x.

Braje, T.J., Dillehay, T.D., Erlandson, J.M., Klein, R.G., Rick, T.C., 2017. Finding the first Americans. Science 358, 592–594. https://doi.org/10.1126/science.aao5473.

Burt, J.M., Tinker, M.T., Okamoto, D.K., Demes, K.W., Holmes, K., Salomon, A.K., 2018. Sudden collapse of a mesopredator reveals its complementary role in mediating rocky reef regime shifts. Proc. R Soc. B 285, 20180553. https://doi.org/10.1098/rspb.2018.0553.

Byrnes, J., Stachowicz, J.J., Hultgren, K.M., Randall Hughes, A., Olyarnik, S.V., Thornber, C.S., 2006. Predator diversity strengthens trophic cascades in kelp forests by

modifying herbivore behaviour. Ecol. Lett. 9, 61–71.

Cánovas, F.G., Mota, C.F., Serrão, E.A., Pearson, G.A., 2011. Driving south: a multi-gene phylogeny of the brown algal family Fucaceae reveals relationships and recent drivers of a marine radiation. BMC Evol. Biol. 11, 371. https://doi.org/10.1186/1471-2148-11-371.

Cho, G.Y., Klochkova, N.G., Krupnova, T.N., Boo, S.M., 2006. The reclassification of *Lessonia laminarioides* (Laminariales, Phaeophyceae): *Pseudolessonia* gen. nov. J. Phycol. 42, 1289–1299. https://doi.org/10.1111/j.1529-8817.2006.00280.x.

Cowman, P.F., Bellwood, D.R., 2013. Vicariance across major marine biogeographic barriers: temporal concordance and the relative intensity of hard versus soft barriers. Proc. R. Soc. B Biol. Sci. 280. https://doi.org/10.1098/rspb.2013.1541.

Cramer, B.S., Toggweiler, J.R., Wright, J.D., Katz, M.E., Wilson, P.A., 2009. Ocean overturning since the Late Cretaceous: inferences from a new benthic foraminiferal isotope compilation. Paleoceanography 24. https://doi.org/10.1029/2008PA001683.

Crisp, M.D., Cook, L.G., 2005. Do early branching lineages signify ancestral traits? Trends Ecol. Evol. 20, 122–128. https://doi.org/10.1016/j.tree.2004.11.010.

Domning, D.P., 1989. Kelp evolution: a comment. Paleobiology 15, 53–56.

Domning, D.P., 1976. An ecological model for Late Tertiary Sirenian evolution in the North Pacific Ocean. Syst. Biol. 25, 352–362. https://doi.org/10.2307/2412510.

Doyle, J.J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. Bot. Soc. Am. 19, 11–15.

Druehl, L.D., Elliot, C.T.J., 1996. Parks Canada Barkley Sound Kelp Distribution. Parks Canada Contract #K3489-3-0006.

Druehl, L.D., Clarkston, B.E., 2016. Pacific Seaweeds. Harbour Publishing. 320 pp.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973. https://doi.org/10.1093/molbev/mss075.

Dubois, A., Iken, K., 2012. Seasonal variation in kelp phlorotannins in relation to grazer abundance and environmental variables in the Alaskan sublittoral zone.

Duggins, D.O., Simenstad, C.A., Estes, J.A., 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science 245, 170–173.

Erlandson, J.M., Graham, M.H., Bourque, B.J., Corbett, D., Estes, J.A., Steneck, R.S., 2007. The Kelp Highway Hypothesis: marine ecology, the Coastal Migration Theory, and the peopling of the Americas. J. Isl. Coast. Archaeol. 2, 161–174. https://doi.org/10.1080/15564890701628612.

Erwin, D.H., 1998. The end and the beginning: recoveries from mass extinctions. Trends Ecol. Evol. 13, 344–349.

Estes, J.A., Burdin, A., Doak, D.F., 2016. Sea otters, kelp forests, and the extinction of Steller's sea cow. Proc. Natl. Acad. Sci. 113, 880–885. https://doi.org/10.1073/pnas.1502552112.

