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Life history of *Hydroclathrus clathratus* (Scytosiphonaceae, Phaeophyta) in the Azores

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Abstract — *Hydroclathrus clathratus* is a common species on the rocky intertidal shores of the Island of São Miguel, Azores, mainly in spring and summer. Fertile saccate thalli appear in spring and disappear from late summer onward. Two types of life history were observed in culture: a direct-type and a heteromorphic, monophasic life history. In the direct type, reproductive cells from erect plants collected in the field developed directly into new erect thalli with plurilocular sporangia. In the heteromorphic life-history pattern, plurispores from the plurilocular sporangia of the erect thalli developed into filamentous prostrate microthalli. These developed unilocular and plurilocular sporangia in both short-day and long-day conditions at 15-22 °C. Unispores released from the unilocular sporangia developed into saccate thalli, whereas plurispores gave rise to filamentous prostrate microthalli.

***Hydroclathrus clathratus* / life history/ morphology/ Phaeophyta/ Scytosiphonaceae**

Résumé — Étude du cycle de vie de *Hydroclathrus clathratus* (Scytosiphonaceae, Phaeophyta) aux Açores. *Hydroclathrus clathratus* est une espèce commune de l'étage littoral sur les côtes rocheuses de l'île de São Miguel, Açores, particulièrement au printemps et en été. Des boules irrégulières fertiles sont visibles dès le début du printemps jusqu'a la fin de l'été. Deux types de cycle vital ont été observés en culture: un type de développement direct et un autre monophasique et hétéromorphe. Dans le cycle monomorphique, des cellules reproductives des échantillons collectés se développent directement en nouvelles boules avec des sporocystes pluriloculaires. Dans le cycle vital hétéromorphique, des plurispores provenant des sporocystes pluriloculaires des thalles creux se développent en microthalles filamenteux. Ceux-ci donnent naissance à des sporocystes uni- et pluriloculaires à 15-22 °C, que le jour soit long ou court. Les monospores libérées des sporocystes pluriloculaires donnent des gamétophytes globuleux tandis que les plurispores se développent en microthalles filamenteux.

Cycle de vie / *Hydroclathrus clathratus* / morphologie / Phaeophyta / Scytosiphonaceae

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INTRODUCTION

Hydroclathrus clathratus (C. Agardh) M. Howe is widely distributed in tropical to warm-temperate seas throughout the world (Lawson & John, 1982). It was first reported for the Azores by South & Tittley (1986), although those authors did not cite a specific locality. It has since been reported for Santa Maria (Neto *et al.*, 1991), Faial (Tittley & Neto, 1994) and São Miguel (Neto, 1997). Characterized by a globular and hollow thallus, *Hydroclathrus* is distinguished from *Colpomenia* by the numerous irregular perforations of its surface.

Hydroclathrus clathratus was first investigated in culture by Clayton (1982) using Australian material. She reported a direct life history, with plurispores released from the plurilocular sporangia on the macroscopic thallus, developing again directly into new erect thalli. However, a heteromorphic, monophasic[1] life history was reported for Japanese *H. clathratus* (Kogame, 1997), in which the erect macrothallus, bearing plurilocular sporangia, alternates with a prostrate microthallus, bearing both uni- and plurilocular organs.

In the present study, the life history of Atlantic material of *H. clathratus* is investigated. Using material collected from the Azores, and a combination of culture studies and field observations, we report on the strategies of reproduction and the growth patterns of this species.

MATERIAL AND METHODS

Fertile specimens of *H. clathratus* were collected in late winter to summer between February and August 2000, from the intertidal at Poças and Praia dos Mosteiros, two sites on the west coast of the island of São Miguel (Fig. 1).

The plants were examined in the laboratory and the entire thallus, portions or thin sections were observed microscopically. Cells and other structures were measured using a micrometer eyepiece. A representative collection of colour slides was made using an OLYMPUS-PM 10-35 AD-1 microphotography system. All specimens were numbered and a reference collection was organized by storing samples of the alga in a 5 % buffered formaldehyde-sea water solution. These collections are deposited in the Department of Biology at the University of the Azores. Code numbers for representative specimens are given in the text. The systematic organization and synopsis of nomenclature used in this study follows that adopted by Silva *et al.* (1996).

