Supplementary information

for

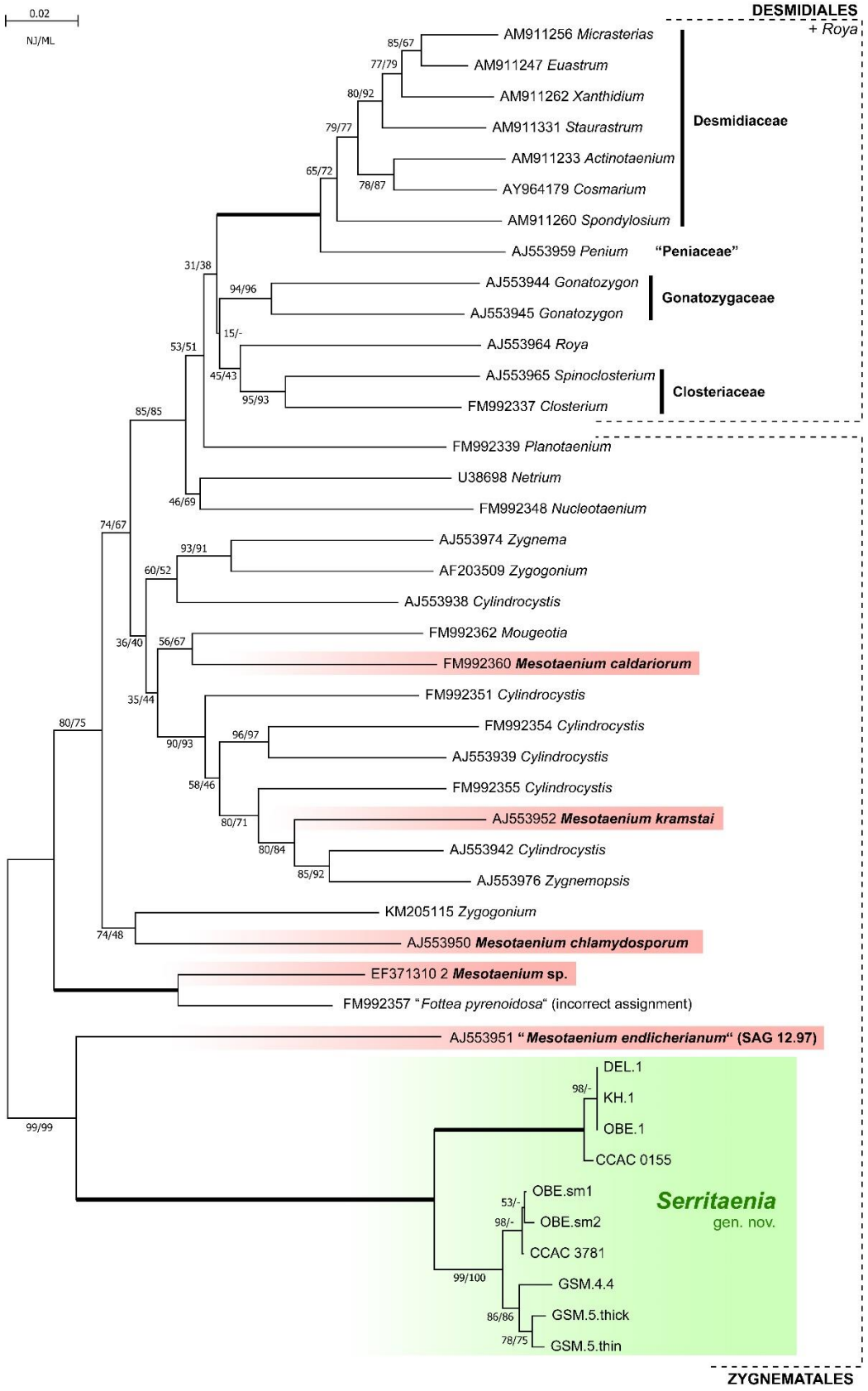
‘Sunscreen mucilage: a photoprotective adaptation found in terrestrial green algae (Zygnematophyceae)’

Busch & Hess, 2021

Supplementary Table S1: Studied *Serritaenia* strains and associated data (collection sites and accession numbers of the Central Collection of Algal Cultures (CCAC) and *rbc*L gene sequences).

