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Green epi-endophytes in *Hymenena falklandica* (Rhodophyta) from the Patagonian coasts of Argentina: Preliminary observations

M. Cecilia Gauna[1,2]* **and Elisa R. Parodi**[1,2]

[1]Laboratorio de Ecología Acuática, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, and [2]CONICET-CCTBBca, Instituto Argentino de Oceanografía (I.A.D.O.), Camino Carrindanga 7,5 km, B8000FTN, Bahía Blanca, Argentina

SUMMARY

The occurrence of epi-endophyte algae in *Hymenena falklandica* J. G. Ag. Ex Kylin (Rhodophyta) is reported in Argentina. *Epicladia heterotricha* (Yarish) Nielsen (Chlorophyceae) revealed a 100% prevalence of occurrence, particularly in the basal region of the host. Low (14%), moderate (28%) and high (58%) severity degrees of infection were also registered for *E. heterotricha. Pseudendoclonium submarinum* Wille (Chlorophyceae) exhibited a lower frequency of occurrence, close to 3%. The developmental morphology and dynamics of *E. heterotricha* and *P. submarinum* were investigated under unialgal as well as bialgal culture conditions. The experimental infection of *H. falklandica* by *E. heterotricha* demonstrated that *E. heterotricha* initially behaves as epiphytic but with endophytic filaments growing into the cortex of the host during late infection. *P. submarinum* was found to be exclusively epiphytic, with no development of endophytic filamentous systems. The present study reports the first lines of evidence of an epidemiological study conducted with the purpose of comparing both the prevalence and effects of algal epi-endophytic organisms in *H. falklandica* in the southern coasts of Argentina.

Key words: Argentina, Chlorophyceae, *Epicladia heterotricha*, epi-endophyte algae, *Hymenena falklandica*, *Pseudendoclonium submarinum*, Rhodophyta.

INTRODUCTION

Endophytism has been defined as a type of symbiosis in which an organism lives within the tissues of a plant host (Lewis 1973; Starr 1975; Goff 1982; Lewin 1982; Smith & Douglas 1987; Douglas & Smith 1989). Among algae, these interspecific relations are a common phenomenon in nature since several species of small, filamentous, green, brown, and red algae have been found living inside the tissues of large algal hosts (Correa 1994). Many studies have been carried out to examine the biological aspects of these associations (White & Boney 1969, 1970; Boney 1972; Garbary 1979; Nielsen 1987; Sánchez *et al*. 1996; Gauna 2005). In general, epi-endophyte pigmented algae are photosynthetically independent and with almost no metabolic relation to their hosts on account of the fact that many of these invading organisms were isolated from their hosts and subsequently cultivated under laboratory conditions. Significant biological information on these interspecific relations results from experimental studies on *Chondrus crispus* Stackhouse – *Acrochaete operculata* Correa & Nielsen and *C. crispus* – *Acrochaete heteroclada* Correa & Nielsen phatosystems (Correa & McLachlan 1991, 1992, 1994; Correa *et al*. 1988).

Hymenena falklandica Kylin is an important component of tidal and subtidal algal communities in the southern coasts of Argentina (Mendoza & Nizovoy 2000). Its fronds are short stiped, boned in the base and repeatedly divided, with the blades brown-reddish and pink at the ends.

Our observations indicate that thalli of *Epicladia heterotricha* (Yarish) Nielsen (Ulvophyceae, Chlorophyta) and *Pseudendoclonium submarinum* Wille (Ulvophyceae, Chlorophyta) frequently infect extensive areas of fronds of *H. falklandica*. Both green algae are also very common as epi-endophytes of different macroalgae, as part of algal crusts on stones, shells, and woods. They are also associated with bryozoids and hydroids (Nielsen & McLachlan 1986; Nielsen 1988). The main objective of this work was to describe, for the first time, the H. *falklandica* and *E. heterotricha* association and the *H. falklandica* and *P. submarinum*

*To whom correspondence should be addressed.
Email: cgauna@criba.edu.ar
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association in populations of *H. falklandica* in the southern coasts of Argentina. To achieve this goal, both the dynamics and the morphology of the symbiosis in nature and under culture conditions were characterized.

The need to undertake this study arose from the paucity of information about algal pathological interactions in Argentina. Further research on this subject would contribute to knowledge, which might be applied to other algal pathosystems integrated by seaweed with commercial interest, many of which develop in important natural populations on our coasts.

MATERIALS AND METHODS

Sampling

Hymenena falklandica fronds were obtained from intertidal and subtidal populations from the coast of Santa Isabel, 43°18′S and 65°06′W in the province of Chubut Argentina (Fig. 1) during December 2004.

