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Spongophloea, a new genus of red algae based on *Thamnoclonium* sect. *Nematophorae* Weber-van Bosse (Halymeniales)

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The red algal order Halymeniales contains a relatively high percentage of sponge-associated taxa. These species are predominantly classified in two genera, *Thamnoclonium* and *Codiophyllum* (and to a lesser extent *Carpopeltis*), and are chiefly distributed in temperate waters along the South African and Australian coasts. Three rare species of *Thamnoclonium* (*T. tissotii*, *T. treubii* and *T. procumbens*), however, were originally described by Weber-van Bosse from tropical localities in Indonesia, the Philippines and northern Australia. These formed her new *Thamnoclonium* sect. *Nematophorae* and differ from typical *Thamnoclonium* in having a pseudoparenchymatous medulla in vegetative tissue and in the production of moniliform chains of cells from the cortex. Recent collections of *T. tissotii* from Western Australia included tetrasporangial and cystocarpic specimens, the latter previously unrecorded for the section. Phylogenetic analyses of *rbc*L sequence data generated from these and other specimens revealed that the genus *Thamnoclonium* is presently polyphyletic. Although the phylogenetic tree was not completely resolved, sponge-algal associations in the Halymeniales seem to have evolved independently at least four times. Specimens of *T. tissotii* formed a sister relationship with *Codiophyllum*. Thus, both morphological and DNA sequence analyses support the segregation of *Thamnoclonium* sect. *Nematophorae* as a new genus, for which the name *Spongophloea* is proposed, in recognition of its seemingly obligate relationship with the sponge that coats the thallus surface.

Key words: Australia, Halymeniales, Rhodophyta, Siboga, *Spongophloea* gen nov., *Thamnoclonium*, *T. tissotii*, *T. treubii*, *T. procumbens*

Introduction

The Dutch Siboga expedition to the Netherlands East Indies [= the Indonesian Archipelago] in 1899–1900 is rightly regarded as one of the great biological voyages (see Van Aken, 2005 for an overview). Led by Max Weber (1852–1937), professor of Zoology at the University of Amsterdam, the six scientific expeditioners also included his wife Anna Weber-van Bosse (1852–1942), who had been a pupil of Dutch botanist Hugo de Vries and had developed an interest in the marine algae. A large number of algal collections were made during the expedition and these eventually formed the basis of numerous monographs, mostly authored by Weber-van Bosse herself, these in combination representing perhaps the greatest phycological contribution of the early 20th century (Barton, 1901; Foslie, 1904; Weber-van Bosse & Foslie, 1904; Weber-van Bosse, 1904a, 1904b, 1913, 1921, 1923, 1928; Gepp & Gepp, 1911). Weber-van Bosse was subsequently awarded an honorary doctorate from the University of Utrecht, the first Dutch woman to receive one.

Prior to the completion of her major monographs, however, Weber-van Bosse (1910) preempted herself when she described two species of sponge-associated red algae, *Thamnoclonium treubii* and *T. tissotii*, which she included in her new section *Nematophorae* of *Thamnoclonium*. The first of these species was based on a Siboga collection from the Sulu Archipelago, the second on a collection from (supposedly) 'Iles Kei' and Thursday Island. These two species differed from typical

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Thamnoclonium (sect. *Anematophorae* Weber-van Bosse = sect. *Thamnoclonium*) in the production of moniliform filaments from the surface of the cortex ('Frondes ramulos cum fili cellularum moniliformibus gerentes'). A third species, *Thamnoclonium procumbens* was later added by Weber-van Bosse in the monographs of the Siboga Expedition (Weber-van Bosse, 1921: 251). All of the species have sponge coatings and produce tetrasporangia (at the time the only known reproductive structures) in small surface leaflets (not known for *T. procumbens*).

Subsequent records of these three species are exceedingly scant. AlgaeBase (Guiry & Guiry, 2010) includes no further records of either *T. treubii* or *T. procumbens*, and the cited records of *T. tissotii* (Lewis, 1984: 21; J.A. Phillips, 1997, 2002, these citing Bailey, 1913: 829; Cotton, 1913: 254; Weber-van Bosse 1921: 251; Lucas 1931: 57) are derived from either the protologue or a collection from Dunk Island (Queensland, Australia) that was made by E.J. Banfield in February 1910 and forwarded by F.M. Bailey to A.D. Cotton at Kew. Cotton (1913) described the distinctive moniliform filaments in the specimens and stated that Weber-van Bosse had confirmed the determination of these specimens, which are now housed at the British Museum (BM) and the Brisbane Herbarium (BRI). The former collection also includes Weber-van Bosse's original correspondence, dated 25 November 1910, in which she states that *T. tissotii* is 'very variable in outward appearance'. Cotton (1913) also felt that the species 'would appear to be frequent', but this has not been borne out by subsequent collections, as none has been reported from the region in close to a century.

A population of plants referable to *Thamnoclonium* sect. *Nematophorae* was first observed by JMH in the late 1980s at Herald Bight, Peron Peninsula, Shark Bay, during a Western Australian Museum expedition to the region commemorating the earlier French exploration (see Berry *et al*., 1990; Huisman *et al*., 1990). Plants at Herald Bight grew in large unattached, entangled clusters, and specimens were also commonly found in the drift at nearby Monkey Mia. All of the specimens were terete and, based on Weber-van Bosse's taxonomy, were initially identified as *T. procumbens*, the only species lacking flattened portions. Unfortunately, all collections were either tetrasporangial or not reproductive and, despite several subsequent visits to the area, no cystocarpic material was ever collected, without which no new morphological observations could be made. As was the practice at the time, all collections were preserved in formalin and were therefore unsuitable for DNA sequencing.

Subsequently, Verheij & Prud'homme van Reine (1993: 465, fig. 6; plate 18:2) collected from the Spermonde Archipelago (near Makassar, Sulawesi Selatan, Indonesia), unattached masses of what they suggested to be a *Eucheuma* species, the entire thallus covered by small papillae and a symbiotic *Prosuberites* sponge. When WFPvR was checking type specimens for the present study, he was reminded of that '*Eucheuma*' and confirmed that it belongs to *Thamnoclonium procumbens*. Unfortunately, all of the specimens they collected were fixed in formalin before drying.

Collections made during June 2009 by JMH from the drift at Monkey Mia, Shark Bay, however, were dried in silica gel and provided ample material for DNA analyses (see Huisman, 2010). The recent collections also yielded cystocarpic specimens, the first known for this group of species. More recently, small specimens of *T. tissotii* were collected *in situ* from an intertidal reef north of Broome, Western Australia, while drift specimens were still present at Monkey Mia in October 2009 (leg. WFPvR). These specimens and the type collections in Leiden Herbarium provided the basis for the present study, which sought to clarify the taxonomic placement of these rare species.

Materials and methods

Recent specimens used in this study were collected by JMH from the drift at Monkey Mia, Shark Bay, or *in situ* at James Price Point, north of Broome, Western Australia. The plants were pressed fresh and some fertile branches preserved in 7% formalin/seawater. Portions of each plant were dried in silica gel for DNA analyses. Slide preparations were made by hand-sectioning preserved material and mounted unstained (to distinguish cell colour), or stained in a solution of 1% aniline blue, 3% 1N HCl, then mounted in 50% Karo® corn syrup/water. Sections of recent and type material in L were prepared by embedding the material in resin and sectioning with a microtome. Macro photographs were taken on a Nikon SMZ800 and microscopic photographs on a Nikon Eclipse 80i, in both cases with a Nikon DS-Fi1 digital camera. Images were arranged into plates using Adobe Photoshop CS2. Type specimens in Leiden were examined and described by WFP. Photographs of the Banfield Dunk Island collections in the BM were taken by Tony Orchard.

DNA sequencing was undertaken by ODC in Ghent. Total genomic DNA was extracted using a standard CTAB extraction method and the *rbc*L exon was amplified and sequenced as outlined in De Clerck *et al.* (2005a, 2005b). Seventeen sequences were newly generated (see Table 1) and complemented with sequences of *Grateloupia filicina* (AJ868475, De Clerck *et al*., 2005a), *Halymenia floresii* (AY772019, De Clerck *et al*., 2005a), *Polyopes tosaensis* (AB096716, Kawaguchi *et al*., 2003) and *Yonagunia tenuifolia* (AB122016, Kawaguchi *et al*., 2004). Given the unstable generic classification of the

Table 1. Specimen details for newly generated sequences.

