Assessment of identity of filamentous fungi colonizing water-damaged building materials

X. Lian[1,2,#], M. Lackner[3,4,#,*], G. S. de Hoog[1], A. H. G. Gerrits van de Ende[1], O. Priha[6], M.-L. Suihko[5], J. Houbraken[1], J. Varga[6], R. A. Samson[1], B. Mälarstig[7], P. Thompson[9], R. Stott[10] and M. D. Richardson[11,12]

[1] CBS-KNAW Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands
[2] Department of Dermatology and Venereology, Union Hospital, Tongji Medical College, China
[3] Federal Institute for Drugs and Medical Devices, Bonn, Germany
[4] Institute of Microbiology, University of Innsbruck, Innsbruck, Austria
[5] VTT Technical Research Centre of Finland, Espoo, Finland
[6] Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[7] Micro Clean Ltd, Virum, Denmark
[8] Anozona Ab, Uppsala, Sweden
[9] Healthy Office Fugenex Ltd., Hull, U.K.
[10] Department of Bacteriology & Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland
[11] Regional Mycology Laboratory, Education and Research Centre, University Hospital of South Manchester, Manchester, U.K.

Lian X., Lackner M., de Hoog G. S., Gerrits van de Ende A. H. G., Priha O., Suihko M.-L., Houbraken J., Varga J., Samson R. A., Mälarstig B., Thompson P., Stott R. & Richardson M. D. (2011) Assessment of identity of filamentous fungi colonizing from water-damaged building materials. – Sydowia 63 (1): 49–66.

Growth of moulds in moisture-damaged buildings has various adverse health effects, such as allergic diseases. To protect the inhabitants being harmed by moulds or their toxins, indoor microbiota need to be investigated; therefore an efficient identification method is needed. To assess the diversity of fungi colonizing temporarily damp indoor building materials in northern European countries (Finland, Sweden, Denmark, and U.K.), 62 samples from 22 houses were taken. In total 234 pure cultures were acquired and identified using morphological and molecular techniques (sequence comparison of rDNA internal transcribed spacer and BT2 [partial β-tubulin]). Comparing CBS-database with GenBank by blasting ITS and BT2 sequences we found correct identification down to species level by GenBank in only 12.8 % and 16.0 %, respectively. GenBank is an inadequate tool for reliable identification of less common fungi. Final identification of fungi is based on internal databases at CBS validated by ex-type strains. Within all samples we identified strains affiliated to 43 species belonging to 20 genera. Predominant were oligotrophic fungi such as *Penicillium, Aspergillus* and *Cladosporium;* some may serve as indicator

Both authors contributed in an equal way.
e-mail: Michaela.Lackner@i-med.ac.at

organisms for indoor moisture problems. Based on final identifications, species-specific probes were developed for fast identification of moulds in indoor environments.

Key words: Indoor fungi, molecular identification, species-specific probes

Indoor environments are an artificial habitat for fungi; similar habitats are not known in the natural environment. While outside air primarily contains the ubiquitous airborne saprobes living on plant debris, the composition of indoor airborne microbiota is significantly different (Lee *et al.* 2006, Meklin *et al.* 2007, Wu *et al.* 2000). These differences are due to fungi growing in and on various materials, such as potted plants, damp basements and moist walls. The composition of microbiota and the number of the fungi present in air is increased after temporary or continuous water damage. Decisive characteristics of the indoor environment of damp building materials are oligotrophism (Satow *et al.* 2008), tolerance of fluctuating temperatures, and relatively high humidity, whereby bacteria require liquid water for growth and the more xerotolerant fungi already do well with the lower water potential of damp supports or moist air (McGregor *et al.* 2008). For the growth on special indoor materials (e.g., materials containing formaldehyde) fungi need a specialized metabolism and certain protective mechanisms to overcome the toxicity of these materials (Sea *et al.* 2008).

In relation to the high numbers of recognized fungi, only few are found in the man-made indoor environments. Water-damaged building materials are colonized by a relatively specific mycobiota (Andersson *et al.* 1997, Gravesen *et al.* 1999, Hyvärinen *et al.* 2002, Torvinen *et al.* 2006). Based on 20 years of experience, Samson *et al.* (2001) recognized only about 90 prevalent taxa in indoor environments. But the number of prevalent fungi in indoor environments is increasing today, due to novel isolation techniques (Lian & de Hoog 2010) and as a result of fundamental changes in fungal taxonomy after the application of molecular techniques, leading to a great diversity of cryptic species. In many cases these newly circumscribed molecular species appear to have narrower ecological amplitudes and more clearly recognizable preferences than the broadly defined morphological species of the past (de Hoog *et al.* 2007).

Moist sites which are heavily overgrown by fungi usually have a very limited number of fungal colonizers (single or very few species), reflecting the special physiologic abilities of fungi needed to grow on these nutrient-poor substrates. Depending on the features (building material, period of water damage, severity) of the moisture problem, the local mycobiota may differ. Rubber (Linos *et al.* 1996), gypsum board (Pasanen *et al.* 2000), air conditioning systems (Samson *et al.* 1994) or bathrooms (Nishimura *et al.* 1987) all harbor their characteristic fungi. The ability of colonizers to become aerosolized is deter-

mined by the ability to sporulate on specific substrates and their mechanisms of spore dispersal, influenced by relative humidity (McGinnis 2007). Dry, hydrophobic conidia and massive sporulation capacity are found in genera such as *Aspergillus, Penicillium, Alternaria* and *Cladosporium*. Fungi with slimy conidia or yeast-like growth, for example members of *Acremonium, Fusarium, Aureobasidium,* and *Phoma,* colonize wet cells and moist windows, and are aerosolized only after drying of the colonized supports, but may be equally important in terms of indicator function and adverse effects on human health. At isolation, the ubiquitous hydrophobic species may overshadow local hydrophiles, the latter category being systematically underestimated when classical isolation techniques are used for quantitative evaluation. This particularly holds true for the slow-growing, melanized opportunistic fungi that are also regularly found as agents of human skin disease and have been suggested to have their reservoir in bathing facilities (Lian & de Hoog 2010).