A collection of 30 randomly selected fronds was used for the present research. Fronds were collected either randomly or along transects perpendicular to the coastline, at selected or at random intervals.

Fig. 1. Site of sampling (arrow).

Identification of epi-endophytes and disease symptoms

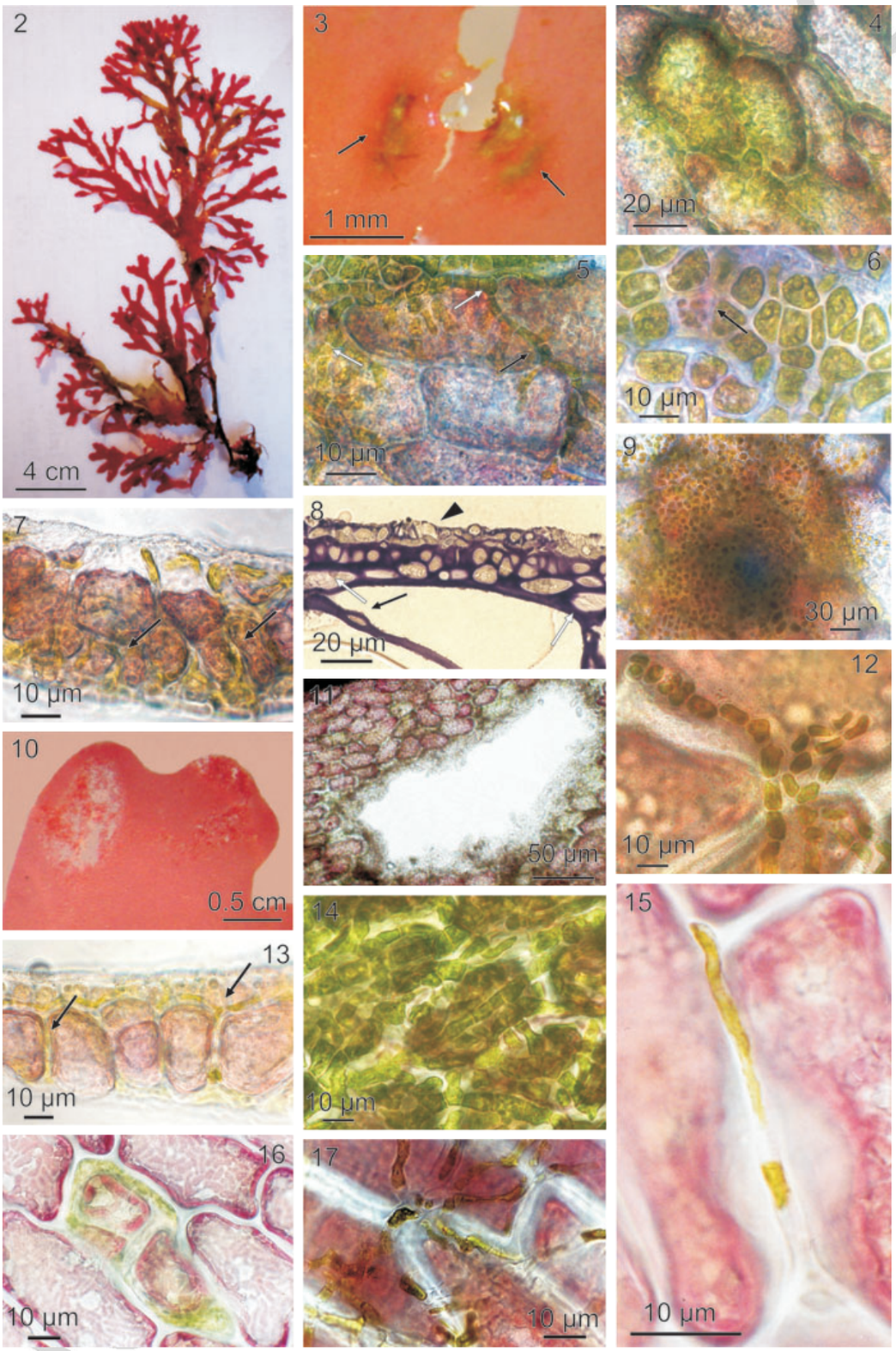
Thalli of *H. flaklandica* (Fig. 2) were examined in apical, intermediate and basal sectors. Size, presence, position and the severity of infection of epi-endophytes were registered in each frond. In order to estimate the degree of severity of infection, a qualitative scale was used (Peters & Schaffelke 1996). This scale resulted from a visual categorization of a dissected frond observed by light microscopy (LM). The degree of infection was considered low when the percentage of host thalli colonized by the epi-endophyte organism ranged from 0% to 10% (i.e. no visible signs of endophytic infection were observed). The degree of infection was categorized as moderate when the percentage of colonized thalli varied from 10% to 70% (i.e. moderate alterations, such as green spots on the lamina, were observed). Finally, the degree of infection was considered high in those cases in which thalli exhibited a percentage of colonized area higher than 70% (i.e. strongly invaded thalli were observed). The distinction between the categories was arbitrary. Only those cases under the categories 'moderate' and 'high' were considered diseased thalli.

