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Diversity, phenomenology and epidemiology of epiphytism in farmed *Gracilaria chilensis* (Rhodophyta) in northern Chile

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This study identified the most common epiphytes infecting the algal host *Gracilaria chilensis* on a farm in northern Chile. Simultaneously, the types of host–epiphyte interfaces were characterized and their relative abundance and temporal variability were monitored. Five types of anatomical relationships were detected. Infection type I included the epiphytes weakly attached to the surface of the host and not associated with damage of host tissues (i.e. *Hincksia mitchelliae*, *H. granulosa* and *Ectocarpus acutus*). Infection type II included those epiphytes strongly attached to the surface of the host but not associated with any host tissue damage (i.e. *Acrochaetium* sp., *Antithamnionella* sp. and *Colpomenia sinuosa*). Infection type III included all the epiphytes that penetrated the outer layer of the host wall without damaging its cortical cells (i.e. *Xenococcus* sp. and *Sahlingia subintegra*). Infection type IV included epiphytes penetrating deep into the host cell wall, disorganizing the cortical tissue (i.e. *Ulva lactuca* and *Acrosorium corallinarum*). Infection type V included epiphytes that penetrated deeply into the cortex, reached the medullary tissue and caused destruction of the host's cells in the area around the infection (i.e. *Ceramium rubrum* and *Polysiphonia harveyi*). Prevalence varied with time and with infection type, with types II and III reaching up to 80% and 90% of the thalli respectively. Severity of epiphyte infection was similar to the distribution of infection prevalence, with crustose epiphytes colonizing up to 80% of the host surface.

Key words: epiphytism, *Gracilaria chilensis*, host, interface, mariculture, Rhodophyta

Introduction

The occurrence of algal species growing on or within other algae has been widely reported (reviews by Goff, 1982; Ducker & Knox, 1984; Correa, 1990). In spite of the evidence indicating that parasitism and epiphytism are common phenomena in marine algae, most of the information available on the micro-anatomy of the interface between the interacting species relates to parasitism (Evans *et al.*, 1973, 1978; Goff, 1976, 1979, 1982; Wetherbee & Quirk, 1982*a*, *b*; Kugrens, 1982; Goff & Zuccarello, 1994, among others). Only a few studies, based on both wild and laboratory-infected material, report on the contact surface established between epiphytes and their hosts (Rawlence, 1972; Rawlence & Taylor, 1972; Ducker & Knox, 1984; González & Goff, 1989; González *et al.*, 1993; Dawes *et al.*, 2000). This information, however, can be of great importance for understanding the patterns of host-specificity, and provide the basic knowledge for unravelling the mechanisms of host recognition and host damage. This is of major interest for farming operations, where it is important to reduce the level of infection and to diminish the direct negative effects of the epiphytes on the host.

A wide variety of algae infect other algae and, from an anatomical point of view, they represent a continuum between epiphytes and endophytes. Epiphytes are usually defined as organisms that grow on plants, but do not derive nutrients from their hosts (Linskens, 1976). According to Linskens (1963), holo-epiphytes are those attached to the outer layers of the host, whereas amphi-epiphytes are deeply anchored in the tissues of their hosts. Linskens (1963) suggested, however, that the type of anatomical contact is highly variable and determined by the nature of the partners. In addition, the damage caused by an epiphyte

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to its basiphyte can be highly variable, and is mainly influenced by the type of anatomical association and the incidence of the epiphyte (Fletcher, 1995). However, in cases where the algal epiphyte is deeply anchored into the host tissue and, even in extreme infections, where infective algae grow almost entirely within the host as an endophyte, the negative effects on the host are not always evident and the classification of such an intruder as a pathogen can be inappropriate (Correa & McLachlan, 1994). Classification of the different types of host–epiphyte interactions is an important step in the development of management tools for any given cultivated algal resource. This may be particularly relevant if the ultimate goal is to advise farmers on cultivation practices, or the implementation of a selection program. Furthermore, classifying infecting algae according to their anatomical relation with their host might be particularly useful in cases where algal hosts are infected by a whole assemblage of algal epiphytes.

In the above context, there is general agreement that epiphytism is one of the major biological problems in *Gracilaria* farms (Pringle *et al.*, 1989, reviewed by Fletcher, 1995), due to the high density of individuals essentially maintained under monoculture conditions. These conditions are known to make the host more susceptible to pests in general and to epiphytes in particular (Friedlander, 1992). Competition between hosts and their epiphytes has been demonstrated under natural and artificial conditions of growth (Arrontes, 1990; Friedlander & Ben-Amotz, 1991; Svirski *et al.*, 1993), and the extent of the damage is clearly determined by the intensity of the infections (Cancino *et al.*, 1987; Buschmann & Gómez, 1993). Farming of *Gracilaria chilensis* is a clear example of how detrimental epiphytes may become (Kuschel & Buschmann, 1991; Pizarro & Santelices, 1993). This study focused on the identification of the most common epiphytes infecting the host in a *G. chilensis* farm in northern Chile. Simultaneously, we characterized the types of host–epiphyte interfaces and monitored their relative abundance and temporal variability.

Materials and methods

Thalli of *Gracilaria chilensis* Bird, McLachlan & Oliveira were collected monthly from April 2002 to June 2003 in a farm located in Caldera (27°04′S, 70°50′W), northern Chile. The farm is located in a protected bay, occupying 9.5 ha of mainly sandy bottom, with scattered rocks, and depths from 3 to 10 m (i.e. subtidal farm). Two sampling strategies were adopted. In order to determine the prevalence of each epiphytic species, 25 host thalli (at least 20 cm in length), were collected by hooka-diving along a single transect in each of 4 pre-defined zones separated by 300–500 m. In each transect, one thallus was collected haphazardly every 1 m in order to avoid sampling the same thallus more than once.

