

## MORPHOLOGY AND LIFE HISTORY OF *SCYTOSIPHON LOMENTARIA* (SCYTOSIPHONACEAE, PHAEOPHYCEAE) FROM THE AZORES<sup>1</sup>

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**Morphological and culture studies of *Scytosiphon lomentaria* (Lyngbye) Link and *Microspongium gelatinosum* Reinke (Scytosiphonaceae, Phaeophyceae) were undertaken on material collected on the Island of São Miguel, Azores, where both species were commonly found. Erect thalli of *S. lomentaria* collected in the field were up to 33 cm long and 2.3 mm wide, tubular, hollow, and commonly constricted at intervals. The plurilocular sporangia were positioned in continuous sori on the thallus surface. Ascocysts were present. In the field, *M. gelatinosum* formed crustose to slightly pulvinate plants, were spongy in texture, and dark brown to black in color, which were circular or irregularly spreading over several centimeters and firmly attached to the substratum. Sessile unilocular sporangia were located in sori on the crust surface. In culture *S. lomentaria* plurisporos developed into *Microspongium*-like crustose prostrate thalli that formed unilocular sporangia. Unisporos developed into new erect thalli that formed plurilocular sporangia. Sexual reproduction was not observed. In culture, *M. gelatinosum* unisporos developed into erect thalli identical with *S. lomentaria*. These results are similar to those reported for other areas and suggest the occurrence in the Azorean plants of a monophasic and heteromorphic life history, involving both entities studied.**

**Key index words:** life history; *Microspongium gelatinosum*; morphology; Phaeophyceae; *Scytosiphon lomentaria*, Scytosiphonaceae

*Scytosiphon lomentaria* (Lyngbye) Link (Scytosiphonaceae, Phaeophyceae) was reported for the Azores by Gain (1914), but *Microspongium gelatinosum* Reinke was only recently reported (Parente et al. 2000). The taxonomy of *Scytosiphon* remains unresolved, with new species described (Kogame 1998) and other species recombined and reduced to synonyms. An example is *S. pygmeus* Reinke, transferred into *S. lomentaria* (Fletcher 1987). Conversely, *S. complanatus* (Rosevinge) Doty, rec-

ognized as an infraspecific taxon of *S. lomentaria* by Wynne (1969) and Clayton (1976), was later recognized as a distinct species by Pedersen (1980). Kogame (1998) suggested the presence or absence of ascocysts to be useful taxonomic character to distinguish *Scytosiphon* species.

*Microspongium gelatinosum* was first described by Reinke (1888) from material collected in the Baltic Sea. Diagnostic features included a monostromatic partly distromatic base, branched erect filaments, one to four plastids per cell, lateral uniseriate plurilocular sporangia, and absence of unilocular sporangia. Later, Reinke (1889) gave a different description of this species in which plants have little branched erect filaments, a single plastid per cell, and unilocular sporangia. According to Fletcher (1974, 1987) and Kristiansen and Pedersen (1979), two entities are involved within Reinke's concept of *M. gelatinosum*, represented by the above two descriptions. Kristiansen and Pedersen (1979) suggested that *M. gelatinosum* should be understood in the original sense of Reinke (1888), whereas the alga with unilocular sporangia is a *Ralfsia*-like prostrate system of *Scytosiphon*. Fletcher (1987), however, disagreed with the description of the prostrate system as *Ralfsia*-like, based on the strength of the erect filaments union. According to him, in the *Ralfsia* spp. the erect filaments are more strongly adjoined.

