



PERSOONIAL Reflections

Editorial Note

A major aim of *Persoonia* is to link fungi to their environment, i.e. to the ecosystems where they occur. Each year mycologists describe around 800–1400 novelties. However, little thought goes to where these fungi were collected. Where are we going to find the remainder of the fungi awaiting description? *Persoonia* aims to highlight the world's incredible fungal diversity, and thus emphasize the importance of supporting fungal biodiversity research. Mycologists are encouraged to submit an environmental picture along with their manuscript. Submitted pictures are to be linked to a paper that appears in the same issue. To be considered, pictures are to be CMYK, 300 dpi, 26.4 cm

wide and 17.6 cm high. You and other mycologists are invited to contribute to this venture.

Editors-in-Chief:

Prof. dr PW Crous
CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT
Utrecht, The Netherlands.

Dr ME Noordeloos
National Herbarium of the Netherlands, Leiden University
branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands.

Book Reviews

Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ. 2008. *Microfungi occurring on Proteaceae in the fynbos*. CBS Biodiversity Series 7. Pp. 166; 93 colour plates, 6 black & white plates, hard cover. Price 50 €. CBS Fungal Biodiversity Centre, ISBN: 978-90-70351-71-7.



This lovely colourful book results from several years work on the fungi in the fynbos which is a unique fire-prone shrubland habitat in South Africa with high plant diversity and a high degree of floral endemism. This book deals with fungi on the Proteaceae which themselves are rather unique stout plants with persistent flowerheads. The book comprises an Introduction which deals with location, the Fynbos, fire, the plants, studies on microfungi and the aims of the study. The latter being to establish whether the diversity of microfungi is similar to the high diversity of plants. There is a Table of plant pathogenic fungi on Proteaceae followed by a list of fungi known to occur on Proteaceae prior to this study.

The materials and methods section lists the study area and host plants, laboratory procedures, and biodiversity analyses. This includes some nice colour photographs of the plants studied. The main section of the book deals with the 141 fungal species found and their taxonomy. There are 59 species and two genera which are new to science, eight of which have previously been published, while 38 of the species collected are new records for South Africa and 48 new to the Proteaceae. The entry for each new species comprises the MycoBank number, a reasonably detailed description and a nice colour plate detailing most characters. In most cases there is a fairly lengthy discussion on the genus and justifying the introduction of the new species.

There is a lot of data here. The authors have carefully identified their taxa and obtained references to justify their naming of taxa or new species. This is a very important book for anyone dealing with ascomycetes and their anamorphs as the taxa are well illustrated and will serve to gain a better understanding of genus concepts.

The final part deals with the diversity of microfungi on fynbos Proteaceae. This is an interesting part and adds further data concerning the ecology and diversity of microfungi which have generally been poorly studied.

As with all books coming out of CBS these days this book is good. It is very well illustrated with colour microphotographs and well written text. The detailed discussion of genera and additions of new species or illustrations of known species will add significantly to our understanding of microfungi and because of this all mycologists should see this book.

I love this book. A huge amount of work has gone into compiling this book and it should be in the library of all mycologists for its wealth of information. The book should be available in all Universities and colleges and any research institute where research in any aspect of mycology is carried out or where mycology is taught.

K.D. HYDE

Faasse PE. 2008. *In splendid isolation: A history of the Willie Commelin Scholten Phytopathology Laboratory 1894–1992*. History of Science and Scholarship in the Netherlands 11. Pp. 296; 40 black & white plates, hard cover. Price 40 €. KNAW Press, www.aksant.nl, ISBN: 978-90-6984-541-8.



Compared to a sick human being, a diseased plant is a simple object. You can cut it into pieces, examine parts of it under a microscope and perform all sorts of experiments with it, without the need for physical precautions or compassion. In other respects, however, physicians who deal with human diseases have an easy life. 'You physicians have only one species of patient, we phytopathologists have hundreds. Today we might be looking at a lily-of-the-valley, tomorrow an elm tree, the next day Java coffee,' explains one of the phytopathologists in this book.

For almost a hundred years, the Willie Commelin Scholten Phytopathology Laboratory (WCS) was the hub of phytopathology research in the Netherlands. Hundreds of students learned the principles of plant pathology there. The laboratory diagnosed and researched dozens of plant diseases, and its scientific reputation spread far beyond the country's borders. *In Splendid Isolation* reconstructs the history of this unique institution, from its beginnings as a small private laboratory in the late nineteenth century to its final days as a renowned university research institute.

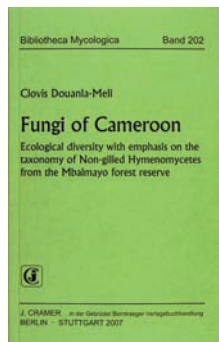
What will prove especially interesting to mycologists is the historic account of the humble beginnings of the Centraalbureau voor Schimmelcultures, which was initially interwoven with the WCS, and eventually became a separate institute in its own right. The appointment of Johanna Westerdijk as director, who was to become the first female professor in the Netherlands, the difficulties experienced in safeguarding the culture collection during World War II, the discovery of Penicillin, research focused on elucidating a devastating disease of elms, later to be known as Dutch elm disease, and much, much more.

In Splendid Isolation provides an interesting overview of the early years of CBS with Westerdijk, Hugo de Vries, Bea Schwarz, Christine Buisman, van Luyk, Van Beyma toe Kingma, and others. About Westerdijk: 'Anecdotes frequently cite her booming laugh, her love for parties, drinking and dancing, her distaste for marriage and other pointless conversations, her short-sightedness, and her expressive eyes, whether mocking, interested or full of sympathy.'

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New Titles in Mycology

Douanla-Mel. C. 2007. *Fungi of Cameroon. Ecological diversity with emphasis on the taxonomy of Non-gilled Hymenomyces from the Mbalmayo forest reserve*. Bibliotheca mycologica 202; J. Cramer, Berlin-Stuttgart. Pp 410; 172 line drawings. ISBN 978-3-43-59104-5. Price € 89.



This book describes in detail the Aphylophorales of the Mbalmayo forest reserve in Cameroon. 271 species have been recognised, including eleven being new to science. Keys are given to families, genera and species, as well as detailed descriptions and line drawings, habit and distributions data, as well as taxonomic notes. This work provides many new data from the African continent, where this kind of meticulous and detailed studies on Aphylophorales are very much needed.

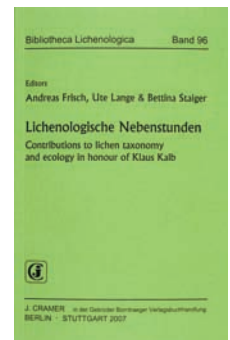
Vánky K, Shivas RG. *Fungi of Australia. The Smut Fungi*. CSIRO Publishing/ABRS. Pp 276 + CD-Rom. ISBN 978-0-643-09536. Aus\$ 130.



