

# Non-monophyly of *Bostrychia simpliciuscula* (Ceramiales, Rhodophyta): Multiple species with very similar morphologies, a revised taxonomy of cryptic species

Giuseppe C. Zuccarello,<sup>1\*</sup> John A. West<sup>2</sup> and Mitsunobu Kamiya<sup>3</sup>

<sup>1</sup>School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand, <sup>2</sup>School of Biosciences 2, University of Melbourne, Parkville, Victoria, Australia, and <sup>3</sup>Faculty of Marine Bioscience, Fukui Prefectural University, Fukui, Japan

## SUMMARY

The discovery of a plethora of cryptic species in many algal groups has led to speculation as to the causes of this observation and has affected taxonomy, with reluctance to give names to species that look identical. While this is defensible for monophyletic cryptic species complexes, both our understanding of similar morphologies (cryptic) and nomenclature is challenged when we encounter non-monophyletic 'cryptic' species. *Bostrychia simpliciuscula* is a wide-ranging species in which multiple cryptic species are known. Our increased sampling shows that this species consists of four lineages that do not form a clade, but lineages are sister to species with different morphologies. Careful morphological examination shows that characters, especially branched monosiphonous laterals and rhizoid morphology in haptera, are able to distinguish these four lineages into two groups, that are still not monophyletic. The similar morphologies in these lineages could be due to convergence, but not developmental constraints or lack of time to diverge morphologically; or possibly maintenance of a generalized body plan. These lineages appear to have specific biogeographic patterns and these will be used to propose a new taxonomy. *B. simpliciuscula* is now confined to the tropics. Another of these lineages matches a previously described species, *B. tenuissima*, that was synonymized with *B. simpliciuscula* and is from cold temperate Australasia, and is resurrected. Another lineage is found in Japan in which a previous name is also available, *B. hamana-tokidae*; the last lineage is found in central New South Wales, morphologically it resembles *B. tenuissima*, with which it overlaps in distribution around Sydney, and is named as a new species, *B. kingii* sp. nov.

Key words: Australia, *Bostrychia kingii* sp. nov., convergence, Japan, morphological stasis, Rhodomelaceae.

## INTRODUCTION

The discovery of cryptic species (i.e., distinct genetic/reproductive/ecological entities that are not distinguishable morphologically) is becoming commonplace in many algal groups. These species can be inferred from a variety of molecular markers including mitochondrial, nuclear and plastid genes. Cryptic species are nearly always found in what was previously thought to be one wide-ranging species, which now appears to be composed of several, to many, distinct evolutionary

lineages with narrower ranges. Many of these cryptic species are found to be sisters to each other within a diverse clade.

The hypotheses proposed that the high proportion of species that are not morphologically distinguishable, especially in some macroalgae, are many but, include: (i) diversification (speciation) without concomitant morphological change, possibly due to developmental constraints or stabilizing selection (Charlesworth *et al.* 1982) (morphological stasis); or (ii) the low number of morphological characters that can be used to distinguish between species (low complexity problem; Verbruggen 2014).

Cryptic species are being reported very frequently in many well sampled algal studies and in all algal groups (e.g., Conklin & Sherwood 2012; Payo *et al.* 2013; Skaloud & Rindi 2013; Niwa *et al.* 2013; Kogame *et al.* 2015; Zuccarello *et al.* 2015; Lyra *et al.* 2016; Montecinos *et al.* 2017). These cryptic species are usually inferred by several 'species delimitation' methods (Leliaert *et al.* 2014), but also by phylogenetic methods indicating strong support for lineages that are morphologically indistinguishable and therefore 'putative species' (e.g., Boo *et al.* 2015). In some cases these cryptic species have been shown to differ in other ways including ecological specialization (Skaloud & Rindi 2013; Muangmai *et al.* 2016) and reproductive isolation (Zuccarello & West 2003; Peters *et al.* 2010).

Cryptic species are often found in wide-ranging species that upon molecular inspection are shown to be composed of multiple species with more limited distributions. Many cryptic species complexes have been found in the tropics (Conklin & Sherwood 2012; Payo *et al.* 2013; Vieira *et al.* 2014), but also temperate species can have multiple cryptic species of more limited distribution (Muangmai *et al.* 2014; Zuccarello & Martin 2016). This limited, often non-overlapping, distribution suggests that speciation is due to allopatric processes enhanced by the low dispersal ability of many algae (e.g., Krueger-Hadfield *et al.* 2015).

In nearly all cases, cryptic species are in a well-supported clade, indicating that while speciation has occurred the 'ancestral' morphology has been maintained. This could be due to stabilizing selection on particular morphologies that

\*To whom correspondence should be addressed.

Email: joe.zuccarello@vuw.ac.nz

Communicating Editor: Wendy Nelson

Received 22 June 2017; accepted 18 September 2017.

may have advantages in certain environments (Davis *et al.* 2014); due to a developmental constraint on the alga in which novel morphologies are not possible; or just a lack of time for morphologies to change.

While cryptic species have been commonly uncovered, taxonomic changes have lagged behind. Sometimes this is due to the intricacies of nomenclatural history. Applying type names to individual lineages can be problematic, without corresponding sequence data from the type specimen (Hughey & Gabrielson 2012), assuming the type is even available. Also a long history of synonymies makes assigning old names to any of the different lineages difficult and therefore discourages proposing new names (see De Clerck *et al.* 2013). This may not be a problem if the cryptic species complex is monophyletic as all the species are more closely related to each other than any morphologically distinct species, and the taxonomy is therefore clade-based.

