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Morphological convergence in the inter-holdfast coalescence process among kelp and kelp-like seaweeds (*Lessonia, Macrocystis, Durvillaea*)

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Abstract: In brown macroalgae, intraspecific holdfast coalescence has only been studied in two species of *Lessonia* (*Lessonia spicata* and *Lessonia berteroana*). In both species coalescence followed the same general pattern: once the connection between holdfasts was established, the contact areas showed significant cellular morphological modifications. Typical epidermal cells became polygonal and similar to cortical cells. In addition, coalescence involved the *de novo* formation of secondary plasmodesmata, establishing a direct cytoplasm connection within neighbouring cells, where dense materials, vacuoles and organelles can be observed. In the present study, we demonstrate intraspecific holdfast coalescence in two additional kelp species, *Lessonia trabeculata* and *Macrocystis pyrifera*, as well as in the kelp-like seaweed, *Durvillaea antarctica*. The process of holdfast fusions in these species is similar to that described previously and suggests that this is a generalized phenomenon among kelp and kelp-like brown algae. In addition, the formation of cytoplasmic connections between genetically different brown algal individuals is shown for the first time.

Key Words: Brown algae, Coalescence, *Durvillaea, Lessonia, Macrocystis*, Plasmodesmata

INTRODUCTION

Kelps are dominant organisms in temperate coastal environments, forming seaweed forests, and providing a diversity of habitats for many marine forms (Vásquez 1992; Graham *et al.* 2007). They also protect coastlines against erosion (Dayton *et al.* 1984) and contribute significantly to the marine carbon cycle (Kelly 2005; Thiel *et al.* 2007; Vásquez *et al.* 2014). Along the Chilean coast, not only do kelp forests have ecological value, but they are also the source of raw materials for the country's alginate and biofuels industries (Vásquez *et al.* 2014; Vega *et al.* 2014), as well as providing food for invertebrate culture (e.g. abalony and sea urchins; Buschmann *et al.* 2013). Kelp harvesting in Chile has a considerable social, economic and ecological importance, providing about 10% of the biomass of brown algae worldwide (FAO 2012), with an annual harvested weight of up to 300,000 dry tons (Vásquez *et al.* 2008; Anuario Sernapesca 2014; Vásquez *et al.* 2012; Rodriguez *et al.* 2014). Five kelp or kelp-like species are found along the central Chilean coast (18 to 42°S), namely, *Lessonia berteroana* Montagne, *Lessonia spicata* (Suhr) Santelices, *Lessonia trabeculata* Villouta & Santelices, *Macrocystis pyrifera* (Linnaeus) C. Agardh and *Durvillaea antarctica* (Chamisso) Hariot (Santelices 1989; Ramírez & Santelices 1991; González *et al.* 2012). They differ in morphology, ecophysiology, longevity and life cycles and show distinct patterns of vertical distribution on the shore (Villouta & Santelices 1986).

Numerous studies have shown that large algal holdfasts *in situ* can fuse with one another. This was described as 'sporadic' or 'frequent' in the fucalean species *Sargassum muticum* (Yendo) Fensholt (Critchley 1983) and the laminarialean species *Macrocystis pyrifera* (Linnaeus) C. Agardh (Dayton *et al.* 1984), *Ecklonia maxima* (Osbeck) Papenfuss (Anderson *et al.* 1997), *Ecklonia radiata* (C. Agardh) J. Agardh (Paine 1990; Wernberg 2005) and *Lessonia berteroana* (Segovia 2009). Recently González *et al.* (2014) showed that intraspecific inter-holdfast fusion in the two intertidal kelps *Lessonia spicata* and *Lessonia berteroana* involved morphological, histological and cytological changes. In these species, the fused discs had a slight scar on the inter-holdfast contact zone. Epidermal cells in the fusion areas became polygonal, increased in size and displayed a reduction in cell wall thickness and in the number of subcellular organelles. Plasmodesmata then developed across thinner cell walls between coalesced cells, enhancing intercellular and inter-holdfast communications (González *et al.* 2014).

The adaptation of cells to form a coalescent organism among kelp holdfasts probably confers many ecological costs and benefits, similar to those suggested for red coalescing algae (Santelices 2004). For example, a major advantage of red algal coalescence resides in ensuing short- and long-term increases in seaweed size (Santelices *et al.* 1989). As for kelps, longer thalli from fused discs were described in *Ecklonia radiata* (Wernberg 2005), as well as larger holdfasts in *Lessonia berteroana* (Segovia 2009; Segovia *et al.* 2014). The formation of a genetically heterogeneous organism was another feature of red coalescing algae (Santelices 2004). In the kelps, *Lessonia spicata* and *L. berteroana*, there was a direct relationship between genetic heterogeneity and the number of stipes (González *et al.* 2014). The benefits of coalescence can be translated into ecological advantages. In the case of coalescent red algae, they may enhance performance in the competition for space. In the case of brown algae, aggregated holdfasts in *E. radiata* were more frequent in wave-exposed areas than solitary holdfasts. These fronds also showed decreased drag that

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reduced the rate of fatal kelp dislodgment and decreased consumption by marine grazers (Denny 1985, 1988; Wernberg 2005).

In this study, we examined the possibility that inter-holdfast fusion or coalescence in kelp and kelp-like seaweeds may result in equivalent morphological changes such as interspecific changes among the Laminariales (i.e., *Lessonia* and *Macrocystis*) and Fucales (*Durvillaea antarctica*). Specifically, we described the cellular events occurring with inter-holdfast fusions in *Lessonia trabeculata*. These observations complement the fusion process described for *Lessonia spicata* and *Lessonia berteroana*. We then examined (1) the frequency of coalescence in paired discs cultured in close proximity in *L. trabeculata*, *Macrocystis pyrifera* and *D. antarctica*; (2) the cellular modification involved in the coalescence of each species and (3) the ultrastructural similarity of plasmodesmata developed *de novo* between coalesced cells in the three genera. These selected taxa represent three families in two phylogenetically distant orders in the Phaeophyceae (Silberfeld *et al.* 2010; Martin & Zucarello 2012).