Estes, J.A., Duggins, D.O., 1995. Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. Ecol. Monogr. 65, 75–100. https://doi.org/10.2307/2937159.

Estes, J.A., Steinberg, P.D., 1989. Response to Domning. Paleobiology 15, 57–60.

Estes, J.A., Steinberg, P.D., 1988. Predation, herbivory, and kelp evolution. Paleobiology 14, 19–36.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R., Wardle, D.A., 2011. Trophic downgrading of planet Earth. Science 333, 301–306. https://doi.org/10.1126/science.1205106.

Filbee-Dexter, K., Wernberg, T., 2018. Rise of turfs: a new battlefront for globally declining kelp forests. BioScience 68, 64–76. https://doi.org/10.1093/biosci/bix147.

Fritsch, F.E., 1935. The Structure and Reproduction of Algae. Cambridge University Press. 939 pp.

Givnish, T.J., 2015. Adaptive radiation versus 'radiation' and 'explosive diversification': why conceptual distinctions are fundamental to understanding evolution. New Phytol. 207, 297–303. https://doi.org/10.1111/nph.13482.

Graham, M.H., 2004. Effects of local deforestation on the diversity and structure of southern California giant kelp forest food webs. Ecosystems 7, 341–357.

Graham, M.H., Kinlan, B.P., Druehl, L.D., Garske, L.E., Banks, S., 2007. Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. Proc. Natl. Acad. Sci. 104, 16576–16580. https://doi.org/10.1073/pnas.0704778104.

Guenther, C.M., Lenihan, H.S., Grant, L.E., Lopez-Carr, D., Reed, D.C., 2012. Trophic cascades induced by lobster fishing are not ubiquitous in Southern California Kelp forests. PLOS ONE 7, e49396. https://doi.org/10.1371/journal.pone.0049396.

Guiry, M.D., Guiry, G.M., n.d. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org.

Hunter, M.D., Price, P.W., 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology 73, 724–732. https://doi.org/10.2307/1940152.

Ingram, T., 2011. Speciation along a depth gradient in a marine adaptive radiation. Proc. R. Soc. B Biol. Sci. 278, 613–618. https://doi.org/10.1098/rspb.2010.1127.

Ivany, L.C., Patterson, W.P., Lohmann, K.C., 2000. Cooler winters as a possible cause of mass extinctions at the Eocene-Oligocene boundary. Nature 407, 887–890. https://doi.org/10.1038/35038044.

Jackson, C., Salomaki, E.D., Lane, C.E., Saunders, G.W., 2017. Kelp transcriptomes provide robust support for interfamilial relationships and revision of the little known Arthrothamnaceae (Laminariales). J. Phycol. 53, 1–6. https://doi.org/10.1111/jpy.12465.

Joly, S., McLenachan, P.A., Lockhart, P.J., 2009. A statistical approach for distinguishing hybridization and incomplete lineage sorting. Am. Nat. 174, E54–E70. https://doi.org/10.1086/600082.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.

Kawai, H., Hanyuda, T., Ridgway, L.M., Holser, K., 2013. Ancestral reproductive structure in basal kelp *Aureophycus aleuticus*. Sci. Rep. 3, 2491. https://doi.org/10.1038/srep02491.

Kawai, H., Hanyuda, T., Uwai, S., 2016. Evolution and biogeography of Laminarialean kelps. In: Hu, Z.-M., Fraser, C. (Eds.), Seaweed Phylogeography. Springer Netherlands, Dordrecht, pp. 227–249. https://doi.org/10.1007/978-94-017-7534-2_9.

Kawai, H., Takeaki, Hanyuda, Gao, Xu., Makoto, Terauchi, Masahiko, Miyata, Lindstrom, Sandra C., Klochkova, Nina G., Ann, Miller Kathy, Lane, C., 2017. Taxonomic revision of the Agaraceae with a description of *Neoagarum gen. nov.* and reinstatement of *Thalassiophyllum*. J. Phycol. 53, 261–270. https://doi.org/10.1111/jpy.12511.

