

Extremotolerance and Resistance of Lichens: Comparative Studies on Five Species Used in Astrobiological Research I. Morphological and Anatomical Characteristics

J. Meeßen · F. J. Sánchez · A. Brandt · E.-M. Balzer ·
R. de la Torre · L. G. Sancho · J.-P. de Vera · S. Ott

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Abstract Lichens are symbioses of two organisms, a fungal mycobiont and a photoautotrophic photobiont. In nature, many lichens tolerate extreme environmental conditions and thus became valuable models in astrobiological research to fathom biological resistance towards non-terrestrial conditions; including space exposure, hypervelocity impact simulations as well as space and Martian parameter simulations. All studies demonstrated the high resistance towards non-terrestrial abiotic factors of selected extremotolerant lichens. Besides other adaptations, this study focuses on the morphological and anatomical traits by comparing five lichen species—*Circinaria gyrosa*, *Rhizocarpon geographicum*, *Xanthoria elegans*, *Buellia frigida*, *Pleopsidium chlorophanum*—used in present-day astrobiological research. Detailed investigation of thallus organization by microscopy methods allows to study the effect of morphology on lichen resistance and forms a basis for interpreting data of recent and future experiments. All investigated lichens reveal a common heteromerous thallus structure but diverging sets of morphological-anatomical traits, as intra-/extra-thalline mucilage matrices, cortices, algal arrangements, and hyphal strands. In *B. frigida*, *R. geographicum*, and *X. elegans* the combination of pigmented cortex, algal arrangement, and mucilage seems to enhance resistance, while subcortex and algal clustering seem to be crucial in *C. gyrosa*, as well as pigmented cortices and basal thallus protrusions in *P. chlorophanum*. Thus, generalizations

J. Meeßen (✉) · A. Brandt · E.-M. Balzer · S. Ott
Institut für Botanik, Heinrich-Heine Universität (HHU), Universitätsstr. 1, 40225 Düsseldorf, Germany
e-mail: joachimmeessen@gmx.de

F. J. Sánchez · R. de la Torre
Instituto Nacional de Técnica Aeroespacial (INTA), Ctra. de Ajalvir km. 4, Torrejón de Ardoz,
28850 Madrid, Spain

L. G. Sancho
Departamento de Biología Vegetal II, Facultad de Farmacia,
Universidad Complutense de Madrid (UCM), 28040 Madrid, Spain

J.-P. de Vera
Institut für Planetenforschung, Deutsches Zentrum für Luft- und Raumfahrt (DLR),
Rutherfordstraße 2, 12489 Berlin, Germany

on morphologically conferred resistance have to be avoided. Such differences might reflect the diverging evolutionary histories and are advantageous by adapting lichens to prevalent abiotic stressors. The peculiar lichen morphology demonstrates its remarkable stake in resisting extreme terrestrial conditions and may explain the high resistance of lichens found in astrobiological research.

Keywords *Buellia frigida* · *Circinaria gyrosa* · Lichen morphology · Lichen anatomy · Extremotolerance · BIOMEX

Abbreviations

CLSM	Confocal laser scanning microscopy
EPS	Extracellular polymeric substances
LEO	Low Earth orbit
LTSEM	Low temperature scanning electron microscopy
PAR	Photosynthetically active radiation (400–700 nm)
PSII	Photosystem II
SEM	Scanning electron microscopy
SLC	Secondary lichen compound
UVR	Ultra-violet radiation (100–400 nm)

Introduction

Since the millennium lichens became valuable model organisms in astrobiological research (Sancho et al. 2008). Remarkable progress to fathom their limits and limitations in resisting harsh environmental conditions was made in recent experimental approaches. This includes space exposure experiments as LICHENS II on BIOPAN 5/FOTON M2, LITHOPANSPERMIA and STONE on BIOPAN6/FOTON M3, and LIFE on EXPOSE-E/EuTEF (Sancho et al. 2007; de la Torre et al. 2007, 2010a; de Vera 2012; Raggio et al. 2011; Onofri et al. 2012; Scalzi et al. 2012) and experiments simulating various space conditions such as vacuum and UVR, (de Vera et al. 2003, 2004a, b, 2007, 2008, 2010; de la Torre et al. 2002, 2004, 2007, 2010a, b; Sánchez et al. 2012) as well as hypervelocity impacts (Stöffler et al. 2007; Horneck et al. 2008; de Vera and Ott 2010). An overview of previous astrobiological studies on lichens is given in Table 1. All experiments demonstrated high resistance towards space and Martian environmental parameters of those lichens that are adapted to harsh abiotic factors in their natural habitat. Furthermore, in April 2014 two lichen species will be exposed to LEO-space conditions and on-board simulated Mars conditions for 15 to 18 months during the BIOMEX-experiment on EXPOSE-R2/Zvezda (ESA call ILSRA-AO 2009). The viability after exposure was assessed by various methods, as determination of photosynthetic activity by chlorophyll- α -fluorescence of PSII and by light dependent gas exchange analyses, live/dead-staining of both symbionts utilizing CLSM (apoplasma as well as eusymbiotic), LTSEM, cultivation experiments and germination capacity analyses of the fungal spores (see references above).

Lichens are symbiotic associations formed by two organisms, the heterotrophic fungal partner (mycobiont) and the photoautotrophic partner (photobiont) which is an eukaryotic green alga or a prokaryotic cyanobacterium. Many lichens tolerate extreme environmental conditions, such as scarcity of soil substratum and nutrients, high levels of (toxic) metal ions (review by Bačkor and Fahsel 2008), cold and long-term snow coverage (Kappen 1993; Dyer and Crittenden 2008), heat, intensive insolation with high levels of UVR (Lange 1992; Nybakken et al. 2004), low water availability, infrequent water supply and extreme drought

Table 1 Overview of past and recent astrobiological investigations on lichens

Experiment	Lichen	Facility	Duration	Tested parameters or exposure conditions	Viability analysis	Reference
Lichens I	<i>R. geographicum</i> , <i>X. elegans</i>	BIOPAN 4/ FOTON M1	short	several samples recovered after explosion during launch	PSII activity	de la Torre et al. 2004
Lichens II	<i>R. geographicum</i> , <i>X. elegans</i>	BIOPAN 5/ FOTON M2	16 d	space exposure, $\lambda > 110$, > 200 , > 290 , > 400 nm, (UVA + UVB at 2.2×10^4 kJ m ⁻²)	PSII activity, LTSEM	Sancho et al. 2007; Sancho et al. 2008
Lithopanspermia	<i>C. gyrosa</i> , <i>R. geographicum</i> , <i>X. elegans</i>	BIOPAN 6/ FOTON M3	10 d	space exposure, $\lambda > 110$, > 200 , > 290 , > 400 nm	PSII activity, LTSEM, light dependent gas exchange analysis	de la Torre et al. 2010a; Raggio et al. 2011
Stone	<i>R. geographicum</i> ,	BIOPAN 6/ FOTON M3	10 d	space exposure, followed by atmospheric re-entry	samples totally destroyed during re-entry	de la Torre et al. 2010a
Life	<i>R. geographicum</i> , <i>X. elegans</i>	EXPOSE-E/ EuTEF	559 d	LEO temp. + irradiation (> 110 nm, 7×10^3 – 5×10^6 kJ m ⁻²), UV (200–400 nm, 9.2×10^2 – 6.3×10^5 kJ m ⁻²), GCR (≤ 190 mGy), vac. (10^{-4} – 10^{-7} Pa) or Mars atm. (100 % CO ₂ , 7 mbar)	Life/Dead staining (Fumi, Bac Light), PSII activity, fxSEM, TEM, biont growth assays, photoproducts	Onofri et al. 2012; Scalzi et al. 2012, analysis in progress
Biomex	<i>B. frigida</i> , <i>C. gyrosa</i>	EXPOSE-R2/ Zvezda	12–18 months	LEO temp. + irradiation (> 190 nm, up to 8×10^5 kJ m ⁻²), vac. (10^{-4} – 10^{-7} Pa) or Mars atm. (100 % CO ₂ , 7 mbar, 100 % rH)	Life/Dead staining (Fumi, Bac Light), PSII activity, fxSEM, TEM, LTSEM, biont growths assays,	scheduled 2014–2015, EVT, SVT in progress
Simulation	<i>F. bracteata</i> , <i>X. elegans</i> , <i>X. parietina</i>	Space Sim. Test Beds, DLR Cologne	4, 8, 16 h	1) vac. (10^{-5} Pa), 2) UV (160–400 nm, $2.8 \times 8.8 \pm 0.7$ W m ⁻²), 3) vac. + UV (10^{-5} Pa, 160–400 nm, $2.8 \times 8.8 \pm 0.7$ W m ⁻²)	Life/Dead staining (Fumi, Sytox Green), ascospore germination rate	de Vera et al. 2003; de Vera et al. 2004a
Simulation	<i>F. bracteata</i> , <i>X. elegans</i>	Space Sim. Test Beds, DLR Cologne	1, 5, 15, 30 min, 1, 2, 4, 8, 16 h	1) vac. (10^{-5} Pa), 2) UV (200–400 nm; 40, 466, 3,347 kJ m ⁻²) 3) vac. + UV (10^{-5} Pa), 200–400 nm; 40, 466, 3,347 kJ m ⁻²) 4) vac. + UV (10^{-3} Pa, 160–400 nm, 8.1 W m ⁻²)	Life/Dead staining (Fumi, Sytox Green), ascospore germination rate	de Vera et al. 2004b
Simulation	<i>B. frigida</i> , <i>P. aphthosa</i>	Space Sim. Test Beds, DLR Cologne	5, 15, 30, 60, 120, 240, 480 min	UVC (254 nm, 2.1–201.8 J m ⁻²)	Life/Dead staining (Fumi, Sytox Green)	de Vera and Ott 2010

