Mycorrhiza (2011) 21:231–236
DOI 10.1007/s00572-010-0328-0

ORIGINAL PAPER

Structural changes to a mycothallus along a latitudinal transect through the maritime and sub-Antarctic

Kevin K. Newsham

Received: 21 April 2010 / Accepted: 27 June 2010 / Published online: 14 July 2010

Abstract Previous studies have shown the leafy liverwort *Cephaloziella varians* to associate consistently with fungi, typically the ericoid mycorrhizal symbiont *Rhizoscyphus ericae*, across a wide latitudinal gradient in the maritime and sub-Antarctic. Hitherto, however, there are no quantitative data on the intensity of colonisation of *C. varians* by fungal structures in the natural environment and how colonisation might vary with changing environmental conditions. A study is hence reported showing that the frequency of colonisation by fungal structures of *C. varians* alters along a latitudinal transect from South Georgia (54° S, 38° W) to Moutonnée Valley on Alexander Island (71° S, 68° W). The percentage of stem length colonised by dark septate (DS) hyphae increased significantly along the transect, from 30% at South Georgia to 97% at Moutonnée Valley. In contrast, the percentage of stem length colonised by hyaline hyphae decreased significantly, from 85% at South Georgia to 13% at Moutonnée Valley, and that colonised by hyphal coils similarly decreased from 71% at the former location to 15% at the latter. The frequencies of DS hyphae were negatively associated with mean annual and seasonal air temperatures, whereas those of hyaline septate hyphae and hyphal coils were positively associated with air temperatures. Coils at northerly locations were more convoluted than those at southerly locations. The data indicate that hyphal coils, usually associated with nutrient exchange between partners in ericoid mycorrhizas, do form in *C. varians* tissues in the maritime and sub-Antarctic, but that the frequency of these structures diminishes in colder habitats.

Keywords *Cephaloziella varians* · Dark septate endophytes · Hyphal coils · Leafy liverwort · *Rhizoscyphus ericae*

Introduction

The leafy liverwort *Cephaloziella varians* (Gottsche) Steph., the commonest and most widespread of Antarctic hepatics (Bednarek-Ochyra et al. 2000), is routinely colonised by fungal structures in the natural environment (Newsham 2010). Analyses of internal transcribed spacer (ITS) region sequences of ribosomal DNA (rDNA) of fungi isolated from *C. varians* collected from Antarctica have shown these hyphae to be chiefly formed by the ericoid mycorrhizal symbiont *Rhizoscyphus ericae* (D.J. Read) W. H. Zhuang & Korf. (Chambers et al. 1999; Upson et al. 2007). Koch's postulates experiments have shown that, in addition to forming dark septate (DS) hyphae on stem surfaces and hyaline hyphae within epidermal cells, *R. ericae* also forms hyphal coils in the bases of rhizoids and in the adjacent epidermal cells of *C. varians*, similar to those formed by *R. ericae* in the hair roots of ericaceous plant species (Upson et al. 2007). However, although bearing structural resemblances to ericoid mycorrhizas, in the absence of evidence of reciprocal benefits to both partners, associations such as that formed between *C. varians* and *R. ericae* are termed mycothalli, denoting potentially mutualistic associations between lower plants and fungi (Boullard 1988; Read et al. 2000).

In plants sampled directly from the natural environment, Williams et al. (1994) reported the formation of loose

K. K. Newsham (✉)
British Antarctic Survey, Natural Environment Research Council,
High Cross, Madingley Road,
Cambridge CB3 0ET, UK
e-mail: kne@bas.ac.uk

hyphal coils in the bases of rhizoids of *C. varians* at Granite Harbour in the continental Antarctic. Hyphal coils have also been shown to form in the bases of rhizoids and in the adjacent epidermal cells of plants sampled from Rothera Point on Adelaide Island and kept hydrated at a favourable temperature (4°C) for several weeks (Upson et al. 2007). However, there are no data in the literature on the frequency of hyphae or hyphal coils in the tissues of *C. varians* in the natural environment, and no information on whether fungal colonisation of *C. varians* tissues might vary with latitude or other environmental factors, such as air temperature. Knowledge of the frequency of fungal structures in the tissues of *C. varians* in the natural environment, and how the mycothallus responds to its environment, is thus limited. The study reported here hence addressed these issues by measuring the frequency of fungal structures in plants of *C. varians* sampled from six locations over a 1,875-km latitudinal transect through the maritime and sub-Antarctic.

