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Short communication

The role of the secondary attachment disc in the vegetative propagation of *Chondracanthus chamissoi* (Gigartinales; Rhodophyta)

Felipe Sáez, Juan Macchiavello, Erika Fonck, Cristian Bulboa *

Departamento de Biología Marina, Facultad Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

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Abstract

The reattachment and vegetative growth of tetrasporophytic and cystocarpic fronds of *Chondracanthus chamissoi* by means of secondary attachment discs was evaluated *in vitro*. Our results show that both the reattachment of fronds and sprouting of new shoots from secondary attachment discs, occurred in both life cycle phases. We did not observe statistical differences between cystocarpic and tetrasporophytic fronds, which have a similar ability to reattach and produce new shoots. Reattachment was observed after 5 d and reached $54 \pm 9\%$ (mean ± S.D.). The new shoots started sprouting 20 d after reattachment, with a growth rate of $5 \pm 1\%\ d^{-1}$ (mean ± DS). Our results show that the formation of secondary attachment discs is not only an effective form of reattachment for drifting fronds but also an important strategy to generate new individuals.

Q2 *Keywords:* Carrageenan; Chile; *Chondracanthus chamissoi*; Propagule; Seaweed; Vegetative propagation

1. Introduction

The fragmentation of thalli can be produced by abiotic and biotic factors and is a common event occurring in benthic marine algae (Thomsen and Wernberg, 2005). It has been demonstrated in various species of algae, that fragmentation is an effective method for vegetative dispersal (Smith and Walters, 1999). In macroalgae where sexual reproduction has not been registered, or is reduced to brief time intervals, fragmentation has been mentioned as the primary means of population maintenance (Perrone and Cecere, 1997). However, the success of this method as a reproductive mechanism would depend on the reattachment capacity of the detached thalli.

Reattachment of thalli can occur in a number of ways, including the formation of rhizoids (Smith and Walters, 1999) or secondary attachment discs, which are formed if the apical regions of lateral branches come in contact with the substratum (Perrone and Cecere, 1997; Pacheco-Ruiz et al., 2005). For a number of Gigartinales, secondary attachment discs have been described to be functioning as anchoring structures, as well as participating in the formation of new individuals. In this case, they produce perennial discs with the capacity to form erect axes during the growing season and thereby providing an extended temporal and spatial persistence of the species (Pacheco-Ruiz et al., 2005).

Chondracanthus chamissoi (C. Agardh) Kützing is a red alga, distributed along the coast of Chile from Iquique (20°S) to Ancud (42°S), inhabiting the low intertidal and subtidal to a depth of 15 m. It is harvested and exported, principally to the Asian market. It has been observed that reattachment occurs when detached thalli are trapped in hard substrate (Macchiavello et al., 2003), after which the rapid formation of secondary attachment discs occurs. This mechanism was suggested as an important strategy for the reattachment of detached *C. chamissoi* thalli in northern Chile (González et al., 1997), suggesting that the thalli are capable of growing and restoring their individual condition after reattachment (Macchiavello et al., 2003).

In this study we propose that the formation of secondary attachment discs takes part not only in the reattachment of thalli, but is also an important mechanism involved in the vegetative propagation of *C. chamissoi*, once the secondary attachment discs have the capacity to grow independently from the thalli which originated them. Here we evaluated *in vitro* the reattachment of thalli of *C. chamissoi* and the ability of secondary attachment discs, to produce new shoots.

Q1 * Corresponding author. Tel.: +56 51 209931; fax: +56 51 209812.
E-mail address: crbulboa@ucn.cl (C. Bulboa).

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2. Materials and methods

The fronds were obtained by diving at depths of 4–5 m, in La Herradura Bay (28°58′4.1″S; 71°21′43.1″W). Tetrasporophytic and cystocarpic thalli were separated and washed with microfiltered seawater. Fifteen thalli were placed in 1000 mL aquaria (three aquaria for each phase) with sterilized seawater, enriched with von Stosh medium diluted to 50%. A layer of *Argopecten purpuratus* shell fragments was placed in the aquaria, upon which the thalli were deposited. The cultivation conditions were maintained constant throughout the experiment at 15 ± 1 °C, 12 h photoperiod, 60 ± 10 μmol photons m^{-2} s^{-1} and permanent aeration. The cultivation medium was changed weekly. After 20 d of culture reattachment was evaluated and expressed as percentage of attached fragments in relation to the total number of fragments contained in each aquarium for each reproductive phase. Once the formation of secondary attachment discs was observed, they were separated from the rest of the thalli, taking care not to detach them from the substratum. The secondary attachment discs were maintained in the same cultivation conditions previously described. New erect axes were observed and photographed at 25 and 45 d after reattachment and the length was registered with the help of the Image Pro Plus (Version 4.5) software. Growth rates (GR) of the erect newly formed axes were calculated following the equation: $GR = [(L_t/L_o)^{1/t} - 1] \times 100$, where L_o = initial length, L_t = final length and t = 20 d. A one-way ANOVA was used to evaluate differences in the reattachment percentages and growth rates between life cycle phases.