MATERIAL AND METHODS

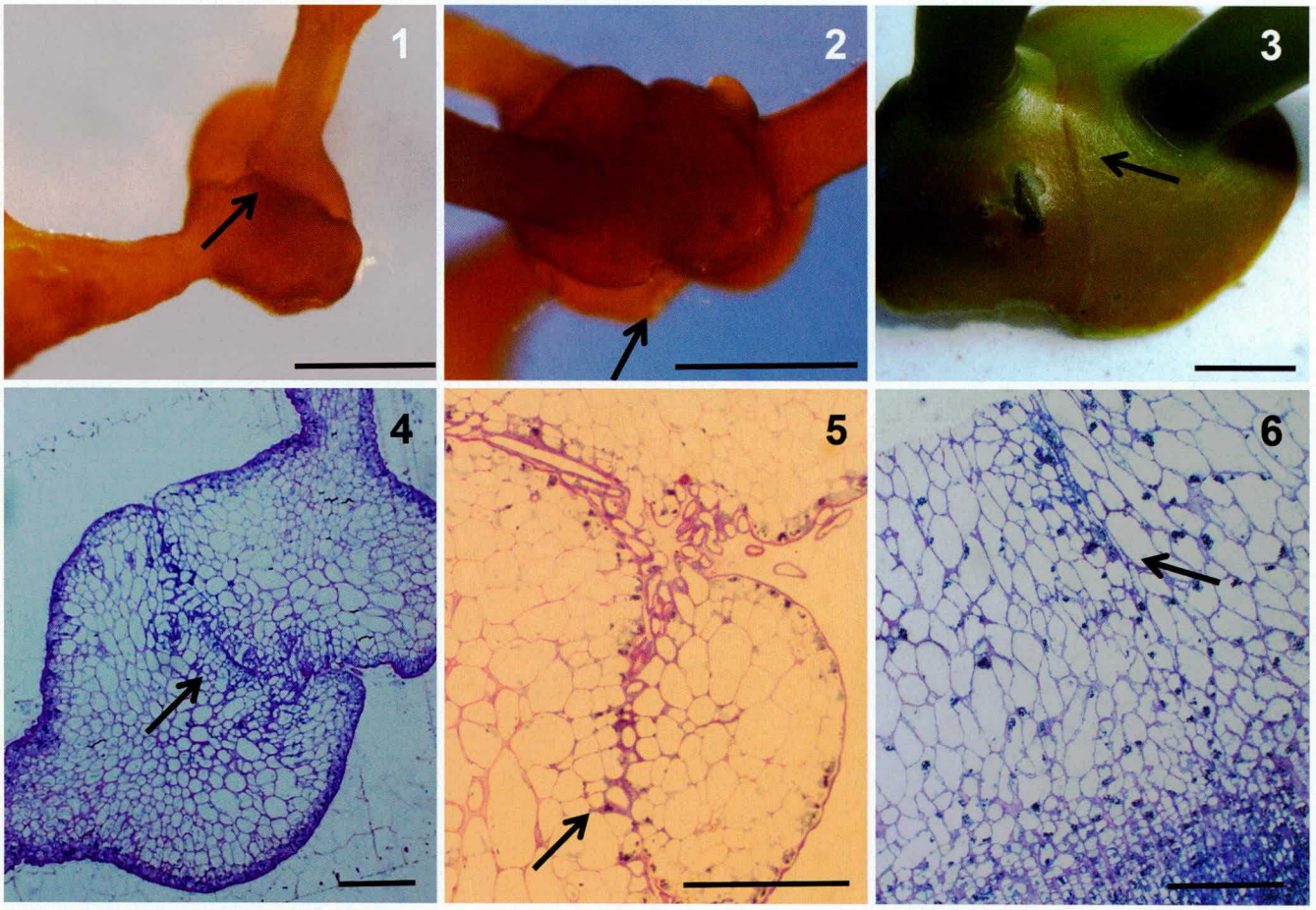
Specimens of *Lessonia trabeculata* (Lessoniaceae, Laminariales) were collected in Punta de Tralca (33°25′S, 71°41′W), 30 km to the north of San Antonio Port, central Chile. Specimens of *Macrocystis pyrifera* (Laminariaceae, Laminariales) were collected at Punta Choros (24°14′S, 71°27′W), 60 km north of Coquimbo, while the individual bull-kelp samples of *Durvillaea antarctica* (Durvillaeaceae, Fucales) were obtained from Caleta Montemar (32°57′S, 71°33′W), 30 km to the north of Valparaíso Port. Ten reproductive specimens of each species were collected at each site and transported to the laboratory in wet plastic bags maintained at 10°C. In the laboratory, fertile tissues were gently cleaned with running tap water and rinsed several times with sterile seawater.

To obtain sporophytes from *Lessonia trabeculata* and *Macrocystis pyrifera*, we followed the method of González *et al.* (2014). Fragments of fertile sporophylls were dehydrated at 14°C for 2 h, placed in 10 Petri dishes (140 × 20 mm) with a glass slide placed at the bottom and then rehydrated with cold (5–8°C) seawater. To obtain gametophytes, the Petri dishes were incubated for spore release for 2–4 h at 14°C under cool-white fluorescent tubes (20′w/54 RSS day, Philips, Sao Paulo, Brazil) with a photon flux density of 35–45 μmol photons m^{-2} s^{-1}. After 2 to 3 d, gametophytes appeared on the glass slide. These were then incubated in beakers with 200 ml medium [Provasoli's enriched seawater (PES), Provasoli 1968] at 12° ± 2°C, at an irradiance of 20 ± 10 μmol photons m^{-2} s^{-1}, with a 12:12 light–dark (LD) photoperiod. The enriched PES medium was changed twice per week. After 10–13 d, sporophytes appeared ($N = 100 \pm 20$ per glass slide). Thirty days later, under controlled conditions, 1–1.2 mm diameter discs were observed in the incubated sporophytes.

Juveniles of *Durvillaea antarctica* were obtained following Collantes *et al.* (2002). Sampled female and male tissues were dehydrated at 14°C for 2 h and placed in 10 centrifuge tubes (Isolab, Wertheim, Germany) with a glass slide at the bottom and 50 ml of sterile seawater. After 24 h, we observed oospores and mature antheridia in the same centrifuge tubes; after 2 d, we observed zygotes ($N = 150 \pm 20$ per glass slide); these were cultured as described above for *Lessonia trabeculata* and *Macrocystis pyrifera*, with the PES medium being changed twice per week. In *D. antarctica*, a basal disc formed only after 60 d of culture.

Coalescence was induced for the three species by placing two sporophytes (*Lessonia trabeculata* and *Macrocystis pyrifera*) or diploid thalli (*Durvillaea antarctica*) in Petri dishes in close proximity with one another. A total of 150 paired basal portions were placed in culture chambers under standard culture conditions (12° ± 2°C, 20 ± 10 μmol photons m^{-2} s^{-1}, 12:12 LD), with the PES medium being changed twice per week. We observed coalescing sporophytes of *L. trabeculata* and *M. pyrifera* after 10 d. However, unitary as well as coalescent basal portions of *D. antarctica* only formed numerous rhizoids, without a consistent holdfast. Coalescent samples maintained themselves by entangling their rhizoids. We therefore collected 30 young thalli from a wild population (Caleta Montemar), with holdfast size ranging from 2 to 4 cm in diameter, characterized by the presence of a fusion scar on the surface and only two erect axes. The samples were then transported to the laboratory inside wet plastic bags maintained at 10°C. Additionally, we selected a 4 × 20 m^2 intertidal platform in the field and estimated the frequency of holdfasts with coalescing scars by random sampling 30 quadrats of 50 × 50 cm^2. In the sampled area (75,000 cm^2) there were 313 plants. A total of 63 plants (20%) showed fused holdfasts with a single scar and two erect axes. Two plants (0.6%) had three erect axes. All other holdfasts lacked scars and had only one erect axis. We used the plants with one scar and two axes as representative of coalesced individuals.

