

LIFE HISTORY OF THE MARINE RED ALGA PSEUDOGLOIOPHLOEA HALLIAE (NEMALIONALES, CHAETANGIACEAE) IN CULTURE¹

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ABSTRACT: The life history of the marine red alga *Pseudogloioiphloea halliae* (Setchell) Joly & Cordeiro collected from eastern Venezuela was studied under controlled environmental conditions in the laboratory. Carpospores germinated to form microscopic, uniseriate, branched filaments with an acrochaetoid morphology. Many of the terminal cells of these branched filaments developed into tetrasporangia with zonate, decussate or irregularly divided tetraspores. The liberated tetraspores germinated and developed in a manner similar to that of the carpospores resulting in profusely branched filaments. Approximately 2 weeks following germination, many cells of the branched filaments issued numerous small buds or button like structures covered with copious mucilage. In approximately 2 months, these buds developed into erect, fleshy plants, up to 2 cm high, very similar in external appearance and thallus structure to the field collected gametophytes. The erect fleshy plants grown in the laboratory did not reach reproductive maturity. Based on our observations, it is concluded that the *in vitro* life history of *P. halliae* is heteromorphic and is very similar to *P. confusa* (Ramus, 1969) from the Californian coast. The present study constitutes the first complete life history report *in vitro* for a member of Chaetangiaceae from the Caribbean Sea.

RESUMEN: Se siguió el ciclo vital completo bajo condiciones controladas del laboratorio del alga marina roja *Pseudogloioiphloea halliae* recolectada en el Oriente de Venezuela. Las carposporas al germinar formaron filamentos microscópicos, ramificados y uniseriados con morfología muy similar al género *Acrochaetium*. Muchas de las células terminales de estos filamentos se transformaron en tetrasporangios con las tetrasporas ordenadas de manera zonada, decusada o irregular. Las tetrasporas liberadas germinaron y formaron filamentos ramificados muy similar a las carposporas. Aproximadamente dos semanas después de la germinación, los filamentos ramificados comenzaron a originar numerosas yemas pequeñas ("botones") cubiertas de abundante mucílago. En dos meses, estas yemas desarrollaron plantas pequeñas erectas de hasta 2 cm de altura, muy similar en apariencia externa y estructura del talo a los gametofitos recolectados en el campo. Estas plantas erectas, carnosas, cultivadas en el laboratorio no alcanzaron la madurez reproductiva. De acuerdo con nuestras observaciones, se concluye que el ciclo vital "*in vitro*" de *P. halliae* es heteromórfico y muy similar al de *P. confusa* (Ramus, 1969) de la costa de California. El presente estudio constituye el primer ensayo completo del ciclo vital para un miembro de Chaetangiaceae del mar Caribe.

INTRODUCTION

The family Chaetangiaceae (Rhodophyta, Nemalionales) to which *Pseudogloioiphloea* belongs, includes 7 other genera and members of this family are reported from tropical to warm-temperate latitudes

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(excepting *Whidbeyella*) (KRAFT, 1981). For the tropical and subtropical western Atlantic ocean, TAYLOR (1960) records 3 genera i.e., *Scinaia*, *Galaxaura* and *Pseudogloioiphloea* and all the available information on species of these genera from this area (see also GANESAN, 1974; EISEMAN, 1979) are restricted to field collected materials and no member has been hitherto subjected to culture studies under controlled laboratory conditions to elucidate life history patterns. The genus *Pseudogloioiphloea* includes about 8 species

and only one species *P. confusa* (RAMUS, 1969) from the Pacific coast (California) has been studied in detail for the developmental sequence of its life-history under controlled environmental conditions. Hence, we present in this paper our observations "in vitro" on the life history on a second species *P. halliae* from the tropical western Atlantic ocean. Recent literature on culture studies of Chaetangiaceae is critically analyzed and evaluated by WEST & HOMMERSAND (1981).

MATERIALS AND METHODS

Abundant, quite fresh and fertile specimens were collected from floating and washed ashore material in the beach of Playa Caribe, Margarita Island, eastern Venezuela on 26.x.'78 and 25.viii.'79. The live material was brought to the laboratory in a plastic ice chest with abundant seawater from the place of collection. During transport, seawater was changed every 3 hours. The plants were maintained alive in the air-conditioned laboratory for a few days with vigorous aeration by aquarium aerators and changing of the seawater every day until cultures were established.

