

A Mediterranean population of *Spongites fruticulosus* (Rhodophyta, Corallinales), the type species of *Spongites*, and the taxonomic status of *S. stalactitica* and *S. racemosa*

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Tetrasporangial male and female/carposporangial plants of a Mediterranean population of *Spongites fruticulosus* are described and compared with the type material of *S. fruticulosus*, *Spongites racemosa* and *Spongites stalactitica*. The three species were established by Kützting on the basis of Mediterranean collections. The type material of *S. fruticulosus* is a rhodolith composed of a tetrasporangial plant possessing uniporate conceptacles, multistratose noncoaxial hypothallium and ovoid epithallial cells, growing on another unidentified sterile coralline with different vegetative characters. *Spongites fruticulosus* is lectotypified here with the tetrasporangial plant. The original material of *S. racemosa* represents a male plant of *Neogoniolithon*, here identified as *Neogoniolithon racemosum* (Kützting) comb. nov. The type material of *S. stalactitica* is a rhodolith composed of a mixture of two species belonging to different genera: one, here selected as lectotype of *S. stalactitica*, is a female plant regarded as conspecific with *S. fruticulosus*. The second is the tetrasporophyte of a *Neogoniolithon* species. Detailed morphological-anatomical accounts of the species are presented, and their features are documented and discussed. The comparison between the Mediterranean types and new collections and the Australian population of *S. fruticulosus* revealed that Mediterranean plants have smaller sexual and asexual conceptacles and lack trichocytes. However, overlap in conceptacle dimensions and the rarity of trichocytes in the Australian specimens do not allow us to separate the Mediterranean and the Indo-Pacific populations into different species at this time.

KEY WORDS: *Spongites fruticulosus*, *Spongites racemosa*, *Spongites stalactitica*, *Neogoniolithon*, Mediterranean, nomenclature, Kützting

INTRODUCTION

Spongites fruticulosus Kützting is the type species of *Spongites* (Woelkerling 1985). The original material described by Kützting (1841) was collected at an unspecified locality in the Mediterranean Sea. Kützting (1841) also established *Spongites racemosa* and *Spongites stalactitica* on the basis of Mediterranean collections. The three species were originally distinguished on the basis of their external morphology (Woelkerling 1985). The type material of *S. fruticulosus*, *S. racemosa* and *S. stalactitica* was examined by Woelkerling (1985), who retained the three species as separate entities until further investigations of Mediterranean populations of *Spongites* were undertaken.

The anatomical and morphological variability of Mediterranean *S. fruticulosus* is unknown, with only the tetrasporangial holotype plant having been adequately described (Woelkerling 1985). The species was later identified in southern Australia (Penrose 1991), and the vegetative and reproductive anatomy of Australian and Red Sea populations of *S. fruticulosus* were described (Penrose 1996). However, Penrose's conclusions can be questioned for two reasons: the first is the extremely wide geographical distribution of *S. fruticulosus* on

the basis of her work; the second is the smaller size of the presumed tetrasporangial conceptacles of the type specimen compared with those from the Australian population. Thus, both biometrical and biogeographical considerations introduce uncertainty concerning the conspecificity of the Australian and Mediterranean plants, thereby necessitating a better circumscription of the species from its type locality.

In this paper, we describe *S. fruticulosus* from several localities along the Mediterranean Sea and compare the anatomical and morphological data with Kützting's original collections and with extra-Mediterranean populations. We also re-examine the systematic position of *S. racemosa* and *S. stalactitica*.

MATERIAL AND METHODS

We collected specimens of *S. fruticulosus* on Elba Island, on the Pontian Islands, at Egads (Tyrrhenian Sea, western Mediterranean), and in Iskenderun Bay (Turkey, eastern Mediterranean). Specimens and slides were deposited in MI. We examined the type material of *S. fruticulosus*, *S. racemosa* and *S. stalactitica*, currently housed at L, and compared it with our collections (abbreviations after Holmgren *et al.* 1990).

Specimens were air dried, fractured and mounted on alu-

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minium stubs with Leit-C conductive carbon cement-306 for scanning electron microscopy (SEM). The samples were gold coated and examined in a Cambridge Stereoscan 360 SEM at 15 or 20 kV (Cambridge Instrument Co, London; now Leica, Wetzlar, Germany).

Specimens for light microscopy (LM) were preserved in 4% commercial formalin in seawater. Pieces of thallus were decalcified with Tellyesniczky's solution (Bressan 1974) for 12–48 h, washed in distilled water, dehydrated through a graded ethanol series and embedded in methacrylate resin (Technovit 7100; Heraeus Kulzer, Wehrheim, Germany). Serial sections obtained with a rotary microtome (Leica, Wetzlar, Germany) were stained with toluidine blue O in sodium tetraborate (Woelkerling & Ducker 1987), washed in distilled water, dried and mounted in Eukitt (O. Kindler GmbH & Co; Freiburg, Germany). Permanent slides for LM were examined and photographed with a Leica DMRB photomicroscope.

Anatomical terminology follows Adey & Adey (1973) and Woelkerling (1988) and that for growth form follows Woelkerling *et al.* (1993). Conceptacle measurements follow the system of Adey & Adey (1973) and were made from the SEM or under LM with a calibrated eyepiece micrometer. Cell measurements follow Basso *et al.* (2004).

RESULTS AND OBSERVATIONS

Spongites fruticulosus Kützting 1841: 33

Figs 1–26; Table 1

TAXONOMIC (HETEROTYPIC) SYNONYMS: *Spongites stalactitica* Kützting 1841: 33 (see below); *Lithophyllum verrucosum* (Foslie) Foslie 1901: 21; *Goniolithon verrucosum* Foslie 1900: 24–25.

LECTOTYPE: L (Rijksherbarium, Leiden, Netherlands L943.8.134).

ILLUSTRATIONS OF TYPE MATERIAL: Kützting 1869: 35, pl. 99d; Woelkerling 1985: 135–139, 150, figs 23–32; Penrose 1991: 440, figs 1–3.

ILLUSTRATIONS OF AUSTRALIAN PLANTS: Penrose 1991: 438, figs 4–24.

REPRESENTATIVE SPECIMENS EXAMINED: **Mediterranean:** lectotype of *S. fruticulosus*; lectotype of *S. stalactitica* (see below). **Tyrrhenian Sea (western Mediterranean):** R/V *Minerva* cruises—TSM Project (Taphonomy and Sedimentology of the Mediterranean shelf)—as rhodoliths: Pontine Islands, W Palmarola Island, 69 m (min89abe103, 12 November 1989, DB209, DB213); SW Palmarola Island, 60 m (min89abe105, 12 November 1989, 2692b9, DB224); Ponza-Palmarola channel, 61 m (min89ebe134–136, 10 July 1989, 2526892b5, b12). Tuscan Archipelago, Capraia Island, 45–63 m (min88edg24, 30 June 1988, DB04); Elba Island, 45 m (min90abe120–121, 1 December 1990, DB/MM rr2, unip2, unip8, unip11, lmu4, b4), 60 m (min90abe96, 30 November 1990, GR/EP17), 75 m (min90abe97, 30 November 1990, GR/PB6). Egads, Marettimo Island, 45 m (25 August 1993). **Eastern Mediterranean:** Yakacik, Iskenderun Bay (Turkey), 12 m (cruise AV195, station 20, 29 January 1995).

HABITAT AND PHENOLOGY: This species is apparently widespread within the Mediterranean Sea, occurring as rhodoliths on soft bottoms, in sediment composed of a mixture of biodegradable coarse fraction and terrigenous mud, down to a depth of 75 m. Specimens collected in late autumn (November) consist of tetrasporangial, gametangial and carposporangial plants. In winter, only tetrasporangial plants have been identified, whereas tetrasporangial and female/carposporangial plants coexist in summer.

DISTRIBUTION: Western and eastern Mediterranean Sea, NE Atlantic Ocean, Indian Ocean (Red Sea), Pacific Ocean (southern Australia).

HABIT AND VEGETATIVE STRUCTURE: Thallus encrusting or warty to fruticose, several millimetres thick, epilithic, fixed by cell adhesion or growing unattached as rhodoliths (Fig. 1). Uniporate conceptacles crowded at the apices of protuberances (Fig. 2). Structure pseudoparenchymatous; organisation dorsiventral, monomerous (Figs 3, 4). Young thalli can locally show a dimerous organisation (Figs 5, 6). Simple protuberances up to 2 mm in diameter (Fig. 1). Cells of adjacent filaments connected by cell fusions; secondary pit connections not seen (Figs 3–8). Trichocytes not seen.

Hypothallium one to four cells thick, composed of filaments running more or less parallel to the substrate (Figs 3–6). Hypothallial cells oval to rectangular in sections, $18.9\ \mu\text{m}$ ($s = 4.4$) long \times $10.2\ \mu\text{m}$ ($s = 2.1$) in diameter (mean values, $n = 30$). Each cell filament of the hypothallium bends toward the thallus surface to form the perithallium, where the cells grow in a direction perpendicular to the substrate. Perithallial cells are oval to elliptical in section and have a mean length of $14.7\ \mu\text{m}$ ($s = 4.3$) and a diameter of $10.5\ \mu\text{m}$ ($s = 3.5$, $n = 35$; Figs 7, 8). The perithallium is zoned by several growth lines (arrows, Fig. 4).

