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The Occurrence of *Laminarionema elsbetiae* (Phaeophyceae) on *Rhodymenia pseudopalmata* (Rhodophyta) from the Patagonian Coasts of Argentina: Characteristics of the Relationship in Natural and Experimental Infections, and Morphology of the Epi-endophyte in Unialgal Free Cultures

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The occurrence of *Laminarionema elsbetiae* (Ectocarpaceae, Phaeophyceae), as epi-endophyte of *Rhodymenia pseudopalmata* (Rhodymeniales, Rhodophyta), described from Santa Isabel, Rawson, Argentina. *L. elsbetiae* grows in the host tissues forming epi-endophytic relationship in the epidermal, cortical and medullar layers. Epiphytic thalli of *L. elsbetiae* were unbranched filaments emerging from hostís surface. Reproductive structures of *L. elsbetiae* on the host were absent. On the contrary, free cultured individuals formed different reproductive structures. Macrozoosporangia containing a single large motile zoospore originated from vegetative cells, they were conical to cylindrical in shape, 30-50 μm in length and 18-20 μm in wide. Uniseriate plurilocular zoosporangia were cylindrical shape, 40 μm in length and 10-13 μm in wide. Sexual fusion was not seen. In mixed cultures of *L. elsbetiae* with *R. pseudopalmata* fronds, *L. elsbetiae* infected the host, grew as in natural host and, formed macrosporangia between host subcortical cells. Gametophytes of *L. elsbetiae* were filaments with diffuse growth, branched with a branch pattern alternate or opposite. Gametangia were plurilocular, uni or biseriate and lateral. When mature they contained 2 to 6 isogametes. The presence *L. elsbetiae* on *R. pseudopalmata* could be defined as an epi-endophytic relationship. The percentage of infection of *R. pseudopalmata* thalli by *L. elsbetiae* was 34%. A 25% of the infected thalli presented a low, non-symptomatic level infection, whereas a 62% and a 13% of them exhibited respectively moderate and high indexes of infection.

Key Words: Ectocarpales, epi-endophytes, infection, *Laminarionema elsbetiae*, Patagonian coasts, *Rhodymenia pseudopalmata*

INTRODUCTION

The genus *Rhodymenia* Greville includes species with traditional used in human nutrition in Ireland and Brittany and more recently marketed as a health food (Le Gall *et al.* 2004). *Rhodymenia palmata* (L.) Kuntze contains as much as 35% proteins and is a rich source of vitamins and eicosapentanoic acid (Mishra *et al.* 1993) and was identified as one of the three red algal species with the best potential for seaweed cultivation in the northeastern United States and Canada (Cheney 1999). Due to its high protein content (Morgan *et al.* 1980; Fleurence 1999), *Rhodymenia* sp. was considered as a good food source for abalone aquaculture (Evans and Langdon 2000; Rosen *et al.* 2000). *Rhodymenia pseudopalmata* (Lamouroux) Silva is an important species of tidal and subtidal algal communities in the southern coasts of Argentina (Mendoza and Nizovoy 2000). Its fronds are flattened, fan-shaped, rather stiff, light brownish – red fronds, with long or short stipes arising from a discoid base. Fronds are repeatedly dichotomously lobed, with axils wide, apices rounded and margins smooth.

This study is a part of a more general study in which we are focused on the identification of the most common epiphytes infecting the host *R. pseudopalmata* on southern coasts of Argentina. We found the occurrence of *Laminarionema elsbetiae* Kawai & Tokuyama. *L. elsbetiae* was first described on *Laminaria japonica* Areschoug from Japan (Kawai and Tokuyama 1995) and it is known in

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Europe only from Helgoland (Peters and Ellertsdóttir 1996).

Algal species growing on or within other algae has been widely reported (Goff 1982; Ducker and Knox 1984; Correa *et al.* 1988, 1993; Correa 1990; Correa and McLachlan 1991, 1992, 1994). Are goals of the present paper to assess the kind the symbiosis interactions and the relative abundance including the estimation of the severity index of infection of *L. elsbetiae* on *R. pseudopalmata*. It was also a goal to study the biology of *L. elsbetiae* under both nature and culture conditions.

MATERIALS AND METHODS

Sampling

Rhodymenia pseudopalmata fronds were obtained from subtidal populations from the coast of Santa Isabel, (43°18'S-65°06'W) in the province of Chubut, Argentina during December, 2004. A collection of 30 randomly selected fronds was used for the present research.

