

The red algal genus *Callithamniella* (Rhodophyta, Ceramiaceae) in Australia, including two new species, *C. cygnora* and *C. carolyniae*

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Abstract

Huisman, J.M., McLay, T.G.B. & Metti, Y. The red algal genus *Callithamniella* (Rhodophyta, Ceramiaceae) in Australia, including two new species, *C. cygnora* and *C. carolyniae*. *Nuytsia* 37: 120–132 (2026). *Callithamniella* Feldm.-Maz. comprises five currently accepted species characterised by a filamentous, uncorticated thallus with prostrate and erect axes, each with at most only a single vegetative lateral branch per cell. The genus is widely distributed but seemingly rarely encountered, and the only known reproductive structures are tetrasporangia, spermatangia and carpogonial branches. Post-fertilization development has not been recorded. *Callithamniella flexilis* Baardseth is currently the only species of this genus recognised in Australia, recorded from north of Coffs Harbour in New South Wales. The present study was prompted by the collection of an unknown species from the Swan Estuary, Perth, Western Australia, here named as *C. cygnora* Huisman, McLay & Metti. Additionally, an unnamed taxon from the south coast distinguished by the presence of relatively large vesicular cells, is also confirmed as distinct and named *C. carolyniae* Huisman, McLay & Metti. DNA sequence analyses of *C. cygnora* confirm the inclusion of *Callithamniella* in the Dohrniielleae tribe of the Ceramiaceae, closely related to *Antithamnionella* Lyle. An updated description for *C. flexilis* is provided along with a key to the Australian species of the genus.

Introduction

Callithamniella Feldm.-Maz. is one of only a handful of filamentous red algal genera that are primarily distinguished based on their vegetative morphology, with post-fertilisation development and carposporophytes unknown. Crucately divided tetrasporangia and spermatangia in specialised branches have been described for some species, but these are not informative in assessing relationships with other algae and the higher-level classification of the genus. Carpogonial branches were described by Stegenga (1988) for specimens he attributed to *C. capensis* Simons (but regarded as *C. tingitana* (Schousb. ex Bornet) Feldm.-Maz by Athanasiadis 1996). The carpogonial branches were borne on the basal cell of a two-celled branch, these arising at intervals in the apical region of indeterminate axes. Stegenga (1988) suggested that the genus is probably most closely related to *Antithamnionella* Lyle, but in that genus there are no species with only one determinate lateral per segment. Both *Callithamniella* and *Antithamnionella* are currently place in the Dohrniielleae tribe of the Ceramiaceae, but as noted by Guiry (2017), ‘confirmation of relationships awaits study of female reproduction.’ Nevertheless, the combination of a filamentous

thallus with prostrate and erect uncorticated axes, the erect axes and lateral branches arising singly on bearing cells, serves to distinguish the genus.

In 2024, a small filamentous alga was encountered as an epiphyte on other algae in the shallow subtidal of the Swan Estuary, at Blackwall Reach adjacent to the suburb of Bicton. The first collection in April was not reproductive, but its unique morphology pointed conclusively to *Callithamniella*. A second collection in November included tetrasporangial thalli, their features also compatible with the genus. Comparison of this entity with published descriptions of the five known species indicated that it represents an undescribed species, which we formally name here as *Callithamniella cygnora* Huisman, McLay & Metti. While female reproduction was not observed, the collection was sufficient to enable a DNA analysis and assess its affinities with other genera.

As part of this study, we also examined specimens that formed the basis of earlier Australian records of the genus. One taxon (*S. Smith*, NSW 1327490 plus others), collected from eastern Australia at Coffs Harbour, was referred to *Callithamniella flexilis* Baardseth by Athanasiadis (1996), despite some reproductive dissimilarities that potentially warrant recognition. A second, different taxon, from southern Australia (*S.A. Shepherd*, AD-A38705), was attributed to *Callithamniella* by Bryan Womersley (in an undated note), but assigning a name was deferred in the expectation that reproductive specimens would eventually be collected. As the genus is characterised by vegetative features, and this latter entity conforms but displays features distinguishing it from other species, we also formally name it here as *Callithamniella carolyniae* Huisman, McLay & Metti.

Materials and Methods

Western Australian specimens of *Callithamniella* were collected by snorkel in the shallow subtidal. The *in situ* image was taken with an Olympus TG-6 in a housing, with a Backscatter strobe. Specimens were pressed as an herbarium voucher and also mounted while fresh on slides in estuary water, unstained, then examined and photographed with a Nikon Eclipse 80i compound microscope, with a Nikon DS L4 digital camera (Nikon Corporation, Japan). Slide preparations were then made permanent by the addition of 30–50% corn syrup. Eastern Australian specimens were obtained on loan from AD and NSW and were all slide preparations stained with aniline blue and mounted in a corn syrup solution. DNA extraction was undertaken by the CSIRO National Biodiversity DNA Library (NBDL.), using a NucleoMag Plant Kit (Macheray-Nagel, Germany), and samples were sequenced using a miniaturised genome skimming approach centred around the QiaSeq FX DNA library kit (Qiagen, The Netherlands) on a NovaSeq S1 flowcell (Illumina, USA) at the Australian National University's Biomolecular Resource Facility (BRF). Paired-end reads were assembled using SPAdes v.4.0 with default settings (Prjibelski *et al.* 2020), and the target DNA loci were identified with a blastn search in BLAST v. 2.17.0 (Altschul *et al.* 1990).

Phylogenetic analyses

Independent phylogenetic analyses were undertaken for each of the three gene regions (28S rRNA, COI, and *rbcL*). Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed for all three loci. Maximum parsimony (MP) analyses with pairwise distance calculations were also performed for *rbcL*. Concatenation of loci was not possible since there was insufficient sequence overlap due to so few sequences being available publicly. All analyses included two newly generated sequences of the Western Australian *Callithamniella* from this study (PERTH 10005331; PERTH 09763570, sequences available from CSIRO Data Access Portal). A total of 21 sequences were used for the *rbcL* analyses (outgroups *Melanothamnus* Bornet & Falkenb. and *Polysiphonia* Grev.), 14 sequences were used for COI (outgroups also *Melanothamnus* and *Polysiphonia*), and 13 sequences for 28S rRNA (outgroups *Polysiphonia* and *Wilsonosiphonia* D.Bustamante, Won & T.O.Cho).