*Bostrychia* is a genus in which significant research has revealed many aspects of its evolution (Zuccarello & West 2011). Cryptic species are known to occur in several species complexes (*B. tenella* (J.V. Lamouroux) J. Agardh/*B. binderi* Harvey, Zuccarello *et al.* 2015; *B. radicans* (Montagne) Montagne/*B. moritziana* (sonder ex Kützinger) J. Agardh, Zuccarello & West 2003; and *B. intricata* (Bory) Montagne, Fraser *et al.* 2013, Muangmai *et al.* 2014). While some cryptic species have relatively wide distributions, some are more limited in their distribution. For example, in *B. intricata*, some cryptic species are localized to certain continents (e.g., South Africa, Australia), while other cryptic species have broader distributions (e.g., New Zealand and Chile; Muangmai *et al.* 2014).

Another morphospecies that appears to contain cryptic species is the *B. simpliciuscula* Harvey ex J. Agardh/*B. tenuissima* R.J. King & Puttock species complex. The taxonomy of *Bostrychia* was revised in a monograph by King and Puttock (1989). They proposed several new species and formally synonymized several names. *Bostrychia tenuissima* was a species proposed (King & Puttock 1989) as a segregate from *B. simpliciuscula* Harvey ex J. Agardh. They based their decision mostly on the lack of monosiphonous determinate branch tips in *B. tenuissima* (type locality: Port Fairy, Victoria, southern Australia), while they are found in *B. simpliciuscula* (King & Puttock 1989). The distribution of *B. tenuissima* was reported from southern Queensland (Noosa Heads) to Tasmania, Australia and New Zealand. While *B. simpliciuscula* has a wider distribution not only in Australia, but also the Pacific Islands, and south-east Asia (King & Puttock 1989). King and Puttock also synonymized several previous names into *B. simpliciuscula*, including *B. andoi* Okamura found in tropical southern Japan (Ryukyu Island) and *B. hamana-tokidae* E. Post from Kyushu Island, Japan.

Later research showed that samples of *B. simpliciuscula* from the southern end of this range, south of Sydney, Australia were a distinct plastid RuBisCo spacer haplotype (designated haplotype H1). These samples only had sorbitol as an osmolyte, while north of Sydney two distinct sequences were found (H2 and H3), which contained both sorbitol and dulcitol as an osmolyte (Zuccarello *et al.* 1999a). Further research, using other genetic markers, confirmed that these three haplotypes were distinct, and H3 was found as far north as Singapore (Zuccarello & West 2006). As these lineages (H1–H3) were not monophyletic and monosiphonous branches did not seem to distinguish the lineages, a

taxonomic decision was made to synonymize *B. tenuissima* with *B. simpliciuscula* until further research could resolve the lineages (Zuccarello & West 2006). These results, plus our further sampling and analyses of the *B. simpliciuscula*, suggests that the taxonomy of this entity needs refining.

## MATERIALS AND METHODS

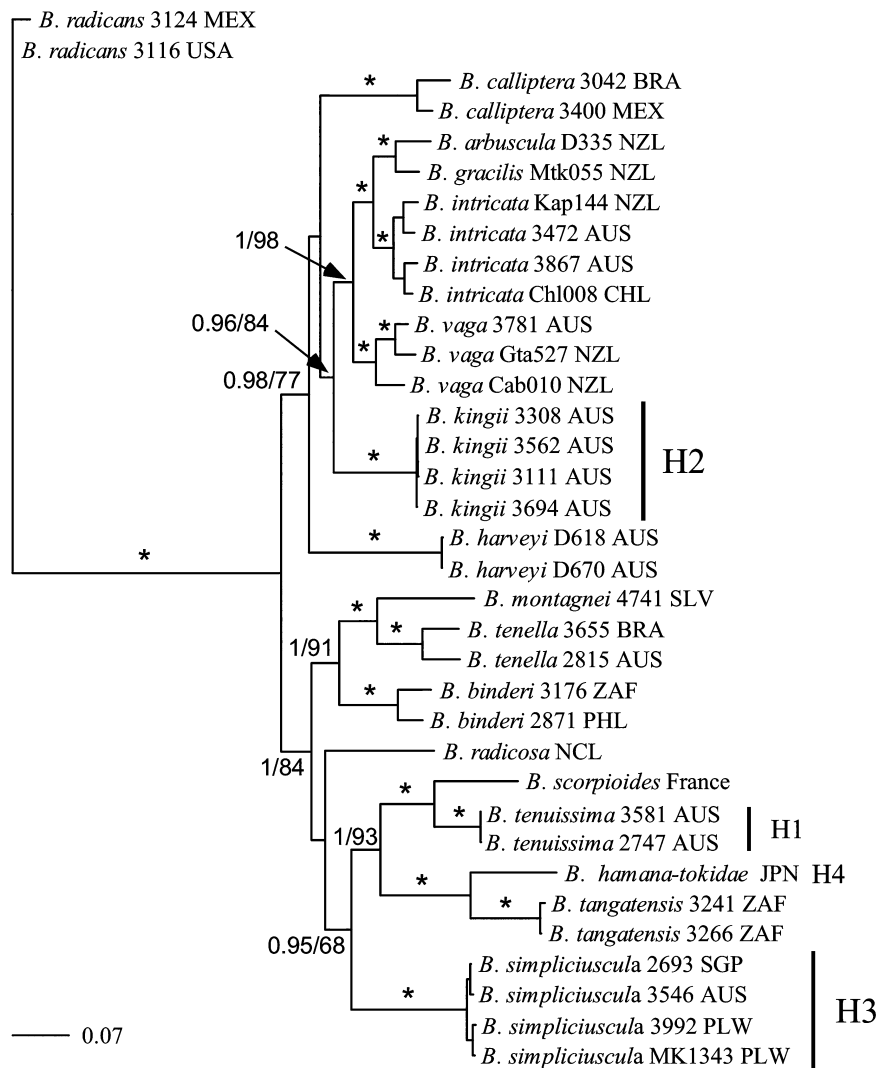
Samples have been collected over many years from many sites around the Pacific Ocean (Table S1 in the Supporting Information). Collection, isolation and maintenance of cultures are as presented in West and Zuccarello (1999) and West (2005).