Table 1 (continued)

Experiment	Lichen	Facility	Duration	Tested parameters or exposure conditions	Viability analysis	Reference
Simulation	<i>X. elegans</i>	Space Sim. Test Beds, DLR Cologne	5 min, 2, 4, 8, 16 h	vac. (2.8×10^{-1} – 3.6×10^{-5} Pa) and UV (200–400 nm, 2.8 W m^{-2})	Life/Dead staining (Funtl, Sytox Green), biont growths assays	de Vera et al. 2008
Simulation	<i>X. elegans</i>	HUMIDITY LAB, DLR Berlin	22 d (1), 1 d (2–4)	1) Mars atm. (95 % CO ₂ , 6 mbar, 100 % rH, –55–+20 °C) 2) Mars atm. (95 % CO ₂ , 6 mbar, 100 % rH, +15 °C) 3) Mars atm. (95 % CO ₂ , 6 mbar, 100 % rH, +5 °C) 4) Earth atm. (100 % rH, +15 °C)	PSII activity, Life/Dead staining (Funtl)	de Vera et al. 2007; de Vera et al. 2010
Simulation	<i>X. elegans</i>	Shock Recovery Exp., EMI Freiburg	< 1 min	hypervelocity impact simulations 5, 10, 15, 20, 30, 41.5, and 50 GPa)	Life/Dead staining (Funtl, Sytox Green)	Stöffler et al. 2007; Horneck et al. 2008
Simulation	<i>P. chlorophanum</i>	Mars Sim. Chamber, DLR Berlin	35 d	Mars radiation (200–2,500 nm, 2.7×10^2 – $6.3 \times 10^3 \text{ kJ m}^{-2}$), Mars atm. (95 % CO ₂ , 6 mbar, –49–+25 °C, 0–80 % rH)	PSII activity	de Vera et al. 2012
Simulation	<i>R. georgaphicum</i> , <i>X. elegans</i>	BIOPAN Sim. Test, INTA Madrid	10, 20 h	1) UV + VIS + NIR (200–900 nm) 2) vacuum + UV + VIS + NIR (3.0×10^{-7} bar, 200–900 nm)	PSII activity	de la Torre et al. 2004
Simulation	<i>C. gyrosa</i>	PASC at CAB, INTA-CSIC Madrid	120 h	1) Mars UV (200–400 nm, 30 mW cm^{-2} , –93 °C) 2) Mars atm. (95 % CO ₂ , 0.6 % H ₂ O, 7 mbar), –93 °C 3) Mars UV (200–400 nm, 30 mW cm^{-2} , vac. (10^{-6} bar), –93 °C	PSII activity, light dependent gas exchange analysis	Sánchez et al. 2012

as observed in cold and hot deserts like the dry valleys in continental Antarctica (Marchant and Head 2007; Harańczyk et al. 2008; Sun et al. 2010) and the Andean Atacama Desert (McKay et al. 2003).

Several adaptive strategies are discussed to contribute to the resistance of lichens towards extreme environmental conditions and therefore they are of interest for astrobiological research. Among other factors, such as the photobiont's capacity to protect its photosynthetic apparatus during and regenerate it after stressful conditions (e. g. drought and cold, Sadowsky et al. 2012), three aspects constituting the extremophile character of many lichen species should be highlighted: the first one is the poikilohydric nature of lichens. Poikilohydry allows lichens to tolerate extreme desiccation—but also high UVR/PAR-levels (Nybakken et al. 2004) and high or low temperatures that may accompany drought—by passing into an ametabolic state which is referred to as anabiosis or, more specifically, anhydrobiosis (Kranner et al. 2005). Re-hydration activates the lichen metabolism, what usually occurs under more moderate and therefore physiologically favourable conditions. It is known that morphological properties affect thallus water-uptake and water-retention, which influence the duration of physiological activity (refer to Lange et al. 1999). A second aspect is the vast variety of secondary lichen compounds (SLCs) formed in high amounts almost exclusively in the symbiotic state (Henssen and Jahns 1974). They cause PAR- and UVR-shielding by absorption (Solhaug and Gauslaa 1996; Huneck and Yoshimura 1996; Solhaug and Gauslaa 2004; Mc Evoy et al. 2006) and are proposed to protect particularly the photobiont.

The present study focuses on a third aspect: the morphological and anatomical traits that shape a lichen thallus and adapt it to the habitat's dominant (micro)climatic and orographic factors. As all fungi, mycobionts are modular organisms (Carlile 1995) characterized by poor differentiation of cell types and plectenchymatic tissues; the photobiont cells show no differentiation. The low degree of functional cell differentiation is compensated on the next higher level of organismic organization by an intriguing complexity of lichen morphology. This is displayed by various growth types (e. g. crustose, placoid, foliose, fruticose, umbilicate) as well as functional morpho-anatomical structures (e.g. heteromerous thallus stratification, pseudocypheallae, rhizines, cephalodia, and reproductive structures as apothecia, perithecia, pycnidia, soredia, and isidia). Valuable introduction into lichen morphology is provided by Jahns (1988) or Büdel and Scheidegger (1996) while the link between morphological traits and their adaptative or eco-physiological significance is still an issue of vivid debate in lichenology. Variation of morphological and anatomical traits unquestionably represents lichen adaptation to the environmental conditions of the respective ecological niche (Jahns 1988; Büdel and Scheidegger 1996). Such traits directly influence major physiological aspects of the lichen's biology as effectiveness of photosynthesis, nutrient uptake, nutrient distribution, gas exchange, but also water uptake and water retention which are fundamental aspects of the poikilohydric life strategy (Lange et al. 2001). Consequently, morphology and anatomy are crucial factors in understanding the extremotolerance of many lichen species.

To date, astrobiological studies were performed with eight lichen species. The mycobiont of all investigated species is an ascomycetes (class Lecanoromycetes). Seven lichens harbour a coccal green alga of the genus *Trebouxia* sp. as a photobiont (phylum Chlorophyta, class Trebouxiophyceae) while *Peltigera aphthosa* harbours *Coccomyxa* sp. (Trebouxiophyceae). All lichens are composed of two eukaryotic symbionts, making the selected species valuable models to investigate the capacity of eukaryotes and of symbioses to resist non-terrestrial environmental factors. The species preferentially used in astrobiological research are *Circinaria gyrosa*, *Rhizocarpon geographicum*, *Xanthoria elegans*, and currently *Buellia frigida*. Recently, promising Martian environment simulations were performed with

Pleopsidium chlorophanum (de Vera et al. 2012). Simulation studies were also performed with *Fulgensia bracteata* and *Xanthoria parietina* (de Vera et al. 2004a, b) but due to their anatomical analogies with *X. elegans*, both are not addressed by the present study. *P. aphthosa* as a mere control to *B. frigida* in UVC-exposure experiments (de Vera and Ott 2010) was also excluded.