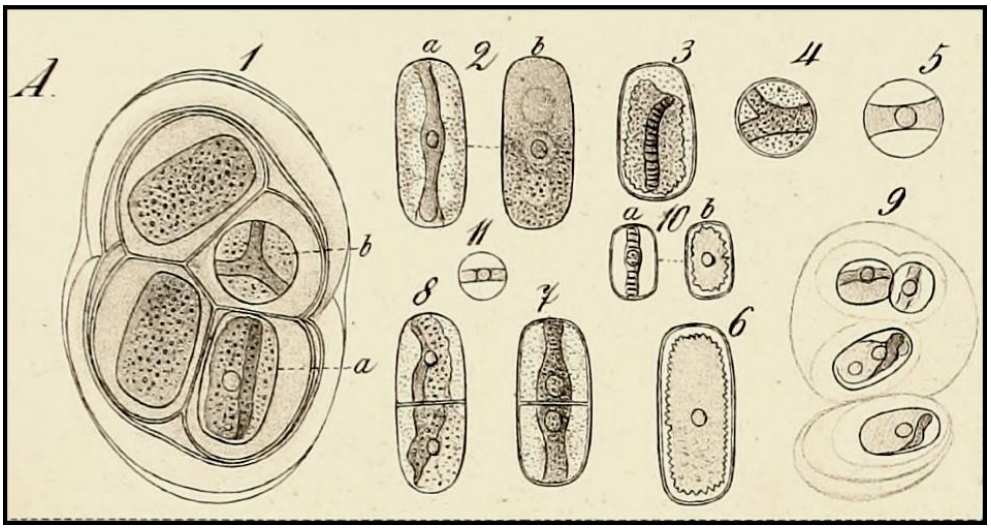
Strain	Taxonomy	Collection site / coordinates	Sample information	CCAC	*rbc*L
DEL.1	*Serritaenia* sp.	Dellbrück, Cologne (DE)	Blackish bryophytes on tree bark (forest)	CCAC 9318	MW159370
		50.974429, 7.091823			
KH.1	*Serritaenia* sp.	Frei-Laubersheim, Bad Kreuznach (DE)	Blackish bryophytes on dead wood (forest)	CCAC 9319	MW159371
		49.806280, 7.882115			
OBE.1	*Serritaenia* sp.	Monsau, Wiehl (DE)	Blackish forest soil and bryophytes	CCAC 9320	MW159369
		50.958607, 7.581137			
CCAC 0155	*Serritaenia* sp.	Strohner Maarchen, Strohn (DE)	Slurry with various desmids	CCAC 0155	FM992358
		50.122289, 6.928597			
OBE.sm2	*Serritaenia* sp.	Wohlsberg, Wiehl (DE)	Blackish bryophytes on dead wood (forest)	CCAC 9321	MW159373
		50.961667, 7.578056			
OBE.sm1	*Serritaenia* sp.	Wohlsberg, Wiehl (DE)	Blackish bryophytes on dead wood (forest)	CCAC 9322	MW159372
		50.961667, 7.578056			
CCAC 3781	*Serritaenia* sp.	Naafbachtal (DE)	Epiphytic bryophytes on tree bark	CCAC 3781	MW159374
		n/a			
GSM.5.thin	***S. testaceovaginata***	Clingmans Dome Rd, Great Smoky Mountains, NC (USA)	Red-brown biofilm on wet rock surface	CCAC 9324	MW159377
		35.568104, -83.481939			
GSM.5.thick	*Serritaenia* sp.	Clingmans Dome Rd, Great Smoky Mountains, NC (USA)	Red-brown biofilm on wet rock surface	n/a	MW159376
		35.568104, -83.481939			
GSM.4.4	*Serritaenia* sp.	Forney Ridge Trail, Great Smoky Mountains, NC (USA)	Bryophytes with brown mucilage	CCAC 9323	MW159375
		35.555917, -83.497417			

Supplementary Fig. S1: Unrooted Neighbour-Joining phylogeny of 43 zygnematophycean *rbc*L gene sequences displaying the polyphyly of *Mesotaenium* (red) and the position of the new genus *Serritaenia* (green). Support values are shown on the respective branches (NJ/ML). Branches with maximum support (100/100) are bold. The scale bar represents 0.02 nucleotide substitution per site.

