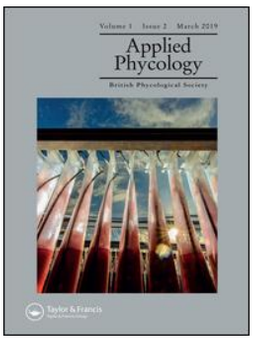

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Mitochondrial and plastid genome variability of *Corallina officinalis* (Corallinales, Rhodophyta)

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ABSTRACT

Corallina officinalis is a calcifying red alga, common in tide pools in the North Atlantic with occasional reports from the north-east Pacific. It is an important habitat-forming alga, providing shelter and substrata to many other organisms. To date there are only five published organellar genomes for *Corallina*, including *C. chilensis* and *C. ferreyrae*. This study reports the first four published plastid genomes for *C. officinalis*, along with three new mitogenomes from samples in the United Kingdom, Spain and Iceland. The plastid genome is 178 kbp and 99.9% of bases are identical for all samples. The mitogenomes are more variable than the plastid genomes, with lengths varying from 26.2 to 26.7 kbp and 99.0% base identity. Structure and length of both of the genomes are consistent with other published *Corallina* genomes. The most variable mitochondrial gene is *sdh*D (3.3% variability), while all plastid genes have <1% base variability, with the most variable being *psb*30 (0.95% variability). The stability of the plastid genome means it is not useful for examining intra-specific variability within *Corallina*. We discuss whether the ratio of mitogenome and plastome sequences recovered in the readpool of NGS sequencing is indicative of relative copy number.

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Introduction

The red coralline alga *Corallina officinalis* (Corallinales) is a calcified seaweed that is widespread in the North Atlantic (Brodie, Walker, Williamson, & Irvine, 2013) with a few reports from the north-east Pacific (Hind, Gabrielson, Lindstrom, & Martone, 2014; Magill, Maggs, Johnson, & O'Connor, 2019). It inhabits tide-exposed rock-pools, where it provides vital ecosystem services such as substrata provision and a habitat that facilitates invertebrate recruitment (Brodie et al., 2016; Nelson, 2009; Perkins et al., 2016). *Corallina officinalis* has a high-magnesium-calcite skeleton, the most soluble polymorph of calcium carbonate deposited by marine calcifiers, making it potentially vulnerable to the global decrease in ocean pH (*i.e.* ocean acidification), which can critically undermine the structural integrity of calcifying organisms (Brodie et al., 2014). To-date, studies have reported a wide tolerance of this species to fluctuating tide-pool conditions, including ambient carbonate chemistry, water temperature and light regime (Williamson et al., 2014; Williamson, Perkins, Voller, Yallop, & Brodie, 2017; Williamson et al., 2018). However, recent work has demonstrated that physiological responses in *C. officinalis* vary over its distribution in the NE Atlantic, with populations from the UK and Spain showing markedly different responses to conditions (Kolzenburg et al., 2019).

We know from population genetic analysis that there is significant genetic structure (based on nuclear SNPs) within *C. officinalis* over a wide latitudinal gradient, from Iceland to Spain (Yesson, Jackson, Russell, Williamson, & Brodie, 2018). There is evidence of gene-flow between the British Isles and Spain, but Icelandic populations appear more isolated (Yesson et al., 2018). We also know there is substantial genetic variability within and between *Corallina* species based on widely used mitochondrial and plastid DNA "barcode" sequences (Williamson et al., 2015). However, genome-level assessment has yet to be undertaken.

Genome analysis can provide valuable insight into the relationships of many organisms including red algae (Iha et al., 2018). However, although relatively few florideophycean algae (the largest class of red algae) have had both organellar genomes sequenced, of the 102 florideophycean plastid sequences available (Cho, Choi, Lam, Kim, & Yoon, 2018), 40 are accompanied by a mitogenome (Bustamante, Calderon, & Hughey, 2019; Salomaki & Lane, 2017). To date three species of *Corallina* have complete mitogenomes published: *C. officinalis* (Williamson, Yesson, Briscoe, &

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Supplemental data for this article can be accessed here.

Brodie, 2016), *C. chilensis* (Alejo et al., 2019) and *C. ferreyrae* (Bustamante et al., 2019); the latter two species also have complete plastid genomes (Alejo et al., 2019; Bustamante et al., 2019). This study examines the mitochondrial and plastid genomes of four samples of *Corallina officinalis* from three countries to assess organellar genome variability within this species and assess the value of these organellar genomes for assessing intra-specific patterns.

