Are long-living lichen thalli an arena for photobiont variation?

Lucie VANCUROVA[a], Lucia MUGGIA[b], Pavel ŠKALOUD[a], Ondrej PEKSA[a,c], Martin GRUBE[b]

[a]*Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, CZ-12801, Praha 2, Czech republic*
[b] *Institute of Plant Sciences, Karl-Franzens-University Graz, Holteigasse 6, 8010 Graz, Austria*
[c] *The Westbohemian Museum in Pilsen, Kopeckého sady 2, CZ-30100 Plzeň, Czech Republic*

Introduction. Previous studies of symbiont selectivity in lichens often relied on the uniformity of the algal partner in individual thalli. Fingerprinting analyses of whole thallus DNA extractions, however, reveal presence of multiple *Trebouxia* strains within individuals of different species. Lichen fungi with an ecologically wide distribution, however, can associate with a wide range of *Trebouxia* photobionts[(2,5)]. A mixture of algal genotypes may be an adaptive advantage, because their different physiological performances can mediate tolerance to varying environmental conditions.

It is known that the lichen *Protoparmeliopsis muralis*, which has a broad ecological tolerance, can host different algae in the same habitat[(4)]. In our preliminary observation we noticed that regenerative outgrowths (lobes or areoles) in the centre of large thalli occasionally contain different algae than found in the peripheric margins.

We hypothesize that the lichen thallus of *Protoparmeliopsis muralis* could be an “arena” for combinations of the mycobiont with several algal strains, in which new fungal combinations with algal strains can arise by secondary growth. Those combinations with faster growth would be fitter under the prevailing ecological conditions and may out-compete other combinations to dominate the re-growing thallus center.

Fig. 1. Habit of *Protoparmeliopsis muralis*; central (C) and external (E) areoles are marked by arrows. Bar = 1 cm.

Material and Methods. We compared the photobiont diversity of marginal lobes (E in Fig. 1) and regenerative central outgrowths (C in Fig. 1) in thalli of *Protoparmeliopsis muralis* collected in eight ecologically different localities among Austria (3), Czech Republic (2), Germany (1) and Italy (2). The specimens were growing on different substrates: calcareous rocks and concrete. According to the availability of lichen material, we sampled for each locality from 2 to 15 thalli. For each thallus one central and one external lobes were prepared for DNA extraction. For those samples which presented external algal colonies on the thallus surface the analyses were performed with both washed and unwashed thalli. The washing was performed with sterile water in order to remove any contaminant algae on the thallus surface, and compare in this way the photobiont composition. The algal DNA was amplified for the nuclear ITS1 fragment (primers ITS2, nuSSU-1780´) [(1,6)] and the PCR products were analysed by using the single strand conformation polymorphism technique (SSCP). Representative bands were excised from the gel and the identity of the photobionts was determined by sequencing. The two bigger populations of *P. muralis* collected in Austria [Mt. Schöckl, Austria (1) in Fig. 2] and in Czech Republic [Kladno, Czech Republic (1) in Fig. 2] under different ecological conditions were thoroughly analysed in all their individuals and the diversity of the ITS1 sequences was analysed by haplotype analysis (program TCS)[(3)].