DNA extraction, PCR amplification and sequencing are described in Zuccarello *et al.* (1999b) for the RuBisCo spacer and Zuccarello and West (2006) for *rbcl* and partial large-subunit ribosomal RNA (LSU). Three datasets were produced using available sequences from Genbank and new sequences: *rbcl*, LSU, and combined *rbcl*–LSU. Phylogenetic trees were constructed with maximum-likelihood (ML) using RAxML 7.2.8 (Stamatakis 2006) under the GTRCAT model with partitioned codons for *rbcl*. Support for individual nodes was determined by 1000 bootstrap replicates. Bayesian trees were inferred using MrBayes 3.2 (Ronquist *et al.* 2012) under the GTR + gamma model for 3 million generations with two independent runs, a sampling frequency of 1000 and a burn-in of 300 trees. The *rbcl*–*rbclS* spacer (RuBisCo spacer) was used to place many samples into one of the four recognized lineages. Networks were created for the RuBisCo spacer data set, where within lineage variation was seen, using TCS 1.21 (Clement *et al.* 2000) to which the sequences could be joined with 95% confidence.

Morphological characters were investigated in field specimens. The characters explored were: number of pericentral cells per tier (all specimens had two tier cells be axial cell); maximum length of monosiphonous laterals; whether the monosiphonous lateral branched, position of peripherohaptera-adjacent to lateral branches (nodal) or not (intermodal); whether the peripherohaptera are stalked at their bases (a coalescence of rhizoids adjacent to the axis) or had loose rhizoids (sessile), following King and Puttock (1989).

## RESULTS

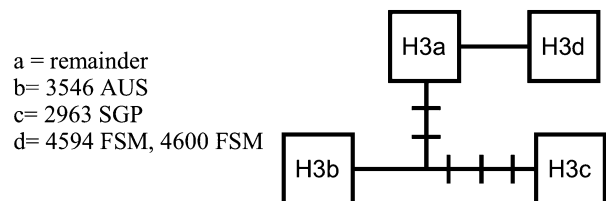
We expanded the distribution of collections to include the central Pacific (e.g., Federated States of Micronesia, Palau) and north Pacific (Japan)). In total, 55 samples were sequenced with one or more molecular markers (Table S1 in the Supporting Information). Our combined phylogenetic analyses of samples, all initially identified as *B. simpliciuscula*, indicates that this morphology is polyphyletic with four distinct lineages (presently called H1–H4) (Fig. 1). The *rbcl* (Fig. S1 in the Supporting Information) and partial LSU phylogenies (Fig. S2 in the Supporting Information) while less supported did not contradict this conclusion. A clade, with high support in the combined analysis, contains two *B. simpliciuscula* lineages (H1 and H4) plus two other morphologically distinct species (*B. tangatensis* E. Post and *B. scorpioides* (Hudson) Montagne). Within this clade there is a supported grouping of *B. scorpioides* and *B. simpliciuscula* H1; plus in the LSU analysis these two species group with *B. flagellifera* E. Post with strong support; and *B. tangatensis*



**Fig. 1.** A majority-rule consensus tree generated from the MrBayes analysis of combined *rbcL* and partial LSU of species of *Bostrychia*. Values on branches = Bayesian posterior probabilities (PP) followed by ML bootstrap percentages (BP). \* = full support of branch (1.0 PP, 100% BP). Outgroup the clado-haptera containing species *Bostrychia radicans*. 3-Letter country codes used. H1-H3 = refer to '*B. simpliciuscula*' lineages from Zuccarello *et al.* (1999aa) and Zuccarello and West (2006). H4 = Japanese lineage of '*B. simpliciuscula*'.

with *B. simpliciuscula* H4. Our RuBisCo spacer analysis, of more samples, shows that samples of *B. simpliciuscula* H1 have a restricted cold temperate distribution and only one haplotype is found. H1 is found in New Zealand, southern Australia (Tasmania, South Australia) and from Botany Bay, Sydney southward on the east and south coast of Australia. This southerly distribution pattern corresponds to previous research on this lineages distribution (Zuccarello *et al.* 1999a). H4 is found in Japan including the main islands of Honshu, Kyushu and Shikoku. Lineage H3 is sister to the clade containing *B. tangatensis* and *B. scorpioides* and lineages H1 and H4 with moderate support. H3 is found wide-ranging throughout the tropical Pacific from northern New South Wales, Australia (Brunswick Heads) to Singapore and in the Federated States of Micronesia and Palau (Table S1 in the Supporting Information). This wide-ranging lineage also has four RuBisCo spacer haplotypes (Fig. 2), with most samples with haplotype H3a, with distinct haplotypes for samples from Australia and Singapore and two of the samples from Micronesia (Table S1 in the Supporting Information). These three lineages of *B. simpliciuscula* (H1, H3, H4) also form a moderately supported clade with other mostly corticated

*Bostrychia* species (*B. binderi*, *B. tenella*, *B. montagnei*). The phylogenetic position of *B. simpliciuscula* lineage H2 is very different. It is not closely related to the other three lineages (Fig. 1), but groups with species mostly from the cold temperate southern hemisphere (*B. arbuscula* W.H. Harvey, *B. gracilis* (R.J. King & Puttock) Zuccarello & J.A. West, *B. intricata*, *B. vaga* J.D. Hooker & Harvey), this group also contains the species *B. harveyi* Montagne and *B. calliptera* (Montagne) Montagne.



