Thallus morphology of two Antarctic foliose lichens evaluated by a digital optical microscopy approach

Short Communication

Rastislav Ošťádal[1], Jana Hazdrová[2]

[1]*Keyence International (Belgium) NV/SA, Keyence Microscope, Europe, Na Strži 65/1702, 140 62 Praha 4, Czech Republic*
[2]*Department of Experimental Biology, Laboratory of Photosynthetic Processes, Faculty of Science, Masaryk University, University Campus – Bohunice, Kamenice 5, 62500 Brno, Czech Republic*

Abstract
Digital microscopy is an emerging technique that combines the tools of classic light microscopy with a computerized imaging system. The main components of digital microscopy is image formation by optics of the system, image registration by a digital camera, saving of image data in a file format that enables advanced image analysis. In this paper, we bring first data on application of digital microscopy approach in lichen thallus morphology study. Two Antarctic lichen species (*Xanthoria elegans*, *Umbilicaria decussata*) with a foliose morphotype of their thallus were studied. Both experimental species had an irregularly round or eliptic shape of a thallus that enabled to measure its diameter. After magnifition, images were taken in dry and fully-hydrated state of thallus in order to evaluate hydration-dependent size changes in thallus size and structures. It has been demonstrated that hydration-dependent size increment depend on thallus size and particular part of thallus. Mean increment of thallus diameter reached 15.1% and 13.8% for *X. elegans* and *U. decussata*, respectively. Higher value of diameter increment (26 %) was found for the upper projection area of apothecia, fruiting bodies developed over the upper thallus surface of *X. elegans.* Size and volume increment in thallus parts is discussed as a consequence of water holding capacity of lichens, and a capability of lichens to hold intra- and extracellular water upon full hydration of a thallus. Finally, a potential of digital microscopy for future studies is discussed as well as some processing techniques such as *e.g.* metrics of profile lines through 3-D objects like apothecia.

Key words: *Xanthoria elegans*, *Umbilicaria decussata*, thallus hydration, thallus dimension, morphometry

DOI: 10.5817/CPR2016-1-8

Received June 26, 2016, *accepted* July 31, 2016.
*Corresponding author: Rastislav Ošťádal <r.ostadal@keyence.eu>
Acknowledgements: Company Keyence is proud that prof. Barták's research group decided to use our digital microscope VHX-5000 and that we could help within research activities. Thank you for your trust. The authors are gratefull to CzechPolar2 infrastructure to work in the Extreme Environments Life laboratory (EEL, Masaryk University, Brno).

Introduction

Lichens are poikilohydric symbiotic organisms. Their water status changes passively with natural variation in environmental conditions, water and moisture availability in particular. Therefore, lichens must cope with frequent hydration and desiccation. During desiccation, lichen decrease their relative water content from 100% (fully wet) to 0% (fully dry). Therefore, thallus water potential decreases from water potential of 0 MPa (fully wet and photosynthetically active) to critical (minimum) water potential found at about -25 MPa (dry, photosynthetically inactive). At full hydration, however, phenomenon of water suprasaturation effect can be found in some lichens which can result in depressed photosynthesis (Lange et al. 2001). At low water potential (typically below -15 MPa), partial or full inhibition of photosynthetic processes is evidenced. For two species included into our experiments, previous studies reported critical -25 MPa for *Xanthoria elegans* (Barták et al. 2005), and -18 MPa for *Umbilicaria decussata* (Jupa et al. 2012). Numerous other studies reported how lichens of different morphology respond to a frequently and rapidly changing availability of water (*see* e.g. Kappen et Valladares 1999). Upon uptake of water by a dry lichen thallus, changes in thallus morphological characteristics happen thanks to increased amount of intracellular water both in fyco- and mycobiont forming lichen association. Those changes comprise an increase in thallus thickness and volume. In many lichen species with foliose morphology, they are accompanied by changes in structural features, such as *e.g.* thallus density, upper surface area, anatomy (Valladares et al. 1998 for *Umbilicariaceae* family). Lichen species differ in their ability to store intrathalline water and thus exhibit species-specific water holding capacity (Gauslaa et Coxson 2011). Several studies have been conducted to study various aspects of water-holding capacity in lichens such as *e.g.* thallus size (Gauslaa et Solhaug 1998), surface characteristics, growth forms (*see* e.g. Olsson 2014) for thin, filamentous alectorioid lichens), interspecific differences (Dahlman et Palmqvist 2003), and limitation of photosynthetic processes in highly-hydrated lichen thalii (Lange et al. 1996). Changes in thallus volume and hydration-dependent changes of thallus morphology are, however, studied less frequently. Hygrochasic movements of thalli parts with dehydration are reported in lobate lichens and considered a photoprotective mechanism (Barták et al. 2006).

