

Progress Report

2006–2007

Edited by

Pedro W. Crous
and
Robert A. Samson



CBS Fungal Biodiversity Centre

An Institute of the Royal Netherlands Academy of Arts and Sciences



CBS Fungal Biodiversity Centre

Visiting and courier address: Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

Postal address: P.O. Box 85167, 3508 AD Utrecht, The Netherlands.

Telephone +31 (0)30 2122600. Telefax +31 (0)30 2512097. Email: info@cbs.knaw.nl

Homepage: <http://www.cbs.knaw.nl>

Studies in Mycology: www.studiesinmycology.org

Persoonia: www.persoonia.org

Fungal Planet: www.fungalplanet.org

Deposit new names and data in MycoBank: www.mycobank.org



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Preface

The CBS Fungal Biodiversity Centre, also known as the Centraalbureau voor Schimmelcultures, is one of 19 institutes of the Royal Netherlands Academy of Arts and Sciences (RNAAS). The CBS is unique in its scope and international significance, curating the world's most diverse living collection of fungi. The collection, which grows at approximately 3000 strains per year, includes organisms of crucial importance to diverse sectors of industry, as well as to agriculture and medicine. In our previous biennial report (May, 2003), we adopted the motto to Collect, Study and Preserve. Two years have gone by, and it is thus prudent to reflect on our vision and mission, and simultaneously assess what we have accomplished to date.

To Collect Biodiversity: In the *Studies of Mycology* volume 50, David Hawksworth provided fresh arguments for his original estimate of 1.5 million species of fungi (now accepted by many as a vast underestimate), and drew our attention to the fact that of the 7% of these species that are currently known from scientific description (approximately 100 000 species), only a subset amounting to 16% are known from culture, i.e., 1.1% of the original estimated 1.5 million species. Although the CBS collection expands more rapidly than any other, similar genetic resource centre in the world, one could ask what new projects have been initiated to further promote the collection of the "silent majority" of as yet unknown and uncultured fungi? To address this concern, the CBS, in collaboration with Agriculture and Agri-Food Canada, will officially launch the **Fungal Planet** (www.fungalplanet.org), which will aim to add a further 1% to the world's currently known fungal biodiversity, by describing 1000 new species of fungi. The concept is that descriptions of new taxa will be published and distributed on a monthly basis, and will be freely available on the web. They will also be linked via MycoBank to vouchers in herbaria, DNA banks, and culture collections world-wide. With this initiative, we hope to highlight the world's incredible fungal diversity, and to underline the importance of funding fungal biodiversity research. A major aim is to **link fungi to their environment**, i.e. the ecosystems where they occur. High quality digital colour photographs capturing the essence of each collection site as an environment are thus a prerequisite for the publication of each species description. The Big Book of Fungi, "Fungal Planet" will be compiled using material selected from the descriptions, as well as unpublished illustrations and text intended to provide a broad perspective on fungi. Our goal is to produce a book with a compelling design, as well as one full of stimulating concepts that can be used to market mycology as a serious component of biodiversity.

To Study Biodiversity: The CBS has chosen to establish various online databases via its unique BioloMICS software. A good example of such a database can be found by consulting MycoBank (www.MycoBank.org), where names of all new fungal taxa published in reputable journals will be deposited, along with the corresponding descriptions, illustrations, and voucher information (for herbarium specimens, DNA sequences and banked DNA specimens, cultures, literature citations, etc.). The CBS has chosen for a more **public engagement with science**, and is thus establishing research programmes to address issues of relevance to society. A good example of this is the inception of new post-doctoral positions for the creation of DNA barcodes to facilitate rapid recognition of fungi in various sectors such as agriculture, medicine, indoor air and food microbiology. CBS will strive for a situation where it will have a DNA sequence and barcode for each strain in the collection. This project has been initiated by means of financial support of the RNAAS, but will need considerable additional funding to attain the goal stated. As an official partner of the Consortium for the Barcode of Life (CBoL), the CBS has chosen to add DNA data to its identifications in its striving to attain a gold standard in fungal identification, and to promote a better understanding of ecological interactions where microorganisms play a role. CBS also strives to support and participate in international ventures aimed at attaining this goal, such as the US National Science Foundation (NSF)-funded *Assembling the Fungal Tree of Life* (AFToL) project.

To Preserve Biodiversity: Since our previous biennial report, the genetic resource centre has been experiencing a phase of rapid expansion. Although this is partly due to policy, it is also due to the fact that the CBS is emerging as an international collection of choice both for mycologists and for editors of high impact journals promoting the preservation of the critical voucher material and DNA extracts upon which important published identifications are based. The original mandate of CBS, when it was officially established in 1904, was based on a recommendation of the *Association Internationale des Botanistes* that an international repository must be established for fungal cultures. Soon, this mandate was broadened to include biosystematic research, and the collection and its research group were for several decades the twin pillars of CBS. In the last two years, to further strengthen the collection and international biosystematic research on fungi, we have established MycoBank (www.MycoBank.org), the registry of new

taxon names, and also have begun actively to collaborate with CABI Bioscience and Landcare New Zealand in the curation of both existing and new fungal names (www.species-fungorum.org), linking these to unique Life Science Identifiers (LSIDs), which are supplied to GBIF. Via its MycoBank and Mycoheritage (www.cbs.knaw.nl/mycoheritage) sites CBS will be making a steadily increasing number of descriptions and illustrations available for existing names. In the coming period we will be actively developing and further improving the MycoBank software in an attempt to provide a further improved and updated service to society. CBS has also chosen for open access to scientific information. This policy gives the internet user maximal access not only to CBS databases, but also to its journal, *Studies in Mycology* (www.cbs.knaw.nl/simonline), which is now published in full colour.



In the coming two years CBS will be playing an increasingly active role in EU research programmes, striving to promote mycology and science for global public good. We will be actively expanding our culture and DNA databases, and will be establishing additional databases for specific fungal groups of interest. CBS will also take active steps to become a major training ground for young mycologists, a role that will be promoted by strengthening the interaction with top-ranking Dutch universities as well as international research bodies. In our previous report I mentioned that CBS represents a wonderful scientific opportunity as a living fungal DNA bank. Young mycologists should make it part of their education to visit the CBS. If you are within the EU, you could apply to SYNTHESYS (www.synthesys.info) for financial support to facilitate such a research visit. If you are in the U.S.A., your research professor's NSF grant

will make it possible to obtain financial support for such a visit, as CBS is a member of CETAF (Consortium of European Taxonomic Facilities), which has an existing exchange with the NSF. If you are a student in a developing country, consult the web page of the Academy (www.knaw.nl), or contact us to hear about possible collaborative ventures.

Lets make the link, lets promote our science together!

Pedro W. Crous

Director,
*CBS Fungal Biodiversity Centre,
an institute of the Royal Netherlands Academy of Arts
and Sciences (CBS-RNAAS)*

Structure and Research Programmes

The mission of the CBS Fungal Biodiversity Centre as an institute for fungal biosystematics research, is to collect, study, preserve, and educate. Although the primary aim of its research programmes is to enhance its unique living collection of fungi by adding valuable new data and cultures, the education component has been added during the last five years to support the training of mycologists in fungal biodiversity. The CBS has chosen to transform itself from simply being the international culture collection of choice to being the trendsetter and gold standard of mycology. To attain this goal, we have strengthened our research ties with several other institutes and universities in the Netherlands, as well as internationally.

CBS is an active partner in numerous national and international collaborative projects. We aim to use these projects to broaden our scope not just in biosystematics studies but also to study functional fungal biodiversity. As a partner of the Consortium for European Taxonomic Facilities (CETAF), CBS has been a participant in obtaining EU projects facilitating scientific exchange (programme called SYNTHESYS), as well as setting standards for taxonomy at European taxonomic institutes (European Distributed Institute of Taxonomy; EDIT). Within EDIT, the main task for CBS as a partner of the Netherlands Biodiversity Information Network (NL-BIF) concerns the establishment of a European network

to facilitate the DNA barcoding of life. CBS coordinates the European Consortium for the barcode of Life (www.ecbol.org; ECBOL), is affiliated as partner to the Consortium for the Barcode of Life (www.barcoding.si.edu; CBOL), is the European node of the International Barcode of Life campaign (www.dnabarcoding.org; IBOL), and is also an active partner in the Netherlands Barcode of Life campaign (www.dnabarcoding.nl; NBOL). The CBS also represents the Netherlands in a programme developed by the Organisation for Economic Co-operation and Development (OECD), the "Biological Resource Centres" task force of the Working Party on Biotechnology. In addition, CBS as a partner within NL-BIF forms a component of the Global Biodiversity Information Network (GBIF).

Our core business is the collection, to which our research programmes should add value. Each research programme consists of several projects. While some projects represent "discovery science", focusing on discovering biodiversity, others are focused on understanding processes, such as striving to unravel metabolomic, proteomic or genomic complexities of specific fungal groups or species. Additional information about these projects can be found further on in this document under the descriptions of specific research programmes.

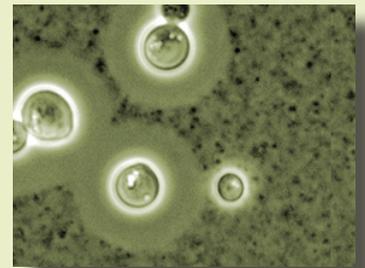


Evolutionary Phytopathology

Origins of Pathogenicity in Clinical Fungi



The Collection



Yeast and Basidiomycete Research

Applied and Industrial Mycology





The Collections

The CBS Collection of Fungi has more than 60.000 strains in its public collection, making it the largest mycological culture collection in the world. CBS is unchallenged as a reference collection for mycological research, as practically all culturable groups of the Fungal Kingdom are represented. Annually, thousands of CBS strains are delivered to some 50 countries. To underpin its dedication to quality in materials and services, CBS set up a quality management system, for which it obtained an ISO 9001:2000 certificate in November 2007. CBS strains are also selected for DNA sequencing projects in the framework of global initiatives, such as the Fungal Tree of Life and DNA Barcoding. The CBS Bacterial Collection (NCCB) consists of another 10.000 strains, including a unique Plasmid and Phage Collection. The high quality of CBS strains is ensured by the practice of having identities and typical features authenticated by specialists of CBS and elsewhere. Scientific and other data related to the strains are constantly added. Much attention is given to expanding and improving CBS web-services, not only by digitising publications, but also by allowing clients to use various types of collection data. CBS has developed web-based polyphasic identification for specific groups such as yeasts, and plans to also include additional economically important groups. Moreover, CBS developed MycoBank, an on-line registration system for new fungal taxonomic names now under the auspices of the International Mycological Association (IMA).

Collection holdings

In the period of 2006–2007, CBS acquired 3500 strains, representing a large number of species new to science or new to the public collection. CBS is also an International Depository Authority (IDA) under the regulations of the Budapest Treaty, holding now over 1000 Budapest Treaty deposits of fungi and bacteria. Furthermore, CBS also holds almost 300 Safe deposits in the restricted collection.

CBS preserves practically all cultures in metabolically inactive condition, so that they remain as much as possible in the pristine original condition in which they were received. The most important preservation methods are cryopreservation and freeze-drying (lyophilisation), for which cutting-edge equipment is installed. Almost all strains are cryo-preserved and stored in liquid nitrogen containers, where the samples are preserved in a dynamic gas phase, at temperatures that are constantly below $-180\text{ }^{\circ}\text{C}$ anywhere in the container. Most yeasts and bacterial strains, and about 60 % of the non-yeast fungal strains are also preserved in a freeze-dried state. CBS uses state-of-the-art freeze-drying equipment, such as Christ-

Epsilon 2-80. All crucial parameters are recorded during the freeze-drying process, and stored for later reference. Some organisms that are recalcitrant to both cryopreservation and freeze-drying need to be maintained in actively growing condition on agar. However, over the past two years the number of fungal strains requiring maintenance on agar was further reduced to about 2900.

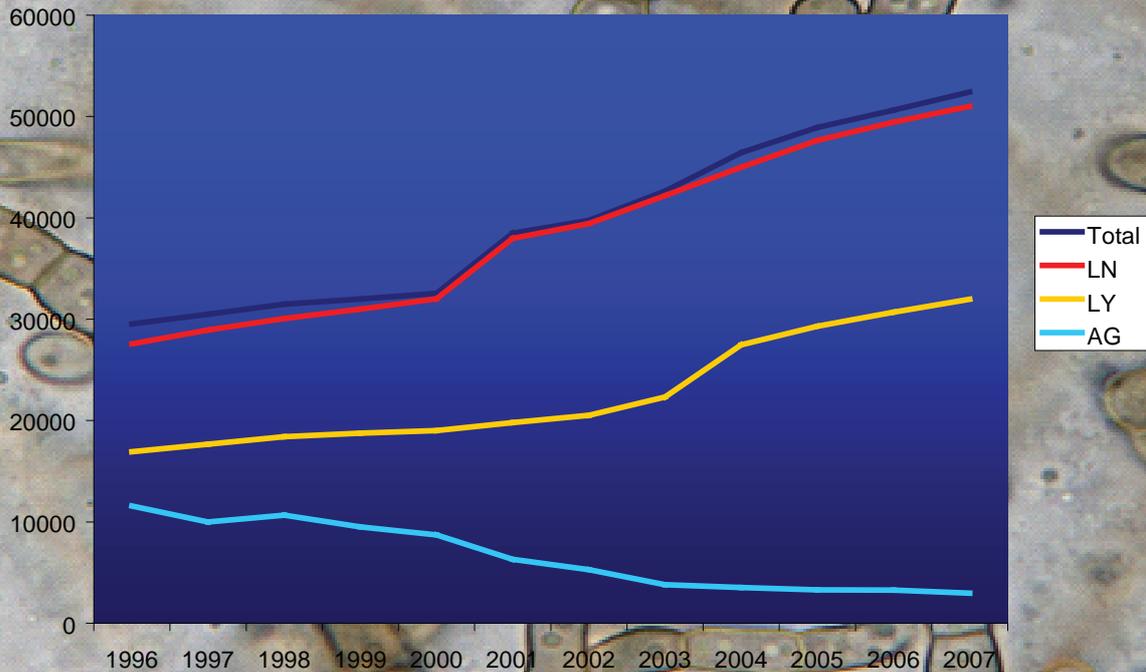
The CBS collection currently contains over 5000 ex-type and authentic strains of yeasts and filamentous fungi and many more reference strains. An ex-type strain fixes the undisputed application of a fungal name, while reference strains, which have been authenticated by a specialist or group of specialists, have a similar function and can also serve as reference material for identification and characterization of a fungal taxon. Moreover, type and reference strains are the preferred material to generate DNA barcodes.

Rigorous identity control of strains is fundamental to good collection management. The identity of new accessions is principally checked by curators or specialist taxonomists based

on morphological characters. Subsequent transfers are checked by other qualified staff, who will consult the specialist in case of doubt. Strains that are sterile on artificial media can be checked for obvious infections, but a positive ID is difficult or impossible. In recent times, however, the coverage by specialists of the various fungal groups in the collection has decreased. Yet, most new accessions are already



Developments in the Collection of Fungi and Yeasts



Trends in the collections of filamentous fungi and yeasts according to the preservation methods. LN = cryopreserved, LY = lyophilized, AG = agar.

characterised at the molecular level by the depositors. CBS performs standard ITS sequencing for all new (ex-)type strains. A routine molecular check for all new acquisitions requires considerable resources presently not available. However, due to the RNAAS grant for barcoding, numerous strains will be sequenced and barcoded. Generally, sequence data and barcodes can only be used to check the identity at the species level, and are rarely strain specific. In collaboration with industrial partners in Germany, the collection is currently investigating the possibilities to use MALDI-ToF spectrophotometric analysis as a tool for positive identity control of strains.

ISO 9001:2000 Certificate for the CBS Collection

CBS Collections have a primary mission to preserve and supply strains of microorganisms to researchers and other user groups world-wide. Thanks to the constantly high quality of the material that is distribut-

ed and customer-friendly services, CBS has built up a good reputation as a supplier of cultures within the scientific community. In order to strengthen its position particularly in the fields of applied science and technology, CBS decided to implement a Quality management system according to the international standard of ISO 9001:2000, and seek to obtain a certificate. In November 2007, Kema issued an ISO 9001:2000 certificate to CBS for the following activities: "Accession, preservation, storage and supply of micro-organisms (public deposits, safe deposits and maintenance deposits) and related information".

DNA-Barcoding the Collection

The term DNA barcoding was coined by Paul Hebert in 2003. The general idea was to create a fast and simple, unified species identification system based on DNA sequence data. An international consortium (CBoL, Consortium for the Barcode of Life) was formed to come up with binding sugges-

tions as what markers and protocols would be accepted for DNA barcoding and to act as mediator between the DNA barcoding community and other parties, i.e. GenBank, potential users, or technology developers. A coordinated approach in the DNA barcoding of fungi (or any other group of organ-



isms) requires the careful selection and agreement upon a suitable marker region. ITS region of the ribosomal DNA has been widely used as a marker in fungal studies, but additional marker regions are needed for many groups of fungi, as the ITS region does not provide sufficient resolution at the species level. Collections such as the CBS can play a major role in the DNA barcoding effort. They are not only depositories for specimens and vouchers, well studied collections are also summaries of the current state of taxonomic knowledge. DNA barcoding of such specimens makes this information accessible and usable for all kinds of molecular studies. Type strains or specimens contained in collections are indispensable for the taxonomic validation of DNA barcodes. It has become obvious in recent years, that many presumed biological species in microfungi cannot be unambiguously identified by morphological or physiological methods. Therefore, DNA barcoding of the CBS collection will enhance the value of the collection enormously while at the same time increasing the body of DNA barcoding reference data. This in turn will put CBS in a position to be a sought-after partner for the development of identification tools or to develop such tools itself. CBS aims at producing at least an ITS sequence for all of its type strains within the coming two years. Apart from this effort, many CBS strains are being sequenced in other research projects providing state-of-the-art molecular identifications.

DNA Bank

The primary purpose of the DNA-Bank is to store genomic DNA extracts of CBS strains selected for DNA-Barcoding or other research projects. Central storage in the DNA-Bank is more economic, and can safe-guard genomic DNA for future use, even after a strain loses viability. A secondary purpose of the DNA-Bank is to keep DNA of fungi that cannot be cultivated,

and DNA that has been isolated from herbarium specimens.

Each DNA extract obtains a unique identifier (CBS D-XXXXX), independent of the unique identifiers connected to the source. For long-term storage, a sample of DNA in buffer is maintained for each accession in the dynamic gas-phase of a liquid nitrogen container, at a temperature constantly below -180 °C. This is relatively expensive compared to other DNA storage methods, but the advantage for CBS is that it is compatible with the system already in place for cryopreservation of strains. For most accessions, additional DNA samples are also maintained in mechanical freezers at -80 °C. The latter samples will normally be used first as stock for the distribution of DNA aliquots. A paid service to distribute DNA extracts from CBS strains will be set up in the near future. The RNAAS provided CBS with start-up funding for this DNA-Bank, and additional funding is currently being actively sought in collaboration with other partners in NL-TAF.

Research projects and collaboration

CBS co-organised a 2-day symposium on Fungal taxonomy in Goslar 2007. Moreover Stalpers visited a microbial collection in Bogor in the framework of the Committee for Endangered Collections. Current items which may have a considerable impact on the functioning of Culture Collections concern biosecurity; it is important to prevent that oncoming new regulations will make the distribution of strains unnecessarily difficult. Also the problems around the required risk analyses have to be formulated and solved.

CBS also played a major role in the development of a standard minimum Material Transfer Agreement by ECCO, necessary to oblige the requirements of the CBD and to safeguard Culture Collections' interests. This proposal was discussed at the ECCO meeting in Goslar (2007) and will be taken up by WFCC.

CBS represented the Dutch government in two working parties of the task force Biological Resource Collections of the OECD, followed by that on the Global Biological Resource Centres Network (GBRCN). This was concluded with a 2-day workshop in Paris at OECD headquarters, where the German government offered to host and support a pilot GBRCN for three years.

Ongoing concern about bioterrorism strongly increased the interest of politicians in culture collections. Attention is being paid to the organisms that are maintained and curated, and to the way the distribution of these organisms is managed, with special focus on the security measurements instituted to prevent undesired use. For these reasons CBS participates in the OECD Working Party on Biotechnology, dealing with issues related to biosecurity (bioterrorism) in the context of the development of a global Biological Resource Centre. Especially the possible threats to agriculture and environment create problems in risk assessment, which is a basic responsibility of BRC's.

The collection participated in several EU projects. European Biological Resources Centres Network (EBRCN), for the establishment of a framework to maximize complementarities and minimize duplications among European Biological Resource Centres (BRCs). CBS analysed the amount of duplication between the European collections and the reasons for duplication. It could be concluded that most duplication was justified (e.g. ex-type strains and tester strains) and the actual percentage of unnecessary duplication was below two percent.

CBS is also involved in the EDIT project (European Distributed Institute of Taxonomy), an FP6 Network of Excellence, for which NL-TAF is coordinating workpackage 3 on integration of the infrastructure basis for leading taxonomic facilities in Europe. The main task of CBS in WP3 is the coordination of DNA

ECBOL
EUROPEAN CONSORTIUM FOR THE BARCODE OF LIFE

ECBOL.org	ECBOL initiative	Resources	About us	Partners
<p>Welcome!</p> <p>What is ECBOL.org?</p> <p>What is DNA Barcoding?</p> <p>What are people doing?</p> <p>How can I get involved?</p> <p>Funding groups</p> <p>DNA5ME meeting 2007</p> <p>Latest developments</p> <p>European campaigns</p>	<p>Introduction</p> <p>Rationale</p> <p>Calibrating Europe's biodiversity using DNA barcodes</p> <p>Interview</p> <p>Professor Pedro Crous</p> <p>EuroBioForum 2008</p>	<p>How do I start DNA barcoding?</p> <p>Protocols</p> <p>DNA Barcoding Databases</p> <p>Publications</p> <p>Media Coverage</p> <p>Meetings</p> <p>Workshop and Meeting Reports</p>	<p>Vision</p> <p>Background</p> <p>Governance</p>	<p>Barcode of Life Data Systems (BOLD)</p> <p>Barcode of Life NL (NBOL)</p> <p>Catalogue of Life (Species 2000)</p> <p>Consortium for the Barcode of Life (CBOL)</p> <p>Canadian Barcode of Life Network (BOLNET)</p> <p>Canadian Centre for DNA Barcoding</p> <p>Global Biodiversity Information Facility (GBIF)</p> <p>International Barcode of Life (IBOL)</p> <p>Get involved in IBOL now!</p>

Welcome!

A new European infrastructure is urgently needed to firmly link names and thus all information to a rapidly developing new standard in biology: the identification of species via DNA sequence signatures, a process known as "DNA barcoding".

Although DNA sequence-based species identification has been utilized previously in connection with phylogenetic studies such as *Assembling the Tree of Life* (ATOL), the development that has made DNA barcoding (of just one or two standardized gene loci per organism) an emerging gold-standard for species recognition has been very recent, with the first publication in 2003 (Hebert et al. 2003), the setting up of the master international Consortium for the Barcoding of Life (CBOL) courtesy of a USD 2 million grant from the Sloan Foundation in 2004, and the first European-based conference at London's Natural History Museum in March, 2005. This development has already shown itself to have unprecedented power for clarifying species identities and limits, uncovering new and often cryptic species, and allowing species identification of difficult specimens such as larvae, spores, tissue fragments, seeds, spores, and fossils. At the same time, however, it has also

Sponsors

EDIT
European Database of Invertebrate Taxonomy

Cetof
Consortium of European Taxonomic Facilities

Barcode of Life Initiative

Barcoding. The National History Museum Naturalis, Leiden, is leading this workpackage, and Gerard Verkley is the Team Leader for CBS. In October 2007, CBS organised the DNA-Barcoding in Europe Meeting in collaboration with the other Dutch partners in the EDIT consortium. Over 150 delegates attended this successful meeting. CBS also maintains a website for the European Consortium for the Barcode of Life (www.ecbol.org) which is intended as a platform for European institutes involved in Barcoding of all organism groups.

