

***Amoenothamnion planktonicum* (Heterothamnieceae, Ceramiaceae, Rhodophyta), an annual drift event along sandy beaches of southern Australia and its affinities**

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Womersley & Norris (1959: 828) gave a fascinating natural-history account of a free-floating red alga, referred to as “*Antithamnion* sp.” at Bridgewater Bay, near Portland Victoria, in April 1959, which formed “algal balls” cast up in a “band ... up to 25 ft. broad and several hundred yards long.” A picture taken at the time graces the cover of Womersley (1998).

Wollaston (1968: 376) described the new genus *Amoenothamnion* E.M.Wollaston with three new species, the type, *A. planktonicum* E.M.Wollaston (type from Bridgewater Bay, Victoria, the “*Antithamnion* sp.” of Womersley & Norris above), *A. elongatum* E.M.Wollaston (type from Kangaroo I., South Australia), and *A. minimum* E.M.Wollaston, type also from Kangaroo I.). A further species, *A. lycopodioides* Stegenga was later described from South Africa by Stegenga (1996, type from Waenhuiskrans [Arniston], Western Cape).

Amoenothamnion planktonicum is now known from Dongara, Western Australia to Gabo I., Victoria, and south-eastern Tasmania to Twofold Bay, New South Wales (Wollaston 1968; Womersley 1998).

Athanasiadis (1996: 184) established a new genus *Leptoklonion* Athanasiadis based upon *L. elongatum* (E.M.Wollaston) Athanasiadis, later referred by Womersley (1998: 190) to *Leptoklonion fastigiatum* (Harvey) Womersley (type from Georgetown, Tasmania).

Amoenothamnion was referred to the tribe *Heterothamnieceae* E.M.Wollaston by Wollaston (1968: 407) and by Womersley & Wollaston in Womersley (1996: 156). The tribe *Heterothamnieceae* currently includes *Acrothamniopsis* Athanasiadis & Kraft, *Amoenothamnion*, *Elisiella* Womersley, *Heterothamnion* J.Agardh (the type), *Tetrathamnion* E.M.Wollaston and *Trithamnion* E.M.Wollaston (Guiry & Guiry 2023). However, no molecular information is currently available for the types of any of these genera.

Samples of *Amoenothamnion planktonicum* were collected on 10 January 2021 by John Miller, at Bridgewater Bay, Victoria. The samples were collected in waist-deep water about 10 m from the shore using a small, hand-held dip-net. Collecting in the water rather than beach-washed specimens ensured that they were fresh, intact, and largely free of sand and other debris. The specimens were then transferred to a small seawater-filled dish on the beach where the samples were further sorted and cleaned of any obvious detritus, and prepared in four ways for transport:

1. Some specimens were added to vials with 5% Formalin in seawater for morphological examination.
2. Some were pat-dried with tissues and placed in small plastic clip-lock bags containing silica crystals.
3. Some specimens were dried and mounted as voucher specimens.
4. Some specimens were added to small vials containing just seawater from the site.

All specimens were processed and packaged the same day and mailed to J.A. West. Living specimens were observed and photographed on the stereomicroscope and compound scope. Silica-dried specimens were prepared for molecular analyses.

Fig. 1A shows *Amoenothamnion* epiphytes on the *Plocamium* sp. host. Fig. 1B is larger view of a single thallus showing overall branching patterns. The main characters are whorl-branchlets, abundant hair cells and absence of gland cells. Fig. 1D shows the upper ends of axial cells (up to about 200 μm diam.) with 1–3 whorl-branchlets with rounded basal cells bearing short branches with acute terminal cells. Fig. 1C shows dense clusters of obovate spermatangia (4–5 μm wide) visible on basal cells of whorl-branchlets. No female thalli were found. Fig. 1E shows ovoid-subovoid tetrasporangia (50–60 μm diam.) with decussate divisions, mostly solitary and sessile on whorl-branchlets. Figs 1D & E show numerous elongate, narrow, undivided hair cells (about 6 μm wide and up to 350 μm long) arising from whorl-branchlets. Hair-cell formation is common among red algae. In *Spyridia filamentosa* (Wulfen) Harvey, nutrient levels and light intensities influenced formation and morphology of hair cells (O'Connor & West 1991). Figs 1D & E show axial cells containing many parallel, peripheral, narrow (4–7 μm diam.) plastids of variable lengths, each divided or branched at the upper end as they elongated.

