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Mitogenome analysis of a green tide forming Ulva from California, USA confirms its identity as Ulva expansa (Ulviceae, Chlorophyta)

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ABSTRACT
An unknown species of marine sea lettuce was observed forming green tides consecutive years from 2014 to 2016 in Seaside, California. This Ulva sp. was similar in thallus size and shape to Ulva expansa. To confirm this identification, whole genome sequencing was performed on the bloom-forming species of Ulva and the holotype specimen of Ulva expansa. The complete green tide Ulva mitogenome is 64,143 bp in length, contains 65 genes, and displays high gene synteny with Ulva pertusa Kjellman. The mitogenome was incomplete for the holotype of Ulva expansa, but the analysis yielded the mitoexome, plastid, and nuclear genetic markers. These data verify that the native Ulva expansa is responsible for the blooms in central California.

Green tides are blooming events of green algae that result from eutrophication and warming seawater (Fletcher 1996, Shi & Wang 2009, Yoshida et al. 2015, Gao et al. 2017). They have been reported worldwide and their occurrences, particularly members of the genus Ulva, are on the rise (Smetacek & Zingone 2013). During the month of July in consecutive years 2014–2016, green tides of Ulva at Seaside, California were observed. To determine the identity of this Ulva species, genome sequencing was performed on a 2014 collection and on the holotype of Ulva expansa (Setchell & N.L.Gardner (type locality: Monterey, California). Plastid markers were also sequenced from the 2015 and 2016 green tide collections to confirm their identities.

DNA was isolated from 5 × 5 mm² snippets from the holotype specimen of Ulva expansa (specimen voucher UC 98481) and from a herbarium specimen of the bloom-forming Ulva from 2014 (UC 2050480) following the protocols of Lindstrom et al. (2011) and Hughey and Gabrielson (2012). The holotype was processed in 2016 using 76 bp paired-end Illumina library construction and sequencing, and the modern material in 2018 using 150 bp Illumina sequencing by myGenomics, LLC (Alpharetta, GA). The data were assembled using the default de novo settings in CLC Genomics Workbench 11 (©2018 CLC bio, a QIAGEN Company, Waltham, MA), and by mapping reads and contigs from the de novo analysis against the mitogenomes of U. linza, U. pertusa, and U. prolifera using default settings in Geneious R8 (Biomatters Limited, Auckland, New Zealand). The genes were annotated with Blastx and NCBI ORFFinder. The mitogenome data were aligned to other Ulva species with MAFFT (Katoh & Standley 2013). The RaxML analysis was executed using complete mitogenome sequences at Trex-online (Boc et al. 2012) with the GTR + gamma model and 1000 fast bootstraps, then visualized with TreeDyn 198.3 at Phylogeny.fr (Dereeper et al. 2008).

The complete mitogenome of the bloom-forming Ulva is 64,143 bp in length and contains 65 genes. The mitogenome of the holotype of Ulva expansa was incomplete; however, the genomic analysis yielded its mitoexome (GenBank numbers MH730977-MH731006). The two Ulva genomes contain cob, 2 rRNAs, 3 cox, 3 rpl, 5 ATP synthases, 7 orfs, 8 nad, 9 rps, and 27 tRNAs. Gene content, organization, and length of the blooming Ulva was similar to Ulva pertusa (Liu et al. 2017). Phylogenetic analysis of the two mitogenomes indicates a sister relationship to Ulva pertusa (Figure 1). Analysis of rbcL sequences of the 2014–2016 bloom-forming Ulva from Seaside (GenBank numbers 2014 – MH730975, 2015 – MH730976, 2016 – MH746437) found three identical sequences, all differing from the holotype of Ulva expansa by...
only 2 bp. Comparison of plastid and nuclear markers of the holotype of *U. expansa* (GenBank numbers *tufa* – MH731007, UPA – MH731008, *rbcL* – MH731009; and SSU/ITS/LSU – MH730160) to the bloom-forming *Ulva* (GenBank numbers *tufa* – MH730973, UPA – MH730974, *rbcL* – MH730975; and SSU/ITS/LSU – MH730161) supports the conclusion that the bloom-forming *Ulva* from Seaside is the native central Californian species *U. expansa*.

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**References**


