Black fungi in lichens from seasonally arid habitats

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Abstract: We present a phylogenetic study of black fungi in lichens, primarily focusing on saxicolous samples from seasonally arid habitats in Armenia, but also with examples from other sites. Culturable strains of lichen-associated black fungi were obtained by isolation from surface-washed lichen material. Determination is based on ITS-DNA sequence data and comparison with published sequences from other sources. The genera Capnobotryella, Cladophialaphora, Coniosporium, Mycosphaerella, and Rhinocladiella were found in different lichen species, which showed no pathogenic symptoms. A clade of predominantly lichen-associated strains is present only in Rhinocladiella, whereas samples of the remaining genera were grouped more clearly in clades with species from other sources. The ecology of most-closely related strains indicates that Capnobotryella and Coniosporium, and perhaps also Rhinocladiella strains opportunistically colonise lichens. In contrast, high sequence divergence in strains assigned to Mycosphaerella could indicate the presence of several lichen-specific species with unknown range of hosts or habitats, which are distantly related to plant-inhabitants. Similar applies to Cladophialaphora strains, where the closest relatives of the strains from lichens are serious human pathogens.

Key words: Aridity, black fungi, Chaetothyriomycetidae, Dothideomycetidae, phylogeny, symbioses.

INTRODUCTION

“Black fungi” is a practical term to group heterogeneous lineages of Chaetothyriomycetidae and Dothideomycetidae with melanised cell walls. It comprises fungi with diverse ecology and different growth styles, such as black yeasts or meristematic black fungi. In black yeasts, daughter cells form yeast-like multilateral or polar budding. The term meristematic was originally introduced for black fungi by de Hoog and Hermanides-Nijhof (1977) and describes non-disintegrating phenotypes which form aggregates of thick-walled, melanised cells. Meristematic growth is infrequent in the fungal kingdom, yet it was for example found in several fungi that parasitise lichens (Diederich 1990).

Black fungi can occur in extreme environments and under poor nutrient conditions, where they often grow meristematically (Sterflinger et al. 1999). In the last yr dematiaceous fungi with meristematic and yeast-like growth patterns, turned out to be, together with lichens and cyanobacteria, among the most successful inhabitants of marble, limestone, granite, and other rock types in arid and semi-arid environments (Sterflinger & Krümbein 1997, Wollenzien et al. 1997, Sterflinger 1998, Ruibal et al. 2005). They have been found in hot deserts of Arizona (U.S.A.) (Staley et al. 1982; Palmer et al. 1987), in cold Antarctic deserts (Nienow & Friedmann 1993, Selbmann et al. 2005), in Mediterranean countries as e.g. Italy, Greece, Turkey (Gorbushina et al. 2005, Ruibal et al. 2005, Sert & Sterflinger 2005), on stone monuments in Austria (Sterflinger & Pillinger 2001), and on granites of the Ivory Coast (Büdel et al. 2000). Sterflinger & Krümbein (1995) hypothesised that the ability to grow meristematically provides the colonies an optimal surface/volume ratio for enhanced stress tolerance. In particular they resist elevated temperatures, low water availability (Wollenzien et al. 1995), UV radiation (Urzi et al. 1995), high salt concentration (Zalar et al. 1999) or combinations of these factors and further stresses (Selbmann et al. 2005, Scott et al. 2007).

Due to the high selective pressure exerted by these stresses on the microbial community, black fungi are rarely found in complex microbial populations, rather they occur solitary or in communities with similarly stress resistant organisms such as lichens (Onofri et al. 2007a) and cyanobacteria (Sterflinger 2006). This is perfectly shown on rock surfaces where black fungi can form communities with epi- and endolithic lichens, cyanobacteria, chemooorganotrophic bacteria and fungi (Nienow & Friedmann 1993).

In the course of current lichenological explorations of Armenia, the first author frequently found lichen thalli with obscure discolourations. Microscopic study showed that they were caused by fungal colonisation, although not leading to sexual structures or distinctive anamorphs on lichen thalli as characteristic of most lichenicolous fungi (Lawrey & Diederich 2003). Rather, the hyphae grew isolated or as sclerotal aggregates on the thallus surface. Further diagnostic characters of the dematiaceous hyphae were missing. The phylogenetic relationships of lichen-dwelling hymenomycetes are generally unclear, due to the lack of sequence data. Despite some of the described taxa seem to be specific to their host, it is not known if other lichenicolous black fungi can colonise a wider range of hosts with similar habitat ecology.