SYNTHESYS (www.synthesis.info) is another FP6 programme, comprising 20 European natural history museums and botanic gardens, aiming to create an integrated European infrastructure for researchers in the natural sciences. SYNTHESYS is setting standards for collection management and databases, and aims to raise scientists' awareness of best practice by offering improved training and workshop opportunities, and guidelines for the care, storage and conservation of collections. The project will also provide new policies on emerging technologies for storing collections, such as DNA samples or tissue banks.

CBS also participated in Euro-

Cat (European Catalogue of Life), a Species 2000 project, aiming at developing an interactive web-based catalogue.

In 2005-2007 the digitalisation project large-scale support from the National Science Organisation (NWO), the Netherlands of the four major Dutch taxonomic institutes [Naturalis, National Herbarium Netherlands (NHN), Amsterdam University Zoological Museum (ZMA) and CBS] financed by NWO digitised millions of herbarium specimens, and also produced species banks for ecologically or economically important groups of organisms. The Database Managers Committee controlling the process was chaired by CBS. The data for the 20.000 specimens present in the CBS herbarium have all been digitized. A concept for a new service referred to as "species banks" with web-based curating possibilities is being developed.

Fungal Identification Service

CBS offers a service for the identification of fungal, oomycetous and bacterial isolates. In 2006-2007 a total of 1144 fungal and 106 bacterial cultures were identified. The involvement of specialist taxonomists guarantees a state-of-the-art identification of cultures. A correct identification is of the utmost

importance in scientific studies, phytopathology, industrial contamination etc. Yeasts are identified mainly by means of DNA sequencing, while filamentous fungal isolates are identified by morphology in culture on the appropriate media and DNA sequencing. CBS has a significant unpublished database of DNA sequences, based on ex-type and other well-characterised CBS strains, and this information can be used by CBS experts for identification purposes. A secondary effect of the revised procedures is that

the identification service is able to inform a significant minority of clients that the isolate they submitted represents a new species. It is now far easier to fully confirm species as undescribed than it was in the past, when only morphological characters were available. In many cases, approval of the customers was obtained to add the undescribed species and many other interesting isolates to the CBS collection.

Bioinformatics and databasing

Index Fungorum

Index Fungorum (IF) is a nomenclatural database, aiming to contain a complete set of all nomenclatural data pertaining to fungi. Since 2003 IF is managed by the Index Fungorum Partnership consisting of CABI Bioscience (P. Kirk), Landcare New Zealand (J. Cooper) and CBS (J. Stalpers). At the moment the database contains over 400.000 names and it is by far the most complete database in this area. However, the quality of the data still has to be improved. For example, many protologue data are missing, the status of many names is incorrectly indicated, there is some discrepancy in dates of publication, etc. The recently concluded NWO project allowed us to add and update

many records, for example with a list of verified pre-1832 names and the "Sydow lists" (lists published by H.P. Sydow) representing all taxonomic novelties published in the years 1895–1918 and with additional data from the Petrak lists from 1918–1940. IF is the base for the CBS initiative MycoBank, and an agreement has been reached to maintain IF as a web-service, preventing unnecessary double checks.

MycoBank

CBS launched MycoBank in 2004, with the purpose of on-line registration of the mycological nomenclatural novelties and allowing authors to also deposit other data associated with these novelties, for example descriptions, illustrations, sequences, etc. It would allow users/depositors to check if their deposits are unique, and the specialists of CBS check the names on correctness as ordained by the International Code of Botanical Nomenclature. Each name is given a unique MycoBank number that can be used in the publication, and also serves as its LSID (Life Sciences Identifier) with the following structure: urn:lsid:indexfungorum.org:names:nnnnnn..The collabora-

tion with Index Fungorum prevents double issuance of LSID identifiers. Authors are notified of the MycoBank accession number and of possible problems. In the last case they are asked if they want to correct or adapt, but MycoBank will never apply censorship; in all cases the depositor decides on the form of the deposited name.

The main reason to start this service was that the Code hardly contains restrictions where to publish nomenclatural novelties, resulting in a plethora of possibilities that are far from covered by the average taxonomic library. Although the Index of Fungi gives a relatively good coverage, it offers no additional data, while on-line databases connected with MycoBank and Index Fungorum can easily do that, and additionally allow sophisticated searches plus links to other on-line sources of information.

Several important mycological journals have already agreed to make this procedure obligatory for their authors, while others are currently considering following suit.

At the moment MycoBank contains 23.000 descriptions and 8.700 illustrations.

Species banks

The number of taxonomists is rapidly dwindling, and no institute houses up-to-date specialists for all fungal groups. CBS has, partly financed by NWO, set up a number of dynamic species banks in various phases of completeness. A species bank contains full obligate and facultative synonymy of the taxa concerned, one or more descriptions, structured morphological, physiological, ecological and/or molecular databases, references and links to both literature and other web-based data, and allows polyphasic identification. The oldest and most complete species bank is that of the yeasts, but there are also species banks on for example *Aspergillus/Penicillium*, medical fungi, *Mycosphaerella* and its anamorphs and resupinate *Russulales*.

Collaboration will be sought with international specialists or specialist groups to adopt a group of fungi. They will be given full responsibility for the taxonomy, have access to and full rights for the data of the group concerned and will of course be recognized as such. It is hoped that in this way the most adequate use can be made of the still available know-how.

Mycoheritage

Bioheritage is an initiative of SYNTHESIS to make important old works available through the Internet. CBS supports this initiative through its new site "Mycoheritage", in which classic mycological works are displayed. A priority has been given to works containing illustrations that give insight into the historical taxonomic concepts devised by the great-grandfathers of Mycology - for example Persoon, Fries and Saccardo.

Online publications

CBS continues to bring its publications online. Previously, this was done in collaboration with the University of Utrecht and the Royal Netherlands Academy of Sciences information institute NIWI, but in 2005 the management of

MycoBank
www.mycobank.org

Fungal Databases
Nomenclature and Species Banks
Online Taxonomic Novelties Submission
Administered by the International Mycological Association

HOME | SEARCH | LOGIN | REGISTER NEW NAME | TOOLS | NEWS | HELP

MycoBank Fungal Images

Welcome to MycoBank

MycoBank is an on-line database aimed as a service to the mycological and scientific society by documenting mycological nomenclatural novelties (new names and combinations) and associated data, for example descriptions and illustrations. The nomenclatural novelties will each be allocated a unique MycoBank number that can be cited in the publication where the nomenclatural novelty is introduced. These numbers will also be used by the nomenclatural database **Index Fungorum**, with which MycoBank is associated and will also serve as **Life Science Identifiers (LSIDs)**.

Nomenclatural experts will be available to check the validity, legitimacy and linguistic correctness of the proposed names in order to avoid nomenclatural errors; however, no censorship whatsoever, (nomenclatural or taxonomic) will be exerted by MycoBank. Deposited names will remain -when desired- strictly confidential until after publication, and will then be accessible through MycoBank, Index Fungorum, GBIF and other international biodiversity initiatives, where they will further be linked to other databases to realise a species bank that eventually will link all databases of life. MycoBank will (when applicable) provide onward links to other databases containing, for example, living cultures, DNA data, reference specimens and pleomorphic names linked to the same holomorph. Authors intending to publish nomenclatural novelties are encouraged to contribute to this new initiative.

Statistics on MycoBank

Total number of records: 411019
Total number of genera: 16786
Total number of species: 389687

Last update: 10/26/2008 3:27:43 PM

Citations

1. P.W. Crous, W. Gams, J.A.



Data present in the CBS nomenclatural and taxonomic databases:

Data	Number
Names	392.711
Protologue info	254.771
Basionymes (taxa)	272.212
Typeinfo	55.047
Citations	330.000
Descriptions	23.000
Images	8.700

CBS information was moved in-house. New volumes of the journal *Studies in Mycology* are now published simultaneously on the Web (HighWire Press) and on paper, and all but one (copyright elsewhere) previously published volumes have been placed on-line. The relevant data from these books have also been transferred into the CBS descriptions database.

The CBS databases are definitely highly appreciated. A user analysis has indicated that the numbers of visitors who actually perform a search (thus not merely the number of hits, which average 10.000 per day!) in the Index Fungorum is about 30.000 per month. The *Aphyllophorales* database processes about 1000 search requests per

month. The yeast database, which provides both information and also an interactive, polyphasic identification tool (via BioloMICS software), is regularly used by more than 7000 researchers from 96 countries (see figure below). A new collaboration with a goal of creating several databases related to fungal human pathogens was initiated in 2005 with W. Meyer (University of Sydney, Westmead Hospital, Sydney, Australia). The goal of this project (Australian grant #352303; Title: "Phylogeny as a basis for molecular identification of pathogenic fungi") is to allow Internet users to perform online polyphasic identifications that include morphological, physiological, electrophoretic and sequence data.

Evolutionary Phytopathology

Producing food sufficient in quality and quantity remains paramount for sustaining quality of life. Inadvertent introductions of phytopathogenic fungi have had dire consequences to nature and to cultivated crops on various continents in the past. The economic impact of such introductions can be seen in yield loss and in increased input costs for cultivation and disease control, as well as in social impact. To combat these diseases on an international scale, it is important to clarify whether the same species and genotypes occur in various countries, since each different species and genotype can be expected to have different patterns of attack, as well as different responses to fungicides and to climatological conditions. With such pathogens, it is also important to know what their host ranges and mating strategies are, and how this relates to different disease control mechanisms. The global movement of agricultural and forestry produce is inextricably cross-linked, and will continue to be so in future. Knowing which pathogens occur where and on what crops facilitates trade in agricultural produce. In this programme, we address these economically vital matters by investigating the speciation and host adaptation of various important phytopathogenic fungi.

Host specificity and speciation in *Mycosphaerella*

The genus *Mycosphaerella* and its associated anamorph genera represent more than 10,000 species, being associated with diseases on most genera of plants. Most species have been described on the assumption that they are highly host-specific. With the implementation of molecular phylogeny as the basis of modern taxonomy, host relationships and specificity can now be tested. A major aim of our research is to determine how exclusive the host-pathogen relationship of *Mycosphaerella* species is. Investigations based on genomic analysis are in progress on fungal species from a wide range of plant hosts. *Mycosphaerella* has been linked to numerous asexual reproductive states that may have evolved into exclusively asexual species. Such asexual forms were often difficult to trace to a sexual ancestor and were thus historically placed in separate genera. One such example is the genus *Cercospora*, which represents several thousand names, of which roughly a thousand can be recognised based on morphology. During the past evaluation period we have monographed the genus *Cercospora*, and are now in the process of studying its phylogeny and sexual behaviour. To this end we used the celery pathogen, *Cercospora*

apii (with approx. 300 morphologically indistinguishable synonyms), and the sugarbeet pathogen, *Cercospora beticola*, as model to study variation and speciation within *Cercospora*. The genus *Cercospora* appears to be largely asexual: very few species have been reported to have *Mycosphaerella* states. To investigate this matter further, mating type primers were developed to screen populations of *C. beticola*, *C. apii*, *C. zea-maydis* (on maize), and two newly described species, *C. apiicola* (on celery), and *C. zeina* (on maize). The results of this screening indicate that some species are undergoing cryptic sex, and probably have functional *Mycosphaerella* teleomorphs that have yet to be found, while others appear to be truly asexual. A similar situation of cryptic sex was revealed when the *Mycosphaerella* complex of *Pinus* (Dothistroma red band needle disease) was investigated, as was "Cladosporium leaf spot" of tomato (*Passalora fulva*). A DNA phylogeny approach was used to investigate the evolution and inter-relationships of *Mycosphaerella* species causing defoliation and deformation of various hosts. These include species occurring on *Pinus*, *Eucalyptus*, *Acacia* (cultivated for timber, paper and pulp industries), *Olea* (olives), *Protea* (cut-flowers), and *Musa* (eating and cooking bananas). Several examples were

found of *Mycosphaerella* species jumping between hosts (citrus, acacia, eucalypts, proteas, banana, etc.), and although this adaptation is not yet well understood, we will study it further in coming years using novel sequencing technologies. We were also able to demonstrate the presence of several novel species, first reports and / or new hosts for these species. Numerous species of *Mycosphaerella* were found to be associated with the Sigatoka disease complex of banana. Several of these species appear to be confined to certain regions, while others were more global in distribution.

Mycosphaerella mating types and genomics

We found numerous species of *Mycosphaerella* to be associated with the Sigatoka disease complex of banana and the possibility of interaction and hybridisation among these species is being investigated. Analyses of the cloned mating type genes of *M. fijiensis*, *M. musicola* and *M. eumusae* indicated an extraordinary organization of the idiomorphs as well as the presence of *Mycosphaerella* unique genes. Functional analyses of these unique genes and characterisation of the mating type loci of additional *Mycosphaerella* spp. is currently underway. Also, the distribution of the mating type loci

within populations is being determined. This is done to assess the occurrence of sexual reproduction, a factor controlling genetic recombination and genotypic diversity. Further mating type studies that have been completed concern the *Cercospora apii* complex, the Dothistroma needle blight pathogens (*D. pini*, *D. septospora*) of pines, and *Passalora (Cladosporium) fulva*.

Specific TaqMan probes have been developed in collaboration with Plant Research International (Wageningen University), which will facilitate the early detection and monitoring of the disease.

To aid our understanding of the pathology of the genus *Mycosphaerella*, two model species of *Mycosphaerella*, *M. graminicola* and *M. fijiensis*, were selected by the International *Mycosphaerella* Genomics Consortium, in which CBS participates, for complete genome sequencing. The genome sequences for both species are finished and publicly available. The genome sequence of *M. graminicola*

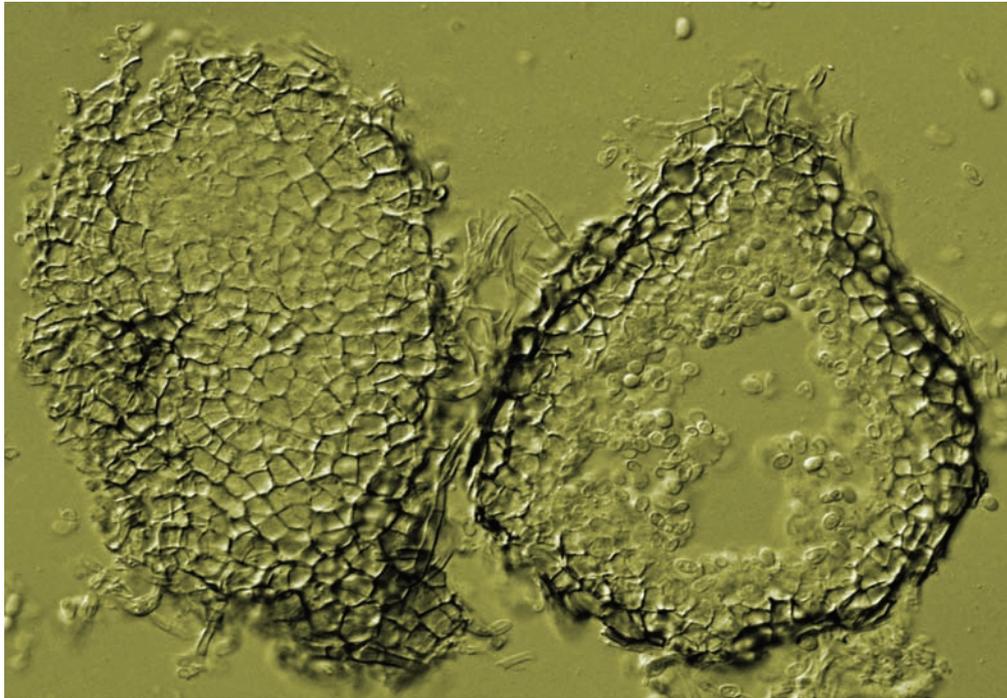
is of extremely high quality and it is expected to be the first completely finished fungal genome (all chromosomes being covered from telomere to telomere and no gaps remaining). Surprisingly, the genome of *M. fijiensis* is almost twice the size of *M. graminicola* (~73MB vs ~41 MB) and contains an extremely high proportion of transposons. Our research group is actively involved in the ongoing annotation of both genomes. The differences observed between the genomes of the two *Mycosphaerella* species illustrates the high genetic variability and flexibility within this genus. Therefore, this project will be further coordinated with sequencing efforts planned for other *Mycosphaerella* species and relatives to greatly increase the power of future comparative genomics analyses.

***Cladosporium* and their *Davidiella* teleomorphs**

The genus *Davidiella* was established for *Mycosphaerella*-like te-

leomorphs with *Cladosporium* anamorphs. Species of *Cladosporium* are common and widespread, and interact with humans in every phase of life, from producing allergens in the indoor environment, to causing fruit decay and plant disease, or being associated with human mycoses. Although *Cladosporium* is one of the largest and most heterogeneous genera of hyphomycetes (700 odd names), only a mere fraction of these species are known from culture, and few have been characterised based on molecular data. By employing a multi-gene phylogenetic approach, integrated with anamorph and teleomorph morphology, *Cladosporium* could be circumscribed, and the family *Davidiellaceae* distinguished from the other genera and families in the Capnodiales. Two species complexes have thus far been elucidated, namely the *C. sphaerospermum* and the *C. herbarum* complex, both of which contained numerous undescribed species. Future attention will now be directed towards the





C. cladosporioides complex, which contains numerous undescribed species, several of which have *Davidiella* teleomorphs. In 2009 we envisage to publish a monograph of *Cladosporium*, in collaboration with Prof. dr U. Braun (Martin-Luther Univ., Halle, Germany), and Dr K. Schubert (Botanische Staatssammlung München, Germany).

Botryosphaeria canker pathogens

Botryosphaeria is a species-rich genus with a cosmopolitan distribution, commonly associated with dieback and cankers of woody plants. As many as 18 anamorph genera have been associated with *Botryosphaeria*, most of which have been reduced to synonymy under *Diplodia* (conidia mostly ovoid, pigmented, thick-walled), or *Fusicoccum* (conidia mostly fusoid, hyaline, thin-walled). However, there are numerous conidial anamorphs with morphological characteristics intermediate between *Diplodia* and *Fusicoccum*, and there are also several records of species outside the *Botryosphaeriaceae* that have anamorphs apparently typical of *Botryosphaeria sensu stricto*. Recent molecular studies have also linked *Botryosphaeria* to species with pigmented, septate ascospores and anamorphs in *Dothiorella*, or to species with hyaline ascospores and *Fusicoccum* anamorphs linked

to *Dichomera* synanamorphs. By employing DNA sequence data for various loci, different lineages, representing 12 clades, could be resolved within the *Botryosphaeriaceae*. Two of these lineages clustered outside the molecularly reconceived *Botryosphaeriaceae sensu stricto*; both were groups with diplodia-like anamorphs occurring on maize. These phylogenetically disparate lineages are best accommodated in *Stenocarpella* (*Diaporthales*) and in an unresolved clade including species of *Camarosporium* / *Microdiplodia*. The ten lineages retained within the *Botryosphaeriaceae sensu stricto* represented different anamorph-teleomorph combinations, many of which are new to science. Further studies are underway to resolve the taxonomic status of many of these generic and species complexes occurring on different woody hosts. In 2009–2010 we envisage to publish a monograph on the species of *Botryosphaeria* known from culture in collaboration with Dr A.J.L. Phillips (Universidade Nova de Lisboa, Portugal), and Prof. dr M.J. Wingfield (FABI, Univ. of Pretoria, South Africa).

Petri disease and phaeohyphomycosis caused by species of *Phaeoacremonium*

Species of *Phaeoacremonium* are involved in Petri disease and esca of grapevines. Additionally, several species of *Phaeoacremonium* also cause phaeohyphomycosis in humans. During this study, *Togninia* (*Calosphaeriales*) was confirmed as teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility, and DNA phylogeny. Three species of *Phaeoacremonium* have been associated with phaeohyphomycosis. These are *Pm. parasiticum*, *Pm. inflatipes* and *Pm. rubrigenum*. Numerous unknown isolates resembling *Phaeoacremonium* spp. have in recent years been isolated from human patients, as well as from woody plants that appear to be the main environmental source of these fungi. New species were identified based on their cultural and morphological characters, and phylogenetic analyses of partial sequences of the actin, β -tubulin and calmodulin genes. A multiple-entry electronic key based on morphological, cultural and β -tubulin sequence data was developed to facilitate routine species identification. The genus *Togninia* was monographed along with its *Phaeoacremonium* anamorphs. Ten species of *Togninia* and 22 species of *Phaeoacremonium* were recognised. Phylogenies of the SSU and LSU rRNA genes were used to determine whether *Togninia* had more affinity with the *Calosphaeriales* or the *Diaporthales*. A rapid molecular identification method was developed for the 22 species of *Phaeoacremonium*. It involved the use of 23 species-specific primers, including 20 primers targeting the β -tubulin gene and three targeting the actin gene. Furthermore, the multiple-entry electronic key was updated to include the new species of *Phaeoacremonium*. Separate dichotomous keys were provided for the identification of the *Togninia* and *Phaeoacremonium* species, and their mating strategies elucidated. Keys for the identification

of phaeoacremonium-like fungi and the genera related to *Togninia* were also provided.

Hybridisation in *Phytophthora* and *Pythium*

Pythium and *Phytophthora* are two highly economically significant genera of fungus-like Oomycetes responsible for many types of crop disease and tree decline. The best known of the crop diseases is potato late blight (*Phytophthora infestans*), the cause of the Irish potato famine and a major agent of crop damage to this day. A study of the organisation of the 5S rRNA gene family was performed for 87 species and varieties of *Pythium*. For the four different patterns of 5S organisation that were found to occur within the genus, studies were conducted to determine how they arose and how evolutionarily stable they were. A number of *Phytophthora* strains were also included in the study as a reference outgroup giving insight into the ancestral organisation of the 5S gene family. The most parsimonious interpretation of the data would be that a contiguously linked arrangement of 5S sequences was the ancestral condition. A DNA array was developed as tool for the rapid identification and detection of *Pythium* species in pure culture, as well as in environmentally mixed samples. Oligonucleotides complementary to specific diagnostic regions of ribosomal internal transcribed spacers (ITS) were designed for more than 100 *Pythium* species and varieties as well as for groups of related species. Specificity was tested in hybridisation experiments with DNA from ex-type strains and other representative strains. BLAST analyses against *Pythium* DNA sequences available in GenBank were used to confirm that species-specific oligonucleotides were unique to all the available strains of each species. In a blind test with 50 additional unidentified *Pythium* isolates from soil, the array hybridisation patterns obtained were found to concur with isolate identifications

obtained via morphological study and ITS sequences. In another blind test, total DNA of soil samples was amplified and hybridised on the array. Results were compared to the results of isolation by soil dilution plating and root baiting. Thirteen species were detected by the DNA array. These species corresponded with those obtained by isolation, though isolation also revealed the presence of one species that was not represented on the array. From these results it can be concluded that the DNA array is a reliable tool for identification and detection of the majority of *Pythium* species in environmental samples. Simultaneous detection and identification of multiple species of soil-borne pathogens such as *Pythium* will be a major step forward for epidemiological and ecological studies. Investigations of a number of atypical *Phytophthora* isolates initially identified as *P. cactorum* disclosed that these isolates were actually inter-species hybrids. Isozyme analysis demonstrated the presence of two alleles rather than the usual single allele for the dimeric malic enzyme (MDHP) in these isolates. One allele of the pair was typical for *P. cactorum* while the other was typical for *P. hedraiaandra*. Sequencing of ribosomal ITS loci showed that this marker was heterogeneous in the atypical isolates, and that the sequences of *P. cactorum* and *P. hedraiaandra* were both present. *Phytophthora* is diploid, and hybrids are expected to combine the genetic characters of both parents as is normally seen in plants and animals (but not most fungi). The mitochondrial genome, however, is inherited maternally and will be present in a single type derived from one of the parents. Indeed, the presumed hybrids were found to possess only one type of the mitochondrial *CoxI* gene, either that of the *P. cactorum* or that of *P. hedraiaandra*. Two iso-



lates showed deviating combinations of the characters mentioned above, suggesting that evolution by genome rearrangement had already taken place in some later-generation progeny of the hybrid lineages. The hybrid *Phytophthora* isolates were found on a variety of plant hosts in public parks all over the Netherlands, making it appear highly likely that they have also become established in natural ecosystems. *Phytophthora hedraiaandra* is a species that has probably only recently been imported into the Netherlands via the use of Mediterranean *Viburnum* shrubs in gardening, while *P. cactorum* is a long-established native phytopathogen. The recent proliferation of hybrids between these species appears to fulfill a long-standing prediction that novel pathogenic Oomycetes would arise as world trade in plant products brought Oomycetes from around the world into interaction with one another. Several of the hosts infected by the hybrids are not known to be infected by either parent species. This suggests that such hybridisations arising from a breakdown in geographic barriers could cause the emergence of novel and unpredictable phytopathogen epidemiologies.

