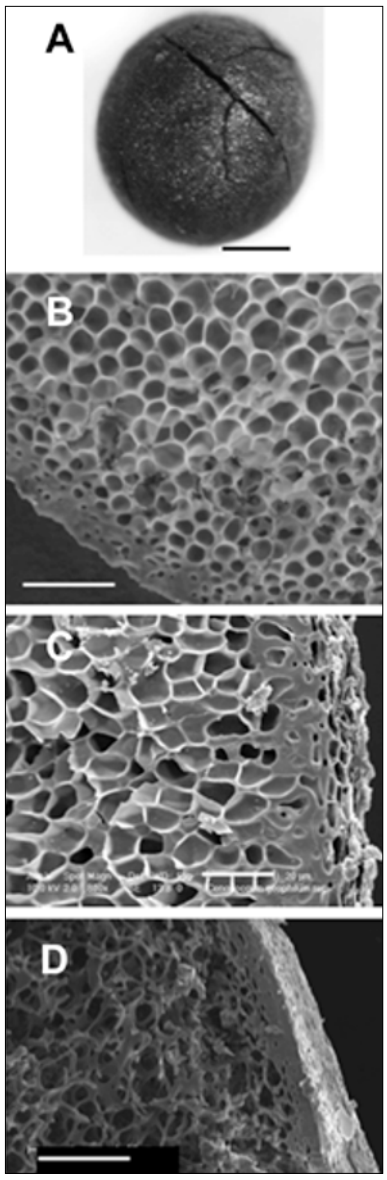
Carbonaceous spherules exposed as fungal sclerotia, are not evidence of the impact of a comet

In the last few years, some palaeoecologists postulated that the onset of the Younger Dryas climate interval at ~ 12,900 yBP was due to an air-bursting or impacting comet which caused intensive wildfires which raged across Europe and North America (Firestone *et al*. 2007; Kennett *et al*. 2008); this hypothesis sparked a controversial debate amongst palaeoclimatologists. Carbonaceous spherules (and similar "elongates") found around the horizon have been interpreted as evidence of intensive wildfires that the postulated comet initiated. However, morphologically and anatomically identical spherules have subsequently also been discovered not only in earlier and later palaeoecological preparations, but further in modern heathland sites where there had been recent fires (notably Thursley Bog in Surrey, UK). When fractured and studied by scanning electron microscopy (SEM), the internal structures of the sphaerules appeared to be pseudoparenchymatous throughout with the exception of a cellularly differentiated outer cortex-like layer. The structures were consequently unquestionably biological in origin, but ascertaining what organism present today they belonged to proved elusive. Now, the question has been answered by the careful SEM studies of Scott *et al*. (2010) who compared the structure of the spherules with that of the sclerotia of species of *Cenococcum* and *Sclerotium* – both fresh and more significantly experimentally charred by fire. The superb illustrations in the paper and accompanying online material leave no doubt that the implicated carbonaceous spherules are fungal sclerotia, while the "elongates" were sclerotia or possibly in some cases coprolites. Especially illuminating was the discovery that at 350 °C the outer cortical cells tended to coalesce, and at 450 °C voids developed inside the sclerotia. Structures that had previously been termed "nanodiamonds" were also evident in the charred sclerotia.

The degree of surface reflectance of the structures and sclerotia was also measured as that is known to be indicative of the temperatures to which organic material has been subjected. As a consequence of the structural comparisons and reflectance data, Scott *et al*. conclude that these carbonaceous spaerules and many of the "elongates" are fungal sclerotia which have been subjected to low-intensity burning, and are not indicative of high-intensity fires as had previously been postulated; i.e. the putative evidence for the catastrophic explanation of the onset of cooling and associated megafaunal extinctions around 12,900 yBP is unsound and alternative explanations must be sought.

Margaret E Collinson and Andrew C Scott kindly prepared the accompanying figure especially for this item.