Name	Collector	Date	Locality	Country	Reference number	rbcL ac.nr.
Carpopeltis phyllophora	N. Goldberg	20/04/2003	Black Island, Esperance Bay, Western Australia	Australia	O255	FN908147
Carpopeltis phyllophora	G. Saunders	27/11/2002	Devil's Hole, south of Eaglehawk Neck, Tasmania	Australia	GWS001494	FN908164
Codiophyllum natalense	O. De Clerck & F. Leliaert	8/06/2003	Protea Banks, Northern Pinnacle, Kwazulu-Natal	South Africa	KZN 2216	FN908160
Cryptonemia lomation	E. Coppejans	10/07/1977	Calvi, Cap de la Revellata	Corsica	HEC 3064	FN908155
Epiphloea bullosa	J. Huisman	30/10/2000	Jurien Bay, Escape Island, Western Australia	Australia	O25	FN908149
Gelinaria ulvoidea	J. Huisman	24/10/2000	Jurien Bay, Escape Island, Western Australia	Australia	O26	FN908154
Thamnoclonium dichotomum	A. Millar & D. Harding	25/10/1995	The Docho, Jervis Bay	Australia	O246	FN908152
Thamnoclonium dichotomum	N. Goldberg	23/10/2002	North Twin Peaks Island, Esperance Bay, Western Australia	Australia	O265	FN908151
Thamnoclonium sp.	N. Goldberg	22/10/2006	Frederick Island, Esperance Bay, Western Australia	Australia	O256	FN908148
Thamnoclonium sp.	N. Goldberg	30/10/2002	Mondrain Island, Esperance Bay, Western Australia	Australia	O264	FN908150
Thamnoclonium sp.	N. Goldberg	6/04/2003	Woody Island, Esperance Bay, Western Australia	Australia	O266	FN908153
Thamnoclonium latifrons	O. De Clerck *et al.*	5/11/2007	5-Mile Reef, Sodwana Bay, Kwazulu-Natal	South Africa	KZN-b 2226	FN908159
Thamnoclonium latifrons	O. De Clerck	22/12/1999	Scottsburgh, Kwazulu-Natal	South Africa	KZN 2134	FN908156
Thamnoclonium latifrons	O. De Clerck & F. Leliaert	7/06/2003	Protea Banks, Southern Pinnacle, Kwazulu-Natal	South Africa	KZN 2190	FN908157
Thamnoclonium latifrons	O. De Clerck & F. Leliaert	7/06/2003	Protea Banks, Southern Pinnacle, Kwazulu-Natal	South Africa	KZN 2191	FN908158
Thamnoclonium lemannianum	M. Hommersand	20/09/1999	Cottesloe, Western Australia	Australia	O241	FN908161
Thamnoclonium tissotii	J. Huisman	10/06/2010	Monkey Mia, Shark Bay, Western Australia	Australia	PERTH 08151229	FN908162
Thamnoclonium tissotii	J. Huisman	10/06/2010	Monkey Mia, Shark Bay, Western Australia	Australia	PERTH 08151210	FN908163

Halymeniaceae, we opted to include only the generitypes of the *Halymenia–Cryptonemia* clade to which the sponge-associated genera of the Halymeniales belong. Sequences were aligned by eye using MEGA v.4.0 (Kumar *et al.*, 2008) and analysed using a likelihood approach. Maximum likelihood (ML) analyses were carried out with PhyML (Guindon & Gascuel, 2003). MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) was used for Bayesian phylogenetic inference (BI). A GTR + I + G model was used to analyse the dataset under ML with parameters estimated by PhyML. Branch support was estimated by non-parametric bootstrapping (500 replicates). Two independent Markov chain Monte Carlo (MCMC) runs, each consisting of four incrementally heated chains, were run for 5 million generations with default priors, chain temperature increments, and other settings. Convergence of the runs was checked visually with Tracer v.1.4 (Rambaut & Drummond, 2007). Trees were sampled every 1000th generation after determining an appropriate burn-in. A majority-rule consensus tree was calculated from the post-burn-in trees with MrBayes' sumt command. Alternative topologies were tested by means of Approximately Unbiased (AU) tests using Consel (Shimodaira & Hasegawa, 2001) with site-specific likelihoods calculated in PAUP (Swofford, 2002).

Results

Morphology and reproduction

***Thamnoclonium* sect. *Nematophorae* Weber-van Bosse, 1910: 587.**

The following descriptions are based on examinations of type and historical specimens in addition to the available fresh material.

***Thamnoclonium tissotii* Weber-van Bosse, 1910: 588, pl. XVI: figs 2, 3; pl. XVII.**

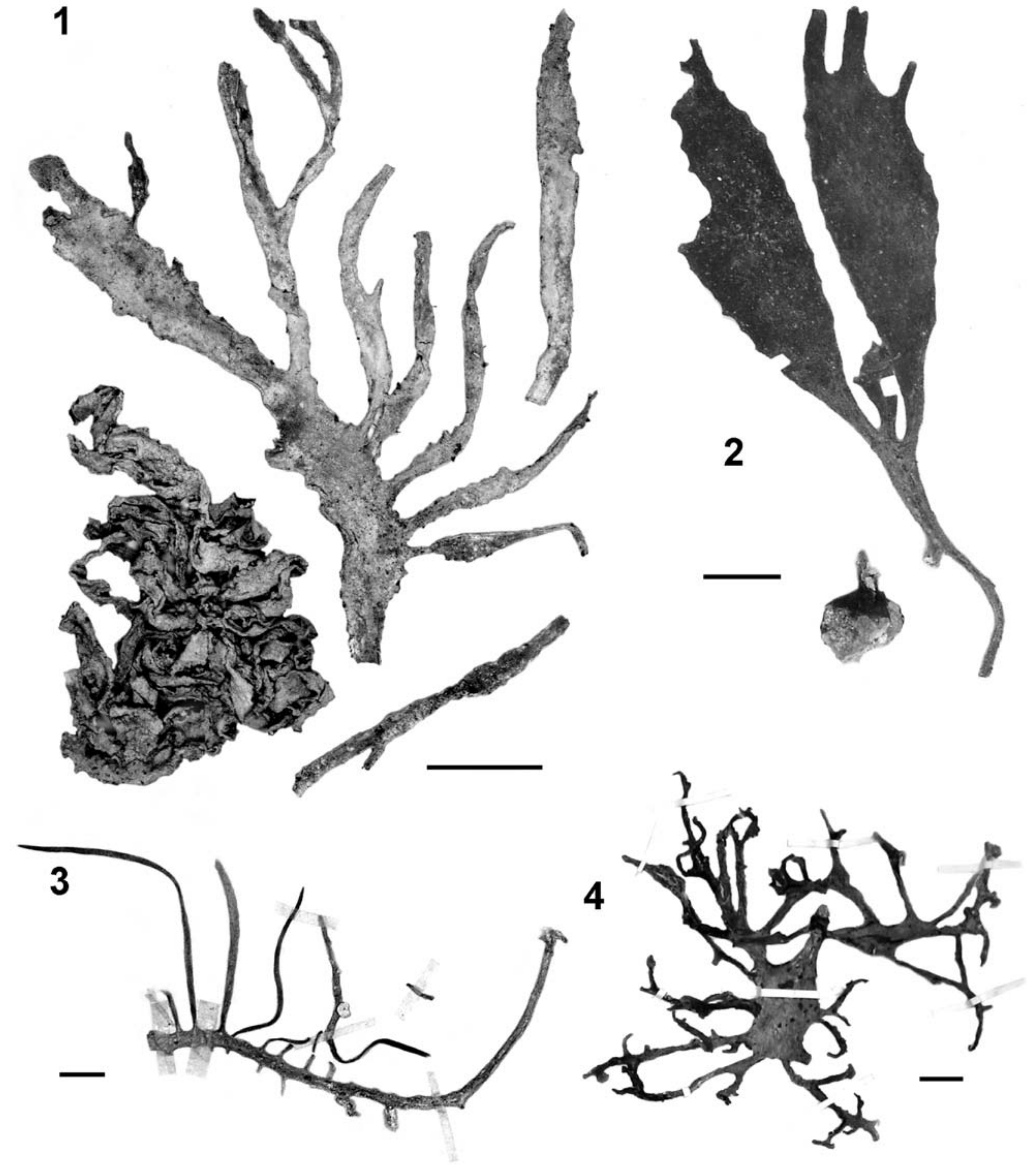
Syntype localities: Given as 'Iles Kei' [Kai Islands, Indonesia] in the protologue, but possibly an error as the only specimens in L that are not from Thursday Island are labelled as from the 'Aroe Eilanden' [Aru Archipelago] (some 160 km to the east of the Kai Islands), [A.] *Tissot van Patot* (L 0535509); Thursday I. [Australia], 18 Nov. 1907, *H.A. Lorentz* (L 0535510). Note: Hendrikus Albertus Lorentz participated in three (two as leader) expeditions into New Guinea, the second in 1907, which also included the botanist/physician Gerard Martinus Versteeg.