Origin of Pathogenicity in Clinical Fungi

Understanding pathogenicity and virulence of fungi causing infections in humans is of prime importance in the hospital, predicting clinical course and applying adequate and cost-effective therapy. The clinical world is focused on the major causative agents of disease, particularly *Candida*, *Cryptococcus* and *Aspergillus*. Outside these groups, a large diversity of potentially harmful fungi exist which may be rare, but frequently have the pathogenic potential to be more important than the common clinical fungi. Several are able to cause fatal disease in otherwise perfectly healthy human hosts. Our objective is to reveal the natural ecology and routes of transmission of such fungi, in order to explain their pathology. We carry out a comparative approach, in order to place known virulence factors in perspective. Since most of the clinically significant fungi are opportunistic, a large share of our work is devoted to extremotolerant fungi that possess several factors in common to fungi of clinical significance.

Black yeasts in *Chaetothyriales*: evolution of human pathogenicity

With the standard application of molecular diagnostics to the black yeasts, that were previously notoriously hard to identify, a new world of fascinating fungi was entered. The ascomycete order *Chaetothyriales* comprises nearly all melanised fungi that are recurrent on human hosts, and some of them cause extreme, fatal infection in otherwise perfectly healthy humans. The nature of these diseases has always been puzzling, as they seem to differ with the species, but sometimes also with the patient population or with geography. *Rhinocladiella mackenziei* only occurs in the Middle East; *Exophiala spinifera* causes severely mutilating, disseminated infections in healthy adolescents only, and brain infections by *Exophiala dermatitidis* are relatively frequent in East Asia only, although this fungus has a world wide distribution. We sequenced large sets of strains from clinical and non-clinical sources from all over the world, and found indeed that diseases by black yeasts are mild or associated with immune disorders in the U.S.A. and Europe, and frequently take a fatal turn in healthy individuals East Asia. We now have sufficient statistics to prove this endemism is a fact. In the case of *Exophiala* we suppose that host-factors may play a role, Asians being more susceptible to this type of infection.

Understanding the origin and routes of transmission of potentially fatal infections is essential for taking preventive measures. The major neurotropic species, *E. dermatitidis*, was found to be present in Turkish steam baths, but infection does not necessarily take place by inhalation. An unique ingestive route has been hypothesised. The fungus may remain asymptotically in the intestinal tract, and it could cause brain infection via intestinal ulcers and subsequent haematogenous dissemination. Ultimate source of infection seems to be wild berries from the tropical rain forest: the fungus was found there in association with frugivorous animals, such as hornbill birds and flying foxes. This occurrence in tropical rain forests has been proven. Two multilocus genotypes are observed, which seem to have separated very recently and which were also recognised with Maldi-tof, and thus differences at the phenetic level are likely. The two genotypes have more or less equal distribution in the natural niche, but their frequencies diverge in the human environment: a thermophilic genotype is prevalent in steam baths, whereas a more mesophilic genotype is preponderant in systemic infection: all brain cases analyzed thus far were of a single genotype.

Chromoblastomycosis is a unique clinical syndrome, caused exclusively by members of the

Chaetothyriales. The etiologic agents are endemic in semi-arid climates, and thus speciation is probably determined by environmental factors. *Cladophialophora carrionii* is known to occur in cactus debris while the related *C. yegresii* was discovered, which is an endophyte of adjacent cactus plants and produces muriform cells in cactus spines. Nevertheless clinical cases are exclusively caused by *C. carrionii*. Differences in virulence were also noted in the plants: *C. carrionii* was found to be less adapted to growth in cactus. An evolutionary step from plant to human seems to have taken place, and thus transmission from the infected human, by skin scales or from the dead body, can be hypothesised. A considerable phylogenetic distance was noted between the two species which are phenetically very similar, which suggests that the step from plant-association to human virulence and opportunism is an exceptional route.

Large sets of *Fonsecaea* strains were obtained from different continents, which enabled us to reveal epidemiological patterns. Populations showed considerable structuring.

The *Cladophialophora* phenotype with simple or branched acropetal conidial chains without supporting conidiophores, is common in the fungal kingdom. All *Cladophialophora* phenotypes with infective potential thus far

were shown to belong to the order *Chaetothyriales*. Within this order a number of new species were described, one of which caused chromoblastomycosis in Polynesia. Other species seemed to be saprobes, some occurring in slightly osmotic foodstuffs such as juices and tea. Host-specific plant pathogens with this morphology were basal to the *Chaetothyriales*. Except for the occasional endophytes mentioned above, plant and human pathogenicity seems to be mutually exclusive.

Early diverging species within the *Chaetothyriales* are mostly involved in mild cutaneous disease and onychomycosis. This phenomenon has thus far been overlooked, because dermatologists tend to discard non-dermatophytes as supposed contaminants. Jointly with clinicians from Germany, Denmark and China we have revealed that a number of chaetothyrialean fungi are consistently found in such clinical samples and are likely to play a role in pathology. New species have been discovered, several of which are limited to human superficial samples. Remarkably, the group also contains meristematic, rock-inhabiting coniosporium-like species, which also predominate among the basal branches of *Chaetothyriales*. Given the fact that the muriform cell of chromoblastomycosis is a virulence factor, the role of the CDC42 gene (cell division cycle) in pathogenic evolution is being analyzed. Suppressive subtractive hybridisation (SSH) techniques are being developed and adapted to black yeasts.

Recent phylogenetic analyses have shown that the order *Chaetothyriales* was sister to the lichen order *Verrucariales*. This relationship between these two ecologically very different orders was puzzling. However, investigations of fungal communities colonising rocks in extreme environments have shown that some slow-growing melanised fungi inhabiting bare rock surfaces belonged to the *Chaetothyriales*. Therefore,

it was hypothesised that the rock-inhabiting habit might be the ecological link between these two orders. Multigene phylogenetic analyses were carried out in order to confirm the affiliation of 25 of these rock isolates. An ancestral state reconstruction was then undertaken to look at the evolutionary history of the rock-inhabiting habit within *Eurotiomycetes*, the class of fungi to which *Chaetothyriales* and *Verrucariales* belong. Results suggest that the ancestor of the lineage including *Verrucariales* and *Chaetothyriales* was likely to be an extremotolerant non-lichenised, rock-inhabiting fungus. *Verrucariales*, a lichenised lineage with mostly species growing on rocks, seems to have evolved from this non-lichenised rock-inhabiting ancestor, independently from other groups of lichens. Within the *Chaetothyriales*, potential virulence factors, such as melanisation and meristematic growth, might have primarily been adaptations for life in extreme habitats in an extremotolerant rock-inhabiting ancestor.

The *Chaetothyriales* are also striking for their ability to grow with toxic monoaromatic alkylbenzenes as sole source of carbon. They are consistently isolated from soil polluted with xenobiotics, and are also derived from natural environments such as berries – containing monoaromatic precursors of tannins – by the use of toluene and benzene enrichment techniques. The species encountered usually are sister species of known pathogenic black yeasts, but rarely the pathogens themselves. This suggests that alkylbenzene assimilation is a symplesiomorph enhancing pathogenic evolution, but ecological specialisation interferes with the alternative ecology. A similar phenomenon has been observed in the Kingdom Fungi with osmotolerance: groups containing osmotolerant and pathogenic species are strongly overlapping, but at the species level the two ecologies are mutually exclusive. The role of hydrocarbon assimilation is being

analyzed in more detail, as it also seems to play a role in some other groups, such as *Pseudallescheria* and *Fusarium*.

The fact that members of *Chaetothyriales*, albeit opportunists, have intrinsic virulence factors is proven by the fact that the order contains a large clade of waterborne species which frequently cause fatal disease in cold-blooded animals such as fish, turtles, sea dragons and toads. Some are even host-specific, causing severe epidemics. A remarkable extending disease was observed in mangrove crabs along the Brazilian coast.

Ochroconis stands out in the Kingdom Fungi, with no known teleomorph species. Although cultural features and morphology of species are highly characteristic, taxa are extremely diverse genetically, suggesting large phylogenetic distances from one species to the next. Ecologically there is similarity with the *Chaetothyriales*, the group containing fish-opportunist and a thermophilic species causing animal and human brain disease. We suppose that the phylogenetic position of the genus must be quite basal to the ascomycetes, diversities in introns and spacers suggesting a long evolutionary history with constancy at the expression level, as we also observed in *Zygomycetes* and some *Hemiascomycetes*.

Black yeasts in *Dothideales*: life in the most extreme environments on earth

Black yeasts of the order *Dothideales* / *Capnodiales* are heavily melanized and show extensive meristematic conversion in response to extreme conditions of dryness and solar irradiation. Numerous species are found colonizing inert surfaces such as glass, metal and rock. In nature these fungi are extremely common with a bewildering biodiversity, which is still to be described. A particularly interesting ecology is that of rock under extremely cold conditions, such as the Antarctic and the Himalaya. Conditions are so harsh that mi-

croorganisms creep into crevices between crystals in order to be subjected to milder conditions, leading to cryptoendolithic communities. Several new genera have been discovered, and many more are still to be described. Morphology is often highly reduced, sometimes expressed in members of the same species, and this has led to a hypothesis of sudden environmental jumps with fixation in a less versatile but highly resistant phenotype. Adaptation of extremotolerant fungi is a matter of loss rather than of gain. A large clade is uncovered merely consisting of species at a reduced expression level and apparently without sexuality. This is in conflict with the hypothesis that anamorphs are unable to survive over time due to Mueller's ratchet. We suggest that extremotolerance by reduction is a highly successful strategy, as long as prevalent conditions remain extreme. Adjacent clades also contain halophilic and acidophilic taxa, able to grow with saturated salt and at pH below 1, respectively.

An intensive cooperation with a research group on extremophilic fungi in Slovenia has led to the description of several new genera and species that are differentially able to survive salty conditions near to the saturation point. The species play different roles during the crystallisation process in salt-erns in subtropical climates. *Hortaea werneckii*, one of the model organisms, was proven by expression of different genes in the salinity range to have a small fitness depression at 17% salt. Better survival was obtained at lower as well as at higher salinities: the HMGR gene, expressed at low and high salinities, played a crucial role. The species is an unambiguous halophile. The clinical syndrome 'tinea nigra', known in adolescents after a beach holiday, is just superficial colonisation of salty hands. The species has no invasive potential and should be listed at a lower BioSafety Level.

Fitness depressions are the leading hypothesis of apparently

sudden adaptations to the extreme of mesophilic *Aureobasidium* species. The common fungus *A. pullulans* occurs worldwide with only two (LSU) genotypes. In a very small sampling area in and around glaciers of Spitsbergen, a stunning diversity of *Aureobasidium* species and populations was found. Through moulins of the glaciers the subglacial ice is reached, which is extremely salty and alkaline. A specific *Aureobasidium* genotype was found, which was not encountered anywhere else. We hypothesise that this is a highly adapted fungus emerged through accelerated evolution and episodic selection, that becomes extinct immediately when it reaches the milder conditions of normal seawater.

Barcoding, detection and microarray techniques

We aim to develop confident, dedicated databases for several taxonomic groups of clinically important fungi, such as filamentous basidiomycetes, Zygomycetes, dermatophytes, black yeasts and the *Pseudallescheria/Scedosporium* complex. This will allow rapid and accurate routine identification based on ITS barcodes. These databases will be publicly available through the CBS website.

The reliability of identification tools strongly relies on comprehensive sampling. The CBS collection contains a large number of type isolates and other well-studied strains that were used to generate the DNA barcodes. In order to assess the intraspecific variability we included strains from different geographic regions, and clinical as well as environmental strains covering a wide range of habitats and substrates. Understanding interspecific divergence ideally includes all species of a clade, and an underlying phylogenetic hypothesis to define the most closely related extant relatives. To fill the gaps in species coverage we therefore cooperate with other institutions. Our databases will allow the user to perform a BLAST analysis for identification

of unknown clinical isolates and a Neighbor joining analysis based on binary alignments. In addition to the position in the NJ tree, the alignments of the unknown sequence with the most similar barcodes, the program will also provide an alignment with the "validated barcode" of the target species. In well studied groups such as anthropophilic dermatophytes we anticipate that currently available barcodes cover the existing ITS diversity. However, databases of poorly understudied groups such as the *Psathyrellaceae* (medically important basidiomycetes) and the black yeasts are likely to be subject to permanent change due to incomplete sampling. For less-known clinical fungi global interaction with clinical networks is mandatory.

The fungal part of the Mould-array project involved a set of 249 isolates, collected by air sampling in indoor environments in Finland, Sweden, Denmark and the U.K. These strains were isolated in order to verify whether the indoor fungal flora deviates from the expected air contaminants that are generally supposed to be common survivors in indoor climates. The panel of species detected by means of the array should match with those that are actually found during routine sampling in northern temperate climates. rDNA ITS has been selected as the fungal barcoding gene, which therefore should be the basis of all fungal diagnostics. However, in many fungal groups the gene is insufficiently polymorphic. Species should then be distinguished by partial sequences of (introns in) tubulin, actin, calmodulin or other genes. This is the case e.g. in *Penicillium*, *Trichoderma* and *Cladosporium*. For a diagnostic mesoarray based on reversed line-blot assays for rapid detection and identification of filamentous fungi, probes based on ITS as well as a second gene should be available. For the current project we have chosen β -tubulin.

With the recent subdivision of *P. boydii* into a set of smaller species,

it became apparent that each of the newly recognized taxa had a particular predilection, which comes from variations on a basic profile. Central species is *Scedosporium apiospermum*, which is equally relatively abundant in the nutrient-rich, often polluted environment as it is found in clinical samples. *P. boydii* inhabits similar environments but is preponderant as an infectious agent, whereas at the other end of the scale *S. dehoogii* is preponderantly environmental and encountered only occasionally as an agent of mild disease. Given the consistent occurrence of *Pseudallescheria* species in hydrocarbon-polluted sites we will study relationship of monoaromatic neurotransmitter assimilation and brain invasion for *P. boydii*, in cooperation with an international consortium. Differential gene and protein expressions will be analyzed between strains growing on neurotransmitters and glucose in vitro, and between strains isolated from brain tissue and other organs of a mouse infection model of *P. boydii*. Key techniques to be used are Differential-Display Reverse Transcription PCR (DDRT-PCR) and Differential Proteomics techniques.

In groups exhibiting poor morphology, such as the black yeasts, the application of molecular methods leads to the description of a large number of novel species and genera. In contrast, as we have seen earlier in the anthropophilic dermatophytes, large-spored and economically important members of *Pleosporales* in part tend to be over-classified. A multilocus, AFLP and pathogenicity study of *Stemphylium* causing disease in pear and other plant hosts showed that some of the major species had to be reduced to synonymy. A model study is being performed in *Alternaria*, focusing on a species complex with known sexuality and taking a somewhat isolated position in the *Pleosporales*. In addition to multilocus sequencing, production of secondary metabolites is applied as parameter for distinction.

Several anthropophilic dermatophytes have adapted to the human host several times independently, emerging since the last few thousand years from domesticated animals. Earlier, the origin of major species could be located in Africa, where the highest diversity of genotypes is observed. Anthropophilic species tend to lose their teleomorphs; the species are adapting as clonal offshoots from sexual complexes. A remarkable diversity is found in species associated with wild animals, as was recently proven with a new species specifically associated with badgers. Teleomorphs are commonly produced in zoophilic and geophilic species, and are likely to be environmental. With the mode of transmission being truly human-based, a remarkable change from environmental to human pathogens has been achieved.

Separation of the two currently recognised *Coccidioides* species has thus far been based on multilocus analysis and microsatellite typing, but no diagnostic criteria were available for clinical practice. We found consistent differences in the rDNA ITS domain. Application to strains from the CBS culture collection proved that *C. posadasii* had several older synonyms. A proposal for conservation of this name has been submitted in the light of its listing as a U.S. Select Agent for bioterrorism.

Our large ITS database of black yeasts enabled to reconstruct a secondary structure for ITS2. Four domains are generally recognizable in lower as well as in higher organisms, but due to compensatory mutations in stems and occasional absence of domain IV the structure was not always apparent. With the secondary structure at hand, a very small number of base changes in the stems of domain IV enabled reliable recognition of nearly all clinical species of *Chaetothyriales*. Several of our studies have shown that in these fungi multilocus analyses of all currently used genes are concordant

with results of ITS, and sexuality is nearly absent. Thus the domain is an excellent target for the development of specific probes, and is very useful for barcoding. Since species of the order may be too distant to allow confident alignment of ITS, a program for tree reconstruction without alignment was tested in cooperation with the makers of the program.

Ectomycorrhiza refers to association between basidiomycetes and ascomycetes with higher plants, which form fungal mantle and intercellular hyphal networks on roots. EcM inoculum fungi persist in the soil after forest change into other land uses, such as rubber agroforests (i.e. smallholder rubber cultivation managed by farmers with low agricultural practices), oil palm plantation and agriculture. A field study showed that inoculation of EcM in the nursery provides a small increase in seedling survival rate but is not essential, since EcM inoculum potential persists in the soil after forest was changed into rubber agroforests. Based on ITS sequences, members of *Basidiomycota* as well as of *Ascomycota* are commonly involved in colonization of roots of *Shorea* seedlings (nursery stage). Further analysis on EcM roots of *S. leprosula* trees and sporocarps collected in the field is in progress.



Yeast and Basidiomycete Research

Fungi are closely related to animals, making them excellent model organisms for basic cell biological and developmental studies that are directly relevant to human biology. They have therefore become one of the most intensively studied eukaryotic groups in the rapidly expanding field of genomics, and the number of complete genome sequences available for fungal species is rapidly increasing. This unprecedented quantity of information will make an unparalleled contribution to our understanding of fungal phylogeny and evolution, as well as to our understanding of how fungal cells, in our case of some selected species of human pathogens, function. In this research programme we explore fungal genomic data from a perspective of understanding functional biodiversity related to disease potential, susceptibility, population structure, and reproduction.

Human pathogenic yeasts

These research projects aim to understand the biodiversity as well as the virulence properties of selected clinically important yeast species, namely *Cryptococcus neoformans*, *C. gattii*, and *Malassezia*, *Trichosporon* and *Candida* species.

***Cryptococcus neoformans*:** Normally, *C. neoformans* and *C. gattii* reproduce asexually, but in some cases mating can occur. The existence of hybrids between *C. n. var. grubii* and *C. n. var. neoformans* demonstrates that mating occurs in nature. Recently, we have also documented a number of unique hybrids between *C. neoformans* and *C. gattii*. The recognition of the isolates involved as hybrids with this unexpected genetic background was supported by numerous analyses and was a breakthrough in the thinking of many people dealing with *Cryptococcus*. The full extent is not yet known, but very recent data suggest that these *gattii* × *neoformans* hybrids occur much more common than previously anticipated. Our observations also suggest that the microspecies within the complex are genetically isolated by postzygotic isolation. We have also observed mitochondrial recombination which indicates that possible hybridization events may occur even more frequent than currently known based on the presence of hybrids. This is important because it allows the possibility of introgression of “aspecific” genetic material,

which may have an effect on e.g. virulence [Note: this is one of our hypotheses to explain the sudden outbreak of a subtype of *C. gattii* at Vancouver island that recently spread into the U. S. Northwest]. Moreover, studies on hybridization and mitochondrial recombination may also contribute to a better understanding of species concepts in these organisms, which are topics of an ongoing debate [Note at the 2005 International Congress of *Cryptococcus* and cryptococcosis, Boston, we participated in a round table debate on the topic featured in the ISHAM newsletter]. Genomic diversification within the complex is also studied using a CBS-developed Agilent microarray, based on the genome of *C. neoformans* isolate JEC 21 (serotype D). Results confirmed that *C. n. var. grubii* and *C. n. var. neoformans* are relatively closely related to each other, but distinct species, and the four genotypes of *C. gattii* could also be distinguished. Interestingly the hybridisation patterns of the AD hybrid differed widely from that of the BD hybrid. This implies that considerable differences exist among the serotype D backgrounds found in both hybrids, thus supporting the notion that the hybrids may be highly aneuploid. We coined the term ‘Taxogenomics’ for this type of investigations.

Isolates of a genetic subgroup of *C. gattii* referred to as AFLP genotype 6 (= PCR-fingerprint group VGII) have been recognised as re-

sponsible for a major outbreak of cryptococcosis in Vancouver Island, Canada [see our paper in PNAS, commented on Faculty of 1000], which recently extended to the Canadian and US mainland territories, thus enlarging its area of distribution and gaining continental access. This outbreak mainly affects otherwise healthy people, but animals, including marine mammals, can be affected. Ecological sampling has indicated that the same genotype occurs on many native tree species, which collectively may represent the main environmental reservoir involved in the outbreak. Recently, a Danish tourist travelling to Canada affected by the outbreak developed cryptococcal pneumonia. Detailed genotypic analysis demonstrated that the isolate obtained from this patient was identical to those from Vancouver Island. Hence, this investigation documented the first known tourist-mediated intercontinental transmission of this disease and pathogen [see our paper in EID; topic of podcast by CDC Atlanta, USA <http://www2a.cdc.gov/podcasts/player.asp?f=3927>].

To trace the origin of this ongoing and expanding outbreak, we used comparative genomic approaches, including comparative AFLP to search for novel, highly variable, molecular markers useful to develop an MLST scheme. Six DNA regions were selected to be sequenced for ca. 120 genotype AFLP 6 isolates, including many

from the Vancouver outbreak. A microsatellite typing system was developed for this genotype in collaboration with C. Klaassen and J. Meis (CWZ, Nijmegen). In addition, our set of strains was studied by an alternative MLST scheme developed at Duke (J. Heitman). This set will be used to analyse the geographical site of origin of the outbreak by coalescence analysis, recombination and further population studies (collaboration P. Ceresini, Zürich, Switzerland). Knowing the origin of the outbreak is not only scientifically important, but it will also provide a possible clue as to understand whether a genetic event caused the hypervirulence (e.g. an introgression, a transposition, or a mating event). Furthermore, it may give insight in those isolates that are genetically most closely related to the ancestor of the outbreak population. Finally, it may unravel whether external factors, such as climate change, have contributed to the outbreak, or if a combination of these different mechanisms caused the outbreak. We presently have identified a low virulent strain that is genetically closest to the outbreak isolates. Comparative genomics approaches such as Comparative Genome Hybridisation (CGH) and resequencing of this strain of low virulence will be performed to identify genomic differences (e.g. SNPs, introgressions) between the outbreak isolates and its closest neighbour of low virulence [PhD project F. Hagen, funding OvV].