Dr. Vánky is the world's leading specialist in smut fungi and author of several monographs. The present publication, with Shivas as co-author, gives an account of the smut fungi of Australia. A key to the genera is followed by an alphabetical arrangement of generic descriptions and keys to the species per host plant. Distributional data are also given. The accompanying CD-Rom contains an interactive identification

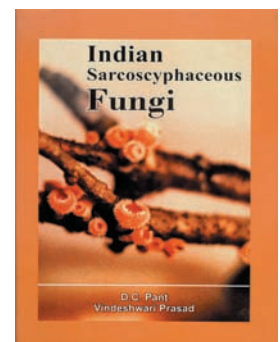
key and a wealth of illustrations, fact sheets and distributions maps. A very useful overview of this economically important group of fungi.

Frish A, Lange U, Staigerr B. 2007. *Lichenologische Nebenstunden. Contributions to lichen taxonomy and ecology in honour of Klaus Kalb*. Bibliotheca lichenologica 96. J. Cramer, Berlin-Stuttgart. Pp 343. ISBN 978-3-443-58075-9. Price € 74.



This festschrift for Klaus Kalb contains twenty-seven contributions by fifty-one authors devoted to lichenology, in a wide-range of topics from morphological-taxonomic, ecological to molecular studies, covering various parts of the world in particular the tropics (Indonesia, Thailand) and the Southern Hemisphere. A number of new species is described, nine of which in honour of Klaus Kalb. In an appendix the lichenological publications of Kalb are listed, and an enumeration is given of all taxa and new combinations described by or named after Klaus Kalb.

Pant DC, Vindeshwari Prasad. 2008. *Indian Sarcoscyphaceous Fungi*. Scientific Publishers, Jodhpur, India. Pp 124. ISBN 978-81-7233-525-0. Price US\$ 34.

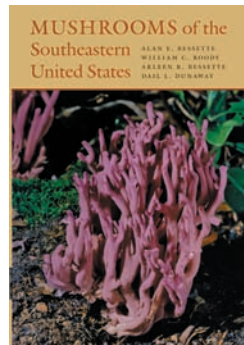


The present work is a monograph of all Sarcoscyphaceae of India. Introductory chapters deal with the history of the classification of

the group concerned and an outline of the morphology and characters used for the delimitation of taxa. The taxonomic part contains generic and species descriptions, accompanied by photographs and line-drawings of microscopic structures. Unfortunately identification keys are missing. The quality of the micrographs and print is poor.

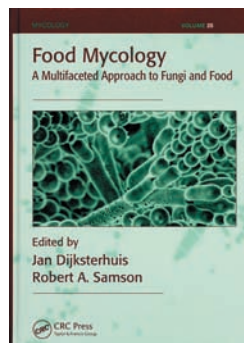
Bessette AE, Roody WC, Bessette AR, Dunaway DL. 2007. *Mushrooms of the Southeastern United States*. Syracuse University Press. Pp 375; numerous coloured photographs. ISBN 978-0-8156-3112-5. Price US\$ 68.50

The authors present a rather comprehensive guide to the mushrooms of the Southeastern United States, which forms as such a compliment to their companion to mushrooms of the Northeastern United States. More than 450 species are presented with coloured photographs and macroscopic descriptions. The microscopy is very concise and restricted to spore characters. Comments usually deal with similar species to facilitate recognition. A key to the major groups of mushrooms is presented, but no key to the species. This book will therefore serve keen amateurs only to a limited extent, but the high quality photographs make this attractive book worthwhile.

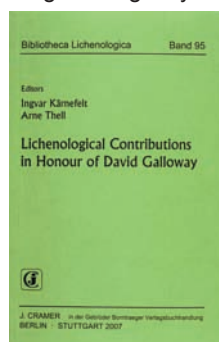


Dijksterhuis J, Samson R (eds). 2007. *Food mycology, A multifaceted approach to fungi and food*. CRC Press, Atlanta. Pp 403; numerous illustrations. ISBN 978-0-8493-9818-6. Price US\$ 169.95.

A team of internationally renowned specialists has been invited to bring together the current state of knowledge on fungi and food, dealing with all aspects of food mycology. Seven chapters deal with fungi and living crops, the fungal spore in food mycology, Fungi and mycotoxins, fungi as hyperproducers, fungal spoilage, and fungi as food, respectively. Novel techniques to manage fungal invasions are presented. As such it forms a unique comprehensive and up-to-date overview of all facets of the role of fungi in food, from fungal threats to crops and postharvest spoilage through mycotoxins and collecting edible mushrooms in the field. A very important textbook for educational purposes and for those working with food and fungi.



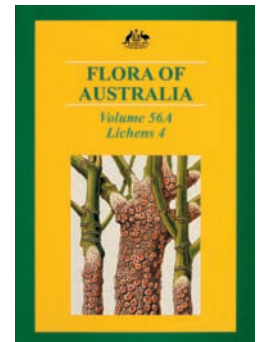
Kärnefelt I, Thell A. 2007. *Lichenological contributions in honour of David Galloway*. Bibliotheca Lichenologica 95. J. Cramer, Stuttgart. Pp 604; numerous illustrations. ISBN 978-3-443-58074-2. Price € 98.



This volume contains 36 papers by 55 authors in honour of the well-known lichenologist David Galloway. It contains two parts, the first being devoted to the history of Lichenology with a focus on four famous lichenologists, viz. Galloway, Lindsay, Acharius, and Spruce. Part II, forming the main body of this publication, is focussed on the Southern Hemisphere and tropical lichenology with molecular phylogenetic studies, phytogeography, studies of the lichens of remote areas, and a considerable number of new taxa.

McCarthy PM, Mallett K (eds). 2004. *Flora of Australia Vol. 56A Lichens 4*. CSIRO Publishing. Pp. 204. ISBN 0-6430-9056-8. Price AU\$ 95.

In this fourth volume devoted to the Lichens of Australia, the orders Lecanorales, Pertusariales, and Teloschistales, which encompass a.o. the genera Pertusaria and Lecanora, which range among the species-richest genera in Australia. Keys are given to genera and species. Full descriptions are accompanied with reference illustrations and geographic distribution, including distribution maps. Fifty six coloured photographs facilitate the identification of these taxa.



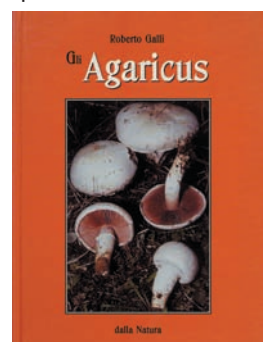
Didukh M, Wasser SP, Nevo E. 2004. *Impact of the family Agaricaceae (Fr.) Cohn on nutrition and medicine*. Gartner Verlag, Koenigstein. Pp 205; 22 colour plates. ISBN 3-906166-19-8. Price € 68.