Estimation of the severity index of infection

Host thalli of *R. pseudopalmata* (Fig. 1) were divided in apical, intermediate and basal sections for their examination. Size, presence and position of individuals of *L. elsbetiae* and severity degree of infection were registered for each frond. In order to evaluate the severity degree of infection, a qualitative scale was used (Peters and Schaffelke 1996). This scale resulted from the visual categorization of a dissection observed by light microscopy. Prevalence (i.e., percentage of thalli that were infected) and severity of infection (i.e., mean abundance of *L. elsbetiae* on each host thallus) were then estimated. A 'severity index' was based on a semi-quantitative estimation of *L. elsbetiae* 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. The severity degree of infection was categorized as low when the percentage of host thalli colonized by *L. elsbetiae* ranged from 1% to 10% (i.e., no visible signs of endophytic infection were observed). The severity degree of infection was categorized as moderate when the percentage of colonized thalli varied from 11% to 70% (i.e., moderate alterations, such as brown spots on the lamina, were observed). Finally, the severity degree of infection was categorized as high in those cases in which thalli exhibited a colonized area percentage higher than 71% (i.e., strongly invaded thalli were observed). The distinction between the categories was arbitrary. Only those thalli under the categories 'moderate' and 'high' were considered diseased.

Isolation of *Laminarionema elsbetiae* and unialgal cultures

After being collected, fronds were kept on ice, retained in labeled plastic bags until they were examined in the laboratory, usually within 5 h after 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. A 2-min sonication was subsequently applied to 5 mm x 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.

L. elsbetiae 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 an illumination regime of 12 : 12 h light-dark (LD), 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 (Across Organics, Gell, Belgium) dissolved in water was added to avoid diatom contamination (Lewin 1966; Christensen 1982).

Experimental infections

Infections of *R. pseudopalmata* by selected isolates of *L. elsbetiae* initially established from swarmers were experimentally carried out. Eight to ten 1-1.5 cm long fragments of fronds of the host were placed into plastic Petri dishes. Two replicates of each isolate were incubated under laboratory conditions during a period from 2 to 3 weeks.

Morphological studies and semi-thin sections

Cytomorphometry was carried out using a stereoscopic microscope Wild-Herbrugg and an inverted microscope Nikon Eclipse TE 300, with anoptral phase contrast and differential interference contras) and with an incorporated camera Nikon FDX 35. Either the presence or absence of epi-endophyte filaments was determined under light microscopy in semi-thin sections of thalli of

R. pseudopalmata. In order to obtain semithin sections, thalli were fixed in 2.5% glutaraldehyde (Fisher Bioreagents, Fair Lawn, Ny, USA) in seawater for 2 hr at 4°C, and postfixed in 1% (OsO_4) Osmium tetroxide (Across Organics, Gell, Belgium) in sea water for 2 hr at 4°C. The material was dehydrated in a graded acetone series (Merck, Darmstadt, Germany) and embedded in Spurr's low viscosity resin (SPi Supplies Divisi, of Strutur pProe, Inc., West Chester, PA, USA). Sections were obtained with glass knives on a Reicher Ultracut OM U2 ultramicrotome (Reicher, Vienna, Austria). The resin was removed using a metallic sodium, benzene and methyl alcohol solution (Hayat 1986). Sections were stained with a combination of colorants, namely haematoxiline (Biopur, Rosario, Argentina) - malachite green (Biopack, Buenos Aires, Argentina) - basic fucsine (Britania, Buenos Aires, Argentina) (1 : 1 : 1) (Berkowitz *et al.* 1968).

Chromosome counts

Chromosome counts were made using unialgal cultures of *L. elsbetiae*. Thalli were fixed either in 1 : 3 mixture glacial acetic acid (Cicarelli Laboratorios, San Lorenzo, Argentina) /absolute ethanol (Cicarelli Laboratorios) or in 6 : 3 : 1 mixture formaldehyde (Cicarelli Laboratorios) - absolute ethanol (Cicarelli Laboratorios) - glacial acetic acid (Cicarelli Laboratorios) at 5°C during a period of 2 to 24 h. Postfixation was carried out with 70% ethylic alcohol (Cicarelli Laboratorios). The material was subsequently hydrolyzed for 30 min in 1 N hydrochloric acid (HCL) (Cicarelli Laboratorios) at room temperature, stained with Schiff stain in darkness for 2 h (Johansen 1940), bleached during 20 min in a 1 : 3 : 3 mixture of sodium metasulphite (Cicarelli Laboratorios): 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 haematoxylin (Biopur) with added iron acetate (Biopur) (Núñez 1968).

Scanning electron microscopy

L. elsbetiae filaments were fixed in 2.5% glutaraldehide (Fisher Bioreagents)-seawater at 5°C in cacodilate buffer (Biopur) 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 during 1 h, coated with gold, and observed with a Jeol 35 CF scanning electron microscope (Jeol, Tokyo, Japan).

