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## *Bostrychia* (Rhodomelaceae, Rhodophyta) species of New Zealand, and relationships in the Southern Hemisphere

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**Abstract** *Bostrychia* is a widespread genus and has recently received considerable molecular attention, leading to insights into its evolution, and nomenclatural changes. We investigated the species of *Bostrychia* in New Zealand, to produce an identification key and highlight the nomenclatural changes of these species. We incorporated into a molecular phylogeny two species prominent in New Zealand (*B. gracilis*, *B. vaga*) that have not been investigated phylogenetically before. Our results show that three species of *Bostrychia* (*B. arbuscula*, *B. gracilis*, *B. vaga*), mainly restricted to New Zealand, share a common ancestor. Only *B. vaga* is known outside New Zealand. This restricted distribution is unusual for species within this genus in which many are widespread. A phylogeographic study of another New Zealand and Southern Hemisphere species, *B. intricata*, indicates that this species consists of several lineages that are found locally. These lineages, and physiological difference between lineages, suggest that cryptic species may be found within this species, as is common in many other red algal species. Our data highlight the unique nature of the New Zealand flora within this common genus and suggest continued investigation is warranted.

**Keywords** cryptic species; identification; phylogeny; *Stictosiphonia*; key

### INTRODUCTION

The mainly mangrove-associated algal genus *Bostrychia* Montagne has been well characterised taxonomically. Falkenberg (1901) was the first to revise *Bostrychia* on the basis of number of tier cells derived from pericentral cells, resurrecting the genus *Stictosiphonia* J.D. Hooker & Harvey (Hooker & Harvey 1845, 1847) for species with more than two tier cells per pericentral cell associated with each axial cell. The division of the group into two genera based on tier-cell numbers was not, however, accepted by Post (1936, 1939) the leading researcher of *Bostrychia* and other mangrove-associated algae during the period from 1936 to 1968. She emphasised the type of hapteron (attachment structure) and on this basis divided the genus into two sections (Post 1936, 1939). The two-genus system (*Bostrychia*, *Stictosiphonia*) was again resurrected in the latest monographic study of King & Puttock (1989), and this nomenclatural system was used by Adams (1994) in her guide of the marine flora of New Zealand. Recent molecular data (Zuccarello & West 2006) also showed that the monophyly of the two genera is not supported and returned all the species to the genus *Bostrychia*. This molecular work also revealed that some “species” are found in multiple divergent clades, that at present cannot be resolved morphologically (e.g., *B. simpliciuscula* Harvey ex J. Agardh, *B. moritziana*/*B. radicans* species complex), and several lineages had wide geographic distributions (Zuccarello & West 2002, 2003). The new nomenclature from Zuccarello & West (2006) and biogeographic and molecular knowledge from Zuccarello & West (2003, 2006) were used to understand the diversity and evolution of *Bostrychia* in New Zealand.

At present, four species of *Bostrychia* are recognised in New Zealand. *Bostrychia moritziana* (Sonder ex Kützinger) J. Agardh belongs to a

genetically highly diverse clade that also includes *B. radicans* (Montagne) Montagne (Zuccarello et al. 1999a; Zuccarello & West 2003). The group is characterised by an attachment structure, termed the cladohapteron, which is produced as a specialised branch from the second axial cell of lateral branches. *Bostrychia moritziana* is found in mangroves or saltmarsh vegetation and is reported from North Island and Marlborough Sounds, South Island (King & Puttock 1989). *Bostrychia harveyi* Montagne has been confused with *B. scorpioides* (Hudson) Montagne from the Northern Hemisphere and South Africa (Prud'homme van Reine & Sluiman 1980), but *B. harveyi* is a distinct Southern Hemisphere species (Zuccarello & West 2006), often found in brackish and freshwater habitats (King & Puttock 1989). Another alga reported in King & Puttock (1989) and Adams (1994) is *B. tenuissima* R.J. King & Puttock. However, molecular analyses by Zuccarello & West (2003) indicated that this species consists of three unrelated lineages that include the older named species *B. simpliciuscula*. Consequently *B. tenuissima* was placed in synonymy with *B. simpliciuscula*. *Bostrychia tenella* f. *flagellifera* (Post) King & Puttock is not reported in Adams (1994), but is noted to occur on Waitangi River, North Island (King & Puttock 1989). Molecular evidence shows it is not closely related to *B. tenella* and should be maintained as a distinct species, *B. flagellifera* Post (Zuccarello & West 2003).

