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Original Article

Analysis of organellar genomes in brown algae reveals an independent introduction of similar foreign sequences into the mitochondrial genome

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ABSTRACT

Kelp species (Laminariales, Phaeophyceae) are globally widespread along temperate to Polar rocky coastal lines. Here we analyse the mitochondrial and chloroplast genomes of *Laminaria rodriguezii*, in comparison to the organellar genomes of other kelp species. We also provide the complete mitochondrial genome sequence of another endemic kelp species from a Polar habitat, the Arctic *Laminaria solidungula*. We compare phylogenetic trees derived from twenty complete mitochondrial and seven complete chloroplast kelp genomes. Interestingly, we found a stretch of more than 700 bp in the mitochondrial genome of *L.rodriguezii*, which is not present in any other yet sequenced member of the Phaeophyceae. This stretch matches a protein coding region in the mitochondrial genome from *Desmarestia viridis*, another brown seaweed. Their high similarity suggests that these sequences originated through independent introduction into the two species. Their origin could have been by infection by yet unknown similar mitoviruses, currently only known from fungi and plants.

1. Introduction

Seaweeds are multicellular photoautotrophic macroalgal species. All brown algae (Phaeophyceae) are part of this non-taxonomic classification, since so far no unicellular members of this group were found [1]. Thus, multicellularity is one of the founding factors of brown algal evolution and factors enabling this were recruited early in the evolution of the branch. Similar functions are often established by using the same toolbox [2]. Therefore, we might learn from the analysis of one group of organisms, how and why other groups evolved. Intriguingly, similar factors seem to have been used to establish multicellularity in green plants and brown algae [3]. Thus, by comparative analyses of genomes from distantly related clades we might learn about common mechanisms of differentiation, habitat occupation, or pathogen susceptibility.

Kelps are a subgroup of brown algal seaweeds, which constitute the order Laminariales in the Phaeophyceae. They form large marine forests which provide habitat and food to other species [4], analogue to land-based tree forests. They thus have a high impact on local environments and need protection as a hot spot of biodiversity. Kelps are also of high economic value as providers of alginates and as a food source [5]. Despite their economical and ecological importance only the nuclear genomes of *Saccharina japonica* (Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders [6], including different cultivars [7], and recently of *Undaria pinnatifida* (Harvey) Suringar [8] were published so far. This fact prompted the Phaeoexplorer project (https://www.france-genomique.org/projet/phaeoexplorer/?lang=en) aiming at whole breadth genomics of brown algae. However, several organellar genomes of brown algae have been published. This is partly due to their over-representation regarding their copy numbers in eukaryote cells compared to the nuclear genome. Thus, reconstruction of organellar genomes needs far less sequencing reads than constructing a draft nuclear genome. Moreover, the small size of organellar genomes enables their completion without the need of long read sequences from nanopore [9] or pacbio [10] sequencing. Thus, several genomes from kelp chloroplasts and mitochondria are available: 42 kelp mitochondrial genomes are stored in public databases, kelp chloroplast genomes are rarer with only yet 9 available at the NCBI nucleotide database. A number of chloroplast genomes were studied and published [11–14] yet only one

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[1] Sadly Gernot Glöckner passed away a few weeks after submission of this manuscript.

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complete mitochondrial genome was so far analysed in more detail [15]. Other mitochondrial genomes were made publicly available but seemingly used for barcoding purposes only, e.g. [16,17]. In a previous study we analysed variation in kelp chloroplast genomes [11]. Only minor rearrangements took place during kelp chloroplast genome evolution and therefore available kelp chloroplast genomes appear collinear. This resembles the situation in land plants, which also mostly possess collinear chloroplast genomes [18,19]. However, kelp species are much younger than land plants with major speciation events occurring only 35 million years ago [20]. As collinearity describes the maintenance of genes in corresponding orders over time we could analyse the abundance of single nucleotide polymorphisms over the complete length of available chloroplast genomes [11]. This analysis showed that likely incomplete lineage sorting contributed to chloroplast evolution. The phylogenetic relationships of genera within the kelp lineage were resolved recently using partial organellar and nuclear genome data [20]. Additional species can easily be fitted into this phylogenetic tree as they become available. Yet it is unclear whether these data are sufficient to finally get a robust tree topology.

There are some kelp species, which are especially rare or growing in remote areas and thus are not easily collectable and readily available for analysis. Especially the endemic deep-water kelp species growing below the thermocline in deep warm-temperate to subtropical waters, such as *Laminaria rodriguezii* Bornet (Mediterranean Sea [21,22]), *Laminaria abyssalis* A.B.Joly & E.C. Oliveira (S-Atlantic) [23] or *Laminaria philippinensis* J.E.Petrov & M.V. Suchovejeva (Pacific Ocean), [24] have seldom been collected. Another habitat difficult to assess is the Arctic sublittoral where *Laminaria solidungula* (J. Agardh) thrives in shaded habitats [22]. Despite their differences in latitudinal distribution and temperature environment, these species all are subjected to especially low light conditions (e.g. [25,26]) and their environment seems to be rather constant compared to species with a wide horizontal or vertical distribution gradient (e.g. [27]).