Lectotype: L 941.182-181 (barcode L 0535509); several specimens are mounted on the sheet and the one in the top right corner (shown as the central specimen in our Fig. 1) is the specimen depicted in Weber-van Bosse (1910: plate XVII, fig. 1) as the 'probably typical form'. It is herein designated as the lectotype.

Etymology: The species was named for Mr Tissot van Patot, who 'first collected the alga on the beach of the Kei Islands and had the amiability to send it to me' (Weber-van Bosse, 1910: 588). This is probably A. Tissot van Patot, a geographer who published about maps of New Guinea and surroundings.

Recent specimens: Drift at Monkey Mia, Shark Bay, Western Australia, 10 June 2009, *J.M. Huisman* (PERTH 08151229, Fig. 5; PERTH 08151210, Fig. 6; PERTH 08151202; PERTH 08151180; 08151199). Same locality: 18 October 2009, *W.F. Prud'homme van Reine* (L). Quondong Point, Broome, Western Australia, from 2 m depth, 16 June 2001, *J.M. Huisman & M. Van Keulen* (PERTH 08188440). North of James Price Point (north of Broome), Western Australia, on intertidal rock, 8 October 2009, *J.M. Huisman* (PERTH 08188084).

Description: Thallus upright, with a small basal attachment disc and a short stipe less than 1 cm long, or with a spreading holdfast to 2.5 cm diam. bearing several upright axes, cylindrical at the base and soon becoming flattened distally, forming an extremely variably branched, flattened frond to 40 cm high, becoming broader towards the tip which is usually branched with obtuse apices, or with flattened primary axes bearing terete lateral branches from the margins (Fig. 6), or with terete branches throughout (Fig. 5). Fronds often somewhat canaliculate in narrower distichous parts. The surface of the thallus is totally covered by the tissue of a *Prosuberites* sponge (Figs 7, 8) incorporating a symbiotic unicellular green alga (Figs 9, 10), with the exception of fertile leaflets (Fig. 11). Structurally, the thallus consists of a central core of spherical to often somewhat flattened, pseudoparenchymatous medullary cells (thickness 20–80 µm, length and width 20–150 µm, Figs 7, 12) surrounded by a pseudoparenchymatous inner cortex of large, rounded cells, up to 150 µm in diameter, grading to a small-celled outer cortex with cells 10–15 µm in diameter (Figs 7, 8). The cortex is sparsely to densely covered by small protuberant branches, these simple or rarely branched (Figs 7, 8). Moniliform chains of up to 20 cells occur at the apices and laterally on the protuberant branches and also directly on the cortex (Figs 8, 9). These chains are generally simple or rarely branched, basally narrow for 1–2 cells but then moniliform, with cells 5 µm in diameter when just formed, but swelling to a diameter of more than 40 µm. Structurally, the protuberant branches are initially uniaxial and arise in a similar pattern to the moniliform chains, but become secondarily corticated. No stellate cells occur in vegetative tissue. Reproductive structures borne in specialized fungiform to subspherical leaflets to 5 mm tall and 7 mm in diameter, which are borne laterally and are clear of the sponge coating (Fig. 11). Structurally, the leaflets have a more loosely



Figs 1–4. Habit of type specimens and historical collections of *Spongophloea*. Fig. 1: *Spongophloea tissotii*, fragments of the lectotype specimen of *Thamnoclonium tissotii* (L). Fig. 2: *Spongophloea treubii*, the holotype specimen (L). Fig. 3: *Spongophloea procumbens*, the lectotype specimen (L). Fig. 4: A stunted specimen of *S. tissotii* from Dunk Island, Queensland (BM). Scales represent: Fig. 1 = 4 cm; Figs 2–4 = 1 cm.

arranged medulla (Fig. 13). Tetrasporangial leaflets with a medulla comprised of stellate hyaline cells, with spherical cell bodies 12–50 µm in diameter and arms extending to 60 µm long, grading to a small-celled pigmented cortex with sub-epidermal cells spherical and 3–5 µm in diameter; the epidermal layer of obliquely dividing anticlinal cells forming a palisade (Fig. 18). Medulla of cystocarpic leaflets more dense, without obvious stellate cells, grading to a cortex of dichotomously divided filaments, the outermost filaments in short chains (Fig. 14). Carpogonial branches 2-celled, borne on a proximal cell of an ampullae filament (Fig. 19). Connecting filaments arising directly from (presumably) fertilized carpogonia (Fig. 19), traversing the inner cortex and fusing with auxiliary cells that are proximal cells of ampullae (Fig. 20). Gonimoblast arising from distal surface of auxiliary cell (Fig. 20), forming immersed cystocarps that are spherical to slightly ovoid, 100–200 µm in diameter, comprised entirely of tightly packed carposporangia 5–8 µm diam. (Figs 15, 16, 21). Tetrasporangia scattered in outer cortex of leaflets, transformed from outermost cells, clavate to ellipsoidal, irregularly cruciately divided, 15–18 × 4–6 µm (Fig. 18).

Remarks: The Western Australian plants are structurally pseudoparenchymatous, are symbiotic with a sponge and have surface protuberances and (most importantly) surface moniliform filaments. There can therefore be no doubt that these specimens belong to Weber-van Bosse's section *Nematophorae* of *Thamnoclonium*. The appropriate specific identity is more problematic. As the Shark Bay specimens are mostly terete, they appear to agree with Weber-van Bosse's concept of *T. procumbens*. However, the June 2009 collections yielded both wholly terete specimens (Fig. 5) and a single plant with a distinctly flattened primary axis bearing terete lateral branches (Fig. 6). This latter plant, although considerably larger, is similar in appearance to one of the Dunk Island collection (in BM) that Weber-van Bosse identified as *T. tissotii*. This specimen is shown in Fig. 4 and clearly has terete as well as flattened portions. Furthermore, the flattened and terete Shark Bay collections are structurally similar and, tellingly, yielded virtually identical DNA sequences. Thus we regard all the Shark Bay collections as representative of *T. tissotii*, which now encompasses terete as well as flattened specimens.

That *T. tissotii* is a morphologically variable species was also recognized by Weber-van Bosse. Some of the specimens from the Dunk Island collection (in BM) are large and comparable with the type of *T. tissotii*, whereas others are much reduced. Weber-van Bosse, in a letter to Cotton dated 25 Nov. 1910, remarked: 'The algae you sent me are indeed *Thamnoclonium tissotii* and I feel inclined to think that Ns 248. 255 are stunted forms of the same species. *Thamnoclonium tissotii* is very variable in outward appearance but a slide through N. 245 showed exactly the same anatomical structure'.

Thamnoclonium treubii **Weber-van Bosse, 1910: 587, pl. XVI: fig. 2**.

Type: North Ubian Island, Sulu Archipelago, Philippines (Siboga Exped. Stat. 99), 28 June 1899, *A. Weber-van Bosse*; holotype: L 941.182-178 (barcode L 0535511) (Fig. 2).

Etymology: Named for the distinguished Dutch botanist Melchior Treub (1851–1910), director of the Bogor Botanical Gardens in Buitenzorg, Java, for his contributions to the knowledge of the flora of the region.

Specimens examined: Known only from the type collection, on which the following description is based.

