

Observations on *Bostrychia radicata* comb. nov. (Rhodomelaceae, Rhodophyta)

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SUMMARY

Only two genera in the Rhodomelaceae share the morphological character of transverse division of periaxial cells into two or more tier cells in which the pit connection is retained between the lower cell and the axial cell: *Bostrychia* and *Rhodolachne*. One species, *Rhodolachne radicata* Itono, has been reported from mangroves, a common habitat for *Bostrychia*. Many collections of an entity similar to *Rhodolachne radicata* have been made from localities around the Indo-Pacific. Culture observations show a *Polysiphonia*-type sexual life history in Malaysia and New Caledonia isolates that produce self-compatible bisexual gametophytes. The New Caledonia isolate also has unisexual gametophytes. An isolate from New South Wales (Australia) reproduces asexually through successive generations of tetrasporophytes. The Thailand isolate has successive generations of mixed-phase tetrasporophytes. The tetrasporangial stichidia also bear male spermatangial sectors, but female structures are lacking. Western Australia and Madagascar isolates do not reproduce in culture. Molecular evidence, based on sequencing of the *rbcL* and the large subunit ribosomal RNA genes, shows that these isolates belong to the genus *Bostrychia*. Low molecular weight carbohydrate analysis reveals high levels of digeneaside in all isolates. The sugar hexitol sorbitol, an osmolyte characteristic of *Bostrychia*, occurs in all isolates, whereas the Madagascar and New Caledonia isolates have very low levels of dulcitol. Molecular, low molecular weight carbohydrate and morphological evidence show that *Rhodolachne radicata* belongs within the genus *Bostrychia*. We transfer *Rhodolachne radicata* to *Bostrychia radicata* (Itono) West, Zuccarello and Hommersand.

Key words: asexuality, *Bostrychia*, low-molecular-weight-carbohydrate, mixed-phase, phylogeny, *Polysiphonia*-type-sexuality, *Rhodolachne*.

INTRODUCTION

The mainly mangrove associated algae of the genus *Bostrychia* Montagne have been well characterized taxonomically (King & Puttock 1989), whereas nomenclatural changes and phylogenetic relationships have been elucidated using molecular data (Zuccarello & West 2006). A main characteristic for the genus is the transverse division of periaxial cells into two or more tier cells in which the pit connection is retained between the lower cell and the axial cell. This character is shared with only one other ecorticate genus in the Rhodomelaceae, *Rhodolachne* Wynne (Womersley & Bailey 1970; Wynne 1970a,b). The affinity of *Rhodolachne* with *Bostrychia* has been noted but the two genera are still considered distinct. Since the original description of the type species, *Rhodolachne decussata* (Wynne 1970a), a second species was described, *Rhodolachne radicata* Itono (Itono 1985). Although characters distinctive of *Rhodolachne* were not evident, this species was still placed in the genus.

During field collections around Australia, Madagascar, Malaysia, New Caledonia and Thailand we obtained and isolated into culture an interesting alga characteristic of the genus *Bostrychia* (i.e. with two tier cells per periaxial cell). Although it is clearly different from other *Bostrychia* species, it is similar to the descriptions of *R. radicata* from Fiji and Japan (Itono 1985) in that it lacks clearly defined cladophylls or peripherophylls. In this paper we describe some of *R. radicata*'s morphological and reproductive features and present a molecular phylogeny of our isolates. In addition, a chemical analysis on the low molecular weight carbohydrates (LMWC) is carried out, because the genus *Bostrychia* is known to synthesize and accumulate the isomeric hexitols sorbitol and dulcitol as main photosynthetic products, which are otherwise very

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Communicating editor: G. H. Kim

Received 15 March 2005; accepted 22 July 2005.

unusual for higher red algae (Karsten *et al.* 1992, 1995a).

MATERIALS AND METHODS

Culture methods

Details about the specimens collected for culture are listed in Table 1. The methods for collection, isolation and culture are those outlined in West and Calumpang (1988) and West (2005).

DNA analysis methods

The methods for DNA extraction, amplification (of *rbcl* and partial nuclear-encoded ribosomal large subunit RNA gene (26S) and sequencing, are identical to those described in Zuccarello and West (2006). Amplification of the RuBisCo spacer follows procedures outlined in Zuccarello *et al.* (1999).

Sequences were assembled using the computer software supplied with the Applied Biosystems sequencer (Foster City, CA, USA), and aligned with Clustal X (Thompson *et al.* 1997). All sequences were compiled in Se-AL version 2a11 (Rambaut 1996). Phylogenetic relationships were inferred with PAUP*4.0b10 (Swofford 2002). Outgroups used were *Centroceras clavulatum* (C. Agardh) Montagne (GenBank accession AF259490)

and *Caloglossa vieillardii* (Kützing) Setchell (AY150327) for the *rbcl*, and *C. clavulatum* (AF259414) and *Caloglossa leprieurii* (Montagne) G. Martens (AF522217) for the large subunit (LSU) data. All new sequences are deposited in GenBank (accession numbers DQ087404–DQ087411).

Maximum-parsimony (MP) trees were constructed in PAUP*, using the heuristic search option, 500 random sequence additions, tree-bisection-reconnection branch swapping, unordered and unweighted characters, and gaps treated as missing data. For *rbcl* analysis rearrangements were run to completion whereas for the 26S data only 100 trees were saved per replicate. The program Modeltest version 3.6 (Posada & Crandall 1998) was used to find the model of sequence evolution that was least-rejected by the hierarchical likelihood ratio test for each dataset. When the best sequence evolution model had been determined, maximum likelihood (ML) was performed in PAUP* using the estimated parameters (substitution model, gamma distribution, proportion of invariable sites, transition–transversion ratio) (1–5 random additions). Distance trees were constructed using neighbor-joining reconstruction (NJ) either using the Modeltest parameters (*rbcl* dataset) or the LogDet distance calculations (Lockhart *et al.* 1994) (26S dataset).

Support for individual internal branches was determined by bootstrap analysis (Felsenstein 1985), as

Table 1. Collection data, reproduction and low molecular weight carbohydrates of culture isolates of *Bostrychia radicata*

Collection locality, date	Culture #	Reproduction	Sorbitol mmol/kg DW	Dulcitol mmol/kg DW	Digeneaside mmol/kg DW
On <i>Rhizophora</i> , Streeters Jetty, Broome, Western Australia, Australia, 17°57'S, 122°14'E. 18.vi.1997	3744	Vegetative in field and culture, tetraspores formed once (28 × 1997) but not released	10.7 ± 1.4	0	306.1 ± 2.2
On <i>Rhizophora</i> , Tempusak (near Kota Belud), Sabah, Malaysia, 06°22' 83"S, 116°20' 87"E. 13.viii.2000	4086	Vegetative in field. <i>Polysiphonia</i> -type life history with self compatible bisexual gametophytes in culture.	5.5 ± 4.9	0	347.6 ± 40.5
On <i>Avicennia</i> , Plage de Foué Fishing Village, New Caledonia. 21°06'27"S, 164°49'76"E, 02.vii.2001	4178	Vegetative in field. <i>Polysiphonia</i> -type life history with self compatible unisexual and bisexual gametophytes in culture.	45.2 ± 10.0	4.3 ± 0.3	472.5 ± 66.2
On <i>Xylocarpus</i> , Krabi, Thailand, 8°2'N, 98°80'E, 17.iii.2002	4207	Vegetative in field. Successive generations of asexual tetrasporangial plants in culture. Some stichidia with spermatangial sectors below tetrasporangial sector.	16.4 ± 0.9	0	683.4 ± 1.3
On <i>Lumnitzera</i> , Mooball Creek, New South Wales, Australia, 28°25.205'S, 153°33.637'E, 28.x.2002	4271	Vegetative in field. Successive generations of asexual tetrasporangial plants in culture.	15.0 ± 0.9	0	352.0 ± 8.8
On <i>Sonneratia</i> , Chenal d'Ampanarata, Belo sur Mer, Madagascar, 20°44.821'-S, 43°59.654'-E. 27.v.2004	4458	Vegetative in field and culture.	43.5 ± 0.4	2.9 ± 0.5	522.5 ± 2.7

DW, dry weight.

implemented in PAUP*. For bootstrap analysis, 1000 bootstrap datasets were generated from resampled data (5 random sequence additions).