Materials and methods

Four samples of *C. officinalis* were selected for analysis from three countries. These samples are lodged in the Natural History Museum algal collections under accession numbers: BM001215284 – Heybrook Bay, South Devon, UK (50°19′09.4”N 4°06′46.3”W), BM013828925 – Combe Martin, North Devon, UK (51°12′33.9”N 4°02′27.1”W), BM013828927 – Comillas, Cantabria, Spain (43° 23′37.3”N 4°17′26.6”W) and BM013828926 – Þorlákshöfn, Ölfus, Iceland (63°50′40.0”N 21°22′23.7”W).

Total genomic DNA was extracted from 0.5 to 1 cm^2 of each frond sample, using a modified CTAB extraction method (Williamson et al., 2015). Double-stranded DNA was quantified with a Qubit fluorometer 2.0 (Invitrogen, Waltham, MA). Index libraries were constructed with a TruSeq Nano DNA sample preparation kit using the recommended ~100 ng of gDNA (Illumina Inc., San Diego, CA) and sequenced on an Illumina MiSeq flowcell, v 3 chemistry (2x300 paired end reads). Two runs were performed. Run one contained equal amounts of the UK samples, run 2 sequenced the Spanish & Icelandic samples. These are the same data used in constructing the mitochondrial genome of sample BM001215284 (Williamson et al., 2016).

After sequencing, the read pools were assessed for quality using FastQC Version 0.11.8 (Babraham Bioinformatics, Cambridge, UK). Adaptors were removed from the sequences using cutadapt (Martin, 2011), which also trimmed poly-A tails, and the end 10 base-pairs were trimmed from all reads. After trimming, reads shorter than 35 bp were discarded.

The genome assembler NOVOplasty (Dierckxsens, Mardulyn, & Smits, 2019) was used to reconstruct the organelle genomes.

Seed sequences were used for the assembly process. For mitogenomes, the *cox1* region from accession KU641510 was used. For the plastid genomes the *rps1* region was used based on *C. ferreyrae* (NC_041636). The seed-and-extend algorithm was run until the contig could be circularized.

Annotations were transferred from published *Corallina* genomes (Alejo et al., 2019; Bustamante et al., 2019; Williamson et al., 2016) in Geneious ver. 2019.1.327. Gene orders were validated by manual comparison with the template and by coverage. Annotations of transfer RNAs (tRNAs) were verified by tRNAscan-SE 2.028 (Chan & Lowe, 2019). Gene boundaries were extended/contracted to the widest matching open reading frame (ORF).

Genomic sequence alignments were made for both genomes, using available Corallinophycidae genomes (see Supplementary table S1 for accession numbers). Alignments were made with ProgressiveMauve (Darling, Mau, & Perna, 2010), and masked with Gblocks (Castresana, 2000). Maximum likelihood (ML) phylogenies were constructed using RaxML (Stamatakis, 2014) under the general time-reversible (GTR+gamma) substitution model after (Bustamante et al., 2019).

Results

All four samples produced millions of short sequence reads (c. 300 bp). Post-filtering, the size of the four read-pools varied from 17 to 57 m (see Table 1). Coverage for the full genomes averaged in the hundreds for all samples/genomes. The Icelandic sample (the northern-most sample) contained a lower ratio of plastid genome reads relative to mitochondrial reads, while the southern-

Table 1. Sequence statistics from the four samples of *Corallina officinalis* used in this study. Original reads refer to unfiltered output from sequencing. Filtered reads are post-cleanup. PM ratio refers to a ratio of plastome (chloroplast) to mitogenome reads defined as (% chloroplast reads/chloroplast length) ÷ (% mitogenome reads/mitogenome length). Coverage for the UK (S Devon) is based on reads from just the first sequencing run.

	Iceland	UK (N Devon)	UK (S Devon)	Spain
Original reads	31,425,418	17,568,280	59,444,142	20,730,640
Filtered reads	29,457,953	17,274,162	57,280,774	17,275,937
Mitogenome Length	26,265	26,700	26,573	26,535
Coverage Mean (min. – max.)	728 (122–3448)	272 (49–515)	335 (21–1610)	196 (10 – 292)
% Mitogenome reads	0.26%	0.14%	0.06%	0.09%
Plastome Length	178,183	178,484	178,170	178,182
Coverage Mean (min. – max.)	1170 (149–1745)	582 (54–1142)	853 (74–1720)	383 (42–540)
% Plastome reads	2.96%	2.31%	1%	1.56%
PM-ratio	1.68	2.47	2.49	2.58

Table 2. Summary of annotations for both mitogenome and chloroplast genomes of *Corallina officinalis*.