Cultures were established from reproductive cells of plurilocular organs of a total of 16 field-collected plants. Segments of thalli bearing mature plurilocular organs were excised, washed in sterile seawater, and set up to release spores following Wynne's (1969) isolation technique. Two sets of environmental regimes were used (22 °C, 16:8 h light:dark, and 15 °C, 8:16 h light:dark, under fluorescent lighting of approximately 30-50 µmol photons.$m^{-2}.s^{-1}$), corresponding approximately to summer and winter intertidal conditions of São Miguel Island. For each cultured plant, a total of 4 petri dishes were set up, two for each of the regimes.

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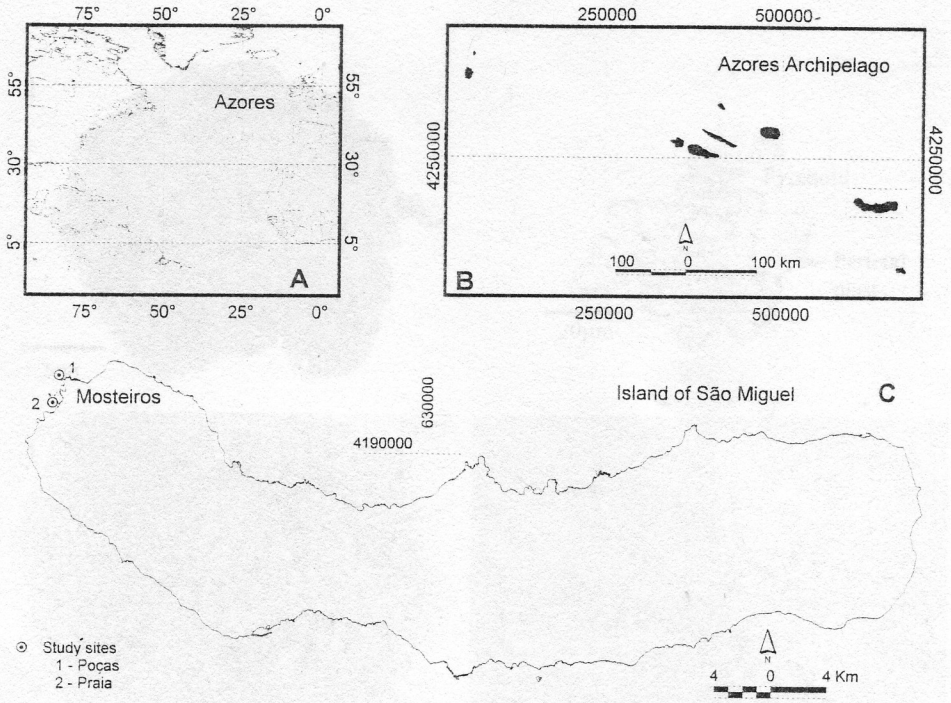