Isolation of epi-endophytes and unialgal culture

After being collected, fronds were kept on ice, and retained in labeled plastic bags until they were examined in the laboratory, usually within 5 h of collection. Fronds were brushed and rinsed under running tap water. Small portions of infected fronds were sectioned, then immersed in fresh 0.5% solution of sodium hypochlorite for 30 s, and finally rinsed three times, 5 min each, in sterile seawater (Correa 1995). A 2-min sonication was subsequently applied to 5×5 mm portions in sterile seawater, renewing the seawater after each burst. This cleaning procedure was followed in order to remove diatoms as well as other epiphytes.

Epicladia heterotricha crude cultures were initiated by inoculating portions of cleaned fronds in plastic Petri dishes containing provasoli enriched seawater (PES) medium (Provasoli 1968). Cultures were maintained at 21 ± 1°C with a light : dark regime of 12:12 h, with a photon flux density of 15 µmol/m^2/s^1. Germlings were obtained either from swarmers or from outgrowths of the endophytes from infected thalli. They were subsequently segregated into unialgal cultures and maintained under the above-mentioned conditions with weekly changes of the medium. A 2.5% germanium dioxide solution dissolved in distilled water was added to avoid diatom contamination (Lewin 1966; Christensen 1982).

Hair production was induced applying the methodology used by Nielsen (1988), which consisted in the



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adding sterilized natural seawater to the dish culture. Strains were maintained in this condition for 4 weeks.

Experimental infection

Infections of *H. falklandica* by selected isolates of *E. heterotricha* and *P. submarinum* previously established from zoospores were experimentally carried out. Eight to ten 1.0–1.5 cm long fragments of host's fronds were placed into plastic Petri dishes. Two replicates of each isolate were incubated under laboratory conditions over 2–3 weeks.

Morphological studies and semi-thin sections

Cytomorphometry was carried out using a stereoscopic microscope Wild-Herbrugg (Gais, Switzerland) and an inverted microscope Nikon Eclipse TE 300 (Tokyo, Japan) with an incorporated camera Nikon FDX 35, with anoptral phase contrast and differential interference contrast (DIC). Either the presence or absence of epi-endophyte filaments was determined under LM in ultramicrotome 6 µm semi-thin sections of thalli of *H. falklandica*. In order to obtain semi-thin sections, thalli were fixed in 2.5% glutaraldehyde in seawater for 2 h at 4°C, and postfixed in 1% OsO4 in seawater for 2 h at 4°C. The material was dehydrated in a graded acetone series and embedded in Spurr's low viscosity resin. Glass knives were used on a Reicher Ultracut OM U2 ultramicrotome (Vienna, Austria). The resin was removed using a metallic sodium, benzene and methylic alcohol solution (Hayat 1986). Sections were stained with a combination of colorants, namely hematoxiline/malachite green/basic fucsine (1:1:1) (Berkowitz *et al*. 1968).

Cytology

Chromosome counts were made on unialgal cultures of *E. heterotricha,* derived from biflagellate zoospores. Thalli were fixed either in 1:3 mixture glacial acetic acid/absolute ethanol or in 6:3:1 mixture formaldehyde/absolute ethanol/glacial acetic acid at 5°C during a period of 2–24 h. Postfixation was carried out with 70% ethylic alcohol. The material was subsequently hydrolyzed for 30 min in 1 N chloridic acid (HCL) at room temperature, stained with Schiff stain in darkness for 2 h (Johansen 1940), bleached for 20 min in a 1:3:3 mixture of sodium metasulfite: 1 N HCL: distilled water, washed with distilled water for 30 min, and finally mounted in a drop of a 2% acetic acid solution of ferric hematoxylin with added iron acetate (Núñez 1968).