Materials and methods

Three samples of healthy *C. varians* were collected from Schlieper Bay on South Georgia (54° 03′ S, 37° 52′ W), Foca Point and Factory Bluffs on Signy Island (60° 42′ S, 45° 39′ W and 60° 43′ S, 45° 36′ W, respectively), Rothera Point on Adelaide Island (67° 34′ S, 68° 07′ W) and Ablation and Moutonnée Valleys in the Ganymede Heights region of Alexander Island (70° 49′ S, 68° 25′ W and 70° 51′ S, 68° 25′ W, respectively; Fig. 1). There are wide differences between the plant communities at each of these locations, with extensive vascular plant cover (typically *Acaena magellanica, Callitriche antarctica, Deschampsia antarctica, Juncus scheuchzerioides, Parodiochloa flabellata* and *Rostkovia magellanica*) at Schlieper Bay on South Georgia, and extensive bryophyte cover (typically *Calliergon sarmentosum* and *Sanionia uncinata*) with scattered vascular plants (*D. antarctica* and *Colobanthus quitensis*) at the sites on Signy Island. Vascular plants are absent from the other sites, but there is extensive bryophyte cover at Rothera Point, where *C. varians* occurs with *S. uncinata* and scattered *Bryum pseudotriquetrum*, and very sparse bryophyte cover at Ablation and Moutonnée Valleys, where *C. varians* occurs with *B. pseudotriquetrum, Campylium polygamum* and *Hypnum* and *Plagiothecium* spp. (Smith 1988). Mean annual precipitation in the sub-Antarctic, where South Georgia is situated, is >900 mm per annum, and in the northern maritime Antarctic, where Signy Island is located, it is 350–500 mm per annum (Smith 1984). The majority of precipitation at these locations is in the form of rain during the summer. Precipitation at Adelaide and Alexander Islands, in the southern maritime Antarctic, is <350 mm per annum, with the majority falling as snow (Smith 1984). After collection, the plants were placed into clean polythene bags and were frozen at −20°C within several hours. After their return to the UK at this temperature, they were defrosted and the plants removed from the substrate on which they were growing.

Plant material was washed in water to remove debris, cleared in 10% potassium hydroxide for 72 h, washed five times in water, acidified for 1 h in 5% lactic acid and then stained for 24 h with 0.01% aniline blue in lactic acid. The stain was removed on absorbent paper, and the plants were destained for at least 24 h in 80% lactic acid. Thirty-six plants from each sample were mounted on glass slides in 80% lactic acid, were lightly squashed and examined for fungal structures at ×400 magnification under UV epifluorescence (BX51 microscope, Olympus UK Ltd.). Using the line intersection method described by McGonigle et al. (1990), the percentages of stem length colonised by (1) DS hyphae on stem surfaces (2) hyaline septate hyphae in stem epidermal cells and (3) hyphal coils in rhizoid bases and stem epidermal cells were calculated. In order to obtain accurate measurements, between 160 and 280 intersections were scored in each of the 18 samples (McGonigle et al. 1990). In addition, the percentage of all rhizoids observed that were colonised by hyaline hyphae, and the mean number of rhizoids mm^{-1} length of stem, were recorded.

Air temperature data, recorded over 8 years between 1988 and 2009 at Grytviken on South Georgia and at Signy and Rothera research stations, were obtained from the READER dataset (http://www.antarctica.ac.uk/met/reader/). The data were recorded at locations within 90 km of the sampling site on South Georgia and within 4 km of the sites on Signy and Adelaide islands. Air temperature data for the two sites on Alexander Island were recorded between 2000 and 2005 by an automatic weather station at Mars Oasis, located 115 km and 110 km to the south of Ablation and Moutonnée Valleys, respectively.

