Bostrychia australiana from Australia, a new species segregated from *B. kelanensis* Grunow ex Post (*Rhodomelaceae, Rhodophyta*)

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The genus *Bostrychia* Montagne, 1842 has been studied extensively (e.g. Post 1936, King & Puttock 1989, Zuccarello & West 2011), yet many questions remain. Numerous taxonomic changes have been proposed since the comprehensive monograph of *Bostrychia* and *Stictosiphonia* by King & Puttock (1989), mainly due to the increased usage of gene sequences to understand diversity. A molecular baseline of the overall phylogeny of *Bostrychia* was provided by Zuccarello & West (2006), who recommended including the genus *Stictosiphonia* J.D.Hooker & Harvey (1847) in *Bostrychia* Montagne, *nom. cons*.

Careful observations of *Bostrychia*, especially in unialgal culture, have led to the description of several morphologically distinct new species such as *B. radicosa* (Itono) J.A.West, Zuccarello & Hommersand (West & al. 2006); *B. anomala* J.A.West, Loiseaux & Zuccarello (West & al. 2013), or reinstated previously synonymised species such as *B. binderi* Harvey (Zuccarello & al. 2015) and *B. tenuissima* R.J.King & Puttock (Zuccarello & al. 2018) based on morphological characters that match evolutionary lineages.

Genetic studies of morphospecies within the genus have found that many morphological species are composed of multiple cryptic species (Zuccarello & West 2003, 2006; Muangmai & al. 2021). Some of these cryptic-species complexes are monophyletic, such as *B. intricata* and *B. radicans/B. moritziana*, but others are not. In non-monophyletic cryptic (or pseudo-cryptic) species complexes species are more closely related to species of different morphologies, indicating that a degree of convergent evolution has occurred. This is the case for an entity known as "*Bostrychia simpliciuscula* Harvey ex J.Agardh" in which the morphology has converged in four different clades, and this led to the recognition of four named species that are not entirely distinguishable without molecular data (Zuccarello & al. 2018).

In other cryptic species complexes individual lineages have not always been named (Zuccarello & West 2002, 2003; Muangmai & al. 2014). Within the *B. radicans/B. moritziana* species complex, which contains at least seven lineages (Zuccarello & West 2003), species have not been named, due to the similarity or plasticity of morphological characters, except for a lineage with an unusual morphology that was described as a new species (West & al. 2013). In *Bostrychia intricata* (Bory) Montagne there are multiple cryptic species, some with very localized distributions, but some that are more widely distributed (Fraser & al. 2013; Muangmai & al. 2015b, 2022), and these cryptic species have also not been named. Tabulation of species is critical for measures of geographical diversity (e.g. indices of alpha diversity), but also in relation to the endangered status of species, the naming of cryptic species is important (e.g. Delić & al. 2017).

The distribution of these cryptic species complexes is not random geographically at both large (e.g. Zuccarello & West 2003; Zuccarello & al. 2006; Muangmai & al. 2015b) and small scales (e.g.



Muangmai & al. 2016), and in many areas two or more cryptic species can be sympatric (Zuccarello & al. 2003, 2006, 2011). These cryptic species are also known to differ physiologically, while retaining indistinguishable morphologies (e.g. Karsten & al. 1994; Muangmai & al. 2015a) and differing chemically (e.g. Orfanoudaki & al. 2020). It may be important to recognize these species, and their distribution, for example, in studies examining responses to climate changes of specific morphospecies.

Bostrychia kelanensis Grunow ex Post is an ecorticate, alternately branched species distinct in having more than two pericentral cell rows longitudinally along the axial cells. This character of pericentral cell numbers previously placed *B. kelanensis* in the genus *Stictosiphonia*, later subsumed into Bostrychia when it was shown to be a character that did not produce monophyletic groupings (Zuccarello & West 2006). Cladohaptera, specialized attachment structures subterminal on lateral branches, are reported in *B. kelanensis*, and this makes it distinct from other species previously placed in *Stictosiphonia* (King & Puttock 1989). This is a characteristic of species such as B. radicans and B. moritziana (Sonder ex Kützing) J.Agardh. Bostrychia kelanensis is profusely branched in field and culture isolates. Peripherohaptera develop from multiple pericentral cells along main axes and branches and occur in B. tenella, B. binderi, B. scorpioides and other species, but not in B. kelanensis. Field specimens can be sparsely branched, and this feature was used by Tseng (1943) to describe two formae ("Bostrychia kelanensis f. typica" C.K.Tseng nom. inval. and Bostrychia kelanensis f. elegans C.K.Tseng), referring to less or more branched thalli, but Tseng did not appreciate the variation in branching in different environmental conditions. These formae were not accepted taxonomically by King & Puttock (1989: 49) who concluded that this "...variation is normal within populations and does not warrant recognition." Bostrvchia kelanensis often produces a rosette of branch shoots emanating from a thick stipe arising from a robust basal disc, also regularly seen in culture. Attached rosettes are also common in cultures of B. moritziana, B. radicans and others and they attach well to the substrate. Molecular analysis indicates that B. kelanensis is sister to cladohaptera-forming species of Bostrychia, such as B. radicans (Montagne) Montagne, and B. pilulifera Montagne, although this relationship is not well supported (Zuccarello & West 2006). Distribution of B. kelanensis is essentially tropical and subtropical (Guiry & Guiry 2022), with recent reports from Micronesia (West & al. 2013), Thailand (Seangkaew & al. 2016), and India (Ganesan & al. 2018).