MATERIALS AND METHODS

Sampling

Lichen material originates from Armenia. The samples were collected at different altitudes (up to ca. 2800 m), and on different substrates (basalt, calcareous and siliceous rocks, and few also bark) representing comparatively dry habitats (precipitation mostly between 400–600 mm/yr¹). Additionally, we used samples from the mountain and the Mediterranean belts of Austria, Italy and Spain.
Table 1. List of investigated specimens with collecting sites, herbarium numbers, GenBank accessions of the ITS sequences of the black fungal strains.

| Lichen species                          | Origin                                                                 | Black fungi isolated                         |
|-----------------------------------------|------------------------------------------------------------------------|----------------------------------------------|
| *Caloplaca erodens* Tretiach, Pinna & Grube | Austria, Styria, Mt. Hochlantsch, c. 1620 m a.s.l., on limestone, 2005, Muggia (TSB 36936). | Capnobryella sp. | FJ265744 |
| *C. erythrocarpa* (Pers.) Zwackh        | Armenia, Syunik, Goris, c. 1550 m a.s.l., on rock, 2006, Harutyunyan (GZU 23-1006). | Cladophialophora sp. | FJ265749 |
| *C. gomerana* J. Steiner                | Spain, Canary Islands, Tenerife, Punta Roja, 15 m a.s.l., on basalt, 2005, Muggia (TSB 36862). | Coniosporium sp. | FJ265755 |
| *C. holocarpa* (Ach.) A.E. Wade         | Armenia, Kotayk, Jvej, c. 1300-1400 m a.s.l., on Prunus sp., 2005, Harutyunyan (GZU 36-105). | Mycosphaerella sp. | FJ265756 |
| *C. saviola* (Hoffm.) Nordin            | Armenia, Kotayk, Garni gorge, c. 1180 m a.s.l., on basalt, 2006, Harutyunyan & Mayrhofer (GZU 05-1000). | Rhinocladiella sp. | FJ265760 |
| *Dermatocarpon miniatum* (L.) W. Mann   | Austria, Styria, Grazer Bergland, Kaschisteig, 800 m a.s.l., on limestone, 2005, Muggia & Hafellner (TSB 39921). |                | FJ265766 |
| *Fulgensia fulgida* (Nyl.) Szatala      | Italy, Sardegna, Nuoro, Mt. Albo Massiv, 1000 m a.s.l., on limestone, 2006, Muggia (TSB 37496). |                | FJ265762 |
| *Protoparmeliopsis muralis* (Schreb.) M. Choisy | Armenia, Kotayk, Garni gorge, c. 1180 m a.s.l., on basalt, 2006, Harutyunyan & Mayrhofer (GZU 05-1001). |                | FJ265750 |
| *Physcia dimidiata* (Arnold) Nyl.       | Armenia, Syunik province, Goris, c. 1550 m a.s.l., on Quercus sp., 2006, Harutyunyan (GZU 23-727). |                | FJ265751 |
| *Tephromela atra* (Huds.) Hafellner     | Italy, Toscana, Mt. Labbro, 1150 m a.s.l., on silicate, 2004, Tretiach (TSB 37086). |                | FJ265759 |
| *Xanthoria elegans* (Link) Th.FR.       | Armenia, Kotayk, Tsaghkadzor, c. 1750 m a.s.l., on basalt, 2005, Harutyunyan (GZU 34-206). |                | FJ265763 |
to explore a wider geographic range. The selected lichen species, origins, and corresponding fungal strains isolated from the lichen thalli are listed in Table 1. Both lichen material without symptoms and with black discolorations on the thalli were selected for the isolations.

Optical analysis

Lichens were analysed for visible colonisation with black fungi using a stereomicroscope. Hand-made transversal sections (using Gillette razor-blades) were examined in water with a Zeiss Axioscope compound microscope (Zeiss, Vienna).

Isolation of fungal strains in culture

We used young areoles or lobes, respectively, of freshly collected lichen material. The isolations were performed following the "lichen tissue culture method" as described by Yamamoto et al. (2002), with some modifications as applied in Stocker-Wörgötter (2002). To remove attached debris and substrate, small pieces of lichen thalli were cleaned mechanically using a forceps. Additionally the pieces were washed in water and in diluted Tween 80 to eliminate other organisms loosely attached to the thallus surface (Bubrick & Galen 1986). Fragments were homogenised using a mortar and pestle. The suspensions, containing small pieces of cortex, medulla and algal layer, were filtered through two sieves of 500 and 150 µm mesh size, respectively. Single fragments of about 150 µm in size were picked up with sterile bamboo sticks under a dissecting microscope and transferred on slanted agar in test tubes.