For microscopic observations, infected thalli were collected in the area of the farm with high prevalence of epiphytes. Samples were kept refrigerated during transport to the laboratory (less than 20 h), where epiphytic load was quantified and pieces of thalli were processed for light and transmission electron microscopy (TEM). For light microscopy, material was fixed in 3% formaldehyde in seawater for at least 36 h at room temperature, followed by freezing and sectioning with a cryo-microtome. Sections, *c.* 20 μm thick, were observed directly or after staining with aniline blue. Alternatively, material was fixed in 3% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) containing 0.25 M sucrose, infiltrated in paraplast, sectioned (5–10 μm) and stained with toluidine blue. For TEM observations, fragments of infected tissue were fixed in 3% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) containing 0.25 M sucrose for at least 2 h. Fragments were trimmed and transferred to a fresh fixative solution containing 1.5% paraformaldehyde. Fixation was followed by a series of rinses in cold 0.1 M Na-cacodylate buffer with gradually decreasing concentrations of sucrose, post-fixation for 2 h in 2% OsO_4 in 0.1 M Na-cacodylate buffer, dehydration in acetone, and infiltration in Spurr's resin over 4 days. Sections were stained with aqueous uranyl acetate followed by lead citrate and observed in a JEOL 100CX-II TEM operated at 80 Kv.

For the epidemiological work, epiphytic load was quantified under a binocular microscope by recording the different infection types on three 10–20 mm-long fragments (i.e. apical, medium and basal) of each sampled thallus. Prevalence (i.e. percentage of thalli in the farm that were infected) and severity of infection (i.e. mean abundance of epiphytes on each host thallus) were then estimated. A "severity index" was based on a semi-quantitative estimation of epiphyte cover on host thallus using four categories, where 0 = a total absence of epiphytes, 1 = 1–30% cover, 2 = 31–70% cover and 3 = 71–100% cover. For filamentous species, cover was estimated by subdividing the analyzed fragments into 10 sections of equal length and counting the proportion of these sections that included at least one epiphyte.

Results

The fine structure of cortical cells in non-infected thalli of *Gracilaria chilensis* (Fig. 1) showed a central nucleus, floridean starch granules usually around the nuclear membrane and numerous chloroplasts with parallel thylakoids, occupying most of the cytoplasm. The epidermal cell wall consisted of an outermost layer or deck-lamella (*sensu* Dawes *et al.*, 2000), and outer and inner wall strata.

Table 1. Epiphytes of cultivated *Gracilaria chilensis* in Caldera and their infection types.

	Epiphyte	Infection type
Rhodophyta		
Erythropeltidales	*Sahlingia subintegra* (Rosenvinge) Kornmann	III
Batrachospermales	*Acrochaetium* sp.	II
Ceramiales	*Antithamnionella* sp.	II
Ceramiales	*Chondria californica* (Collins) Kylin	II
Ceramiales	*Acrosorium corallinarum* (Nott) Kylin	IV
Ceramiales	*Ceramium rubrum* (Hudson) C. Agardh	V
Ceramiales	*Ceramium secundatum* (Lyngbye) C. Agardh	V
Ceramiales	*Polysiphonia harveyi* Bailey	V
Ceramiales	*Polysiphonia flaccidissima* Hollenberg	V
Corallinales	*Fosliella* sp.	III
Phaeophyceae		
Ectocarpales	*Ectocarpus acutus* Setchell & Gardner	I
Ectocarpales	*Hincksia mitchelliae* (Harvey) Silva	I
Ectocarpales	*Hincksia granulosa* (J. E. Smith) Silva	I
Scytosiphonales	*Colpomenia sinuosa* (Roth) Derbés & Solier	II
Chlorophyta		
Ulvales	*Ulva lactuca* L.	IV
Ulotrichales	*Ulothrix flacca* (Dillwyn) Thuret	II
Cyanophyta		
Chamaesiphonales	*Xenococcus* sp.	III

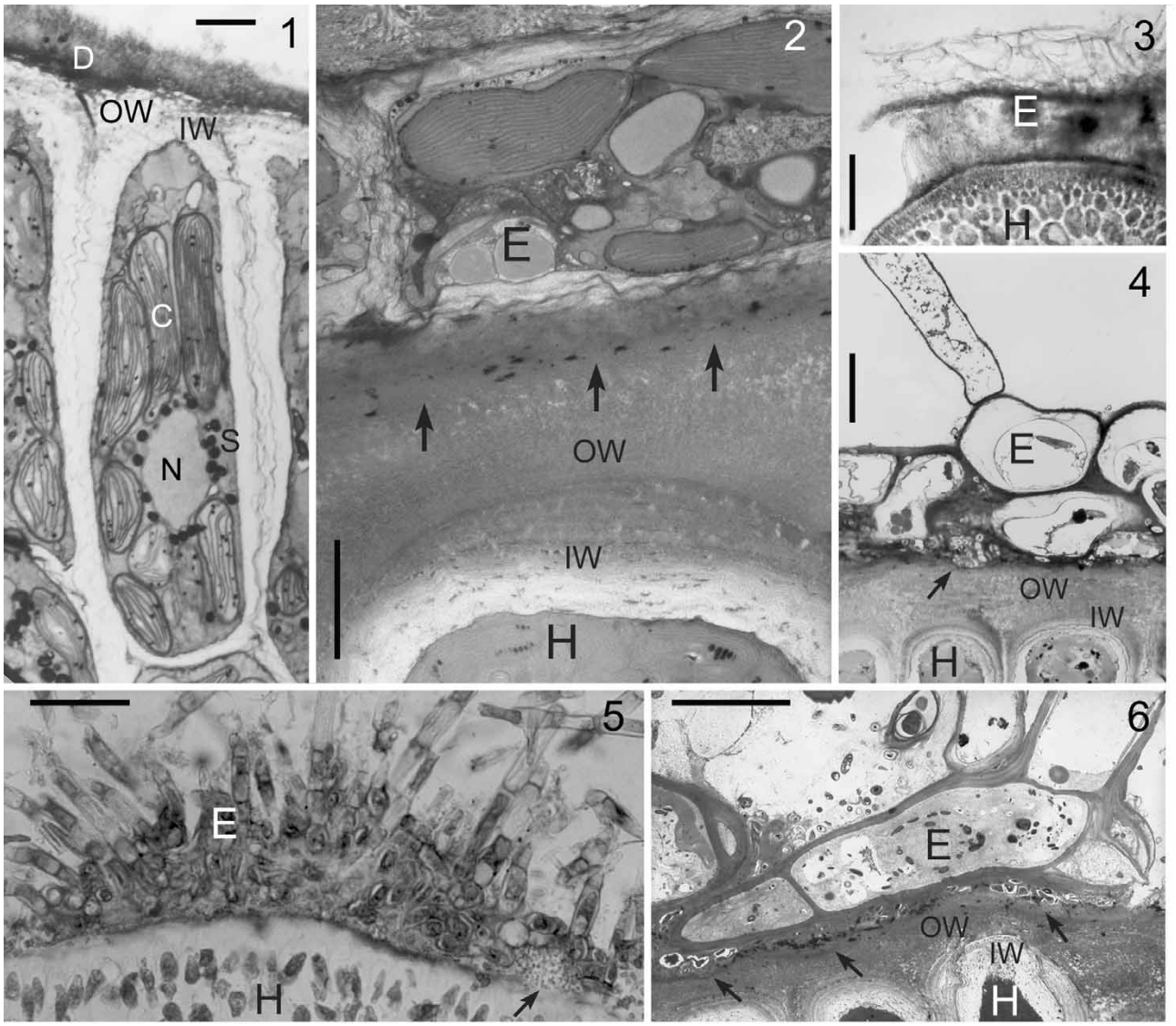
The epiphytes found on farmed *Gracilaria chilensis* included members of the Rhodophyta, Phaeophyceae, Chlorophyta and Cyanophyta (Table 1). Based on the invasiveness of the attaching structure displayed by the epiphytes and the extent of the host damage at the host/epiphyte interface, the infections were classified into five groups.