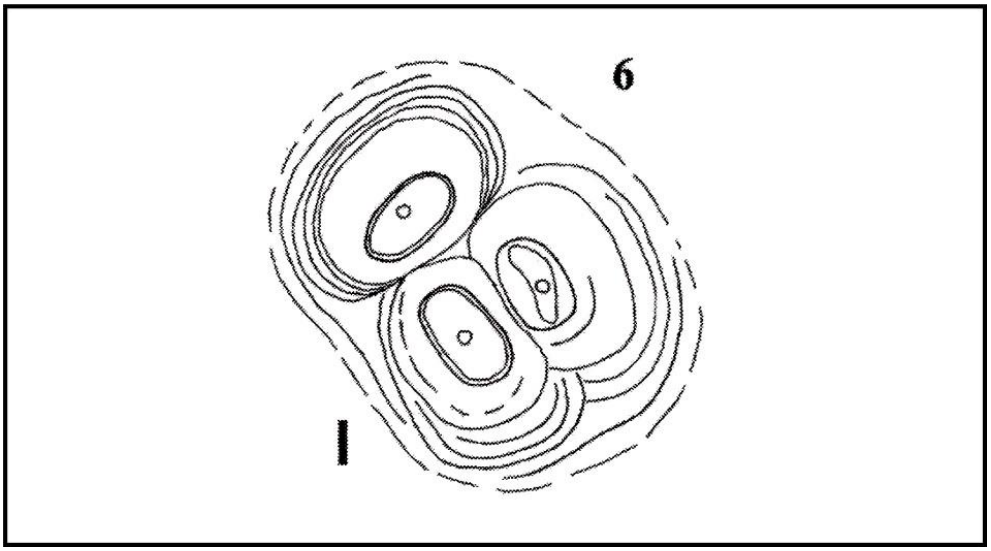


Supplementary Figs S2 and S3: Illustrations published with the original descriptions of *Mesotaenium braunii* (S2: *A, 1-8*), *M. braunii* var. *minus* (S2: *A, 9-11*), and *M. testaceovaginatum* (S3). The illustration of *M. testaceovaginatum* (S3) is designated as lectotype for this species.