Our culture collections identified as *Bostrychia kelanensis* indicate that we have isolates that are highly divergent from one another and suggest that a new species should be recognised. We formally describe this new species below. Samples were collected from the field and transported alive for unialgal cultures or dried in the field for DNA analysis. Culture methods follow West (2005). Samples shown in Table 1 with JAW culture number or field-collection designation. DNA extraction, PCR, sequencing, chromatogram editing and alignment followed Zuccarello & D'Archino (2022). We used three sets of markers: *rbc*L (primers–F8, F753, R753, RbcS-start, Freshwater & Rueness 1994, Wang & al. 2000); COI (primers–GazF1 and GazR1, Saunders 2005), and the RuBisCo spacer (primers–rbcF1, rbcR2, Kamiya & al. 1999). Maximum-likelihood (ML) analyses used IQ-TREE 2.2.0 (Minh & al. 2020) following procedures as described by Zuccarello & D'Archino (2022). Haplotype networks were produced in PopART v1.7 (University of Otago, available from http://popart.otago.ac.nz) following procedures in Zuccarello & D'Archino (2022).

Our *rbc*L dataset of the genus *Bostrychia* was 1161 bp in length and produced a ML phylogeny matching previous studies (Zuccarello & West 2006). Samples identified as *B. kelanensis* were again sister to other species with cladohaptera (e.g. *B. radicans, B. moritziana*), but without bootstrap support (64%) (Fig. 1 A). Within "*B. kelanensis*" there were two clear and supported groups, the first



clade containing some samples from eastern and northern Australia (e.g., JAW 2988, 3350), and other parts of the world (e.g. Micronesia). The second lineage contained isolate JAW3810 from Western Australia (Table 1, Fig. 1 A). Phylogenetic analysis of a smaller fragment of rbcL (690 bp alignment, 18 samples) showed that most isolates grouped in the first group, including samples from New Ireland, Papua New Guinea (close to the type locality of *B. kelanensis*) but also including samples from Thailand, Japan, and India, and the second group, containing isolate JAW 3810, also included another sample from Australia (JAW 3748 from the Northern Territory). This latter clade is well supported (Fig. 1 B). The cytochrome oxidase subunit 1 (COI) alignment was 660 bp long and produced a similar phylogeny, with isolate 3810 distinct from the other isolates (Fig. 2). There was variation within the larger group of samples with a mean of 20bp (3%) within the group and between this group and isolate 3810 there were 97 bp (14.7%) differences. The RuBisCo spacer haplotype network (32 sequences, 312 bp alignment) also indicated that isolates JAW 3810 and 3748 were different from most of the B. kelanensis samples by 19 base pairs. There was also a field sample from NSW, Australia (E479) that was also different from most samples by 19bp and different from the JAW 3810/3748 by 15 bp (Fig. 3.). A ML phylogeny of the RuBisCo spacer sequences also indicated that these three samples were different form the remainder of the *B. kelanensis* samples (phylogeny not shown).

Comparisons of reproduction and patterns of longitudinal pericentral cells (also called tier cells) per axial cell formation: *Bostrychia kelanensis* cultures (3075, 3348 and 3350) showed the general habit and details of the species. JAW 3075 showed a normal basal disc (Fig. 4 A, arrow) and had numerous elongate axial shoots with alternate bilateral branching. The disc had a coalescent complex of branched uniseriate filaments adherent to the substrate and secondary shoots arose from the main stipe complex (Fig. 4 B).

To distinguish patterns of pericentral cell formation it was necessary to place filaments in distilled water and treat with 10 s of 800-W microwave to shrink cells before arranging filaments on microscope slides to view pericentral and axial cells clearly. In Fig. 4 C, the most frequent pattern was 3 longitudinal pericentral cells along each axial cell of a filament (see vertical bar on left side of one axial cell). In Fig. 4 D, an atypical pattern was seen along a main shoot of successive longitudinal 3 longitudinal pericentral cells per axial cell, 2 longitudinal pericentral cells per axial cell. In Fig. 4 E, a branch junction showed a node with 2 longitudinal pericentral cells per axial cell above and below the branch point, and 3 longitudinal pericentral cells per axial cell further down. In Fig. 4 F, the apex showed pericentral cell formation in the first 4 axial cells. Just below the branch node three longitudinal pericentrals per axial cell. There were 5 pericentrals around each axial cell in mature filaments.