The pathogenicity of *C. gattii* genotype AFLP 6 was further studied using the nematode worm *Caenorhabditis elegans*, interaction with macrophages and a mouse model system (in collaboration with Dr. R. May, Birmingham, UK; I. Polacheck, Jerusalem, Israel, respectively). The absence of an adaptive immune system in *C. elegans* allows this model to be used to dissect out “basic” cryptococcal virulence factors. Interestingly, considerable differences were observed among isolates from the Vancouver Island

outbreak that could not be distinguished otherwise. PhD student H. Ma (University of Birmingham) performed interaction studies between selected isolates and macrophages, using our phylogenomics data for strain selection. F. Hagen (PhD student OvV) is closely interacting with H. Ma and R. May. During a research visit to CBS, H. Ma assessed virulence attributes, such as protease activity, phospholipase activity, melanisation rates, and capsule sizes (all at both 25 and 37 °C). Virulence of a selection of these isolates was further assessed in a mouse model by I. Polacheck (Jerusalem, Israel). In what may turn into one of the highlights of our research during the past years an astonishing correlation was observed between behaviour in macrophages, mouse and origin of the isolates. This is the very first time that *in vitro* tests fully predicted the virulence potential of cryptococcal cells in a cell based model. This is not only interesting from the point of view to develop alternatives for animal models, but it also clearly showed that the isolates causing the Vancouver outbreak possess some unique immunomodulating properties. These results led us to propose that an introgression event may have caused the hypervirulence of these isolates. We also hypothesise that the origin of this genetic element may belong to a different haplotype of *C. gattii* than that causing the outbreak. Support for the possibility for inter-haplotype matings is obtained by incongruent mitochondrial phylogenies [M. Bovers, PhD thesis 2007]. This research will be taken further as the main topic of Ferry Hagen, a PhD student [OvV funding], who is just in his second year. Further collaboration on this topic will be with Jim Kronstad (molecular mycology, University of British Columbia, Vancouver, Canada), U. Himmelreich (NMR, Leuven, Belgium) and G. Janbon (molecular biology, Paris, France).

A functional genomics analysis performed in collaboration with

F. Coenjaerts (UMC Utrecht) concerned a serotype D strain of *C. neoformans* var. *neoformans* that had a mutant form of the *Skn7* gene (listed by the *Saccharomyces* genome database as a nuclear response regulator and transcription factor required for optimal induction of heat-shock genes in response to oxidative stress). Preliminary data suggest that only few genes were upregulated after exposure of the mutant to oxidative stress, including a putative transcription factor. Further research will include a more detailed bioinformatics analysis, as well as additional genetics and microarray experiments (K. Khayhan, PhD student from Thailand, 2008–2012).

Malassezia: The genetic diversity within the lipid-dependent species *Malassezia furfur* was investigated in collaboration with Roma Batra (presently Milwaukee, U. S. A.) and F. Cabañes (Barcelona, Spain). In AFLP analysis, we found several subclusters within the species. Additional techniques have been used to analyse this complex further, e.g., pulsed field gel electrophoresis as well as sequencing of the LSU and ITS regions of the rDNA and part of the chitin synthase gene. As part of the results, one of the *M. furfur* subclusters was shown to have a mixture of markers suggestive of a hybrid origin, even though no sexual mechanisms are known so far for any species clustering within the *Malassezia* lineage. Interestingly, the genome sequence of *M. globosa* revealed a mating type locus (see below).

In collaboration with J. Cabañes (Barcelona, Spain) two new species were described. The landmark in *Malassezia* research was the publication of the genome of *M. globosa* and *M. restricta* [see our paper in PNAS, commented on Faculty of 1000]. Interesting life-style adaptations were observed. Part of the genome was less close to that of the phylogenetically related *Ustilago maydis*, a plant pathogen, but was found to be closer related (at

least in gene composition) to the distantly related *Candida albicans*, another inhabitant of skin. The release of this paper received substantial press coverage all over the globe [including Dutch radio shows and newspapers]. It is estimated that this information was covered by an audience of > 77 million! This paper is also important as it allows the further development of molecular tools in this field. A challenge already undertaken by research groups in Duke (Durham, U. S. A.) and Vancouver (Canada), respectively. The detection of a mating type locus suggested that sex may be possible (already suggested previously on molecular data and the presence of hybrid genotypes, see above), and we are currently looking for diversity in mating types in *M. globosa*, in collaboration with P&G, and J. Kronstad. A further spin off of this project will be the study of the mitochondrial genome of *M. globosa*. Within the ISHAM working group on *Malassezia* we will participate in the set up of a MLST typing scheme. Besides, the first monograph of this genus will be edited within the frame of the ISHAM working group.

Phylogenomics, Tree of Life (TOL) and Yeast Biodiversity

Phylogenetic studies are generally based on comparing DNA or protein sequences that, though found in a wide range of organisms, all arose from the same ancestral genes that occurred millions of years ago in a hypothetical common ancestor species. Such genes that occur among many organisms, but that all have a common ancestral root, are referred to as "orthologous" (= "directly related"). Orthologous groups of proteins (KOGs, or "eukaryotic clusters of Orthologous Groups of proteins") from complete fungal genomes species were analysed in order to resolve their phylogenetic relationships. Conflicting data were obtained on the phylogenetic position of *Schizosaccharomyces pombe* when using different set of cophenetical-

ly similar orthologues. In collaboration with B. Dutilh (PhD student, University of Nijmegen) a bioinformatics study compared various phylogenetic tools [see our paper in Bioinformatics]. Cophenetics as an analysis tool was developed to mine phylogenetically informative single copy orthologues [see our highly accessed paper in BMC Evol. Biol.]. M. van Passel, a post-doctoral guest worker (6 months), performed a bioinformatics study on the genome signature, a genome compositional parameter in prokaryotes and fungi (see our highly accessed paper in BMC Evol. Biol.).

A main asset in the area of Yeast Biodiversity is the co-editing of the 5th edition of "The Yeast, a taxonomic study". This book will set the stage for another ten years and will cover all c. 1500 known yeast species. Descriptions will include molecular phylogeny, morphology, physiology, biochemistry, gene accession numbers for the type strains, and discussions on Systematics, Ecology, Biotechnology, Food and Agriculture, and Clinical importance. This book will appear in 2009.

During the process of editing "The Yeasts" it became very clear that the present phylogenetic classification of the basidiomycetous yeasts urgently needs to be revised. In order to realize this, we are in the process of establishing a global network (a so-called wiki) in order to analyze ca. 20 genes among all ca. 500 accepted species and, revise the phylogenetic classification accordingly. Interest in this project is shown by groups in China, Japan, Malaysia, Brazil, Portugal, Germany, USA and The Netherlands. Among these are also groups with genomics facilities. When realised, this new taxonomic system will result in major conceptual changes, and, it can be expected will result in highly cited papers. Postdocs E.K. Kuramae [KNAW renewal fund 2003–2007] and Carlos Echavarrí [CURIE GRANT CT-2006-036584, 2006–2009] have contributed to the design of this TOL analysis, by

selecting candidate single copy orthologues using comparative genomics approaches.

Yeast diversity was further studied in collaboration with various researchers on an ad hoc base. Clinically important yeasts from neonates and HIV-infected persons were studied from Jakarta (Indonesia) in collaboration with Retno Wahyuningsih [SPIN Mobility grant]. Interestingly, the recently described species, *Candida nivariensis*, a close relative of *Candida glabrata*, was found [paper JCM]. Type strain of current synonyms of *C. albicans* were studied by MLST in collaboration with F. Odds (Aberdeen, UK). Interestingly, *C. stellatoidea*, long time recognised as a separate and clinically important species, but presently interpreted as a synonym under *C. albicans*, was found to be divergent from the remainder of *C. albicans*. A recently obtained grant for a three-year postdoc position will merge phylogenomics knowledge and the development of innovative detection tools for *Candida* yeasts [grant EuroTransBio, EU, SenterNovem, 1 postdoc vacancy, 3 yr, 2008–2011]. This project will be run in collaboration with academia, hospitals and small enterprises in Flanders (Belgium) and The Netherlands. Innovative diagnostics tools were further developed using Luminex XMap technology for *Cryptococcus* and *Malassezia* yeasts in collaboration with M. Diaz and J. Fell (Key Biscayne, USA) [M. Bovers, PhD Thesis, two publications]. A new clinically important species of *Trichosporon* was identified using molecular tools in collaboration with Dr Saad J. Taj-Aldeen (Doha, Qatar). With V. Passoth (Uppsala, Sweden) a new species of *Cryptococcus* will be described. Biological control agents on mites and powdery mildews were described as *Meira* and *Acaromyces* gen. nov. with U. Gerson and A. Szejnberg (Rehovoth, Israel). Three new species were described and recently, two more by other researchers. A paper on dual biocontrol capabilities published in Crop

Science was highly accessed. With Z. Kahn (Kuwait) a new species of *Cryptococcus* (*C. rhandawi*) will be described. Together with K. Seifert (Ottawa, Canada) a new thermophilic species of *Rhodospiridium* (*R. concentricum*) is in the process of being described. B. Turchetti, a postdoc guest (one year) from Perugia (Italy) is studying psychrophilic yeasts from (disappearing) Alpine glaciers and found a number of new species of *Mrakia* and *Leucosporidium* [funding FEMS, Synthesys, University of Perugia].

A functional study of the septal pore caps (SPC) in basidiomycetes and phylogeny of the *Rhizoctonia solani* complex

The septal pore cap (SPC) or parenthesome is a membranous structure associated with endoplasmic reticulum covering both sides of the dolipore septum and is restricted to *Basidiomycetes*. Although this structure was already described in 1958 and well studied at an ultrastructural level, no functional studies have been done so far. Therefore, the composition and the precise function of the SPC at the dolipore is still unclear. The aim of this study was to isolate the SPC and to partly characterise the proteins present, so that a start can be made in understanding the function of this organelle. We used the plant pathogen *Rhizoctonia solani* as a model organism, because it has relatively well-studied, large SPCs. We have been the first ever, to isolate and biochemically study the SPC of basidiomycetes. Consequently, we have opened a research field, that probably will be entered by other research groups soon.

Laser microdissection with a P.A.L.M. microscope (P.A.L.M. Micro-laser Technologies GmbH, Bernried, Germany) was used successfully to isolate the SPC-dolipore region. We could identify the septal regions using lectin-gold labelling of antibodies specifically targeting the septa; this analysis was done with a scanning electron microscope. In addition, we successfully enriched

SPCs from *R. solani* cell fractions by isopycnic (buoyant density or equilibrium) centrifugation. In electron microscopic studies, we observed that plug material at the orifice of the septal pore channel remained attached via filamentous material to the SPCs. This tight connection between SPCs and pore-occluding material implicates a key role of SPCs in the process of plugging septal pores in *Basidiomycetes*. Such plugging is often connected to maintaining hyphal integrity in situations where some cells are damaged or otherwise strongly stressed.

Protein electrophoresis showed that a 18 kDa glycoprotein (SPC18) was present in the SPC-enriched fraction. This protein was N-terminally sequenced and afterwards the complete gene sequence was obtained. No homologue could be identified using the available sequences in genome databases. Western blot analysis, however, suggests that the protein may be limited to the *R. solani* lineage. Attempts are ongoing to study the nucleotide diversity of the gene within the *Rhizoctonia* lineage and to compare it with standard D1/D2 and ITS variable region sequences of the ribosomal DNA. Polyclonal antibodies raised against the 18 kDa glycoprotein were labelled using the immunogold technique and then used to perform immunodetection studies. The labelled antibodies were found to be localized in the SPC, the SPC membrane and plug material. Thus suggesting that SPC18 may be involved in plugging the pore upon stress and that the SPC may have a repository function for SPC to make it possible that the process of pore plugging can start directly after the cells have encountered a serious stress (prefab model).

The observed heterogeneity of SPC morphology in some of the major lineages of the Basidiomycetes, notable the *Hymenochaete* and *Cantharellus-Rhizoctonia* clades, was confirmed by analysing the SPC of *Rickenella* spp. and *Can-*

tharellus sp. It appeared that the observed heterogeneity in SPC morphology is characteristic of basal lineages among the basidiomycetes. This may suggest that genes involved in both types of SPC morphology may be present in these basal lineages, and that the basal organisms may thus manifest a genetic condition that existed in the ancestors of other *Basidiomycetes* prior to the occurrence of lineage sorting.

When all manuscripts from this project will be published, we will investigate possibilities for new funding to continue this intriguing research line.

PhD student A. Nakatani, studied > 300 global isolates of *Rhizoctonia solani* by sequence analysis of the ITS 1 and 2 regions. A good correlation was observed with the anastomosis groups. Per ITS clade a smaller selection of isolates was made and studied by three genes (data analysis still in progress).

Bioprospecting of fungi

Thescreeningshavebeensetupand are running smoothly. One patent has been filed (decision pending). In the first half year of the project we established an efficient collaboration between CBS, Hubrecht Institute and the Department of Pharmacy at Utrecht University.



Filamentous fungi are playing an important role in our daily life as well as in the context of food products in indoor situations. The area of the research group is to study the biodiversity, phylogeny and cell biology of fungi with special relation to food and indoor mycology, with an emphasis on the genera *Penicillium* and *Aspergillus*. These genera are significant as spoilage agents of food and beverages, as producers of toxic compounds and as the dominant fungi in human dwellings. They even occasionally occur as human and animal pathogens. On the other hand, many species of these genera are very important industrial microorganisms in food fermentation and biotechnology. The mission of the program is to reach a deeper understanding of applied and fundamental insights of fungi related to food-association and indoor situations. These include a novel polyphasic classification of the subgroups of the genera *Penicillium* and *Aspergillus*, and the development of tools for a practical barcoding system of these groups. To understand the biology of the important fungi in these applied fields, the cell biology of the fungal cells, including fungal survival structures (spores) is studied. The research is focussed on the nature of heat resistance of ascospores and the sensitivity of fungal spores for antifungal compounds. The research of the group has always been intertwined with numerous (smaller) projects with external parties (industrial companies and governmental institutions) that request expertise with problems related to food spoilage, indoor environments and industrial applications. This is illustrated by the fact that several large fundamental or applied research projects have been directly initiated as a result of these smaller projects.

Biodiversity of *Penicillium* and *Aspergillus*

A major achievement in *Penicillium* research was the publication of the monograph of subgenus *Penicillium*, published together with J.C. Frisvad in *Studies in Mycology* 49 (2004). Studies on other subgenera were started and it is expected that a major part of subgenus *Furcatum* will be finished in 2009. Phylogenetical analysis of *Penicillium* subgenus *Biverticillium* were collected and expanded by sequencing more genes. The taxonomic place-

ment of this subgenus will lead to a separation from *Penicillium* and final experiments will be completed in 2008.

The taxonomic research on *Aspergillus* was greatly stimulated by the appointment of Janos Varga as post-doc. In April 2007 an International workshop on *Aspergillus* systematics in the genomic era was organised and recommendations and contributions were published in *Studies in Mycology* 59, and the book *Aspergillus* in the genomic era which was published in January 2008. Several papers dealing with the various sections of *Aspergillus* have been completed and it is anticipated that in 2008 and 2009 most of the genus *Aspergillus* will be completed.

DNA barcoding

DNA barcoding is a taxonomic method which uses a short genetic marker in an organism's (mitochondrial) DNA to quickly and easily identify it as belonging to a particular species. A DNA sequence should meet several criteria to be used successfully for species identification. DNA sequences should be orthologous in the examined organisms, and variable enough to allow species identification, with

low levels of intraspecific variation. A DNA barcode should be easily accessible (universally amplified/sequenced by standardized primers from a wide set of organisms), relatively short ($\leq 5-600$ bp), simple to sequence, easily alignable, with no recombination. The mitochondrial cytochrome oxidase subunit 1 (*cox1*, usually referred to as COI in barcoding studies) was proposed to be a good candidate for barcoding animal species including birds, fishes and Lepidopteran insects.

To evaluate the usefulness of the *cox1* gene for DNA barcoding, we gathered altogether 47 *cox1* sequences and examined their properties for species delimitation. Our data indicate that *cox1* is not appropriate to be used as DNA barcode in aspergilli since none of the eight species of the *Aspergillus niger* species complex could be identified unequivocally. The phylogenetic tree constructed based on the *cox1* sequences also shows an overlap between intra- and interspecific variation possibly due to past mitochondrial DNA recombination events as suggested earlier. Although a high degree of heterokaryon incompatibility was observed among isolates of the



A. niger species complex, mtDNA transfers occur readily even between incompatible isolates. Comparing the phylogenies based on *cox1*, ITS, β -tubulin and calmodulin sequences, either β -tubulin and calmodulin could serve as a suitable region for species identification among black aspergilli. Recent attempts to use the *cox1* gene for species identification in other fungal groups including *Fusarium* species and basidiomycetes have also met with limited success (K. Seifert, pers. comm.). Recently, the fungal community decided to use the ITS region as the first choice for DNA barcoding the Fungal Kingdom. If this region does not distinguish all species, a second region could be used to resolve the taxa. In the case of aspergilli, our opinion is to use either β -tubulin or calmodulin sequences for accurate species identification.

Food Mycology

Various aspects such as the fungi producing mycotoxins represented an integral part of the research of the group. Several studies were completed in which a polyphasic taxonomy led to the descriptions of new taxa together with data on their mycotoxin analysis. In this respect the ecological studies of food-borne fungi are integrated in the taxonomic studies of the biodiversity of *Penicillium* and *Aspergillus* as described above.

In August 2006 the group was involved in the organisation of the International Food Mycology Workshop in Cairns (Australia), and in June 2007 they organised together with the International Commission of Food Mycology a three day workshop at Key West (U.S.A.) followed by a two day symposium for the Industry.

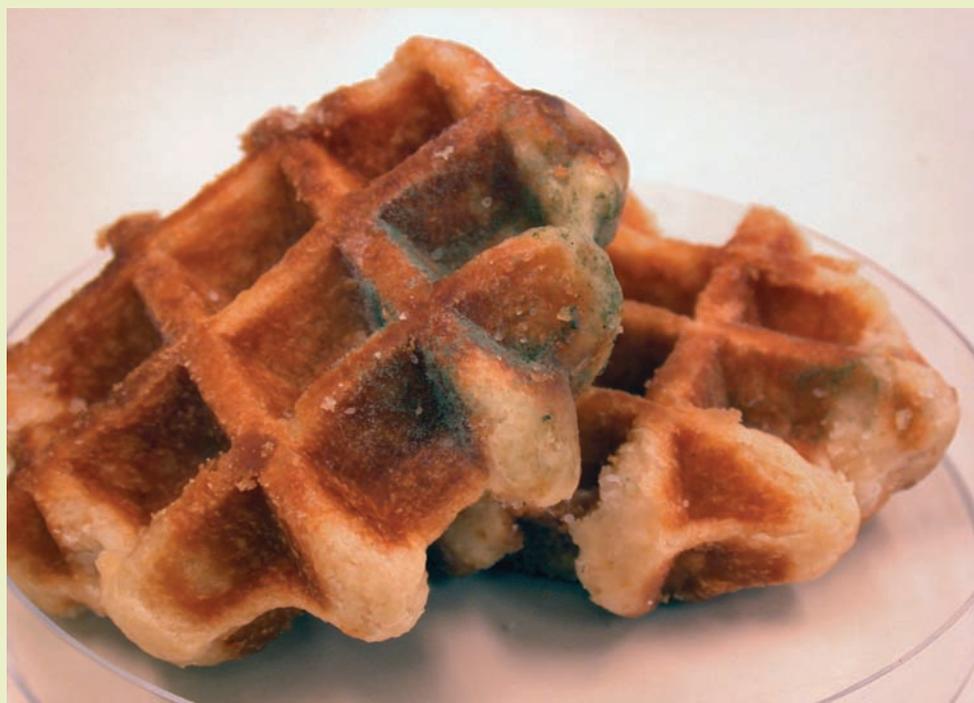
Fundamental aspects of spore biology of food-relevant fungi

From 2002 onwards, the biology of the extremely stress-resistant ascospores of the fungus *Talaromyces macrosporus* was addressed. These spores are comparable with some bacterial spores in their tolerance for high temperatures, drought and high pressure. Moreover, short rigorous environmental triggers (as a pasteurisation treatment or a pressure at 6000 Bar) can activate germination of the dormant cells. Activation is correlated with different changes in the cell wall of the spore as for instance the release of a protein and an increase of permeability. Upon activation, trehalose is degraded and the degradation product, glucose, is released from the cell. Then a sudden ejection of the spore through a very thick outer cell wall occurs which is dubbed "prosilition" (*prosilire* (Latin), means "to jump out"). The process occurs within seconds and is accompanied by an increase in respiration. This process was identified in more species of the genus *Talaromyces*. Spin probe studies (ESR) indicated that the cytoplasmic parameters as microviscosity and anisotropy changed most dramatic after prosilition. These data indicate that prosilition is an important process to increase the uptake of nutrients and oxygen (water?) to enable the

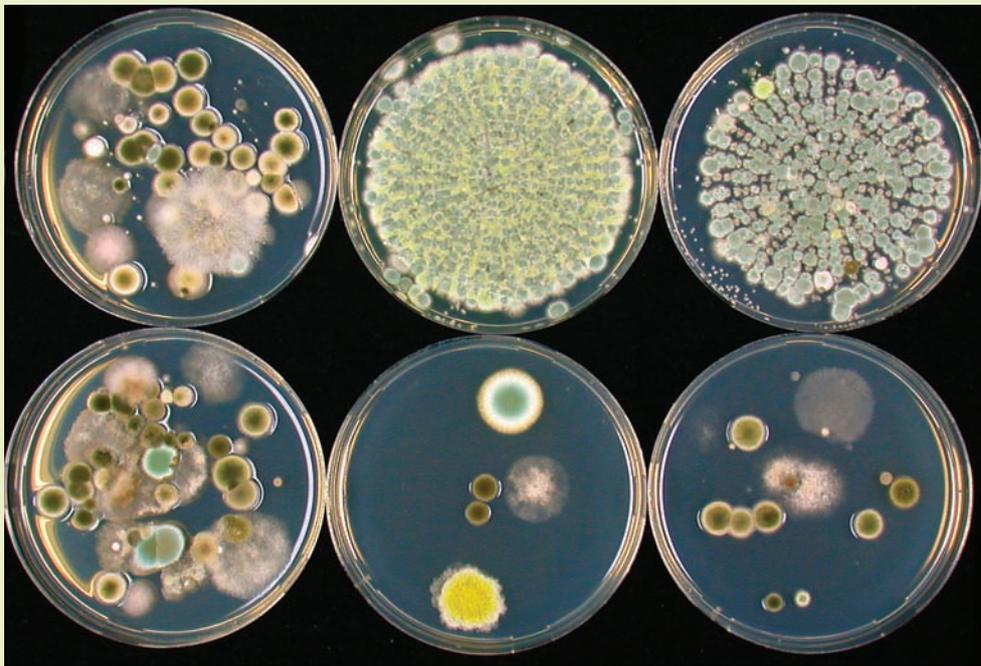
spore to swell and form a germ tube which was not possible inside the encasement of the isolating outer cell wall.

Further studies have addressed the biology of conidia, air- or water-borne survival structures that are very important for the distribution of food-related fungi. The identification that 1-octen-3-ol, one of the most notable flavour compounds in mushrooms, is also a volatile self-inhibitor of germination of conidia of *Penicillium paneum* is regarded as a highlight here.

In addition, germination of multicellular conidia has been studied, and it became clear that compartments of these spores initially are alike, but do differentiate and communicate during early germination. When germinating compartments collapse due to an adverse condition, the non-germinating compartments take over and form germ tubes by themselves. In collaboration with the Swedish Agricultural University, the morphology of the sporangiospores of the *Rhizopus microsporus* groups was studied and novel leads to identification of the members of this important food-related group of fungi were found. Finally, a number of studies were completed on fungal growth and development as a result of collaboration with other groups (including University



Cake contaminated with the xerophilic mould *Aspergillus penicillioides*.



of Edinburgh (U.K.), University of Wageningen, and the University of Utrecht).

More recently we have studied germination of conidia of *P. discolor*, and identified an ergosterol-enriched area of the plasma membrane on the site of germ-tube formation. This is part of a STW-funded project on the mode of action of natamycine on fungal spores. The sensitivity of these spores for the polyene-antibiotic during several stages of germination has been studied and several manuscripts are in preparation. We have also done a large study (as part of a SENTER-funded project) on post-harvest diseases, with as a

model system the infection of tulip bulbs by the fungus *Fusarium oxysporum* with the aim to find novel treatments or procedures.