Among the most common adverse effects of airborne fungi on human health (Mitchell *et al*. 2006) are irritation of mucous membranes and allergic diseases, leading to, e.g., bronchial asthma, allergic rhinitis/conjunctivitis, atopic dermatitis (AD), allergic bronchopulmonary mycosis (ABPM) or extrinsic allergic alveolitis (Burge *et al*. 1989, Fisk *et al*. 2007, Lacey *et al*. 1994). These types of chronic disorders may severely impair the quality of life of patients. In a large multicenter investigation by Gray *et al*. (2003), mould-exposed patients reported a greater frequency and intensity of symptoms, particularly of neurological and inflammatory nature. Higher levels of various autoantibodies were detected compared to the controls. Another study done by Campbell *et al*. (2003) showed that exposure to moulds in water-damaged buildings increased the risk for development of neural autoantibodies, peripheral neuropathy (such as numbness, tingling, tremors and muscle weakness in the extremities), and neurophysiologic abnormalities in exposed individuals. With the increasing number of immunocompromised patient populations in hospitals, opportunistic pathogens in indoor environments are notorious for causing systematic and fatal infections, particularly as a result of construction works that lead to dust from building materials (Kennedy *et al*. 1995, Nihtinen *et al*. 2007, Oren *et al*. 2001). Waterborne fungi may be involved in cutaneous infections. This is well known for dermatophytes, which are easily dispersed by adherence to the skin of the foot (Watanabe *et al*. 2001) and are picked up from wet cell, such as bathrooms and swimming pools (Hilmarsdottir *et al*. 2005). There are indications that melanized skin pathogens are distributed in a similar fashion (de Hoog and Saunte; unpublished data).

The development of efficient methods to detect and identify fungi in the indoor environment has been greatly enhanced by the application of molecular methods such as microarrays and Realtime-PCR

(e.g., Haugland & Vesper 2007, Haugland *et al.* 2002, Meklin *et al.* 2007, Wang *et al.* 2004). Given the precise ecological role of each species, correct identification is essential. With the rapid developments in molecular taxonomy, continuous updating is mandatory in numerous areas, where micro- or sibling species with highly specific ecologies are introduced regularly. Standard molecular identification of filamentous fungi is done by sequencing rDNA Internal Transcribed Spacers (ITS), supplemented with data from more variable gene regions, such as introns in the BT2 gene region (partial β-tubulin). Routinely, sequences are compared with those deposited in GenBank. However, GenBank depositions are poorly supervised and for less common fungi this system is insufficient. One major flaw is the inconsistent use of reference to sequences derived from ex-type strains. In addition, depositions rarely represent the intra-specific variability of species. In the present paper we describe procedures for more reliable identification using dedicated databases validated by comparison with ex-type material and representing the full range of variability per gene in each species, assessed by genealogical concordance studies.

Materials and Methods

Isolation

A total of 62 building material samples were taken from 22 different water-damaged houses between January to June 2006, of these were 31 from Finland, 15 from Sweden, 8 from Denmark, and 8 from the United Kingdom. Samples were packed separately in sterile plastic or paper bags and sent to laboratory. Soft or small sample materials like insulation wool or wood crumbs were weighed, and the surfaces of hard, large samples, such as gypsum board, wood, stone and linoleum, were carefully scraped with a sterile knife into an Erlenmeyer flask (0.01–7.00 g of sample depending on total sample volume) containing Ringer or 0.9 % NaCl solution (to obtain a dilution of 1/10 [w/v] in most cases). The suspension was allowed to stand for 30 min and was then shaken for 10 min in a laboratory blender (Stomacher 400, Seward, West Sussex, U.K.). The cotton stick samples were placed into 5 mL of Ringer solution and after settling were mixed with a Vortex for 2 min. A dilution series of samples was plated on the following media: (1) Potato Dextrose Agar (PDA, Difco, Becton Dickinson, Sparks, MD, U.S.A.) supplemented with 0.01 % chloramphenicol (Sigma) and 0.01 % chlortetracycline (Sigma) (to inhibit bacterial growth) and 0.02 % Triton x-100 (Fluka) (controlling the spreading of colonies), (2) Malt Extract Agar (2 % MEA, LAB M, Lancashire, U.K.) with 0.01 % chloramphenicol; (3) Dichloran-Glycerol Agar (DG18, Oxoid, Hampshire, U.K.) with 0.01 % chloramphenicol. Culture plates were incubated at 25 °C, monitored daily during the first week and subsequently every two days for up to 14 days. All morphological distinct colonies of

each sample were subcultured to gain pure cultures. A total of 234 isolates was subsequently gained for morphological and molecular identification.

Morphological identification

Fungi were preliminarily identified to genus level by morphological characteristics colony morphology (stereomicroscopy) and microscopic morphology (light microscopy).

Molecular identification

DNA extraction

Approximately 1 cm^2 of fungal material was transferred to a 1.5 mL Eppendorf tube with glass beads (Sigma G9143) and 400 µL TEX buffer (composition of the lyses buffer: 10 mM Tris-Cl [ph 8.0], 5mM EDTA, and 1 % [v/v in water] Triton X-100). The fungal material was homogenized for 1 min on a MoBio Vortex. After adding 120 µL of 10 % SDS and 10 µL of 10 mg/mL proteinase K, followed by incubation at 55 °C for 30 min. Subsequently the mixture was vortexed again for 3 min; 120 µL of 5 M NaCl solution was added. 1/10 volume cetyltrimethylammonium bromide (CTAB) buffer 10 % was added, followed by incubation for 60 min at 55 °C. Samples were vortexed for another 3 in and one volume chloroform-isoamylalcohol (v/v = 24/1) was added and mixed carefully by hand. The top layer was transferred to a sterile Eppendorf tube the mixture was spun for 5 min at 4 °C, 20 400 rcf. The supernatant was mixed with 225 µL 5 M NH_4-acetate and incubated 30 min on ice. After centrifugation at 20400 rcf at 4 °C for 5 min, the top layer was transferred again to a clean Eppendorf tube and mixed with 0.55 vol ice-cold isopropanol. After incubation at –20 °C for 30 min and centrifugation for 5 min, 20400 rcf, the supernatant was decanted and the pellet washed with ice-cold ethanol 70 % twice and dried in a vacuum dryer. The powder was re-suspended in 100 µL TE-buffer, incubated at 37 °C for 15–30 min and stored at –20 °C until used.

