

Mycological Research News¹

This month *Mycological Research News* features a report of an extraordinary richness of undescribed truffle-like fungi in Australia, and an explanation of why mycologists should always deposit voucher specimens and cultures to enable their work to be validated, co-authored by 18 mycologists.

Amongst the 21 papers included in this issue are three on the biocontrol of insect pests by *Erynia* and *Metarhizium* species, including methods of application and longevity of conidial preparations. Biomass estimations by ergosterol in decomposing leaves in the Everglades, and by chitin in *Crinipellis* infections on cacao are described. Variation in *Phytophthora infestans* provides evidence of heterokaryons, conidial production in *Colletotrichum acutatum* is favoured by monsoon conditions, and the conidia of *Pestalotiopsis neglecta* inhibit other fungi. Six papers on mycorrhizas address competition with saprobic fungi and other ectomycorrhizal species, the characterization of a chitin synthase, variation at the molecular level in two endomycorrhizal fungi, a culture technique, and the early response of tobacco roots to colonization. Amongst other papers are ones characterizing a protease in *Schizophyllum*, describing incompatibility groups in *Pleurotus tuberregium*, and showing different *Venturia* species attack different pears.

The following new scientific names are introduced: *Pseudohelicomyces* gen. nov.; *Anthostomella acuminata*, *A. applanata*, *A. cafrariae*, *A. colligata*, *A. meerenensis*, *A. palmae*, *A. raphiae*, *A. spiralis*, *Pseudohelicomyces albus*, *Ramulispora cerealis*, and *Trichoderma stromaticum* spp. nov.

IN THIS ISSUE

This issue includes several papers with new results on the use of fungi as biocontrol agents of insects. Granular and mycelial mat preparations of *Erynia neoaphidis* applied to soil had similar effects to foliar *Beauveria bassiana* applications against *Macrosiphum euphorbiae* (pp. 645–652). In field situations, rain can remove considerable amounts of *Metarhizium* inoculants, but the extent to which this occurs varies markedly with different inoculum formulations (pp. 653–661). The longevity of *Metarhizium* conidia to be used in biocontrol applications is improved if they are dried slowly (pp. 662–665).

Ergosterol has proved efficacious in estimating fungal biomass in soils, which did not vary under *Cladium* or *Typha* in the Everglades, but 70% of the respiration in decaying leaves was from fungi and other eukaryotes not bacteria (pp. 666–670). However, the amount of chitin has proved a sensitive marker for the biomass of infections of *Crinipellis perniciosus* in *Theobroma cacao* tissues at different stages of infection (pp. 671–675).

Two phenotypes of *Phytophthora infestans* from a garden in Wales had different mating types; some were self-sterile and others self-fertile, the self-fertile strains being heterokaryons (pp. 676–680). Conidial production in another plant pathogen, *Colletotrichum acutatum*, has been studied at different humidities and temperatures which suggest that monsoon conditions will

favour its spread (pp. 861–685). In *Pestalotiopsis neglecta*, the conidial matrix has been found to inhibit spore germination in some other mitosporic fungi (pp. 686–690).

Six papers focus on mycorrhizal fungi. Interactions between three ectomycorrhizal basidiomycetes, two saprophytic species of *Collybia*, and a range of soil fungi were examined at different ammonium concentrations; the ectomycorrhizal species tended to be the most competitive (pp. 691–697). *Tuber borchii*, when inoculated onto *Pinus* seedlings together with other fungi, was found to out-competed other species by using specific DNA primers (pp. 698–702). The chitin synthase gene in *T. magnatum* crucial to its growth has been characterized (pp. 703–707). A comparison of the ITS sequences in *Glomus mosseae* and *Gigaspora margarita* revealed variation even within a single ascospore and demonstrate a high degree of variation in the field of these fungi (pp. 708–715). The successful culture of *Gigaspora margarita* on transformed carrot roots is also reported (pp. 716–721). *Nicotiana tabacum* roots are stimulated to form catalase and ascorbate peroxidase just as appressoria of *Gigaspora mosseae* form on the root surface (pp. 722–725).

A small serine protease formed under nitrogen limiting conditions has been discovered and characterized in *Schizophyllum commune* (pp. 726–731). The edible *Pleurotus tuberregium* has been found to represent a unique intersterility group within its genus by compatibility studies of isolates from several tropical countries (pp. 732–737). Mating experiments also revealed that *Venturia nashicola* on Japanese and Chinese pears is distinct from *V. pirina* (pp. 755–759).

Several new taxa are introduced, including the new genus and species *Pseudohelicomyces albus* for an anamorph of *Psilocybe*

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merdaria (pp. 738–741), eight new species of *Anthostomella* from South Africa (pp. 742–754), a new *Trichoderma* parasitic

on witches broom of cacao (pp. 755–759) and a new *Ramulispora* from cereal stems in the UK (pp. 765–768).

