PHYCOLOGIA
https://doi.org/10.1080/00318884.2022.2090122

Genetic diversity of sympatric *Schizymenia dubyi* and *S. apoda* (Schizymeniaceae, Rhodophyta) in Wellington harbour, *New Zealand*

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ABSTRACT

Schizymenia dubyi is reported as a new introduction in Wellington, which already has introduced *S. apoda*. These species coexist in tidepools and the crustose sporophyte is for the first time reported from this population. The diversity and species status of these two species was explored using mitochondrial, plastid and nuclear markers. Haplotypes of *rbc*L in New Zealand samples of *S. dubyi* match a haplotype found in Australia that was considered as 'introgressed' into *S. apoda*. COI haplotypes of *S. dubyi* from New Zealand are also similar to specimens from Australia and Europe, although New Zealand *S. apoda* samples have a unique haplotype. ITS ribotypes of *S. dubyi* from New Zealand also are shared with Australia and Europe, while again *S. apoda* ribotypes are unique. Phylogenetic and network analyses show that these two species are very closely related, and the hypothetical 'introgressed' nature of specimens is not supported by phylogenetic analyses. In addition, species delimitation methods are not congruent and do not clarify the species status of these two entities. Morphologically, these species are not distinct, except that in *S. dubyi* the cystocarp ostiole may be in a depression. The apparent incongruence between markers, leading to the hypothesis of introgression, is probably due to the early stages of the speciation process during which species have a 'paraphyletic' phase, in which stochastic incomplete lineage sorting leads to ancestral haplotypes being retained differentially, versus hybridization, introgression and non-maternal inheritance of organelles. Whether these two entities are distinct species needs to be reconsidered, as many species criteria are not met (e.g. monophyly) or need to be tested (e.g. reproductive barriers).

ARTICLE HISTORY
Received 14 February 2022
Accepted 13 June 2022
Published online xx xxx xxxx

KEYWORDS
COI; Introduction; ITS; *rbc*L; Speciation; Sympatry; Taxonomy

INTRODUCTION

Cryptic anthropogenic introductions are difficult to detect but may be common. Not only are these introductions likely to be overlooked in floras that are not well studied (i.e. what is native is not well known so 'introductions' are classed as previously unnoticed species), but red algae are known to consist of many cryptic or near-cryptic species, e.g. *Portieria* Zanardini (Payo *et al.* 2013) and *Bostrychia* Montagne (Muangmai *et al.* 2014), making morphological detection of new incursions problematic.

Species delimitation in algae, and in most other organisms, is difficult. Part of the difficulty derives from the plethora of species definitions (Hey 2006; Leliaert *et al.* 2014; Manoylov & Graham 2014). Other sources of difficulty are the speciation process itself, in which newly diverged species contain ancestral characters (including alleles), the lack of available data needed to delimit species of many algae (beyond morphological variability), or the adoption of a narrow definition of species (e.g. percentage of base pair differences in one gene) not based on biological processes (e.g. reproductive barriers). Seaweed species delimitation has moved from describing species on the basis of fixed morphological distinctness to a greater reliance on DNA sequence data. Species delimitation, especially in recently diverged species, is still confounded by incomplete lineage sorting (i.e. maintenance of ancestral polymorphism), which can make monophyly of 'species' incongruent between markers or compared to morphology (Leliaert *et al.* 2014).

DNA-based taxonomy, while having limitations, has led to the discovery of great genetic variation within apparently morphologically similar individuals, and further investigation has uncovered characters that correlate with the resulting genetic groupings (Diaz-Tapia *et al.* 2017), but can often reveal the truly cryptic nature of some species (Schneider *et al.* 2017), or only partially resolve the taxonomy (Zuccarello *et al.* 2015).

The red algal genus *Schizymenia* J. Agardh (Nemastomatales, Schizymeniaceae) currently includes 11 species distributed world-wide (Guiry & Guiry 2022). New Zealand has three native species of *Schizymenia: Schizymenia novae-zelandiae* J. Agardh and two undescribed species from remote locations, *Schizymenia* sp. A (WELT A016511) from the subantarctic Bounty Islands and *Schizymenia* sp. B (WELT A009419) from the Kermadec Island (Adams 1994). In addition, a non-indigenous species, *Schizymenia apoda* (J. Agardh) J. Agardh (type locality: South Africa), was discovered in New Zealand (D'Archino & Zuccarello

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2014) and it is probably introduced in other locations around the world (Gabriel *et al.* 2011; Kim *et al.* 2012; Saunders *et al.* 2015).

Schizymenia dubyi (Chauvin) J. Agardh, the type species of the genus, has been widely reported around the world. Its type locality is Cherbourg, France (Womersley 1994) and it is considered native in Europe, the Mediterranean, Japan, Korea and Australia (Gabriel *et al.* 2011; Saunders *et al.* 2015). However, molecular studies have identified previous records of *S. dubyi* from the Azores and China as *S. apoda* (Gabriel *et al.* 2011) while records from Iceland resulted in the establishment of a new species, *S. jonssonii* K. Gunnarsson & J. Brodie (Gunnarsson *et al.* 2020). *Schizymenia apoda* was also reported from Britain and Ireland suggesting that many records of *S. dubyi* could represent *S. apoda*, and vice versa (Saunders *et al.* 2015; Gunnarsson *et al.* 2020). *Schizymenia dubyi* has been considered introduced in Argentina (Ramirez *et al.* 2012) and California (Hughey & Miller 2009).

A previous study suggested that introgression had occurred between *S. apoda* and *S. dubyi* (Saunders *et al.* 2015) as found in Australia. They found that some samples that grouped with *S. dubyi* on the basis of a mitochondrial marker (COI) and a nuclear marker (ITS), grouped with *S. apoda* based on a plastid marker (*rbc*L), although these samples also had unique *rbc*L sequences.

We report the discovery of *Schizymenia dubyi* in Wellington harbour, where it is growing together with *S. apoda*, and explore the variation of these samples in comparison with samples from around the world, especially with reference to species designation and the 'introgressed' state of some samples.

MATERIAL AND METHODS

Samples of foliose *Schizymenia* and several crustose samples were mostly collected at low tide at Whairepo Lagoon (Frank Kitts Lagoon) (41°17.2977′S, 174°46.7877′E) in Wellington harbour (Table S1). Some samples were pressed and deposited at Te Papa Tongarewa (WELT; Thiers 2022). Subsamples were preserved in silica gel for molecular analysis and in 5% formalin seawater for morphological observations. Hand sections were stained with 1% aniline blue acidified with 1% HCl and mounted in 50% Karo® syrup (Englewood Cliffs, New Jersey, USA).

