Short communication

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Tetraspore germination of two vulnerable marine algae, *Gelidium canariense* and *G. arbusculum* (Rhodophyta, Gelidiales)

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Abstract: *Gelidium canariense* and *Gelidium arbusculum* coexist in the upper sublittoral zone in the Canary Islands and are the only Gelidiales registered as vulnerable species. Spore germination and the formation of rhizoids are vital steps for the successful growth of new plants. We investigated the initial germination stages of tetraspores and the growth of the primary rhizoid in these two vulnerable species. Both Gelidiales exhibited *Gelidium*-type germination. However, liberated tetraspores of *G. canariense* and *G. arbusculum* germinated earlier than those of other species of the Gelidiales. The growth curves of primary rhizoids were similar in both species.

Keywords: agarophytes; endemic species; germ tube; growth curve; initial stages.

Gelidium canariense (Grunow) Seoane Camba *ex* Haroun, Gil-Rodríguez, Díaz de Castro *et* Prud'homme van Reine is an endemic macroalga of the Canary Islands that coexists in the upper sublittoral zone with *G. arbusculum* Bory ex Børgesen. The latter species has been documented in the Canary Islands and Northwest Africa (Lawson and John 1987, Harper and Garbary 1997, John et al. 2003), but doubt has been cast on the validity of records from outside the Canaries (Price et al. 1988, John et al. 2004). These benthic macroalgae are canopy-forming species of the exposed rocky shores in the Canarian archipelago (Lawson and Norton 1971, Betancort and González 1991, Pinedo and Afonso-Carrillo 1994, Sangil et al. 2004, Alfonso 2016) and are registered as vulnerable species in the catalogue of threatened species of the Canary Islands (BOC 2010). Previous studies have focused on their reproductive phenology (Polifrone et al. 2012, Alfonso et al. 2017) and other aspects of the reproductive process, such as spore culture in axenic conditions (García-Jiménez et al. 1999) and the variability in sporangial types (Rico et al. 2005). However, no study has yet described the initial stages of tetraspore germination or the development of primary rhizoids.

The attachment to and colonization of a new substratum by spores is one of the most important processes in the life cycle of benthic marine algae (Fletcher and Callow 1992). Spores (mainly haploid tetraspores and diploid carpospores) are the natural form of dispersal in most Rhodophyta, and several factors may affect the germination process. Studies of spore germination patterns have been important for taxonomic approaches (Chemin 1937, Guiry 1990). In Gelidiales, the settlement of spores triggers the germination process, which begins with the reorganization of cell organelles, undergoes a polar migration, and ends with the formation of the germ tube (Macler and West 1987, Santelices 1988, Rico and Guiry 1997, Bouzon et al. 2005, 2006). This characteristic pattern of division is known as *Gelidium*-type germination (Chemin 1937, Inoh 1947).

The spores represent a critical stage in the life cycles of algae (Agrawal 2009). Thus, studies of the initial stages of spores in vulnerable species are crucial. The spore germination pattern is common in the Gelidiales species according to old references (Chemin 1937, Inoh 1947). However, the time intervals of the initial stages of spores for different Gelidiales has not been examined. In this study, we describe and compare the duration of initial stages of tetraspore germination of *G. canariense* and *G. arbusculum* and the growth curve of the primary rhizoids for 7 days.

Tetrasporophytes of *G. canariense* and *G. arbusculum* were collected at Puerto de la Cruz on the north rocky shore of Tenerife in March 2017. The samples were transported to the laboratory at ambient temperature in dark

