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Diversity of non-reducing polyketide synthase genes in the Pertusariales (lichenized Ascomycota): A phylogenetic perspective

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Abstract

Lichenized fungi synthesize a great variety of secondary metabolites. These are typically crystalline compounds, which are deposited extracellularly on the fungal hyphae. While we know a lot about the chemical properties and structures of these substances, we have very little information on the molecular background of their biosynthesis. In the current study we analyze the diversity of non-reducing polyketide synthase (PKS) genes in members of the lichenized Pertusariales. This order primarily contains fully oxidized secondary metabolites from different substance classes, and is chemically and phylogenetically well studied. Using a degenerate primer approach with subsequent cloning we detected up to five non-reducing PKS sequences in a single PCR product. Eighty-five new KS sequence fragments were obtained for this study. Analysis of the 157 currently available fungal KS sequence fragments in a Bayesian phylogenetic framework revealed 18 highly supported clades that included only lichenized taxa, only non-lichenized taxa, or both. Some Pertusarialean groupings of PKS sequences corresponded partly to phylogenetic groupings based on ribosomal DNA. This is reasonable, because a correlation between well-supported phylogenetic lineages and the occurrence of secondary metabolites in the Pertusariales has been observed before. However, no clear linkage was found between the PKS genes analyzed and the ability to produce a particular secondary substance. Several PKS clades did not reveal obvious patterns of secondary compound distribution or phylogenetic association. Compared with earlier phylogenetic analyses of KS sequences the increased sampling in the current study allowed us to detect many new groupings within the fungal non-reducing PKSs.

Keywords: *Pertusaria*; Pertusariaceae; Ascomycetes; Symbiosis; PKS; Bayesian phylogeny; Depsides; Depsidones; Depsones; Xanthones

1. Introduction

Lichens are symbiotic organisms consisting of a fungal, an algal and/or a cyanobacterial partner. They are well known for their ability to produce large amounts and a great variety of secondary metabolites (e.g., Elix, 1996; Huneck and Yoshimura, 1996). For more than 150 years these so-called lichen products have been used for identification and taxonomy (Culberson and Culberson, 1970; Hawksworth, 1976; Lumbsch, 1998; Nylander, 1866), and therefore lichens constitute one of the chemically best studied groups of organisms today.

Within the lichen thallus carbohydrates move from the photobiont to the mycobiont (Ahmadjian, 1993). The photosynthetic partners excrete sugars (cyanobacteria) or different types of polyols (green algae), which are used by the mycobiont to form, for example, aromatic or aliphatic polyketides. These crystalline substances are typically deposited extracellularly on the fungal hyphae (e.g., Honegger, 1986). There are only few reports

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of secondary metabolites from lichen cyanobionts (Oksanen et al., 2004; Yang et al., 1993).

It is experimentally difficult to establish the biological functions of secondary metabolites in the slow growing and ecologically sensitive lichens. Among the functions which have been proposed are: irradiation screen by cortical substances such as parietin (Gauslaa and Solhaug, 2001; Solhaug and Gauslaa, 1996; Solhaug et al., 2003; Rundel, 1978), protection against herbivore grazing by the production of bitter tasting compounds (Emmerich et al., 1993; Fröberg et al., 1993; Lawrey, 1980), competitive advantages against bacteria or other fungi through antibiotic or antifungal substances (Whiton and Lawrey, 1982, 1984), defense against parasitic fungi (Lawrey, 1995), weathering of rocks for better attachment to the substrate (Purvis et al., 1987), improvement of gas exchange by hydrophobic properties of some lichen substances (Huneck, 2003), and influence on the permeability of the cell walls and maintenance of the symbiontic equilibrium (Kinrade and Ahmadjian, 1970; Schimmer and Lehner, 1973). It has also been proposed that they are stress substances comparable to those produced by some vascular plants under extreme environmental conditions (Huneck and Höfle, 1978; Lange, 1992).

Lichens have been acknowledged in traditional herbal medicines for their high content of active substances (Llano, 1944; Richardson, 1988; Schindler, 1988; Schöller, 1997), and some biological activities, such as antibiotic and toxic effects of usnic acid, could be confirmed (Han et al., 2004; Lauterwein et al., 1995; Pramyothin et al., 2004; Vartia, 1973). Depsides and depsidones, for which lichens are a particularly rich source, are pharmaceutically interesting because of their antioxidant activity (Hidalgo et al., 1994; Neamati et al., 1997). However, pharmaceutical exploitation and drug development from lichens is hampered by low growth rates of lichens and the difficulty of sustaining mycobiont cultures. Furthermore, cultivated mycobionts often do not produce the same metabolites that are typically found in the intact symbiosis. They may produce less, different, or no products, often depending on culture conditions (e.g., Hamada, 1984, 1989; Miyagawa et al., 1993, 1994, 1997; Stocker-Wörgötter, 2001; Yamamoto et al., 1993). The fact that cultivated mycobionts are capable of producing substances typically not detected in natural lichens suggests that these organisms have an extensive, largely undescribed biosynthetic potential.

An alternative approach to analyzing lichen metabolites is to study aspects of the biosynthetic genes that are involved in their production (Miao et al., 2001). Recently, several studies dealing with the assessment of PKS diversity and phylogenetic relationships of such genes in fungi and bacteria have been published (Grube and Blaha, 2003; Kroken et al., 2003; Lee et al., 2001; Metsä-Ketelä et al., 1999; Moffitt and Neilan, 2004; Sauer et al., 2002). The diversity of PKS genes of uncultivated bacteria in environmental samples has been assessed by Ginolhac et al. (2004) and Piel et al. (2004).

Fungi synthesize polyketides via type I PKS systems, which consist of a single protein complex that contains all necessary catalytic sites. These domains are arranged on a single module and may be used reiteratively. A minimal module carries ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP) domains to perform one chain elongation cycle. Optional additional domains responsible for successive reduction steps are ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER). PKSs which lack KR, DH, and ER domains produce fully oxidized polyketides.

The order Pertusariales contains primarily non-reduced metabolites from various substance classes, such as chlorinated and non-chlorinated xanthones, orcinol depsides, orcinol depsidones, β-orcinol depsidones, and depsones (Archer, 1993, 1997; Brodo, 1991; Dibben, 1980; Hanko, 1983; Hanko et al., 1985; Lumbsch et al., 1999; Schmitz et al., 1994). There are also reports of fatty acids (e.g., Hanko, 1983). Recent molecular studies based on nuclear and mitochondrial ribosomal DNA have established a robust phylogenetic estimate of the group (Lumbsch and Schmitt, 2001; Schmitt et al., 2001, 2003; Schmitt and Lumbsch, 2004), which is schematically reproduced in Fig. 1. We have shown that monophyletic clades within the Pertusariales correspond well with the occurrence of secondary metabolites (Lumbsch and Schmitt, 2001; Schmitt and Lumbsch, 2004). For example, depsones are restricted to the *Variolaria*-group, while the depsidone lecanoric acid occurs only in the *Varicellaria*-group. Chlorinated xanthones are exclusively synthesized by members of the *Pertusaria* s.str.-group. These phylogenetic patterns make the Pertusariales an ideal group to study the diversity and distribution of non-reducing PKS genes.

In the current study we use a degenerate primer approach with subsequent cloning of PCR products to address the following questions: (1) How many different non-reducing PKS genes can we find in the Pertusariales? (2) What are the closest relatives of Pertusarialan PKS genes in non-lichenized and other lichenized fungi? (3) Are there groupings of exclusively Pertusarialean PKS genes? and (4) Are these groupings correlated with the ribosomal gene phylogeny of Pertusariales?