For culture studies, 2 growth media, VON STOSH and PROVASOLI (see MCLACHLAN, 1973) were used. Small pieces of fertile thalli were thoroughly washed with abundant seawater and examining under a dissecting microscope, small epiphytes were removed by a pair of sharp needles. Thallus surface was generally free of microscopic epiphytes. The cleaned bits with the mature cystocarps were placed on coverslips in a Petri-dish containing 20-30 ml of the culture medium. The Petri-dishes were incubated in a growth chamber LAB-LINE AMBI HI-LO CHAMBER at 24°C with a photoregime of 12:12 LD with a light intensity of 230 foot candles. After liberation, the carpospores were sown with the help of a Pasteur disposable pipette the tip of which was previously drawn into a capillary tube. Carpospores were sown on coverslips in Petri-dishes containing the respective culture medium. Similarly, tetraspores were also transferred and sown on coverslips in Petri-dishes containing the growth medium.

All developmental stages were observed "in vitro" with a compound microscope and photographed with a LEITZ COMBIPHOT AUTOMATIC micro-

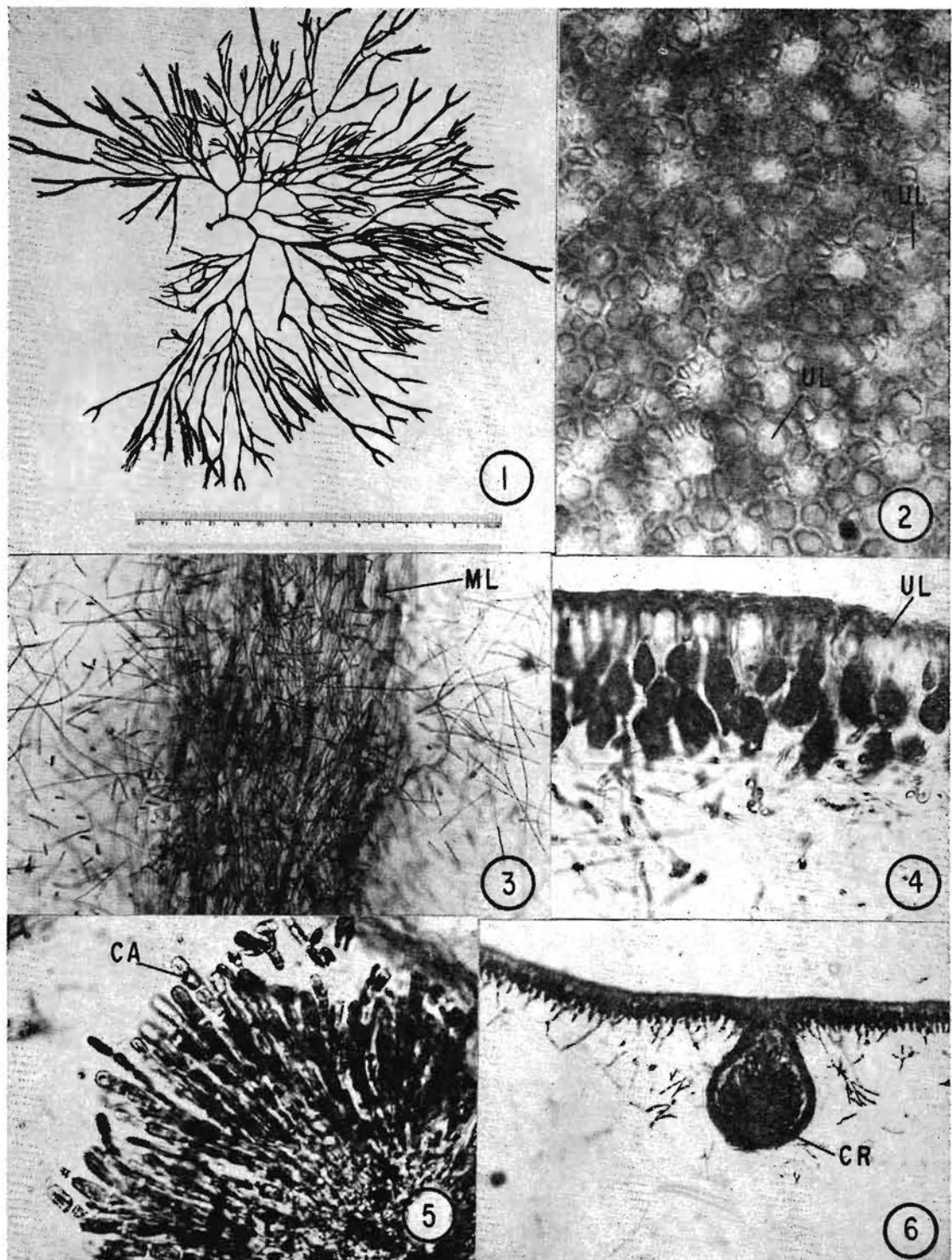
photographic equipment with a 125 ASA B & W film. All cultures were maintained unialgal and culture medium was replenished every 10 days. To eliminate diatoms growth, a 1ml/L solution of germanium dioxide (LEWIN, 1966) was used. For each Petri dish, 1-2 drops of this solution was added. Light intensity was measured with a visual and cosine-corrected Wetson (model 756) illumination meter. All cultures were maintained at 24°C, 12:12 LD with 230 foot candles of light intensity.