Linear regression was used to analyse associations between variables, initially with latitude as a predictor and annual and seasonal air temperatures (means of 5 or 8 years) as responses. Regression analyses were also made with latitude and mean air temperatures as predictors and the percentages of stem length colonised by fungal structures, the percentage of rhizoids colonised by hyphae, and the number of rhizoids mm^{-1} length of stem as responses. Further analyses were made using the number of rhizoids mm^{-1} length of stem as a predictor and the percentages of stem length colonised by fungal structures as responses. Precipitation was not included as a predictor or response variable in these analyses because accurate precipitation measurements are unavailable for each of the locations studied. One value for each of the six sampling locations was entered into regression analyses.

Results

There were significant reductions in air temperatures at higher latitudes along the transect from which *C. varians* was sampled: mean annual air temperature decreased from 2.5°C at South Georgia to −10.0°C at Moutonnée Valley (Fig. 1a, inset). Similar associations were found between latitude and seasonal air temperatures, with mean spring, summer, autumn and winter air temperatures respectively of 3.3°C, 6.1°C, 0.6°C and −0.7°C at South Georgia, compared with −7.9°C, −1.8°C, −11.3°C and −21.5°C at Moutonnée Valley, and significant decreases in each of these four parameters at higher latitudes along the transect (r^2 adj.=80.6–88.8%; all $P<0.010$).

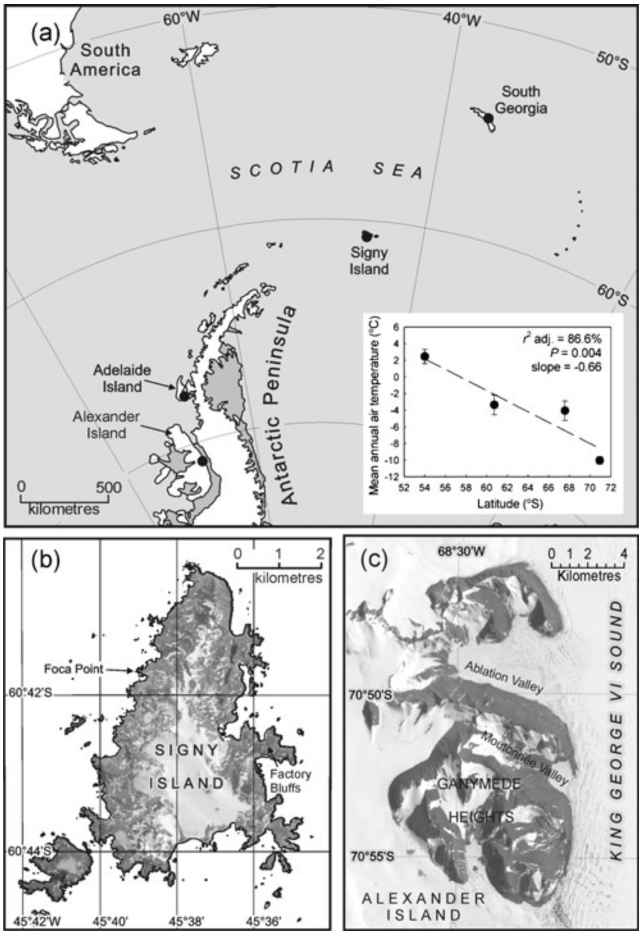


Fig. 1 Maps showing the locations of the sampling sites. **a** Map showing positions of South Georgia, Signy Island, Rothera Point on Adelaide Island and the Ganymede Heights on Alexander Island. **b** Map of Signy Island, showing positions of Foca Point and Factory Bluffs. **c** Map of the Ganymede Heights, showing positions of Ablation and Moutonnée Valleys. *Inset* in **a** shows mean annual air temperature at each sampling site as a function of latitude. Regression details are shown in *panel* and the *dashed line* is best fit. Values are means of 5 or 8 years±SD