Bayesian analyses were performed using MrBayes version 3.0b4 (Huelsenbeck & Ronquist 2001) following the procedure in Zuccarello and West (2006).

TCS 1.18 (Clement *et al.* 2000) was used to construct a haplotype network based on RuBisCo spacer sequence data.

Carbohydrate analysis methods

For polyol analyses the algal samples (10–15 mg dry weight (DW)) were extracted in 1 mL 70% ethanol (v/v) for approximately 3 h in a waterbath at 70°C. Following extraction, samples were centrifuged at 5000 *g* for 5 min, and 700 µL of the supernatant was evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H, Savant). Dried extracts were re-dissolved in 700 µL distilled water, vortexed for 30 s and centrifuged at 10 000 *g* for 5 min prior to injection onto the high performance liquid chromatography (HPLC) column. Samples were analyzed for sugar alcohols with an Agilent HPLC system with a refractive index detector according to the method of Karsten *et al.* (1991). Digeneaside was separated on a stainless-steel Phenomenex Rezex ROA-Organic Acid column (300 × 7.8 mm outside diameter) protected with a Phenomenex Carbo-H⁺ guard cartridge (4 × 3 mm inside diameter) according a newly developed method (Karsten *et al.* 2005). The mobile phase was 5 mM H₂SO₄ run isocratically at a flow rate of 0.5 mL/min and a temperature of 65°C. All low molecular weight carbohydrates were identified by comparison of retention times with those of standard compounds, and quantified by peak areas. All concentrations are expressed as mmol/kg on a DW basis.

RESULTS

Morphology and reproduction

Western Australia isolate (3744)

In field material this specimen was mixed with *Caloglossa ogasawaraensis* Okamura and *Bostrychia moritziana* (Sonder ex Kützing) J. Agardh on *Rhizophora* prop roots. In culture 3744 plants reach up to 80 mm in overall length. Stoloniferous growth is visible with most erect shoots having recurved tips. Nodes of mature stolons are spaced 0.5–2.5 mm apart. Each node initially has a single branch and subsequently develops 1–4 rhizoids and up to three shoots of variable length (Fig. 1). Occasionally a multicellular rhizoid arises first at the node. Nodal branches are all derived from a slightly enlarged axial cell. Mature branches are 55–60 µm wide with 4 periaxial cells per axial cell. Each

periaxial cell is divided once vertically, forming two tier cells that are connected along the axis by single pit connections. Tier cell formation occurs at the 10th axial cell from the apex and vertical secondary pit connections between tier cells of adjacent axial cells are formed by the 15th axial cell. Mature tier cells are 20–30 µm wide and 35–60 µm long.

Rhizoids develop from tier cells and are uniseriate and variably branched. Cells are 20–30 µm wide and 75–80 µm long, each with a single central nucleus (7–8 µm wide) surrounded by a halo of starch grains. The rhizoids are clearly separated and not cohesive as seen in cladohaptera and peripherohaptera in most *Bostrychia* species. Rhizoids are commonly over 1 mm long and can directly produce erect shoots and secondary rhizoids at various intervals.

This isolate was vegetative in the field and grows vigorously in culture but has remained non-reproductive except for a brief time in October 1997 when it formed stichidia with abortive tetrasporangia.

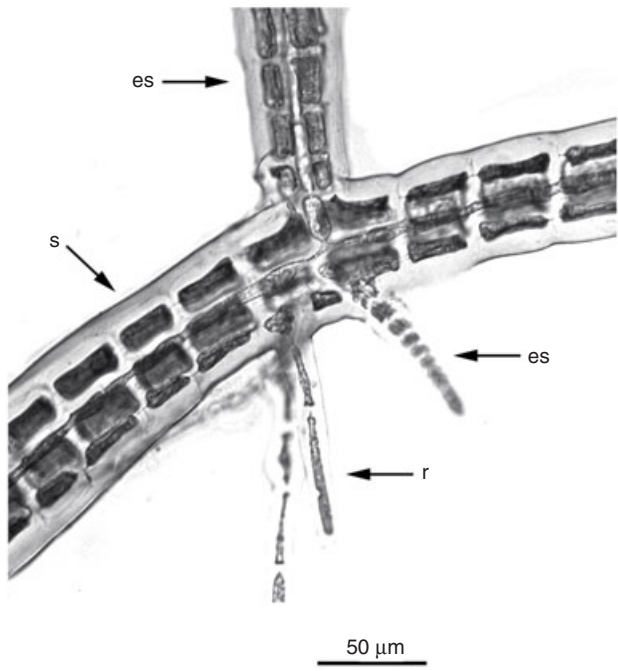
Madagascar isolate (4458)

Plants in culture are up to 45 mm long with stoloniferous growth. Erect shoots arise at intervals of 0.4–2 mm on the stolons. The nodes are similar to those of 3744. Mature shoots are up to 70–75 µm wide and tier cells are 30–40 µm wide and 60–80 µm long. As seen in the other isolates the rhizoids give rise to erect shoots at variable intervals and secondary rhizoids also (Fig. 2). Other features are also similar to those of 3744. No reproduction was evident in the field or in culture.

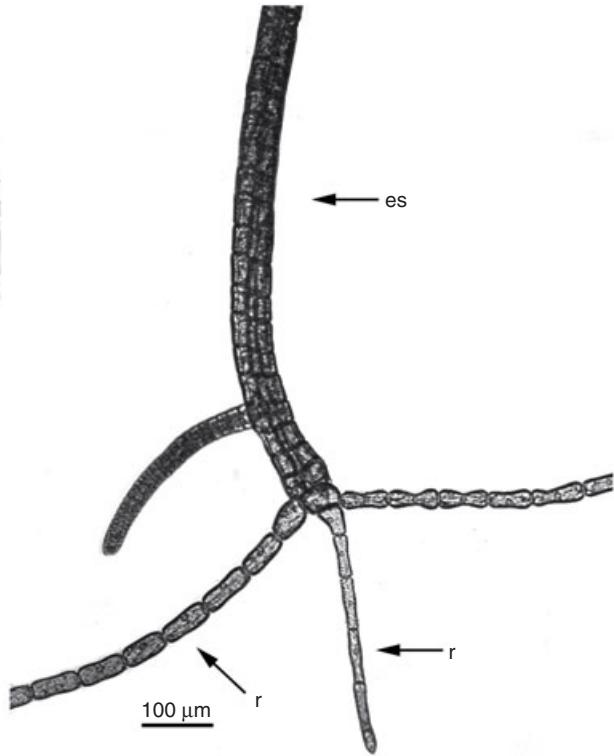
Sabah, Malaysia isolate (4086)

Bostrychia calliptera (Montagne) Montagne, *Caloglossa leprieurii* (Montagne) J. Agardh and *Hypnea* sp. were also present in the collection. The field plant was non-reproductive but in culture formed tetrasporangial stichidia with a characteristic falcate appearance (Fig. 3). The vegetative characters are similar to those of the other isolates.