2. Results and discussion

We obtained 85 KS sequence fragments from 37 species of the Pertusariales. Sequence identity was checked with Blast Searches in GenBank and only those sequences with high similarity (>70%) to other fungal KS sequences were used for the analysis. A single PCR product amplified with degenerate primers de-

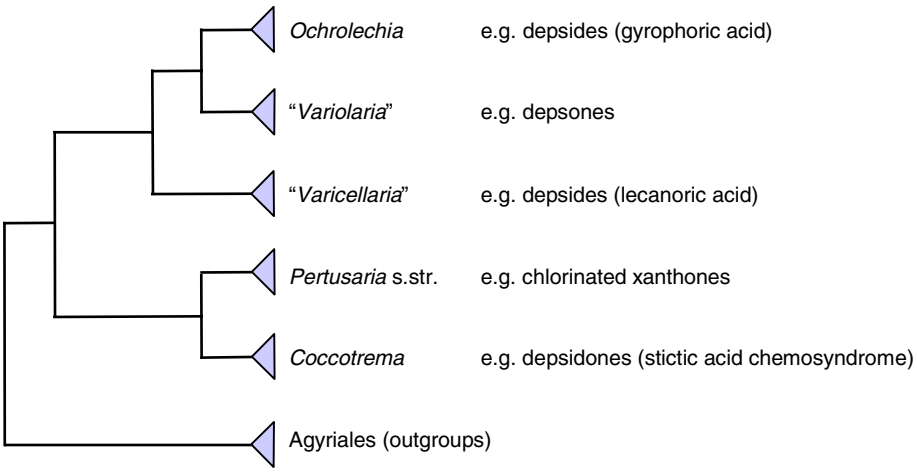

Fig. 1. Phylogeny of major groups in the Pertusariales based on nuclear and mitochondrial rDNA sequences (adapted from Schmitt and Lumbsch, 2004; Schmitt et al., 2001). Characteristic secondary compound classes are indicated for each clade.

signed to target non-reducing PKSs (Bingle et al., 1999) yielded 1–5 different KS sequences (Table 1). The sequences were aligned with fungal PKS sequences retrieved from GenBank, including all sequences from the "non-reducing clades I–IV" identified by Kroken et al. (2003), non-reducing lichen PKSs published by Grube and Blaha (2003), and all further non-reducing PKS sequences found by BLASTx searches in GenBank (Fig. 2(a) and (b)). From a total of 157 sequences we produced an alignment of 690 nucleotide positions, excluding the primer sequences. Thirty-three introns (46–60 bp) were removed from the newly generated sequences prior to the analysis. They were not further analyzed in the current study. Four KS sequences of reducing PKSs were selected as outgroups, because this group was shown to be the sister group of the non-reducing PKSs (Kroken et al., 2003; Nicholson et al., 2001).

The 50% majority rule consensus tree of the Bayesian analysis using nucleotide data partitioned into 1st, 2nd and 3rd codon positions is shown in Figs. 2(a) and (b). Only the external major clades receiving significant support (posterior probabilities >94) will be discussed in the following. The most derived clade (Clade I) contains 48 sequences of lichenized and non-lichenized species, which form seven sub-clades (I a–g). This clade corresponds to "non-reducing clade I" in the study by Kroken et al. (2003). All characterized genes included in this group are either involved in aflatoxin or pigment biosynthesis. Interestingly, the sub-clades in this group, which predominantly contain lichenized taxa, are congruent with the main clades of the ribosomal gene phylogeny. Clade I-a contains 12 members of the *Variolaria*-group, including two identical sequences of different collections of *Pertusaria subventosa*, but also two sequences of different collections of *Pertusaria amara*, which are slightly different. Clade I-b contains only members of the *Pertusaria* s.str.-group, including two identical sequences of *Pertusaria flavida*. Clade I-c consists of two members of the *Varicellaria*-group with three identical KS sequences found in three different collections of *Pertusaria hemisphaerica*. Three collections of *Ochrolechia* species form clade I-d. In this case two collections of the same species, *Ochrolechia parella*, have deviating KS sequences differing in several amino acid positions. Clade I-e consists only of non-lichenized fungi. It contains a PKS of *Aspergillus fumigatus* (AFY17317), which has been shown to be involved in conidial pigment biosynthesis and virulence (Langfelder et al., 1998), one of *A. nidulans* (X65866) which codes for naphtopyrone (Watanabe et al., 1996), and two not further characterized KS sequences from *A. parasiticus* and *Penicillium patulum* (Bingle et al., 1999). Clade I-f contains lichenized taxa from the *Pertusaria* s.str.-group (*P. coronata*) and *Variolaria*-group (the remaining sequences), with two KS sequences of non-lichenized fungi nested in between. These are: (a) an uncharacterized PKS from *Aspergillus terreus* (AB072445), and (b) a PKS from *Nectria haematococca* involved in the synthesis of a perithecial red pigment (Graziani et al., 2004). Interestingly, three taxa from the *Variolaria*-group (*P. erythrella*, *P. scaberula*, *P. subventosa*) form a closely related group, which can also be observed in clade I-a. Also in phylogenetic analyses based on ribosomal DNA the three taxa are closely related (Schmitt and Lumbsch, 2004). Similar PKS genes in closely related species were also found in the lichenized genus *Lecanora* (Grube and Blaha, 2003).

Clade I-g contains one PKS from a lichenized fungus (*Xanthoria parietina*) and 10 non-lichenized fungi. These are involved in norsolorinic acid/aflatoxin biosynthesis (AB076803, AF441403, AY371490), sterigmatocystin biosynthesis (AACD01000132), and sirodesmin biosynthesis (AY553235). Also in this clade are two PKSs of