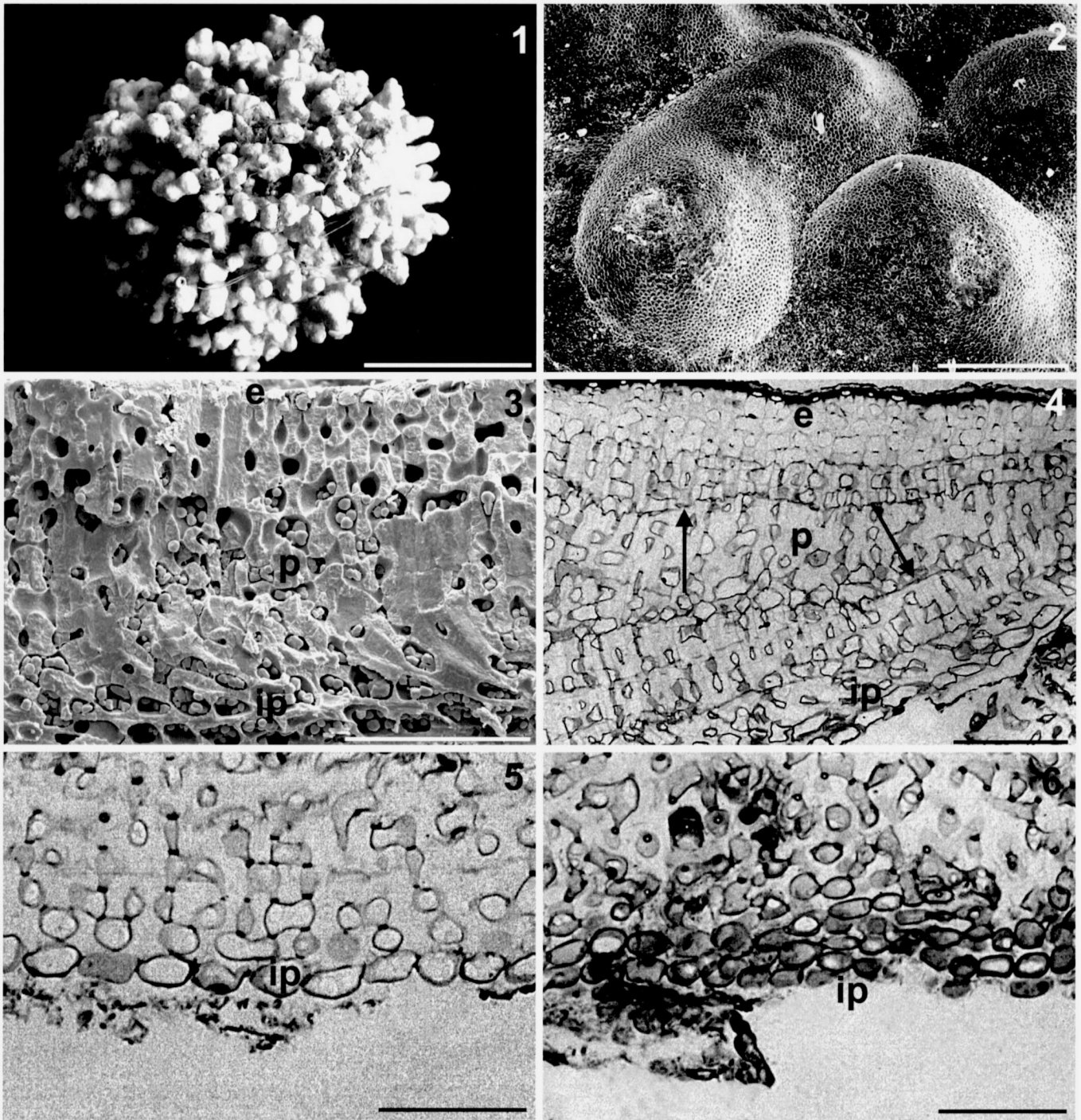
Each cell filament terminates at the thallus surface with a single epithallial cell. Epithallial cells are elliptical to dome-shaped and covered by a thick cuticular layer (Figs 9, 10). They have a mean length of $6.3\ \mu\text{m}$ ($s = 1.0$) \times $9.8\ \mu\text{m}$ ($s = 1.6$) in diameter ($n = 30$). In surface view, they appear hemispheric or elongate (Fig. 9).

REPRODUCTIVE ANATOMY: Gametangial plants dioecious. Male conceptacle chambers can become buried within the thallus and are 190 – $230\ \mu\text{m}$ in diameter and 40 – $70\ \mu\text{m}$ high (excluding the pore canal length; Figs 11–14). Pore canals are 70 – $100\ \mu\text{m}$ in length and 40 – $60\ \mu\text{m}$ in diameter. The male conceptacles protrude slightly above the thallus surface. Simple spermatangial branches are confined to the chamber floor (Figs 12–14).

Female conceptacles protrude above the thallus surface as conical domes. Conceptacle chambers are 125 – $345\ \mu\text{m}$ in diameter and 170 – $308\ \mu\text{m}$ high and can be buried in the thallus (Fig. 15). The female conceptacle contains 20 or more supporting cells across the chamber floor, each bearing two two-celled carpogonial branches. Only the central carpogonial branches form trichogynes, whereas the peripheral ones appear sterile (Figs 16–18).

After presumed karyogamy, carposporophytes develop within female conceptacles and produce flask-shaped conceptacle chambers that protrude above the thallus surface, 409 – $496\ \mu\text{m}$ in diameter and 340 – $360\ \mu\text{m}$ high with a pore canal 220 – $223\ \mu\text{m}$ long and 111 – $161\ \mu\text{m}$ in diameter (Fig. 19).

Tetrasporangial plants with uniporate conceptacles. Conceptacles are often concentrated at the apex of protuberances, with chambers 320 – $480\ \mu\text{m}$ in diameter and 130 – $250\ \mu\text{m}$ high (excluding the pore canal length). Remnants of a columella have been seldom observed. Pore canal 44 – $120\ \mu\text{m}$ in diameter and 90 – $200\ \mu\text{m}$ long. Conceptacle roofs slightly protruding above the surrounding thallus surface (Fig. 20). The pore canal is lined by cells protruding laterally into the canal and oriented parallel to the roof surface (Fig. 21). Conceptacles become buried within the thallus, and no in-filling by vegetative cells occurs (Figs 20, 21). Each tetrasporangium contains four zonate tetraspores (Figs 21, 22). We observed only very small tetrasporangia, probably immature, about 50 –



Figs 1–6. Recent Mediterranean collections of *Spongites fruticosus*. External morphology and anatomy of the vegetative thallus.

Fig. 1. Morphology and structure of a fruticose rhodolith of *Spongites fruticosus* (sample min89abe105). Scale bar = 2 cm.

Fig. 2. Uniporate tetrasporangial conceptacles, surface view, SEM photograph (sample min89abe103, stub 2692b5). Scale bar = 200 μ m.

Fig. 3. Thallus structure under SEM (sample min90abe120–121, DB/MM unip8, stub29699b2). e, epithallial cells; p, perithallium; ip, hypothallium. Scale bar = 100 μ m.

Fig. 4. As above under LM. Note growth zones (arrows) of the thallus (sample min90abe96, GR/EP17, slide 8). Scale bar = 100 μ m.

Fig. 5. LM photograph of LS (longitudinal section) of a dimerous hypothallium in a juvenile thallus (sample min90abe 97, GR/PB6, slide 10). Scale bar = 50 μ m.

Fig. 6. LM photograph of monomerous hypothallium composed of two- to four-celled filaments (sample min90abe97, GR/PB6, slide 5). Scale bar = 50 μ m.

Table 1. *Spongites fruticosus* Kützinger. Comparative data for the type material of *S. fruticosus* and *S. stalactitica* (both Mediterranean, this work), the Mediterranean population (this work) and the Australian population (Penrose 1991, 1996). Unless specified otherwise, all measurements are in micrometers.¹

	<i>S. fruticosus</i> lectotype	<i>S. fruticosus</i> Mediterranean	<i>S. stalactitica</i> lectotype	<i>S. fruticosus</i> southern Australia
Thallus thickness	up to 2000	up to 3000	up to 2000	300 µm–30 mm
Protuberances	D 1.5–4 mm up to 5 mm long	variable D 1–5 mm, up to 8 mm long	< 1 mm	variable
Hypothallial cells (L × D)	17–28 × 10–17	10–32 × 5–17	11–32 × 7–18	5–35 × 5–16
Perithallial cells (L × D)	10–28 × 8–22	6–28 × 5–22	11–36 × 6–22	
Epithallial cells (D × L)	11–14 × 6–11	6–18 × 4–11	7–14 × 5–9	not given
Trichocytes	not observed	not observed	not observed	not buried, absent from most plants, single or in horizontal fields
Tetrasporangial conceptacle chamber (D × H)	up to 480 × 209	320–480 × 130–25	not observed	350–595 × 275–410 including the pore canal
Pore canal (L × D)	up to 190 × 110	90–200 × 44–120		
Tetrasporangium (L × D)	52 × 25	50–70 × 30	Not observed	85–225 × 65–130
Male conceptacle chamber (D × H)	Not observed	190–230 × 40–70	Not observed	235–335 × 145–245
Pore canal (L × D)		70–100 × 40–60		
Female conceptacle chamber (D × H including pore canal)	Not observed	125–345 × 170–308	153–198 × 170	Usually 515–580 × 255–275 but figured also 313 × 220 ²
Carposporophyte chamber (D × H)	Not observed	409–496 × 340–360	Not observed	575–595 × 295–320
Pore canal (L × D)		220–223 × 111–161		

¹ D, diameter; L, length; H, height.² Value calculated from Penrose (1991, fig. 20) and not reported in her text.