DNA was extracted using a 5% Chelex method (Zuccarello *et al.* 1999). Three markers were sequenced in various samples: partial mitochondrial cytochrome oxidase subunit one, COI (using primers GazF1, TCAACAAATCATAAAGATATTGG, and GazR1, ACTTCTGGATGTCCAAAAAAYCA; Saunders 2005), partial plastid-encoded ribulose bisphosphate carboxylase, *rbc*L (using primers F765, TGAAAGAGCTGAATTYGCTAA; Freshwater & Rueness 1994; and R1381, ATCTTTCCATAAAT CTAAAGC; Wang *et al.* 2000). A subset of samples were sequenced for the almost complete *rbc*L using the previous primers plus F8, GGTGAATTCCATACGCTAAAATG (Wang *et al.* 2000), and R753, GCTCTTTCATACATATCTTCC (Freshwater & Rueness 1994). Finally, the nuclear-encoded internal transcribed spacer (ITS1–5.8S–ITS2) of the ribosomal cistron (using primers ITS1, TCCGTAGGTGAACCTGCGG, and ITS4, TCCTCCGCT TATTGATATGC; White *et al.* 1990) was sequenced in a subset of the samples. PCR conditions used an annealing temperature of 45°C for the organellar genes and 50°C for the nuclear marker.

Maximum Likelihood analyses were implemented using IQTREE 2.1.3 (Minh *et al.* 2020). Genes were partitioned by codon where appropriate. Model selection (Kalyaanamoorthy *et al.* 2017) and partitioning to select the best partitions (Chernomor *et al.* 2016) were implemented in IQTREE. Three branch support methods were used: non-parametric bootstrapping (500 replicates; Felsenstein 1985); ultrafast bootstrapping (UF) (2,000 replicates; Hoang *et al.* 2018) and the approximate likelihood ratio test (aLRT) (1,000 replicates; Guindon *et al.* 2010). These support methods provide different approaches to determining branch reliability and have different confidence thresholds in determining branch support (see methods papers above).

Haplotype/ribotype networks were produced using TCS (statistical parsimony) as implemented in PopART v1.7 (University of Otago, available from http://popart.otago.ac.nz).

A survey of two tidepools at Wherepo Lagoon Frank Kitts Park, consisting of man-made substrate (cement and small boulders), was performed regularly when blades were visible, mostly in spring-summer. Samples were taken from blades with a holepunch (5-mm diameter) and the remainder of the blade left in place. The disk was dried in silica gel. A set of primers was developed from *rbc*L sequence data for species identification based on two independent PCR reactions. One set of primers amplified *S. apoda* individuals (Sa_796forward, ACAATTAGGGACTATTATC, plus Schizy_974reverse, GGTCTACTCCAGCCATACGC) and a second primer combination amplified *S. dubyi* individuals (Sd_774forward, CTAAACAATTAGGGACTGTC, plus Schizy_974reverse). Each sample was assessed with each set of primers (2.5 mM MgCl, annealing temperature 56°C). The PCR products were electrophoresed in 2% agarose, and samples scored on whether a band was present with one or the other PCR primer combination.

Three genetic species delimitation methods (putative species) were applied using the 'barcode' data set of the COI gene of all available *S. apoda* and *S. dubyi* samples: 1) distance-based method (Assemble Species by Automatic Partitioning, ASAP; Puillandre *et al.* 2021); 2) a tree-based method (Poisson-Tree processes, PTP; Zhang *et al.* 2013) and 3) a model-based method (Generalized Mixed Yule Coalescent, GMYC; Pons *et al.* 2006; Monaghan *et al.* 2009; Fujisawa & Barraclough 2013). All analyses were run with both the full COI dataset or a reduced dataset, with identical sequences removed.

RESULTS

The Wherepo Lagoon tidepool samples collected between November 2019 and April 2021 showed that both species were present, except for 13 May 2020 and April 2021 when only *S. apoda* was found (Fig. S1). Follow up sequencing of a subset of these samples showed that primers did correctly identify the intended species.

Phylogenetic analyses of the nearly complete *rbc*L (alignment length 1299 bp) showed that *S. apoda* and *S. dubyi* were sister species (Fig. 1; GenBank Accession numbers ON128271–ON128274). While samples of *S. dubyi* formed a well-supported clade (100% BS/92% aLRT/98% UF), the monophyly of *S. apoda* was not supported (values below significant levels: 51%/64%/62%). There was a supported subclade of *S. apoda* containing samples from New Zealand (e.g. H957) plus samples that were previously described as 'introgressed' *S. dubyi* from Australia (e.g. KP733870). One GenBank sample of *S. obovata* (AY294401) clearly grouped within the *S. apoda* clade (Fig. 1).

A haplotype network of partial *rbc*L (546 bp) showed 12 haplotypes (Fig. 2) with two haplotypes in New Zealand, R1 and R2. R1 consisted of 21 samples from New Zealand plus the previously mentioned 'introgressed' *S. dubyi* from Australia (Table S1). R2 consisted of sample designated as *S. dubyi* from New Zealand (25 samples) plus samples from other locations in the world (Argentina, France, Italy, Japan) and was 8 bp different from R1, but 2 bp different from