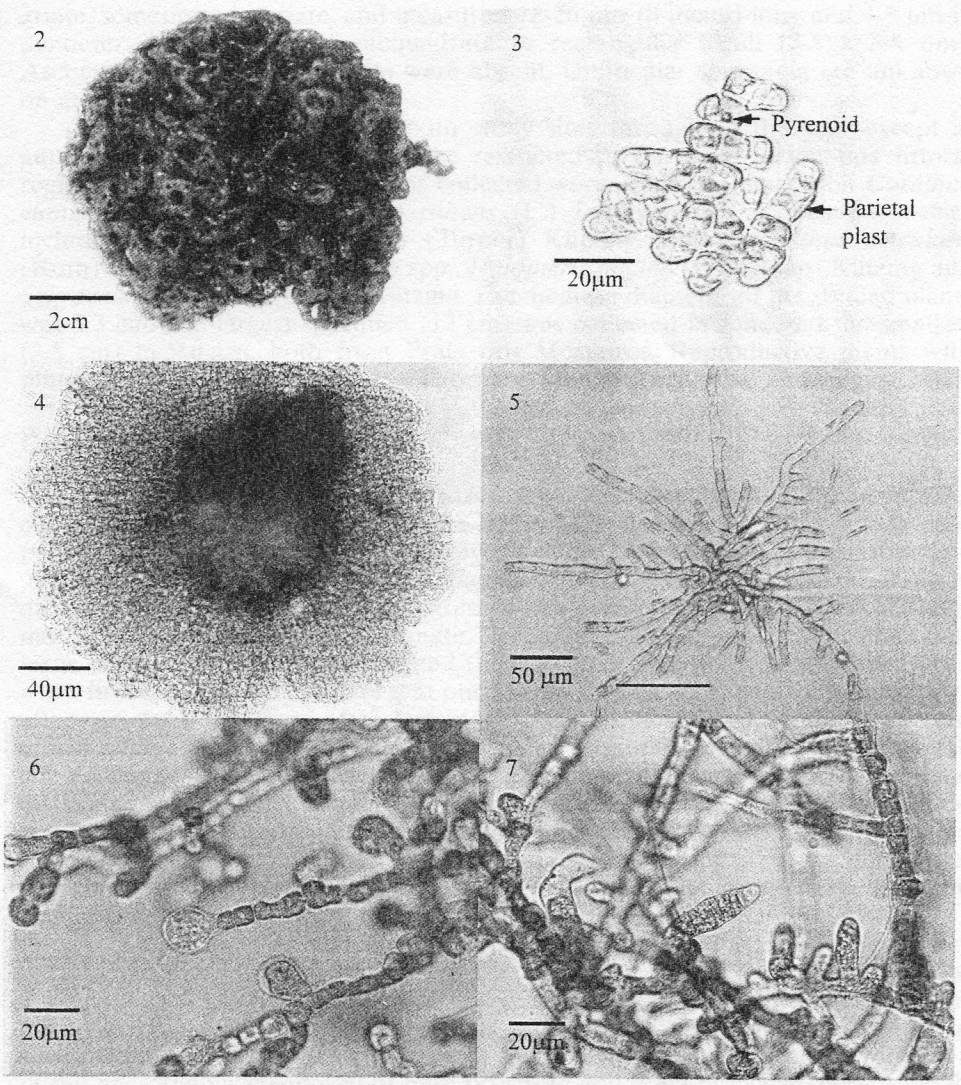
Fig. 1. Location of the Azores archipelago in the North Atlantic (A); position of the island of São Miguel in the archipelago (B), and the location of the study sites (C).

When a subsequent generation was required, fertile material was sub-cultured. Grund culture medium (von Stosch, 1963) was used and was changed every week. All cultures were examined every 4-5 days. Special attention was given to the different phases of development of the life cycle and the occurrence of reproductive organs.

RESULTS

Morphology and phenology

The following plants were studied (SMG meaning plants collected in São Miguel Island, followed by the year of collection and the number of each specimen in the algal collections): SMG-00-34; SMG-00-35; SMG-00-36; SMG-00-49; SMG-00-50; SMG-00-51; SMG-00-86; SMG-00-87; SMG-00-88; SMG-00-173; SMG-00-176; SMG-00-177; SMG-00-182; SMG-00-183; SMG-00-246; SMG-00-247. Erect thalli were irregularly globular, hollow, sessile, and exhibited numerous circular or oval perforations and convolutions (Fig. 2) usually bordered by inrolled edges. In surface view, cells were irregularly arranged, rectangular to polygonal, 5-15 μm in diameter, with a single, plate-like, parietal plastid containing a single pyrenoid. In transverse section, the cortex comprised two layers of small, pigmented