PCR and sequencing

PCR was performed in 25 µL vol of a reaction mixture containing 10 mM Tris-HCl, 10 pmol of each primer, 50 µM concentrations of each dNTP, 1.5 U of Taq polymerase, and 1 µL of template DNA. V9G (5'-TTA CGT CCC TGC CCT TTG TA-3') and LS266 (5'-GCAT TCC CAA ACA ACT CGA CTC-3') were used to amplify ITS region of rDNA and the program was performed as follows: 95 °C for 5 min, followed by 35 cycles consisting of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 70 s. Primers for BT2 PCR were Bt-2a (5'-GGT AAC CAA ATC GGT GCT GCT TTC) and Bt-2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') and the program was changed to 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 45 s and 72 °C for 2 min. The PCR products were

cleaned with GFX-columns. Sequence-PCRs were performed as follows: 95 °C for 1 min, followed by 30 cycles of 95 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min with primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') for ITS sequencing, and Bt-2a and Bt-2b for BT2. DNA was purified with Sephadex G-50 Superfine and sequenced using DYE-ET terminator. Sequences obtained were adjusted using SeqMan P of Lasergene software (Dnastar, Inc.) and aligned iteratively using Ward's averaging in the BioNumerics package v. 4.0 (Applied Maths, Kortrijk, Belgium).

Sequence comparison

ITS and BT2 sequences were compared in GenBank using blastn algorithm (www.ncbi.nlm.nih.gov/blast/Blast.cgi). The first three scores with the highest 'Max identity' were maintained. If below 95 %, the identification was listed as 'no match'. For most of the species a second comparison was performed in databases maintained at CBS for research purposes using BioNumerics package v. 4.0. These databases contain sequences of ex-type strains and reference where available, and enable multilocus sequence comparison in view of genealogical concordance-assisted determination of specific borderlines. Databases available for identification and further refinement were dedicated to *Eurotiales* (*Penicillium, Aspergillus, Doratomyces, Eurotium*), melanized fungi (*Aureobasidium, Cladosporium, Exophiala, Ulocladium*), *Hypocreales* (*Acremonium*), coelomycetes (*Phoma, Epicoccum*) and Zygomycetes (*Rhizopus*). The *Trichoderma* database (www.isth.info/) was used for identification of *Trichoderma* strains. For 10 ITS and 10 BT2 (partial β-tubulin region) sequences, GenBank results were final because no validated database was available. Results of 234 ITS and 204 BT2 sequences were compared in GenBank as well as in BioNumerics, and deviations were scored qualitatively.

Design of specific probes

After ITS identification, sequences were aligned in BioNumerics, aided by iterative tree reconstruction with the Ward's averaging option, and arranged according to overall similarity. For every group of species belonging to the same genus, small motifs (18–50 bases) that deviated significantly from remaining species were selected, and species-specific oligos were designed. Predictivity and specificity of probes was confirmed by blasting in GenBank, and quality of the motifs to be used as probes was verified in PrimerSelect of Lasergene software (Dnastar, Inc.). Specificities of these oligos were tested in GenBank. If the correct species was found with both 100 % of 'Query coverage' and 100 % of 'Max identities', the oligo was judged to be highly specific and was maintained for later use. If no specific motif was obtained with ITS, BT2 sequences were used instead.

Tab. 1. – Building material samples of the same type positive for fungal growth, number of fungal genera/species identified per sample type. Predominant genera/species are listed according to their frequencies in sample types; only taxa found more than twice are included. Data on organic building materials are overlaid in grey, swab samples are not included.

Types of building material	Fungal positive [%]	Genera [N]	Predominant genera	Species [N]	Predominant species
Wood/ plywood [N = 4]	100	7	*Aspergillus* *Acremonium* *Penicillium* *Cladosporium* *Trichoderma*	12	*A. versicolor* *Acremonium sp.* *P. corylophilum* *C. langeronii/psychrotolerans T. atroviride*
Wood chip [N = 10]	70	7	*Penicillium* *Aspergillus* *Epidermophyton Epicoccum*	8	*P. corylophilum* *A. versicolor – E. rubrum Epicoccum nigrum* *P. chrysogenum*
Chipboard [N = 2]	100	2	*Penicillium*	2	*P. corylophilum*
Insulator paper/ wallpaper [N = 5]	80	5	*Aspergillus* *Ulocladium* *Phoma*	7	*A. versicolor* *U. botrytis*
Gypsum board [N = 10]	100	9	*Aspergillus* *Penicillium* *Phoma* *Ulocladium* *Cladosporium*	10	*P. corylophilum* *A. versicolor* *P. chrysogenum* *C. cladosporioides*
Insulator wool [N = 17]	88	12	*Penicillium* *Cladosporium* *Trichoderma* *Phoma* *Ulocladium*	17	*P. corylophilum* *P. chrysogenum* *C. langeronii/psychrotolerans* *T. atroviride*
Stone materials [N = 2]	50	2	*Aspergillus*	2	*A. niger*
Paint/ plaster [N = 2]	50	1	*Penicillium*	1	
Flooring materials [N = 3]	100	2	*Penicillium* *Trichoderma*	3	*P. corylophilum* *T. atroviride*
Unspecified swab samples [N = 7]	100	5	*Aspergillus* *Cladosporium* *Penicillium* *Ulocladium* *Gliomastix*	7	*A. versicolor* *P. corylophilum* *C. sphaerospermum* *P. chrysogenum –* *U. botrytis- G. murorum*

Results

Fungal diversity on and in building materials

Fungi were present in 54 out of 62 samples (87.1 %). All samples were found to be fungus-positive in Denmark (n = 8), Sweden (n = 15), and the U.K. (n = 8), while in Finland, 74.2 % samples revealed fungi (23 of 31 samples). Different diversities of fungal genera were obtained from nine types of building materials (Tab. 1). Wood/plywood showed the highest diversity of fungi, with an average of 3.5 genera per sample and a maximum six fungal genera (seven species) within a single sample. The widest total diversity was found in insulation wool: 12 genera and 17 species were identified from 17 samples. Subsequently, gypsum board, wood chips and insulating paper/wallpaper also proved to be easily colonized by divergent fungi with rather high numbers of genera and species. Numbers of genera were relatively low on flooring materials, chipboard, stone materials and paint/plaster.