3. Results

Reattachment of lateral branches was observed for some branches beginning at day 5. After this, secondary attachment discs increased gradually in diameter, forming disc shaped crusts (Fig. 1A–C). The mean reattachment percentage was 54 ± 9% (mean ± S.D.), and no differences were observed between cystocarpic and tetrasporophytic thalli (Table 1). In some cases more than one secondary attachment disc was observed in both reproductive phases.

After 25 d, the formation of erect axes, located in the periphery of the discs, was observed in all secondary attachment discs (Fig. 1D and E). Twenty days after their appearance (45 d of culture), the erect axes reached mean lengths of 0.8 ± 0.3 mm (mean ± S.D.), with growth rate of 5.1 ± 1.3% (mean ± S.D.). Growth rate was not statistically different between cystocarpic and tetrasporophytic thalli (Table 1).

Table 1
One-way ANOVA used to evaluate differences between two life cycle phases of *C. chamissoi*, for reattachment of the thalli and growth rate of new shoots

Source	d.f.	Mean square	*F*-value	*P*-value
Reattachment				
Life cycle phases	1	266.666	0.4	0.561
Growth rate				
Life cycle phases	1	4.570	1.861	0.244

4. Discussion

It is known that *C. chamissoi* presents two reproductive strategies, the production of spores and the reattachment of fragmented thalli. The spores are produced seasonally in the spring and summer months (González et al., 1997; Macchiavello et al., 2003), with clear synchronization between the fertility and the period of greatest vegetative growth (Bulboa and Macchiavello, 2001), incorporating new individuals into the population, when there is a higher chance of survival (Bulboa, 2006). On the other hand, the fragmentation of thalli in this species, as well as its reattachment capacity, has been observed during the entire year, although with greater intensity in the period of maximum biomass accumulation (Macchiavello et al., 2003).

Until now, it has been assumed that the only function of secondary attachment discs was to provide anchorage for detached fronds of *C. chamissoi*. However, our results show that the formation of secondary attachment discs also plays an important role as a vegetative propagation strategy, because they have the capacity to persist, once they have been isolated from the original thallus, and generate growing fronds that can develop independently from the mother branch. These new individuals can act as propagation structures which colonize new areas, as has been described for other Gigartinales elsewhere (Perrone and Cecere, 1997; Pacheco-Ruiz et al., 2005).

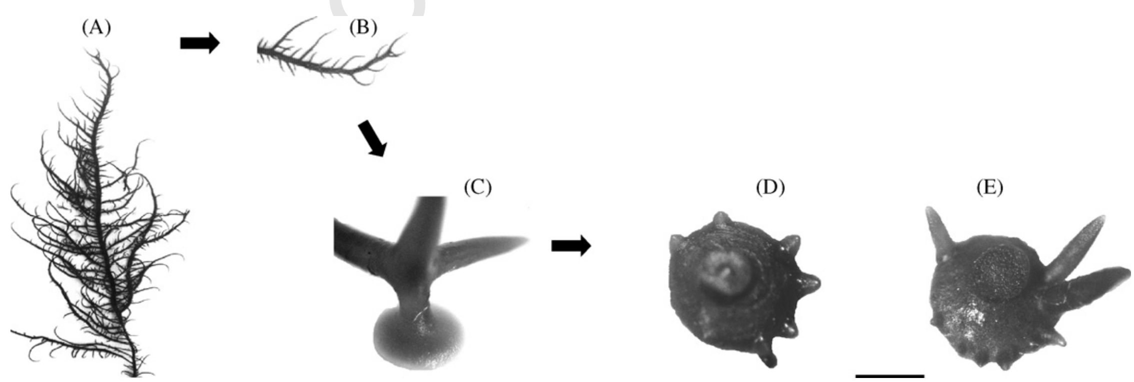

Fig. 1. Sequence involved in the vegetative propagation of *C. chamissoi*. (A and B) thallus detachment. (C) Thallus reattachment by means of secondary attachment discs formation. (D and E) Growth of new fronds from secondary attachment discs. Bar, 2 mm.

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We believe that *C. chamissoi* presents a high potential for secondary attachment discs formation, because its thalli have numerous lateral ramifications which are potential generators of secondary attachment discs. In addition, a high number of detached fronds in situ, up to 129 fronds m^{-2}, has been reported (Macchiavello et al., 2003). If we consider that each frond can reattach in more than one secondary attachment disc and that each disc can produce more than one shoot, the potential to produce new individuals by this mechanism may become highly important for the propagation of this species.

The growth rates of the erect axes generated from secondary attachment discs were similar to those described previously by Bulboa and Macchiavello (2001) for both life cycle phases. We believe that the relatively rapid formation of secondary attachment discs in experimental systems could be utilized as a source of biomass propagation for commercial cultures (Bulboa et al., 2005) or restoration programs of *C. chamissoi* based on propagation by means of secondary attachment discs, in the same way as it has been reported for *Solieria filiformis* (Perrone and Cecere, 1997).

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