A. Modern charred fungal sclerotium (Thursley, Surrey, UK). **B.** SEM of section through charred fungal sclerotium from the Younger Dryas, 12 900 Cal BP (Santa Rosa Island, CA, USA). **C.** SEM of section through uncharred *Cenococcum* (Peace River Canada; photo: A Heiss). **D.** SEM of section through charred sclerotium of the genus *Sclerotium*, charred at 350 °C for 5min. Bars: A = 200 µm, B = 50 µm, C–D = 20 µm.

Firestone RB *et al*. (2007) Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. *Proceedings of the National Acedemy of Sciences, USA* **104**: 16016–16021.

Kennett DJ, Kennet JP, West GJ, Erlandson JM, Johnson JR, Hemdry IL, West A, Culleton BJ, Jones TL, Stafford TW jr (2008) Wildfire and abrupt ecosystem disruption on California's Northern Channel Islands at the Allerød—Younger Dryas boundary (13.0-12.9 ka). *Quarterly Science Review* **27**: 2350–2545.

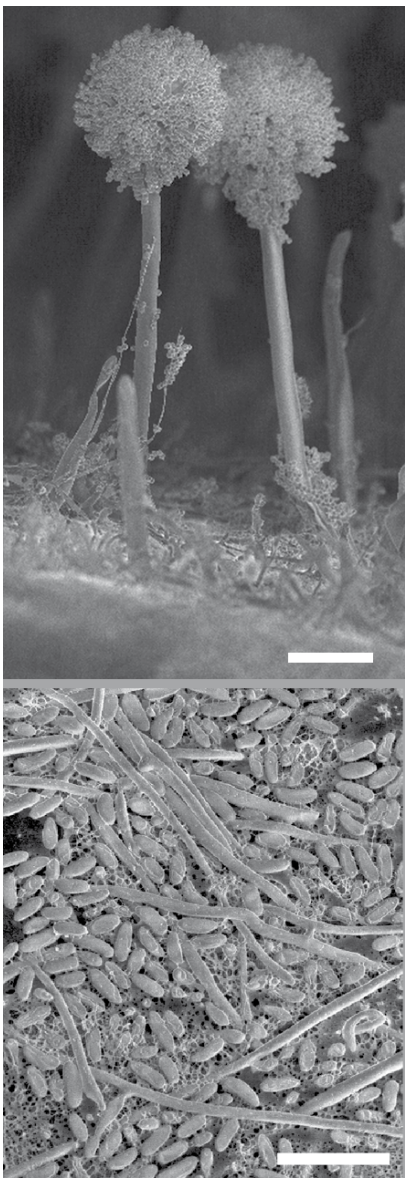
Scott AC, Pinter N, Collinson ME, Hardiman M, Anderson RS, Brain PR, Smith SY, Marone F, Stampanoni M (2010) Fungus, not comet or catastrophe, accounts for carbonaceous spherules in the Younger Dryas "impact layer". *Geophysical Research Letters* **37**: <doi: 10.1029/2010GL043345>.

Physiological differences between wet- and dry-distributed conidia

The distinction between fungi which produce conidia in slimy heads ("wet-spored" and waterborne) and those which form them in powdery masses or chains ("dry-spored" and airborne) has been recognized as of fundamental importance in the characterization of genera since the early 20th century. Now, some clues as to the basis of this distinction have been generated by van Leeuwen *et al.* (2010). The freshly harvested condia of two wet-spored (*Fusarium oxysporum* and *Lecanicillium fungicola*[1]) and two dry-spored species (*Aspergillus niger* and *Penicillium discolor*) were compared physiologically using electron spin resonance (ESR) to test for cytoplasmic viscosity and staining with the fluorescent dye filipin to indicate the presence of ergosterol. Striking differences were found; the wet-spored species had lower cytoplasmic viscosity than the dry-spored pair, and the staining showed ergosterol was present in the plasma membrane only of the wet-spored ones. Whether this correlation can be used as a generalization must, however, await studies on a much more diverse range of fungi.

