Fungal diversity and its implications for genetic resource collections

David L. Hawksworth*

The Yellow House, Calle Aguila 12, Colonia La Maliciosa, Mataelpino, ES-28492 Madrid, Spain

**Correspondence*: David L. Hawksworth, myconova@terra.es

Abstract: The extent of fungal diversity is reviewed, with respect to revised estimates of the numbers of plant species, and recent data on the extent of novelty in tropical forests, unexplored habitats, and numbers of orphaned, cryptic, and collected but yet undescribed species. Collections of fungal cultures are considered to be better referred to as "genetic resource collections" rather than "culture collections" to mesh with current terminology in other groups of organisms. The extent of holdings relative to the numbers of known and estimated species are reviewed and compared with those of vascular plants in botanic gardens and seed banks. The role of collections in supporting fungal genomics and molecular biology, and as a source of vouchers to vindicate published work in all aspects of mycology, is highlighted. Information is presented on the extent to which collections worldwide document and conserve the Earth's fungal genetic resource. Finally, the special role and responsibilities of CBS, as the major centre for the conservation of fungal genetic resources worldwide, is emphasized.

Key words: biodiversity, biological resource centres, Centraalbureau voor Schimmelcultures, conservation, culture collections, numbers of fungi, undescribed species.

INTRODUCTION

The issue of fungal diversity, its extent and conservation, has attracted more attention in the last 10–15 years than in any period of history. But what implications do the recent debates have for collections of fungal cultures, especially in the genomic age? The centenary of the Centraalbureau voor Schimmelcultures (CBS), the Fungal Biodiversity Centre, now in Utrecht, provides an appropriate occasion to consider the emerging issues, the extent of the problems, and implications for the role of such collections. Just as living organisms evolve to meet environmental challenges, so the scientific infrastructure needs to adapt. In particular, institutions must both meet the immediate needs of successive generations of scientists, and also position themselves to be able to fulfil anticipated future demands. Having been privileged to be entrusted with the management of one of the world's leading mycological centres for 14 years, through a period of major change and relocation (Aitchison & Hawksworth 1993), I am acutely aware of the need for pragmatic approaches. Here I consider the current state of our knowledge of fungal diversity, existing fungal genetic resource collections, and the challenges collections face in supporting the needs of fungal genomics and molecular biology, as well as those of conservation.

THE EXTENT OF FUNGAL DIVERSITY

Since the number of fungi present on Earth was conservatively estimated at 1.5 million species (Hawksworth 1991), alternative estimates ranging from 0.5–9.9 million have been published by other authors (Hawksworth 2001). Schmitt & Mueller (2004) calculated that there must be a minimum of 0.6 million species, using criteria that most mycologists would regard as excessively conservative to establish a lower boundary figure. In addition, a major comparative metadata analysis of macrofungi and plant diversity has been undertaken for the first time. Data from 25 studies in different parts of Asia, Europe and North America were analyzed statistically by Schmitt *et al*. (2004); the results showed that fungal species richness was much higher than that of the plants, demonstrated that tree species diversity was a good predictor of macrofungal diversity, supported the use of ratio estimates to measure fungal species richness, and were consistent with the high estimates of species numbers made by Hawksworth (1991).

Extrapolations made from the numbers of fungi and plants growing in particularly well-studied countries to the global scale are heavily influenced by the number of plant species recognized. Hawksworth (1991) used a figure of 270 000 plants worldwide. Since then larger estimates have been made, which would imply that the extrapolated numbers of fungi could be too low. For example, Prance *et al*. (2000) estimated 300–320 000 plant species, taking note of those that remained to be described from the tropics, while Govaerts (2001) argued that there were probably already 420 000 accepted and known seed plant species, based on the number of published scientific names and synonymy rates. However, using results from selected monographs, Scotland & Wortley (2003) considered Govaert's estimate had used too

low a synonymy rate and that his figure could be an overestimate by more than 200 000 species. Developing this approach further, Wortley & Scotland (2004) extrapolated from synonymy rates in 17 monographs (mean 66 %, i.e. about two in three of the published names are synonyms) that, with 95 % confidence, the number was in the range 117 734–575 320. These last authors did not provide a revised estimate, however, recognizing the need to analyse many more monographs. What is evident from these debates is that the 270 000 figure used in my original extrapolations (Hawksworth 1991) would be regarded as low by many plant taxonomists who have subsequently addressed this question, with Raven (2004) settling on 300 000. This is a further reason for retaining the 1.5 million species figure as the current working hypothesis, which remains widely accepted by mycologists (Mueller *et al*. 2004b).