70 µm long × 30 µm in diameter (insets, Figs 21, 22). Bisporangial conceptacles not observed.

OBSERVATIONS OF TYPE MATERIAL: Although Woelkerling (1985) provided a detailed account of the type collection of *S. fruticosus* (L 943.8.134), including relevant historical information, we re-examined this material to confirm the identity of our collections with it. The type material is a single rhodolith made of conjoined, branch-like protuberances up to 5 mm long, 1.5–4 mm in diameter, variously developed and irregularly furcate. At the surface of the rhodolith the fruticose thallus shows a noncoaxial multistratose medulla (= hypothallium) made of cells with a mean length of 19.6 µm ($s = 3.3$) × diameter of 14.4 µm ($s = 2.3$, $n = 12$), passing to a cortex (= perithallium) made of cells with mean length 17.8 µm ($s = 5.4$) × diameter 13.6 µm ($s = 4.4$, $n = 21$; Figs 23, 24). Adjacent cell filaments are connected by cell fusions, which are frequently multiple. Each cell filament terminates at the thallus surface with a rounded to ellipsoidal (transversely compressed) epithallial cell with mean length 8.4 µm ($s = 1.8$) × diameter 11.7 µm ($s = 0.8$, $n = 15$). Fusions are absent between epithallial cells. The thallus surface is covered by a thick cuticle (Fig. 25). Trichocytes were not seen, despite a diligent search of about 100 serial sections. Several uniporate conceptacles with mean diameter 340 µm ($s = 84$) × height 150 µm ($s = 30$, $n = 7$) occur at the thallus surface and are buried in the perithallium. Some of them show remnants of small tetrasporangia and a pore canal lined by cells oriented parallel to the thallus surface and protruding laterally into the canal (Fig. 26).

Another sterile multilayered thallus occasionally occurs below *S. fruticosus*, separated by evident discontinuities. It possesses cell fusions between perithallial cells of adjacent filaments, rounded epithallial cells and common trichocytes,

solitary or in vertical rows, becoming buried in the perithallium (Figs 27, 28). Because the identification of this plant would require further analyses, possibly compromising the integrity of the type material of *S. fruticosus*, and because this problem is beyond the scope of this study, we exclude this sterile plant from the type of *S. fruticosus*.

Because two different coralline species occur in the rhodolith that constitutes the type collection of *S. fruticosus*, lectotypification is necessary. In agreement with Woelkerling's intentions and with Article 9.9 of the *International Code of Botanical Nomenclature* (ICBN; Greuter *et al.* 2000) and to favour nomenclatural stability, we lectotypify *S. fruticosus* with the superficial tetrasporangial plant in the type collection (Figs 23–26 only), explicitly excluding the underlying sterile thallus (Figs 27, 28).

***Spongites stalactitica* Kützinger 1841: 33 (= *Spongites fruticosus* Kützinger)**

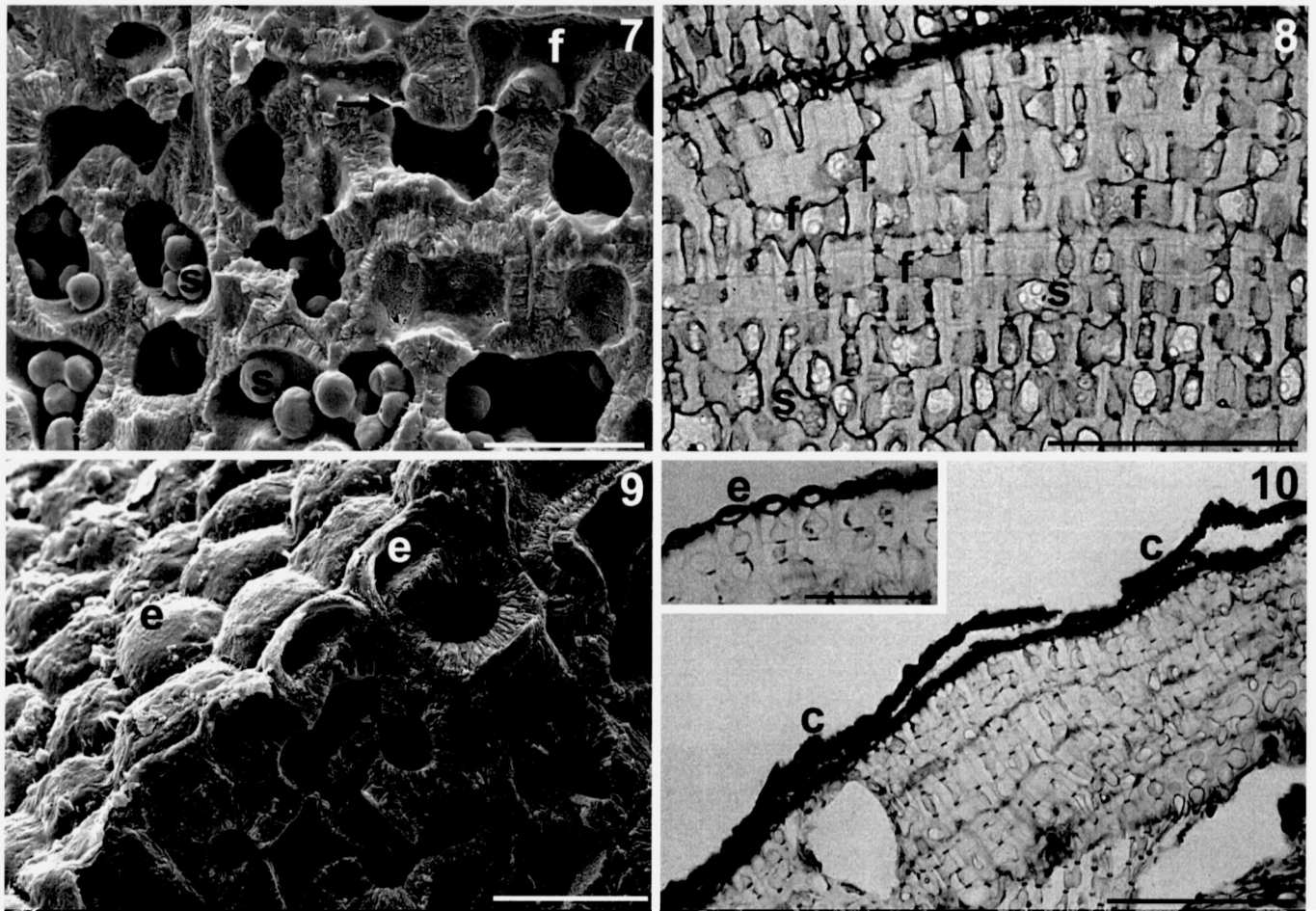
Figs 29 (pro parte)–34; Table 1

LECTOTYPE: L (Rijksherbarium, Leiden, Netherlands, L 943.7.76, portion A only).

LECTOTYPE ILLUSTRATIONS: Woelkerling 1985: 144, fig. 41 pro parte; 145, figs 47, 48.

TYPE LOCALITY: Mediterranean Sea.

The holotype collection of *S. stalactitica* (L 943.7.76) consists of a single rhodolith (Fig. 29) that, as already described and illustrated (Woelkerling 1985), shows a heterogeneous external form with variously developed protuberances. Under a binocular microscope, the portion of rhodolith surface with dense, nodular and conjoined excrescences showed large conceptacles along thallus fractures, whereas another portion of



Figs 7–10. Recent Mediterranean collections of *Spongites fruticulosus*. Perithallial and epithallial anatomy.

Fig. 7. SEM photograph of perithallial cells with primary pit connections (arrows) and wide fusions (f); s, starch grains (sample min90abe120–121, DB/MM unip11, stub29699b3). Scale bar = 25 μm .

Fig. 8. LM photograph of perithallium showing wide fusions (f) and primary pit connections (arrows); s, starch grains (sample min90abe97, GR/PB6, slide 5). Scale bar = 100 μm .

Fig. 9. SEM photograph of thallus surface. Each cell filament terminates with one dome-shaped epithallial cell (e) (sample min90abe120–121, DB/MM unip2, stub29699b1). Scale bar = 20 μm .

Fig. 10. LM photograph of LS of thallus showing growth layers and a thick outer cuticle (c) partially sloughed off. Scale bar = 100 μm . Inset. Detail of epithallial cells (sample min90abe96, GR/EP17, slide 8). Scale bar = 50 μm .

the same rhodolith with submillimetric low protuberances showed inconspicuous conceptacle chambers. Because the holotype description (Woelkerling 1985) does not contain any specification on the precise fragment of the rhodolith that was examined and illustrated and because the holotype contains two apparently different entities (on the basis of external, macroscopic and reproductive morphology), a separate examination and comparison was performed on the two forms, indicated as B and A, respectively, in Fig. 29.