Kawamata, S., 2001. Adaptive mechanical tolerance and dislodgement velocity of the kelp *Laminaria japonica* in wave-induced water motion. Mar. Ecol. Prog. Ser. 211, 89–104. https://doi.org/10.3354/meps211089.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Klochkova, T.A., Klochkova, N.G., Yotsukura, N., Kim, G.H., 2017. Morphological, molecular, and chromosomal identification of dwarf haploid parthenosporophytes of *Tauya basicrassa* (Phaeophyceae, Laminariales) from the Sea of Okhotsk. ALGAE 32, 15–28. https://doi.org/2017.32.1.15.

Kober, K.M., Bernardi, G., 2013. Phylogenomics of strongylocentrotid sea urchins. BMC Evol. Biol. 13, 88. https://doi.org/10.1186/1471-2148-13-88.

Kremen, C., 2005. Managing ecosystem services: what do we need to know about their ecology? Ecol. Lett. 8, 468–479.

Krumhansl, K.A., Okamoto, D.K., Rassweiler, A., Novak, M., Bolton, J.J., Cavanaugh, K.C., Connell, S.D., Johnson, C.R., Konar, B., Ling, S.D., Micheli, F., Norderhaug, K.M., Pérez-Matus, A., Sousa-Pinto, I., Reed, D.C., Salomon, A.K., Shears, N.T., Wernberg, T., Anderson, R.J., Barrett, N.S., Buschmann, A.H., Carr, M.H., Caselle, J.E., Derrien-Courtel, S., Edgar, G.J., Edwards, M., Estes, J.A., Goodwin, C., Kenner, M.C., Kushner, D.J., Moy, F.E., Nunn, J., Steneck, R.S., Vásquez, J., Watson, J., Witman, J.D., Byrnes, J.E.K., 2016. Global patterns of kelp forest change over the past half-century. Proc. Natl. Acad. Sci. 113, 13785–13790. https://doi.org/10.1073/pnas.1606102113.

Lago-Lestón, A., Mota, C., Kautsky, L., Pearson, G.A., 2010. Functional divergence in heat shock response following rapid speciation of *Fucus* spp. in the Baltic Sea. Mar. Biol. 157, 683–688. https://doi.org/10.1007/s00227-009-1348-1.

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. Syst. Biol. 62, 789–804.

Lane, C.E., Lindstrom, S.C., Saunders, G.W., 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. Mol. Phylogenet. Evol. 44, 634–648. https://doi.org/10.1016/j.ympev.2007.03.016.

Lane, C.E., Mayes, C., Druehl, L.D., Saunders, G.W., 2006. A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. J. Phycol. 42, 493–512. https://doi.org/10.1111/j.1529-8817.2006.00204.x.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773. https://doi.org/10.1093/molbev/msw260.

Leigh, E.G., Paine, R.T., Quinn, J.F., Suchanek, T.H., 1987. Wave energy and intertidal productivity. Proc. Natl. Acad. Sci. 84, 1314–1318.

Lindberg, D.R., Marincovich, L., 1988. New species of limpets from the Neogene of Alaska (Patellogastropoda. Mollusca). ARCTIC 41.

López-Cristoffanini, C., Tellier, F., Otaíza, R., Correa, J.A., Contreras-Porcia, L., 2013. Tolerance to air exposure: a feature driving the latitudinal distribution of two sibling kelp species. Bot. Mar. 56, 431–440. https://doi.org/10.1515/bot-2013-0042.

Lüning, K., 1991. Circannual growth rhythm in a brown alga, *Pterygophora californica*. Bot. Acta 104, 157–162. https://doi.org/10.1111/j.1438-8677.1991.tb00211.x.