This publication forms the fifth in the series on biodiversity of Cyanoprocyotes, Algae and Fungi of Israel, produced by the University of Haifa. It contains chapters on the nutritional and medical value of the Agaricaceae, including the economically important genus *Agaricus*. Much attention is paid to *Agaricus brasiliensis*, a relatively newcomer on the market of commercially grown mushrooms, with information on taxonomy, cultivations techniques, medical properties, antiviral activity and other aspects are extensively described. Beside *Agaricus*, also the genera *Melanophyllum*, *Chlorophyllum*, *Leucocoprinus*, *Leucoagaricus*, *Macrolepiota*, and *Lepiota* are treated. The numerous photographs are of poor quality.



Galli R. 2004. *Gli Agaricus* Ed. Dalla Natura, Milano, Italian. Pp 216; 216 coloured photographs. ISBN none. Price € 110.

This is the fifth in a series of monographs, after *Russula*, *Tricholoma* and *Boletes*, the author has tackled the genus *Agaricus*, an important



genus of edible and cultivated mushroom. Despite several attempts, the taxonomy of *Agaricus* still offers problems as to species delimitation and distinction. We therefore do not think that this rather uncritical monograph offers solutions. But it is an attractive work, and gives a good representation of current species concepts, finely illustrated with good quality coloured photographs, usually several per species. The text is in Italian, with English summary, and bilingual keys. It is very suitable as an introduction to the genus, although the price seems rather high.

Pegler D, Freedberg D. 2005. *Fungi*. The paper museum of Cassiano dal Pozzo. Three volumes. Pp 1028, 925 coloured plates. ISBN 1-905375-05-0. Price GBP 237.

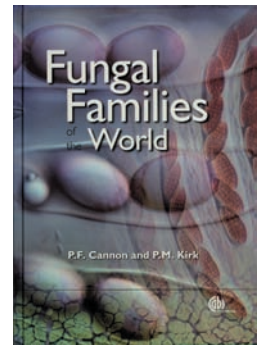
This unique publication in three volumes gives an overview of the extensive collection of mycological drawings in the Paper Museum of Cassiano dal Pozzo, commissioned by Federico Cesi, Prince of Acquasparta (1585–1630). The drawings were thought to be lost but rediscovered in the 1980s in Paris. The pictures are made between 1625 and 1630 in the vicinity of Rome and in southern Umbria, Italy. The drawings are also very unique because they represent the first of its kind where microscopic structures also were included. The initiative to publish these wonderful plates with comment by a professional mycologist is greatly welcomed, as it offers a delight for the reader to see the wonderful paintings and read the informative text. The introductory chapters give a picture of the scientific environment at the time the paintings were drawn, and a background for the painters and paintings. This publication is of high artistic and mycological level and will find its place in public and private libraries.

Cannon P, Kirk P. 2007. *Fungal families of the world*. CABI publishing. Pp. 456. ISBN 780-85199-827-5. Price € 150.

In the age of rapidly changing insight in fungal classification, due to molecular phylogenetic analyses, an up to date account of the fungal families has been much wanted. This book is meant

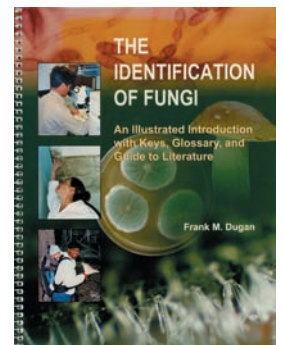


to cover this need, supplementary to the information that can be found in the Dictionary of Fungi by the same authors. An alphabetical account is given of all families recognised in the kingdom Fungi. An extensive glossary of more than 50 pages is added to facilitate the use of this book. Every family diagnose is followed by an enumeration of the most important genera and references to literature. Coloured photographs are added to illustrate some of the most important genera. A useful compilation at first sight, but on closer inspection one is confronted with omissions and mistakes, in particular with regard to higher basidiomycete families. Representative genera are not always carefully chosen, and the authors seem to have missed most of the recent literature, in particular important monographs. Some of the coloured pictures are evidently wrongly named, which could easily have been avoided. One wonders whether this kind of information could not be better provided electronically, with the advantage of continuously updating the information.



Dugan FM. 2006. *Identification of Fungi: An illustrated introduction with keys, glossary, and guide to literature*. APS press, St. Paul, USA. Pp 182; 520 illustrations. ISBN 0-89054-336-4. Price US\$ 65.

This manual is meant for students in mycology. After a short introduction one finds an enumeration of the most important characters, arranged per main division: lower fungi, Zygomycota, Ascomycota, Basidiomycota and Deuteromycetes. Artificial keys are given to distinguish the main families. Within these divisions families are listed alphabetically with a short diagnosis and one or more line-drawings. References to literature are given. An illustrated glossary and extensive list of references provide further information. The quality of the line drawings generally is rather poor. The keys are often too artificial to be of great help.



Editors choice: Best Student Biodiversity paper of 2008

The following paper was selected by the editorial board as best student paper for the year 2008 (volumes 20, 21). The decision was chiefly based on the innovative approach used in the paper to discover novel niches of fungal biodiversity, and the integration of molecular (DNA barcoding) and cultural (extinction plating) techniques to achieve this goal.

Ruibal C, Platas G, Bills GF. 2008. High diversity and morphological convergence among melanised fungi from rock formations in the Central Mountain System of Spain. *Persoonia* 21: 93–110.

Profile – Constantino Ruibal Villaseñor

In 1998, I received my Chemistry degree from the Universidad Autónoma de Madrid, specialising in Biochemistry and Molecular Biology. In 1999, while looking for more applied training, I became 'Specialist in Applied Biochemical Methodology', (2nd grade in the Spanish system) at the Universidad de Alcalá.