Material and Methods

Xanthoria elegans and *Umbilicaria decussata* were collected at northern part of the James Ross Island in February 2016. Collecting site of *X. elegans* was located close to Big and Small Lachman lakes on North-East slopes of the Berry Hill mesa (63° 47′ 57′′ S, 57° 49′ 04′′ W). *U. decussata* was collected from the upper parts of volcanic boulders located N of the Berry Hill mesa (43° 48′ 31′′ N, 57° 50′ 30′′ W). The thalli of collected lichens were dried under natural outside conditions and then stored at 5°C. They were transported in dry state into the Czech Republic where stored in a refrigerator until used for wetting experiment.

In this preliminary study, we applied a new method of measurements hydration-dependent changes in dimensions of lichen thallus. The method allows accurate identification and quantification of changes in size of particular surfaces using digital op-

tical microscopy approach. First, dry thalli of *U. decussata* and *X. elegans* were analysed using a digital VHX-5000 microscope with a maximum resolution of 18 megapixels (Keyence, Japan) powered by microscope controller unit with integrated 23" LED monitor and Keyence software. The microscope had built-in LED lights and magnification adjustable from 20 to 200×, thus allowing accurate measurements of lichen surface viewed in real time depth composition. The microscope was equipped with a versatile stand and stage that allowed 360 degree views of an object.

Upper surface of dry thallus was lit by a high brightness LED lamp and then documented by a camera with CMOS image sensor and software resulting in a digital photograph file. Then, water was added to the experimental thalli by regular spraying by demineralized water and the thalli were measured repeatedly each 30 min. Each measurement was documented by recording a digital image. The images were of high-resolution quality thanks to a short-wavelength light used for illumination of a thallus, and the HDR (high dynamic range) function that captures high-color gradation images at different exposures and then compiles them into a single image. The images were stored in a VHX (Keyence, Japan) integrated system, that enabled observing, capturing and measuring of morphometrical parameters of the experimental thalli. In this preliminary study, we used images of dry and fully wet thalli and measured hydration-induced changes in size of particular thallus parts, apothecia using tools for metrical measurements in the Keyence software.

Results and Discussion

X. elegans forms foliose thallus organized in a rosette up to 6 cm in diameter (*see* Fig. 1). Marginal parts shows dorsiventral lobes with rotund to truncate upper surface (Nash et al. 2004). The species does possess apothecia. Hydration of *X. elegans* thallus led to an increase in thallus size (diameter), as well as diameter of apothecia. We measured hydration-dependent diameter change only in those particular apothecia located in perpendicular plane to optical axis. Mean diameter increase was 26.0 % ± 6 %. Overall thallus size increased by 14.5 % ± 1 %. Hydration of *U. decussata* thallus led to an 13.8 % ± 1 % increase in thallus size (diameter). In *X. elegans* an increase in apothecia diameter was 27.0 ± 6.0 μm (*see* Fig. 2). Relative increment of the diameter is apothecium-size dependent. The smaller apothecium, the lower diameter increase.

Generally, the increase of thallus size and particular morphological parts is caused by water uptake into fungal and autotrophic partners forming lichen association. In lichens, maximum amount of water that can be absorbed by a thallus is called the water-holding capacity. Thanks to differences in anatomical and morphological properties, water-holding capacity is species-specific. Lichens, however, do not have any active mechanism to regulate their water content and their water holding capacity. Water content in lichen thalli, however, can be controlled through morphological traits. Several factors influence the water-holding capacity of lichens. The first is the ratio between surface area and weight. The second is thallus surface characteristics, and the third relates to different growth forms.