For histological and ultrastructural observations, we fixed 10 coalescing holdfasts from the culture of *Lessonia trabeculata* and *Macrocystis pyrifera* and 10 coalescing holdfasts from the natural population of *Durvillaea antarctica*. Fixation followed the protocol described in previous studies (Santelices *et al.* 1996, 1999; González *et al.* 2014). Post-fixation was carried out using 2% O_5O_4 tetroxide and 1% potassium ferrocyanide followed by dehydration in an ethanol series. Samples were then embedded in Spurr's resin. Semi-thin and thin sections of coalescing individuals were stained according to Reynolds (1963) and Santelices *et al.* (1996). Observations and cell characterization were made in cells at the contact zone of coalescing discs using a Nikon Optiphot 2 microscope (Nikon, Melville, New York USA), and a Phillips Tecnai 12 transmission microscope (Philips Electron Optics, Eindhoven, Holland). Additionally, in order to follow the line of cell fusion between two individuals, we studied serial sections (semi-thin and thin sections) in holdfasts at different stages of fusion in *L. trabeculata*, in order to characterize the interface zone, the pattern of cell modifications during the fusion process and the establishment of simultaneous connections between neighbouring cells. Cell size, the number of plasmodesmata, and the area of plasmodesmata in the coalescing zone for all



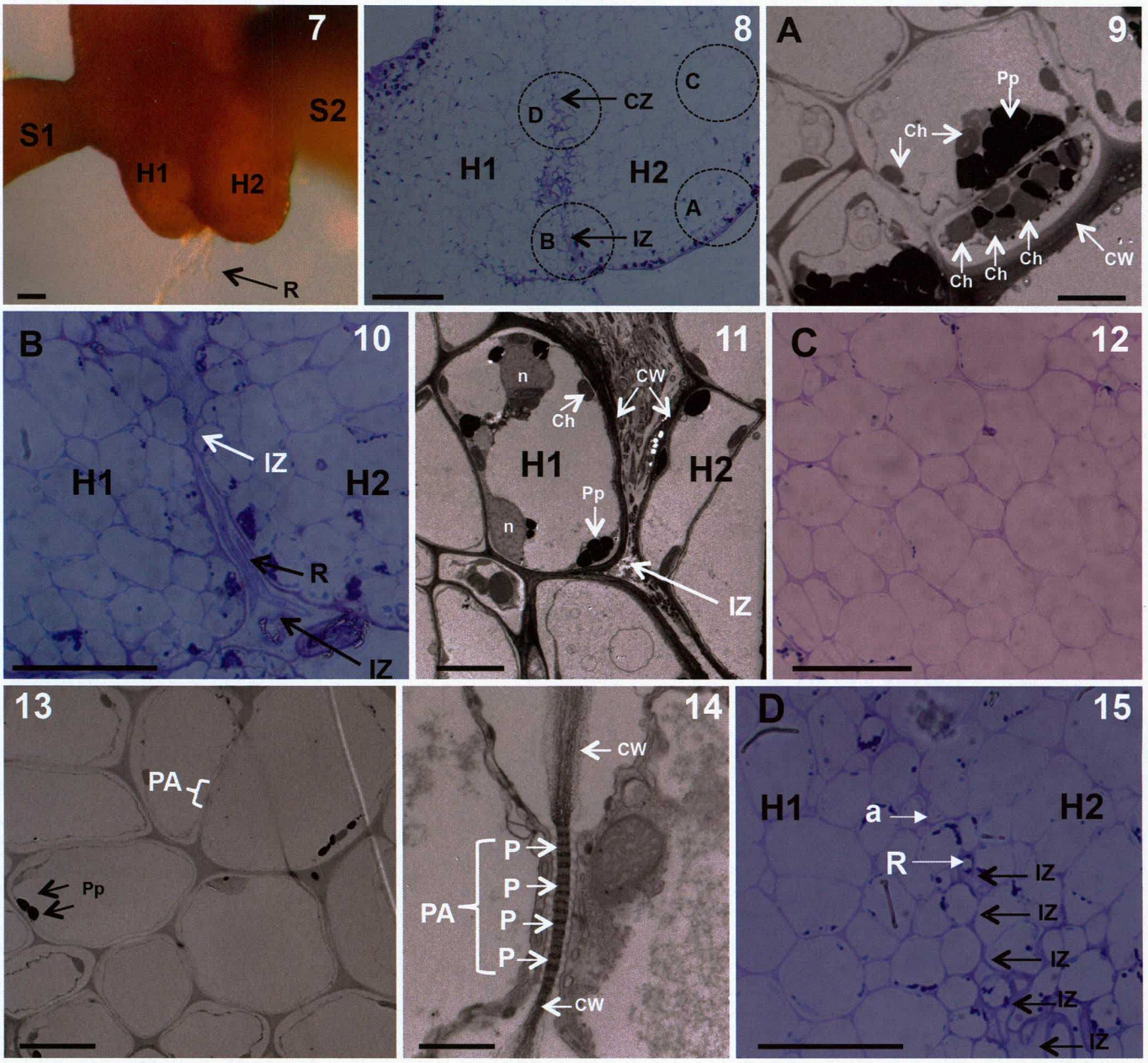
Figs 1–6. Holdfast disc fusions in *Lessonia trabeculata, Macrocystis pyrifera* and *Durvillaea antarctica.*
Fig. 1. Holdfast disc fusions in *L. trabeculata* after 15 ± 2 d under culture conditions. Arrow shows the contact zone between paired holdfasts. Scale bar = 1 cm.
Fig. 2. Holdfast disc fusions in *M. pyrifera* after 15 ± 2 d under culture conditions. Arrow shows contact zone between fused holdfasts. Scale bar = 1 cm.
Fig. 3. Holdfast disc fusions in *D. antarctica* collected from the field. The arrow shows the surface interface zone indicating the existence of fusion between holdfasts. Scale bar = 1 cm.
Figs 4–6. Semi-thin transections through fused holdfasts in *L. trabeculata, M. pyrifera* and *D. antarctica.* The arrows indicate the interface zone between holdfasts. At this level, the interface zone was composed of numerous rounded epidermal cells with thick cell walls. The continuous tissues near the contact zone are formed by polygonal-shaped cells of larger sizes and reduced wall thickness. Scale bar = 100 μm.

species were compared using the Kruskal–Wallis analysis of variance (Sokal & Rohlf 1995).

RESULTS

About 10 to 50 d after placing the paired discs of *Lessonia trabeculata*, *Macrocystis pyrifera* and *Durvillaea antarctica* in close proximity, holdfast fusion was observed in 100% of replicates for each species. In the case of *L. trabeculata* and *M. pyrifera*, fused discs formed a larger shared holdfast, which initially showed an interface zone as evidence of the fusion. The fused discs subsequently grew, and the size of the interface zone eventually reduced until contact was made between neighbouring cells, facilitating holdfast fusion (Figs 1 and 2). In contrast, the basal portion of *D. antarctica* only developed rhizoids, without forming a consistent holdfast. Accordingly, paired samples maintained contact by entangling their rhizoids. However, fused holdfasts collected from the field also showed a surface interface area, indicating the existence of contacting zones among different holdfasts (Fig. 3). All three species had a uniform pattern of cellular modification during holdfast fusion. This was a gradual process until complete holdfast fusion had occurred, when it became impossible to distinguish which cell corresponded to which individual. Semi-thin transections at the initial stages of holdfast fusion exhibited an interface zone composed of numerous epidermal cells with very thick cell walls, providing evidence of the contact zone between holdfasts (arrows Figs 4–6).

