A MORPHOLOGICAL AND CYTOLOGICAL
STUDY OF AUDOUINELLA PORPHYRAE
AND A. VAGA (RHODOPHYTA)

By

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B. SC., The University of British Columbia, 1982

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE.

in

THE FACULTY OF GRADUATE STUDIES
(Department of Botany)

We accept this thesis as conforming
to the required standard.

THE UNIVERSITY OF BRITISH COLUMBIA
August, 1985

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ABSTRACT

A comparative study was made between two similar red algal endophytes, *Audouinella porphyrae* (Drew) Garbary and *Audouinella vaga* (Drew) Garbary, Hansen et Scagel, (Acrochaetiaceae, Acrochaetiales) and their red algal hosts *Porphyra* spp. and *Pterosiphonia bipinnata*, respectively. Both endophytes have axial, stellate chloroplasts with a central pyrenoid and reside in their host's cell walls, producing erect portions outside the host that may bear monosporangia. The endophytes were cultured "free" from their hosts and morphological and cytological features of the free-living forms were compared with field material. Although the two endophytes differ significantly in cell dimensions and branching patterns, the free-living forms do not retain these differences. Cell dimensions, branching patterns and developmental patterns are identical in the two free-living forms. Asexual reproduction with regeneration by monosporores was observed. Sexual reproduction was not observed in either endophytic or free-living forms of the endophytes. Free-living forms were used for re-infection and cross-infection experiments. Under all experimental conditions, the endophytes showed only epiphytic growth. The hosts seem to have some effect on both of the endophytes. Epiphytes, *Audouinella porphyrae* and *A. vaga* were not selective and grew on both hosts, *Porphyra torta* and *Pterosiphonia bipinnata*. Both epiphytes growing on blades of *Porphyra* tended to branch and have more extensive prostrate portions (3-5 cells) whereas both epiphytes
on *Pterosiphonia bipinnata* tend not to branch and have only 1-2 cells in the prostrate portions. Ultrastructural studies of both endophytes showed typical florideophycean features. Ultrastructural features of field material of the two endophytes were similar and free-living, cultured endophytes were similar to field material. A large vacuole was observed in sections of the field material of both prostrate and erect portions whereas this was not observed in cultured material. Based on the results of this study it is proposed that the two endophytes are conspecific. *Audouinella vaga* is referred to synonymy in *Audouinella porphyrae* (Drew) Garbary, Hansen et Scagel.
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Acknowledgements

I am indebted to Dr. K. Cole for all of her wisdom, guidance and financial support throughout this thesis. Her constant encouragement and enthusiasm were greatly appreciated through my years as a graduate student. I would also like to thank Dr. D. Garbary for the initiation of this project, and for introducing me to the field of Phycology. Editorial comments were gratefully accepted from Dr. K. Cole, Dr. D. Garbary and Dr. P. Gabrielson. Warm gratitude is expressed to Bev. Hymes for all of her help and advice in the laboratory. Appreciation is also expressed to Kermit Ritland for his support and computer knowledge throughout my thesis. A special thanks is given to Karen Morin for drawing figure one. Thanks also go to Herb. Vandermeulen, Bev. Hymes, Ellen Rosenberg, Dawn Renfrew, Ira Borgmann, Sandy Lindstrom and Larry Golden for their company during many collecting trips to Point No Point. I would like to acknowledge Mr. L. Veto for technical assistance in the E.M. laboratory.
Dedicated with love to my family:

Mom, Dad, Stella and Glory.
INTRODUCTION

Red algae may be found growing on other algae (epiphytic), growing on the surfaces of animals (epizoic), and partially inside the cell walls of other algae (endophytic) as well as on non-organic substrata. Epi/endophytic algae may grow parasitically, or just on or within the host cell walls without affecting the physiology or morphology of the host cells. Many symbiotic associations between parasitic red algae and their hosts have been well studied (for review see Goff, 1982), but the relationship between red algal endophytes and their red algal hosts has not received much attention (Goff, 1983).

Traditionally, many red algal endophytes have been classified as distinct entities based on their association with a particular host (White and Boney, 1969, 1970). These "obligate" interactions have been inferred from observations of field-collected materials and have been only recently subjected to experimental analysis. Culture studies of some species of Audouinella (Bory) Dixon et Irvine, using re-infection and cross-infection studies, have shown that they may not be as host-specific as presumed (White and Boney, 1969, 1970; Garbary, 1979b). White and Boney (1969, 1970) studied A. endophytica (Batters) Dixon, and A. asparagopsis (Chemin) Dixon (both as Acrochaetium Naegeli), in their hosts, Heterosiphonia plumosa (Ellis) Batters, and Bonnemaisonia hamifera Hariot, respectively. They measured cell dimensions of endophytes in their hosts and compared these with cultured, "free-living"
endophytes concluding that the two taxa were distinct species. They also showed that the endophytes infect other hosts and do not show any substrate preference.

Garbary (1979b) studied several endophytic species of *Audouinella*, *A. endophytica*, *A. bonnemaisonae* (Batters) Dixon, *A. tetraspora* Garbary et Rueness, from red algal hosts as well as an endozoic species from hydroids *A. asparagopsis*. He concluded that all isolates established some degree of relationship with a given host (both the original and alternate hosts). Based on observations of cell size and shape, Garbary (1979b) noted that the endophytic and free-living forms were morphologically different species. The free-living forms tended to be larger in cell length and smaller in cell diameter, and to branch less frequently. He found that only *A. endophytica* grew endophytically into given hosts and that when growing endophytically its morphology was altered. He speculated that the hosts caused modifications of the cell morphology of the endophyte and emphasised the importance of comparing culture and field material before delimiting species of endophytic and endozoic members of Acrochaetiaceae.

To contribute to our knowledge of red algal endophyte/host relationships, field and cultured material of two endophytic species of *Audouinella*, *A. porphyrae* (Drew) Garbary and *A. vaga* (Drew) Garbary, Hansen et Scagel (Rhodophyta, Acrochaetiales, Acrochaetiaceae) associated with *Porphyra* spp. and *Pterosiphonia bipinnata* (Postels et Ruprecht) Falkenberg, respectively, were studied at one site in British Columbia.
Members of the Acrochaetaceae are small, uniseriate branched filaments. The algae vary in height from approximately 20 urn to 10 mm and their colour ranges from dark steel blue to green and violet red to rose. The order Acrochaetiales is distinguished by the two layered pit plugs, absence of carpogonial branches, and apical growth of uniseriate filaments that are not elaborated into pseudoparenchymatous thalli.

This group of algae has exploited a wide range of environments and adapted to many different niches. Different species occur in fresh and marine habitats; however, they are primarily marine. Although some species are epilithic, most taxa are symbiotic and either epibiotic or endobiotic. With regard to generic classification, at least 24 different schemes incorporating one to eight genera have been used since the early 20th century with over a dozen schemes employed since 1970 (Woelkerling, 1983). Criteria for generic segregation include features of chloroplasts, life histories and cell morphology. A complete sexual life history has been reported from only about 23 of the 390 described species of which 7 species are from field collected populations. Many species are known only to produce monosporangia (White and Boney, 1969) and/or tetrasporangia (West, 1979; Garbary and Rueness, 1980). Four distinct sexual histories are known: 1) Triphasic, dimorphic - with similar morphological gametophyte and tetrasporophyte; 2) Triphasic, trimorphic - with a diminutive gametophyte and a large tetrasporophyte; 3) Diphasic, dimorphic - with a large tetrasporophyte and no carposporophyte and 4) Diphasic, dimorphic - with a diminutive carpotetrasporophyte and no tetrasporophyte,
(for more details, see Woelkerling, 1983). Because of the few life history studies and inadequate distinct morphological characters it is difficult to utilize these features as generic criteria.

Drew (1928) proposed that all members of the Acrochaeticeae be included in the genus *Rhodochorton* (Naegeli). She suggested that chloroplast and sporangia features were too variable to be used to distinguish genera. Dixon and Irvine (1977) and Garbary (1979a) all concurred with Drew (1928) that a one genus system should be used. Dixon and Irvine, however, proposed the genus *Audouinella* since it is the older name. In this thesis I also recognize only one genus *Audouinella*. Although many phycologists agree with this classification (Garbary et al., 1982; Woelkerling, 1983), the opposing schemes of Papenfuss (1945), Feldmann, (1962), Stegenga and van Wissen (1979), Bold and Wynne (1985) and their variants also continue to be used.

Within *Audouinella* a variety of characters is used to differentiate species. These include: 1) basal systems: uni- or multicellular, 2) chloroplast structure: parietal vs stellate, and one to many in number, 3) mature cell: diameter and length, 4) pyrenoid: present or absent, and one or more in number, 5) branching patterns: irregular, secund or opposite, 6) habitat: epibiotic, endobiotic or epilithic, 7) morphology, size, shape and clustering of monosporangia and/or tetrasporangia, 8) spore germination pattern, 9) morphology of gametangia and 10) post fertilization development. For review of characters nine and ten, see Hansen and Garbary (1984).
The two species of endophytic *Audouinella* studied in this thesis have been placed in several different genera. Smith (1944) transferred *Rhodochorton porphyrae* Drew from *Rhodochorton* into *Acrochaetium* because the plants produced monosporangia. Similarly, Jao (1937) transferred *Rhodochorton vagum* Drew from *Rhodochorton* to *Acrochaetium*. Papenfuss (1945) transferred *Rhodochorton porphyrae* to *Chromastrum* Papenfuss, and made the combination *Chromastrum porphyrae* (Drew) Papenfuss, based on the presence of a stellate chloroplast. However, he retained *Acrochaetium vagum* Jao in *Acrochaetium* based on the supposed presence of parietal chloroplast in the original described material (Drew, 1928). Papenfuss (1947) again revised the classification of this family and replaced *Chromastrum* with *Kylinia* Rosenvinge. Stegenga and Mulder (1979) transferred *Acrochaetium vagum* to *Chromastrum* based on the presence of a stellate chloroplast and life history presumed to be similar to other species of *Chromastrum*. However, due to a lack of stable generic characters Garbary (1979a) has chosen the one genus concept and both species are now referred to *Audouinella* (Garbary et al., 1982).