For anatomical studies, some specimens of the field collected gametophytes were also fixed in 4% seawater formalin. Bits of the preserved material were stained with 1% aniline blue in distilled water, acidified with a dilute HCL solution and sectioned (30 µm thick) with a freezing microtome. Sections were mounted in a 50% solution of Karo syrup.

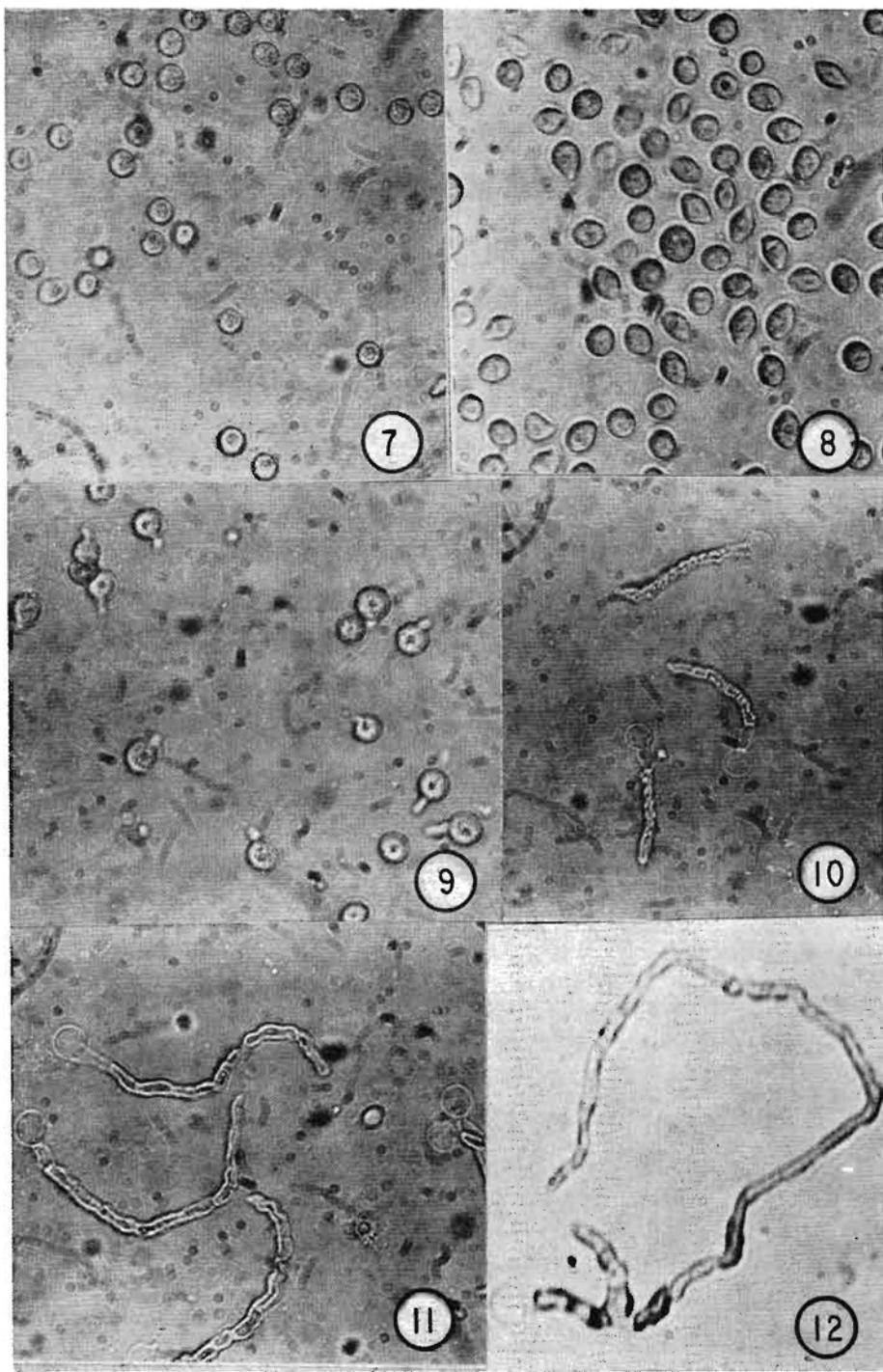
Duplicate specimens (liquid preserved and herbarium mounts) are deposited in the Herbarium, Department of Marine Biology, Institute of Oceanography, Cumaná, Venezuela.