(i) Infection type I: Epiphytes weakly attached to the surface of the host and not associated with any host tissue damage. This was the case for the ectocarpoids *Hincksia mitchelliae*, *H. granulosa* and *Ectocarpus acutus*. Although the filamentous prostrate thalli of *Hincksia* spp. and *Ectocarpus* appeared to be clearly in contact with the deck-lamella of *G. chilensis*, they were never observed penetrating the host. The contact between the host and these epiphytes was so close that the interface was indistinct (Fig. 2). Furthermore, neither the wall nor the cytoplasm of the cortical host cells showed any alteration.

(ii) Infection type II: Epiphytes strongly attached to the surface of the host but not associated with any host tissue damage. Although always firmly attached to the host surface, the rhizoidal portion of *Colpomenia sinuosa* (Figs 3, 4), the basal portion of *Antithamnionella* sp., the crustose calcareus thallus of *Fosliella* sp. and the prostrate filaments of the basal portion of *Acrochaetium* sp. (Figs 5, 6), never penetrated into the host. Bacteria and electron-dense material were commonly found at the interface (Figs 4, 6). In spite of the fact that the deck-lamella of the host showed some degradation at the host–epiphyte interface, the outer and inner layers of the cell wall, as well as the cytoplasm of cortical cells, were normal. *Chondria californica* was also classified in this infection type, although bacteria at the host–epiphyte interface were more abundant than for other species described as type II infection (Fig. 7). Furthermore, a distinctive feature was a spongy aspect of the outer layer of host wall, including small and numerous electron-translucent areas (Fig. 7).

(iii) Infection type III: Epiphytes breaching the deck-lamella and penetrating the outer layer of the host wall without damaging its cortical cells. This was the case for the cyanophyte *Xenococcus* sp. (Fig. 8), which developed as colonies embedded in the outer wall layer of *G. chilensis*. Cortical cells at the infecting site were normal. A similar pattern of infection was observed in the red alga *Sahlingia subintegra* (Fig. 9).

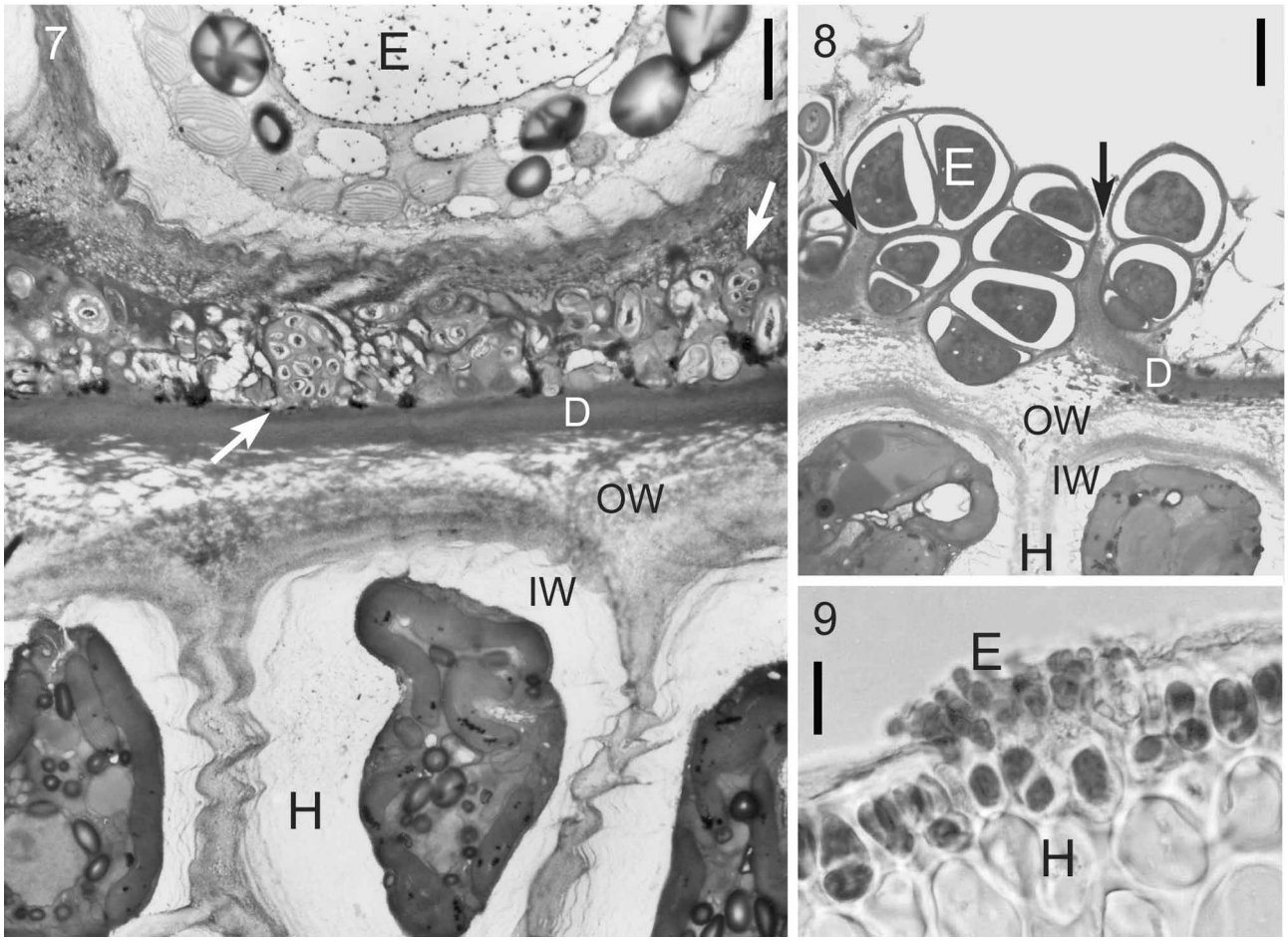
(iv) Infection type IV: Epiphytes penetrating the deck-lamella and outer layer of the host cell wall, disorganizing the cortical tissue. This category included the green alga *Ulva lactuca*, in which numerous hyaline rhizoids penetrated the host cell wall (Figs 10–11). Even though deeper penetration of the rhizoids was not recorded, the cortex of the