DNA was extracted from the dried *Amoenothamnion* sample in silica using a CTAB method described by Cremen & al. (2016). An Illumina sequencing library was prepared with the VAHTS Universal DNA kit and ca. 3 gigabases of sequencing data was generated on the Novaseq platform (paired-end, 150 bp) by Azenta. Raw sequencing data were assembled with megahit v.1.2.9 (Li & al. 2016). Plastid genome contigs were identified and gene predictions were made as described elsewhere (Costa & al. 2016; Marcelino & al. 2016). The *rbcL* gene was extracted from the contig and used in downstream analysis. The SSU gene was extracted from the same dataset.

For phylogenetic analyses, sequences of the *rbcL* and SSU were added to sequences downloaded from GenBank and aligned using MAFFT in Geneious Prime® 2019.2.3. Phylogenetic analyses were performed using the maximum likelihood (ML) method, the best-fit model of each gene sequence was determined with IQTREE2 (Minh & al. 2020) and ModelFinder (Kalyaanamoorthy & al. 2017). The *rbcL* was partitioned by codon position and separate DNA substitution models were chosen for each codon. The SSU was not partitioned. Support for individual internal branches was determined by non-parametric bootstrap (500 replicates) (Felsenstein 1985).

Bayesian inference analysis also partitioned *rbcL* by codon position and implemented in MrBayes3 (Ronquist & Huelsenbeck 2003). The model partitions were unlinked, with variable rates, and six rate categories. Two parallel runs of four Markov chain Monte Carlo were performed for 3,000,000 generations, sampling every 1,000 generations. Estimated samples size, split frequencies, and stationarity were checked after each run. Post-analysis, 10% of generations were removed as a burn-in and posterior probabilities visualized. Figtree v1.1.4 (Rambaut 2009) and Canvas X Draw (Canvas GFX, Inc., Boston, MA, USA) were used to manipulate trees for presentation. Species of *Rhodomelaceae* were used as outgroups (e.g. *Melanothamnus*) for *rbcL* and *Schimmelmanniaceae* (e.g. *Schimmelmannia*) for the SSU datasets.

With our *rbcL* dataset (1467 bp alignment, model used: 1st codon–GTR+F+I+G4; 2nd codon: TIM+I+G4; 3rd codon: TIM +F+I+G4), the sample of *Amoenothamnion planktonicum* from Bridgewater Bay groups with members of the *Ceramieaceae* (e.g. *Antithamnion*, *Ceramium*, *Pterothamnion*; 74% ML BS, 1.0 PP). Its relationships within this clade are unsupported with this dataset. It is excluded, for example, from the clade containing *Antithamnion* and *Hollenbergia*. Many of the generic relationships seen in this dataset indicate that further taxonomic work is needed as many genera are not monophyletic (*Ceramium*, *Hollenbergia*, *Griffithsia*).

Our SSU dataset (1819 bp alignment, model used: TN+F+I+G4), again shows that *Amoenothamnion planktonicum* does not group with any particular genus with any support. It is in an unsupported polytomy with *Tetrathamnion* sp. GWS464 (and *Ceramium* and *Centroceras*), but is excluded from the clade containing *Heterothamnion* sp. GWS460 (and *Antithamnionella* spp.). Both *Heterothamnion* and *Tetrathamnion* are currently assigned to the tribe *Heterothamnieae* (Guiry & Guiry 2023), along with *Amoenothamnion*. The monophyly of this tribe is thus questionable and further work is needed, as several genera have not been sequenced, plus many backbone relationships within the *Ceramieaceae* are unsupported with the SSU data set, as they are with *rbcL*. Nevertheless there are indications in Figs 2 and 3 that the tribe *Heterothamnieae* is sister to the tribe *Ceramieae*.