*Scytosiphon lomentaria* is usually reported to have a heteromorphic life history with small crustose and/or tufted stages (e.g. *Microspongium*, *Stragularia*, *Ralfsia*) associated with the erect blade (Nakamura 1965, Lund 1966, Tatewaki 1966, McLachlan et al. 1971, Fletcher 1974, Nakamura and Tatewaki 1975). Wynne (1969) reported that *Ralfsia californica* Setchell et Gardner represented the crustose phase in the life history of the pacific *S. lomentaria* and *Petalonia fascia*, and Rhodes and Connell (1973) reported *Ralfsia clavata* (Carmichael) Crouan *sensu* Farlow as the crustose stage of nonidentified species of Atlantic *Scytosiphon* and *Petalonia*. Conversely, Lund (1966), studying material from Denmark, considered that the microthallus and the *Scytosiphon* thallus were both diploid structures and suggested that Atlantic *S. lomentaria* may not be conspecific with algae of the Pacific. He identified the

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sporophyte microthallus as *M. gelatinosum*. Similar results were obtained by other researchers also working with Atlantic material (McLachlan et al. 1971, Fletcher 1974, Kristiansen and Pedersen 1979).

Here we describe the morphology of Azorean plants of *S. lomentaria* and *M. gelatinosum* and investigate the life history connection between these two entities in this region of the Atlantic Ocean.

#### MATERIALS AND METHODS

Algal material used in the present study was collected monthly from May 1998 to May 1999 at seven sites around the island of São Miguel, Azores (Fig. 1). Collecting continued until May 2001 to provide material for unialgal cultures.

All specimens were numbered, and a reference collection was made and deposited in the Department of Biology, University of the Azores. The code numbers of representative specimens are given in the text. The systematic organization and nomenclatural synopsis used generally follows Silva et al. (1996). Measurements of cells and other structures were made using a micrometer eyepiece. Unialgal cultures were established. Small segments of thalli bearing unilocular sporangia (*M. gelatinosum*, 29 specimens) or plurilocular sporangia (*S. lomentaria*, 70 specimens) were placed in drops of Grund culture medium (von Stosch 1963), following the isolation technique of Wynne (1969). After settlement, the coverslips with attached spores were placed into Petri dishes containing the same medium. Two sets of environmental regimes were used (15° C, 8:16-h light:dark, and 22° C, 16:8-h light:dark, under fluorescent lighting of approximately 30–50  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), corresponding approximately to the winter and summer intertidal conditions of São Miguel Island. A minimum of four replicates was used under each culture regime. If a subsequent generation was required, fertile material was subcultured. All cultures were examined at least every 5 days.

#### RESULTS

**Morphology and phenology of *Scytosiphon lomentaria*.** Specimens studied were SMG-98-752, SMG-98-931, SMG-99-440, SMG-99-653, SMG-99-798, and SMG-99-800. Plants comprised a tubular thallus, simple, cylindrical, hollow, and commonly constricted at intervals, and measured up to 33 cm (–40 cm) long and 2.3 mm wide. In surface view, cells ( $5\text{--}11 \times 4\text{--}6 \mu\text{m}$ ) were irregularly placed. Each cell had one plate-like parietal plastid with one to two pyrenoids. In section (Fig. 2A), thalli comprised a cortex of two layers of small pigmented cells (outer cells,  $8\text{--}10 \times 5\text{--}8 \mu\text{m}$ ; inner cells,  $9\text{--}15 \times 7\text{--}9 \mu\text{m}$ ) and an inner medulla. The medulla was formed of four to five layers of irregular and colorless cells, each with a thick wall (outer cells,  $10\text{--}40 \times 10\text{--}24 \mu\text{m}$ ; inner cells,  $42\text{--}73 \times 30\text{--}50 \mu\text{m}$ ). Hairs ( $6\text{--}12 \mu\text{m}$  in diameter) arose from superficial cells. The plurilocular sporangia were formed in continuous sori on the thallus surface and arose from the cortical cells in closely packed vertical rows. They were commonly uniseriate, sometimes biseriate,  $23\text{--}43 \mu\text{m}$  (seven loculi) long and  $4\text{--}5 \mu\text{m}$  in diameter, with subquadrate to rectangular loculi ( $5\text{--}6 \times 4\text{--}5 \mu\text{m}$ ). Ascocyst-like cells were elongate to pyriform in shape,  $22\text{--}29 \times 6\text{--}11 \mu\text{m}$ . Unilocular sporangia were not observed on erect thalli.