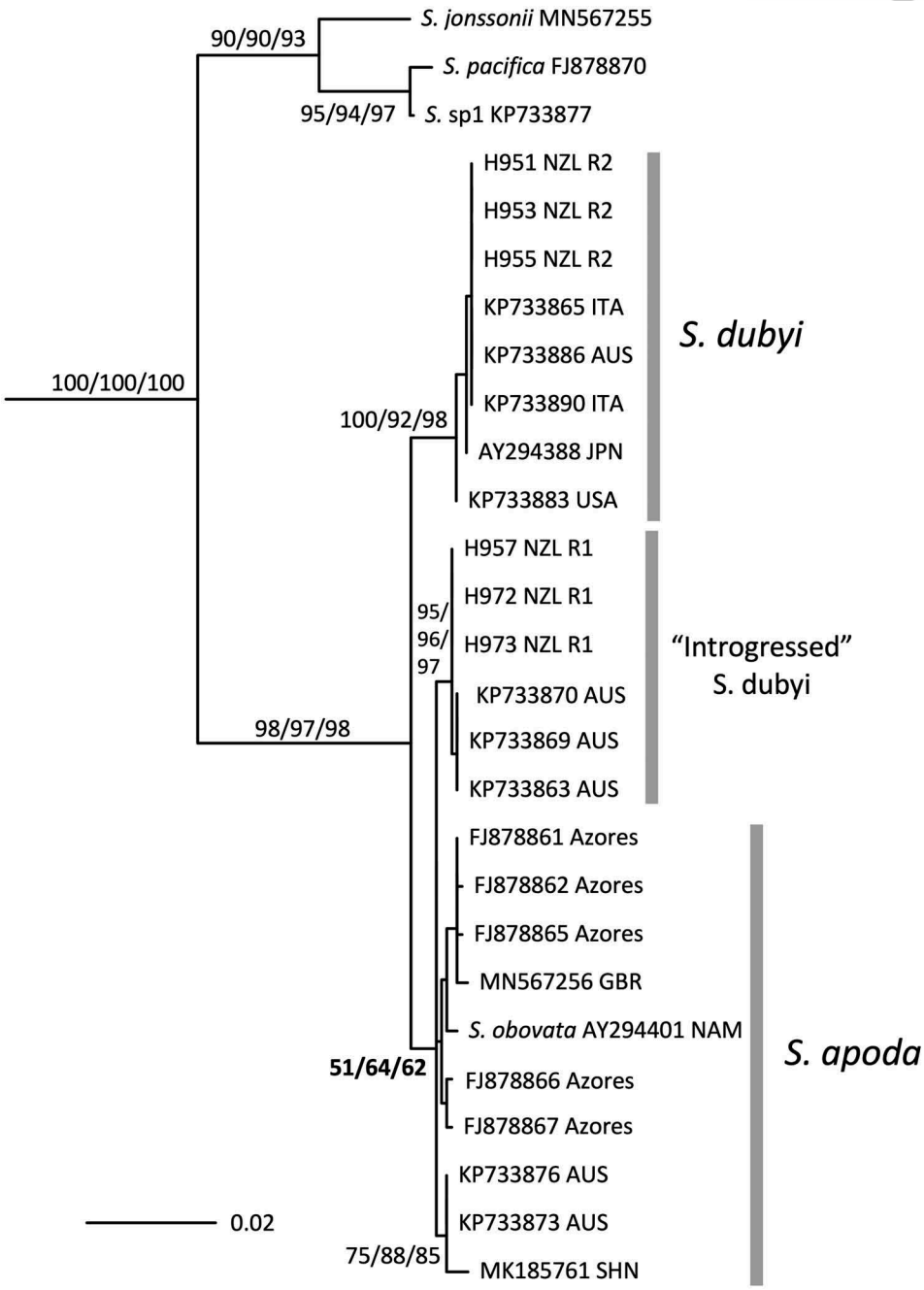


Fig. 1. Maximum Likelihood (ML) phylogeny of *Schizymenia* samples using almost complete *rbc*L sequences. GenBank Accession numbers shown. Information on New Zealand *Schizymenia apoda* and *S. dubyi* samples is given in Table S1. Model used: TN+F +I–codon 1; F81 +F–codon 2; TN+F+G4–codon 3. Support values on branches are non-parametric bootstrap percentage/aLRT percentage/Ultrafast bootstrap percentage. *Schizymenia apoda, S. dubyi* and 'introgressed' *S. dubyi* lineages are indicated. *Titanophora* species (AY294364, FJ878881), used as outgroups, were removed for clarity. Locations of samples shown with three-letter country designations, where known. Scale bar indicates number of substitutions per site.

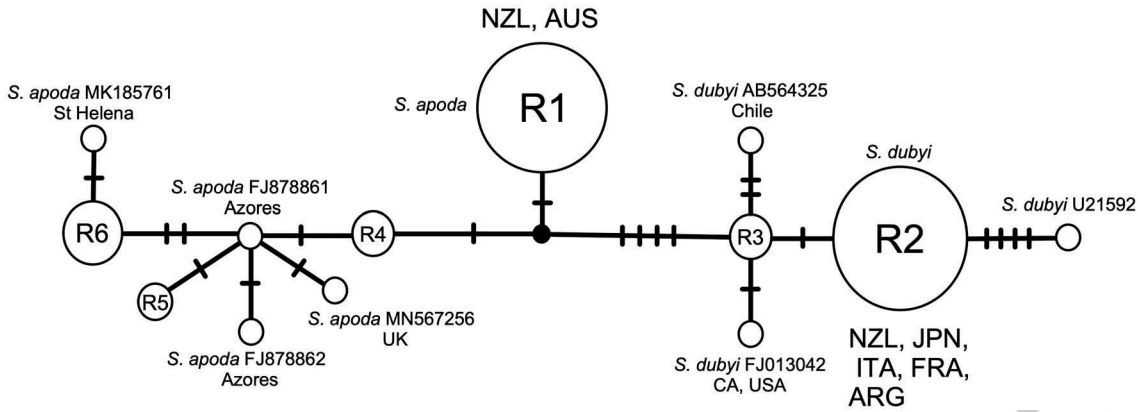


Fig. 2. Statistical parsimony network of variation in partial *rbc*L sequence data of *Schizymenia apoda* and *S. dubyi* samples. Haplotype designations of more than single sequences shown, as found in Table S1. Hatch in line represents a mutation step, filled circle is a missing haplotype. Locations of samples shown with three-letter country designations, where known.

a haplotype R3 consisting of samples of *S. dubyi* from Peru and California. R4 is *S. apoda* from the Azores and was 3 bp different from R1. Other haplotypes were found from other locations (*S. apoda*: R5, Azores; R6, Australia) or only found in one sample (Fig. 2). A ML phylogeny using this data was fairly unresolved, but there was a moderately supported grouping of haplotype R1 (87%/85%/84%) and a grouping of R2 and other samples of *S. dubyi* (95%/98%/99%) (Fig. S2). The remainder of the *S. apoda* samples formed an unsupported paraphyletic grade.

A haplotype network based on COI sequences (603 bp) of samples of *S. dubyi* and *S. apoda* showed six haplotypes, with four found in New Zealand (GenBank Accession numbers ON128275–ON128278). C1 contained samples from New Zealand (21 samples), which was 8 bp from Australian samples of *S. apoda* (haplotype C3). Haplotype C2 contained samples from New Zealand (20 samples) plus samples of *S. dubyi* from Australia and Italy (Fig. 3; Table S1). Haplotype C2 and C1 differed by 11 bp. The two remaining New Zealand haplotypes were found in single samples, one related to C1 (C4, sample H971), and one related to C2 (I111) (Fig. 3). The ML phylogeny of these haplotypes produced relationships that showed moderate to low support for the distinction between *S. apoda* and *S. dubyi* (e.g. 80% or 64% nonparametric bootstrap support; Fig. S3). A ML phylogeny of all sequences of COI (Fig. 4) showed that there is strong support for a clade containing samples of *S. apoda* from Australia (corresponding to haplotype C3), sister to a poorly support clade (60%/83%/62%) of the samples from New Zealand (i.e. haplotype C1). The monophyly of these two clades of *S. apoda* was not supported (63%/47%/63%). There was a moderately supported clade of *S. dubyi* samples (corresponding to the New Zealand haplotypes C2 and I111), plus the sequence of *S. dubyi* from California (KM254443) (Fig. 4).