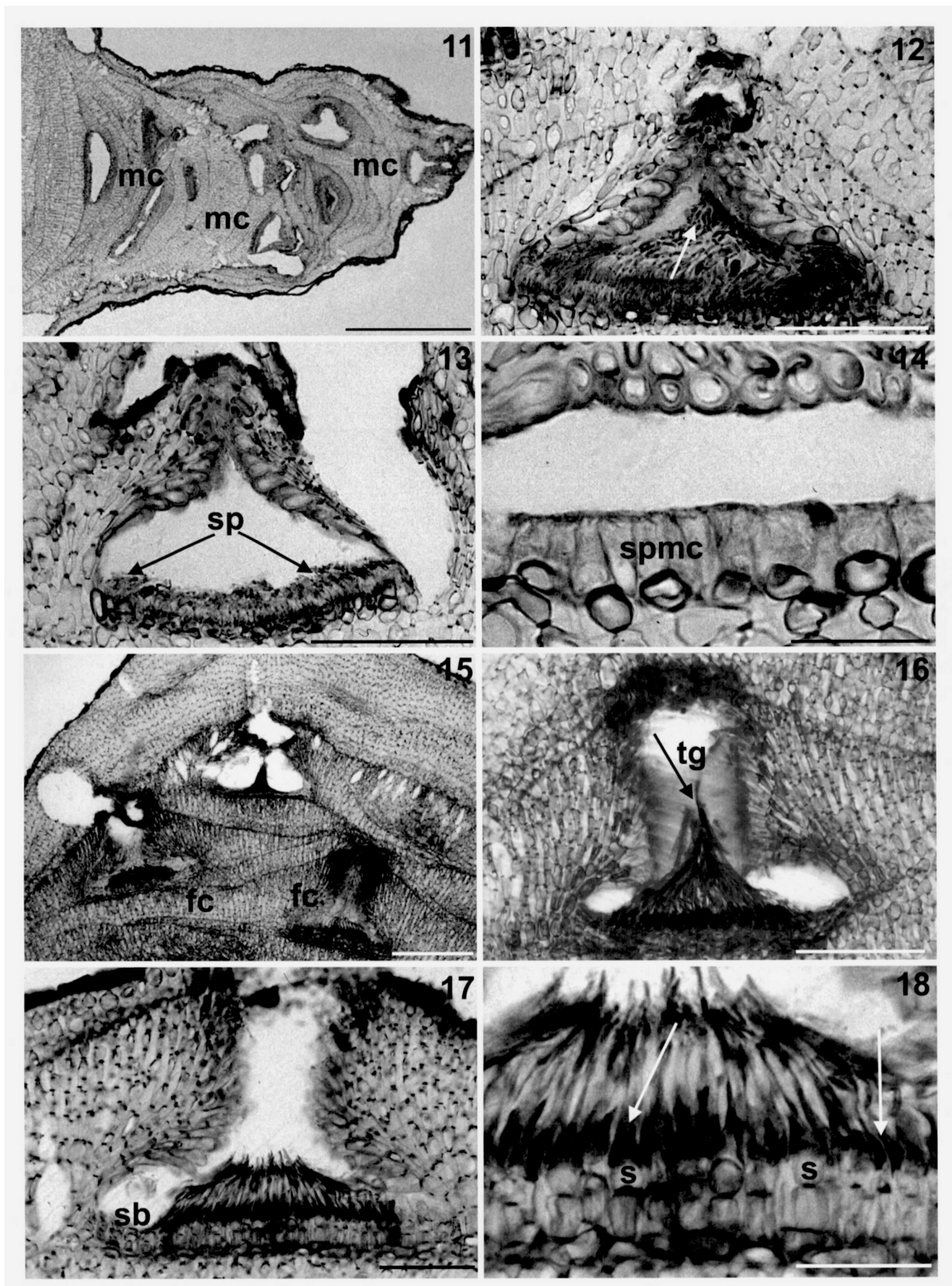
The portion of type material showing inconspicuous conceptacles and submillimetric protuberances (Fig. 29, arrow A) is made of a thallus up to 2000 μm thick. The noncoaxial hypothallium is composed of cells 25.7 μm ($s = 5.3$) long \times 13.5 μm ($s = 3$) in diameter ($n = 16$), bending toward the thallus surface to form the perithallium. The perithallium is zoned and composed of cells 19.7 μm ($s = 5.8$) long \times 12.9 μm ($s = 3.5$) in diameter ($n = 57$; Figs 30–32). Cell filaments of the hypothallium and perithallium are connected by cell fusions. Epithallial cells are rounded or compressed, 6.6 μm

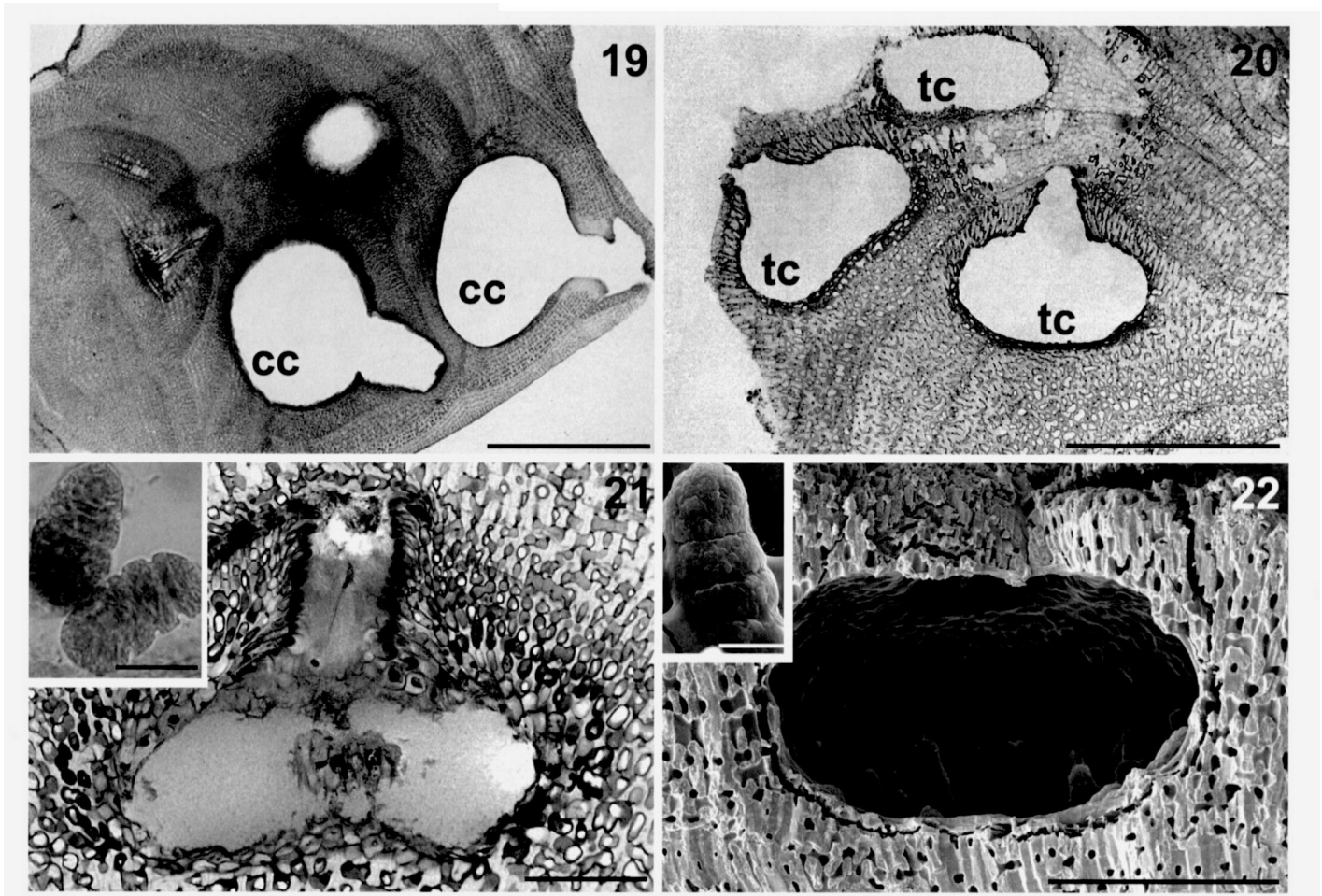
($s = 1.4$) long \times 10.4 μm ($s = 2.4$) in diameter ($n = 12$), frequently hidden by a thick, dark cuticle and lacking lateral anastomoses (Figs 32, 33). Trichocytes have been not observed. Uniporate conceptacles occur at the thallus surface and buried in the thallus. Some conceptacle chambers 174.6 μm ($s = 18.7$) \times 40.4 μm ($s = 6.5$, $n = 5$) show long trichogynes developing from the centre of the conceptacle floor toward the pore canal, which is 108–117 μm long (Fig. 34; Table 1).

Neogoniolithon sp.