Sequences were edited and aligned using BioEdit v7.2.0 (Hall 1999). ML and BI analyses were run using the online CIPRES Gateway (Miller *et al.* 2010). MP analyses and pairwise distance calculations were undertaken in PAUP* v4.0b10 (Swofford 2003).

ML analyses were run using RAxML v8.2.12 (Stamatakis 2014) to infer phylogenies with the evolutionary model set to a GTR substitution model, including a gamma-distributed rate variation. Node support was estimated with RAxML rapid bootstrapping and subsequent ML search. The number of bootstrap replicates was determined automatically using the autoMRE bootstrapping criterion (Pattengale *et al.* 2010) in RAxML, which was limited to maximum 1000 replicates. This resulted in 250 replicates for the *rbcL* dataset and 100 replicates for COI. The 28S rRNA dataset bootstrapping required the maximum number of replicates which was 1000. Bootstrap support values of 75% or greater were considered supported.

MrBayes v3.2.7a (Ronquist *et al.* 2012) was used for running BI analyses. The substitution model also was set to GTR + Γ including four gamma rate categories. For each gene region, two independent runs of four Markov chains (three hot, one cold, including heating parameter $T = 0.20$) were undertaken for up to 1,000,000 generations, with one tree sampled every 1,000 generations. The first 25% of trees were discarded as a burn in. Runs were terminated when the average standard deviation of split frequencies (ASDSF) reached below 0.01. For the *rbcL* gene, this occurred at 200,000 generations, 785,000 generations for COI, and 565,000 generations for 28S rRNA. Posterior probabilities (PP) equal to or greater than 0.95 were considered supported.

The software PAUP for PC (v.4.0 beta10, Swofford 2003) was used for MP analyses. Node support was estimated from 1,000 bootstrap replicates. Pairwise distances (p-distances) were calculated using uncorrected 'p' distances.

Newly generated sequences, a list of GenBank sequences, and trees inferred from COI and 28S rRNA analyses are available from the CSIRO Data Access Portal (DAP – [<https://data.csiro.au/collection/csiro:74974>]).

Results

Molecular analysis

Molecular, *rbcL*: The final length of the *rbcL* alignment was 1317 base pairs (bp), which included 319 parsimony-informative characters. The *rbcL* ML tree is shown in Figure 1 and includes the ML bootstrap values followed by the BI posterior probabilities. The clade corresponding to the tribe Dohrnielleae, which in these analyses included the two genera *Callithamniella* and *Antithamnionella*, resolved as well supported across all three analyses (ML bootstrap = 100%, BI posterior probability = 1.00 and MP bootstrap = 99%). Two other resulting clades are also clearly separated and corresponding to tribes. One was the clade Antithamnieae (100%, 1.00, 100%), which was fully supported and represented by two *Antithamnion* Nägeli sequences, and the other clade was Ceramieae, represented by three *Reinboldiella* De Toni and three *Ceramium* Roth taxa. The Ceramieae clade showed variable support across the analyses (92%, 1.00, 63%).

The Western Australian *Callithamniella*, represented by two sequences newly generated from this study, formed a fully supported clade across all analyses (100%, 1.00, 100%), and consistently showed as sister to the *Antithamnionella* clade within the Dohrnielleae tribe. The intraspecific pairwise distance within the *Callithamniella* clade was 0.00%. Intraspecific distances within the *Antithamnionella spirographidis* (Schiffn.) E.M.Woll. clade were also very small (0.00%–0.15%). The interspecific distances within the *Antithamnionella* clade ranged from 5.77% to 6.99%. Pairwise distances between the *Callithamniella* clade and *Antithamnionella* sequences, all within the larger Dohrnielleae clade, ranged from 6.07%–7.37% showing large enough distances between the two clades to consider them separate taxa. Specifically, the pairwise distances between the Western Australian *Callithamniella* and the generitype *A. ternifolia* (Hook.f. & Harv.) Lyle was 6.07%. The distance between *Callithamniella* and the *Antithamnion* clade, which is sister to the Dohrnielleae clade, was 10.48%. Although there are two sequences within the *Antithamnion* clade both resulted in this same distance. All other taxa resulted in even greater distances from *Callithamniella*.

The results from all analyses were consistent and only the *rbcL* is shown (Figure 1).

Molecular, COI: The final length of the COI alignment was 665 bp. In both the ML and BI analyses, the larger Dohrnielleae clade was fully resolved (ML bootstrap = 100%, BI posterior probability = 1.00). Again, the clade *Callithamniella*, containing both newly generated sequences, was fully supported (100%, 1.00), was within the Dohrnielleae clade and consistently resolved as sister to *Antithamnionella*. The *Antithamnionella* sequences themselves also nested within the Dohrnielleae and were shown to be polyphyletic.

Molecular, 28S ribosomal RNA: The 28S rRNA alignment was a total of 585 bp long. Again, as for the other two loci, the *Callithamniella sp. nov.* sequences were fully supported (100%, 1.00) within the Dohrnielleae clade, which again was also supported (100%, 1.00). The *Callithamniella* clade resulted as sister clade to the *Antithamnionella* sequences. The two *Antithamnionella* sequences in these analyses did not form their own clade, but were still well supported within the tribe. In this analyses *Ceramium* was recovered as polyphyletic as well.