Figs. 1–6. Cross-sections through non-infected thalli of *Gracilaria chilensis* and thalli infected with different epiphytes. Fig. 1. Fine structure of normal cortical cells of *G. chilensis*. Fig. 2. Infection type I represented by a prostrate portion of *Ectocarpus acutus* attached to the host. The arrows indicate the host–epiphyte interface. Scale bar: 2 μm (Figs 1, 2). Figs 3–6. Infection type II. Figs 3, 4. Light and TEM micrographs of rhizoids of *Colpomenia sinuosa* attached to the host. Arrow in Fig. 4: bacteria at the interface. Scale bars: 70 μm (Fig. 3); 4 μm (Fig. 4). Figs 5, 6. Light and TEM micrographs of prostrate filaments of *Acrochaetium* sp. attached to the host. Bacteria and electron-dense material appear at the interface (arrows). Scale bars: 30 μm (Fig. 5); 4 μm (Fig. 6). Abbreviations: C: chloroplast; D: deck-lamella; E: epiphyte; H: host; IW: inner wall; N: nucleus; OW: outer wall; S: floridean starch granule.

host thickened, changing from the normal 2–3 layers of cells in non-infected thalli to 7–8 layers in the area of the cortex underneath the epiphyte (Fig. 10). Intracellular disorganization was evident in the cortical host cells closer to the epiphyte. In the apical portion of these cells, the plasmalemma adopted an irregular outline and the chloroplasts appeared deformed and disorganized (Fig. 12). As disorganization progressed, the inner host cell wall presented a stratified structure where fibrous layers alternated with compressed cell remains (Fig. 13). In fully developed infections, cortical cells of the host were grossly compressed and difficult to identify, as they became part of the inner wall (Fig. 11). The red alga *Acrosorium corallinarum* was also classified as a type IV infection, even though the rhizomatous holdfast that surrounded the host thallus did not penetrate it. At some well-defined points of the host–epiphyte interface, however, the outermost cell wall of the host appeared degraded and the cortical cells underwent hypertrophy and hyperplasia (Fig. 14).

(v) Infection type V: Epiphytes penetrating deeply into the cortex and reaching the