Shortly thereafter, in January of 2000, the FUAM (Universidad Autónoma de Madrid General Foundation) awarded me a formation scholarship at the Centro de Investigación Básica, Merck, Sharp & Dohme de España (CIBE) in the Microbiology

and Bacterial Physiology Group. As my stay at CIBE continued, I was offered a FUAM PhD scholarship in the Fungal Research Group, in January 2001. This PhD scholarship was followed by another from the Universidad Complutense de Madrid until January 2005, when I awarded my Ph.D. in Sciences from the Universidad Autónoma de Madrid where I defended my thesis: *Isolation and characterisation of melanised, slow-growing fungi from semiarid rock surfaces of central Spain and Mallorca.*

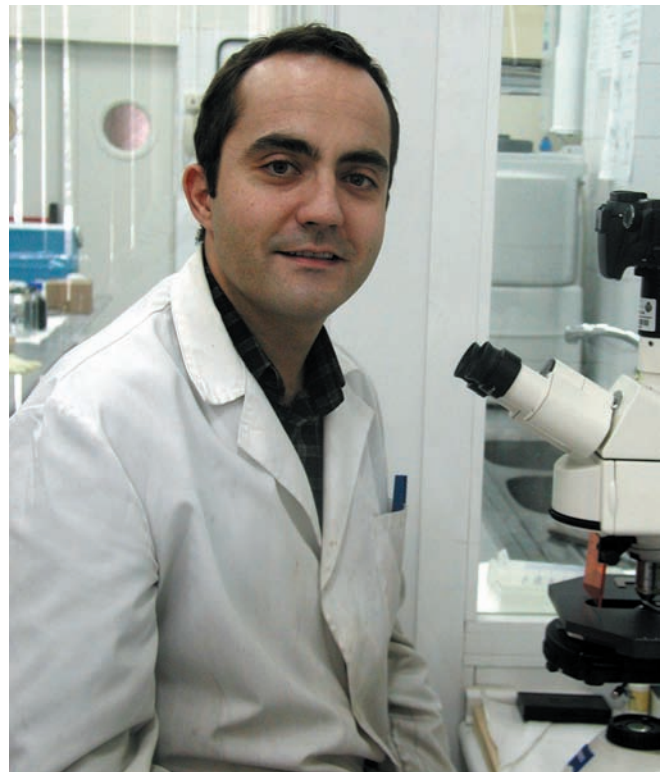
The thesis project was supervised by Drs Gerald F. Bills and Gonzalo Platas Mochales. The idea for the project originated from the lab's ongoing mission to explore new kinds of fungi for their bioactive secondary metabolites. When Gerald arrived in Spain in 2001, he decided that we needed to take advantage of the local environment to explore for new fungi. However, because the native vegetation of Spain is either highly disturbed or converted into agriculture, options for exploring fungal diversity are limited. So after reading about observations of new fungi on ancient monuments in the Mediterranean region, we decided that it might prove interesting to exploit the rocky arid landscape to see if we could find more of these fungi. Gerald's intuition was correct; when I starting isolating fungi from rocks we were blown away by how many different organisms were present on what appeared to be barren rock!

Current and future research

After finishing my thesis, I worked briefly at the Polytechnical University of Madrid, in the Biodeterioration Laboratory of their Materials Engineering and Sciences Department, identifying and characterising fungi isolated from cave paintings in Cantabria (Spain). I then travelled to the Centraabureau voor Schimmelcultures where, under the direction of Prof. dr Sybren de Hoog, I continued to immerse myself in the study of the melanised, rock fungi.

In September of 2006, I went to Duke University to improve my knowledge and skills with the phylogenetics of Pezizomycotina, under the direction of Dr François Lutzoni. In his laboratory I focused on the orders that have members of the black, rock fungi, namely, Chaetothyriales, Dothideales and Capnodiales. The research carried out at CBS and Duke suggests the black, rock fungi could be a model for tracing of the origin and evolution of these orders, including the origin of lichenisation in the Eurotiomycetes, within the ancestors of the Chaetothyriales and Verrucariales.

Since January 2007, I am back at the Biodeterioration Laboratory of the Polytechnical University of Madrid under the direction of Dr Diego A. Moreno Gómez. I'm carrying out research



Dr Constantino (Tino) Ruibal.

on the potential biodegradation of used rubber tyres by both bacteria and fungi, having achieved so far provisional data which could yield promising results in the future, provided that funding continues to be available for this challenging and almost unexplored field.

My current research has not prevented me from working with black, rock fungi and, when circumstances allow it, I keep collaborating with colleagues at the CBS and other laboratories working in the field, as most of the fungi I isolated are novel, and still need to be named! We hope shortly to submit a new paper for publication as a special issue of *Studies in Mycology*, devoted to black fungi. A study, written and coordinated by Dr Cécile Gueidan, entitled 'An extremotolerant rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages', where the possibility of black, rock fungi as ancestors for both lichenised Verrucariales and human-pathogenic Chaetothyriales is being proposed.

Dr C. Ruibal

Integrating DNA Barcoding into the Mycological Sciences

Introduction

Mycology has a proud taxonomic tradition, and mycologists were early to adopt molecular techniques such as DNA sequencing for identifying fungi. The recently completed Assembling the Fungal Tree of Life (AFTOL) project was a community effort, and provided a robust phylogenetic framework for mycologists wishing to 'leaf out the tree' of species. The Fungi are a huge

kingdom, with challenges that parallel those of the invertebrate animal world that has given us DNA barcoding. Many fungi are pleomorphic; many are miniscule and morphologically similar; there are few trained taxonomists to identify them; and there is a large reservoir of undescribed species awaiting discovery. Mycologists should be enthusiastic proponents of DNA barcoding. Why has this not happened?

Mycological barcoding discussions are preoccupied with selection of the most appropriate barcode marker. This is not a trivial matter, but my observation is that mycologists generally overlook other aspects of the emerging barcode standards (Lorenz et al. 2005), unaware of important details and nuances that will affect common practice far more in the long term. 'DNA barcoding' is no longer a vague phrase to be tossed around as a marketing device in grant applications. The term has precise meanings and implications for those who intend to conduct this kind of research.

The most critical detail is the acceptance of the BARCODE keyword in GenBank (Hanner & Gregory 2007). To qualify for this keyword, a DNA sequence must meet the following criteria:

1. It must represent the DNA barcode marker accepted for the taxonomic group in question.
2. A voucher for the barcode must be available.
3. The organism that the barcode represents must be correctly identified.
4. The DNA sequence itself must be correct, which is assured by storing sequence chromatograms in on-line repositories.

These criteria are discussed below. If any are not met, the BARCODE keyword will not be appended to a GenBank accession. If the conditions associated with any of these criteria change, for example if either the identification or sequence are shown to be incorrect, then the BARCODE keyword will be removed, with or without the authority of the original depositor. When this happens, the original sequence record remains otherwise unchanged, but it is excluded from BLAST or other searches restricted to the BARCODE keyword. This is presently the only permitted third-party alteration of GenBank records. The decision to remove the keyword can be made by GenBank staff, or by the CBOL Scientific Advisory Board.

DNA barcoding markers

The Consortium for the Barcode of Life (CBOL, not to be confused with the Canadian Barcode of Life Network, BOLNET) is an international body with more than 160 members, with offices in the Smithsonian Institution in Washington, D.C. CBOL promotes DNA barcoding internationally, and facilitates the development of DNA barcoding projects. It regulates the barcode standard through its Scientific Advisory Board. CBOL has the authority, negotiated with GenBank, to sanction DNA barcode markers for all kingdoms of life, and thus oversees the application of the BARCODE keyword. CBOL adopted the mitochondrial gene cytochrome oxidase I, *COX1* (also known as *CO1*), as the default barcode for animals, where it has received a lot of attention, particularly with invertebrates. Other markers may be proposed as DNA barcodes for particular taxonomic groups. First, proponents must demonstrate that *COX1* is ineffective for the taxonomic group in question. Then, the utility of the alternative marker must be demonstrated. The procedures and requirements for these proposals, and the detailed barcoding standards, are published on the CBOL website (www.barcoding.si.edu).