Serial sections of holdfasts at different stages of fusion in *Lessonia trabeculata* (Figs 7–22) allowed us to follow the path of cell fusion between two coalescing individuals and illustrated the pattern of cell modification during the fusion process. This was similar in each of the three species. Accordingly, the fused discs (H1 and H2, Fig. 7), relating to



Figs 7–15. Serial sections in holdfast discs with different stages of fusion in *Lessonia trabeculata*.

Fig. 7. Holdfast with different stages of holdfast discs fusion. S1, Sporophyte 1; S2, Sporophyte 2; H1, Holdfast disc of sporophyte 1; H2, Holdfast disc of sporophyte 2; R, Rhizoids. Scale bar = 100 μm.

Fig. 8. Semi-thin section across the holdfast discs fusion. Circle A, epidermal cells of H2 far from the fusion zone; Circle B, epidermal cells in the interface zone between H1 and H2; Circle C, cortical cells of H2, far from the fusion zone; and Circle D, meristematic cells in the interface zone between H1 and H2. Scale bar = 100 μm.

Fig. 9. A fine section in the Circle A, shows epidermal symmetrical cells, with numerous chloroplasts (Ch), polyphenols (Pp) and a thick cell wall (CW) on the front of the cell that is in contact with the external environment. Scale bar = 5 μm.

Fig. 10. A close-up of Circle B, shows the interface zone (IZ) between discs H1 and H2, with numerous rhizoids (R) and epidermal cells that are beginning to undergo morphological changes at the edges. Scale bar = 50 μm.

Fig. 11. A fine section of the interface zone (IZ), where the thickness of the epidermal cell walls (CW) is beginning to reduce. The number of chloroplasts (Ch) and polyphenols (Pp) is also reduced, despite slightly increasing in size, and exhibiting similar morphology to the typical cortical cells. n = nucleus. Scale bar = 5 μm.

Fig. 12. A close-up of Circle C, shows the cells from the cortex area, far from the contact zone. Scale bar = 50 μm.

Fig. 13. A fine section of Circle C, shows the cortical cells with large size and the scarce number of polyphenols (Pp) but also that the cell wall (CW) is more flexible and reduced at certain points between neighbouring cells, with a plasmodesmata area (PA) among them. Scale bar = 10 μm.

Fig. 14. A close-up in the plasmodesmata area (PA) shows numerous channel-type connections or plasmodesmata (P) between two cortical cells far from the contact zone. Scale bar = 0.5 μm.

sporophytes S1 and S2 (lower part of Fig. 7), showed an interface zone with numerous rhizoids (R) at the periphery, where disc fusion had not yet occurred. Meanwhile, at the more advanced fusion stages (upper part of Fig. 7), the tissue appeared continuous, making it impossible to distinguish between H1 and H2. Semi-fine sections allowed us to characterize four zones at this fusion stage (see circles in Fig. 8). Each is separately described as follows: Circle A, epidermal cells of H2 far from the fusion zone; Circle B, epidermal cells in the interface zone between H1 and H2; Circle C, cortical cells of H2, far from the fusion zone; and Circle D, meristematic cells in the interface zone between H1 and H2. A fine section in Circle A (Fig. 9) shows that the epidermal cells in the areas where fusion had not yet occurred were symmetrical, with numerous chloroplasts (Ch), polyphenols (Pp) and a thick cell wall (CW) on the face of the cell in contact with the external environment. A close-up of the interface zone (Circle B, Fig. 10) showed that the widest part of the interface zone between discs H1 and H2 (lower part of Fig. 10, black IZ) contained numerous rhizoids (R) and epidermal cells that were undergoing morphological changes at the edges. At the more advanced fusion stages (upper part of Fig. 10), there was a reduction in the size of the interface zone between the holdfast discs (see white IZ) but there was still no contact between cells, making it possible to distinguish between H1 and H2. In a fine section of this area (Fig. 11), substantial numbers of bacteria were observed in the interface zone (white IZ, Fig.11) where the thickness of the epidermal cell walls (CW) was beginning to reduce. The numbers of chloroplasts (Ch) and polyphenols (Pp) were also reduced. However, the epidermal cells were slightly larger and exhibited morphology somewhat similar to typical cells found in the cortex, far from the contact zone (see close-up of zone C, Fig. 12). A fine, transversal section of these cortical cells showed not only their large size and paucity of organelles (Fig. 13) but also that the cell wall (CW) was more flexible and reduced at certain points between neighbouring cells, from where numerous (between 5 and 25) channel-type connections or plasmodesmata (P) emerge between them, thus generating an area of plasmodesmata (PA), which could be observed with transmission electron microscopy (Fig. 14).

Similarly, as the discs H1 and H2 came into contact with each other (close-up of zone D, in Fig. 15), the visible interface zone (IZ) from initial stages of the fusion began to disappear, leaving only traces, with the remains of rhizoids (R) and cells (an example is shown as 'a'). These appeared to be at the centre of the H1 and H2 disc fusion, culminating in the disappearance of the separation between both sporophytes.

Tracking through fine, serial sections in the interface zone (Figs 16–22) not only showed the remains of the contact zone (IZ in Fig. 16) but also the location of cell a with two cells (b and c) on one side and two cells (f and g) on the other. The first two cells (b and c) belong to disc H1, while cells f and g belong to disc H2. The continuity of the cell wall remained, and the position of the rhizoid (R) indicated the border area between the coalescing sporophytes. Cells d and e could also be observed, which, according to the position of the rhizoid (R) and the cell walls, related to H2. In the initial fine, serial sections (Figs 17 and 18), the cells of the two different discs were in contact with each other (b, a and g). In places where wall thickness of cell a was reduced by three to four times, contact was established with the neighbouring cells b and g, via secondary areas of plasmodesmata (PA, Fig. 19). Here, several pores were formed *de novo* that crossed over into the walls of cells, a, b and g (see white braces in Fig. 19). The same pattern of secondary connections or areas of plasmodesmata (PA) were observed in subsequent sections, between cell b of disc H1 and cell g of disc H2 (Fig. 20). Similarly, areas of plasmodesmata (PA) were recorded between cell c of the H1 sporophytes and cell d (see white braces in Fig. 21), and also between cells d and e (see white braces in Fig. 22), which belonged to H2. In summary, the cells that belonged to different holdfast discs (H1 and H2) ended up in contact with each other. To achieve this, the cells underwent morphological changes, reducing their cell wall until secondary connections were formed *de novo* as plasmodesmata, with the sporophytes S1 and S2 fusing completely. At the final stages of fusion, a continuous cortical tissue was observed, where it was impossible to determine whether the cells belonged to H1 or H2. Moreover, it seems likely that numerous plasmodesmata maintain a cytoplasmic connection between coalescing individuals.