Interestingly, the 66 % synonym figure for seed plants reached by Wortley & Scotland (2004) is virtually identical to the 65 % names : accepted species in 15 fungal monographs analyzed earlier (Hawksworth 1992). Applying this synonymy rate to the 300 000 species names in the *Index Fungorum* database (Kirk 2000; www.indexfungorum.org/names), implies that some 105 000 fungi may already be known. A somewhat larger figure of 120 000 was derived from the same data set when only directly ascribed synonyms and no excluded taxa were considered (Hawksworth 2001). These figures for currently known fungal species are, however, higher than that obtained by summing the figures in the eighth edition of *Ainsworth & Bisby's Dictionary of the Fungi* (Hawksworth *et al.* 1995) and making an allowance for subsequently described species, which gave a figure of 74 000 (Hawksworth 2001), or the 80 000 total in the ninth edition, where adjustments were not made in all entries (Kirk *et al*. 2001). These differences are primarily attributable to "orphaned" names, i.e. probably sound taxonomic entities whose generic placements have not been reassessed, but suggest that we have actually described around 100 000 "good" fungal species, although the position of perhaps 25 % of these is yet unresolved.

Accepting the 1.5 million estimated and 100 000 described species implies that only 7 % of the world's fungi have so far been described; a small increase on the 5 % hypothesized by Hawksworth (1991). The next issue is: "Where are all the undescribed fungi?" Hawksworth & Rossman (1997) identified the following categories: (1) fungi in tropical forests; (2) fungi in unexplored habitats; and (3) lost or hidden species. The last category included cryptic species, those lost within broadly circumscribed species, named but orphaned species, and those collected but yet unidentified. It is pertinent to consider progress in each of these three categories.

Fungi in tropical forests

There has been some, but less progress than would be ideal, in the exploration of the fungi in tropical forests in the last 10–15 years. The most important studies are from Hong Kong, where the number of fungi known has quadrupled in a decade, with over 150 of the species being new to science (Hyde 2001); many were from poorly studied host plants and special ecological niches such as plant litter in tropical streams. Whenever detailed studies are made, either by teams on short visits (e.g. Aptroot *et al*. 1997) or by students based in a tropical region (e.g. Homchantara & Coppins 2002), significant numbers of hitherto undescribed fungi continue to be found and described.

Over the last decade, an unexpected reservoir of fungal diversity in tropical forests has been recognized, mycorrhizas associated with leguminous tropical trees. In a transect study in Guyana, 75 species of putatively ectomycorhizal fungi were recorded of which only 19 could be confidently identified to species (Henkel *et al*. 2002). Even the wood decay corticioid and polyporoid fungi in tropical forests are proving to be much less well-known than previously thought; a recent issue of *Synopsis Fungorum* included descriptions of three new genera and 20 new species in these groups, almost all from the neotropics (Ryvarden 2004).

The situation with microfungi in tropical forests is currently unclear. While new species can be found apace, the extent of host restriction is generally unknown. In order to obtain more information on this problem in the case of palms, Taylor & Hyde (2003) studied the microfungi associated with three palm species in areas where they were native and where they had been introduced. Their results suggested that 26 of the 288 species found were host-specific, and demonstrated that the fungal diversity was greater in the natural habitats of the palms. There is a parallel situation with endophytes, where enormous numbers of isolates can be obtained from tropical trees, to the extent that these fungi have been considered potentially "hyperdiverse" (Arnold *et al*. 2000). While it is clear that these fungi are an important component of fungal diversity, uncertainty remains as to the extent to which many endophytic fungi are host-restricted; more molecular studies are needed on the lines of that by Pandey *et al.* (2003) who showed that the same *Phyllosticta* foliar endophyte occurred in tropical trees of different families in India. However, a single study need not be representative of the general pattern or tropical host specificity in general. For example, Beilharz & Cunnington (2003) established by molecular methods that morphologically indistinguishable *Pseudocercospora*'s on two different hosts in Australia were separate species.

Fungi in unexplored habitats

The habitats which fungi can occupy are extraordinarily diverse. This point is made exemplarily by Mueller *et al*. (2004a), who compiled information on the methods used for the inventorying of fungi in different habitats. The numbers of fungi known in little-studied habitats continue to soar, as indicated by the following examples.

Perhaps most surprising has been the case with hypogeous fungi in Australia, where 152 of 209 species found were new to science (Claridge *et al*. 2000). The number of such fungi in Australia alone is now estimated at about 1500 species, that is one third of the 4500 hypogeous fungi predicted to occur worldwide (James A. Trappe, pers. comm.). Also spectacular is the continuing rise in the numbers of lichenicolous fungi, the fungi obligately occurring on lichens, where the total of known species has risen from 457 species in 1976 to 1559 (Lawrey & Diederich 2003), with 3–4000 species now being estimated worldwide (Hawksworth 2001, Gams *et al*. 2004). Yet that might be the tip of the iceberg; Miadlikowska *et al*. (2004) obtained 325 isolates from surface-sterilized *Peltigera* thalli in Costa Rica and North Carolina which represented 96 unique genotypes phylogenetically related to endophytic fungi known from plants, and termed them "endolichenic" fungi.

New and hardly explored habitats which are rich sources of undiscovered fungal biodiversity continue to be discovered. For example, over 200 new species of yeasts have been found amongst 650 isolates from the guts of beetles, species accumulation estimating suggesting that at least one third more species are present in the sampled sites (Suh *et al*. 2004*,* Suh & Blackwell 2005). Insect guts are also the home of trichomycete fungi which are proving more diverse than formerly supposed; aquatic insect larvae collected over one 40 day period in Norway yielded 25 species including one new genus and nine new species (White & Lichtwardt 2004). Sadly, the high degree of novelty in tropical entomogenous laboulbeniaceous fungi reported by Weir & Hammond (1997) has yet to be transcribed into formally named taxa or pursued further by other workers.