*Audouinella porphyrae*, an endophyte which grows in the cell walls of a number of species of *Porphyra* (Bangiophyceae), is found primarily in the mid- to lower intertidal regions. Endophytic *Audouinella porphyrae* in species of *Porphyra* are widely distributed, in western North America from Yakutat Bay, Alaska, U.S.A. (Hansen et al., 1981) to Baja, California, Mexico (Garbary et al., 1982), New Zealand (South and Adams, 1976), Australia (Woelkerling, 1971) and Tristan da Cuhna
Audouinella vaga, an endophyte in two florideophycean genera, Pterosiphonia, and Polysiphonia, is reported from Alaska to California but is unknown elsewhere (Garbary et al., 1982).

Audouinella porphyrae and A. vaga are morphologically and ontogenetically similar (Garbary et al., 1982). Both have cells with a single stellate chloroplast that contains one pyrenoid, and similar patterns of spore germination. Based on differences in hosts, branching patterns, presence or absence of hair cells and tetrasporangia, Garbary et al. (1982) maintained them as different species (Table 1), but suggested that the two taxa might be conspecific, the morphological differences between them being a reflection of the influence of the hosts on their development.

Using culture studies and light and electron microscopy, a comparative study was initiated on Audouinella porphyrae and Audouinella vaga. The following features were investigated: 1) morphological and reproductive features of both field and cultured material, 2) life histories of the endophytes in unialgal culture, 3) the relationship between the endophytes and their hosts and 4) the question of conspecificity.
Table I: Similarities and Differences between *Audouinella porphyrae* and *Audouinella vagae* according to Garbary et al. (1982).

<table>
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<th><em>A. porphyrae</em></th>
<th><em>A. vagae</em></th>
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<tr>
<td><strong>Similarities:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Inside host cell wall</td>
<td>Inside host cell wall</td>
</tr>
<tr>
<td>Chloroplast</td>
<td>Single, axial, stellate</td>
<td>Single, axial, stellate</td>
</tr>
<tr>
<td>Pyrenoid</td>
<td>One</td>
<td>One</td>
</tr>
<tr>
<td>Spore germination</td>
<td>Internal division</td>
<td>Internal division</td>
</tr>
<tr>
<td><strong>Differences:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Host</td>
<td><em>Porphyra</em> spp.</td>
<td><em>Pterosiphonia</em> sp.</td>
</tr>
<tr>
<td>Cell length</td>
<td>18-25μm</td>
<td>Less than 18μm</td>
</tr>
<tr>
<td>Tetrasporangia</td>
<td>Unknown</td>
<td>Reported to be rare</td>
</tr>
<tr>
<td>Hair cells</td>
<td>Reported to be rare</td>
<td>Unknown</td>
</tr>
<tr>
<td>Branching pattern</td>
<td>Irregular</td>
<td>At right angles</td>
</tr>
</tbody>
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MATERIALS AND METHODS

Field Material

Monthly field trips May 1984 - April 1985, were made to Point No Point on the west coast of Vancouver Island, British Columbia (48° 23'N, 123° 59'W). *Audouinella porphyrae* and *Audouinella vaga* var. *vaga* (henceforth *A. vaga*) were usually collected at the same time. Collections were also made at Eagle Cove (48° 28'N, 123° 01'W) and Cattle Point (48° 27'N 22° 58'W) on San Juan Island, Washington, U.S.A., in May 1984, in order to compare material from different sites. Collections in the herbarium of the University of British Columbia (UBC) containing *A. porphyrae* and *A. vaga* were examined to determine previous collection sites and dates of collection.

*Audouinella porphyrae* tends to grow in the holdfast region of *Porphyra* spp. and is detected by red patches. Care was taken to remove holdfasts of *Porphyra* spp. when these were collected. Infected host specimens (*Porphyra* spp. and *Pterosiphonia bipinnata*), which tended to be desiccated following exposure at low tide, were brought into the laboratory on ice directly from the field. Some were rehydrated in large quantities of seawater overnight for light and electron microscopic studies, and others were kept dry and cold in the refrigerator (4°C) for further examination of field characters.

In contrast, it is not possible to identify *Pterosiphonia bipinnata* infected by *Audouinella vaga* with the unaided eye; this requires microscopic examination (Fig. 4). Consequently,
several handfuls of *P. bipinnata* were collected from a number of sites in the mid- to lower intertidal zone. There are other *Audouinella* species epiphytic on *P. bipinnata*, but their chloroplast shapes and growth patterns are different from those of *A. vaga*. Such taxa include: *A. densa* (Drew) Garbary, *A. arcuata* (Drew) Garbary, Hansen and Scagel and *A. thuretii* (Bornet) Woelkerling (Garbary et al., 1982).

Some infected specimens *Porphyra* species and of *Pterosiphonia bipinnata* were examined with dissecting and compound microscopes to determine the extent of infection by the two *Audouinella* species. Measurements of the endophytes included: (cell dimensions) length and diameter of cells in primary axes and of fully elongated apical cells of a filament; maximal extent of infection (length and width); and distance of the endophyte from the host's holdfast. The extent of growth outside the host and the reproductive nature of both the host and the endophyte were also noted. Three types of statistical analyses were applied to the data. A linear regression analysis was used to relate the size of *Porphyra* to the area of primary endophyte infection (Sokal and Rohlf, 1973). Secondary infections were not considered in this analysis. Another statistical analysis was a 2X2 test of independence and a Chi square test to verify its significance (Sokal and Rohlf, 1973). A "t-test" was used to compare the significance of cell dimensions in the two species, *A. porphyrae* and *A. vaga* from field material (Sokal and Rohlf, 1973).

For permanent collections, infected *Porphyra* blades were dried on herbarium sheets. Portions of infected *Pterosiphonia*
bipinnata plants were mounted on slides in 30% Karo. Other material was preserved in 5% formalin/seawater and where possible the Porphyra species were identified. Voucher specimens of field material were deposited in the University of British Columbia phycological herbarium (UBC).

Cultures

Establishing cultures of free-living endophytic species.

Some heavily infected host specimens were used to establish free-living cultures of the endophytes (Table II). Ten millimeter discs of non-reproductive, heavily infected Porphyra blades from close to the holdfast region were placed in 30-40 ml of PES medium (Pravosoli, 1968) with (5-10 mg/l) GeO₂ to stop diatom growth on the hosts thallus (Lewin, 1966). Discs were screened to ensure no epiphytes were present. The material was then kept at 6°C 16:8 h Light:Dark (L:D), photon flux density of 5-6 microeinsteins m⁻² sec⁻¹ in PES medium to induce sporulation. Conditions were chosen to simulate field conditions. Some stock cultures were also maintained at 10°C 8:16 h (L:D) photon flux density of 4-5 microeinsteins m⁻² sec⁻¹ in PES medium. When spores were observed on the bottom of culture dishes, host material was removed. Spores were then grown in 60 X 20 mm plastic dishes, with or without coverslips, and in 125 ml flasks containing 75 ml of medium. Re-isolation of sporelings of Audouinella porphyrae into new vessels was necessary to achieve unialgal cultures, and penicillin levels of about 150 mg/l (Hoshaw and Rosowski, 1973) were sometimes used in cultures that
were overly infected by bacteria and fungi. The medium was changed every three weeks, and the cultures were covered with cheesecloth to reduce the amount of light. It was observed that a high photon flux density of 25 microeinstein m\(^{-2}\) sec\(^{-1}\) tended to reduce pigmentation if the medium was not replaced weekly. Pieces of infected *Pterosiphonia bipinnata* were treated similarly to establish free-living cultures of *Audouinella vaga*.

Free-living specimens of both endophytes were then used to compare their general morphologies, developmental patterns, cross-infection abilities and life histories. Dimensions of the initial spore, filament lengths, cell dimensions (length and width), and branching types were recorded.

Developmental and Life History Studies.

To study developmental patterns, filaments of free living *Audouinella porphyrae* and *A. vaga* were grown on coverslips at 6°C 16:8 h (L:D), photon flux density of 4-5 microeinstein m\(^{-2}\) sec\(^{-1}\) in PES medium. Various stages of the life cycle, such as spore germination, filament initiation branching pattern, monospore production and spore release were photographed using brightfield as well as Nomarski modulation contrast microscopy.

Attempts were made to induce the sexual phase of the life history. Filaments of both free-living *Audouinella* species were grown on coverslips and placed under various temperature and daylength conditions with the same photon flux density (2 microeinstein m\(^{-2}\) sec\(^{-1}\)). The following conditions were tried: 6°C 8:16 h (L:D), 6°C 16:8 h (L:D), 15°C 8:16 h (L:D) and 15°C 16:8 h (L:D).
Re-infection and Cross-infection Studies.