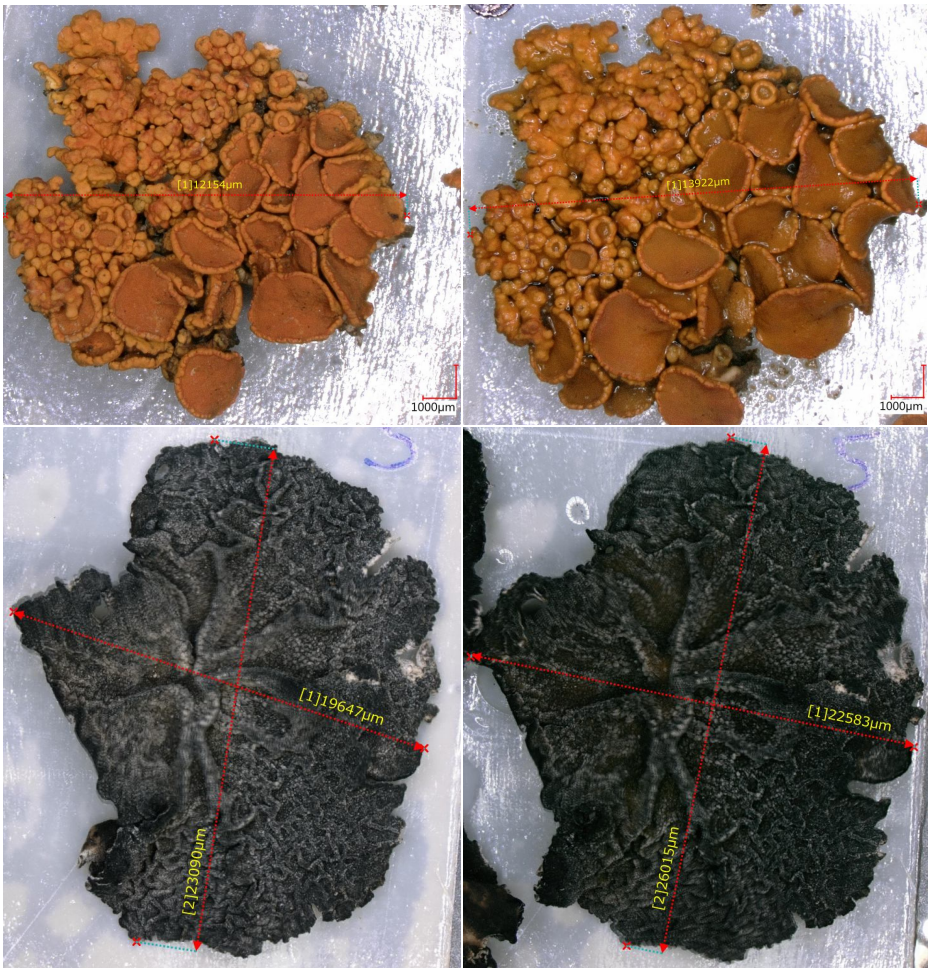

Fig. 1. Lenghts measured across thalli in dry (left column) and wet (right column) of *Xanthoria elegans* (upper row) and *Umbilicaria decusata* (lower row).

Size and volume increment upon lichen thallus rehydration is dependent on thallus anatomy, morphology (Fos et al. 1999) and considered species-specific. It is, however, still an open question whether extracellular water may be found in a thallus when fully hydrated. Several microscopic studies addressed the problem using cross sections of frozen thalli. In these studies (*see* e.g. de los Ríos et al. 1999), the techniques such as low temperature scanning electron microscopy (LTSEM) and confocal laser scanning microscopy (CLSM) were used. Souza-Egipsy et al. (2000) reported presence of extracellular water in *Xanthoria parietina* and attributed to hygroscopicity of the upper cortex of lichen thallus. Accumulation of water in intrathalline extracellular spaces may have negative consequences to CO_2 exchange in fully-hydrated lichen thallus since it may be a physical barrier to CO_2 diffusion into photobiont cells (Scheidegger 1994, Scheidegger et al. 1995).