The other species of *Bostrychia* reported from New Zealand have all been treated by King & Puttock (1989) and Adams (1994) as species of *Stictosiphonia*, and have recently been transferred back to *Bostrychia* (Zuccarello & West 2006). *Bostrychia vaga* (J.D. Hooker & Harvey) was reported from both the North and South islands (King & Puttock 1989; Adams 1994) and was found commonly at Doubtful Sound (Boyle et al. 2001). The species has also been reported from Kerguelen Islands (Hooker & Harvey 1847) and Falkland Islands (King & Puttock 1989). *Stictosiphonia gracilis* R.J. King & Puttock has only been reported from northern New Zealand (Titahi Bay northwards). *Bostrychia arbuscula* J.D. Hooker & Harvey, the largest *Bostrychia* species, is common in the South Island high intertidal but has also been reported from Cook Strait, North Island (Adams 1994). The most widespread species is *B. intricata* (Bory de Saint-Vincent) Montagne, that has been reported throughout the Southern Hemisphere (Australia, South Africa, South America, Falkland Islands, subantarctic islands) but also from more northern

locations (India, Indonesia) (King & Puttock 1989; Adams 1994; Silva et al. 1996).

The latest phylogenetic study of the genus *Bostrychia* (Zuccarello & West 2006) lacked two species common to New Zealand (*B. gracilis*, *B. vaga*). The present study was undertaken to clarify the taxonomy of *Bostrychia* in New Zealand using molecular data, to provide an updated key to these species, and to investigate the phylogeographic patterns of one widespread species, *B. intricata*.

## MATERIALS AND METHODS

Some material was collected and maintained in culture following procedures of West & Zuccarello (1999), other samples were collected in the field and dried onto herbarium sheets. Voucher specimens and DNA sequences are deposited at WELT and GenBank, respectively (see Table 1). Some samples were prepared for microscopy by soaking dried samples in sea water for 10 min and then simultaneously staining and preserving the specimens in 0.1% acidified aniline blue in 50% Karo® syrup on a microscope slide. Photographs were taken on an Olympus AX-70 microscope with an Olympus DP-70 camera.

From cultured material, or dried field samples, DNA extraction followed a modified Chelex extraction method (Zuccarello et al. 1999b). Amplification of an approximately 900–1000 basepair region of the nuclear large subunit of ribosomal RNA (LSU), corresponding to the middle third of the molecule (Y-fragment, Harper & Saunders 2001) followed the procedure of Zuccarello & West (2002). Amplification and sequencing of the plastid-encoded large subunit of the ribulose biphosphate carboxylase/oxygenase gene (*rbcL*) used amplification primers presented by Nam et al. (2000) and additional sequencing primers listed in Freshwater & Rueness (1994). The polymerase chain reaction procedure followed Zuccarello et al. (2002). Sequences were assembled using the computer software VectorNTI (Invitrogen), and aligned with Clustal X (Thompson et al. 1997). All sequences were compiled in Se-A1 v.2a11 (Rambaut 1996). Phylogenetic relationships were inferred with PAUP\*4.0b10 (Swofford 2002). Other sequences were selected from available GenBank deposits (Table 1). *Centroceras clavatum* and *Caloglossa vieillardii* were used as outgroups and have been used previously in the phylogenetic analysis of *Bostrychia* (Zuccarello & West 2006).

**Table 1** Samples used in this study. Collection information include: location, date collected, and voucher information deposited at WELT, if applicable. Culture numbers refer to J. West's (University of Melbourne) culture collection, number preceded by a letter indicates DNA extraction number. Genbank accession numbers used. New sequences in bold type.