A total of 234 pure cultures were obtained: 32 from samples from Denmark, 89 from Finland, 83 from Sweden, and 45 from the U.K. The set represented 22 genera and 43 species. The dominant genus was *Penicillium*, with 41.5 % of the entire set of strains. The remaining generic ranking of widespread entities comprised *Aspergillus* (14.5 %) and *Cladosporium* (11.5 %). *Trichoderma, Acremonium, Phoma* and *Ulocladium* were less distributed. *Paecilomyces, Gliomastix, Doratomyces, Chaetomium, Eurotium, Rhizopus, Aureobasidium, Epicoccum, Plectosphaerella, Stachybotrys, Phanerochaete*, *Fusarium, Geomyces,* and *Verticillium* species were occasionally recovered with one to three isolates.

Penicillium isolates belonged to six species: *P. corylophilum* Dierckx, *P. chrysogenum* Thom, *P. brevicompactum* Dierckx, *P. commune* Thom, *P. roseopurpureum* Dierckx, and *P. biourgeianum* K.M. Zalessky. *Penicillium corylophilum* was the most frequently encountered species, with a total of sixty-five colonies and a percentage of 27.8 % of all strains. Other frequent species were *Aspergillus versicolor* (Vuill.) Tirab. and *Penicillium chrysogenum*, with percentages of 12.8 % and 9.8 %, respectively. *Cladosporium langeronii* (Fonseca, Leão & Nogueira) Vuill., *C. sphaerospermum* Penz., *Trichoderma atroviride* P. Karst., and *Ulocladium botrytis* Preuss were also very common species (Tab. 2).

Molecular identification using GenBank

Of the total of 234 isolates, ITS and BT2 sequence data were both available for 204 strains. GenBank identification for both genes resulted in the same species name in 78 strains (38.2 %). When only ITS sequences were available (Tab. 3), 30 strains (12.8 %) yielded an unambiguous answer, while with 9 isolates (3.9 %) no match was obtained. In 184 isolates the correct identification (see below) in GenBank was

Tab. 2. – Identification of fungal pure cultures by means of morphological and molecular identification. Absolute number of strains affiliated to one species and frequency of species; regarding a total number of 234 isolates. Number of false identifications are listed blasting ITS or BT2 sequences in GenBank.

Identification [N = 42]	**Isolates [N]**	**Frequency [%]**	**False ID blasting ITSa**	**False ID blasting BT2a**
Penicillium corylophilum	65	27.8	> 10	> 10
Aspergillus versicolor	30	12.8	> 10	6
Penicillium chrysogenum	23	9.8	> 10	7
Trichoderma atroviride	15	6.4	5	3
Cladosporium langeronii	13	5.6	8	5
Ulocladium botrytis	10	4.3	5	3
Acremonium sp.	10	4.3	3	4
Cladosporium sphaerospermum	9	3.8	4	5
Phoma herbarum	5	2.1	7	6
Penicillium brevicompactum	4	1.7	6	3
Stachybotrys chartarum	3	1.3	1	1
Penicillium commune	3	1.3	6	3
Phoma fimeti	3	1.3	3	2
Trichoderma longibrachiatum	3	1.3	4	2
Chaetomium globosum	3	1.3	2	5
Cladosporium cladosporioides	2	0.9	3	1[b]
Gliomastix murorum	2	0.9	1	2
Aspergillus niger	2	0.9	3	3
Eurotium rubrum	2	0.9	4	2
Cladosporium tenuissimum	2	0.9	3	1
Paecilomyces variotii	2	0.9	3	5
Phoma sp.	2	0.9	3	1
Acremonium kiliense	2	0.9	3	no tubulin
Cladosporium bruhnei	1	0.4	> 10	1[b]
Doratomyces stemonitis	1	0.4	2	no match
Acremonium aff. furcatum	1	0.4	3	3
Aureobasidium pullulans	1	0.4	1	no tubulin
Aspergillus sclerotiorum	1	0.4	3	3
Epicoccum nigrum	1	0.4	1	1[b]
Aspergillus restrictus	1	0.4	1	no match
Leptosphaerulina chartarum	1	0.4	1	no match
Penicillium biourgeianum	1	0.4	2	2
Penicillium roseopurpureum	1	0.4	1[b]	3
Phanerochaete chrysosporium	1	0.4	2	no match
Phoma narcissi	1	0.4	> 10	7
Plectosphaerella cucumerina	1	0.4	1	3
Rhizopus sexualis	1	0.4	4	no tubulin
Rhizopus oryzae	1	0.4	2	no match
Ulocladium chartarum	1	0.4	2	no match
Verticillium nigrescens	1	0.4	1	1b
Geomyces pannorum	1	0.4	3	no tubulin
Fusarium sp.	1	0.4	7	no tubulin
Total:	**234**	**100**		

[a] Numbers of false results in GenBank.

[b] Obtained in GenBank, but different from validated database.

Table abbreviations: no match (= maximum identity in GenBank < 95%); no tubulin (= no tubulin sequence in GenBank); > 10 (= > 10 isolates of a certain species misidentified using GenBank); ID (= Identification); isolates [N] (= absolute number of isolates); frequency (percentage of isolates affiliated to the species).

listed among the names with identical similarity scores: the ITS regions of other species or genera deposited in GenBank were invariable between these taxa. In a number of cases an obviously incorrect deposition was concerned. For example, after blasting the ITS sequence of isolate dH16932 in GenBank, *Aspergillus versicolor, Aspergillus nidulans* (Eidam) G. Winter, *Penicillium* sp. and *Hyalodendron* sp. all showed 100 % coverage and identity, but *Aspergillus* and *Penicillium* are two widely different ascomycete genera and *Hyalodendron* is known to belong to the basidiomycetes. Final identification of this strain using the validated database below was *Aspergillus versicolor*.

When only BT2 sequences were available (Tab. 3), the percentage of correctness with GenBank Blast was slightly higher (15.7 %) than that obtained with ITS, but 36 sequences (17.7 %) did not match with any GenBank accession, because appropriate depositions were unavailable. Compared to final identification with validated databases below, the percentage of above-species misidentification, i.e. at genus, family, ordinal or class level, was also higher than with ITS (31.6 % and 20.5 %, respectively).