The gametophytes are always bisexual with either separate stichidia bearing procarps and spermatangia or mixed on the same stichidium (Fig. 6). The male stichidia form tier cells at the fourth axial cell and spermatangial mother cells at the eighth axial segment. Spermatangial sectors are variable in length, 250–1300 µm long and 60–70 µm wide. There is one, sometimes two, procarps per segment scattered along the stichidium and they are four-celled or three-celled. Mature trichogynes are 100–200 µm long. This isolate is self-compatible and forms many globose to subglobose cystocarps, 200–300 µm wide and 350–400 µm long, with a pericarp of conjoined filaments in one layer (Fig. 6). Approximately 20–30 elongate carpospores (100–120 by 30–40 µm) are evident in most cystocarps.



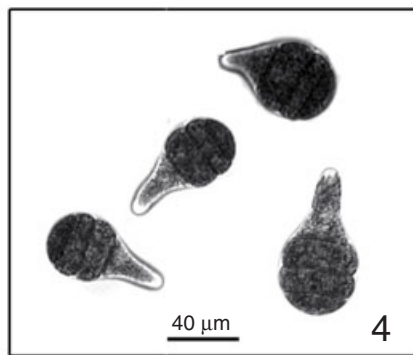
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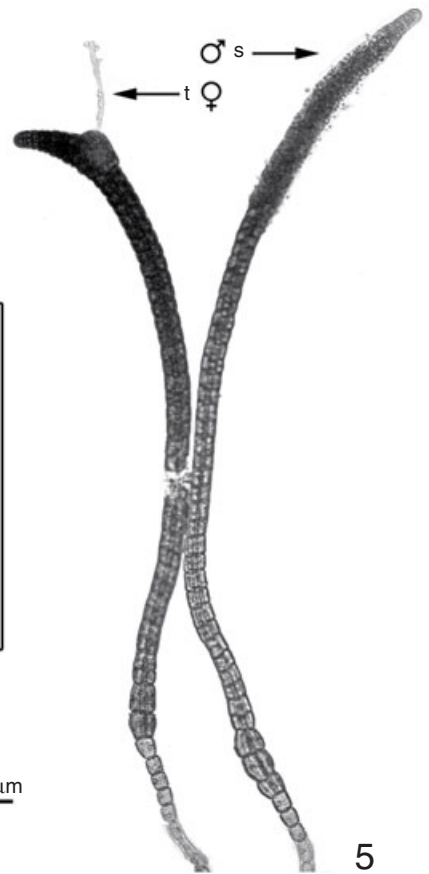
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Figs 1–5. Morphological observations of *Bostrychia radicata* in culture. 1. Isolate 4086. A node with long multicellular rhizoids (r) arising from tier cells, and erect shoots (es) forming from the axial cell of the stolon (s). Formalin fixed specimen stained with aniline blue. 2. Isolate 4458. Elongate rhizoid (r) with erect shoot (es) and secondary rhizoids. 3. Isolate 4178. Characteristic falcately curved tip of a tetrasporangial stichidium seen in all reproductive isolates. 4. Isolate 4178. 2-day old tetrasporelings of 4 cells in each. 5. Isolate 4178. 3-week old tetrasporelings approximately 1.2 mm long. The male tetrasporeling has a spermatangial sector (s) visible along the upper third of the shoot. The female tetrasporeling has a trichogyne (t) with spermatia attached and shows early development of a carposporophyte.

New Caledonia isolate (4178)

In the field samples *B. moritziana* and *Caloglossa vieillardii* were closely associated with this isolate. In culture the 4178 tetrasporophytes and gametophytes are similar to those of 4086. Tetrasporophytes often reach 2–3 cm in length and have characteristic erect shoots in pairs with the recurved upper part bearing a series of tetrasporangia (Fig. 9). The rhizoids are borne below and give rise to secondary nodes (Fig. 9). The tetraspore germlings show the characteristic bipolar spore germination of the Ceramiales (Fig. 4). As young unbranched germlings become 1–2 mm long they are unisexual (Figs 5,10), but when larger many become bisexual like 4086. The spermatangial stichidia and the carposporophytes are similar to those of isolate 4086.

Thailand isolate (4207)

In the field this specimen occurred with *Bostrychia kelanensis* Grunow ex Post on roots of the mangrove *Xylocarpus*. The mature plants of this isolate are smaller overall than those of most other isolates, usually 5–8 mm. Erect shoots are 38–42 μm wide and are borne at variable intervals on the stolons. The four periaxial cells per axial cell form two tier cells at the fifth to sixth axial cell. Secondary pit connections are seen first between tier cells of vertically adjacent axial cells at the 10th axial cell. Nodes are spaced between 0.5 and 3 mm. Initial nodes have just a lateral branch or a branch and rhizoid but older nodes might have up to four branches and three rhizoids. Many rhizoids are long and also produce erect shoots at regular intervals. Rhizoid cells are 23–37 μm wide and 55–75 μm long, each with a single nucleus surrounded by a halo of starch grains.

Mature shoots bear recurved tetrasporangial stichidia 0.2–3.5 mm long and 85–95 μm wide. Either one or two tetrahedral sporangia (Figs 7,8) are present in each segment and measure 55–75 μm wide. Free spores are 55–60 μm wide. On some stichidia adventitious branches develop from the sporangial cover cells.

All tetraspore germlings observed here develop into plants that bear normal tetrasporangial stichidia. Mixed-phase tetrasporophytes are frequently seen with spermatangial sectors produced below the sporangial

sector and these discharge free spermatia (Fig. 8). No female structures are seen in these cultures.

Most sporelings are quite small, 1–2 mm tall with a single unbranched shoot, when they form a slightly recurved terminal tetrasporangial stichidium (Fig. 7). Only one sporangium per segment is seen in the stichidia of sporelings. The stichidia are shorter and have fewer sporangia on sporelings compared with larger plants. Some sporangia produce only two spores and some do not divide completely before discharge (Fig. 7). No male sectors have been seen on these sporelings. This isolate is mixed-phase-asexual but might have the potential to form sexual gametophytes as well.

New South Wales isolate (4271)

The original material was a small plant (<5 mm) mixed with *B. moritziana*, *C. vieillardii*, and *Purpureofilum apyrenoidigerum* J. A. West, Zuccarello et J. D. Scott. This specimen is morphologically similar to the Western Australia isolate and plants in culture reach 50–70 mm length with node structure and spacing on the stolons similar to 3744, although mature axes are larger (70–80 vs 55–65 μm wide).

Reproduction is similar to that of the Thailand isolate with tetraspores giving rise to successive generations of tetrasporophytes. No male sectors are observed on the tetrasporangial stichidia and no female gametophytes are observed among the tetrasporelings.