As reviewed in the last part of this article, *COX1* has hardly been studied in mycology, and it was not included among the genes sequenced for the AFTOL project. The majority

of mycologists clearly favour the nuclear ribosomal internal transcribed spacer (ITS) for DNA barcoding of fungi, with a smaller constituency, especially yeast specialists, favouring the D1/D2 region of the nuclear large ribosomal subunit (LSU, or 28S). A formal proposal to CBOL to designate the ITS as the primary fungal barcode is expected shortly (see below), and if it is accepted, the BARCODE keyword will begin to be applied to fungal sequences that meet the other barcode criteria. Yeast workers, who have a more complete database for the LSU, may independently elect to propose a primary or secondary barcode for those fungi.

Mycologists are not alone in rejecting *COX1* as a barcode for a kingdom. Botanists have also been searching for alternative barcodes, and will probably adopt a multi-gene approach (see Pennisi 2007). However, in this kingdom also, formal proposals to CBOL are pending.

Vouchering and identification

Maintenance of voucher specimens is already common practice for most taxonomic mycologists, and barcoding vouchering requirements are similar to those enforced by AFTOL. The preservation of vouchers allows identifications associated with barcode records to be reassessed, and this will probably be the most important factor for the eventual removal of the BARCODE keyword from GenBank records.

The barcode standard allows flexibility in the definition of a voucher. In mycology, a voucher normally would be a culture or a herbarium specimen deposited in a recognized collection. In some circumstances, the voucher could be a photograph. The main requirement is that the voucher should allow determinations to be verified, long after an initial identification was made.

A recent shift in barcode voucher requirements will have implications for some mycological collections. The first version of the barcode standard required only that vouchers be deposited in recognised collections, but recent modifications require that the voucher meta-data be available on-line. This means that herbaria and culture collections lacking on-line databases may not be recognised as acceptable repositories for barcode vouchers. Furthermore, the acronyms used by some mycological collections may have to be altered if there are zoological collections using the same ones. CBOL has initiated an on-line registry of biological collections to eliminate identical acronyms, and to provide a portal between GenBank and the collections holding specimen meta-data (www.biorepositories.org).

Beyond this, culture collections and other biorepositories seem ready to adopt barcoding as a part of their quality control regimes (Hanner & Gregory 2007). The American Type Culture Collection (ATCC) has recently implemented barcoding to assure that its animal cell culture lines are correctly identified (Cooper et al. 2007), and starting in 2009, the CBS Fungal Biodiversity Centre will initiate a bold project to generate DNA barcodes for its entire culture collection.

Ensuring sequence quality

On-line archiving of DNA sequencing chromatograms is the final requirement of the DNA barcoding standard. The standard does

not proscribe what archive is used, as long as it is possible to hyperlink from a GenBank record. GenBank itself maintains a chromatogram archive (www.ncbi.nlm.nih.gov/Traces/home), which is open to barcode chromatograms. The Barcode of Life Data Systems (BOLD), described in more detail below, requires the deposit of chromatograms for barcode records stored in that database. Supplementary information, such as primer sequences used to generate barcodes, may also be required. The chromatogram archive of AFTOL would presumably satisfy this barcode requirement.

Most taxonomically oriented mycologists would welcome on-line accessibility of chromatograms. Mycologists have been vocal critics of the GenBank policy of forbidding third-party annotation of sequence records, generating various statistics that indicate a high error rate either in sequences themselves, or in the identification of the organisms from which they originate (Bridge et al. 2007, Bidartondo et al. 2008). At least when using GenBank as an identification tool, the possibility of removing the BARCODE keyword will be an effective method of policing sequencing and identification errors.

The issue of legacy data and sequence traces is considered near the end of this article.

Other aspects of DNA barcoding and mycology

The goal of DNA barcoding is to develop a sequence-based approach for identifying all life. This requires cooperation of taxonomists working with all kingdoms to develop a system that will work for all kingdoms. The eventual DNA barcode database of fungi is not just for mycologists, but for all biologists. It will be a mechanism for including the fungi in a much broader range of biological studies than could ever be undertaken by those who self-identify as mycologists. But only mycologists can credibly assemble the fungal barcode database tool, acknowledging that its heaviest users may be other scientists.

Standardisation is critical. We cannot expect biologists with limited knowledge of fungi to know that to identify species of a particular genus they must sequence a particular gene that is not part of the barcode regime. Universality is critical. We cannot expect investigators to include large numbers of PCR primers in order to identify a few fungi in their research system. However, rigorous standardisation and insistence on universality impose limitations that need to be clear to users of the DNA barcode databases. No single-gene barcode system will be capable of identifying all fungi to species, and the limitations of ITS sequences for identifying species in some groups, and the failure of the 'universal' ITS primers to work in other groups, will have to be carefully documented.

DNA barcoding exploits high-throughput technologies, and a core barcoding laboratory can generate hundreds of thousands of barcodes per year. Invertebrate barcoders have exploited this, increasingly including thousands of barcodes in a single study (e.g. Hajibabaei et al. 2006). Animals may seem easier to work with in high volumes than fungi. Individuals can be collected and photographed quickly, tissue can be extracted for molecular analysis, and the remainder of the animal used as a voucher. The cost of sequencing is low, and animal barcoders consider it an acceptable expense to sequence hundreds or

thousands of individuals to find a few new or cryptic species. After all, taxonomic expertise is the limiting factor in species discovery and description, not a shortage of people with molecular technical skills.

Fungal taxonomy is still mostly engaged with what might be called the botanical collecting model, that is, the collection, identification and processing of single specimens. Common species are usually not gathered, and collectors focus on species that are either unknown to them, that are of interest because of ongoing research projects, or that capture their curiosity for other reasons. But perhaps the adoption of the high-throughput practices employed by animal barcoders is feasible for macrofungi. Collectors could photograph and collect *all* fruiting bodies they find, put a tissue sample in an extraction vial in the field for each fruiting body, accession the specimens from one collecting location *en masse*, do the barcoding, and then sort out identifications and specimens of taxonomic interest afterwards. Putative new species discovered by barcoding could then be verified by Geneological Concordance Phylogenetic Species Recognition, morphological studies, or any other preferred taxonomic process. Apart from facilitating species discovery, our knowledge of the geographical distribution of all fungal species would be extended in a way that conventional collecting cannot do.