Table 1
Material used in this study

Organism	Source	Major secondary substances found in sample	Phylogenetic group	DNA #	# of PKSs found	GB accession number and clone number
Coccotrema coccophorum	Argentina, Messuti 2001 (F)	const, cryptost, st	C	299	1	AY918715 (0299A)
Coccotrema cucurbitula	Argentina, 12 Dec. 2003 Messuti & Wirtz (F)	const, st, unknown	C	1439	1	AY918716 (1439A)
Coccotrema maritimum	Canada, 13 June 2004, Schmitt (F)	const, st, unknown	C	1471	1	AY918717 (1471A)
Coccotrema pocillarium	Alaska, Printzen (ESS 20863)	st, const, unknown	C	202	4	AY918718 (0202A) AY918719 (0202B) AY918720 (0202D) AY918721 (0202F)
Coccotrema pocillarium	Canada, Brodo 29916 (CANL)	st, const, unknown	C	432	1	AY918722 (0432X)
Ochrolechia androgyna	Germany, 15 Apr. 2004, Schmitt (F)	gyr	O	1368	3	AY918723 (1368A) AY918724 (1368C) AY918725 (1368E)
Ochrolechia balcanica	Greece, Schmitt (ESS 20968)	gyr	O	640	1	AY918726 (0640X)
Ochrolechia oregonensis	Canada, 11 June 2004, Schmitt (F)	gyr, fatty acids	O	1494	1	AY918727 (1494A)
Ochrolechia pallescens	Spain, 05 June 2003, Schmitt (F)	gyr, var	O	1023	2	AY918728 (1023A) AY918729 (1023B)
Ochrolechia parella	Corsica, May 2001, Schmitt (F)	gyr, var	O	573	1	AY918730 (0573X)
Ochrolechia parella	Antarctica, Lumbsch 19018f (F)	gyr	O	1441	2	AY918731 (1441A) AY918732 (1441B)
Pertusaria albescens	Germany, 15 Apr. 2004, Schmitt (F)	aliphatic compounds	VA	1377	1	AY918733 (1377C)
Pertusaria albescens	Spain, 2 June 2003, Schmitt (F)	aliphatic compounds	VA	1017	1	AY918734 (1017B)
Pertusaria amara	Germany, 15 Apr. 2004, Schmitt (F)	pic, protocet	VA	1473	2	AY918735 (1473A) AY918736 (1473B)
Pertusaria amara	Germany, 15 Apr. 2004, Schmitt (F)	pic, protocet	VA	1366	1	AY918737 (1366B)
Pertusaria amara	Slovakia, 24 May 2003, Schmitt (F)	pic, protocet	VA	1031	1	AY918738 (1031A)
Pertusaria amara	Canada, 20 Aug. 2003, Lumbsch, Schmitt, Wirtz (F)	pic, protocet	VA	1066	3	AY918739 (1066A) AY918740 (1066F) AY918741 (1066L)
Pertusaria aspergilla	Sweden, Aug. 2001 Schmitt (F)	protocet, fum, suc	VA	585	1	AY918742 (0585X)
Pertusaria aspergilla	Corsica, Schmitt (ESS 21505)	protocet, fum, suc	VA	580	1	AY918743 (0580X)
Pertusaria corallophora	Antarctica, Lumbsch 19013d (F)	protocet	VA	1443	2	AY918744 (1443B) AY918745 (1443F)
Pertusaria corallophora	Antarctica, Lumbsch 19026e (F)	protocet	VA	1446	1	AY918746 (1446B)
Pertusaria coronata	Czech Republic, Schmitt & Palice (ESS 21494)	4,5-dichl, st, norst, const	PS	427	1	AY918747 (0427X)
Pertusaria coronata	Slovakia, 24 May 2003, Schmitt (F)	4,5-dichl, st, norst, const	PS	1033	1	AY918748 (1033B)

Pertusaria erythrella	Australia, Archer (ESS 20866)	norst	VA	326	2	AY918749 (0326A) AY918750 (0326G)
Pertusaria excludens	Spain, 4 June 2003, Schmitt (F)	norst, sal, unknown fatty acid	VA	1021	2	AY918751 (1021A) AY918752 (1021A)
Pertusaria flavida	Germany, 15 Apr. 2004, Schmitt (F)	thio, unidentified	PS	1371	2	AY918753 (1371J) AY918754 (1371L)
Pertusaria flavida	Spain, 05 June 2003, Schmitt (F)	thio, unidentified	PS	1029	1	AY918755 (1029A)
Pertusaria gibberosa	Australia, March 2003 Archer (NSW)	4,5-dichl, 2'-O-met	PS	1061	1	AY918756 (1061A)
Pertusaria graphica	New Zealand, Wright 7561 (HB Wright)	norst	PS	1070	2	AY918757 (1070D) Y918758 (1070K)
Pertusaria hemisphaerica	Germany, Schmitt (ESS 21065)	lec	VC	452	1	AY918759 (0452X)
Pertusaria hemisphaerica	Germany, 15 Apr. 2004, Schmitt (F)	lec	VC	1367	1	AY918760 (1367A)
Pertusaria hemisphaerica	Spain, 5 June 2003, Schmitt (F)	lec	VC	1028	1	AY918761 (1028B)
Pertusaria hymenea	Germany, 15 Apr. 2004, Schmitt (F)	thio, gyr	PS	1365	4	AY918762 (1365A) AY918763 (1365C) AY918764 (1365D) AY918765 (1365F)
Pertusaria kalelae	Argentina, Messuti 2004 (F)	4,5-dichl	PS	341	1	AY918766 (0341G)
Pertusaria lactescens	Scotland, Coppins (ESS 21496)	norst	PS	629	1	AY918767 (0629D)
Pertusaria lecanina	USA, 2000, Tucker (Santa Barbara Botanical Garden)	thio, gyr	PS	345	4	AY918768 (0345C) AY918769 (0345I) AY918770 (0345L) AY918771 (0345J)
Pertusaria leioplaca	Germany, Schmitt (ESS21502)	4,5-dichl	PS	448	1	AY918772 (0448X)
Pertusaria mammosa	Greece, Sipman & Raus 47130 (B)	fum, suc	VA	610	1	AY918773 (0610X)
Pertusaria mesotropa	Mexico, Herrera Campo S39P9 RL5 (MEXU)	2′-*O*-met, 4,5-dichl	PS	1064	2	AY918774 (1064A) AY918775 (1064L)
Pertusaria mesotropa	Mexico, Herrera Campo S39P9 RL5 (MEXU)	2′-*O*-met, 4,5-dichl	PS	1064		AY918776 (1064X)
Pertusaria oculata	Sweden, Kanz & Printzen 5453 (HB Printzen)	gyr, protocet	PS	457	5	AY918777 (0457A) AY918778 (0457C) AY918779 (0457D) AY918780 (0457E) AY918781 (0457G)
Pertusaria ophthalmiza	Scotland, Coppins (ESS 21498)	aliphatic compounds	VA	631	1	AY918782 (0631X)
Pertusaria panyrga	Canada, Printzen 5718 (HB Printzen)	aliphatic compounds	VA	599	1	AY918783 (0599X)
Pertusaria pertusa	Germany, 15 Apr. 2004, Schmitt (F)	4,5-dichl, st	PS	1369	3	AY918784 (1369A) AY918785 (1369E) AY918786 (1369L)

(*continued on next page*)

Table 1 (*continued*)

Organism	Source	Major secondary substances found in sample	Phylogenetic group	DNA #	# of PKSs found	GB accession number and clone number
Pertusaria scaberula	Australia, Archer (ESS20867)	lich, tham	VA	327	1	AY918787 (0327C)
Pertusaria scaberula	Australia, Archer P932 (NSW)	lich, tham	VA	1448	2	AY918788 (1448B)
						AY918789 (1448C)
Pertusaria subventosa	Australia, Elix (ESS 15602)	lich, tham, pic	VA	385	1	AY918790 (0385B)
Pertusaria subventosa	Australia, Lumbsch 19070a (F)	lich, tham, pic	VA	1078	2	AY918791 (1078A)
						AY918792 (1078G)
Pertusaria subverrucosa	New Zealand, Wright 7560 (HB Wright)	norst	PS	1067	2	AY918793 (1067A)
						AY918794 (1067C)
Pertusaria subverrucosa	New Zealand, Wright 7560 (HB Wright)	norst	PS	1068	1	AY918795 (1068X)
Pertusaria velata	Australia, Archer (ESS 21500)	lec, lich	VC	632	1	AY918796 (0632X)
Pertusaria xanthoplaca	Austalia, Lumbsch 19070b (F)	4,5 dichl	PS	1079	1	AY918797 (1079A)
Varicellaria rhodocarpa	Austria, Soukup & Türk 29416 (HB Türk)	lec	VC	528	2	AY918798 (0528A)
						AY918799 (0528B)

Herbarium acronyms follow Holmgren et al. (1990).