Molecular phylogeny

The *rbcL* dataset consisted of 1163 characters, 403 of which were parsimony-informative. The evolutionary model least-rejected by the hierarchical likelihood test was: general time reversible model (Lanave *et al.* 1984) (substitution rate matrix: a = 7.64, b = 7.24, c = 3.16, d = 4.64, e = 80.74, f = 1.00) plus proportion of invariable sites set to 0.4419 and a gamma distribution of 0.5749. The MP analysis produced 10 MP trees of 1788 steps. ML analysis produced 2 trees of $-ln L$ score of 9510.84913 (Fig. 11). The ML, NJ and MP trees did not differ in any supported relationships. The detailed analysis of the relationships within the genus have been presented elsewhere (Zuccarello & West 2006); the present analysis did not differ from these

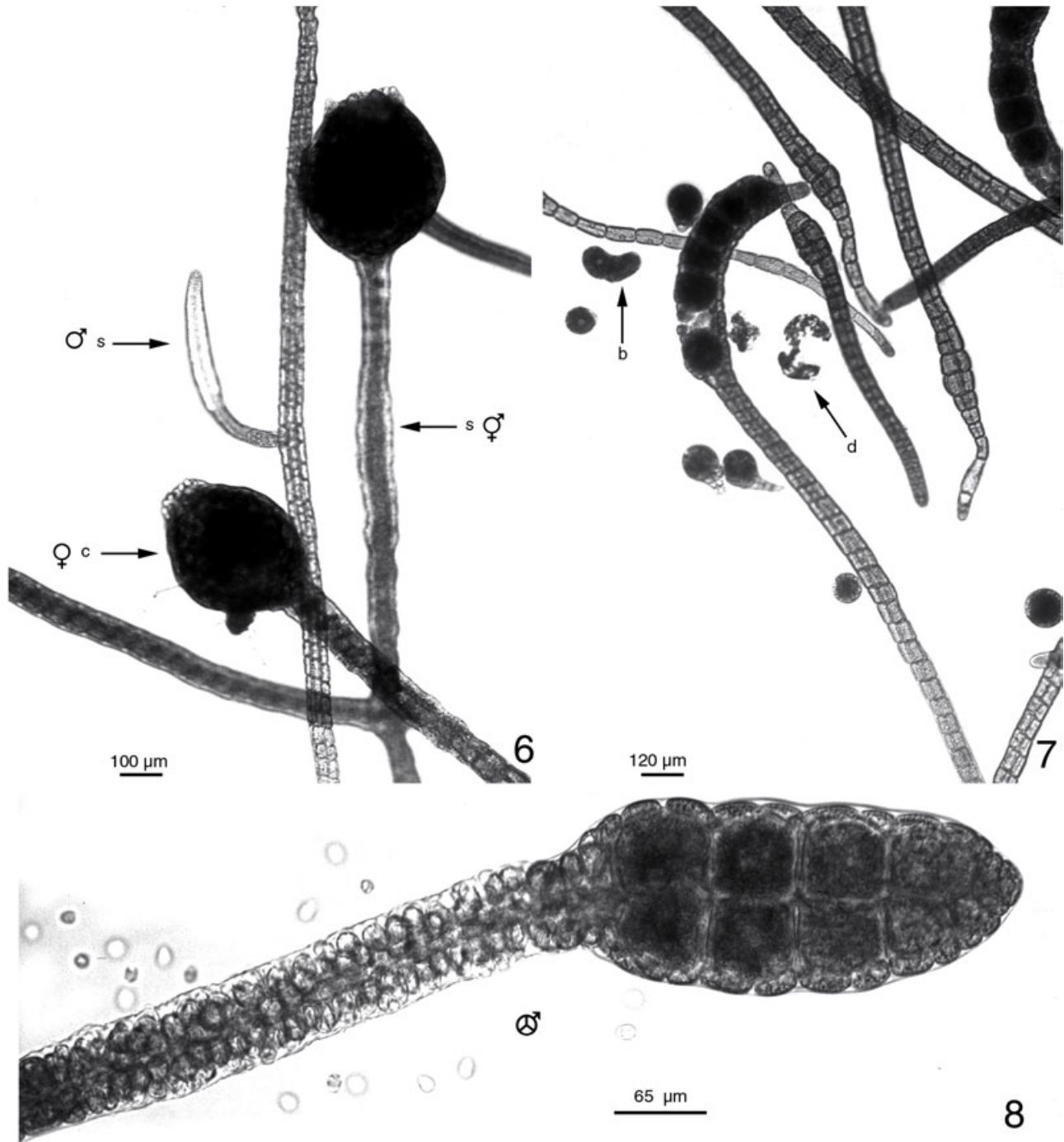


Fig. 6–8. Reproductive development of *Bostrychia radicata* in culture. 6. Isolate 4086. Mature bisexual gametophytes with mature cystocarps (c) and spermatangial sectors (s). A long spermatangial sector is evident below one cystocarp. 7. Isolate 4207. Tetrasporangial stichidia discharging spores. One spore is incompletely divided (b) and two have lysed (d). 8. Isolate 4207. Shoot with tetrasporangial stichidium at tip and spermatangial sector below it actively releasing spermata.

data. Two isolates investigated morphologically (4086 and 4178) nested within the peripherohaptera-containing subgroup with the genus *Bostrychia* (for further details see Zuccarello & West 2006), along with the corticate species *Bostrychia tenella* (Lamouroux) J.

Agardh, *Bostrychia scorpioides* (Hudson) Montagne, and the ecorticate species *Bostrychia tangatensis* E. Post and *Bostrychia simpliciuscula* Harvey ex J. Agardh, although clearly distinct from them ($\geq 95\%$ BP (bootstrap percentages) and posterior probabilities (PP).