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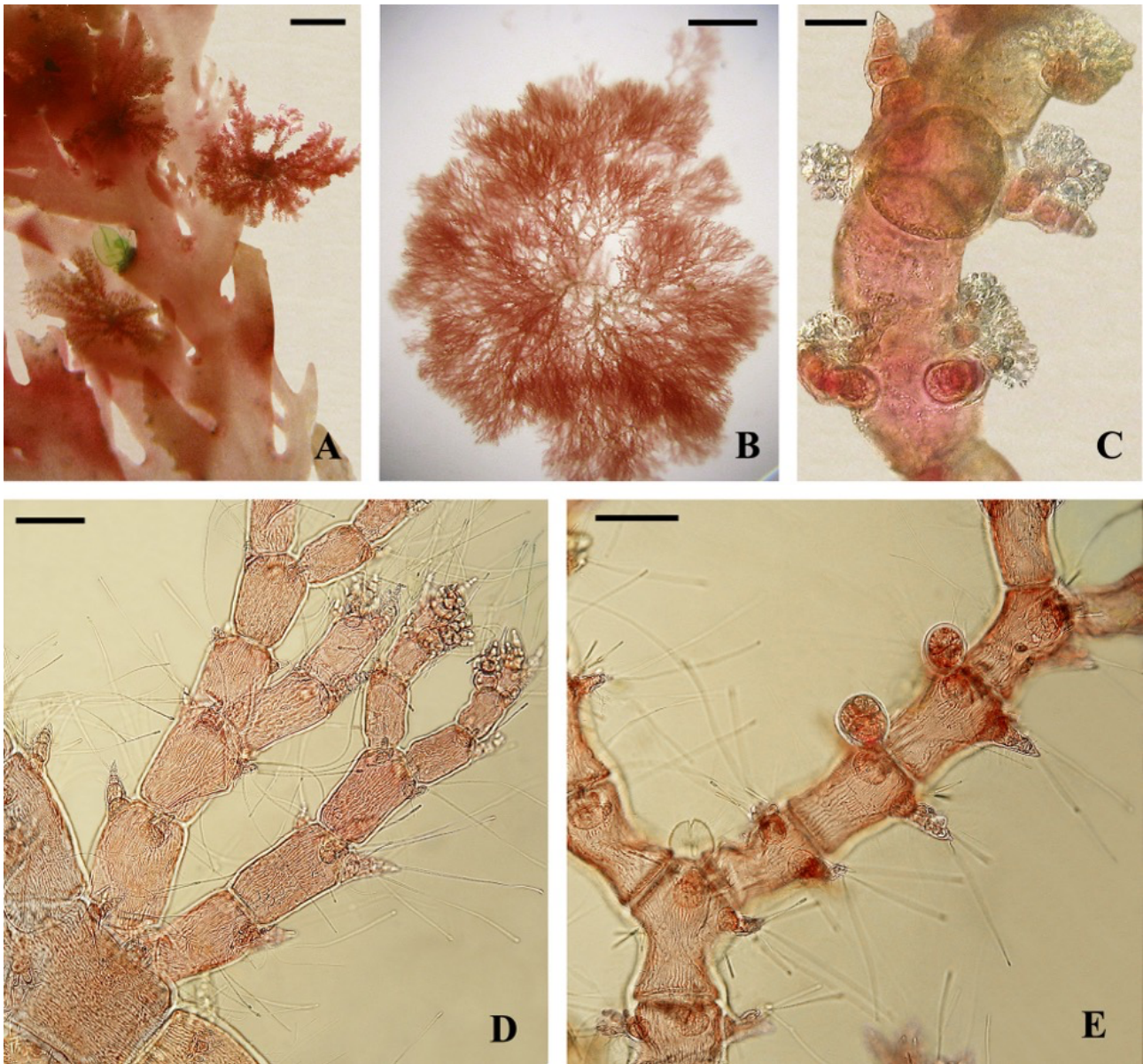


Fig. 1A. *Amoenothamnion planktonicum* epiphytes on *Plocamium* sp. from drift collection. Scale bar = 1.0 cm. **Fig. 1B.** Whole *Amoenothamnion* thallus. Scale bar = 0.6 mm. **Fig. 1C.** Male branch axis with whorl-branchlets bearing spermatangial clusters on basal cells. Scale bar = 50 μm . **Fig. 1D.** Vegetative branches with axial cells bearing 1–3 whorl-branchlets and many hair cells from branchlet cells. Narrow plastids showing intercalary divisions as plastids elongate. Scale bar = 100 μm . **Fig. 1E.** Tetrasporophyte with uppermost two decussately divided tetrasporangia visible on whorl-branchlets, lowermost discharged empty sporangium. Scale bar = 100 μm .