Tetraspores of *Pterosiphonia bipinnata* (collected June, 1984) were grown in the laboratory at 10°C 8:16 h (L:D) with PES medium in 60 X 20 mm plastic dishes. Filaments with rhizoids were grown to approximately 2-3 cm in length and then individual filaments were placed in 250 ml flasks containing 100 ml of PES medium at a photon flux density of 55 microeinstein m\(^{-2}\) sec\(^{-1}\) with continuous aeration. Clumps of material approximately 4-5 cm long were used for subsequent studies.

Young uninfected blades of *Porphyra torta* Krishnamurthy and *P. abbottae* Krishnamurthy (collected Feb. 1985) were used in the re- and cross-infection experiments. To ensure that these blades were clean, a method modified from Polne-Fuller and Gibor (1984) was employed. Young blades of *Porphyra* (3-8 cm in length) were sonicated gently in sterile distilled water for 15 seconds in an Artek Sonic Dismembrator Model 300 at the 30 position, then rinsed in sterile seawater. The procedure was repeated three more times using sterile seawater. To eliminate protozoans and bacterial epiphytes, blades were next soaked for 7 minutes in a solution of 1 Betadine (TM): 100 seawater and rinsed in sterile seawater until no traces of the brown Betadine were visible. Blades were also rinsed with antibiotics (Hoshaw and Rosowski, 1973). Several isolates of *A. porphyrae* (see Table II) were made to ensure that all isolates were the same species. Antibiotics (Hoshaw and Rosowski, 1973) were also used for one week when establishing cultures.

Infection experiments involved three series of flasks: 1) control flasks in which pieces of the host plants were cultured
without any endophytes, 2) re-infection flasks in which
*Audouinella porphyrae* grown on 18 X 18 mm² coverslips was placed
in the flasks with cleaned pieces of *Porphyra* and *A. vaga* was
placed in flasks with *Pterosiphonia bipinnata* and 3) cross-infection experiments in which *A. porphyrae* was placed in
a 250ml flask with *P. bipinnata* and *A. vaga* in a 250ml flask
with *Porphyra*. All conditions were established in triplicate.
Flasks were placed at photon flux density of 55 microeinstein m⁻² sec⁻¹ at 10°C 8:16 h (L:D) and aerated using an air pump.
Observations were made weekly for signs of infection.

Since epiphytic growth only was observed in the infection
experiments, an attempt was made to induce endophytic growth.
*Pterosiphonia bipinnata* together with free-living endophytes
were placed in 250 ml erlenmeyer flasks. The medium was poured
out daily and plants were allowed to desiccate from 2-48 hours
with desiccation times increasing over a four week period. Two
replicates of this experiment were performed. Control flasks
were not desiccated. The experimental conditions were 15°C,
16:8 h (L:D), photon flux density of 55 microeinstein m⁻²
sec⁻¹.

Light and Electron Microscopy Studies

For light microscopic studies, rehydrated and infected
*Porphyra fucicola* Krishnamurthy and *Pterosiphonia bipinnata* were
fixed with 2-3% glutaraldehyde/phosphate buffer (0.067M; pH =
7.2) or 5% formalin/phosphate buffer (0.067M; pH = 7.2).
Specimens were dehydrated in a methanol series, followed by a
propylene oxide series, and then embedded in glycol methacrylate
plastic from Polysciences, Warrington, Wa. (Feder and O'Brien, 1968).

Serial sections, approximately 2-2.5 um thick were cut using glass knives on a Sorvall Porter-Blum microtome. Serial sections were obtained by applying a small amount of rubber cement along the width of the blocks (Henry, 1977). Sections were picked up, dried on glass slides, and placed in various histochemical stains: alcian blue/periodic acid schiff (AB/PAS) for various polysaccharides (Kiernan, 1981), fast green (FG) for proteins (McCully et al., 1980), IKI for starch (O'Brien and McCully, 1981), periodic acid/schiff (PAS) for polysaccharides and starch (O'Brien and McCully, 1981), sudan black (SB) for lipids (O'Brien and McCully, 1981), Calcofluor White (CW) ST for B-1,3 or B-1,4 polysaccharides (Hughes and McCully, 1975) and toluidine blue-0 (0.05%, pH = 4.4) (TBO) was used as a general stain (O'Brien and McCully, 1981).

For transmission electron microscopy (T.E.M.) studies of rehydrated field material of Porphyra fucicola/Audouinella porphyrae and Pterosiphonia bipinnata/Audouinella vaga, as well as free-living A. porphyrae and A. vaga were fixed in 2-3% glutaraldehyde/ phosphate buffer (0.067 M; pH = 7.2) (Hymes and Cole, 1983a). Several other fixatives were found to be unsatisfactory on field material: phosphate-0.1M cacodylate-0.2M sucrose (pH = 7.2)/ 2-3% glutaraldehyde (Pueschel and Cole, 1985), seawater-NaCl/ 2-3% glutaraldehyde (Pueschel, pers. comm.). Free-living specimens were fixed with PIPES/2-3% glutaraldehyde (Hayat, 1981), but the phosphate buffer (0.067M; pH = 7.2)/ 2-3% glutaraldehyde (Hymes and Cole, 1983a) fixation
proved most satisfactory for free-living specimens. All materials was postfixed in 2% OsO₄/phosphate buffer (0.067M; pH = 7.2) at 4°C for 2 hours, except free-living A. porphyrae and A. vagæ, which were postfixed for 1 hour at 4°C to reduce dark contrast in the cytoplasm. Specimens were dehydrated in a graded series of methanol followed by graded series of propylene oxide and embedded in Spurr's plastic (fresh plastic was replaced every 24 hours for 7 days) that was hardened at 70°C for 7-8 hours (Spurr, 1969). Sections of 60-90nm were cut using glass knives on a Reichert OmU3 ultramicrotome. Sections were picked up on uncoated copper grids, stained for 45 mins with uranyl acetate (Watson, 1958) and restained for 15 mins with lead citrate (Reynold, 1963). Sections were viewed in a Zeiss EM-10 transmission electron microscope.
RESULTS

Field material:

Monthly collections at Point No Point showed that host plants, *Porphyra* spp. and *Pterosiphonia bipinnata*, were present from February to October. The endophytes *Audouinella porphyrae* and *Audouinella vaga* were observed from May to October, and their initial appearance coincided with the maturation of the host plants in late spring. Endophytes and host plants were not present during the winter. A study of the herbarium collections in UBC indicated that both endophytes occurred in their hosts from February to September and were most frequently reported from June and July. The abundance of herbarium collections from these months may be a reflection of collection effort, and current observations suggest that plants are abundant throughout the periods of May - June and September - October. The two endophytes were more difficult to collect in July and in late fall when host plants became less common in the intertidal zone. Host/endophytes collected from San Juan Island (Washington) and Triple Island, B.C. (collected by L. Golden) were morphologically identical to those collected from Point No Point.

*Audouinella porphyrae*:

*Audouinella porphyrae* is a filamentous endophyte, growing in the host cell wall, just under the surface. It branches randomly and penetrates the wall between the cells of the host.
Cells of *A. porphyrae* tend to be cylindrical in older portions of the filaments and to taper towards the apex. Erect filaments project outward from the host surface where the asexual reproductive structures, monosporangia, are formed (Fig. 2).

*Audouinella porphyrae* is not species specific. It grows in several *Porphyra* species when they are present (Table II), most frequently in the holdfast regions (Table III and Fig. 1). Other parts of the vegetative blade may also be infected, but these infections are usually less extensive than the main infection site in the holdfast area. In April when the endophyte first appeared, it was observed only in the holdfast area. By September many other areas of infection were also noted on more distal vegetative parts of the blade. It was noted that these later infections were generally smaller than the original infection. No endophytes were seen in the reproductive areas of the host blades. The area occupied by the primary infection of *A. porphyrae* ranged from 0.1 cm$^2$ to 22.5 cm$^2$ (Table III). No correlation ($b = 0.032; r^2 = 0.006$) was found between the size of the host blades and the size of the primary infection areas. The infection is restricted to vegetative portions of *Porphyra* spp. Most of the infected blades were reproductive (Table III). The endophyte produces monosporangia outside the host cell wall when the area of infection is greater than 3.0 cm$^2$.

*Audouinella vaga*

*Audouinella vaga* is an abundantly branching filamentous endophyte that grows just beneath the surface of the host,
Table II: Monthly field collections of *Audouinella porphyrae* in various species of *Porphyra*.

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Location of collection</th>
<th>Species of Porphyra</th>
<th>Presence of endophyte in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 16, 1984</td>
<td>Eagle's Cove, S.J.</td>
<td><em>P. schizophylla</em></td>
<td>+</td>
</tr>
<tr>
<td>May 16, 1984</td>
<td>Eagle's Cove, S.J.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>June 14, 1984</td>
<td>Triple Is., B.C.*</td>
<td><em>P. schizophylla</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P. B.C.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. schizophylla</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. sanjuanensis</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. abbottae</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. schizophylla</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>June 29, 1984</td>
<td>P.N.P.</td>
<td><em>P. fucicola</em></td>
<td>+</td>
</tr>
<tr>
<td>July 15, 1984</td>
<td>P.N.P.</td>
<td><em>P. schizophylla</em></td>
<td>+</td>
</tr>
<tr>
<td>Aug. 15, 1984</td>
<td>P.N.P.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>Oct. 12, 1984</td>
<td>P.N.P.</td>
<td><em>P. kanakaensis</em></td>
<td>+</td>
</tr>
<tr>
<td>Oct. 12, 1984</td>
<td>P.N.P.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>Oct. 12, 1984</td>
<td>P.N.P.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>Oct. 12, 1984</td>
<td>P.N.P.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
<tr>
<td>Dec. 19, 1984</td>
<td>P.N.P.</td>
<td>No <em>Porphyra</em></td>
<td>-</td>
</tr>
<tr>
<td>Feb. 4, 1984</td>
<td>P.N.P.</td>
<td>Juvenile</td>
<td>-</td>
</tr>
<tr>
<td>Mar. 4, 1984</td>
<td>P.N.P.</td>
<td>Juvenile</td>
<td>-</td>
</tr>
<tr>
<td>Apr. 10, 1984</td>
<td>P.N.P.</td>
<td><em>P. torta</em></td>
<td>+</td>
</tr>
</tbody>
</table>

S.J. = San Juan Island, Washington  
P.N.P. = Point No Point, British Columbia  
* = collected by L. Golden
Table III: Dimensions of *Audouinella porphyrae* growing *in situ*, field collections of *Porphyra*, and general host features.