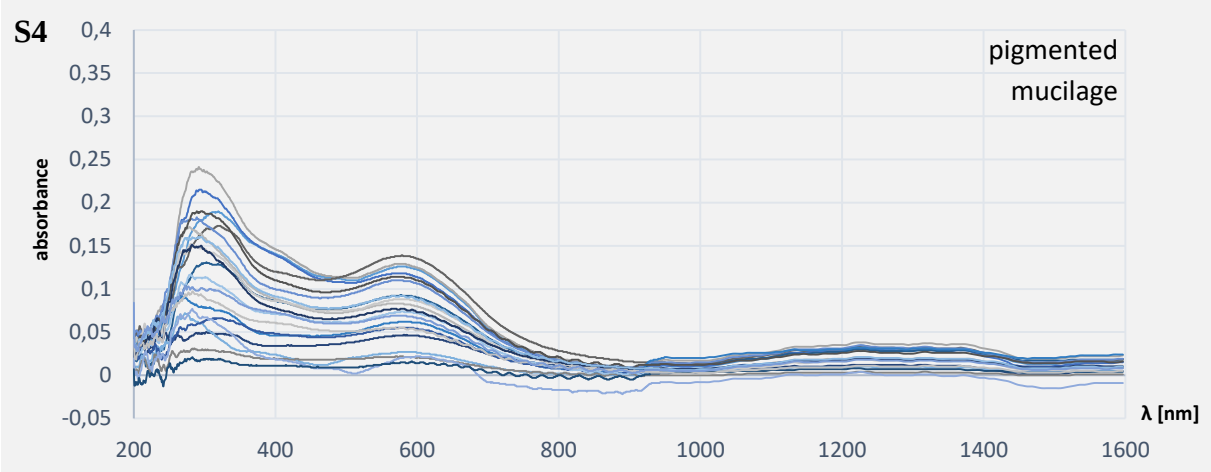
S2

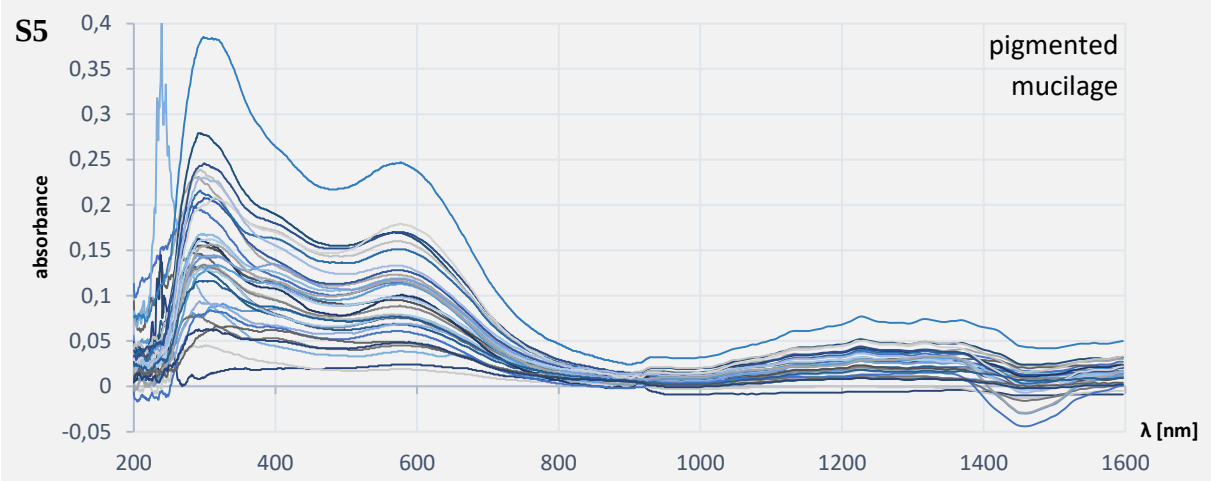


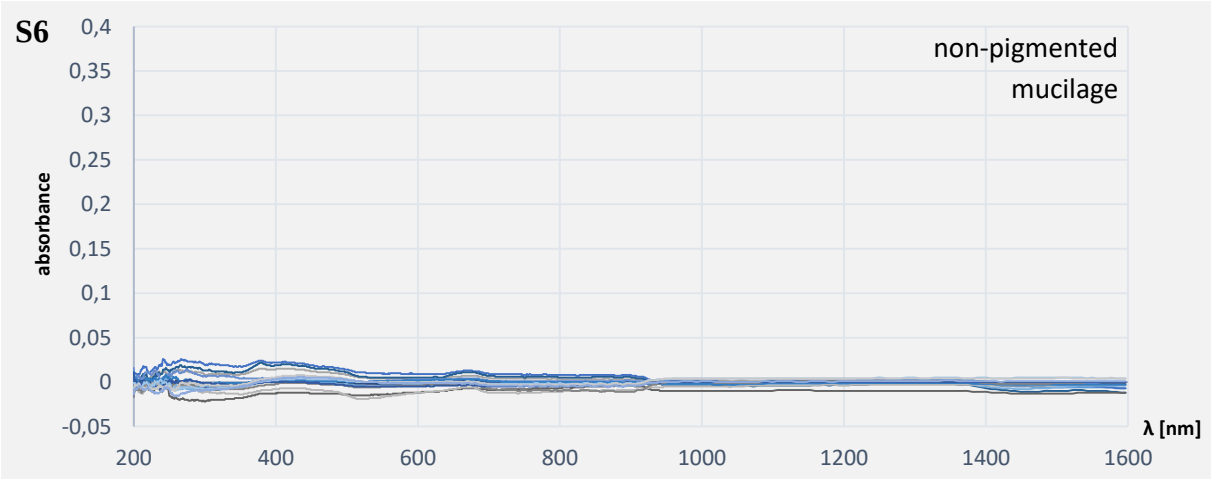
S3



Supplementary Figs S4-S6: Microspectrophotometric measurements taken from the mucilage of *Serritaenia testaceovaginata* (strain GSM.5.thin) over a spectral range of 200–1600 nm. S4 and S5 display absorbance spectra of mucilage with varying degree of pigmentation from two independent wet mounts with 21 and 33 measurements, respectively. S6 displays 15 absorbance measurements of non-pigmented mucilage for comparison.







Supplementary Text:

Rationale for the new genus *Serritaenia* and taxonomy of its members

The studied algae resemble certain species of the ill-defined and polyphyletic genus *Mesotaenium* NÄGELI, which – according to AlgaeBase – contains 29 recognised species and 39 infraspecific taxa (Guiry & Guiry, 2020). As revealed by former molecular inferences (Gontcharov *et al.*, 2004; Gontcharov & Melkonian, 2010) and shown in our *rbc*L phylogeny as well, some of these *Mesotaenium* species fall in several (at least four) not directly related evolutionary lineages of the Zygnematophyceae. Although the members of these lineages exhibit clear differences in cellular details (Gontcharov, 2008), all of them are – until now – referred to as *Mesotaenium*, and a taxonomic revision of these algae is pending.

The following species resemble the algae studied in this work (with homotypic synonyms; ≡):

M. braunii DE BARY

M. braunii* var. *minus DE BARY
≡ *M. macrococcum* var. *minus* (DE BARY) COMPÈRE
≡ *Palmogloea macrococca* var. *minor* (DE BARY) RABENHORST

M. macrococcum (KÜTZ.) J.ROY & BISSET
≡ *Palmogloea macrococca* KÜTZ.

M. testaceovaginatum FUČÍKOVÁ, J.D.HALL, J.R.JOHANS. & R.L.LOWE