Indoor Mycology

The research on indoor fungi focuses mostly on the taxonomy and biodiversity of the species occurring in dwellings, buildings, archives, etc. Two major studies on aspergilli were completed. In March 2006 an international workshop was organised bringing together the various research disciplines working with fungi in indoor environments. The contributions and recommendation are being prepared for a book "Molds, water and the built

environment". Together with Prof. Olaf Adan, studies on the behaviour of fungi on building materials with emphasis on the role of water were initiated, and this has led to an application for a research grant at STW. During 2002–2007 Rob Samson taught 3 courses per year, particularly in Germany, dealing with the identification and detection of indoor fungi. Together with the Landesgesundheitsamt in Stuttgart and some German reference laboratories, a proficiency testing for the identification of common indoor moulds was initiated and performed. This testing is carried out twice a year and between 70 to 80 laboratories working in the field of food and indoor mycology participate.



Programmes, Themes and Projects

1. Yeast and Basidiomycete Research (T. Boekhout)

Project YBRP 1.01.01: Comparative Fungal genomics

2003–2007: T. Boekhout, E. Kuramae (postdoc), B. Snel (Bio-informatics, Utrecht University, The Netherlands), L. Stougie, P. Vitanyi & R. Cilibrasi (CWI, University of Amsterdam, The Netherlands), T. van der Lee, C. Waalwijk, G. Kema, R. van der Ham, J. Leunissen, (PRI, Wageningen University, Wageningen, The Netherlands), A. van der Burgt (aio PRI), Arnold Kuzniar (aio Wageningen University), M. Weiss, University Tübingen, Germany).

Funding: KNAW Renewal fund, Genomics fund.

Project YBRP 1.01.02: Functional diversity of human pathogenic yeasts (including evolution, virulence and pathogenesis)

2003–2007: T. Boekhout, E. Kuramae (postdoc), V. Robert, B. Theelen, F. Hagen, M. Bovers (Ph.D. student), I. Hoepelman, F. Coenjaerts (Department of Internal Medicine and Infectious Diseases, UMC, Utrecht University, The Netherlands), R. May (University of Birmingham, UK), H. Hoogveld (NIOO-KNAW, Nieuwersluis, The Netherlands), E.J. Kuijper (Department of Medical Microbiology, LUMC, University of Leiden, The Netherlands), L. Spanjaard, (Department of Medical Microbiology, AMC, University of Amsterdam, The Netherlands), F. Hochstenbach (Department of Biochemistry, AMC, University of Amsterdam, The Netherlands), M. Lazera (Fundação Oswaldo Cruz, Brazil), C. Paula (University of São Paulo, Brazil), I. Polacheck (Haddassah Medical Centre, Israel), J. Heitman (Duke University, USA), W. Meyer (University of Sydney, Australia), U. Himmelreich (MPI, Cologne, Germany), G. Janbon (Institut Pasteur, Paris, France), S. Oliver (University of Manchester, UK), J. Fell & M. Diaz, (RSMAS, University of Miami, USA), R. Wahyuningsih (Schools of Medicine, Universitas Indonesia and Universitas Kristen Indonesia, Jakarta, Indonesia), A. Botha (University of Stellenbosch, S. Africa), F.J. Cabañes, Autonomous University of Barcelona, Spain), T. Dawson (Procter & Gamble, USA), R. Batra (Milwaukee, USA), E. Guého (Mauves sur Huisnes, France), L. Ball (LUMC, University Leiden, The Netherlands), H. Korporaal (Leids Cytologisch en Pathologisch Laboratorium, Leiden, The Netherlands), S. Taj-Aldeen (Hamad Medical Corporation, Doha, Qatar), I. Okoli (Awka, Nigeria).

Funding: Odo van Vloten, KNAW Renewal Fund, KNAW Indonesia-Netherlands SPIN mobility grant.

Project YBRP 1.01.03: Biodiversity of yeasts and selected basidiomycetes

Project YBRP 1.01.03.01: 'The yeasts, a taxonomic study 5th edition'

2005–2007: T. Boekhout, V. Robert, J.W. Fell, G. Scorzetti (RSMAS, Miami, USA), C.P. Kurtzman (USDA-NCAUR, Peoria, USA), T. Nakase (NITE, Tokyo, Japan), M. Hamamoto (Meiji University, Higashimita, Japan), A. Fonseca, J.P. Sampaio (New University of Lisbon, Caparica, Portugal), R.J. Bandoni (Vancouver, Canada), F.J. Bai (Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, The Chinese Academy of Sciences, Beijing, China).

Project YBRP 1.01.03.02: 'Studies on the microbiological and biochemical properties of masau (*Ziziphus mauritiana*) fruits fermentation and prospects for the development of starter cultures to produce masau wine and/or beverage'

2004–2008: T. Boekhout, L. Nyanga (Ph.D student Wageningen University), R. Nout, M. Zwietering (Laboratory of Food Microbiology, Wageningen University, The Netherlands).

Funding: McGillavry fund, International Foundation of Science.

Project YBRP 1.01.03.03: The septal pore complex (SPC) in Basidiomycetes (*Rhizoctonia solani*)

2003–2007: T. Boekhout, E.E. Kuramae, K.G.A. van Driel (Ph.D. student), W.H. Müller & A. Verkleij (Department of Molecular Cell Biology, Utrecht University, Utrecht, The Netherlands), H. Wösten & A.F. van Peer (Department of Molecular Microbiology, Utrecht University, The Netherlands), A. Nakatani (PhD student, UNESP, São Paulo, Brazil), D. Rosa (M.Sc student, USP, Piracicaba-SP, Brazil).

Funding: Odo van Vloten, Utrecht University

Project YBRP 1.01.03.04: The *Rhizoctonia solani* Tree of Life.

2005–2006: T. Boekhout, E.E. Kuramae, J.A. Stalpers, K.G.A. van Driel (Ph.D. student), A. Nakatani (PhD student, UNESP, São Paulo, Brazil), N. de Souza (UNESP, Botocatu, Brazil),

1. Yeast and Basidiomycete Research (T. Boekhout)

Theme: Evolutionary genomics of fungi

Funding: CNPq (Brazil).

Project YBRP 1.01.04.05: Fungal biodiversity in regenerating tropical lowland forests, Colombia. 2002–2006: T. Boekhout, R. Summerbell, C. Lopez-Q. (Ph.D student) (CBS / Universidad de Antioquia, Medellin, Colombia), A. M. Cleef, J. Duivenvoorden & J. Sevink (University of Amsterdam, The Netherlands), A.E. Franco Molano & A. Vasco-P. (University of Antioquia, Colombia), J. Frisvad (Technical University, Denmark).

Funding: NWO-Wotro.

2. Applied and Industrial Mycology (R.A. Samson)

Theme 1: Biodiversity and ecology of food and airborne fungi

Theme 2: Biology of food spoilage fungi

2. Applied and Industrial Mycology (R.A. Samson)

Project IFA 2.01.01: Biodiversity of *Penicillium*, *Aspergillus* and related genera 2003–2010: R. Samson, E. Hoekstra, J. Houbroken, J.C. Frisvad (Lyngby, Denmark), K.A. Seifert (Ottawa, Canada).

Project IFA 2.01.02: Biodiversity and strain selection of fungi in indoor environments for quality management

2003–2007: R.A. Samson, E.S. Hoekstra, T. Gabrio, (Landes Gesundheitsamt Baden-Württemberg, Stuttgart, Germany), K. Senkpiel, R. Keller (Medizinischer Universität zu Lübeck, Germany).

Project IFA 2.01.03: Taxonomy and phylogeny of food borne Zygomycetes

2003–2007: R. Samson, J. Dijksterhuis, A. Kuijpers, J. Houbroken, J. Jenneson, J. Schnurer (University of Agriculture, Uppsala, Sweden).

Project IFA 2.02.04: Image analysis macroconidia of *Fusarium culmorum*

2002–2005: J. Dijksterhuis, G. Chitarra, T. Abee, F. Rombouts (Food Microbiology, University of Wageningen, The Netherlands).

Project IFA 2.02.05: Volatile compounds as germination regulators in *Penicillium paneum*

2003–2005: J. Dijksterhuis, G. Chitarra, T. Abee, F. Rombouts (Food Microbiology, University of Wageningen, The Netherlands).

Project IFA 2.02.06: Germination of heat resistant ascospores of *Talaromyces macrosporus*

2003–2006: J. Dijksterhuis, F. Hoekstra, E. Golovina, J. Nijse (Plant Physiology, University of Wageningen, The Netherlands).

Project IFA 2.02.07: The cell wall of ascospores of *Talaromyces macrosporus* before and after heat activation

2003–2006: J. Dijksterhuis, M. Hanssen, T.T. Wyatt, L. Lugones, H. Wösten (Molecular Microbiology, University of Utrecht, The Netherlands), J.H. Sietsma (Molecular Biology of Plants, University of Groningen, The Netherlands).

Project IFA 2.02.08: Role of natamycin as a membrane pertubator in fungal conidia and hyphae

2005–2008: J. Dijksterhuis, R.A. Samson, E.J. Breukink, B. de Kruijf (Biomembranes, University of Utrecht, The Netherlands).

Project IFA 2.02.09: Sustainable control of fungal diseases of flower bulbs.

2005–2008: J. Dijksterhuis, J. Houbroken, T. van Doorn, W. van der Krieken (PRI, Wageningen), A. van der Lans, M. de Boer (PPO, Lisse), J. Stark, F. van Rijn (DSM, Delft), H. de Vries (Innoventis, Breezand), G. Top (Profyto, Emmeloord).

3. Evolutionary Phytopathology (P.W. Crous)

Theme 1: Evolutionary patterns and host adaptation

Theme 2: Mating strategies and speciation

3. Evolutionary Phytopathology (P.W. Crous)

Project EPP 3.01.01: Hybridisation in *Phytophthora*

2006–2010: A.W.A.M. de Cock, H. Brouwer, W.A. Man in 't Veld (Plant Protection Service, Wageningen), C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada).

Project EPP 3.01.02: Genetics of host specificity and speciation within *Cercospora*

2003–2009: J.Z. Groenewald, P.W. Crous, U. Braun (Martin-Luther University, Germany), H-D. Shin (Korea University, Seoul).

Funding: Volkswagenstiftung.

Project EPP 3.01.03: Genetic diversity of *Mycosphaerella* species associated with Sigatoka disease on bananas

2003–2008: P.W. Crous, J.Z. Groenewald, M. Arzanlou (Ph.D. student), G. Kema (Plant Research International, The Netherlands), J. Carlier (CIRAD, France).

Project EPP 3.02.04: *Mycosphaerella* spp. occurring on *Eucalyptus*
2003–2010: P.W. Crous, J.Z. Groenewald, G. Hunter, M.J. Wingfield, B.D. Wingfield, T. Coutinho (University of Pretoria, South Africa).
Funding: NRF, TPCP, University of Pretoria, South Africa.

Project EPP 3.02.05: Circumscription and detection of the *Cylindrocarpon* black foot rot complex of grapevines
2002–2010: P.W. Crous, J.Z. Groenewald, H.-J. Schroers (Agricultural Inst., Slovenia), F. Halleen, P.H. Fourie (University of Stellenbosch, South Africa), A. Cabral (Ph.D. student), H. Oliveira (Fundação para a Ciência e a Tecnologia, Portugal).
Funding: Winetech, ARC-Nietvoorbij, South Africa; Fundação para a Ciência e a Tecnologia, Portugal.

Project EPP 3.02.06: Speciation in *Cylindrocladium*
2003–2009: P.W. Crous, L. Lombard (Ph.D. student), M.J. Wingfield, B.D. Wingfield, T. Coutinho (University of Pretoria, South Africa).
Funding: TPCP, NRF, South Africa.

Project EPP 3.02.07: Circumscription and detection of *Phaeoacremonium* and *Phaeomoniliella* in grapevines
2003–2010: P.W. Crous, J.Z. Groenewald, L. Mostert, U. Damm (Univ. of Stellenbosch), S. Esakhi (University of Florence, Italy)
Funding: Odo van Vloten.

Project EPP 3.02.8: Circumscription, detection and infection strategies of *Botryosphaeria* spp.
2003–2010: P.W. Crous, J.Z. Groenewald, J. van Niekerk, P.H. Fourie (University of Stellenbosch, South Africa), F. Halleen (ARC-Nietvoorbij, South Africa), M.J. Wingfield, B. Slippers (University of Pretoria, South Africa), A. Phillips (Univ. of Lisboa, Portugal).
Funding: Winetech, NRF, South Africa.

Project EPP 3.02.9: Phylogeny and population genetics of *Alternaria* and related Pleosporales
2002–2010: G.S. de Hoog, P.W. Crous, B.M. Pryor (Tucson, USA), T.L. Peever (University of Washington State, USA), E.G. Simmons (Amherst, USA), B. Anderson (Lyngby Technical University, Denmark).

Project EPP 3.02.10: Phylogeny in the genus *Pythium* and development of a molecular identification and detection system
1996–2010: A.W.A.M. de Cock, H. Brouwer, C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada).
Funding: FES

Project EPP 3.02.11: Species delimitation in *Pythium*
1996–2010: A.W.A.M. de Cock, H. Brouwer, C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada), G.R. Klassen, J.E.J. Bedard, A.M. Schurko (University of Manitoba, Winnipeg, Canada).
Funding: FES

Project EPP 3.02.12: Phylogeny in the genus *Phytophthora* and development of a molecular identification and detection system
2001–2010: A.W.A.M. de Cock, H. Brouwer, C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada), R.C. Hamelin, G. Bilodeau (Natural Resources Canada, Canadian Forest Service, Québec, Canada).
Funding: FES

Project EPP 3.02.13: Species delimitation in *Phytophthora*
2001–2010: A.W.A.M. de Cock, H. Brouwer, C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada), Man in 't Veld, W.A. (Plant Protection Service, Wageningen, The Netherlands).
Funding: FES

Project EPP 3.02.14: Taxonomy, phylogeny and biology of *Cladosporium*
2003–2009: P.W. Crous, J.Z. Groenewald, G.S. de Hoog, H.-J. Schroers, P. Zalar (Agricultural Inst., Slovenia), U. Braun, K. Schubert (Martin-Luther University, Germany),

Project EPP 3.02.15: Phytopathogenic *Phoma* complexes
2002–2011: P.W. Crous, M. Aveskamp (Ph.D. student), G. Verkley, J.Z. Groenewald, J. de Gruyter (Ph.D. student; PPS, Wageningen, The Netherlands), S.T. Koike, K. Subbarao (University of California, USA), T. O'Neill (ADAS, UK).

Project EPP 3.02.16: Taxonomy and phylogeny of *Septoria*
2000–2011: G. Verkley, M. Starink-Willemse, A. van Iperen, S. Vanev (Botanical Institute, Sofia).

Project EPP 3.02.17: Genomic comparisons of the mating type loci of *Mycosphaerella* spp.
2006–2011: L.-H. Zwiers, M. Arzanlou (Ph.D. student), G. Hunter, P.W. Crous

Project EPP 3.02.18: Functional analysis of the mating type loci of *Mycosphaerella* spp associated with Sigatoka disease on banana
2006–2011: L.-H. Zwiers, M. Arzanlou (Ph.D. student), G.H.J. Kema (Plant Research International, the Netherlands)

Project EPP 3.02.19: Establishment of a transformation system for *Mycosphaerella* spp associated with Sigatoka disease on banana
2008–2011: L.-H. Zwiers, P.W. Crous, G.H.J. Kema (Plant Research International, the Netherlands)

Project EPP 3.02.20: Annotation of the *Mycosphaerella graminicola* and *M. fijiensis* genomes
2006–2011: L.-H. Zwiers, P.W. Crous, International *Mycosphaerella* consortium

4. Origins of pathogenicity in clinical fungi (G.S. de Hoog)

Theme 1: Evolution of human-virulence in filamentous fungi

Theme 2: Evolution of extremotolerance and host interactions in filamentous fungi

Theme 3: Barcoding of medical fungi

4. Origins of pathogenicity in clinical fungi (G.S. de Hoog)

Project OPC 4.01.01: Natural life cycle and selective sweeps of *Exophiala dermatitidis*
2002–2008: G.S. de Hoog, A.H.G. Gerrits van den Ende, M. Sudhadham (Ph.D. student), T. Matos (Fac. Medicine, Slovenia), S. Sivichai (Chulalongkorn Univ., Thailand), G. Haase (RWTH-Aachen, Germany), A. van Belkum (Bacteriology, Erasmus Univ., Rotterdam), S.B.J. Menken (IBED, Amsterdam, The Netherlands), Y. Gräser (Charité, Berlin, Germany), A. Mayr (Hautklinik, Univ. Innsbruck, Austria).
Funding: WOTRO.

Project OPC 4.01.02: Antimycotic susceptibility of herpotrichiellaceous black yeasts
2008–2010: G.S. de Hoog, R. Vitale (Buenos Aires, Argentina), G. Haase (RWTH-Aachen, Germany), V. Vicente (Microbiol., Univ. of Curitiba, Brazil), R. Caligiorne (Fiocruz, Belo Horizonte, Brazil).

Project OPC 4.01.03: Black oligotrophs in indoor water systems and their impact on human health
2002–2008: G.S. de Hoog, A.H.G. Gerrits van den Ende, J. Harrak (PhD student), M. Nauta (Rijksherbarium, Leiden, The Netherlands), S.B.J. Menken (IBED, University of Amsterdam, The Netherlands), E. Göttlich (LUFA Augustenberg, Karlsruhe, Germany), A. Velegriki (Fac. Medicine, Athens, Greece), N. Hageskal (Vet. Faculty, Oslo, Norway), W. Boeger (Curitiba, Brazil), S. Frasca (Frasca).
Funding: CBS/IBED.

Project OPC 4.01.04: Atlas of Clinical Fungi
2002–2010: G.S. de Hoog, K.F. Lijsterburg, J. Guarro, J. Gené, M.J. Figueras (University Rovira i Virgili, Reus, Spain), J. Albert (Inst. Informatik, Würzburg, Germany), T. Weniger (Med. Mikrobiol. University of Münster, Germany).

Project OPC 4.01.05: Phylogenetic position and taxonomy of *Ochroconis* and *Scolecobasidium*
2007–2008: G.S. de Hoog, R. Horré (Bundesanst. Arzneimittel, Bonn, Germany), H.-J. Choi (Mikrobiol. Univ. Bonn, Germany), K. Samerpitak (Thailand), H.-j. Choi (Evangelisches Krankenhaus, Wesel, Germany).

Project OPC 4.01.06: Evolution of virulence in chaetothyrialean black yeasts: between pathology and bioremediation
2007–2009: G.S. de Hoog, C. Gueidan, A. Ram (University of Leiden, The Netherlands), S.B.J. Menken (IBED, University of Amsterdam, The Netherlands), Dongming, Shuwen Deng, Ruoyu Li (Beijing / Xinjiang, P.R. China), D. Attili, M. Satow (Rio Claro, Brazil), F. Prenafeta-Boldu (Barcelona, Spain), T. Matos (Ljubljana, Slovenia), D. Saunte (Lyngby, Denmark).
Funding: KNAW and Chinese Academy.

Project OPC 4.01.07: The biodiversity of para-Hypocrealean fungi in human and animal disease
2008–2012: C. Gueidan, G.S. de Hoog, H.-J. Schroers (Ljubljana, Slovenia), K. O'Donnell (Peoria, USA), L. Sigler (University of Alberta, Canada), A.A. Padhye, M. Brandt (CDC, USA), S. Moser (Univ. Alabama), P. Kammeyer (Loyola Univ. Med Ctr, Maywood IL, USA), D. Sutton, M.G. Rinaldi (Fungus Testing Center, San Antonio, USA), W. Merz (Johns Hopkins, Baltimore USA),

M. Hayden (Rush Med. Ctr., Chicago, USA), A. Goldschmit-Reuveni, G. Rahav (Tel Hashomer Hospital, Tel-Aviv, Israel), S. Krajden (St. Joseph's Hospital, Toronto, Canada).

Project OPC 4.01.08: Chromoblastomycosis: from opportunism to pathogenicity
2006-2010: H. Badali (PhD student), M.J. Najafzadeh (PhD student), G.S. de Hoog, L. Xi, J. Sun (Ghazou, China), F. Queiroz Telles, V. Vicente (Curitiba, Brazil).

Funding: Ministry of Health. Iran.

Project OPC 4.02.01: Evolution of extremotolerance in dothidealean black yeasts
2008-2010: G.S. de Hoog, A.H.G. Gerrits van den Ende, N.A. Yurlova (St. Petersburg, Russia), N. Gunde-Cimerman, P. Zalar (Ljubljana, Slovenia), M. Grube (Graz, Austria), L. Selbmann (Viterbo, Italy), F. Lutzoni (Duke, U.S.A.), M. Gorbushina (Oldenburg, Germany), F. de Leo (Messina, Italy).

Project OPC 4.02.02: The diversity of ectomycorrhizal and root endophyte fungi on dipterocarp trees

2004-2008: H. Tata (PhD student), M. Weger (Utrecht, The Netherlands), M. van Noordwijk (Bogor, Indonesia), R.C. Summerbell (Toronto, Canada).

Funding: NUFFIC.

Project OPC 4.02.03: Barcoding of medical fungi

2006-2009: G. Walther, V. Robert, G.S. de Hoog, Y. Graeser (Berlin, Germany), K. Voigt (Jena, Germany), M. Weiß (Tübingen, Germany), D.A. Sutton (Texas, USA), A. Alastruey (Madrid, Spain), J. Wang (Guangzhou, China), J.S. Choi (Korea), G. Jacon (Brussels, Belgium), J.S. Rodrigues-Tudela (Salamanca, Spain), J. Kaltseis (Innsbruck, Austria).

Funding: EDIT.

5. Collection, Preservation and Digitalisation (J. Stalpers)

Project CPD 5.02.01: Sequencing and characterisation of ex-type strains

2003-2010: J. A. Stalpers, G. J. M. Verkley, G.S. de Hoog, R. A. Samson, IJ. Vlug, P. W. Crous, T. Boekhout.

Funding: EU-EBRCN project.

Project CPD 5.02.02: DNA-Bank

2006-2009: G. J. M. Verkley, J. A. Stalpers, G. van Haalem, P. W. Crous.

Funding: KNAW Stimuleringsfonds.

Project CPD 5.02.03: DNA-Barcoding of selected fungi from the CBS Collection

2006-2008: U. Eberhardt, G. Verkley, J. A. Stalpers, G. van Haalem.

Project CPD 5.02.04: Species delimitation, infrageneric relationships and DNA barcoding in selected fungal genera

2008-2009: U. Eberhardt, K. Dukich; J. Vesterholt (Natural History Museum of Denmark, Copenhagen), H.J. Beker (Brussels, Belgium)

Funding: Henry J. Beker Charitable Trust, Belgium.

Project CPD 5.02.05: DNA-Barcoding and taxonomy of *Vararia* and *Peniophora*

2007-2008: U. Eberhardt, J. A. Stalpers, G. van Haalem, G. Verkley; S.-G. Li (Agricultural Collection of China, Beijing).

Project CPD 5.02.06: Yeast, a taxonomic study. Taxonomic revision of the yeast clades (for Springer Verlag)

2007-2011: V. Robert, M. Smith, M. Groenewald; H.M. Daniel (MUCL, Belgium).

Project CPD 5.02.07: Fungal phylogenomics and finding of universal barcoding genes

2006-2010: V. Robert, U. Eberhardt, E. Kuramae (Netherlands Institute of Ecological Research, NIOO, Heteren), A. Levesque, K. Seifert (Agriculture and Agri-Food Canada, Ottawa), T. Bruns (University of California, Berkeley).

Project CPD 5.03.01: Digitalisation and accessibility of nomenclatural and taxonomical data

2003-2010: V. Robert, J. A. Stalpers, G. J. Stegehuis; P. Romano (ABC, Italy).