Scanning electron microscopy

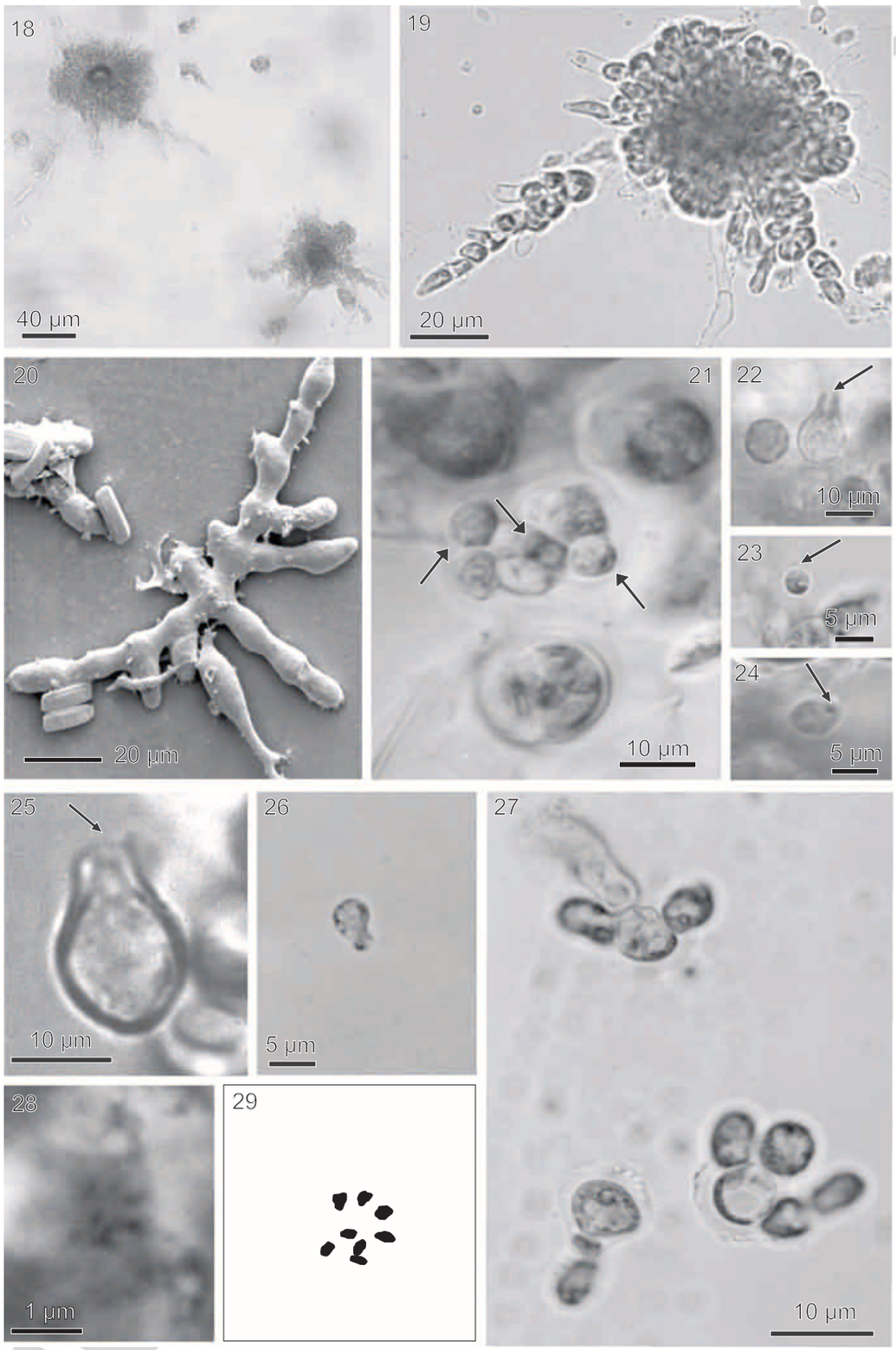
Filaments of *E. heterotricha* were fixed in 0.01 M sodium cacodilate (pH 7.2) buffer containing 2.5% glutaraldehide at 5°C for 2 h. They were subsequently mounted on slides covered with 0.5% poly D-lysine and dehydrated in a graded acetone series. Samples were finally critical-point dried for 1 h, coated with gold, and observed with a Jeol 35 CF scanning electron microscope (SEM).

