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[Note]

Unusual Rhizoidal Development in *Bangia* (Bangiales, Rhodophyta) – Another Form of Vegetative Reproduction?

Christian Boedeker[1]*, Tracy J. Farr[2] and Wendy A. Nelson[2]

[1]*National Herbarium of the Netherlands, Leiden University Branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands*
[2]*National Institute of Water and Atmospheric Research, Private Bag 14-901, Kilbirnie, Wellington, New Zealand*

The gametophytic filaments of two genetically distinct taxa of *Bangia* from New Zealand showed unusual rhizoidal development in comparative culture experiments. In the past *Bangia* has been reported to possess simple, colourless rhizoids that extend from the basal cells of the unbranched filaments, whereas in this study the rhizoids observed became pigmented and multicellular. A reversal of growth direction occurred and filamentous extensions developed from the rhizoids under some culture conditions. These extensions were either prostrate or resembled new gametophytic filaments. This is the first report for filamentous members of the Bangiales of the development of such stolon-like rhizoids, apparently serving as a form of vegetative reproduction.

Key Words: Bangia, perennial phase, stolon-like rhizoids, vegetative reproduction

Filamentous algae currently placed in the genus *Bangia* Lyngbye (Bangiales, Rhodophyta) are found worldwide on hard substrates in marine habitats in the upper intertidal zone (Lüning 1990; Brodie and Irvine 2003; Broom *et al.* 2004). The macroscopic gametophytic phase consists of superficially simple, unbranched, uniseriate filaments (Fig. 1) that can become multiseriate by radial cell divisions at maturity (Figs 2, 3). Members of the Bangiales have heteromorphic sexual life histories involving a filamentous (the recently erected freshwater genus *Bangiadulcis* (Nelson 2007), *Bangia*, *Dione*, *Minerva*, *Pseudobangia*), or leafy (*Porphyra*) gametophyte and a microscopic sporophyte called conchocelis phase (Figs 4, 6) (Drew 1956; Garbary *et al.* 1980). The gametophytic phase of both genera can reproduce asexually through neutral spores/archaeospores released by vegetative cells of the thallus (terminology follows Nelson *et al.* 1999).

In *Bangia* the first cell division of a haploid spore results in two daughter cells, one of which becomes the basal cell. All cell divisions in *Bangia* spp. are intercalary: a uniseriate filament develops initially and the modified basal cell does not undergo any further cell divisions (Sommerfeld and Nichols 1970). Only the upper part of the basal cell is pigmented and a protrusion of the lower part forms the primary rhizoid, attaching the filament to the substratum (Geesink 1973). These rhizoids are colourless and show no internal structures. Secondary rhizoidal outgrowths may grow intramatrically from additional lowermost cells within a filament (Kylin 1921; Smith 1969), and can lead to a branched appearance of the holdfast when penetrating the surrounding sheath (Sommerfeld and Nichols 1970). The development of secondary rhizoids from intercalary cells also has been observed (Gargiulo *et al.* 2001).

In this study the unusual stolon-like development of the rhizoids of two genetically distinct taxa of *Bangia* from New Zealand is reported. Field material was collected in marine intertidal habitats on rocky shores on the west coast of the South Island of New Zealand in 2000 for each of the two taxa *Bangia* sp. BMW (culture BCB2, S41°46.47′, E171°11.63′) and *Bangia* sp. BGA (cultures B1HH and BCS, S42°26.31′, E171°11.63′ and S41°35′, E171°53.7′, respectively). Unialgal stock cultures were established in the algal culture collection at the Museum of New Zealand Te Papa Tongarewa (WELT, Holmgren *et al.* 1990), now transferred to the National Institute of Water and Atmospheric Research, Wellington, New Zealand. Collections were identified as *Bangia* in the field, and DNA sequencing was carried out as part of a larger project examining diversity in Bangiales. From the results of nSSU sequencing, strains

*Corresponding author (boedeker@nhn.leidenuniv.nl)