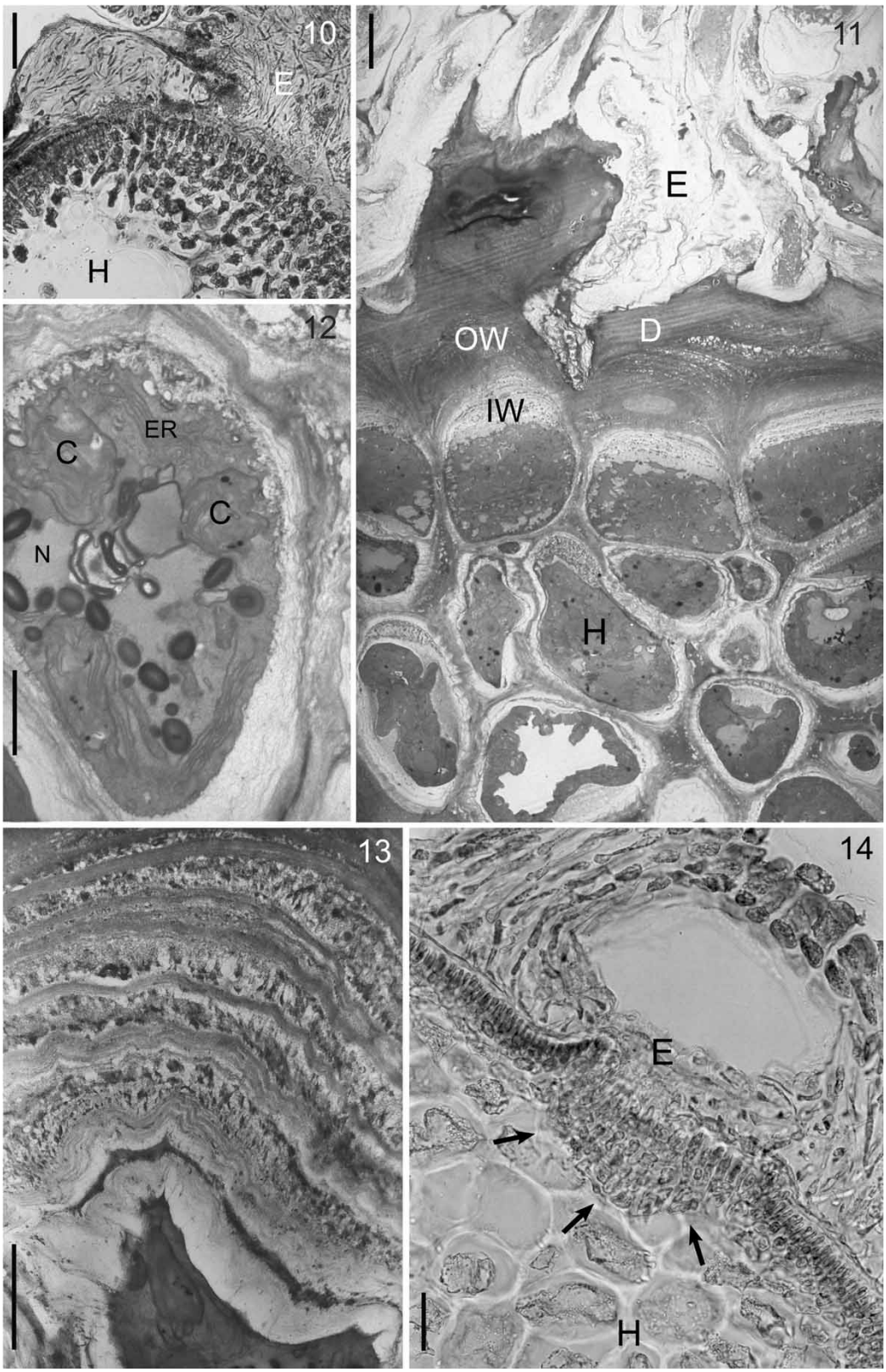


Figs. 7–9. Cross-sections through the interface of *Gracilaria chilensis* with different epiphytes. Fig. 7. Infection type II showing *Chondria californica* attached to the host. Numerous bacteria appear at the interface (arrows). Scale bar: 2 µm. Figs 8, 9. Infection type III. Fig. 8. Detail of *Xenococcus* sp. protruding from the deck-lamella (arrows) and growing into the outer wall of the host. Scale bar: 2 µm. Fig. 9. Light micrograph of thallus of *Sahlingia subintegra* attached to the host. Scale bar: 20 µm. Abbreviations: D: deck-lamella; E: epiphyte; H: host; IW: inner wall; OW: outer wall.

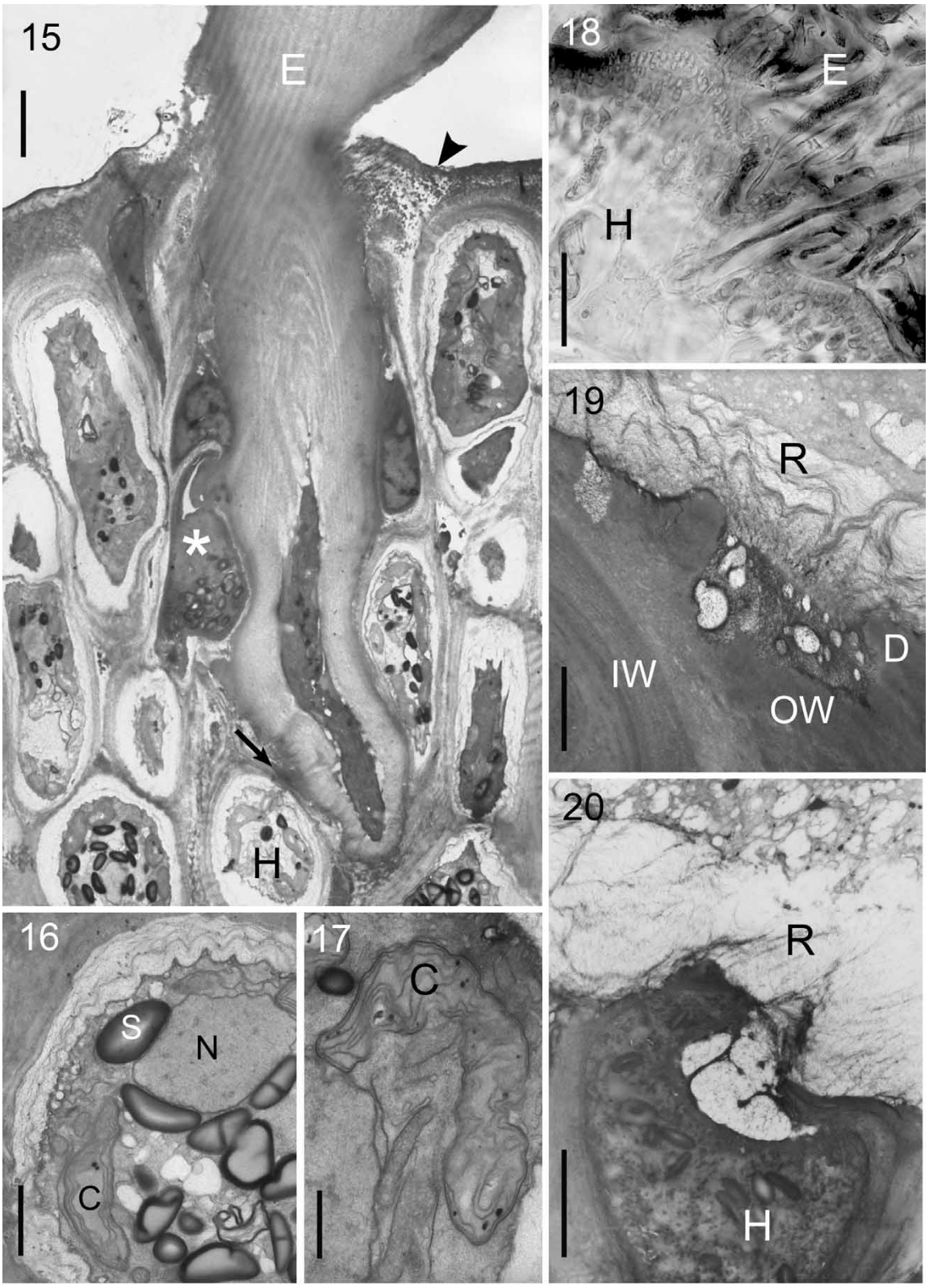
medullary tissue. This infection type is associated with destruction of the host's cells in the area directly affected by the penetrating structures and was recorded in infections by the red algae *Ceramium rubrum* and *Polysiphonia harveyi*. Rhizoids of *C. rubrum* breached the deck-lamella and penetrated deeper into the host using the intercellular cortical walls (Fig. 15) and, in some cases, reached the outer medullary tissue. Changes in the host wall around the penetrating rhizoid consisted of small and numerous electron-translucent areas (Fig. 15). Disruption of the pit plugs in cortical cells was sometimes observed (Fig. 15). Cortical cells of *G. chilensis* located around the rhizoid of *C. rubrum* presented various degrees of damage. Cells in contact with the rhizoid appeared severely compressed, and only remains of floridean starch granules were recognizable (Fig. 15). Cells of the host cortex not in direct contact with the invasive rhizoid had cell walls and plasmalemma with wavy profiles (Figs 15, 16). In these cells, chloroplasts appeared disorganized and the number of floridean starch granules increased (Figs 16, 17). *Polysiphonia harveyi* displayed a similar pattern of host invasion (Fig. 18). However, TEM revealed digested areas in the host cell wall (Fig. 19) and, occasionally, of the cellular content (Fig. 20).