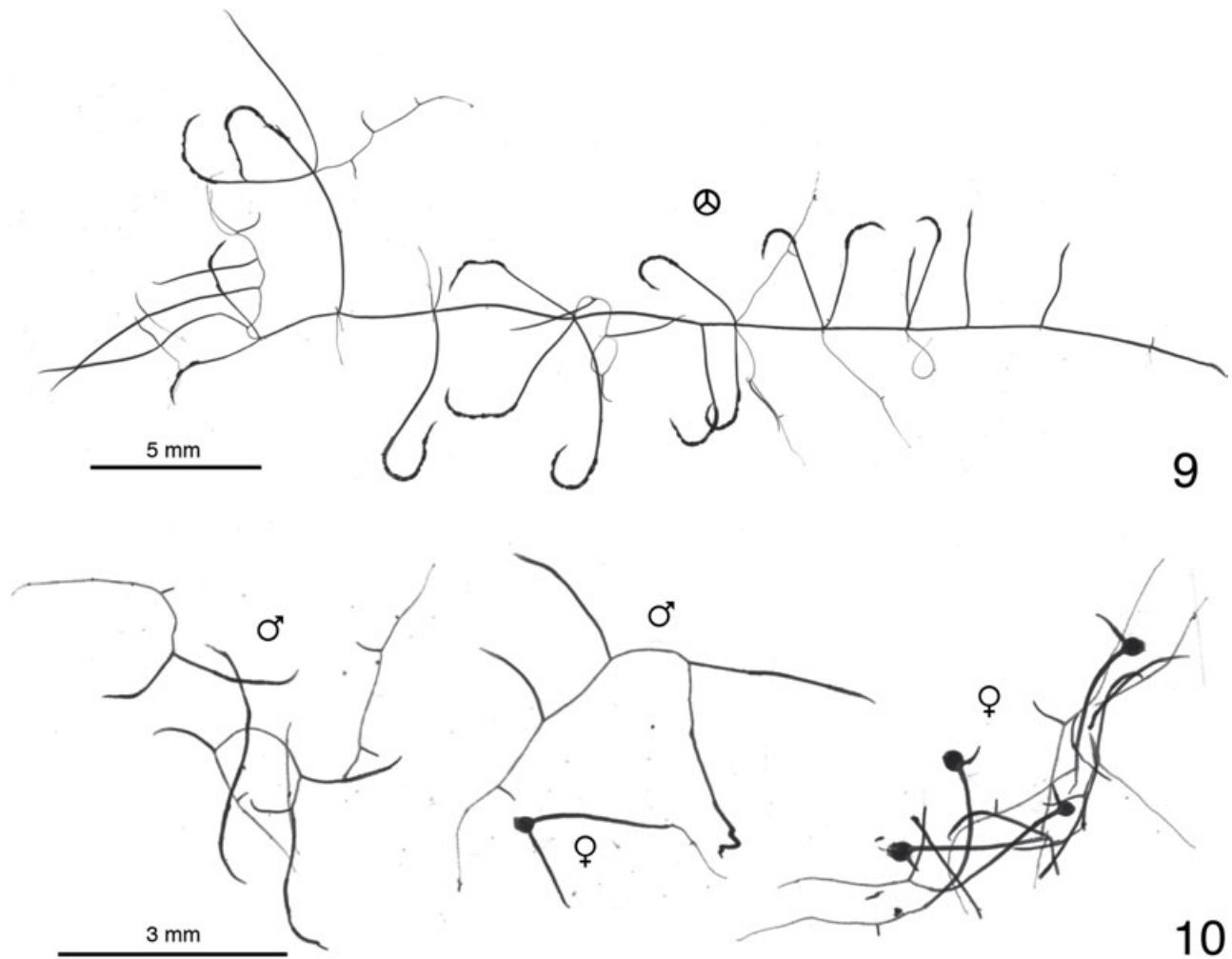


Fig. 9–10. Habit of young plants of *Bostrychia radicata* in culture. Isolate 4178. 9. Habit view of a tetrasporophyte with an elongate stolon bearing pairs of erect shoots opposite rhizoids. Series of tetrasporangia are visible on the recurved upper portion. 10. Isolate 4178. Habit view of young male gametophytes bearing erect spermatangial stichidia and young unbranched female gametophytes with subapical carposporophytes.

The partial sequence of the 26S gene consisted of 787 characters, 236 of which were parsimony-informative. The evolutionary model least-rejected by the hierarchical likelihood test was: TrN (Tamura & Nei 1993) (substitution rate matrix: $a = 1.0$, $b = 2.78$, $c = 1.0$, $d = 1.0$, $e = 4.55$, $f = 1.00$) plus proportion of invariable sites set to 0.356 and a gamma distribution of 0.572. The MP analysis produced 147 MP trees of 699 steps. ML analysis produced 1 tree of $-\ln L$ score of 4632.86186. As a result of the long-branch produced by the sequence of *B. kelanensis* the position of this species is different in the ML topology from the other analysis methods (see Zuccarello & West 2006), so only the LogDet-neighbor joining (NJ) topology is shown (Fig. 12), beside the placement of this species the MP, ML and NJ topologies did not differ significantly. Again the relationships did not differ in sup-

ported relationships from the *rbcl* analysis or from the dataset presented elsewhere (Zuccarello & West 2006). The two isolates investigated morphologically (4086 and 4178) again grouped with the periphero-haptera-containing subgroup of the genus *Bostrychia*, its relationship to other species was not supported, although it was clearly distinct from other species ($\geq 95\%$ BP and PP).

The RuBisCo spacer of five isolates was investigated (3744, 4086, 4178, 4207 and 4458); these sequences did not differ by more than four base pair substitutions (Fig. 13). These RuBisCo spacer sequences were not confidently alignable to other *Bostrychia* species. There does seem to be a grouping of southern hemisphere samples (Madagascar, Malaysia, Western Australia and New Caledonia). Also, the isolates that do not reproduce in culture

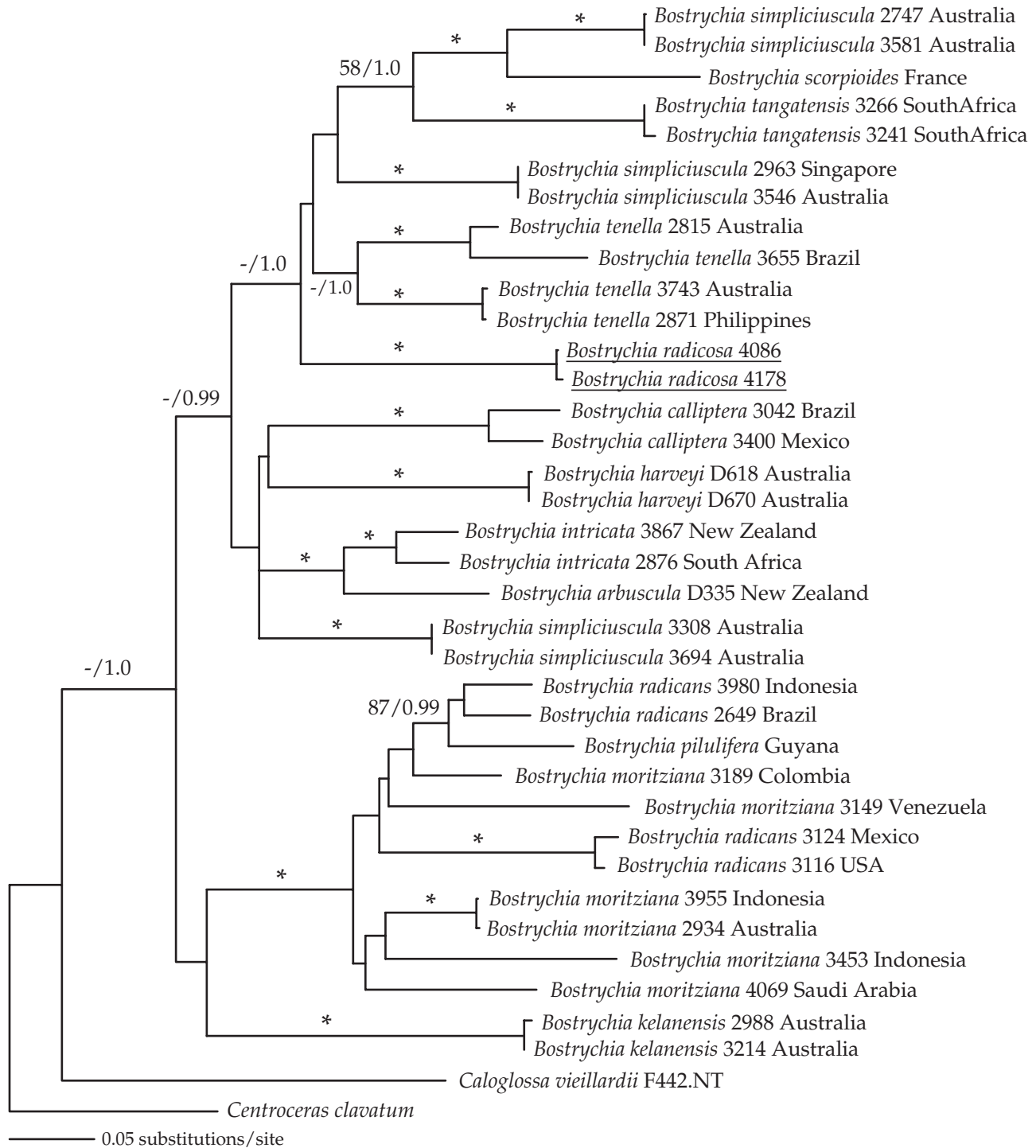


Fig. 11. Maximum-likelihood topology of *rbcL* DNA sequence data of species of *Bostrychia*. * = bootstrap support ≥ 95% and posterior probabilities (PP) ≥ 0.95. Other branch supports listed in the order (bootstrap percentages (BP)/PP). Outgroup used *Centrocercus clavatum* and *Caloglossa vieillardii*. For further information on samples see Zuccarello and West (2006). Isolates of *Bostrychia radicata* are underlined.