<table>
<thead>
<tr>
<th>Endophytes</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td><strong>Width</strong></td>
</tr>
<tr>
<td>(cm)</td>
<td>(cm)</td>
</tr>
<tr>
<td>7.5</td>
<td>3.0</td>
</tr>
<tr>
<td>8</td>
<td>21.5</td>
</tr>
<tr>
<td>3.8</td>
<td>4</td>
</tr>
<tr>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
<td>2.4</td>
</tr>
<tr>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>2.5</td>
<td>1.8</td>
</tr>
<tr>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

+ = reproductive structures were present  
- = reproductive structures were absent

Note: all infections begin at the holdfast of the host and other subsequent smaller infections begin further up the host blade.

a = *Porphyra fucicola*

b = *Porphyra aboitaiae*

c = *Porphyra sanjuanensis*

d = *Porphyra tertia*

e = *Porphyra schizophylla*
mainly in the pericentral cell walls (Fig. 4). The endophyte grows parallel to the primary axis of the longitudinal axis of the host (Fig. 5). Cells tend to be cylindrical in the older portion of the endophyte and taper toward the growing point. Erect filaments are exterior to the host wall and usually abundant, arising from almost any cell of the endophytic filaments. Monosporangia are formed from the external, erect filaments.

*Audouinella vaga* grows most frequently in the wall of the main axis and in larger branches of its host and only rarely in young apical tips and rhizoids (Table IV and Fig. 1). Infection areas of *A. vaga* ranged from 180 \( \mu m^2 \) to 7.5 mm\(^2\) (Table IV). Monosporangia usually attach to the host wall in the area between two adjacent pericentral cells. As for *A. vaga*, the size of individual infection areas is positively correlated \( (x^2 = 3.03; p = 0.05) \) with the host being reproductive. Thus, the age of the host is probably a determining factor for infection by the endophyte. This is supported by the observation that older portions of the host tend to become heavily infected before secondary infections occur. The larger the infection area (greater than 250 \( \mu m^2 \)), the more likely the endophyte will be reproductive (Table IV).

Development of endophytes: *Audouinella porphyrae*

Infection of both hosts by the two endophytes commences in the same way. Monosporangia attach to outer walls of their respective hosts and divide internally (Fig. 6). A germination tube is then produced from one of the two daughter cells (Fig.
Table IV: Measurements of *Audouinella vaga* in its host *Pterosiphonia bipinnata* from field collections.

<table>
<thead>
<tr>
<th>Endophyte Length (µm)</th>
<th>Width (µm)</th>
<th>Area (µm²)</th>
<th>Reprod. Erect portions outside host</th>
<th>Reprod. of host</th>
<th>Location on host</th>
</tr>
</thead>
<tbody>
<tr>
<td>151.5</td>
<td>50</td>
<td>7575</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>138</td>
<td>42</td>
<td>5796</td>
<td>+</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>165</td>
<td>28.5</td>
<td>4702.5</td>
<td>+</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>129</td>
<td>25.5</td>
<td>3289.5</td>
<td>+</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>49.5</td>
<td>60</td>
<td>2970</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>198</td>
<td>13.5</td>
<td>2673</td>
<td>+</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>90</td>
<td>27</td>
<td>2430</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>75</td>
<td>21</td>
<td>1575</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>77.1</td>
<td>18</td>
<td>1387.8</td>
<td>+</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>69</td>
<td>18</td>
<td>1242</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>54</td>
<td>20</td>
<td>1080</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>43</td>
<td>24</td>
<td>1032</td>
<td>+</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>45</td>
<td>18</td>
<td>810</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>42</td>
<td>15</td>
<td>630</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>7.5</td>
<td>63</td>
<td>472.5</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>450</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>39</td>
<td>9</td>
<td>351</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>52.5</td>
<td>4.5</td>
<td>236.25</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>37.5</td>
<td>6</td>
<td>225</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>34.5</td>
<td>6</td>
<td>207</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>13.5</td>
<td>9</td>
<td>121.5</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>90</td>
<td>20</td>
<td>180</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
</tbody>
</table>

Total: 2 13 6 1

+ = reproductive structures (carpogonia, or spermatangia or tetrsporangia) are present

- = reproductive structures are absent

/ = location of endophyte on host according to a schematic drawing of *Pterosiphonia bipinnata* (Fig. 1) (see Fig. 1 for explanation of location on host.)
7) and the resulting filament tapers as it penetrates the host cell wall. The initial spores remain outside the hosts' cell walls throughout development.

Settled monospores of *Audouinella porphyrae* measured 12.0 μm ±0.3 in length and 9.0 μm ±0.3 in width (n=50) after the initial cell division (Table V). Cells in the primary filament were 13.5 μm ±2.3 in length and 7.5 μm ±0.8 in diameter (n=50). All cells have a single, stellate chloroplast and one prominent pyrenoid (Fig. 8). As the endophyte grows within the host wall, it branches irregularly, radiating out from the initial point of infection; eventually a somewhat circular reddish pink area is formed. These features could be seen easily using Hoffman microscopy (Figs. 6-9). As the endophyte grows areas of infection become larger and larger, eventually giving the blade a red blotchy appearance. There were no indications of the endophyte penetrating host cells. Erect filaments are formed external to the host wall. These developed perpendicularly to the host surface, extended to about 25 μm, and had two to six cells. In general, one to two monosporangia were present on each erect axis. No tetrasporangia or hair cells were noted in material examined in this study.

Development of endophytes: *Audouinella vaga*

Settled monospores *Audouinella vaga* were 12 μm ±0.3 in length and 8 μm ±0.3 in diameter (n = 50). The apical cell of growing filaments measured 21 μm ±0.4 in length and 3.0 μm ±0.3 in diameter (n = 50). The narrow diameter of apical cells compared to older vegetative cells was a consistent feature of
both endophytes. There was a significant difference in the lengths of cells on primary filaments of the two species and *A. vaga* had significantly smaller diameters (Table V). No differences in the chloroplast and pyrenoid were noted between the two species (Table V). Filaments of *A. vaga* tend to grow parallel to the main axis and to the longitudinal dimension of pericentral cells of the host. In the prostrate portions of the endophyte branching tends to occur at right angles (Fig. 5). Erect portions arise from the prostrate filaments and protrude through the host cell walls. Erect axes generally consist of one to two cells and one to two monosporangia are produced on these erect portions. The infection did not show macroscopic patterns or blotches in the host filaments, and it was only by close examination with a compound microscope that the endophytic growth could be resolved. Microscopically, the endophyte forms numerous parallel filaments that branch at right angles (Fig. 5). Neither tetrasporangia nor hair cells were observed in field material of *A. vaga*.

Culture material

Establishment of free-living endophytes

*Audouinella porphyrae* and *A. vaga* grew readily as free-living endophytes in culture without the presence of host material and therefore are not obligate endophytes.

Commencing with monospores released from mature plants, the developmental pattern of both endophytes was similar, and the free-living forms were morphologically identical. Upon
Table V: Cell dimensions and branching patterns of *Audouinella porphyrae* and *Audouinella vaga* in their respective hosts from Point No Point. 
(* p = <0.05; all cases n = 50*)

<table>
<thead>
<tr>
<th></th>
<th><em>A. porphyrae</em></th>
<th><em>A. vaga</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell dimension:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Settled spore**</td>
<td>12µm (l)</td>
<td>12µm (l)</td>
</tr>
<tr>
<td></td>
<td>9µm (d)*</td>
<td>8µm (d)*</td>
</tr>
<tr>
<td>Main filament:</td>
<td>13.5µm (l)*</td>
<td>15µm (l)*</td>
</tr>
<tr>
<td></td>
<td>7.5µm (d)*</td>
<td>6µm (d)*</td>
</tr>
<tr>
<td>Mature apical cell:</td>
<td>15µm (l)*</td>
<td>21µm (l)*</td>
</tr>
<tr>
<td></td>
<td>3µm (d)</td>
<td>3µm (d)</td>
</tr>
<tr>
<td><strong>Host:</strong></td>
<td><em>Porphyra</em> spp.</td>
<td><em>Pterosiphonia</em> sp.</td>
</tr>
<tr>
<td><strong>Branching pattern:</strong></td>
<td>Irregular</td>
<td>At right angles</td>
</tr>
</tbody>
</table>

(l) = length  (d) = diameter  
* indicate significant differences at p = <0.05.  
** measurements after internal division.
attaching to a variety of substrata such as plastic, glass or nylon thread, monosporangia were formed later from the second cell of the original spore. A small prostrate filamentous plant was formed that gave rise to erect filaments (Fig. 13). At no time did either of the two initial cells inside the spore wall give rise to erect filaments. Basal cells were 14.0 μm in length and 9.0 μm in diameter, whereas the cells in the primary erect filament were 16.5 μm in length and 9.0 μm in diameter (Fig. 14). Up to four erect axes could be formed from a single basal system (Figs. 13). Apical cells were 18.0 μm in length and 9.0 μm in diameter. Erect filaments branch frequently; branching was primarily unilateral. Cells in lateral branches were smaller than those of the primary axis (Figs. 15, 18) and occasionally, the lateral branches, rebranched. These secondary branches always developed adaxially (Fig. 18). Most of the plant is comprised of erect portions (50-100 cells); very few basal cells are formed (5-9 cells). Growth in the flasks became quite dense and plants grew on the sides of the vessels.