Laminaria rodriguezii is restricted to deep water habitats stretching between 70 and 120 m and rarely up to 50 m or down to 260 m [22,27,28]. Temperatures there seem to be quite constant year round at 13–14 °C [27]. *L. solidungula* thrives in Arctic habitats where temperatures typically vary between −1.5 and 5 °C and populations at their southern distribution boundary in south east Canada subduct to zones with year round cold temperatures ([29] and references therein). Both species endure low light conditions over most of the year. Besides its endemism *L. rodriguezii* also is distinctive from all other kelp species, except *Laminaria sinclairii* (Harvey ex J.D.Hooker & Harvey) Farlow, Anderson & Eaton [30], in its ability to form stolons and produce clonal sporophytes by vegetative propagation [31] thereby being able to bypass the obligate sexual life history of Laminariales [32].

To be able to compare available chloroplast and mitochondrial genomes with those of endangered *L.rodriguezii* and *L. solidungula*, we sampled sporophytes of *L. rodriguezii* from its Mediterranean habitat via the GOMBESSA expedition on the 7-9th of July in 2019 at Banc de Magaud, a rocky ledge between 68 and 80 m depth off Le Levant Island (Provence, France) [33]. In addition, we cultivated Arctic *L. solidungula* from stock cultures to comparatively study their organellar genomes. We were interested to see whether these two species from extreme habitats and despite their phylogenetic difference [27], might share common characters or show signs of special adaptation in their organellar genomes that deviate from each other or from kelp species with broad distribution ranges.

2. Material and methods

2.1. Sampling

Cultivated sporophytes propagated from clonal gametophyte samples of *L. solidungula,* originally sampled at Spitsbergen were used for DNA extraction and all cultivation details are given in [11]. Several samples of *L. rodriguezii* were taken from the Mediterranean Sea on the 7-9th of July 2019 at Banc de Magaud, a rocky ledge between 68 and 80 m depth off Le Levant Island (Provence, France; 6°0.67375 E - 43°0.05624 N) [33]. The *L. rodriguezii* samples were from in situ sporophytes, silica dried and then used for DNA extraction.

2.2. DNA extraction and PCR analysis

Freeze dried juvenile sporophytes of *L. solidungula* and silica dried sporophytic meristem discs of *L. rodriguezii* were grinded under liquid nitrogen to yield a fine powder, where all cell walls were destroyed. The DNA then was extracted according to Doyle and Doyle modified cetyl trimethyl ammonium bromide method (CTAB) [34]. PCR analysis with total DNA from *L. rodriguezii*, *Laminaria digitata (Hudson) J.V.Lamouroux*, and *L. solidungula* was essentially performed as described [35]. The external primers were specific for the rpl31 and 16S rRNA genes, respectively, which are located directly up- and downstream of the *L. rodriguezii* ORF. The internal primers were specific for the 5′ and 3′ region of the *L. rodriguezii* open reading frame (ORF), respectively. Primers are listed in Table S1.

2.3. Sequencing and assembly

Total DNA (5 μg) was converted to an Illumina sequencing library and analysed on an Illumina Hiseq machine. Read sizes in the paired end library were 150 bases with a mean distance of reads of 235 bases. Trimming and further processing were done with the Illumina software suit. De novo assembly was performed with abyss-pe [36] using kmers 40, 45, and 55. The resulting contigs were converted to a BLAST Table database and queried with the *L. digitata* chloroplast and mitochondrial genome sequences. Matching contigs were used to reconstruct the complete chloroplast and mitochondrial genomes by closing gaps with Gapfiller [37].

2.4. Annotation, alignment and phylogenetic analyses

The coding sequences (CDS) of the recently published *L. digitata* mitochondrial and plastid genomes [11] were used to find the corresponding CDS on the completed organellar genomes. Furthermore, with the algorithm implemented in tRNAscan [38] we detected the tRNA genes. Detection of the rRNA genes was done by BLASTing the respective *L. digitata* nucleotide sequences against the whole organellar genomes. We then scanned the sequence portions larger than 100 bases without any annotation for presence of additional open reading frames (ORFs). The completed annotated organellar genome annotation was converted to a GenBank file and visualized with OGDRAW [39].

Sequences of other mitochondrial and chloroplast genomes from kelp species used for phylogenetic analysis were retrieved from the NCBI database (Table 1). Collinearity of the assembled kelp chloroplast and mitochondrial genomes was tested with the nucmer tool of mummer [40]. All sequences were edited to start at the same position and then complete genomes were aligned with Clustal Omega [41,42]. Further manual inspection of the sequences was done to ensure proper alignment for phylogenetic analyses.