Species delimitation methods were not congruent with each other, nor with the two species as presently understood. bPTP grouped all samples as one hypothesized 'species'. ASAP and multi-threshold GMYC (mGMYC) divided the samples into four or five hypothesized 'species' that roughly corresponded to the main haplotype groups (Fig. 4). Single-threshold GMYC (sGMYC) grouped all *S. dubyi* samples into a 'species', and split *S. apoda* into two 'species', corresponding to haplotype C3 from Australia and C1 from New Zealand.

Finally, a network based on nuclear-encoded ITS sequences (519 bp) showed four ribotypes (Fig. 5). I1 (GenBank Accession ON125004) contained samples from New Zealand (11 samples) and was 5 bp different from ribotype I2 (GenBank Accession ON125005) that also

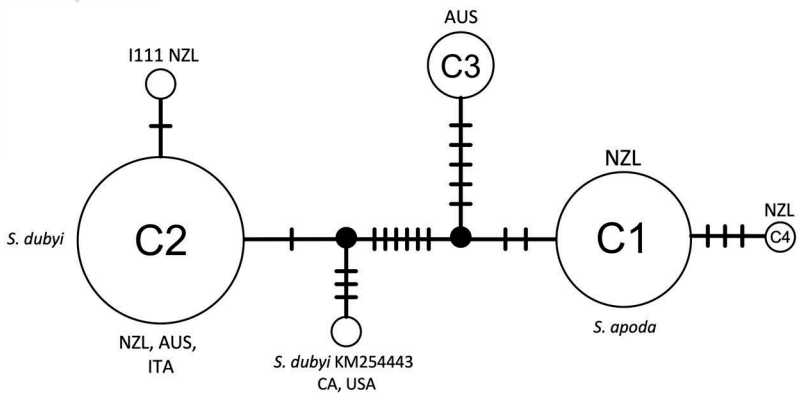


Fig. 3. Statistical parsimony network of variation in partial COI sequence data of *Schizymenia apoda* and *S. dubyi* samples. Haplotype designations of more than single sequences shown, as found in Table S1. Hatch in line represents a mutation step, filled circle is a missing haplotype. Locations of samples shown with three-letter country designations, where known.

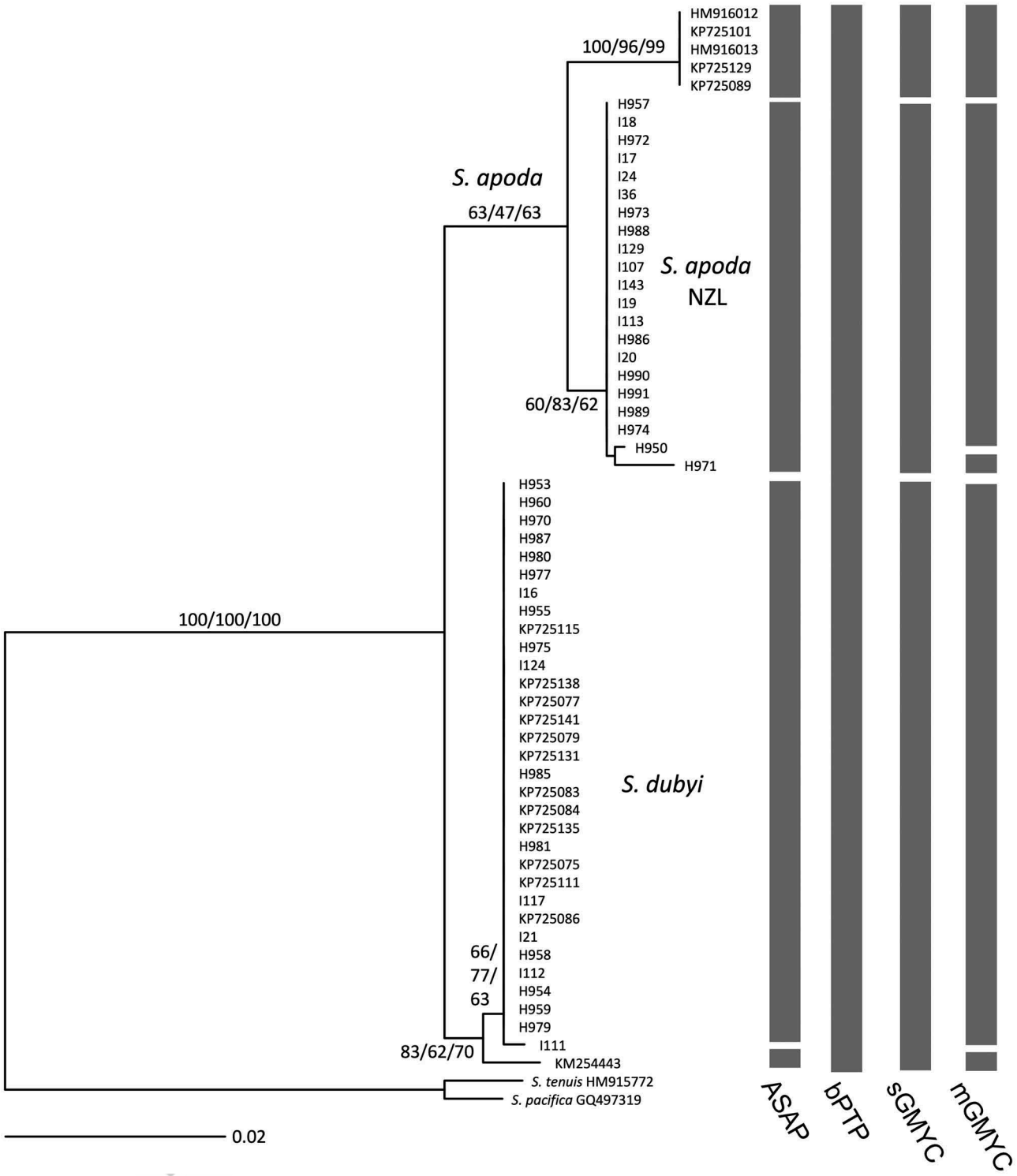


Fig. 4. Maximum Likelihood (ML) phylogeny of samples of all *Schizymenia* samples using partial COI sequences. GenBank Accession numbers shown. Information on *Schizymenia apoda* and *S. dubyi* samples is given in Table S1. Support values on branches are non-parametric bootstrap percentage/aLRT percentage/Ultrafast bootstrap percentage. Four species delimitation outputs shown: ASAP distance methods split *S. apoda* and *S. dubyi* into two species each, closely matching the haplotype variation; bPTP indicates a single species for all samples; single-threshold GMYC (sGMYC) suggests splits *S. apoda* as two species; multi-threshold GMYC (mGMYC) suggests splits *S. apoda* into three species and *S. dubyi* into two species. *Schizymenia pacifica* and *S. tenuis* used as outgroups. Scale bar indicates number of substitutions per site.

contained samples from New Zealand (21 samples) plus samples of *S. dubyi* from Australia, USA and Italy (Table S1). These ribotypes were between 8 and 10 bp different from samples of *S. apoda* from Australia (VIC1 and VIC2). A ML phylogeny showed three supported clades (corresponding to ribotypes I1, I2, VIC1–VIC2), but the relationships between these clades were not well supported (71%/72%/66%) (Fig. S4).