Two isolates JAW 3810 and JAW 3748 from field and culture were selected for the best vegetative and reproductive examples of the new species for comparison with *B. kelanensis* shown above.

In isolate JAW 3810 tetrasporophytes had three longitudinal pericentrals per axial cell and 5 pericentrals around each axial cells. A 4-months old female specimen (Fig. 5 A), from a tetraspore germling, had typical alternate lateral branching and lacked cladohaptera but was attached by a well-developed basal disc (not seen in Fig. 5A). A male specimen (Fig. 5 B) had well developed spermatangial stichidia with 4 longitudinal pericentral cells per axial cell in basal section and 3 longitudinal pericentral cells per axial cell in upper sections and showed well developed spermatangia releasing spermatia. In Fig. 5 C, the vegetative lower axis had 4 longitudinal pericentral cells per axial cell in the upper vegetative section. Both figures show an expanded extracellular matrix following microwave treatment. Females had

conspicuous procarps with persistent trichogynes extending from the apex to lower lateral branches and intercalary regions of main axes (Figs 5 D & E). A self-cross of JAW 3810 yielded limited carposporophytes that released few carpospores (Fig. 5 F).

A rehydrated field specimen from which culture JAW 3748 (Fig. 6 A-H) was isolated showed a female thallus adherent on the mangrove prop root bark had many cystocarps in various developmental stages (Fig. 6 A). A carefully removed basal disc shows erect branched axes and parts of horizontal stolons developed from the disc (Fig. 6 B, arrowheads). The female isolated in culture formed many procarps at the apices and they remained visible as persistent trichogynes along the main branch axes (Fig. 6 C). The cystocarps of field specimens produced numerous carposporangia and released carpospores through an apical ostiole (Fig. 6 D). Field specimens of the female developed visible procarps subapically (Fig. 6E). The pericentral cell formation pattern in the intercalary parts of axes consistently formed four longitudinal pericentral cells per axial cell and five pericentrals around each axial cell (Fig. 6 E). At branch nodes the first axial cell above node had three or four longitudinal pericentral cells per axial cell and five pericentrals around each axial cell. All axial cells below the node had four longitudinal pericentral cells per axial cell and five pericentrals around each axial cell (Fig. 6 F). In cultured specimens, intercalary sections occasionally had six longitudinal pericentral cells per axial cell, which is surprising but stable (Fig. 6 G). At the nodes of branches the first axial cell above a node had three longitudinal pericentral cells per axial cell and the first axial cell below the node had four longitudinal pericentral cells per axial cell (Fig. 6 H).

Our results clearly show that at least two clades of "*B. kelanensis*" differ substantially, one clade containing most of the samples and all samples collected outside Australia. The second clade includes JAW 3810 and 3748 from Australia. We propose that these latter samples, exclusive to Australia, be formally described as a species new to science as follows.

Bostrychia australiana J.A.West, Loiseaux-de Goër & Zuccarello, sp. nov.

- Description: Thallus of prostrate and suberect indeterminate axes, slender, turf forming; attachment often stipitate; axes 25 mm long, unbranched or with one order of determinate laterals; 3-4 tiers of pericentral cells per axial cell, 5-6 pericentral cells around the indeterminate axes, essentially ecorticate throughout, with the exception of irregular cortication of the stipe; cladohaptera not seen; carpogonial branches borne within the apical or intercalary axial cells; Cystocarps small, with few spores in culture. Spermatangial stichidia as typical of the genus.
- Holotype: Cossack, Western Australia, 24°53'S. 117°11'E, 9 xii 1997, on a mangrove (*Avicennia* sp.), dried specimen from culture, JAW 3810 (female gametophyte), UC 2050473. Holotype (JAW3810)

Accession Numbers: *rbc*L: OQ291113; COI: OQ291130; Rubisco Spacer: OQ297673.

Paratype: Mandorah, Northern Territory, Australia, 12°26'S 130°46'E, 21 vi 1997, on a mangrove (*Rhizophora* sp.), dried specimen from culture JAW 3748, UC 2050596.
Physic Park Registration: 102500

PhycoBank Registration: 103599.