The results of astrobiological research depicted above justify continuing research on the resistance of lichens and to focus on the questions, if and to what extend the morphological organization of the symbiotic association contributes to its potential of resistance. Therefore, detailed investigations of thallus structure and anatomical organization were made by light-microscopic analysis and fixed scanning electron microscopic analysis. The presented results give decisive insight into the morphology and anatomy of five out of eight lichens used to date in astrobiological studies. They allow determining the portion of thallus organization in the lichens' potential of resistance and will give a valuable basis for interpreting the data of recent and future experiments. The results demonstrate that the peculiar morphology and anatomy of lichens have a remarkable stake in their resistance towards extreme environmental conditions on Earth as well as towards space and Mars conditions.

Material and Methods

Buellia frigida Darb. (1910) is a frequent, endemic, crustose lichen of maritime and especially continental Antarctica, colonizing habitats down to 84°S. The thalli grow on rocks being fully exposed to wind, low temperatures and high irradiation levels during Antarctic summer and in altitudes up to 2015 m a.s.l. and intact thalli of up 15–20 cm are frequent found on Inexpressible Island (Øvstedal and Lewis Smith 2001). For the present study, *B. frigida* was collected on two sites in continental Antarctica: In 1996/1997 at Inexpressible Island (74°54'S, 163°43'E) in Terra-Nova-Bay, Ross Sea (by R. L. Smith), and in 2009/2010 in the vicinity of the German Gondwana Station at Gerlache Inlet (74°38' S, 164°13'E), in North Victoria Land (by S. Ott). Air-dried samples were stored at –25 °C until use. For BIOMEX on EXPOSE-R2/Zvezda samples from Gondwana Station will be used.

Circinaria gyrosa Sohrabi (2012) was recently revised from *Aspicilia fruticulosa* (Sohrabi 2012) and used under its previous name for several space and simulation experiments (de la Torre et al. 2010a; Raggio et al. 2011; Sánchez et al. 2012). *C. gyrosa* originates from continental deserts and arid areas of Middle Asia, Eurasia, North America and Northern Africa. It is adapted to harsh environmental conditions, including heat, drought, and high levels of solar UVR (Sancho et al. 2000). Samples were collected from clay soils in high basins of central Spain: Guadalajara, Zaorejas, 1260 m a.s.l. (40°45'40"N, 02°12'08"E). The samples were collected in June 2010 and kept under dark and dry conditions until testing.

Rhizocarpon geographicum (L.) DC (1805) (*R. geo.*) is a widespread, bipolar lichen, mostly distributed on northern hemispherical arctic and alpine habitats but also found in maritime and continental Antarctica. It is found on exposed, often dry, siliceous or granite, non-calciferous rock in montane, subalpine and alpine or polar zones. The specimen investigated in this study are collected on two distinct montaneous, respectively alpine sites: In Spain at the Sierra de Guadarrama mountains near Navacerrada (about 40°47'20"N, 04°00'12"W, at about 1400 m a.s.l.), and in Valais, Switzerland at Col du Sanetsch, (46°20'01"N, 07°17'11"E, at 2140 m a.s.l.) as well as in the vicinity of Zmutt (46°00'N, 07°71'E, at 1950 m a.s.l.).

Xanthoria elegans (Link) Th. Fr. (1860) is a cosmopolitan lichen colonizing open sites on diverse substrata in various habitats, including harsh environments as alpine (up to 7,000 m.s.l. in Himalaya), peninsular Antarctic (Berry Hills, Cape Lachman, James Ross Island, 64°S) and continental Antarctic inland sites (near Wood Bay, Victoria Land, 74°S, Øvstedal and Lewis Smith 2001). It is frequent on volcanic, silicate and limestone rock, at nitrophilic sites, and occasionally found on concrete and other anthropogenic substrata. Habitats of *X. elegans* are usually exposed to high levels of UVR (de Vera 2005). For the present study, thalli of *X. elegans* were collected in June 2005 at Col du Sanetsch, Valais, Switzerland (46°21'48"N, 07°17'51"E, at 2140 m a.s.l.) and stored at -25 °C until investigation. Thalli of *X. elegans* from this and from adjacent collection sites (Zermatt, 46°00'N, 07°71'E, at about 1950 m a.s.l.) were used before in astrobiological studies (de Vera et al. 2003, 2004a, b, 2007, 2008, 2010; Stöffler et al. 2007; Horneck et al. 2008) and in the LIFE experiments on EXPOSE-E/EuTEF (see de Vera et al. 2012; Onofri et al. 2012).

Pleopsidium chlorophanum (Wahlenb.) Zopf (1855) is a bipolar distributed species found in North Europe and North America, but also throughout maritime and continental Antarctica (Øvstedal and Lewis Smith 2001). It is most frequent in continental Antarctica, where it is widespread down to far inland nunataks at altitudes of up to 2500 m a.s.l. *P. chlorophanum* colonizes dry, shaded rocks and cracks. It forms hyphal strands which penetrate 10–15 mm into the rock and facilitate bio-weathering. The samples used for the present investigation were collected in 2009/2010 next to Gondwana Station at Gerlache Inlet, North Victoria Land (74°38'S, 164°13'E). The air-dried samples were stored at -25 °C until further use.

Explanation of lichenological terminology: *heteromerous*: internally stratified; *pseudocypellae*: cortical pores; *hypo-/prothallus*: basal/marginal layer of crustose thalli; *plectenchyma*: general term for interwoven hyphal tissues; *paraplectenchyma*: tissue of isodiametrical hyphae; *prosoplectenchyma*: tissue of elongated periclinial hyphae; *apothecium*: fungal fruitingbody; *hymenium*: asci-bearing layer in apothecia; *epi-/hypothecium*: layer above/below the hymenium; *ascus*: ascospore-bearing cell; *paraphyse*: sterile hymenial hypha; *pycnidium*: conidiospore-forming fungal structure.

Light-Optical Microscopic Studies and Stratification Measurements

Five to twelve specimen of each lichen species were screened on their morphological properties and representative ones were prepared for further investigations under a stereoscopic microscope (M8, Wild Heerbrugg AG). Photo-documentation of the respective lichen morphology were performed with a Nikon D80 (AF Micro-NIKKOR macro lense, 60 mm focal length). For each lichen species thin layer sections (12–20 µm) were obtained from at least 4 representative specimen at marginal (distal) and central (proximal) lobes or areolae, respectively (Table 2), by using a freezing microtome (ca. -30 °C, Frigomobil 1206, Reichert-Jung) and subsequently stained with 5 % lactoglycerol cotton blue. Analysis of thallus anatomy and further photo documentation were performed under a digital lightmicroscope (Axio imager A1). and stratification measurements were done by proprietary software tools (AxioVision Rel. 4.8.2).

Scanning Electron Microscopy of Fixed Thallus Samples

For each lichen species, thin layer sections (50–80 µm) of 2–3 different lobes/areolae of 2–4 representative thalli were prepared (as described above), transferred into 2.5 % glutaraldehyde in 200 mM cacodylate-buffer, fixed under vacuum and under ambient pressure (30 min

each), and washed for 10 min in pure cacodylate-buffer. Afterwards the samples were mounted in microporous capsules (plano GmbH) and dehydrated by subsequent dilution series of graduated ethanol (in water) and acetone (in ethanol). Submerged in acetone, the thallus sections were brought into screw cap containers (Dr. W. Hert Mikrotechnik), closed with 3 mm copper grids, transferred to a critical point drying device (CPD 020, Balzers Union), washed thrice, and dried at the critical point temperature of 34.5 °C. The dehydrated lichen sections were transferred to specimen holders, air dried for 8 h, and gold-sputter coated for 180 s at 35 mA (Sputter Coater, Agar Scientific Ltd.), followed by detailed examination of thalline morphology and anatomy by scanning electron microscopy (SEM; LEO 1430(VP), LEO Electron Microscopy Ltd.).