The fungal cultures were grown on MY, TM (Ahmadjian 1967), and LBM (Lilly & Barnett 1951) media for 4–5 mo at 15–20 °C, with a cycle of 14 h of light and 10 h of dark. As soon as the mycelia reached ca. 0.5 cm in diam, a small part was sub-cultured and another was used for DNA isolation. Subcultures were prepared on up to five small Petri dishes having either the same original agar media or different media. The fungus was transferred on fresh media for subcultures after 3–4 mo.

Molecular analysis: DNA-isolation, PCR-amplification and sequencing

DNA isolation was performed on a part of the mycelium grown in the slant tubes, following Cubero et al. (1999). Identity of the cultured strains were checked with sequences of the ITS regions, amplified with the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR reactions were prepared for a 30 µL final volume containing 4.05 µL double-distilled water, 3 µL 10 x Tag polymerase reaction buffer (10 mM Tris pH 8.3), 1.8 µL MgCl₂ (25 mM), 3 µL of 2.5 mM dNTPs, 0.15 µL Tag DNA polymerase, 1.5 µL for each of the 10 µM primers. PCR amplification were performed under the following conditions: an initial heating step of 2 min at 94 °C, 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 53 °C, and 2 min of extension at 72 °C, and one final extension step of 7 min at 72 °C, after which the samples were kept at 4 °C. PCR products were cleaned using Qiaquick spin columns (Qiagen, Vienna, Austria). Both complementary strands were sequenced with the BigDye Cycle Sequencing Ready Reaction Kit (Applied, Vienna, Austria) according to the manufacturer’s instructions, and sequences were run on an ABI310 automated sequencer (Applied, Vienna, Austria).

Alignment and phylogenetic analysis

Sequences of the isolated fungal strains were subjected to BLAST search (Altschul et al. 1990, http://www.ncbi.nlm.nih.gov/BLAST/), and were assigned to genera that appeared as first matches in the GenBank. Sequences that matched closer to our data were selected from GenBank and included in the analyses.

All fungal strains corresponding to the same genus were grouped together and for each of them a small alignment was produced automatically with ClustalW (Thompson et al. 1994) as implemented in BioEdit v. 5.0.6 (Hall 1999, http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html) and then manually adjusted. Small phylogenetic analyses were carried out to outline the genetic similarity of the isolated strains with the closest matches resulted from the BLAST search. The maximum parsimony (MP) algorithm was used in PAUP v. 4.0b10 (Swoford 2002). A heuristic search using 100 random addition replicates was conducted with tree-bisection-reconnection (TBR), branch swapping, and MulTree options. Bootstrapping was performed on 1000 pseudoreplicates (Felsenstein 1985). Ambiguously aligned positions were excluded and no root was selected. The phylogenetic trees were drawn using the program TreeView (Page 1996).

RESULTS

Several lichen species used in the present study (Caloplaca erythrocarpa, C. saxicola, some samples of Protoparmelopsis muralis, Physconia americana and Xanthoria elegans) showed visual signs of fungal colonisation in the form of minute black hypheae or dots. Attempts to determine the colonising species using available literature that includes keys to several hyphomycetous genera occurring on lichens (e.g. Hawksworth 1979, Clauzade et al. 1989, etc.) did not give clear results. Other samples, such as C. holocarpa, some samples of P. muralis and Physcia dimidiata were free of any visual contaminants.