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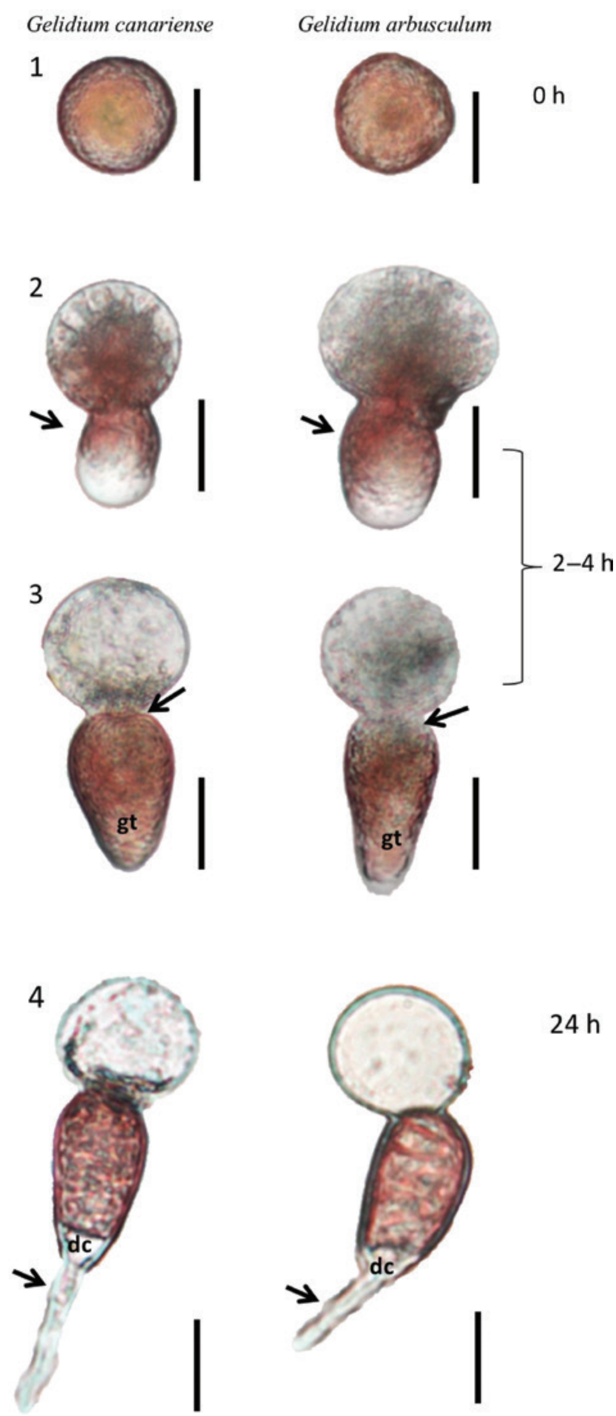
containers, and they were meticulously cleaned of macroepiphytes with a brush and filtered seawater.

Individuals bearing tetrasporangial sori were incubated in the dark at 20°C for 12 h in beakers with filtered seawater in order to release tetraspores. The released tetraspores were cultivated on slides in Petri dishes under the same conditions explained above and exposed to fluorescent lightning [Philips TL-D 36W 840 Super 80 (MASTER), Madrid, Spain] at 40 μmol photons $m^{-2}s^{-1}$ and a 12 h:12 h light:dark photoperiod. The growth of the primary rhizoid was studied by measuring the length of the hyaline distant cell of the germ tube. Morphological changes in the early stages of development of both species were analysed with a Leica DM1000 light microscope (Leica Microsystem, Wetzlar, Germany), and photographs were taken with a Canon EOS1200D (Canon Inc., Madrid, Spain) fitted to this microscope. Rhizoid length was measured on days 1 (*G. canariense*, *G. arbusculum* n = 5), 3 (*G. canariense*, *G. arbusculum* n = 10), 5 (*G. canariense* n = 13, *G. arbusculum* n = 8) and 7 (*G. canariense* n = 4, *G. arbusculum* n = 10) after spore release using the open source image processing program ImageJ2 (Schindelin et al. 2015). The filtered seawater of the Petri dishes was changed every 2 days.

After 12 h of incubation in dark conditions, spores with no cell wall were released in both species. Tetraspores were deep red and spherical, measuring 29.02 ± 3.2 μm (mean ± SD, n = 13) and 27.75 ± 2.2 μm (mean ± SD, n = 12) in diameter for *G. canariense* and *G. arbusculum*, respectively (Figure 1). The median diameters of tetraspores of *G. canariense* and *G. arbusculum* were similar to other Gelidiales species, such as *G. pusillum* (Stackhouse) Le Jolis (28 μm), *G. crinale* (Hare ex Turner) Gaillon (26.8 μm), and *Gelidiophycus divaricatus* (G. Martens) G.H. Boo, J.K. Park *et* S.M. Boo (30.5 μm) (Katada 1955, Chihara and Kamura 1963, Ngan and Price 1979).