Could such a model also be applied to microfungi? For fungi with the potential to grow in culture, this seems feasible, although culture maintenance is more labour intensive than managing herbarium specimens. Collado et al. (2007) described a high-throughput method for isolating fungi from leaf litter (adaptable to other kinds of substrates), which facilitates the isolation of fastidious and slow-growing fungi by inoculating highly diluted particle suspensions onto agar media compartmentalised in 48-well microplates. With such a method, barcoding of fungal colonies before deciding to purify and maintain conventional cultures could reduce associated labour costs by allowing redundant or unwanted isolates to be discarded.

Eventually, it will be important to separate the barcodes that comprise the identification database from DNA sequences that have been identified using that database. Once the barcode sequence variation, including that associated with geography, has been elucidated, it would be counterproductive to keep populating the barcode database with redundant sequences. This leads us to environmental DNA, a topic of great excitement in our science, and a frequently cited topic in discussions of fungal DNA barcoding. Presently, sequences derived from environmental samples that contain mixtures of species, such as soil, would probably be unacceptable as DNA barcodes. Mixed samples cannot serve as voucher specimens because their components cannot be unequivocally matched with individual barcodes. Similarly, sequences cloned directly from substrates or hosts would not qualify as barcodes unless a one to one relationship between a sequence and an organism on a voucher could be established. These issues will be especially critical for fungi, if it is true that the majority of fungi cannot be isolated into pure culture and do not produce conspicuous fruiting bodies that can be collected. The majority of fungi may be invisible to us, detectable only by their DNA. Inclusion of such organisms in the fungal DNA barcode database will require modification

of the barcoding standard, and mycologists should take the lead in this discussion.

DNA barcoding in fungi to date

Presently, the only funded DNA barcoding network is in Canada, centred at the University of Guelph, and overseen by Paul Hebert and his colleagues at the Biodiversity Institute of Ontario. The network received seed funding from the Gordon and Betty Moore Foundation, and the Alfred P. Sloan Foundation. In 2005, it received three years of support as a research network from the Natural Sciences and Engineering Research Council of Canada (NSERC) and four years from Genome Canada (through Genome Ontario).

The public face of BOLNET (www.bolnet.ca) is the BOLD website (Barcode of Life Data Systems, www.barcodinglife.org, Ratnasingham & Hebert 2007). For the participants in the network, BOLD acts as a Laboratory Information Management System (LIMS), in which specimen data and meta-data is archived, sequences derived at the high-throughput sequencing facility in Guelph are deposited, and various taxonomic tools, such as tree building algorithms (which function easily because *COX1* lacks indels and alignment is not required) and an automated geographical distribution mapper, are available. Projects from the LIMS are published in much the same fashion as sequences are made public in GenBank, after work is published or the scientist involved decides to make the data available to the public. External users can use the identification engine of BOLD to identify unknowns using their own barcode sequences, and verify the results by comparison with an extensive library of on-line images of voucher specimens. BOLD is also available for use by scientists who are not funded by the network.

Mycology was included as an exploratory element in the first Canadian network. The participants agreed to assess the suitability of *COX1* as a DNA barcode for fungi, while expressing their opinion that mycologists would be reluctant to accept this marker, because of the predominance of ITS as a species-level marker. From the small amount of *COX1* data then available for fungi, it seemed likely that frequently reported introns could interfere with successful amplification. Similarly, there did not seem to be conserved regions sufficient for universal primer design. The features of the ideal barcode marker have been discussed exhaustively, and the reader is referred to Geiser et al. (2007) for an overview with respect to fungi.

The first intensive assessment of *COX1* in fungi was published by Seifert et al. (2007). They developed PCR primers for *COX1* that worked for the ascomycetes family Trichocomaceae, and sequenced the barcode region for 360 strains of *Penicillium* sub-genus *Penicillium*. *COX1* provided species specific barcodes for about 2/3 of this taxonomically intractable group, superior to the resolution of ITS, but inferior to that of the protein-coding gene *BenA*. Surprisingly, introns were detected in only about 1 % of the sequenced strains, and had little impact on the barcode utility of *COX1* in this group. A recent study of a smaller genus, *Leohumicola*, by Nguyen & Seifert (2008), using new primers designed for members of the Pezizomycotina, showed similar species resolution between *COX1* and ITS among the seven known species. Introns were detected in about 5 % of the strains.

The results of other studies of *COX1* have been less encouraging. Geiser et al. (2008) included *COX1* in their study of the *Aspergillus niger* complex. They detected multiple copies, with insufficient variation to allow recognition of species, and rejected the marker as a suitable barcode in this complex. Seifert et al. (in prep.) found similar problems in *Fusarium*. Paralogs occurred within individual strains, with double bases at some positions, or with otherwise variable sequences among copies. When apparently homologous copies were compared among species, taxa assigned to different taxonomic sections had identical sequences. *COX1* results from other mycologists in the Canadian network have yet to be published, but indicate a similar pattern of success in some groups, and intron issues in others.

In May 2007, against the backdrop of the recently published article on *Penicillium* (Seifert et al. 2007), a discussion among 37 mycologists from 12 countries was held at the Smithsonian's Conservation and Research Centre at Front Royal, Virginia, organised by Amy Rossman and Mary Palm. The meeting was funded by the A.F. Sloan Foundation under the auspices of CBOL, and the conclusions were summarised by Rossman (2007). Not surprisingly, the participants at the meeting unanimously endorsed the ITS. Unfortunately, the meeting concluded without a defined plan to propose the ITS to CBOL as the fungal marker. Following this meeting, CBOL attempted to maintain momentum by encouraging the appointment of an *ad hoc* committee to promote DNA barcoding in mycology. Eventually, Pedro Crous, Keith Seifert and John Taylor agreed to act as interim co-chairs. Following the 2nd Science Symposium of the Canadian Barcode of Life Network in Toronto in May 2008, the co-chairs met with the mycologists participating in BOLNET, and several participants from the Front Royal Meeting, including three from Europe. The selection of the ITS as the fungal marker was endorsed once more, and a plan was tabled to prepare the formal proposal to CBOL. The writing of the proposal is led by Dr Ursula Eberhardt at CBS, and the intention is to publish the proposal in the peer reviewed literature prior to presenting it to CBOL Scientific Advisory Board for its decision. Anyone interested in participating in this proposal is welcome to contact Dr Eberhardt at u.eberhardt@cbs.knaw.nl. The *ad hoc* committee on fungal barcoding, which presently goes by the acronym FUN-BOL, may continue to function as an *ad hoc* body or may evolve into a subcommission of the International Commission on the Taxonomy of Fungi (www.fungaltaxonomy.org) with formal election of officers, members and drafting of statutes.