Figs 29 (pro parte), 35–40; Table 2

The rhodolith portion with large conceptacles and nodular protuberances (Fig. 29, arrow B) is composed of a thallus up to 5000 μm thick made of cell filaments connected laterally by cell fusions. Hypothallial cells are 30.5 μm ($s = 3.6$) long \times 16.7 μm ($s = 3$) in diameter ($n = 15$) and form a non-coaxial hypothallium. The perithallium is made of ovoid or





Figs 19–22. Carpogonial and tetrasporangial plants in recent Mediterranean collections of *Spongites fruticulosus*.

Fig. 19. LM photograph of a female/carpogonial plant showing the shape of the female conceptacle after the development and release of carpospores (sample min90abe120–121, DB/MM lmu4). CC, empty carposporangial conceptacles. Scale bar = 600 μ m.

Fig. 20. Several empty tetrasporangial conceptacle chambers (tc) at the apex of a protuberance (sample min90abe96, GR/EP17b, slide 14). Scale bar = 500 μ m.

Fig. 21. Detail of an empty tetrasporangial conceptacle showing an arched floor with possible remnants of the columella. Note the cells protruding laterally into the pore canal and oriented parallel to the roof surface. LM photograph (sample min90abe96, GR/EP17, slide 9). Scale bar = 100 μ m. Inset. A couple of tetrasporangia. Scale bar = 25 μ m.

Fig. 22. SEM photograph of a tetrasporangial conceptacle showing small tetrasporangia over the floor. Scale bar = 200 μ m. Inset. Detail of a tetrasporangium. Scale bar = 20 μ m (sample min90abe120–121, DB/MM unip2, stub29699b1).

isodiametric to subrectangular cells $19.2 \mu\text{m}$ ($s = 3.9$) \times $16.2 \mu\text{m}$ ($s = 3$, $n = 40$; Figs 35–38). Very abundant trichocytes, solitary or in vertical rows, are interspersed in the perithallus and at the thallus surface (Figs 37, 39). Epithallial cells are

ovoid to transversely flattened, $7.8 \mu\text{m}$ ($s = 2.2$) \times $17.1 \mu\text{m}$ ($s = 2.5$, $n = 16$) and lack lateral anastomoses (Figs 38, 39).

Numerous empty conceptacle chambers, $1032 \mu\text{m}$ ($s = 55$) in diameter and $393 \mu\text{m}$ ($s = 10.4$) high ($n = 3$) with a pore

←

Figs 11–18. LM photographs of gametangial plants in recent Mediterranean collections of *Spongites fruticulosus*.

Fig. 11. A male plant with buried spermatangial conceptacles (mc) (sample min90abe97, GR/PB6, slide 9). Scale bar = 500 μ m.

Fig. 12. Detail of a male conceptacle in Fig. 11. The spermatangia (arrow) are distributed over the floor of the male conceptacle. Scale bar = 100 μ m.

Fig. 13. Detail of a male conceptacle in Fig. 11. sp, spermatangia (arrows) on the floor of a male conceptacle. LM photograph. Scale bar = 100 μ m.

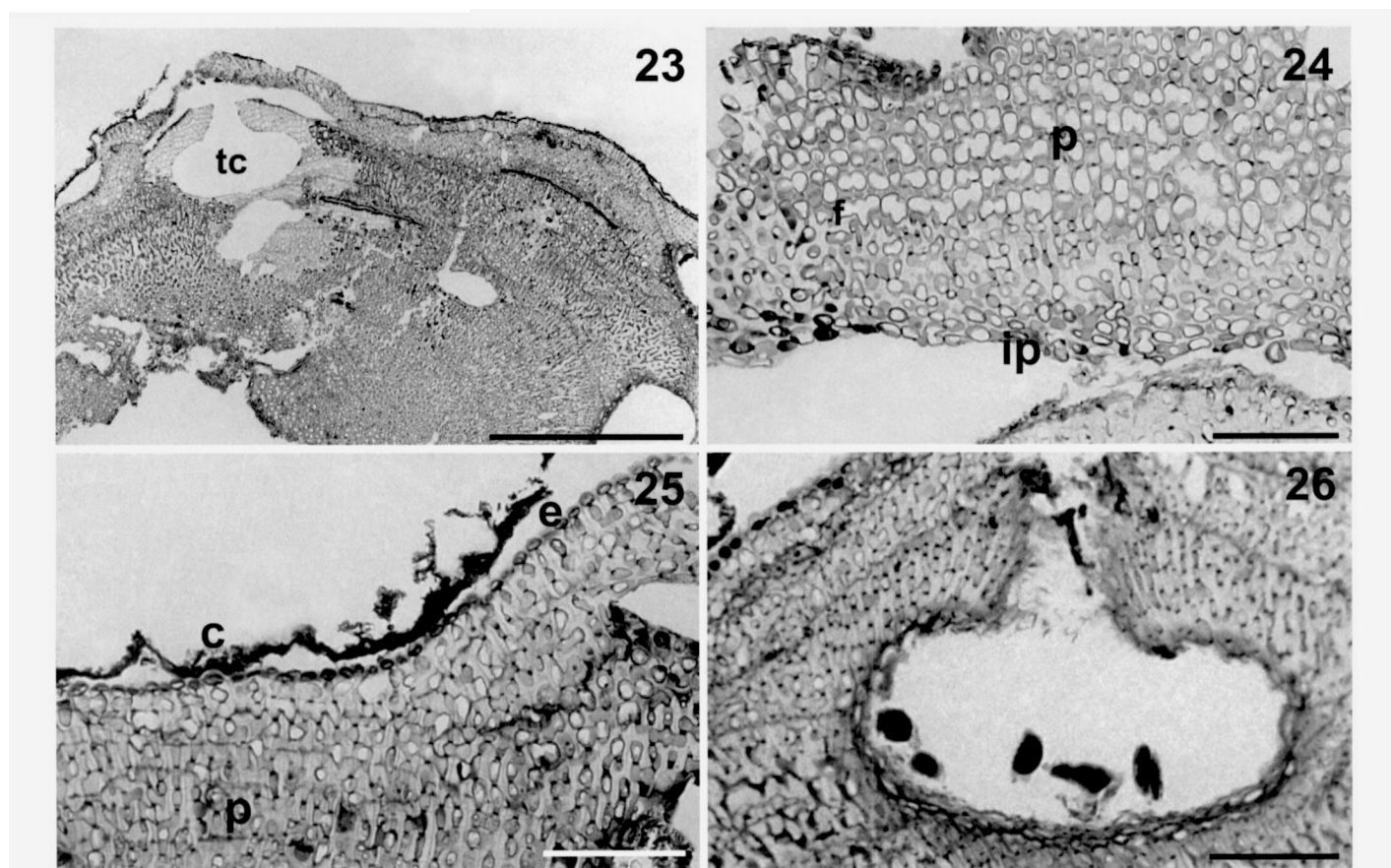
Fig. 14. Detail of a male conceptacle in Fig. 11, showing a layer of spermatangial mother cells (spmc). Scale bar = 50 μ m.

Fig. 15. A female plant. Several female conceptacles buried in the thallus (fc) (sample min90abe120–121, DB/MM b4). Scale bar = 250 μ m.

Fig. 16. Detail of Fig. 15. The female conceptacle shows fertile carpogonial branches with long trichogynes (tg, arrow) confined to the centre of the conceptacle. Scale bar = 100 μ m.

Fig. 17. Female conceptacle with sterile branches (sb) not forming trichogynes at the periphery of the conceptacle floor (sample min90abe120–121, DB/MM b4). Scale bar = 100 μ m.

Fig. 18. A detail of the floor of the female conceptacle in Fig. 17. Two carpogonial branches (e.g. arrows) developing from a single supporting cell (s). Scale bar = 50 μ m.



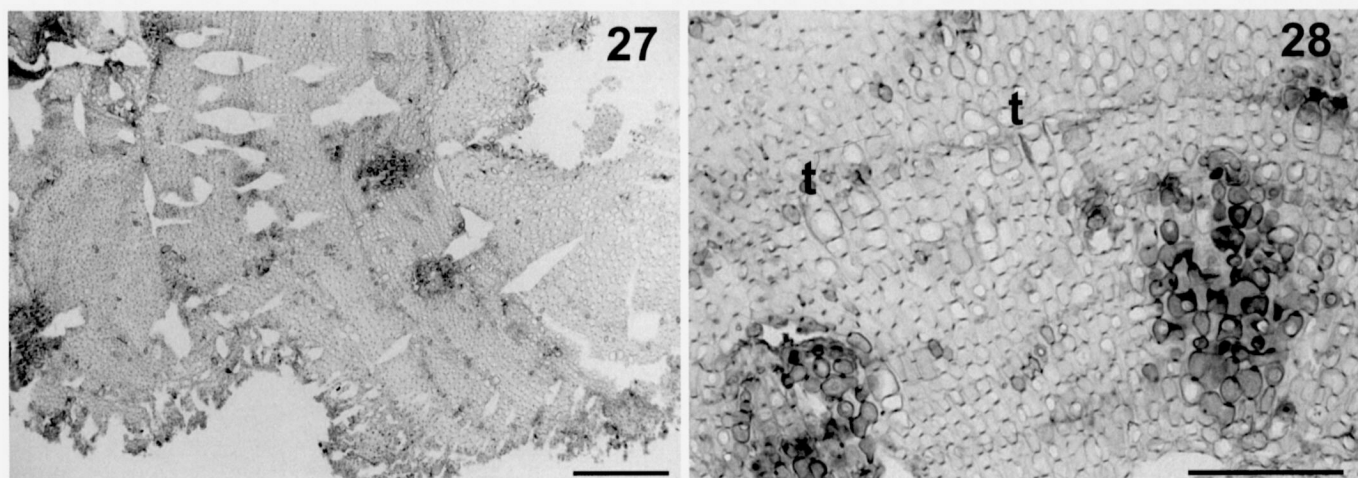
Figs 23–26. LM photographs of the lectotype of *Spongites fruticulosus* (L 943.8.134).