*Scytosiphon lomentaria* was observed from February to July at all sites, on stones, bedrock, and other hard substrata in the mid to low-tide region of exposed shores and also in tide pools. All specimens collected were reproductive.

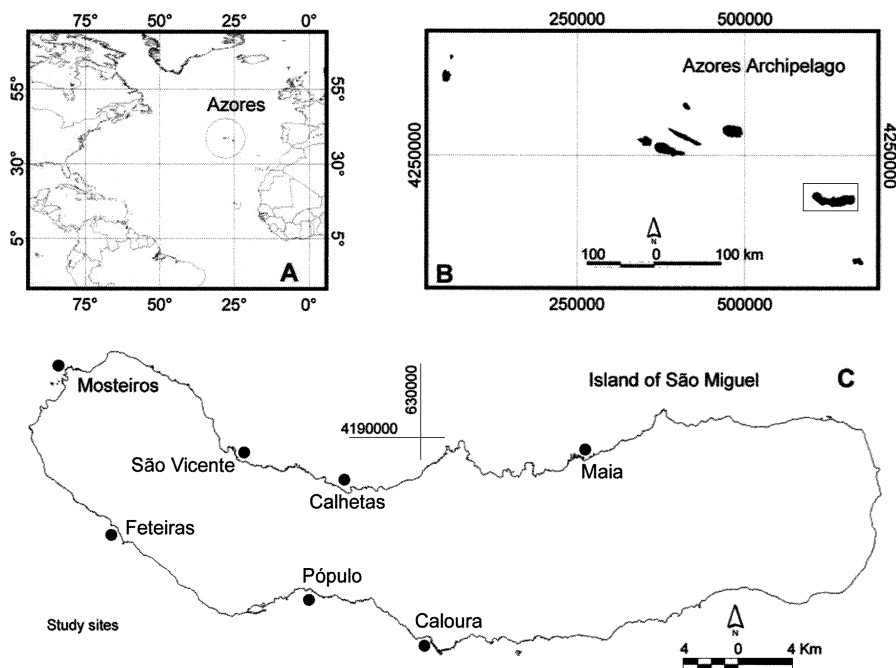


FIG. 1. (A) Location of the archipelago of the Azores in the North Atlantic. (B) Position of the island of São Miguel in the archipelago. (C) Location of the study sites.

*Morphology and phenology of Microspongium gelatinosum.* Specimens studied were SMG-98-620, SMG-98-754, SMG-98-811, SMG-98-829, SMG-98-880, and SMG-98-947. Plants of *Microspongium* were epilithic, crustose to slightly pulvinate in appearance, spongy in texture, dark brown to black in color, circular or more commonly irregularly spreading over several centimeters, firmly attached to the substratum, and

usually without obvious rhizoids. Crusts comprised a monostromatic base, which gave rise to erect usually unbranched coherent filaments up to 12 cells (108  $\mu\text{m}$ ) long, easily separable under pressure (Fig. 2B). Basal cells (10–26  $\times$  6–10  $\mu\text{m}$ ) were commonly rectangular; upper cells (8–34  $\times$  8–12  $\mu\text{m}$ ) were quadrate, elongate, or slightly pyriform. Cells possessed in the upper region a single, parietal, plate-like plastid