Fungal structures were frequent in the tissues of *C. varians* sampled from all points along the latitudinal transect. DS hyphae of 1.6-(1.9)-2.2-μm diameter formed a network of mycelia on stem surfaces. These hyphae were continuous with hyaline septate hyphae of the same diameter in epidermal cells. Hyaline septate hyphae of the same diameter were frequently observed in the shafts of rhizoid cells, and these hyphae were continuous with intracellular septate hyphal coils that formed in the bases of these cells (Fig. 2a). Hyphal coils which formed in epidermal cells were usually located adjacent to colonised rhizoid cells (Fig. 2a, inset). Coils were not observed in leaf cells and were typically absent from epidermal cells close to the apical meristem. The morphology of hyphal coils differed markedly along the transect, with highly convoluted coils that tightly packed the rhizoid cell bases and epidermal cells of plants from South Georgia (Fig. 2b), more open coils in the cells of plants from Signy Island and Rothera Point, and relatively unconvoluted, sparse coils in the cells of plants from Ablation Valley and Moutonnée Valley (Fig. 2c).

The colonisation of stem tissues by fungal structures was strongly associated with latitude. A significant positive association between latitude and the percentage of stem length colonised by DS hyphae was recorded, with the frequency of DS hyphae increasing from 30% at South Georgia to 97% at Moutonnée Valley (Fig. 3a). In contrast, significant negative associations between latitude and the percentage of stem length colonised by hyaline septate hyphae and hyphal coils were recorded, with the frequency of hyaline septate hyphae decreasing from 85% at South Georgia to 13% at Moutonnée Valley (Fig. 3b), and the frequency of hyphal coils decreasing from 71% at the former location to 15% at the latter (Fig. 3c). In contrast, the percentage of rhizoids colonised by hyaline hyphae, which varied between 90% and 72%, was not associated with latitude (Fig. 3d).

The frequencies of colonisation of *C. varians* tissues by fungal structures along the latitudinal transect were associated with air temperature. The percentage of stem length colonised by DS hyphae was negatively associated with mean annual air temperature (Fig. 3a, inset) and the percentages of stem length colonised by hyaline septate hyphae and hyphal coils were each positively associated with mean annual air temperature (Fig. 3b, c, insets). There were also significant negative associations between the frequency of DS hyphae and mean seasonal air temperatures (r^2 adj.=64.6–73.1%; all $P<0.034$), and significant positive associations between the frequencies of hyaline septate hyphae and hyphal coils and mean seasonal air temperatures (r^2 adj.=84.9–98.2%; all $P<0.006$). The