Abbreviations for secondary substances: const = constictic acid; cryptost = cryptostictic acid; fum = fumarprotocetraric acid; gyr = gyrophoric acid; lec = lecanoric acid; lich = lichexanthone; norst = norstictic acid; pic = picrolichenic acid; protocet = protocetraric acid; sal = salazinic acid; st = stictic acid; suc = succinprotocetraric; tham = thamnolic acid; thio = thiophaninic acid; var = variolaric acid; 2′-*O*-met=2′-*O*-methylperlatolic acid; 4,5-dichl = 4,5-dichlorlichexanthone. Abbreviations for phylogenetic groups: PS = *Pertusaria* s.str., VC = ”*Varicellaria*”-group, VA = ”*Variolaria* ”-group, O = *Ochrolechia*, C = *Coccotrema*.

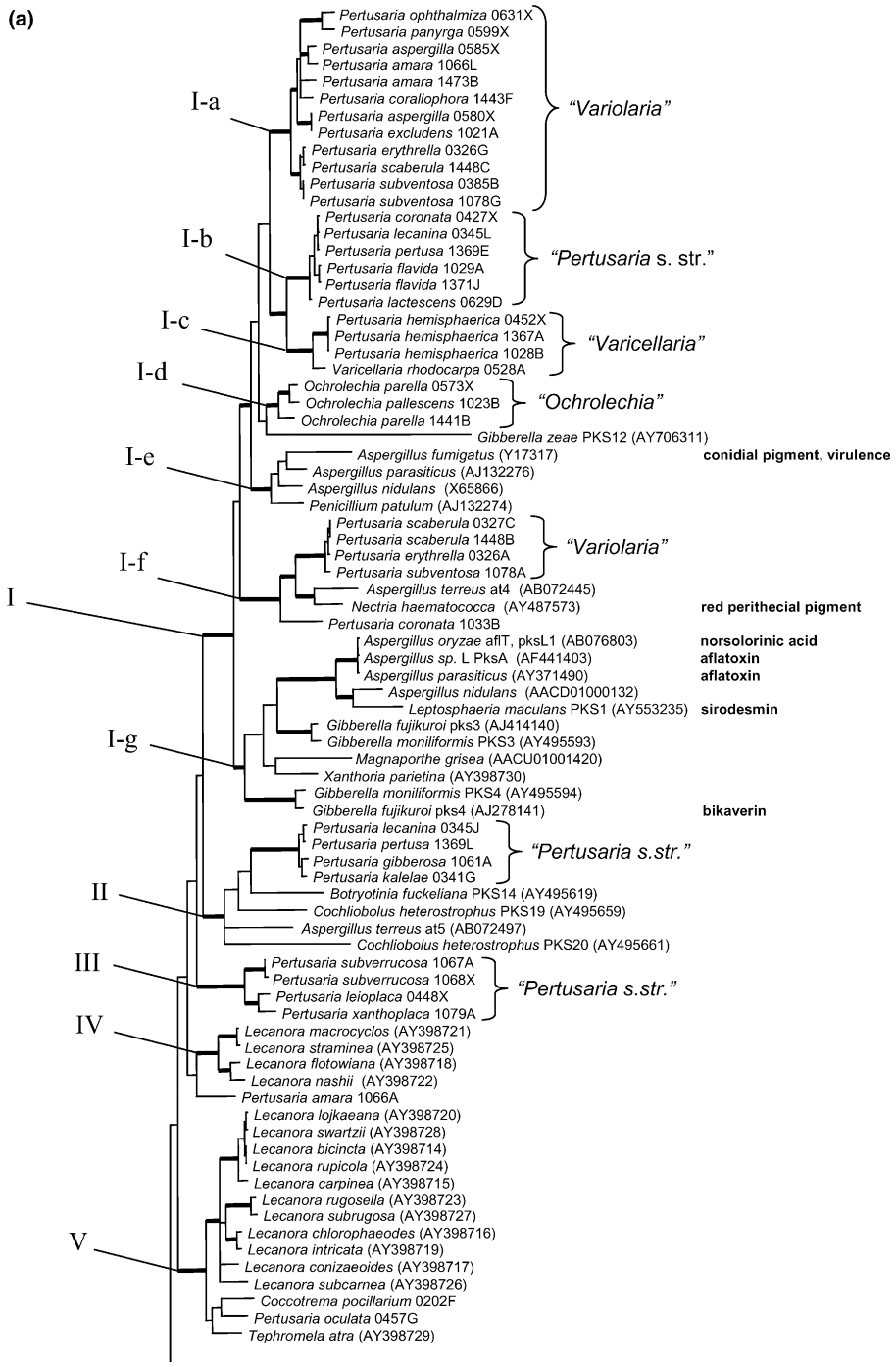

Fig. 2. Phylogeny of non-reducing KS sequences from lichenized and non-lichenized fungi. Branches in bold print indicate significant statistical support (posterior probabilities >0.94). Sequences retrieved from GenBank are indicated by GB accession numbers. Sequences generated for the current study are labeled with species name, DNA extraction number (see Table 1), and name of clone (capital letter). Secondary metabolites of characterized PKSs are typed in bold print behind the respective taxa. Groupings of Pertusarialean PKS genes that correspond to phylogenetic clades found in rDNA phylogenies are bracketed and named. (a) Upper part of phylogenetic tree, (b) lower part.

Gibberella sp. (AY495593, AJ414140) and *Magnaporthe grisea* (AACU01001420) with unknown functions, and *Gibberella fujikuroi* PKS4 (AJ278141), which encodes a an enzyme for the biosynthesis of the red polyketide pigment bikaverin (Linnemannstöns et al., 2002). The close relationship of PKSs involved in the synthesis of mycotoxins and pigments has been observed before (Graziani et al., 2004).

Clade II contains four Pertusarialean fungi from the *Pertusaria* s.str.-group (*P. pertusa*, *P. kalelae*, *P. gibberosa* and *P. lecanina*), which all produce chlorinated xanthones, plus uncharacterized PKSs of *A. terreus*,