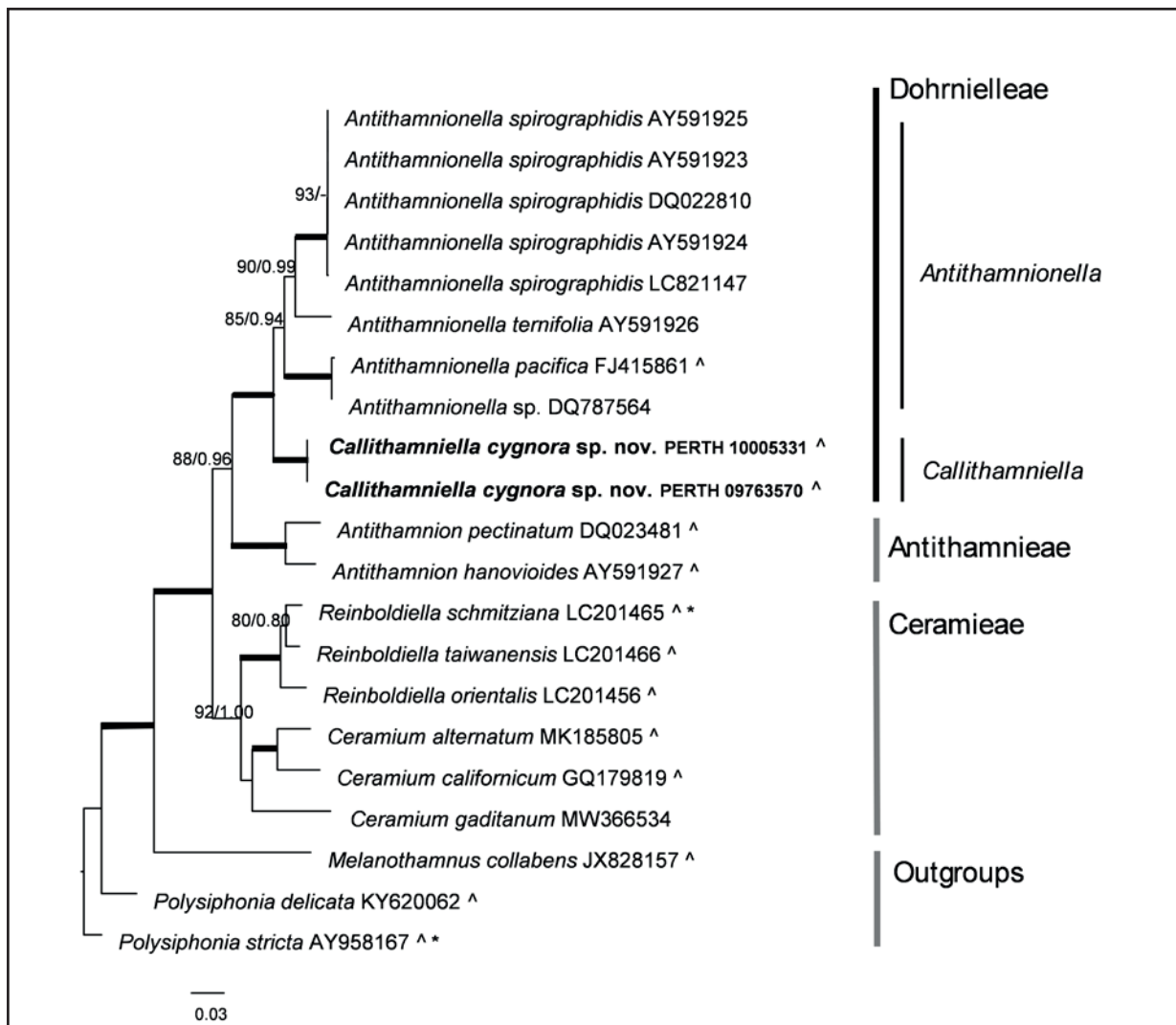


Figure 1. Maximum likelihood tree (RAxML) inferred using *rbcL* sequence data. The tree shows ML bootstrap values (250 replicates) and Bayesian posterior probabilities. Values lower than 75/0.75 are not shown. Bold lines indicate full support values of 99/0.99 or greater. Newly generated sequences from this study are indicated by bold font. Sequences from material collected near type localities are indicated by ^ and generitype sequences by *.

The *Callithamniella* clade was consistently fully supported, as was the Dohrnielleae clade it nested within. This result was consistent in analyses of all gene regions (*rbcL*, COI and 28S rRNA) and strongly suggesting it is correctly placed within the family Ceramiaceae. Pairwise distances also showed strong support for the Western Australian *Callithamniella* to be considered as a separate taxon, and sister to *Antithamnionella*.

Taxonomy

Key to the Australian species of *Callithamniella*

1. Determinate branches consistently simple, vesicular cells absent ***C. flexilis***
- 1: Determinate branches occasionally simple but more typically branched; vesicular cells present or absent **2**
2. Determinate branches simple or with a lateral branch on the third cell from the base; vesicular cells inconspicuous and sometimes absent ***C. cygnora***
- 2: Determinate and indeterminate branches similarly much branched; vesicular cells obvious and common ***C. carolyniae***

Callithamniella cygnora Huisman, McLay & Metti, *sp. nov.*

Type: Blackwall Reach, Swan Estuary, Western Australia, epiphytic on *Prionitis* sp., 12 November 2024, J.M. Huisman s.n. (*holo:* PERTH 09763570).

Thallus filamentous, with prostrate and erect axes, spreading laterally for several millimetres to the extent of the host, erect axes to 3–4 mm tall. Prostrate axes with a single lateral or upright branch per cell, attached by a hyaline rhizoid 1–2 cells long arising from the basal cell of a lateral branch. Indeterminate lateral branches arising directly from cells of prostrate axes or from the basal cell of a determinate lateral, with one lateral branch per cell. Determinate lateral branches to 11–15 cells and 340–485 µm long, simple or once divided from the third proximal cell from the base, occasionally with a second branch, basal cell trapezoidal and conspicuously shorter and often broader than the suprabasal cell. Cells of prostrate axes and larger indeterminate axes 40–50 µm diam., 200–250 µm long, near apices *c.* 36 µm diam. and 180 µm long. Cells of determinate branches 12–15 µm diam., 36–41 µm long, L/B mostly 3–4, becoming shorter near branch apices. Apical cell of determinate branches rounded distally or bullet-shaped. Plastids ellipsoidal to elongate, sausage-shaped. Vesicular cells inconspicuous, often absent, lens-shaped and lateral/adaxial on the basal cell or suprabasal cell of determinate branches or lateral branch arising from determinate laterals, 8–15 µm long, *c.* 5 µm diam. Tetrasporangia pedicellate, with a 1 (or 2)-celled stalk, borne on indeterminate branches adjacent to a determinate branch, ellipsoidal, 35–45 µm long, 25–30 µm diam., L/B 1.4–1.5, cruciately divided. Other reproductive structures not observed. (Figure 2)

Diagnostic features. *Callithamniella cygnora* is the only species of the genus recorded for Western Australia and its small, filamentous habit is similar to other genera in the Ceramiaceae, but it can be distinguished by its prostrate axes, upright axes with a single lateral branch per cell, lateral branches simple or once-divided from the third cell from the base, and position of vesicular cells.

Additional specimen examined. WESTERN AUSTRALIA: Blackwall Reach, Swan Estuary, epiphytic on *Colpomenia* at 1 m depth, 21 Apr. 2024, J.M. Huisman s.n. (PERTH 10005331).

Distribution and habitat. Known only from the type locality, epiphytic in shallow subtidal areas.