Common defining characters of these species and our strains are cylindrical cells, a length-to-width ratio of maximum 2–3 (depending on the species), and a plate-like chloroplast in the centre of the cell (not parietal) with serrate or crenate edges. Furthermore, the cells are typically surrounded by mucilage (often in form of layered capsules) and thrive in terrestrial habitats (de Bary, 1858; West & West, 1904; Fučíková *et al.*, 2008). With that, these algae differ fundamentally from *M. endlicherianum* NÄGELI (the type species of the genus *Mesotaenium*), whose cells are more slender (length-to-width ratio 3–4), contain a chloroplast with smooth margins, and seem to lack conspicuous extracellular mucilage (Nägeli, 1849). In addition, *M. endlicherianum* is not reported to show the angled cell arrangement observed in our strains shortly after cell division, and, instead, divides in a chain-like manner.

Unfortunately, at present there is no algal strain available that closely matches the description of *M. endlicherianum*. This also applies to strain SAG 12.97 (the closest known relative of the algae studied here), which was probably misidentified and repeatedly referred to as '*M. endlicherianum*' (Gontcharov *et al.*, 2003, 2004; Gontcharov, 2008; Matasci *et al.*, 2014; Cheng *et al.*, 2019). Hence, we lack important phylogenetic information about the type species of *Mesotaenium* and cannot place this generic name with certainty. However, the marked phenotypic differences between *M. endlicherianum* NÄGELI and the algae studied here justify a separate genus name for the latter. In the context of potential genus names, it also has to be noted that *Mesotaenium macrococcum* was first described as *Palmogloea macrococca* KÜTZ. (Kützing, 1845). The genus *Palmogloea*, however, was established earlier (Kützing, 1843) with *P. protuberans* (SM.) KÜTZ. as the only species, which hence represents the type species of

the genus. This type species is considered a chlorophycean green alga (Fott & Nováková, 1971), so that the genus *Palmogloea* is not appropriate for zygnematophyceaen algae. Consequently, the studied algae require a new genus name and we here introduce *Serritaenia* gen. nov. (see main text for formal description).