The 'incongruence' between phylogenies and named species can be seen with two examples. Sample I282,

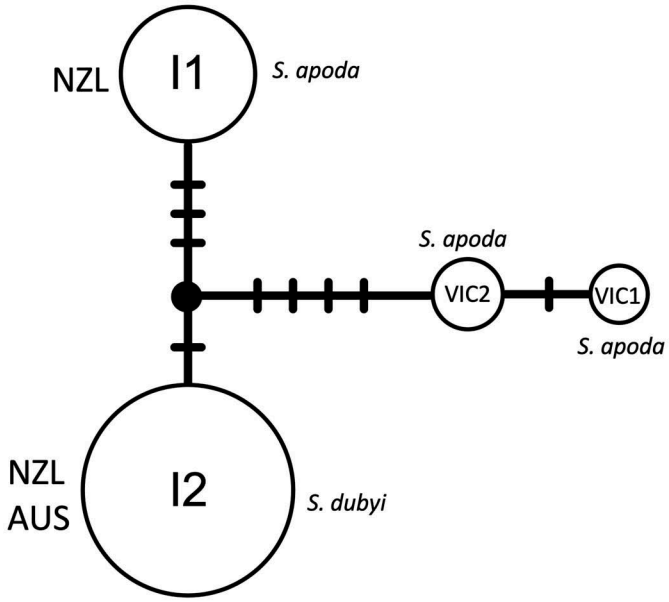


Fig. 5. Statistical parsimony network of variation in ITS sequence data of *Schizymenia apoda* and *S. dubyi* samples. Ribotype designations of more than single sequences shown, as found in Table S1. Hatch in line represents a mutation step, filled circle is a missing haplotype. Locations of samples shown (three-letter country designations) where known.

a tetrasporophytic crust collected from Wharepo tidepools, contained *rbc*L haplotype R2 that in a phylogeny formed a clade with *S. dubyi* samples with support, but this clade was in a trichotomy with *S. apoda* samples (R1 and other *S. apoda* samples). R1 and R2 differ by 8 bp. Sample I282 has COI haplotype C2 and was in a moderately supported phylogenetic clade containing *S. dubyi* samples that was sister to an unsupported clade of *S. apoda* samples (including haplotype C1). These two haplotypes differ by 11 bp. The ITS ribotype for sample I282 (I2) grouped it with other samples of *S. dubyi*, and this clade was sister to a clade with samples of *S. apoda* (ribotype I1 from New Zealand), but this relationship is unsupported. These two clades differ by 5 bp. All markers suggest that I282 belongs to *S. dubyi* but support is weak both based on phylogenetic support and on low numbers of base pair differences.

Sample H950 was in a supported *rbc*L clade (haplotype R1) that was sister to an unsupported clade of *S. apoda* (inferred 'introgressed' samples), therefore forming a trichotomy with a supported clade of *S. dubyi* samples. With COI, H950 was in a clade with samples of *S. apoda* (haplotype C1), but this clade is not supported. With ITS, H950 (ribotype I1) was in a clade sister to *S. dubyi* (though this relationship was unsupported) and distinct form *S. apoda* samples. Whereas *rbc*L data suggests common ancestry of H950 with *S. apoda* (not supported), with COI and ITS H950 is grouped with *S. dubyi*, but again without support and with small bp differences.

Morphological analysis of *S. dubyi*

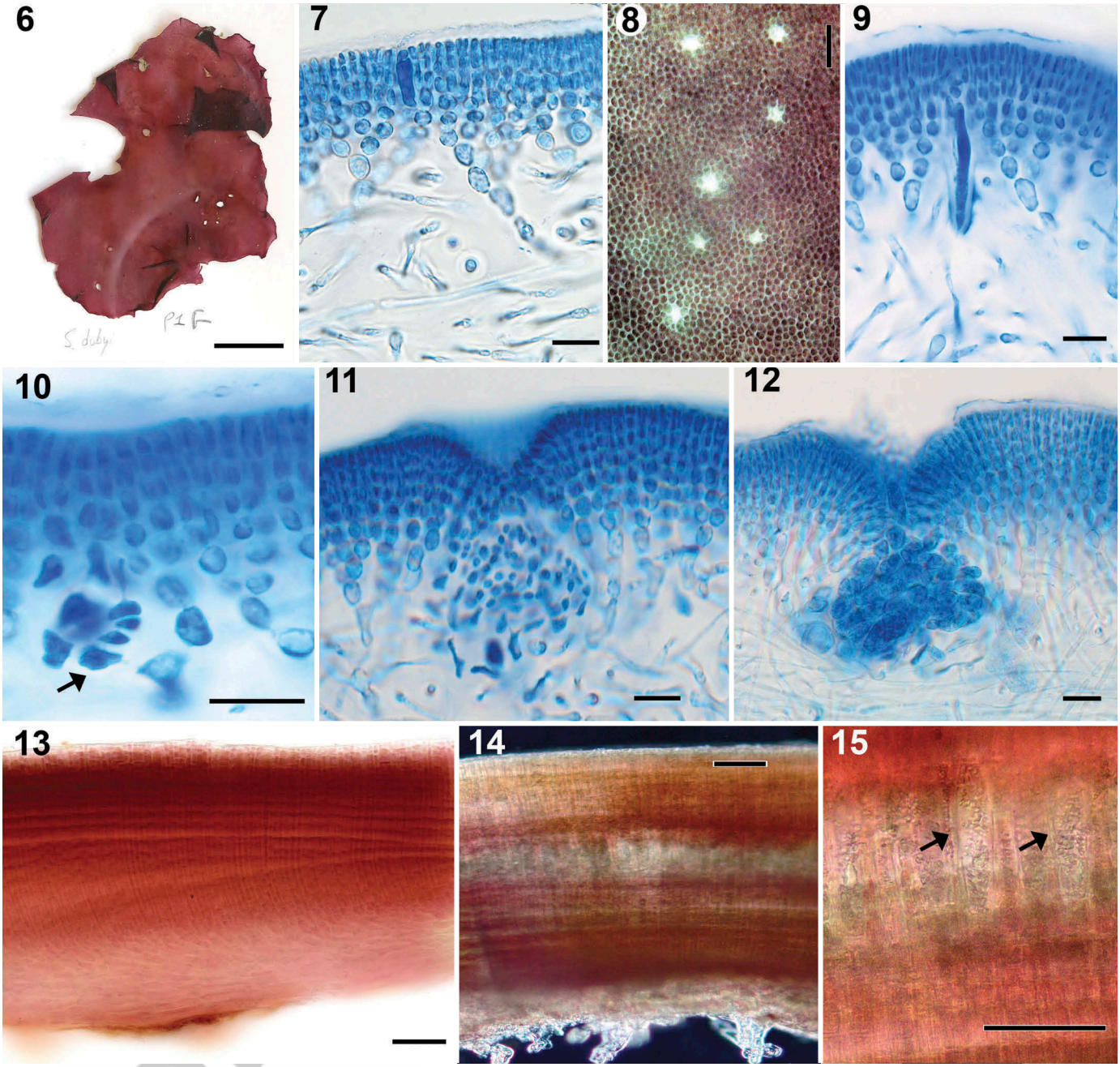
Gametophytic thalli were foliose, with margins smooth or slightly undulate (Fig. 6), up to 6 cm high and 5 cm wide, dark red to crimson in colour and firm in texture. Thalli were multiaxial, 300–350 µm thick, including a cortex 40–60 µm thick, consisting of sub-dichotomously branched filaments (6–8 cells long) and a medulla of entangled filaments (Fig. 7). Prominent gland cells, 25–90 × 8–27 µm, were immersed in the cortex but visible on the surface (Fig. 8). Gland cells were occasionally deeply immersed in the inner cortex (Fig. 9). Gland cells were not visible in some specimens. Carpogonial branches were three-celled, borne on an inner cortical cell, with a short straight trichogyne (Fig. 10). Rudimentary auxiliary ampullae, 60–65 × 70–75 µm (Fig. 11), and carposporophytes, 130–115 × 80–90 µm, were immersed in the medulla. Cystocarps had a distinct depressed ostiole (Fig. 12). Carposporangia were ovoid, 13–16 × 9–11 µm.