RESULTS

Morphology of thalli in nature

Hymenena falklandica infected fronds exhibited green spots (Fig. 3) as symptoms of the presence of *E. heterotricha*. Thalli of *E. heterotricha* formed networks of epiphytic and endophytic filaments in epidermic and cortical host cells. Epiphytic thalli (Fig. 4) showed a pseudoparenchymatous central area made up of cells either spherical 7.5 (4–11) µm in diameter or ovoid 11.5 (5–18) µm wide, irregularly surrounded by branched filaments composed of cylindrical cells, 5.75 (3.0–8.5) µm wide, three to four times longer than they were wide (Fig. 5). Cells contained a single chloroplast with one or two pyrenoids (Fig. 5). Most of the cells of the pseudoparenchymatous part of the

Figs 2–17. 2. General view of a thallus of *Hymenena falklandica* infected by *Epicladia heterotricha* and *Pseudendoclonium submarinum*. 3. Detail of a surface view of the host to show green spots (arrows) and patches indicating sites of infection. 4. View of an epiphytic thallus of *E. heterotricha* showing a pseudoparenchymatous central area formed by spherical or ovoid cells. 5. Detail of a lateral ramification of *E. heterotricha* (black arrow) showing cylindrical cells, with a single chloroplast with one or two pyrenoids (white arrows). 6. Mature sporangium of *E. heterotricha* (arrow) with up to eight swarmers. 7. A field-collected frond of *H. falklandica*, with endophytic filaments of *E. heterotricha* deeply embedded in the medulla (arrows). 8. Semithin section of thallus infected by *H. falklandica* showing epiphytic thalli of *E. heterotricha* (arrowhead), in the medullar region (black arrow) and sporangia between medullar and cortical regions (white arrows). 9. Case of a high severity degree of infection, associated with secondary bacterial infections. 10–11. Cases of highly severe degrees of infection where cuticles exhibit perforations. 12. Young thallus of *E. heterotricha* on the cuticle of a *H. falklandica* frond, under culture conditions. 13. Cross section of a *H. falklandica* frond that shows an endophytic filament of *E. heterotricha*. 14. Advanced stage of infection when *E. heterotricha* filaments fully cover the host thallus. 15. Developing thalli from recently settled zoospores showing a parietal chloroplast with one pyrenoid. 16. Thallus in a more advanced stage of development surrounding hosts' epidermic cells. 17. Stage in which the infection of *P. submarinum* is more consolidated. Note the filaments covering the entire cellular surface, forming an epiphytic dense network on the hosts' cells.



Figs 18–29. Development of *Epicladia heterotricha* thalli under culture conditions. 18. Cultured pseudoparenchymatous thallis of *Epicladia heterotricha.* Young plant attached to the substratum. 19. Detail of the *E. heterotricha* thalli showing prostrate and erect filaments out from the pseudoparenchyma. 20. Scanning electron microscopy photomicrography of *E. heterotricha* young thallus, both alternate and oppositely branched, with isodiametric cells. 21. Detail of a sporangium with mature zoospores with stigmata (arrows). The thallus had been recently transferred into fresh medium. 22. Detail of a mature sporangia that has developed a conical expulsion papilla (arrow). 23. Quadriflagellate zoospore. 24. Biflagellate zoospore with stigma (arrow). 25. Detail of a sporangium, showing the apical pore (arrow). 26. Zoospore during the single-pole germination. Note it has already lost flagella. 27. Young thalli, with early ramifications. 28. Photomicrography of a haploid metaphasic plate with eight chromosomes. 29. Schematic representation of the haploid metaphasic plate of Figure 28.

thalli became sporangia (Fig. 6). Each sporangium with similar form and size to those of vegetative cells, gave rise to up to eight swarmers. In transverse sections, networks of *E. heterotricha* were observed in the cortical region. Filaments were made up of spherical and cylindrical cells 3–8 μm wide, and 3–10 times longer than they were wide (Fig. 7). Each cell contained a chloroplast with one to two pyrenoids. Occasionally, when the endophytic filaments reached the host medullar region, cells were up to 4–8 μm width and 10 μm in length (Fig. 8). Endophytic filaments developed in intercellular spaces located in cortical and medullar areas, without penetrating the host cells (Fig. 8). In addition, the development of sporangia was occasionally observed in the filaments surrounded by cortical cells (Fig. 8).

Also epiphytic vegetative filaments of *E. heterotricha* were widely distributed on the cuticle of *H. falklandica* fronds. They formed a layer composed of either one or two rows of cells. The innermost cells (those in contact with the cuticle) were mainly cylindrical, whereas the external ones were shorter and had a tendency to be ovoid (Fig. 8). In a few cases, erect, short filaments composed of one to three cells were observed (not illustrated).

The host cuticle remained, in general, intact, except in regions of the thallus with high degrees of infection, where cuticles exhibited perforations, and which were also associated with secondary bacterial infections (Figs 9, 10).