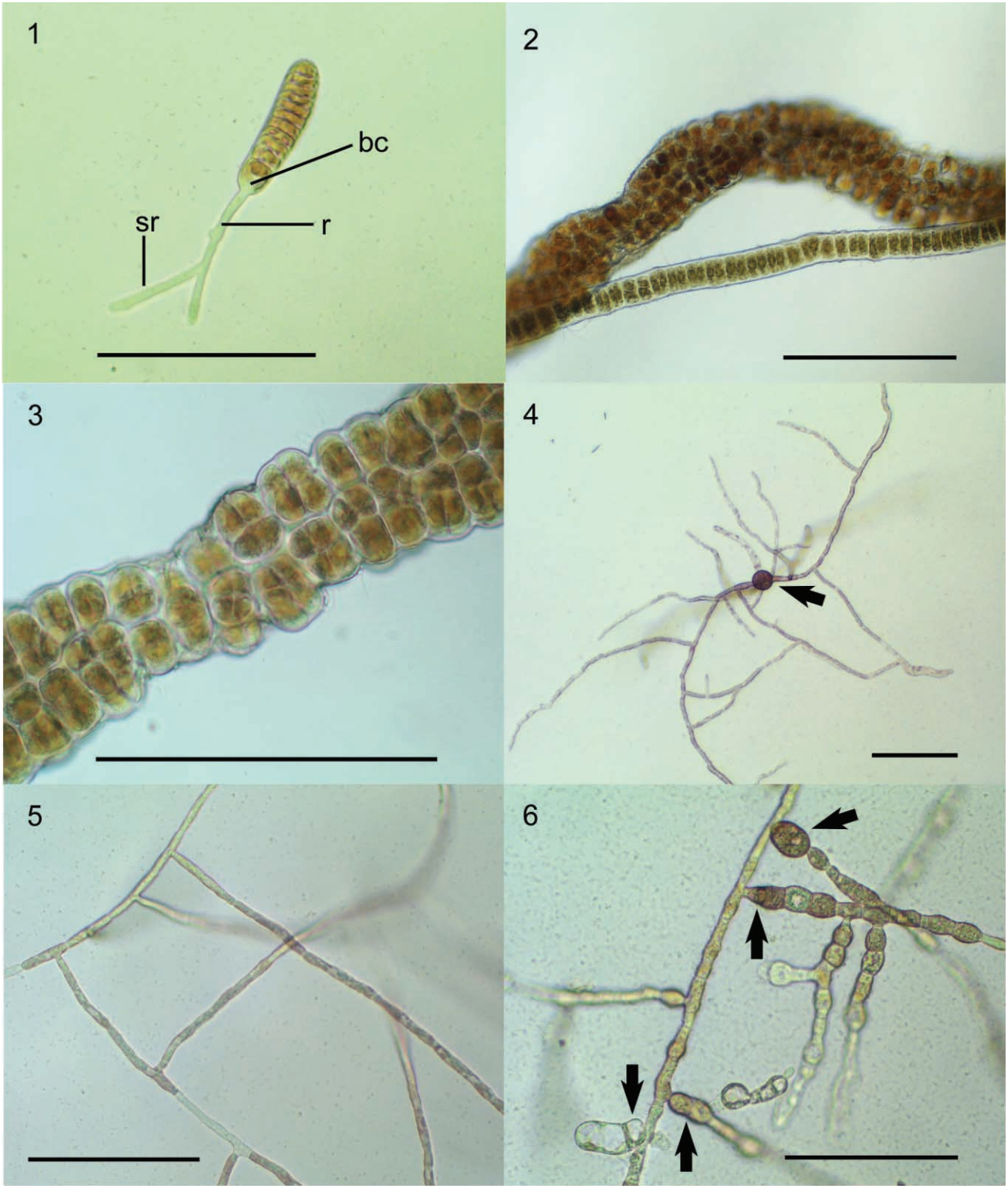

Fig. 1. Filament of *Bangia* sp. BMW displaying normal rhizoidal development (cultured for 14 days at 12°C, 12:12 h L:D, 34 psu).
Fig. 2. Comparison of uni- and multiseriate filaments (*B. sp.* BFK, see Broom *et al.* 2003 for details).
Fig. 3. Female multiseriate filament consisting of zygotosporangia (*B. sp.* BFK).
Fig. 4. Sporophytic conchocelis phase (*B. sp.* BTS, see Broom *et al.* 2003 for details) developing from a phyllospore (arrow).
Fig. 5. Rectangular branching pattern of sterile conchocelis filaments of *B. sp.* BGA after 77 days at 12°C, 12:12 h L:D, 34 psu.
Fig. 6. Conchocelis filaments of *B. sp.* BMW with conchosporangial branches (arrows) after 70 days at 12°C, 12:12 h L:D and another 21 days at 10°C, 9:15 h L:D, 34 psu.

