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Observations on *Chnoospora minima* (HERING) PAPENFUSS (Phaeophyta, Scytosiphonales) in the field and in culture

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Examination of the brown alga *Chnoospora minima* (HERING) PAPENFUSS from Hawaii, provides cytological evidence for its placement in the Scytosiphonales. Cortical cells possess one large plastid and a single pyrenoid, and the erect blades bear only plurilocular sporangia. The thallus structure is similar to that of the other genera within the Scytosiphonales. However, the blade shape and subapical meristem are characteristic features of the alga. *C. minima* is perennial; although a seasonal decline of large thalli occurs in areas exposed to autumn and winter wave action, small thalli persist throughout the year.

Culture study reveals the presence of a discoid phase, which can independently reproduce itself for generations, in the vegetative development of the erect thallus.

Key Index Words: *brown alga*; Chnoospora; *cytology*; *morphology*; *phaeophycean life history*; *Phaeophyta*; *Scytosiphonales*.

In 1949 FELDMANN proposed a new phaeophycean order, the Scytosiphonales, to accomodate genera in the Scytosiphonaceae, Chnoosporaceae and the Phaeosaccionaceae (since transferred to the Chrysophyceae). The order was circumscribed using the following features: production of only plurilocular sporangia on the erect blade, the presence of a single large plastid and a single pyrenoid in the cortical cells, and an isomorphic life history. However, it is now well established (TAKEWAKI 1966, WYNNE 1968, NAKAMURA and TATEWAKI 1975, CLAYTON 1979) that crustose or filamentous microthalli alternate with the erect blade in Scytosiphonalean species. Nonetheless, FELDMANN's cytological criteria are still valid (COLE 1970).

The genus *Chnoospora* J. AGARDH is a tropical alga, with compressed, elongated, dichotomously branched fronds arising from a basal disc. It was placed in its own family, the Chnoosporaceae, by SETCHELL and GARDNER (1925). These authors also suggested that *Chnoospora* represented an evolutionary development of cylindrical thalli. Placement of the Chnoosporaceae in the Scytosiphonales (FELDMANN 1949) was based only on morphological features. Cytological evidence of a single plastid and pyrenoid was not available.

This study was conducted to obtain cytological information on one of the species found in Hawaii, *Chnoospora minima* (HERING) PAPENFUSS, to confirm its placement within the Scytosiphonales. The morphology of the thallus was examined and its habitat investigated. A culture study of the life history of *C. minima* was undertaken to determine the presence of a crustose stage.

Materials and Methods

Thalli of *Chnoospora minima* were collected from three sites on Oahu island, Hawaii, over a period of two years, from 1976-1978. The inclination of the substratum

slope at each site was measured with a protractor. Thalli used for culture were washed in sterile seawater, trimmed 4 cm from the blade apex, soaked in an antibiotic mixture (GUILLARD 1973) and placed in petri dishes containing seawater-algal agar. Petri dishes contained tips from a single thallus only. All cultures were maintained at 18-26°C (ambient temperature) under a 16 hour light regime at 600 foot candles, using cool white flourescent tubes. Thalli obtained through culture were kept in covered culture dishes containing 250 m*l* of filtered seawater to which PROVASOLI's Enriched Medium (MCLACHLAN 1973) was added. Groups of immature F_2 and F_3 thalli were kept in a growth chamber at 20°C with 10 hours of light. Germanium dioxide (LEWIN 1966) and the antibiotic mixture were added during the weekly medium change to surpress contaminants. Culture dishes were agitated at 100 RPM on rotary shakers.

Material for cytological examination was sectioned on a freezing microtome and stained with analine blue. Material examined for nuclear dimensions was fixed with alcohol-acetic acid and stained with aceto-carmine.

A dense field population at Kaloko, Oahu was surveyed monthly from August, 1977 through January, 1978. A sampling ring 0.18^2 M was placed in the densest portion and all visible thalli were harvested, counted and measured. Dry weights of each sample were recorded. Three size range categories were established for thalli harvested: small, less than 2.5 cm; medium, from 2.6 to 5.5 cm; and large, over 5.6 cm.

Results

Ecology: *Chnoospora minima* is a common intertidal alga in Hawaii, growing in tufts on low angled (less than 50°) wave washed shorelines protected from strong waves except during winter storms or southwesterly winds. The species is restricted to a belt in the upper littoral zone. Although the thalli are exposed to air at low tide, and may be submerged for only a few hours a day during high tide, they are continually wetted by wave action. None are found above the zone of constant surge. The lower limits are also sharply defined. The second species, *C. implexa*, also occurs in Hawaii, but is subtidal and has a round, cushion-like habit.