**Fig. 2.** Statistical parsimony network inferred from RuBisCo data of samples of *B. simpliciuscula* (lineage H3). Lines indicate one mutational step; cross lines indicate inferred missing or intermediate haplotypes.

Inspection of field material did not find any characters that could separate all four species (Table S2 in the Supporting Information). The morphological characters that have been used to separate species within the *B. simpliciuscula* complex have been few, but King and Puttock (1989) suggested that the presence of monosiphonous lateral branches and the morphology of peripherohaptera, stalked versus free rhizoids, could separate *B. simpliciuscula* (with monosiphonous laterals and free rhizoids) from *B. tenuissima* (polysiphonous throughout and stalked rhizoids). Our measurements of field samples (with variable sampling within clades) suggest that two main groupings can be found. Monosiphonous lateral branches do not seem to define particular clades but branching of those monosiphonous laterals does seem to fall into two groupings. Lineages H1 and H2 with short monosiphonous tips to lateral branches (2–7 cells) and no monosiphonous branching of these laterals, and lineages H3 and H4 with longer monosiphonous tips to lateral branches (3–30 cells) and monosiphonous branching of these laterals. Also, the morphology of the peripherohaptera appears to be distinct between these two groupings. Lineages H1 and H2 have mostly stalked peripherohaptera, while lineages H3 and H4 have free rhizoids for their peripherohaptera. Samples of lineages H1 and H2, while not monophyletic, conform closely to the morphology of *B. tenuissima*. Samples of H3 and H4 are similar to *B. simpliciuscula* (sensu King & Puttock 1989). While there is no morphology that unambiguously separates these species, i.e. there is some variation in haptera morphology within species, and no characters seem to even tentatively separate the groups into four species, we propose that the species be defined by their biogeographic distribution. We propose that the following taxonomic changes be made:

New circumscription: *Bostrychia simpliciuscula* Harvey  
ex J. Agardh 1863: 854.

*Type locality*: (Friendly Islands) Tonga

For samples belonging to lineage H3, which seems to be found throughout, and possibly restricted to, the tropics (Federated States of Micronesia, Guam, Palau, Singapore, central eastern Australia), have peripherohaptera mostly with free rhizoids and branched monosiphonous laterals.

Resurrected species: *Bostrychia tenuissima* R.J. King & Puttock 1989: 18.

*Type locality*: Port Fairy, Victoria, Australia

Plants with morphology as stated in King and Puttock (1989) but with a more cold temperate distribution. For samples belonging to lineage H1, found in temperate Australia, from Sydney NSW southward, South Australia, Victoria, Tasmania, and New Zealand. This follows the distribution cited by King and Puttock (1989) for the most part, but they proposed a northern distribution to 26°S, which include the distribution of lineage H2. A small diagnostic amendment to the description, occasionally lateral branch tips are short monosiphonous, 2–7 cells, but this is probably environmentally variable.

Resurrected species: *Bostrychia hamana-tokidai*  
E. Post (1941): 208.

*Type locality*. Kedo River, Makurazaki, 'Satsuma Province' Kagoshima Prefecture, Kyushu, Japan.

For samples belonging to lineage H4 with morphology of *B. simpliciuscula* but found in the main islands of Japan. This species is found in Honshu, Shikoku and Kyushu. An older species name conforming to the morphology of *B. simpliciuscula* (and *B. hamana-tokidai*) is found in the Japanese tropical island of Okinawa (Ryukyu Islands), *B. andoi* Okamura (1907). At present we do not know if this species belongs to lineage H4 or is part of the tropical species H3 (*B. simpliciuscula*). Samples from Okinawa, which we were unable to amplify, morphologically could be H3 or H4 (Table S2 in the Supporting Information). Collection of this 'species' from Okinawa is needed to resolve if this name is appropriate.

New species: *Bostrychia kingii* Zuccarello, J.A. West & M. Kamiya sp. nov. (Fig. 3a–f).

For samples belonging to lineage H2 and showing the morphology of *B. tenuissima*.

*Diagnosis*. Morphology undistinguishable from *B. tenuissima*. Mostly ecorticate, branching irregular, with a few cortical cells in some older branches. Two tiers of pericentral cells per axial cell with 4–5 pericentral cells per tier. Very short monosiphonous tips (2–7 cells) on lateral branches but no monosiphonous branching. Peripherohaptera mostly with 'stalked' rhizoids (a coalescence of rhizoids adjacent to the axis).

*Type locality*. Forster, New South Wales, Australia (32°10'S 152°30'E).

*Holotype*. MEL 2409237.

*Isotype*. MICH701905, dried JAW3562 culture.