Species	Collection locations	Collection dates	Culture no.	GenBank accessions nos.	
				<i>rbcL</i>	LSU rRNA
<i>Bostrychia arbuscula</i> J.D. Hooker & Harvey	Brighton Beach, South Island, New Zealand	11 July 1998	D335	AY920845	AY920894
<i>B. calliptera</i> (Montagne) Montagne	Rio Sitio Grande, Ilha do Cardoso, Brazil	18 January 1990	3042	AY920805	AF382926
<i>B. calliptera</i>	Sontecomapan, Vera Cruz, Mexico	10 February 1994	3400	AY920806	AF382925
<i>B. gracilis</i> (R.J. King & Puttock) Zuccarello & West	Kakamatua Point, Manukau Harbour, New Zealand (coll. M. D. Wilcox), WELT A023194	16 May 2007	F562	—	<b>EU886785</b>
<i>B. harveyi</i> Montagne	Havelock, Tasmania, Australia	7 July 1998	D618	AY920807	AY920857
<i>B. harveyi</i>	New Norfolk, Derwent River, Tasmania, Australia	14 October 1998	D670	AY920808	AY920858
<i>B. intricata</i> (Bory de Saint-Vincent) Montagne	Kommetjie, Cape Province, South Africa	17 February 1988	2876	AY920846	AY920895
<i>B. intricata</i>	Brighton Beach, South Island, New Zealand	11 July 1998	3867	AY920848	AY920897
<i>B. kelanensis</i> Grunow ex E. Post	Sadgroves Creek, Darwin, Northern Territories, Australia	4 June 1989	2988	AY920849	AY920898
<i>B. kelanensis</i>	Bowling Green Bay, Queensland, Australia	28 September 1991	3214	AY920853	AY920899
<i>B. moritziana</i> (Sonder ex Kützing) J. Agardh	Buenaventura, Colombia	10 September 1991	3189	AY920812	AY920861
<i>B. moritziana</i>	Rio Guire, Edo Sucre, Venezuela	11 April 1991	3149	AY920811	AY920862
<i>B. moritziana</i>	Millers Landing, Wilsons Promontory, Victoria, Australia	17 December 1988	2934	AY920809	AY920863
<i>B. moritziana</i>	Nusa Dua, Bali, Indonesia	10 May 1999	3955	AY920815	AY920866
<i>B. moritziana</i>	W. Sawang, Sulawesi, Indonesia	16 November 1994	3453	AY920813	AY920865
<i>B. moritziana</i>	Farasan Islands, Saudi Arabia	8 July 2000	4069	AY920816	AY920867
<i>B. pilulifera</i> Montagne	West Branch, Demerara River, Guyana	30 January 2002	4200	AY920817	AY920868
<i>B. radicans</i> (Montagne) Montagne	São Sebastião, Brazil	2 July 1982	2649	AY920818	AY920869
<i>B. radicans</i>	Teluk Awang, Lombok, Indonesia	27 May 1999	3980	AY920824	AY920874
<i>B. radicans</i>	Estero Coyote, Bahia San Ignacio, Baja California Sur, Mexico	26 March 1991	3124	AY920821	AY920872
<i>B. radicans</i>	Cape Fear Estuary, North Carolina, United States	27 January 1991	3116	AY920820	AY920871
<i>B. radicata</i> (Itono) West, Zuccarello & Hommersand	Tempusak, Sabah, Malaysia	13 August 2000	4086	DQ087406	DQ087404
<i>B. radicata</i>	Plage de Foué, New Caledonia	2 July 2001	4178	DQ087407	DQ087405
<i>B. scorpioides</i> (Hudson) Montagne	Roscoff, France	1999	E130	AY920825	AY920875
<i>B. simpliciuscula</i> Harvey ex J. Agardh	Mandai, Singapore	13 June 1989	2963	AY920826	AY920876
<i>B. simpliciuscula</i>	Ballina, New South Wales, Australia	12 February 1997	3694	AY920828	AY920878
<i>B. simpliciuscula</i>	Western Port Bay, Victoria, Australia	25 October 1986	2747	AY920839	AY920889
<i>B. simpliciuscula</i>	Williamstown, Victoria, Australia	29 February 1996	3581	AY920844	AY920893
<i>B. simpliciuscula</i>	Brunswick Heads, New South Wales, Australia	23 October 1995	3546	AY920843	AY920889
<i>B. simpliciuscula</i>	Broughton Creek, New South Wales, Australia	11 January 1993	3308	AY920840	AY920890
<i>B. tangatensis</i> E. Post	St. Lucia, Natal, South Africa	20 January 1992	3241	AY920850	AY920901
<i>B. tangatensis</i>	St. Lucia, Natal, South Africa	22 December 1991	3266	AY920851	AY920902
<i>B. tenella</i> (Lamouroux) J. Agardh	Tawi-Tawi, Mindanao, Philippines	15 January 1988	2871	AY920832	AY920882
<i>B. tenella</i>	Bouncing Stones Beach, Queensland, Australia	13 June 1987	2815	AY920831	AY920881
<i>B. tenella</i>	Ilha do Itaparica, Bahia, Brazil	11 November 1996	3655	AY920837	AY920887
<i>B. tenella</i>	Mangrove Trail, Broome, Western Australia, Australia	18 June 1997	3743	AY920838	AY920888
<i>B. tangatensis</i> E. Post	St. Lucia, Natal, South Africa	20 January 1992	3241	AY920850	AY920901
<i>B. tangatensis</i>	St. Lucia, Natal, South Africa	22 December 1991	3266	AY920851	AY920902
<i>B. vaga</i> (J.D. Hooker & Harvey)	Tawharanui Marine Park, Anchor Bay, New Zealand (coll. M. D. Wilcox) WELT A023193	16 May 2007	F560	<b>EU886771</b>	<b>EU886784</b>
Outgroup					
<i>Caloglossa vieillardii</i> (Kützing) Setchell	—			AY150327	AF522217
<i>Centroceras clavatum</i> (C. Agardh) Montagne	—			AF259490	AF259414