Table 1. Prevalence, severity index and localization of infection of *R. pseudopalmata* thalli by *L. elsbetiae*

Infection	% thalli	Region thalli	% thalli
Low	25	Basal	41
Moderate	62	Media	21
High	13	Apical	38

RESULTS

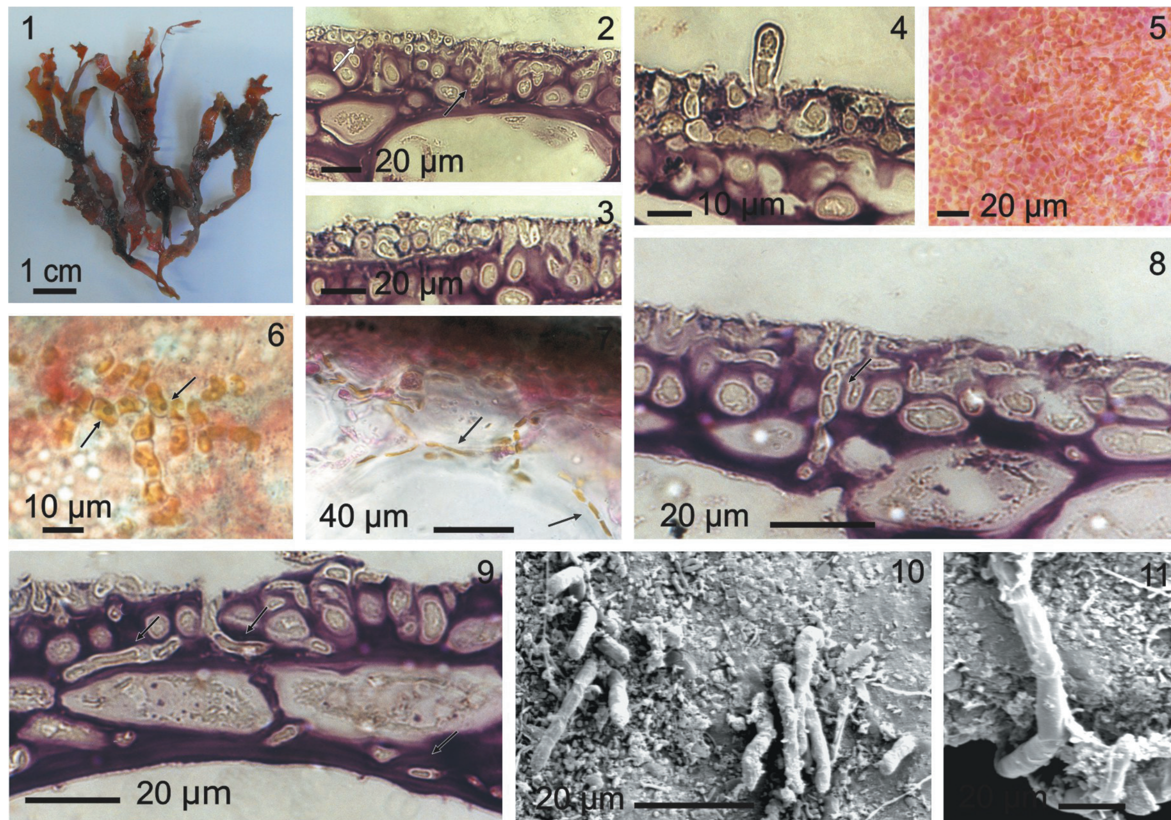
Prevalence and severity index infections

The percentage of infection of *R. pseudopalmata* thalli by *L. elsbetiae* was 34%. A 25% of the infected thalli presented a low, non-symptomatic level infection, whereas a 62% and a 13% of them exhibited respectively moderate and high indexes of infection and presented dark patches on the host's surface as a visible symptom of the presence of dense bushes of *L. elsbetiae* filaments (Table 1).

A 41% of the infected fronds R. pseudopalmata presented *L. elsbetiae* individuals in the basal region, whereas a 21% and a 38% of them exhibited them in the median and the apical regions respectively (Table 1).

Morphology of *Laminarionema elsbetiae* individuals growing on *Rhodymenia pseudopalmata* thalli both in nature and after experimental infections

Vegetative filaments of *L. elsbetiae* that infected *R. pseudopalmata* thalli were uniseriate. Those growing on the cuticle (Fig. 2) were postrate, regularly arranged with thick cellular walls and in some sectors observed in 2 or 3 levels (Fig. 3). Also erect filaments were observed formed by few cells (Fig. 4). Those developing amid epidermic cells of the host (Figs 5, 6) also formed a fine net flanked by both cortical and medullar cells (Fig. 7). In both areas, cortical and medullar, vegetative filaments did not penetrate into the host' s cells. Filaments were irregularly branched and showed apical growth (Fig. 8). They were formed by cylindrical cells (Fig. 7) 50-60 μm in long and 7-13 μm in diameter. These cells contained 1 or 2 discoid or irregularly elongated chloroplasts with a single pyrenoid. Filaments invaded the host tissues penetrating through intercellular spaces of the cortical region (Fig. 9) up to the medulla (Fig. 8). Reproductive structures of *L. elsbetiae* on the host were absent. Under scanning electron microscope the cells of *L. elsbetiae* exhibited delicate surface ornamentation on the walls (Figs 10, 11).