At the ultrastructural level, comparative analysis showed the same pattern in all three species, with transformed polygonal cells located in the fusion area and an area of plasmodesmata (PA), where the thickness of the cell wall decreased by 78% in *Lessonia trabeculata*, 82% in *Macrocystis pyrifera* and 94% in *Durvillaea antarctica* (Table 1). The area of plasmodesmata showed morphological similarity with cell wall reduction and numerous pores traversing the cell wall as simple plasmodesmata (see black arrows in Figs 23–25). All of these most likely corresponded to secondary plasmodesmata, which were unbranched pores extending between two adjacent cells. Comparisons among species showed that *D. antarctica* had a significantly larger number, (H test = 6.52, $\nu = 2$, $P = 0.03$) and a larger area of plasmodesmata (H test = 7.13, $\nu = 2$, $P = 0.02$) across the cell wall (Table 1), probably related to the larger size of cells compared with *L. trabeculata* and *M. pyrifera*. The morphology of these secondary plasmodesmata was identical and exhibited dense materials, with vacuoles and organelles located close to the plasmodesmata. The similarity in cell structure suggests equivalent cellular processes and intercellular movements of various-sized molecules.

DISCUSSION

This work demonstrated intraspecific coalescence in kelp and a kelp-like species from Chile. This coalescence followed a

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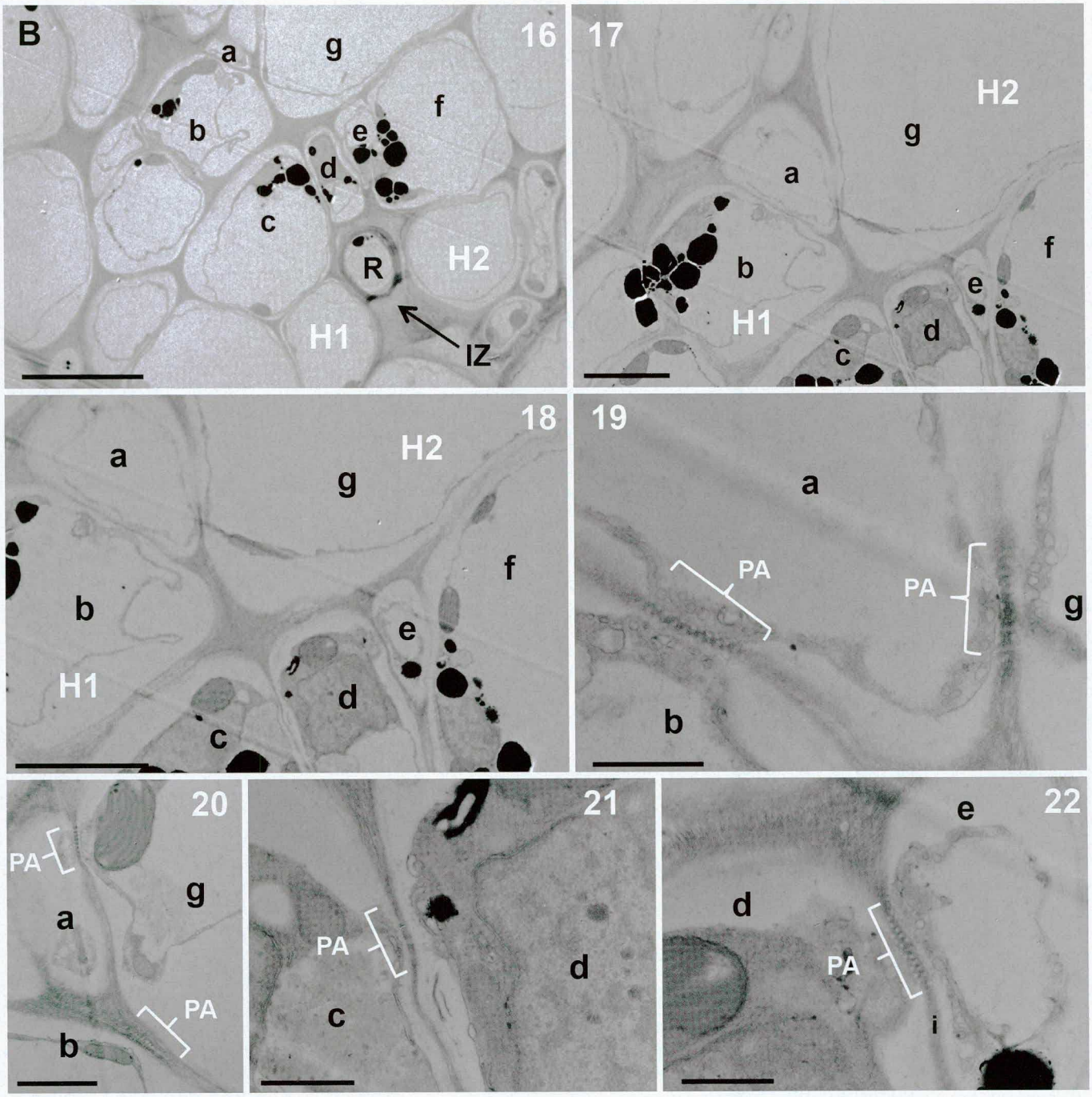
Fig. 15. A close-up of zone D shows the visible interface zone (IZ) from initial stages of the fusion beginning to disappear, leaving only traces, with the remains of rhizoids (R). Cell a appears to be at the centre of the H1 and H2 disc interface, culminating in the disappearance of the separation (interface zone: IZ) between both sporophytes. Scale bar = 50 μm.



Figs 16–22. Tracking through fine, serial sections in the Circle B, shows the interface zone (IZ) in *Lessonia trabeculata*, characterizing the cells, and the establishment of simultaneous connections among neighbouring cells.

Fig. 16. The remains of the interface zone (IZ) but also the location of cell a with cells b and c on one side, and cells f and g on the other. The first two cells (b and c) belong to disc H1, while cells f and g belong to disc H2. The continuity of the cell wall remains, and the position of the rhizoid (R) indicates the border area between the coalescing sporophytes. Scale bar = 10 μm.

Fig. 17. Close-up view at the beginning of the serial sections. Scale bar = 5 μm.

Fig. 18. A magnification of this zone shows the initial close proximity among cells of the two sporophytes. Scale bar = 5 μm.

Fig. 19. A cross section in other level of the tissue shows the cells a, b (of disc H1) and g (of disc H2) contacted by plasmodesmata area (PA). Scale bar = 1 μm.

Fig. 20. A close-up in other level of the tissue shows secondary connections or areas of plasmodesmata (PA) between cells b of disc H1 and cell g of disc H2. Scale bar = 2 μm.

Fig. 21. A cross section of other level of the tissue shows the cells c of disc H1 and d of disc H2 in contact with each other via the area of plasmodesmata area. Scale bar = 1 μm.

Fig. 22. A cross section of other level of the tissue shows the cells d and e (both from disc H2) in contact with the area of plasmodesmata. Scale bar = 1 μm.