RESULTS

Field collected gametophytes: Plants of *Pseudogloiocephoea halliae* studied in the present investigation grew up to a height of 8-15 cm, with a cylindrical thallus and fleshy consistency, repeatedly dichotomously branched up to 7-11 times (Fig. 1). Branches are about 1 mm in the basal part and between 2-2.5 mm in the upper portions. Structurally, the thallus is multiaxial in construction with a well-defined axial strand (Fig. 3). In transections, utricles are more or less rectangular and below the layer of utricles are found 2-3 layers of pyriform, densely pigmented cells, which are connected to the medulla by loosely packed, long colorless cells (Fig. 4). In surface view of the thallus, the utricles are circular in form and are surrounded by pigmented cells (Fig. 2). Externally and internally, the material studied in the present paper agreed well with the descriptions given by SETCHELL (1914), TAYLOR (1960) (see also GANESAN, 1974) for *P. halliae*. Also, by having monoecious sexual plants, carpogonial branch structure and post-fertilization developments, the present material agreed well with an early study by GANESAN (*loc. cit.*).



Figs. 1-6. *Pseudogloiocephloea balliae* (field collected material); Fig. 1. Habit of a gametophyte. Fig. 2. Surface view of part of the thallus showing the disposition of the colorless utricles X777; Fig. 3. Transection of a mature thallus showing the prominent axial strand. X 5; Fig. 4. Transection of a mature thallus showing the shape of the utricles and the subepidermic pigmented layers X 600. Fig. 5. Goniomblast filaments showing mature terminal carposporangia X 750; Fig. 6. Section of the gametophyte to show the immersed nature of the cystocarp X 8.
(UL: Utricle; CA: Carposporangium; CR: Mature cystocarp; ML: Medulla (Axial strand)).



Figs. 7-27. *Pseudogloioiphloea balliae* (Culture studies); Fig. 7. Liberated carpospores X 800; Fig. 8. Liberated carpospore showing a more irregular form X 800; Fig. 9. First stage in the germination of carpospores showing the initiation of a lateral protuberance X 800; Figs. 10, 11 & 12. Filaments 3, 6 & 7 days old respectively resulting from the germination of carpospores X 800.