The data sets of the two genomic regions (*rbcL* and LSU) were tested for incongruence using the partition homogeneity test (PHT) (Farris et al. 1994) as implemented in PAUP\* (1000 replicates, 10 random additions, 100 trees per addition saved). The data were combined as no incongruities were detected ( $P = 0.28$ ). Maximum-parsimony trees (MP) were constructed in PAUP\*, using the heuristic search option, 500 random sequence additions, tree-bisection-reconnection (TBR) branch swapping, and unordered and unweighted characters, with gaps treated as missing data. The program Modeltest version 3.06 (Posada & Crandall 1998) was used to find the model of sequence evolution that best fit each data set by an Akaike Information Criterion (AIC) (Posada & Crandall 2001). When the best sequence evolution model had been determined, maximum-likelihood was performed in PAUP\* using the estimated parameters (substitution model, gamma distribution, proportion of invariable sites, transition–transversion ratio) (1–5 random additions).

Support for individual internal branches was determined by bootstrap analysis (Felsenstein 1985), as implemented in PAUP\*. For MP bootstrap analysis, 1000 bootstrap data sets were generated from resampled data (5 random sequence additions).

Bayesian trees were inferred using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) using a partitioned data set with three partitions for the

codon positions for *rbcL*, with a GTR model with gamma distribution with eight variable rate category, two parallel runs each of three heated chains and one cold one, and  $5 \times 10^6$  generations with sampling every 1000 generations. A burn-in of 2000 trees was removed before constructing the consensus tree. The analyses were submitted to the Computational Biology Service Unit (CBSU, Cornell University, <http://cbsuapps.tc.cornell.edu/mrbayes.aspx>).

RuBisCo spacer sequence amplification followed procedures outlined in Zuccarello et al. (1999b). (Location of sequenced samples, mainly of *Bostrychia intricata*, are in Table 2.) MP trees were constructed in PAUP\*, as above.