AUSTRALIA: A GOLD-MINE FOR NOVEL TRUFFLES

Australia has long been known to be a source of endemic truffle-like fungi. In recent decades, the work of the amateur mycologist Gordon W. Beaton in the late 1970s and mid-1980s, spurred on by and working with various colleagues inside and outside Australia, started to alert the mycological world to a potential Pandora's box (e.g. Beaton & Weste 1977, Beaton, Pegler & Young 1984). In the 1990s the American mycologist James M. Trappe, the worlds leading authority on hypogeous fungi, started to pay attention to the continent. A fresh series of papers prepared jointly with Australian mycologists started to flow, including a stream of new genera and species of both ascomycetes and basidiomycetes hypogeous fungi (e.g. Trappe, Castellano & Malajczuk 1992, 1996).

These studies scarcely prepared the mycological community for the richness reported by Claridge, Cork & Trappe (2000). The stream is becoming a tidal wave. Claridge *et al.* examined 136 sites in Victoria and New South Wales, and collected 7451 fruit-bodies in one year which proved to represent 209 species. Of those species, only 57 had been previously described; 152 (73%) were found to be new to science. But the richness was not just in terms of species; two new genera of ascomycetes, and six of basidiomycetes will be needed to accommodate some of the novelties. The focus of this paper is the extent of the diversity, and a companion one considers the factors influencing their occurrence (Claridge *et al.* 2000). Names are coined for the new genera, but not validly published here; formal descriptions of the new genera and also the species will be published separately.

More fruit-bodies were collected in the autumn (5938) than the spring (1513), and the number found at each site varied from 3 to 129. However, as sampling was carried out only once in each season, the authors recognize that the full extent of the diversity present will not have been discovered. They note that Hunt & Trappe (1987) were still finding additional

hypogeous species after sampling every month for three years in one site in Oregon.

The hypogeous fungi reported appear to be mycorrhizal with eucalypts; and so are not just a of taxonomic interest but directly relevant to ecosystem function and maintenance. Future holistic studies on ecosystems dominated by eucalypts will have to take note of these fungi so it is to be hoped that the necessary formal descriptions and keys will be prepared shortly to expedite their identification.

The discovery of such a high percentage of novel macrofungi in one habitat in part of Australia in a single year also has implications for species richness estimates².

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² See the Mycological Research News item 'How many fungi are there?' in *Mycological Research* **104**(1): 4–5 (2000). Also note that in that item the journal in which Aptroot (1997) appeared should read '*Abstracta Botanica*'.

ALWAYS DEPOSIT VOUCHERS

To help minimize invalid publication of newly proposed scientific names of fungi, Korf (1995) provided advice on how to guarantee valid publication, and offered a few simple guidelines for authors, reviewers, and editors. He regretted that 'unfortunately many of the errors are committed by highly respected mycologists, and published in thoroughly respectable journals' and emphasised that 'although the ultimate responsibility for publishing correct names lies with authors, clearly reviewers and editors are shirking their duties to advise authors of such errors prior to publication'.

In order to be published validly, names must be introduced according to requirements of the International Code of Botanical Nomenclature (ICBN; Greuter *et al.* 1994, 2000).

Since 1990 it has been compulsory to deposit the vouchers for new species and infraspecific taxa, the name-bearing types, in an herbarium or other collection. It is generally accepted that such voucher specimens should be deposited in publicly accessible reference collections such as herbaria.

However, voucher collections are invariably necessary, not only when new fungi are described, but also in connection with any scientific study, whether by taxonomists, systematists, physiologists, chemists, molecular biologists, pathologists, ecologists, clinicians, etc., dealing with organisms. It is essential to preserve voucher specimens as dried material or, where possible, in addition as permanently preserved living cultures. When none of the investigated material is preserved,

it is impossible to confirm the identity of the investigated fungi. If species concepts have changed, it is particularly crucial to be able to re-identify the organism at a later time. There are several examples of entities once thought to be single species but now revealed as species complexes, where the species concept has been or will be changed, including *Pisolithus tinctorius* (Burgess *et al.* 1995) and *Paxillus involutus* (Fries 1985, Hahn & Agerer 1999). In such cases, re-identification of the original material is indispensable in order to know which organism was studied so that previous work will continue to be relevant. In recent years molecular biological studies have a tremendous impact on systematics, taxonomy, and ecology. DNA-sequences are frequently obtained from fungal cultures. Too often there is no record either of an exact citation of the fungal material used, such as an unequivocal number referring to collection accession data and the voucher culture, or reference to the institution where the material has been deposited. Frequently, only personal or laboratory strain numbers are given, which make it hard to trace the origin of the fungal material. Only accession numbers allocated by permanent public or other open institutional collections can ensure the retrieval of voucher material over the long-term. It is not yet common practice to publish complete collection or isolation data, or to deposit vouchers, except in taxonomic articles.

Conservation of dried fruit-bodies from which cultures are made is also indispensable in order to allow checking of anatomical and morphological features that cannot be reproduced in culture. The cultures also can be checked using molecular methods after prolonged preservation, in order to exclude the possibility of contamination. While it is rarely possible to culture fungi from dried specimens, the associated collection details are indispensable not only to clarify the geographical and ecological source, but also to facilitate the possibility of recollecting the fungus in the same site. This requires as detailed and exact a description of the sampling location as possible, preferably including geographic coordinates – something now facilitated by hand-held or wrist-band global positioning devices.