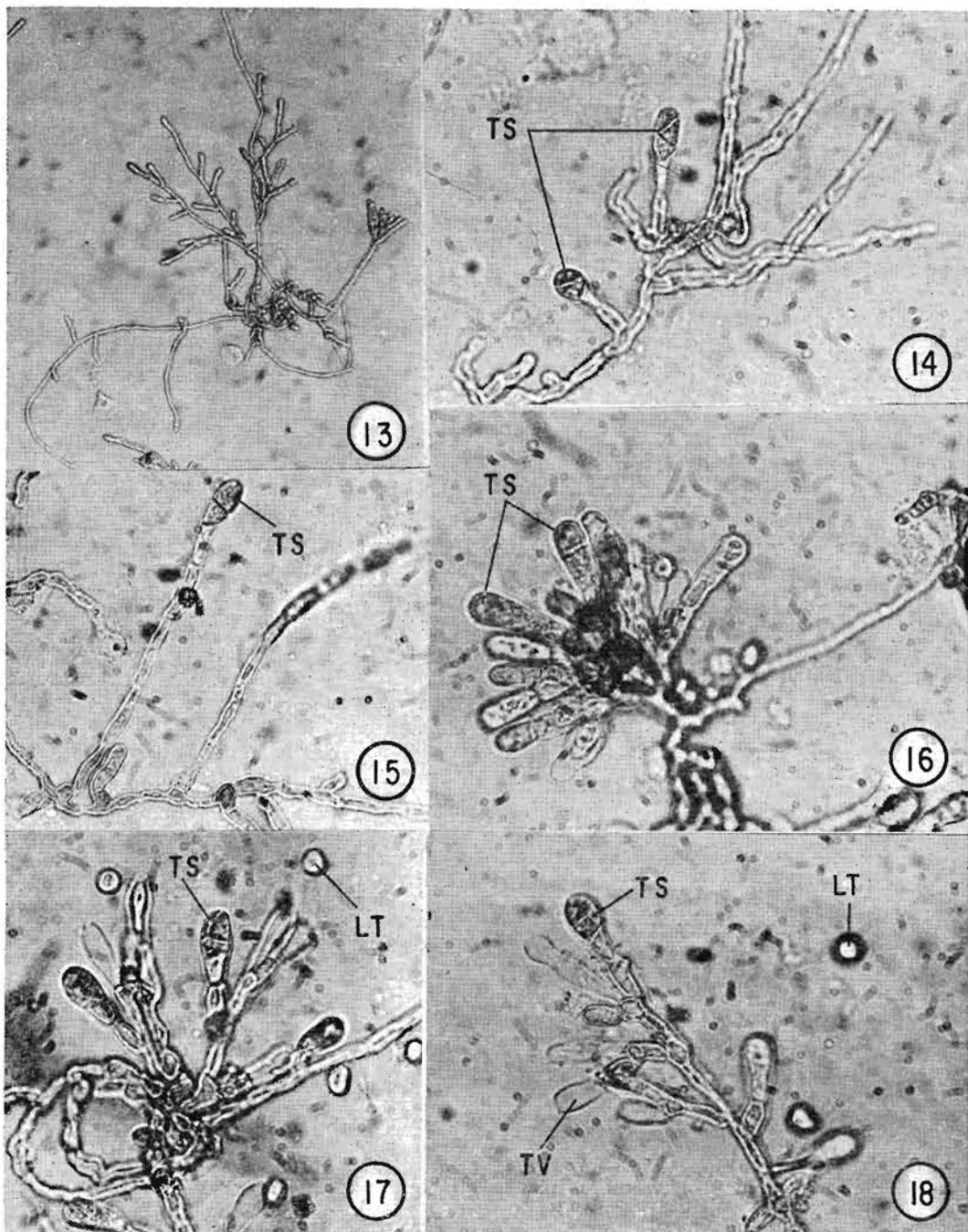


Fig. 13. A more or less fully developed and mature tetrasporophyte 16 days old X250; Fig. 14. Part of a tetrasporophyte enlarged to show two developing tetrasporangia. Note the 2 irregular divisions in the upper one X 400. Fig. 15. A young tetrasporangium, borne on a long branch with the first transverse division X 400; Fig. 16. A cluster of tetrasporangia in various stages of development and borne on a short lateral X 400; Fig. 17. Two mature tetrasporangia with cruciate divisions. Note also the empty tetrasporangia with the released tetraspores X 400; Fig. 18. A group of empty tetrasporangia after liberation of their contents. Note the zonate division in the tetrasporangium at the top X 400.
(TS: Tetrasporangium; LT: Liberated tetraspore; TV: Empty tetrasporangium).