- Crosses attempted with a shaker (70 rev min⁻¹) for 4 months of a self-cross (JAW 3810 male x JAW 3810 female) showed spermatia on about 10% of trichogynes, but no pseudocystocarps (i.e. enlarged pericarp but no sporangial development) were produced and only one cystocarp (Fig. 5 F) developed. This cystocarp developed about 10 carpospores that released but did not germinate. A cross between two different isolates (JAW 3810 male x JAW 3348 female) produced over 10 pseudocystocarps but no viable cystocarp. A cross between a JAW 3810 male and a JAW 4261 female produced no pseudocystocarps or cystocarps.
- Reproduction in culture: Reproduction in culture was variable in *B. kelanensis*. Some isolates produced no viable tetraspores (JAW 3075, 3076); Isolate JAW 3349 released tetraspores but

sporelings did not develop; Some isolates released tetraspores and produced male and female gametophytes (JAW 3000, 3860, 4589, 4632, 4635), males often died, but occasionally a surviving female switched to male; Some released tetraspores and only produced female gametophytes (JAW 3213, 3214, 3348, 4261). *B. australiana*: JAW 3810 also released tetraspores that produced male and female gametophytes. JAW 3748 was a female (derived from a tetrasporophyte) that was rarely bisexual, but carposporophytes were never produced. This unusual reproduction in culture is found in some other isolates of *Bostrychia*. For example, *Bostrychia tenella* isolates have mixed-phase patterns (see West & Calumpong 1988) and some *B. moritziana* isolates recycle tetrasporophytes (see West & al. 1992, West & Zuccarello 1999). *Bostrychia radicosa* recycles tetrasporophytes and has mixed-phase reproduction, either males or females on tetrasporophytes (see West & al. 2006). Many other isolates of *Bostrychia* have regular *Polysiphonia*-type sexual cycles. What leads to these unusual patterns and how they are manifested in the field, if they are, needs further investigation.

Note: While cladohaptera are said to be less common in *Bostrychia kelanensis* (e.g. King & Puttock 1989), none were observed in field or culture specimens of this species, and basal discs were the only attachment structures seen. Cladohaptera was also lacking in *B. australiana*.

In conclusion, *Bostrychia australiana sp. nov.* had a different and less variable pattern of longitudinal pericentral cell development (3-4 longitudinal pericentral cells per axial cell) compared to *B. kelanensis* (3-5 longitudinal pericentral cells per axial cell). Cladohaptera are absent in our field and culture specimens, even though they are stated to be a key character of *B. kelanensis* by King & Puttock (1989). While *B. australiana* shows less variability than *B. kelanensis*, the morphological characters of the two species overlap and *B. australiana* can be considered a true cryptic species. The naming of cryptic species is important for cataloguing diversity in various areas. While this is easily tractable in cryptic species complexes with only a few species, as in "*B. kelanensis*", this may be more onerous in species complexes with many cryptic species (Payo & al. 2013, Vieira & al. 2014, Muangmai & al. 2022). The problem of naming is exacerbated when cryptic species overlap in distribution: *B. kelanensis* and *B. australiana* are found in close proximity around Darwin (Northern Territory). Only sequencing of types will clarify the correct name assignment and lead to naming the new species (Hughey & al. 2001). To date, *B. australiana* has been found only in Australia, but further sampling (e.g. neighbouring Indonesian islands) may reveal it to be more widespread, although we did not find it in Papua New Guinea (unpubl. data).

- Delić, T., Trontelj, P., Rendoš, M. & Fišer, C. (2017). The importance of naming cryptic species and the conservation of endemic subterranean amphipods. *Scientific Reports* 7: 1–12.
- Fraser, C. I., Zuccarello, G. C., Spencer, H. G., Salvatore, L. C., Garcia, G. R. & 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.
- Freshwater, D. W. & Rueness, J. (1994). Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* 33: 187–194.
- Ganesan, E.K., West, J.A. & Necchi, O., Jr. (2018). A catalogue and bibliography of non-marine (freshwater and estuarine) Rhodophyta (red algae) of India. *Phytotaxa* 364: 1–48.
- Guiry, M.D. & Guiry, G.M. 2022. AlgaeBase. World-wide electronic publication, University of Galway. https://www.algaebase.org; searched on 18 December 2022.
- Kamiya, M., Tanaka, J., King, R. J., West, J. A., Zuccarello, G. C. & Kawai, H. (1999). Reproductive and genetic distinction between broad and narrow entities of *Caloglossa continua* (Delesseriaceae, Rhodophyta). *Phycologia*. 38: 356–67.