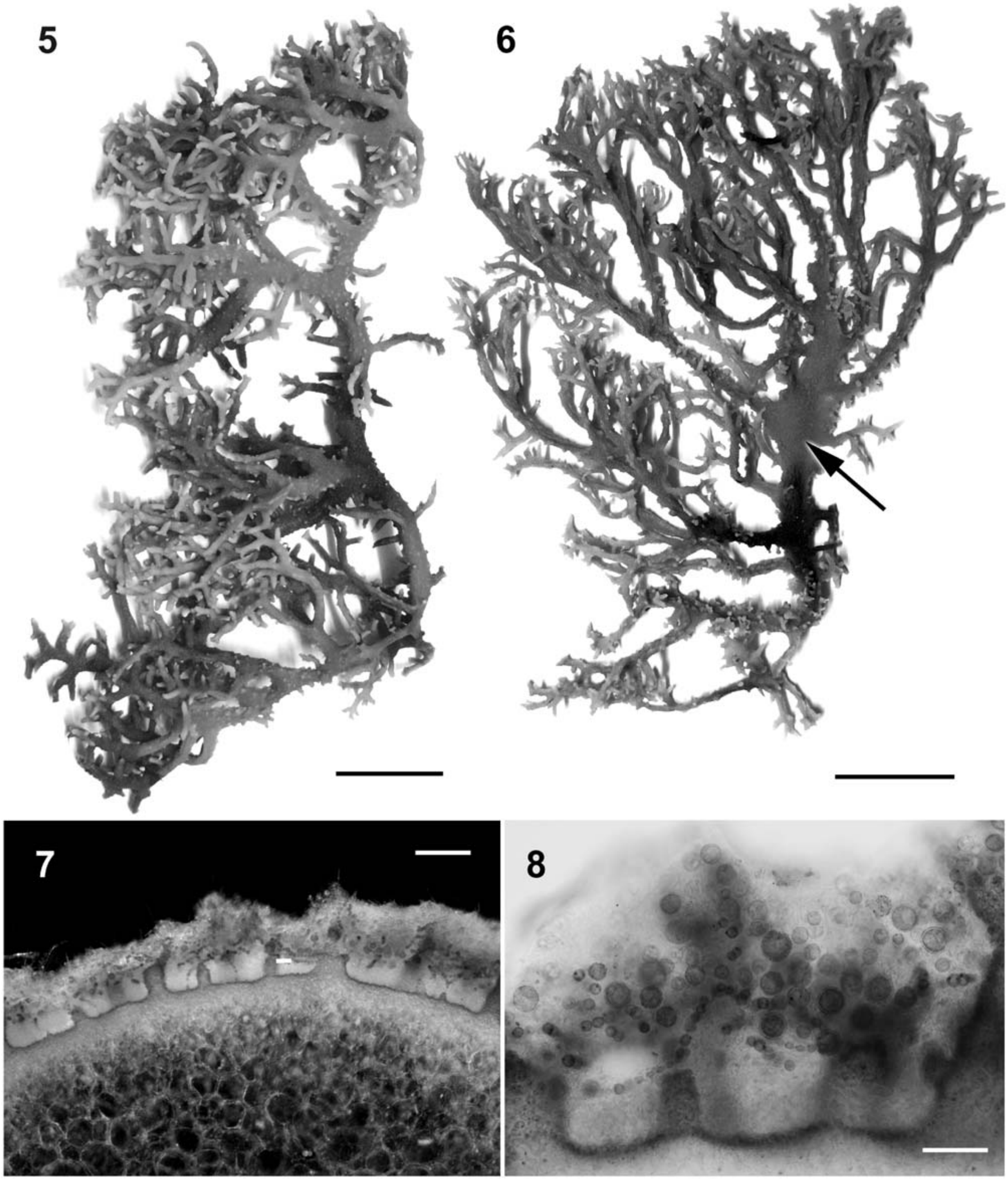
Description: Thallus upright, with a small basal attachment disc and stipe to 4 cm in length, cylindrical at the base and gradually becoming flattened distally, forming flat, leafy fronds, 5–6 cm high, becoming broader towards the tip which is branched or unbranched, with obtuse apices. The surface of the thallus is totally covered by the tissue of a *Prosuberites* sponge. Fronds are shallowly and coarsely dentate at the margins and the outer cortex produces numerous small wart-like unbranched or slightly branched laterals, which form chains of up to 10 moniliform cells at their tips (Fig. 17). The moniliform cells are 5 µm in diameter when just formed, but swell to a diameter of more than 40 µm. The thallus consists of a central core of often somewhat flattened pseudoparenchymatous medullary cells (thickness 20–80 µm, length and width 20–90 µm) surrounded by a pseudoparenchymatous inner cortex of large, rounded cells, 70–100 µm in diameter, grading to a small-celled outer cortex with cells 10–20 µm in diameter. The wart-like laterals also consist of these small cortex cells. In the medulla a few stellate cells occur (Fig. 22), with a body of 50 µm diameter and with filamentous shoots of 10–20 µm.

Remarks: This species is apparently known only from the type specimen and is distinguished by its flattened blade and the presence of a stipe. Whether this latter feature is sufficient to maintain the species as distinct from *T. tissotii* requires further study, ideally with fresh material from the type locality. The present study has noted the presence of stellate cells in the medulla of *T. treubii* that were not observed in material of *T. tissotii*, but the consistency of this feature cannot be assessed. While stellate cells do not occur in the medulla of *T. tissotii*, they are found in fertile leaflets where there is a gradual transition at the base from the vegetative pseudoparenchyma to the fertile stellate cells.

Thamnoclonium procumbens **Weber-van Bosse, 1921: 251, text figs 78, 79**.

Type: Sulu Archipelago, Philippines, anchorage off Kapul [Capual] Island, dredged (Siboga Exped. Stat. 106), 4 July 1899, *A. Weber-van Bosse*; lectotype: L, 941.182-180 (barcode: L0061146). Nine specimens (or portions of specimens) are mounted on the single herbarium sheet. Of these, the top right specimen is the one depicted by Weber-van Bosse (1921: fig. 78) and is herein designated as the lectotype (Fig. 3).

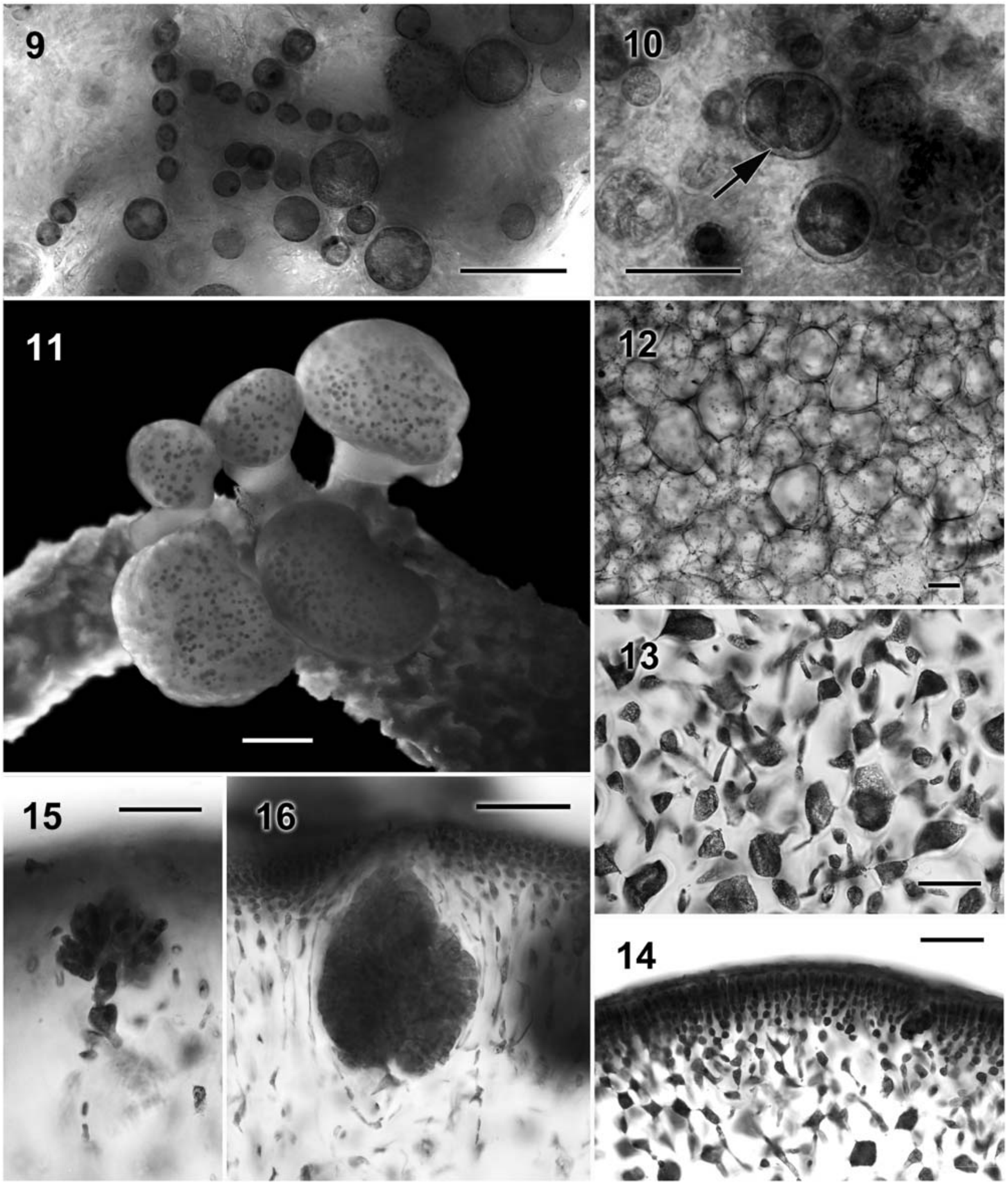
Etymology: Named for the procumbent habit.