Molecular identification in validated databases

All sequences were compared in validated databases containing ex-type strains and providing specific borderlines having been assessed by genealogical concordance studies. Relevant databases were available at CBS for *Alternaria, Aspergillus, Aureobasidium, Cladosporium, Paecilomyces, Penicillium* and relatives, whereas a warded database of *Trichoderma* is available at www.isth.info. For 10 ITS and 10 BT2 sequences no appropriate databases were available. When comparing identifications from GenBank and validated databases, only 30 ITS sequences (12.8 %) and 32 BT2 sequences (15.7 %) yielded exactly the same results. Remaining sequences matched with GenBank at levels below class (species, genus, family, order). The levels of deviation (Tab. 3) were calculated on the basis of the highest difference between results of the two databases.

Comparison with morphological identification

Prior to sequencing, strains had been provisionally identified on the basis of morphology. Most of them were diagnosed at least down to the genus level, with the exception of twenty-three strains which could not be recognized by morphological means because of lack of sporulation or pure yeast-like growth. After sequencing the strains were found to be species of *Acremonium, Aureobasidium*, *Chaetomium, Cladosporium, Epicoccum, Leptosphaerulina, Phanerochaete, Phoma* and *Verticillium*.

Tab. 3. – Absolute number [N] and frequency percentage of correct identifications using GenBank blasting function for ITS and BT2 sequences and deviations of identification results gained by GenBank from final identification.

	ITS		BT2	
Deviation of ID	**Isolates [N]**	**Frequency**	**Isolates [N]**	**Frequency**
False species	147	62.8	72	35.3
False genus	18	7.7	16	7.8
False family	1	0.4	1	0.5
False order	3	1.3	3	1.5
False class	26	11.1	43	21.1
False division	0	0.0	0	0.0
No match*	9	3.9	36	17.6
Correct	**33**	**16.2**	**33**	**16.2**
Total	234	100.0	204	100.0

*No match = maximum identity in GenBank < 95 %.

Probes for species differentiation

Thirty-nine species with final, validated identification could be characterized by at least one specific probe, either based on ITS or on BT2 (Tab. 4). For *Ulocladium botrytis, Penicillium chrysogenum, P. roseopurpureum* Dierckx, and four *Cladosporium* species (*C. langeronii, C. cladosporioides* (Fresen.) G.A. de Vries, *C. bruhnei* Linder and *C. tenuissimum* Cooke), motifs could not be selected from ITS sequences, and hence BT2 sequences were used. In some cases specific motifs were based on data from validated databases only. For example, the probe for *Ulocladium botrytis,* which was chosen from BT2, proved to be specific in the CBS database, but there was no record of this species with BT2 in GenBank (data available upon request).

Discussion

The percentage of samples positive for fungi growing on different kinds of building materials (87.7 %) was high when compared to previous studies (Andersson *et al.* 1997, Gravesen *et al.* 1999, Hyvärinen *et al.* 2002, Torvinen *et al.* 2006). As expected, fungi were able to colonize all kinds of organic materials, especially wood/plywood. Also insulation wool and gypsum board, while stone and paint/plaster remained significantly less affected. Probably the texture of the supporting material plays a role, in addition to nutritional constraints. Because in all materials nutrient availability is very limited, and no appreciable disintegration of the material was observed, it may be stated that the major factor promoting fungal colonization is moisture. Excessive fungal growth was primarily observed on materials with higher water activity, under chronically moist conditions or after severe water-damage.

The identifications listed in Tab. 2 are considered as confident based on the current state of taxonomy, reflected in the used validated

Tab. 4. – Specific ITS (*) or BT2 (#) probes for species/genus recognition.

Species	Specific probes
Acremonium sp.*	TGGCATTATCTGAGTGGCAC
Aspergillus sclerotiorum #	GATCGGCGAGAAATTTTCGGCAACG
	AAATCCACCATGCCATCTTC
Aspergillus niger *	TGCCGACGTTTTCCAACCATTCTTTCC
Aspergillus restrictus *	CTCCAACATTGAACACTG
Aspergillus versicolor *	CGACGTCTCCAACCATTTTT
Aureobasidium pullulans *	TTTCAGTCGGCAGAGTTCCT
Chaetomium globosum *	GGCCTCTCTGAGTCTTCTGTACTGAATAAG
Cladosporium cladosporioides #	CGCATACACCGATTGACAAC
	GTCAGTGTGTGGACGTGGAG
Cladosporium langeronii #	ACTGCGAGTCATTGATGG
Cladosporium sphaerospermum *	TATTCGCTAAAGGGTGCCAC
Cladosporium tenuissimum #	GAGGGAATTCATCTGACATGCGACAGG
Cladosporium bruhnei #	AGGTATGTACTCTCAAGGCTCCAGCATACGCACG-
	-GCCCATGTTCAATCAC
Doratomyces stemonitis *	GCCCCCTGCGTAGTATAACA
Epicoccum nigrum *	TTTGTAGACTTCGGTCTGCTACCTCTTA
	TTTGTAGACTTCGGTCTGCT
Eurotium sp. *	GTGGCCACGGCCCGCCGAAGACTAACA
	GCCCGCCGAAGACTAACA
Fusarium sp. *	CACAGCTTGGTGTTGGGACT
Geomyces pannorum *	CAGTAGTCATCCGGGTTG
Gliomastix murorum *	GGCCGTCCCCTAAATCCAGTGGCGACCA
Leptosphaerulina chartarum *	AGGGGCTACGGCTCGCGTCCAACAAGCACATTTAC
Paecilomyces variotii *	TGGAAGGTTGCCGTCTGAGTATA
Penicillium biourgeianum *	CTTGCCGATCAACCAAACTT
Penicillium brevicompactum *	CTGCCGATCAACCAAACTTT
Penicillium commune *	TTACGTATCGCATTTCGCTG
Penicillium corylophilum *	CCTCCCACCCATGTTTATTG
Penicillium chrysogenum #	ATTTGGAGCCCGGTACCATGGATGCTGT
Penicillium roseopurpureum #	CTTCTGGTAACGAAAAGGTGCTGC
	CTTCTGGTAACGAAAA
Phanerochaete chrysosporium *	CAGGTTGTAGCTGGCCTCTC
Phoma fimeti #	GGACAACCTTATAACCTATTT
Phoma herbarum *	ATTGCTTGGTGTTGGGTGTTTGTCTCGCCTTTGCGTGT
Phoma narcissi *	GATGTACTGCGCTCCGAAAT
	ATGTACTGCGCTCCGAAATC
Plectosphaerella cucumerina *	AGTAGCATCAGCCTCGCATT
Rhizopus sexualis *	CAATAATATACCTTGAAATTCAGATTGTAAAATA
Rhizopus oryzae *	AGAGACTCAGACTGGTCA
Stachybotrys sp. *	ACAACTCCCAAACCCTTATGTGAACC
	CAGTATTCTCTGAGTGGCAAACGCAAA
Trichoderma atroviride *	CCTCGGGAGCCCCTAAGACGGGAT
	CTCTTTTCTGTAGTCCCCTCGC
Trichoderma longibrachiatum *	GGCTCCCGTCGCGGCTCTGTTTTATTTTTGC
	GTTACCAATCTGTTGCCTCGGCGGGATTCTCTT
Ulocladium botrytis #	TGCGTCACCCGACTGTCCATA
Ulocladium chartarum *	TTTGGGGTTACAGCCTTG
Verticillium nigrescens *	GATCTCTTGGCTCCAGCATC
	TATAACTCATAACCCTTTGTGAACCT-TTATACCTGTTGCTT