- Karsten, U., West, J. A., Zuccarello, G. C. & Kirst, G. (1994). Physiological ecotypes in the marine red alga *Bostrychia radicans* (Ceramiales, Rhodophyta) from the east coast of the USA. *Journal of Phycology* 30: 174-182.
- King, R. J. & Puttock, C. F. (1989). Morphology and taxonomy of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae/Rhodophyta). *Australian Systematic Botany* 2: 1-73.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Haeseler, A. von & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534.
- Muangmai, N., West, J. A. & Zuccarello, G. C. (2014). Evolution of four Southern Hemisphere Bostrychia (Rhodomelaceae, Rhodophyta) species: phylogeny, species delimitation and divergence times. *Phycologia* 53: 593–601.
- Muangmai, N., Preuss, M. & Zuccarello, G. C. (2015a). Comparative physiological studies on the growth of cryptic species of *Bostrychia intricata* (Rhodomelaceae, Rhodophyta) in various salinity and temperature conditions. *Phycological Research*. 63: 300–306.
- Muangmai, N., Fraser, C. & Zuccarello, G. C. (2015b). Contrasting patterns of population structure and demographic history in cryptic species of *Bostrychia intricata* (Rhodomelaceae, Rhodophyta) from New Zealand. *Journal of Phycology* 51: 574–585.
- Muangmai, N., Ammon, U. von, & Zuccarello, G. C. (2016). Cryptic species in sympatry: nonrandom small-scale distribution patterns in *Bostrychia intricata* (Ceramiales, Rhodophyta). *Phycologia* 55: 424-430.
- Muangmai, N., Preuss, M., West, J. A. & Zuccarello, G. C. (2022). Cryptic diversity and phylogeographic patterns of the *Bostrychia intricata* species complex (Rhodomelaceae, Rhodophyta) along the coast of southeastern Australia. *Phycologia* 61: 27–36.
- Orfanoudaki, M., Hartmann, A., Ngoc, H.N., Gelbrich, T., West, J., Karsten, U. & Ganzera, M. (2020). Mycosporine-like amino acids, brominated and sulphated phenols: Suitable chemotaxonomic markers for the reassessment of classification of *Bostrychia calliptera* (Ceramiales, Rhodophyta). *Phytochemistry* 174:112344.
- Payo, D.A., Leliaert, F., Verbruggen, H., D'hondt, S., Calumpong, H.P. & DE Clerck, O. 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. *Proceeding of the Royal Society, B Biological Sciences* 280: 20122660.
- Post, E. (1936.) Systematische und pflanzengeographische Notizen zur *Bostrychia-Caloglossa*-Assoziation. *Revue Algologique* 9: 1–84.
- Saunders, G.W. (2005). Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society* B 360: 1879–1888.
- Seangkaew, J., Bovonsombut, S. & Peerapornpisal Y. (2016). Species diversity and distribution of mangrove-associated red alga *Bostrychia* (Rhodomelaceae, Rhodophyta) from southern Thailand. *International Journal of Applied Environmental Studies*. 11: 55–71.
- Vieira, C., D'hondt, S., Clerck, O. D. & Payri, C. E. (2014). Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *Journal of Phycology*. 50: 1101–1119.
- Wang, H. W., Kawaguchi, S., Horiguchi, T. & Masuda, M. (2000). Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and *rbcL* sequences. *Phycologia* 39: 228–237.
- West, J. A. (2005). Long Term Macroalgal Culture Maintenance, Chapter 11, pp. 157-163, (ed.) R. A. Andersen, *Algal Culturing Techniques* Academic Press, New York.
- West, J. A. & Calumpong, H. P. (1988). Mixed-phase reproduction of *Bostrychia* (Ceramiales, Rhodophyta) in culture. I. *B. tenella* (Lamouroux) J. Agardh. *Japanese Journal of Phycology*. 36: 292–310.

- West, J. A., Zuccarello, G. C. & Calumpong, H. P. (1992). *Bostrychia bispora* sp nov. (Rhodomelaceae, Rhodophyta), an apomictic species from Darwin, Australia: reproduction and development in culture. *Phycologia* 31: 37–52.
- West, John A. & Zuccarello, G. C. (1999). Biogeography of sexual and asexual reproduction in *Bostrychia moritiziana* (Rhodomelaceae, Rhodophyta). *Phycological Research* 47: 115-123.
- West, J. A., Zuccarello, G. C., Hommersand, M., Karsten, U. & Görs, S. (2006). Observations on *Bostrychia radicosa* comb. nov. (Rhodomelaceae, Rhodophyta). *Phycological Research* 54:1–14.
- West, J. A., Loiseaux de Goër, S. & Zuccarello, G. C. (2013). Monosiphonous growth and cell-death in an unusual *Bostrychia* (Rhodomelaceae, Rhodophyta): *B. anomala* sp. nov. *Algae*. 28: 161–171.
- Zuccarello, G. C. & D'Archino R. (2022). Genetic diversity of sympatric *Schizymenia dubyi* and *S. apoda* (Schizymeniaceae, Rhodophyta) in Wellington Harbour, New Zealand. *Phycologia* **61**: 549–557.
- Zuccarello, G. C., Muangmai, N., Preuss, M., Sanchez, L., Loiseaux de Goër, S. and West, J.A. (2015). *Bostrychia tenella* species complex: morphospecies and genetic cryptic species with resurrection of *B. binderi*. *Phycologia* 54: 261–270.
- Zuccarello, G. C. & West, J.A. 2002. Phylogeography of the *Bostrychia calliptera/B. pinnata* complex (Rhodomelaceae, Rhodophyta) and divergence rates based on nuclear, mitochondrial and plastid DNA markers. *Phycologia* 41: 49–60.
- Zuccarello, G. C. & 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. *Journal of Phycology* 39: 948–959.
- Zuccarello, G. C. & 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.
- Zuccarello, G. C. & West, J. A. (2011). Insights into evolution and speciation in the red alga *Bostrychia*: 15 years of research. *Algae*. 26: 3–14.
- Zuccarello, G. C., West J. A. & Kamiya M. (2018). Non-monophyly of *Bostrychia simpliciuscula* (Ceramiales, Rhodophyta): multiple species with very similar morphologies, a revised taxonomy of cryptic species. *Phycological Research* 66: 100–107.
- Zuccarello, G. C., West, J. A. & Loiseaux-de Goër S. (2006). Diversity of the *Bostrychia radicans/Bostrychia moritziana* species complex (Rhodomelaceae, Rhodophyta) in the mangroves of New Caledonia. *Cryptogamie Algologie* 27: 245–254.