## RESULTS

Eight species of *Bostrychia* are currently recognised in New Zealand. The most obvious characters separating species of *Bostrychia* are number of tier cells per axial cell, presence/absence of cortication, and the attachment structure. The species characterised by the character number of tier cells are divided into two groups: species with two tier cells per axial cell (Fig. 1A), with the lower tier cell attached to the axial cell by a pit connection (“*Bostrychia*-type”); and species with more than two, usually 3–5 tiers of cells per axial cell (“*Stictosiphonia*-type”)

**Table 2** *Bostrychia arbuscula*, *B. gracilis*, and *B. intricata* samples used for RuBisCo spacer sequence analysis. (Falkland Islands samples collected by Louise Phillips.)

Species	Collection location (codes for DNA extractions)	GenBank accession nos.
<i>B. arbuscula</i>	Brighton Beach, Dunedin, New Zealand (D331, D336, D337)	EU886783
<i>B. gracilis</i>	Tawharanui Marine Reserve, Flat Rock, Anchor Bay, New Zealand (F559, F576)	EU886782
<i>B. gracilis</i>	Kakamatua Point, Manukau Harbour, New Zealand (F562)	EU886782
<i>B. intricata</i>	Millers Point, Cape of Good Hope, South Africa (E404)	EU886774
<i>B. intricata</i>	Oude Kraal, Cape Town, South Africa (E405)	EU886775
<i>B. intricata</i>	Cape Point, Cape of Good Hope, South Africa (E406)	EU886776
<i>B. intricata</i>	Kommetjie, Cape Province, South Africa (2875)	EU886774
<i>B. intricata</i>	Kommetjie, Cape Province, South Africa (2876)	EU886774
<i>B. intricata</i>	Cochoaque, Concepcion, Chile (E588)	EU886772
<i>B. intricata</i>	Falkland Islands (Louise Phillips 306) (E867)	EU886773
<i>B. intricata</i>	Falkland Islands (Louise Phillips 64) (E868)	EU886772
<i>B. intricata</i>	Falkland Islands (Louise Phillips 139) (E869)	EU886773
<i>B. intricata</i>	Falkland Islands (Louise Phillips 247) (E879)	EU886772
<i>B. intricata</i>	Pambula Beach, New South Wales, Australia (E216)	EU886779
<i>B. intricata</i>	Tooradin, Victoria, Australia (E399)	EU886777
<i>B. intricata</i>	Whiskey Bay, Victoria, Australia (3472)	EU886779
<i>B. intricata</i>	Millers Landing, Wilsons Promontory, Victoria, Australia (3781)	EU886778
<i>B. intricata</i>	Waikowhai, Manukau Harbour, New Zealand (F561), WELT A023195	EU886781
<i>B. intricata</i>	Brighton Beach, Dunedin, New Zealand (3867)	EU886780

**Key to *Bostrychia* species in New Zealand**

- 1 Plants with distinct cortication..... 2  
Plants ecorticate throughout, or at least ecorticate in ultimate regions (cortication light)..... 5
- 2 Two tier cells per axial cell..... 3  
More than two tier cells per axial cell..... 4
- 3 With monosiphonous laterals, and well developed peripherohaptera..... *B. flagellifera*  
Without monosiphonous laterals, peripherohaptera as swellings at base of most laterals ..... *B. harveyi*
- 4 Branches terminating in colourless acute tips, intricate thalli..... *B. gracilis*  
Branches not with acute tips, upright thalli..... *B. arbuscula*
- 5 Two tier cells per axial cell..... 6  
More than 2 tier cells per axial cell..... 7
- 6 Attached by peripherohaptera, mostly polysiphonous throughout..... *B. simpliciuscula*  
Attached by cladohaptera, often with ultimate monosiphonous laterals..... *B. moritziana*
- 7 Erect branches rarely with lateral branches, some minor cortication ..... *B. vaga*  
Erect branches regularly branched..... *B. intricata*