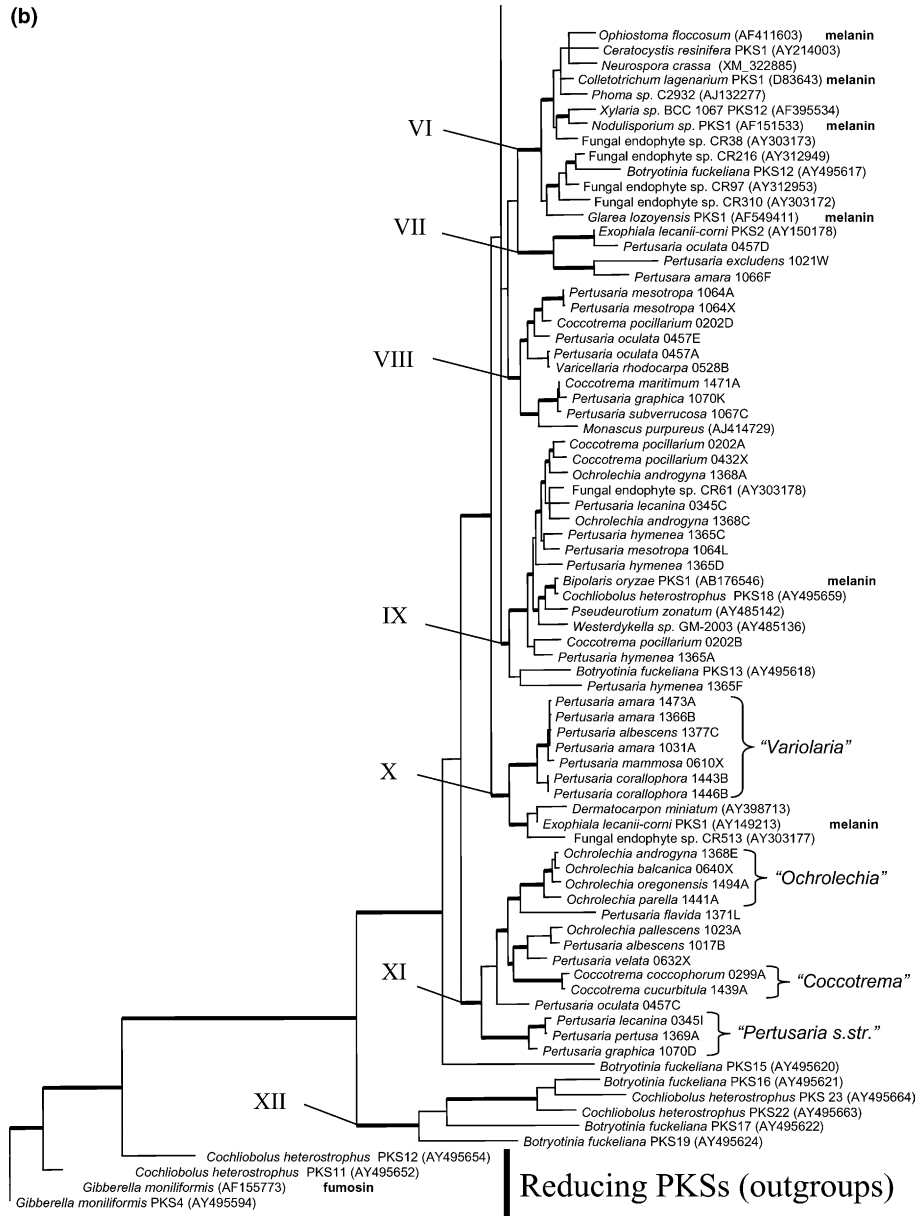

Fig. 2 (*continued*)

Botryotinia fuckeliana, and *Cochliobolus heterostrophus*. Clade III contains three *Pertusaria* s.str. taxa (*P. leioplaca*, *P. xanthoplaca* and *P. subverrucosa*), the first two of which contain chlorinated xanthones.

Clade IV was also detected in the study by Grube and Blaha (2003) and includes four *Lecanora* species. Clade V consists of the second *Lecanora*-group and additionally includes the lichenized taxa *Tephromela atra* and *Pertusaria oculata*, which are both characterized by dark pigmented disks, and *Coccotrema pocillarium.* No discrete chemical pattern is observed in these species.

Clade VI includes three characterized PKSs from non-lichenized fungi involved in melanin biosynthesis: *Colletotrichum lagenarium* PKS1 (D83643) (Takano et al., 1995), *Glarea lozoyensis pks1* (AF549411) (Zhang et al., 2003), and *Nodulisporium* sp. *pks1* (AF151533) (Fulton et al., 1999). It further contains uncharacterized PKSs of *Ceratocystis resinifera* (AY214003), *Neurospora crassa* (XM 322885), *Ophiostoma floccosum* (AF411603), *Phoma* sp. (AJ132277), *Xylaria* sp. (AF395534), fungal endophytes (AY303173, AY312949, AY312953, AY303172), and *B. fuckeliana pks12* (AY495617). There are no PKSs from lichenized taxa in this clade.

Clade VII includes PKS2 found in *Exophiala lecanii-corni*, which is similar to the melanin producing PKS1 in this species, but which was shown not to be involved in melanin biosynthesis (Cheng et al., 2004). At the time of their study, Cheng et al. (2004) could not find any

similar PKSs in public data bases, however, the current study reveals that there are three very similar PKSs in Pertusarialean fungi: *P. excludens*, *P. amara*, and *P. oculata*.

Clade VIII includes nine PKSs of Pertusariales, which do not show any phylogenetic patterns, plus one PKS of *Monascus purpureus* (AJ414729) which has the domain order KS-AT-ACP, but is not further characterized.

Clade IX is composed of a variety of lichenized and non-lichenized taxa. *Bipolaris oryzae* PKS1 is involved in melanin biosynthesis (AB176546) (Moriwaki et al., 2004), while all other PKSs in this group are not characterized: *Pseudeurotium zonatum* (AY485142), *Westerdykella* sp. (AY485136), *B. fuckeliana* (AY495618), *C. heterostrophus* (AY495659), and fungal endophyte (AY303178). The Pertusarialean taxa in this group do not correspond to phylogenetic relationships. *Pertusaria hymenea* is represented by four slightly differing KS sequences from the same PCR product, *C. pocillarium* and *Ochrolechia androgyna* by two each.

Clade X includes *Exophiala lecanii-corni* PKS1 involved in melanin biosynthesis (Cheng et al., 2004), a non characterized fungal endophyte (AY303177), and the lichenized fungus *Dermatocarpon miniatum*, which contains an amorphous dark compound in the cell walls but lacks crystalline substances (Grube and Blaha, 2003). Additionally there are three pertusarialean taxa with almost identical sequence (*P. amara*, *P. albescens*, *P. mammosa*), and two identical sequences of different collections of *P. corallophora* (all *Variolaria*-group).

Clade XI consists solely of lichenized taxa. There is no clear phylogenetic pattern, however, four *Ochrolechia* species, two *Coccotrema* species and three *Pertusaria* s.str. species (*P. graphica*, *P. lecanina*, *P. pertusa*) each form a group. The typical lichen compounds depsides and depsidones are dominant secondary metabolites of the species included in this clade, but it should be stressed that there is no clear linkage between a particular compound and PKSs in this clade.

Clade XII corresponds to "non-reducing clade III" in the study by Kroken et al. (2003) and contains no lichenized taxa.

Some Pertusarialean specimens in this study are represented by multiple PKS sequences. These include sequences appearing in different clades and those clustering in the same major clade, but on different branches. Sequences from the same species found in different clades in the PKS phylogeny, e.g., *Pertusaria amara* (clade I-a, IV, X), *P. subventosa*, *P. erythrella*, *P. scaberula* (clade I-a, I-f), or *P. pertusa* (I-b, II, XI) most likely encode paralogous PKS genes. This is to be expected, because (a) most Pertusariales produce more than one secondary compound, and (b) it is likely that the biosynthetic potential of these fungi is higher than the measurable metabolite content. For example, several secondary metabolites were recorded for mycobiont cultures that have not been found in lichen thalli (Ernst-Russell et al., 1999; Kon et al., 1997; Miyagawa et al., 1993, 1994, 1997; Moriyasu et al., 2001). Some PKS sequences found in the same species are not identical, but occur in the same major clade, e.g., *Pertusaria hymenea*, *C. pocillarium* and *O. androgyna* in clade IX. This "scattering" of PKS sequences of the same species can also be observed in PKSs from the same species, but different collections, e.g., *O. parella* (clade I-d). Some caution should be exercised with these sequences, since it cannot be excluded that they represent cryptic species, or PKSs from contaminating fungi. Cryptic species have been described in lichen-forming fungi, such as the genus *Letharia* (Kroken and Taylor, 2001), and endophytic fungi, which may occur in natural lichens, could be a source of non-mycobiontal PKSs. Lichen material containing lichenicolous fungi or other visible parasites was not used for the molecular study.

The phylogenetic estimate presented in the current study does not allow a clear linkage between a particular secondary metabolite and a PKS clade. However, those clades containing only sequences from lichen-forming fungi (clade I-a, b, c, d, III, IV, V, XI) are potential candidates for PKSs responsible for the production of typical lichen metabolites, such as depsones, depsides or depsidones. Clades including sequences from lichen-forming and non-lichenized ascomycetes (I-f, g, II, VII, VIII, IX, X) presumably represent the cryptic, mostly undescribed biosynthetic potential of lichen fungi, which may be capable of supporting a variety of complex polyketides. The genes responsible for their production would not necessarily bear a close relationship to the generally simpler biosynthetic requirements of, e.g., depsides or depsidones, and this would explain the placement of some PKS genes very close to those of non-lichenized fungi.