Fig. 23. Panoramic view of the lectotype plant with an empty tetrasporangial conceptacle (tc) (slide SF2B-4). Scale bar = 450 μ m.

Fig. 24. Multistratose, noncoaxial hypothallium (ip) and multistratose perithallium (p) showing cell fusions (f) (slide SF2B-6). Scale bar = 100 μ m.

Fig. 25. Perithallium (p) terminating in a layer of ellipsoidal epithallial cells (e). A thick cuticle (c) is also visible (slide SF2A-13). Scale bar = 100 μ m.

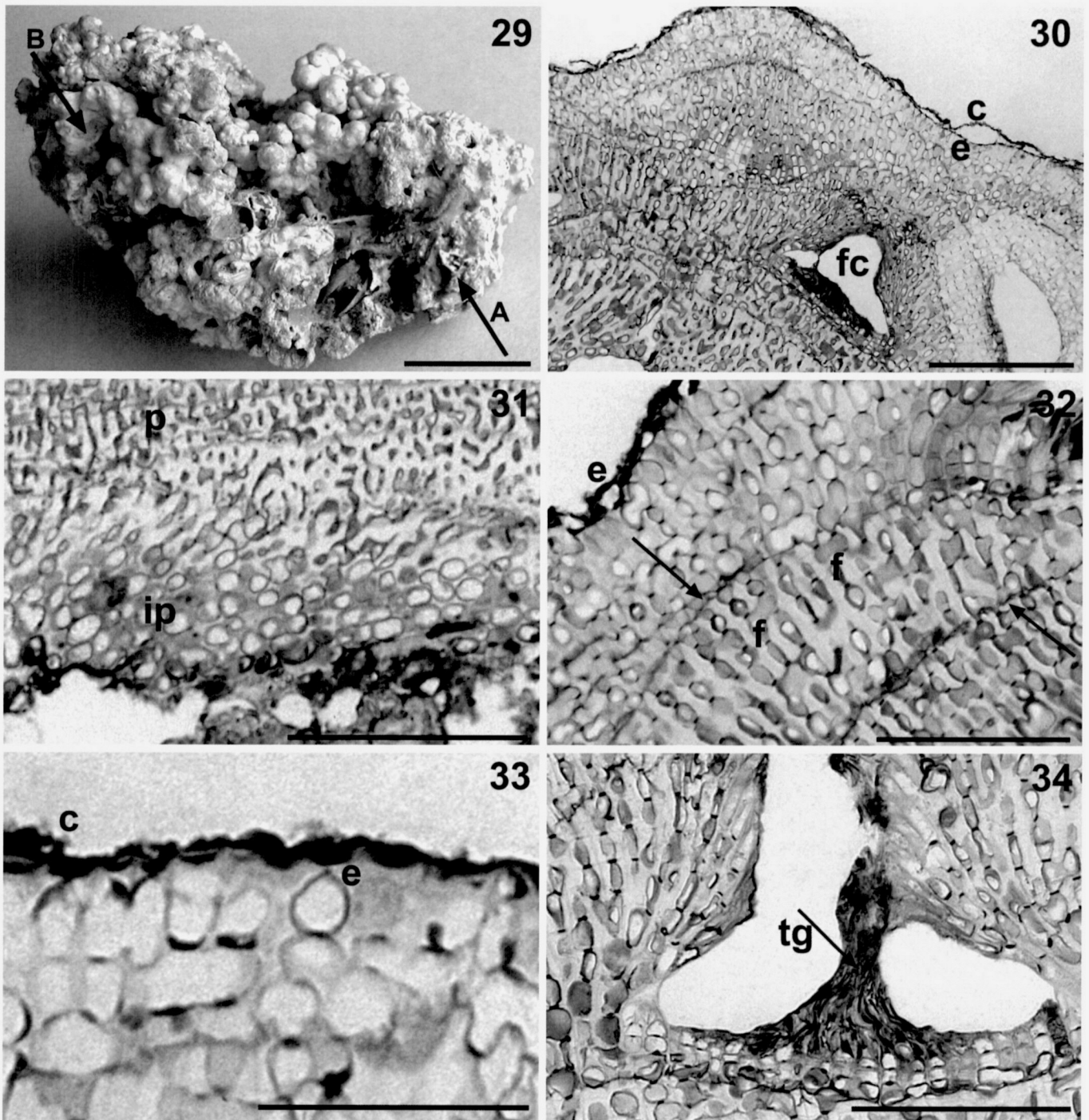
Fig. 26. Uniporate tetrasporangial conceptacle showing cells protruding into the pore canal and remnants of small tetrasporangia (slide SF2A-9). Scale bar = 100 μ m.



Figs 27, 28. LM photographs of the sterile plant in the type material of *Spongites fruticulosus*, here excluded from the lectotype.

Fig. 27. Panoramic view of the sterile plant (slide SF2C-7). Scale bar = 200 μ m.

Fig. 28. Detail of the perithallium, showing trichocytes (t), solitary and in vertical rows (slide SF2C-7). Scale bar = 100 μ m.



Figs 29–34. The type collection of *Spongites stalactitica*.

Fig. 29. Original collection of *S. stalactitica* in Leiden (L 943.7.76). This rhodolith is composed of at least two different coralline species. Arrow A, lectotype of *S. stalactitica*; arrow B, *Neogoniolithon* sp.

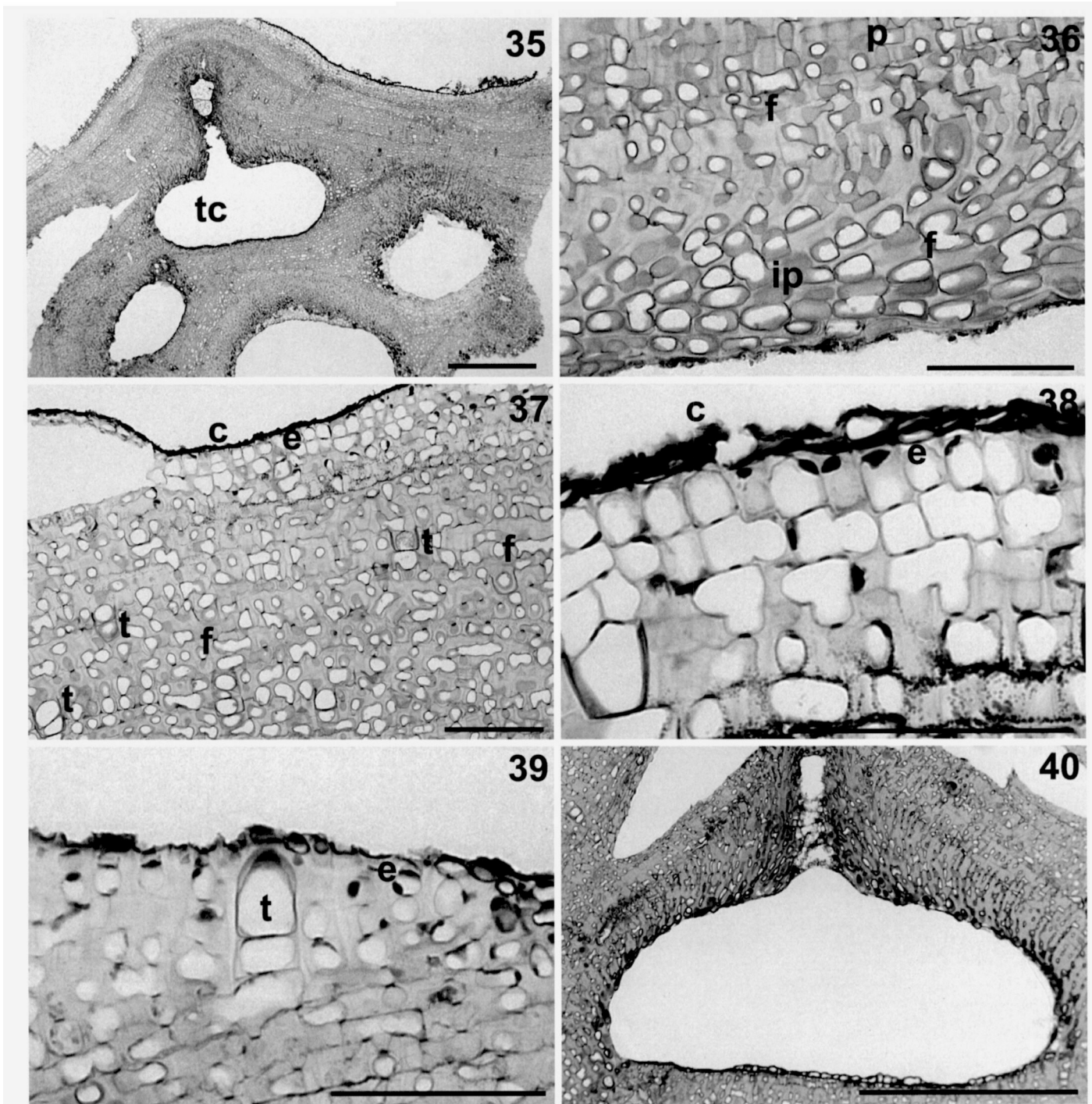
Fig. 30. LS of lectotype showing a buried female conceptacle (fc) with remnants of carpogonial branches, zoned perithallium and epithallial cells (e) covered by a thick cuticle (c). (Slide SS1–5). Scale bar = 200 μ m.