Temporal variation in epiphytic load

Prevalence varied with time and with the infection type (Fig. 21). Infection types II and III were most frequently observed, with up to 80% and 90%, respectively, of the sampled thalli displaying some degree of infection. These values resulted from the high and homogeneous distribution, within the farm and throughout the year, of crustose epiphytes such as *Sahlingia subintegra*, *Fosliella* sp. and *Xenococcus* sp.



Figs. 10–14. Cross-sections through *Gracilaria chilensis* thalli with different epiphytes belonging to infection type IV. Figs 10–13. *Ulva lactuca*. Figs 10, 11. Light and TEM micrographs of interface between *Ulva* rhizoids and the host. Fig. 10. *Gracilaria* cortex cells increase from 2–3 layers to 7–8 layers in area underneath site of attachment. Scale bar: 50 μm. Fig. 11. Detail of rhizoid penetration through the host outer wall. *Gracilaria* cortical cells appear disorganized and compressed. Scale bar: 5 μm. Fig. 12. Detail of a *Gracilaria* cortical cell in progressive disorganization; the plasmalemma is irregular in profile and chloroplasts appear deformed and disorganized. Scale bar: 3 μm. Fig. 13. Apical portion of a *Gracilaria* cortical cell showing compressed cell remains alternating with fibrous layers of inner wall. Scale bar: 3 μm. Fig. 14. Light micrograph of *Acrosorium corallinarum* attached to the host. Scale bar: 50 μm. Arrows: hypertrophy and hyperplasia of *Gracilaria* cortical cells in well-defined point of the host–epiphyte interface. Abbreviations: C: chloroplast; D: deck-lamella; E: epiphyte; ER: endoplasmic reticulum; H: host; IW: inner wall; N: nucleus; OW: outer wall.



Figs. 15–20. Cross-sections through *Gracilaria chilensis* with different epiphytes belonging to infection type V. Figs 15–17. *Ceramium rubrum*. Fig. 15. Fine structure of *Ceramium* rhizoid penetrating intercellularly into host. *Gracilaria* wall around *Ceramium* rhizoid penetration has numerous small electron-translucent areas (arrowhead); host cells adjacent to rhizoid are reduced in size and show compacted cytoplasm in which only starch granules can be recognized (asterisk). Arrow: disrupted pit plug. Scale bar: 5 µm. Fig. 16. Portion of host cortical cell with numerous floridean starch granules, and wall and plasmalemma with wavy profiles. Scale bar: 2 µm. Fig. 17. Detail of partially disorganized chloroplast from cortical cell of *Gracilaria*. Scale bar: 1 µm. Figs 18–20. *Polysiphonia harveyi*. Fig. 18. Light micrograph of a rhizoid reaching the medullary tissue. Scale bar: 70 µm. Fig. 19. Detail of portion of *Polysiphonia* rhizoid and *Gracilaria* showing digestion of host wall. Scale bar: 2 µm. Fig. 20. Detail of *Gracilaria* cortical cell with contents partially digested. Scale bar: 2 µm. Abbreviations: C: chloroplast; D: deck-lamella; E: epiphyte; H: host; IW: inner wall; N: nucleus; OW: outer wall; R: rhizoid; S: floridean starch granule.

The lowest frequency of these two infection types (0–10%) was observed in April–June, a situation that was associated with a dense red tide that affected the entire area from February to April in both 2002 and 2003. Infection type V showed a clear seasonal pattern, with a high prevalence during winter and a low prevalence during summer and early autumn. On the other hand, prevalence of infection types I and IV was generally low, with a maximum from June to August 2002, but absent or very low during the remaining sampling period. The severity of epiphyte infection followed a similar pattern to infection prevalence (Fig. 22). Infection types II and III were the most abundant, mainly due to the presence of crustose epiphytes. Crustose epiphyte cover could reach 80% of the host thalli, while the cover of the other infection types was always lower (less than 30%, data not shown).

Discussion

This study has demonstrated that, under normal farming conditions, *Gracilaria chilensis* can carry a wide range of algal species as epiphytes, and that these species display diverse types of anatomical relationships with their host. Characterization of the epiphyte–host interface allowed a classification of the different epiphytes into one of five types of anatomical interactions, ranging from those where the epiphytes were restricted to the surface of the host (Types I and II), to associations in which the epiphytes penetrated deeply into the host tissues, as in Type V infection. In general, higher levels of host cell damage were associated with the more invasive types of infection. This classification should be considered complementary to that into holo-epiphytes and amphi-epiphytes proposed by Linskens (1963), which is also based on the level of host penetration.