Severity degree of infection on the field

The size of the fronds of *H. falklandica* varied between 2.0 and 4.5 cm in length and 2.0–3.6 cm width. Thalli of *H. falklandica* of all sizes were susceptible to be invaded by *E. heterotricha.* Every single collected plant was infected, which indicated a prevalence of infection of 100%. Thalli showed different degrees of infection. Of the fronds analyzed, 14% showed evidence of a low severity degree of infection, 28% a moderate severity degree of infection, and 58% a high severity degree of infection. The frequency of infection of *H. falklandica* varied in the different regions of the fronds. The basal zone was the mostly affected section since 92% of the fronds analyzed were not only profusely invaded in this zone but also exhibited the most severe damage, namely, massive depigmentation, cellular disorganization, cuticle rupture and tallus perforations (Figs 10,11). The mid-area showed a clearly lower frequency of infection, on account of the fact that only 3% of the total fronds analyzed were infected in this region. Finally, infections in the apical zone were observed in only 5% of the examined fronds, with different severity degrees of infection varying from low to moderate but not with damage symptoms.

Pseudendoclonium submarinum was also found in *H. falklandica*, but as an exclusively epiphytic organism, showing no development of an endophytic system. It exhibited an approximately 3% frequency of occurrence in the total number of fronds examined.

Other epiphytic taxa were also registered in *H. falklandica* fronds, namely the diatom *Cocconeis* sp. colonizing particularly the basal region of the thalli and evidencing a 41% frequency of occurrence of the 30 fronds examined. Colonial diatoms were also observed mainly in the basal region.

Experimental infections

Only the vegetative growth of the epiphytes was observed during the experiments conducted in the present study. *E. heterotricha* initially developed an epiphytic stage. During the initial infection stages, the endophyte produced no severe damage. These thalli were irregularly branched filaments formed by cylindrical or contoured cells exhibiting one chloroplast with one to two pyreinods (Fig. 12).

Filaments immediately became endophytic by invading internal tissues of the host (Fig. 13). Vegetative young filaments rapidly reached massively the outer medulla in spite of the fact that the inoculation experiments conducted on fronds of *H. falklandica* demonstrated that some filaments of *E. heterotricha* could penetrate into the innermost tissues of the host. Once infection was consolidated, green spots were found scattered on the surface of the fronds (Fig. 14).

Tetrasporophytic and gametophytic fronds of *H. flaklandica* were also experimentally infected with isolates of *P. submarinum.* The colonization of fronds was induced by zoospores. Zoospores containing a parietal chloroplast with one pyrenoid adhered to the substrate and then filaments were observed around epidermic cells of *H. falklandica* (Fig. 15). In more advanced stages the epiphytic filaments surrounded the superficial cells of the host (Fig. 16). Once infection was consolidated, the filaments covered the entire cellular surface, forming an epiphytic dense network (Fig. 17).

Development and morphology of *E. heterotricha* thalli in culture

Under culture conditions growth in all thalli of *E. heterotricha* was initially prostrate, whereas the erect system prevailed subsequently. Young thalli were initially filamentous, uniseriate with opposite and alternate branching. When they were adhered to the substratum, they immediately became pseudoparenchymatous (Fig. 18). Thalli subsequently developed prostrate as well as erect filaments out of the pseudoparenchyma (Fig. 19) and they fused and covered the substratum. The distal cells of the prostrate ramifications were cylindrical, 3.5 µm in diameter and two to three times as long as the cell diameter, whereas the central cells were isodiametric and 5–8 µm in diameter.

Uniseriate, erect branches immediately developed from the central zone of the basal system. Their cells exhibited a similar shape to that of the prostrate system but they were larger and 2.5–5.0 times longer than they were wide. Some young plants were observed either non-adhered or adhered to the substratum and unattached or attached at the end of culture time. In the non-adhered plants, uniseriate branches were formed, similar to the vertical branches of the fully attached thalli. Cells from the uniseriate ramifications were cylindrical, 4 (5) µm in diameter and two to three times longer than wide. Cells possessed a typical parietal chloroplast, with one (two) pyrenoids. Short secondary branches composed of cylindrical cells, 7–9 (11) µm in diameter and one to three times as long as the cell diameter, were formed from vertical, erect branches. These secondary branches were densely aggregated, forming a cushion on the prostrate thalli and a large tuft in the free-floating plants.

Scanning electron microscopy observations revealed that young plants of *E. heterotricha* were not only alternate but also oppositely branched (Fig. 20).

Their cellular surface evidenced a minor roughness, which was only discernible at high magnifications (Fig. 20).