Fig. 31. Detail of hypothallial cell filaments (ip) of lectotype bending toward the surface to form the perithallium (p) (slide SS1–6). Scale bar = 200 μ m.

Fig. 32. Detail of perithallium of lectotype showing zonation (arrows), cell fusions (f) and terminal epithallial cells (e) (slide SS1–5). Scale bar = 200 μ m.

Fig. 33. Upper perithallium of lectotype. Each cell filament terminates with an epithallial cell (e) covered by a darkly stained cuticle (c) (slide SS1–5). Scale bar = 50 μ m.

Fig. 34. Female conceptacle of lectotype showing carpogonial branches and long trichogynes (tg) protruding into the pore canal. Note the fertile area confined at the centre of the chamber floor (slide SS1–6). Scale bar = 100 μ m.



Figs 35–40. LM photographs of *Neogoniolithon* sp. in the type material of *Spongites stalactitica* in Fig. 29 (arrow B).

Fig. 35. LS of a protuberance showing several large, ovoid, empty conceptacle chambers (tc), presumably belonging to a tetrasporangial plant. Note the long pore canal (slide SS2–8). Scale bar = 500 µm.

Fig. 36. LS of a primary hypothallium (ip) passing upward to the perithallium (p). Note the noncoaxial arrangement of the hypothallium and cell fusions (f) in hypothallium and perithallium (slide SS2–16). Scale bar = 100 µm.

Fig. 37. Perithallium with fusion (f) between cells of adjacent filaments and common buried trichocytes (t), also in vertical rows. Note dark-stained cuticle (c) covering the epithallial cells (e) (slide SS2–7). Scale bar = 100 µm.

Fig. 38. Perithallial cell filaments, each terminating with an epithallial cell (e), partially hidden by the thick cuticle (c) (slide SS2–8). Scale bar = 100 µm.

Fig. 39. Trichocyte (t) occurring at the thallus surface. Epithallial cells (e) are also visible (slide SS2–11). Scale bar = 100 µm.

Fig. 40. Presumed tetrasporangial conceptacle with flat chamber floor and straight pore canal (slide SS2–8). Scale bar = 500 µm.

Table 2. Summary of biometric data for *Neogoniolithon racemosum* (lectotype of *Spongites racemosa*) from Spalato, Adriatic Sea, eastern Mediterranean and *Neogoniolithon* sp. (from type material of *Spongites stalactitica* from the Mediterranean Sea). Unless specified otherwise, all measurements are in micrometers.¹

	<i>N. racemosum</i>	<i>Neogoniolithon</i> sp.
Thallus thickness	up to 4000	up to 5000
Protuberances (D × L)	3–8 mm × up to 8 mm	up to 5 mm × 5 mm
Hypothallial cells (L × D)	12–22 × 11–19	25–36 × 11–22
Perithallial cells (L × D)	7–25 × 9–18	11–29 × 9–22
Epithallial cells (D × L)	9–22 × 5–13	13–22 × 4–11
Trichocytes	solitary or in horizontal fields, buried	solitary or in vertical rows, superficial or buried
Tetrasporangial (presumed)		
conceptacle chamber (D × H)	not observed	972–1080 × 387–405
Pore canal (L × D)		315–522 × 63–99
Male conceptacle chamber (D × H)	270–630 × 72–234	not observed
Pore canal (L × D)	144–198 × 36–108	

¹ D, diameter; L, length; H, height.

canal 315–522 µm long are buried in the thallus (Figs 35, 40; Table 2).

TAXONOMIC IMPLICATIONS: Two different coralline species occur at the surface of the rhodolith holotype collection of *S. stalactitica* Kützinger.

Woelkerling (1985) described the holotype of *S. stalactitica* as a single torulose rhodolith with a fruticose-spongiose surface. He observed some inconspicuous, uniporate conceptacles occurring on some excrescences, with fusiform to depressed hemispherical chambers up to 164 µm in diameter and 38 µm high with ostioles up to 125 µm long. He described conceptacle roofs containing 10–15 or more layers of cells with fusions, chamber floors lined with carpogonial branches and long trichogynes protruding into the ostiole. Solitary trichocytes are also reported at the thallus surface and buried in the cortex (= perithallium).

In the present revision, we identify the portion of the type specimen with inconspicuous conceptacles and submillimetric protuberances (Fig. 29, arrow A) as possessing the vegetative anatomy and uniporate conceptacles described by Woelkerling as *S. stalactitica* (1985, p. 143–145, figs 47, 48; compare with our Figs 30, 34). However, a major difference exists in the absence of trichocytes, which we were unable to locate despite diligent effort (Figs 30–34). Because abundant trichocytes have been documented in the perithallium of *Neogoniolithon* sp. and because Woelkerling did not realise the occurrence of another coralline species in the same type specimen, it is likely that the trichocytes illustrated in his work did not belong to *S. stalactitica* but to the neighbouring thallus of *Neogoniolithon* sp. (Woelkerling 1985, p. 145, figs 44, 46; compare with our Figs 37, 39).

In agreement with Woelkerling's intentions and with Article 9.9 of the ICBN (Greuter *et al.* 2000), and to favour nomenclatural stability, we lectotypify *S. stalactitica* with the female plant in the type collection (Fig. 29, arrow A only), explicitly excluding the thallus described above as *Neogoniolithon* sp. Moreover, because the vegetative and reproductive features of *S. stalactitica* correspond completely to those of the female plants of the freshly collected Mediterranean material of *S. fruticosus* (compare Woelkerling 1985, figs 47, 48 with our Figs 15–18, 34), including the absence of trichocytes, and because the type specimen of *S. stalactitica* was also collected

in the Mediterranean, we consider *S. stalactitica* as a heterotypic synonym of *S. fruticosus*.

Neogoniolithon racemosum (Kützinger) comb. nov.

Figs 41–46; Table 2

BASIONYM: *Spongites racemosa* Kützinger (1841: 32).

LECTOTYPE: L (Rijksherbarium, Leiden, Netherlands, L 943.7.78).

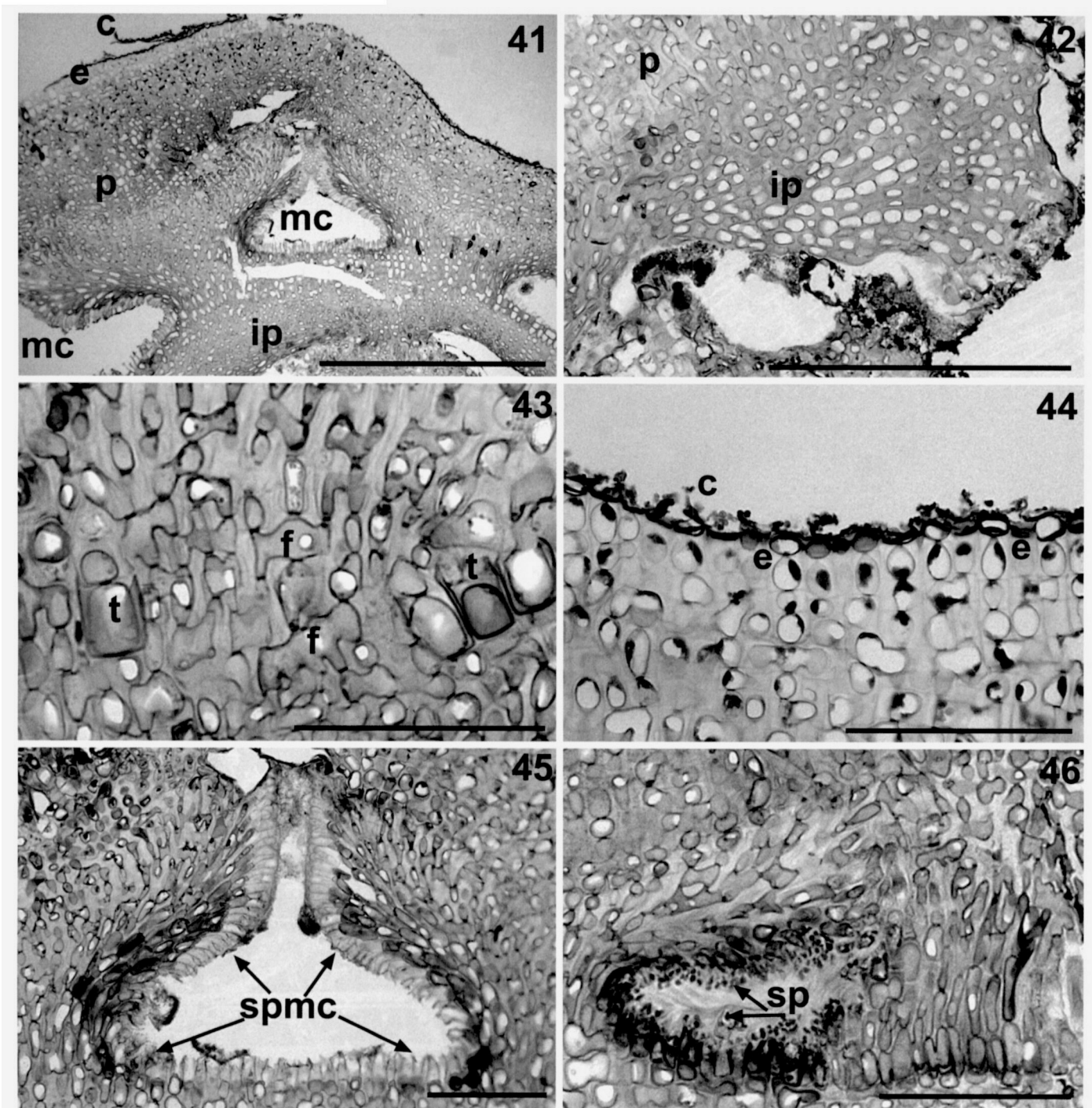
LECTOTYPE ILLUSTRATIONS: Woelkerling 1985: 140, figs 33, 34; 142, figs 35–40.