(Fig. 1B). Cortication is absent in *B. moritziana*, *B. simpliciuscula*, *B. intricata*, and not seen in our specimen of *S. vaga* (Fig. 1C). Cortication is well developed in *B. harveyi*, *B. arbuscula*, and *B. gracilis*, but is often less well developed in lateral branches of *B. flagellifera* and the ultimate branches in this species are conspicuously monosiphonous (Fig. 1D). *Bostrychia arbuscula* and *B. gracilis* are distinguished only by the presence of acute tips in *B. gracilis* (Fig. 1E cf. 1F), the more upright and less intricate growth of *B. arbuscula* and the geographic range of *B. arbuscula*, which is found from Cook Strait south, whereas *B. gracilis* is more common north of Poverty Bay. The other character used to distinguish species is the type of attachment structures of which there are also two main types with one pronounced modification. Peripherohaptera, found in species such as *B. vaga*, are derived from tier cells, or cortical cells, usually associated with the bases of indeterminate laterals. The apical cells elongate and often form attachment pads on the substrate (see Fig. 1C). A modification is seen in *B. harveyi* in which peripherohaptera do not develop beyond small lumps at branch axes (Fig. 1G). Cladohaptera are derived from transformed branches that develop from the first axial cell of an indeterminate lateral (Fig. 1H).

*Bostrychia simpliciuscula*, reported as *B. tenuissima* in New Zealand, is a non-monophyletic species of three molecular lineages (Fig. 2). A sample of *B. simpliciuscula* from New Zealand (collected Havelock, South Island, 7 December 2005, data not shown) is of the H1 lineage. New Zealand samples also lack monosiphonous branch tips.

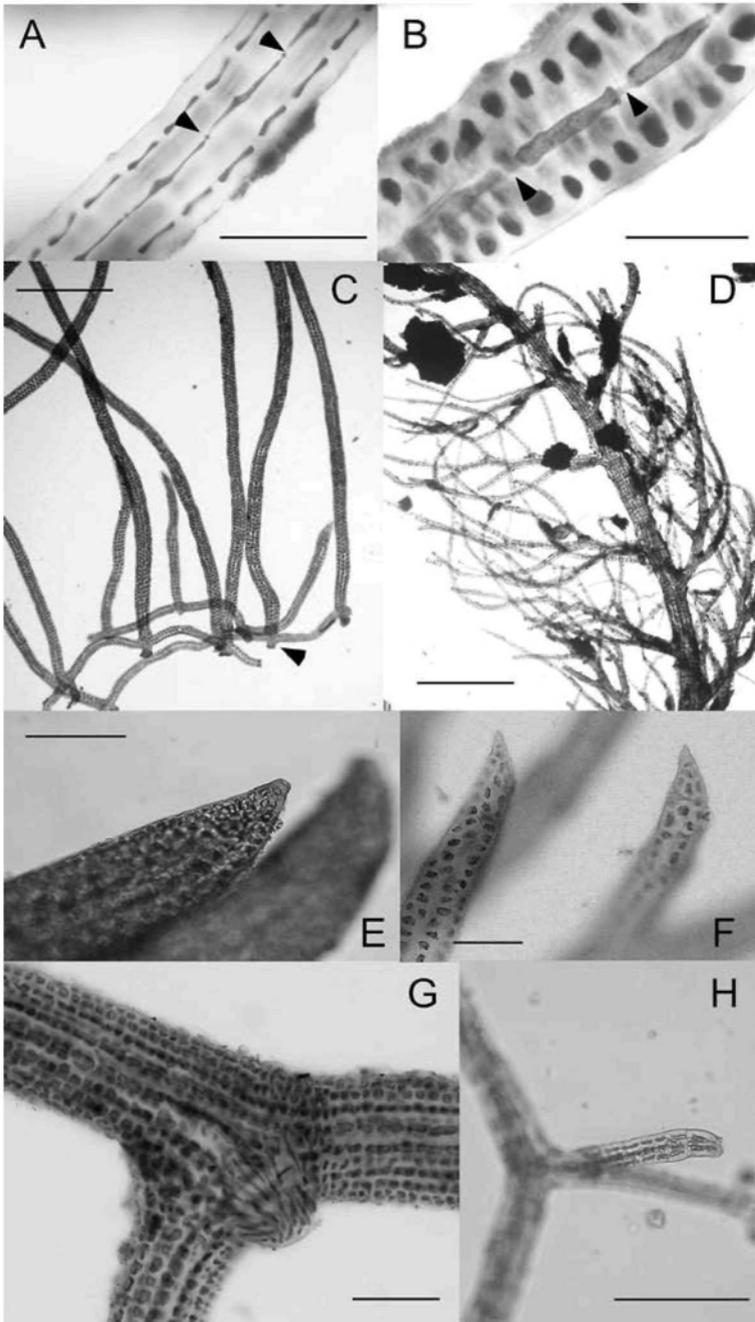
Two species not previously used in a phylogenetic analysis of the genus are *B. gracilis* and *B. vaga*.

