
Mycological Research News¹

This contribution of Mycological Research News features: Publication in *Mycological Research* accelerates, and Multiples of eight in *Cordyceps* ascospores.

This issue contains 14 original research papers. Fast-tracked are papers on the transformation of the genome of *Verticillium fungicola*, and the occurrence of double-stranded RNA mycoviruses in *Rhizoctonia solani*. Other molecular papers in this issue concern the development of primers for the identification of *Alternaria* species, the diversity and molecular strain characterization of *Lentinula edodes*, genotypic variation within *Beauveria bassiana*, compare *Coniothyrium zuluense* isolates from South Africa and Thailand, show that the causal agent of *Rhododendron* anthracnose is *Colletotrichum dematium*, and suggest a new placement for the genus *Monosporascus*.

The contents of ergocaliferol (vitamin D₂) in dried fruit bodies of *Cantharellus cibarius* examined, a new floating culture technique for mycelia of ectomycorrhizal fungi is described, the growth of *Epichloë/Neotyphodium* endophytes in grasses has been studied microscopically, and the production of lignin-modifying enzymes in white-rot fungi compared, and the effect of clay minerals on the morphology of fungal pellets explored.

One new scientific name is introduced: *Monosporascus ibericus* sp. nov.

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PUBLICATION IN MYCOLOGICAL RESEARCH ACCELERATES

One of my primary objectives when entrusted with the role of Executive Editor for *Mycological Research* in January 2000, was ‘to reduce the time taken from acceptance to publication to under six months’ (Hawksworth 2000). Of the fifteen papers in this present issue, four were accepted four months ago, five five months ago, and two six months ago. The other three took slightly longer to be issued due to special circumstances. This means that *Mycological Research* is now able to offer authors of regular articles publication within six months of acceptance. This is in addition to the service offered for fast-tracked papers which always appear 12–16 weeks from acceptance. *Mycological Research* is now publishing original research papers more quickly than any other major international journal in the field. During 2002, I and my fellow Editors plan to cut the time between the receipt of papers and acceptance, although often those delays are mainly due to the time authors take to revise papers in the light of

the reports of Referees and Editors. If you have something really exciting to say to the mycological community at large, don’t hesitate to make *Mycological Research* your first choice.

During 2001, 187 papers, 25 news items, and 20 book reviews appeared in *Mycological Research*, so maintaining the journal’s position as the premier world outlet for original research in all aspects of mycology. An innovation for 2002 is a fresh print typeface, Times New Roman, which is much easier to read when small than the previous Palatino typeface. Some papers already typeset will, however, continue to appear in the older style for the next 1–2 issues.

I am grateful to Trevor Burling and Mike Adams for effecting this transition.

Hawksworth, D. L. (2000) *Mycological Research* in the new millennium. *Mycological Research* **104**: 1.

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IN THIS ISSUE

Two papers have been fast-tracked for publication in this issue. The first reports the transformation of the

genome of *Verticillium fungicola* using PEG- and *Agrobacterium*-mediated methods; both were successful

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and provide a new tool for the study of the epidemiology of this important pathogen of cultivated *Agaricus bisporus* (pp. 4–11). In the second, double-stranded RNA mycovirus elements have been found in *Rhizoctonia solani* anastomosis group three (AG 3) and its transmission and elimination investigated (pp. 12–22).

Other molecular papers in this issue describe the development of primers for the identification of *Alternaria* species in carrots and compare the approach with other techniques (pp. 23–33); the diversity and molecular strain characterization of *Lentinula edodes* in Japan (pp. 34–39); the genotypic variation within *Beauveria bassiana* has been examined by a variety of methods and eight phylogenetic clusters found – which were not significantly correlated with the groups of insects from which they were obtained (pp. 40–50); the *Eucalyptus* pathogen *Coniothyrium zuluense* is established as present in Thailand, the first time the fungus has been found outside South Africa (pp. 51–59); and molecular data has been used to show that the causal agent of *Rhododendron* anthracnose is *Colletotrichum dematium* (pp. 60–69).

The contents of ergocaliferol (vitamin D₂) in dried fruit bodies of *Cantharellus cibarius* vary significantly

and challenge figures often given in dietary tables (pp. 70–73).

A new floating culture technique has been developed for mycelia of ectomycorrhizal fungi and used to compare nitrogen source utilisation in different subtropical species – most of which were unidentifiable and may well represent undescribed fungal species (pp. 74–85). The growth of *Epichloë/Neotyphodium* endophytes in grasses has been studied microscopically showing evidence of synchronized growth by examining patterns of growth within host tissues (pp. 93–106). The production of lignin-modifying enzymes in ten white-rot fungi in relation to different carbon and nitrogen regimes is compared and a cellulose-low nitrogen medium is recommended for screening procedures (pp. 74–85). The effect of clay minerals on the morphology of fungal pellets, something crucial to the effectiveness of fungi as absorbants of toxic metals, is also explored (pp. 107–117).

A new species of *Monosporascus* has been discovered and molecular studies carried out for the first time on the genus indicate affinities with the *Xylariales* rather than the *Sordariales* (pp. 118–127).

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MULTIPLES OF EIGHT IN *CORDYCEPS* ASCOSPORES

Darwin (1872) drew attention to Haeckel's concept of a phylogenetic approach to classification – 'the lines of descent of all organic beings'. The intervening 130 years have seen an increasing interest in this approach. Especially, the last 10–15 years have witnessed the rise of molecular phylogenetics to a point where some have considered phylogenetics only from a molecular perspective. The following aims to highlight how the morphology-based approach to phylogenetics must work in concert with the molecular approach to solve basic problems in systematics, with *Cordyceps* as an example.