were assigned to *Bangia* taxa as described in Broom *et al.* (2004, see also for the corresponding GenBank accession numbers). Stock cultures of the taxa were maintained at 12°C, 12:12 h light:darkness (L:D), 10-50 μmol photons m^{-2} s^{-1}, 34 psu.

Gametophytic filaments were grown from neutral spores/archaeospores released from filaments in culture after drying overnight and subsequent submersion. About 40 spores per treatment for each taxon were transplanted to cover slips, allowed to settle overnight, and then placed in petri dishes with f/2 growth medium (Guillard and Ryther 1962). The growth of the developing filaments was investigated under five different culture conditions in laboratory experiments. Conditions were: (1) 10°C, 9:15 h L:D, 34 psu; (2) 12°C, 12:12 h L:D, 34 psu; (3) 15°C, 15:9 h L:D, 34 psu; (4) 20°C, 16:8 h L:D, 34 psu; (5) 12°C, 12:12 h L:D, 5 psu. Photon flux densities in all treatments were ca. 35 μmol photons m^{-2} s^{-1}. The development of the filaments was followed for 28 days. On days 1, 3, 7, 10, 14, 21 and 28 of the experiments the filaments were examined and measured using an Olympus CK2 inverted microscope and digital micrographs were taken of each filament with an Olympus DP10 digital camera (Olympus America Inc.).

A reversal of growth direction was observed in the filaments of the two *Bangia* taxa, with new growth occurring in the rhizoidal parts. In addition to extending apically due to intercalary cell divisions in the main filament, the rhizoidal part started extending in the opposite direction. The rhizoids were extending and became pigmented under some conditions (Fig. 7). After approximately 14 days, cell walls were visible in pigmented parts of the rhizoids of both taxa (Figs 8-10). These rhizoids continued to extend from the original filaments, and showed further cell divisions. After ca. 28 days some of the rhizoids showed stolon-like outgrowths resembling new gametophytic filaments (Figs 11, 12). One taxon (BMW) showed this unusual development of rhizoids only under the low salinity regime of condition 5 (5 psu), while the other taxon (BGA) displayed stolon-like rhizoids under conditions 2-4, with a higher percentage of filaments displaying stolon-like rhizoids at higher temperatures. After 28 days all filaments of BGA cultured at 20°C (condition 4) and all filaments of BMW at 5 psu (condition 5) had developed rhizoids with filamentous outgrowths.

The observed rhizoidal extensions have a lumpy appearance with an irregular outline. These rhizoids are 5-15 μm in diameter and have a thin cell wall (< 1 μm). The shape and length of cellular compartmentation in the rhizoidal parts is very variable, ranging from rounded or triangular cells (2-10 μm wide) to stretched rhizoidal parts (many times longer than broad). The extent of overall pigmentation in the rhizoids increased gradually with time, with short cells in the stolon-like parts being dark and densely pigmented while the the long rhizoidal parts were irregularly pigmented with stretched strands of connected cytoplasm.