Figs 2-7. Habit of *H. clathratus* collected from the field and its development in culture. Fig. 2. Habit of field-collected thallus. Figs 3, 4. Progeny derived from zooids of plurilocular sporangia from erect thalli. Fig. 3. 1-week old germling showing cells with a large parietal plate-like plastid and a prominent pyrenoid (Arrows). Fig. 4. Saccate thallus arising from the centre of a disc. Figs 5-7. progeny derived from unfused female gametes of plurilocular organs of erect thalli. Fig. 5. 2-3-week-old filamentous germling; Fig. 6. Terminal and lateral unilocular sporangia on erect filaments; Fig. 7. Tuft with ectocarpoid plurilocular sporangia.

cells, measuring 5-16 wide x 4-9 µm in height. The medulla comprised 3-4 layers of large, colourless cells; the outer region cells measuring up to 44 µm in diameter, mid-region cells measuring up to 120 µm in diameter and the inner cells measuring up to 200 µm in diameter. Hairs, 10-16 µm in diameter, were common, grouped, and originated from the cortical cells.

Extensive sori of plurilocular sporangia closely packed in vertical rows formed in the surface cortical cells. In vertical section, they were commonly uniseriate, sometimes biseriate, and measured 18-26 µm (8 loculi) long and 4-9 µm in diameter. They comprised subquadrate to rectangular loculi (3-5 × 3-4 µm). Ascocyst-like cells (paraphyses) were absent. Unilocular sporangia are unknown on erect thalli.

Plants were present at both study sites throughout the year except in autumn. Never abundant, they were restricted to the mid- to low-tide littoral region and to pools. All specimens collected were epiphytic, mainly on *Corallina elongata* Ellis *et* Sol. and *Jania rubens* (L.) J. V. Lamour. Associated species included *Caulacanthus ustulatus* (Turner) Kützing, *Chondracanthus acicularis* (Roth) Fredericq, *Enteromorpha* spp., *Halopteris filicina* (Grateloup) Kützing and *Stypocaulon scoparia* (Linné) Kützing. The medium diameter of the studied plants was 5.3 cm. The largest specimen (12 cm) was collected in June and the smallest (2.5 cm) in August, both from Praia dos Mosteiros. Reproductive plants with plurilocular organs were found in February, March, April, June and August 2000.

Culture studies

The reproductive cells released from the erect thalli differed in size, colour and ultimate development. The larger cells measured 6–8 µm in diameter, possessed one prominent red spot eye, a large yellow, plate-like plastid, one pyrenoid, and two laterally inserted flagella. The smaller cells measured 3-4 µm in diameter, were lighter in colour, possessed one eye spot and, after a few hours of mobility, settled but did not germinate. The larger cells exhibited two distinct patterns of development. Some attached themselves directly to the coverslips within a few hours and within 2-3 days sent out a small germ tube. They formed germlings after one week that were often terminated by a multicelullar hair. Each cell contained a large parietal, plate-like plastid with a prominent pyrenoid (Fig. 3). Small circular monostromatic discs were formed directly by transverse and longitudinal divisions of the original germling. From the centre of these discs and pseudodiscs, erect saccate thalli arose (Fig. 4) which later developed holes on the surface. No reproductive organs were observed on the discs/pseudodiscs, or on the young saccate thalli. This development into new erect plants, similar to the parental thalli, occurred under both culture regimes.

Other larger reproductive cells, about 2-3 days after attaching themselves to the coverslips, started to germinate by projecting a small germ tube. In about 2 weeks they developed into filamentous germlings (Fig. 5) in which each cell contained a large parietal plate-like plastid with a prominent pyrenoid. Transverse and longitudinal divisions in the oldest part of the original germling formed a tufted microthallus composed of both prostrate and erect branched, uniseriate filaments. Each cell of these structures contained one large parietal plate-like plastid with a prominent pyrenoid. When these tufts were 2 months old, rounded unilocular sporangia developed apically and/or laterally on the erect filaments (Fig. 6). In addition, nine-week-old tufts developed ectocarpoid plurilocular sporangia (Fig. 7), both unilocular and plurilocular sporangia occurring on the same tufted microthallus. This process, by which the unfused cells gave rise to filamentous prostrate tufts bearing unilocular and plurilocular sporangia, was observed under both culture conditions.