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Fig. 1 A. Phylogenetic relationships based on ML analysis of the *rbc*L data set (1161 characters,) of *Bostrychia* species. Outgroups *Polysiphonia, Chondria* and *Laurencia*. Only ML bootstrap values >70% shown. GenBank Accession numbers shown and 4-number JAW culture number, where applicable. 3-letter country code shown, plus Australian state designation where applicable. Further information in Table S1. Scale bar = substitutions per site. **Fig. 1 B.** Phylogenetic relationships based on ML analysis of partial *rbc*L data set (690 characters,) of *Bostrychia kelanensis sensu lato*. Outgroups *B. radicans* and *B. moritziana*. Only ML bootstrap values >70% shown. Genbank

Accession numbers shown and 4-number JAW culture number, where applicable. 3-letter country code shown, plus Australian state designation where applicable. Further information in Table S1. Scale bar = substitutions per site.



Fig. 2. Phylogenetic relationships based on ML analysis of the COI data set (660 characters,) of *Bostrychia kelanensis sensu lato*. New species *B. australiana* shown. Outgroups *B. radicans* and *B. moritziana*. Only ML bootstrap values >70% shown. 4-number JAW culture number, where applicable. 3-letter country code shown, plus Australian state designation where applicable. Further information in Table S1. Scale bar = substitutions per site.



Fig. 3. Statistical parsimony network of variation in RuBisCo spacer sequence data (312 characters) of *Bostrychia kelanensis sensu lato*. The new species, *B. australiana*, represented by JAW isolates 3810 and 3748. Field collected samples E479 distinct. All *B. kelanensis* with identical RuBisCo spacer haplotypes except for JAW 4217. Hatch in line represents a mutation step, filled circle is a missing haplotype.

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Figs 4 A-F. *Bostrychia kelanensis* **Fig. 4-A.** JAW3075 Guam. Culture four months after transfer, thallus 3.6 cm long with typical alternate branching, filaments 90-100 μ m diam., no cladohaptera evident, basal disc (arrowhead) 450 μ m diam. Scale bar = 1mm. **Fig. 4-B.** JAW 3075 Guam. Closeup view of basal disc (600 μ m overall), branching uniseriate filaments (8-10 μ m diam.) adhering to glass substrate, with multiple erect shoots (90-110 μ m diam.). Scale bar = 100 μ m. **Figs 4-C-F.** Microwave



treated for 10 secs to shrink cells & show pit connections & variation of pericentral cells (pc) numbers per axial cell (ac). **Fig. 4-C.** JAW 3348 NSW, AUS. Typical filament (100 μ m diam.) showing linear sequence of 5 acs with 3 longitudinal pericentral cells per axial cell and 5 pericentral cells around each axial cell. Scale bar = 100 μ m. **Fig. 4-D.** JAW 3348 NSW, AUS. Atypical filament showing linear sequence of 3-2-3 tiers of pericentral cells for 3 adjacent axial cells. Scale bar = 100 μ m. **Fig. 4-E.** JAW 3348 NSW, AUS. At branch nodes of this filament the 2 axial cells above & 2 below have only 2 longitudinal pericentral cells per axial cell changing to 3 longitudinal pericentral cells per axial cell away from branch point. Scale bar = 100 μ m. **Fig. 4-F.** JAW 3350 NSW, AUS. Branch apex shows development of transverse divisions of pc into tier cells 8 cells from apical cell. After branch point 3 longitudinal pericentral cells per axial cell and extending continuously for 11 successive axial cells. In transverse view 5 pericentral cells around each axial cell. Scale bar =100 μ m.