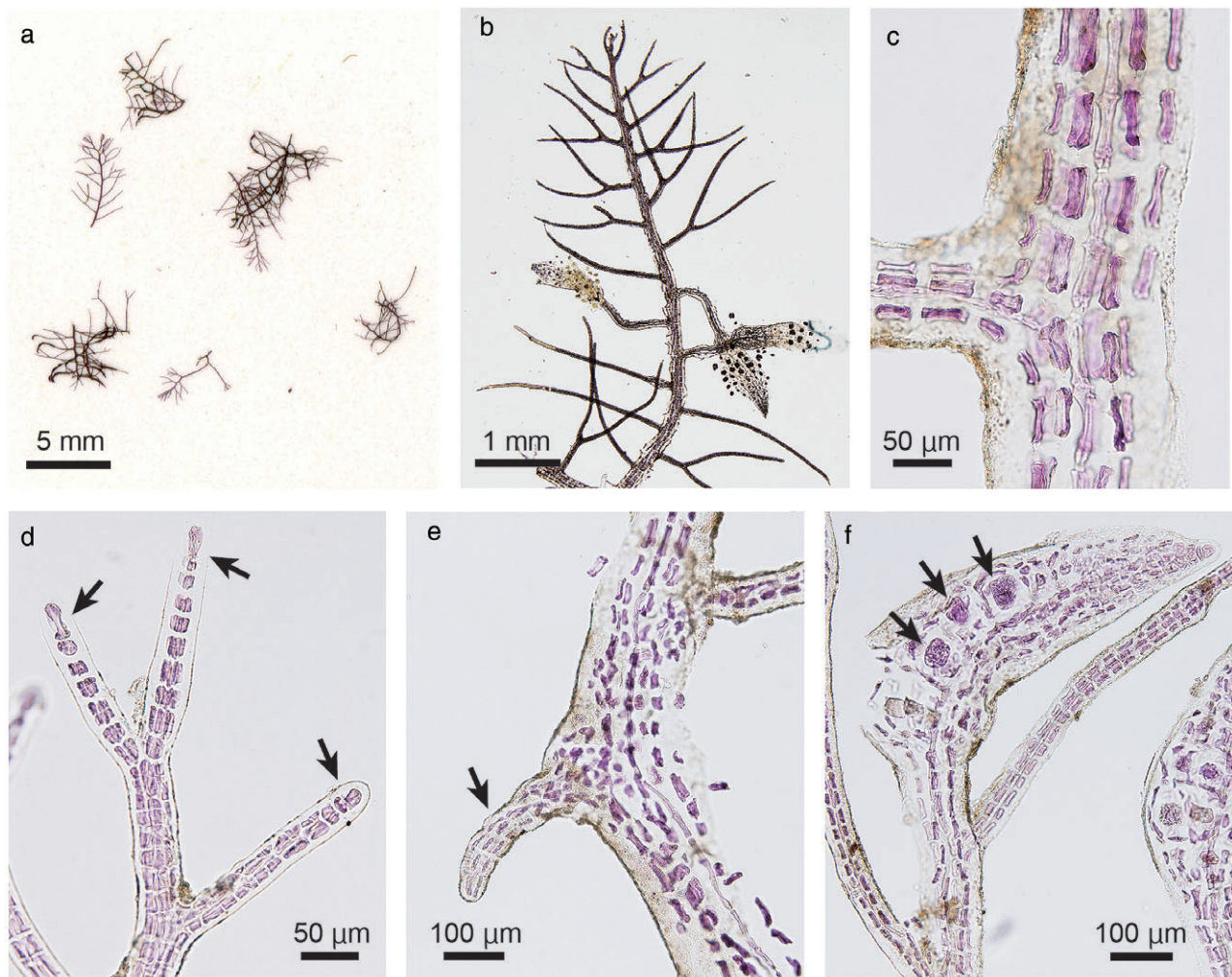
*Genbank accession numbers*. *rbcL*: AY920827; LSU: AY920877.

*Distribution*. This species has a limited distribution in central NSW, Australia (28–34°S), but overlaps with *B. tenuissima* in its southern range (e.g., Wooloware, Botany Bay, NSW) and may also overlap with *B. simpliciuscula* in its northern range, more sampling in these 'edge' areas is needed.

*Etymology*. *kingii* = in honor of Robert J. King for his pioneering work on the taxonomy of mangrove algae, especially *Bostrychia*, and appropriately this species appears to be confined to NSW where he spent his scientific career.

#### Description

Plants with prostrate axis and uprights up to 6 mm (Fig. 3a, b). Two tier cells per axial cell and 4–6 pericentral cells per tier around the main axial cell (Fig. 3c). Plant mostly ecorticate, but occasional cortical cells in older axes. Determinate lateral branches unbranched or with 1–2 order of branching (Fig. 3b). Occasionally laterals with, up to six cell monosiphonous tips (Fig. 3d). Peripherohaptera, adjacent or not to lateral branches, rhizoidal cells in coalescence branch (stalked) (Fig. 3e). Tetrasporangia (50–75 µm) in unbranched stichidia, tetrahedrally divided (Fig. 3f).