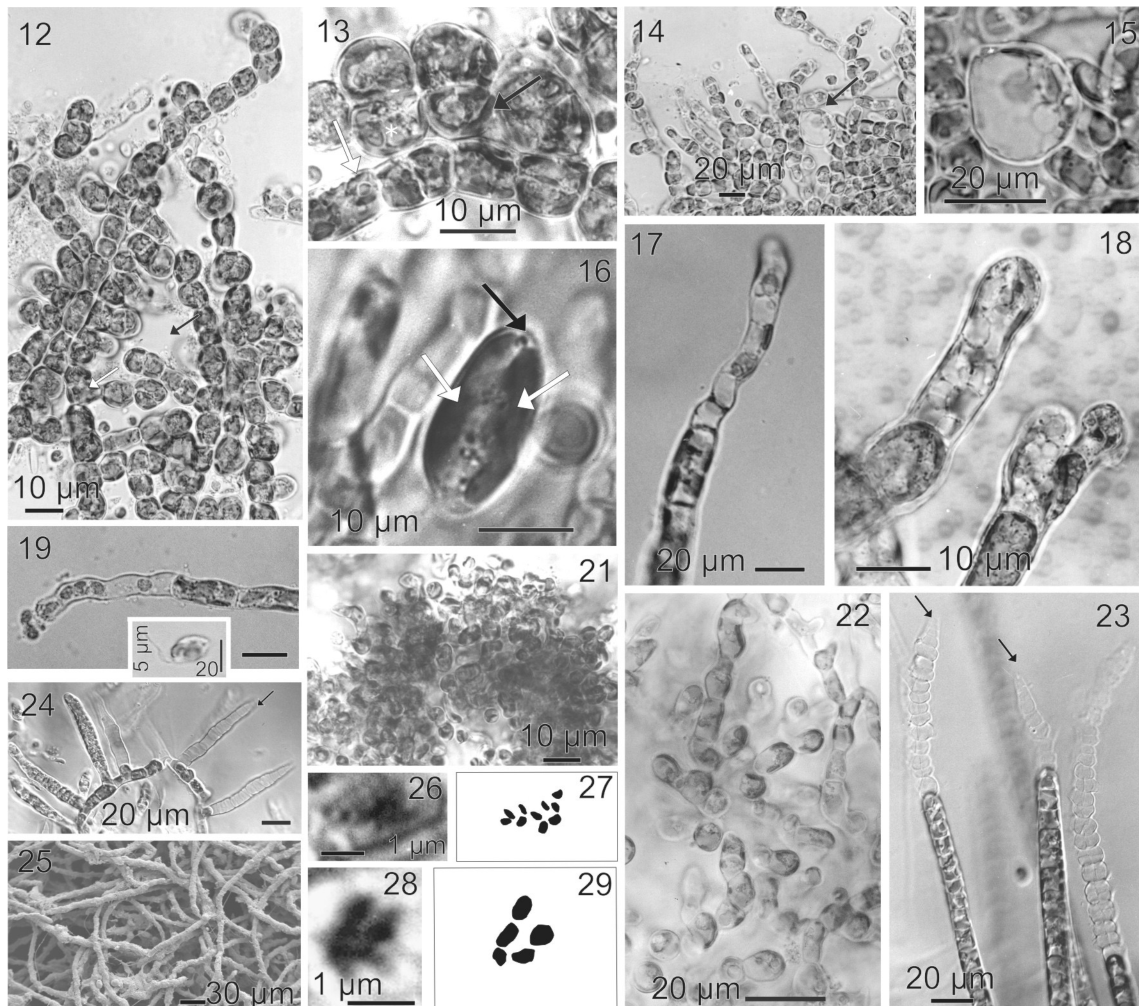
Figs 1-11. Fig. 1. *Rhodymenia pseudopalmata*. Thallus recollected from the nature. **Fig. 2.** Semi-thin section of *R. pseudopalmata* thallus, showing both epiphytic monostromatic (arrow white) and endophytic (arrow black) *L. elsbetiae* filaments. **Fig. 3.** Semi-thin section of *R. pseudopalmata* thallus, with 2 or 3 layers of *L. elsbetiae* cells on your surface. **Fig. 4.** Epiphytic *L. elsbetiae* thallus with erect filaments formed with few cells. **Fig. 5.** *Laminarionema elsbetiae* on host thalli. **Fig. 6.** Detail of *L. elsbetia*e thalli. Epiphytic filaments showing the pyrenoids (arrows). **Fig. 7.** Transversal section of *R. pseudopalmata* showing endophytic filament of *L. elsbetiae* between both cortical and medullar regions (arrows). **Fig. 8.** Endophytic filaments *L. elsbetiae* irregularly branched (arrow), penetrating directly crossing cortical region to medullar area. **Fig. 9.** Endophytic filament *L. elsbetiae*, formed by short, curved filaments formed by two cells (arrows). **Fig. 10.** Photomicrograph under scanning electron microscopy (SEM) showing shorten filament epiphytic *L. elsbetiae* on *R. pseudopalmata's* surface. **Fig. 11.** Detail of wall cell of epiphytic filament *L. elsbetiae* under SEM.

Development and morphology of free thalli of Laminarionema elsbetiae in culture conditions

After a week of growth, sporophytes exhibited a postrate basal system and also a less developed erect system (Fig. 12). Both system presented diffuse growth.