Figs 5A-F. *Bostrychia australiana sp. nov.* Holotype JAW 3810 WA, AUS. **Fig. 5-A.** Live female thallus 4-months old, over 25 mm long, showing typical usually alternate lateral branching with procarps, no cladohaptera; basal disc was accidentally removed. Scale bar of 1 mm units. **Figs 5-B, C** & E before mounting on slide specimens were treated with 800-watt microwave for 10 secs in distilled water to shrink cells, to show pit connections with variation of vertical number of tiers of pericentral cells (pc) per axial cell (ac). Microwaving lead to expansion of the outer extracellular matrix. **Fig. 5-B.** Microwave-treated male with mature spermatangial stichidium with discharged



spermatia in upper area. Below in basal region, 4 longitudinal pericentral cells per axial cell, and in upper region 3 longitudinal pericentral cells per axial cell . Scale bar = 90 μ m. **Fig. 5-C.** Microwave-treated male lower vegetative axes. Four longitudinal pericentral cells per axial cell and 3 longitudinal pericentral cells per axial cell in different axes. Scale bar = 90 μ m. **Fig. 5-D.** Live female axis and lateral branches both with persistent trichogynes evident. Older trichogyne protoplasts collapsed. Scale bar = 100 μ m. **Fig. 5-E.** Microwave-treated female showing with procarps with trichogynes. longitudinal pericentrals per axial cell in upper branch & four in lower branches. Scale bar = 100 μ m. **Fig. 5-F.** Live female carpospore release. At lower right is apex of branch bearing cystocarp. Scale bar = 100 μ m.

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Figs 6A-H. *Bostrychia australiana sp. nov.* Isotype JAW 3748 NT, AUS. Figs **6 A, B, D, E & F** are pressed field specimens, the others are culture specimens. **Fig. 6 A.** Habit of female thallus on bark surface of mangrove prop root. Various stages of cystocarps visible. Scale bar = 1 mm. **Fig. 6 B.** Basal disc removed from bark shows erect branched axes & parts of horizontal stolons (arrowheads) derived from the basal disc. Scale bar = 240 μ m. **Fig. 6 C.** Pressed cultured females have numerous trichogynes visible sub-apically and persistent along main axes. Scale bar = 240 μ m. **Fig. 6 D.** Cystocarp of pressed field specimen with numerous carposporangia and apical ostiole. Scale bar =

100 μ m. Fig. 6 E. Apex of pressed female field specimen with developing procarps. Axis below with 4 longitudinal pericentral cells per axial cell and 5 pericentral cells around each axial cell. Scale bar = 120 μ m. Fig. 6 F. Pressed field specimen lower axis with 4 longitudinal pericentral cells per axial cell and 5 pericentral cells around each axial cell. First ac above node has 3-4 longitudinal pericentral cells per axial cell around each axial cell. All axial cells below with 4 longitudinal pericentral cells per axial cell and 5 pericentral cells around each axial cell. All axial cells below with 4 longitudinal pericentral cells per axial cell and 5 pericentral cells per axial cell and 5 pericentral cells around each axial cell. All axial cells below with 4 longitudinal pericentral cells per axial cell and 5 pericentral cells around each axial cell. Scale bar = 120 μ m. Fig. 6 G. In contrast to field specimens, the culture specimens had intercalary axial cells with 6 longitudinal pericentral cells per axial cell. Scale bar = 120 μ m. Fig. 6 H. In cultured specimen, first axial cells above node has 3 longitudinal pericentral cells per axial cell. Scale = 120 μ m.