We obtained 70 black fungal cultures from 20 different thalli belonging to 14 lichen species, representing crustose, foliose or fruticose growth (Table 1). On the same media, black fungi grew slowly but clearly faster than lichen mycobionts (ca. 1 cm diameter black fungal colony is reached after about 3 mos, whereas most of the lichen mycobionts show only initial development of compact mycelium after this period). Sequencing of the complete ITS regions from cultures, from which DNA was successfully extracted, revealed 31 distinct strains. According to results of the BLAST searches, the obtained strains were classified as representatives of the subclasses Chaetothyriomycetidae and Dothideomycetidae. In particular, they belong to five genera: Capnobotryella, Cladophialophora, Coniosporium, Mycosphaerella and Rhinocladiella. Molecular data of 16 cultures were not subjected to phylogenetic analyses because we so far obtained incomplete sequences from these strains. According to BLAST searches these latter were mostly closely assigned to uncultured soil fungi (isolated from Protoparmelopsis muralis, Teloschistus contortuplicatus and Xanthoria elegans), melanised limestone ascomycetes (from Caloplaca saxicola, C. holocarpa, Fulgensia fulgida, Leptogium curculiatum and P. muralis), Capronia and Exophiala (representing Chaetothyriomycetidae on Physconia americana, P. muralis and X. elegans). In addition to black fungal groups we also found a species showing sequence similarity with Nectria (representing Hypocreales) in Physcia scopolorum.
Strains belonging to the genera Cladophialophora (8) and Rhinocladiella (11) were found more frequently in lichen thalli than Capnobotryella (4), Coniosporium (3), and Mycosphaerella (5). Cladophialophora and Rhinocladiella did not show any preference to elevation and were isolated from six lichen species at different altitudes. Not supporting a species-specific occurrence, the same fungal strains representing Coniosporium were isolated from co-occurring Caloplaca saxis cola and Protoparmeliopsis muralis in Armenia (h11 and h6). Fungal strains identified as Capnobotryella, Cladophialophora, and Rhinocladiella did not show any specificity for the lichen growth habit. All of them were isolated from crustose, foliose and fruticose lichens, respectively. Fungal strains assigned to Coniosporium and Mycosphaerella were so far isolated only from crustose lichens. Among all the five genera only Cladophialophora was isolated also from the two epiphytic lichens included, i.e. Physcia dimidiata and Physconia americana. These strains represented distinct lineages.

In the phylogenetic analyses (Fig. 1A–E), all fungal strains identified as Rhinocladiella, are grouped in a single clade with the exception of one rock-inhabiting strain from Turkey (AJ972799). Except for the latter, these strains are genetically rather similar although they were isolated from different lichen species. In the remaining genera, by contrast, sequences from lichens are genetically rather heterogeneous and are scattered among strains coming from other different sources. Two lineages of lichen-colonisers were so far found in Mycosphaerella and more lineages colonising lichens are present in Cladophialophora. Several distinct strains were also found in Capnobotryella.

Multiple strains of black fungi were isolated from the same thallus of four crustose lichen species (Caloplaca holocarpa, C. saxi cola, Protoparmeliopsis muralis and Xanthoria elegans) (Table 1). The most diverse of these was C. saxi cola, yielding four isolates representing the genera Capnobotryella, Coniosporium, Mycosphaerella and Rhinocladiella. P. muralis contained Capnobotryella, Mycosphaerella and Rhinocladiella, and X. elegans hosted Cladophialophora, Mycosphaerella and Rhinocladiella sp. occurring simultaneously. The thallus of C. holocarpa was a substrate for Capnobotryella and Mycosphaerella. Two separate
lichens (Table 1). To the sequencing results with several isolates retrieved from these genera, only one culturable fungal strain from the above-mentioned genera erodens, Dermatocarpon minutum, Fulgensia fulgida, Physcia dimidiata, Physconia americana, and Tephromela atra according to the sequencing results with several isolates retrieved from these lichens (Table 1).

**DISCUSSION**

High diversity of life-styles characterises the Chaetothyriomycetidae and Dothideomycetidae (Ruibal et al. 2008). In these two subclasses, genera can comprise animal and human pathogens, endophytes or epiphytes of living plants and fungi (de Hoog 1994, Geiser et al. 2006, Schoch et al. 2006). On the other hand, phylogenetic studies indicate a rather scattered distribution of lichenised fungi in Dothideomycetidae (Del Prado et al. 2006, Muggia et al. 2007), whereas in Chaetothyriomycetidae, lichenised forms belong to the large monophyletic orders Pyrenulales and Verrucariales (Geiser et al. 2006). Repeated loss of lichenisation and lichenicolous habit occurred in Verrucariales (Navarro-Rosínés et al. 2007). However, black fungi detected by us do not belong to any of the lineages of lichenised fungi in Chaetothyriomycetidae or Dothideomycetidae. The BLAST analysis revealed indeed high sequence similarity primarily with the genera Capnobotryella, Cladophialophora, Coniosporium, Mycosphaerella, and Rhinocladiella. Within these genera genetic similarities were found with undetermined fungal strains isolated directly from rock surfaces, or with plant and human pathogenic species.