This is the first time that stolon-like development of rhizoids of *Bangia* has been documented. The pigmentation, cell divisions and outgrowths of the rhizoids suggest a role beyond merely attaching filaments to the substrate; the stolon-like rhizoids and associated filaments produced in this way may contribute to population maintenance and growth through vegetative reproduction. In studies of other members of the Bangiales, various observations of asexual reproduction have been recorded. Dixon and Richardson (1969) reported "perennating basal fragments" of *Porphyra perforata* J. Agardh and *Bangia* at a site where the gametophyte phase was absent and no trace of the microscopic sporophyte phase had been detected. These perennating structures were described as pigmented cells which released vegetative cells that grew into new gametophytic thalli (*loc. cit.*). To our knowledge, similar stolon-like development of the rhizoids is not known from other red algae. The propagating rhizoids of the ulvophyte *Blidingia minima* var. *stolonifera* differ by being colourless and containing no nuclei or chloroplasts (Garbary and Tam 1989). Gargiulo *et al.* (2001) documented resting cells in both the macroscopic thalli and microscopic conchocelis stage of freshwater *Bangiadulcis* (as *Bangia atropurpurea*), as well as *in situ* germination of spores released by the conchocelis giving rise to new gametophytic filaments. We have observed both *in situ* germination of spores within *Bangia* filaments, and the development of conchocelis filaments in culture. Both of these clearly differ from the rhizoidal growth we report here. Furthermore, the conchocelis stage of the two *Bangia* taxa used here shows a very different overall morphology (Figs 4-6) from the described stolon-like rhizoids. Conchocelis filaments have relatively long and regular cells, more or less uniform in diameter in sterile branches, branched at right angles and having one to several ribbon-shaped parietal chloroplasts.

The stolon-like rhizoids reported here may function as perennial structures during unfavourable conditions: their prevalence at high temperature and/or low salinity indicates a taxon-specific response to stress, since the