For phylogenetic analyses we used MEGAX [43] and MrBayes [44]. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model [45]. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. We performed 500 bootstrap repetitions on the data sets. In case of the MrBayes analysis we set the evolutionary model to GTR with gamma distributed rate variation and a proportion of invariable sites. We used 80,000 generations to get the standard deviation of split frequencies below 0.01 and then used a burn-in of 1000.

Table 1
The organellar genomes used in this study. The IDs are the NCBI accession numbers.

	Species name	ID	Sequence length [bp]	Reference
Mitochondrial genomes	*Desmarestia viridis*	AY500367.1	39,049	[47]
	Lessonia spicata	NC_044181.1	37,097	[66]
	Nereocystis luetkeana	NC_042395.1	37,399	[67]
	Macrocystis integrifolia	NC_042669.1	37,366	[68]
	Laminaria solidungula	MT732098	37,862	This study
	Laminaria rodriguezii	MT732097	38,047	This study
	Laminaria hyperborea	JN099683.1	37,976	[69]
	Laminaria digitata	AJ344328.1	38,007	[15]
	Saccharina latissima	KM675818.1	37,659	[70]
	Saccharina longissima	JN099684.1	37,628	[69]
	Saccharina japonica Dongfang No.6	MG712776.1	37,657	[71]
	Saccharina japonica SJAPO	AP011493	37,657	[72]
	Saccharina japonica var. *religiosa*	AP011494.1	37,657	[72]
	Saccharina longipedalis	AP011497.1	37,657	[72]
	Saccharina japonica var. *diabolica*	AP011496.1	37,657	[72]
	Saccharina japonica var. *ochotensis*	AP011495.1	37,656	[72]
	Saccharina cichorioides f. *coriacea*	AP011499.1	37,500	[72]
	Saccharina angustata	AP011498.1	37,605	[72]
	Saccharina sculpera	KR350664.1	37,627	[73]
	Costaria costata	KF384641.1	37,461	[74]
	Undaria pinnatifida	KF319031.1	37,402	[75]
Chloroplast genomes	*Lessonia spicata*	NC_044182.1	130,305	[66]
	Laminaria solidungula	MH784528.1	130,784	[11]
	Costaria costata	NC_028502.1	129,947	[14]
	Undaria pinnatifida	NC_028503.1	130,383	[13]
	Laminaria digitata	MH784527	130,376	[11]
	Saccharina japonica	JQ405663.1	130,584	[12]
	Laminaria rodriguezii	MT732096	131,092	This study

3. Results

3.1. New organellar genome sequences

We completely reconstructed the mitochondrial genomes of *L. rodriguezii* and *L. solidungula* and the chloroplast genome of *L. rodriguezii* from short read sequencing of extracted total DNA from the two species (Fig. 1). The organellar contigs were retrieved using related sequences. We closed the remaining gaps (one gap in the *L. solidungula* mitochondrial genome; three gaps in the *L. rodriguezii* chloroplast genome) from the assembly procedure using available raw read information (see methods). All these genomes were of similar length as their related counterparts from other kelp (Table 1).

3.2. Phylogeny of kelp species using complete mitochondrial and chloroplast genomes

After annotation of the newly assembled genomes we checked all brown algal organellar genomes from the NCBI database for collinearity and retrieved 18 of the 42 collinear kelp mitochondrial genomes and 6 of the 9 collinear kelp plastid genomes (Table 1; Tables S2 and S3). According to this analysis all Laminariales (kelp) organellar genomes are collinear. Additionally, the mitochondrial genome of the brown alga *Desmarestia viridis* (O.F.Müller) J.V. Lamouroux [46,47], which is not a kelp species, turned out to be collinear to kelp mitochondrial genomes, providing a suitable outgroup for phylogenetic analyses. Other known brown algal mitochondrial and chloroplast genomes exhibit structural recombination and are therefore not compatible with the analyses performed here. In a previous study [11], we only used the coding sequences (CDS) for phylogenetic analyses of brown algal chloroplast genomes. As we here focus on the brown algal species with collinear organellar genomes, we can make use of this collinearity and employ complete organellar genome alignments directly for a phylogenetic analysis irrespective of their coding potential, thus also including tRNAs, rRNAs, and intergenic regions. Here, the alignment of intergenic regions is crucial to the outcome of the analysis. We therefore manually checked the alignments for incorrectly aligned portions and used a refined alignment for further analyses. The alignment of the mitochondrial genomes can be found in the supplement (Supplemental alignment 1). The chloroplast phylogenetic reconstruction recapitulated the previously published topology of kelp species obtained with concatenated CDS [11], albeit without the possibility to root them with other brown algae (Fig. S1). We used 18 complete mitochondrial genomes of kelps for the calculation of the mitochondrial tree together with the newly sequenced mitochondrial genomes from *L. solidungula* and *L. rodriguezii* and *D. viridis* as an outgroup. We found no differences in species placements between the two organellar trees (Fig. 2 and Fig. S1) in cases where both trees contained the species. Thus, we could place the newly added species in the trees with high confidence. As the mitochondrial maximum likelihood tree was not well resolved in the *Saccharina* species complex due to scarce phylogenetic signal, we additionally used MrBayes (see Methods) to compare its outcome with the maximum likelihood tree. This approach confirmed the tree topology of the maximum likelihood method and delivered even higher support at the deeper branches with cumulative probability of 100% at all nodes (Fig. S2). MrBayes also yielded two alternative topologies with lower cumulative probability affecting the placement of *S. japonica* var. *diabolica* (Miyabe) and *S. japonica* var. *ochotensis* (Miyabe) [48] (not shown).