Project CPD 5.03.02: Index Fungorum

2003-2007: J.A. Stalpers, G. J. Stegehuis; P. Kirk (CABI Bioscience, UK); J. Adams (Landcare New Zealand, Auckland).

Project CPD 5.03.03: Input of CBS data in CBS database

The databases will be linked with other, non-CBS databases, as PubMed and GenBank

2003-2008: J. A. Stalpers, G. J. Stegehuis, V. Robert, M. Vermaas; P. Romano (ABC, Italy).

5. Collection, Preservation and Digitalisation (J. Stalpers)

Theme 1: Preservation research

Theme 2: Sequencing and characterisation of type strains

Theme 3: Online access to nomenclatural and taxonomic databases

Project CPD 5.03.04: CBS publications on the Web
2003–2008: J. A. Stalpers, G. J. Stegehuis, D. Stalpers-den Brinker.

Project CPD 5.03.05: Global Biological Research Centres Network (GBRCN)
2007–2010: J. A. Stalpers, V. Robert, G. J. Stegehuis; D. Smith (CABI, UK), E. Stackebrand (DSMZ, Germany), C. Bizet (Institut Pasteur, France), P. Romano (ABC, Italy), D. Janssens (LMG, Belgium).
Funding: EU, Germany.

Project CPD 5.03.06: European Distributed Institute of Taxonomy (EDIT), coordinating WP 3.4
2006–2011. U. Eberhardt, R. van der Linden, G. J. M. Verkley, and various collaborators at National Herbarium Netherlands (Leiden, Wageningen), Zoological Museum Amsterdam, and L. M. Kriegsman, WP-leader, Naturalis (Leiden, the Netherlands).

Project CPD 5.03.07: Mycoheritage. Reproduction of important classical illustration books on the Web
2005–2010: J. A. Stalpers, G. J. Stegehuis, L. Reijers.

Project CPD 5.03.08: Digitalisation of collection data and Species Banks
2005–2007: J. A. Stalpers, V. Robert, G. J. Stegehuis, P. W. Crous, R. A. Samson, G. S. de Hoog, L. Reijers, D. Stalpers-den Brinker, P. Meredith, S. Vanev, and various collaborators in National Herbarium Netherlands (Leiden, Wageningen), Zoological Museum Amsterdam, and Naturalis (Leiden, the Netherlands).
Funding: NWO.

Project CPD 5.03.09: Dictionary of the Fungi
2005–2009: J. A. Stalpers; P.M. Kirk, P.F. Cannon, J.C. David (CABI Bioscience, UK).

Project CPD 5.03.10: Integrated phytopathogenic databases and species identification systems
2007–2010: V. Robert, L.-H. Zwieters, M. M. Aveskamp; H. Huttinga (independent consultant); E.Y.J. van Heese, L.C.H. van Valkenburg; J.L.J. van de Bilt, P. van den Boogert, A.J.M. Loomans (Plant Protection Service, Wageningen, the Netherlands); M. Verbeek, R. van der Vlugt (Plant Research International, Wageningen, the Netherlands); L. Duistermaat (National Herbarium Netherlands, Leiden); J. van Tol (Naturalis, Leiden, the Netherlands); Y. de Jong (Zoological Museum Amsterdam); B. Jabas, S. Szoke (BioAware, Belgium).

Scientific Output (2006–2007)

SCIENTIFIC PUBLICATIONS

2006

- Aptroot A, Geel B van (2006). Fungi of the colon of the Yukagir Mammoth and from stratigraphically related permafrost samples. *Review of Palaeobotany and Palynology* 141: 225–230.
- Aptroot A, Wirth V (2006). A new saxicolous *Arthothelium* from the Namibia desert. *Lichenologist* 38: 123–126.
- Ayala-Escobar V, Yáñez-Morales M de Jesús, Braun U, Groenewald JZ, Crous PW (2006). *Pseudocercospora opuntiae* sp. nov., the causal organism of cactus leaf spot in Mexico. *Fungal Diversity* 21: 1–9.
- Baddley JW, Mostert L, Summerbell RC, Moser SA (2006). *Phaeoacremonium parasiticum* infection. *Journal of Clinical Microbiology* 44: 2207–2211.
- Balajee SA, Nickle D., Varga J, Marr KA (2006). Molecular studies reveal frequent misidentification of *A. fumigatus* by morphotyping. *Eukaryotic Cell* 5: 1705–1712.
- Bedard JEJ, Schurko A, Cock AWAM de, Klassen GR (2006). Diversity and evolution of 5S rRNA gene family organization in *Pythium*. *Mycological Research* 110: 86–95.
- Beer ZW de, Begerow D, Bauer R, Pegg GS, Crous PW, Wingfield MJ (2006). Phylogeny of the Quambalariaceae fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. *Studies in Mycology* 55: 289–298.
- Boekhout T, Gildemacher P, Theelen B, Müller WH, Heijne B, Lutz M (2006). Extensive colonization of apples by smut anamorphs causes a new post-harvest disorder. *FEMS Yeast Research* 6: 63–76.
- Bovers M, Hagen F, Kuramae EE, Diaz MR, Spanjaard L, Dromer F, Hoogveld HL, Boekhout T (2006). Unique hybrids between fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*. *FEMS Yeast Research* 6: 599–607.
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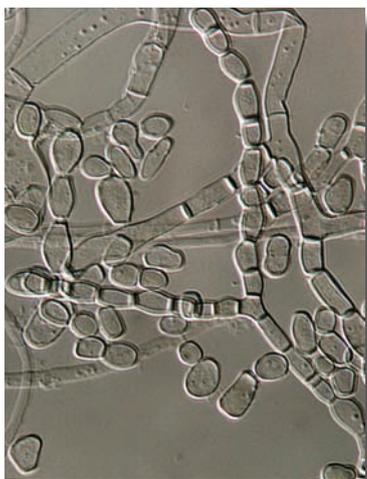
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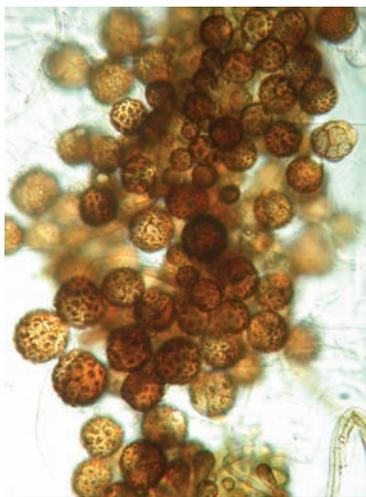
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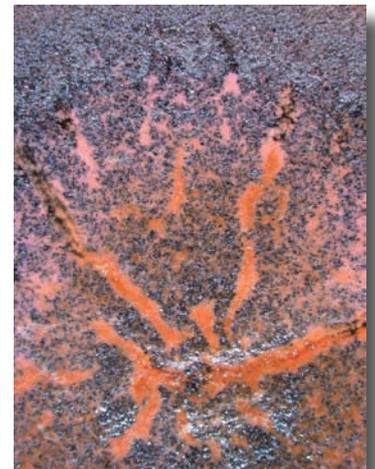
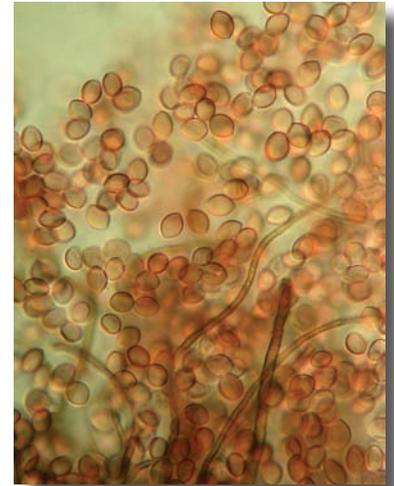
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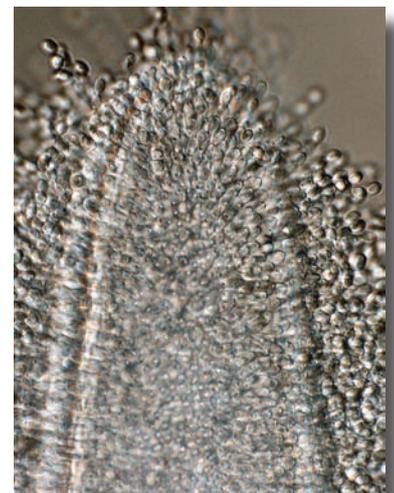
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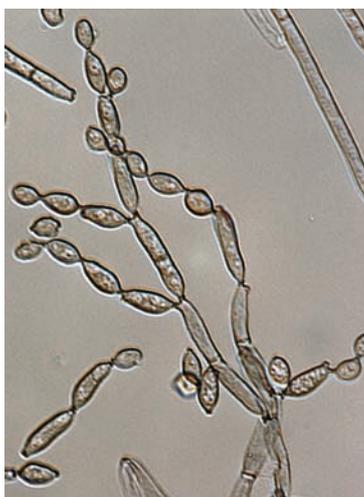
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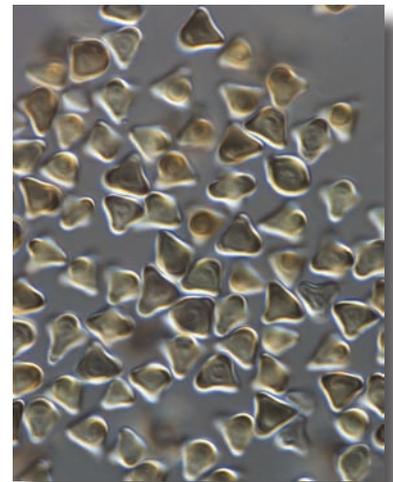
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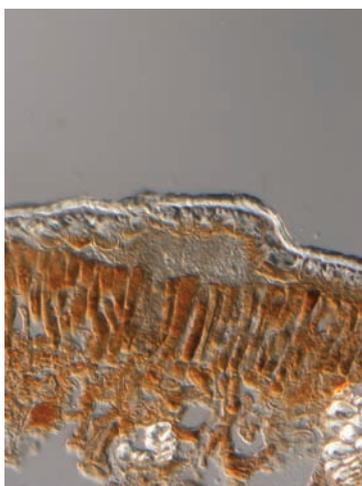
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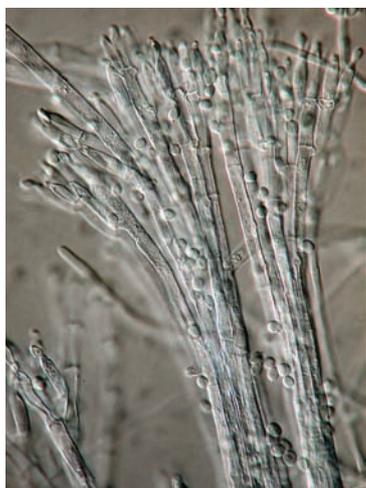
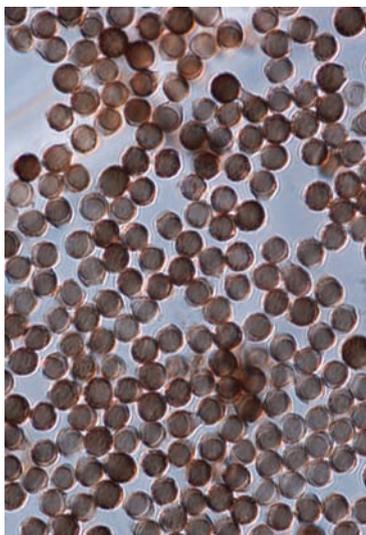
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- Hong SB, Frisvad JC, Shin H, Samson RA (2007). Species delimitation in *Aspergillus* Section *Fumigati* based on Polyphasic Taxonomy. *Aspergillus* systematics in the genomics era. April 12–14, Utrecht, The Netherlands.
- Hoog GS de (2006). Evolution of virulence in fungi, exemplified by black yeasts. 12th scientific meeting of the European Society of Chemotherapy, Infectious Diseases (ESCID). March 16–19, Aachen, Germany.
- Hoog GS de (2006). Host shifts and evolution of pathogenicity in black yeasts. Workshop Extremotolerant Black Yeasts. June 1, Graz, Austria.
- Hoog GS de (2006). Networking for the benefit of clinical science. Joint Meeting of the Netherlands Society for Medical Mycology and the Paul Ehrlich Gesellschaft for Chemotherapy. November 22, Nijmegen, The Netherlands.
- Hoog GS de (2006). Wirtswechsel und Evolution der Pathogenität der schwarzen Hefen. 30th Jahrestagung der Oesterreichischen Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin. May 29 May – June 1, Linz, Austria.
- Hoog GS de, Borman A, Ahmed AOA, Aveskamp M (2006). Phylogeny of *Madurella*-like agents of black-grain mycetoma. 16th Congress of the International Society for Human and Animal Mycology. June 25–29, Paris, France.
- Hoog GS de, Horr  R (2006). ECMM Working Group *Pseudallescheria* and *Scedosporium* infections. Paul Ehrlich Gesellschaft f r Chemotherapy. May 11–12, Bonn, Germany.
- Hoog GS de, Zeng J (2006). Diagnosis of the genus *Exophiala*, agents of human mycoses. 16th Congress of the International Society for Human and Animal Mycology. June 25–29, Paris, France.
- Horr  R, Schnitzler N, Grueger T, Hoog GS de, Guarro J (2006). Review of infections due to *Pseudallescheria boydii*. 16th European Congress of Clinical Microbiology and Infectious Diseases. April 1–4, Nice, France.
- Houbraken J, Samson RA (2007). Ascospore formation in the heat resistant fungus *Paecilomyces variotii*. International Commission on Food Mycology. 2007 Workshop. June 4–6, Key West, Florida, U.S.A.
- Houbraken J, Samson RA (2007). Detection of airborne molds and yeasts. Workshop for Food Industry. Emerging Mold Problems and Spoilage in Food and Beverages. June 7–8, Key West, Florida, U.S.A.
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- Illnait-Zaragozi MT, Curfs-Breuker I, Martinez GF, Fernandez CM, Boekhout T, Meis JF (2006). In vitro activity of the new azole isavuconazole BAL 4815 compared with six other antifungal agents against 155 *Cryptococcus neoformans* isolates from Cuba. 46th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, ICAAC, September 27–30, San Francisco, U.S.A.
- Illnait-Zaragozi MT, Mart nez GF, Fern ndez CM, Perurena MR, Vald s EA, Jerez LE, Boekhout T, Klaassen CH, Meis JF (2007). First report of *Cryptococcus gattii* environmental isolates in Cuba. 3rd Trends in Medical Mycology, October 28–31, Turin, Italy. *Journal of Chemotherapy* 19(S3): 55.
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- Klaassen CHW, Balajee SA, Samson RA, Varga J, Valk HA de, Meis JFGM (2007). AFLP and STR analysis for inter- and/or intraspecies discrimination of *Aspergillus* spp. *Aspergillus* systematics in the genomics era. April 12–14, Utrecht, The Netherlands.
- Klaassen CHW, Valk HA de, Hagen F, Boekhout T, Meis JFGM (2006). Microsatellite based

typing of fungi and yeasts. 2nd Scientific Meeting of the Nederlandse Vereniging voor Medische Mycologie. November 21, Utrecht, The Netherlands.

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liver transplantation. 16th Congress of the International Society for Human and Animal Mycology. June 25–29, Paris, France.

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Man in 't Veld WA, Cock AWAM de, Summerbell RC (2006). Two different natural hybrids of *Phytophthora cactorum* occupy two different ecological niches. APS-CPS-MSA Joint Annual Meeting. July 29 – August 2, Québec city, Québec, Canada.

Man in't Veld WA, Cock AWAM de, Summerbell RC (2007). Natural hybrids of resident and introduced *Phytophthora* species proliferating on multiple new hosts in Europe. 4th IUFRO meeting phytophthoras in Forests & Natural Ecosystems, August 26–31, California, U.S.A.

Manikandan, Kocsubé S, Dóczy I, Narendran V, Varga J, Antal Z, Vágvölgyi C, Revathi R, Nagy E, Kredics L (2007). *Aspergillus keratitis*: epidemiological features, molecular identification, antifungal susceptibility and clinical outcome at the Aravind Eye Hospital in South India. *Acta Microbiologica et Immunologica Hungarica* **54**: 80–81.

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Robert V (2006). Data integration and analyses: a challenge for culture collections. NLBIF-NWO Groot Symposium. February 2, Naturalis, Leiden, The Netherlands.

Robert V (2006). Data management and identification. Institut Pasteur Course on Medical Mycology. March 20, Paris, France.

Robert V (2006). Invited speaker at 25th ECCO meeting: “How many genes do we need to sequence? And which ones?”. June 8–9, Budapest, Hungary.

Robert V (2006). MycoBank: linking names to genomes. 8th International Mycological Congress. August 20–25, Cairns, Australia.

Robert V (2007). CABI-Biosciences. BioloMICS software for the management and the analyses of biological data. August 16, Egham, U.K.

Robert V (2007). Canadian Culture Collections CRTI Workshop in Ottawa. Information systems: BioloMICS. February 13–14, Ottawa, Canada.

Robert V (2007). CBS Course on Mycology: biology, phylogenies and polyphasic identification. February 9, Utrecht, The Netherlands.

Robert V (2007). Corpoica. Conference on: Bioinformatics for Biological Collections. February 28, Bogota, Colombia.

Robert V (2007). Course on BioloMICS software Institut Nacional de Salud. February 26– March 3. Bogota, Colombia.

Robert V (2007). DNA barcode meeting, The MycoBank system, collections, information systems and their applications. October 4, Leiden, The Netherlands.

Robert V (2007). Institut Pasteur Course on Medical Mycology. Data management and identification. March 20, Paris, France.

Robert V (2007). Invited speaker at DNA barcode meeting, The MycoBank system. October 5, Leiden, The Netherlands.

Robert V (2007). NCIMB. BioloMICS software for the management and the analyses of biological data. November 14, Aberdeen, Scotland.

Robert V (2007). PhD course University of Wageningen; Bests genes for phylogeny. October 19, Wageningen, The Netherlands.

Robert V (2007). The International Workshop on *Aspergillus* systematics in the genomic era. Development of web-based initiatives and tools for analysis: Experience from yeasts. April 13, Utrecht, The Netherlands.

Robert V (2007). The MSA meeting symposium “Getting fungal nomenclature into top gear”: Deposit of new fungal names in MycoBank. August 9, Baton Rouge, Louisiana, U.S.A.

Robert V (2007). TIMM ISHAM meeting, Possible databasing and bioinformatic solutions for *Malassezia* associated data. October 28, Torino, Italy.

Robert V (2007). WFCC meeting, MycoBank, past, present and future. October 8, Goslar, Germany.

Robert V, Kuramae E, Boekhout T (2006). Bioinformatics for phylogenomics. 8th International Mycological Congress. August 20–25, Cairns, Australia.

Robert V, Kuramae E, Boekhout T (2007). Institut Nacional de Salud. Conference on: Bioinformatics for phylogenomics. March 2, Bogota, Colombia.

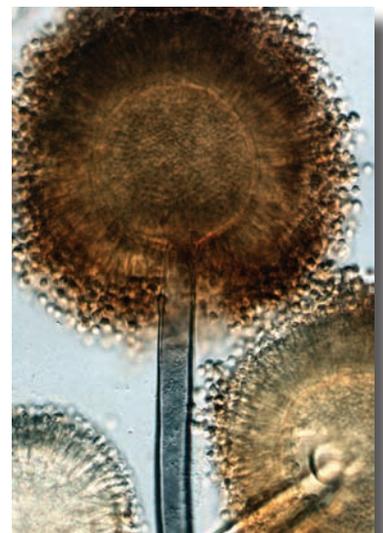
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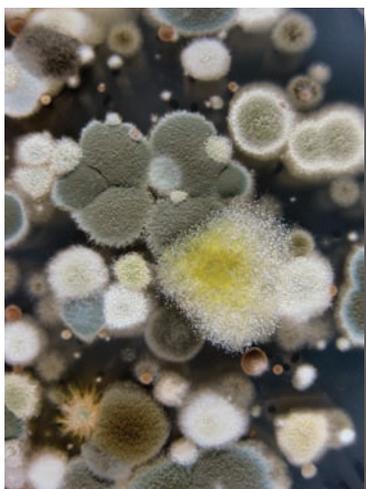
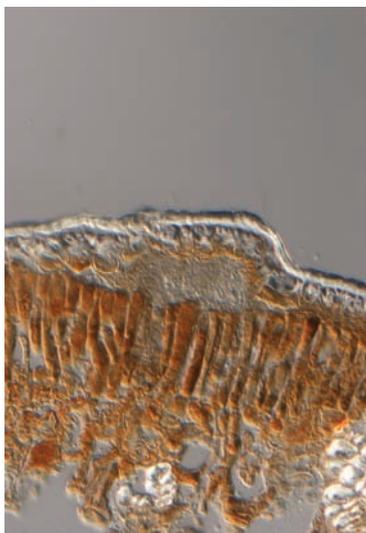
Robert V, Stalpers J, Verkley G (2006). Culture collections, genomics and bioinformatics, the golden triangle. APS-CPS-MSA Joint Annual Meeting. July 29 – August 2, Québec city, Québec, Canada.

Roets F, Dryer L, Crous PW, Wingfield MJ (2006). Fungus-arthropod mutualism and dispersal biology of *Ophiostoma* spp. inhabiting *Protea* flower-heads. 8th International Mycological Congress. August 20–25, Cairns, Australia.

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- Mycological Congress. August 20–25, Cairns, Australia.
- Ruibal C (2006). Isolation and characterization of melanized, slow-growing fungi from semiarid rock surfaces of central Spain and Mallorca. Workshop Extremotolerant Black Yeasts, June 1, Graz, Austria.
- Ruibal C, Selbmann L, Gerrits van den Ende AHG, Hoog GS de (2006). The emergence of a highly successful extremotolerant clade of melanized fungi. Workshop Extremotolerant Black Yeasts, June 1, Graz, Austria.
- Ruibal C, Selbmann L, Gerrits van den Ende AHG, Hoog GS de (2006). Advances in the phylogeny of the *Chaetothyriales*. Workshop Extremotolerant Black Yeasts, June 1, Graz, Austria.
- Safodien S, Halleen F, Crous PW, Botha A, Smit WA (2007). *Eutypa dieback* of grapevines in South Africa. 45th Congress of the Southern African Society for Plant Pathology, January 20–23, Gauteng, South Africa.
- Samson R, Noonim P, Varga J (2007). Overview of the black Aspergilli in food and beverages. International Commission on Food Mycology workshop. June 4–6, Key West, U.S.A.
- Samson RA (2006). Biodiversity of the genus *Aspergillus* in view of new taxonomic concepts. 9th International Symposium of the Research Center for Pathogenic Fungi and Microbial Toxicoses (RC-PFMT), Chiba University. December 15, Chiba, Japan.
- Samson RA (2006). Foodborne Fungi and Mycotoxins - VLAG Course. September 18 – 22 September, Wageningen, The Netherlands.
- Samson RA (2006). Nachweis, bewertung, sanierung und qualitätssicherung aus internationaler sicht. - Berufsverband Deutscher Baubiologen VDB, 10. Pilztagung. June 19–20, Dessau, Germany.
- Samson RA (2006). Overview of the mycology of indoor fungi. ICF workshop. 8th International Mycological Congress. August 19–20, Cairns, Australia.
- Samson RA (2006). Schimmelpilze in Innenräumen. Ein internationaler Überblick – DECHEMA. January 12, Frankfurt, Germany.
- Samson RA (2006). Schimmels in voedsel en dranken Friesland Foods. December 7, Ede, The Netherlands.
- Samson RA (2006). Standardization of methods of detection of fungi in indoor environments. 16th Congress of the International Society for Human and Animal Mycology. June 25–29, Paris, France.
- Samson RA (2007). Food borne Fungi and Mycotoxins. VLAG Course. November 19–23, Wageningen, The Netherlands.
- Samson RA (2007). Molds in Food and Indoor environments. Seminar PURAC, July 20, Nunspeet, The Netherlands.
- Samson RA (2007). Protocol for naming new *Aspergillus* species. *Aspergillus* systematics in the genomics era. April 12–14, Utrecht, The Netherlands.
- Samson RA (2007). The *Aspergillus* initiative of web data bases. *Aspergillus* systematics in the genomics era. April 12–14, Utrecht, The Netherlands.
- Samson RA (2007). Xerofiele schimmels in 'intermediate moisture' zoetwaren. Workshop Zoetwaren met intermediair vochtgehalte. De puntjes op de i's van microbiologie. November 29, Gent, Belgium.
- Samson RA Hong SB, Frisvad JC (2006). New and old concepts of species differentiation in *Aspergillus*. 2nd Advance Against Aspergilloses. February 23–25, Athens, Greece.
- Samson RA, Dijksterhuis J, Houbraken J, Rico E, Johnson S (2007). Food Mycology 2007: Emerging mold problems and spoilage in food and beverages. Proceedings of Food Mycology 2007, June 7–8, Key West, Florida, U.S.A.
- Selbmann L, Onofri S, Hoog GS de, Ruibal C, Zucconi L, Ruisi S, Grube M (2006). Antarctic rock inhabiting fungi. Workshop Extremotolerant Black Yeasts, June 1, Graz, Austria.
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- Sudhadham M, Sihanonth P, Gerrits van den Ende AHG, Hoog GS de (2006). Host shift in the neurotropic black yeast *Exophiala dermatitidis*: a steam bath colonizer emerging from the tropical rain forest. Meeting of the Netherlands Society for Medical Mycology and the Netherlands Society for Medical Microbiology. April 11, Arnhem, The Netherlands.
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- Supabandhu J, Hoog GS de, Vanittanakom N (2006). Comparison of physiological characteristics between environmental and clinical isolates of human pathogenic *Pythium insidiosum*. 8th International Mycological Congress. August 20–25, Cairns, Australia.
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- Verkley GJM, Kempen I van (2006). Foliar endophytes versus leaf litter saprobes: Annual cycle of an ascomycete community associated with oak leaves. Invited lecture, Symposium 41, 8th International Mycological Congress, August 20–25, Cairns, Australia.
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- Zwiers L-H (2007). Genomics of sex in *Mycosphaerella*. 5th NHN-day (Nationaal Herbarium Nederland), April 24, Wageningen, The Netherlands.
- Zwiers L-H (2007). Genomics of sex in *Mycosphaerella*. CBS-Phytopathology day, June 22, Wageningen, The Netherlands.