Conservation status. The species is known from limited collections but is unlikely to be under threat.

Etymology. The epithet is from the Latin *cygnus* (a swan), in reference to the Swan Estuary type locality.

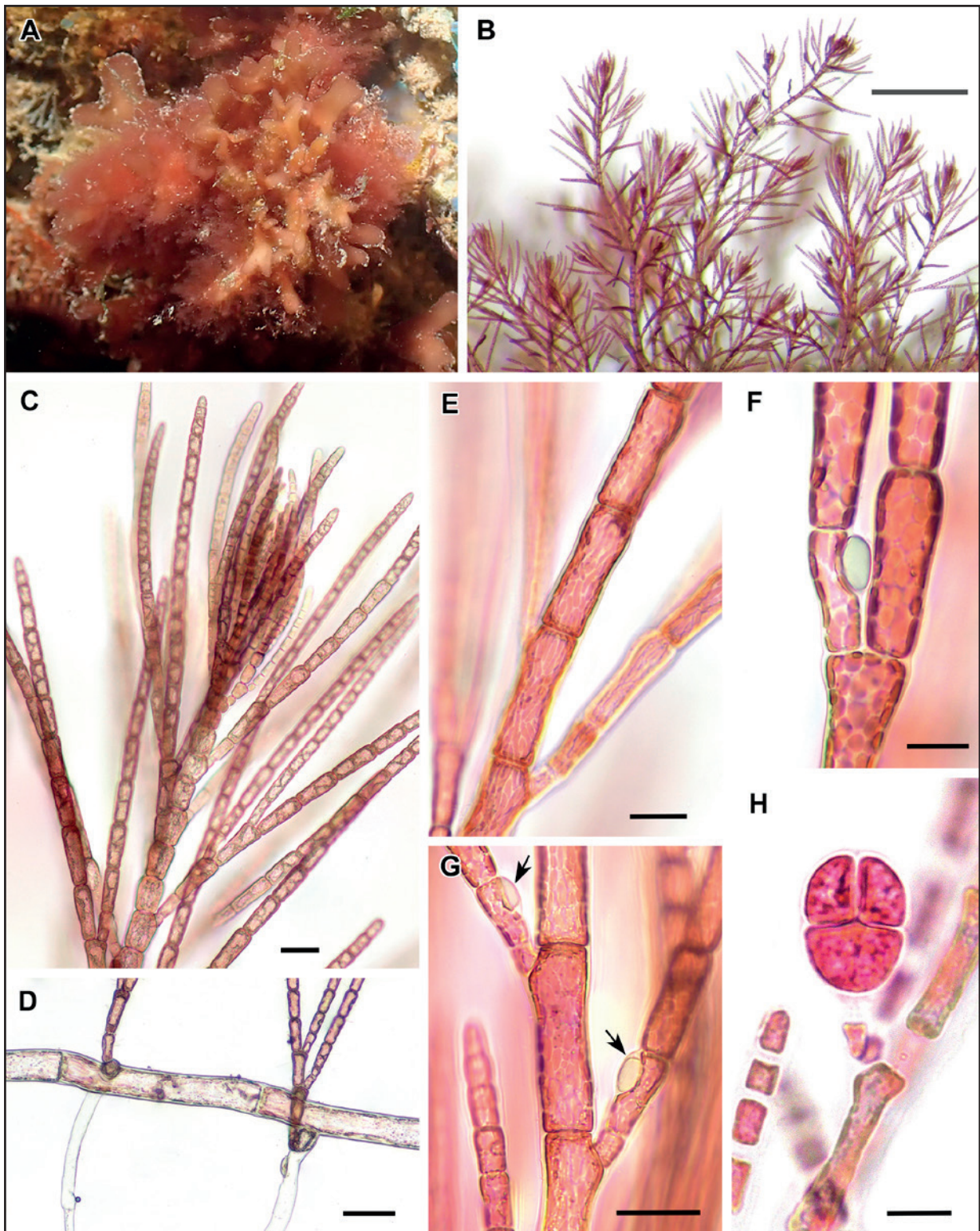


Figure 2. *Callithamniella cygnora*. A – alga *in situ* at Blackwall Reach, epiphytic on *Prionitis* sp.; B – detail of tufted thallus; C – apex of primary axis; D – prostrate axis with hyaline attachment rhizoids; E – closer view of cells showing sausage-shaped plastids; F, G – vesicular cells (arrows); H – cruciately divided tetrasporangium. Scale bars = 500 µm (B); 60 µm (C, D); 30 µm (E, G); 15 µm (F, H). Images from PERTH 09763570. Photographs by J.M. Huisman.

Notes. Similar to *Callithamniella pacifica* I.A. Abbott & R.E. Norris in habit, number of cells in determinate branches, and the presence of inconspicuous vesicular cells, but differing in greater length of determinate branches (340–485 µm vs up to 250 µm long), smaller size of tetrasporangia (35–45 µm long vs ‘up to 90 µm long’, Abbott & Norris 1993: 459) and the truncated cone shape of the basal cells of laterals

(described as ‘hourglass’ in *C. pacifica* by Abbott and Norris, 1993). Tetrasporangia in *C. pacifica* are also proportionally more elongate, L/B c. 4, whereas those of *C. cygnora* are L/B 1.4–1.5, and appear to have a cup-shaped subtending cell (Abbott & Norris 1993, Fig. 17) that is absent in *C. cygnora*. Kim and Lee (2012) recorded *C. pacifica* from Korea, but tetrasporangia in their specimens are not as elongate as those described by Abbott and Norris (1993), and with different proportions (shorter and broader); however, they were still longer than those of *C. cygnora* (60–65 µm vs 35–45 µm). Whether the Korean species should be retained in *C. pacifica* will probably require molecular analyses. The new species is clearly related to the generitype *C. tingitana* (Schousb. ex Bornet) Feldm.-Maz., especially in the branching of determinate laterals from the third cell from the base. Originally described from Tangier, Morocco, *C. tingitana* has subsequently been recorded from numerous locations including nearby in Biscay, Spain (Secilla 2012), and disparate locations including North Carolina, U.S.A. (Schneider 1984), Bermuda (Schneider & Searles 1997), Venezuela (García & Huérfano 2006), Japan (Kitayama 2015), and possibly South Africa (Kitayama 2015). The South African record is based on the synonymy of *C. capensis sensu* Stegenga 1988: 376, proposed by Athanasiadis (1996). None of these records report vesicular cells for *C. tingitana* (see also Cormaci *et al.* 2023). Other species include the South African *C. capensis* Simons (1970), *C. silvae* Searles (in Searles & Schneider 1989) from southeastern U.S.A., and *C. flexilis* Baardseth (1941) from Tristan da Cunha in the South Atlantic. Again, none of these species have been described with vesicular cells (see also Stegenga 1986, 1988; Stegenga *et al.* 1997; Anderson *et al.* 2016).