The current study is the first phylogeny of PKS sequences with a broad sampling of taxa, which is confined to only one kind of PKS, the non-reducing fungal type I. The overall similarity of the sequences allowed us to produce a completely unambiguous alignment, and analyze the data at the nucleotide level. The number of informative characters is thus greatly increased compared to alignments based on amino acid sequences, and improves the resolution of the phylogenetic estimate. Over-saturation at third codon positions and homoplasy effects are not severe problems in this data set, which is indicated by a tree based on amino acid sequences of the same data set (data not shown), which has the same supported groups, but less overall resolution. While the study by Grube and Blaha (2003) showed that there are at least two groups of functionally different non-reducing PKSs in one genus of lichenized fungi, the current study indicates that there are many more groups of non-reducing PKSs. Since PKSs typically catalyze the formation of a single product, each of the highly sup-

ported PKS groups found in the current study is putatively involved in the synthesis of a different polyketide. However, at this stage of the work it is not possible to make predictions about the functions of the recovered Pertusarialean PKS genes. Primer design for particular clades, which will be facilitated by the broad sampling of this study, and analysis of unrelated mycobionts that produce the same substances, may help us to answer this question in the future.

3. Experimental

3.1. Materials

Specimens used in this study are compiled in Table 1. We included multiple collections of the same species in almost one-third of the taxa.

3.2. Chemical analysis

Presence of secondary compounds in lichen samples was verified using gradient-elution high performance liquid chromatography (HPLC) as described in Feige et al. (1993) and Lumbsch (2002). The HPLC system (Shimadzu SPD-M10A) used for the analysis is equipped with a photodiode array detector (Yoshimura et al., 1994). A Spherisorb OSS2 column (5.0 μm, 250 × 4.6 mm) was used at room temperature. Two solvent systems were employed: 1% orthophosphoric acid and methanol. Small thallus fragments of the examined lichens were extracted in acetone for 1 h, and 20 μl of this extract were injected. The run was programmed as described in Lumbsch (2002), but with a flow rate of 1 ml/min.

3.3. Molecular methods

Total genomic DNA was extracted using the QIAGEN Plant Mini Kit (Qiagen, Hilden, Germany) or E.Z.N.A. Fungal MiniPrep Kit (Omega-Biotech, Doraville, USA) following the instructions of the manufacturers. KS fragments were amplified with the primers LC1/LC2c (Bingle et al., 1999), or modified versions thereof (LC1-Im 5′-GAC CCG MGG TTY TTY AAY ATG-3′ and LC2c-Im 5′-GTG CCG GTG CCR TGC ATY TC-3′) using Ready-to-Go® PCR Beads (Amersham-Biosciences, UK) as mentioned in Winka et al. (1998). Amplification products were cleaned using QIAquick Gel (Qiagen, Hilden, Germany) and sequenced directly with primers mentioned above. If sequening failed due to the presence of multiple products, PCR products were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) or pGEM-T easy vector II cloning kit (Omega Biotech, USA). We picked 3–12 clones of each cloning reaction. Cloned products were sequenced with universal primers specific to the plasmids: M13for and M13rev (TOPO TA) and T7 and SP6 (pGEM-T). Sequencing was done with the BigDye Terminator Ready mix (Applied Biosystems), and an ABI 3100 or 3730 automatic sequencer (Applied Biosystems). Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and manually adjusted. The alignment was produced in BioEdit (Hall, 1998).

3.4. Phylogenetic analysis

We employed a Bayesian approach (Huelsenbeck et al., 2001; Larget and Simon, 1999) with Markov Chain Monte Carlo (MCMC) tree sampling to infer a phylogenetic estimate. Posterior probabilities of each node were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. The program MrBayes (Huelsenbeck and Ronquist, 2001) was employed to sample the trees. The analyses were performed assuming the general time reversible model (Rodriguez et al., 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR + I + Γ). Data were partitioned in first, second and third codon positions, and α-shape parameters were calculated for each partition individually. No molecular clock was assumed. The MCMC process was set so that 12 chains ran simultaneously for 2,000,000 generations. Trees were sampled every 100th generation for a total of 20,000 trees. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) and determined that stationarity was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist, 2001). The initial 1000 trees were discarded as burn-in before stationarity was reached. Using the "sumt" option in MrBayes, a majority-rule consensus tree was calculated from 19,000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. Phylogenetic trees were drawn using TREEVIEW (Page, 1996).

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References

Ahmadjian, V., 1993. The Lichen Symbiosis. Wiley, New York.

Archer, A., 1993. A chemical and morphological arrangement of the lichen genus *Pertusaria*. Bibl. Lich., 1–17.

Archer, A., 1997. The lichen genus *Pertusaria* in Australia. Bibl. Lich. 69, 1–249.

Bingle, L.E.H., Simpson, T.J., Lazarus, C.M., 1999. Ketosynthase domain probes identify two subclasses of fungal polyketide synthase genes. Fun. Gen. Biol. 26, 209–223.

Brodo, I.M., 1991. Studies in the lichen genus *Ochrolechia*. 2. Corticolous species of North America. Can. J. Bot. 69, 733–772.

Cheng, Q., Kinney, K.A., Whitman, C.P., Szaniszlo, P.J., 2004. Characterization of two polyketide synthase genes in *Exophiala lecanii-corni*, a melanized fungus with bioremediation potential. Bioorg. Chem. 32, 92–108.

Culberson, W.L., Culberson, C.F., 1970. A phylogenetic view of chemical evolution in the lichens. Bryologist 73, 1–31.

Dibben, M., 1980. The chemosystematics of the lichen genus *Pertusaria* in North America north of Mexico. Publ. Biol. Geol. 5, 1–162.

Elix, J.A., 1996. Biochemistry and secondary metabolites. In: Nash, T. (Ed.), Lichen Biology. Cambridge University Press, Cambridge, pp. 154–180.

Emmerich, R., Giez, I., Lange, O., Proksch, P., 1993. Toxicity and antifeedant activity of lichen compounds against the polyphagous herbivourous insect *Spodoptera littoralis*. Phytochemisty 33, 1389–1394.

Ernst-Russell, M.A., Elix, J.A., Chai, C.L.L., Willis, A.C., Hamada, N., Nash III, T.H., 1999. Hybocarpone, a novel cytotoxic naphthazarin derivative from mycobiont cultures of the lichen *Lecanora hybocarpa*. Tetrahedron Lett. 40, 6321–6324.

Feige, G.B., Lumbsch, H.T., Huneck, S., Elix, J.A., 1993. Identification of lichen substances by a standardized high-performance liquid chromatographic method. J. Chromatogr. 646, 417–427.

Fröberg, L., Baur, A., Baur, B., 1993. Differential herbivore damage to calcicolous lichens by snails. Lichenologist 25, 83–95.

Fulton, T.R., Ibrahim, N., Losada, M.C., Grzegorski, D., Tkacz, J.S., 1999. A melanin polyketide synthase (PKS) gene from *Nodulisporium* sp. that shows homology to the pks1 gene of *Colletotrichum lagenarium*. Mol. Gen. Genet. 262, 714–720.