3.3. Special features in organelle genomes

Overall, all kelp species organellar genomes contain the same gene sets, i.e. no gene was missing or additionally present in any species with two exceptions: i) In a previous sequencing effort we found, that *L. solidungula*, in comparison to other Laminariales, lacks an ORF (YCF37) in its plastid genome [11]. YCF37 is also present in cyanobacteria and it was reported that a deletion mutant shows enhanced susceptibility to high light [49]. *L. solidungula* is a low light adapted kelp and one of the reasons to sequence low light adapted *L. rodriguezii* and high light tolerant *L. digitata* was to investigate whether *L. rodriguezii* also lacks the ORF. This is, however, not the case and we also found no other common markers in the organellar genomes of the two low light species, *L. solidungula* and *L. rodriguezii*, which distinguishes them from the high light tolerant ones. ii) In this study we found in *L. rodriguezii* an additional ORF in the mitochondrial genome. This ORF is embedded in an approximately 700 bp stretch of DNA, is 456 bases or 152 amino acids long and, according to IPRscan [50], contains no recognizable domain.

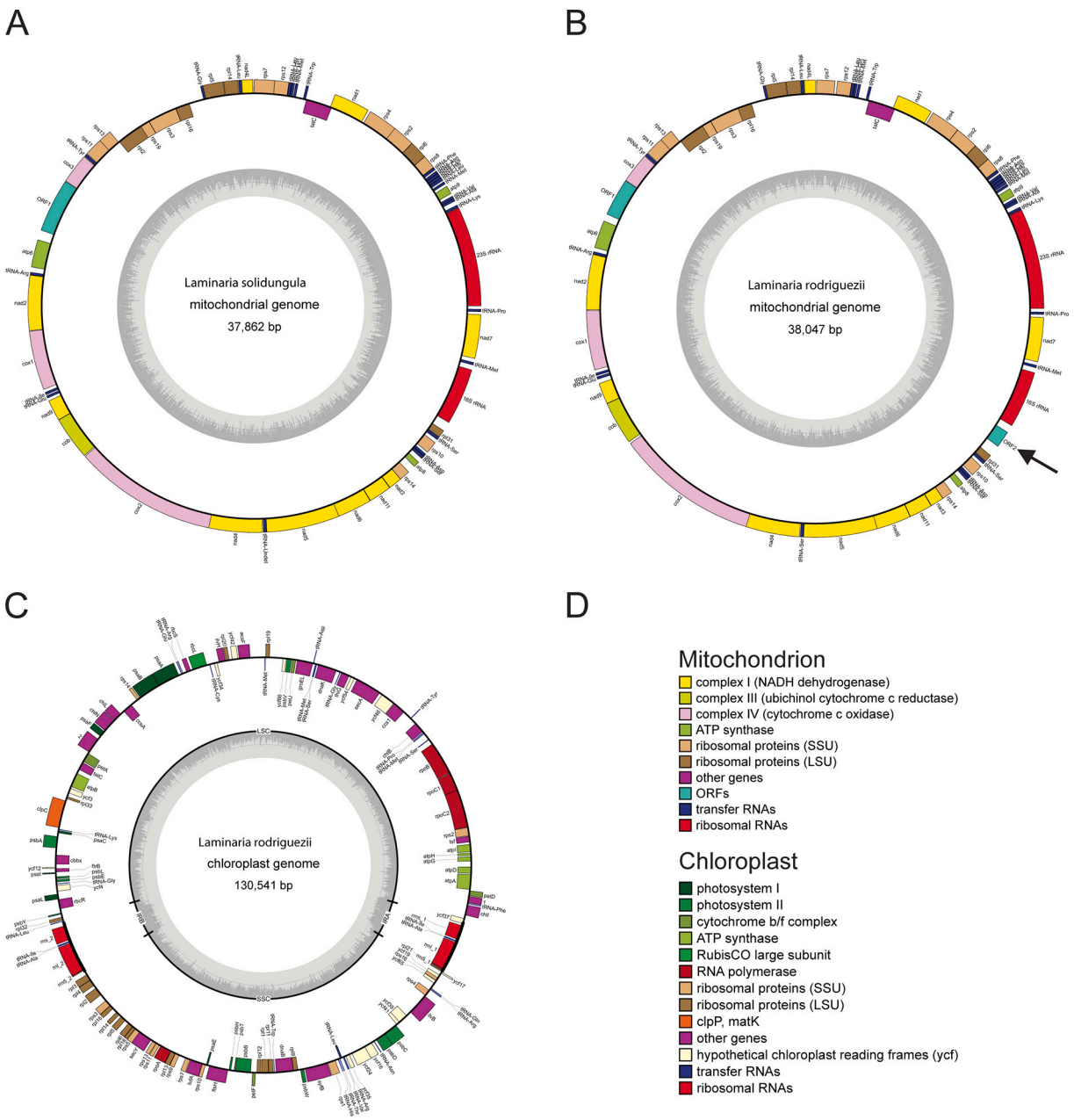


Fig. 1. Newly sequenced organellar genomes. A: *Laminaria solidungula* mitochondrial genome. B: *Laminaria rodriguezii* mitochondrial genome. C: *L. rodriguezii* chloroplast genome. The arrow in B indicates the position of the ORF discussed in the text. The figures were made with OGdraw [39] and the legends are depicted in D.