TYPE LOCALITY: Spalato (Adriatic Sea, eastern Mediterranean).

The original collection of *S. racemosa* was illustrated by Woelkerling (1985). The large plant of *S. racemosa* has a thallus up to 4000 µm thick with a multilayered perithallium of filaments connected by cell fusions (Figs 41, 43; Table 2). Perithallial cells measure 15.8 µm ($s = 4.5$) long × 13.2 µm ($s = 2.3$) in diameter ($n = 42$). We were able to identify only a secondary hypothallium, showing a noncoaxial arrangement, made of cells 18.9 µm ($s = 3.5$) long × 15 µm ($s = 2.5$) in diameter ($n = 10$; Fig. 42). Trichocytes are common in the perithallium, solitary or in horizontal fields (Fig. 43). The epithallium is composed of a single layer of ovoid or flattened cells capped by a thick cuticle (Figs 41, 44). The mean size of epithallial cells is 8 µm ($s = 2.1$) long × 15.1 µm ($s = 3$) in diameter ($n = 24$).

In addition to Woelkerling's (1985) description, we identified many uniporate conceptacles, both at the thallus surface and buried in the perithallium, showing spermatangial mother cells (Figs 41, 45) or simple spermatangia (Fig. 46) distributed on the floor and roof of conceptacle chambers. Conceptacle chambers have a flat to concave floor with a mean internal diameter 437.5 µm ($s = 141.7$) × 136.4 µm ($s = 50.1$) high ($n = 13$) and a straight pore canal 171.6 µm ($s = 19.3$) long × 75.1 µm ($s = 25.6$) in diameter ($n = 7$; Table 2). Spermatangial mother cells apparently also line the pore canal (Fig. 45).

TAXONOMIC IMPLICATIONS: Following the present delimitation of *Spongites* (Woelkerling 1985; Penrose 1991, 1996), this genus includes those Mastophoroideae that (1) are nonendophytic and lack haustoria; (2) lack a ventral region composed of palisade cells; (3) lack a ventral region composed of cells arranged in a coaxial manner; (4) possess a multilayered



Figs 41–46. LM photographs of *Neogoniolithon racemosum*, lectotype of *Spongites racemosa* (the larger plant of L 943.7.78).

Fig. 41. LS of a protuberance showing cell filaments more or less parallel to the substrate (ip, hypothallium), bending toward the surface to form the perithallium (p) and terminating with a surface epithallial cell (e) covered by a thick cuticle (c). Some male conceptacles (mc) are buried in the perithallium (slide SR2). Scale bar = 500 μ m.

Fig. 42. A secondary hypothallium (ip), produced as a lateral expansion of the perithallium (p) (slide SR7). Scale bar = 500 μ m.

Fig. 43. Trichocytes (t) and multiple cell fusions (f) in the perithallium (slide SR2). Scale bar = 100 μ m.

Fig. 44. Epithallial cells (e) covered by cuticle (c) (slide SR2). Scale bar = 100 μ m.

Fig. 45. A male conceptacle showing floor and roof lined by spermatangial mother cells (spmc, arrows) (slide SR5). Scale bar = 100 μ m.

Fig. 46. Section near edge of male conceptacle showing simple spermatangial branches (sp, arrows) on floor and roof of chamber (slide SR3). Scale bar = 100 μ m.

thallus; (5) possess simple spermatangia confined to the floor of male conceptacles and (6) possess gonimoblast filaments arising only from the margin of a continuous fusion cell.

Characters (5) and (6) allow the delimitation of *Spongites* from *Neogoniolithon* because the latter includes plants with spermatangia distributed on the floor and roof of male conceptacles and gonimoblast filaments arising dorsally from fusion cells (Penrose 1992). The occurrence of a coaxial hypothallium was listed among the distinctive features of the genus *Neogoniolithon* (Woelkerling 1988); however, further observations on the type species *N. fosliei* demonstrated that the hypothallium can be arranged in a coaxial or a noncoaxial manner (Penrose 1992), preventing the use of this feature to characterise the genus *Neogoniolithon*.

Previous studies identified only some empty conceptacles in the type collection of *S. racemosa*, which were presumed to be female gametangia (Woelkerling 1985). Our findings demonstrate that the lectotype of *S. racemosa* is a mastophoroid male plant with spermatangia distributed both on floor and roof of conceptacle chambers. On the basis of this feature, this species must be placed in the genus *Neogoniolithon*. The lack of general agreement about species delimitation within the genus *Neogoniolithon* prompts us to retain this material as a separate entity under the new combination *N. racemosum* (Kützinger) Basso & Rodondi.

DISCUSSION

The external morphology of newly collected Mediterranean plants of the fruticose rhodolith *S. fruticulosus* is variable, from flat thalli to those with protuberances up to several millimetres in length. All characters evident in the lectotype material are consistent with those in the other Mediterranean tetrasporangial plants of our collection.

We were unable to find trichocytes in the lectotype or in other Mediterranean plants of *S. fruticulosus*, including the lectotype of *S. stalactitica*, herein considered a heterotypic synonym of *S. fruticulosus*. This is despite the report of occasional trichocytes at the thallus surface of type material of *S. fruticulosus* (Woelkerling 1985). Previous examination of the type material of *S. fruticulosus* and *S. stalactitica* failed to recognise the heterogeneous composition of the specimens. The lectotype of *S. fruticulosus* has overgrown a sterile, unidentified coralline with trichocytes that are solitary or in vertical rows. The type material of *S. stalactitica* contains, in addition to the portion here designated as lectotype (Fig. 29, arrow A), a species of *Neogoniolithon*, also with trichocytes. This confusion of material is likely to be the reason for the previous report of trichocytes in *S. fruticulosus* and *S. stalactitica*, which more probably belonged to the neighbouring species. Trichocytes are absent from most plants of the Australian population of *S. fruticulosus*, and their rare occurrence is limited to the thallus surface (Penrose 1991; Table 1).

Several authors have questioned the reliability of trichocyte occurrence as a diagnostic character, and recent papers do not consider it useful for genera delimitation, although a consensus is yet to be reached (Chamberlain 1985, 1994; Penrose & Woelkerling 1988; Woelkerling 1988; Penrose 1996). Because trichocytes are absent or rare and never buried in the perithallium of all specimens of *S. fruticulosus* in which they have

been reported so far, their utility in the circumscription of the genus within the mastophoroids appears to be doubtful. Further investigations are needed to clarify the value of trichocyte occurrence as a diagnostic character.

Comparison of the Mediterranean (this paper) and Australian (Penrose 1991) populations shows that Mediterranean plants have smaller tetrasporangial and gametangial conceptacles and lack trichocytes. However, a statistical comparison of data from the Australian population is not available. Penrose (1991) provided the "usually" recorded size of the anatomical and reproductive features of the Australian population, omitting extreme values: this is particularly evident for gametangial conceptacles, for which some pictures (Penrose 1991, figs 19, 20) reveal a much smaller size than that described. Moreover, because most Australian plants are reported to lack trichocytes, this character appears to be almost useless when trying to segregate the Australian and Mediterranean populations into different species. Because of these considerations, we conclude that the dimensional overlap and the common absence of trichocytes prevents a separation of the Australian population from the Mediterranean one on the basis of existing data (Table 1).

The lectotype of *S. racemosa* is a mastophoroid male plant with spermatangia distributed both on the floor and roof of conceptacle chambers; therefore, it is assigned to the genus *Neogoniolithon*. We cautiously retain this material as a separate species under the new combination *N. racemosum* until further studies clarify the morphological variability within freshly collected populations of related species of *Neogoniolithon*.

Because precise information concerning the provenance of the type collection of *S. stalactitica* is not available (other than the generic "Mediterranean Sea") and because unequivocal diagnostic characters are lacking, we cannot confidently establish any relationship between *Neogoniolithon* sp. and *N. racemosum* nor achieve any more precise identification of this specimen at this time.

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