databases. Comparing the spectrum of species identified in our study with lists of commonly occurring indoor fungi based upon many years of practical experience and published by several authors (Gravesen *et al*. 1997, Gravesen et al, 1999, Lugauskas *et al*. 2004, Pitkäranta *et al*. 2008, Samson *et al*. 2001), dominant genera invariably are *Penicillium* and *Aspergillus*. Several species can be regarded as resident mycobiota of indoor environments, colonizing the building materials themselves rather than primarily being blown in from outside. *Penicillium corylophilum* and *Aspergillus versicolor* were among the predominant species. These species were also found to be very common in settled dust in family dwellings (Miller 1995). As in several other studies, *Cladosporium, Trichoderma, Acremonium* and *Ulocladium* were also frequent inhabitants of water-damaged buildings. In contrast, the genera *Chaetomium, Mucor,* and *Fusarium,* which were frequently mentioned in other studies, and commonly encountered fungi, such as the xerophilic species *Aspergillus penicillioides* (Vesper *et al*. 2005) and *Wallemia sebi* (Fr.) Arx (Wang *et al*. 2004), and the toxigenic mould *Stachybotrys chartarum* (Ehrenb.) S. Hughes (Tiffany & Bader 2000) were not isolated in the course of our study, despite the use of appropriate media for isolation. *Stachybotrys chartarum* is known to grow well at room temperature and with humidity above 93 % (Chapman 2003). Among the sporadically isolated genera, *Doratomyces, Gliomastix, Phanerochaete,* and *Plectosphaerella* were found, to our knowledge, for the first time in indoor environments. The recently reclassified species *Cladosporium langeronii* (Zalar *et al*. 2007) was not reported in any previous indoor study. It was segregated from the *Cladosporium sphaerospermum* Penz. complex and found to be somewhat osmotolerant and oligotrophic, while the remaining species are invaders of dead plant material. In our investigation it had a rather high frequency with 13 strains obtained.

In summary, despite the supposed relatively small spectrum of indoor fungi we witness much variability in actual microbiota, due to differences in prevalence, ambient conditions, and detection methods. It may be questioned whether complete evaluation of indoor fungal presence may be clinically relevant. For example, clone libraries reveal a high prevalence of basidiomycetes, which have rarely been associated with respiratory problems. The use of standardized oligonucleotide arrays for identification may provide an excellent means to collect comparative data (Haugland *et al*. 2002). However, a problem is caused by the rapid developments in molecular fungal taxonomy and phylogeny, species names and concepts change faster than ever. Many newly circumscribed molecular entities tend to be ecologically restricted, and thus continual updating according to latest taxonomic standards is relevant to understanding of the biology of fungi colonizing indoor environments. Among these are xerophilic and indoor *Aspergillus* species (Houbraken *et al*. 2007, Varga *et al*. 2007), osmotoler-

ant *Cladosporium* species occurring in wet cells (Zalar *et al*. 2008), toxigenic *Stachybotrys* (Andersen *et al*. 2003) and xerophilic *Wallemia* (Zalar *et al*. 2005). We experienced large problems with identification of indoor fungi using semi-automated blasting with GenBank. Unambiguous and correct identification was rarely obtained. When they were found correct, frequently there were competing species listed at equal similarities, so that decisions on final IDs were difficult to make. Part of this problem has intrinsic reasons, because particularly ITS may be insufficiently variable at the species level, neighboring species having the same sequence. With the more variable BT2 sequences even more problems were encountered, as a result of which the gene yielded less reliable identifications than ITS. This could largely be ascribed to insufficient depositions being available., A particular and significant problem is, however, the fact that much of the taxonomic deviation was due to incorrect depositions in GenBank. Interpretation of multiple identification results requires specialized taxonomic expertise. GenBank does not validate sequences on the basis of ex-type strains, and hence incorrect depositions may multiply when a first deposition was incorrect. As a result, GenBank is useful for identification of common clinical fungi, e.g., for diagnosis of diseases and for susceptibility testing, but holds insufficiently verified entries to allow reliable identification of environmental fungi. The number of incorrect depositions in GenBank is considerable and is likely to increase with growing numbers of sequences deposited of less common environmental fungi are deposited, where taxonomic validation on the basis of ex-type strains is frequently lacking. For this reason we developed an identification schedule involving three phases: (1) provisional morphological identification, at least at the generic level, which is to be used as control to sort out accidentally sequenced contaminants; (2) provisional GenBank identification using both ITS and BT2 or one of these loci; (3) precise identification by alignment with ex-type isolates, using databases where all fungal names had been validated on the basis of ex-type sequences. Species borderlines in such databases have been assessed using multilocus genealogical concordance analysis, and thus the intra-specific variability of such species is known. Species-specific motifs should be based on sequences thus verified.

Based on the validated identifications above, motifs unambiguously characterizing the species encountered were selected. Since ITS is most widely used as a barcoding and array gene for fungal identification (Hinrikson et al. 2005, Jackson *et al*. 1999, Ratnasingham & Hebert 2007), specific motifs were first chosen from this region. Most species can be characterized by specific ITS-based oligos that reliably distinguish them from neighboring species. For others, e.g., *Cladosporium* species, which cannot be differentiated by ITS, the partial β-tubulin BT2 gene can be used additionally.