Results

Buellia frigida

Morphology *B. frigida* is an epilithic crustose to placoid lichen (Physciaceae) which forms greyish to black circular thalli up to \varnothing 50 cm (Fig. 1a). The thallus margin is composed of rectangular areolae (max. 0.6×3.0 mm) of black colour and represents the growth zone. The older central region is formed by angular grey to black coloured areolae and numerous, black, convex and lecideine apothecia (max. \varnothing 1 mm). The apothecia bear apically thickened and pigmented paraphyses and club-shaped asci containing eight highly melanized, two-celled ascospores each. Asexual reproductive structures—as soredia and isidia—are not formed while immersed pycnidia are frequently formed in central areolae.

In the hydrated state, the thallus is significantly swollen by water uptake, reducing the density of its blackish melanin pigmentation in the cortex (Fig. 4a). Consequently, the photobiont in the algal layer becomes effectively exposed to light in the wet state. During dehydration, the thallus shrinks, leading to densification of the melanin pigmented cortex (Fig. 4b). This effect is most obviously observed in the algal rich marginal zones of *B. frigida* thalli where the number of algae is highest (Fig. 2a). The thallus surface is mostly covered by a mucilageous epicortex (Fig. 5) which may appear white in the dehydrated state (Fig. 1a), usually more prominent in the depigmented central parts of the thallus (Fig. 2a, bottom).

Anatomy The stratification is measured at thalli from two collection sites (Inexpressible Island and Gondwana Station) and for the specimen of the latter habitat in marginal (MS) and central (CS) thallus sections (Table 2). Despite a decrease of algal layer thickness and pigmentation in central thallus sections, the differences between sites and sections are low. Below the epicortex, which is occasionally interrupted in more central areolae, a paraplectenchymatous cortex of swollen and melanin-pigmented apical cells is located. From the marginal (e.g. younger) towards the central (e.g. older) thallus sections the cortex structure remains stable while its pigmentation ceases and the conglutination of the cortical cells increases, leading to a patchy pattern of pigmented and unpigmented areas. Below, the algal layer is composed of homogeneously dispersed algal clusters and interwoven hyphae, both strongly gelatinized (Fig. 3a). The algal layer is more pronounced at the margin but reduced to singled, patchy clusters in the centre. The occurrence of algal clusters in the central parts is clearly correlated to the residual melanin-pigmented areas above (Fig. 2a). Three morphological aspects, (i) depigmentation, (ii) increase of mucilage in the epicortex, and (iii) ceasing of the algal layer, point to thallus degeneration in the central (e.g. older

Table 2 Stratification of five lichen species relevant in astrobiological research

Lichen (from)	Thickness of thalline strata in [μm] \pm standard deviation							
	Mucilage layer	Cortex pigm.	parapl.	Sub-cortex	Algal layer	Medulla	Lower cortex	Hypo-thallus
<i>B. frigida</i> (from Gondwana Station, North Victoria Land and Inexpressible Island, both Antarctica)								
Gondwana	9.4 \pm 2.9	13.3 \pm 2.9	–	–	104.6 \pm 51.5	206.1 \pm 35.5	–	–
Inexpr. Isl.	12.4 \pm 2.9	15.8 \pm 5.5	–	–	68.0 \pm 15.3	204.8 \pm 43.8	–	–
Marginal	8.2 \pm 1.8	14.5 \pm 1.8	–	–	116.9 \pm 52.4	205.5 \pm 37.2	–	–
Central	12.6 \pm 2.8	14.8 \pm 5.6	–	–	67.1 \pm 14.3	205.3 \pm 41.9	–	–
<i>C. gyrosa</i> (from Zaorejas, Guadalajara, Central Spain)								
Proximal	–	20.8 \pm 0.9	18.3 \pm 1.3	125.7 \pm 13.0	69.2 \pm 12.0	385.9 \pm 12.2	–	–
Distal	–	24.3 \pm 9.5	21.4 \pm 2.4	147.1 \pm 29.0	78.3 \pm 39.7	346.3 \pm 111.0	–	–
<i>R. geographicum</i> (from Navacerrada, central Spain and Col du Sanetsch, Valais, Switzerland)								
Valais	–	28.1 \pm 3.5	–	–	94.7 \pm 15.1	159.1 \pm 61.3	–	66.0 \pm 37.7
Navacerr.	5.0 \pm 1.5	16.1 \pm 11.9	–	–	123.6 \pm 4.4	71.7 \pm 12.2	–	61.9 \pm 15.5
<i>X. elegans</i> (from Col du Sanetsch, Valais, Switzerland)								
Marginal	–	22.1 \pm 4.5	18.8 \pm 8.2	–	65.4 \pm 10.6	146.6 \pm 46.8	24.2 \pm 3.7	–
Central	–	35.7 \pm 20.2	20.5 \pm 6.5	–	96.6 \pm 72.6	230.0 \pm 150.3	29.5 \pm 14.2	–
<i>P. chlorophanum</i> (from Gondwana Station, North Victoria Land, Antarctica)								
Gondwana	4.1 \pm 1.8	15.0 \pm 5.0	11.7 \pm 2.3	–	228.2 \pm 102.7	n.d.	–	–

Numbers indicate the mean of $n=44$ to 110 independent measurements of thallus stratification with standard deviation. For *B. frigida*, marginal and central stratification properties were examined with samples from Inexpressible Island, Antarctica. marginal = lobe sections at the margin of a thallus, central = lobe sections from centrally located lobes, proximal = sections from the inward parts of a thallus, distal = sections near the branch tips of *C. gyrosa*