Tetrasporophytic crustose thalli were 370–480 µm thick, attached by multicellular rhizoids, consisting of 4–6 basal layers of cells, 41–58 × 8–11 µm, lying almost parallel to the substrate and several layers of periclinal cells, 16–11 × 4–6 µm (Fig. 13). Tetrasporangia were not observed. However, mature thalli (Figs 14, 15) showed a paler band, consisting of larger cells 45–50 × 10–13 µm that could represent the discharged tetrasporangial layer (Fig. 15).

DISCUSSION

The identification of many foliose red algal species relies on molecular data for accurate identification, and this is especially true for the two sister species *Schizymenia dubyi* and *S. apoda*. The introduction of *S. dubyi* has probably gone unnoticed for some years in New Zealand following the discovery of *S. apoda* in Wellington (D'Archino & Zuccarello 2014). Both species are common, indistinguishable morphologically without careful examination, and coexist in Wellington harbour. Their abundance seems to be seasonal (common in spring-summer) but differences in phenology have not been established. These samples have been mixed in physiological studies (Gambichler *et al.* 2021a, b), but these preliminary physiological data suggest that they do not differ in their physiological response to salinity and ultraviolet light, and produce similar mycosporine-like amino acids and osmolytes.

Previous studies on these two *Schizymenia* species in Australia indicated that some samples of *S. dubyi*, as determined by mitochondrial (COI) and nuclear markers (ITS), grouped them with *S. apoda* with a plastid marker (Saunders *et al.* 2015), while other samples of *S. dubyi* grouped with other *S. dubyi* samples with all markers. This led to the hypothesis that these former samples, which showed an incongruent pattern, were 'introgressed' *S. dubyi* and were produced by an ancestral hybridization with *S. apoda* and differential inheritance of organelles (plastid from *S. apoda* and mitochondrion from *S. dubyi*) and backcrossing of these hybrids to one parent, *S. dubyi*, leading to nuclear markers (ITS) of one parent only. Organelles in red algae appear to be exclusively inherited maternally (Zuccarello *et al.* 1999; Choi *et al.* 2008; Niwa *et al.* 2010; Zuccarello & West 2011; Wang *et al.* 2020), which is a common pattern in plants (Greiner *et al.* 2015), but not many red algal species have been studied. Our phylogenetic analyses shows that this 'introgressed'



Figs 6–15. Vegetative and reproductive anatomy of *Schizymenia dubyi*, LM.
Fig. 6. Habit of a specimen collected in the Whairepo Lagoon (WELTA034385). Scale bar = 2 cm.
Fig. 7. Cross section of the thallus showing a darkly stained surface gland cell. (WELTA034386). Scale bar = 20 µm.
Fig. 8. Surface view of the thallus with visible gland cells (WELTA034387). Scale bar = 20 µm.
Fig. 9. Cross section of the thallus showing a darkly stained gland cell deeply sunken in the inner cortex (WELTA034387). Scale bar = 20 µm.
Fig. 10. Carpogonial branch borne on an inner cortical cell (arrow) (WELTA034387). Scale bar = 20 µm.
Fig. 11. Rudimental auxiliary ampulla (WELTA034387). Scale bar = 20 µm.
Fig. 12. Carposporophyte with immersed ostiole (WELTA034387). Scale bar = 20 µm.
Fig. 13. Tetrasporophyte. Young crust (Crust I2). Scale bar = 50 µm.
Fig. 14. Tetrasporophyte. Mature crust (Crust C2). Scale bar = 50 µm.
Fig. 15. Detail of the discharged tetrasporangial compartments (arrows) (Crust C2). Scale bar = 50 µm.

relationship is poorly supported (no or only moderate support with the statistical methods used) for this incongruous plastid grouping, and that the plastid haplotype found in these 'introgressed' *S. dubyi* is unique and not found in other *S. apoda* samples. So, while ancestral hybridization is a possibility, it has not been confirmed. A more likely scenario is a well-known phenomenon found in early stages of the speciation process called incomplete lineage sorting or maintenance of ancestral polymorphism (Leliaert *et al.* 2014). These two species are very closely related, as the poor monophyletic support of the species, the incongruence of species delimitation methods and the number of base pair differences seen in the haplotype networks clearly indicate. It is likely that these two groups are still in early stages of speciation, and ancestral

organelle haplotypes are randomly assorting, producing this apparent species incongruence. Therefore, evidence for introgression is lacking.