Figs 5–8. Recent collections of *Spongophloea tissotii* from Western Australia. Fig. 5: A large drift specimen from Shark Bay with terete branches (PERTH 08151229). Fig. 6: A plant from the same collection with flattened central branches (arrow) (PERTH 08151210). Fig. 7: Section of thallus showing pseudoparenchymatous medulla and cortex with short branches and sponge coating. Fig. 8: Detail of cortex and sponge layer, showing chains of moniliform cells and larger, probably green algal, cells. Scales represent: Figs 5, 6 = 5 cm; Fig. 7 = 300 μm; Fig. 8 = 100 μm.

Specimens examined: The description given below is based primarily on an examination of the type collection, but additional specimens (both Siboga and recent) are housed in Leiden.

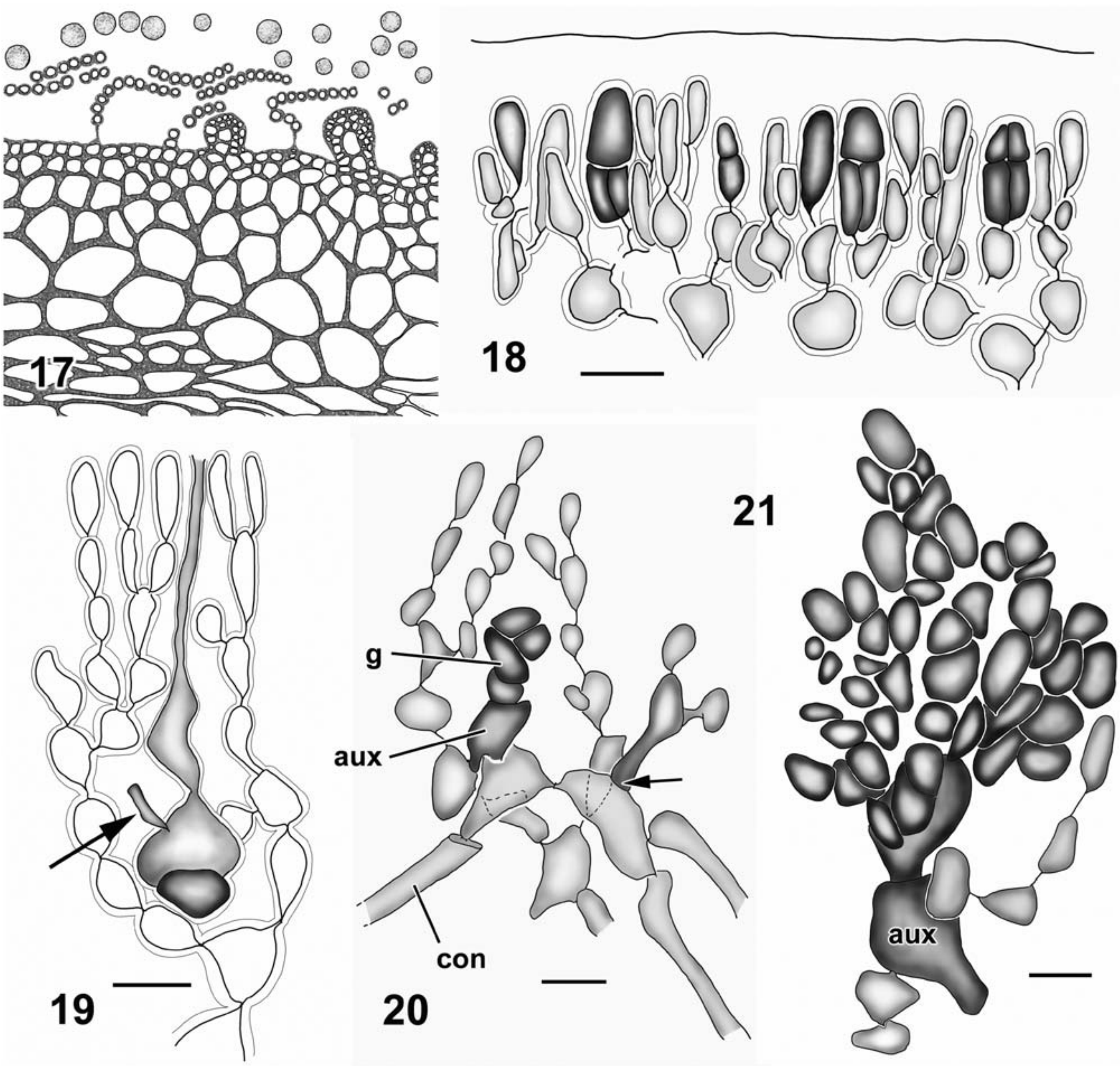
Other specimens: Selayar, Indonesia, reef (Siboga Exp. stat. 213), 26 October 1899, *A. Weber-van Bosse* (L.941.182-156); Sarasa, Postillon Islands, Indonesia (Siboga Exp. stat. 43), 4–5 April 1899, *A. Weber-van Bosse* (L. 941.182-157); Tanah Djampea, Indonesia, depth 30 m (Siboga Exp. stat. 64), 4–5 May 1899, *A. Weber-van Bosse* (L. 941.180-155);



Figs 9–16. *Spongophloea tissotii*. Fig. 9: Detail of moniliform cells and larger, thick-walled green cells. Fig. 10: A green cell dividing transversely (arrow). Fig. 11: Surface cystocarpic fungiform leaflets arising clear of the sponge layer. Fig. 12: Pseudoparenchymatous medulla of vegetative branches. Fig. 13: Loosely arranged medulla of reproductive leaflets. Fig. 14. Cortex of reproductive leaflets. Fig. 15: Young cystocarp. Fig. 16: Mature cystocarp. Scales represent: Figs 9, 10, 14, 15 = 50 µm; Fig. 11 = 1 mm; Figs 12, 14, 16 = 100 µm.

Barng Lompo, Kudingareng Keke, Lae Lae and Langkai, Spermonde Archipelago, Sulawesi Selatan, Indonesia, at the bases of reefs at depths of 10–35 m, 1988–1990, *E. Verheij* (L) (Figs 23–25).

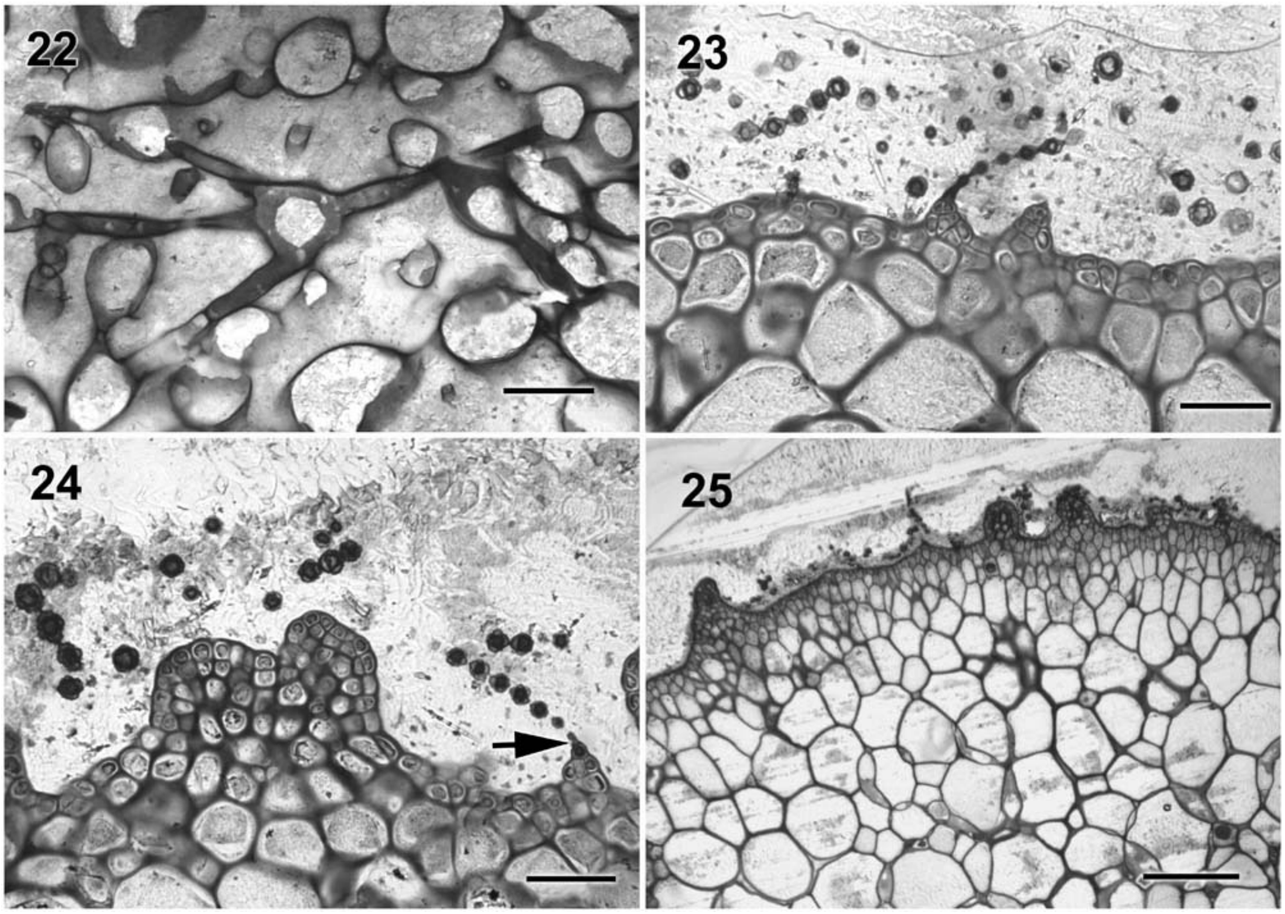
Description: Thallus without a basal attachment disc but attached by rhizoids which develop from the prostrate, cylindrical, terete or somewhat flattened axis to 40 cm long and 5–7 mm in diameter. Branching irregular: subdichotomous, unilateral or subdistichous. Lateral branches divaricate, branched or unbranched, originally terete, but later often somewhat flattened. The thallus