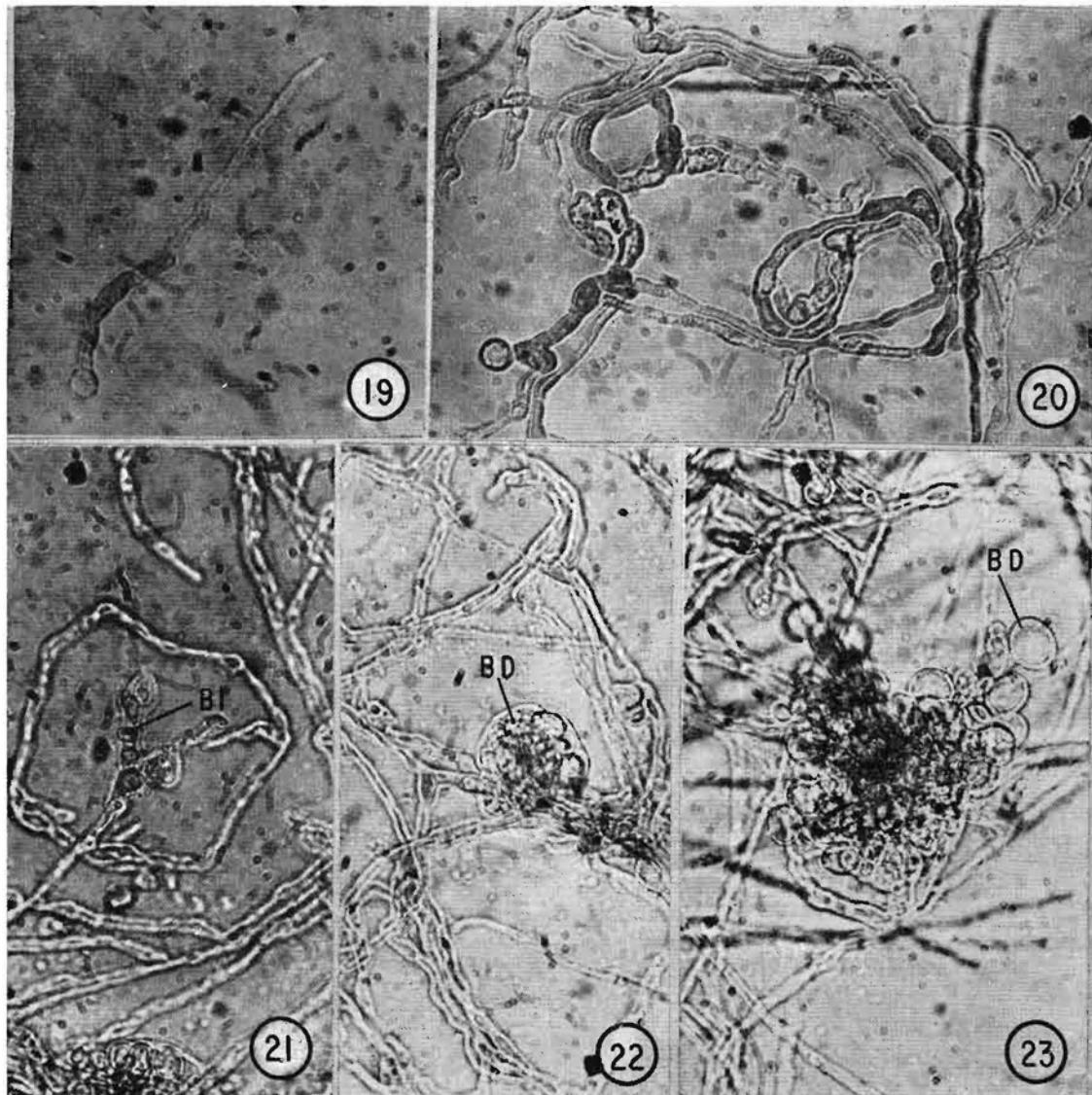


Fig. 19. A uniseriate filament, 6 days old, resulting from the germination of a tetraspore X 800. Fig. 20. Irregularly branched filaments with long, irregular cells and undulating walls, 2 weeks old, resulting from the germination of a tetraspore X 800. Fig. 21. Formation of bud initial from the cells of the branched filament X 500. Figs. 22 & 23. Bud initials more developed and organized with rounded cells and covered with abundant mucilage X 500.
(BI: Bud initial; BD: Developing buds).