The megagenus *Cordyceps* is primarily pathogenic to insects (but also to spiders, mites and other fungi) and was based on *C. militaris*. There are about 300 species known with many more synonyms to add to confusion. As such, there has been an understandable interest in developing meaningful subdivisions of the genus. Kobayasi (1941, 1982) in particular sought to establish an infrageneric structure.

The asci in *C. militaris* produce eight long, filiform ascospores that break into part-spores $3\text{--}3.5 \times < 1 \mu\text{m}$. Historically, part-spore size and shape was used as one morphological character for separating species. Notably, *C. myrmecophila* and *C. irangiensis* are pathogens of ants almost identical in gross morphology but easily separated by part-spore characteristics. *C. myrmecophila* has barrel-shaped, truncate part-spores

6–7.5 μm long while *C. irangiensis* has fusoid part-spores 8.5–12.5 μm long (Hywel-Jones 1996).

Not all species of the genus have ascospores that divide into part-spores. Consequently, Petch (1931) established the genus *Ophiocordyceps* for *Cordyceps* species that produce mature, whole ascospores. The type species, *O. blattae*, has not been recollected, and the best known example of an *Ophiocordyceps* as conceived by Petch is *C. unilateralis*. Petch's genus was not accepted by subsequent workers and Kobayasi (1941) soon relegated it to a subgenus where it remains. Within *Cordyceps* three other subgenera are accepted: *Neocordyceps* and *Eucordyceps* (Kobayasi 1982), and *Bolacordyceps* (Eriksson 1986). Molecular work is demonstrating that of these subgenera only *Neocordyceps* is a strong grouping (J. Spatafora & J. Mitchell, pers. comm.) while the status of *Bolacordyceps* is still unclear. *Neocordyceps*, which includes *C. myrmecophila* and *C. irangiensis*, is restricted to *Hymenoptera* (ants, bees and wasps).

Hywel-Jones (1996) reported a novel discharge pattern for *C. myrmecophila* and *C. irangiensis* where part-spores were oozed onto the fertile head rather than being forcibly ejected. However, more recent observations show this was an artefact due to keeping specimens in a semi-airtight humid chamber. Natural forest has a fluctuating humidity and air pressure, which seem to govern ascospore discharge in *Cordyceps*.

Providing more natural conditions for spore discharge in the laboratory showed that *C. myrmecophila* and *C. irangiensis* discharge ascospores as a still-connected chain of part-spores. If discharged onto glass slides, heat-fixed and stained, it is possible to count the number of part-spores that constitute a whole ascospore. For all species of *Neocordyceps* that number is 64. No examples were seen of ascospores splitting into more than 64 part-spores in members of this subgenus. Where less than 64 part-spores were counted, this was attributable to division failure with a resultant part-spore twice the normal length. Natural release of ascospores is now routinely encouraged in the laboratory. Within *Cordyceps s. lat.* a pattern has developed based on part-spore development. Although subgenus *Neocordyceps* invariably produces 64 part-spores, species of subgenus *Eucordyceps* have a more variable pattern of part-spore production.

Some *Eucordyceps* produce 64 part-spores. However, *Cordyceps s. str.* (*C. militaris* and its relatives) ascospores routinely divide into 128 part-spores. These are pathogens of *Lepidoptera* or occasionally *Coleoptera*. Other species divided into 32 part-spores (especially species on cicadas, *Homoptera*), or 16 (several species on spiders with purported *Akanthomyces* anamorphs). No species have been found whose spores divide into two or eight part-spores, although one new species from *Coleoptera* has ascospores that divide into 4. Significantly, no species were seen to make the next progression beyond 128, namely 256 part-spores. Those species that divided into 128 part-spores had spores typically 2–5 µm long. Halving this size must reach limits where a fungal spore could not adequately contain a nucleus and the reserves to sustain it.

Nobody can look at the relatively short squat fusoid ascospores of *C. unilateralis* (100–160 x 2–3 µm) and consider these the same as the filiform ascospores of *C. khaoyaiensis* and *C. pseudomilitaris* (typically 400–600 x < 1 µm) (Hywel-Jones 1994). However, using current morphology-based subgeneric divisions all three species were classified in *Ophiocordyceps*. But, in their overall morphology and host affinity *C. khaoyaiensis* and *C. pseudomilitaris* were closer to *C. militaris* than to *C. unilateralis*. The major difference was that *C. khaoyaiensis* and *C. pseudomilitaris* produced and discharged whole ascospores while *C. militaris* produced ascospores that separated into 128 part-spores. Given the host affinities, it is posited that *C. khaoyaiensis* and

C. pseudomilitaris are whole-spored ancestors of *C. militaris*. Similarly, it is logical to consider *C. unilateralis* as a whole-spored ancestor of subgenus *Neocordyceps*.

A molecular phylogenetic approach provides a powerful tool for understanding the relationships within megagenera such as *Cordyceps*. Already, the small number of species sequenced to date raise many questions regarding the subgeneric divisions established by Kobayasi. But importantly, morphology must still be used to add real meaning to phylogenetic trees based on molecular sequences. Darwin (1872) was writing in the pre-dawn of Mendelian genetics. His thoughts were wholly based on morphology but with an understanding of how continued selection through breeding (natural or artificial) could effect change at the species level and, over time, at the genus level (see Darwin's figure between pp. 90 and 91). Darwin's conclusion on the matter of the phylogenetic approach was this: 'Professor Hackel in his *Generelle Morphologie* ... has thus boldly made a great beginning, and shows us how classification will in the future be treated'. Molecular phylogenetics, classical morphology and field observation must be used together to provide a holomycological approach to fungal classification. Without this approach confusion can only ensue, especially in respect of megagenera such as *Cordyceps*.

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