	Culture			Collection			RuBisCo
Species	number	Lat. / Long.	Localities	Date	COI	<i>rbc</i> L	spacer
<i>B</i> .		12°26'35.9"S					
australiana	3748	130°46'01.0"E	Mandorah, NT, AUS	21 vi 1997		Х	Х
<i>B</i> .							
australiana	3810	24 ° 53 S. 117 ° 11'	Cossack, WA, AUS	9 xii 1997	Х	Х	Х
			Sadgroves Creek,				
B. kelanensis	2988	12° 27'S 130° 51'E	Darwin, NT, AUS	4 vi 1989		AY920849	
	• • • • •		Dehpehk Island,	26 viii			
B. kelanensis	3000		Pohnpei, FSM	1989	Х	X	X
B. kelanensis	3075	13° 20' N 144° 44'E	Talofofo River, GUM	8 vii 1990			Х
B. kelanensis	3076	13° 20' N 144° 44'E	Talofofo River, GUM	8 vii 1990			Х
			Chunda Bay, QLD,				
B. kelanensis	3210	19°6'S 147°03'E	AUS	28 ix 1991	Х		Х
			Bowling Green Bay,				
B. kelanensis	3213	11° 27'S 147° 10'E	QLD, AUS	28 ix 1991	Х	Х	Х
			Bowling Green Bay,				
B. kelanensis	3214	11° 27'S 147° 10'E	QLD, AUS	28 ix 1991	Х	AY920853	Х
			Chunda Bay, QLD,				
B. kelanensis	3218	19°6'S 147°03'E	AUS	28 ix 1991			Х
		32°06'S 47° 152'	Darawank, Walamba				
B. kelanensis	3348	29"E	R., NSW, AUS	12 iii 1993	Х	Х	Х
		32°06'S 47° 152'	Darawank, Walamba				
B. kelanensis	3349	29"E	R., NSW, AUS	12 iii 1993	Х	Х	Х
		32°06'S 47° 152'	Darawank, Walamba				
B. kelanensis	3350	29"E	R., NSW, AUS	12 iii 1993	Х	Х	Х
			Port Macquarie, NSW,				
B. kelanensis	3549	25° 15'S 153° 10'E	AUS	22 x 1995		Х	
		24°21'18.1"N	Isobe River., Ishigaki				
B. kelanensis	3860	124°12'11.1"E	I., JPN	23 i 1998	Х	X	Х
B. kelanensis	4217		Krabi, THA	17 iii 2002	Х	Х	Х
		N 11° 25.844' E 79°	Pichavaram, Tamil				
B. kelanensis	4261	46.944'	Nadu, IND	12 x 2002	Х	Х	Х
		24°21'18.1"N	Puthen I., Ashtamudy				
B. kelanensis	4290	124°12'11.1"E	Estuary, Kerala, IND	14 ii 2003	Х	Х	Х
		13° 15.371' N 144°	Achang Bay Resort,				
B. kelanensis	4589	41.063' E	GUM	12 ii 2006	Х	Х	Х
		07°26. 925' N 151°	Peniyak Village, Weno				
B. kelanensis	4632	53.313' E	I., Chuuk, FSM	11 ii 2006		Х	Х

Table 1. Samples of *Bostrychia* used in phylogenetic analyses. JAW cultures numbers given, plus localities and collection date. X= genetic regions sequenced in this study.

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	Culture			Collection			RuBisCo
Species	number	Lat. / Long.	Localities	Date	COI	<i>rbc</i> L	spacer
		07°26. 925' N 151°	Peniyak Village, Weno				
B. kelanensis	4635	53.313' E	I., Chuuk, FSM	11 ii 2006		Х	Х
		13° 15.371' N 144°	Achang Bay Resort,				
B. kelanensis	4637	41.063' E	GUM	12 ii 2006			Х
			Lewver River, Karu,				
B. kelanensis	1538		Kaguso, NI, PNG	4/06/13	Х		
			Lewver River, Karu,				
B. kelanensis	1540		Kaguso, NI, PNG	4/06/13	Х		
		N 11º 25.844' E 79º	Pichavaram, Tamil				
B. kelanensis	E846	46.944'	Nadu, IND		Х		
		3°28'59"S	Bilwo River, Kaguso,				
B. kelanensis	G374	152°15'14"E	NI, PNG	6/06/13		Х	Х
		3°28'59"S	Bilwo River, Kaguso,				
B. kelanensis	G372	152°15'14"E	NI, PNG	6/06/13		Х	Х
B. kelanensis	D947	08° 06'S 114° 30'E	Benoa East , Bali, IDN				Х
B. kelanensis	D948	08 ° 48 S. 115 ° 13' E	Nusa Dua, Bali, IDN				Х
			Port Douglas, QLD,				
B. kelanensis	E997		AUS	12/10/03			Х
		07°26. 925' N 151°	Peniyak Village, Weno				
B. kelanensis	F383	53.313' E	I., Chuuk, FSM	11/04/06			Х
B. kelanensis	G322		Sarawak, MYS				Х
		2°48'07.5"S					
B. kelanensis	G332	151°11'00.4"E	Cape Sass, NI, PNG	4/06/13			Х
			Woolooware, NSW,				
<i>B. sp.</i>	E479	34° 01'S 151° 09'E	AUS	22/03/01			Х
			upper Tomkinson R.,				
		12 ° 11' .98" S 134 °	Arnhem Land, NT,	22 viii			
B. radicans	3994	15' .52" E	AUS	1999	Х		
		23° 48' S. 42 ° 25'W	São Sebastião, São				
B. radicans	2649		Paulo, BRA	2 vii 1982		AY920818	
В.			Brunswick Heads,				
moritziana	3695	28° 31'S 153° 33'E	NSW, AUS	12 ii 1997	Х		
			Millers Landing,				
В.			Wilson's Promontory	17 xii			
moritziana	2934	38° 54'S 146° 18'E	VIC, AUS	1988		AY920809	
<i>B</i> .			W. Sawang, N.				
moritziana	3453		Sulawesi, IDN	16 x 1994		AY920813	