The almost complete lack of variation in *S. dubyi* and *S. apoda* New Zealand samples, compared to Australia and Europe where most sampling has been done, and their harbour location suggests that they are introduced to New Zealand. Red algae are well known to be introduced to New Zealand, often from Asia (D'Archino *et al.* 2013, 2015; Nelson *et al.* 2015). The haplotypes of *S. dubyi* are also found in other parts of the world, also suggesting that these samples are probably introduced. Sampling of these species is poor in Asia. Further sampling will elucidate potential areas of origin to New Zealand.

Before the finding of *S. dubyi*, the presence of the gland cells, spotted in surface view, was a sufficient character to distinguish *Schizymenia* from other foliose genera (e.g. *Tsengia* K.C. Fan & Y.P. Fan and *Grateloupia* C. Agardh) occurring in New Zealand harbours. However, some samples of *S. dubyi* appear to lack gland cells, although this has not been observed in *S. apoda*. This apparent lack of gland cells could be due to the observation of buried gland cells in some sections of *S. dubyi*. The cystocarp's ostiole seems to differ, with it being depressed in *S. dubyi* (Womersley 1994; our observations) and superficial in *S. apoda* (Stegenga *et al.* 1997; D'Archino & Zuccarello 2014). Whether these or other characters are consistent enough to distinguish the two species needs further study. The crustose tetrasporophyte has been reported for the first time in New Zealand. Of the four crusts sequenced from Wellington all were *S. dubyi* and genetically identical to foliose gametophytes in that area, with no evidence of hydridization between the two species obtained from the ITS nuclear marker in their sympatric locations (Hodge *et al.* 2010).

What the status of these two species is needs to be reassessed. As can be seen in this study, species in the process of speciation cannot be easily determined without an integrated approach, which may involve studies on reproductive barriers, which can only be studied in the field when plants are in sympatry, physiological differentiation, difference in phenology, genomic differentiation and morphological variation.

ACKNOWLEDGEMENTS

We thank Maren Preuss for help in collecting. We also thank António José Calado for valuable help.

DISCLOSURE STATEMENT

Q7 No potential conflict of interest was reported by the authors.

FUNDING

Q10 This work was supported via strategic research funds from the School of Biological Sciences at Victoria University of Wellington and a Strategic Science Investment Fund (SSIF) from the National Institute of Water and Atmospheric Research (NIWA).

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REFERENCES

Adams N.M. 1994. *Seaweeds of New Zealand*. Canterbury University Press, Christchurch, New Zealand. 360 pp.

Chernomor O., von Haeseler A. & Minh B.Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 65: 997–1008. DOI:10.1093/sysbio/syw037.

Choi S.-J., Park E.-J., Endo H., Kitade Y. & Saga N. 2008. Inheritance pattern of chloroplast and mitochondrial genomes in artificial hybrids of *Porphyra yezoensis* (Rhodophyta). *Fisheries Science* 74: 822–829. DOI:10.1111/j.1444-2906.2008.01594.x.

D'Archino R. & Zuccarello G.C. 2014. First record of *Schizymenia apoda* (Schizymeniaceae, Rhodophyta) in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 48: 155–162. DOI:10.1080/00288330.2013.847849.

D'Archino R., Neill K.F. & Nelson W.A. 2013. Recognition and distribution of *Polysiphonia morrowii* (Rhodomelaceae, Rhodophyta) in New Zealand. *Botanica Marina* 56: 41–47. DOI:10.1515/bot-2012-0183.

D'Archino R., Nelson W.A., Yang M.Y. & Kim M.-S. 2015. New record of *Hypnea flexicaulis* in New Zealand and description of *Calliblepharis psammophilus sp. nov.* *Botanica Marina* 58: 485–497. DOI:10.1515/bot-2015-0053.

Diaz-Tapia P., McIvor L., Freshwater D.W., Verbruggen H., Wynne M.J. & Maggs C.A. 2017. The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray constitute well-defined clades of the red algal tribe Polysiphonieae (Rhodomelaceae, Ceramiales). *European Journal of Phycology* 52: 1–30. DOI:10.1080/09670262.2016.1256436.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. DOI:10.1111/j.1558-5646.1985.tb00420.x.

Freshwater D.W. & Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. *Phycologia* 33: 187–194. DOI:10.2216/i0031-8884-33-3-187.1.

Fujisawa T. & Barraclough T.G. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. *Systematic Biology* 62: 707–724. DOI:10.1093/sysbio/syt033.

Gabriel D., Schils T., Parente M.I., Draisma S.G.A., Neto A.I. & Fredericq S. 2011. Taxonomic studies in the Schizymeniaceae (Nemastomatales, Rhodophyta): on the identity of *Schizymenia* sp. in the Azores and the generic placement of *Nemastoma confusum*. *Phycologia* 50: 109–121. DOI:10.2216/09-67.1.

Gambichler V., Zuccarello G.C. & Karsten U. 2021a. Physiological responses to salt stress by native and introduced red algae in New Zealand. *Algae* 36: 137–146. DOI:10.4490/algae.2021.36.6.10.

Gambichler V., Zuccarello G.C. & Karsten U. 2021b. Seasonal changes in stress metabolites of native and introduced red algae in New Zealand. *Journal of Applied Phycology* 33: 1157–1170. DOI:10.1007/s10811-020-02365-0.

Q8

Greiner S., Sobanski J. & Bock R. 2015. Why are most organelle genomes transmitted maternally? *Bioessays* 37: 80–94. DOI:10.1002/bies.201400110.

Guindon S., Dufayard J.-F., Lefort V., Anisimova M., Hordijk W. & Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321. DOI:10.1093/sysbio/syq010.

Guiry M.D. & Guiry G.M. 2022. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. https://www.AlgaeBase.org; searched on February 2022.

Gunnarsson K., Russell S. & Brodie J. 2020. *Schizymenia jonssonii sp. nov.* (Nemastomatales, Rhodophyta): a relict or an introduction into the North Atlantic after the last glacial maximum? *Journal of Phycology* 56: 324–333. DOI:10.1111/jpy.12957.

Hey J. 2006. On the failure of modern species concepts. *Trends in Ecology and Evolution* 21: 447–450. DOI:10.1016/j.tree.2006.05.011.

Hoang D.T., Chernomor O., von Haeseler A., Minh B.Q. & Vinh L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522. DOI:10.1093/molbev/msx281.

Hodge F.J., Buchanan J. & Zuccarello G.C. 2010. Hybridization between the endemic brown algae *Carpophyllum maschalocarpum* and *Carpophyllum angustifolium* (Fucales): genetic and morphological evidence. *Phycological Research* 58: 239–247. DOI:10.1111/j.1440-1835.2010.00583.x.