**Fig. 3.** Morphology of *Bostrychia kingii* sp. nov. holotype specimen. (a) morphology of voucher specimen. (b) Close up of lateral branching, 0–1 orders of branching, with tetrasporangial stichidia. (c) Higher magnification of vegetative axis. Two tiers cells per axial, lack of cortication and six tiers of pericentral cells around the axial cell. (d) Short monosiphonous tips (arrows) to determinate and indeterminate branches (2–4 cells long). Arrows = monosiphonous tips. (e) Peripherohapteron (arrow) with coalesced rhizoids (stalked), not associated with a lateral branch. (f) Tetrasporangial stichidium showing tetrasporangia (arrows). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## DISCUSSION

We find a very similar morphology in four non-monophyletic lineages that have all been previously identified as *B. simpliciuscula*. While cryptic, or pseudocryptic, species are reported commonly, most of these cryptic species complexes are monophyletic (Zuccarello & West 2003; Payo *et al.* 2013; Muangmai *et al.* 2014). This monophyly of cryptic species suggests that morphological diversification has not kept pace with genetic differentiation and species most likely retain their ancestral morphology. The reasons for this could be constraints on morphological diversification (developmental constraints), maintenance of morphology due to natural selection or lack of any force, for example, drift, leading to diversification. While these are possible for monophyletic cryptic species, our present results may need other explanations.

Careful examination of our available field collected molecularly identified specimens suggests that two morphologies could

be discerned. One of these morphologies correspond to the species described by King and Puttock (1989) as *B. tenuissima*, the other morphologically matches the more wide ranging species *B. simpliciuscula*. While these groups are recognizable they have few diagnostic characters that don't overlap, and still these characters do not define all four species. The only morphological character that appears to be diagnostic is branching in the monosiphonous lateral branches which appears to be confined to *B. simpliciuscula* and *B. hamana-tokidae* and not found in *B. tenuissima* and *B. kingii*. This character is probably not seen in all specimens. Along with this diagnostic character, there appears to be differences in peripherohaptera morphology with free rhizoids in *B. simpliciuscula* and *B. hamana-tokidae*, while the rhizoidal cells are fused into a stalk in the other two species. This character is not consistent within species groups and could be environmentally plastic. There are slightly more tiers of pericentral cells in *B. tenuissima* and *B. kingii* (4–6) than the other two species (4–5).



While these morphologies help in differentiating some of these species lineages they still do not define four species, nor are they found in monophyletic species groups. For example, branched monosiphonous branches are found in *B. simpliciuscula* (lineage H3) and *B. hamana-tokidae* (H4), these two species form a clade with *B. tenuissima* (lineage H1), plus two morphological distinct species *B. scorpioides* and *B. tangatensis*. While these two species also have peripheropoda for attachment, *B. scorpioides* is heavily corticated, and *B. tangatensis* has 3 (–4) tiers of cells per axial cell, a character previously used for generic separation (King & Puttock 1989). *B. kingii* which is indistinguishable from *B. simpliciuscula*, is found in a larger clade not closely related to these previous species. So what could be causing these similar morphologies in so many clades? While the slight morphological difference between these four species could be attributable to some sort of constraints on morphology if these lineages were monophyletic, that they are sister to species that are morphologically quite distinct, in the context of the genus *Bostrychia*, indicates that the inability to change morphologically is probably not a reason. The lack of time for morphologies to diverge is also unlikely as they again are sister to species that are quite distinct morphologically.

It is possible that convergence could have made these four species so similar. Convergence usually is invoked when phenotypic similarity is found in distantly related taxa and is considered evidence that natural selection has produced these evolutionary changes, usually leading to convergent adaptations (Losos 2011). What the adaptation for the particular morphology of these species is, which would have led to their convergence, is unknown. A comparative phylogenetic approach could be useful, but in this case it is difficult to evoke a selective pattern. *B. simpliciuscula* and *B. kingii* are both associated with mangrove habitats, especially on the bark and pneumatophores of mangrove trees. It is possible that these environments have driven this morphological convergence. Yet, these two species are very similar to *B. hamana-tokidae* and *B. tenuissima*, respectively, which are mostly not associated with mangroves but are found in rivers (in central Japan) or salt marshes (in colder areas—e.g., New Zealand) where different selection forces may be operating.

It is also possible that what we have is a group of species that have been misidentified (lumped) because they have not evolved diagnostic characters, perhaps by retaining a general body plan. While a combination of characters in *Bostrychia* has been used to define species, these four studied species seem to have a basic undifferentiated plan. For example, *B. tenuissima* is sister to *B. scorpioides*, which has evolved dense cortication and circinate apices. *B. hamana-tokidae* is sister to *B. tangatensis* but the latter has three tiers of pericentrals per axial cell. *B. kingii* is sister to a group of species found in the cold temperate southern hemisphere that have both cortication and three or more tiers of pericentral cells per axial cell, while still retaining peripheropoda. It is possible that what we have here is a lack of morphological evolution in a genus with few characters in the first place, it is possible that this ‘nondescript’ morphology is ancestral or a generalized body plan without any apparent change over time.

We have proposed to name these cryptic species, even though they are not morphologically distinguishable. While

maintaining a single descriptor name for a clade composed of many cryptic is defensible (i.e., all cryptic species are more closely related to each other than to any other morphologically distinct species; e.g., Muangmai *et al.* 2014; Boo *et al.* 2015), in this case we do not believe that maintaining a polyphyletic species aids understanding of diversity. We believe that in order to move taxonomy forward the names proposed should be applied to these taxa. At present the biogeographic distribution of these species is mostly quite distinct and this should be used to ‘identify’ the species in a particular location. The changes in distribution that may occur with further sampling should not hinder these taxonomic changes. The taxonomic changes also are minimally disruptive. Many references to *B. simpliciuscula* are from tropical regions (Silva *et al.* 1996; Atmadja & Prud 2012), so these records will not need updating. Cold temperate regions have the species *B. tenuissima* which has been used since 1989 (Adams 1994; Womersley 2003). Records from New South Wales, Australia are problematic, if the species is found in the south central region it could be *B. tenuissima* or *B. kingii* if it lacks branched monosiphonous laterals and has stalked peripheropoda. In central northern New South Wales it could be *B. kingii* if it has the previous morphology or *B. simpliciuscula* if it has branched monosiphonous laterals and free rhizoids. In the cool temperate northern hemisphere the issue of distribution of *B. hamana-tokidae* versus the extent of *B. simpliciuscula* needs to be investigated. For example, which species is found in China (Liu 2008) or Korea (Nam & Kang 2012)?