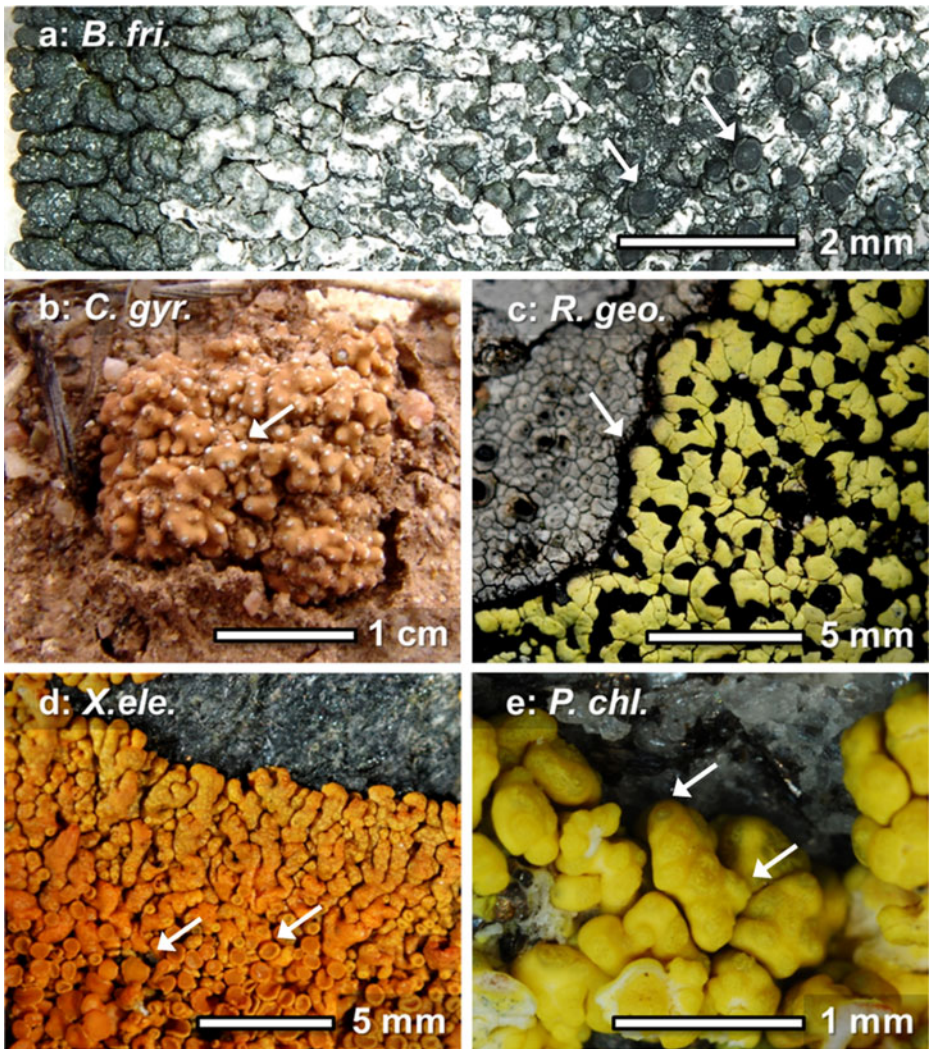


Fig. 1 Habitus macrographs of the five investigated lichens. **1a** thallus detail of *B. frigida* from marginal zones (left) to the center (right) with black rectangular areolae at the margin and angular areolae and black apothecia (arrows) in the center. **1b** vagrant, spherical, fruticose, and compact thallus of *C. gyrosa* with pseudocypbellae as white tips (arrow) at the end of sympodial branchings. **1c** habitus of a *R. geographicum* thallus with yellowish areolae, black interspersed apothecia and black prothallus (left margin, arrow). **1d** placoid thallus of *X. elegans* with protruding, branched, and narrow lobes at the margin (upper part) and disc-like apothecia in the center (lower part, arrows). **1e**: thallus detail of *P. chlorophanum*, showing yellow, irregular, distinct, convex to pulvinate areolae with verrucose openings of the pycnidia (arrows)

parts) of the thallus. Thalline lobes, pycnidia, and apothecia are frequently formed in the inner parts. The medulla is the largest stratum of *B. frigida* consisting of strongly interwoven hyphae, stabilizing the thallus, and acting as a layer of water retention and gas exchange. The thallus is tightly fixed to the substratum by medullary hyphae, often incorporating small rock particles. A lower cortex or rhizine strands were not observed.

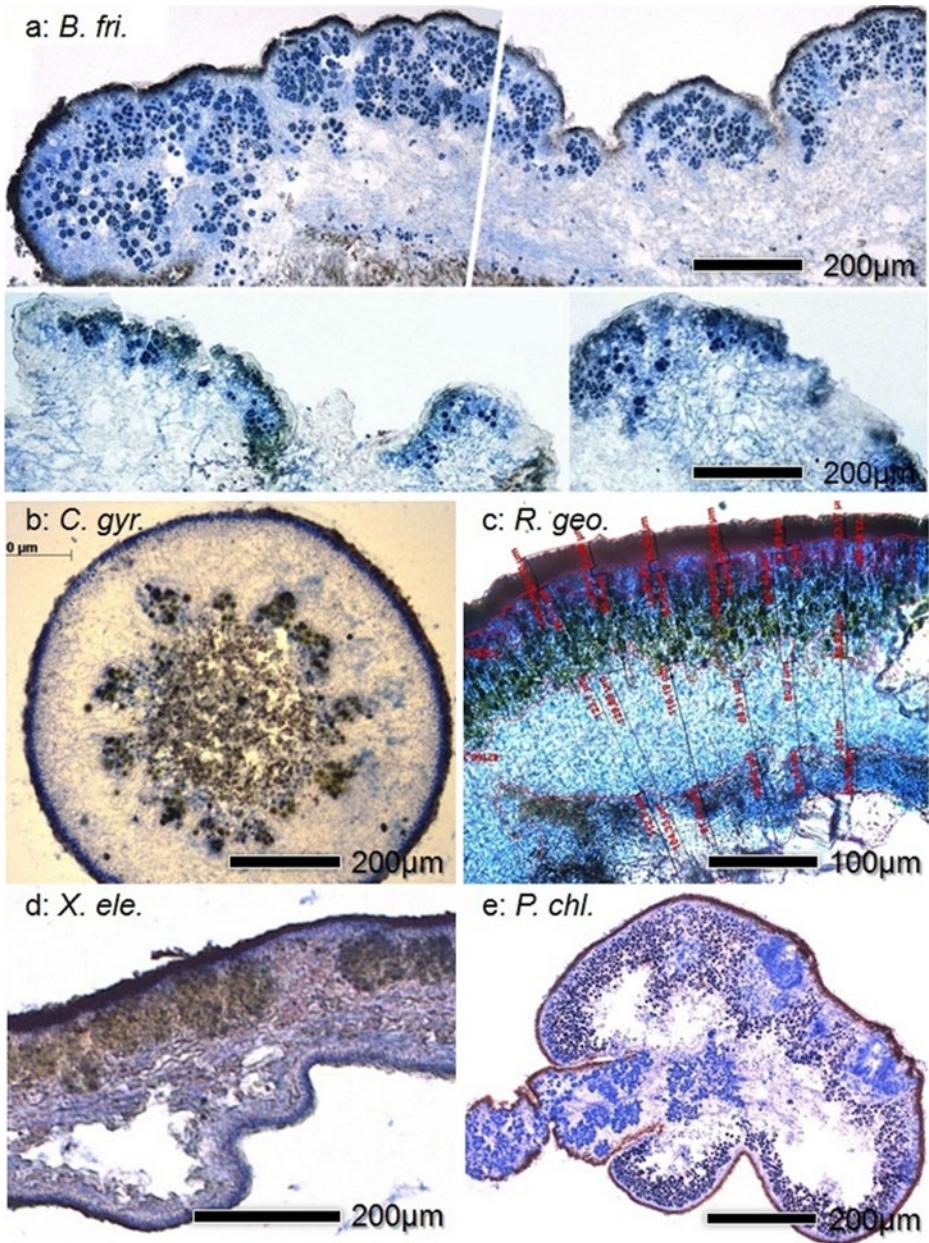


Fig. 2 Thin sections of five investigated lichens. **2a** *B. frigida*, high amounts of algal cells below a pigmented cortex at younger marginal areolae (upper row) and deceased algal numbers below the depigmented cortex in older, central areolae (lower row). **2b** *C. gyrosa*, distal cross section, stratification from outside to the center: pigmented and paraplectenchymatous cortex, pronounced subcortex, evenly arranged algal clusters and loose central medulla. **2c** section through an areola of *R. geographicum*, showing the algae arranged in vertical lines below the highly pigmented cortex. **2d** heteromerous lobes of *X. elegans* **2e** lobe section of *P. chlorophanum*, two types of photobionts, in the algal layer of the lobe (a) and at the base of the rhizine-like strand (left, b)

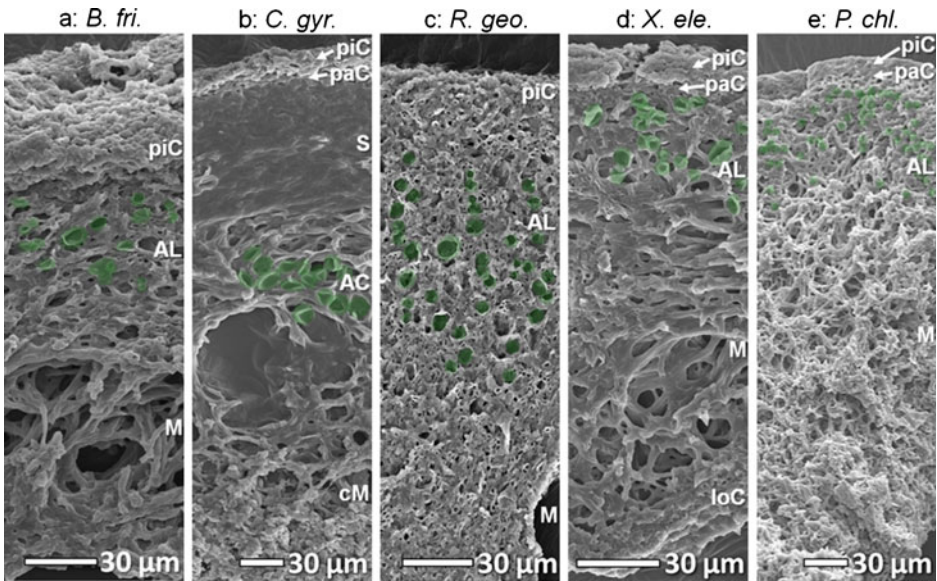


Fig. 3 Fixed SEM micrographs of the five investigated lichens. Algal cells highlighted with transparent green spots. **3a** *B. frigida* **3b** *C. gyrosa* **3c** *R. geographicum* **3d** *X. elegans* **3e** *P. chlorophanum*. Legend as follows: **AC**: algal cluster (in *C. gyrosa*), **AL**: algal layer, **cM**: medullary hyphae, sheathed with whewellite crystals (in *C. gyrosa*), **loC**: lower cortex (in *X. elegans*), **M**: medulla, **paC**: paraplectenchymatous Cortex, **piC**: pigmented cortex, **S**: subcortex (in *C. gyrosa*). Mucilageous epicortex (*B. frigida*, *R. geographicum*, *P. chlorophanum*) not visible in the chosen micrographs