Hughey J.R. & Miller K.A. 2009. Noteworthy collection: *schizymenia dubyi* (Chauvin ex Duby) J. Agardh, Rhodophyta, new to California. *Madroño* 56: 64. DOI:10.3120/0024-9637-56.1.64a.

Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A. & Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. DOI:10.1038/nmeth.4285.

Kim S.-Y., Seo T.-H., Park J.-K., Boo G.-H., Kim K.-M. & Boo S.-M. 2012. *Cryptonemia rotunda* (Halymeniales) and *Schizymenia apoda* (Nemastomatales), two new records of red algae from Korea. *Algae* 27: 1–8. DOI:10.4490/algae.2012.27.1.001.

Leliaert F., Verbruggen H., Vanormelingen P., Steen F., López-Bautista J. M., Zuccarello G.C. & Clerck O.D. 2014. DNA-based species delimitation in algae. *European Journal of Phycology* 49: 179–196. DOI:10.1080/09670262.2014.904524.

Manoylov K.M. & Graham L. 2014. Taxonomic identification of algae (morphological and molecular): species concepts, methodologies, and their implications for ecological bioassessment. *Journal of Phycology* 50: 409–424. DOI:10.1111/jpy.12183.

Minh B.Q., Schmidt H.A., Chernomor O., Schrempf D., Woodhams M. D., von Haeseler A., Lanfear R. & Teeling E. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534. DOI:10.1093/molbev/msaa015.

Monaghan M.T., Wild R., Elliot M., Fujisawa T., Balke M., Inward D.J., Lees D.C., Ranaivosolo R., Eggleton P., Barraclough T.G. *et al.* 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology* 58: 298–311. DOI:10.1093/sysbio/syp027.

Muangmai N., West J.A. & Zuccarello G.C. 2014. Evolution of four Southern Hemisphere *Bostrychia* (Rhodomelaceae, Rhodophyta) species: phylogeny, species delimitation and divergence times. *Phycologia* 53: 593–601. DOI:10.2216/14-044.1.

Nelson W.A., Neill K.F. & D'Archino R. 2015. When seaweeds go bad: an overview of outbreaks of nuisance quantities of marine macroalgae in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 49: 472–491. DOI:10.1080/00288330.2015.1064975.

Niwa K., Kobiyama A. & Sakamoto T. 2010. Interspecific hybridization in the haploid blade-forming marine crop *Porphyra* (Bangiales, Rhodophyta): occurrence of allodiploidy in surviving F_1 gametophytic blades. *Journal of Phycology* 46: 693–702. DOI:10.1111/j.1529-8817.2010.00853.x.

Payo D.A., Leliaert F., Verbruggen H., D'hondt S., Calumpong H.P. & Clerck O.D. 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. *Proceedings of the Royal Society B, Biological Sciences* 280: Article 20122660.

Pons J., Barraclough T.G., Gomez-Zurita J., Cardoso A., Duran D.P., Hazell S., Kamoun S., Sumlin W.D., Vogler A.P. & Hedin M. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595–609. DOI:10.1080/10635150600852011.

Puillandre N., Brouillet S. & Achaz G. 2021. ASAP: assemble Species by Automatic Partitioning. *Molecular Ecology Resources* 21: 609–620. DOI:10.1111/1755-0998.13281.

Ramirez M.E., Nuñez J.D., Ocampo E.H., Matula C.V., Suzuki M., Hashimoto T. & Cledón M. 2012. *Schizymenia dubyi* (Rhodophyta, Schizymeniaceae), a new introduced species in Argentina. *New Zealand Journal of Botany* 50: 51–58. DOI:10.1080/0028825X.2011.642887.

Saunders G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B* 360: 1879–1888. DOI:10.1098/rstb.2005.1719.

Saunders G.W., Birch T.C. & Dixon K.R. 2015. A DNA barcode survey of *Schizymenia* (Nemastomatales, Rhodophyta) in Australia and *British Columbia* reveals overlooked diversity including *S. tenuis sp. nov.* and *Predaea Borealis sp. nov. Botany* 93: 859–871. DOI:10.1139/cjb-2015-0122.

Schneider C.W., Quach P.K. & Lane C.E. 2017. A case for true morphological crypsis: pacific *Dasya anastomosans* and Atlantic *D. cryptica sp. nov.* (Dasyaceae, Rhodophyta). *Phycologia* 56: 359–368. DOI:10.2216/16-79.1.

Stegenga H., Bolton J.J. & Anderson R.J. 1997. *Seaweeds of the South African West Coast*. Creda Press, University of Cape Town, Cape Town, South Africa. 655 pp. Q9

Thiers B. 2022. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium [continuously updated]. http://sweetgum.nybg.org/science/ih/; searched on 1 February 2022.

Wang H.W., Kawaguchi S., Horiguchi T. & Masuda M. 2000. Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and *rbc*L sequences. *Phycologia* 39: 228–237. DOI:10.2216/i0031-8884-39-3-228.1.

Wang L., Wang J., Zhu Y., Cui Z., Kong F., Tang X. & Mao Y. 2020. Development of organelle single nucleotide polymorphism (SNP) markers and their application for the identification of cytoplasmic inheritance patterns in *Pyropia yezoensis* (Bangiales, Rhodophyta). *Journal of Oceanology and Limnology* 39: 1447–1457. DOI:10.1007/s00343-020-0298-9.

White T.J., Bruns T. & Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *A guide to molecular methods and applications* (Ed. by M.A. Innis, D.H. Gelfand, J.J. Sninsky & J.W. White), pp 315–322. Academic Press, New York, USA.

Womersley H.B.S. 1994. The marine benthic flora of southern Australia – part IIIA – bangiophyceae and Florideophyceae. In: *(Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato). Australian biological resources study*, pp 508. Canberra, Australia. Q10

Zhang J., Kapli P., Pavlidis P. & Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29: 2869–2876. DOI:10.1093/bioinformatics/btt499.

Zuccarello G.C. & West J.A. 2011. Insights into evolution and speciation in the red alga *Bostrychia*: 15 years of research. *Algae* 26:21–32. DOI:10.4490/algae.2011.26.1.021.

Zuccarello G.C., West J.A., Kamiya M. & King R.J. 1999. A rapid method to score plastid haplotypes in red seaweeds and its use in determining parental inheritance of plastids in the red alga *Bostrychia* (Ceramiales). *Hydrobiologia* 401: 207–214. DOI:10.1023/A:1003706931897.

Zuccarello G.C., Muangmai N., Preuss M., Sanchez L.B., de Goër S.L. & West J.A. 2015. The Bostrychia tenella species complex: morphospecies and genetic cryptic species with resurrection of B. binderi. *Phycologia* 54: 261–270. DOI:10.2216/15-005.1.