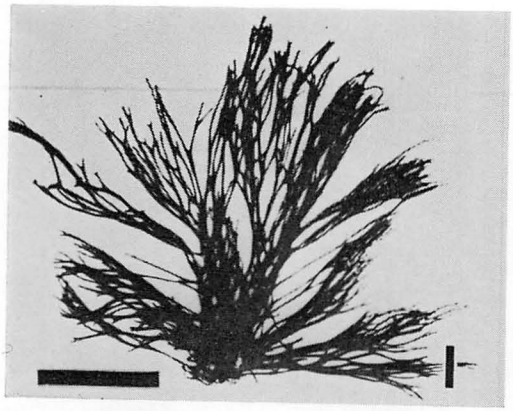
C. minima is perennial. However, field populations were observed during this study to decline sharply from September, with large and medium length thalli found only in sheltered locations. However, small thalli and discs with blade initials were present at all sites throughout the year. In April, field populations again became conspicuous, with medium and large thalli in abundance.

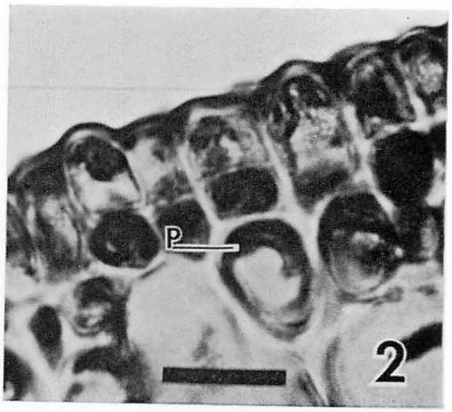
Morphology and cytology: Mature thalli occur in clumps, consisting of a large, irregular basal crust, composed of coalesced discs and erect fronds which arise from the discs (Fig. 1) Young thalli are often found singly. Blades may grow to a maximum of 16 cm. Although each blade usually branches dichotomously several times, specimens have been collected with 4 or 5 blades emerging from a single junction. Regeneration of blades from basal crusts as well as from eroded blade tips is common.

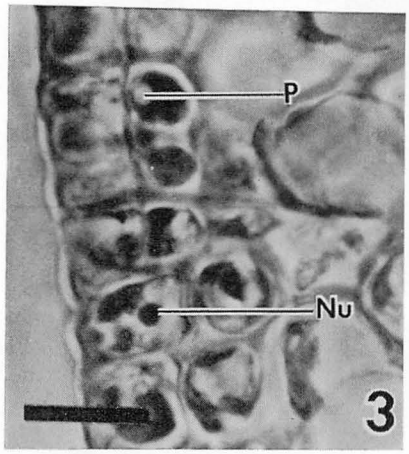
Both blade and holdfast consist of a cortex of small, pigmented cells and a medulla of large colorless cells. Cortical cells are oval, averaging 10×15 μm and contain a single, large, irregularly shaped plastid lying against the portion facing the blade periphery (Fig. 2). Below the plastid is a round nucleus, averaging 5 μm in diameter. A single large pyrenoid is embedded in or adjacent to the plastid (Fig. 3).

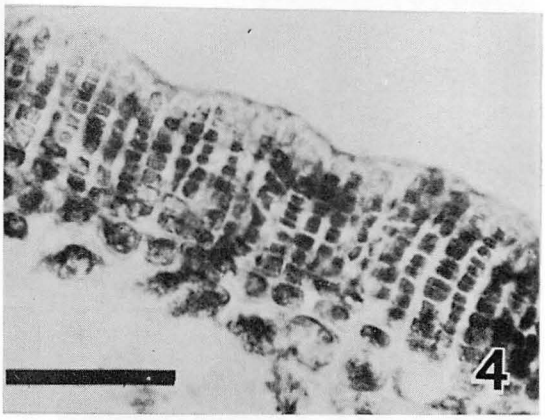
Young medullary cells are circular, becoming distorted and ovoid with age, the largest averaging 34×70 μm. Cell walls of both cortical and medullary cells are thick and irregular, with lamellate regions.