Affinities. *Callithamniella cygnora* occupies similar habitats in the Swan Estuary is another filamentous red alga, *Antithamniella ternifolia*, which differs in producing three lateral branches per axial cell (vs one), vesicular cells in various positions on lateral branches (vs restricted to the basal cell or suprabasal cell), and sessile tetrasporangia (vs stalked).

Callithamniella carolyniae Huisman, McLay & Metti, *sp. nov.*

Type: Waldegrave I., South Australia, on *Cystophora monilifera*, 23 m depth, 11 May 1971, *S.A. Shepherd s.n.* (*holo:* AD-A38705, slides 3584, 3585).

Thallus filamentous, with prostrate and erect axes, spreading laterally for several millimetres, erect axes to 1–2 mm tall. Prostrate axes with a single lateral or upright branch per cell, attached by a hyaline rhizoid 1–4 cells long arising from the basal cell of a lateral branch, often branched at the first cell, occasionally joining with adjacent rhizoids. Indeterminate lateral branches arising directly from cells of prostrate axes or from the basal cell of a determinate lateral, with one lateral branch per cell, apices congested, the primary cell divisions slightly oblique. Determinate and lesser lateral branches to 12–20 cells and 500–1000 µm long, branched several times, similar to indeterminate branches, basal cell conspicuously shorter and often broader than the suprabasal cell, which is also often shorter than more distal cells. Cells of prostrate axes and larger indeterminate axes 35–40 µm diam., 100–110 µm long, near apices 12–20 µm diam. and 25–50 µm long. Cells of determinate branches 10–25 µm diam., 35–100 µm long, L/B mostly 3–4, becoming shorter near branch apices. Apical cell of determinate branches rounded distally or bullet-shaped. Plastids ellipsoidal to elongate, sausage-shaped. Vesicular cells numerous, cut off laterally from apical and other cells, ellipsoidal to kidney-shaped, 26–34 µm long, 17–22 µm diam. Reproductive structures not observed. (Figure 3)

Diagnostic features. Distinguished from other species of *Callithamniella* by the abundant presence of conspicuous vesicular cells that are often lateral on apical and subapical cells.

Additional specimen examined. VICTORIA: Lorne, on *Gelidium asperum*, drift, 23 Jan. 1967, *H.B.S. Womersley s.n.* (AD-A31532, slide 1489).

Distribution and habitat. Known only from the type locality and a single collection from Lorne, Victoria. Both collections epiphytic, the type collected from 23 m depth.