To exclude contamination or an assembly error we confirmed the presence of the ORF in *L. rodriguezii* and its absence in *L. solidungula* and *L. digitata* by PCR (Fig. S3). BLAST searches with the ORF against all published 90 complete mitochondrial genomes of Phaeophyceae (Table S4) did not result in any match. However, a search against all brown algae revealed significant similarity to a mitochondrial ORF in *D. viridis* [47], where the similarity covers the 3′ end of the *L. rodriguezii* ORF and at the 5′ end of the *D. viridis* ORF over a length of 234 bases or 78 amino acids (Fig. 3B). Also, a short stretch of 45 bases upstream of orf211 in *D. viridis* is similar to ORF 2 in *L. rodriguezii* (Fig. 3A, C). Despite overall collinearity of the two mitochondrial genomes the two ORFs do not share the same relative position within the mitochondrial genome. While the *D. viridis* ORF is located between tRNA-K and tRNA-V adjacent to the 23S rRNA gene replacing tRNA-A, the *L. rodriguezii* ORF is placed between the 16S rRNA gene and rpl31 (Fig. 1B). The foreign sequences in both, *L. rodriguezii* and *D. viridis,* show no detectable similarity to any known sequences including brown algal or kelp nuclear genomes, bacteria associated with brown algae, or viruses. We also found no evidence for transcriptional activity of the foreign sequences as there are no similar sequences in any currently available brown algal transcriptome dataset [51–54].

4. Discussion

4.1. Phylogenetic analysis

Since currently no complete nuclear data are available for most kelp species our phylogenetic analysis was restricted to that of the organellar genomes. Only 9 kelp chloroplast genomes are yet sequenced (Table S3), but these are collinear with only minor rearrangements at the inverted

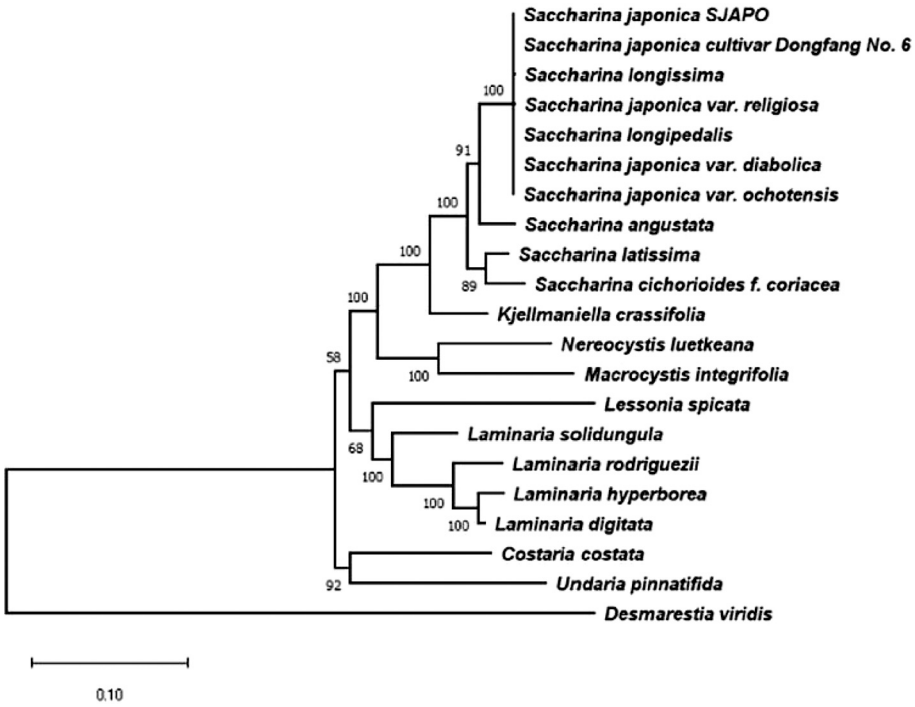


Fig. 2. Phylogenetic analysis of kelp species with complete mitochondrial genomes. Maximum likelihood tree of aligned mitochondrial genomes. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model [45].