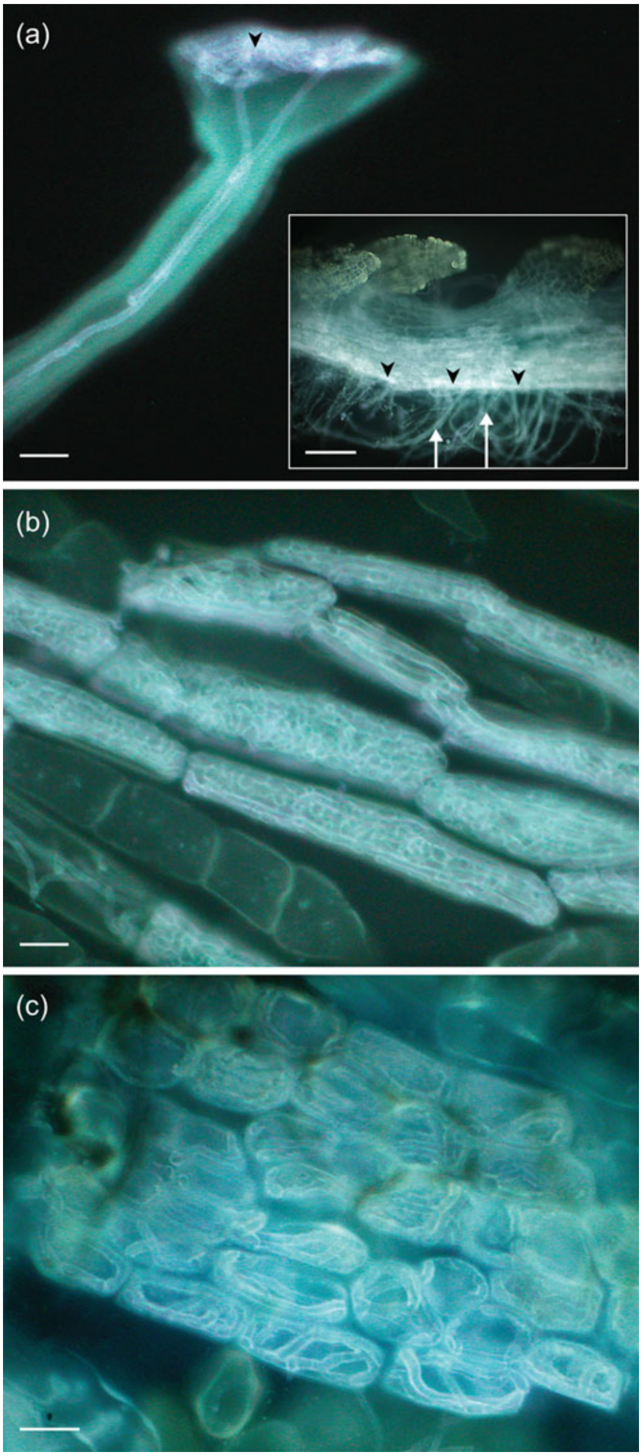


Fig. 2 UV epifluorescent micrographs of **a** detached rhizoid cell from South Georgia colonised by hyaline hyphae in its shaft and a hyphal coil (*arrowhead*) in its base, and hyphal coils in the epidermal cells of *C. varians* from **b** South Georgia and **c** Moutonnée Valley. *Inset* in **a** shows a stem of *C. varians* from South Georgia with blocks of epidermal cells colonised by fluorescing coils (*arrowheads*) adjacent to rhizoids colonised by hyphae (*arrows*). Note the absence of fluorescing coils from the upper part of the stem, distant from rhizoids. *Scale bars* in **a**–**c** are 10 µm, that in *inset* is 100 µm

percentage of rhizoids colonised by hyaline hyphae was not associated with mean annual or seasonal air temperatures (r^2 adj.=3.5–25.8%; all $P>0.05$).