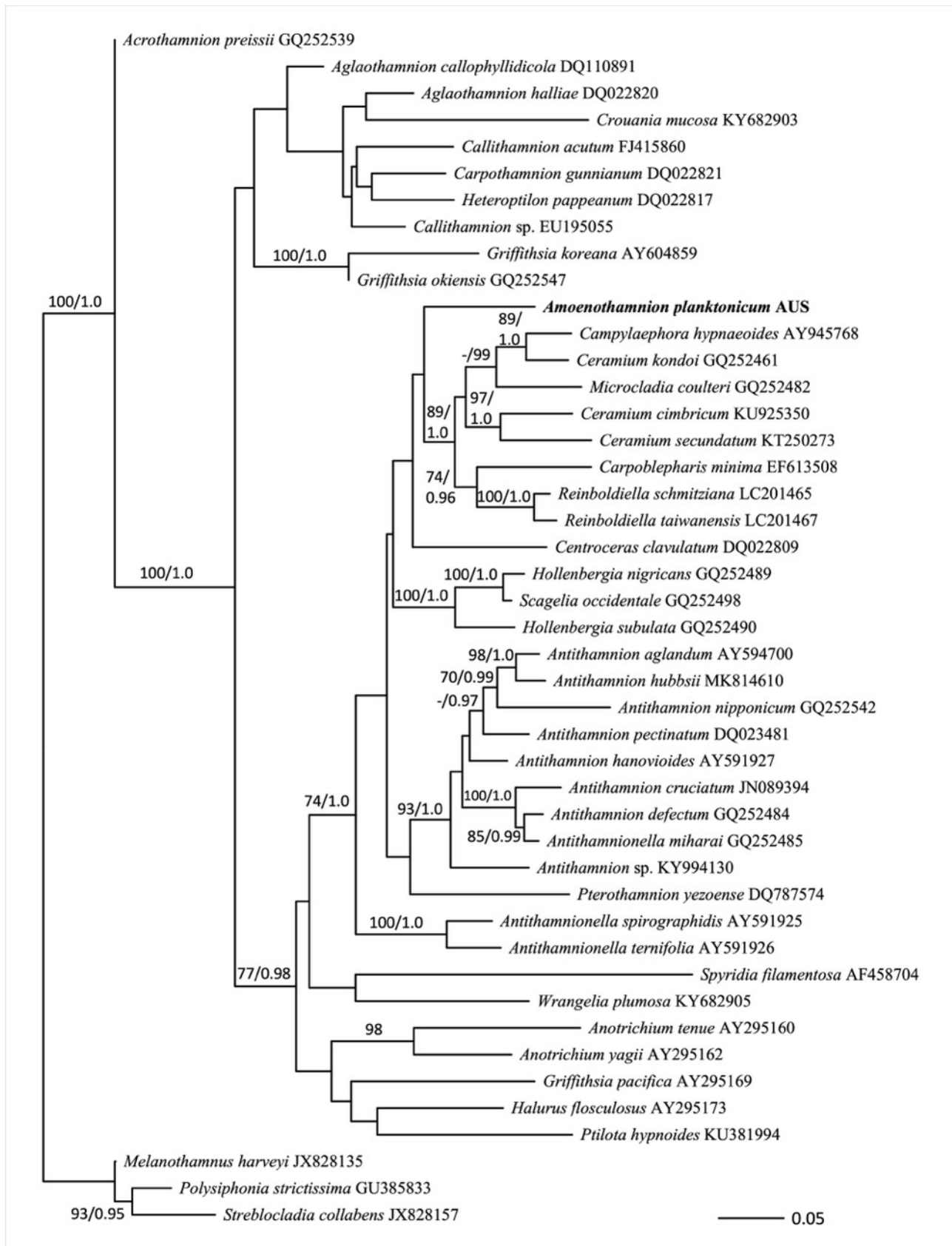


Fig. 2. Phylogenetic relationships based on ML analysis of the *rbcL* data set (1467 base pairs) of *Amoenothamnion planktonicum* from Bridgewater Bay, Victoria (in bold).