Gauslaa, Y., Solhaug, K.A., 2001. Fungal melanins as a sun screen for symbiotic green algae in the lichen *Lobaria pulmonaria*. Oecologia 126, 462–471.

Ginolhac, A., Jarrin, C., Gillet, B., Robe, P., Pujic, P., Tuphile, K., Bertrand, H., Vogel, T.M., Perriere, G., Simonet, P., Nalin, R., 2004. Phylogenetic analysis of polyketide synthase I domains from soil metagenomic libraries allows selection of promising clones. Appl. Environ. Microbiol. 70, 5522–5527.

Graziani, S., Vasnier, C., Daboussi, M.J., 2004. Novel polyketide synthase from *Nectria haematococca*. Appl. Environ. Microbiol. 70, 2984–2988.

Grube, M., Blaha, J., 2003. On the phylogeny of some polyketide synthase genes in the lichenized genus *Lecanora*. Mycol. Res. 107, 1419–1426.

Hall, T., 1998. Bioedit. Department of Microbiology, North Carolina State University, Raleigh, NC, USA.

Hamada, N., 1984. The content of lichen substances in *Ramalina siliquosa* cultured at various temperatures in growth cabinets. Lichenologist 16, 96–98.

Hamada, N., 1989. The effect of various culture conditions on depside production by an isolated lichen mycobiont. Bryologist 92, 310–313.

Han, D., Matsumaru, K., Rettori, D., Kaplowitz, N., 2004. Usnic acid-induced necrosis of cultured mouse hepatocytes: inhibition of mitochondrial function and oxidative stress. Biochem. Pharmacol. 67, 439–451.

Hanko, B., 1983. Die Chemotypen der Flechtengattung *Pertusaria* in Europa. Bibl. Lich. 19, 1–297.

Hanko, B., Leuckert, C., Ahti, T., 1985. Beiträge zur Chemotaxonomie der Gattung *Ochrolechia* (Lichenes) in Europa. Nova Hedwigia 42, 165–199.

Hawksworth, D.L., 1976. Lichen chemotaxonomy. In: Brown, D.H., Hawksworth, D.L., Bailey, R.H. (Eds.), Lichenology: Progress and Problems. Academic Press, London, pp. 139–184.

Hidalgo, M.E., Fernandez, E., Quilhot, W., Lissi, E., 1994. Antioxidant activity of depsides and depsidones. Phytochemistry 37, 1585–1587.

Holmgren, P.K., Holmgren, N.H., Barnett, L.C., 1990. Index Herbariorum Part I, eighth ed. New York Botanical Garden Press, New York.

Honegger, R., 1986. Ultrastructural studies in lichens. II. Mycobiont and photobiont cell wall surface layers and adhering crystalline lichen products in four Parmeliaceae. New Phytol. 103, 797–808.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES, Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.

Huneck, S., 2003. Die wasserabweisende Eigenschaft von Flechtenstoffen. Bibl. Lich. 85, 9–12.

Huneck, S., Höfle, G., 1978. Struktur und ^{13}C-NMR-Spektroskopie von chlorhaltigen Flechtenxanthonen. Tetrahedron 34, 2491–2502.

Huneck, S., Yoshimura, I., 1996. Identification of Lichen Substances. Springer, Berlin, Heidelberg.

Kinrade, W.T.B., Ahmadjian, V., 1970. The effect of usnic acid on the physiology of two cultured species of the lichen alga *Trebouxia* Puym. Lichenologist 4, 234–247.

Kon, Y., Kashiwadani, H., Wardlaw, J.H., Elix, J.A., 1997. Effects of culture conditions on dibenzofuran production by cultured mycobionts of lichens. Symbiosis 23, 97–106.

Kroken, S., Taylor, J.W., 2001. A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. Mycologia 93, 38–53.

Kroken, S., Glass, N.L., Taylor, J.W., Yoder, O.C., Turgeon, B.G., 2003. Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. Proc. Natl. Acad. Sci. USA 100, 15670–15675.

Lange, O., 1992. Pflanzenleben unter Stress. Flechten als Pioniere der Vegetation an Extremstandorten der Erde. Rostra Universitatis Wirceburgensis, Würzburg.

Langfelder, K., Jahn, B., Gehringer, H., Schmidt, A., Wanner, G., Brakhage, A.A., 1998. Identification of a polyketide synthase gene (pksP) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. Med. Microbiol. Immunol. 187, 79–89.

Larget, B., Simon, D.L., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Lauterwein, M., Oethinger, M., Belsner, K., Peters, T., Marre, R., 1995. In vitro activities of the lichen secondary metabolites vulpinic (+)-usnic acid, and (−)-usnic acid against aerobic and anaerobic microorganisms. Antimicrob. Agents Chemother. 39, 2541–2543.

Lawrey, J., 1980. Correlations between lichen secondary chemistry and grazing activity by *Pallifera varia*. Bryologist 83, 328–334.

Lawrey, J., 1995. The chemical ecology of lichen mycoparasites. Can. J. Bot. 73, 603–608.

Lee, T., Yun, S.H., Hodge, K.T., Humber, R.A., Krasnoff, S.B., Turgeon, G.B., Yoder, O.C., Gibson, D.M., 2001. Polyketide synthase genes in insect- and nematode-associated fungi. Appl. Microbiol. Biotechnol. 56, 181–187.

Linnemannstöns, P., Schulte, J., Mar Prado, M., Proctor, R.H., Avalos, J., Tudzynski, B., 2002. The polyketide synthase gene pks4 from *Gibberella fujikuroi* encodes a key enzyme in the biosynthesis of the red pigment bikaverin. Fun. Gen. Biol. 37, 134–148.

Llano, G.A., 1944. Lichens. Their biological and economic significance. Bot. Rev. 10, 1–65.

Lumbsch, H.T., 1998. The use of metabolic data in lichenology at the species and subspecific levels. Lichenologist 30, 357–367.

Lumbsch, H.T., 2002. Analysis of phenolic products in lichens. In: Kranner, I., Beckett, R., Varma, A. (Eds.), Protocols in Lichenology. Springer, Berlin, pp. 281–295.

Lumbsch, H.T., Nash III, T.H., Messuti, M.I., 1999. A revision of *Pertusaria* species with hyaline ascospores in southwestern North America (Pertusariales, Ascomycotina). Bryologist 102, 215–239.

Lumbsch, H.T., Schmitt, I., 2001. Molecular data suggest that the lichen genus *Pertusaria* is not monophyletic. Lichenologist 33, 161–170.

Metsä-Ketelä, M., Salo, V., Halo, L., Hautala, A., Hakala, J., Mantsala, P., Ylihonko, K., 1999. An efficient approach for screening minimal PKS genes from *Streptomyces*. FEMS Microbiol. Lett. 180, 1–6.

Miyagawa, H., Hamada, N., Sato, M., Ueno, T., 1993. Hypostrepsilic acid, a new dibenzofuran from the cultured lichen mycobiont of *Evernia esorediosa*. Phytochemistry 34, 589–591.

Miyagawa, H., Hamada, N., Sato, M., Ueno, T., 1994. Pigments from the cultured lichen mycobionts of *Graphis scripta* and *G. desquamescens*. Phytochemistry 36, 1319–1322.

Miyagawa, H., Yamashita, M., Ueno, T., Hamada, N., 1997. Hypostrepsilalic acid from a cultured lichen mycobiont of *Stereocaulon japonicum*. Phytochemistry 46, 1289–1291.

Miao, V., Coeffet-LeGal, M.F., Brown, D., Sinnemann, S., Donaldson, G., Davies, J., 2001. Genetic approaches to harvesting lichen products. Trends Biotechnol. 19, 349–355.