	No. of genes/regions	Length (bp)	Base variability	Longest	Shortest	Most variable
mtDNA						
Protein coding	23	17,979	1.13%	*nad*5 (2015 bp)	*atp*9 (231 bp)	*sdh*D (3.3%)
Transfer RNAs	25	1866	1.06%	*trn*L1(tag) (85 bp)	*trn*T(tgt) (58 bp)	*trn*I(tat) (3.2%)
Ribosomal RNAs	2	3966	0.33%	*rrl* (2567 bp)	*rrs* (1399 bp)	*rrl* (0.47%)
Unannotated regions	-	3087	28.83%	-	-	-
cpDNA						
Ribosomal protein	47	19,035	0.03%	*rpl*2 (828 bp)	*rpl*36 (114 bp)	*rpl*32 (0.53%)
Photosystem I and II	30	15,051	0.07%	*psa*A (2265 bp)	*psa*M (93 bp)	*psb*30 (0.95%)
ycf	26	16,455	0.10%	*ycf*45 (1692 bp)	*ycf*17 (204 bp)	*ycf*52 (0.55%)
ORFs (>100 bp)	10	6168	0.08%	ORF 700 (2121 bp)	ORF 260 (108 bp)	ORF 13 (0.46%)
Other named genes	93	93,369	0.05%	*glt*B (4593 bp)	*pet*N (90 bp)	*cpc*A (0.3%)
Transfer RNAs	31	2360	0.21%	*trn*S(gct) (91 bp)	*trn*D(gtc) (70 bp)	*trn*E(ttc) (2.74%)
Ribosomal RNAs	3	4473	0.00%	*rrl* (2878 bp)	*rrf* (117 bp)	-
Non-coding RNAs	2	456	0.00%	*rnp*B (361 bp)	*ffs* (95 bp)	-
Unannotated regions	-	20,816	0.20%	-	-	-

most sample (from northern Spain) showed the highest ratio of chloroplast to mitochondrial reads.

Mitogenome lengths ranged from 26,265 to 26,700 bp, which represents a 1.7% length variability. GC content was consistently 30.0%. The structure of the mitogenomes were conserved over all samples and code for 23 protein-coding genes, 25 tRNA genes, and two rRNA genes (Table 2, Supplementary figure S1). Each mitogenome included a large (1999–2434 bp) intergenic region (IGR) containing just transfer RNAs with the longest stretch of unannotated sequence of the IGRs being between the *trn*Y and *trn*N regions (up to 898 bp). We note that this section contains long, inverted repeats at either end. Contained within this large IGR, and between the two long repeats were the only two features which differed in length between populations: *trn*S1 (76–88 bp), and *trn*Y (83–88 bp). All other regions were fully conserved in length. The most variable region is *sdh*D, which shows 3.3% (8/243) base variability, while overall there is a 0.99% variability on annotated regions (Table 2). Supplementary table S2 provides a detailed list of the annotated regions of the mitogenomes.

The plastid genome lengths ranged from 1,78,170 to 1,78,183 bp, a much lower length variability than the mitogenome (0.2%). GC content was consistently 30.2%. Each plastid genome codes for 205 protein-coding genes (including 27 hypothetical conserved proteins, and 10 unassigned ORFs), 2 non-coding RNAs, 31 transfer RNA genes, and 3 ribosomal RNAs (Fig 1, Table 2). A singular group II intron is situated within the *chlB* gene, along with an intron-encoded ORF (ORF 456). Overall, the four plastid genomes were highly conserved, with only 102 variable bases (99.9% conserved) of these variable bases 91 are in coding regions and 61 in named/recognized genes (excluding ycf and ORF). The majority of these are synonymous substitutions with only 14 resulting in a change of amino acid (Table 2). Supplementary table S3 gives a detailed list of the annotated regions of the plastid genomes.