Lichens can host a wide range of associated fungi with rather varied ecologies, specificities, and biological behaviours (Lawrey & Diederich 2003). Some fast-growing lichenicolous species (e.g. Athelia, Marchandiomycetes) with often low host specificity can rapidly eradicate lichen vegetation, whereas many others grow slowly without expressing any or showing only local pathogenic symptoms on their specific hosts. Pathogenic and commensalistic interactions with their hosts appear to be corners of an ecological continuum, yet, known lichenicolous fungi have clear affinity to lichens as hosts and are not found without their hosts.

In this publication we find indirect evidence that some lineages of black fungi can opportunistically grow on lichens. Generally lichen-colonising forms did not form monophyletic groups, although in Rhinocladiella (Arnanlou et al. 2007), only one published marble-colonising strain was found among a genetically homogeneous group of lichen inhabitants. This pattern might nevertheless be incomplete because only few strains from rock are published so far. We suspect that Rhinocladiella is only a facultative lichen coloniser (Fig. 2), also because the same strains can be found in different co-occurring lichen species. More evidence for this hypothesis is found in Coniosporium and Capnobotryella, where lichen-inhabiting strains are scattered among groups of rock-inhabitants. This clearly contrasts with the ecological relationships found in Mycosphaerella and Cladophialophora. Mycosphaerella strains from lichens form two clades which are related to plant-associated fungi. Sequence divergence suggests that the lichen associates could represent distinct species with so far undetermined host specificity. The lichenicolous genus Stigmidium is phenotypically recognised in the mycological literature by the similar phenotypic features as the phytopathogenic Mycosphaerella (Mycosphaerella Johanson being a younger name than Stigmidium Trevis.). The understanding of relationships between these two genera will clarify whether some our Mycosphaerella-like strains could in fact represent Stigmidium species. If this is the case, it will be interesting to assess whether they represent new species or individuals growing cryptically in a suboptimal host. Stigmidium species are regarded as highly specialised for their lichen host species but they are only recognised by their fertile structures. Cladophialophora isolates from lichens are represented in several distinct lineages. Some of them are related to the human pathogens C. bantiana, C. boppi and C. carrionii, while others are related to the moss-inhabitant C. minutissima (and a strain isolated from rocks).

Microscopic observations of the interactions between black fungi and their host lichens are difficult. Some black fungi, including the ones found by us, are well pigmented only at the surface of the thallus. Their hyphal walls can lose their pigmentation when they stretch downwards through lichen cortex plectenchyma into the thallus, and then become unapparent. At present we cannot assess how extensively hyphae can invade the thallus and how they interact with the lichen symbionts. Only Intralichen (Hawksworth & Cole 2002) species form mycelia that also visibly extend deep into the thallus. Clear mycoparasitic or algal-parasitic behaviour is not evident and the hyphae of Intralichen grow well between fungal plectenchyma, which might be evidence for an affinity to the fungal partner in lichens. However, this is perhaps not a general feature of lichen-colonising black fungi. A direct involvement of black fungi in fungal-algal interactions was earlier described for Coniosporium aeroaigicum, which seems to establish a balanced stage of algal parasitism (Turian 1977). Also, co-culture of various rock-inhabiting microcolonial fungi with lichen algae can develop into lichenoid structures after 2–12 mo, with the fungi contacting algae by haustoria- and appressoria-like structures (Gorbushina et al. 2005). Finally, Brunauer et al. (2007) showed that a lichen-associated black fungus (with unclear position at the basis of Chaetothyriomycetidae) discovered on Lecanora rupicola forms lichenoid structures with a range of coccoid green algae in vitro.

As some Cladophialophora strains are involved in degradation of aromatic hydrocarbons (Prenafeta-Boldú et al. 2006), it might be possible that black fungi can take benefits from the numerous aromatic polyketide secondary metabolites found in lichens, but there is so far no clear evidence for this from our observations. Parts of the lichens colonised by black fungi are not necessarily bleaching or devoid of secondary compounds. Moreover, aromatic hydrocarbons can also originate from other sources. Turian (1975) noticed that Coniosporium aeroaigicum was tolerant to high levels of air pollution, and was abundant at urban places where hydrocarbons originate from traffic exhausts. High incidence of black fungi is also noticed on roadside trees in Armenia (de Hoog, unpubl. data).