Lüning, K., tom Dieck, I., 1990. The distribution and evolution of the Laminariales: North Pacific—Atlantic relationships. In: Evolutionary Biogeography of the Marine Algae of the North Atlantic. Springer, pp. 187–204.

Maddison, W.P., Knowles, L.L., Collins, T., 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. 55, 21–30. https://doi.org/10.1080/10635150500354928.

Mahler, D.L., Revell, L.J., Glor, R.E., Losos, J.B., 2010. Ecological opportunity and the rate of morphological evolution in the diversification of greater antillean *Anoles*. Evolution 64, 2731–2745. https://doi.org/10.1111/j.1558-5646.2010.01026.x.

Martin, P., Zuccarello, G.C., 2012. Molecular phylogeny and timing of radiation in *Lessonia* (Phaeophyceae, Laminariales). Phycol. Res. 60, 276–287. https://doi.org/10.1111/j.1440-1835.2012.00658.x.

Matson, P.G., Edwards, M.S., 2006. Latitudinal variation in stipe hollowing in Eisenia arborea (Phaeophyceae, Laminariales). Phycologia 45, 343–348.

Matzke, N.J., 2013. BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts. R Package Version 02 (1), 2013.

McDevit, D.C., Saunders, G.W., 2010. A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. Phycol. Lawrence 49, 235–248.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2011. The CIPRES Science Gateway: A community resource for phylogenetic analyses, in: Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery, TG '11. ACM, New York, NY, USA, pp. 41:1–41:8. https://doi.org/10.1145/2016741.2016785.

Miller, R.J., Lafferty, K.D., Lamy, T., Kui, L., Rassweiler, A., Reed, D.C., 2018. Giant kelp,

Macrocystis pyrifera, increases faunal diversity through physical engineering. Proc R Soc B 285, 20172571. https://doi.org/10.1098/rspb.2017.2571.

Neiva, J., Paulino, C., Nielsen, M.M., Krause-Jensen, D., Saunders, G.W., Assis, J., Bárbara, I., Tamigneaux, É., Gouveia, L., Aires, T., Marbà, N., Bruhn, A., Pearson, G.A., Serrão, E.A., 2018. Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. Sci. Rep. 8, 1112. https://doi.org/10.1038/s41598-018-19620-7.

Oudot-Le Secq, M.-P., Kloareg, B., Loiseaux-De Goër, S., 2002. The mitochondrial genome of the brown alga *Laminaria digitata*: a comparative analysis. Eur. J. Phycol. 37, 163–172.

Palumbi, S.R., 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu. Rev. Ecol. Syst. 25, 547–572. https://doi.org/10.1146/annurev.es.25.110194.002555.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412.

Parker, B., Dawson, E., 1965. Non-calcareous marine algae from California Miocene marine deposits. Nova Hedwig. 10, 273–295.

Pfister, C.A., Berry, H.D., Mumford, T., 2017. The dynamics of kelp forests in the Northeast Pacific Ocean and the relationship with environmental drivers. J. Ecol. 106, 1520–1533. https://doi.org/10.1111/1365-2745.12908.

Phillimore, A.B., Price, T.D., 2008. Density-dependent cladogenesis in birds. PLOS Biol. 6, e71. https://doi.org/10.1371/journal.pbio.0060071.

Power, M.E., 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology 73, 733–746. https://doi.org/10.2307/1940153.

Rabosky, D.L., 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLOS ONE 9, e89543. https://doi.org/10.1371/journal.pone.0089543.

Rabosky, D.L., 2013. Diversity-dependence, ecological speciation, and the role of competition in macroevolution. Annu. Rev. Ecol. Evol. Syst. 44, 481–502. https://doi.org/10.1146/annurev-ecolsys-110512-135800.

Rai, H.S., O'Brien, H.E., Reeves, P.A., Olmstead, R.G., Graham, S.W., 2003. Inference of higher-order relationships in the cycads from a large chloroplast data set. Mol. Phylogenet. Evol. 29, 350–359.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6. Available from http://beast.bio.ed.uk/Tracer.