Other Scientific Activities

Staff served on the following societies, foundations, committees, etc.

- Africa Fund for Fungal Biodiversity and Mycotic Infections, de Hoog GS (founder and member of board).
- Australasian Plant Pathology, Crous PW (member of editorial board).
- CBS Biodiversity Series, Crous PW, Gams W, Samson RA, Summerbell RC (members of editorial board).
- Centre of Excellence in Tree Health Biotechnology, South Africa, Crous PW (member).
- Centro de recursos microbiológicos, Universidade Nova de Lisboa, Boekhout T (member of scientific advisory board)

Christine Buisman Stichting, Crous PW (member of board).

Consortium of European Taxonomic Facilities, Crous PW (member of board).

DNA barcoding in Europe Meeting, Leiden, The Netherlands, 3–5 Oct 2007, Eberhardt U (Main organizer; head of scientific committee, head of organizing committee, abstract volume).

ECCO meeting, Portugal, Stalpers JA (co-organizer & chair).

ECMM Working Group Pseudallescheria Scedosporium Infections, Hoog GS de (co-ordinator).

ESF-EMBO Symposium on Comparative Genomics of Eukaryote Microorganisms: Eukaryotic Genome Evolution, Sant Filu de Guixols, Spain, October 2007, Boekhout T (organizer / vice chair).

European Culture Collection's Organization (ECCO), Stalpers JA (member of board).

FEMS Yeast research, Boekhout T (Editor in chief).

Fungal Diversity, Aptroot A, Crous PW (members of editorial board).

GBIF (Global Biodiversity Information Facility), Stalpers JA (member of technical committee).

Gewasbeschermingsmiddelen stuurgroep, Crous PW (member).

International Commission for the Taxonomy of Fungi, Crous PW (member and co-ordinator of the Mycosphaerella subcommission), Samson RA (member).

International Commission on Food Mycology, Samson RA (treasurer)

International Commission on Indoor Fungi, Samson RA (chairman).

International Commission on Penicillium and Aspergillus, Samson RA (chairman).

International Mycological Association, Crous PW (member of executive committee).

International Mycological Congress (IMC8), Boekhout T (member of scientific committee)

International Society for Human and Animal Mycology (ISHAM), de Hoog GS (President-elect).

International Union of Microbiological Societies. Samson RA (Secretary General).

International Workshop on Esca and grapevine decline (ICGTD 4), South Africa, Crous PW (co-organiser).

Johanna Westerdijk Stichting, Crous PW (member of board).

Journal of Plant Pathology, Crous PW (member of editorial board).

KREM (Dutch working group for Scanning Electron Microscopy), Dijksterhuis J (member of board).

Masterclass Fungal Ecology, Curitiba, Brazil, Nov. 16, 2005, de Hoog GS (organiser & lecturer).

Medical Mycology – The African Perspective, Hartenbosch, South Africa, Jan 25, 2005, de Hoog GS (organiser).

MSc Committee, Bester W (2006) University of Stellenbosch, Crous PW (co-supervisor).

MSc Committee, Carsens E (2006) University of Stellenbosch, Crous PW (co-supervisor).

MSc Committee, Lubbe C (2004) University of Stellenbosch, Crous PW (co-supervisor).

MSc Committee, Pretorius MC (2004) University of Stellenbosch, Crous PW (co-supervisor).

MSc Committee, Van Coller GJ (2004) University of Stellenbosch, Crous PW (co-supervisor).

Mycological Progress, Hoog GS de (member of editorial board).

Mycological Research, Boekhout TB (members of editorial board).

Mycological Society of America, Crous PW (chair and member of culture collections committee).

Mycoses, de Hoog GS (managing editorial board).

Mycosphaerella leaf diseases of eucalypts, Australia, Geelong Crous PW (co-organiser).

National Museum of Natural History Naturalis, Crous PW (member of scientific advisory board).

National Research Foundation, South Africa, Boekhout T & Crous PW (peer reviews).

Natural Sciences and Engineering Research Council, Canada, Boekhout T & Crous PW (project reviews).

Netherlands Society for Medical Mycology, de Hoog GS (scientific secretary).

Netherlands Society for Microbiology, Boekhout T (chair of mycology section).

Netherlands Society for Microbiology, Boekhout T (member of board).

NL-BIF, the Dutch National Organisation Participating in GBIF (Global Biodiversity Information Facility), Stalpers JA (member of board).

NMV (Dutch Mycological Society), Stalpers JA (member of scientific committee).

Nomenclature: Committee for Fungi, Gams W (member of committee, member of Committee Art. 59).

Nomenclature: Committee for Fungi, Gams W (secretary until Aug. 2005).

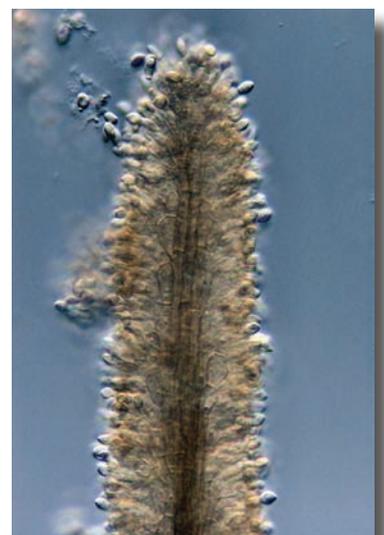
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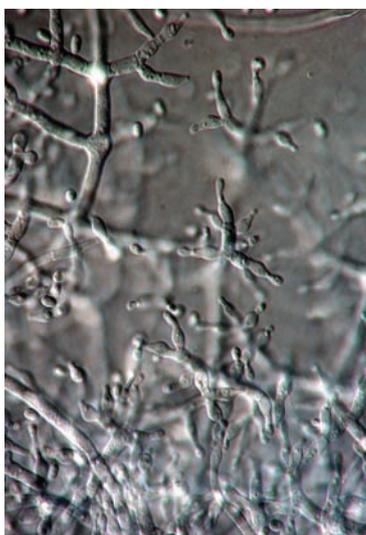
Odo van Vloten Stichting, Crous PW (member of board).

OECD (Organisation for Economic Co-operation & Development), Stalpers JA (Dutch representative on Biotechnology for Biological Research Centres).

Pan-African Medical Mycology Society (PAMMS), de Hoog GS (founder and co-organiser).

PhD Committee, Bovers M (2007). *Cryptococcus neoformans* and *Cryptococcus gattii*: speciation in progress. Utrecht University, November 9, 2007. (Boekhout T, co-promotor).





- PhD Committee, Driel KGA van (2007). Septal Pore caps in basidiomycetes – Ultrastructure and Composition. Utrecht University, December 17, 2007. (Boekhout T, co-promotor).
- PhD Committee, Frøslev T (2007). MISSING THESIS NAME AND DATE OF PROMOTION Copenhagen University (Eberhardt U, opponant).
- PhD Committee, Groenewald M (2007). Molecular characterization of *Cercospora beticola* and its relatives. University of Wageningen, Netherlands. February 19, 2007. (Crous PW, promoter).
- PhD Committee, Mostert L (2006) University of Wageningen, Crous PW (promoter).
- PhD Committee, Niskanen T (2007). MISSING THESIS NAME AND DATE OF PROMOTION Helsinki University (Eberhardt U, external examiner).
- PhD Committee, Roets F (2006) University of Stellenbosch, Crous PW (co-promoter).
- PhD Committee, Roohparvar R (2007). Drug transporters of the fungal wheat pathogen *Mycosphaerella graminicola*. PhD Committee, University of Wageningen, February 26, 2007. (Zwiers L-H, co-promoter).
- PhD Committee, Zalar P (2007). MISSING THESIS NAME AND DATE OF PROMOTION (Hoog GS de, co-promotor).
- PhD Committee, Zeng J (2007). Developing species recognition and diagnostics of rare opportunistic fungi. Amsterdam University, September 18, 2007. (Hoog GS de, promoter).
- PhD Committee, Zijlstra J (2006) University of Wageningen, Gams W (committee member).
- Proctor and Gamble, Cincinnati, U.S.A., Boekhout T (consultant).
- Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba, Japan, Hoog GS de (member of evaluation committee).
- Research School of Biodiversity, Crous PW (member of board).
- Studies in Mycology, Crous PW, Gams W, Samson RA, Summerbell RC (members of editorial board).
- Systematic and Applied Microbiology, Samson RA (member of the editorial board).
- The yeasts, a taxonomic study: 4th edition (expected 2008) Boekhout T (editor).
- UNITE database, Eberhardt U (contributor and group member).
- University Katsetsart Bangkok, Thailand, Samson RA (adjunct professor).
- University of Amsterdam, Hoog GS de (extraordinary professor).
- University of Pretoria, Crous PW (extraordinary professor).
- University of Stellenbosch, Crous PW (extraordinary professor).
- University of Wageningen, Crous PW (extraordinary professor).
- Willie Commelin Scholten Stichting, Crous PW (member of board).
- World Federation of Culture Collections (WFCC), Stalpers JA (member of board).

Software and Manuals

- Robert V (2007). BioloMICS Web Software. Version 3. Centraalbureau voor Schimmelcultures, September, Utrecht, The Netherlands.
- Robert V (2007). BioloMICS Software. Version 7.5. Centraalbureau voor Schimmelcultures, October, Utrecht, The Netherlands.
- Robert V, Stegehuis G (2007). MycoBank Web software. Version 3. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
- Robert V (2007). BioloMICS 7.5. manual and course. Version 1. Centraalbureau voor Schimmelcultures, December, Utrecht, The Netherlands, 160 pp.

CBS SEMINAR SERIES 2006

- January 2: Jamal Harrak, Katia Cruz, Sybren de Hoog - *Xophiala* species causing disease in cold-blooded animals.
- January 9: Eiko Kuramae - Phylogenomics reveal a robust Fungal Tree of Life.
- January 23: Richard Summerbell - DNA barcoding: a strategic overview.
- January 30: Mahdi Arzanlou - Molecular-based diagnostics in the Sigatoka complex disease of banana.
- February 6: Teun Boekhout - On the crossroad between biodiversity and comparative genomics.
- February 13: Ewald Groenewald - The value of molecular data for *Mycosphaerella* phylogenies.
- February 20: Marizeth Groenewald - Mating type genes in *Cercospora*. Does sex occur in apparently asexual species?
- March 27: Ferry Hagen - Where is the origin of the Vancouver Island *Cryptococcus gattii* outbreak?
- April 3: Jos Houbroken, Rob Samson & Jan Dijksterhuis - Applied research 2005: Diamonds in the dirt.
- April 24: Carlos Echavarrri-Erasun - The yeast *Xanthophyllomyces dendrorhous* a fun, colorful fellow.
- May 1: Lute-Harm Zwiers - Fungal transporters: to pump or to drown!
- May 15: Marjan Bovers - Welcome to the Wonderful World of *Cryptococcus*...
- May 22: Tino Ruibal - Advances in the phylogeny of the Chaetothyriales.
- May 29: Jingsi Zeng - Pathogenicity and antifungal susceptibility of clinical isolates of the genus *Exophiala* from North America and East Asia.
- June 12: Carlos Lopez-Quintero - Macro-fungal diversity in different types of forests in Colombian Amazonia.
- June 19: Gerard Verkleij - Challenges and opportunities in coelomycete taxono-

my.

- June 26: Jan Dijksterhuis - A volatile self-inhibitor of *Penicillium paneum*.
- Juli 3: Cecile Gueidan - Molecular phylogeny of the Verrucariales (lichenized ascomycetes) and evolution of lifestyles in the Chaetothryiomycetidae.
- September 4: Janos Varga - DNA barcoding of *Aspergillus* species The first steps.
- September 11: Bart Theelen - Digging up *Candida*.
- September 18: Sybren de Hoog - Arctic glaciers: A hot spot for fungal speciation.
- September 25: Eiko Kuramae - Conflicting phylogenetic position of *Schizosaccharomyces pombe*.
- October 2: Wolf Becker - AFLP diversity in *Scedosporium*.
- October 9: Ursula Eberhardt - Species delimitation and biogeography in the *Xerocomus subtomentosus* complex.
- October 16: Mahdi Arzanlou - Sex Evolution in *Mycosphaerella*.
- October 23: Kenneth van Driel - Overview of 4 years of septal pore cap research.
- October 30: Ewald Groenewald - Towards a better understanding of the genus *Mycosphaerella*.
- November 6: Marizeth Groenewald - Molecular characterization of *Cercospora beticola* and its relatives: an Overview.
- November 13: Teun Boekhout - Functional biodiversity in *Cryptococcus neoformans*.
- November 27: Shuwen Deng - Molecular genetic studies of *Cdc42-1* in black yeasts.
- December 11: Hamid Badali - New agents of chromoblastomycosis.
- December 18: Maikel Aveskamp - A primer on *Phoma* systematics.

CBS SPECIAL SEMINARS 2006

- March 20: Corné Klaassen (Molecular Biology Unit, Canisius Wilhelmina Hospital, Nijmegen) - Exact fungal genotyping.
- May 8: Bas Dutilh (Center for Molecular and Biomolecular Informatics, - - Radboud University Nijmegen) - From phylogenetics to phylogenomics at successive levels: super- and orthology approaches tested on the Fungi.
- November 20: Artur Alves (Department of Biology - University of Aveiro - Portugal) - Phylogenetic relationships in *Diplodia* and *Lasiodiplodia*.
- December 4: Hermann Voglmayr (System & Evol. Botany, Univ. Vienna, Austria). - Aeroaquatic fungi - fungi from amphibious habitats.

CBS SEMINAR SERIES 2007

- January 15: Timon Wyatt - PLAY- a factor in heat resistance of *Talaromyces macrosporus*?
- January 29: Lute-Harm Zwieters - The Twin

- Peaks of *Mycosphaerella fijiensis*
- February 5: Richard van Leeuwen - Conidia of *Penicillium discolor* display an ergosterol enriched cap at the site of polarity establishment and elongation.
- February 19: Laura Selbmann, G.S. de Hoog, C. Ruibal, L. Zucchini, S. Onofri - Drought meets acid: new genera in two dothidealean subclades of extremotolerant fungi
- February 26: Marjan Bovers - *Cryptococcus neoformans* & *Cryptococcus gattii*; a story of nuclear and mitochondrial genes
- March 5: Ewald Groenewald, Biological Safety Official ("BVF") - Biosafety at CBS
- March 12: Rob Samson - *Aspergillus* and Peanuts
- April 16: Jan Dijksterhuis - Cytoplasmic parameters in fungal spores
- April 23: Janos Varga - Outcomes of the *Aspergillus* workshop: New taxa, new ideas
- May 14: Gerard Verkley - Quality Management for CBS Culture Collections
- May 21: Bert Gerrits van den Ende - Modeltest
- June 11: Ursula Eberhardt - DNA barcoding in and outside of CBS
- June 18: Mahdi Arzanlou - Secret of Sex in Banana Field; Sigatoka Disease Complex (Part II)
- June 25: Vincent Robert - The quest for the best genes
- July 9: Joost Stalpers - Mycobank and species banks, prospects and problems
- September 3: Grit Walther - Molecular and morphological species recognition and clinical relevance of the ink cap family (Psathyrellaceae)
- September 10: Ewald Groenewald - Precies zoals het hoort / Exactly right
- September 17: Paramee Noonim - Ecology of ochratoxin-producing fungi from Thai coffee beans
- September 24: Maikel Aveskamp - *Phoma* on a tree: Phylogenetic relationship of the genus and related coelomycetes
- October 8: Gerrit Stegehuis - MycoBank: status and developments
- October 15: Jos Houbraken - Ascospore formation in the heat resistant fungus *Paecilomyces variotii*
- October 22: Bart Theelen - Microarray applications in comparative mycology
- October 29: Henk Brouwer - Interspecific hybridization in *Phytophthora*
- November 5: Gavin Hunter - Global movement and population biology of the Eucalyptus leaf pathogen *Teratosphaeria nubilosa*
- November 12: Richard van Leeuwen - Shedding light on fungal endocytosis
- November 26: Josef Kaltseis, Johannes Rainer & Sybren de Hoog = Accumulation of *Pseudallescheria* and *Scedosporium* in urban areas - the promotion of pathogenic fungi by humans.
- December 3: Hansong Ma (Molec. Pathobiology, University of Birmingham, UK) -

Macrophage 'hijacking' by the human pathogen *Cryptococcus*

- December 10: Marcela Satow - Understanding selective method for black yeast isolation
- December 17: Ferry Hagen - Micro-satellite analysis as a tool to trace the origin of the *Cryptococcus gattii* Vancouver Island outbreak,

CBS SPECIAL SEMINARS 2007

- January 22: Jos Vaessens (Head of the IT Department) - Facelift for the IT Infrastructure at CBS/NIOB. Upcoming changes to the networking environment, reasons and results.
- March 26: Fons J. Verbeek (Leiden Institute of Advanced Computer Science Leiden University) Classification of yeast cells from image features; evaluation of pathogen conditions
- April 2: Barbara Dunn (Dept. of Genetics, Stanford Univ. Med. Center) - Genome rearrangement differences among hybrid lager yeasts revealed by microarray karyotyping correlate with DNA sequence and phenotype differences.
- November 19: Keith Seifert (Agriculture and Agri-Food Canada) - The Genera of *Hyphomycetes* - A Tour.



Contract Research and Services

Food and Feed Mycology

Heat-treated products: Heat resistant fungi are moulds which can survive a pasteurization step and therefore cause spoilage in heat treated products. The source of contamination can often be traced back to the (raw) ingredients, which are associated with soil. Many samples were received in 2006 and 2007 for the analyses on the presence of heat resistant fungi. The majority of these were samples pectin (61), which is used as an ingredient in various food products. In most cases no heat-resistant fungi could be detected in the pectin; occasionally, *Byssochlamys nivea* was isolated. Also other samples were analysed, such as coconut milk and related (raw) ingredients (13), sports drinks (12) and water ice (2). The most frequently encountered species were *Neosartorya glabra* and *Byssochlamys nivea*. The presence of the fungus *Lasiodiplodia theobromiae* in the heat treated coconut milk was remarkable and studies have been conducted to determine the degree of heat resistance.

Vegetables and fruits: Various fruit samples were analyzed, including several samples pear,

where the rot causing fungus could be identified as *Cadophora luteo-olivacea*. Furthermore, avocados with *Colletotrichum gloeosporioides* and mandarins with the blue and green mould *Penicillium italicum* and *P. digitatum* respectively, were received for analyses. Also different types of melons were investigated. Fungal growth was occurring on these melons during the refrigerated transport from South-America to Europe. The predominant fungi on these melons were *Cladosporium* species, *Alternaria* species and species belonging to the *Fusarium equiseti* and *F. chlamydosporum* complex.

Bakery products: Many different bakery products, like noodles, (imitation) almond paste, rye bread, (energy) bars, tortillas and modified-atmosphere-packaged (MAP) bread were analysed. As expected, *Penicillium roqueforti* was mostly occurring on products containing preservatives like sorbate or acetate, members of the xerophilic genus *Eurotium* were often detected on products with low water activity, and the chalk fungus, *Endomyces fibuliger*, was frequently encountered of MAP bread. However, also other species were detected

on some bakery products, like the xerophiles *Chrysosporium xerophilum*, *Aspergillus penicillioides* and *Wallemia sebi* on energy bars or *Monascus ruber* and various *Penicillium* species (eg *P. crustotum*, *P. paneum*, *P. griseofulvum*, *P. polonicum*) on tortillas. In order to prevent fungal growth on the tortillas, different packaging systems and recipes were challenged by inoculation of different fungi on the tortillas.

Dairy products: Various dairy products, including butter, cheese, yoghurts and drinks were investigated. On the investigated butter samples *Cladosporium herbarum*, *Epicoccum nigrum* and *Penicillium* species were often detected. The natamycin resistant fungus *Penicillium discolor* was often detected on the outer surface of hard cheeses, while yeasts, *Geotrichum candidum* and *Penicillium* species were present on the parts where the cheese was cut. The presence of fungi on these cheeses was often due to low hygienic conditions during manufacturing.

Feed: Pelleted feed was received on several occasions and the extreme xerophile *Xeromyces bisporus* was often detected. Besides this species, also other less xerophilic fungi, like *Eurotium* and *Aspergillus penicillioides*, were detected. Silage samples were investigated and *Penicillium roqueforti* was detected. *Fusarium verticillioides* (= *Gibberella moniliformis*) was detected in samples chicken feed.

Surveys, audits and expert reports

The majority of the surveys have been conducted in bakeries producing modified-atmosphere-packaged bread. The reason for these surveys was because (small) white spots occurred on bread before the end of the shelf life. These spots were caused by the chalky mould *Endomyces fibuliger*. The surveys showed that this chalky mould is commonly occurring in ingredients, air and on surfaces of the production process before the baking step. This should not cause any problems, since the baking process serves as a





decontamination step and after the baking the fungal load of product is very low. However, in the surveyed bakeries the chalky mould was also detected in the air and/or on certain surfaces after the baking process. Improving the hygienic situation and/or application of sterile air, reduced the degree of spoilage.

Expert reports and audits were made for various companies. Literature searches were performed on the biodegradation of hard synthetic materials like polyethylene and expert opinions were given in legal cases.

Indoor Mycology

Fungi in indoor environments: Numerous samples of building materials such as wallpaper and



plaster were examined, as well as wall scrapings. In addition, swab samples and cellotape impression samples from indoor surfaces were received. Samples came from museums, archives and private dwellings. About 25 wood samples were analyzed for the presence of wood rot fungi. In only three cases the extremely damaging dry rot fungi *Serpula lacrymans* or *Coniophora puteana* was detected. In other cases *Phellinus contiguus*, *Asterostroma cf. cervicolor*, *Postia* species, *Perenniporia medulla-panis*, *Peziza domiciliana*, *Asterostroma cervicolor* and *Antrrodia serialis* were detected.