Figs 17–21. *Spongophloea treubii* and *S. tissotii*. Fig. 17: *S. treubii*: Vegetative structure, redrawn from Weber-van Bosse (1910). Figs 18–21: *S. tissotii*. Fig. 18: Cruciately divided tetrasporangia borne in the outer cortex. Figs 19: Two-celled carpogonial branch and young connecting filament (arrow). Fig. 20: Diploidization of auxiliary cell and production of gonimoblast. After fusing with the auxiliary cell (aux), the connecting filament has then fused with an additional auxiliary cell (on right, arrow). Fig. 21: Young cystocarp with gonimoblast composed entirely of carposporangia. Scales represent: Figs 17–21 = 10 μm. Abbreviations: aux = auxiliary cell; con = connecting filament; g = gonimoblast.

is totally covered by the tissue of a *Prosuberites* sponge, with the exception of reproductive branchlets which are naked. The algal surface is sparsely covered by small protuberant unbranched or slightly branched laterals, which form chains of up to 8 moniliform cells at their tips (Figs 23, 24). The moniliform cells are 5 μm in diameter when just formed, but swell to a diameter of more than 40 μm. Structurally, the thallus consists of a central core of pseudoparenchymatous medullary cells (Fig. 25) with a diameter of 20–160 μm, surrounded by a pseudoparenchymatous inner cortex of cells up to 100 μm in diameter, grading to a small-celled outer cortex with cells 5–10 μm in diameter. The protuberant laterals also consist of these small cortex cells. No stellate cells were observed.

Remarks: This species is structurally similar to *T. tissotii* and may be synonymous, given that herein we include both terete and flattened specimens in that species. *Thamnoclonium procumbens* has prostrate axes, however, and for the moment is maintained as an independent species based solely on that feature. As with *T. treubii*, *T. procumbens* requires further study, ideally with fresh material from the type locality.



Figs 22–25. Morphological structure of type and recent collections. Fig. 22: A stellate cell in the medulla of the type of *T. treubii* (L.). Figs 23–25: From Kudingarang Keke, Spermonde Archipelago, Indonesia. L 992.274-230. Fig. 23: Cortex of *T. procumbens* showing chains of moniliform cells. Fig. 24: Similar view of *T. procumbens* showing the tapered proximal region of the surface filaments. Fig. 25: Transverse section of *T. procumbens* showing pseudoparenchymatous medulla and cortex with short branches. Scales represent: Figs 22–24 = 50 µm, Fig. 25 = 200 µm.

DNA sequencing

The *rbc*L alignment included 23 sequences of 1259 bases and the model selection procedure selected a GTR + I + G model with the following parameters as estimated by PhyML: base frequencies A = 0.30, C = 0.16, G = 0.22, T = 0.31; substitution matrix A-C = 0.931, A-G = 7.601, A-T = 0.821, C-G = 1.011, C-T = 16.767, G-T = 1.0; gamma distribution shape parameter alpha = 0.274; proportion of invariable site = 0.273. The burn-in of the Bayesian analysis was determined at 10^6 generations, resulting in a dataset containing 8000 trees from both parallel runs.

The ML tree was identical to the Bayesian consensus tree, and we present only the latter with branch support values from both analyses. Terminal and subterminal clades are highly supported in both ML and BI analyses, but the backbone of the tree remains unsupported (Fig. 26). The two *Thamnoclonium tissotii* (sect. *Nematophorae*) samples from Western Australia have nearly identical sequences. They form a sister clade to *Codiophyllum natalense* from South Africa. The other sponge-associated taxa are clustered in three separate clades. One clade comprises samples here referred to as *Thamnoclonium* sp. from Esperance Bay in southern Western Australia, as well as *Carpopeltis phyllophora*, the generitype of *Carpopeltis*. A second clade, which is highly supported, unites South African *Thamnoclonium* specimens with *T. lemannianum*, collected at Cottesloe in Western Australia. Two remaining *Thamnoclonium* specimens, one from Esperance Bay and the other from Jervis Bay in New South Wales, Australia, form a third well-supported clade. The monophyly of all *Thamnoclonium* lineages, a monophyletic clade uniting *Thamnoclonium* with *Thamnoclonium tissotii*, as well as a sister relationship of *Carpopeltis* to *Thamnoclonium*, were all significantly rejected using AU tests.

Discussion and taxonomic proposals

Multiple origins of sponge associations

Sponge–seaweed associations have been reported for a number of macroalgal genera (Price *et al.*, 1984; Scott *et al.*, 1984; Norris, 1987, 1991;