Reed, D.C., Rassweiler, A., Carr, M.H., Cavanaugh, K.C., Malone, D.P., Siegel, D.A., 2011. Wave disturbance overwhelms top-down and bottom-up control of primary production in California kelp forests. Ecology 92, 2108–2116.

Repenning, C.A., 1976. Adaptive evolution of sea lions and walruses. Syst. Zool. 25, 375–390. https://doi.org/10.2307/2412512.

Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x.

Roberson, L., Coyer, J., 2004. Variation in blade morphology of the kelp *Eisenia arborea*: incipient speciation due to local water motion? Mar. Ecol. Prog. Ser. 282, 115–128. https://doi.org/10.3354/meps282115.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029.

Rothman, M.D., Lydiane, M., Anderson, R.J., Bolton, J.J., Verbruggen, H., 2017. A phylogeographic investigation of the kelp genus *Laminaria* (Laminariales, Phaeophyceae), with emphasis on the South Atlantic Ocean. J. Phycol. 53, 778–789. https://doi.org/10.1111/jpy.12544.

Rothman, M.D., Mattio, L., Wernberg, T., Anderson, R.J., Uwai, S., Mohring, M.B., Bolton, J.J., 2015. A molecular investigation of the genus Ecklonia (Phaeophyceae, Laminariales) with special focus on the southern hemisphere. J. Phycol. 51, 236–246.

Saunders, G.W., Druehl, L.D., 1992. Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): implications for kelp evolution. J. Phycol. 28, 544–549. https://doi.org/10.1111/j.0022-3646.1992.00544.x.

Saunders, G.W., McDevit, D.C., 2014. A DNA barcode survey of Haida Gwaii kelp (Laminariales, Phaeophyceae) reveals novel ecological and distributional observations and *Saccharina druehlii sp. nov*. Botany 92, 821–826. https://doi.org/10.1139/cjb-2014-0119.

Saunders, G.W., McDevit, D.C., 2012. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. DNA Barcodes. Springer 207–222.

Schaffelke, B., Lüning, K., 1994. A circannual rhythm controls seasonal growth in the kelps *Laminaria hyperborea* and *L. digitata* from Helgoland (North Sea). Eur. J. Phycol. 29, 49–56. https://doi.org/10.1080/09670269400650471.

Schluter, D., 2000. The Ecology of Adaptive. Radiation. OUP, Oxford.

Schluter, D., 1996. Ecological causes of adaptive radiation. Am. Nat. 148, S40–S64. https://doi.org/10.1086/285901.

Setchell, W.A., Gardner, N.L., 1925. The Marine Algae of the Pacific Coast of North.

America: Malanophyceae. University of California Press.

Shackleton, N., Boersma, A., 1981. The climate of the Eocene ocean. J. Geol. Soc. 138, 153–157. https://doi.org/10.1144/gsjgs.138.2.0153.

Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17, 1246–1247. https://doi.org/10.1093/bioinformatics/17.12.1246.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic Inference. Mol. Biol. Evol. 16, 1114. https://doi.org/10.1093/oxfordjournals.molbev.a026201.

Silberfeld, T., Leigh, J.W., Verbruggen, H., Cruaud, C., de Reviers, B., Rousseau, F., 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the "brown algal crown radiation". Mol. Phylogenet. Evol. 56, 659–674. https://doi.org/10.1016/j.ympev.2010.04.020.

Smale, D.A., Burrows, M.T., Moore, P., O'Connor, N., Hawkins, S.J., 2013. Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. Ecol. Evol. 3, 4016–4038.

Stadler, T., Rabosky, D.L., Ricklefs, R.E., Bokma, F., 2014. On age and species richness of higher taxa. Am. Nat. 184, 447–455. https://doi.org/10.1086/677676.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Starko, S., Bailey, L.A., Creviston, E., James, K.A., Warren, A., Brophy, M.K., Danasel, A., Fass, M.P., Townsend, J.A., Neufeld, C.J., 2019. Environmental heterogeneity mediates scale-dependent declines in kelp diversity on intertidal rocky shores. PLOS ONE 14, e0213191. https://doi.org/10.1371/journal.pone.0213191.