If the proposal to CBOL to accept the ITS for fungal barcoding is accepted, then the BARCODE keyword can begin to be applied to ITS accessions in GenBank. The BARCODE keyword now applied to the few *COX1* sequences for fungi will be stripped, and newly deposited ITS sequences that meet the barcoding criteria outlined in this article will begin to carry the keyword instead. Presently, BOLD is the only on-line database accepting ITS sequences as barcodes, in anticipation of the adoption of this marker as the fungal marker by CBOL.

How much of the legacy ITS data for fungi will qualify for the BARCODE keyword is open to question. Perhaps a grandfathering exemption can be negotiated between CBOL, GenBank

and mycologists, but if this fails, only sequences with accessible on-line chromatograms will be eligible. By this criterion, only the AFTOL ITS data and the limited amount of fungal ITS data deposited in BOLD will be sanctioned as barcodes.

Current estimates of the ITS sequences now in GenBank usually quote a number of about 70 000 sequences. If the chromatogram requirements are waived, how many would qualify as DNA barcodes? Nilsson et al. (2006) estimated that 82 % of the 51 000 fungal ITS sequences they examined were not associated with explicitly identified vouchers. Thus, about 13 000 of the remaining c. 70 000 ITS sequences could be eligible for the BARCODE keyword. Whether or not the traces are required, this is the volume of legacy data that will have to be re-evaluated sequence by sequence. Which represent types? Which are possibly misidentified or identified only to species? Most laboratories have their chromatograms archived, but retrieving them and preparing them for database submission will be time-consuming, and require at least some bioinformatics support. CBOL has pledged its assistance as mycologists attempt to find a pragmatic solution to the legacy data issue.

Final thoughts

Once a DNA barcode marker has been approved for the fungi by CBOL, the real barcoding work for mycologists will begin. Our taxonomic community will need to develop a coordinated strategy for populating the fungal barcode database efficiently.

At this writing, a new acronym has appeared on the horizon that may have a huge impact. The International Barcode of Life Network, with the acronym iBOL, is now in the planning stages (www.dnabarcoding.org). If funded, this five year, \$150 million network will encompass three or four central nodes with high-throughput barcoding facilities and BOLD mirrors (Canada, China, Europe, United States), nine regional node countries and six smaller national node countries. Mycology is included in the network proposal in Canada, but the research plan has not yet been finalised. For mycologists in other countries, now is the time search to search out your national iBOL representatives and make the case for including fungi in national or regional networks. Don't be discouraged by a first response that 'fungi are not included'; because the initiative has largely been developed and led by zoologists, and they sometimes are unaware that other kingdoms are to be included! If, as expected, iBOL is launched in the last half of 2009, we would like to have a strong mycological presence.

Barcoding needs mycology. Mycology needs barcoding. An important train is getting ready to leave the station. Now is the time to jump on.

Acknowledgements I am grateful for funding from the Canadian Barcode of Life Network and Genome Canada through the Ontario Genomics Institute, NSERC, and other sponsors listed at www.bolnet.ca. My thanks to

Robert Hanner (Associate Director, BOLNET) for his patient explanations of the nuances of DNA barcoding over some very pleasant meals, to Paul Hebert (Director, Canadian Centre for DNA Barcoding) for his exuberance and willingness to allow this shy mycologist to ride on his coat tails, and to David Schindel (Executive Secretary, CBOL) for his persistence in trying to motivate mycologists to take part in barcoding. My colleagues André Lévesque, Richard Hamelin, Jean-Marc Moncalvo, Donal Hickey, Mehrdad Hajibabaei, Amy Rossman, Mary Palm, Pedro Crous and John Taylor have shared this ride (or parts of it) with me, and I am grateful to them for their passion, compassion and exasperation as we stumble our way towards what seems to us all a critical objective: BARCODE THE FUNGI.

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Keith A. Seifert
Biodiversity (Mycology & Botany), Eastern Cereal and Oilseed Research Centre, Agriculture & Agri-Food Canada, 960 Carling Ave., Ottawa, Ontario K1A 0C6 Canada;
e-mail: seifertk@agr.gc.ca

Are we losing the battle in describing fungal biodiversity?

Number of novel fungal species described per year¹

Year	Total	Seq. ²	PubMed ³	Strains ⁴
2003	1268	176	74	141
2004	1522	336	134	281
2005	1008	267	113	207
2006	846	210	71	172
2007	853	162	35	134
Total	5497	1151 (21%)	427 (7.8%)	935 (17%)

¹ Data taken from MycoBank.

² Novelty for which DNA sequence data were located in GenBank.

³ Species published in journals indexed by PubMed.

⁴ Ex-type strains located in long term Biological Resource Centres.

Estimates for the number of undescribed fungi vary from one to several million, and where these fungi are 'hiding', has frequently proven to be a topic of many hotly debated sessions, workshops and papers (Hawksworth 1991, 2004, Crous et al. 2006). If all goes according to plan, we will be celebrating the international year of biodiversity in 2010, which will hopefully also generate attention for the kingdom Fungi. However, as shown above, not all is well with fungal taxonomy. For one, we are describing less than the commonly perceived number of 1200–1400 species per year. That the decline is happening even after the employment of molecular techniques, which

enables us to easily recognise cryptic taxa, is alarming. It is generally known that describing novelties does not guarantee highly cited papers, and thus these biodiversity papers do not fare well with funding agencies, again fuelling momentum away from basic fungal systematics. Another worrisome fact is that close to 80 % of the yearly harvest of novelties are not known from their DNA, and thus not compatible with modern BLAST or DNA Barcode approaches. A further interesting point is that of the strains deposited in Biological Resource Centres, most were also subjected to DNA sequence analysis and these data deposited in GenBank.

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P.W. Crous, V. Robert

CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

Mycological awards and honours

On 13–14 November 2008 the CBS Fungal Biodiversity Centre celebrated the 200th anniversary of the Royal Netherlands Academy of Arts and Sciences (KNAW), to which CBS is affiliated as research institute. A special symposium, '*Fungi and Health*', was organised at the Academy headquarters, Trippenhuis, in Amsterdam. The symposium, which consisted of six sessions, was attended by close to 200 mycologists representing 18 countries. To commemorate the KNAW, a new species of fungus was named after the Academy as *Sporidesmium knawiae*, which was published and distributed on 13 November 2008 (see www.fungalplanet.org). A special framed copy of the formal description of *S. knawiae* was handed to Prof. dr Robbert Dijkgraaf (President of the KNAW) by the director of CBS, Prof. dr P.W. Crous.

The symposium also represented the first occasion on which two special mycological awards were made, namely the *Johanna Westerdijk Award* and the *Josef Adolf von Arx Award*.