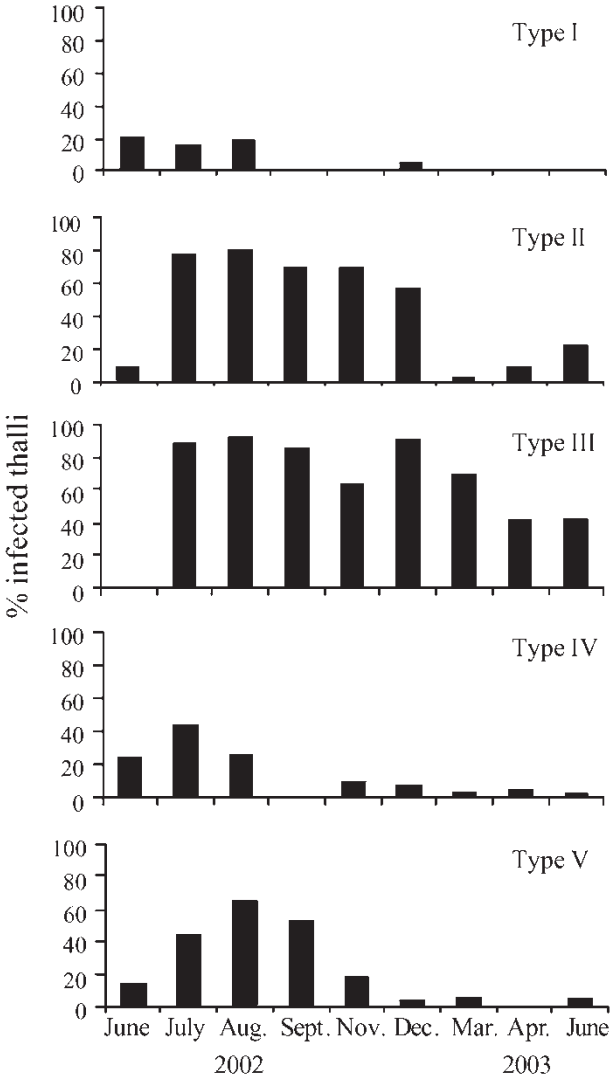


Fig. 21. Temporal variation of prevalence for epiphytes of the five infection types affecting *Gracilaria chilensis* in the Caldera farm.

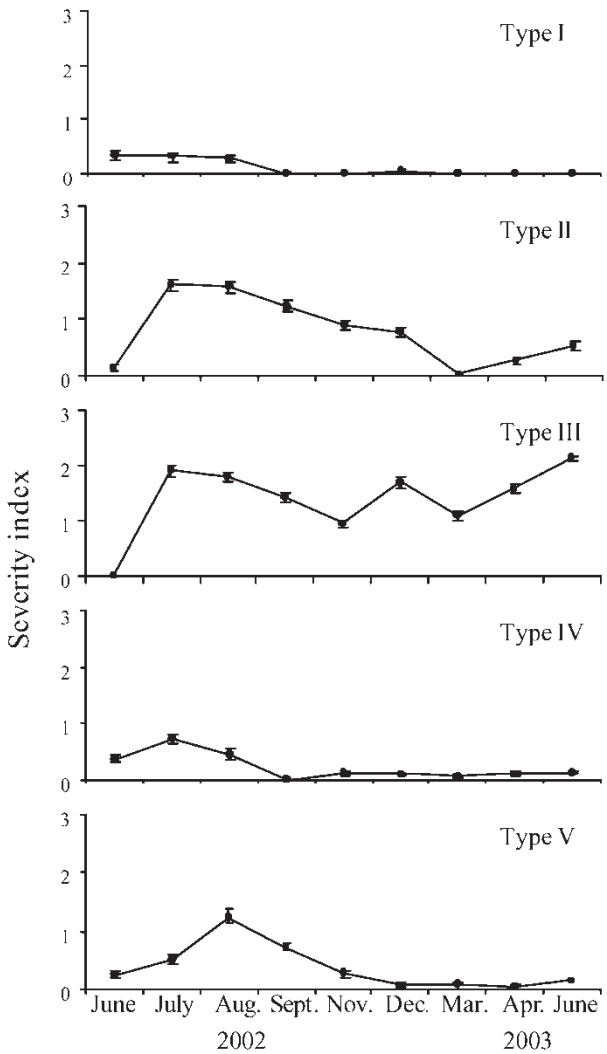


Fig 22. Temporal. variation of infection severity for epiphytes of the five infection types affecting *Gracilaria chilensis* in the Caldera farm.

In spite of the high consistency of our observations, wide generalizations about infection types and their relationship with the type and degree of host damage should be made with caution. For example, infection type I was represented by ectocarpoid filaments with a weak attachment, even though the ultrastructural observations showed a close contact with the thallus of *G. chilensis*. On the other hand, some related species, such as *Ectocarpus elachistaeformis*, are known to have a penetrating basal portion (Taylor, 1985). In this context, Ducker and Knox (1984) indicated that most members of the Ectocarpales seem to have no specific host requirements, a feature that supports the hypothesis of a non-specific host–epiphyte relationship based on a labile anatomical dependence. What seems clear from the above is that, if a degree of host-specificity is involved in infections by ectocarpoids, it is unlikely to be related to the extent of host penetration.

Infection types II and III could represent a transitional type of host–epiphyte anatomical relationship. In both types, the epiphytes are strongly attached to the host and, regardless of the size of the epiphytes, we did not observe them penetrating host tissues. Our observations indicated that the smallest species (i.e. *Sahlingia* and *Xenococcus*) could breach the outer cell wall of the host, but never reached the cytoplasm. In spite of their small size and undetectable anatomical damage to the host, *Sahlingia* and *Xenococcus* were by far the most common and abundant epiphytes in the *Gracilaria* farm. In this context, there is certainly a need for better understanding of these small cryptic epiphytes and their effects on their hosts.