Starko, S., Boo, G.H., Martone, P.T., Lindstrom, S.C., 2018. A molecular investigation of *Saccharina sessilis* from the Aleutian Islands reveals a species complex, necessitating the new combination *Saccharina subsessilis*. ALGAE 33, 157–166. https://doi.org/10.4490/algae.2018.33.2.157.

Starko, S., Martone, P., 2016a. An empirical test of "universal" biomass scaling relationships in kelps: Evidence of convergence with seed plants. New Phytologist 212, 719–729. https://doi.org/10.1111/nph.14120.

Starko, S., Martone, P., 2016b. Evidence of an evolutionary-developmental trade-off between drag avoidance and tolerance strategies in wave-swept intertidal kelps (Laminariales, Phaeophyceae). J. Phycol. 52, 54–63. https://doi.org/10.1111/jpy.12368.

Steinberg, P.D., 1985. Feeding preferences of *Tegula funebralis* and chemical defenses of marine brown algae. Ecol. Monogr. 55, 333–349. https://doi.org/10.2307/1942581.

Steinberg, P.D., Estes, J.A., Winter, F.C., 1995. Evolutionary consequences of food chain length in kelp forest communities. Proc. Natl. Acad. Sci. 92, 8145–8148. https://doi.org/10.1073/pnas.92.18.8145.

Steneck, R.S., Graham, M.H., Bourque, B.J., Corbett, D., Erlandson, J.M., Estes, J.A., Tegner, M.J., 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ. Conserv. 29, 436–459.

Teagle, H., Hawkins, S.J., Moore, P.J., Smale, D.A., 2017. The role of kelp species as biogenic habitat formers in coastal marine ecosystems. J. Exp. Mar. Biol. Ecol. Ecol. Resp. Environm. Change Marine Syst. 492, 81–98. https://doi.org/10.1016/j.jembe.2017.01.017.

Van Alstyne, K.L., McCarthy III, J.J., Hustead, C.L., Duggins, D.O., 1999. Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. Mar. Biol. 133, 371–379. https://doi.org/10.1007/s002270050476.

Vermeij, G.J., 2012. The evolution of gigantism on temperate seashores. Biol. J. Linn. Soc. 106, 776–793. https://doi.org/10.1111/j.1095-8312.2012.01897.x.

Vermeij, G.J., Banker, R., Capece, L.R., Hernandez, E.S., Salley, S.O., Vriesman, V.P., Wortham, B.E., 2018. The coastal North Pacific: Origins and history of a dominant marine biota. J. Biogeogr. https://doi.org/10.1111/jbi.13471.

Wernberg, T., Bennett, S., Babcock, R.C., de Bettignies, T., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C.J., Hovey, R.K., Harvey, E.S., Holmes, T.H., Kendrick, G.A., Radford, B., Santana-Garcon, J., Saunders, B.J., Smale, D.A., Thomsen, M.S., Tuckett, C.A., Tuya, F., Vanderklift, M.A., Wilson, S., 2016. Climate-driven regime shift of a temperate marine ecosystem. Science 353, 169–172. https://doi.org/10.1126/science.aad8745.

Wernberg, T., Smale, D.A., Tuya, F., Thomsen, M.S., Langlois, T.J., de Bettignies, T., Bennett, S., Rousseaux, C.S., 2012. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. Nat. Clim. Change 3, 78–82. https://doi.org/10.1038/nclimate1627.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693. https://doi.org/10.1126/science.1059412.

Zhang, L., Wang, X., Liu, T., Wang, H., Wang, G., Chi, S., Liu, C., 2015. Complete plastid genome of the brown Alga *Costaria costata* (Laminariales, Phaeophyceae). PLOS ONE 10, e0140144. https://doi.org/10.1371/journal.pone.0140144.