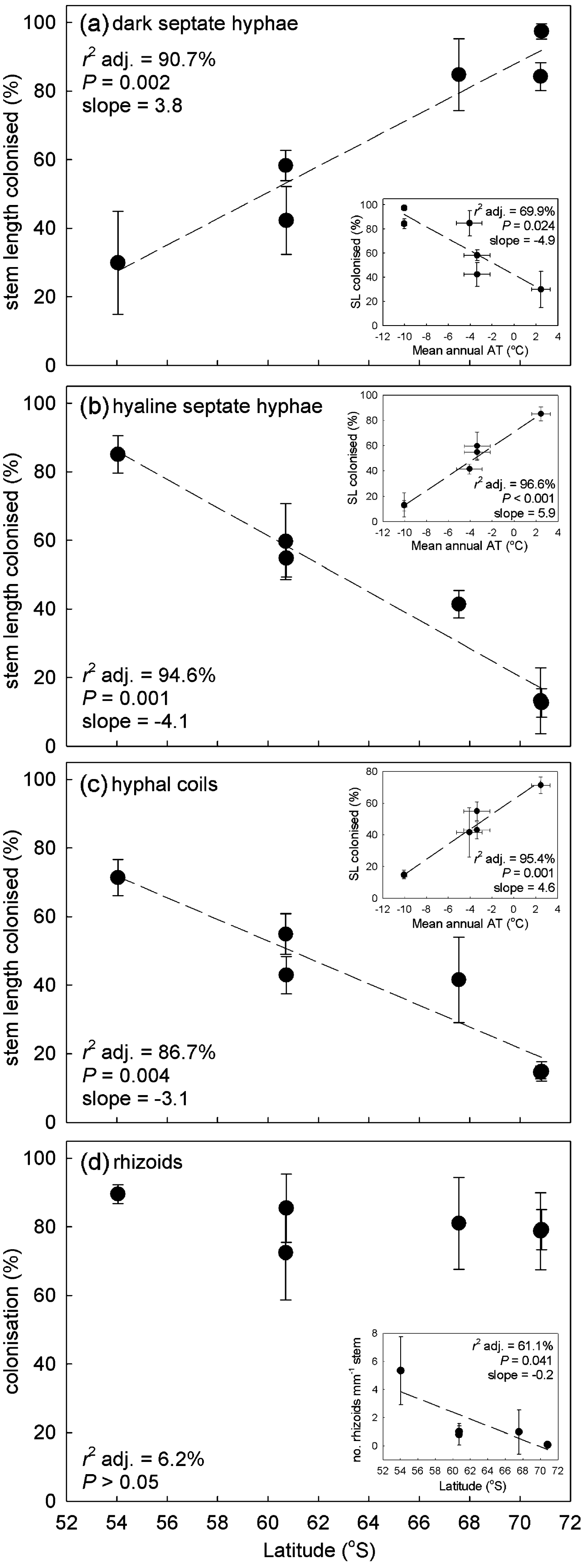
The frequency of rhizoids declined significantly at higher latitudes, with the mean number of rhizoids mm^{-1} stem declining from 5.3 at South Georgia to 0.1 at Moutonnée Valley (Fig. 3d, inset). The mean number of rhizoids mm^{-1} stem was also positively associated with mean annual air temperature (r^2 adj.=68.7%; $P=0.026$). The percentage of stem length colonised by hyaline septate hyphae was positively associated with the mean number of rhizoids mm^{-1} stem (r^2 adj.=60.3; $P=0.043$), and there was a marginally significant positive association between the percentage of stem length colonised by hyphal coils and the frequency of rhizoids per unit length of stem (r^2 adj.=57.0%; $P=0.051$). There was also a marginally significant negative association between the percentage of stem length colonised by DS hyphae and the frequency of rhizoids (r^2 adj.=46.6%; $P=0.081$). The percentage of rhizoids colonised by hyphae was not associated with the mean number of rhizoids mm^{-1} stem (r^2 adj.=40.4%; $P=0.104$).

Discussion

In the present study, hyaline hyphae were observed in the shafts of rhizoid cells of *C. varians*, with septate hyphal coils forming in the bases of these cells and in the adjacent epidermal cells of the stem. Networks of DS hyphae of 1.6–2.2 µm diameter, which were continuous with hyaline septate hyphae of the same diameter in the epidermis, were also observed on stem surfaces of the liverwort. These patterns of fungal colonisation are the same as those observed in plants of *C. varians* grown from spores and inoculated with *R. ericae* under axenic conditions (Upson et al. 2007). Furthermore, analyses of ITS region sequences of rDNA of fungal isolates from *C. varians* show *R. ericae* to be present in the liverwort sampled from South Georgia, Signy Island, Rothera Point and Moutonnée Valley (Upson et al. 2007). Thus, although other fungi will undoubtedly have colonised the tissues of *C. varians* sampled in the current study, as members of the Chaetothyriales do at Rothera Point (Jumpponen et al. 2003), it is reasonable to assume that the majority of the fungal structures observed were formed by *R. ericae*.