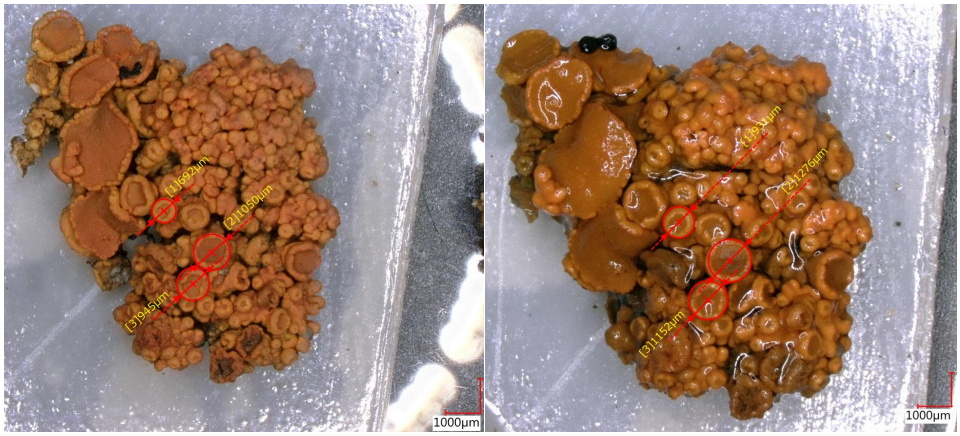

Fig. 2. Dry (left) and wet (right) thallus of *Xanthoria elegans* with indication of apothecium diameter using a software tools. An example shows 3 particular apothecia oriented in a plane rependicular to the optical axis of a microscope. Particular diameters are indicated on the photographs as red circles and their numerical values reported in yellow characters.

Although many studies have been devoted to water holding capacity (Gauslaa et Solhaug 1998, Merinero et al. 2014), maximum storage of water and surface to volume ratio (Snelgar et Green 1981) in hydrateted lichens, only little attention has been devoted to size and voluntometric changes in lichens upon hydration of thallus. In lichens with fruticose thallus anatomy, however, the study of Esseen et al. (2015) addressed this problem and related the changes to different morphological characteristics such as *e.g.* density of branching and thallus area overlap ratio. In foliose lichens, however, size and volume increase by a thallus and/or its morphological structures is less studied. The method described in this study provides an efficient approach to study hydration dependent changes in a great variety of lichens and morphological parts forming thallus structure.

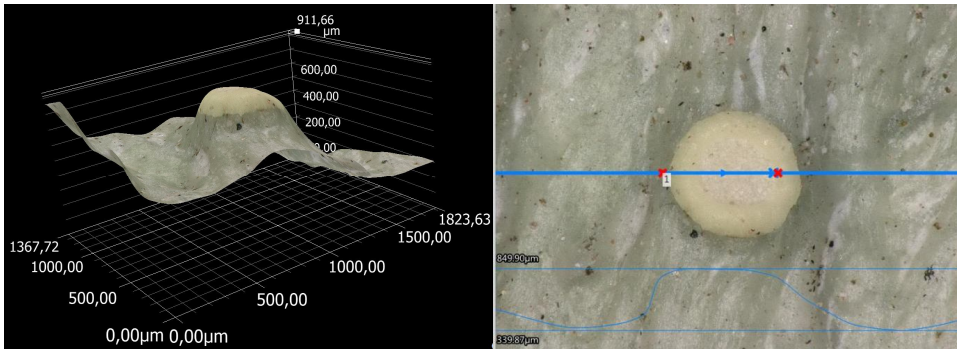

Fig. 3. A 3-D image of a nearly stage of apothecium of *Ramalina* sp. showing the height of the upper flat area of apothecium above thallus surface (about 510 µm) – left. Measurements of diameter of apothecium and a profile of cross section through an apothecium of *Ramalina* sp. – right.

Concluding remarks

Hydration-dependent changes in lichen morphology can be studied by a new technique of digital optical microscopy presented in this study. In this approach, reflected light capacity enables whole lichen specimens or particular surface structures such as *e.g.* apothecia to be observed, saved as a 3-D image and processes using different image processing tools. Such as *e.g.* height profile along a selected line placed across a thallus from a margin through centre to an opposite margin or through an apothecium (*see* Fig. 3). Another option is to use DFP method (Depth From Defocus) which compiles an image from images taken at different focal planes (typically from bottom to the top of investigated object). This approach might be applied in upper thallus structures like pustulas or apothecia and supplemented by advanced techniques like *e.g.* calculation of cross-sectional area of pustulas and their volume.

References

BARTÁK, M., GLOSER, J. and HÁJEK, J. (2005): Visualized photosynthetic characteristics of the lichen *Xanthoria elegans* related to daily courses of light, temperature and hydration: A field study from Galindez Island, maritime Antarctica. *The Lichenologist*, 37: 433-443

BARTÁK, M., SOLHAUG, K. A., VRÁBLÍKOVÁ, H. and GAUSLAA, Y. (2006): Curling during desiccation protects the foliose lichen *Lobaria pulmonaria* against photoinhibition. *Oecologia*, 149: 553-60.