As the plants matured, monosporangia (Figs. 16, 17) were produced. Monosporangia were either terminal or lateral on erect branches, and either sessile or on a one-celled pedicell (Figs. 16, 17). In most cases only a single monosporangium was formed on a supporting cell; infrequently sporangia were formed
in pairs (Fig. 21). Similar to the formation of lateral branches, monosporangia were formed unilaterally. Two to six sporangia may be formed in succession within the original sporangium wall (Figs. 19, 20). Remnants of empty sporangium walls could be easily seen with Hoffman contrast microscopy (Fig. 20). Monosporangial production was greater in long days 16:8 h (L:D) vs 8:16 h (L:D)), at a low temperature (6°C vs 15°C) and at low photon flux densities (4-5 vs 22 vs 55 microeinstein m$^{-2}$ sec$^{-2}$). Only a few monosporangia were produced under other experimental conditions. Four months after inoculation the bottom of a dish was covered with plants and monosporangia. The monosporangial cycle took three to six weeks.

Attempts to induce sexual reproductive structures by varying culture conditions were unsuccessful. This corroborates the absence of gametangia observed in field material.

Infection experiments

In the control series, *Pterosiphonia bipinnata* plants grown from tetraspores were large enough to be used for infection experiments within three weeks. Under the culture conditions provided, they branched and grew larger during the entire time of the experiment (Figs. 22-24). *Porphyra torta*, on the other hand, did not grow much larger than the original field material and there was no evidence of thallus deterioration (Fig. 25). In re-infection experiments, both endophytes showed epiphytic growth on *Porphyra* and *Pterosiphonia bipinnata* but in none of the re- and cross-infection experiments was there evidence of endophytic growth. Monosporangia appeared to be partially embedded
in the host cell wall (Fig. 28). All parts of *Pterosiphonia bipinnata* were infected (Figs. 29-33). The results of cross-infection experiments were identical to those of re-infection experiments. The epiphytic growth was similar to free-living plants in that spores showed the same initial internal division (Figs. 26, 27) and a similar monosporangial reproductive cycle. However, several differences were evident in both *Audouinella* species between the free-living cultures versus those growing epiphytic on *Porphyra torta* or *Pterosiphonia bipinnata*. In the epiphytic plants there was less development of the basal system (three to six vs. five to nine cells). Typically, one (rarely two to three) erect axis was produced that always arose from one of the two cells in the initial spore (Figs. 30, 32-33), and most erect axes were unbranched except for the production of lateral monosporangia (Figs. 31, 32-33).

Monospores released from the free-living cultured *Audouinella porphyrae* and *A. vaga* attached to host cell walls. Numerous plants were soon observed covering the host plants. Both endophytes completely covered the *Pterosiphonia bipinnata* plants within 18 days except for the actively growing apical portions. Endophytes did not kill the *Pterosiphonia bipinnata* plants in culture, but rather slowed growth compared to control cultures. After 65 days, the host still grew and produced new shoots even though it was totally covered by epiphytes (Fig. 34). When growing on *Porphyra torta*, epiphytic populations took much longer to develop (two weeks to three to five days) than on *Pterosiphonia bipinnata*. In addition, the population was less
dense on *Porphyra torta*.

*Audouinella porphyrae* and *A. vaga* showed differences in their basal portions and branching patterns when growing on *Porphyra torta* and *Pterosiphonia bipinnata*. On *Pterosiphonia bipinnata* the basal system was limited to the initial two cells within the spore and occasionally to one other cell. This is different from epiphytes on *Porphyra torta* where up to five basal cells might be produced (Figs. 28, 35, 37). In addition the erect axes were more commonly branched when plants were growing on *Porphyra torta* (compare Figs. 32 and 36). Various conditions including lack of aeration, reduction in light intensity and desiccation of host were used in an attempt to induce epiphytic plants to become endophytic in *Pterosiphonia bipinnata*. The only effect of these experiments was a reduction in the rate of epiphytic growth which was noted when plants were desiccated.

Light and transmission electron microscopy

Using sections from methacrylate embedded field material, various observations were made on the cytological relationships between the endophytes and their hosts. Many endophytic prostrate filaments were seen in the hosts' cell walls. *Audouinella porphyrae* tended to branch irregularly in the *Porphyra fucicola* cell walls (Figs. 38-39, 43), whereas the prostrate portions of *Audouinella vaga* tended to grow parallel to the longitudinal axis of *Pterosiphonia bipinnata*. Thus, when the host wall curved, the endophyte also appeared to grow in an arc (Figs. 47, 49-50). Internally divided spores were observed
on top of the wall of both hosts. It was noted that the germinating filament but not the spore penetrated the host cell wall. There were many filaments within the outer cell wall layer (Figs. 42,43) of Porphyra. Both endophytes produced erect portions outside their hosts, and monospores were formed from these erect filaments (Fig. 41). Filaments of A. porphyrae were not seen to penetrate the cytoplasm of the Porphyra cells. However, on two occasions pericentral cells of Pterosiphonia bipinnata were penetrated by A. vaga (Fig. 48).

There were no differences between Audouinella porphyrae and Audouinella vaga in their reactions to the histochemical stains used in this study. Staining with Toluidine blue-0 gave a red-purple metachromatic reaction with the cell walls and cytoplasm of the two hosts and the two endophytes. This is interpreted to indicate the presence of polyphosphates, polysulfates and polycarboxyl acids (O'Brien and McCully, 1981). Fast green showed a positive reaction for proteins in the cytoplasm of all cells as well as in the cuticle of Porphyra fucicola (Figs. 44-46). When endophytic filaments were producing erect axes, the host cell walls adjacent to the emerging filament showed a positive reaction with fast green (Figs. 45-46). In addition localized areas of staining were observed immediately above certain endophytic cells (Fig. 44-46, 52-53). This suggests that some proteinaceous material may be released into the host wall prior to upright axis initiation. Audouinella vaga showed a similar reaction except there was no cuticle present that stained darkly on the Pterosiphonia bipinnata host (Figs. 52,56). IKI and Sudan black did not show
any reaction. No differences between the cell walls of the two endophytes were shown by the Alcian blue/PAS reaction. Both showed purplish coloration indicating a mixture of polysaccharides (Kiernan, 1981). The two hosts, however, showed differences in their cell wall. *Porphyra fucicola* stained pink-pinkish red in the cytoplasm and cuticle whereas the outer walls stained bluish, this reaction was also observed in *Bangia* (Cole et al., 1985). The differences indicate different types of polysaccharides. *Pterosiphonia bipinnata* walls showed a purplish coloration indicating a mixture of polysaccharides (Kiernan, 1981). Lack of fluorescence with Calcofluor White ST test indicated the absence of detectable B-1,3 or B-1,4 polysaccharides in the walls of the endophytes and their hosts.

Transmission electron microscopy studies showed that cells of the two endophytes *in vivo* are ultrastructurally identical (Figs. 69-71, 72-75). They have the "Nemalion" type chloroplast (Hara and Chihara, 1973) - a single, stellate, axial chloroplast with one pyrenoid that has an irregular pattern of thylakoids within the pyrenoid matrix (Figs. 54, 56, 62). Pit plugs have two cap layers and the nucleus is surrounded with abundant starch (Figs. 69-71). Mitochondria and dictyosomes are not abundant in vegetative cells (Figs. 69, 71-73); when present, they occur in the peripheral cytoplasm of cells. A large central vacuole is often observed within endophytic cells in field material (Figs. 54, 56) which was not observed in free-living material. Further ultrastructural details will be discussed later when comparing these taxa to other acrochaetioid algae.
Preliminary ultrastructural studies on endophytes growing in situ were carried out. There were no major effects on host plants (Figs. 57-60), however, some of the fibers within the walls of *Porphyra* became disorganized during infection by *Audouinella porphyrae* (Figs. 61, 63-64). The structural organization of walls of *Pterosiphonia bipinnata* was unaffected by growth of *A. vaga* (Figs. 65-68). A summary of all results is recorded in Table VI.
Table VI: Summary of differences and similarities of morphological, reproductive and cytological characters of field and cultured *Audouinella porphyrae* and *Audouinella vaga* obtained in the current study.