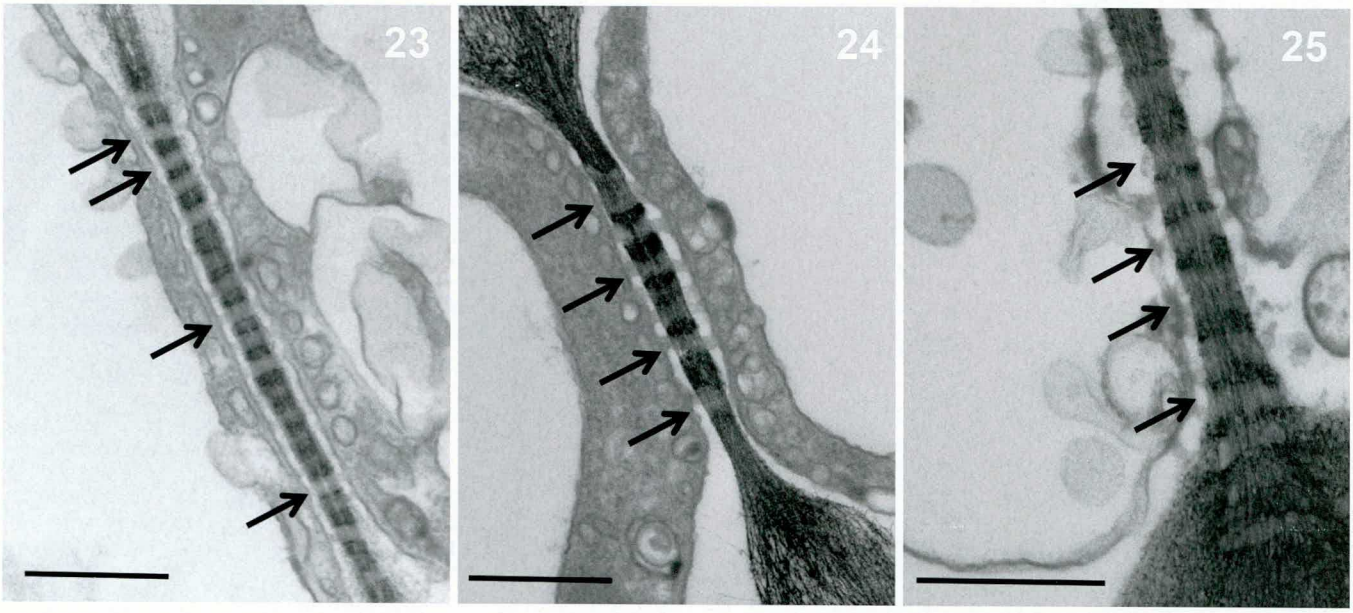
Table 1. Cell, plasmodesmata (P) and size of plasmodesmata area (PA) in the coalescing zone in *Lessonia trabeculata*, *Macrocystis pyrifera* and *Durvillaea antarctica*, from the central Chilean coast.

	Laminariales		Fucales
	L. trabeculata ($N = 4$)	*M. pyrifera* ($N = 4$)	*D. antarctica* ($N = 4$)
Cell length (μm)	20.4 ± 0.4	24.0 ± 0.2	43.0 ± 8.9
Cell width (μm)	13.7 ± 1.2	13.4 ± 0.9	30.8 ± 6.5
Cell wall thickness (nm)	342.3 ± 137.9	588.5 ± 490.3	2383.3 ± 1789.7
Cell wall thickness in the PA (nm)	74.4 ± 17.0	108.5 ± 17.0	150.3 ± 37.0
Maximum PA length (nm)	1.7 ± 0.4	2.0 ± 0.5	7.9 ± 0.9
Numbers of P per PA	20.3 ± 5.1	16.0 ± 6.2	56.0 ± 9.5

consistent pattern, regardless of the phylogenetic affinities of the species concerned. In addition, we demonstrated that the process of coalescence between holdfast discs not only entails their adhesion to one another but also that the cells involved were prepared for contact by undergoing morphological changes. The observed changes were an increase in size and a reduction in both the number of organelles and the cell wall thickness. This process culminated in the *de novo* formation of plasmodesmata that resulted in cytoplasmic connections among the cells. This study opens up new avenues for research into the biological processes that appear to be common in non-phylogenetically related species. This is the first time these processes have been demonstrated. It is also the first time evidence has been provided for the *de novo* formation of secondary plasmodesmata.

All organisms have evolved numerous strategies for intracellular and intercellular signalling and communication. Intercellular communication is common in several kingdoms, from bacteria to algae, mosses, plants and animals (see Lucas & Lee 2004 for reviews). Indeed, it plays a crucial role in the organized development and differentiation of many different multicellular organisms, since it mediates the exchange of metabolites, ions and small, signalling molecules. Since the formation and pattern of these cytoplasmic channels are genetically controlled, the potential arises for the evolution of symplasmic domains (Lucas & Lee 2004) through gap junctions in animals cells (Makowski *et al.* 1984; Kumar & Gilula 1996), unbranched plasmodesmata in land plants (Oparka *et al.* 1999), mosses (Cook *et al.* 1997), green and brown algae (Franceschi *et al.* 1994; Terauchi *et al.* 2012), branched plasmodesmata in land plants (Oparka *et al.* 1999) and pit-plug connections in red algae (Raven 2003; Maggs *et al.* 2007; Ueki *et al.* 2008).

Primary plasmodesmata are commonly produced during cytokinesis. Information on the structure of these primary plasmodesmata in brown algae has been reported in numerous genera of Phaeophyceae, including *Laminaria* (Schmitz & Srivastava 1974; Schmitz & Kuhn 1982), *Alaria* (Schmitz & Srivastava 1975), *Nereocystis* (Schmitz & Srivastava 1976), *Cutleria* (La Claire 1981; Katsaros *et al.* 2009), *Scytosiphon* (Nagasato & Motomura 2002), *Ascophyllum* (Xu *et al.* 2008), *Silvetia* (Nagasato *et al.* 2010) and *Dictyota* (Terauchi *et al.* 2012). However, since plasmodesmata are essential for establishing communication between neighbouring cells, the formation of secondary plasmodesmata is only possible among cells that are not derived from



Figs 23–25. Close-up view of the areas of plasmodesmata in *Lessonia trabeculata*, *Macrocystis pyrifera* and *Durvillaea antarctica*, respectively. The black arrows indicate unbranched plasmodesmata (P) traversing the cell wall, and extending between two adjacent cells. Vacuoles and organelles are seen in the protoplasts area near the plasmodesmata. Scale bar = 5 μm.

the same cell lineage or precursor cell. These secondary plasmodesmata are typically branched in land plants, and many are interconnected by a cavity in the region of the middle lamella. Nevertheless, in grafted tobacco plants, for example, simple secondary plasmodesmata (similar to that observed in our study) maintain an interconnection between the two different lineages (Xiao-cai *et al.* 1995). This also allows the exchange of genetic information via either large DNA pieces or entire plastid genomes (Stegemann & Bock 2009). In brown macroalgae, primary plasmodesmata produced during cytokinesis (Terauchi *et al.* 2012) appear to have the same unbranched morphology as secondary plasmodesmata, described here as being produced during coalescence of *Lessonia trabeculata, Macrocystis pyrifera* and *Durvillaea antarctica,* as well as *Lessonia spicata* and *Lessonia berteroana* (González *et al.* 2014).