Circinaria gyrosa

Morphology *C. gyrosa* is a vagrant, spherical, fruticose, and compact lichen of brownish to ochre colour with a diameter of max. \varnothing 2.5 cm (Fig. 1b). It has been recently assorted to the family of the Megasporaceae (Sohrabi 2012). The outer branches end in nearly circular pseudocyphellae that appear as white tips, exposing the medulla directly to the atmosphere (Fig. 1b, as described in Sancho et al. 2000). The surface is formed by a brownish epinecral layer containing no detectable amounts of SLCs (Raggio et al. 2011). The detailed study of morphological-anatomical traits reveals insight that might explain its high potential of

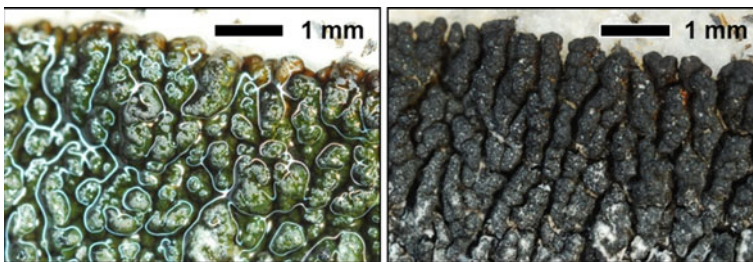
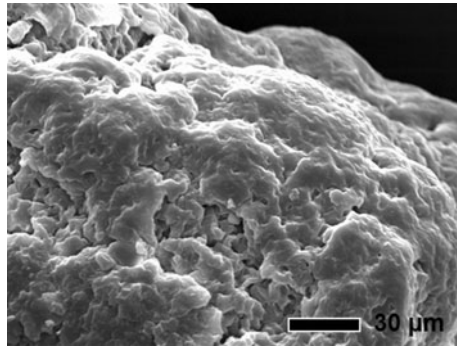


Fig. 4 Macroscopic top view on the thallus margin of *B. frigida* under wet and dry conditions. **4a** In the wet and swollen state (left) the green colour of the algal layer is dominant, while in the dry and shrunken state (**4b**, anhydrobiosis) the black melanin pigmentation of the upper cortex shields lower strata of the thallus from excess irradiation

Fig. 5 SEM-micrograph (*top view*) on the surface of a marginal *B. frigida* areola. The smooth areas are covered with mucilage, the rugged areas in between show the unsheathed hyphae of the cortex



resistance—as demonstrated in previous astrobiological studies (de la Torre et al. 2010a; Raggio et al. 2011; Sánchez et al. 2012). The porous pseudocyphellae at the tips facilitate gas exchange between the atmosphere and the interior gas space (Sánchez et al. 2012). Apothecia are rare and no subject of astrobiological studies; asexual reproduction was not observed.

Anatomy Thin sections (15 μm) of proximal and distal parts of sympodial branches revealed particular anatomical structures and internal stratification of *C. gyrosa* (Table 2, Figs. 2b and 3b). Minor differences between distal and proximal parts were detected. The outer stratum is formed by a brown epinecral layer, followed by a vivid paraplectenchymatous layer of roughly isodiametric cells. Below this layer, *C. gyrosa* forms a particular, extended, and compact periclinal prosoplectenchymatous subcortex. This pronounced thallus structure consists of tightly arranged fungal hyphae that are conglutinated by high amounts of extracellular mucilage (Fig. 3b). It is supposed to significantly contribute to the lichen's mechanical stability, to act as a diffusion barrier for gas exchange, and to contribute to the high resistance of *C. gyrosa*. Below, singled, dense, and evenly distributed clusters of photobiont cells are located which are lowly abundant and do not form a continuous algal layer as it is observed in other lichens (Figs. 2b and 3b; e. g. *Xanthoria*- and *Peltigera*-species). The algal clusters are more frequent in distal parts next to the pseudocyphellae. The spaces between the clusters consist of fungal tissue similar to the subcortex while inward, loose medullary hyphae connect the medulla to the clusters. The central branch tubes are formed by medullary fungal tissue that is rich in inner aerial spaces and connected to the atmosphere by apical pseudocyphellae. To prevent complete soaking with water under wet conditions and to enable efficient gas exchange when wet, the medullary hyphae were covered with extracellularly deposited whewellite-crystals (Fig. 6, Böttger et al. unpubl.).

Rhizocarpon geographicum

Morphology *R. geographicum* (Rhizocarpaceae) forms epilithic, crustose thalli of lime-green, angular to rounded, flat to convex areolae, situated upon a well-developed black hypothallus surrounded by a marginal prothallus (Fig. 1c). Usually several thalli fuse into large colonies. The frequently formed apothecia between the areoles are black and disc-shaped, with a black epithecium, a thin margin and up to \varnothing 1 mm. The asci form eight large (25–35 μm), melanized and septate spores. Asexual reproduction was not observed.

Anatomy Thalli samples from the Spanish location Navacerrada occasionally show an interrupted gelatinous epicortex that is missing in samples from the Swiss Alps (Table 2,

Fig. 6 SEM-micrograph, cross section of the medulla of *C. gyrosa*. The medullary hyphae are dense ensheathed with whewellite crystals (arrows)

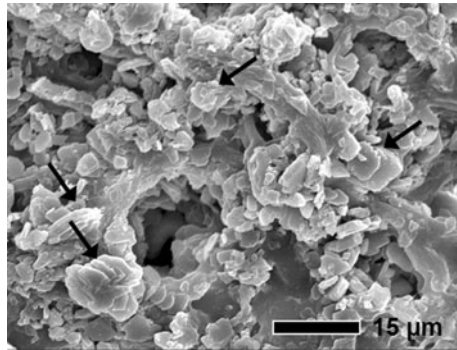
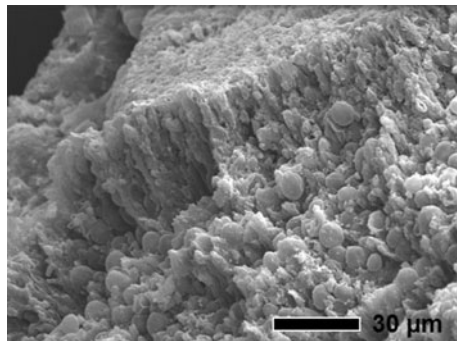


Fig. 2c). The samples collected in Spain were covered by a gelatinous layer upon the cortex which is thinner compared to samples from the Alps while the algal layer is thicker and the medulla is thinner. Samples from both locations display densely arranged cortical cells (Fig. 7) that are intensely coloured and incrustated with SLCs. The algal layer below is characterized by rows of algal cells and interjacent hyphae both arranged antiklin to the surface and highly gelatinized (Figs. 2c and 3c). Upon the blackish prothallus, the medulla is formed by densely aggregated and highly gelatinized hyphae of antiklin orientation with few interior gas spaces (Fig. 3c).

Xanthoria elegans

Morphology *X. elegans* (Teloschistaceae) is a placoid to crustose lichen (Fig. 1d) that may cover large areas of the substrate. At the margin, the thallus protrudes narrow, convex, densely arranged, and overlapping lobes that are lifted above the substrate or attached to it by rhizine-like strands. Thalli are often fusing to form large colonies. The bright yellow-orange to red colour is produced by SLCs in the upper cortex. The intensity of the colour depends on the degree of insolation in the respective habitat (Nybakken et al. 2004) and is effectuated by superficial formation of parietin-crystals—a SLC also found in abundant, orange, lecanorine apothecia (\varnothing 1–3 mm) in central thallus parts. The asci are formed among straight to branched paraphyses and bear eight elliptic ascospores. Asexual reproductive structures are missing.