Ruibai *et al.* (2004) isolated 117 strains of melanized fungi from limestone surfaces in Mallorca, which represented 39 genotypes only three of which could be identified with certainty; the sequences did not match any publicly available, and the strains could be of unknown genera not corresponding to any well-defined ascomycete order. Further, Schadt *et al*. (2003) reported what they interpreted as three novel fungal clades in under-snow tundra soils in Colorado that represented "major new groups of fungi (divergent at the subphylum or class level)".

However, it has to be recognized that so many known fungi remain to be sequenced (see below) that some of these molecular profiles may relate to already known, but as yet unsequenced, families, genera and species. The significance of such molecular studies in respect of overall fungal diversity is therefore difficult to assess at this time.

Morphological studies of little-studied plants continue to generate novel fungi. For example, of 117 species found on *Juncus roemerianus* in the U.S.A., 68 were undescribed (Kohlmeyer & Volkmann-Kohlmeyer 2001). Similarly, a project to examine the fungi on selected endemic plants in Mauritius has revealed over 200 species of saprobic microfungi which include one new genus and 38 new species (Dulymamode *et al*. 2001). However, in such studies, it is possible that some of the novel species described may in the future be found to have wider host ranges. Again, the significance of such studies for overall species numbers of fungi has to be tempered against the background of our inadequate knowledge.

Lost or hidden species

Here I will focus on cryptic species, those biological entities hidden within already named and, with hindsight, too-widely circumscribed morphospecies. Almost any single "species" of fungus studied by molecular or incompatibility methods proves to comprise several biological species, many of which then are found with hindsight to have differentiating morphological and (or) ecological features. Examples can be found in almost all groups of fungi, ranging from macromycete genera such as *Armillaria* (Pegler 2001), *Cantharellus* (Dunham *et al*. 2003) and *Ganoderma* (Hong & Jung 2004), to micromycetes such as *Trichoderma* (e.g. Chaverri & Samuels 2003), and lichen-forming fungi (Kroken & Taylor 2001, Molina *et al*. 2004). The case of the well-studied *Fusarium graminearum* is especially illuminating, which is now recognized as comprising nine separate species (O'Donnell *et al*. 2004).

Even without molecular data, more critical work on anamorph cultures, ascospore details, and secondary metabolites can also reveal hitherto hidden species, as in *Daldinia* (Stadler *et al*. 2004).

The numbers of species lost as unjustified synonyms, or languishing in the drawers of specialists with insufficient time to formally describe them, further swell the numbers of lost or hidden species. Hawksworth & Rossman (1997) estimated that worldwide there were probably more than 20 000 already collected fungal species still awaiting formal description.

FUNGAL GENETIC RESOURCE COLLECTIONS

Institutions or activities must reconsider and reinterpret their objectives in the language of the day; failure so to do may endanger their survival. The single act of re-labelling "culture collections" as "genetic re-

source collections" would immediately make the link with agendas of the 158 governments who have so far ratified the Convention on Biological Diversity[1], and overview assessments of global genetic resources (e.g. Heywood 1995). The Culture Collection of the then International Mycological Institute (now part of CABI Bioscience) made the name-change to Genetic Resource Collection in 1992[2] .

The label "culture collections" is a barrier to communication. It has hindered the forging of links with the rise of interest and funding devoted to the *ex situ* conservation of plants and animals during the last two decades. Interdisciplinary meetings on "genetic resources" have only exceptionally considered microbial groups, and it is proving a slow process to change the perception that fungi and microorganisms are something apart from "genetic resources".

Article 2 of the Convention on Biological Diversity defines genetic resources as "genetic material of actual or potential value"; "genetic material" being "any material of plant, animal and microbial or other origin containing functional units of heredity" (United Nations Environment Programme 1992). Article 9 (b) directs the contracting parties to the Convention to "Establish and maintain facilities for ex-situ conservation of and research on plants, animals and microorganisms, preferably in the country of origin of genetic resources". A dictionary definition of a resource is "a source or possibility of help"; that immediately conveys a positive message in contrast to "culture" which makes no value statement.

In addition to the value of using the phrase "genetic resource collections" with respect to the Convention on Biological Diversity, it would also make a second natural link: to the concept of "biological resource centres", on which recommendations for actions by governments have been made by the countries of the Organization for Economic Cooperation and Development (OECD 2001, Ryan & Smith 2004).