Sporangial development

Sporangia developed from intercalar or terminal cells after 10 days of the transfer of thalli to the fresh medium. Most of them were formed from the basal prostrate layer, whereas only a few developed from the vertical branches (Fig. 21). Sporangial mother cells elongated at the first stages of development of sporangia and mature sporangia produced a conical expulsion papilla (Fig. 22). Zoospores were individually released through a colorless tube (Figs 22, 25). Both quadriflagellate and biflagellate swarmers were observed.

Quadriflagellate swarmers varied from pyriform to nearly spherical, and they were 4–5 × 5–6 µm, with an anterior papilla. They contained a parietal cup-shaped chloroplast with a single pyrenoid and an eyespot (Figs 23,24).

Biflagellate swarmers were ovoid, 2–3 µm long, and with a conspicuous band-shaped, parietal chloroplast having an eyespot (Fig. 24). Both bi- and quadriflagellate swarmers showed positive phototaxis. After swimming for 1–5 min, swarmers adhered to the substratum and became spherical.

Swarmers germinated unipolarly forming a germination-tube (Fig. 26) towards which all of the cytoplasm migrated. New germlings developed subsequently (Fig. 27). The development of plants from biflagellate swarmers was identical to that of quadriflagellate swarmers.

Diffuse growth occurred by simultaneous mitosis in several cells. Interphase nuclei were spherical, approximately 1 µm in diameter. Eight chromosomes were observed in a tight metaphase plate of small size (Figs 28, 29).

DISCUSSION

Epi-endophytism on *Hymenena falklandica*

Two species of green seaweeds were found in our study of *Hymenena falklandica* J. G. Ag. *ex* Kylin, namely *Epicladia heterotricha* (Yarish) Nielsen and *Pseudendoclonium submarinum* Wille living together in the same frond. This multiple epi-endophytism phenomenon was also observed in *Chondrus crispus*, a species epiphyted by several green seaweeds (Correa *et al.* 1987, 1988; Correa & McLachlan 1991, 1992, 1994), Phaeophyceae and Rhodophyceae (Correa *et al.* 1987).

Our study revealed experimentally that both epi-endophytes have the ability to grow and reproduce *in vitro* outside the host, under appropriate culture conditions (Gauna 2005), thus indicating their nutritional independence.

The morphology of individuals of the Argentine population of *E. heterotricha* agrees, in general, with that of the populations from southern Finland, which have been studied by Nielsen (1988), except that the latter was reported to have larger thalli than those of the populations from the Patagonian coasts. Another significant difference between these two populations lies in the life habits. The Argentine thalli were found to be epi- and endophytic organisms in *H. falklandica,* whereas the European thalli were found to be exclusively epiphytes in higher plants such as *Phragmites australis* (Cav.) Trin. and *Potamogeton pectinatus* L. (Nielsen 1988) growing in the Baltic Sea (Nielsen *et al*. 1995) and the Canary Islands (John *et al*. 2004), respectively. In view of the above, our study reports the first lines of evidence on the ability of *E. heterotricha* to colonize red macroalgae on the one hand, and to invade internal tissues as an endophytic organism, on the other.

The dissimilar distribution of thalli of *E. heterotricha* in the different zones of the studied thalli of *H. falklandica* may lead us to hypothesize that the basal region of the fronds is the area of primary invasion of the epi-endophytes. In contrast, in *C. crispus–Acrochaete operculata* algal pathosystem, the endophyte spores directly penetrate into the cortical tissue next to the apical regions, where the cuticle is absent as a result of growth (Correa *et al*. 1987). Moreover, it has also been observed that in the *H. falklandica–E. heterotricha* association, the endophyte filaments reach the medulla also in the basal area, whereas in the *C. crispus–Acrochaete* association the medulla is invaded only in the apical region of the thalli with no cuticle (Chen & Taylor 1976; Tveter-Galagher & Mathieson 1980; Sieburth & Tootle 1981). But on the other hand, similarities have also been observed with *C. crispus–A. operculata* pathosystem (Correa *et al*. 1987) in the way in which *E. heterotricha* penetrates into *H. falklandica*.

Taking into account the high degree of prevalence of *E. heterotricha* and the effects of epi-endophytes on fronds of *H. falklandica*, it can be concluded that the most severe injurious effects are produced after complete replacement of the cortical tissue of the host.

In *E. heterotricha* an initial epiphytic stage was observed during infection similar to the case of *Acrochaete heteroclada* growing on *C. crispus* (Correa *et al*. 1988). Thus, this mechanism seemed not to be particular for genera, on account of the fact that a different pattern occurs in *A. operculata*, a species evolutionarily very close to A. *heteroclada* (Correa 1990), which is exclusively an endophytic organism.