The morphologically 'simple' *Mesotaenium* species have mostly been described on the basis of the cell shape and size of vegetative material. The lack of meaningful original descriptions in several cases and the resulting uncertainties led to a convoluted taxonomic history with a high number of infraspecific taxa and synonyms (Guiry & Guiry, 2020). At present there are a few names used for Zygnematophyceae that are here included in the genus *Serritaenia*. As detailed below, the available information about these taxa varies, and there are still some questions to be solved. Based on a careful comparison of our *Serritaenia* strains with published taxa (including original material), we follow a conservative approach and form only two new combinations (Art. 6.10. and 41 in Turland *et al.*, 2018) in this study. As the holotype of one species (*M. testaceovaginatum*) was evidently lost, we designate a cited illustration published along with the original description as lectotype (Art. 7.3. and 9.3. in Turland *et al.*, 2018) and provide a supporting epitype connected to genetic information (Art. 9.9. in Turland *et al.*, 2018). Some details about relevant species follow.

***Mesotaenium macrococcum* (KÜTZ.) J.ROY & BISSET** is a widely accepted name for Zygnematophyceae that closely resemble *Serritaenia* species (Lenzenweger, 2003; Coesel & Meesters, 2007; Brook & Williamson, 2010; Ettl & Gärtner, 2014). As already mentioned above, it is a nomenclatural synonym of *Palmogloea macrococca* KÜTZ., a rather ill-defined species that caused much debate about its identity (Archer 1864, 1866; Hicks 1864). As far as we know, the question whether *P. macrococca* is a zygnematophycean green alga is still not solved, but the name *M. macrococcum* found its way in contemporary taxonomic literature. The current circumscription of this taxon in monographs and identification guides is quite broad and probably based on a variety of biological species as indicated by the stated cell sizes (e.g. cell width of 5–19 µm in Ettl & Gärtner, 2014). We here show that genetically separate *Serritaenia* strains have a relatively narrow and stable cell width range, emphasising the need to reflect the observed phenotypic diversity on the taxonomic level. Unfortunately, the description of *P. macrococca* is fairly meagre (Kützing, 1845), and the associated drawing difficult to interpret (Kützing, 1847). To assess whether *P. macrococca* is a *Serritaenia*-like alga and can be considered for a new combination, we studied the original material of that species (deposited at the Naturalis Biodiversity Center in Leiden, NL). Based on our microscopic examination alone, we cannot solve this question with certainty (genetic work in progress), so that we refrain from establishing a new combination of *P. macrococca* at this point. However, the cells of *P. macrococca* turned out to be markedly smaller than expected (about 10 µm wide) and, thus, are not identical with *M. braunii* (cells 15–19 µm wide). Consequently, the synonymy of these taxa seems to be unjustified and the name *M. braunii* becomes relevant for the large-celled representatives of the *Serritaenia*-clade.

***Mesotaenium braunii* DE BARY** (illustrated in Supplementary Fig. S2) was established in a very detailed description and there is little doubt that this species belongs to the *Serritaenia*-clade. De Bary clearly depicted the chloroplast morphology, the mucilage capsules, and even mentioned the presence of an extracellular (violet) pigment (de Bary, 1858). The stated cell dimensions of *M. braunii* (15–19 µm) closely match those of our *Serritaenia* strains DEL.1, KH.1 and OBE.1. Here, we introduce a new combination, *Serritaenia braunii* comb. nov., but for now refrain from selecting a reference strain, as there might be the option to study original material of *M. braunii* in future (currently inaccessible to us).