Cells of the first system were ovoid with a length of 10-20 μm (12.6 ± 2.5 μm, n = 30) and a wide of 7-13 μm (10 ± 1.5 μm, n = 30). These cells presented various parietal, discoid or ribbon-shaped plastids with pyrenoids (Fig. 13). The second system were formed by cylindrical cells, 5.4-21 μm (11 ± 33 μm) (n = 30) in length, 7.7-13 μm (8.7 ± 1.4 μm, n = 30) in wide. Macrozoosporangia originated from vegetative cells of the postrate system were observed (Fig. 14). They were both intercalar and terminal, presented an ovoid shape and a length of 30-50 μm and a wide of 18-20 μm (Fig. 15). In each macrosporangium a single macrozoospore was formed (Fig. 16), which was released through an apical aperture. Macrozoospores presented positive phototaxis. They were pyriform, 18-20 μm in length and 10-13 μm in wide and exhibited two plastids and stigma (Fig. 16). Flagella were of equal lengths and were laterally inserted adjacent to the stigma.

In most of the thalli uniseriate plurilocular zoosporangia were observed (Fig. 17). They were sessile with ovoid to cylindrical shape and they presented ca. 40 μm in length and 10-13 μm in wide. These zoosporangia were formed in terminal vegetative cells. In them unispores or microzoospores were observed (Fig. 18). The unispores were released from loci through an apical pore (Fig. 19) they presented an ovoid chloroplast with a stigma in its anterior end (Fig. 20). They were biflagellate and 4-8 μm



Figs 12-29. Fig. 12. Sporophytic filaments *L. elsbetiae* constituted by both basal system with postrate cells and erect system. **Fig. 13.** Detail de cells showing plastid (arrow black) and pyrenoid (arrow white). **Fig. 14.** *L. elsbetiae* thalli arising macrosporangium (arrow) in its postrate system. **Fig. 15.** Detail of macrosporangium. **Fig. 16.** Single macroid. **Fig. 17.** Uniseriate zoosporangia. **Fig. 18.** Unilocular sporangium showin unispores inside. **Fig. 19.** Release of unispores through an apical pore. **Fig. 20.** Detail of biflagellated unispore. **Fig. 21.** *L. elsbetiae* gametophytic thalli formed by microscopic branched filaments. **Fig. 22.** Detail of cells of gametophytic thalli. **Fig. 23.** *L. elsbetiae* plurilocular biseriate gametangia of lateral position. **Fig. 24.** *L. elsbetiae* plurilocular uniseriate gametangia of lateral position, with apical pore. **Fig. 25.** Photomicrograph showing gametophytic thalli under SEM. **Fig. 26.** Metaphasic plate with 10 chromosomes. **Fig. 27.** Representation of Fig. 26. **Fig. 28.** Metaphasic plate with 5 chromosomes. **Fig. 29.** Representation of Fig. 28.

in wide. More of 18 microspores per every unilocular sporangium were formed (Fig. 18). These microspores were smaller than macrospores and presented a faster motion. Macrosporangia and uniseriate plurilocular zoosporangia were formed in different thalli.

Gametophytes of *L. elsbetiae* were filaments with diffuse grow, branched with a branch pattern alternate or opposite (Figs 21, 25). Basal cells were 8-26 μm (11.7 ± 4 μm, n = 50) in length and 3.7-11 μm (9.6 ± 3.7 μm, n = 50) μm in wide. Distal cells were 3.7-43.6 μm (15.2 ± 3.5 μm, n = 50) in length and 3.7-25.9 μm (8.9 ± 1.7 μm, n = 50) in wide (Fig. 22). Both type of cells had two or three plastids with pyrenoids (Fig. 22).

Gametangia were plurilocular, uni or biseriate and lateral. They were 31.4 μm (15-46 μm) in length and 12.9 μm (7.7-25 μm) in wide (Figs 23, 24). Gametangia loci were 5-6 μm in length and 7-8 μm in wide. When mature they contained 2 to 6 isogametes with a size 4-6 x 4-6 μm. Gametes presented 1 or 2 plastids and stigma. They were released from a pore located in the apical end of gametangia (Fig. 23). Gametes showed positive phototaxis.

Chromosome counts

Chromosomes were ovoid. *L. elsbetiae's* sporophytes presented a number 2n = 10 (Figs 26, 27) and gametophytes n = 5 (Figs 28, 29).

DISCUSSION

Kawai and Tokuyama (1995) considered *L. elsbetiae* a representative of the order Ectocarpales because of its morphology and plastid type. Peters and Ellertsdóttir's (1996) observations of sexuality, a slightly heteromorphic life history and isogamy are congruent with this criterion.

This work represents the first report of *L. elsbetiae* in America, since this species only had been reported for Japan (Kawai and Tokuyama 1995) and Germany (Peters and Ellerrsdóttir 1996). In 1995, Kawai and Tokuyama described *L. elsbetiae* as a new endophyte kelp of northern Japan. In Europe *L. elsbetiae* was detected infecting *Laminaria saccharina* (Linnaeus) Lamouroux with high prevalences (Ellertsdóttir and Peters 1997). In Muroran, Japan, *L. elsbetiae* is common in adult Laminaria sporophytes, although they were not found in Costaria Greville and Undaria Suringar, which also grow in the same locality (Kawai and Tokuyama 1995).