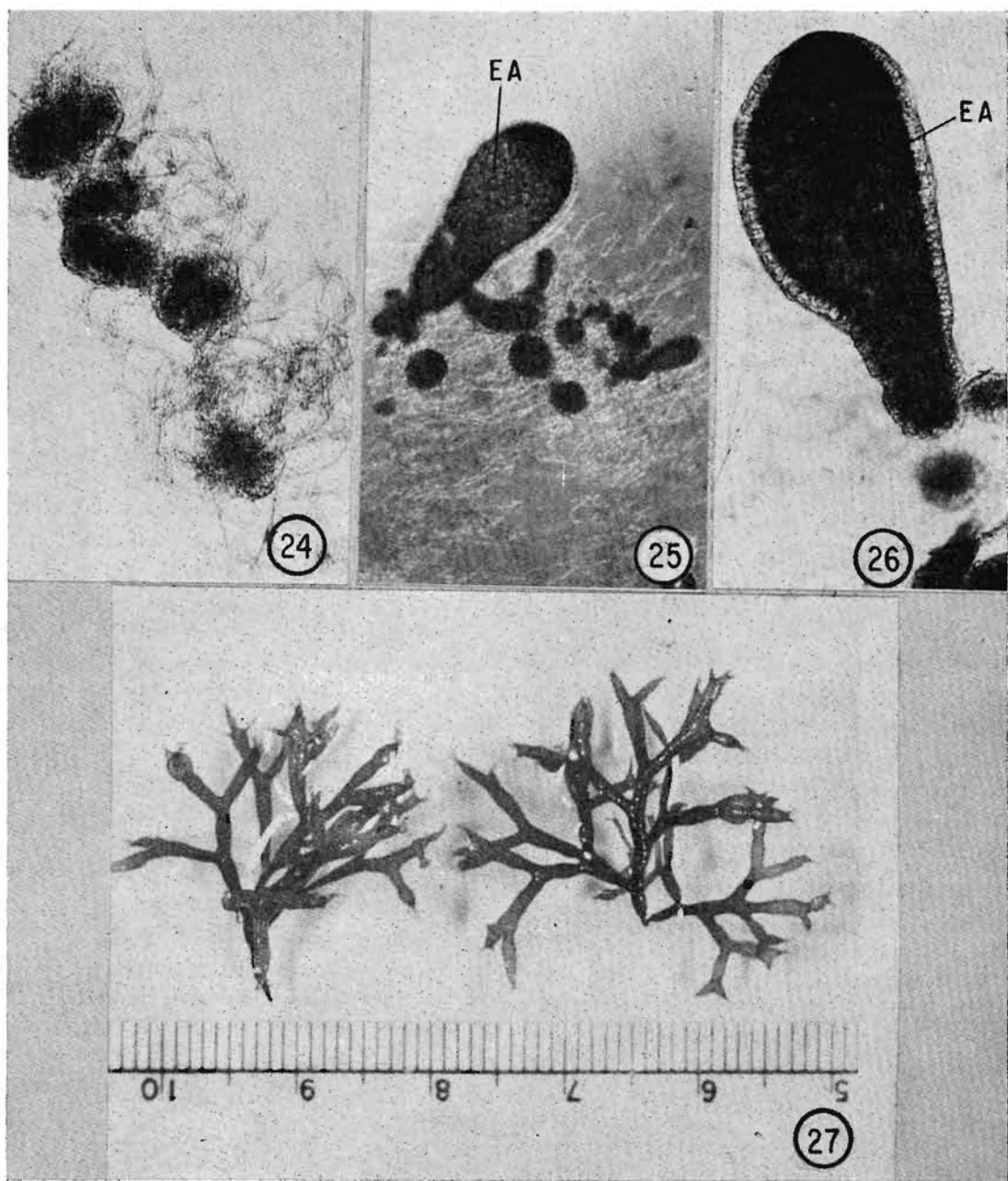


Fig. 24. Buds in more advanced stages of development with the protonematal basal portion X 204; Figs. 25-26. Two young, erect plants. Note in Fig. 26 differentiation of the peripheral colorless utricles and the elongate pyriform shape of the young erect axis. Fig. 2: X 3; Fig. 3: X 5; Fig. 27. Two erect plants grown in the laboratory (about 2 months old) resulting from the germination of tetraspores. Although these plants did not reach sexual maturity in the laboratory, in external and internal aspects closely resembled the field collected gametophytes.
(EA: Erect axis).

Culture studies: When small pieces of a few mm long fertile thalli containing mature cystocarps (Figs. 5,6) were incubated in growth chambers (see materials and methods), liberation of carpospores took place after approximately 12-24 hours. Liberated carpospores were more or less circular (Fig. 7) or irregular (Fig. 8), 6-9 μm broad with a perforated chloroplast. Germination was initiated after 24 hours following liberation with the formation of a small protuberance (Fig. 9). A septum at the base separated the protuberance from the carpospore and this newly formed cell divided subsequently to give rise to an uniseriate filament of few cells. In about 3 days, a uniseriate filament with 5-8 cells with the empty carpospore still attached at the base is formed (Fig. 10). Carpospore germination is unipolar. In about a week, the filament is about 12-17 cells long, the walls of which are undulating and subsequently small lateral uniseriate branches were also initiated (Fig. 12).