In some cases, under both culture regimes, plurispores released by the same erect plant exhibited different patterns of development, some giving rise to erect thalli whereas others produced prostrate microthalli.

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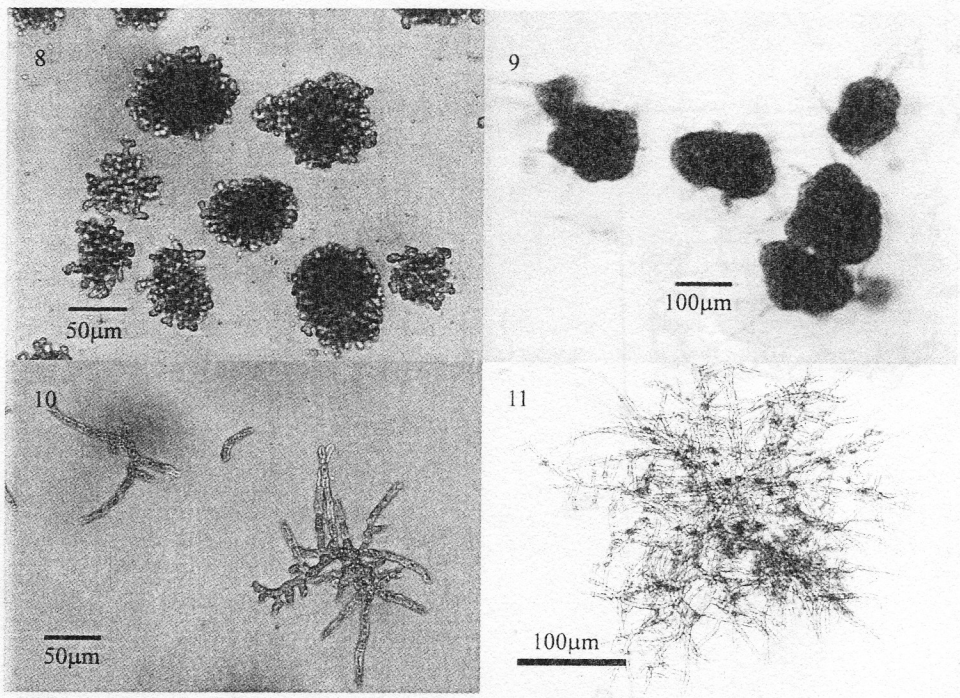


Figs 8-11. Development of *H. clathratus* in culture. Figs 8, 9. progeny derived from unispores of unilocular sporangia on tufted microthalli. Fig. 8. Small germlings Fig. 9. Young saccate plants. Figs 10, 11. progeny derived from plurispores of plurilocular sporangia on tufted microthalli. Fig. 10. Uniseriate germlings. Fig. 11. Tufted microthalli.

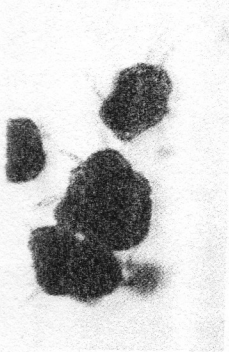
Unispores released from the unilocular sporangia of the tufted microthalli developed into small filamentous germlings (Fig. 8), each cell containing one plate-like plastid with one pyrenoid. After approximately 2 weeks, these germlings developed into a parenchymatous tissue from which small saccate plants were differentiated (Fig. 9).

Plurispores released from plurilocular sporangia of the tufted microthalli developed into short uniseriate germlings (Fig. 10). These gave rise, after 3-4 weeks, to small, branched, tufted microthalli (Fig. 11) in which each cell contained one plate-like plastid with one pyrenoid.

Only asexual reproduction was observed in Azorean *H. clathratus* regardless of developmental pathway (Fig. 12). The life history, therefore, comprises: a heteromorphic, monophasic life history in which erect thalli bearing plurilocular sporangia produce prostrate thalli bearing unilocular and plurilocular sporangia, and a direct-type of life history in which erect thalli bearing plurilocular sporangia develop into new erect thalli with plurilocular sporangia.