***Mesotaenium braunii* var. *minus* DE BARY** (illustrated in Supplementary Fig. S2) was described as a smaller variety that reaches only half the size of *M. braunii* (= cell width 8–9.5 µm). Thus, it conforms with the small-celled *Serritaenia* strains OBE.sm1, OBE.sm2, CCAC3781 and GSM4.4, which show marked genetic distances to the large-celled *Serritaenia* strains. The small-celled strains are, however, not monophyletic, complicating the application of the name *M. braunii* var. *minus*. In addition, we found that the type material of *Palmogloea macrococca* resembles *M. braunii* var. *minus* in size, questioning the justification of the latter name due to priority (in case these organisms are indeed identical; pending genetic analyses). Because of this uncertainty, we refrain from creating any new combination at this point. *M. braunii* var. *minus* is also known under its homotypic synonyms *M. macrococcum* var. *minus* (DE BARY) COMPÈRE and *Palmogloea macrococca* var. *minor* (DE BARY) RABENHORST.

***Mesotaenium testaceovaginatum* FUČIKOVÁ, J.D.HALL, J.R.JOHANS. & R.L.LOWE** (illustrated in Supplementary Fig. S3) was most recently described from the Great Smoky Mountains National Park (North Carolina, USA) on a purely morphological basis (Fučíková *et al.*, 2008). In search of this species at its type locality, we found two morphotypes* (GSM.5.thin and GSM.5.thick), which differed in morphology, *rbc*L gene sequence, and their ability to grow under laboratory conditions. Both morphotypes matched the original description of *M. testaceovaginatum* to some extent: The cell dimensions provided in the written description (cell width 12–14 µm) and the drawing (Fig. 6 on p. 55 in Fučíková *et al.*, 2008) correspond to GSM.5.thin, while the cells depicted in the micrographs (Figs 26–28 on p. 55 in Fučíková *et al.*, 2008) rather resemble GSM.5.thick. As confirmed by one of the authors, a mix-up of the two co-occurring morphotypes in the description is, indeed, possible (pers. comm. K. Fučiková). Unfortunately, the holotype of *M. testaceovaginatum*, the aldehyde-fixed natural sample *GSM 10/23/04 J5A* (John Carroll University, Ohio), was lost. Furthermore, the holotype is very likely to contain both morphotypes, along with other algal taxa, so that the designation of a lectotype from the cited illustrations (as part of the original material) seems reasonable (Art. 9.3. in Turland *et al.*, 2018). In agreement with the cell dimensions given in the written description, we designate the drawing (Fig. 6 on p. 55 in Fučíková *et al.*, 2008; reproduced in Supplementary Fig. S3) as lectotype (see also Art. 9.14. in Turland *et al.*, 2018), and fixed cells of strain GSM.5.thin as supporting epitype. In addition, we establish a new combination, *Serritaenia testaceovaginata* comb. nov., and emend the written description of that species (see main text). As *S. testaceovaginata* currently has the most detailed (and unambiguous) description, we designate it as the type species of the genus *Serritaenia*.

Furthermore, there are some infraspecific taxa to be discussed. *M. macrococcum* var. *micrococcum* (KÜTZ.) WEST & G.S. WEST, synonymous with *M. micrococcum* (KÜTZ.) KIRCHN., is not considered for inclusion in the genus *Serritaenia*. Although these names were sometimes regarded as synonyms of *M. braunii* var. *minus* (Krieger, 1937), they are clearly based on *Palmogloea micrococca* KÜTZ. (see West & West 1904). The latter species, however, differs drastically from the algae studied here and rather represents *Coccomyxa* (Trebouxiophyceae, Chlorophyta) or relatives (Kützing, 1847, 1849). The varieties *M. macrococcum* var. *lagerheimii* WILLI KRIEG. and *M. macrococcum* var. *truncatum* (WEST & G.S. WEST) WILLI KRIEG. both display major morphological differences to *Serritaenia* and are not considered for inclusion in this genus as well. Finally, there is evidence of a species named *Palmogloea macrococca* var. *nigrescens* C. CRAMER in the *Index Nominum Algarum* database (Silva Center for Phycological Documentation, The University Herbarium at UC Berkeley, USA), but we were unable to locate its original description so far. Given the meaning of the name (Latin: *nigrescens* = blackening, darkening), it might be possible that Cramer's variety is a *Serritaenia*-clade member (name pointing to blackish extracellular pigmentation?). Future in-depth studies may shed some light on these orphan taxa.

* The term ‚morphotype' is here used in the broad sense and does not denote a taxonomic level.

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