It is possible that continued study of these morphologically similar species will uncover morphological differences, but it is clear that these characters will not be readily accessible to most biologist, and the real possibility that no characters will be found must be accepted in organisms with low morphological diversity and available characters that are environmentally plastic (Verbruggen 2014).

We believe our proposed changes update the taxonomy of *Bostrychia* to more closely reflect the evolution and biodiversity of the group. Interestingly there appears to be one other polyphyletic species in the genus, samples identified as *B. calliptera* (Zuccarello & West 2006), resolving this morphospecies will require further study.

## ACKNOWLEDGMENTS

We thank the Japanese Society for the promotion of Science (JSPS) for a fellowship to GCZ. We also thank our many collectors that have sent samples over the years.

## REFERENCES

- Adams, N. M. 1994. *Seaweeds of New Zealand*. Canterbury University Press, Christchurch, New Zealand.
- Agardh, J. G. 1863. *Species, genera et ordines floridearum, seu descriptiones succinctae specierum, generum et ordinum*, Vol. 2. Gleerup, Lund, pp. 701–1291.
- Atmadja, W. S. and Prud, W. F. 2012. *Checklist of the Seaweed Species Biodiversity of Indonesia with their Distribution and Classification: Rhodophyceae*. Coral Reef Rehabilitation

- and Management Programme. Indonesian Institute of Sciences (LIPI), Jakarta.
- Boo, G.-H., Nelson, W. A., Preuss, M., Kim, J. Y. and Boo, S. M. 2015. Genetic segregation and differentiation of a common subtidal alga *Pterocladia lucida* (Gelidiales, Rhodophyta) between Australia and New Zealand. *J. Appl. Phycol.* **28**: 2027–34.
- Charlesworth, B., Lande, R. and Slatkin, M. 1982. A neo-Darwinian commentary on macroevolution. *Evolution* **36**: 474–98.
- Clement, M., Posada, D. and Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657–9.
- Conklin, K. Y. and Sherwood, A. R. 2012. Molecular and morphological variation of the red alga *Spyridia filamentosa* (Ceramiales, Rhodophyta) in the Hawaiian archipelago. *Phycologia* **51**: 347–57.
- Davis, C. C., Schaefer, H., Xi, Z., Baum, D. A., Donoghue, M. J. and Harmon, L. J. 2014. Long-term morphological stasis maintained by a plant-pollinator mutualism. *Proc. Natl. Acad. Sci. U.S.A.* **111**: 5914–9.
- De Clerck, O., Guiry, M. D., Leliaert, F., Samyn, Y. and Verbruggen, H. 2013. Algal taxonomy: a road to nowhere? *J. Phycol.* **49**: 215–25.
- Fraser, C. I., Zuccarello, G. C., Spencer, H. G., Salvatore, L. C., Garcia, G. R. and Waters, J. M. 2013. Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the Southern Hemisphere. *PLoS One* **8**: e69138.
- Hughey, J. R. and Gabrielson, P. W. 2012. Comment on “Acquiring DNA sequence data from dried archival red algae (Florideophyceae) for the purpose of applying available names to contemporary genetic species: a critical assessment.”. *Botany* **90**: 191–203.
- King, R. J. and Puttock, C. F. 1989. Morphology and taxonomy of *Bostrychia*. *Aust. Syst. Bot.* **1**: 1–73.
- Kogame, K., Ishikawa, S., Yamauchi, K., Uwai, S., Kurihara, A. and Masuda, M. 2015. Delimitation of cryptic species of the *Scytosiphon lomentaria* complex (Scytosiphonaceae, Phaeophyceae) in Japan, based on mitochondrial and nuclear molecular markers. *Phycol. Res.* **63**: 167–77.
- Krueger-Hadfield, S. A., Roze, D., Correa, J. A., Destombe, C. and Valero, M. 2015. O father where art thou? Paternity analyses in a natural population of the haploid-diploid seaweed *Chondrus crispus*. *Heredity* **114**: 185–94.
- Leliaert, F., Verbruggen, H., Vanormelingen, P. *et al.* 2014. DNA-based species delimitation in algae. *Eur. J. Phycol.* **49**: 179–96.
- Liu, J. Y. (Ed.). 2008. *Checklist of Biota of Chinese Seas*. Science Press, Academia Sinica, Beijing.
- Losos, J. B. 2011. Convergence, adaptation, and constraints. *Evolution* **65**: 1827–40.
- Lyra, G. d. M., Gurgel, C. F. D., Costa, E. D. S. *et al.* 2016. Delimitating cryptic species in the *Gracilaria domingensis* complex (Gracilariaceae, Rhodophyta) using molecular and morphological data. *J. Phycol.* **52**: 997–1017.
- Montecinos, A. E., Couceiro, L., Peters, A. F., Desrut, A., Valero, M. and Guillemain, M.-L. 2017. Species delimitation and phylogeographic analyses in the *Ectocarpus* subgroup *siliculosi* (Ectocarpales, Phaeophyceae). *J. Phycol.* **53**: 17–31.
- Muangmai, N., von Ammon, U. and Zuccarello, G. C. 2016. Cryptic species in sympatry: nonrandom small-scale distribution patterns in *Bostrychia intricata* (Ceramiales, Rhodophyta). *Phycologia* **55**: 424–30.