SUPPORTING FUNGAL GENOMICS AND MOLECULAR BIOLOGY

Sequences representing 16 421 fungal "species names" are now available in GenBank (www.ncbi.nlm.nih.gov/taxonomy[3]). While this may suggest that at least some part of the genome of 16 % of the currently known 100 000 fungal species has been examined, this figure is an overestimate as it has to be seen in the context of issues of separately named anamorphs, synonyms, and misidentifications (see above). The extent of the overestimate is difficult to assess, but it may be reasonable to assume that this will be about the same proportion as in the World Data Center (WDC) records (see below); i.e. 1.43 %, suggesting the true representation is around 11 480 species, 11.5 % of the estimated known species. This figure compares reasonably favourably with the GenBank data on green plants; 40 629 species names of green plants are represented, amounting to about 14 % of the known around 300 000 species (see above).

These data are now enabling major phylogenetic analyses to be conducted. Tehler *et al*. (2003) published a full-length phylogenetic tree of 1551 SSU rRNA sequences, representing about 1260 species names of fungi; the names used in GenBank were adhered to, so this figure will also be an overestimate. This aspect is less of a problem in the Assembling the Fungal Tree of Life (AFTOL) project, launched only in January 2003, and aiming to obtain sequences from eight loci in 1500 selected fungi in four years, most newly collected especially for the project. The first paper from the study focussed on the analysis of already available data, but when four-locus data sets were combined, multiple deep relationships that had not previously been revealed were resolved (Lutzoni *et al*. 2004). Such major analyses depend on having access to material in genetic resource collections, and in turn need collections in which to deposit vouchers of additional material sequenced so that it is available for future studies and its identity can be verified.

However, cases of misidentifications in deposited sequences are repeatedly being uncovered. Bridge *et al*. (2003) argued that the level of misidentifications of deposited fungal sequences could be "up to 20 %". Notwithstanding flaws in how this figure was arrived at (Hawksworth 2004a, Holst-Jensen *et al*. 2004), it could well be true.

The long-term preservation of voucher specimens and (or) cultures is essential to validate entries in sequence and genomics databases. Indeed, this should be made a mandatory requirement before sequences are accepted for inclusion in databases or used in published reports (see below). The CBS collections perform a significant role in this respect, with 18S sequences on 4496 strains, 28S on 4502, and ITS data on 4724 (Joost A. Stalpers, pers. comm.).

DOCUMENTING AND CONSERVING THE FUNGAL GENETIC RESOURCES

The World Data Center for Microrganisms currently holds data on 370 251 strains of fungi (including

[1] As of 22 September 2004.

[2] The Herbarium was also relabelled as the Biosystematics Reference Collection at the same time. These changes were made when the Institute moved from Kew to Egham to make their roles clear to the government representatives on CAB International's Executive Council, not all of whom had scientific backgrounds.

[3] Data accessed 22 September 2004.

yeasts) (Hideaki Sugawara, pers. comm.[4]). The number of names to which these are assigned is about 24 000. No critical revision of the names to eliminate synonyms and teleomorph/anamorph duplications has been carried out. However, when an analysis of the holdings was last carried out, the proportion of strains held to checked and accepted species names was 22:1 (Hawksworth 1991). Assuming that this overall ratio applies to the current holdings, this suggests that around 16 830 species may really be represented. While this is a significant increase on the 11 500 species represented in the last analysis, and constitutes about 30.5 % of the estimated culturable species, it amounts to only 16 % of the estimated already known 100 000 species of fungi, and 1.1 % of the estimated 1.5 million on Earth. It is salutary to reflect that these last two figures were 17 % and 0.8 % respectively in the last analysis (Hawksworth 1991). This implies that, collectively, the world's fungal culture collections are scarcely even keeping abreast of the new species continually being discovered, let alone making significant inroads into conserving a substantially greater proportion even of the known fungi.

In the case of flowering plants, there is an amazing 6 million accessions of plant genetic resources worldwide, although around half relate to major crops; 90 % of these accessions are in seed banks, with 85 000 species in cultivation, primarily in botanic gardens (Guerrant *et al*. 2004). This implies that about 28 % of the estimated 300 000 known plant species are secured in genetic resource collections. Against this benchmark, the 16 % of known fungi safeguarded is commendable, bearing in mind the disparity between resources devoted to botanic gardens and seed banks and those available for fungal collections. For example, there are about 2000 botanic gardens dispersed through 148 countries (Guerrant *et al*. 2004), compared with 483 fungal genetic resource collections spread through only 61 (Hideaki Sugawara, pers. comm. [4]). It has been estimated that as many as half of the world's plant species may qualify as threatened with extinction under the criteria used by IUCN-The World Conservation Union (Pitman & Jørgensen 2002), and botanic gardens and seed banks now focus on endangered species. Knowledge of the distribution of most fungal groups is too poor to enable endangered fungi to be targeted in the same way, with the exception of some macrolichens and hydnaceous fungi. Because so many fungi are obligate associates of particular plants, it may well be that more than half of the Earth's fungi are also entering endangered categories. In order to safeguard the global fungal genetic resource for posterity, collections consequently need to endeavour to secure material of as many different species as possible in a viable state. If this is not treated as a matter of urgency, there may be no second chance.