In 2006 and 2007 many surveys in indoor environments were performed. These surveys were conducted in 34 private dwellings and 15 museums, depots and archives. The main reason for surveying dwelling was because of health complaints of the residents and these surveys were mainly conducted in collaboration with the public health services, property rental companies and building and construction companies. Sensitized individuals, particularly asthmatic individuals, might experience strong allergic reactions in contact with high fungal allergen levels. In various cases high numbers of viable fungal particles were detected in the air, and often no visible mould growth could be observed in the surveyed houses. The presence of crawling spaces with poorly isolated wooden floors linked all these dwellings and in many cases the same mycobiota could be detected in the crawling spaces and living areas. In contrast, these high numbers

of viable fungal particles were not present in dwellings with concrete floors where a good separation between the crawling space and the indoor house environment existed or in dwellings without crawling spaces (apartments). Remarkable was that the detected mycobiota in the dwellings with crawling spaces, was deviating from what is usually detected in other dwellings in the Netherlands and *Aspergillus ochraceus*, *A. versicolor*, *Tritirachium* species and *Sistotrema brinkmanii* were predominating in these airs.

The main reason for surveying museums, storage depots or archives was the possible presence of mould growth on objects or walls. In many cases this mould growth was also observed, however, also sometimes no fungi could be detected and dust or crystals were the reason of this "mould growth". The xerophilic moulds *Aspergillus restrictus*, *A. penicillioides* and/or *A. versicolor* were often present in archives with poor temperature and/or humidity control, while other species like *Chaetomium globosum* or *Stachybotrys chartarum* were more predominant in building where water leakages had occurred. In one case also extremely high numbers of *Paecilomyces variotii* were detected in the air of a museum. The source of this fungus was wood chips used in art object which was exhibited in a museum. As a consequence of this heavily moulded object, extremely high numbers of fungal propagules could be detected in this museum. By replacing the wood chips for grit, numbers dropped within a few days and were back to normal levels.

Applied research

Many experiments were performed to establish the effect of preservatives on tulip bulbs. Tulip bulb rot is often caused by *Fusarium oxysporum*, *Penicillium hirsutum*, *P. tulipae* or *Aspergillus niger*. Both laboratory and field tests were conducted. Also investigations have been carried out to study the effect of UV light in combination with ozone (dry disinfection). These investigations were carried out in collaboration with a bakery and the manufacturer of these systems. In 2006 and 2007 investigations have been performed in order to test the effect of gamma irradiation on the fungi *Aspergillus versicolor*, *A. niger*, *Chaetomium globosum* and *Eurotium herbariorum*.

Mycotoxin analyses

Fungi are used for the industrial production of enzymes or acids and therefore should not produce mycotoxins. Twenty-three fungal strains (mainly *Aspergillus niger* and *A. oryzae*) were screened for toxin production and 12 fermentation broths and concentrates were investigated in 2006 and 2007. This research was performed in collaboration with Biocentrum, Danish Technical University, Lyngby, Denmark.

Miscellaneous

Many other projects were conducted in 2006 and 2007. Spore suspensions of different fungi were prepared and biomass of fungi was produced for an allergen producing company. Also a company, which grows plants in-vitro, had problems with fungal contamination in their agar cultures. Their main problem causing fungus was *Nigrospora sphaerica* and an on-site survey showed that this fungal contamination was spread by mites.

In collaboration with the Dutch Food and Consumer Product Safety Authority, samples of tattoo paint were investigated for moulds. High numbers of moulds were detected, predominantly consisting of *Fusarium solani*, *Aspergillus sydowii* and *Scopulariopsis brevicaulis*.



Fungal courses

- Medical Mycology course (2006 and 2007). This course was attended by more than 75 participants from all over the world.
- Introduction to Food and Airborne Fungi Course (2006 and 2007). This course was attended by participants from China, Germany, Italy, Hungary, Sweden, Belgium, and the Netherlands. The course was also given in 2006 and 2007 in Ottawa in collaboration with Agriculture Canada.

Special one-day courses were organized for two bakeries and a dairy industry.

A three-day course intended for industrial hygienists and others working in indoor environments (hospitals, building industries, analytical labs, etc.) was given in Stuttgart in collaboration with the Landesgesundheitsamt. A short mycology course on indoor fungi was given in Lübeck, Germany in 2006.

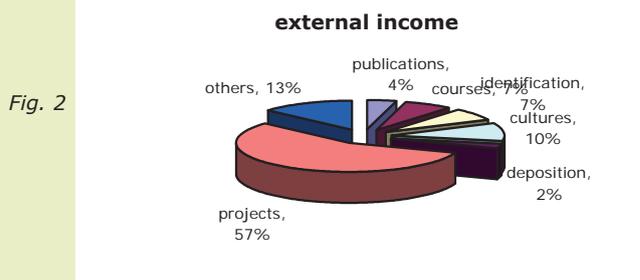
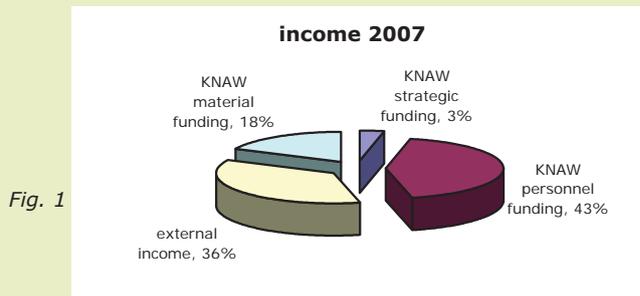


Finances and Staff

Income

The Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre (CBS-KNAW) has a total income of 4.1 million Euros (Fig. 1). Approximately 69 % of this amount is KNAW funding. A further subsidy recently obtained from the KNAW strategic-fund is destined to be used for initiating innovative research projects. CBS has chosen to establish a DNA bank (NL-Bank), and to strengthen its DNA Barcoding projects by appointing a third post-doc to barcode the type strains of the CBS collection.

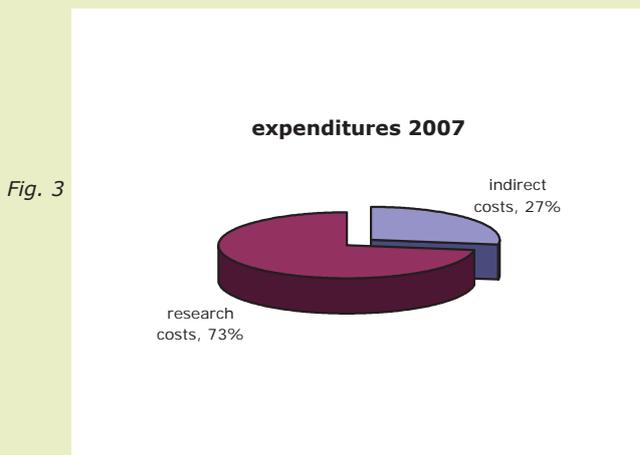
The external income (Fig. 2) of 1.3 million euros is profit earned mainly from research projects and regular activities, such as the sale of books, courses and the training of scientists and students, identification/sales of fungi and bacteria. The "Odo van Vloten" Foundation currently finances four Ph.D. research projects.



Expenditures

The total costs of the CBS-KNAW consist mainly of salaries (70 %). Non-personnel costs are costs of materials and depreciation of durable equipment.

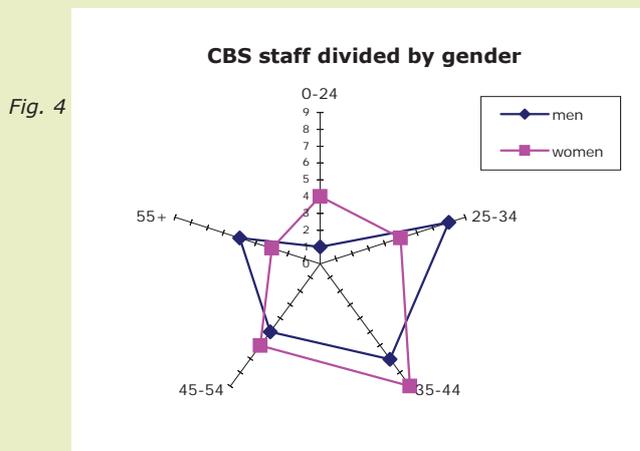
Three-quarters of the expenditures (Fig. 3) can be indicated as direct research costs. Indirect costs are for management and for the collective supporting division of the CBS-KNAW and its neighbouring institute, the Hubrecht Laboratory (HL).



Staff

The CBS had 53 employees on January 1 2006, with an equivalent of 46,1 full-time staff (fte). The staff consists of 23 researchers and 30 analytical/technical support staff. A considerable proportion of the support staff is involved in the applied research division, fungal preservation, and digitalisation of data pertaining to the collection. Approximately 20 additional persons, guest researchers, researchers with an official appointment other than the CBS and students have been working at the CBS. Within CBS 53 % of the employees are female, and 47 % male (Fig. 4).

The collective support division CBS/HL employs 27 people with a full-time equivalent of 24,1 fte, with approximately 7,8 fte effectively working for the CBS.



CBS staff (2006–2007)

Directie	Crous PW Koelman JBJ Verweij MJ	Groep Crous	Crous PW Aveskamp MM Brouwer H Gerritzen CHA Groenewald JZ Iperen van AL Linden van der M Merwe van der M Willemse M Woudenberg JHC Zwiers LH
Groep Samson	Samson RA Dijksterhuis J Doorn van TM Houbraken JAMP Leeuwen van MR Meijer M Varga J		
Collectie	Stalpers JAJM Verkleij GJM Claus FB Eberhardt U Epping WWM Figge MJ Groenewald M Haalem van G Hendrikse ED Holtman J Kleyn D Kuyt CJ Linden van der R Merkx BPM Mul E Nooijer de ABE Robert VARG Setropawiro M Snippe J Stegehuis GJ Vlug YA Wilde de NR	Groep De Hoog	Hoog de GS Gerrits Van Den Ende AHG Luijsterburg KF Walther G
		Bibliotheek	Vermaas MT
		Support services	Freund ECA Velzen van DAJ Bruin de JCM Kroon van der TJ Maas ASSM Norbruis J Raghoebir S Versluis I Yamini S Geers WNM Have van der AJ Heinen J Lagemaat van de G Vaessens GJ Duivenbode van EW Rodriguez Seco JA Verboekend RS Davids R Deel JL Domselaar van ME Voorst van R Brantsma L Breul van den AL Vermeulen RC Waals van der R
Service Unit	Cock de AWAM Ketelaar AMH Visser CJ		
Groep Boekhout	Boekhout T Echavarri-Erasun C Hagen F Boesten R Quast QJ Theelen BJF Wever C		

CBS Publications (2006–2007)

Studies in Mycology:

Studies in Mycology is an international journal that publishes systematic monographs of filamentous fungi and yeasts, and on occasion the proceedings of special meetings related to all fields of mycology, biotechnology, ecology, molecular biology, pathology and systematics. Since 2004, it has been an open-access journal that is freely available on the internet, though the hard copy version is still available reasonably priced. The journal now has a full colour format, and is directly linked to MycoBank, with all papers linked to strains in the CBS collection that are available to the international scientific community. (<http://www.studiesinmycology.org>).

SIM 59: *Aspergillus* systematics in the genomic era - Robert A. Samson, Janos Varga (editors): 206 pp., 2007.

SIM 58: The genus *Cladosporium* and similar dematiaceous hyphomycetes - Pedro W. Crous, Uwe Braun, Konstanze Schubert and Johannes Z. Groenewald (editors): 253 pp., 2007.

SIM 57: Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi - Gi-Ho Sung, Nigel L. Hywel-Jones, Jae-Mo Sung, J. Jennifer Luangsa-ard, Bhushan Shrestha and Joseph W. Spatafora 63 pp., 2007.

SIM 56: *Hypocrea* and *Trichoderma* studies marking the 90th birthday of Joan M. Dingley - W. Gams (editor): 177 pp., 2006.

SIM 55: 100 Years of Fungal Biodiversity in southern Africa - Pedro W. Crous, Michael J. Wingfield, Bernard Slippers, Isabella H. Rong and Robert A. Samson (editors): 305 pp., 2006

SIM 54: Taxonomy and Pathology of *Togninia* (*Diaporthales*) and its *Phaeoacremonium* Anamorphs. - Lizel Mostert, Johannes Z. Groenewald, Richard C. Summerbell, Walter Gams and Pedro W. Crous: 115 pp., 2006.

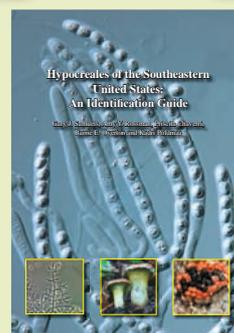
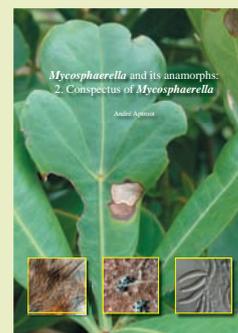
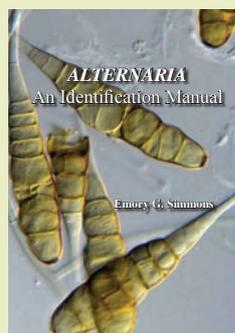
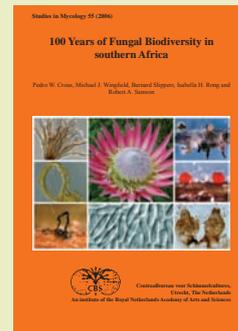
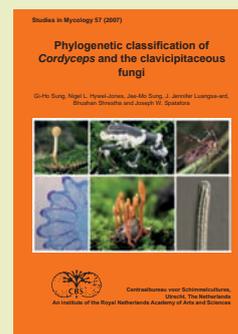
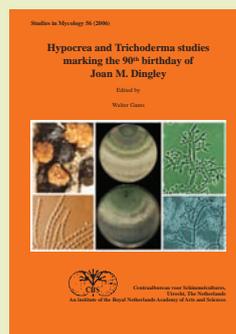
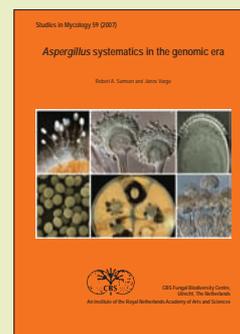
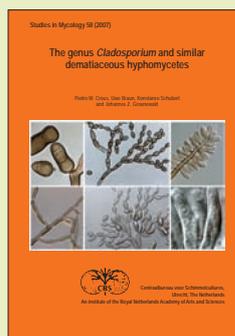
CBS Biodiversity Series:

The CBS Biodiversity Series is an international publication on filamentous fungi and yeasts and publishes systematic monographs related to all fields of mycology including biotechnology, ecology, molecular biology, pathology and systematics.

CBS Biodiversity Series 6: *Alternaria*. An Identification Manual - Emory G. Simmons: 775 pp. 288 B&W Line Drawings. (A 4 format), hardcover, 2007.

CBS Biodiversity Series 5: *Mycosphaerella* and its anamorphs: 2. Conspectus of *Mycosphaerella* - André Aptroot: 231 pp., 2006.

CBS Biodiversity Series 4: Hypocreales of the Southeastern United States: An Identification Guide - Gary J. Samuels, Amy Y. Rossman, Priscila Chaverri, Barrie E. Overton and Kadri Pöldmaa: 145 pp., 2006.



Popular Scientific Activities

Moulds and mycology have been subject of many articles in the press and the research at the Fungal Biodiversity Centre has attracted much interest. The CBS website has also attracted much attention. In 2006-2007 the site has been visited numerous times with an average page view per day of about 12.000. Many visitors consult the CBS collections and databases, while the PDF's of the Studies in Mycology are often downloaded.

Conferência Desteque do Infocus 2007

Prof. Dr. Flavio de Queiroz Telles*

*Professor Adjunto de Engenharia de Alimentos - Hospital de Clínicas, Universidade Federal do Paraná - Curitiba

Conforme realizado na edição anterior, o Infocus 2007, fórum de reflexões acerca da prática científica, teve entre seus convidados internacionais o Prof. Sylvain de Hoog, que fez palestra a Conferência Magna de abertura, intitulada "The Ecology of the Pathogenic Fungus". Sylvain de Hoog é professor e atual Presidente do Fórum de Cientistas para o Desenvolvimento da Comunidade para o Desenvolvimento da Comunidade (CBS) e Professor do Instituto de Biotecnologia e Ordenação de Ecossistemas, na Universidade de Amsterdam, Holanda. Sua produção científica inclui 310 artigos internacionalmente indexados, principalmente relacionados à área de taxonomia e biologia molecular de fungos, principalmente de fungos negros ou dematiás. A maioria de seus trabalhos relaciona-se à epidemiologia e origem de fungos patogênicos.



Prof. Dr. Flavio de Queiroz Telles

O Centraalbureau voor Schimmelcultures ou CBS, localizado em Utrecht, próximo a Amsterdam, é uma renomada instituição internacionalmente conhecida por manter uma grande coleção de referências de fungos filamentosos, leveduras e bacterias. Seu principal foco de pesquisa inclui, além da taxonomia de fungos, sua ecologia e adaptação a diversos ecossistemas, incluindo animais, vegetal e ambiental. Aproximadamente 100 espécies de fungos são mantidas em sua coleção de referências de fungos filamentosos e dematiás. O CBS atualmente emprega cerca de 60 pessoas. 17 áreas científicas de vasta produção científica.

Sua coleção de microrganismos compreendendo a cerca de 60.000 espécies, representando a maioria das amostras de

todos os grupos de fungos, principalmente de fungos negros ou dematiás, do mundo. O CBS é um banco de dados de mais atualizadas informações micológicas disponíveis na atualidade.

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bioSCOPE

Missie: Schimmels in kaart brengen

door Liesbeth Bouter en Lucien Scholten

Het Centraal Bureau voor Schimmelcultures (CBS) van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.



In het lab

Van nature is het CBS een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.

Ghostbuster

door Liesbeth Bouter en Lucien Scholten

Het Centraal Bureau voor Schimmelcultures (CBS) van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.



In het lab

Chiquita met schimmel

door Liesbeth Bouter en Lucien Scholten

Het Centraal Bureau voor Schimmelcultures (CBS) van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.



In het lab

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Haarrooschimmel eet vet op de huid

door Liesbeth Bouter en Lucien Scholten

Het Centraal Bureau voor Schimmelcultures (CBS) van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.



In het lab

16

16

EXTRACT

Haarrooschimmel leeft van huidvet

Een schimmel (*Malassezia globosa*) die een rol speelt bij het ontstaan van haarroos, leeft van huidvetten. De schimmel kan zelf niet de benodigde vetzuren maken, maar beschikt wel over een serie vetzuchtende enzymen waarmee hij op de hoofdhuid zijn kostje bij elkaar kan scharrelen. Dat blijkt uit een onlinepublicatie van diverse onderzoekers uit verschillende landen in het Amerikaanse tijdschrift *Proceedings of the National Academy of Sciences* (PNAS). Al heel lang is bekend dat de schimmel betrokken is bij haarroos, maar hoe dat precies in zijn werk gaat, was niet duidelijk. De onderzoekers hebben nu de complete genomvolgorde van de schimmel onttrafd. Daaruit blijkt dat hij enzymen uitscheidt waarmee hij vetzuren van de hoofdhuid kan gebruiken voor zijn eigen groei. Uit het onderzoek blijkt verder dat er verrassende overeenkomsten zijn met andere bekende huidschimmels, de *Candida albicans*. Deze is geen directe familie, maar produceert eveneens vet splitsende enzymen. Bij het onderzoek waren ook Nederlandse onderzoekers betrokken: Teun Boekhout, verbonden aan onder meer het Centraalbureau voor Schimmelcultures (CBS) van de Koninklijke Nederlandse Akademie van Wetenschappen in Utrecht, en Eiko Kuramae, die eveneens aan het CBS is verbonden en werkt voor het Nederlands Instituut voor Ecologie in Heteren. Aan de studie werd ook meegewerkt door onderzoekers van Procter & Gamble in Cincinnati (VS), fabrikant van antiroosshampoo.



Koninklijke Nederlandse Akademie van Wetenschappen

Uitgeverij: Koninklijke Nederlandse Akademie van Wetenschappen

Website: www.knaaw.nl

Telefoon: +31 (0) 43 38 70 00

Fax: +31 (0) 43 38 70 01

E-mail: info@knaaw.nl

Zoeken in nieuws

Zoek

Tijd voor overzicht

19-10-2006

Reconstructie van de vroegste evolutie van schimmels op basis van een gen

Op 19 oktober 2006 zijn in het tijdschrift *Nature* de resultaten gepubliceerd van het Fungal Tree of Life project (FTAL). Dit project is een internationaal samenwerkend team van onderzoekers van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) en de Universiteit van Amsterdam (UvA).

Aan de samenstelling van de Fungal Tree of Life heeft onder meer de Nederlandse mycoloog Teun Boekhout van het Centraalbureau voor Schimmelcultures van de KNAW in Utrecht, geleidde meerdere jaren geleden. De onderzoekers hebben nu de complete genomvolgorde van de schimmel onttrafd. Daaruit blijkt dat hij enzymen uitscheidt waarmee hij vetzuren van de hoofdhuid kan gebruiken voor zijn eigen groei.

Het lab van de CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.

Taakomschrijving van de CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld. Het CBS is een van de meest bekende en meest uitgebreide culturele instituten ter wereld.

Informatie over het CBS-KNAW vindt u op de website.

Personenlijst van de CBS

RESOURCE

30 SEPTEMBER 2007

SCHIMMEL MOET AF VAN DUF IMAGO

Willem Koert

Zieke gebouwen, extremofiele en de volgende fase in de menselijke evolutie. Het zijn thema's in het vak *Magical mushrooms, slayers and sex*. De cursus moet een ondergeschoven kindje in de wetenschap voor het voetlicht halen. Schimmels zijn heel veel, maar niet - de extremofiele uitgedroerd - saai.

De grootste extremisten die de evolutie op onze planeet heeft voortgebracht leven op Antarctica. Daar bij temperaturen van tientallen graden onder nul, voelen sommige schimmels zich het best. Vertelt dr. Sylvain de Hoog van het Centraal Bureau voor Schimmelcultures (CBS). Ze leven een centimeter diep in rotsen, tussen de kristallen die ze beschermen tegen de ergste kou. Om te overleven verloopt hun stofwisseling zo traag mogelijk. Ze hebben twintig jaar nodig om een miljoenste meter te groeien!

Biologen noemen organismen die onder zulke extreme omstandigheden kunnen groeien extremofiele. Nu ruimtevaartorganisaties werken aan missies naar Mars, bloeit de belangstelling voor extremofiele op. Als je leven op Mars voorbikt, dan zou dat wel eens kunnen lijken op de taai schimmels op Antarctica. Maar wie denkt dat die extremofiele over opwindende eigenschappen beschikken, komt bedrogen uit. "Eigenlijk kunnen ze niet zoiets", zegt Dr. Hoog. "Fabrikanten van wasmiddelen hebben wel eens gedacht dat zulke schimmels interessante enzymen kunnen aanmaken die je kunt gebruiken voor wasmiddelen die op een lage temperatuur werken. Maar een project dat de enzymen moest vinden liep op niets uit. In de hondert miljoen jaar dat de extremofiele hebben overleefd zijn zij slechts vrede hebben geproceerd in het overleven onder barre omstandigheden, hebben ze alles opgeofferd wat ze niet strikt nodig hebben. Als je het DNA van extremofiele vergelijkt met dat van verwante maar normale schimmels, zie je dat er bij extremofiele heel sequenties zijn verdwenen. Strikt genomen zijn extremofiele tamelijk saai. Ze kunnen vooral heel veel niet!"

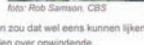


Foto: Rob Samman, CBS