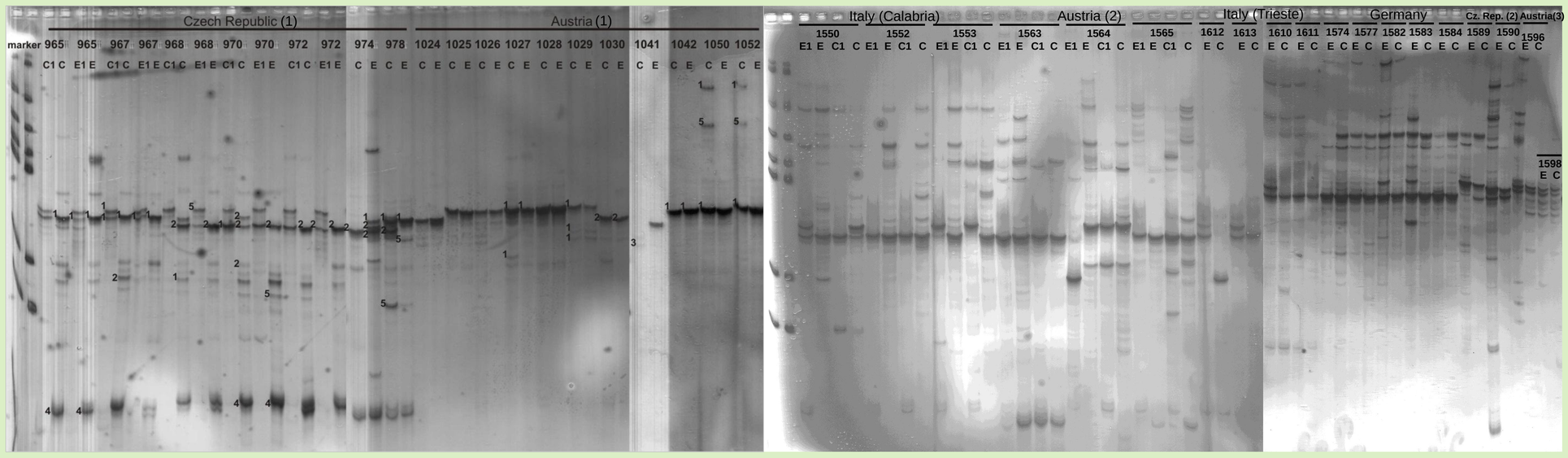
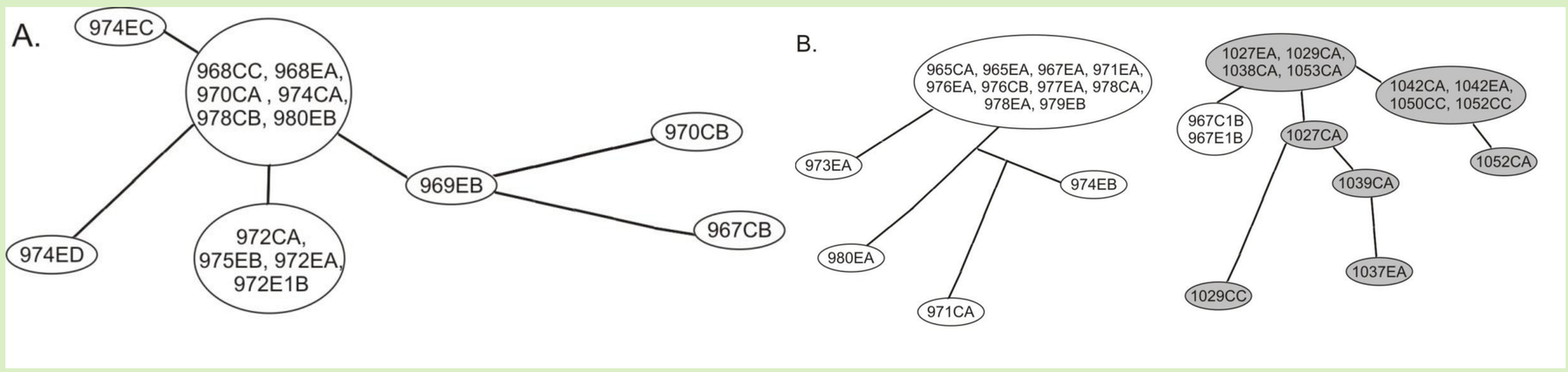


Fig. 2. SSCP analyses of selected samples of *P. muralis*. For each sample one central (C) and one external (E) lobe were analyzed. The lobes of those samples which were cleaned from contaminant algae on the thallus surface are marked as C1 and E1. The samples are reported with their DNA extraction number. Sequenced bands are labeled by a number corresponding to their identity according NCBI blast similarity: 1) *Trebouxia* sp. (uncultured); 2) *Trebouxia incrustata*; 3) *Asterochloris*; 4) *Chlorella*; 5) others.



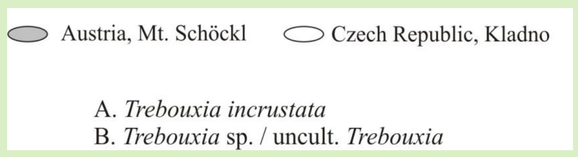


Fig. 3. Haplotype analyses of all *Trebouxia* spp. sequences recovered in two population of *P. muralis* [Mt. Schöckl, Austria (1), Kladno, Czech Republic (1)].

Results and Discussion. The SSCP analysis revealed a high intrathalline variation of algae in thalli of *Protoparmeliopsis muralis* coming from different environments. Samples of the same origin were homogeneous in their photobiont composition, whereas clear differences of banding pattern were observed among different sampling localities.
The washing removed many contaminant algae from the lichen thalli, as the number of SSCP bands is clearly lower in washed samples than in the corresponding unwashed ones (Fig. 2, comparison between E vs. E1 and C vs. C1).
The sequencing results confirmed the presence of four major photobionts within and superficially the thalli of *P. muralis*, identified as *Trebouxia* sp. (uncultured), *Trebouxia incrustata*, *Asterochloris* and *Chlorella*. We assume that the predominant photobionts, *Trebouxia* sp. (uncultured) and *T. incrustata a*re the true photobionts, as they are present in both washed and unwashed specimens and are represented by the thickest band in the SSCP analyses.
The haplotype analysis performed for the two Austrian and Czech populations revealed that *Trebouxia* sp. photobionts can be distiguished in two groups correlating geographic origins (Fig. 3).
We do not recover, however, any striking differences in photobiont composition between central and external lobes of the same thallus. We demonstrate that the cosmopolitan *P. muralis* associates with different photobionts, and these multiple associations may promote longevity and robustness, and be responsible for its wide ecological range.

References. 1) Beck A, Friedl T, Rambold G. 1998. *New Phytology* **139**:709–720; **2)** Blaha J, Baloch, E, Grube, M. 2006. *Biological Journal of Linnean Society* **88**:283–293; **3)** Clement M, Posada D, Crandal KA, 2000. *Molecular Ecology* **9**: 1657–1659; **4)** Guzow-Krzeminska B. 2006. *Lichenogist* **38**:469–476; **5)** Muggia L, Grube M, Tretiach M. 2008. *Mycological Progress* **7**:147–160; **6)** Piercy-Normore MD, DePriest PT. 2001. *American Journal of Botany* **88**: 1490 – 1498.

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