Thalli of *E. heterotricha* did not severely compress *H. falklandica* cells. On the contrary in the system *C. crispus–A. operculata,* the cells of the latter not only compressed but also injured host cells thus greatly affecting both their shape and distribution as a result of the penetration (Correa 1990).

In advanced stages of *E. heterotricha* infections, thalli of *H. falklandica* were perforated by strong bacterial invasions. This phenomenon has also been observed in other macroalgae such as (i) *Laminaria* (Dou *et al*. 1981) in which *Pseudomonas* sp. injured sporophytes; (ii) *Nereocystis* in whose fronds *Acinetobacter* sp. not only induced necrosis but also caused an injury known as 'white rotting' (Andrews 1977); and (iii) *Porphyra,* whose thalli were injured by *Pseudomonas* sp. and *Vibrio* sp. (Fujita *et al*. 1972). A similar phenomenon was also observed in the system *C. crispus–A. operculata*, where microorganisms were present as secondary invaders in the last stage of infection (Correa 1990; Correa & McLachlan 1992).

Under culture conditions, the filaments of *E. heterotricha* developed to adult thalli in a few weeks. In contrast, host thalli collected from nature were not highly infected by *E. heterotricha*. This may be indicative of either a low rate of growth in nature or a putative ability of herbivory to control the density of the invading thalli in a similar way to that observed by Bidwell *et al.*'s (1985) observations in *C. crispus–Endophyton ramosum* Gardner pathosystem.

Experimental studies would be necessary to confirm one of these hypotheses, but we predict that herbivory plays a more important role. This phenomenon was studied by Correa and McLachlan (1992) in the *A. operculata* and *A. heterochada–C. crispus* pathosystems. It is suspected that the grazers may be attracted to infected fronds as they become softer during algal infections. Also, preferential feeding may involve the release of metabolic substances from the endophytes, which are phagostimulants.

Epicladia heterotricha lyfe cycle

The populations of *E. heterotricha* studied in the present research regularly produce bi- and quadriflagellate swarmers that originate both new sporophytic thalli. It has not yet been assessed if biflagellate swarmers correspond to partenogenetic isogametes. In the study of European populations of *E. heterotricha,* Nielsen (1988) made no reference to the presence of biflagellate swarmers but only that of tetraflagellate swarmers. Nielsen (1988) reported the same sporangia characteristics as those reported in the present research, with the difference being that he observed sporangia to form two to four zoospores instead of the eight swarmers observed in the Patagonian species.

Our experimental results indicate that the internal propagation of thalli of *E. heterotricha* in individuals of

H. falklandica, from one frond to another, may occur through swarmers. Indeed, our results clearly revealed that zoospores of *A. heterotricha* have the ability to germinate in either organic or inorganic substrates and under different culture conditions. This aptitude is indicative of a low specificity in the colonization capability of the species. The same occurs in swarmers of *A. operculata* (Correa & McLachlan 1991). Also a similar low specificity in the early stages of infection has been registered in endophytic species of *Audouinella* (White & Boney 1969; Boney 1972; Tam *et al*. 1987) and in pathogens of different red seaweeds (Nonomura 1979; Goff 1982).

Epicladia heterotricha cytology

Only a few members of *E. heterotricha* have been examined cytologically to date. Godward (1966) reported a list showing the haploid chromosomal numbers varying from 8 to 20, with many species with 12 or 14. In addition, Kapraun (1993) reported haploid numbers between four and eight and diploid ones between 11 and 15.

Epicladia heterotricha, a species belonging to Ulvophyceae, exhibited small metaphase plates with chromosomes similar to those observed in other representatives of the group, such as *Acrochaete repens* Pringsheim (Kermarrec 1970), *Bolbocoleon piliferum* Pringsheim (Moestrup 1969; Kermarrec 1970), *Acrochaete viridis* = *Entocladia viridis* (Reinke) Nielsen (O'Kelly & Yarish 1981), *Acrochaete wittrockii* (O'kelly & Yarish 1981), *Phaeophila dendroides* (P.L. Crouan & H.M. Crouan) Batters (O'Kelly & Yarish 1980), *Pringsheimiella scutata* (Reinke) Marchewianka (Nielsen & Pedersen 1977) and *Pseudopringsheimia confluens* (Rosenvinge) Wille (Perrot 1969).

CONCLUSION

This preliminary study is the first step to analyze new associations between symbiotic organisms of *Hymenena falklandica* to determine the sanitary state of this population. Research now in progress in our lab, with efforts concentrated on ultrastructural and ecophysiological aspects of the species involved and using *H. falklandica* pigmented endophytes as an experimental system in laboratory, will provide information on the nature of these symbiotic associations.

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