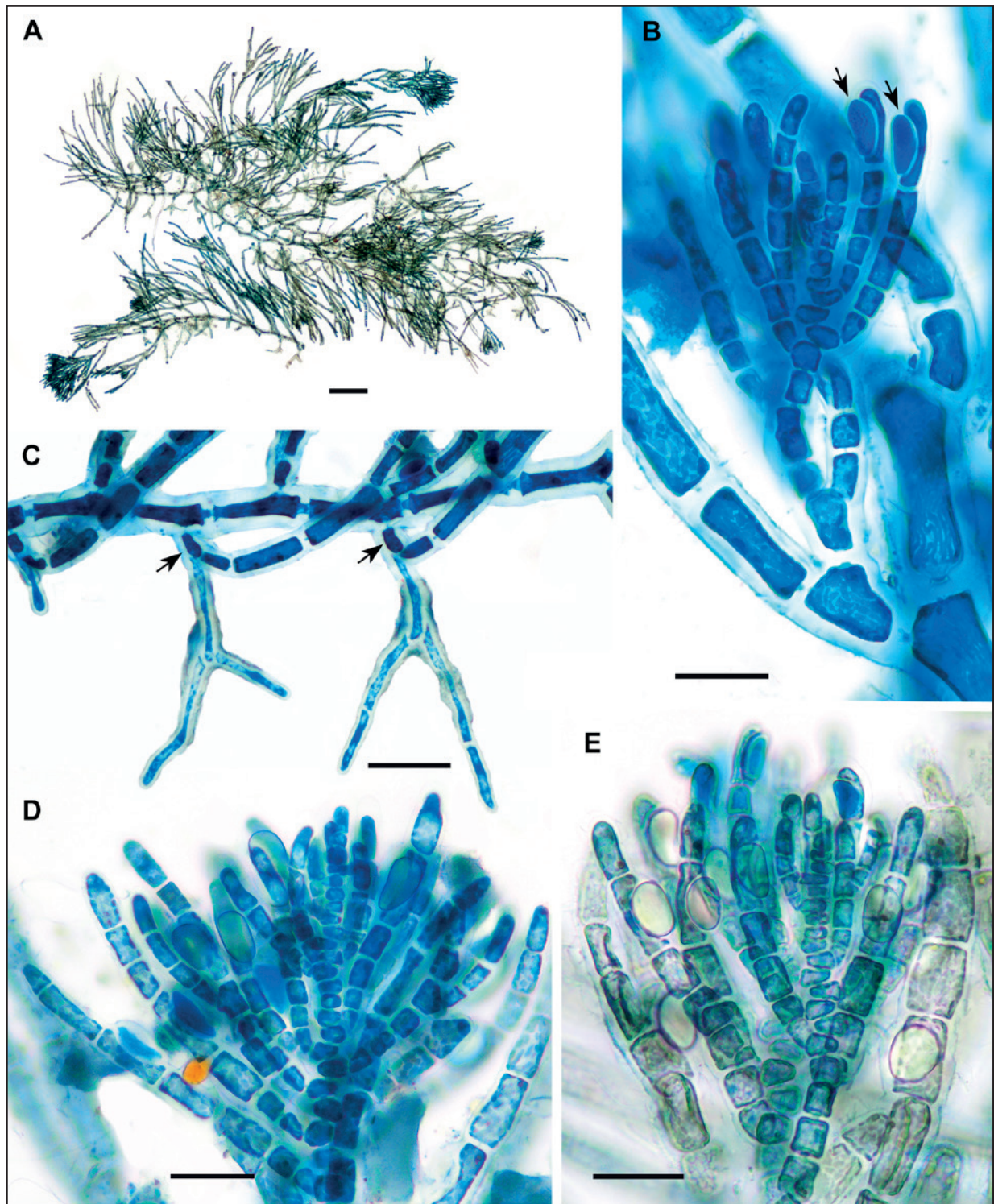


Figure 3. *Callithamniella carolyniae*. A – holotype specimen (slide preparation) showing filamentous habit; B – apex of primary axis with developing vesicular cells (arrows); C – prostrate axis with hyaline attachment rhizoids arising from basal cell of lateral branch (arrows); D – branch apex; E – branch apex with developing and mature vesicular cells. Scale bars = 300 µm (A); 30 µm (B, D, E); 60 µm (C). Images from AD-A38705, slide 3584 (A, E); AD-A38705, slide 3585 (B, C, D); Photographs by J.M. Huisman.

Conservation status. The species is known from limited collections but is unlikely to be under threat.

Etymology. Named for Carolyn Ricci, acknowledging her long career as Technical Officer (Phycology) at the State Herbarium of South Australia.

Notes. These south-eastern Australian specimens differ from *Callithamniella cygnora* in the relative uniformity of cell diameters between prostrate and upright axes, and the rarity of simple determinate lateral branches, with most laterals branched several times and not in a consistent pattern. The plants also differ by the abundant presence, location, and size of vesicular cells. These are often lateral on apical and subapical cells of lateral branches, in addition to other locations. Vesicular cells are also very conspicuous, 26–34 µm long, 17–22 µm diam., whereas those of *C. cygnora* are rarely present and 8–15 µm long, c. 5 µm diam. These features also distinguish the south-eastern Australian plants from all other species of *Callithamniella*. Wynne and Ballantine (1985) regarded unicellular rhizoids as one of the defining features of *Callithamniella*, which would exclude *C. carolyniae* from the genus; however, multicellular rhizoids have been reported for *C. sylvae*, *C. tingitana* and *C. flexilis* (Schneider 1984; Searles & Schneider 1989).

Callithamniella flexilis Baardseth, *Mar. alg. Tristan da Cunha*: 88, fig. 46. (1941).

Type: Seal Bay, Tristan da Cunha [st. 171] (Baardseth 1941: 89); (*lecto:* O [University of Oslo], two slides, tetrasporic and male, designated by Athanasiadis (1996: 89), *n.v.*).

Thallus filamentous, with prostrate and erect axes, spreading laterally for several millimetres, erect axes to 1–2 mm tall. Prostrate axes attached by hyaline rhizoids arising from the basal cells of lateral branches, mostly single celled and terminating in a digitate pad. Prostrate and indeterminate axes with a single indeterminate or determinate lateral branch per cell. Indeterminate lateral branches arising directly from cells of prostrate axes or from the basal cell of a determinate lateral, with one lateral branch per cell, apices congested, the primary cell divisions slightly oblique. Determinate branches to 25–30 cells and 500–1000 µm long, unbranched, basal cell conspicuously shorter and often broader than the suprabasal cell, which is also often shorter than more distal cells. Cells of prostrate axes and larger indeterminate axes 35–50 µm diam., L/B $\pm$ 2–3. Cells of determinate branches 10–20 µm diam., L/B mostly 1–3, becoming shorter near branch apices. Apical cell of determinate branches rounded distally or bullet-shaped. Plastids ellipsoidal to elongate, sausage-shaped. Vesicular cells absent. Tetrasporangia terminal on a 1–4-celled pedicel, adjacent to a lateral branch, initially elongate and clavate, 7–15 µm diam., 35–36 µm long, becoming ellipsoidal when mature, c. 22 µm diam., 44 µm long, most sporangia immature with a single transverse division, becoming irregularly or cruciately divided when mature. Other reproductive structures not observed. (Figure 4)

Diagnostic features. *Callithamniella flexilis* can be distinguished from other Australian species of the genus by the presence of unbranched determinate lateral branches and the absence of vesicular cells.

Specimens examined. NEW SOUTH WALES: Look-at-me-now Headland, near Coffs Harbour, epilithic on intertidal rocks, 30 Oct. 1990, *S. Smith s.n.* (NSW 1327490, 1327494, 1327495, 1327496, 1327497, 1327499, 13274500, 1327501); *loc. id.* 18 Dec. 1990, *S. Smith s.n.* (NSW 1327502, 1327503, 1327504, 1327505).

Distribution and habitat. In Australia, known only from the present collections from northern New South Wales, epilithic on intertidal rocks. Elsewhere from disjunct locations including Spain, Brazil and possibly South Africa (Guiry 2015).

Conservation status. The species is known from limited collections in Australia but is unlikely to be under threat.

Notes. Millar and Kraft (1993: 37) tentatively referred these specimens from Coffs Harbour, New South Wales, to *C. flexilis* based on the presence of unbranched determinate laterals but noted that further study was required. Based on morphological congruence with the type collection, Athanasiadis (1996) identified these specimens as *C. flexilis*, despite only observing bisporangia, not tetrasporangia. Our examination of the same collections has shown the rare occurrence of irregularly or cruciately divided tetrasporangia, supporting their identification as *C. flexilis*.