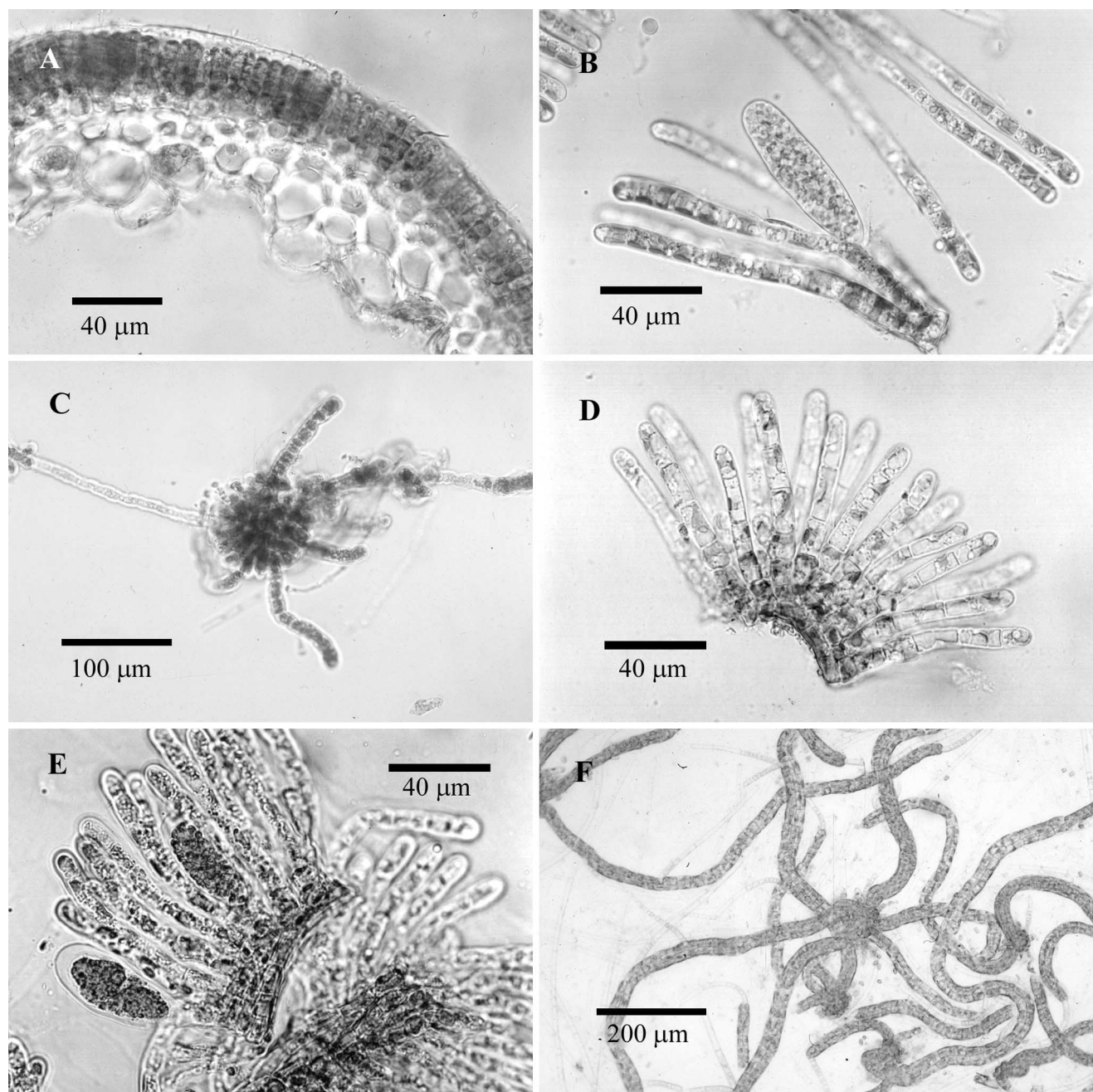


FIG. 2. Cross-section of mature thallus of field *Scytosiphon lomentaria* bearing plurilocular sporangia. (A) Portion of fertile thallus of field *Microspongium gelatinosum* with mature unilocular sporangia. (B) Development of *Scytosiphon lomentaria* in culture. (C–E) Progeny derived from plurispores. (C) Knotted structure; (D) multi-stratose crust derived from squash of knotted structure; (E) squashed crust with lateral unilocular sporangia emerging from the base of paraphyses. (F) Cylindrical blades derived from unispores of crustose thalli.

with one pyrenoid. Unilocular sporangia ( $46\text{--}102 \times 14\text{--}32 \mu\text{m}$ ) were in extensive spongy sori on the crust surface. They were elongate-pyriform or elongate-cylindrical and sessile (Fig. 2B). The associated paraphyses were linear in shape and up to 10 cells long. Plurilocular sporangia were not observed.