DISCUSSION

The material studied corresponds well with the descriptions and illustrations given by Hamel (1937), Fritsch (1945) and Abbott & Hollenberg (1976).



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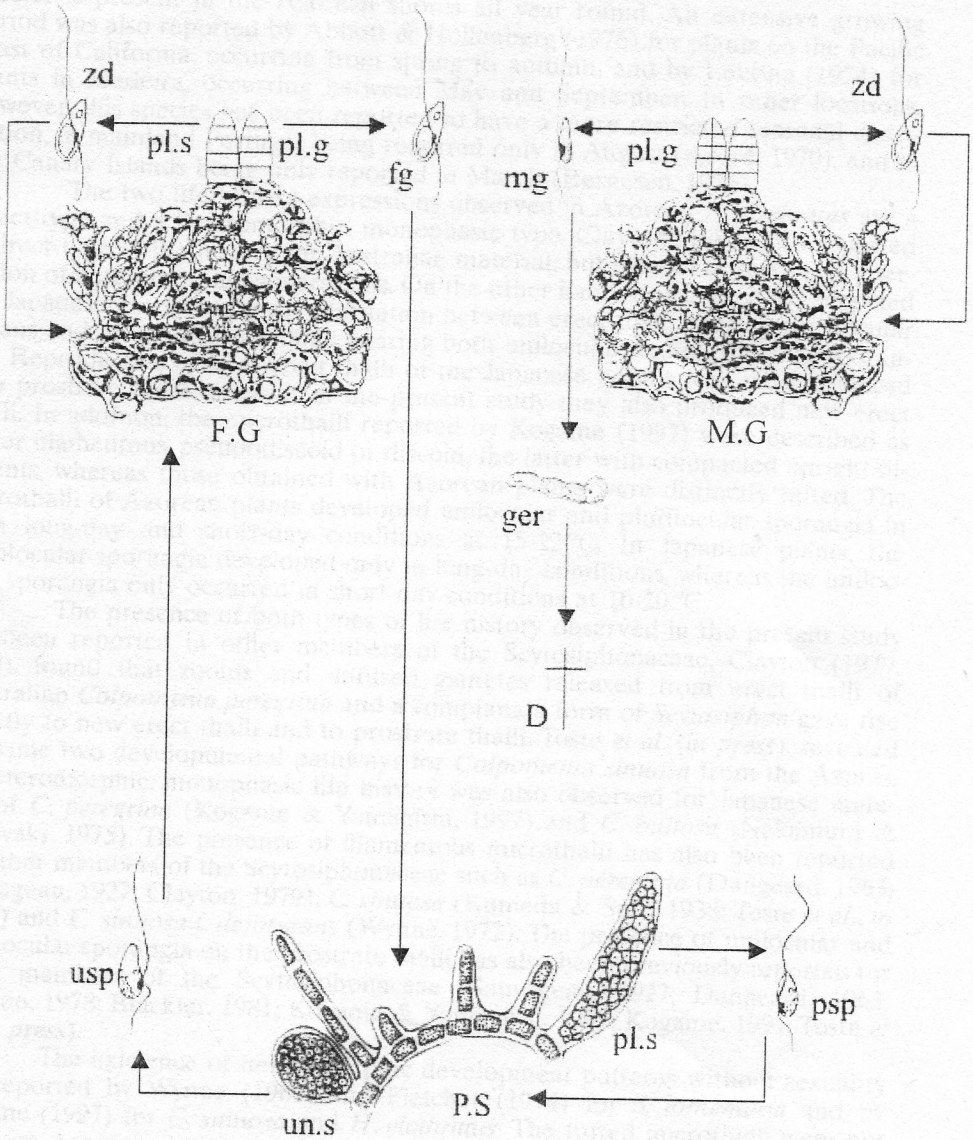

Fig. 12. Diagrammatic illustration of the life history of *H. clathratus* in culture. F.G. female gametophyte; M.G. male gametophyte; fg. female gamete; mg. male gamete; pl.s. plurilocular sporangium; psp. plurispore; un.s. unilocular sporangium; usp. unispore; P.S. partheno-sporophyte; ger. germling; D. death; pl.g. plurilocular gametangium; zd. zooid.