In about 12-14 days, the apical cells of the main filament and of the lateral branches became notably enlarged and their contents more dense than the surrounding vegetative cells. These enlarged cells are claviform or ovoid and when mature are about 17-25 μm long. These could be recognized as tetrasporangia since the contents were divided to form 4 spores. Division in the tetrasporangia is not uniform and it may be irregular (Fig. 14) zonate (Fig. 18) or decussate (Fig. 17). In about 16 days, the microscopic tetrasporophyte reaches its maximum development, the vegetative part many times irregularly branched and bearing numerous tetrasporangia (Fig. 13).

Liberation of tetraspores generally took place by the dissolution of the wall in the distal part of the sporangium (Fig. 17). Liberated tetraspores were slightly smaller (5-7 μm) than the carpospores. Formation of new tetrasporangia within the empty ones were also frequently observed. Mature tetrasporangia may occur singly or frequently grouped in a dense cluster with different sporangia in various stages of development (Fig 16). In our material, we have carefully looked for the presence of monosporangia on the tetrasporophyte and these were not encountered. In the numerous sporangia we have examined, the contents were always seen to be divided more than once.

Germination of tetraspores:

The liberated tetraspores were spherical (Fig. 17) and germinated after 4-5 days following liberation. The first stages of germination of the tetraspores were quite similar to those of the carpospores, resulting in the formation of an uniseriate filament with the empty sporangial wall still attached at the base (Fig. 19). This uniseriate filament is morphologically indistinguishable from the filament of the same age originating from the carpospore. In about 2 weeks, the uniseriate filament became profusely branched with cells of various shapes and sizes (Fig. 20). On the cells of these filaments are initiated at different points, many buds or button-like structures (Fig. 21), initially made up of 3-4 isodiametric cells and covered with abundant mucilage. Although numerous buds may be initiated, not all of them develop completely. Three different stages of bud development are shown in figs 22, 23 & 24. In about 20-30 days, cells at the basal part of the buds are differentiated into a basal disc and a small erect axis (Fig. 25). At this stage, in the distal part, the apical cells are arranged within an apical pore and the utricles were well differentiated as a colorless peripheral layer (Fig. 26). In 2 months, these erect axes grew up to a height of 1-2 cm (Fig. 27). By the fleshy consistency, mode of branching, a paler color and internal anatomy, these plants grown entirely in the laboratory from tetraspores were similar to the field collected gametophytes. However, these plants did not reach sexual maturity. Formation of monosporangia were not observed in the filamentous stage of the gametophyte. With the initiation of the buds and differentiation of erect axes, the basal filamentous or protonematal part became obliterated.

DISCUSSION AND CONCLUSION

Culture studies in the laboratory to elucidate the life history patterns of marine red algae from the tropical waters, particularly from the Caribbean sea, are very few. As pointed out earlier in the introduction, no member of Chaetangiaceae has ever been studied in this regard from the tropical western Atlantic ocean. References to earlier works on Chaetangiaceae (see WEST & HOMMERSAND, 1981) deal with species from the Mediterranean, Pacific (California) and the

Japanese coasts. Since many red algal families have been increasingly shown to have a mixture of isomorphic and heteromorphic life history, laboratory culture studies on members from different geographical latitudes are perhaps important to make meaningful comparisons and generalizations.

In general, our observations on the life history of *P. halliae* from Venezuela closely resembled the developmental sequence reported for the Jalama beach isolate (California) of *P. confusa* studied in great detail by RAMUS (1969). Like the Jalama beach isolate, the Venezuelan *P. halliae* also has a heteromorphic life history i.e. an erect, fleshy, macroscopic gametophyte alternating with a microscopic, filamentous, acrochaetoid sporophyte. RAMUS (*loc. cit.*) reported in his study that the filamentous sporophytic phase and the protonematal stage of the gametophyte reproduced by monospores. In our material, formation of such spores was not found. In having a heteromorphic life history, *P. halliae* also resembles *Scinaia* for which 3 species have been studied (*S. furcellata*, BOILLOT, 1968, 1969, 1972; *S. complanata*, VAN DEN HOEK & CORTEL-BREEMAN, 1970 and *S. japonica*, UMEZAKI, 1971).

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