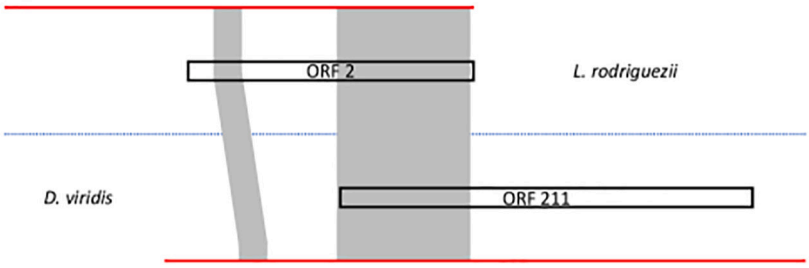
repeat (IR) regions. Similarly, all 44, including the two new ones from *L. rodriguezii* and *L. solidungula,* available mitochondrial genomes from kelp species are collinear (Table S2) and we have used 18 for the phylogeny (Table 1). Even the mitochondrial genome from *D. viridis*, a more distantly related brown alga from the sister order Desmarestiales, which was used as outgroup in the phylogenetic analysis, has the same gene order as the kelp species. Thus, we conclude that rearrangements of mitochondrial genomes in kelps are rare if not completely absent. The chloroplast and mitochondrial genome sequences can be aligned completely irrespective of coding or intergenic region. Thus, for phylogenetic analyses we have slightly more data per species at our disposal than previous studies [20,27], but limited to organellar genomes only. With this data set it was impossible to resolve the *Saccharina* species complex with the maximum likelihood method indicating weak phylogenetic signal in mitochondrial genomes within this group. The MrBayes analysis however resolved the topology with high confidence values (99% each).

We compared our mitochondrial tree with the previously published comprehensive overview on kelp radiation [20] and found no difference. Our tree includes other kelp species than Starko *et al.* [20], but the species used in both studies are placed at alike positions in the tree. We therefore are confident that the placement of *L. solidungula* and *L. rodriguezii* within our tree is also correct. Interestingly the close relationship between *L. digitata* and *L. rodriguezii* is also reflected in their temperature tolerance profiles which are more similar to each other than to *L. ochroleuca* Bachelot Pylaie, a southern European kelp species [55]. The tree topology within the Laminariaceae conforms to that, which was previously published using roughly 2 kb of data from internal transcribed spacer (ITS; nuclear), Rubisco spacer (chloroplast) and mitochondrial cytochrome *c* oxidase sequences [27]. The number of informative sites present in organellar genomes thus seems to be sufficient to resolve the kelp phylogenetic tree with high confidence. When further kelp organellar genomes are being produced they can be readily added to the alignment. The phylogenetic position of *L. rodriguezii* presented here builds the framework, on which our further interpretation of results relied.

4.2. Deviations from collinearity in kelp organellar genomes

In light of the overall collinearity of kelp mitochondrial genomes it is surprising that we observed a long stretch of DNA in the *L. rodriguezii* mitochondrial genome adjacent to the 16S rRNA gene without counterparts in 43 other kelp species and 48 other Phaeophyceaen mitochondrial genomes (Table S4). We can exclude a technical (sequencing or assembly) problem here since neither the cultivating nor the sequencing laboratory ever handled *D. viridis* and the sequences in the two species differ enough to exclude an accidental co-cultivation with assembly errors afterwards. Nevertheless, we confirmed the presence of the ORF in the mitochondrial DNA of *L. rodriguezii* and its absence in *L. digitata*, and *L. solidungula* by PCR (Fig. S3). Part of the additional DNA in *L. rodriguezii* can be translated into an ORF and yields a potential protein of 152 amino acids. The encoded protein of this ORF has an overlap with high identity of 72% and a similarity of more than 88% to part of a protein encoded by an ORF in the *D. viridis* mitochondrial genome (Fig. 3B). The nucleotide identity is even higher than the amino acid sequence identity indicating mutational changes affecting all codon positions. Possible reasons for this could be fast evolution (positive selection) or degradation. The nucleotide sequence similarity of the region in which ORF211 from *D. viridis* resides, extends 5′ to its start codon (Fig. 3A, C), indicating that the annotated start codon of this ORF is not the original one. Furthermore, a stretch of 45 bases in the 5′ region of ORF211 has also high similarity of 80% to ORF2 of *L. rodriguezii*. These similarities, extending from the originally defined ORF, indicate that the ancient coding gene in *D. viridis* was longer than the extant one. Similarities on nucleotide level are very unlikely to have occurred by chance and should have originated from independent lateral introduction of the same gene from an unknown source. A vertical transfer between the two species is unlikely since they are separated by several other brown algae in the tree (Fig. 2). In case of vertical inheritance some traces of this transfer should have remained in at least some of the other mitochondrial genomes of kelps. Moreover, the differing locations of the two ORFs argues against a vertical transfer in the light of the otherwise strictly collinear genomes. The different location together with the only