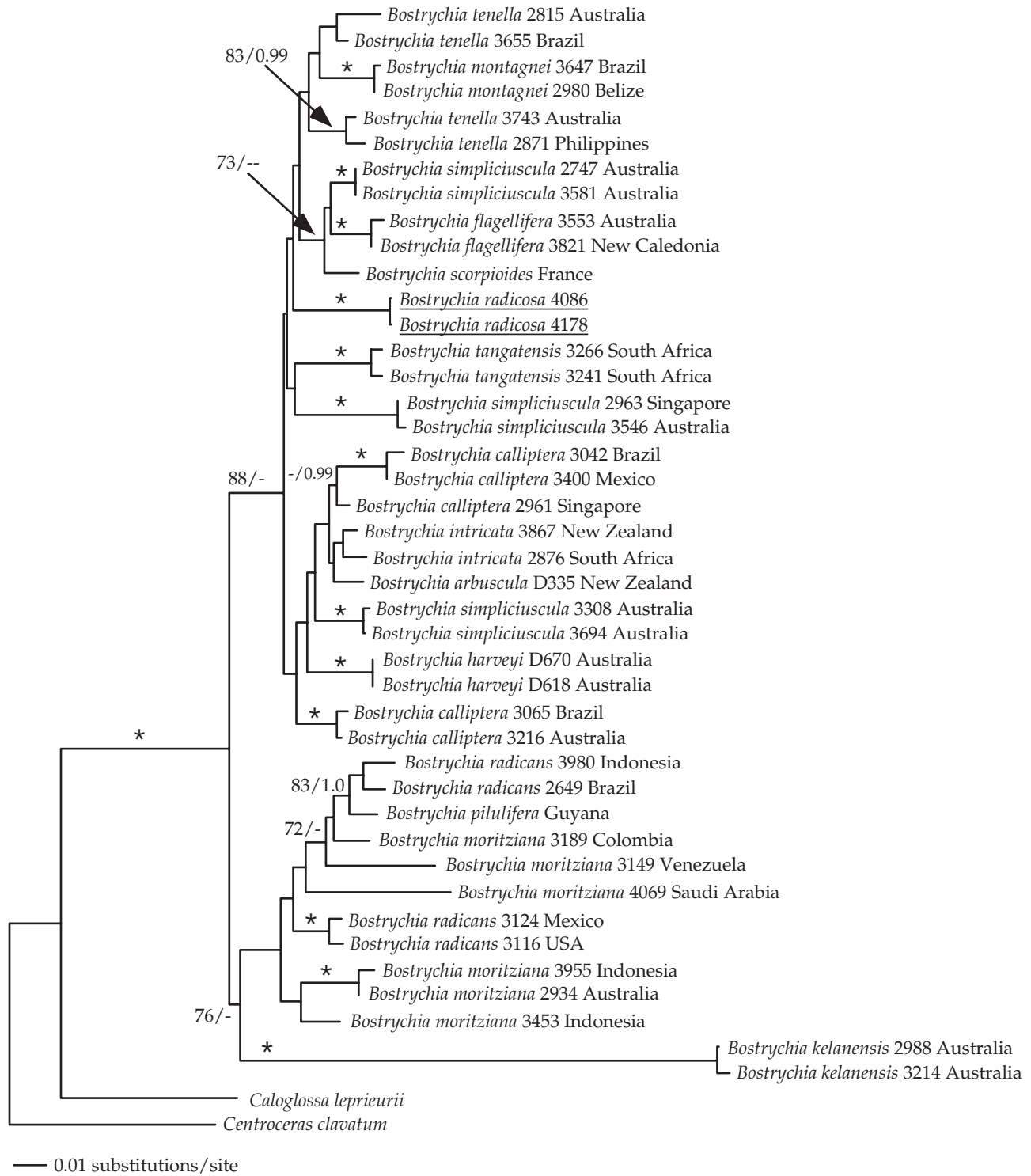


Fig. 12. Neighbor-joining topology (LogDet distances) of partial large-subunit ribosomal DNA sequence data of species within the subfamily Bostrychioideae. * = bootstrap support $\geq 95\%$ for both maximum-parsimony (MP) and neighbor joining (NJ) analysis plus posterior probabilities ≥ 0.95 . Other branch supports listed in the order (BP–MP/BP–NJ/PP), or as specified. The outgroup used was *Centroceras clavatum* and *Caloglossa leprieurii*. For further information on samples see Zuccarello and West (2006). Isolates of *Bostrychia radicata* are underlined.

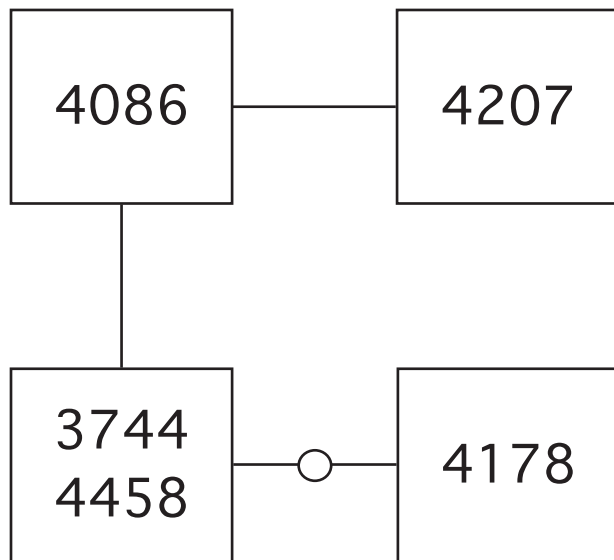


Fig. 13. Haplotype network of the chloroplast RuBisCo spacer sequence data for *Bostrychia radicata*. Straight line indicates one base pair change between sequences. O = missing haplotype in RuBisCo spacer network. Numbers refer to isolates listed in Table 1 and results.

(3744 and 4458) have the same RuBisCo spacer sequence.

Low molecular weight carbohydrates

Sorbitol is present in all strains and ranges from 5.5 to 45.2 mmol/kg DW (Table 1). It is noteworthy that most isolates, except New Caledonia and Madagascar, lack dulcitol. Digeneaside is present in all isolates.

Neotype Designation and Modified Description

Based on the description given by Itono (1985) and the morphology of our cultured isolates we believe they are the same as *R. radicata* and include these in *Bostrychia radicata* *comb. nov.*

Bostrychia radicata (Itono) J. A. West, G. C. Zuccarello et M. H. Hommersand *comb. nov.*

Basionym: *Rhodolachne radicata* Itono (1985) pp. 53–64, figs 1–18.

A neotype is designated and based on culture isolate 4178 from New Caledonia: NSW 714892, Royal Botanic Gardens, Mrs Macquaries Road, Sydney, New South Wales 2000, Australia.

A culture of 4178 is deposited as CCAP 1357/7 with the Culture Collection of Algae and Protozoa, SAMS Research Services, Dunstaffnage Marine Laboratory, Dunbeg, Argyll, PA37 1QA, UK.

GenBank Accession numbers (*rbcL*: DQ087407; LSU: DQ087405; RuBisCo spacer: DQ087408). SEMICOLON

The description below is a composite from the original description by Itono (1985) and our observations on the isolate 4178.