Acknowledgements

This work was carried out with financial support from the Commission of the European Communities, contract no 017626. We thank Maikel Aveskamp for his help with the identification of *Phoma* species and Irina S. Druzhinina for helping us to identify sequences of *Trichoderma*. Tarja Nordenstedt is thanked for skillful technical assistance in morphological identification of fungi. The SMEs are thanked for taking and providing the samples for this study, and R. Pöder for improving the manuscript.

References

Andersen B., Nielsen K. F., Thrane U., Szaro T., Taylor J. W., Jarvis B. B. (2003) Molecular and phenotypic descriptions of *Stachybotrys chlorohalonata* sp. nov. and two chemotypes of *Stachybotrys chartarum* found in water-damaged buildings. *Mycologia* **95:** 1227–1238.

Andersson M. A., Nikulin M., Köljalg U., Andersson M. C., Rainey F., Reijula K., Hintikka E. L., Salkinoja-Salonen M. (1997) Bacteria, molds, and toxins in water-damaged building materials. *Applied and Environmental Microbiology* **63:** 387–393.

Burge H. A. (1989) Airborne allergenic fungi. *Immunology and Allergy Clinics of North America* **9:** 307–319.

Campbell A. W., Thrasher J. D., Madison R. A., Vojdani A., Gray M. R., Johnson A. (2003) Neural autoantibodies and neurophysiologic abnormalities in patients exposed to molds in water-damaged buildings. *Archives of Environmental Health* **58:** 464–474.

Chapman J. A. (2003) *Stachybotrys chartarum* and other problems caused by allergenic fungi. *Allergy and Asthma Proceedings* **24:** 1–7.

De Hoog G. S., Nishikaku A. S., Fernandez Zeppenfeldt G., Padin-Gonzalez C., Burger E., Gerrits van den Ende A. H. G. (2007) Molecular analysis and pathogenicity of the *Cladophialophora carrionii* complex, with the description of a novel species. *Studies in Mycology* **58:** 219–234.

Fisk W. J., Lei-Gomez Q., Mendell M. J. (2007) Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. *Indoor Air* **17:** 284–296.

Flannigan B. (1989) Airborne deteriogens and pathogens. *The Biodeterioration Society*. Kew Surrey, UK.

Flannigan B., Samson R. A., Miller J. D. (2001) *Microorganisms in Home and Indoor Work Environments*. Taylor and Francis, London.

Gravesen S., Nielsen P. A., Nielsen K. F. (1997) *Microfungi in Water Damaged Buildings*. SBI report No. 282. Danish Building Research Institute, Copenhagen, Denmark.

Gravesen S., Nielsen P. A., Iversen R., Nielsen K. F. (1999) Microfungal contamination of damp buildings – examples of risk constructions and risk materials. *Environmental health perspectives* **107:** 505–508.

Gray M. R., Thrasher J. D., Crago R. , Madison R. A., Arnold L., Campbell A. W., Vojdani A. (2003) Mixed mold mycotoxicosis: immunological changes in humans following exposure in water-damaged buildings. *Archives of Environmental Health* **58:** 410–420.

Haugland R. A. , Brinkman N. E., Vesper S. J. (2002) Evaluation of rapid DNA extraction methods fort he quantitative detection of fungal cells using real time PCR analysis. *Journal of Microbiological Methods* **50**: 319–23.

Haugland R. H., Vesper S. J. (2007) Genetics-based analytical methods for bacteria and fungi in the indoor environment. In: Yang C. S., Heinsohn P. A. (eds) *Sampling and Analysis of Indoor Microorganisms*. Wiley.

Hilmarsdottir I., Haraldsson H., Sigurdardottir A., Sigurgeirsson B. (2005) Dermatophytes in a swimming pool facility: difference in dermatophyte load in men's and women's dressing rooms. *Acta Dermato-Venereologica* **85:** 267–268.

Hinrikson H. P., Hurst S. F., De Aguirre L., Morrison C. J. (2005) Molecular methods for the identification of *Aspergillus* species. *Medical Mycology* **43:** 129–137.

Houbraken J., Due M., Varga J., Meyer M., Frisvad J. C., Samson R. A. (2007) Polyphasic taxonomy of *Aspergillus* section *Usti*. *Studies in Mycology* **59:** 107–128.

Hyvärinen A., Meklin T., Vepsäläinen A., Nevalainen A. (2002) Fungi and actinobacteria in moisture-damaged building materials — concentrations and diversity. *International Biodeterioration and Biodegradation* **49:** 27–37.

Jackson C. J., Barton R. C., Evans E. G. (1999) Species identification and strain differentiation of dermatophyte fungi by analysis of ribosomal-DNA intergenic spacer regions. *Journal of Clinical Microbiology* **37:** 931–936.

Kennedy H. F., Michie J. R., Richardson M. D. (1995) Air sampling for *Aspergillus* spp. during activity in a paediatric hospital ward. *The Journal of Hospital Infection* **31:** 322–325.

Kozak P. P., Gallup J., Cummins L. H., Gillman S. A. (1980) Current available methods for home mold surveys. II. Examples of problem homes surveyed. *Annals of Allergy* **45:** 167–176.

Lacey J., Dutkiewicz J. (1997) Bioaerosols and occupational lung disease. *Journal of Aerosol Science* **25:** 1371–1404.

Lee T., Grinshpun S. A., Martuzevicius D., Adhikari A., Crawford C. M., Luo J., Reponen T. (2006) Relationship between indoor and outdoor bioaerosols collected with a button inhalable aerosol sampler in urban homes. *Indoor Air* **16:** 37–47.

Lian X. & de Hoog G. S. (2010) Indoor wet cells harbour melanized agents of cutaneous infection. *Medical Mycology* **48:** 622–628.

Linos A., Steinbüchel A. (1996) Investigations on the microbial breakdown of natural and synthetic rubbers. In: *Proceedings of Tenth International Biodeterioration and Biodegradation Symposium*, DECHEMA, Frankfurt am Main, 279–286.