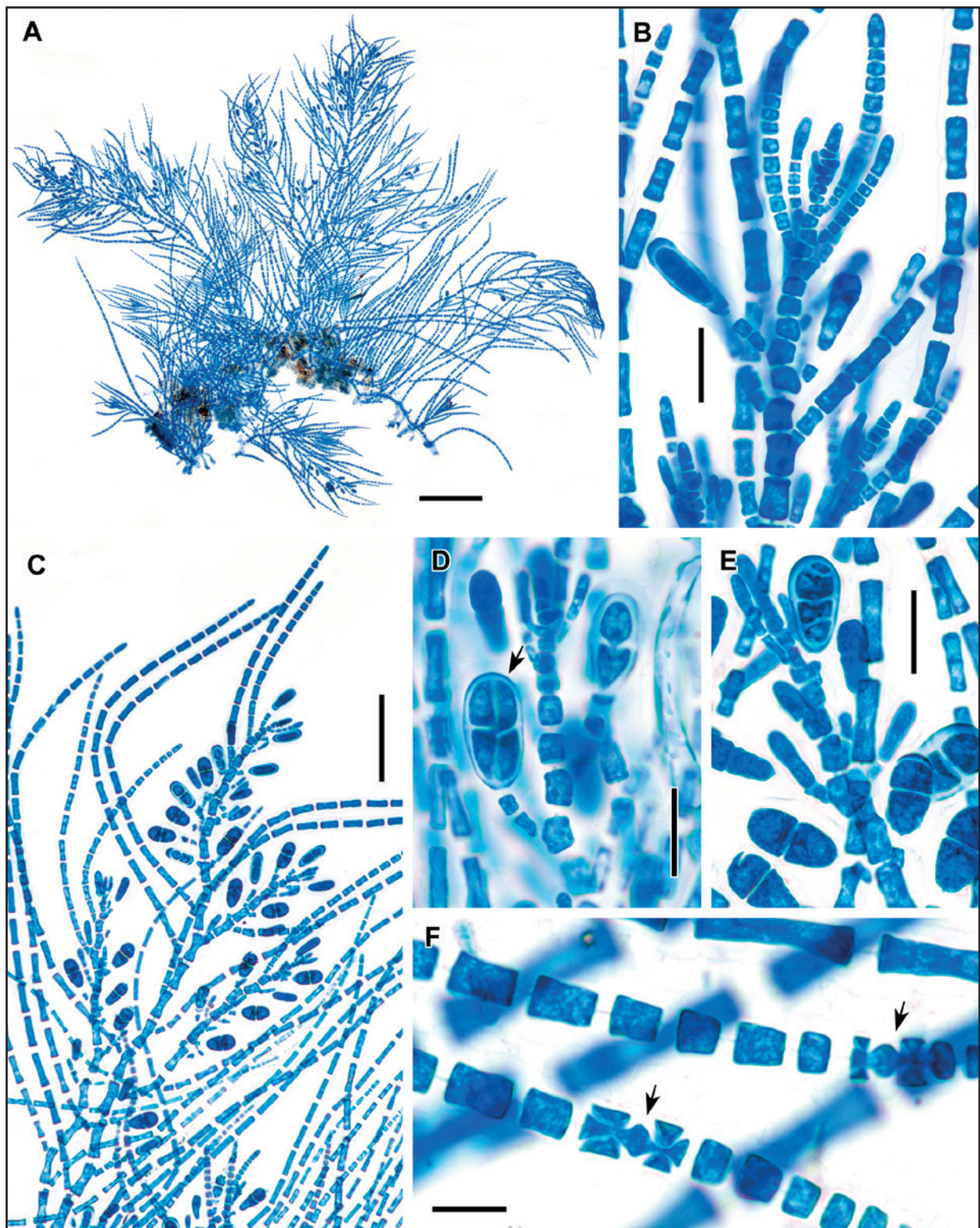


Figure 4. *Callithamniella flexilis*. A – slide preparation showing filamentous habit; B – apex of primary axis with developing tetrasporangia (arrows); C – tetrasporangial thallus; D – detail of a cruciately divided tetrasporangium (arrow); E – irregularly divided bisporangia and tetrasporangia; F – immature spermatangial branch (arrows). Scale bars = 300 µm (A); 30 µm (B, D, E); 100 µm (C); 15 µm (F). Images from NSW 1327494 (A); NSW 1327504 (B, D); NSW 1327503 (C, E); NSW 1327502 (F). Photographs by J.M. Huisman.

Athanasiadis (1996) also suggested that the South African *Callithamniella capensis* could represent a heterotypic synonym of *C. flexilis*. Indeed, Simons (1970), when describing *C. capensis*, noted in relation to *C. flexilis* that it is ‘so similar to the plant in hand [*C. capensis*] that their relationship seems obvious’ but does not offer any features that may separate the two. Based on published records, species

of *Callithamniella* have seemingly been introduced to widely separated locations. Athanasiadis (1996) suggested that the Australian plants of *C. flexilis* were located ‘near a harbour’; however, the collection came from a headland some distance from the town of Coffs Harbour, which, while coastal and with a marina, is regarded as a minor port and does not experience significant international boat traffic.

Discussion

Post-fertilization development is unknown in *Callithamniella*, leading to some uncertainty regarding its higher-level relationships. Its vegetative similarity to *Grallatoria* M.Howe prompted Abbott (1976) to subsume *Callithamniella* into that genus, but this was not accepted by Schneider (1984) and Wynne and Ballantine (1985), and subsequently Abbott herself, who later described a new species in *Callithamniella* (Abbott & Norris 1993). Schneider (1984) indicated that the genera differ in features of the origin of branching, stating that in *Grallatoria reptans* M.Howe indeterminate erect axes never arise on determinate axes, whereas this is a regular developmental feature for prostrate axes of *Callithamniella*. This was refuted by Wynne and Ballantine (1985), who nevertheless maintained the distinction between the two genera based on the number of lateral branches per axial cell (singly or in irregular pairs in *Callithamniella*, two or three in a precise pattern in *Grallatoria*), pattern of tetrasporangial division (cruciate in *Callithamniella*, tetrahedral in *Grallatoria*), and the presence of unicellular (*Callithamniella*) versus multicellular (*Grallatoria*) rhizoids. This last feature is compromised by the inclusion of *C. silvae* Searles (in Searles & Schneider 1989) with multicellular rhizoids, and the report by Schneider (1984) of both uni- and multicellular rhizoids in *C. tingitana* and *C. flexilis*. Baardseth (1941) also described 1–3-celled rhizoids in the protologue of *C. flexilis*. The position of the four-celled carpogonial branches in *C. tingitana* was first described by Stegenga (1988, as *C. capensis*) as borne on the basal cell of a two-celled branchlet, the branchlets arising at short intervals in the apical region of the indeterminate erect axes. Huisman (2018) described a somewhat similar carpogonial branch structure in Australian *G. reptans* as ‘4-celled, curved, borne on periaxial supporting cell (the basal cell of a determinate branch) near the apex of fertile filament’. The structure of carposporophytes in *Grallatoria*, as described by Abbott (1976), suggested to Wynne and Ballantine (1985) a close relationship with *Wrangelia* C.Agardh and the genus was included in the Wrangeliaceae J.Agardh by Huisman (2018). Unfortunately, carposporophytes in *Callithamniella* remain unknown. Stegenga (1988) suggested that the carpogonial branch structure was similar to that of the tribe Heterothamnieae and most closely related to *Antithamnionella*, a genus currently included in the tribe Dohrnielleae of the Ceramiaceae (Athanasiadis 1996).