Thalli composed of prostrate indeterminate axes bearing sparse unbranched or once or more branched erect determinate laterals up to 6 mm high and 48–59 μm in diameter; prostrate axes attached to the substratum by separate, rarely branched, multicellular uniseriate rhizoids arising from periaxial cells and bearing one to three (four) erect determinate axes produced endogenously at intervals from a single axial cell; prostrate and erect axes composed of axial cells surrounded by 4 periaxial cells each of which divides transversely into a basal cell that retains the pit connection to the axial cell and an anterior cell (tier cell); periaxial cells in a segment each linked to the tier cells in the segment below by a secondary pit connection and all cells surrounding the axis arranged in four longitudinal rows; ecorticate throughout; fertile segments, like the sterile segments, bearing only four periaxial cells. Procarys formed near the tips of the determinate laterals, one to two per segment, if two then on opposite sides of the axis, consisting of a single 2- to 3-celled sterile group and a 3- or 4-celled carpogonial branch bearing a terminal trichogyne; cystocarps terminal or subterminal, ellipsoid to ovoid (250–400 μm long by 250–350 μm wide) and containing 20–30 carposporangia surrounded by a one layered pericarp. Spermatangia formed in stichidia-like clusters and either terminal on determinate branches or produced in groups irregularly along the axis in bisexual and mixed phase male-tetrasporophytes; spermatangial filaments issuing from each of the periaxial and tier cells in a segment, multiply branched in two to three layers with each surface cell bearing one to three spermatangia covered by a continuous outer cuticular layer. Tetrasporangia tetrahedrally divided, 36–43 μm in diameter, borne in terminal stichidia in longitudinal rows with one sporangium or two adjacent sporangia per segment; each tetrasporangium surrounded by two horizontally divided presporangial cover cells and one horizontally divided postsporangial cover cell.

DISCUSSION

Our morphological, molecular and biochemical results show that the isolates investigated are a new species of *Bostrychia*. It is very similar to *R. radicata* as described by Itono (1985) from mangroves of Fiji and Japan. Unfortunately, the type of this specimen is unavailable for morphological or molecular analysis. When Itono's specimens were transferred to the herbarium collection at Toho University in Japan

(following his death) they were lost (M. Yoshizaki, 2002, pers. comm.). Therefore, a neotype is designated. This species differs from other *Bostrychia* species by long ungrouped hapteral rhizoids and recurved erect shoots.

Rhodolachne radicata was amply described and illustrated by Itono (1985). Although Itono saw some similarities between *R. radicata* and *R. decussata*, the type species of *Rhodolachne* Wynne (1970a), he recognized important differences and suggested that the two species might not belong in the same genus. *R. decussata* is an alga of coral reefs, whereas *R. radicata* is known only from mangroves. More significantly, the tetrasporangia are strictly opposite in *R. decussata*, whereas they are adjacent to one another in *R. radicata*. The similarities between the two that led Itono (1985) to place *R. radicata* in *Rhodolachne* are: (i) transverse division of the pericentral cells into 2, with the lower retaining pit connection with the axial cell; (ii) spiral arrangement of the decussate pairs of the pericentral cells and their derivatives; (iii) exogenous branching of erect branches and endogenous branching of prostrate branches; (iv) radial symmetry of erect branch apices; (v) complete lack of trichoblasts; and (vi) presence of two tetrasporangia per segment in the stichidia-like branches. Characters (i), (iii) and (v) also hold for *Bostrychia*. As already mentioned, character (vi) is different in detail: opposite versus adjacent position of the pairs of tetrasporangia in the stichidia. The central problem has to do with the apparent spiral arrangement of the decussate pairs of the pericentral cells and the radial symmetry of the erect branch apices. Both of these characters require re-examination in *R. decussata*, but whatever the outcome of future studies, the condition in *R. radicata* is not affected. The fact that the periaxial cells and tier cells lie in straight rows is clearly illustrated by Itono, especially in his figure 1. Long branches might be difficult to interpret because they are often curved and might become twisted, but a careful examination of short branches (10–20 segments long) clearly shows that the first periaxial cell is cut off abaxially and slightly to one side, that the second is formed laterally on the same side as the first, that the third is cut off adjacent to the first opposite the second, and that the fourth is formed adaxially on the same side as the third. This is the dorsiventral pattern that is characteristic of *Bostrychia*. It is not as obvious as in some *Bostrychia* species because there are only 4 periaxial cells. The presumed spiral arrangement of the decussate pairs of periaxial cells is not convincing in Itono's drawings and was not seen in our material. Instead, the periaxial cells and associated tier cells are slightly offset during enlargement, probably as a spatial accommodation to crowding. Even the pit connections change their levels as the axial cells elongate. All of this is a

secondary effect seen in many species of *Bostrychia*. Likewise, the overall symmetry is probably dorsiventral and not radial.

Careful examination of numerous axes showed that one to three branches might originate from a single axial cell in a prostrate axis in *B. radicata*. Each of these is connected by a primary pit connection to the anterior half of the elongated axial cell and the branches emerge between the anterior tier cells. This is the cause of the more or less regular opposite branching and the occasional clusters of three erect branches that arise at the same point at intervals along the prostrate axis. Itono (1985) points out that there might be four such branches (see his fig. 14); however, the fourth branch corresponds to the continuation of the original axis and is not truly a branch. Rhizoidal filaments may arise anywhere along an axis, but are most abundant in the vicinity of the erect axes. They always originate from tier cells and are never produced endogenously from axial cells. Such a cell forms a single rhizoid, never two, and the rhizoids grow freely and separately from one another and never cluster to form a hapteron of any type. This is a distinction emphasized by Itono, and one that might be diagnostic for *B. radicata*. Rhizoids were uniseriate, multicellular and very rarely branched in cultured material, but did produce lateral polysiphonous axes from time to time. These were formed from intercalary cells in apparent correspondence to endogenous branching from axial cells.

Itono (1985; figs 4,5) records and illustrates the exogenous branching of erect axes. These figures resemble the exogenous branches seen in other species of *Bostrychia*; however, they were not seen in our material. In contrast, exogenous branches were common at the ends of broken axes in cultured material and might provide a method for regenerating the growth of damaged axes.

Spermatangia, procarps and cystocarps are described here from cultured material for the first time. Although such structures are rarely seen in nature, their form and development is typical for species of *Bostrychia*. Procarps appeared to originate opposite one another from periaxial cells 2 and 3 and never from the first periaxial cell. A single sterile group was present in *B. radicata*, as has been described elsewhere in *Bostrychia*. The carpogonial branches were 3-celled in the sterile bisexual plants of isolate 4086 from Sabah, Malaysia, an uncommon feature in Rhodomelaceae, but one that was recorded earlier by West and Calumpong (1988) in *B. tenella* and in *Bostrychia flagellifera* Post (Kumano 1988). Our observations of tetrasporangial development confirm every detail of the studies of Itono (1985). The tetrasporangia are formed in rows with paired tetrasporangia adjacent to one another and the number and sequence of divisions leading to the for-

mation of cover cells is exactly as Itono describes. Itono (1985) points out that the tetrasporangia are probably associated with particular pericentral cells. Although precise studies are needed, it appears from our observations that periaxial cells 1 and 2 are sterile and that the adjacent tetrasporangia are produced exclusively from periaxial cells 3 and 4.