*Microspongium gelatinosum* was common throughout the year at all sites visited except Calhetas and Mosteiros on rocks and in pools at low-tide level. Reproductive plants were found all year round.

*Cultures established from the field* *Scytosiphon lomentaria*. The plurispores were pear shaped, laterally biflagellated, contained a large plastid and a red eye spot, and measured after settlement  $5\text{--}8 \mu\text{m}$  in diameter. Within 1–3 days, they developed into small germlings. Transverse divisions along these germlings produced short filaments usually terminated by a multicellular hair. Each cell of the filament contained one large parietal plate-like plastid with a prominent pyrenoid. Some germlings continued to develop and formed branched prostrate filaments. In some cases the cells of these filaments formed secondary longitudinal walls and became knotted (Fig. 2C). These knotted structures possessed numerous hairs and when squashed revealed a central base with radial erect filaments that resembled a three-dimensional crust (Fig. 2D). Other germlings gave rise directly to disks strongly adherent to the substratum, which by continuous divisions of

the cells gave rise to a multistratose crust. The basal cells of this crust ( $5\text{--}16 \times 5\text{--}14 \mu\text{m}$ ) gave rise to erect and tightly bound together unbranched filaments up to  $82 \mu\text{m}$  (13 cells long), composed of rectangular cells ( $8\text{--}13 \times 7\text{--}11 \mu\text{m}$ ). Unilocular sporangia ( $17\text{--}63 \times 9\text{--}25 \mu\text{m}$ ), elongate-pyriform in shape (Fig. 2E), were formed laterally on the erect filaments of many knotted structures and crusts. They were protected by linear paraphyses ( $78\text{--}100 \times 6\text{--}8 \mu\text{m}$ ) up to 10 cells long. The unispores released were pear shaped, laterally biflagellated, and had a prominent plate-like plastid with one pyrenoid. After approximately 2 weeks, the germlings produced small erect filaments, with longitudinal divisions and terminal hairs, which within 2 months had become parenchymatous, producing erect blades (Fig. 2F). Blades were also produced directly as extensions of the surface cells of the initial germlings, knotted structures, and polystromatic crusts and initially resembled filaments terminated by a true hair. A series of transverse and longitudinal divisions of these small filaments produced parenchymatous erect blades identical to *Scytosiphon* specimens, which after 1 month developed plurilocular sporangia on their surface.

*Cultures established from the field* *Microspongium gelatinosum*. The unispores ( $6\text{--}8 \mu\text{m}$  in diameter after settlement) had a short period of motility (about 5–10 min) and rapidly settled around the empty unilocular sporangia. Within 1–2 days, they developed into small