A combined data set of *rbcL* and partial LSU data contained 1951 aligned characters and 637 parsimony-informative sites. MP produced 3 MP trees of 2497 steps. Although ML produced one tree of  $-ln = 14399.44596$  (AIC parameters: GTR + gamma distribution (0.5704) + proportion of invariable site (0.3605), Rate matrix: a = 2.171, b = 4.9787, c = 3.2184; d = 1.6853; e = 15.4520; f = 1.00), the trees did not differ in supported branches. The two newly sequenced species, *B. gracilis* and *B. vaga*, from New Zealand are in a moderately supported lineage with another New Zealand species, *B. arbuscula* (Fig. 2). These three species have a sister group relationship to another Southern Hemisphere species, *B. intricata*.

RuBisCo spacer analysis of 16 samples of *B. intricata* (298 aligned bases, 48 parsimony-informative characters including outgroups *B. arbuscula* and *B. gracilis*, produced 1 MP tree of 47 steps) indicates that there is a high degree of regional variation between samples, with samples from Australia in two supported clades, as are samples from New Zealand and most samples from South Africa (Fig. 3).

**DISCUSSION**

Our results present a key to species of *Bostrychia* found in New Zealand and update the taxonomy based on molecular insights and morphology (Zuccarello & West 2006). Additional data on their morphology and distribution can be found in King & Puttock (1989) and Adams (1994). Eight species of *Bostrychia* are currently recognised in New Zealand, that are distinguishable based on a few