The current study found substantial changes in the fungal colonisation of *C. varians* tissues along the latitudinal transect between South Georgia and the Ganymede Heights on Alexander Island. The frequency of DS hyphae on stem surfaces increased significantly along the transect, from 30% at South Georgia to 97% at Moutonnée Valley, the most southerly location from which *C. varians* was sampled. In contrast, colonisation by hyaline septate hyphae in stem

Fig. 3 Percentage of stem length colonised by **a** dark septate (DS) hyphae, **b** hyaline septate hyphae and **c** hyphal coils, and **d** percentage of rhizoids colonised by hyaline septate hyphae, in the tissues of *C. varians* as a function of latitude. *Insets* in **a**–**c** show the percentage of stem length colonised by DS hyphae, hyaline septate hyphae and hyphal coils as a function of mean annual air temperature (AT). *Inset* in **d** shows mean no. rhizoids per mm^{-1} length of stem as a function of latitude. Regression details are shown in each *panel* and the *dashed lines* are best fits. Values are means of three and five or eight replicates±SD for fungal structures and air temperatures, respectively



tissues decreased from 85% at South Georgia to 13% at Moutonnée Valley, with similar patterns being observed for septate hyphal coils, which decreased in frequency from 71% at the former location to 15% at the latter. Changes to the morphology of hyphal coils at higher latitudes were also apparent, with coils that tightly packed the epidermal cells of *C. varians* being observed at South Georgia, and relatively loose, unconvoluted coils being observed at Ablation and Moutonnée Valleys. This corroborates the findings of Williams et al. (1994), who reported the presence of loose coils in the rhizoid bases of *C. varians* from Granite Harbour in the continental Antarctic, the southernmost location from which *C. varians* is known (Newsham 2010).

As suggested by previous studies (Williams et al. 1994; Upson et al. 2007), rhizoids appeared to be the main point at which fungi entered the epidermal tissues of *C. varians*: the colonisation of epidermal cells by hyphal coils was most frequent in the proximity of rhizoid cells that were colonised by hyaline hyphae. The substantial reduction in the frequency of rhizoids per unit length of stem at higher latitudes, and the positive association between the frequency of rhizoids and the frequency of hyaline septate hyphae, might thus explain the reduction in hyaline hyphae at more southerly locations. However, the analyses here suggest that the main factor responsible for changes in the frequencies of hyaline hyphae, and of hyphal coils and DS hyphae, was air temperature, since closer associations were found between the frequencies of these structures and mean annual air temperature than with the number of rhizoids per unit length of stem. It remains a possibility, however, that factors other than air temperature, such as water or soil nutrient availability, may also have been associated with latitude, and could thus explain changes in the frequencies of fungal structures in *C. varians* between South Georgia and Alexander Island. Nevertheless, the data here corroborate those from studies showing positive associations between air temperatures and the mycorrhizal colonisation of plant species along a transect through the sub- and high Arctic (Newsham et al. 2009), and reductions in the mycorrhizal colonisation of roots in habitats at higher altitudes or latitudes (Read and Haselwandter 1981; Olsson et al. 2004).

The finding here that DS hyphae increase in abundance at higher latitudes is consistent with the hypothesis that fungal hyphae become more melanised under colder and drier environmental conditions (Robinson 2001). Increased melanisation of hyphae under such conditions is thought to protect hyphae from extreme temperatures and desiccation, broadening the ecological niches of fungi (Jumpponen and Trappe 1998). However, clear demonstrations of this association are apparently absent from the literature. For example, in a study on *Deschampsia antarctica* and *C. quitensis*, the two native Antarctic higher plant species, no relationship was found between increasing latitude and the frequency of DS hyphae in roots (Upson et al. 2008).