The *Westerdijk Award* is given on special occasions to an individual who has made an outstanding contribution to the culture collection of the CBS, marking a distinguished career in mycology. Nominees for the award are evaluated on the basis of quality, originality, and quantity of their contributions to the collection, and on the basis of associated mycology research in general. The first *Westerdijk award* went to Dr Emory G. Simmons, who is the world authority on the genus *Alternaria*, and has had a close link with CBS from the days of Westerdijk until present. Dr Simmons, who recently published his *Alternaria identification*



President of the KNAW, Prof. dr Robbert Dijkgraaf, accepting a framed copy of the fungus named after the Academy, *Sporidesmium knawiae*, which was published on the 13th of Nov. 2008 in the Fungal Planet (www.fungalplanet.org).

manual (for review, see Persoonia 20: 103), also deposited the associated fungal strains in CBS, ensuring that they remain available for the international research community.

The *von Arx Award* is given on special occasions to an individual who has made an outstanding contribution to taxonomic research of fungal biodiversity, marking a distinguished career in mycology. Nominees for the award are evaluated on the basis of quality, originality, and quantity of their contributions in the field of fungal taxonomy. The first von Arx award was given to Dr Cletus P. Kurtzman (Peoria, USA), who contributed largely to the field of systematics of ascomycetous yeasts. He introduced sequencing of the ribosomal DNA for a better understanding of yeast phylogeny. Thanks to this work (and that of his colleagues) a nearly complete database of D1–D2 Large subunit rDNA sequences exists for all yeast species, which is widely used for e.g. identification of yeast isolates from industry, agriculture and medicine. Recently, he explored the usefulness of phylogenies based on multiple genes and this has resulted in a major rearrangement of important genera such as *Saccharomyces* and *Kluyveromyces*. Dr Kurtzman is also an editor of

the major yeast monograph “*The yeasts: a taxonomic study*”, of which the 5th edition is to appear in 2009.



Dr Kurtzman (awarded the *Josef Adolf von Arx Award*) congratulating Dr Simmons (awarded the *Johanna Westerdijk Award*), during the symposium ‘Fungi and Health’ hosted at the Royal Dutch Academy for Arts and Sciences (13–14 November 2008).

Taxonomic novelties in this issue

Species

Species	Gene loci sequenced
<i>Barriopsis</i> A.J.L. Phillips, A. Alves & Crous, gen. nov. (p. 39)	ITS, TUB, EF, LSU, SSU
<i>Barriopsis fusca</i> (N.E. Stevens) A.J.L. Phillips, A. Alves & Crous, comb. nov. (p. 39)	ITS, TUB, EF, LSU, SSU
<i>Cortinarius mahiquesii</i> Vila, A. Ortega & Suár.-Sant., sp. nov. (p. 154)	ITS
<i>Cyathus subglobisporus</i> R.L. Zhao, Desjardin, K. Soyong & K.D. Hyde, sp. nov. (p. 74)	ITS, LSU
<i>Dothidothiaceae</i> Crous & A.J.L. Phillips, fam. nov. (p. 35)	ITS, TUB, EF, LSU, SSU
<i>Leohumicola atra</i> Nguyen & Seifert, sp. nov. (p. 65)	ITS, COX1
<i>Leohumicola incrustata</i> Nguyen & Seifert, sp. nov. (p. 65)	ITS, COX1
<i>Leohumicola levissima</i> Nguyen & Seifert, sp. nov. (p. 66)	ITS, COX1
<i>Leptographium bhutanense</i> X.D. Zhou, K. Jacobs & M.J. Wingf., sp. nov. (p. 6)	ITS2, LSU
<i>Mycosphaerella irregulari</i> Cheewangkoon, K.D. Hyde & Crous, sp. nov. (p. 82)	ITS, LSU
<i>Mycosphaerella pseudomarksii</i> Cheewangkoon, K.D. Hyde & Crous, sp. nov. (p. 83)	ITS, LSU
<i>Mycosphaerella quasiparkii</i> Cheewangkoon, K.D. Hyde & Crous, sp. nov. (p. 85)	ITS, LSU
<i>Neodeightonia phoenicum</i> A.J.L. Phillips & Crous, sp. nov. (p. 43)	ITS, TUB, EF, LSU, SSU
<i>Penidiella eucalypti</i> Cheewangkoon, K.D. Hyde & Crous, sp. nov. (p. 86)	ITS, LSU
<i>Phaeoacremonium croatiense</i> Essakhi, Mugnai, Surico & Crous, sp. nov. (p. 127)	TUB, ACT
<i>Phaeoacremonium hungaricum</i> Essakhi, Mugnai, Surico & Crous, sp. nov. (p. 127)	TUB, ACT
<i>Phaeoacremonium sicilianum</i> Essakhi, Mugnai, Surico & Crous, sp. nov. (p. 131)	TUB, ACT
<i>Phaeoacremonium tuscanum</i> Essakhi, Mugnai, Surico & Crous, sp. nov. (p. 131)	TUB, ACT
<i>Phaeobotryon mamane</i> Crous & A.J.L. Phillips, sp. nov. (p. 45)	ITS, TUB, EF, LSU, SSU
<i>Phaeobotryon quercicola</i> (A.J.L. Phillips) Crous & A.J.L. Phillips, comb. nov. (p. 45)	ITS, TUB, EF, LSU, SSU
<i>Phaeobotryosphaeria citrigena</i> A.J.L. Phillips, P.R. Johnst. & Pennycook, sp. nov. (p. 50)	ITS, TUB, EF, LSU, SSU
<i>Phaeobotryosphaeria porosa</i> (Van Niekerc & Crous) Crous & A.J.L. Phillips, comb. nov. (p. 51)	ITS, TUB, EF, LSU, SSU
<i>Phaeobotryosphaeria visci</i> (Kalchbr.) A.J.L. Phillips & Crous, comb. nov. (p. 47)	ITS, TUB, EF, LSU, SSU
<i>Phaeomoniella capensis</i> Crous & A.R. Wood, sp. nov. (p. 137)	ITS, LSU
<i>Pseudocercospora Chiangmaiensis</i> Cheewangkoon, K.D. Hyde & Crous, sp. nov. (p. 87)	ITS, EF, LSU
<i>Saccharata capensis</i> Crous, Marinc. & M.J. Wingf., sp. nov. (p. 116)	ITS, EF
<i>Saccharata kirstenboschensis</i> Crous & A.R. Wood, sp. nov. (p. 138)	ITS, LSU
<i>Spencermartinsia</i> A.J.L. Phillips, A. Alves & Crous, gen. nov. (p. 51)	ITS, TUB, EF, LSU, SSU
<i>Spencermartinsia viticola</i> (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous, comb. nov. (p. 51)	ITS, TUB, EF, LSU, SSU
<i>Teratosphaeria altensteinii</i> Crous, sp. nov. (p. 139)	ITS, LSU
<i>Teratosphaeria encephalarti</i> Crous & A.R. Wood, sp. nov. (p. 140)	ITS, LSU
<i>Thecaphora capensis</i> Roets & L.L. Dreyer, sp. nov. (p. 151)	LSU