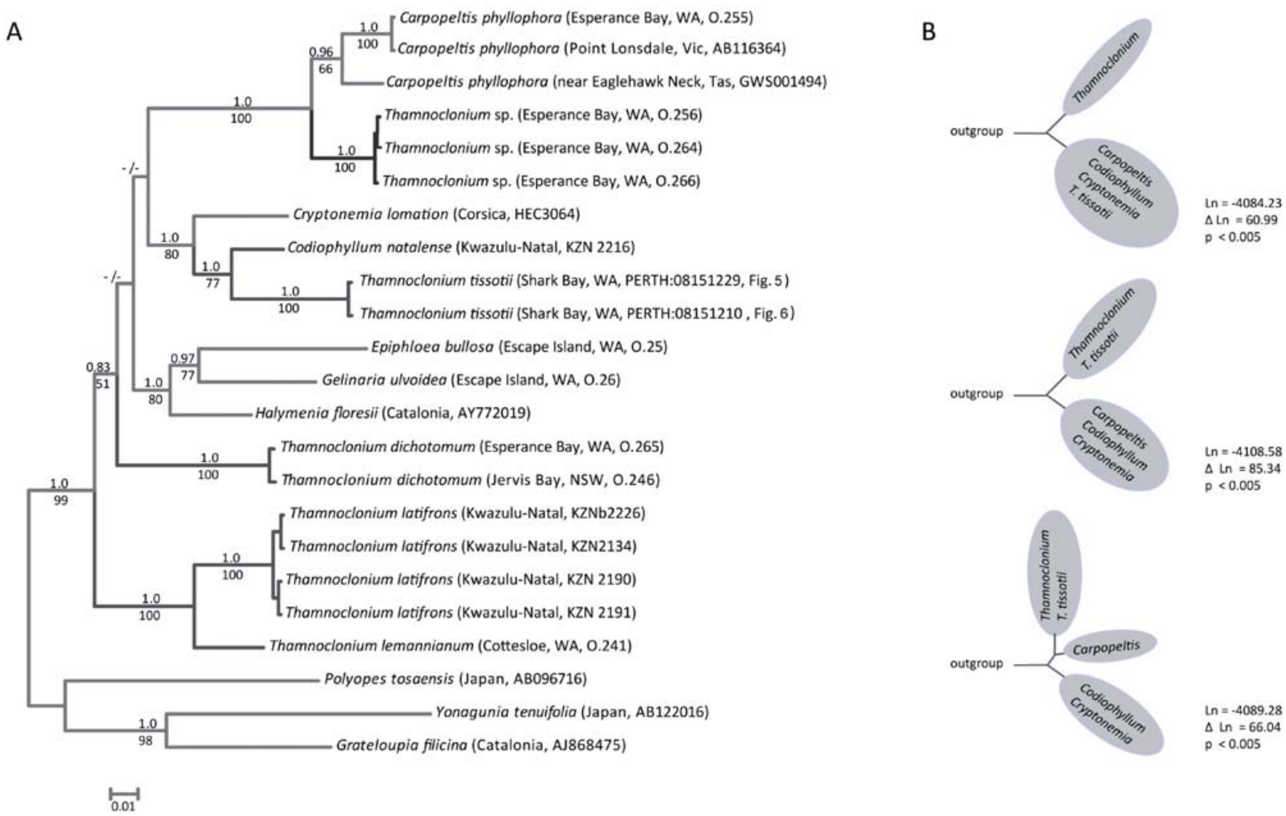


Fig. 26. A. Phylogenetic hypothesis ($-\ln L = 4023.24$) obtained by maximum likelihood inference of the *rbc*L dataset (1259 bp). Bayesian posterior probabilities and ML bootstrap values are indicated above and below the branches, respectively; values below respectively 50 and 0.7 are not shown. Bold branches represent sponge-associated lineages. B. Results of the AU tests for assessing the likelihood of alternative topologies using various constraint trees.

Rützler, 1990; Zea & de Weerdt, 1999; L.E. Phillips, 2002) and these associations can take many forms. Filamentous algae may be simply embedded in sponge tissue (e.g. *Ostreobium* and *Audouinella*). More specific associations include sponges that reinforce their own skeleton by the incorporation of geniculate coralline algae (e.g. *Jania*). In the case of *Ceratodictyon spongiosum* Zanardini (Lomentariaceae), which lives in the tissue of *Haliclona cymiformis* Esper (Chalinidae), the alga has a stiff, branched thallus that provides the underlying rigid skeletal structure of the association, but also governs the shape of the sponge (Price *et al*., 1984; Price & Kraft, 1991; Trautman *et al*., 2000, 2003). Associations wherein algae are embedded in sponge tissue contrast with situations wherein sponges grow epiphytically on the algal surface. Several *Ptilophora* species (Gelidiaceae) as well as *Epiglossum smithiae* and *Osmundaria prolifera* (both Rhodomelaceae) are usually coated in sponge tissue, the extent and thickness of which can vary considerably (Norris, 1991; L.E. Phillips, 2002; Tronchin *et al*., 2004, 2006). In these cases the establishment of the sponge is apparently facilitated by proliferations on the surface of the alga, which presumably promote attachment. This is also true for the genera of the Halymeniales that are invariably associated with sponges, *Thamnoclonium* and *Codiophyllum* (Scott *et al*., 1984). In *Thamnoclonium* the fronds are covered by irregularly contoured excrescences that are covered by sponges. Species of *Codiophyllum* J.E. Gray produce networks of anastomosing filiform laterals that create compartments analogous to those of *Thamnoclonium*. It is clear, therefore, that these intimate, mostly obligate sponge associations have evolved on numerous occasions within the algae.

Multiple origins of sponge–algal associations followed by morphological convergence are perhaps the rule rather than the exception. Studies in the green algal order Siphonocladales by Leliaert *et al*. (2009) revealed that *Cladophoropsis vaucheriiformis*, which is typically identified as such due to its seemingly obligate association with a halichondrine sponge, is in fact comprised of several independent taxa that are spread out over a phylogenetic tree, intermixed with free-living species. Most *Cladophoropsis* species occur without a sponge association, in which case they adopt a genuine filamentous *Cladophoropsis*-like morphology. Only when the species live in association with the halichondrine sponge does this result in the tough, irregular clump-like thalli typical of

'*C. vaucheriiformis*'. Although not observed in our dataset, the possibility that '*Thamnoclonium*' species may be able to grow without sponges cannot be ruled out. A denser taxon sampling of the related genera (e.g. *Carpopeltis*) could reveal closer relationships between sponge-associated and free-living taxa.

Our phylogenetic analyses of *rbc*L gene sequences demonstrate that sponge-associations have also evolved multiple times in the Halymeniales. At least four lineages contain specimens that, based on morphological evidence, would be classified as *Thamnoclonium*: viz, the thallus is flattened to terete and from the surface of the axes project excrescences that become filled by sponge tissue. Two clades, sitting on relatively long branches, are entirely composed of specimens conforming to this *Thamnoclonium* morphology. The taxonomic implications of these results are discussed below.

Thamnoclonium

The type species of *Thamnoclonium* is *T. hirsutum* [Type: 'Neuholland: Sieber (Lucae!)', Kützing 1843: 392] but this is generally regarded as a synonym of *T. dichotomum* [Type: 'Ad Novam Hollandiam (orientalem?) Sieber!, Agardh 1876: 169]. Both type specimens were therefore collected by F.W. Sieber, whom Womersley & Lewis (1994: 214) and Orchard (1999) indicate collected from the Sydney region in NSW for seven months from 1 June 1823 until December 1823. Of the two clades shown in Fig. 26 that include specimens with *T. dichotomum* morphology, one includes specimens from Jervis Bay, NSW, and Esperance Bay in the Recherche Archipelago (on the south coast of Western Australia), and the second has specimens from Esperance only. We therefore regard the first as representing *T. dichotomum* and thus the genus *Thamnoclonium*.

The second clade, including only specimens from Esperance that also conform to the '*Thamnoclonium'* morphology and were initially also identified as *T. dichotomum*, appears, however, to be closely related to the genus *Carpopeltis* and far removed from true *Thamnoclonium*. The final taxonomic placement of this clade awaits further study of the several species currently considered synonymous with *T. dichotomum*, most importantly *T. proliferum* Sonder [Type; 'Ad litus occidentale Novae Hollandiae. Herb. Preiss, no. 2620', Sonder 1848: 186], which has a type locality in Western Australia.

An additional consequence of our *rbc*L analyses is that *Thamnoclonium latifrons* Endlicher *et* Diesing 1845: 289 [Type: Port Natal (Durban), South Africa], generally regarded as a taxonomic synonym of *T. dichotomum* (Guiry & Guiry, 2010), is shown to be an independent species. It is closely related to the Western Australian *T. lemannianum* and our analyses place these two species in a clade separate to *T. dichotomum*, suggesting that an independent genus is warranted.

Thamnoclonium sect. Nematophorae

The primary focus of the present study is the placement of *Thamnoclonium* sect. *Nematophorae* species. Our phylogenetic analyses of *rbc*L gene sequences show that specimens of *T. tissotii* are far removed from true *Thamnoclonium* and are most closely related to *Codiophyllum*, which is represented in the dataset by the South African species *C. natalense*, the generitype.

Morphologically, the three species of *Thamnoclonium* sect. *Nematophorae* differ from typical *Thamnoclonium* (sect. *Anematophorae* Weber-van Bosse = sect. *Thamnoclonium*) in the production of moniliform filaments from the surface of the cortex, which was a key feature of Weber-van Bosse's section. The present study has highlighted additional differences, including the presence of a pseudoparenchymatous medulla as opposed to the filamentous medulla of *Thamnoclonium*. While the affinity with *T. dichotomum* (the generitype) appears reasonable based on gross morphology, it is clear that these distinctive structural differences and our DNA analyses strongly indicate that sect. *Nematophorae* should not be retained in *Thamnoclonium* and is worthy of recognition at the generic level. Based on these results, we propose the erection of a new genus to accommodate *T. tissotii*, *T. treubii* and *T. procumbens*. Raising Weber-van Bosse's sect. *Nematophorae* to genus is not an option given the prior existence of *Nematophora* J. Agardh (Dasyaceae), so we are therefore describing the new genus *Spongophloea* to accommodate these three species.