Between 2 and 4 h after spore release and adhesion, the protoplasmic content began to migrate and initiated the formation and elongation of the germ tube (Figures 2 and 3) in both *G. canariense* and *G. arbusculum*. A cell wall was formed around the germ tube during its development. The distant cell of the multicellular tetrasporeling elongated and developed into the primary rhizoid (Figure 4), whereas the proximal cells became the erect thallus after successive divisions. The pattern of spore division in *G. canariense* and *G. arbusculum* exhibits *Gelidium*-type germination (Chemin 1937, Inoh 1947, Chihara and Kamura 1963, Macler and West 1987, Santelices 1988, Rico and Guiry 1997, Bouzon et al. 2005, 2006). However, specific time intervals of the initial stages of spore germination have only been described in *G. floridanum* W.R. Taylor (Bouzon et al. 2005, 2006, Simioni et al. 2014, Filipin



Figures 1–4: Stages of tetraspore germination in *Gelidium canariense* and *G. arbusculum*.
(1) 0 h after release (0 h). Tetraspore after release and settlement, without cell wall. (2 and 3) Between 2 and 4 h after tetraspore release (2–4 h): (2) Formation of the germ tube, showing the migration of the protoplasmic content and the protrusion of the germ tube (arrows). (3) Germ tube (gt) surrounded by cell wall and separated from the spore (arrows). (4) 24 h after tetraspore release (24 h). Multicellular sporeling showing the elongation of the distal cell (dc) and the differentiation of the primary rhizoid (arrows). Scale bars 20 μm.

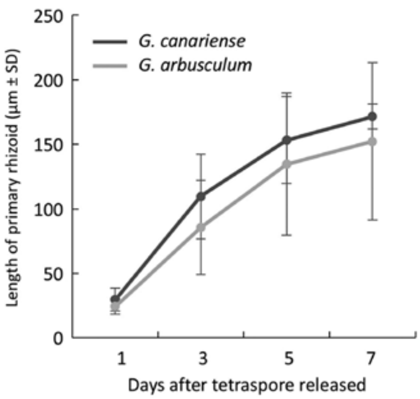


Figure 5: Growth curve of the primary rhizoid in *Gelidium canariense* and *G. arbusculum* during 7 days after tetraspore released. Day 1 (*G. canariense*, *G. arbusculum* n = 5), 3 (*G. canariense*, *G. arbusculum* n = 10), 5 (*G. canariense* n = 13, *G. arbusculum* n = 8) and 7 (*G. canariense* n = 4, *G. arbusculum* n = 10).

et al. 2016). In contrast to *G. floridanum*, in which the germination process begins 6 h after spore release, the tetraspore germination of *G. canariense* and *G. arbusculum* is triggered only 2–4 h after spore release. The success of spore and zygote attachment and further development determines recruitment, growth, vertical distribution and hence, survival of the species (Scariot et al. 2013). In addition, tetraspores lack the resistant vegetative tissues of terrestrial plants (Wiencke et al. 2006, Roleda et al. 2007, Navarro et al. 2010, Scariot et al. 2013), such that faster germination produces a higher chance of success for these vulnerable species.

The primary rhizoid displayed the same pattern in both species. The size increased more than 4 times between day 1 and day 7 after tetraspore release (Figure 5). In most Gelidiales species, the primary rhizoids play a key role in adhering plants to substrates and consolidating them for later growth (Perrone et al. 2006). Previous studies have shown the sporeling stage several days after germination (Chemin 1937, Inoh 1947, Macler and West 1987, Santelices 1988, Rico and Guiry 1997); however, this study shows the rhizoidal growth curve of two species of Gelidiales for the first time.

The early germination of *G. canariense* and *G. arbusculum*, together with the rapid growth of rhizoids, could be a survival strategy for these upper subtidal species.

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Bionotes

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