Given the abundance of fungal hyphae and coils in the tissues of *C. varians* recorded in the current study, it seems likely that these structures exert significant effects on the growth of the hepatic in the natural environment. Whether these effects are positive or negative is currently unknown, but the health of the plants sampled in this study suggests that the fungi are not pathogenic on *C. varians*. Despite the formation of these structures in the rhizoid and epidermal cells of other leafy liverworts, both in the Antarctic and at lower latitudes (Boullard 1988; Read et al. 2000; Newsham and Bridge 2010), their functional significance in hepatics has yet to be elucidated. In particular, it remains a possibility that hyphal coils, which are the interface at which nutrients, and in particular nitrogen, are exchanged between *R. ericae* and plants in ericoid mycorrhizas (Smith and Read 2008), may have similar beneficial functions in leafy liverworts. Given the frequencies of hyphal coils in the tissues of leafy liverworts, and the abundance of these plants in ecosystems at high latitudes, experiments under controlled conditions into the influence of fungal endophytes on the nitrogen assimilation and growth of *C. varians* and other members of the Jungermanniales are now required.

Acknowledgements Funding was supplied by the Natural Environment Research Council through the British Antarctic Survey's long-term monitoring and survey programme. Kat Snell kindly collected the samples of *C. varians* from Ablation Valley, Roger Worland, Paul Geissler and Helen Peat supplied the air temperature data recorded at Mars Oasis, and Peter Fretwell and Jamie Oliver helped to prepare figures. Katarzyna Turnau gave advice on staining procedures and three anonymous referees supplied helpful comments. All are gratefully acknowledged.

References

Bednarek-Ochyra H, Váňa J, Ochyra JVR, Smith RIL (2000) The liverwort flora of Antarctica. Institute of Botany, Polish Academy of Sciences, Cracow, p 236

Boullard B (1988) Observations on the coevolution of fungi with hepatics. In: Pyrozynski KA (ed) Coevolution of fungi with plants and animals. Academic, London, pp 107–124

Chambers SM, Williams PG, Seppelt RD, Cairney JWG (1999) Molecular identification of *Hymenocyphus* sp. from rhizoids of the leafy liverwort *Cephaloziella exiliflora* in Australia and Antarctica. Mycol Res 103:286–288

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Jumpponen A, Newsham KK, Neises DJ (2003) Filamentous ascomycetes inhabiting the rhizoid environment of the liverwort *Cephaloziella varians* in Antarctica as assessed by direct PCR and cloning. Mycologia 95:457–466

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swann JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 155:495–501

Newsham KK (2010) The biology and ecology of the liverwort *Cephaloziella varians* in Antarctica. Antarct Sci 22:131–143

Newsham KK, Bridge PD (2010) Sebacinales are associates of the leafy liverwort *Lophozia excisa* in the southern maritime Antarctic. Mycorrhiza 20:307–313

Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate endophytes in polar regions. Fungal Ecol 2:10–20

Olsson PA, Eriksen B, Dahlberg A (2004) Colonization by arbuscular mycorrhizal and fine endophytic fungi in herbaceous vegetation in the Canadian High Arctic. Can J Bot 82:1547–1556

Read DJ, Haselwandter K (1981) Observations on the mycorrhizal status of some alpine plant communities. New Phytol 88:341–352

Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal associations in 'lower' land plants. Phil Trans R Soc Lond B 355:815–831

Robinson CH (2001) Cold adaptation in Arctic and Antarctic fungi. New Phytol 151:341–353

Smith RIL (1984) Terrestrial plant biology of the sub-Antarctic and Antarctic. In: Laws RM (ed) Antarctic Ecology vol 1. Academic, London, pp 61–162

Smith RIL (1988) Bryophyte oases in ablation valleys on Alexander Island, Antarctica. The Bryologist 91:45–50

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Upson R, Read DJ, Newsham KK (2007) Widespread association between the ericoid mycorrhizal fungus *Rhizoscyphus ericae* and a leafy liverwort in the maritime and sub-Antarctic. New Phytol 176:460–471

Upson R, Newsham KK, Read DJ (2008) Root-fungal associations of *Colobanthus quitensis* and *Deschampsia antarctica* in the maritime and sub-Antarctic. Arct Antarct Alp Res 40:592–599

Williams PG, Roser DJ, Seppelt RD (1994) Mycorrhizas of hepatics in continental Antarctica. Mycol Res 98:34–36