A



B

```
L. rodriguezii  MGTYHPQGCITSSGLSSPGCIVGITNVLNIHPSGMLSKFLFIQILEQLTE
D. viridis      --------------------------------------------------

L. rodriguezii  SILGIIHKYETTFPSSIFKNKPSSDPILISLLEQIVQSQEDQRHFVREMS
D. viridis      -------------------------------MEQVVQSQEDQRRFVKEIS
                                              :**:********:**:*:*

L. rodriguezii  NRQNILLESLNDVVMRQDKISLDLKQMFYKDNNSWYYSHLSTTLQYLQII
D. viridis      NRQNILLESLNDVVIRQDKMSLDLKHLSSGKSHSWHY-LSTTLQYLQVI
                **************:****:*****::   ..:**:*  *********:*

L. rodriguezii  NV-------------------------------------------------
D. viridis      SIFTPYIAKILPTTLIENIPLVNKVVWFFSPGQPEWVGPMRNMETNINTL
                .:

L. rodriguezii  --------------------------------------------------
D. viridis      AAALNQTNQGLAGVHTQVTALNETARGLVESQTNIQRIHENGLAEQLARD

L. rodriguezii  ---------------------------------------------
D. viridis      LNTLATQTPYTPPVPTAEVTSAVDNSTPMDRPRLQNTAHLFRRT
```