Fig. 7 SEM-micrograph (side view) on the surface of a *R. geographicum* areola. The cortex is partially removed, exposing the algal layer and illustrating the clear vertical orientation of the hyphae in the cortex and the algal layer



Anatomy The anatomy of *X. elegans* is investigated with marginal (i. e. younger) and central (i.e. older) lobes of representable thallus samples (Table 2). The inner structure is heteromerous (Figs. 2d and 3d). The upper surface is coated by a well-developed upper cortex, which is formed by iso-diametric anticlinal paraplectenchymatous cells and pigmented by parietin in the outer parts. The algal layer is composed of distinct but evenly arranged clusters of photobiont cells with gelatinized interjacent hyphae (Fig. 3d). The medulla consists of a spongy, loose network of long periclinal prosoplectenchymatous hyphae forming a large, gas filled interior space (Fig. 3d). In contrast to the other investigated lichens *X. elegans* forms a lower cortex. The comparison of marginal and central sections indicates an extension of cortical, algal and medullary layers with age.

Pleopsidium chlorophanum

Morphology *P. chlorophanum* (Acarosporaceae) is a crustose, effigurate, morphologically variable lichen with irregular, distinct, convex to pulvinate areolae (\varnothing 10–20 mm), and a smooth to verrucose surface (Fig. 1e). The colour is sulphuric yellow on mature or exposed areolae to lime green on young or shaded areolae. Apothecia of up to \varnothing 2.5 mm are reported to be frequent (Øvstedal and Lewis Smith 2001), but not found in samples collected at Gondwana Station. This might be correlated to extreme environmental conditions preventing sexual reproduction by extremely short periods of favourable growth conditions. Nonetheless, large numbers of pycnidia are formed in the thalli releasing the pycnospores through bottle-neck apertures in verrucose elevations. Penetration of rock fissures by outgrowing hyphal strands is a common observation; it fixes the thallus to the substratum and promotes rock colonization as well as bio-weathering.

Anatomy The cortex is divided into a pigmented upper layer (Table 2, Fig. 3e). The pigmentation—which is missing in premature areolae but develops by time—is due to extracellular deposits of needle-shaped yellow crystals while the unpigmented layer is constituted of paraplectenchymatous and intensively gelatinized hyphae. The cortex is covered by an epicortex which is more pronounced above the pycnidia. Below this layer, the large numbers of photobiont cells are situated (Fig. 2e). They are not consistently arranged in a distinct and uniform algal layer, but fill large areas of the globose areolae more or less densely or clustered. Depending on that, the extremely loose medulla is irregularly shaped. In the vicinity of and within the basal thallus strands the aggregation of hyphae becomes denser and more gelatinized. In these basal parts of the areolae, a second type of photobiont is found which is smaller, blueish-green, and shows a different proliferation pattern (Fig. 2e). For clarification of photobiont identity molecular phylogenetics are in progress.

Discussion

As represented by the different growth types (crustose, placoid, and fruticose) and the diverse morphological and anatomical traits (i. a. the prevalence of different strata, Table 2), it is not a peculiar growth type or trait but an individual set of features that enables lichens to brave harsh environmental conditions and explains the high potential in resisting extreme environmental factors. Protection against excess PAR and UVR is often considered one of the most crucial factors in research on lichen extremotolerance (Solhaug and Gauslaa 2004), therefore the following paragraphs pre-dominantly focus on photoprotective effects.

By the comparative approach, it is possible to identify some features that contribute to the resistance of astrobiologically relevant lichens. While the discussion focuses on thalline structures, the fruiting bodies of the investigated lichens reveal additional features to protect the fungal spores inside: melanized paraphyses, a gelatinous matrix in and on the hymenium, deposits of SLCs in the epithecium, the hypothecium and the apothecial margins (parietin in *X. elegans*, melaninic substances in *B. frigida* and *R. geographicum*). In the case of *B. frigida* and *R. geographicum* the spores themselves are highly melanized implying that they are not only protected within the apothecium but also beyond, being an advantage for successful establishment at highly insolated habitats.

Mucilage Matrices The formation of extracellular polymeric substances (EPS, i. e. mucilageous or gelatinous matrices) is a basic property of the investigated lichens. Besides being the basic biont contact interface (Honegger 1992), mucilage covers the surface, conglutinates cortical and subcortical cells, ensheaths algal clusters and covers medullary hyphae. Two predominant appearances of gelatinous substances are observed: the formation of a gelatinous epicortex (partially in *P. chlorophanum* and *X. elegans*, site-dependent in *R. geographicum*, frequently in *B. frigida*) and the formation of gelatinous substances in peculiar strata (in the subcortex of *C. gyrosa* or the algal layer of *B. frigida*). Besides aspects of water-uptake and -retention, the mucilage in the epicortex and the (sub)cortex might promote resistance: It was discussed that gelatinous substances have UVR-screening properties (Lütz et al. 1997; Belnap et al. 2001; de Vera et al. 2003, 2010; Flemming et al. 2007; Ortega-Retuerta et al. 2009) and that mucilage might act as a radiation-protective layer. Studies with bacterial exopolymer biofilms show that they are only transmitted by minor proportions of UVR (13 % of UVC, 31 % of UVB, 33 % of UVA), protecting the cells from exposure and suggesting that EPS is a natural defense against UVR (Elasri and Miller 1999). With up to 12.6 µm in *B. frigida*, 5.0 µm in *R. geographicum*, and 4.1 µm in *P. chlorophanum* the mucilageous epicortices of these extremotolerant lichens are more extended than in the more temperate distributed Parmeliaceae (0.6–1.0 µm, Büdel and Scheidegger 1996). The epicortex may also change the reflection properties of the surface—due to refractive and dispersive effects—and reduce the intensity of PAR and UVR in the thallus. The remarkable amounts of gelatinous substances in the subcortex of *C. gyrosa* as well as in the algal layer of *B. frigida* and *R. geographicum* may cause additional shielding against PAR and/or UVR.

Cortices As in most lichens, a pigmented and conglutinated cortex is found in all five investigated species followed by an unpigmented paraplectenchymatous cortex in *C. gyrosa*, *X. elegans*, and *P. chlorophanum*. In these cortices, the vivid fungal cells are found in the lower part while the upper pigmented part occasionally lacks vivid cell lumina, forming an epinecral layer of pigment incrustated dead cell remnants (as observed in *X. elegans* and *P. chlorophanum*). In all cases the pigmentation is confined to fungal cell walls of the apical hyphae, ceasing with increasing depth. The SLCs of astrobiologically relevant lichens are addressed elsewhere (Meeßen et al., unpubl.), but also the cortical morphology contributes to resistance. Besides physiological limitation, herbivore defence, and mechanical stabilization, protection of the photobiont is considered a main function of the cortex (Ertl 1951; Jahns 1988; Kappen 1988). In general, lichen cortices are able to absorb 26–43 % of the incident light while shade- and light-adapted thalli of the same species may vary in cortical organization due to the different light regimes (Ertl 1951; Büdel and Scheidegger 1996).

Hydrated, physiologically active thalli of *B. frigida* are coloured intensively green by the algal layer below the cortex (Fig. 2a). The swelling of cortical cells by water-uptake reduces

the density of the cortical pigmentation, and exposes the algae to higher light intensities. If *B. frigida* passes into anhydrobiosis, the thallus becomes intensively coloured black by shrinking cortical cells and densifying melanin incrustations in the upper cortex (Fig. 4b). This effect of increasing cortical absorbance substantially reduces excess light levels reaching the photobiont and might be an adaptation comparable to the pruina, a superficial layer of crystalline deposits or dead cells that increases reflection when dry (Jahns 1988; Büdel and Scheidegger 1996) and resembles a protective adaptation (Kappen 1973). Both effects protect lichens most effectively in anhydrobiosis, in which it experiences considerably long periods of insolation (Lange et al. 1999), its repair mechanisms are dormant, and harmful effects of excess PAR/UVR are accumulative (Solhaug and Gauslaa 2004).