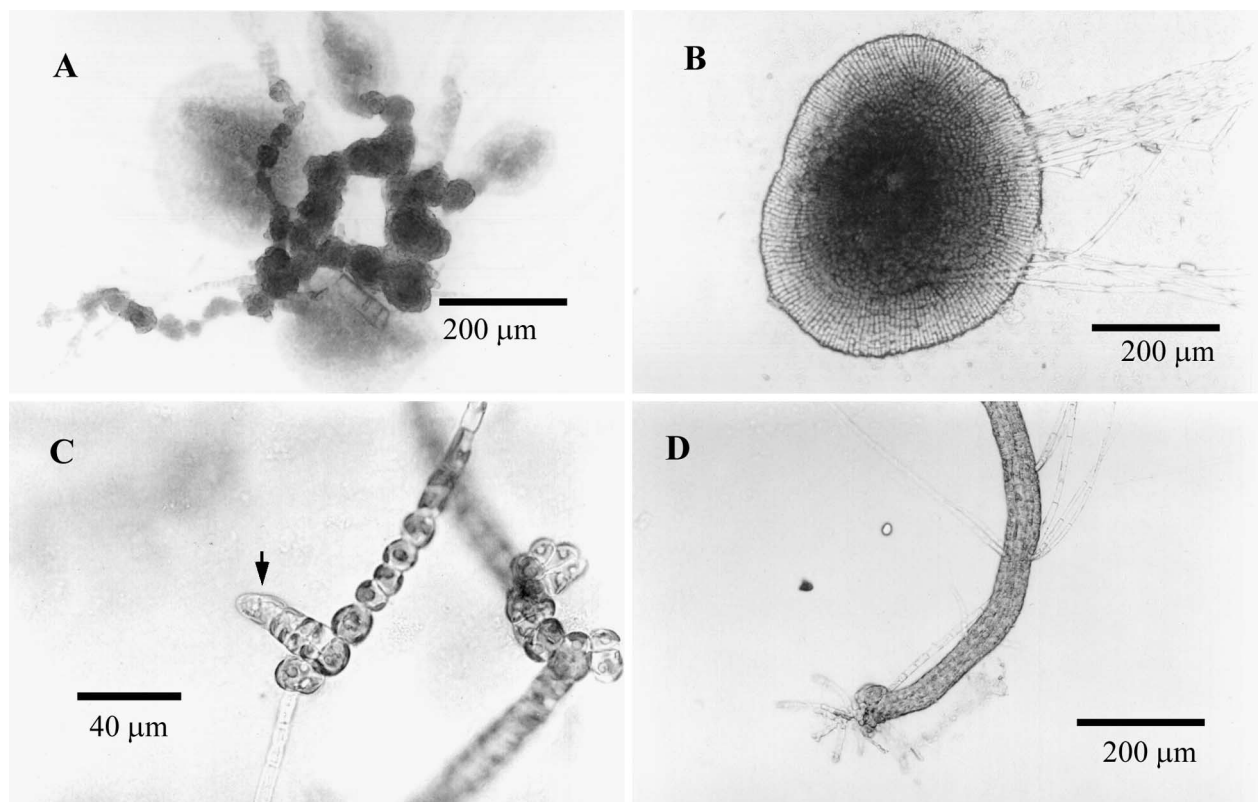


FIG. 3. Development of *Microspongium gelatinosum* in culture (progeny derived from unispores). (A) Prostrate growth and disk formation from 20-day-old knotted structure. (B) Crust with true hairs. (C) Initial formation of erect blade (arrow) from a 10-day-old germling filament. (D) Cylindrical thallus showing rhizoidal filaments and true hairs.

germlings, with cells containing one large plate-like plastid with one pyrenoid. In some germlings cell divisions continued to form filaments, which by further divisions produced knotted structures and discs (Fig. 3A). Disks were also produced directly from the knotted structures when these had good contact with the substratum. The disks grew by transverse divisions of the apical cells of the confluent filaments. Some remained monostromatic and irregular in outline. Oth-

ers became bigger and thicker, forming polystromatic crusts (Fig. 3B). In squash preparations, these crusts revealed adjoined erect filaments and one or two layers of basal cells. True hairs were present on all structures. Erect blades, resembling *Scytosiphon*, were produced vertically from cells of the initial germling filaments, the knotted structures, and the monostromatic disks. They were usually initiated as small uniseriate filaments (Fig. 3C), with a terminal colorless hair,