Kawai and Tokuyama (1995) observed under culture condition that *L. elsbetiae* also infected sporophytes of *Saccorhiza* Bachelot de la Pylaie, which is less related to *Laminaria* than *Costaria* and *Undaria*, and suggested that probably *L. elsbetiae* did not show high host specificity and was potentially able to infect many other Laminarialean species in nature. The presence of *L. elsbetiae* on *R. pseudopalmata* confirmed their predictions of low specificity of this species, not only by its capacity of infection to Laminarialean species, but also to seaweeds of different algal classes. Nevertheless here, in Argentina *L. elsbetiae* was never observed on *Undaria pinnatifida* (Harvey) Suringar sporophytes whose fronds, in turn, were infected by another brown endophyte, *Laminariocolax aecidioides* (Rosenvinge) Peters (Gauna *et al.* 2009).

L. elsbetiae infection is widespread throughout different populations of *R. pseudopalmata* from Argentina (Gauna 2005). In this particular Patagonian population, the infection levels observed did not reach the high intensities reported by Peters and Ellertsdóttir (1996) in populations *L. saccharina*, with prevalences there of ca. 93% in the 53% of the population. In fact, here only a 13% of the *R. pseudopalmata* population presented the highest level of prevalence.

We found here differences with the representatives of *L. elsbetiae* of Japan (Kawai and Tokuyama 1995) and Germany (Peters and Ellertsdóttir 1996). Given the great plasticity of members of Ectocarpales (Müller 1967) we considered them inside a normal intraspecific variability. Vegetative cells of sporophytes of *L. elsbetiae* isolated from *L. saccharina* (Peters and Ellertsdóttir 1996) were a little shorter than those of the Patagonian isolates and their cellular features are in agreement with those described by Kawai and Tokuyama's (1995) in Japan. On the contrary, when comparing reproductive structures we found conspicuous differences, i.e., unilocular sporangia of *L. elsbetiae* growing on *R. pseudopalmata* were decidedly smaller than those of *L. elsbetiae* from Helgoland (Peters and Ellertsdóttir 1996) and Japan (Kawai and Toyuyama 1995). In addition, in the sporangia from the Argentinian sporophytes not more than 18 microspores were observed, while in sporophytes from *L. saccharina* (Peters and Ellertsdóttir 1996) zoospores are more than 68. And finally, macrosporangia of thalli isolated from *R. pseudopalmata* are smaller than those observed by Kawai and Tokuyama (1995) and consequently macrozoids of the Patagonian thalli were shorter than those observed in Japan. With relation to the cellular dimensions of gametophytes, they are in agreement with the dimensions of central cells of the thalli of Patagonian *L. elsbetiae*. The loculi of the gametangia observed in our samples were larger than those observed in *L. elsbetiae* from Helgoland.

We have not observed the formation of reproductive structures of *L. elsbetiae* in nature during summer. No fertile structures were also observed during summer and other seasons (winter and autumn) by Peters and Ellertsdóttir (1996). These authors observed the formation during May (spring in the North hemisphere) of macrosporangia that protruded slighty from the meristoderm of the host and they hypothesize that during this season *L. elsbetiae* rapidly spreads in the host population by means of direct reproduction of sporophytes.

Despite that we did not observe fusion of gametes, by means of chromosome counts we could find out in the present populations of *L. elsbetiae* the presence of two heteromorphyc generations with both haploid and diploid levels of ploidy indicative of a diplobiontic life cycle. Gametophytes were not clearly observed in the field but they were experimentally isolated from hosts' fronds. The firm adherence of them to the substrate and the fact that they never developed from isolates started with filaments from internal tissues of infected hosts

may indicate that the idea of Peters and Ellertsdóttir (1996) in the sense that gametophytes of *L. elsbetiae* has epilithic or epiphytic but not endophytic growth habit is correct.

Field and experimental observations indicate that the infection of *L. elsbetiae* is transferred from one frond of *R. pseudopalmata* to another by spore discharge into the surrounding medium. Collected material, on which recently settled zoospores and germlings were found, the subsequent development of the infection in areas of the fronds took place. As Heesch and Peters (1999) did, we observed that *L. elsbetiae* successfully enter into the host and developed as interstitial filaments, but the mode of entry into the host tissue has not been accurately verified.