Leeuwen MR van, Doorn TH van, Golovina EA, Stark J, Dijksterhuis J (2010) Water- and air-distributed conidia differ in sterol content and cytoplasmic microviscosity. *Applied and Environmental Microbiology* **76**: 366–369.

[1]The authors used the name *Verticillium fungicola*, but that species has been transferred to *Lecanicillium* (Zare R, Gams W, *Mycological Research* **112**: 818, 2008).



Formation of conidia by *Aspergillus niger* (top) and *Fusarium oxysporum* (bottom) observed by scanning cryo-electron microscopy. Numerous conidia of *A. niger* are formed on erect conidiophores while conidia of *F. oxysporum* are formed within the mycelium. Bars = 10 μm.

Mobile chromosomes: the clue to pathogenicity in *Fusarium* species

Fungal chromosomes are notoriously difficult to visualize, although a few mycologists have been able to achieve this, including Punithalingam (1975) on *Fusarium*. Now it appears that fungal cytology could have provided the clue to why some fusaria are pathogenic and some are not. Ma *et al.* (2010) sequenced genomes in *F. oxysporum* f. sp. *lycopersici* and *F. verticilliodes*, and compared the results with those of the genome of *F.graminearum* that had previously been sequenced. The three species have different numbers of chromosomes: 15 in *F. oxysporum*, 12 (11 mapped) in *F. verticilloides*, and only four in *F. graminearum*. It was found that the four additional chromosomes in *F. oxysporum* were the ones which were rich in transposons and genes related to pathogenicity. That these chromosomes were indeed the clue to enhanced pathogenicity was shown experimentally

within *F. oxysporum* by transferring two of these chromosomes from a pathogenic to a non-pathogenic strain by co-incubation; that whole chromosomes were involved was demonstrated by electrophoresis. The non-pathogenic strain then became pathogenic to tomato. Over 28 % of the entire *F. oxysporum* genome was composed of repetitive sequences, and while only 20 % of those only found in this species could be functionally classified, they were evidently rich in ones considered related to secreted effectors and virulence factors. The results were also compared with the recently published genome of *F. solani*, which has three "supernumerary" chromosomes (Coleman *et al*. 2009) which lacked the suites of genes involved in the pathogenicity of *F. oxysporum*. As *F. graminearum* and *F. verticilloides* have *Gibberella* teleomorphs, *F. solani* one in *Haematonectria* (not *Nectria* as wrongly stated in the *Nature* paper, and also by Coleman *et al*. 2009), and *F. oxysporum* f. sp. *lycopersici* none (though one in *Gibberella* would be expected as it clusters with *F. verticilloides*), the difference with *F. solani* might have been no surprise to a taxonomist. Perhaps the most exciting aspect of this study, but one that should cause concern to those involved with food security, is the ease with which pathogenicity was transferred by whole chromosome movement between *F. oxysporum* isolates growing together. It also shows that when possible unexpected pathogenicity emerges in fungi that it could be advantageous to try pulse-gel electrophoresis to see whether extra chromosome transfer was involved as a prelude to embarking on similar complex molecular high cost studies – this one involved 63 co-authors distributed through 25 laboratories . . . .

Coleman JJ *et al*. (2009) The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expression. PLos Genetics 5: e10000618.
Ma L-J *et al*. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**: 367–373.
Punithalingham E (1975) Cytology of some *Fusarium* species. *Nova Hedwigia* **26**: 275–303.