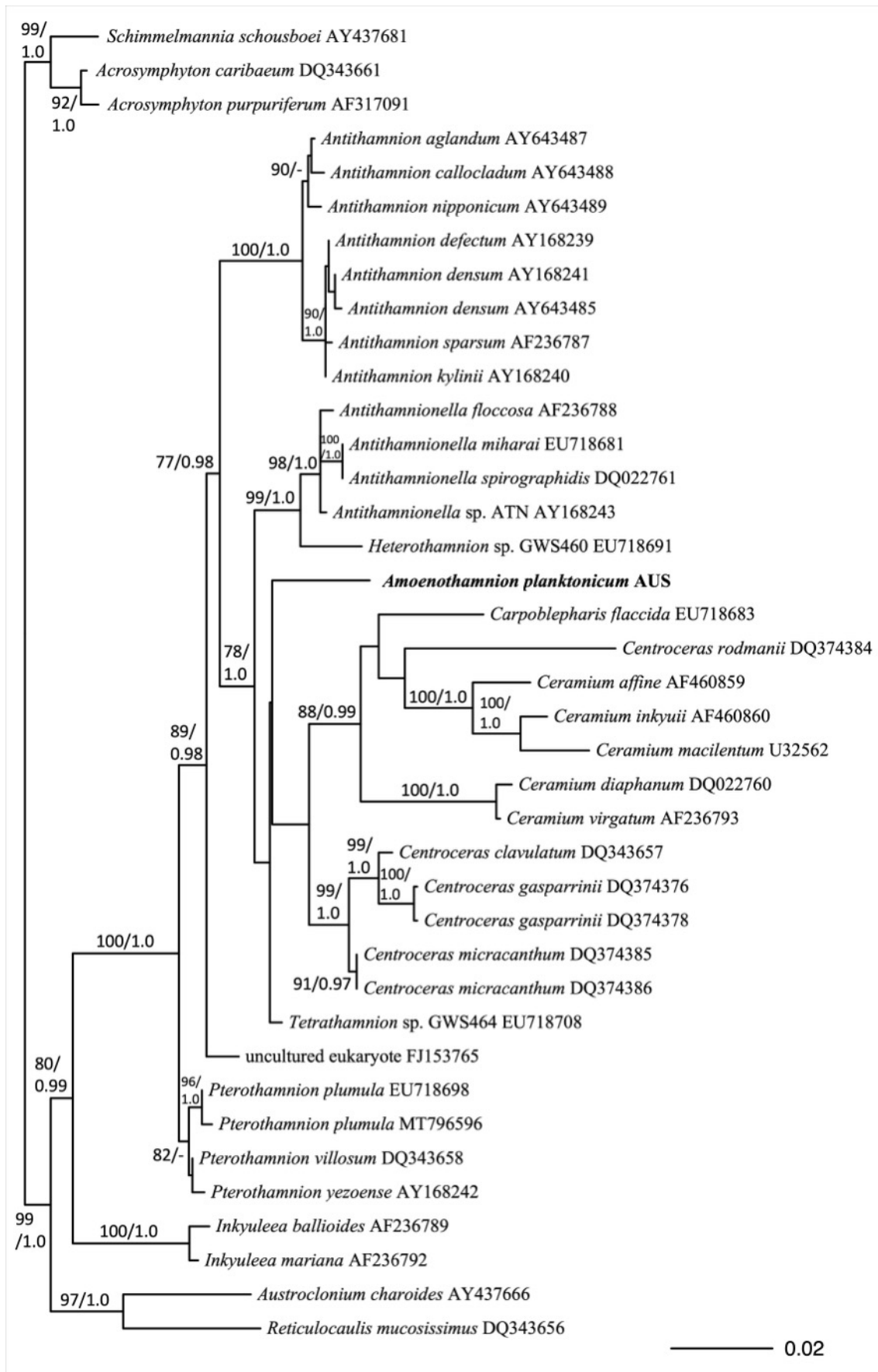


Fig. 3 Phylogenetic relationships based on ML analysis of the SSU data set (1819 base pairs) of *Amoenothamnion planktonicum* (in bold).