<table>
<thead>
<tr>
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<th>Field material</th>
<th>Cultured material</th>
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<tr>
<td></td>
<td><em>A. porphyrae</em></td>
<td><em>A. vaga</em></td>
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<td></td>
<td><em>A. porphyrae</em></td>
<td><em>A. vaga</em></td>
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<td>Morphological features:</td>
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<tr>
<td>Cell dimensions:</td>
<td>12-15μm(l)</td>
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<td></td>
<td>3-7.5μm(d)</td>
<td>3-6μm(d)</td>
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<tr>
<td>Hosts:</td>
<td><em>Porphyra</em> sp.</td>
<td><em>Pierosiphonia</em> bi pinnata</td>
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<tr>
<td>Branching pattern:</td>
<td>irregular</td>
<td>right angular</td>
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<td>Infection cycle:</td>
<td>monospore</td>
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<td>Reproductive features:</td>
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<td>Life history:</td>
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<td>monospore</td>
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<td>Cytological features:</td>
<td>similar ultrastructural features for field and cultured materials (see text)</td>
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DISCUSSION

A study of field material showed that there are similarities and differences between the two endophytes, *Audouinella porphyrae* and *A. vaga*. Many features of the endophytes reported previously by Garbary et al. (1982) have been corroborated in this investigation. Such features include aspects of the seasonality, infection process, reproductive structures, morphology and spore germination in the two taxa.

Some additional characters of the endophytes in field material were noted in this study. They relate primarily to the relationship between the endophytes and their hosts. In neither endophyte/host relationship was there a correlation between the size of endophytic infection areas and the size of the hosts. A positive correlation between the size of the endophyte and when the hosts were reproductive was noted. This could indicate that the endophyte grows as the hosts mature. Both endophytes appear to infect the oldest or older portions of their hosts. Initially, *A. porphyrae* first infects the holdfast region of *Porphyra* spp. whereas *A. vaga* infects the older regions of the primary axis of *Pterosiphonia bipinnata*, closest to the rhizoids. White and Boney (1970) also noted this when examining a series of endophytic and endozoic *Acrochaetium* species, and suggested the basal region of a red alga will usually contain any infections by endophytes.

Unlike parasites which require nutritional needs from their hosts (Goff, 1982) and epiphytes which may require a certain
colonizable substrata (Harlin, 1975), these endophytes do not appear to have any of these requirements. A possible reason for these symbionts to become endophytic is to "escape" environmental stresses such as desiccation, sand scouring and wave action. This has yet to be studied. The phenomenon of wounding by grazers plays an important role in the penetration of parasites (Goff, 1982). This was not observed for the endophyte in this study and no obvious damage of host cell walls was noted in the area of settlement and penetration.

Secondary infections occur in vegetative regions of both hosts probably from germination of monospores produced by the primary infection. In support of this argument, secondary infections are seen only when the primary infections are large and reproductive.

Differences which were previously noted and used to separate the two species (Garbary et al., 1982 and Table I) were also observed in this study. For example, cell dimensions of the endophytes inside their hosts are statistically different, and current cell measurements for the two taxa overlap with those reported by Garbary et al. (1982) (Table I and Table V). However, cell lengths were slightly longer than those reported by Garbary et al. (1982) thus extending the range of cell length for *Audouinella vaga*. The branching pattern of the two endophytes in the host material differs; *A. porphyrae* branches irregularly, whereas *A. vaga* branches at right angles to the main growing filament just inside the host wall. Although Garbary et al. (1982) noted tetrasporangia from one collection of *A. porphyrae* and hair cells from one collection of *A. vaga*
(Table I), neither of these two characters was observed in field or culture material from this study. Since the presence of hair cells and tetrasporangia are so sporadic in field material, they are unsuitable characters for distinguishing these species.

Some differences between the endophytes in addition to those indicated by Garbary et al. (1982) were observed in the current investigation. Occasionally, filaments of *Audouinella vaga* were observed penetrating cells of *Pterosiphonia bipinnata*. This is the first report of this phenomenon. Drew (1928) noted the rare penetration of *A. porphyrae* into *Porphyra* cells, this was not observed in the present study. Since both endophytes have been observed to penetrate their hosts' cells infrequently, it appears that this is not a useful character to distinguish between these two species (Garbary et al., 1982). The rarity of this phenomenon suggests that host cell penetration is not a normal aspect of the growth strategy of the two taxa.

Thus, there are three major differences that could be used in characterizing the two endophytes in their hosts: 1) They reside in separate hosts, *Audouinella porphyrae* occurs in *Porphyra* whereas *A. vaga* is present in *Pterosiphonia bipinnata*, 2) *A. porphyrae* has shorter apical and main filmentous cells than *A. vaga* (Table V), and 3) *A. porphyrae* branches irregularly whereas *A. vaga* branches at right angles to the primary axis.

Other than the differences discussed above, the two endophytes are quite similar morphologically and cytologically. In addition, the monosporangial reproductive cycles observed in the field and the pattern of primary and secondary infections are the same. Chloroplasts were consistent with the "Nemalion"
- type as described by Hara and Chihara (1973). Similar chloroplasts have been shown in other *Audouinella* species, such as *Kylinia* sp. (Gibbs, 1970), *Acrochaetium* sp. (Hara and Chihara, 1974) and *Rhodochorton* sp. (Mitrakos, 1960). Other *Audouinella* spp. have different chloroplast types; this was shown in ultrastructural studies by Hymes and Cole, (1983a) and Lichtlé, (1973) who reported a modified "Batrochospermum" - type of chloroplast. The two species of endophytic *Audouinella* have pit plugs with two cap layers. This is consistent with the observations of Pueschel and Cole (1982); Lee (1971); Hymes and Cole (1983a).

Other than the limited ultrastructural studies of chloroplast and pit plugs (see above), the only papers describing the general fine structure of an acrochaetioid alga (*Audouinella hermannii* (Roth) Duby) are by Hymes and Cole (1983a,b). Like *A. hermannii*, the free-living endophytes showed few mitochondria and dictyosomes; these were located at the periphery of the cells. Furthermore, the cell walls of the free-living endophytes were thick, representing over 30% of the cell diameter. A large vacuole was observed in field material and in older filaments of endophytes in culture, similar to older cells of *A. hermannii* (Hymes and Cole, 1983a).

Unlike *Audouinella hermannii*, both endophytes showed a central prominent pyrenoid with four to five traversing thylakoids. Nuclei of both endophytes were commonly observed near the base of vegetative cells in erect (free-living) axes (Figs. 72-73). This has been observed in one other species of *Audouinella, A. parvula* (Kylin) Dixon (Garbary, 1978). In
contrast, the nuclear position in *A. hermanii* is more centrally located (Hymes and Cole, 1983a).

Culture studies were conducted to determine if the endophytic characters in field material persisted in the free-living state. Field characters were not maintained in the free-living endophytes. The data obtained revealed clearly that the free-living forms of *Audouinia porphyrae* and *A. vaga* are similar in morphology, cell dimensions, branching and growth patterns. They both demonstrate a monosporangial reproductive cycle in their free-living forms, and cell length appears to be within the range encountered in field material. However, the cell diameters of the free-living forms is greater than that of the endophytic forms (9 vs 3-7.5 μm). Garbary (1979b) also noted some significant differences in cell dimensions between the free-living and endophytic forms of *A. endophytica* isolated from *Heterosiphonia plumosa*. In contrast, White and Boney (1970) did not find any significant differences in cell dimensions and morphology when comparing the *in vivo* and *in situ* forms of *A. endophytica*.

It is possible to conclude from the above observations that the differences in cell dimensions and branching patterns in field materials are a consequence of the difference in habitats represented by the host cell walls. *Porphyra* walls are much thicker (50μm vs 20μm) than *Pterosiphonia bipinnata* walls and this could influence the branching and growth patterns of the endophytes. The orientation of microbils in the cell wall matrix of florideophyceae (Young, 1980) and the chemical constituent of the walls of both hosts could influence the
observed differences in the branching pattern of the endophytes. The walls of macrothalli of *Porphyra* are composed of xylan and mannan (Frei and Preston, 1964) whereas members of Florideophyceae have sulfated polysaccharide and cellulose in the wall matrix (Mackie and Preston, 1974).

Re- and cross-infection studies demonstrated that both *Audouinella porphyrae* and *A. vaga* were not host specific. They both grew epiphytically on either host under various culture conditions. Both White and Boney (1969) and Garbary (1979) studied various endophytes, under field and culture conditions. They found that many endophytes such as *Audouinella infestans* and *A. bonnemaisoniae* grew on various substrata including other red algae and hydroids. However, they both found that only *A. endophytica* grew endophytically in various substrata such as shells and other red algae (not its normal host). The other endophytes studied established nonendophytic relationships ranging from simple entanglement to some form of epiphytic growth on given substrata. White and Boney (1969, 1970) noted that only *A. endophytica* grew endozoically in a hydroid and showed different growth responses when given a variety of calcareous substrata. Differences began shortly after spore germination and the germlings formed growth patterns that were determined by the organic matrix of the given substratum. For *A. porphyrae* and *A. vaga*, differences in responses when growing on the hosts, indicate that the nature of the host cell walls also influences the development of the endophytes.

The lack of endophytic growth could result from several factors. The cell walls of the juvenile host plants may not be
"thick" enough for the endophytes to penetrate and grow. This argument is presented by Garbary for some of the *Audouinella* species he studied (1979b). There could be some mechanism such as sand scouring or wave action which could prepare host cell walls for penetration by the endophytes.

Monospore production is the most common form of reproduction in the genus *Audouinella*. Regeneration of monosporangia within remnants of old monosporangial walls was observed for both *A. porphyrae* and *A. vaga*. This appears to be a typical feature of many *Audouinella* spp. (White and Boney, 1970; Boney, 1967; Hymes and Cole, 1983b). White and Boney (1979) noted changes in the abundance of monosporangial production in different culture conditions. Differences in abundance of monosporangial production were also noted in the current experiments carried out in culture conditions. Other morphological features such as hair cells and sexual reproductive structures were not seen under any culture conditions used in the present study. White and Boney (1979) did not find any sexual reproductive structures or hair cells in the endophytes, *A. endophytica*, *A. asparagopsis* and *A. infestans*, even when experimenting with different daylengths and temperatures.