With so many fungi that can be grown in culture still to preserve, the incorporation into collections of living material that cannot be (or at least has not yet been) grown has tended to take a back seat. However, there are increasing numbers of studies that show that cryopreservation of fungal-infected host tissues is effective, for example with rust fungi (Ryan & Ellison 2003). This is an area where there is immense scope for fungal genetic resource collections to undertake basic research, with a view to developing protocols that can be widely used to conserve "unculturable" or "recalcitrant" fungi.

The extent to which the full spectrum of fungi is held in different collections varies considerably (Table 1). CBS is the front-runner in terms of both the numbers of strains and species represented in a single public collection; 25 % of the culturable fungi known, compared with 30.5 % across all 483 collections with fungal holdings. However, the majority of species are represented by very few isolates worldwide, which does not come close to representing the full intraspecific genomes.

Especially important in genetic resource collections are strains that represent the nomenclatural types of fungal species. These can now be cultures preserved in a metabolically inactive state, such as by lyophilization or storage in liquid nitrogen (Greuter *et al*. 2000: Art. 8.4) with cultures derived from them referred to as "ex-type" (*loc. cit*.: Rec. 8B.2). These are strains to which scientific names are permanently attached and provide the critical reference points for the application of those names. As a matter of long-term security, duplicates of cultures that are nomenclatural types should be deposited in several public collections from which they can be obtained for comparative studies.

The deposit of voucher material is, however, a much wider issue in biology, and its importance in mycological publications in particular has been repeatedly emphasized, most strongly by Agerer *et al*. (2000). Original scientific work should be reproducible, and verifiable by the scientific community at large. In the case of biology, this means that the actual material used should be permanently preserved so that it can be made available to other researchers. If it is not, then all kinds of research and records are unverifiable and irreproducible. For example, the availability of strains in collections enabled the identity of *Trichoderma* isolates mainly labelled as *T. harzianum* and used in biocontrol to be reassessed along with ex-type isolates; they represented at least seven species (Hermosa *et al*. 2004).

[4] 27 March 2004.

Table 1. Approximate numbers of fungal (including yeast) strains and species in key genetic resource collections and databases.

Collection		Strains	Species	Proportion of known culturable species (%)	Strains : Species
ATCC	American Type Culture Collection	27 000	7 000	13.5	3.9
CBS	Centraalbureau voor Schimmelcultures	55 000	13 000	25	4.2
IMI	CABI Bioscience UK Centre	26 000	6 000	11.5	4.3
MUCL	Mycothèque de l'Universite Catholique de Louvain	15 700	n/a[b]	n/a	n/a
NRRL	Agricultural Research Service, USDA	45 000	n/a	n/a	n/a
WDCM	World Data Center for Microorganisms	370 251	16 800[a]	30.5	22

[a]Approximately 23 000 names are recorded in the database but this does not allow for synonyms and anamorphs; this figure has been calculated by assuming the ratio of strains : species remains the same as when these were checked for Staines *et al.* (1986; *cfr* Hawksworth 1991). [b]n/a, information not available

I have argued elsewhere that the deposit of vouchers should be condition of acceptance of papers for publication in the biosciences (Hawksworth 2004b). This may not avoid mistakes, but at least would enable questionable research results to be re-evaluated.

Further, genetic resource collections have a key role in relation to intellectual property rights, especially with regard to strains cited in patents, and key national collections can serve as an International Depositary Authority (IDA) under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure 1977 which came into force in late 1980 (Bousfield 1988, Fritze 1994). To be recognized as IDA's, collections must be able to issue viability statements, furnish samples in accordance with the pertinent regulations, and store the organisms for 30 years.

Fungal genetic resource centres have a recognized role in "underpinning the future of life sciences and biotechnology" (OECD 2001). However, they also have a longer-term responsibility, preserving the Earth's resources in trust for future generations and well-being of humankind. Consequently, genetic resource collections need to operate to the highest standards of quality control, good practice, and preservation technology. These issues have been of concern to the World Federation for Culture Collections (WFCC) for over 20 years, and are ably discussed by Smith & Ryan (2004). However, there will be increasing problems of a mismatch between the demands of the scientific community and the resources of collections. This is especially so because at present the various fungal genetic resource collections have independent terms of reference and funding bodies, with no over-arching support or long-term strategy endorsed by the international community.

In the longer term, the method of operation of the UN's Food and Agricultural Organization (FAO) International Board on Plant Genetic Resources (IBPRG) might provide a model to be emulated. However, a parallel to the IBPGR scheme of centres specializing in different crop plant resources (Heywood 1995), could not be established without strong support from governments. A first step could be an outline proposal by the WFCC for discussion with potentially key UN agencies, not least of all UNESCO, which already established the Microbial Resource Centres (MIRCEN) network (Da Silva 1995), but also UNEP (the agency responsible for the Convention on Biological Diversity), and FAO.

THE MANDATE AND ROLE OF CBS

CBS occupies a unique position with respect to collections of fungal cultures. Not only is it claimed to be "the oldest collection of living fungi in the world" (Anon. 1975), its origins were an international initiative, the brainchild of a meeting of the Association Internationale des Botanistes held in Leiden in 1903 (Auger-Barreau 1967, de Hoog 1979). At that time there was no mechanism or organization such as IUBS (established 1919) or UNESCO (established 1946) that could have funded such a body internationally. The collection was established in 1904 and consequently came to be supported by the government of The Netherlands, at first through the Phytopathological Laboratory in Baarn, and from 1968 as an institute of the Royal Netherlands Academy of Arts and Science. The international mycological community is gratified by the record of ongoing and stalwart support provided by the Academy over so many years.