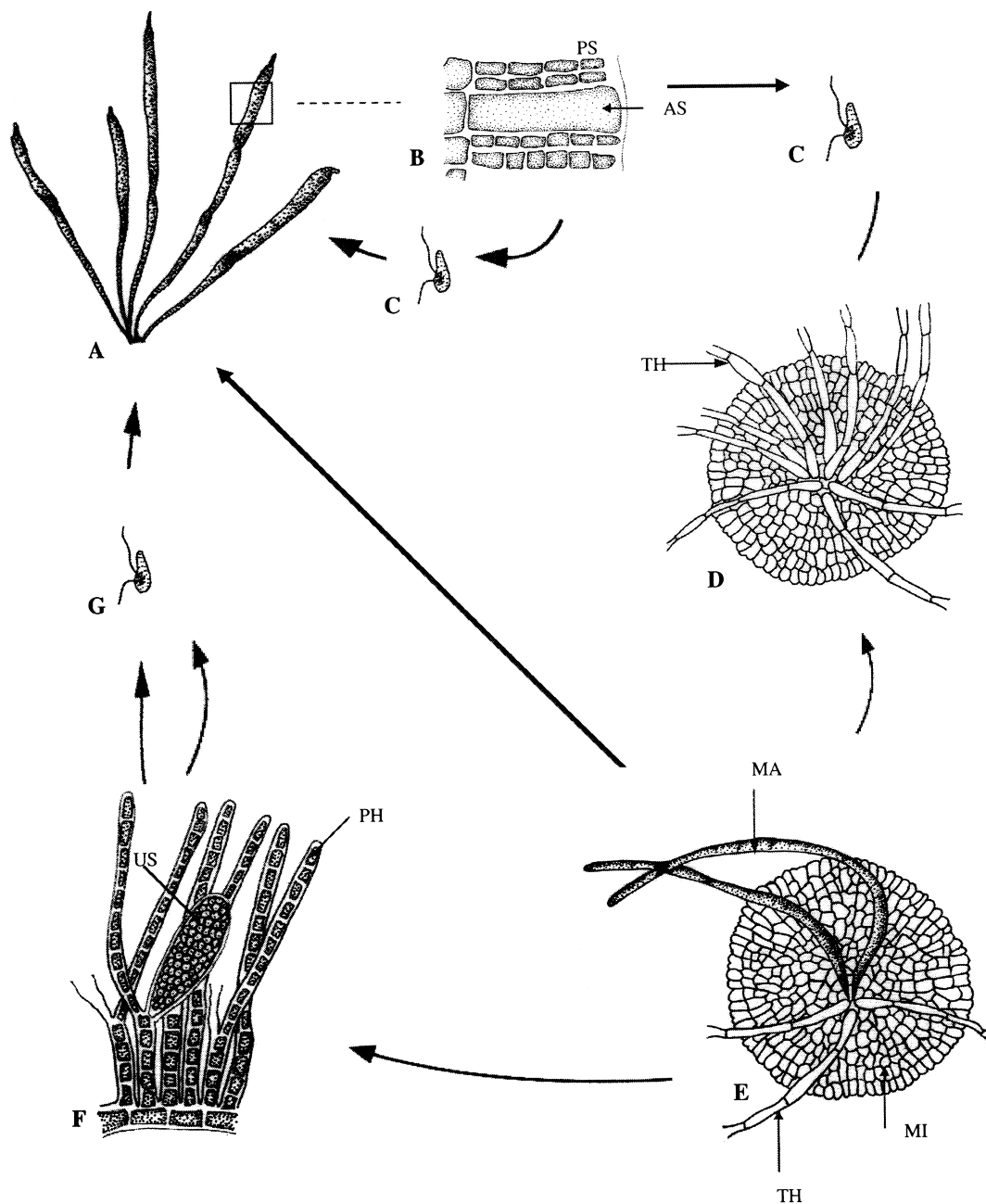


FIG. 4. Diagrammatic illustrations of the life history of Azorean *Scytosiphon lomentaria* in culture. (A) Macrothallus with plurilocular sporangia. (B) Transverse section of a small portion of macrothallus with plurilocular sporangia. (C) Spore from the plurilocular sporangia (plurispore). (D) Crustose microthallus. (E) Crustose microthallus with erect blades and true hairs emerging. (F) Squashed microthallus with unilocular sporangia and paraphyses. (G) Spore from the unilocular sporangia (unispore). AS, ascocyst; MA, macrothallus; MI, microthallus; PH, paraphyses; PS, plurilocular sporangia; TH, true hair; US, unilocular sporangia.

TABLE 1. Comparative measurements of *Microspongium gelatinosum* from the Azores, Denmark, the British Isles, the Mediterranean, and Japan.