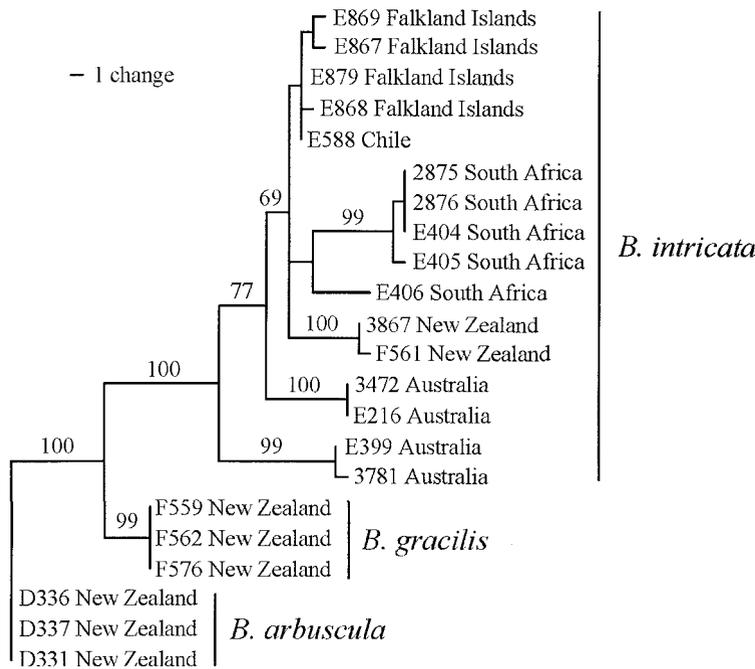


**Fig. 1** **A**, Axis of *Bostrychia simpliciuscula* showing two tier cells per axial cell. Arrowheads indicate start and end of an axial cell. Scale bar = 100  $\mu$ m. **B**, Axis of *B. intricata* showing 4 tier cells per axial cell. Arrowheads indicate start and end of an axial cell. Scale bar = 100  $\mu$ m. **C**, Habit of *B. vaga*. Unbranched upright axis, prostrate axis with periphapteron (arrowhead) visible. Scale bar = 0.5 mm. **D**, Habit of *B. flagellifera*. Monosiphonous ultimate laterals visible (WELT voucher A023191). Scale bar = 0.5 mm. **E**, Apex of *B. arbuscula* showing non-acute tips. Scale bar = 100  $\mu$ m. **F**, Apex of *B. gracilis* showing acute tips. Scale bar = 100  $\mu$ m. **G**, *B. harveyi* with partially developed periphapteron in axis of branch. Scale bar = 200  $\mu$ m. **H**, Cladohapteron of *B. moritziana* (WELT voucher A023192). Scale bar = 100  $\mu$ m.

morphological characters. At present many of these "species" are not monophyletic, and morphologically indistinguishable lineages can only be identified by molecular methods. For example, *B. moritziana*, the only species with cladohaptera found in New

Zealand, is distinguished from samples identified as *B. radicans* by the presence of monosiphonous laterals. However, this character does not define a monophyletic group separate from *B. radicans* (Zuccarello & West 2003, 2006, and Fig. 2).





**Fig. 3** Single most parsimonious tree of RuBisCo spacer sequence data of *Bostrychia intricata*. Designated outgroups *B. gracilis* and *B. arbuscula*. Bootstrap percentage values indicated on branches with over 50%.

*Bostrychia tenuissima*, another New Zealand species (Adams 1994; King & Puttock 1989), was also distinguished from *B. simpliciuscula* by the lack of monosiphonous branch tips (King & Puttock 1989). Three lineages are now recognised containing specimens both with and without monosiphonous branch tips (RuBisCo spacer lineages H1–H3, Zuccarello & West 2006), which appear to have different biogeographic distributions, with H1 the most southerly (Zuccarello et al. 1999c). A New Zealand sample, from the South Island, belongs to this southern lineage.

Our data support the monophyletic origin of the three exclusively (*B. gracilis*, *B. arbuscula*) or predominantly (*B. vaga*) New Zealand species of *Bostrychia*. Monophyly of the New Zealand species would indicate that morphological differentiation and speciation occurred in New Zealand, though the data can not directly address the question of speciation. *Bostrychia arbuscula* and *B. gracilis* are similar morphologically (well developed cortication, peripherohaptera). However, *B. vaga* is an extremely distinctive species (greatly reduced cortication (none in plants examined) and reduced laterals). Genetically the relationships between these species are not well resolved, owing to the low level of genetic variation. Within *Bostrychia*, morphological

change is not reflected by levels of change in commonly used genetic markers. For example, the highly corticated species *B. pilulifera* is genetically similar to some samples of the ecorticate species *B. radicans*, whereas other samples of *B. radicans* are genetically more distant (Zuccarello & West 2006, and Fig. 2). The scenario of an origin of these species within New Zealand would indicate that the presence of *B. vaga* in Falkland Islands, Chile, and Kerguelen Islands is a result of subsequent dispersal to these areas.

*Bostrychia intricata* is the sister species to *B. arbuscula*, *B. gracilis*, and *B. vaga*. It also has the widest distribution (as *B. hookeri* Harvey, *B. mixta* J.D. Hooker & Harvey, or *S. hookeri* (Harvey) J.D. Hooker & Harvey) as it is found throughout the Southern Hemisphere, mostly in temperate water, but also reported from Japan (Yoshida et al. 1990) and tropical water in India and Indonesia (King & Puttock 1989), although this tropical distribution is unusual and these records must be checked. Our analysis of the RuBisCo spacer data indicates that there is a high degree of regional variation between samples. The genetic differentiation of these samples is reflected in the temperature of induction of reproductive development (West et al. 1996), with samples from Australia requiring temperatures of

20–25°C, for South Africa 15°C, and Chile 10°C. These data indicate that more cryptic species may exist in this widespread alga, as has been observed in *B. moritziana*/*B. radicans* (Zuccarello & West 2003). More sampling and culture studies of this species are called for.

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