Especially welcome has been the maintenance of the highest levels of organismal systematic expertise in the molecular era. The synergy resultant from having specialists in traditional and molecular approaches working together is crucial to CBS maintaining its special world role and the quality of naming used in its living collections. Any who doubt the importance of more traditional approaches should read the critique by Wheeler (2004).

Although nationally owned, and still without international legal status[5], from the first CBS has had an international mission. This has been the vision of all its Directors, four of whom I have been privileged to have known, and its special position in the league table of fungal resources held (Table 1) is their legacy. The supplementary name "Fungal Diversity Centre", added from 2001 (Anon. 2001), is a statement of what has come to be CBS's unique focus. It is pleasing to see that focus maintained, and the original vision and enthusiasm reflected in the penultimate progress report to the collection's centenary year (Crous *et al*. 2004). The collection has enormous utility and prospects. CBS is now the strongest centre for fungal systematics in the world, combining molecular phylogenetic approaches with the highest levels of mycological expertise; it merits commensurate national and international support.

ACKNOWLEDGEMENTS

I was honoured to be invited to prepare this contribution in view of the high regard in which I have held CBS since my first visit to Baarn in 1975, and my subsequent involvement with the institute as a collaborator on various projects, and latterly as a member of its Scientific Committee for 1997–2002. I am indebted to CBS and the British Mycological Society for enabling me to participate in the institute's centenary celebrations. Gerald F. Bills, Meredith Blackwell, Thorsten H. Lumbsch, Greg Mueller, Matthew Ryan, and James M. Trappe generously made unpublished data or advance copies of papers accepted for publication still in press available. Hideaki Sugawara kindly provided up-to-date information on the data held by the WDCM, and other information was kindly supplied by David Smith, and Joost A. Stalpers. Amy Y. Rossman kindly reviewed the manuscript. This contribution was prepared whilst I was in receipt of a Programa Ramón y Cajal award from the Ministerio de Ciencia y Tecnología de España held in the Facultad de Farmacía of the Universidad de Complutense de Madrid.

REFERENCES

Agerer R, Ammirati J, Blanz P, Courtecuise R, Desjardin DE, Gams W, Hallenberg N, Halling R, Hawksworth DL, Horak E, Korf RP, Mueller GM, Oberwinkler F, Rambold G, Summerbell RC, Triebel D, Watling R (2000). Always deposit vouchers. *Mycological Research* **104**: 642–644.

Aitchison EM, Hawksworth DL (1993). *IMI: retrospect and prospect.* CAB International, Wallingford.

Anon. (1975). *Centraalbureau voor Schimmelcultures Baarn and Delft (The Netherlands).* Centraalbureau voor Schimmencultures, Baarn.

Anon. (2001). *Centraalbureau voor Schimmelcultures: Fungal Diversity Centre.* Centraalbureau voor Schimmelcultures, Utrecht.

Aptroot E, Diederich P, Sérisiaux E, Sipman HJM (1997). Lichens and lichenicolous fungi from New Guinea. *Bibliotheca Lichenologica* **64**: 1–220.

Arnold AE, Maynard Z, Gilbert GS, Coley OD, Kursar TA (2002). Are tropical fungal endophytes hyperdiverse? *Ecology Letters* **3**: 267–274.

Auger-Barreau R (1967). Une collection de cultures de champignons vivants: le Centraalbureau voor Schimmelcultures de Baarn (Hollande). *Actes de la Société Linnéene de Bordeaux* **104**: 1–7.

Beilharz V, Cunnington J (2003). Two new closely related species of *Pseudocercospora* on unrelated host families from south-east Australia. *Mycological Research* **107**: 445–451.

Bousfield IJ (1988). Patent protection for biotechnological inventions. In: *Living resources for biotechnology: filamentous fungi* (Hawksworth DL & Kirsop BE, eds). Cambridge University Press, Cambridge: 115–161.

Bridge PD, Roberts PJ, Spooner BM, Panchal G (2003). On the unreliability of published DNA sequences. *New Phytologist* **160**: 43–48.

Chaverri P, Samuels GJ (2003). *Hypocrea/Trichoderma* (*Ascomycota, Hypocreales, Hypocreaceae*): species with green ascospores. *Studies in Mycology* **48**: 1–116.

Claridge AW, Cork SJ, Trappe JM (2000). Diversity and habitat relationships of hypogeous fungi. I. Study design, sampling techniques and general survey results. *Biodiversity and Conservation* **9**: 151–173.

Crous PW, Samson RA, Summerbell RC (2004). *Progress Report 2002–2003.* Centraalbureau voor Schimmelcultures, Utrecht.