The nature of interactions between *R. pseudopalmata* and *L. elsbetiae* remains to be undoubtedly established. In some instances *L. elsbetiae* caused little perturbation of the *R. pseudopalmata* tissues. In other cases we have noted the complete replacement of the host cortical tissue. Similar distortions of host have been observed especially by species of *Streblonema* (Andrews 1976; Yoshida and Akiyama 1979). Epiphytes are usually defined as organisms that grow on plants, but do not derive nutrients from their hosts (Linskens 1976). Linskens (1963) named two kings of epiphytes: holo-epiphytes, organisms attached to the outer layers of the host and amphi-epiphytes, organisms deeply anchored in the tissues of their hosts. Also, Linskens (1963) suggested that the type of anatomical contact is highly variable and that it is determined by the nature of the partners. Endophytism has been defined as the type of symbiosis in which an organism lives within the tissues of a plant host (Correa *et al.* 1988). Other authors, such as Lewis (1973); Starr (1975); Goff (1982); Lewin (1982); Smith and Douglas (1987) and Douglas and Smith (1989) have also used the term endophytism in the same sense. A wide variety of algae that infect other algae from an anatomical point of view are a continuum between epiphytes and endophytes. Therefore they are named epi-endophytes. In general, epi-endophytes are pigmented algae photosynthetically independent with almost no metabolic relation with their hosts. For that reason many of them can be isolated from their hosts and subsequently be cultivated under laboratory conditions (White and Boney 1969, 1970; Boney 1972; Garbary 1979; Nielsen 1987; Gauna *et al.* 2009). In this sense the presence *L. elsbetiae* on *R. pseudopalmata* could be defined as an epi-endophytic relationship.

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REFERENCES

Andrews J.H. 1976. The pathology of marine algae. *Biol. Rev.* **51**: 211-253.

Berkowitz L.R., Fiorello O., Kruger L. and Maxwell D.S. 1968. Selective staining of nervous tissue for light microscopy following preparation for electron microscopy. *J. Histochem. Cytochem.* **16**: 808-814.

Boney A.D. 1972. *In vitro* growth of the endophyte *Acrochaetium bonnemaisoniae* (Batt.) J. et G. Feldmann. *Nova Hedwigia* **23**: 173-186.

Cheney D. 1999. Developing seaweed aquaculture in the northeastern United States and Canada: species selection and strain improvement. *Bull. Aquacul. Assoc. Can.* **1**: 23-26.

Christensen T. 1982. *Alger i Naturen og i Laboratoriet*. Ørsted, Nucleus, 136 pp.

Correa J.A. 1990. *Pigmented algal endophytes of* Chondrus crispus *Stackhouse: host specificity, fine structure, and effects on host performance in infections by* Acrochaete operculata *Correa & Nielsen and* A. heteroclada *Correa & Nielsen*. Ph. D. dissertation, Dalhousie University, Halifax, Nova Scotia, Canada, 272 pp.

Correa J.A., Flores V. and Séánchez P. 1993. Deformative disease in *Iridaea laminarioides* (Rhodophyta): gall development associated with an endophytic cyanobacterium. *J. Phycol.* **29**: 853-860.

Correa J.A. and McLachlan J.L. 1991. Endophytic algae of Chondrus crispus (Rhodophyta). III. Host specificity. *J. Phycol.* **27**: 448-459.

Correa J.A. and McLachlan J.L. 1992. Endophytic algae of *Chondrus crispus* (Rhodophyta). IV. Effects on the host following infections by *Acrochaete operculata* and *A. heteroclada* (Chlorophyta). *Mar. Ecol. Prog. Ser.* **81**: 73-87.

Correa J.A. and McLachlan J.L. 1994. Endophytic algae of *Chondrus crispus* (Rhodophyta). V. Fine structure of the infection by *Acrochaete operculata* (Chlorophyta). *Eur. J. Phycol.* **29**: 33-48.

Correa J.A., Nielsen R. and Grund D.W. 1988. Endophytic algae of *Chondrus crispus* (Rhodophyta). II. *Acrochaete heteroclada* sp. nov. *A. operculata* sp. nov., and *Phaeophila dendroides*

(Chlorophyta). *J. Phycol.* **24**: 528-539.

Douglas A.E and Smith D.C. 1989. Are endosymbioses mutualistic? *Trends Ecol. Evolut*. **4**: 350-352.

Ducker S.C. and Knox R.B. 1984. Epiphytism at the cellular level with special references to algal epiphytes. In: Linskens H.F. and Heslop-Harrison J. (eds), *Encyclopaedia of Plant Physiology*. Springer-Verlag, Berlin. pp. 113-133.

Ellertsdóttir E. and Peters A.F. 1997. High prevalence of infection by endophytic brown algae in populations of *Laminaria* spp. (Phaeophyceae). *Mar. Ecol.Prog. Ser*. **146**: 135-143.

Evans F. and Langdon C.J. 2000. Co-culture of dulse *Palmaria mollis* and red abalone *Haliotis rufescens* under limited flow conditions. *Aquaculture* **185**: 137-158.

Fleurence J. 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol*. **10**: 25-28.

Garbary D. 1979. A revised species concept for endophytic and endozoic members of the Acrochaeticeae (Rhodophyta). *Bot. Notiser*. **132**: 451-455.

Gauna M.C. 2005. *Relaciones interespecíficas en macroalgas bentónicas de la costa patagónica: epi-endofitismo algal*. MSc. Thesis. Universidad Nacional del Sur, Bahía Blanca, Argentina, 143 pp.