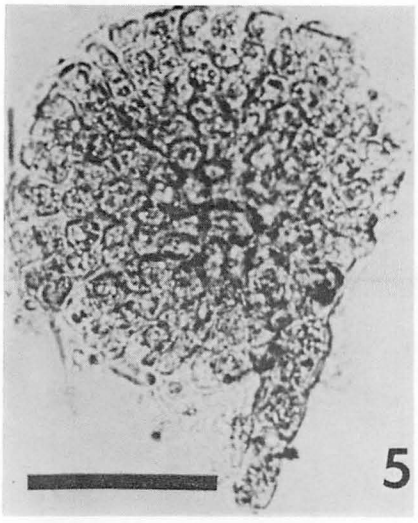
Growth takes place through divisions of a subapical meristem consisting of a layer











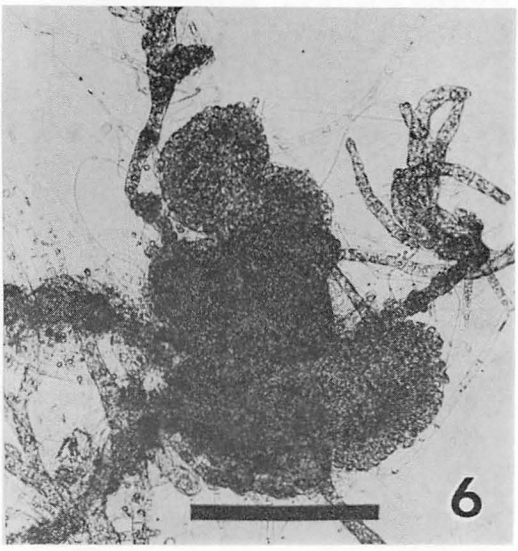
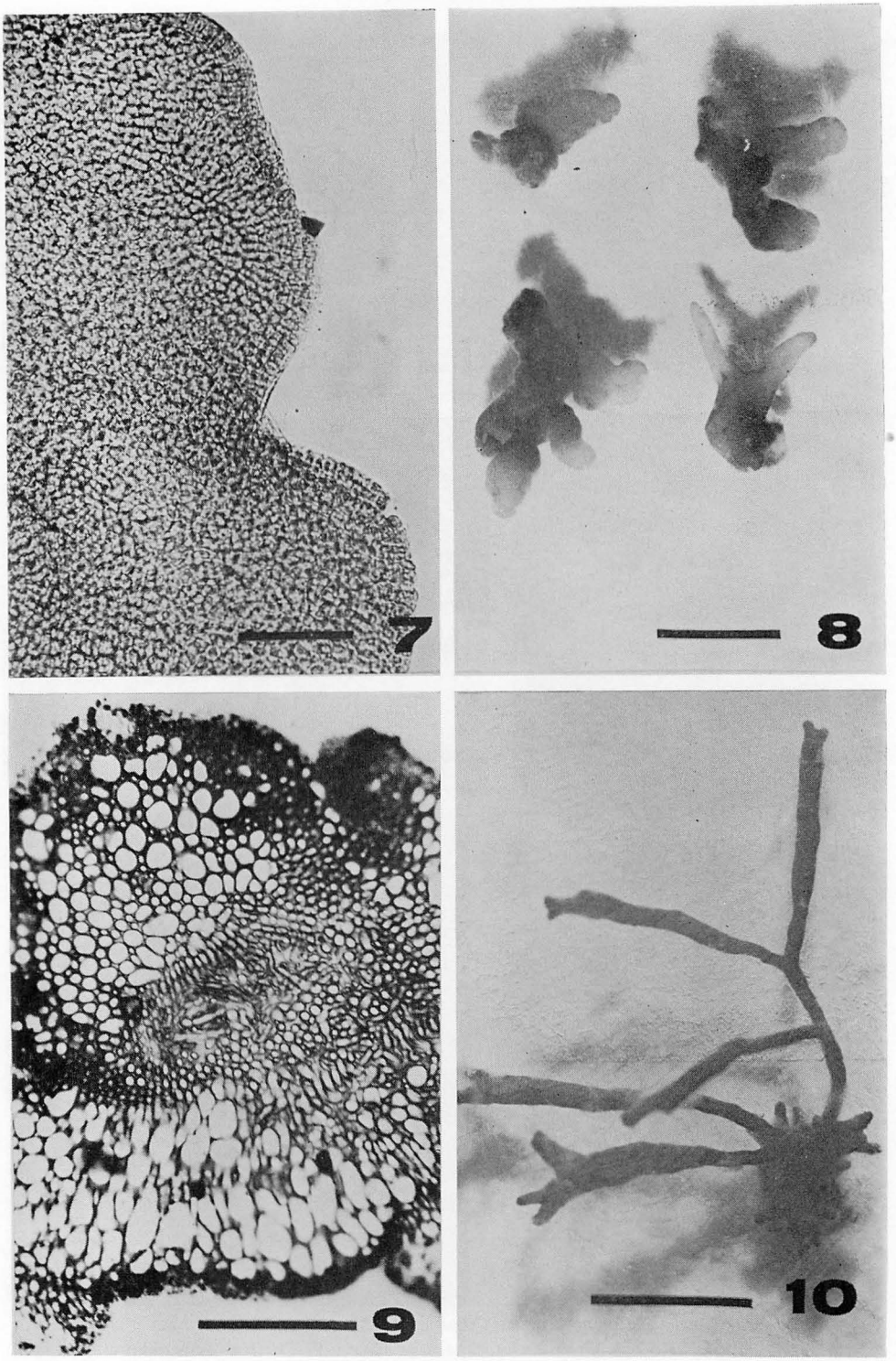