Ectomycorrhizal symbiosis in basidio- and ascomycete fungi have different origins

Whole genome studies are remarkable in what unexpected information can emerge. The sequencing of the whole genome of the Périgord truffle (*Tuber melanosporum*) by French and Italian researchers (Martin *et al*. 2010) is no exception, First, at ~ 125 megabases it is the largest by far of all fungal genomes sequenced to date. Second, the size is a result of a proliferation of transposable elements that account for 58 % of the size and not a consequence of whole-genome duplication. Third, it lacks large sets of carbohydrate-cleaving enzymes, but has some involved with plant cell wall degradation that are induced in symbiotic tissues. And fourth, most fascinatingly, comparison with whole genome data from the ectomycorrhizal basidiomycete *Laccaria bicolor* revealed that strikingly distinct proteomes were encoded; i.e. that different "molecular toolkits" had evolved to enable these two fungi to be become ectomycorrhizal. This implies that the ectomycorrhizal habit has evolved independently in asco- and basidiomycete fungi – just as appears from phylogenenetic studies to have been the case with lichenization.

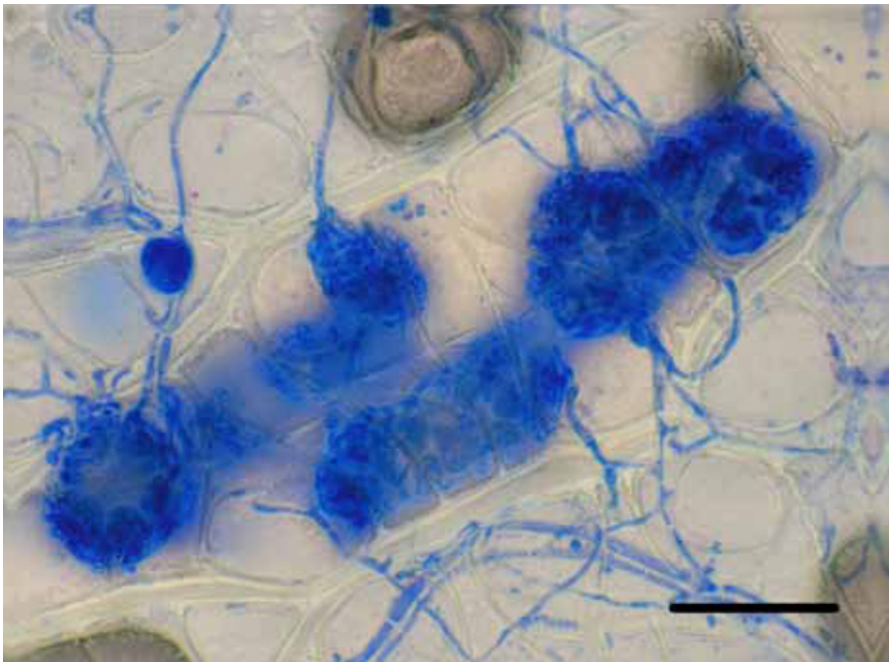
Martin F *et al*. (2010) Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* **464**: 1033–1038.

A basal bryophilous fungus associates with cyanobacteria

For almost 40 years, Peter Döbbeler has, almost single-handedly, been revealing the enormous variety and abundance of specialized ascomycetes that live on bryophytes. The phylogenetic position of many of the genera he discovered has been obscure, but now, in collaboration with Finnish mycological colleagues, a five-gene phylogeny has been constructed for an amazing 61 bryosymbionts. DNA was extracted from cultures prepared from freshly collected specimens – in itself a major achievement. As expected, the fungi were found to be dispersed through different ascomycete lineages, including *Dothideomycetes*, *Eurotiomycetes*, *Lecanoromycetes*, *Leotiomycetes*, *Pezizomycetes*, and *Sordariomycetes*. Most of the fungi are commensals, weak parasites, or saprobes, and "symbiont" is used here in the original sense of organisms living together[2], whether mutualists or not. The results suggest that bryophily has arisen at different times in different lineages of ascomycetes.

However, one of the fungi is of particular phylogenetic and biological interest: the newly described *Trizodia acrobia* which inhabits the living shoot apices of at least eight *Sphagnum*

[2]The term "symbiosis" is attributed here to an 1879 publication of de Bary, but actually originated from one of AB Frank of 1877 who studied lichen anatomy; *viz* Hawksworth (*Nature* **374**: 841, 1995) and Sapp (*Evolution by Association: a history of symbiosis*, 1994). Frank went on to coin the term "mycorrhiza" in 1887.