***Spongophloea* Huisman, De Clerck, Prud'homme van Reine & Borowitzka, gen. nov**.

DIAGNOSIS: *Thallus erectus vel ex parte decumbens, cum vel sine stipitate, ab hapterono unico vel coniunctionibus aliquot secondariis affixus, profuse et irregulariter ramosus, saepe cervicornis; rami teretes vel complanati, tegmento spongioso. Fabrica multiaxialis, pseudoparenchymata; medulla ex cellulis grandibus hyalinis cum cellulis minoribus paucis mixtis constanta, in corticem cellulis minoribus, pigmentiferis transeuntibus; coniunctiones secundariae foveolarum inter cellulas frequentes. Pagina protuberationibus pseudoparenchymatis multis brevibus, et filamentis frequentibus simplicibus (raro ramosis) moniliformibus, ex epidermide exorientibus. Fabricae reproductivae in foliolis fungiformibus* *lateraliter exorientibus portatae, supra tegmentum spongiosum, firmae, cartilagineae, laeves. Fabrica foliolarum reproductivarum cum medulla cellularum stellatarum, his axe sphaerico et ramis radiatis, in corticem filamentum subdichotome ramosum transeunta, in foliolis cystocarpicis saepe ordinibus cellularum, in foliolis tetrasporangialibus cellulis binatis ellipsoidalibus. Tetrasporangia in cortice exteriore dispersa, immersa, ex cellulis exterioribus corticalibus transformata, irregulariter cruciatim divisa. Spermatangia non visa. Ampulla praesens, exigua; filamenta bis vel ter ramosa. Rami carpogoniales bicellulares, in cellula proximali filamenti ampullaris portati. Cellula auxiliaris in ampulla discreta, cellula infima filamenti secondarii ampullaris. Gonimoblastus ex cellula auxiliari extrinsecus exoriens et massam sphaericam ad ovoideam carposporangiarum arcte fasciculatarum formans, ad basim cum cellula parva auxiliari coniungenti. Pericarpium e filamentibus ampullaribus et corticalibus contiguis formatum; carpostomum exiguum, saepe indistinctum, praesens. Res vitae verosimiliter triphasica, gametophytis isomorphicis et tetrasporophytis.*

Thallus upright or partially decumbent, stipitate or not, attached by a single holdfast or with several secondary attachments, profusely and irregularly branched, often cervicorn, branches terete or flattened, with a sponge coating. Structure multiaxial, pseudoparenchymatous, with a medulla of large hyaline cells mixed with occasional smaller cells, grading to a cortex of smaller, pigmented cells, secondary pit connections between cells common. Surface with numerous short pseudoparenchymatous protuberances and frequent simple (rarely branched), moniliform filaments arising from the epidermis. Reproductive structures borne in fungiform leaflets arising laterally and clear of the sponge, these firm and cartilaginous with a smooth surface. Structure of reproductive leaflets with a medulla of stellate cells, these with a spherical core and radiating arms, grading to a filamentous subdichotomously divided cortex, in cystocarpic leaflets often with files of cells, in tetrasporangial leaflets with paired ellipsoidal cells. Tetrasporangia scattered in outer cortex, immersed, transformed from outer cortical cells, irregularly cruciately divided. Spermatangia not observed. Ampullae present, slight, the filaments branched two or three times. Carpogonial branches 2-celled, borne on a proximal cell of an ampullar filament. Auxiliary cell in a separate ampulla, the lowermost cell of a secondary ampullar filament. Gonimoblast arising outwardly from the auxiliary cell and forming a spherical to ovoid mass of tightly clustered carposporangia, basally with a small auxiliary fusion cell. Pericarp formed from ampullar and adjacent cortical filaments, with a slight, often indistinct carpostome present. Life history presumably triphasic with isomorphic gametophytes and tetrasporophytes.

Synonym: *Thamnoclonium* sect. *Nematophora* Weber-van Bosse, 1910: 587.

Etymology: From the Greek *spongia* (a sponge) and *phloios* (bark), in reference to the sponge coating on the surface of the algal thallus.

Type species: *Spongophloea tissotii* (Weber-van Bosse) Huisman, De Clerck, Prud'homme van Reine & Borowitzka.

Spongophloea includes the following three species:

***Spongophloea tissotii* (Weber-van Bosse) Huisman, De Clerck, Prud'homme van Reine & Borowitzka, comb. nov**.

Basionym: *Thamnoclonium tissotii* Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg*, 3 (Suppl.): 588, pl. XVI: figs 2, 3; pl. XVII (1910).

***Spongophloea treubii* (Weber-van Bosse) Huisman, De Clerck, Prud'homme van Reine & Borowitzka, comb. nov**.

Basionym: *Thamnoclonium treubii* Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg*, 3 (Suppl.): 587, pl. XVI: fig. 2, (1910).

***Spongophloea procumbens* (Weber-van Bosse) Huisman, De Clerck, Prud'homme van Reine & Borowitzka, comb. nov**.

Basionym: *Thamnoclonium procumbens* Weber-van Bosse, *Siboga-Expeditie Monographie* 59b: 251, text figs 78, 79 (1921).

The species of *Spongophloea* can be separated using the following key:

1 Thallus procumbent, generally with terete branches throughout although some distal branches slightly flattened . . . *S. procumbens*
1: Thallus upright, wholly terete or wholly or partially flattened . . . 2
  2 Thallus with an elongate stipe to 4 cm long . . . *S. treubii*
  2: Thallus with a short stipe less than 1 cm long . . . *S. tissotii*

The pre- and post-fertilization processes in *Spongophloea* clearly place it in the Halymeniaceae (Rhodophyta, Halymeniales). In this family, species of three genera, *Thamnoclonium*, *Codiophyllum* and *Carpopeltis*, are regularly sponge-associated and produce fertile leaflets that stand proud of the sponge tissue. In *Carpopeltis*, the only sponge-associated species is *C. spongeaplexus* Womersley & J.A. Lewis (1994: 172–175), which differs from *Spongophloea* in its flattened, dichotomously branched fertile branchlets. Species of *Codiophyllum* have a distinctly different habit with anastomosing filiform branches and an internal structure in which the medulla is filamentous with frequent periclinal filaments. A genus with which *Spongophloea* shares many features is *Thamnoclonium*, where Weber-van Bosse (1910, 1921) placed the species originally. Excluding the species treated here, as presently conceived *Thamnoclonium* includes only two species, the type *T. dichotomum* (J. Agardh) J. Agardh (1876: 168), found in southern and eastern Australia and southern Africa, and the seemingly rare *T. lemannianum* Harvey (1855: 538), found only in south-western Australia. Based on the structure of *T. dichotomum*, *Thamnoclonium* differs from *Spongophloea* in the presence of a filamentous medulla with refractive cells. Our phylogenetic analyses of *rbc*L gene sequences (Fig. 26), however, indicate that '*Thamnoclonium dichotomum*' encompasses cryptic diversity at the genus level. At least two segregate genera are warranted, one for the entity from Esperance (southern Australia) and a second for *T. latifrons* (South Africa) and *T. lemannianum* (south-western Australia). Characterization of these genera will require DNA sequence analyses and detailed morphological examination of the many species currently regarded as synonymous with *T. dichotomum*, preferably from their type localities. As our focus here is primarily clarification of *Thamnoclonium* sect. *Nematophorae*, a full revision of the genus was not our intention and we conclude by flagging the need for further study.

Symbiotic green alga

One of the unique features of the *Spongophloea* 'community' is the presence of a second cell type, not attached to the algal thallus and lying amongst the sponge tissue. In fresh material these cells can be seen to have a green colour that contrasts with the cells of the moniliform filaments, which are a purple-red. These green cells are of various sizes, some similar to the cells of the moniliform filaments and others much larger, to 40 µm in diameter in the present collections (Fig. 9), but some cells up to 100 µm in diameter were recorded by Weber-van Bosse (1910). Weber-van Bosse also observed cells of different colours, noting that those of *T. treubii* were purple-grey ['gris violet'] and those of *T. tissotii* were an intense green ['vert intense'].

Weber-van Bosse (1910) felt that these larger cells originated from the moniliform filaments, from where they detached and remained amongst the sponge tissue ['quelquefois les cellules se détachent les unes des autres'], before growing to several times their original size, a process Weber-van Bosse felt was 'remarquable'. Our observations, however, suggest that the green cells are a separate symbiont, unrelated to the red alga. As mentioned previously cells of the moniliform filaments are red-purple; they also have a large centrally placed vacuole and a darkly staining body (possibly a pyrenoid). In green cells (of all sizes), however, the vacuole is displaced to one side and a large crystalline inclusion (the 'crystalloide' of Weber-van Bosse) is present in all cells. Moreover, in one case we observed one of these green cells clearly undergoing division within the thick wall (Fig. 10), in much the same way as a coccoid green alga would do. Thus we believe that *Spongophloea* is a component of a symbiosis between three taxa, itself plus a sponge and a green alga. At present we are unable to suggest a name for the green alga, but given its unusual habitat it is possibly new to science.

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