In the asexual species, *Audouinella proskaueri* (West) Garbary Hansen et Scagel, West (1971) noted that hair cell production was related primarily to light intensity rather than photoperiod or temperature. Field material of *A. porphyrae* has been reported with hair cells (Garbary et al., 1982) but no hairs from free-living cultures of the endophytes were observed.
under different photon flux densities (4-5, 25, 55 microeinstein m$^{-2}$ sec$^{-1}$). The lack of hairs in cultures of *A. vaga* and *A. porphyrae* may result from an absence of suitable culture conditions, or the fact that the entities do not have the genetic potential to produce them. It would appear that varying individual environmental factors in the laboratory may be insufficient to induce certain reproductive and morphological features that were infrequently seen in the field. Perhaps more particular combinations of conditions of photoperiod, temperature and light intensity are needed to induce tetrasporangia, hair cells, and gametangia. It is also possible that such structures are not formed by these entities.

In their studies on *Audouinella arcuata* (Drew) Garbary, Hansen et Scagel, Hansen and Garbary (1984) noted similarities between two species of *Audouinella*: *A. arcuata* and *A. vaga* and suggested that they may represent tetrasporophytic and gametophytic phases of a single sexual life history. There are several examples where epiphytes with unicellular bases and diminutive erect filaments are the gametophytic stages in triphasic, trimorphic life histories. In these life histories, tetrasporophytes are comprised of multicellular bases and more extensive erect filaments (eg. Stegenga and Mulder, 1979; Stegenga and Vroman, 1976; see Woelkerling, 1983). Hansen and Garbary (1984) have observed *A. arcuata* with a few erect filaments, and *A. microscopica* also with a few erect filaments (Naegeli) Woelkerling growing with *A. vaga* on the same host, *Pterosiphonia bipinnata*. I have also observed this in my field collections. *Audouinella arcuata* and *A. microscopica* are
diminutive plants with unicellular bases, one to several erect axes and single, stellate chloroplasts. Hansen and Garbary (1984) suggested that *A. arcuata* might be the gametophytic phase of *A. vaga*. *Audouinella arcuata* from San Juan Island was grown at 10°C 16:8 h (L:D) at a photon flux density of 4-5 microeinsteins m\(^{-2}\) sec\(^{-1}\), and did not observe any sexual structures on this plant. Other culture conditions are needed to induce gametogenesis. Similarly, *A. microscopica* (sensu Garbary et al., 1982 not Stegenga et al., 1979) maybe a possible gametophytic phase for *A. porphyrae* (Garbary et al., 1982). This also needs to be investigated in culture. Thus, if *A. porphyrae* and *A. vaga* are the same entity as shown from the culture studies of this thesis and if they have the suggested gametophytes as part of their sexual life histories, then at least three species of *Audouinella* could be synonymized when the life histories are resolved.

To summarize culture observations, the endophytes are identical in their free-living forms, and both showed similar results in the re- and cross-infection experiments. Based on field and culture data (Table VI), I propose that the two endophytes are conspecific. *Audouinella porphyrae* is chosen over *A. vaga* because of less taxonomical confusion in its original description (Drew, 1928). I propose synonymizing the endophytes under *Audouinella porphyrae*. Another taxon *A. vaga* var. *implicata* (Drew) Garbary, Hansen et Scagel is associated with this complex. It differs from *A. vaga* var. *vaga* only in its' host (*Halosaccion glandiforme* (Gmelin) Ruprecht), and provisionally may be synonymized with *A. vaga* (Garbary et al.,
A complete listing of the taxonomic and nomenclatural synonyms of *Audouinella porphyrae* is as follows:

*Audouinella porphyrae* (Drew) Garbary, Hansen and Scagel, 1982 [1983]: 42

Basionym: *Rhodochorton porphyrae* Drew 1928: 188

Synonyms:  
- *Acrochaetium porphyrae* (Drew) Smith 1944: 179  
- *Chromastrum porphyrae* (Drew) Papenfuss 1945: 325  
- *Kylinia porphyrae* (Drew) Papenfuss 1947: 438  
- *Colaconema porphyrae* (Drew) Woelkerling 1971: 50  
- *Rhodochorton vagum* Drew 1928: 188  
- *Acrochaetium vagum* (Drew) Jao, 1937: 111  
- *Chromastrum vagum* (Drew) Stegenga and Mulder 1979: 305 in Tam  
- *Rhodochorton implicatum* Drew 1928: 190  
- *Chromastrum implicatum* (Drew) Papenfuss 1945: 324  
- *Kylinia implicata* (Drew) Papenfuss 1947: 438  

In conclusion, future studies are needed to provide more information on *Audouinella porphyrae* (including *A. vaga*) that would complement the current knowledge. For example, a complete life history would be useful in understanding the possible alternate life history stages in nature. More extensive ecological studies are needed to determine the location of the endophytes, when the host plants are absent during the winter as well as the source of infection for the young hosts during early spring. There are several possibilities which could be explored. The endophytes could be in a cryptic form in the crevices of rocks since they both infect the oldest portions of the host plants. Another possibility is that they could be residing in an alternate host. For example, *A. vaga* var.
implicata, is described from Halosaccion glandiforme which is abundant during winter months, however, A. vaga var. implicata is a poorly known taxon. It thus becomes important to characterize adequately it to resolve this problem. Such studies may add to our general knowledge of host-endophytic relationships and resolve taxonomic and ecological problems in Audouinella.
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*Rhodophycées: Rhodochorton purpureum* (Lightf.) Rosenvinge


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FIGURE LEGENDS

Fig. 1 Diagrammatic representations of Porphyra sp. and Pterosiphonia bipinnata showing areas of infection.

Fig. 1a Porphyra blade: 1 = holdfast; 2 = vegetative region; 3 = reproductive region.

Fig. 1b P. bipinnata thalli: 1 = holdfast; 2 = main filament; 3 = older branches; 4 = apical growth.
Fig. 2 Cross-section of living *Porphyra torta* (h), with the endophyte, *Audouinella porphyrae*. Note the erect filaments (e) growing from the prostrate portion (p) of the endophyte. Scale bar = 18μm

Fig. 3 *P. torta* heavily infected by *A. porphyrae*. Note the irregular branching pattern of the endophyte (e). Scale bar = 19μm

Fig. 4 *Pterosiphonia bipinnata* primary axis heavily infected by the endophyte *A. vaga* (en). Note growth pattern of the endophyte in the walls of the host pericentral cells. Scale bar = 75μm

Fig. 5 Close up of *A. vaga* (arrow) as it grows and branches in the wall of its host. Note the branching and growth pattern of the endophyte. Scale bar = 25μm
Fig. 6-9: Micrographs of living field *Audoinella porphyrae*/
*Porphyra fucicola* taken with Nomarski Modulation
Contrast Microscopy.

Fig. 6 A spore of *A. porphyrae* with an internal division.
Note germinating cell (arrow). Scale bar = 15μm

Fig. 7 Note germinating cell (arrow) from settled spore.
Scale bar = 11μm

Fig. 8 Prostrate filaments in the host cell walls. Note
prominent pyrenoid in the cell (arrow). Scale
bar = 14μm

Fig. 9 Extensive infection by prostrate filaments in the host.
Note irregular branching pattern. Scale bar = 14μm

Fig. 10 Monospore of free-living *A. porphyrae* settling and
dividing internally (arrow) on a plastic petri plate.
Scale bar = 12μm

Fig. 11 Germinating cell from settled spore (arrow). Scale
bar = 8μm

Fig. 12 Growing filament. Cytoplasm is being
"pushed" towards the growing tip by an expanding
vacuole (arrow). Scale bar = 8μm

Fig. 13 Prostrate cells (p) of *A. porphyrae* with two initial
erect filaments (e) (arrows). Scale bar = 20μm

Fig. 14 Erect filaments of *A. porphyrae* showing a stellate,
axial chloroplast (c) with a central pyrenoid (py) and
a large vacuole (v). Scale bar = 13μm
Figs. 15-21: Culture material of living *A. porphyrae* (since both free-living endophytes are identical, only *A. porphyrae* is shown).

Fig. 15 Erect filaments with short lateral branches which form monosporangia. Scale bar = 14μm

Fig. 16 Erect filaments (clear arrow) with discharged monosporangia. Note production of a new cell in an empty monosporangium (dark arrow). Scale bar = 15μm

Fig. 17 Monosporangia can be lateral and/or terminal on an erect filament. Note pedicels below monosporangium (arrow). Scale bar = 15μm

Figs. 18-21: Nomarski Contrast Modulation Microscopy

Fig. 18 Erect filaments with many lateral filaments. Monosporangia filaments can also be formed on secondary branchlets (arrows). Scale bar = 8μm

Fig. 19 A budding apical cell growing into an empty sporangium. Note the vacuole (arrow). Scale bar = 17μm

Fig. 20 Four to six monosporangia can be produced from one sporangium. Note old wall layers (arrow).

Scale bar = 17μm

Fig. 21 Usually one to two or occasionally three monosporangia are formed from one cell.

Scale bar = 17μm
Figs. 22-25: Uninfected living *Pterosiphonia bipinnata* and *Porphyra* blades used for re-infection and cross-infection experiments.