Trizodia acrobia, hyphae enveloping cyanobacterial colonies on the leaf of *Sphagnum*. Photo: Tomi Laukka. Bar = 30 µm.

species in Finland. The fungus has minute convex ascomata which are translucent white to pale pink when fresh, inoperculate unitunicate asci, and single-celled colourless ascospores. *Trizodia* appeared as a basal clade in *Leotiomycetes*, and is of special interest biologically because it is associated with *Nostoc* cyanobacteria as well as *Sphagnum*. The genus is not "categorized as a lichen *per se* [as] it does not form organized thallus structures" (p. 16), but that is also the situation in some of the fungi traditionally studied by lichenologists, especially pyrenocarpous species on bark! Amongst other fungi that would fall in this category is *Nectria phycophora* (syn. *Calonectria phycophora*), which occurs on the moss *Dawsonia grandis* and has pockets of algal cells in the perithecial wall (Döbbeler 1978, Hawksworth 1988). Many fungi do not fit neatly into biological categories conceived by humans, and the authors describe this as "a primitive form of a bryosymbiotic pyrenolichen" – but does that matter? It is the fungi themselves and the diversity of their intimate associations that are so fascinating – evidently not least amongst the bryosymbionts.

This is a most elegant study that now needs to be emulated for the obligately lichenicolous fungi – which sadly prove more recalcitrant to culture and yield their DNA than those that are bryophilous.

Döbbeler P (1978) Moosbewöhnende Ascomyceten I. Die pyrenocarpen, den gametophyten besiedelnden Arten. *Mitteilungenaus der botanischen Staatsammlung, München* **14**: 1–360.

Hawksworth DL (1988) The variety of fungal-algal symbioses, their evolutionary significance, and the nature of lichens. *Botanical Journal of the Linnean Society* **96**: 3–20.

Stenroos S, Laukka T, Huhtinen S, Döbbeler P, Myllys L, Syrjänen, Hyvönen J (2010) Multiple origins of symbioses between ascomycetes and bryophytes suggested by a five-gene phylogeny. *Cladistics* **26**: 281–300.

Molecular clocks and evolutionary rates

The evolution of fungi is attracting increasing interest as the discovery of fossils and the production of phylogenetic trees with representatives of increasing numbers of families and orders accelerates – with earlier and earlier dates being suggested for major divergences. For example, *Ascomycota* and *Basidiomycota* could have diverged as early as 1.2 Byr ago (Heckman *et al.* 2001). Berbee & Taylor (2010) have now revisited this question, one that has fascinated them since the early 1990s. They used the BEAST program to apply a relaxed lognormal clock analysis to a data set comprising 50 loci from 26 taxa and then endeavoured to ground-truth nodes on the basis of assumptions made as to the systematic position of some well-researched fungal fossils of known age. The assumptions on position had a dramatic effect. In the case of *Palaeopyrenomycites devonicus*, if it can be placed in *Ascomycota* but no further the minimum age of the phylum (and its sister phylum) would be just 452 Myr. However, if is considered to be assignable to *Pezizomycotina* the probable date for the divergence to 843 Myr. And most strikingly if it is in *Sordariomycetes*, as seems very likely, that pushes the *Ascomycota*/*Basidiomycota* divide back to 1489 Myr – a result not so different from the figure computed by Heckman *et al.* Some of the most potentially intriguing fossils that could be pertinent are unfortunately difficult to interpret in modern terms, for example the Lower Devonian *Prototaxites* and the 900 Myr-old Proterozoic *Tappania*. Further, if the Silurian hyphae and especially conidum-like structures reported by Sherwood-Pike & Gray (1985) could be pinned-down to modern taxa, that could materially change the picture pushing orders even further back.