In extremely hostile places on Earth, meristematic fungi are frequently associated with lichens (Onofri et al. 2007b), where they can directly or indirectly benefit from primary production of the algae at very low temperatures and limiting water conditions. Lichens from increasingly arid habitats are often more pronouncedly colonised by dark pigmented fungi, not only at the edges of the thallus areoles and squamules, but also on the more central surfaces. The isolation of black fungi from several visually uninfected thalli suggests that black fungi are ubiquitous in lichens and wait for chances to grow out, e.g. when parts of the host become senescent (Aptroot & Alstrup 1999). Microscopic studies also confirm the frequent presence of dark-walled hyphal fragments in otherwise healthy parts of lichens. We hypothesise that black fungi readily colonise most, if not all, thalli of P. muralis hosted Coniosporium with Rhinocladiella and Cladophialophora with Rhinocladiella respectively.
lichen thalli from dry sites. Moreover, we have shown here that several distinct strains can be present on a single thallus at the same time. Preliminary results using single strand conformation polymorphisms (SSCP, L. Muggia, prelim. data), indicate that up to 8 fungi can be present in a single lichen thallus (incl. the mycobiont). It is possible that we might have been able to isolate additional black fungal strains from the same samples with specific media for xerophilic fungi. However, black discolourations, especially at the thallus or areole margins do not always indicate the presence of black fungi. They may rather represent thallospores or prothalline edges formed by the lichen itself. These dark coloured mitosposes can be found in a various crustose lichens, and are particularly common in arid habitats (Poelt & Obermayer 1990).

We have no evidence that associated black fungi overgrow the host when it is in a wealthy ecological state, but they may become more prominent when the lichen hosts are somewhat affected by sustained aridity. We observed that the upper cortex becomes more brittle in arid habitats than found in samples of the same species collected from more humid situations. Water dropped on such brittle surfaces is usually taken up very quickly. The thalli also do not keep the water for extended time in a gelatinous intercellular matrix, which is often better developed in the samples from more humid locations (unpubl. observation). Some of the lichen-associated meristematic black fungi may be rock-inhabitants, which appear as opportunists on lichens under certain circumstances. We observed more commonly meristematic forms on the brittle surfaces, whereas gelatinous surfaces might more often host filamentous forms of hyphomycetes. Adjacent lichens, which slightly differ in their cortical structures, can contain black fungi that were different in appearance and abundance, but further studies are required to see whether these represent different co-inhabiting species or just represent growth modifications of the lichen-colonisers. We also observed that the algal layers beneath the colonised parts of the host lichens can look rather wealthy, indicating that the algae seem to proliferate under a cover of black fungi rather than being negatively affected. It is still unclear whether black fungi could influence hydration of the lichens or even dissipate excessive sunlight to protect the algae.

However, when black fungal hyphae become abundant on the surface, the thallus structure is severely impaired. Reinfection of Lecanora rupicola with a concentrated inoculum of a black fungus isolated from the same lichen actually led to necrotic symptoms (Brunauer et al. 2007). Moreover, there are also examples for negative interactions and rather aggressive dematiaceous hyphomycetes on lichens. Black fungi apparently interact in various ways with lichens and they likely have different degrees of specificity. Future studies will show which black fungi are facultative opportunists on lichens, and which ones represent obligate and specialised lichen inhabitants. Such studies will also elucidate relationships with described lichenicolous fungi. Growing on their hosts, some of our fungi have at least some similarities with poorly understood lichenicolous species assigned to, e.g., the genera Taeniolella or Torula. Some of these described species are regarded as highly host-specific (e.g. Etayo & Calatayud 2005). This may perhaps be questioned, if future sequencing data may reveal that growth and behaviour of these fungi can be modified in other potential lichen hosts. Future studies may also resolve the relationships with the fertile lichenicolous genus Lichenostigma and poorly known species assigned to the lichenised genus Lichenothelia, which are capable of meristematic growth.

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REFERENCES

Ahmadjian V (1967). The lichen symbiosis. Blaisdell Publishing Company, Massachusetts.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.

Aptom A, Alstrup V (1999). Three hyphomycetes isolated from the necrotic lichen Cladonia rangiformis. Cryptogamie, Mycologie 20: 189–191.

Büdel B, Becker U, Follmann G, Sterflinger K (2000). Algae, fungi and lichens on inselbergs. In: Inselbergs (Porembski S, Barthlott W, eds.). Ecological Studies 146: 69–90.

Bubrick P, Galun M (1986). Spore to spore resynthesis of Xanthoria parietina. The Lichenologist 18: 47–49.

Brunauer G, Blaha J, Hager A, Türk R, Stocker-Würgötter E, Grube M (2007). Lichenoid structures in vitro of a cultured lichenicolous fungus. Symbiosis 44: 127–136.

Clauzade G, Diederich P, Roux C (1989). Neliikenigintaj fungoj likenlogaj. Ilustrita