Moffitt, M.C., Neilan, B.A., 2004. Evolutionary affiliations within the superfamily of ketosynthases reflect complex pathway associations. J. Mol. Evol. 56, 446–457.

Moriyasu, Y., Miyagawa, H., Hamada, N., Miyawaki, H., Ueno, T., 2001. 5-Deoxy-7-methylbostrycoidin from cultured mycobionts from *Haematomma* sp. Phytochemistry 58, 239–241.

Moriwaki, A., Kihara, J., Kobayashi, T., Tokunaga, T., Arase, S., Honda, Y., 2004. Insertional mutagenesis and characterization of a polyketide synthase gene (PKS1) required for melanin biosynthesis in *Bipolaris oryzae*. FEMS Microbiol. Lett. 238, 1–8.

Neamati, N., Hong, H., Sunder, S., Milne, G.W., Pommier, Y., 1997. Potent inhibitors of human immunodeficiency virus type 1 integrase: identification of a novel four-point pharmacophore and tetracyclines as novel inhibitors. Mol. Pharmacol. 52, 1041–1055.

Nicholson, T.P., Rudd, B.A.M., Dawson, M., Lazarus, C.M., Simpson, T.J., Cox, R.J., 2001. Design and utility of oligonucleotide gene probes for fungal polyketide synthases. Chem. Biol. 8, 157–178.

Nylander, W., 1866. Circa novum in studio Lichenum critericum chemicum. Flora 49, 198–201.

Oksanen, I., Jokela, J., Fewer, D.P., Wahlsten, M., Rikkinen, J., Sivonen, K., 2004. Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp. strain IO-102-I. Appl. Environ. Microbiol. 70, 5756–5763.

Page, R.D.M., 1996. Treeview, an application to display phylogenetic trees on personal computers. Comp. Appl. Biosci. 12, 357–358.

Piel, J., Hui, D., Fusetani, N., Matsunaga, S., 2004. Targeting modular polyketide synthases with iteratively acting acyltransferases from metagenomes of uncultured bacterial consortia. Environ. Microbiol. 6, 921–927.

Pramyothin, P., Janthasoot, W., Pongnimitprasert, N., Phrukudom, S., Ruangrungsi, N., 2004. Hepatotoxic effect of (+) usnic acid from *Usnea siamensis* Wainio in rats, isolated rat hepatocytes and isolated rat liver mitochondria. J. Ethnopharmacol. 90, 381–387.

Purvis, O.W., Elix, J.A., Broomhead, J.A., Jones, G.C., 1987. The occurrence of copper-norstictic acid in lichens from cupriferous substrata. Lichenologist 19, 193–203.

Rodriguez, F., Oliver, J.F., Martín, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Richardson, D.H.S., 1988. Medicinal and other economic aspects of lichens. In: M., Galun (Ed.), CRC Handbook of Lichenology, vol. 3. CRC Press, Boca Raton, pp. 93–108.

Rundel, P.W., 1978. The ecological role of secondary lichen substances. Biochem. Syst. Ecol. 6, 157–170.

Sauer, M., Lu, P., Sangari, R., Kennedy, S., Polishook, J., Bills, G., An, Z., 2002. Estimating polyketide metabolic potential among non-sporulating fungal endophytes of *Vaccinium macrocarpon*. Mycol. Res. 106, 460–470.

Schimmer, O., Lehner, H., 1973. Untersuchungen zur Wirkung von Usninsaure auf die Grünalge *Chlamydomonas reinhardtii*. Arch. Mikrobiol. 93, 145–154.

Schindler, H., 1988. Zur Geschichte der Anwendung von Flechten (Lichenes) in der Medizin. Carolinea 46, 31–42.

Schmitt, I., Messuti, M.I., Feige, G.B., Lumbsch, H.T., 2001. Molecular data support rejection of the generic concept in the Coccotremataceae (Ascomycota). Lichenologist 33, 315–321.

Schmitt, I., Lumbsch, H.T., 2004. Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. Mol. Phylogenet. Evol. 33, 43–55.

Schmitt, I., Martín, M.P., Türk, R., Lumbsch, H.T., 2003. Phylogenetic position of the genera *Melanaria*, *Varicellaria* and *Thamnochrolechia* (Pertusariales). Bibl. Lich. 86, 147–154.

Schmitz, K., Lumbsch, H.T., Feige, G.B., 1994. Systematic studies in the Pertusariales II. The generic concept in the Pertusariaceae. Acta Bot. Fenn. 150, 153–160.

Schöller, H., 1997. Flechten. Geschichte, Biologie, Systematik, Ökologie, Naturschutz und kulturelle Bedeutung. Waldemar Kramer, Frankfurt am Main.

Solhaug, K.A., Gauslaa, Y., 1996. Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. Oecologia 108, 412–418.

Solhaug, K., Gauslaa, Y., Nybakken, L., Bilger, W., 2003. UV-induction of sun-screening pigments in lichens. New Phytol. 158, 91–100.

Stocker-Wörgötter, E., 2001. Experimental lichenology and microbiology of lichens: culture experiments, secondary chemistry of cultured mycobionts, resynthesis, and thallus morphogenesis. Bryologist 104, 576–581.

Takano, Y., Kubo, Y., Shimizu, K., Mise, K., Okuno, T., Furusawa, I., 1995. Structural analysis of PKS1, a polyketide synthase gene involved in melanin biosynthesis in *Colletotrichum lagenarium*. Mol. Gen. Genet. 249, 162–167.

Vartia, K.O., 1973. Antibiotics in lichens. In: Ahmadjian, V., Hale, M.E. (Eds.), The Lichens. Academic Press, New York, London, pp. 547–561.

Watanabe, C.M., Wilson, D., Linz, J.E., Townsend, C.A., 1996. Demonstration of the catalytic roles and evidence for the physical association of type I fatty acid synthases and a polyketide synthase in the biosynthesis of aflatoxin B1. Chem. Biol. 3, 463–469.

Whiton, J.C., Lawrey, J.D., 1982. Inhibition of *Cladonia cristatella* and *Sordaria fimicola* ascospore germination by lichen acids. Bryologist 85, 222–226.

Whiton, J.C., Lawrey, J.D., 1984. Inhibition of crustose lichen spore germination by lichen acids. Bryologist 87, 42–43.

Winka, K., Ahlberg, C., Eriksson, O.E., 1998. Are there lichenized Ostropales. Lichenologist 30, 455–462.

Yamamoto, Y., Miura, Y., Higuchi, M., Kinoshita, Y., Yoshimura, I., 1993. Using lichen tissue cultures in modern biology. Bryologist 96, 384–393.

Yang, X., Shimizu, Y., Steiner, J., Clardy, J., 1993. Nostoclide I and II, extracellular metabolites from a symbiotic cyanobacterium, *Nostoc* sp., from the lichen *Peltigera canina*. Tetrahedron Lett. 34, 761–764.

Yoshimura, I., Kinoshita, Y., Yamamoto, Y., Huneck, S., Yamada, Y., 1994. Analysis of secondary metabolites from lichen by high performance liquid chromatography with a photodiode array detector. Phytochem. Anal. 5, 195–205.

Zhang, A., Lu, P., Dahl-Roshak, A.M., Paress, P.S., Kennedy, S., Tkacz, J.S., An, Z., 2003. Efficient disruption of a polyketide synthase gene (pks1) required for melanin synthesis through Agrobacterium-mediated transformation of *Glarea lozoyensis*. Mol. Genet. Genom. 268, 645–655.