Fig. 1. Herbarium specimen of mature thallus of *Chnoospora minima*. Scale equals 4 cm.

Figs. 2-6. Light micrographs of *Chnoospora minima*. 2. Cortical cells, showing large, single plastid (p) lying against upper cell surface. Scale equals 25 μm; 3. Cortical cells, each possessing a single nucleus (nu) and pyrenoid (p). Scale equals 25 μm; 4. Cross section 1 cm from blade apex, showing plurilocular reproductive organs. Scale equals 100 μm; 5. Young disc with pluriseriate section of knot filament arising from it. Scale equals 100 μm; 6. Mature disc with sterile hairs and knot filaments. Scale equals 150 μm.



Figs. 7 and 9. Light micrographs of *Chnoospora minima*. Figs. 8 and 10. Live thalli of *Chnoospora minima*. 7. Surface view of blade apex at zone of developing dichotomy. Scale equals 100 μm; 8. Erect fronds arising from mature F_2 discs. Scale equals 1 cm; 9. Oblique section through the center of mature disc initiating blade. Scale equals 150 μm; 10. Thallus of *C. minima* obtained from F_3 disc. Scale equals 1 cm.

of small rectangular cells lying beneath the top cortical cell layer.

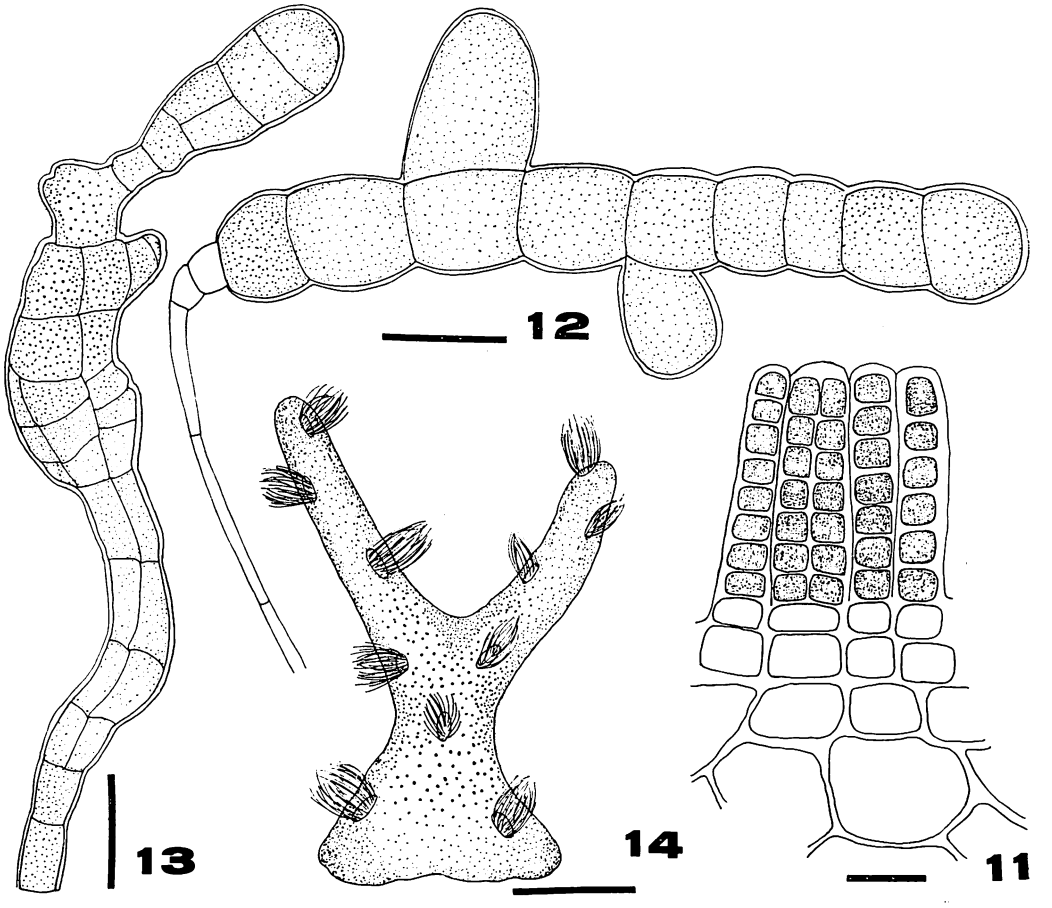
Cells derived from periclinal divisions become medullary cells. Anticlinal divisions of the subapical meristem produce the blade dichotomy (Fig. 7) and the meristoderm layer. This layer increases blade length and width through both periclinal and anticlinal divisions.