- Muangmai, N., West, J. A. and Zuccarello, G. C. 2014. Evolution of four southern hemisphere *Bostrychia* (Rhodomelaceae, Rhodophyta) species: phylogeny, species delimitation and divergence times. *Phycologia* **53**: 593–601.
- Nam, K. W. and Kang, P. J. 2012. *Algal Flora of Korea. Vol. 4, Number 4. Rhodophyta: Ceramiales: Rhodomelaceae: 18 Genera Including Herposiphonia*. National Institute of Biological Resources, Incheon.
- Niwa, K., Kikuchi, N., Hwang, M. S., Choi, H.-G. and Aruga, Y. 2013. Cryptic species in the *Pyropia yezoensis* complex (Bangiales, Rhodophyta): sympatric occurrence of two cryptic species even on same rocks. *Phycol. Res.* **62**: 36–43.
- Okamura, K. 1907. *Icones of Japanese Algae, Vol. 1*. Kazamashobo, Tokyo.
- Payo, D. A., Leliaert, F., Verbruggen, H., D'hondt, S., Calumpong, H. P. and De Clerck, O. 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. *Proc. R. Soc. Lond. B Biol. Sci.* **280**: 20122660.
- Peters, A. F., Van Wijk, S. J., Cho, G. Y. *et al.* 2010. Reinstatement of *Ectocarpus crouaniorum* Thuret in Le Jolis as a third common species of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Western Europe, and its phenology at Roscoff, Brittany. *Phycol. Res.* **58**: 157–70.
- Post, E. 1941. *Bostrychia hamana-tokidai* spec. nov., eine neue süd-japanische *Bostrychia*. *Beih. Bot. Centralbl. Abt B* **61**: 208–10.
- Ronquist, F., Teslenko, M., van der Mark, P. *et al.* 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**: 539–42.
- Silva, P. C., Basson, P. W. and Moe, R. L. 1996. Catalogue of the Benthic Marine Algae of the Indian Ocean. *Univ. Calif. Publ. Bot.* **79**: 1–1259.
- Skaloud, P. and Rindi, F. 2013. Ecological differentiation of cryptic species within an asexual protist morphospecies: a case study of filamentous green alga *Klebsormidium* (Streptophyta). *J. Eukaryot. Microbiol.* **60**: 350–62.
- Stamatakis, A. 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–90.
- Verbruggen, H. 2014. Morphological complexity, plasticity, and species diagnosability in the application of old species names in DNA-based taxonomies. *J. Phycol.* **50**: 26–31.
- Vieira, C., D'hondt, S., De Clerck, O. and Payri, C. E. 2014. Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *J. Phycol.* **50**: 1101–19.
- West, J. A. 2005. Long term macroalgal culture maintenance. In Andersen, R. (Ed.). *Algal Culturing Techniques*. Academic Press, New York, pp. 157–63.
- West, J. A. and Zuccarello, G. C. 1999. Biogeography of sexual and asexual populations in *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* **47**: 115–23.
- Womersley, H. B. S. 2003. *The Marine Benthic Flora of Southern Australia. Part IIID: Ceramiales-Delesseriaceae, Sarcomeniaceae, Rhodomelaceae*. Australian Biological Resources Study, Adelaide, 533 pp.
- Zuccarello, G. and West, J. 2011. Insights into evolution and speciation in the red alga *Bostrychia*: 15 years of research. *Algae* **26**: 21–32.
- Zuccarello, G. C. and Martin, P. 2016. Phylogeography of the *Lissonia variegata* species complex (Phaeophyceae, Laminariales) in New Zealand. *Algae* **31**: 91–103.
- Zuccarello, G. C., Muangmai, N., Preuss, M., Sanchez, L. B., Loiseaux de Goër, S. and West, J. A. 2015. The *Bostrychia tenella* species complex: morphospecies and genetic cryptic species with resurrection of *B. binderi*. *Phycologia* **54**: 261–70.
- Zuccarello, G. C., West, J., Karsten, U. and King, R. 1999a. Molecular relationships within *Bostrychia tenuissima* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* **47**: 81–5.
- Zuccarello, G. C., West, J. A., Kamiya, M. and King, R. J. 1999b. 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**: 207–14.
- Zuccarello, G. C. and West, J. A. 2003. Multiple cryptic species: molecular diversity and reproductive isolation in the *Bostrychia radicans*/*B. moritziana* complex (Rhodomelaceae, Rhodophyta) with focus on North American isolates. *J. Phycol.* **39**: 948–59.
- Zuccarello, G. C. and West, J. A. 2006. Molecular phylogeny of the subfamily Bostrychioideae (Ceramiales, Rhodophyta): subsuming

*Stictosiphonia* and highlighting polyphyly in species of *Bostrychia*.  
*Phycologia* **45**: 24–36.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** A phylogeny of *rbcL* data of species of *Bostrychia*.

**Fig. S2.** A phylogeny of partial LSU data of species of *Bostrychia*.

**Table S1.** Samples used in study.

**Table S2.** Morphological characters from field collected material.