Our newly generated sequences of *Callithamniella cygnora* indicates a relationship with *Antithamnionella ternifolia*, the generitype, which supports the placement of the genus in the Dohrnielleae tribe of the Ceramiaceae, closely related to *Antithamnionella*. When first described (Feldmann-Mazoyer 1941), the Dohrnielleae (as Dohrniellées) included only *Dohrniella* Funk and *Callithamniella*, with *Antithamnionella* added later. Both *Dohrniella* and *Callithamniella* include species with or without vesicular cells.

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References

- Abbott, I.A. (1976). On the red algal genera *Grallatoria* Howe and *Callithamniella* Feldmann-Mazoyer (Ceramiales). *British Phycological Journal* 11: 143–149.
- Abbott, I.A. & Norris, R.E. (1993). New species of Ceramiaceae (Rhodophyta) from the Hawaiian Islands. *Phycologia* 32: 451–461.

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* 215(3): 403–410.
- Anderson, R.J., Stegenga, H. & Bolton, J.J. (2016). Seaweeds of the South African South Coast. World Wide Web electronic publication, University of Cape Town, <http://southafrseaweeds.uct.ac.za> [accessed on 18 August 2025].
- Athanasiadis, A. (1996). Morphology and classification of the Ceramioideae (Rhodophyta) based on phylogenetic principles. *Opera Botanica* 128: [1]–216.
- Baardseth, E. (1941). The marine algae of Tristan da Cunha. Results of the Norwegian Scientific Expedition to Tristan da Cunha 1937–1938. No. 9: 1–173.
- Cormaci, M., Furnari, G., Alongi, G. & Serio, D. coautrice per le Ceramieae (2023). Flora marina bentonica del Mediterraneo: Rhodophyta - Rhodymeniophycidae III: Ceramiales I (RHODOMELACEAE escluse). *Bollettino Accademia Gioenia di Scienze Naturali in Catania* 56(386): 81–621.
- Feldmann-Mazoyer, G. (1941 ‘1940’). Recherches sur les Céramiacées de la Méditerranée occidentale. (Imprimerie Minerva 5, Rue Clauzel: Alger.)
- García, M. & Huérfano, A. (2006). *Callithamniella tingitana* (Schousb. ex Bornet) Feldmann-Mazoyer (Ceramiales, Rhodophyta), nuevo registro de género y especie para la costa Venezolana. *Hoehnea* 33(1): 1–6.
- Guiry, M.D., in Guiry, M.D. & Guiry, G.M. (2015). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <https://www.algaebase.org> [searched on 23 June 2026].
- Guiry, M.D., in Guiry, M.D. & Guiry, G.M. (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <https://www.algaebase.org> [searched on 13 August 2025].
- Hall, T.A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Huisman, J.M. (2018). *Algae of Australia. Marine benthic algae of north-western Australia. 2. Red algae.* (ABRS & CSIRO Publishing: Canberra & Melbourne.)
- Kim, H.-S. & Lee, I.K. (2012). Algal flora of Korea. Volume 4, Number 5. Rhodophyta: Florideophyceae: Ceramiales: Ceramiaceae I (Non-corticate species). (National Institute of Biological Resources: Incheon.)
- Kitayama, T. (2015). First Record of *Callithamniella tingitana* (Schousboe ex Bornet) Feldmann-Mazoyer (Ceramiales, Rhodophyta) from Japan. *Bulletin of the National Museum of Nature and Science. Series B, Botany* 41(4): 147–153.
- Millar, A.J.K. & Kraft, G.T. (1993). Catalogue of marine and freshwater red algae (Rhodophyta) of New South Wales, including Lord Howe Island, South-western Pacific. *Australian Systematic Botany* 6: 1–90.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp 1–8. <https://doi.org/10.1109/GCE.2010.5676129> PDF.
- Pattengale, N.D., Alipour, M., Bininda-Emonds, O.R.P., Moret, B.M.E. & Stamatakis, A. (2010). How Many Bootstrap Replicates Are Necessary? *Journal of Computational Biology* 17(3): 337–354. <https://doi.org/10.1089/cmb.2009.0179>
- Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A. & Korobeynikov, A. (2020). Using SPAdes de novo assembler. *Current Protocols in Bioinformatics*. 70, e102. <https://doi.org/10.1002/cpbi.102>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61(3): 539–542. <https://www.jstor.org/stable/41515220>
- Searles, R.B. & Schneider, C.W. (1989). New genera and species of Ceramiaceae (Rhodophyta) from the southeastern United States. *Journal of Phycology* 25: 731–740.
- Secilla, A. (2012). La familia Ceramiaceae sensu lato en la costa de Bizkaia. *Guineana* 18: 1–369.
- Schneider, C.W. (1984). Studies on *Antithamnionella*, *Callithamniella* and *Calloseris* (Rhodophyta, Ceramiales) from North Carolina, USA. *Phycologia* 23(4): 455–464.
- Schneider C.W. & Searles R.B. (1997). Notes on the marine algae of the Bermudas. 1. New records of Antithamnieae and Dohrnilleae (Ceramiaceae, Rhodophyta), including *Antithamnionella bermudica* sp. nov. *Phycologia* 36: 12–23.
- Simons, R.H. (1970). Marine algae from southern Africa. 1. Six new species from the inter- and infra-tidal zones. *Investigational Report Division of Sea Fisheries South Africa* 88: 1–13.
- Stamatakis, A. (2014). RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

- Stegenga, H. (1986). The Ceramiaceae (excl. *Ceramium*) (Rhodophyta) of the South West Cape Province, South Africa. *Bibliotheca Phycologica* 74: 1–149.
- Stegenga, H. (1988). Notes on Ceramiaceae (Rhodophyta) from the eastern Cape Province, South Africa. I. New records and remarks on morphology. *Blumea* 33: 371–393.
- Stegenga, H., Bolton, J.J. & Anderson, R.J. (1997). *Seaweeds of the South African west coast*. (Bolus Herbarium, University of Cape Town: Cape Town.)
- Swofford, D.L. (2003) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. (Sinauer Associates, Sunderland.)
- Wynne, M.J. & Ballantine, D.L. (1985). Notes on the marine algae of Puerto Rico. IV. The taxonomic placement of *Grallatoria* (Ceramiaceae, Rhodophyta). *Cryptogamie Algologie* 6: 219–229.