This study also suggests that different epiphytes display different mechanisms to penetrate the host thallus. The most superficial host penetration, observed in type III infections, involved an outward deformation of the outer cell walls of the host around the penetrating cells, which seems to indicate a primarily mechanical mechanism of infection. Similar inward bending has been reported during mechanical penetration of *Chondrus crispus* by its non-specific green algal endophyte *Acrochaete heteroclada* (Correa, 1990). Type IV infections involved a deeper invasion of the host, particularly by *Ulva lactuca*. For this infection type, it is not possible to identify only one mechanism as responsible for penetrating the host. For example, the presence of hyaline filaments of *U. lactuca* penetrating the outer cell wall of the host accompanied by compression of host cortical cells strongly suggests the occurrence of a combined mechanical and enzymatic disruption of the *Gracilaria* wall at the site of infection. A similar pattern of infection was suggested for *Gracilaria cornea* and *G. tikvahiae* epiphytized by *U. lactuca* (Dawes *et al*., 2000). Characteristic changes occurred in the host cortex during infections by both *U. lactuca* and *Acrosorium corallinarum*. In spite of the fact that only the former epiphyte clearly breaches the outer cell wall of the host, both species triggered a thickening of the cortex, characterized mainly by an increased number and size of the host cells. These responses are not uncommon in seaweeds, and have been reported in algal hosts infected by heterotrophic bacteria (Apt & Gibor, 1989), cyanobacteria (Correa *et al*., 1993; Faugeron *et al*., 2000), brown endophytes (Apt, 1988; Peters, 1991) and parasitic red algae (Goff, 1982). Similar responses may be the result of different stimuli (e.g. growth factors from bacteria, and mechanical stress imposed by non-specific epiphytes). Direct penetration through intact plant surfaces is probably the most common type of penetration used by fungi and parasitic vascular plants (Agrios, 1997). In fungi, three mechanisms have been identified to be responsible for cuticle penetration: (i) mechanical, (ii) enzymatic and (iii) a combination of both mechanisms (Cooper, 1981; Bailey *et al*., 1992).

Infection type V included red algal species that penetrated between the cells and deep into the host. Even though rhizoids of *Ceramium rubrum* and *Polysiphonia harveyi* were found growing in close contact with host cells, intercellular connections, like those reported for parasitic red algae and their hosts (Goff & Coleman, 1984, 1985; Goff & Zuccarello, 1994), were not detected. Rhizoids of *C. rubrum* and *P. harveyi* showed a similar pattern of penetration into *G. chilensis*. In both species, the outer cell wall of the host partially wrapped around the intrusive rhizoids, which were constricted at this point. Ultrastructural features were similar to those described for the endophytic alga *Acrochaete operculata* growing into *Chondrus crispus* (Correa & McLachlan, 1994), for the parasites *Laminariocolax aecidioides* and *Laminarionema elsbetiae* on their host *Laminaria saccharina* (Heesch & Peters, 1999) and for the penetration of terrestrial plants by pathogenic fungi known to produce enzymes that digest the host tissue (Cooper, 1981; Agrios, 1997; Wharton *et al*., 2001). Afterwards, the penetrating structure of *Ceramium rubrum* seemed to advance into the host mainly mechanically; this is supported by the presence of host cells with undulated cell walls and plasmalemma invaginations when they are in the vicinity of the penetrating rhizoid, and by the absence of digested areas. Mechanical penetration of the host, usually complementary to enzymatic penetration of the cuticle, has been reported in many terrestrial associations

(Mendgen *et al.*, 1996). *Polysiphonia harveyi* appears to be similar because, in addition to host cellular compression, partial digestion of the cell wall and the cytoplasm of the host in contact to the rhizoid was observed. Similar cell wall digestion was described in the walls of *Chondrus crispus* infected by the endophyte *Acrochaete operculata* (Correa & McLachlan, 1994) although, here, the endophyte penetration did not produce cell wall compression. Enzymes would hydrolyse the intercellular polysaccharide matrices of red algal hosts, as suggested for various algal associations (Rawlence, 1972; Rawlence & Taylor, 1972; Goff & Cole, 1976; Goff, 1982; González & Goff, 1989). Enzymatic activity has been clearly demonstrated in parasitic fungi developing in angiosperm tissues (Calonge *et al.*, 1969; McKeen *et al.*, 1969; Agrios, 1997). In *Polysiphonia lanosa*, Rawlence (1972) suggested a chemical rather than a mechanical penetration of rhizoids into *Ascophyllum nodosum*.

From an aquaculture perspective, there are several aspects that need to be taken into account when analysing the problem of epiphytism. One is that, in normal farming conditions, the multi-species epiphytic assemblage includes components that may persist throughout the year and others that fluctuate seasonally. The types of epiphyte prevailing will determine the degree of negative effects on the farmed host (Buschmann & Gómez, 1993; Pizarro & Santelices, 1993; Buschmann *et al.*, 2001). Whereas the main pests for *Gracilaria* farming in the south of Chile are *Polysiphonia*-type epiphytes and tube-forming herbivorous polychaetes, crustacean grazers and fouling mussels (Retamales & Buschmann, 1996; Buschmann *et al.*, 1997*a*, *b*; Buschmann *et al.*, 2001), the studied farm in Caldera, in the north of Chile, was mainly affected by small sized epiphytes. However, epiphytism does not only affect the host tissues, but it can also significantly depress the productivity of *Gracilaria* through reduction in irradiance, depletion of nutrients and the additional weight (Buschmann & Gómez, 1993). These aspects, which were not considered in the present study, are important when the effects of epiphytes on the survival and growth of cultivated seaweeds have to be evaluated. Furthermore, the large-scale effects on the productivity of the *Gracilaria* stands remain to be specifically assessed, and separated from other unforeseen events, such as red and brown tides and blooms of the invasive *Codium fragile* (S. Faugeron, personal observation). The new way of classifying and characterizing epiphyte communities proposed in this study may be a valuable tool to help define strategies to avoid pests and improve productivity in *Gracilaria chilensis* farms.

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