Lugauskas A., Krikstaponis A., Sveistyte L. (2004) Airborne fungi in industrial environments-potential of respiratory disease. *Annals of Agricultural and Environmental Medicine* **11:** 19–25.

McGinnis M. R. (2007) Indoor mould development and dispersal. *Medical Mycology* **45:** 1–9.

McGregor H., Miller J. D., Rand T., Solomon J. (2008) Mold ecology: Recovery of fungi from certain moly building materials. In: Prezant B., Weekes D. M., Miller J. D. (eds): Recognition, Evaluation and Control of Indoor Mold. *American Industrial Hygiene Association Journal*, Fairfax.

Meklin T., Reponen T., McKinstry C., Cho S. H., Grinshpun S. A., Nevalainen A., Vepsäläinen A., Haugland R. A., LeMasters G., Vesper S. J. (2007) Comparison of mold concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously. *The Science of the Total Environment* **382:** 130–134.

Miller J. D. (1995) Quantification of health effects of combined exposures: a new beginning. In Morawska L.L., Bofinger N.D. & Maroni M. (eds*): Indoor Air Quality: An Integrated Approach*. Elsevier, Amsterdam, Netherlands, 515.

Mitchell C. S., Zhang J., Sigsgaard T., Jantunen M., Lioy P. J., Samson R. A., Karol M. H. (2007) Current state of the science: health effects and indoor environmental quality. *Environmental Health Perspectives* **115:** 958–964.

Nihtinen A., Anttila V. J., Richardson M., Meri T., Volin L., Ruutu T. (2007) The utility of intensified environmental surveillance for pathogenic moulds in a stem cell transplantation ward during construction work to monitor the efficacy of HEPA filtration. *Bone Marrow Transplantation* **40:** 457–460.

Murray P. R., Baron E. J., Pfaller M. A., Tenover F. C. (1995) *Manual of Clinical Microbiology*, 6th ed. ASM Press, Washington.

Nishimura K., Miyaji M., Taguchi H., Tanaka R. (1987) Fungi in bathwater and sludge of bathroom drainpipes. *Mycopathologia* **97:** 17–23.

Oren I., Haddad N., Finkelstein R., Rowe J. M. (2001) Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. *American Journal of Hematology* **66:** 257–262.

Pasanen A. L., Kasanen J. P., Rautiala S., Ikäheimo M., Rantamäki J., Kääriäinen H., Kalliokoski P. (2000) Fungal growth and survival in building materials under fluctuating moisture and temperature conditions. *International Biodeterioration and Biodegradation* **46:** 117–127.

Pitkäranta M., Meklin T., Hyvärinen A., Paulin L. (2008) Analysis of fungal microbiota in indoor dust by ribosomal DNA sequence analysis, quantitative PCR and culture. *Applied and Environmental Microbiology* **74:** 233–244.

Ratnasingham S., Hebert P. (2007) The Barcode of Life Datasystem. *Molecular Ecology Notes* Doi: 10.1111/j.1471-8286.2006.01678.

Samson R. A., Flannigan B., Flannigan M. E., Verhoeff A. P., Adan O. C. G., Hoekstra E. S. (1994) *Health Implications of Fungi in Indoor Environments*. Elsevier, Amsterdam.

Samson R. A., Hoekstra E. S., Frisvad J. C. (2004) *Introduction to Food – and Airborne Fungi*. Seventh Edition. CBS. Utrecht: 389.

Satow M. M., Attili-Angelis D., de Hoog G. S., Angelis D. F., Vicente V. A. (2008) Selective factors involved in oil flotation isolation of black yeasts from the environment. *Studies in Mycology* **61:** 157–163.

Sea J., Kato S., Tatsuma T., Chino S., Takada K., Notsu H. (2008) Biosensing of an indoor volatile organic compound on the basis of fungal growth. *Chemosphere* **72:** 1286–1291.

Simon-Nobbe B., Denk U., Pöll V., Rid R., Breitenbach M. (2008) The spectrum of fungal allergy. *International Archives of Allergy and Immunology* **145:** 58–86.

Tiffany J. A., Bader H. A. (2000) Detection of *Stachybotrys chartarum*: the effectiveness of culturable-air sampling and other methods. *Archives of Environmental Health* **62:** 9–11.

Torvinen E., Meklin T., Torkko P., Suomalainen S., Reiman M., Katila M., Paulin L., Nevalainen A. (2006) Mycobacteria and fungi in moisture-damaged building materials. *Applied and Environmental Microbiology* **72:** 6822–6824.

Varga J., Frisvad J. C., Samson R. A. (2007) Polyphasic taxonomy of *Aspergillus* section *Candidi* based on molecular, morphological and physiological data. *Studies in Mycology* **59:** 75–88.

Vesper S. J, Wymer L. J., Meklin T., Varma M., Stott R., Richardson M., Haugland R. A. (2005) Comparison of populations of mould species in homes in the U.K. and U.S.A. using mould-specific quantitative PCR. *Letters in Applied Microbiology* **41:** 367–373.

Wang X. R., Zeng Q. Y., Westermark S. O., Rasmuson-Lestander A. (2004) Detection and quantification of *Wallemia sebi* in aerosols by real-time PCR, conventional PCR, and cultivation. *Applied and Environmental Microbiology* **40:** 7295–7302.

Watanabe K., Taniguchi H, Katoh T. (2001) Adhesion of dermatophytes to healthy feet and its simple treatment. *Mycoses* **43:** 45–50.

Wu P. C., Su H. J., Lin C.Y. (2000) Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. *The Science of the Total Environment* **253:** 111–118.

Zalar P., de Hoog G. S., Schroers H. J., Crous P. W., Groenewald J. Z., Gunde-Cimerman N. (2007) Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum* with description of seven new species from hypersaline environments. *Studies in Mycology* **58:** 157–83.

Zalar P., de Hoog G. S., Schroers H. J., Frank J., Gunde-Cimerman N. (2005) Taxonomy of the osmophilic genus *Wallemia*. *Antonie van Leeuwenhoek* **87:** 311–328.

Zalar P., de Hoog G. S., Schroers H. J., Crous P. W., Groenewald J. Z., Gunde-Cimerman N. (2007) Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with description of seven new species from hypersaline environments. *Studies in Mycology* **58:** 157–183.

(Manuscript accepted 7 October 2010; Corresponding Editor: R. Pöder)