The relatively conserved plastid genome gives much lower genetic distances between samples than the more variable mitogenomes (Fig 2, Table 3). The Icelandic sample is the most genetically distinct according to the plastid genomes, but the north Devon sample shows slightly higher genetic distances according to the mitogenome. Furthermore, the mitogenome and plastid genome phylogenies differ slightly, firstly *C. ferreyrae* is shown as sister to *C. officinalis* (with weak support) based on the mitogenome, while *C. chilensis* is sister (with high support) based on the plastid genome data. The within-species relationships reflect the genetic distances, with the low genetic differentiation of the plastid genome being reflected in weak support values for the recovered relationships.

Discussion

This study reports the first complete plastid genome for the widespread *C. officinalis* from samples in the North Atlantic. Two other *Corallina* species have complete mitochondrial and plastid genomes published, *C. chilensis* (Alejo et al., 2019) and *C. ferreyrae* (Bustamante et al., 2019).

Plastomes

Corallina officinalis plastomes are similar in size to the other *Corallina* genomes, with a < 200 bp length difference from *C. chilensis* for all samples. The gene order amongst *Corallina* is identical for all shared annotations, although the *rnp*B and *pet*J regions are not annotated in either of the previously published *Corallina* sequences. *rnp*B is a non-coding RNA region which

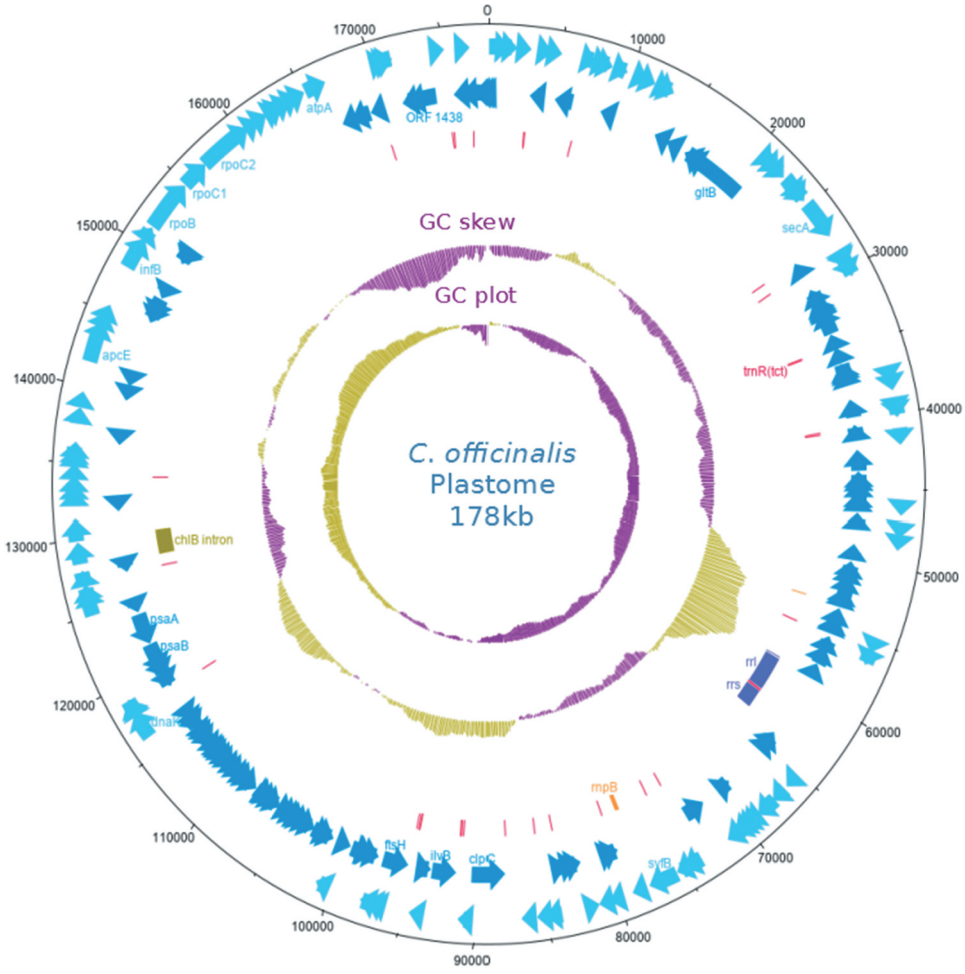

Figure 1. Circular representation of the chloroplast genome of *Corallina officinalis*. Genes are shown in blue light forward, dark reverse (arrows indicate direction of translation), tRNAs in red, rRNAs in orange and introns in green. Numbers around the outer circle indicate base pairs. For GC plot and GC skew graphics green indicates positive, purple negative, based on window size 10,000, step size 200. Graphic generated by DNAPlotter (Carver, Thomson, Bleasby, Berriman, & Parkhill, 2009).