Comparative analyses of coalescence among red and brown macroalgae showed some equivalent processes, including the modification of cell walls, and the establishment of secondary connections between cells derived from two organisms. We can therefore suggest that this similarity is a convergent process among these independent lineages. In addition, at the intra-phylum level, the brown genera, *Lessonia*, *Macrocystis* and *Durvillaea,* showed the same cellular and histological modifications, together with the formation of simple plasmodesmata. It could therefore be concluded that there is a morphological convergence in the coalescence process of all these species, suggesting that this pattern may be more widespread in brown seaweed capable of somatic fusion. Furthermore, coalescence in kelps probably involves costs, benefits, and the formation of chimeric entities similar to those described in red coalescing seaweeds. Since kelps are ecologically and economically important organisms, future research should provide insights into the effects of coalescence on different aspects of macroalgal biology and utilization, including the effects on community structure, changes in biochemical compounds, cultivation advantages of coalescent individuals and the possibility of generating grafted organisms with specific qualities.

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REFERENCES

Anderson R.J., Carrick P., Levitt G.J. & Share A. 1997. Holdfasts of adult kelp *Ecklonia maxima* provide refuges from grazing for recruitment of juvenile kelps. *Marine Ecology Progress Series* 159: 265–273.

Anuario Sernapesca. 2014. Anuario Estadístico de Pesca. Ministerio de Economía Fomento y Reconstrucción, República de Chile. http://www.algaebase.org; searched on 15 February 2013.

Buschmann A.H., Stead R.A., Hernández-González M.C., Pereda S.V., Paredes J.E. & Maldonado M.A. 2013. Un análisis crítico sobre el uso de macroalgas como base para una acuicultura sustentable. *Revista Chilena de Historia Natural* 86: 251–264.

Collantes G., Merino A. & Lagos V. 2002 Fenología de la gametogénesis, madurez de conceptáculos, fertilidad y embriogénesis en *Durvillaea antarctica* (Chamisso) Hariot (Phaeophyta, Durvillaeales). *Revista de Biología Marina y Oceanografía* 37: 83–112.

Cook M.E., Graham L.E., Botha C.E.J. & Lavin C.A. 1997. Comparative ultrastructure of plasmodesmata of *Chara* and selected bryophytes: toward an elucidation of the evolutionary origin of plant plasmodesmata. *American Journal of Botany* 84: 1169–1178.

Critchley A.T. 1983. *Sargassum muticum*: a morphological description of European material. *Journal of the Marine Biological Association of the United Kingdom* 63: 813–824.

Dayton P.K., Currie V., Gerrodette T., Keller B.D., Rosenthal R. & Ven Tresca D. 1984. Patch dynamics and stability of some California kelp communities. *Ecological Monographs* 54: 253–290.

Denny M.W. 1985. Wave forces on intertidal organisms: a case study. *Limnology and Oceanography* 30: 1171–1187.

Denny M.W. 1988. *Biology and the mechanics of the wave-swept environment*. Princeton University Press, Princeton, New Jersey. 329 pp.

Food and Agriculture Organization of the United Nations (FAO). 2012. Fishery and Aquaculture Statistics. Capture production 2010. FAO, Rome. 609 pp.

Franceschi V.R., Ding B. & Lucas W.J. 1994. Mechanism of plasmodesmata formation in characean algae in relation to evolution of intercellular communication in higher plants. *Planta* 192: 347–358.

González A.V., Beltrán J., Hiriart-Bertrand L., Flores V., de Reviers B., Correa J.A. & Santelices B. 2012. Identification of cryptic species in the *Lessonia nigrescens* complex (Phaeophyceae, Laminariales). *Journal of Phycology* 48: 1153–1165.

González A.V., Borras-Chávez R., Beltrán J., Flores V., Vásquez J.A. & Santelices B. 2014. Morphological, ultrastructural, and genetic characterization of coalescence in the intertidal and shallow subtidal kelps *Lessonia spicata* and *L. berteroana* (Laminariales, Heterokonthophyta). *Journal of Applied Phycology* 26: 1107–1113.

Graham M.H., Vásquez J.A. & Buschmann A.H. 2007. Global ecology of the giant kelp *Macrocystis* from ecotypes to ecosystems. *Oceanography and Marine Biology: an Annual Review* 45: 39–88.

Katsaros C., Motomura T., Nagasato C. & Galatis B. 2009. Diaphragm development in cytokinetic vegetative cells of brown algae. *Botanica Marina* 52: 150–161.

Kelly E. (ed.). 2005. *The role of kelp in the marine environment*. Irish Wildlife Manuals, No. 17. National Parks and Wildlife Service, Department of Environment, Heritage and Local Government, Dublin, Ireland. 123 pp.

Kumar N.M. & Gilula N.B. 1996. The gap junction review communication channel. *Cell* 84: 381–388.

La Claire J.W. II. 1981. Occurrence of plasmodesmata during infurrowing in a brown alga. *Biology of the Cell* 40: 139–142.

Lucas W.J. & Lee J. 2004. Plasmodesmata as a supracellular control network in plants. *Nature Reviews Molecular Cell Biology* 5: 712–726.

Maggs C.A., Verbruggen H. & De Clerck O. 2007. Molecular systematics of red algae: building future structures on firm foundations. In: *Unravelling the algae: the past, present, and future of algal systematics* (Ed. by J. Brodie & J. Lewis), pp.103–121. Taylor and Francis, Boca Raton, Florida.

Makowski L., Caspar D.L., Phillips W.C., Baker T.S. & Goodenough D.A. 1984. Gap junction structures. VI. Variation and conservation in connection conformation and packing. *Biophysical Journal* 45: 208–218.

Martin P. & Zucarello G. 2012. Molecular phylogeny and timing of radiation in *Lessonia* (Phaeophyceae, Laminariales). *Phycological Research* 60: 276–287.

Nagasato C. & Motomura T. 2002. Ultrastructural study on mitosis and cytokinesis in *Scytosiphon lomentaria* zygotes (Scytosiphonales, Phaeophyceae) by freeze-substitution. *Protoplasma* 219: 140–149.

Nagasato C., Inoue A., Mizuno M., Kanazawa K., Ojima T., Okuda K. & Motomura T. 2010. Membrane fusion process and assembly of cell wall during cytokinesis in the brown alga, *Silvetia babingtonii* (Fucales, Phaeophyceae). *Planta* 232: 287–298.

Oparka K.J., Roberts A.G., Boevink P., Santa Cruz S., Roberts I.M., Pradel K.S., Imlau A., Kotlizky G., Sauer N. & Epel B. 1999. Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* 97: 743–754.

Paine R. 1990. Benthic macroalgal competition: complications and consequences. *Journal of Phycology* 26: 12–17.