Hydroclathrus clathratus had a late winter to summer occurrence (February to August) in São Miguel and all, but one, of the 16 erect plants bore plurilocular organs. Neto (1997) recorded reproductive plants in São Miguel in late spring to early autumn (from May to October), which suggests that this

species is present in the Azorean shores all year round. An extensive growing period was also reported by Abbott & Hollenberg (1976) for plants on the Pacific coast of California, occurring from spring to autumn, and by Levring (1974) for plants in Madeira, occurring between May and September. In other locations, however, this species has been reported to have a more restricted seasonal distribution, in mainland Portugal being reported only in August (Ardré, 1970), and in the Canary Islands being only reported in March (Børgesen, 1926).

The two life history expressions observed in Azorean *H. clathratus* are a direct-type and a heteromorphic, monophasic type. Clayton (1982) also reported a direct-type of life history for Australian material, but did not observe any alternation of heteromorphic generations. On the other hand, Kogame (1997) reported for Japanese *H. clathratus* an alternation between erect thalli bearing plurilocular organs and prostrate microthalli bearing both unilocular and plurilocular sporangia. Reproductive cells of erect thalli of the Japanese populations only produced new prostrate thalli, whereas in the present study they also produced new erect thalli. In addition, the microthalli reported by Kogame (1997) were described as either filamentous, pseudodiscoid or discoid, the latter with compacted upright filaments, whereas those obtained with Azorean plants were distinctly tufted. The microthalli of Azorean plants developed unilocular and plurilocular sporangia in both long-day and short-day conditions at 15-22 °C. In Japanese plants, the plurilocular sporangia developed only in long-day conditions, whereas the unilocular sporangia only occurred in short-day conditions at 10-20 °C.

The presence of both types of life history observed in the present study has been reported in other members of the Scytosiphonaceae. Clayton (1979, 1980), found that zooids and unfused gametes released from erect thalli of Australian *Colpomenia peregrina* and a complanate form of *Scytosiphon* gave rise directly to new erect thalli and to prostrate thalli. Toste *et al.* (*in press*), revealed the same two developmental pathways for *Colpomenia sinuosa* from the Azores. A heteromorphic, monophasic life history was also observed for Japanese material of *C. peregrina* (Kogame & Yamagishi, 1997) and *C. bullosa* (Nakamura & Tatewaki, 1975). The presence of filamentous microthalli has also been reported for other members of the Scytosiphonaceae such as *C. peregrina* (Dangeard, 1963; Sauvageau, 1927; Clayton, 1979), *C. sinuosa* (Kunieda & Suto, 1938; Toste *et al.*, *in press*) and *C. sinuosa* f. *deformans* (Wynne, 1972). The presence of unilocular and plurilocular sporangia on the prostrate thalli has also been previously reported for other members of the Scytosiphonaceae (Sauvageau, 1927; Dangeard, 1963; Clayton, 1978; Blackler, 1981; Kogame & Yamagishi, 1997; Kogame, 1997; Toste *et al.*, *in press*).

The existence of heteromorphic development patterns without sexuality was reported by Wynne (1969) and Fletcher (1974) for *S. lomentaria* and by Kogame (1997) for *C. sinuosa* and *H. clathratus*. The tufted microthalli were not found on Azorean shores, despite careful searching. Even so, it seems likely that a heteromorphic, monophasic life history pattern, with an alternation between erect and prostrate thalli, is likely to be present in wild Azorean *H. clathratus*.

To our knowledge, this is the third study contributing to an understanding of the life history of *H. clathratus*, and the first based on Atlantic material. The occurrence of two distinct pathways developing simultaneously from the same plant is reported for the first time, complementing the published information on the life cycle of this species. Moreover, a heteromorphic, monophasic life cycle is reported for the first time in an Atlantic population.

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