Astrobiological investigations stress the role of the lichen cortex in protecting fungal and algal bionts: for *R.geographicum* it was found that the removal of the cortex before exposition reduces the relative PSII activity of the photobiont depending on the type of UVR-transmission filter used (de la Torre et al. 2010a). In other experiments the thallus tissue viability decreased at about 15 % in *F. bracteata* and 25 % in *X. elegans* if the cortex is removed before UVR-exposure (de Vera et al. 2003; de Vera and Ott 2010). However, in such experiments the effect of the cortical structure itself was not separated from the effect of the deposited SLCs.

Subcortex In the present study, a subcortex is found in *C. gyrosa* only. It measures up to 150 μm and is characterized by dense fungal hyphae that are highly conglutinated with mucilage but lack any pigmentation. This structure is exclusively formed by the very lichen species that showed no sign of major SLC production (Raggio et al. 2011). The thick and dense fungal cortex was found to protect the algal populations within the cluster while the contribution of the single layers (cortex and subcortex) is not yet quantified. We conclude that the highly conglutinated subcortex does not only deal with the mechanical stress of the lichen's vagrant life style, but also compensates the lack of photoprotective SLCs. Especially, if its location above the algal layer, the lack of SLCs in the cortex (Raggio et al. 2011), and its high resistance towards UVR-exposure (de la Torre et al. 2010a; Sánchez et al. 2012) are taken into account. Several factors may contribute: the sheer thickness, the high amount of mucilage (with the shielding properties discussed above), and the protective effect of the densely packed hyphae themselves.

Algal Layer Investigations on the viability of both symbionts of *X.elegans* and *P. aphthosa* after exposure to UVC_(254nm) at 2.1–201.8 $\text{J}\times\text{m}^{-2}$ (de Vera and Ott 2010) showed a higher decrease in viability of the photobiont compared to the mycobiont, supporting the hypothesis that the photobiont is the more sensitive partner of the symbiosis. Recent observations show that the resistance of lichens to high UVR and vacuum can be attributed to the mycobiont (de Vera et al. 2008; de Vera 2012), while additional results indicate that also the arrangement of the photobiont contributes to resistance: In *B. frigida*, *C. gyrosa*, *P. chlorophanum* and *X. elegans* the algae are clustered in more or less dense aggregates which are enveloped by a layer of gelatinous substances. In *R. geographicum* the algae are surrounded by highly gelatinized hyphae and arranged in rows vertical to the surface of the thallus and thus, in line with the direction of most intensive insolation. Both arrangements—clustering and alignment—can be interpreted as protective strategies to avoid excess insolation. Live/Dead analysis after several simulation experiments supports this hypothesis, clearly showing that inner cells of algal clusters are more vital than outer ones after exposure to UVR or UVR + vacuum (de Vera et al. 2003, 2004a, b), even if isolated photobionts were tested (de Vera et al. 2008).

Basal Thallus Strands Out of the five investigated species, only *P. chlorophanum* forms basal thallus strands penetrating the upper layer of the rock substrate, integrating an endolithic characteristic to an usually epilithic lichen. This feature may not only substantially contribute to the lichen's potential of substrate colonization and bioweathering but may also reflect an adaptation towards extremotolerance. Growing inside the substrate and using its structure as a protection is a strategy of many organisms—including lichens—to colonize the most extreme terrestrial habitats (Sun et al. 2010). For *P. chlorophanum*, the endolithic strands resemble a reservoir of hyphal biomass which might allow regeneration if the epilithic thallus is damaged by stressors as UVR and abrasion. This is stressed by the fact that a second, morphologically distinct type of algal partner is found to be located in the basal zone, suggesting not only a regenerative capacity of the mycobiont but also of an alternative photoautotrophic partner.

General Aspects All investigated lichens reveal the same anatomical blueprint of a heteromerous thallus (Jahns 1988; Büdel and Scheidegger 1996) but show diverging sets of morphological-anatomical traits represented by the presence and properties of different strata and anatomical structures. Besides other factors (poikilohydry, SLCs), the results indicate that these traits help to explain lichen extremotolerance towards abiotic factors as well as their resistance towards space and Mars parameters (de Vera et al. 2003, 2004a, b, 2007, 2008, 2010; de la Torre et al. 2004; 2007; Sánchez et al. 2012). From a morphological point of view, in *B. frigida*, *R. geographicum*, and *X. elegans* the combination of cortex (with varying SLCs), algal arrangement, and mucilage seems to be fundamental to constitute resistance, while in *C. gyrosa* the subcortex seems to play a crucial role, as well as the rhizine-like strands in *P. chlorophanum*.

All lichens tested to date showed high viability in astrobiological experiments. However, experimental attempts to test the protective effects of the distinct morphological and anatomical thallus structures are scarce. In UV-exposure experiments ($\lambda > 160$ nm) with *X. elegans* and *F. bracteata*, lichen thalli with intact and with removed cortex were compared (de Vera et al. 2003). Samples with removed cortex showed a loss in viability of 15–35 % in *X. elegans* and 15–40 % in *F. bracteata* indicating a protective effect of the cortex. In the LITHOPANSERMIA experiment, *R. geographicum* and *X. elegans* were exposed to space with intact and with removed or depigmented cortices, respectively (de la Torre et al. 2010a), revealing a post-flight reduction of PSII activity of 6.9–81 % in *R. geographicum* and of 0.1–43 % in *X. elegans*. Comparing the effect of removed (*R. geographicum*) and depigmented (*X. elegans*) cortices, a more severe effect is found if the cortex is removed. However, such results do not help much to separate the protective effect of the cortex itself and the adjacent SLCs, as both lichens also reveal anatomical differences in terms of cortical structure (Table 2) and algal arrangement. Nonetheless, the high viability in both studies indicates additional protective features, e. g. as discussed above. In *C. gyrosa*, the post-flight reduction of PSII activity was low (0–4.5 %, de la Torre et al. 2010a) what might be correlated to the protective effect of its extended subcortex, as its cortex is supposed to lack sufficient amounts of SLCs (Raggio et al. 2011). The differences in the reduction of PSII activity in *X. elegans* and *R. geographicum* after the LIFE experiment are evident (Onofri et al. 2012). Post-flight dark control samples of *X. elegans* showed a reduction of PSII activity of 2 % and irradiated post-flight samples showed a reduction of about 55 %, in *R. geographicum* the reduction is 97.5 % and 99.5 %, respectively. What morphological-anatomical features of *X. elegans* might help to explain such difference? Especially as both lichens are crustose, form pigmented cortices of about 20 μm thickness, and bear a photobiont of the same genus (*Trebouxia*). Besides the different predominant SLCs in both

lichens (parietin in *X. elegans* compared to rhizocarpic acid in *R. geographicum*), two features may give an explanation: the additional 20 μm -wide paraplectenchymatous cortex and the densely clustered photobiont cells, presumably shielding each other more effectively than the aligned photobiont cells in *R. geographicum*. Nonetheless, a different level of desiccation resistance among the exposed lichen species is also supposed to contribute to the diverging survival rates.

The present study shows that generalizations concerning the resistance of lichens towards extreme conditions have to be avoided. The differences reflect the diverging evolutionary histories of their lineages which led to different adaptations to the respective ecological niches (Jahns 1988). Such adaptations enable the symbiosis to successfully cope with prevalent abiotic stressors and support its persistence in extreme habitat. Alpine and polar regions are characterized by high levels of insolation and the dominance of lichens in these regions can be explained by their ability to endure UVR (Solhaug and Gauslaa 2004). Several studies highlight the mycobiont to be more resistant towards UVR-exposure than the photobiont (de la Torre et al. 2002; de Vera et al. 2008; de Vera and Ott 2010; de Vera 2012), and thus protects the photosynthesizing partner. Nonetheless, studies with isolated mycobionts stress that undifferentiated axenic fungal tissue is more susceptible to the damaging effects of UVR than complete lichen thalli (de Vera and Ott 2010). These results give a clear hint on the importance of distinct differentiated thallus structures—as demonstrated in the present study—rather than mere fungal biomass.

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