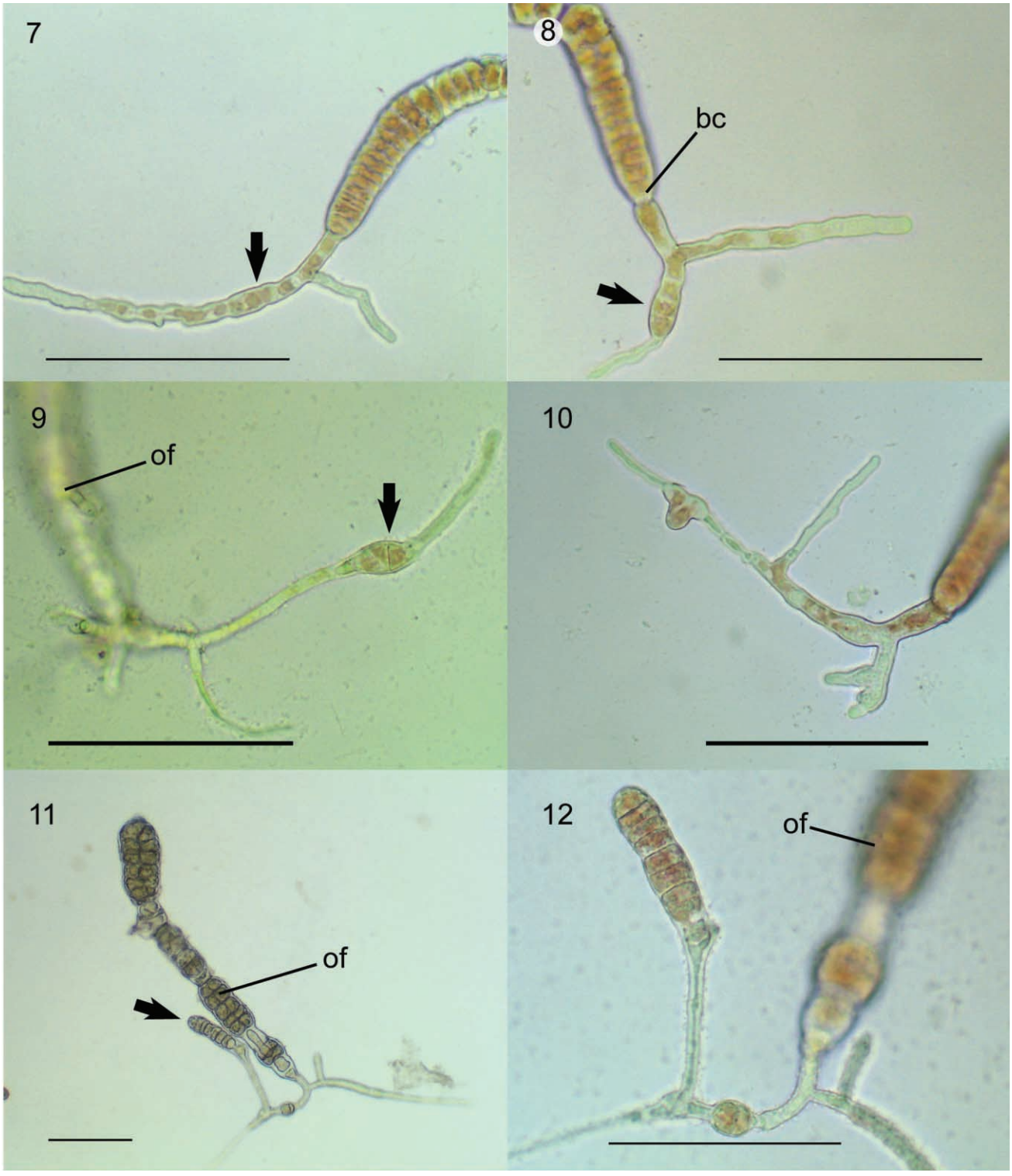

Fig. 7. Pigmented rhizoid (arrow) of *Bangia* sp. BMW after 14 days at 12°C, 12:12 h L:D, 5 psu.
Fig. 8. Visible cell walls (arrow) within rhizoid of *Bangia* sp. BMW after 14 days at 12°C, 12:12 h L:D, 5 psu.
Fig. 9. Clearly visible cells and cell walls (arrow) within rhizoid of Bangia sp. BGA after 21 days at 15°C , 15:9 h L:D, 34 psu.
Fig. 10. Pigmented rhizoid of *Bangia* sp. BMW with visible cell walls and beginning outgrowth (arrow) after 21 days at 12°C, 12:12 h L:D, 5 psu.
Fig. 11. New filamentous outgrowth (arrow) developing from a stolon-like rhizoid of *Bangia* sp. BGA after 27 days at 20°C, 16:8 h L:D, 34 psu.
Fig. 12. Enlargement of same filament.
bc = basal cell, of = original filament.

original material of both taxa had been acclimatised to a temperature of 12°C and full marine growth medium (34 psu). In our experiments the development of filaments extending from the rhizoids was observed early in the experiment (i.e. from day 7-10). Five other taxa of *Bangia* that were investigated (results not presented) did not show similar rhizoidal development under any of the described conditions.

There is no direct link between the geographical distribution of the taxa and the culture conditions under which the stolon-like rhizoids were observed, but the simulated conditions would at least sometimes occur in the natural habitats. *Bangia* sp. BMW only formed the described rhizoids at 5 psu and is often found in sites with lowered salinity, such as freshwater streams influenced by the tides. *Bangia* sp. BGA showed a higher percentage of filaments with rhizoidal extensions at higher temperatures. This taxon occurs further north than BMW, experiencing summer surface temperatures of 20°C or more in the north of the North Island.

Broom *et al.* (2004) examined diversity within the filamentous members of the Bangiales and reported on distinct lineages which they considered warranted consideration as new genera. Subsequently 3 new taxa have been described for filamentous Bangiales (Nelson *et al.* 2005, Müller *et al.* 2005). The taxa considered in this paper (BGA and BMW) both belong to the genus *Bangia* and fall into separate clades in a Bangiales phylogeny as discussed in Broom *et al.* (2004), possibly representing two separate species. The propagation of the rhizoids as described here is not a synapomorphic character, but seems to occurr independently in distant parts of phylogenetic trees.

Asexual reproduction plays an effective role in maintaining *Bangia* populations; some are known to be solely asexual (Sheath and Cole 1980; Sheath *et al*. 1985). Conditions suboptimal for reproductive development may have triggered the rhizoid development in our experiments. Members of the Bangiales possess a range of sexual and asexual reproductive methods, and this capacity is regarded as a major contributor to the success of this widely distributed family (Cole and Conway 1980). The observations reported here suggest a further vegetative mechanism that may be contributing to the perennation of populations of some taxa in this order.

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