shows only 0.6% base variability; in contrast the *pet*J gene shows higher base variability (6.1%). We also note that closely matching unannotated regions are contained within the other published *Corallina* sequences and are both present and annotated in other published plastomes of Corallinaceae (Janouškovec et al., 2013). Another notable feature is a large (1600 bp) insertion in *C. officinalis* (relative to other *Corallina* species) between *omp*R and *rrs* rRNA, which encompasses two substantial (> 400 bp) ORFs (ORF 1811 and ORF 1809). The presence of species-specific regions containing multiple ORFs has been observed in plastid genomes of other red algae (Janouškovec et al., 2013). The group II intron within the *chl*B gene is present in other Corallinaceae (Alejo et al., 2019; Bustamante et al., 2019; Janouškovec et al., 2013). Overall, there is high plastome conservancy observed within and between *Corallina* species, which is indicative of the high plastome conservancy reported within the in general red algae (Iha et al., 2018).

Mitogenomes

As for the mitogenomes, there is at most an 805 bp length difference with other *Corallina*, and the structure and gene order are highly conserved. *C. chilensis* has a 500 bp ORF (orf158) and a similar 507 bp ORF is evident in all *C. officinalis* mitogenomes located between *trn*W and *trn*A. This section of the genome is a highly variable region across the genus *Corallina* with 26.4% of bases showing some variation. This region appears to be a hotspot of variability for the red algae as Iha et al. (2018) found high variability for this region in Gracilariaceae. The *sdh*3 region could also be a potential target for sequencing studies as it showed the highest variability of all genes; however it is noted