Provasoli L. 1968. Media and prospects for the cultivation of marine algae. In: *Cultures and collections of algae* (Ed. by A. Watanabe, A Hattori), pp. 63–75. Japanese Society Plant Physiology, Hakone, Japan.

Ramírez, M.E. & Santelices B. 1991. Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica. *Monografías Biológicas* 5: 1–437.

Raven J.A. 2003. Long-distance transport in non-vascular plants. *Plant, Cell and Environment* 26: 73–85.

Reynolds E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *The Journal of Cell Biology* 17: 208–212.

Rodriguez D.C., Oróstica M.H. & Vásquez J.A. 2014. Coalescence in wild organisms of the intertidal population of *Lessonia berteroana* in northern Chile: management and sustainability effects. *Journal of Applied Phycology* 26: 1115–1122.

Santelices B. 1989. Algas marinas de Chile. Distribución. Ecología. Utilización. Diversidad. Ediciones Universidad Católica de Chile, Santiago. 399 pp.

Santelices B. 2004. A comparison of ecological responses among aclonal (unitary), clonal and coalescing macroalgae. *Journal of Experimental Marine Biology and Ecology* 300: 31–64.

Santelices B., Correa J., Meneses I., Aedo D. & Varela D. 1996. Sporeling coalescence and intraclonal variation in *Gracilaria chilensis* (Gracilariales, Rhodophyta). *Journal of Phycology* 32: 313–322.

Santelices B., Correa J., Aedo D., Hormazábal M., Flores V. & Sánchez P. 1999. Convergent biological processes among coalescing Rhodophyta. *Journal of Phycology* 35: 1127–1149.

Schmitz K. & Kuhn R. 1982. Fine structure, distribution and frequency of plasmodesmata and pits in the cortex of *Laminaria hyperborea* and *L. saccharina*. *Planta* 154: 385–392.

Schmitz K. & Srivastava L. 1974. Fine structure and development of sieve tubes in *Laminaria groenlandica* Rasenv. *Cytobiologie* 10: 66–87.

Schmitz K. & Srivastava L. 1975. On the fine structure of sieve tubes and the physiology of assimilate transport in *Alaria maginata*. *Canadian Journal of Botany* 53: 861–876.

Schmitz K. & Srivastava L. 1976. The fine structure of sieve elements of *Nereocystis lutkeana*. *American Journal of Botany* 63: 679–693.

Segovia N. 2009. Microsatélites de *Lessonia nigrescens* revelan que existe ocurrencia diferencial de fusión de individuos en un mismo disco de adhesión en sectores de alta y baja densidad de plantas. Tesis Biólogo Marino, Facultad de Ciencias del Mar, Universidad Católica del Norte, 40 pp.

Segovia N., Vásquez J.A., Faugeron S. & Haye P.A. 2014. On the advantage of sharing a holdfast: effects of density and occurrence of kin aggregation in the kelp *Lessonia berteroana*. *Marine Ecology* doi: 10.1111/maec.12206.

Silberfeld T., Leigh J. W., Verbruggen H., Cruaud C., de Reviers B. & Rousseau F. 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the 'brown algal crown radiation'. *Molecular Phylogenetics and Evolution* 56: 659–74.

Sokal R.R. & Rohlf F.J. 1995. *Biometry: the principles and practice of statistics in biological research,* 3rd ed. W.H. Freeman, New York. 887 pp.

Stegemann S. & Bock R. 2009. Exchange of genetic material between cells in plant tissue grafts. *Science* 324: 649–651.

Terauchi M., Nagasato C., Kajimura N., Mineyuki Y., Okuda K., Katsaros C. & Motomura T. 2012. Ultrastructural study of plasmodesmata in the brown alga *Dictyota dichotoma* (Dictyotales, Phaeophyceae). *Planta* 236: 1013–1026.

Thiel M., Macaya E.C., Acuña E., Arntz W.E., Bastias H., Brokordt K., Camus P.A., Castilla J.C., Castro L.R., Cortés M., Dumont C.P., Escribano R., Fernandez M., Gajardo J.A., Gaymer C.F., Gomez I., González A.E., González H.E., Haye P.A., Illanes J.-E., Iriarte J.L., Lancellotti D.A., Luna-Jorquera G., Luxoro C., Manriquez P.H., Marín V., Muñoz P., Navarrette S.A., Perez E., Poulin E., Sellanes J., Sepúlveda H.H., Stotz W., Tala F., Thomas A., Vargas C.A., Vasquez J.A. & Vega J.M.A. 2007. The Humboldt current system of northern and central Chile: oceanographic processes, ecological interactions and socioeconomic feedback. *Oceanography and Marine Biology: an Annual Review* 45: 195–344.

Ueki C., Nagasto C., Motomura T. & Saga N. 2008. Reexamination of the pit plugs and the characteristic membranous structures in *Porphyra yezoensis* (Bangiales, Rhodophyta). *Phycologia* 47:5–11.

Vásquez J.A. 1992. *Lessonia trabeculata*, a subtidal bottom kelp in northern Chile: a case of study for a structural and geographical comparison. In: *Coastal plant communities of Latin America* (Ed. by U. Seeliger), pp. 77–89. Academic, San Diego, California.

Vásquez J.A., Tala F., Vega J.M.A., Zúñiga S., Edding M. & Piaget N. 2008. Bases ecológicas y evaluación de usos alternativos para el manejo de praderas de algas pardas de la III y IV regiones. Fondo de Investigación Pesquera, Informe Final No. 2005-22, 220 pp. http://www.fip.cl

Vásquez J.A., Piaget N. & Vega J.M.A. 2012. Chilean *Lessonia nigrescens* fishery in northern Chile: how do you harvest is more important than how much do you harvest. *Journal of Applied Phycology* 24: 417–426.

Vásquez J.A., Zuñiga S., Tala F., Piaget N., Rodríguez D.C. & Vega J.M.A. 2014. Economic valuation of kelp forests in northern Chile: values of goods and services of the ecosystem. *Journal of Applied Phycology* 26: 1081–1088.

Vega J.M.A., Broitman B.R. & Vásquez J.A. 2014. Monitoring the sustainability of *Lessonia nigrescens* (Laminariales, Phaeophyceae) in northern Chile under strong harvest pressure. *Journal of Applied Phycology* 26: 791–801.

Villouta E. & Santelices B. 1986. *Lessonia trabeculata* sp. nov. (Laminariales, Phaeophyta), a new kelp from Chile. *Phycologia* 25: 81–86.

Wernberg T. 2005. Holdfast aggregation in relation to morphology, age, attachment and drag for the kelp *Ecklonia radiata*. *Aquatic Botany* 82: 168–180.

Xiao-cai G., Bo-ji W., Hui J. & Guo-chang Z. 1995. Formation of interfamilial cell symplast during cell co-culture *in vitro*. *Acta Botanica Sinica* 37(5): 339–345.

Xu H., Deckert R.J. & Garbary D.J. 2008. *Ascophyllum* and its symbionts. X. Ultrastructure of the interaction between *A. nodosum* (Phaeophyceae) and *Mycophycias ascophylli* (Ascomycetes). *Botany* 86: 185–193.

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