The entire thallus bears pits containing uniseriate unbranched colorless hairs with elongated cells. Hair production varies with the degree of exposure to air, thalli growing at the uppermost limits often producing copious hairs.

Blades were observed to produce only plurilocular reproductive organs. These are uni-or biseriate (Fig. 4) and average 38 μm in length. Individual locules (Fig. 11) have an average length of 6 μm, with a width of 8 μm. There are from 5 to 8 locules in a uniseriate plurilocular organ.

The plurilocular organs are derived from the top layer of cortical cells and are produced basipetally. Sometimes their development along one blade face preceeds that along the other, but mature organs occur in dense layers over the entire surface of the blade.

After the discharge of swarmers, the



Figs. 11-14. Line drawings. 11. Plurilocular organs of *C. minima*, showing uni- and biseriate forms. Scale equals 10 μm; 12. 7-10 day old germling. Scale equals 10 μm; 13. Knot filament (detail from Fig. 6), showing multiseriate section. Scale equals 15 μm; 14. Young *C. minima* thallus, obtained from F_2 disc. Scale equals 2.5 mm.

plurilocular organs and the cortical cells from which they are derived erode away, exposing layers of medullary cells. The eroded apex then drops off, leaving a blade stub several cm in length. Regeneration of a new blade from the stub often occurs.

Actively swimming, biflagellate swarmers, bearing one plastid and an eyespot, were observed in culture dishes containing field thalli as well as in those holding cultured material. They were 8-10 μm in length and 3-4 μm in width. The releasing process of swarmers from the plurilocular organs was unfortunately not confirmed. Fusion of swarmers in culture dishes was not encountered, probably because dishes contained thalli derived only from one parent. It was not possible during this study to collect swarmers from different thalli and mix them, to test for mating strains and obtain zygotes.

Observations on the life life cycle in culture: Swarmers from the plurilocular organs of field thalli germinated parthenogenetically into uniseriate filaments of 5-10 rounded cells (Fig. 12). Lateral branches developed which coalesced, producing discs, 1-2 mm in diameter and 0.5 mm thick (Fig. 5). After two weeks the discs produced long colorless hairs as well as short branches of limited length containing both uni- and multiseriate sections of darkly staining cells (Fig. 6). These branches are termed knot filaments because of apparent morphological similarities with knot filaments borne on Ralfsioid discs of *Petalonia* type thalli (EDELSTEIN *et al.* 1970) in culture. Knot filaments were also observed on the basal discs of field material of less than 2.5 cm in length.

Swarmers identical to those previously described were observed in culture dishes containing discs with knot filaments and in dishes containing only knot filaments. Although the release of swarmers from knot filament cells was not directly confirmed, it is likely that portions of the knot filament (Fig. 13) function as reproductive organs. No unilocular or plurilocular reproductive organs were observed on the discs or the knot filaments at any time during this study.

Several days after the development of knot filaments a new generation of discs appeared in culture. The pattern of growth of the F_2 discs was identical to that of the F_1 thalli. Several generations of discs were produced in this manner.

Two weeks after the development of knot filaments approximately 80% of all discs gave direct rise to fronds identical in appearance to field thalli of *C. minima* (Figs. 8, 14). An oblique section through a mature disc giving rise to a blade (Fig. 9) shows differentiation into cortex and medulla in the blade initial. The small, round, elongated medullary cells of the emerging blade contrast with the large, irregular medulla of the disc. Blade initiation occurs when the cell layer lying beneath the top cortical cell layer becomes meristematic. A meristoderm layer is produced and its divisions increase blade length and width.