DAHLMAN, L., PALMQVIST, K. (2003): Growth in two foliose tripartite lichens, *Nephroma arcticum* and *Peltigera aphthosa*. *Functional Ecology*, 17:821-831.

DE LOS RÍOS, A., ASCASO, C. and WIERZCHOS, J. (1999): Study of lichens with different state of hydration by the combination of low temperature scanning electron and confocal laser scanning microscopies. *International Microbiology*, 2: 251-257.

ESSEEN, P. A., OLSSON, T., COXTON, D. and GAUSLAA, Y. (2015): Morphology influences water storage in hair lichens from boreal forest canopies. *Fungal ecology*, 18: 26-35.

FOS, S., DELTORO, V. I., CALATAYUD, A. and BARRENO, E. (1999): Changes in Water Economy in Relation to Anatomical and Morphological Characteristics During Thallus Development in *Parmelia acetabulum*. *The Lichenologist*, 31: 375-387.

GAUSLAA, Y., COXSON, D. (2011): Interspecific and intraspecific variations in water storage in epiphytic old forest foliose lichens. *Botany*, 89: 787-798.

GAUSLAA, Y., SOLHAUG, K.-A. (1998): The significance of thallus size for the water economy of the cyanobacterial old-forest lichen *Degelia plumbea*. *Oecologia*, 116: 76-84.

JUPA, R., HÁJEK, J., HAZDROVÁ, J. and BARTÁK, M. (2012): Interspecific differences in photosynthetic efficiency and spectral reflectance in two *Umbilicaria* species from Svalbard during controlled desiccation. *Czech Polar Reports*, 2: 31-41.

KAPPEN, L., VALLADARES, F. (1999): Opportunistic growth and desiccation tolerance: the ecological success of poikilohydrous autotrophs. *In*: F. I. Pugnaire, F. Valladares (eds.): Handbook of functional plant ecology. New York: Marcel Dekker, Inc, pp. 10-80.

LANGE, O. L., GREEN, T. G. A., REICHENBERGER, H. and MEYER, A. (1996): Photosynthetic depression at high thallus water content in lichens: concurrent use of gas exchange and fluorescence techniques with a cyanobacterial and a green algal *Peltigera* species. *Botanica Acta*, 109: 43-50.

LANGE, O. L., GREEN, T. G. A. and HEBER, U. (2001): Hydration-dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance? *Journal of Experimental Botany*, 52: 2033-2042.

MERINERO, S., HILMO, O. and GAUSLAA, Y. (2014): Size is a main driver for hydration traits in cyano- and cephalolichens of boreal rainforest canopies. *Fungal Ecology*, 7: 59-66.

NASH, T. H., RYAN, B. D., GRIES, C. and BUNGARTZ, F. (eds.) (2004): Lichen flora of the Greater Sonoran Desert Region. Vol. 2., 742 p.

OLSSON, T. (2014): Morphological traits in hair lichens affect their water storage. Thesis. University of Umeå, Sweden. 26 p.

SCHEIDEGGER, C. (1994): Low temperature scanning electron microscopy: the localization of free and perturbed water and its role in the morphology of the lichen symbionts. *Cryptogamic Botany*, 4: 290-299.

SCHEIDEGGER, C., SCHROETER, B. and FREY, B. (1995): Structural and functional processes during water vapour uptake and desiccation in selected lichens with green algal photobionts. *Planta*, 197: 399-409.

SNELGAR, W. P., GREEN, T. G. A. (1981): Ecologically-linked variation in morphology, acetylene reduction, and water relations in *Pseudocyphellaria dissimilis*. *New Phytologist*, 87: 403-411.

SOUZA-EGIPSY, V., VALLADARES, F. and ASCASO, C. (2000): Water Distribution in Foliose Lichen Species: Interactions between Method of Hydration, Lichen Substances and Thallus Anatomy. *Annals of Botany*, 86: 595-601.

VALLADARES, F., SANCHO, L. G. and ASCASO, C. (1998): Water storage in the Lichen family *Umbilicariaceae. Botanica Acta*, 11: 1-9.