Gauna M.C., Parodi E.R. and Cáceres E.J. 2009. Epi-endophytic symbiosis between *Laminariocolax aeidioides* (Ectocarpales, Phaeophyceae) and *Undaria pinnatifida* (Laminariales, Phaeophyceae) growing on Argentinian coasts. *J. Appl. Phycol*. **21**: 11-18.

Goff L.J. 1982. The biology of parasitic red algae. *Prog. Phycol. Res*. **1**: 289-369.

Hayat M.A. 1986. *Basic Techniques for Transmission Electron Microscopy*. Academic Press Inc., London, 411 pp.

Heesch S. and Peters A.F. 1999. Scanning electron microscopy observation of host entry by two brown algae endophytic in *Laminaria saccharina* (Laminariales, Phaeophyceae). *Phycol. Res*. **47**: 1-5.

Johansen D.A. 1940. *Plant Microtechnique*. McGraw-Hill Book Company, New York, 523 pp.

Kawai H. and Tokuyama M. 1995. *Laminarionema elsbetiae* gen. et sp. nov. (Ectocarpales, Phaeophyceae), a new endophyte in Laminaria sporophytes. *Phycol. Res*. **43**: 185-190.

Le Gall L., Pien S. and Rusig A.M. 2004. Cultivation of *Palmaria palmata* (Palmariales, Rhodophyta) from isolated spores in semicontrolled conditions. *Aquaculture* **229**: 181-191.

Lewin J. 1966. Silicon metabolism in Diatoms V: Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* **6**: 1-12.

Lewin R.A. 1982. Symbiosis and Parasitism. Definitions and Evaluations. *BioSciences* **32**: 254-259.

Lewis D.H. 1973. Concepts in fungal nutrition and the origin of biotropy. *Biol. Rev*. **48**: 261-278.

Linskens H.F. 1963. Beitrag zur Frage der Beziehungen zwischen Epiphyt und Basiphyt bei marinen Algen. *Publ. Staz. Zool. Napoli*. **33**: 274-293.

Linskens H.F. 1976. Specific interactions in higher plants. In: Wood R.K.S. and Graniti A. (eds), *Specificity in Plant Diseases*. Plenum Press, New York. pp. 311-325.

Mendoza M.L. and Nizovoy A. 2000. *Géneros de Macroalgas Marinas de la Argentina fundamentalmente de Tierra del Fuego: Secretaria de Desarrollo y Planeamiento de la Provincia de Tierra del Fuego*. Ushuaia, Tierra del Fuego, 142 pp.

Mishra V., Temelli F., Ooraikul B., Shacklock P. and Craigie J. 1993. Lipids of the red alga *Palmaria palmata. Bot. Mar*. **36**: 169-174.

Morgan K.C., Wright J.L.C. and Simpson F.J. 1980. Review of the chemical constituents of the red alga *Palmaria palmata* (dulse). *Econ. Bot*. **34**: 27-50.

Müller D.G. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge Ectocarpus siliculosus im Kulturversuch. *Planta* **75**: 39-54.

Nielsen R. 1987. Marine algae within calcareous shells from New Zealand. *N. Z. J. Bot*. **25**: 425-438.

Núñez O. 1968. An acetic - hematoxilin squash method for small choromosomes. *Caryologia* **21**: 115-119.

Peters A.F. and Ellertsdóttir E. 1996. New record of the kelp endophyte *Laminarionema elsbetiae* (Phaeophyceae, Ectocarpales) at Helgoland and its life history in culture. *Nova Hedwigia* **62**: 341-349.

Peters A.F. and Schaffelke B. 1996. *Streblonema* (Ectocarpales, Phaeophyceae) infection in the kelp *Laminaria saccharina* (Laminariales, Phaeophyceae) in the western Baltic. *Hidrobiología* **326/327**: 111-116.

Provasoli L. 1968. Media and prospect for the cultivation of marine algae. In: Watanabe A. and Hatorri A. (eds), *Cultures and Collections of Algae*. Japanese Society for Plant Physiology, Tokyo. pp. 63-75.

Rosen G., Langdon C.J. and Evans F. 2000. The nutritional value of *Palmaria mollis* cultured under different light intensities and water exchange rates for juvenile red abalone *Haliotis rufescens. Aquaculture* **185**: 121-136.

Smith D.C. and Douglas A.E. 1987. *The Biology Symbiosis*. Edward Arnold, London, 320 pp.

Starr M.P. 1975. A generalized scheme for classifying organismic associations. *Symp. Soc. Exp. Biol*. **29**: 1-20.

White E.B. and Boney A.D. 1969. Experiments with some endophytic and endozoic *Acrochaetium* species. *J. Exp. Mar. Biol. Ecol.* **3**: 246-274.

White E.B. and Boney A.D. 1970. In situ and in vitro studies on some endophytic and endozoic *Acrochaetium* species. *Nova Hewigia* **19**: 841-881.

Yoshida T. and Akiyama K. 1979 *Streblonema* (Phaeophyceae) infection in the frond of cultivated *Undaria* (Phaeophyceae). *Proc. Ninth Int. Seaweed Symp*. **9**: 219-223.

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