	Azores	Denmark	British Isles	Mediterranean	Japan
Maximum thickness; $\mu\text{m}$	200	170–250	>430	400	160–300
Diameter of erect filaments; $\mu\text{m}$	6–12	7–8	5–10		7–12
Unilocular sporangia	46–102 $\times$ 14–32	70–100 $\times$ up to 20	48–100 $\times$ 16–27	50–130 $\times$ 13–30	60–150 $\times$ 18–28
No. of cells in paraphyses	5–8		6–8	5–10	
Source	Parente <i>et al.</i> (2000)	Lund (1996)	Fletcher (1987)	Sartoni and Boddi (1990)	Kogame (1998)

and subsequently became parenchymatous by transverse and longitudinal divisions (Fig. 3D). Plurilocular sporangia were observed on their surface.

The present observations indicate that Azorean *S. lomentaria* has a monophasic and heteromorphic life history (Fig. 4) in which the crustose microthallus that bears unilocular sporangia resembles *M. gelatinosum*. Sexual reproduction was not observed.

#### DISCUSSION

The Azorean *S. lomentaria* agrees well with the description given by Fletcher (1987). The Azorean material of *M. gelatinosum* agrees with the material with unilocular sporangia described by Reinke (1889) and corresponds well with the descriptions and illustrations given by many authors for specimens collected elsewhere (Lund 1966, Fletcher 1987, Sartoni and Boddi 1990, Kogame 1998). Differences were only found on the thickness of the thallus, Azorean specimens having thinner thallus when compared with material from the British Isles and the Mediterranean (Table 1).

The occurrence of a monophasic and heteromorphic life history observed in the present study has also been reported by Wynne (1969) and Kristiansen and Pedersen (1979). Sexual reproduction was not observed for Azorean specimens. These results contrast with the studies of Nakamura and Tatewaki (1975) and Clayton (1980), who reported gametic fusion. In the later studies, erect haploid thalli released gametes, which after fusion germinated into diploid crusts. These crusts developed unilocular sporangia, where meiosis occurred, and unispores gave rise to new erect haploid blades. In these cases, the erect thalli represent the gametophyte that alternates with a sporophyte represented by the crustose thalli.

Isolated cultures of plurispores from Azorean *S. lomentaria* produced crustose thalli, bearing unilocular sporangia. These thalli comprised a monostromatic basal layer, later becoming multistratose and cells with only one plastid. These are characteristics common to three different genera—*Ralfsia*, *Stragularia* (separated by some authors from *Ralfsia*), and *Microspongium*—but only the later has filaments easily separated under pressure in squash preparations (Wynne 1969, Fletcher 1974). It is therefore suggested that Azorean *S. lomentaria* is associated with the crust *M. gelatinosum*. The results obtained from the culture studies support this hypothesis: *S. lomentaria* brought from

the shore gave rise in culture to crustose thalli identified as *M. gelatinosum*, and *M. gelatinosum* collected from the shore gave rise to erect *Scytosiphon* blades in culture. Other investigations (Lund 1966 in Denmark, McLachlan et al. 1971 from the northeast coast of Canada, Fletcher 1974 in the British Isles, Kristiansen and Pedersen 1979 in Denmark, Kogame 1998 in Japan) concur with our observations and conclusion in that they found a life history relationship between *Scytosiphon* and *Microspongium*. Still other investigations (Nakamura 1965, Tatewaki 1966 [Japan], Wynne 1969 [California], Clayton 1980 [Australia], Rhodes and Connell 1963 [Atlantic Ocean]) have shown a relationship between crustose *Ralfsia*-like thalli and *S. lomentaria*. Fletcher (1987) noted that the illustrations of the crustose thalli described by Nakamura and Tatewaki (1975) and Clayton (1980) as *Ralfsia*-like resembled *Microspongium*-like thalli. In a recent study with Japanese plants (Kogame 1998), *M. gelatinosum* was recognized as being the crust associated with *S. lomentaria*. This author also showed that the crust identified as *Ralfsia*-like by Nakamura and Tatewaki (1975) was in fact *Microspongium*. Therefore, *Microspongium* is associated with *S. lomentaria* in most of these previous studies. On the other hand, Fletcher (1987) also noted that cultured crusts of Californian *S. lomentaria* shown by Wynne (1969) are clearly *Ralfsia*-like. This suggests that Californian *S. lomentaria* may be needed to be re-examined.

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