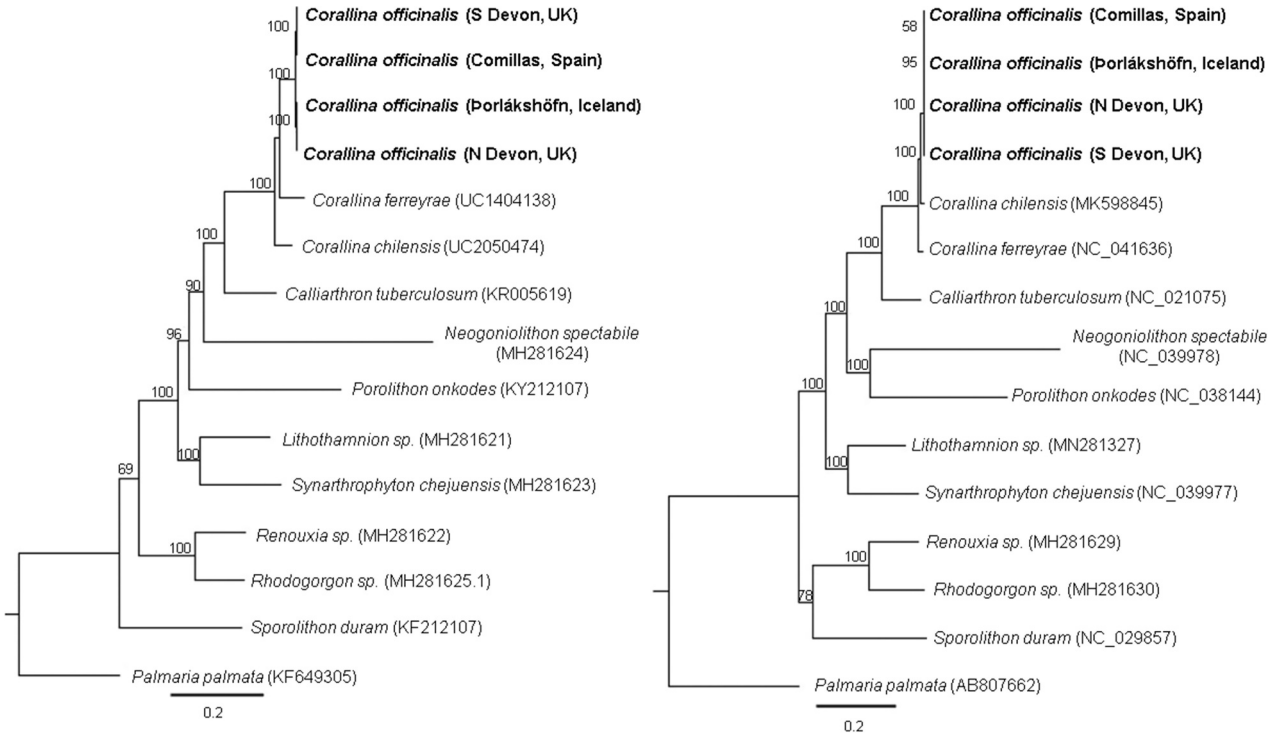

Figure 2. Maximum likelihood phylogenies based on mitogenome (left) and chloroplast (right). Bootstrap support values indicated at nodes. Branch lengths proportional to DNA substitutions. Taxa in bold are samples from this study.

Table 3. Genetic distances between genomes of *Corallina officinalis*. Distances based on pairwise % dissimilarity. Mitogenomes are shown in the upper triangle, with chloroplast in the lower.

	UK (N Devon)	Spain	UK (S Devon)	Iceland
UK (N Devon)	-	1.02	1.03	0.19
Spain	0.02	-	0.03	0.96
UK (S Devon)	0.02	0.01	-	0.96
Iceland	0.03	0.02	0.03	-

that this region is lost in some other Florideophyceae (Yang et al., 2015), potentially limiting its wider use. The *trn*T and *trn*I regions are not annotated for other *Corallina* mitogenomes (Alejo et al., 2019; Bustamante et al., 2019), although these sequence regions are highly conserved across *Corallina*. The *trn*I region is situated alongside *trn*L1 between *cob* and *nad*6, whereas other Corallines see this positioned within a group II intron between *nad*5 and *nad*6 (Lee et al., 2018). The length variability of *trn*S1 (gct) seen within *C. officinalis* is also observed over the Corallinaceae, with the length for *Neogoniolithon* (94 bp) being longer than any of our samples (Lee et al., 2018). *trn*Y also shows length variability across other *Corallina* (*C. chilensis* 84 bp, Alejo et al., 2019; *C. ferreyrae* 83 bp, Bustamante et al., 2019). The number of tRNA (25), CDS (23) and rRNA (2) are in the same range reported for other red algae (Yang et al., 2015), although the tRNA count is the joint highest reported only matched by the Rhodymeniophycidae *Plocamium*. The higher variability within *Corallina* mitogenomes relative to plastid fits the expected pattern reported for many other organisms (Smith & Keeling, 2015).

Ratio of cpDNA to mtDNA

The ratio of cpDNA reads to mtDNA is lowest in the Icelandic samples. It is notable that this is the most northerly population (close to the northern limits of the species in the North Atlantic). Environmental conditions for these northern populations are characterized by lower irradiance, temperature, and carbonate saturation (Brodie et al., 2014). We know that environmental conditions can affect copy number of plastid and mitochondrial genomes in plants (Wang, Anderson, & Griffin, 2004), so it would be worth further investigation to test whether the relative coverage rates in sub-Arctic Iceland are influenced by the colder temperatures and lower light levels, particularly in light of evidence that photoregulatory capacity of *C. officinalis* appears to decrease in more northerly populations (Kolzenburg et al., 2019).

The relative copy number of organellar genomes is a potentially interesting variable characteristic that is not

easily accessible. Previous methods for determining this characteristic are labour intensive (e.g. qPCR-based methods (Palmeira & Rolo, 2015) and western-blotting (Picard et al., 2011)). Currently, the turnover rate of Krebs cycle enzymes is the nearest proxy for absolute mitochondrial number, at least in animal models (Larsen et al., 2012). Assembly of multiple, paired, conspecific organellar genomes allows relativized estimation of copy-number proportions, as the assembler detects relative abundance of organellar sequences in the readpool.

However, it is difficult to draw conclusions based on the low level of sampling in this study, particularly when copy number can change over time (Zoschke, Liere, & Börner, 2007).

In conclusion, this study reports the first four plastomes and three novel mitogenomes for *C. officinalis*. Plastome variability is very low within the species, making it an unlikely target for assessment of intra-specific variability. Mitogenome variability is higher and would make a better target for sequencing-based studies within the group.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Author Contributions

CY: Conceptualization, formal analysis, writing; CW: Conceptualization, writing, lab work; XB: Formal analysis, writing; AB: Lab work; writing; JB: Conceptualization, writing

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