Voucher specimens are equally important for a wide range of other investigations. Dennis' (1960: xxii) remark that 'records that cannot be verified are mere waste paper' applies to numerous aspects of our discipline. Studies of the species composition of any habitat depend on properly determined fungi, and so will require dried vouchers deposited in publicly accessible collections. This applies, for example, not only to fruit-bodies, but indeed to any other form of fungal structure, such as sclerotia or ectomycorrhizas (Agerer 1991), used in scientific work. Ecological, chemical, applied, and physiological studies quite often rely on ecotypes of species, which could later be considered, depending upon the species concepts applied, as separate species. In the 1970s, Hawksworth (1974), Yocum & Simons (1977) and Ammirati (1979) were among the first to point out the importance of voucher material particularly in chemical, but also other physiological and ecological studies. In ecological studies on ectomycorrhizas, the increasing use made of RFLP patterns or DNA sequences for the detection of the symbionts requires comparison with those of identified fruit-bodies. In many studies, the identified

ectomycorrhizas are completely consumed by the extraction and amplification methods. Instead, voucher specimens should be stored, when individual tips of a larger hyphal system have been used. Even more important is the citation and preservation of the fruit-body specimen from which DNA was extracted for comparison with that obtained from ectomycorrhizal hyphae.

Voucher cultures are urgently needed when clinically relevant fungi are investigated and their etiologic data and their impact on human beings have to be evaluated (de Hoog & Guého 1985). Sufficient information on clinical direct microscopy or histopathology results to determine whether an isolate was medically significant or a biomedical contaminant is essential for later evaluations. In cases of apparently exotic fungi, a brief notation of relevant patient travel history is strongly recommended. Further, where cultural or chemical features are crucial for the evaluation of newly described fungi such as yeasts, the non-availability of cultures can make interpretation impossible and frustrate other researchers (Banno *et al.* 1993, Hawksworth 1984).

Additional documentation requirements apply to strains deposited in the major service collections of fungal cultures, such as ATCC (American Type Culture Collection, Manassas, Virginia, USA), CBS (Centraalbureau voor Schimmelcultures, Baarn/Utrecht, The Netherlands), or IMI (CABI Bioscience (UK Centre), Egham, Surrey, UK); these and other culture collections often provide forms for depositors to simplify the documentation process. In such major culture collections, the cultures are safely stored by cryopreservation methods, and may be revived at any time. For sporulating fungi, the citation of the allocated accession number is generally enough to meet the goal of reproducibility of scientific results, i.e. to confirm the identity of the species studied. But a comparison with naturally grown material is only possible when the original collection or isolation details have been cited. A completely different situation arises in cultures which are sterile and thus cannot be identified by normal methods. For such cultures, preservation of vouchers is particularly important together with exact collection data of the fruit-bodies and the herbarium or other collection where they have been deposited. Misidentifications can then be detected, new species concepts applied to the material, and recollection of new living material from the site of the original fruit-body might still be possible.

The addresses of public and open institutional dried reference collections can be found in *Index Herbariorum* (Holmgren, Holmgren & Barnett 1990), and of microbial culture collections in the *World Directory* (Sugawara *et al.* 1993); these works both contain generally applied acronyms, which are convenient and informative enough for citation. Public and institutional collections ensure that the material in their care is preserved in a proper way for centuries, and they usually loan dried material free of charge, subject to certain requirements being met. Whilst the long-term maintenance of private herbaria is often uncertain and the mailing expenses exceed a private budget, nearly all of the international herbaria and other institutions that house fungi will warmly accept properly dried and documented fungal material. Living cultures are normally supplied for a charge to cover the cost of preparation and carriage, again subject to particular regulations

that may apply; details vary and are available from the collections' catalogues and web sites.

Particularly in recent years, the behaviour of the scientific community has set tongues wagging, especially in relation to falsified data in publications concerning human cancer. It is a fundamental principle of science that research work must be reproducible. Reproducibility requires that studies can be made using the same material or cultures as the original study used. As a consequence, publications lacking unambiguous reference to the locations where the critical study materials can be accessed by later researchers should not be accepted for publication. They are of no or limited scientific value in that they cannot be reproduced. Editors and referees in all aspects of mycology are often confronted with such situations and it is therefore necessary to include advice for the deposition of voucher material in instructions for authors (e.g. Hawksworth 2000³) and to regard this as a prerequisite for publication.

All scientists are responsible for their results. This responsibility lies not only in relation to the scientific community, but also in relation to those who support their research – the taxpayer, charities or other funding agencies, and ultimately society at large. The general public expects integrity from the scientific community. It is the responsibility of individual scientists, referees, and editors to rigorously apply the highest standards and make every effort to ensure published research will be reproducible. Reproducibility in mycology is irrevocably and inextricably connected to the unequivocal citation of voucher specimens and cultures.

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³ This policy is being rigorously applied to papers now being accepted for publication in *Mycological Research*. Some papers accepted and typeset under the previous instructions to authors will, however, still be appearing for the next few issues of the journal.