Da Silva EJ (1995). Extent and development of the human resource. In: *Microbial biodiversity and ecosystem function* (D Allsopp, RR Colwell & DL Hawksworth, eds): 427–438. CAB International, Wallingford.

de Hoog GS (ed.) (1979). *Centraalbureau voor Schimmelcultures: 75 years culture collection.* Centraalbureau voor Schimmelcultures, Baarn.

Dulymamode R, Cannon PF, Peerally A (2001). Fungi on endemic plants of Mauritius. *Mycological Research* **105**: 1472–1479.

Dunham SM, O'Dell TE, Molina R (2003). Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. *Mycological Research* **107**: 1163–1177.

Fritze D (1994). Patent aspects of the Convention at the microbial level. In: *The biodiversity of microrganisms and the role of microbial resource centres* (B Kirsop & DL Hawksworth, eds). World Federation for Culture Collections, Braunschweig: 37–43.

Gams W, Diederich P, Põldmaa K (2004). Fungicolous fungi. In: *Biodiversity of fungi: inventory and monitoring methods* (GM Mueller GF Bills & MS Foster, eds). Elsevier Academic Press, Amsterdam: 343–392.

[5] Apart from fungi also maintained incidently at IBPGR centres, the only fungal collection to have international legal status has been that of the erstwhile International Mycological Institute (now part of CABI Bioscience) of CAB International, a body registered with the UN by treaty in 1988, and currently with signatories from 39 governments.

Govaerts R (2001). How many species of seed plants are there? *Taxon* **50**: 1085–1090.

Greuter W, McNeil J, Barrie FR, Burdet HM, Demoulin V, Filgueras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL (eds) (2000). *International Code of Botanical Nomenclature* (*Saint Louis Code*). [Regnum Vegetabile No. 138.] Koeltz Scientific Books, Königstein.

Guerrant EO jr, Havens K, Maunder M (eds) (2004). *Ex situ plant conservation.* Island Press, Washington DC.

Hawksworth DL (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research* **95**: 641–655.

Hawksworth DL (1992). The need for a more effective biological nomenclature for the 21st century. *Botanical Journal of the Linnean Society* **109**: 543–567.

Hawksworth DL (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* **105**: 1422–1432.

Hawksworth DL (2004a). 'Misidentifications' in fungal DNA sequence databanks. *New Phytologist* **161**: 13–15.

Hawksworth DL (2004b). Biological papers without cited voucher material are so much waste paper. *European Science Editing* **30**: 81–83.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DM (1995). *Ainsworth & Bisby's dictionary of the fungi.* 8th edition. CAB International, Wallingford.

Hawksworth DL, Rossman AY (1997). Where are all the undescribed fungi? *Phytopathology* **87**: 888–891.

Henkel TW, Terborgh J, Vilgalys RJ (2002). Ectomycorrhizal fungi and their leguminous hosts in the Pakaraima Mountains of Guyana. *Mycological Research* **106**: 515–531.

Hermosa MR, Keck E, Chamorro I, Rubio B, Sanz L, Vizaíno JA, Grondona I, Monte E (2004). Genetic diversity shown in *Trichoderma* biocontrol isolates. *Mycological Research* **108**: 879–906.

Heywood VH (ed.) (1995). *Global biodiversity assessment*. Cambridge University Press, Cambridge.

Holst-Jensen A, Vrålstad T, Schumacher T (2004). On reliability. *New Phytologist* **161**: 11–13.

Homchantara N, Coppins BJ (2002). New species of the lichen family *Thelotremataceae* in SE Asia. *Lichenologist* **34**: 113–140.

Hong SG, Jung HS (2004). Phylogenetic analysis of *Ganoderma* based on nearly complete mitochondrial small-subunit ribosomal DNA sequences. *Mycologia* **96**: 742–755.

Hyde KD (2001). Where are the missing fungi? Does Hong Kong have any answers? *Mycological Research* **105**: 1514–1518.

Kirk PM (2000). World catalogue of 340 K fungal names. *Mycological Research* **104**: 516–517.

Kirk PM, Cannon PF, David JC, Stalpers JA (eds) (2001). *Ainsworth & Bisby's dictionary of the fungi.* 9th edition. CABI Publishing, Wallingford.

Kohlmeyer J, Volkmann-Kohlmeyer B (2001). The biodiversity of fungi on *Juncus roemerianus. Mycological Research* **105**: 1411–1412.

Kroken S, Taylor JS (2001). A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia. Mycologia* **93**: 38–51.

Lawrey JD, Diederich P (2003). Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist* **106**: 80–120.

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hall B, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida N, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R (2004). Assembling the fungal tree of life: classification and the evolution of their their subcellular traits? *Amerian Journal of Botany*: **91**: 1446–1480.

Miadlikowska A, Arnold AE, Hofstetter V, Lutzoni F (2004). High diversity of cryptic fungi inhabiting healthy lichen thalli in a temperate and tropical forest. *IAL book of abstracts: lichens in focus* (T Randlane, A Saag, eds): 43. Tartu University Press, Tartu.