After 1½ months, the blades reached 2.5 cm in length (Fig. 10) and developed plurilocular reproductive organs. After two weeks a new generation of discs appeared around the base of the mature thalli. These discs also showed a direct return to the blade. Development of neither knot filaments nor blades occurred among approximately 20% of cultured discs. Cultures grown under lower temperatures and shorter day length developed identically to those at regular culture conditions.

Conclusions

Early morphological studies of *Chnoospora* (BARTON 1898, SETCHELL and GARDNER 1925, KUCKUCK 1929) described its flattened, dichotomous blade, subapical meristem, pits of hairs and dense rows of plurilocular sporangia. Whereas the former two features are distinctive to the genus, the distribution of pits and plurilocular sporangia were found to be similar to that of *Colpomenia* DERBES and SOLIERS (BARTON 1898,

SETCHELL and GARDNER 1925, FELDMANN 1949). The specimens of *C. minima* examined show the general morphological and cytological features characterizing the members of the order Scytosiphonales. The erect blades bear only plurilocular reproductive organs and the cortical cells possess a single plastid with a conspicuous pyrenoid. These features support the accomodation of *Chnoospora* within the Scytosiphonales.

However, although dimorphism in the absence of a sexual cycle has been well demonstrated (HSIAO 1969, WYNNE 1969, WYNNE and LOISEAUX 1976) for members of the Scytosiphonaceae, it has been linked to enviromental conditions. The present observations show that decreases in the photoperiod and temperature, conforming to the minimum range of the Hawaiian climate, did not inhibit blade development in *C. minima*. The apparent seasonal decline of large and medium length thalli in the field was not due to surpression of blade development. Large and medium thalli were present throughout the year in sheltered locations and discs with blade initials were found at all sites throughout the year. It is suggested that the decline is due to removal by the mechanical action of autumn and winter storms. Discs and small thalli are less suseptible to removal by wave action and persist.

The occurance of a plethysmothalloid discoid phase which perpetuates itself before producing the erect blade is an unusual finding. However, the existance of knot filaments arising from the microthallus has been recorded for another member of the Scytosiphonales (EDELSTEIN *et al.* 1970) Blades of the genus *Petalonia* were produced by Ralfsioid discs which also bore knot filaments. However, only unilocular reproductive organs were produced on the discs and knot filaments. The erect blades produced only plurilocular organs.

The microthalli of *C. minima* did not produce unilocular organs. This observation differs from other recent findings regarding both haploid and diploid microthalli of other genera in the Scytosiphonales (NAKAMURA and TATEWAKI 1975, CLAYTON 1979). This is possibly due to unfavorable culture conditions, and future work with the genus may show the production of unilocular organs on the discoid phase.

The parthenogenetic development of gametes is common in other Scytosiphonalean genera (see WYNNE and LOISEAUX 1979 for a review), and production of zygotes has been shown in some species to be limited to certain seasons of the year (CLAYTON 1979, 1980). This may be the case with *C. minima* as well. The present study demonstrates the parthenogenetic development of swarmers. Future work with *Chnoospora* species may reveal a heteromorphic alternation of haploid blades with diploid discs, a sexual life history described (NAKAMURA and TATEWAKI 1975, CLAYTON 1979, 1980) for a number of genera in the Scytosiphonales.

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S. S. フォトス：ボウガタムラチドリの野外観察および培養

ほとんど研究が行なわれていない褐藻のムラチドリ属 *Chnoospora* J. Agardh の一種，ハワイ産のボウガタムラチドリ *Chnoosoora minima* (Hering) Papenfuss を詳しく調べたところ，本種がカヤモノリ目に帰属すべきものであることの細胞学的な根拠が得られた。皮膚細胞は1個の大きな葉緑体と1個のピレノイドをもち直立葉片は複（室胞）子嚢のみを有する。葉状体の構造はカヤモノリ目の他の属のそれと同様である。しかしながら葉片の形態と亜頂端の表皮層は *C. minima* に特徴的である。本藻は多年性である。大きな葉状部は秋や冬に波の影響で季節的衰退を示すが，小さな葉状部は年間を通じて存在する。

直立葉状体の栄養的発達過程における一世代として，独立の生殖能力を有する盤状体の相が存在することが培養実験によって示された。(305 茨城県新治郡桜村天王台 1-1-1，筑波大学生物科学系)