C

```
L.rodriguezii   ATATAATAATGTATATATAATGGGGACATATCATCCTCAAGGATGTATAACGTCTTCAGG
D.viridis       ---C...C.AT..G.G..TG.AA.A...C..T.G.T.-.CATCAA..AT.TTAG..ATAT
consensus       ....***.*..**.*.**..*..*.***.**.*.*.*.*......**..*....**....

L.rodriguezii   CCTCTCTTCTCCGGGGTGTATAGTAGGAATCACTAACGTACTTAATATACACCCTAGCGG
D.viridis       ..C..T.C..........C.....T.........G..A...GC..----------------
consensus       **.**.*.**********.*****.*********.**.***..**................

L.rodriguezii   TATGCTTAGCAAGTTTTTATTCATACAAATCCTCGAACAACTCACGGAATCTATACTAGG
D.viridis       ---------------...T...T..T..GG.T...TGA.TTGAG.T..TC...AGA..AA
consensus       ..............***.***.**.**..*.***...*.....*.**..***...**..

L.rodriguezii   CATAATTCACAAATATGAAACTACATTTC---CTTCTAGTATTTTCAAGAACAAGCCTTC
D.viridis       ACCC.AC.GTCTCA.A.GCCA.TTTAAG.CCCT.A.AGTG.CAAAA.CAT.ATGAA..CA
consensus       ....*..*......*.*....*......*....*.*....*.....*...*.....**..

L.rodriguezii   TTCCGATCCCATTCTTATTTCCCTTTTAGAGCAAATCGTGCAGTCCCAAGAAGATCAACG
D.viridis       AA.G.......CC...G.......C..G.......G....C..................G..
consensus       ..*.*******..***.*******.**.******.****.******************.**

L.rodriguezii   TCACTTTGTGAGAGAAATGTCAAATAGACAAAACATTCTTTTAGAAAGCTTAAATGATGT
D.viridis       G.GG.......A......C.....C........T.....................C.....
consensus       .*..*******.******.*****.********.**********************.*****

L.rodriguezii   CGTAATGCGTCAAGATAAGATTTCATTGGACCTTAAACAAATGTTTTATAAGGATAATAA
D.viridis       G.....C..............G..C..............CC...C..C.GGTA.G.G.C.
consensus       .*****.***************.**.***************.***.**.*.....*.*.*.*

L.rodriguezii   CTCCTGGTATTACTCACACCTATCTACTACCCTACAATATTTACAAATAATTAATGTGTA
D.viridis       ...G...C......------..........T.G............G.C....G.A.A.T
consensus       ***.***.******.....***********.*.************.*.****.*.*.*.
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Fig. 3. Alignment of the gene product of ORF2 of *Laminaria rodriguezii* with its partial counterpart from *Desmarestia viridis* (orf211 in NC_007684). A: A sketch of the two ORFs. ORFs are drawn as open rectangles, the unique regions of both species are represented by red lines. The two overlaps are depicted as grey boxes. B: The alignment of the translated protein sequences of the two ORFs. Asterisks denote conserved residues, colons conserved substitutions, and dots semi conserved substitutions. C: The section comprising the two overlaps aligned on nucleotide level. The overlapping parts from A are framed with red lines, the start codon of orf211 is highlighted by a green open box. Asterisks denote conserved residues in the consensus. Identical bases in *D. viridis* are represented by dots. The alignments were done with clustalw [65]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

partial overlap suggests independent introductions of the two ORFs into the genomes and thus the overlapping conserved region.

In our opinion, the conservation of nucleotide sequences could have been achieved by two reasons: Functional conservation, i.e. purifying selection, or recent introductions from a similar source into the two species, so that only a few mutations could accumulate. Yet, in the overlapping stretch the nucleotide conservation is higher than the amino acid conservation, which points to loss of functional constraint and rapid degradation. We thus prefer the idea, that relatively recent independent events introduced these sequences into the mitochondrial genomes of *L. rodriguezii* and *D. viridis,* respectively. The origin of such sequences could lay in the nuclear genome, yet we could not find such a conserved gene in the published genome of a *Saccharina* species [7] and in our draft assembly of *L. digitata*. Thus, a horizontal gene transfer (HTG) of the genes from external sources is currently the best explanation for the presence of these sequences. Since we found no similarities to other sequences the source of this HTG remains unknown. We can, however, speculate, where this sequence came from. A HTG is often achieved via a vector, be it plasmid or virus. We therefore searched for potential candidates of the observed HTG, and found that in certain evolutionary branches so-called mitoviruses exist, which are exclusively found in mitochondria. Such mitoviruses replicate in mitochondria only and encode a single protein on their RNA(+) strand, an RNA-dependent RNA polymerase. They are so far known from fungi [56] and plants [57] only. In fungi they are transmitted via spores, mating or cytoplasmatic mixing, but can also be transmitted between distantly related species by unknown means [56]. The viral RNA polymerases are generally not well conserved [58,59] and therefore it is not surprising that we found no match of the brown algal ORF to publicly available mitovirus sequences. Even the complete mitovirus sequences from plants and fungi cannot be aligned together and branch specific alignments (fungi or plants) yield only a few conserved residues [57]. Moreover, the apparent degradation of the ORFs likely affected the identifiability of the scarce conserved amino acids in RNA-dependent RNA polymerases. Nevertheless, mitoviruses are reasonable candidates for the independent infestation of the mitochondria of the two brown algae. Consequently, we also searched brown algal genome and transcriptome data for similar sequences but without positive match. One explanation for this situation could be that mitoviruses normally do not integrate into DNA and thus are mainly present in transcript data [57]. However, such data are currently scarce for brown algae and unfortunately focussed on polyA+ mRNAs only, which renders the probability to detect RNA viruses marginal.

From gene and virus integration assays it is known that such sequences preferentially integrate into transcriptionally highly active portions of a genome [60,61]. From the analysis of mammalian and plant mitochondrial genomes it is also known that the rRNA genes are normally expressed at a much higher level than the rest of the mitochondrial genome, which seems to be transcribed as one large RNA [62,63], however, sometimes post transcriptional regulation may account for different levels of steady state levels of RNA [64]. Taken together it is intriguing to speculate that the observed locations of the two similar sequences in the vicinity of rRNA genes are due to the higher propensity of DNA pieces to integrate at highly expressed sequences.

5. Conclusion

Here we present three newly sequenced organellar genomes from kelp species together with a thorough analysis of their specific features and phylogenetic information content in comparison to other kelp organellar genomes. Collinearity of the organellar genomes within the kelp species together with their considerable length and thus informative positions make them suitable as very robust phylogenetic markers for the entire order. Our finding of an independent introduction of homologous nucleotide sequences into two brown algal mitochondrial genomes, potentially of mitovirus origin, is unprecedented and should be the beginning of endeavours to search for similar sequences in other brown algae, e.g. via the PHAEOEXPLORER project, and ultimately target complete mitovirus genomes from kelp.

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Author contributions

GG conceived the study and wrote the manuscript. SR and JR carried out the experiments and analysed the data. LE contributed to the experiments and to the final version of the manuscript. IB, AB, LR, and TT contributed material and to the final version of the manuscript. KV contributed to the collection of *L. rodriguezii* and discussions of the results. All authors provided critical feedback.

Data accessibility

The newly described organellar genome sequences have been deposited in the NCBI database under the Accession numbers MT732096, MT732097, and MT732098.

Author statement

We declare, that we have no competing financial interests.

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