Fig. 22 A young branch on the main axis of *P. bipinnata*.  
Scale bar = 375μm

Fig. 23 Apical tips of *P. bipinnata* tend to curve adaxially (arrow).  
Scale bar = 375μm

Fig. 24 The main axis of cultured *P. bipinnata* with a rhizoid (r) and a branch.  
Scale bar = 188μm

Fig. 25 Cleaned *Porphyra* blade.  
Scale bar = 90μm
Figs. 26-34: Cross-infected on *Audouinella porphyrae* on living *Pterosiphina bipinnata*.

Fig. 26 Germlings of epiphytic *A. porphyrae* infecting a rhiziod of *P. bipinnata*. Note internal division (arrow). Scale bar = 10µm

Fig. 27 Note erect filament from settled spore (arrow). Scale bar = 10µm

Fig. 28 Erect filament growing from a monospore. Note the partial penetration by the basal cell (arrow). Scale bar = 12µm

Fig. 29 Erect filaments growing on rhizoid (r) of *P. bipinnata*. Scale bar = 45µm

Fig. 30 Tufts of epiphytes growing on a rhizoid of infected host. Scale bar = 45µm

Fig. 31 Older apical axis of host covered with epiphytes. Scale bar = 45µm

Fig. 32 Epiphytes growing on a branching point of the host. Scale bar = 50µm

Fig. 33 Heavy infection by *A. porphyrae* obscuring the host. Scale bar = 50µm

Fig. 34 A young apical shoot of *P. bipinnata* growing from heavily infected host. The new shoot has already been infected (arrows). Scale bar = 40µm
Figs. 35-37: *Audouinella vaga* cross-infecting a living *Porphyra torta* blade.

Fig. 35 Infection is sparse. Few epiphytes are seen on the vegetative area of the blade. Note the long erect filaments of the epiphytes (arrow). Scale bar = 30μm.

Fig. 36 Erect filaments of *A. vaga* on host. Note the extensive branching of the epiphyte. Scale bar = 30μm.

Fig. 37 Basal cells (p) of epiphytes (e) growing on *Porphyra* blades (h) are more extensive (arrow) than epiphytes growing on *P. bipinnata*. Scale bar = 15μm.
Figs. 38-41: Methacrylate sections of *Audouinella porphyrae*/ *Porphyra fucicola* (2.5μm) stained with Toluidine Blue-O.

Fig. 38 Extensive prostrate filaments (p) in host cell wall.
Scale bar = 40μm

Fig. 39 Prostrate filament growing between host cells of *P. fucicola*. Majority of endophytic growth is within the outer wall of the host.
Scale bar = 36μm

Fig. 40 Close-up of an erect filament as it grows through the wall of the host. Note the inner wall of the host (arrow). Scale bar = 11μm

Fig. 41 An empty monosporangium on an erect filament, outside the host cell wall. Scale bar = 8μm
Figs 42-46: Methacrylate sections of *A. porphyrae/Porphyra fucicola* (2.5μm thick) stained with Toluidine Blue-O (Figs. 42-43) and Fast Green (Figs. 44-46).

Fig. 42 Close up of prostrate portions growing extensively in the outer wall of the host. Scale bar = 7μm  

Fig. 43 Close up of prostrate portions growing between two host cells (arrow) within the inner wall of the host. Erect filaments (e) are also present. Scale bar = 9μm  

Fig. 44 Suggestion of proteinous substance (dark stain) is seen on the cuticle (cu) and in the cytoplasm of both the endophyte and the host. Pit plug (arrow) is also stained. Scale bar = 14μm  

Fig. 45 Protuberance of an erect initial cell (arrow). Note dark stain in the host wall beside the protuberance. Scale bar = 6μm  

Fig. 46 Note the dark stain just above the endophyte close to the outer edge of the host (arrow). Scale bar = 3μm
Figs 47-50: Methacrylate sections of *Audouinella vaga/* *Pterosiphonia bipinnata* (2.5μm thick) stained with Toluidine Blue-O.

Fig. 47 Note the filaments of *Audouinella* growing along the wall of a pericentral cell with a large vacuole (v). Erect filaments are produced from prostrate filaments (arrow). Scale bar = 8μm

Fig. 48 Penetration of *A. vaga* into the cytoplasm of the host (arrow). Note the nucleus (n) and large vacuoles (v) of the host cell. Scale bar = 6μm

Fig. 49 Endophytic growth limited by the host wall (dark arrow). Note the large vacuole (v) and cytoplasm against the periphery of the host cell (clear arrow). Scale bar = 8μm

Fig. 50 Filaments of the endophyte growing between two cells of the host (arrow). Scale bar = 10μm
Figs. 51-53: Methacrylate sections of *A. vaga/Pterosiphonia* (2.5μm thick) stained with Alcian Blue/PAS (Fig. 51) and Fast Green (Figs. 52-53).

**Fig. 51** Endophyte branching within the host cell wall (arrow). Note the angular branching pattern of the endophyte. Scale bar = 10μm

**Fig. 52** Suggestion of proteinaceous substance located within cytoplasm of both endophyte and host. Note the large vacuoles of the endophyte (arrows). Note the darken area of the host wall just above the endophyte. Scale bar = 10μm

**Fig. 53** A few erect filaments (e) outside of host (arrow). Note darkened areas in host wall beside the endophyte. Scale bar = 8μm
Figs 54-56: Ultrastructure of *Audouinella porphyrae*/ *Porphyra fucicola*

Fig. 54 Note the large vacuole (v) and the stellate chloroplast (c) in the endophyte cell. The host cell wall appears to be affected by the endophyte. Note the irregular matrix of the host cell wall (hcw). Scale bar = 2μm

Fig. 55 Section through a growing filament of *A. porphyrae* inside the host. Note the mitochondria (m) and half pit plug (arrow). Scale bar = 1μm

Fig. 56 Note the nucleus (n) of the endophyte towards the end of the cell. Part of the stellate chloroplast (c) is seen within the vacuole (v). Scale bar = 2μm
Figs. 57-60: Ultrastructure of uninfected *Porphyra fucicola*.

Fig. 57 A central pyrenoid (py) with mitochondria (arrow) in the periphery of a *P. fucicola* cell.

Scale bar = 4μm

Fig. 58 A nucleus (n) with mitochondria (m) around it.

Scale bar = 1μm

Fig. 59 Close-up of the cell wall of *P. fucicola*. Note the regular fibrous matrix. Bar = 0.2μm

Fig. 60 Overview of *P. fucicola* inner wall. Note the regular matrix. Scale bar = 1.5μm
Figs 61-64: Ultrastructure of *Audouinella porphyrae/Porphyra fucicola*.

Fig. 61 Section of endophyte growing outside of the host.  
Note the irregular matrix of the host cell wall (hcw).  
A mitochondrion (m) and parts of the chloroplast (c)  
of the endophyte. Scale bar = 0.5μm

Fig. 62 Section through the chloroplast of the endophyte  
showing a central pyrenoid (py) with irregular  
thylakoids (arrow) traversing it. Scale bar = 1μm

Fig. 63 Note the irregular matrix of the host (hcw) compared to  
the wall of the endophyte (ecw). Scale bar = 0.8μm

Fig. 64 Enlargement of endophytic cell wall (ecw) and host cell  
wall (hcw). Scale bar = 0.2μm
Figs. 65-68: Ultrastructure of *Audouinella vaga*/ *Pierosiphonia bipinnata*.

Fig. 65 Section of a part of uninfected host cell showing the regular matrix of the wall (hcw) and part of a vacuole (v) and mitochondrion at the periphery of the cell. Scale bar = 0.8μm

Fig. 66 Section of the endophyte protruding outside the host. Host cell wall (hcw) does not appear to be affected. Scale bar = 0.2μm

Fig. 67 Section of endophyte inside the host cell wall (hcw) showing organelles of the host (h) which do not appear to be affected. Note large vacuole of endophyte (arrow). Scale bar = 2μm

Fig. 68 Note how the growth pattern of the endophyte (e) follows the longitudinal growth of the host. Scale bar = 2μm
Figs. 69-71: Ultrastructure of free-living *Audouinella porphyrae*.

Fig. 69 Note the large chloroplast with a central pyrenoid (py) and the florideophycean starch (s) at the periphery of the cell. Note mitochondria (m) along the periphery of the cell and the thick wall (double arrow). Scale bar = 1μm

Fig. 70 Close up of a pit plug. Note the two cap layers. Scale bar = 0.5μm

Fig. 71 A prominent nucleus (n) with a nucleolous (nu) is frequently located at the base of the cell. Note the starch grains (s) surrounding the nucleus. Scale bar = 1μm
Figs. 72-75: Ultrastructure of free-living *Audouinella vaga*.

Fig. 72 Note the numerous layers (arrow) within the cell wall of a growing filament. Note the nucleus (n) toward the base of the cells. Scale bar = 1\( \mu \text{m} \)

Fig. 73 Close-up of apical tip. Note the nucleus (n), membranous bodies (mb) and parts of a stellate chloroplast (c) within the cytoplasm of the cell. Scale bar = 0.8\( \mu \text{m} \)

Fig. 74 Cross section of a filament. Note the nucleus (n) and starch grains (s) around it. Scale bar = 0.8\( \mu \text{m} \)

Fig. 75 Section through the prostrate portion of the endophyte. Note the central pyrenoid (py) and numerous starch grains (s). Scale bar = 2\( \mu \text{m} \)