Rhodolachne decussata, the type species, is morphologically distinct from *B. radicata* (Table 2) for three primary reasons: (i) in *R. decussata* the tier cells are decussately arranged; (ii) the rhizoids are short and unicellular; and (iii) paired tetrasporangia are opposite rather than adjacent. As already mentioned, the tier cells of *B. radicata* are arranged linearly in rows and are not spiral or clearly decussate, a feature common to most isolates of *Bostrychia*, and the rhizoids are multicellular. In the original description of *R. radicata*, Itono (1985) considered the tier cells of *R. radicata* to be decussately arranged; however, this is not clear from his drawings. It is even possible that the decussate arrangement reported in *R. decussata* (Wynne 1970a,b) is a byproduct of the way in which the tier cells enlarge. Furthermore, the coral reef and open coast rocky habitat of *R. decussata* is quite different from the mangrove habitat of *R. radicata*.

To properly determine the generic relationship of *Rhodolachne* and *Bostrychia* it is essential that molecular analyses be done. Unfortunately, this is not possible because the Wynne, Womersley/Bailey and Abbott specimens of *R. decussata* all were preserved in formalin (pers. comm. 2002 with each author) making the DNA very difficult to analyze. Until critical molecular analyses are carried out with specimens of

R. decussata it is not possible to clarify its taxonomic position.

Womersley and Bailey (1970) provide a good description of *R. decussata* from Guadalcanal, Solomon Islands, including the tetrasporangial stichidia, male spermatangial stichidia and carposporophytes. In general, these structures are similar to those of the sexual plants of *B. radicata* (4086, 4178) that we have observed in culture. Abbott (1999) also briefly describes the tetrasporophytes, male and female. She indicates that two to three sporangia were present in each segment rather than two per segment as described by Wynne (1970a,b) and Womersley and Bailey (1970).

Rhodolachne decussata (the type specimens) occurred in the Seychelles on *Lithothamnion* in shallow coral reef (Wynne 1970a,b), and other specimens in the Solomon Islands were on encrusting corallines (probably *Neogoniolithon*) attached to igneous boulders in the mid-eulittoral (Womersley & Bailey 1970). By contrast, all the specimens we have collected for culture occurred in various types of mangroves (*Avicennia*, *Lumnitzera*, *Rhizophora*, *Sonneratia* and *Xylocarpus*). Itono (1985) found *R. radicata* growing on *Rhizophora* prop roots in the Rewa River, Viti Levu, Fiji and on Ishigaki Island, southern Japan. These habitats are common to most *Bostrychia* species.

Reproduction of the *B. radicata* specimens from different areas is variable. Itono found only tetrasporophytes in his Fiji collection. We observed only vegetative plants in all our field material, and in culture two have remained non-reproductive and two have shown successive generations of asexual tetrasporo-

Table 2. Comparison of morphological characters of *Bostrychia radicata*, *Rhodolachne radicata* and *Rhodolachne decussata*

Character	New isolates†	<i>R. radicata</i> ‡	<i>R. decussata</i> §
Rhizoids	Multicellular from tc	Multicellular from tc	Unicellular from tc
Shoots per nodes	up to 4	up to 4	Up to 5
Erect shoots:	Straight to falcate apices	Straight to falcate apices	Straight to falcate apices
Length (maximum)	to 8–10 mm	to 6 mm	To 7 mm
Width	40–75 µm	48–59 µm	50–60 µm
Branching from stolon	Endogenous from ac and r	Endogenous from ac and r	Endogenous from ac
Periaxial tier cells	4 pa, each with 2 tc	4 pa, each with 2 tc	4 pa, each with 2 tc NOTE CHANGES
Arrangement of tier cells	Linear, partially decussate	Linear, decussate?	Decussate
Tetrasporangial stichidia	1–2 adjacent tetrahedral tsp/segment, 55–75 µm wide	2 adjacent tetrahedral tsp/segment. 36–43 µm wide	2 opposite tetrahedral tsp/segment. 65–100 µm wide
Cystocarps	200–350 µm wide	not seen	200–300 µm wide
Life history	Sexual in some isolates, asexual and vegetative in others	only tetrasporophytes known	Possible sexual life history
Habitat	Mangroves	Mangroves	On coralline red algae, coral and igneous rock, open coasts

ac, axial cello; pa, periaxial cell; r, rhizoid; tc, tier cello; tsp, tetrasporangia.

†Composite data for all isolates (the present paper).

‡Data from Itono (1985).

§Data from Wynne (1970a,b) and Womersley and Bailey (1970).

phytes. One of these has mixed-phase tetrasporophytes with male sectors that release spermatangia, as seen in other *Bostrychia* species (e.g. *B. tenella*, West & Calumpong 1988). Two isolates have shown *Polysiphonia*-type sexual life histories with unisexual and bisexual gametophytes. We have observed other mangrove-associated red algae, *Caloglossa vieillardii* and *B. moritziana* that also have asexual populations and sexual populations in different regions (West & Zuccarello 1999, West *et al.* 2001). It might be that the mangrove habitats with their extreme environmental conditions are suitable to the asexual reproductive mode.

Molecular analysis clearly shows that all isolates belong to one species and that this species is distinct from other *Bostrychia* species. *RbcL* and *26S* analyses place *B. radicata* in the peripherohaptera clade. This indicates that peripherohaptera (haptera derived laterally from tier cells) can be fused (hapteral rhizoids in clusters, in most other *Bostrychia* species, King & Paddock 1989) and unfused (hapteral rhizoids separate, *B. radicata*). RuBisCo spacer analysis of five isolates also indicates that all isolates are probably one species. Four base pair substitutions within the RuBisCo spacer is approximately average for a red algal species (reproductively compatible units) (Zuccarello *et al.* 2002; Kamiya *et al.* 2003), although exceptions occur; that is, reproductively isolated species with less than four base pair substitutions in the RuBisCo spacer (Zuccarello & West 2003). Unfortunately, most isolates of *B. radicata* are not sexual so reproductive compatibility studies are not possible.

The low molecular weight carbohydrate data proved interesting. Digeneaside, a compound typical in most Ceramiales (Lobban & Harrison 1994), occurred in all isolates. No dulcitol was found in most isolates tested and only very low quantities in the New Caledonia and Madagascar isolates. The presence of only sorbitol has been noted in other *Bostrychia* species (Karsten *et al.* 1993; 1995b). In an earlier study, digeneaside was observed in most *Bostrychia* and all closely related *Stictosiphonia* species (Karsten *et al.* 1995a). In addition, some primitive red algae such as *Chroodactylon*, *Rhodorus* and *Stylonema* of the Stylonematales and *Rhodochaete* of the Erythropeltiales in the subclass Bangiophycidae (Karsten *et al.* 2003) contain this heteroside. Although digeneaside is more widely distributed among red algae and, hence, has a limited value as a chemical marker, the presence of sorbitol is restricted to few red algal taxa, and is thought to provide important taxonomic information.

Bostrychia radicata might be found to be quite widely distributed as further field collections are made around the Indo-Pacific, but it is necessary to verify each specimen through careful laboratory culture work and molecular analyses.

ACKNOWLEDGMENTS

We thank Mike Wynne for pointing out the similarity of *Rhodolachne radicata* to *Bostrychia*, for the loan of the type of *R. decussata* and for a photocopy of the Itono paper. Marie West, Susan Loiseaux de Goër, Rosario Braga and Tim Moulton helped in several of the field collections. This research is partially supported by a grant from the Australian Biological Resources Study to JAW & GCZ for 2002–2005, as well as by a grant from the Deutsche Forschungsgemeinschaft (DFG) to UK (Ka 899/8-1/2).

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