Molina MC, Crespo A, Blanco O, Lumbsch HT, Hawksworth DL (2004). Phylogenetic relationships and species concepts in *Parmelia s. str.* (*Parmeliaceae*) inferred from nuclear ITS rDNA and β-tubulin sequences. *Lichenologist* **36**: 37–54.

Mueller GM, Bills GF, Foster MS (eds) (2004a). *Biodiversity of fungi: inventory and monitoring methods.* Elsevier Academic Press, Amsterdam.

Mueller GM, Schmitt JP, Leacock PR, Buyck, B, Cifuentes J, Desjardin DE, Halling RE, Hjortstam K, Iturriaga T, Larsson K-H, Lodge DJ, May TW, Minter D, Rajchenberg M, Redhead SA, Ryvarden L, Trappe JM, Watling R, Wu QX (2004b). Global diversity and distribution of macrofungi. *Biodiversity and Conservation*: in press.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T (2004). Geneological concordance between the mating types and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics and Biology* **41**: 600–623.

OECD (2001). *Biological Resource Centres: underpinning the future of life sciences and biotechnology.* OECD Publications, Paris.

Pandey AK, Reddy MS, Suryanarayanan TS (2003). ITS-RFLP and ITS sequence analysis of a foliar endophytic *Phyllosticta* from different tropical trees. *Mycological Research* **107**: 439–444.

Pegler DN (2001). Taxonomy, nomenclature and description of *Armillaria.* In: *Armillaria root rot: biology and control of honey fungus* (RTV Fox, ed.): 81–93. Intercept, Andover.

Pitman NCA, Jørgensen PM (2002). Estimating the size of the world's threatened flora. *Science* **298**: 989.

Prance GT, Beentje H, Dransfield H, Johns R (2000). The tropical flora remains undercollected. *Annals of the Missouri Botanical Garden* **87**: 67–71.

Raven PH (2004). Foreword. In *Ex situ plant conservation* (Guerrant E O jr, Havens K, Maunder M (eds): xiii–xv. Island Press, Washington DC.

Ruibai C, Platas G, Bills GF (2004). Isolation and characterization of melanized fungi from limestone formations in Mallorca. *Mycological Progress* **3**: in press.

Ryan M, Ellison C (2003). Development of a cryopreservation protocol for the microcyclic rust fungus *Puccinia spegazzini. CryoLetters* **24**: 43–48.

Ryan MJ, Smith D (2004). Fungal genetic resource centres and the genomic challenge. *Mycological Research* **108**: 1351–1362.

Ryvarden L (ed.) (2004). *Synopsis Fungorum.* Vol. 18. Fungiflora, Oslo.

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003). Seasonal dynamics of previously unknown fungal lineages. *Science* **301**: 1359–1361.

Schmitt JP, Mueller GM (2004). An estimate of global fungal diversity. *Biodiversity and Conservation*: in press.

Schmitt JP, Mueller GM, Huang Y, Leacock PR, Mata JL, Wu Q (2004). Assessment of tree species richness as a surrogate for macrofungal species richness. *Biological Conservation*: in press.

Scotland RW, Wortley AH (2003). How many species of seed plants are there? *Taxon* **52**: 101–104.

Smith D, Ryan MJ (2004). Current status of fungal collections and their role in biotechnology. In: *Handbook of fungal biotechnology* (DK Arora, ed.) 2nd edition Marcel Dekker, New York: 527–538.

Staines JF, McGowan VF, Skerman VDB (1986). *World directory of collections of cultures of microorganisms.* 3rd edition World Data Center, Brisbane.

Stadler M, Læssøe TL, Simpson JA, Wollweber H (2004). A survey of *Daldinia* species with large ascospores. *Mycological Research* **108**: 1025–1041.

Suh S-O, Blackwell M (2005). Beetles as hosts for undescribed yeasts. In: *Insect-fungal associations: ecology and evolution* (FE Vega, M Blackwell, eds). Oxford University Press, Oxford: 244–256.

Suh S-O, McHugh JV, Blackwell M (2004). Expansion of the *Candida tanzawaensis* clade: 16 new *Candida* species from basidiocarp-feeding beetles. *International Journal of Systematics and Evoilutionary Microbiology*: in press.

Taylor JE, Hyde KD (2003). *Microfungi of tropical and temperate palms.* Fungal Diversity Press, Hong Kong.

Tehler A, Little DP, Farris JS (2003). The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, *Fungi. Mycological Research* **107**: 901–916.

United Nations Environment Programme (1992). *Convention on Biological Diversity. June 1992*. United Nations Environment Programme, Nairobi.

Weir A, Hammond PM (1997). *Laboulbeniales* on beetles: host utilization patterns and species richness of the parastites. *Biodiversity and Conservation* **6**: 701–719.

Wheeler QD (2004). Taxonomic triage and the poverty of phylogeny. *Philosophical Transactions of the Royal Society of London, B* **359**: 571–583.

White MM, Lichtwardt RW (2004). Fungal symbionts (Harpellales) in Norwegian aquatic insect larvae. *Mycologia* **96**: 891–910.

Wortley AH, Scotland RW (2004). Synonymy, sampling and seed plants. *Taxon* **53**: 478–480.

.