Berbee & Taylor recognize that the rates of divergence, i.e. the speed of the molecular clock, could well vary in different fungal groups. This issue has been examined by Wang *et al.* (2010) who studied the rates of substitution in 21 protein-coding genes in the lichen-forming *Rhizoplaca chrysoleuca* and used them to test for discrepancies in rate between *Ascomycota* and *Basidiomycota* by comparing them with sequences in GenBank – in all 299 taxa were considered, 13 of which had data on 105 protein-coding genes. Significant differences emerged between the phyla, the higher rate being in *Ascomycota*. Within *Ascomycota*, the fastest evolutionary rates detected were in *Sordariomycetes*. They speculate that the high level of speciation in the class is due to founder-effects rather than the development of mutualisms, ecological adaptations, shorter generation times or metabolic rates;

i.e. isolated populations undergoing rapid change as a result of genetic drift.

This is becoming a fascinating area of enquiry which would benefit from much more involvement of palaeontologists with both morpho-mycologists and molecular systematists. For example, there are enormous numbers of fossil fungal propagules now documented and given scientific names (Kalgutkar & Jansonius 2000), which are rarely considered by mycologists . . . . .

Berbee ML, Taylor JW (2010) Dating the molecular clock in fungi – how close are we? *Fungal Biology Reviews* **24**: 1–16.
Heckman DS, Geiser DM, Eudell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. *Science* **293**: 1129–1133.
Kalgutkar RM, Jansonius J (2000) *Synopsis of Fossil Fungal Spores, Mycelia and Fructifications*. [AASP Contributions Series No. 39.] Dallas, TX: American Association of Stratigraphic Palynologists.
Sherwood-Pike MA, Gray J (1985) Silurian fungal remains: probable records of the class *Ascomycetes*. *Lethaia* **18**: 1–20.
Wang H-Y, Guo S-Y, Huang M-R, Lumbsch HT, Wei J-C (2010) *Ascomycota* has a faster evolutionary rate and higher species diversity than *Basidiomycota*. *Science China* (*Life Sciences*) **53**: 1163–1169.

Numbers of fungi in China

Dai & Zhuang (2010) have systematically scoured publications dealing with fungi recorded from China. They located reports of 16 046 species and 297 varieties from the Chinese territories as a whole; the mainland had 14 846 species reported, Taiwan 6 207, and Hong Kong 2 122. The overall total included 300 chromistan (straminipilous) fungi, and 340 protozoan fungi (myxomycetes). However, as far as I can gather from the Chinese text and especially the literature cited, these totals omit the lichen-forming species. An unfortunate oversight which would have added at least 1766 species to the Chinese mainland total (Wei 1991), 261 to that for Hong Kong (Aptroot & Seaward 1999), and 559 to that for Taiwan (Lai 2000); allowing for species in common to these three regions the known lichens actually amount to some 2200 species (J Wei *in litt*.). This oversight is most unfortunate as the names applied to lichen associations strictly refer to the fungal partners alone!

As to the number of fungi to be expected in a total inventory for China, as 32 200 plants are known in China according to the World Resources Institute database portal (<earthtrends.wn.org/>), the now often cited 6:1 model (Hawksworth 1991) would suggest the real figure could be as much as 193 200 species. This would imply that only around 9 % of the fungi of China have yet been recognized and catalogued.

The authors note that 2849 species new to science and 5260 new records of non-lichenized fungi were added over the years 1978–2010, i.e. the rate of accrual was 89 and 164 species per year respectively. However, they suggest that the totals of new taxa be reduced because of possible synonymy, proposing a reduction of 10 % – much less than the 70 % that would be predicted by the 1:2.6 accepted species to synonym ratio derived from an analysis of 15 monographs of fungal genera (Hawksworth 1992). Such an estimate is perhaps pessimistic as the 1:2.6 ratio covered the whole period since 1753 when formal fungal nomenclature is now considered to have started, and does not allow for the diligence of mycologists in their endeavours to discover possible candidate names in often obscure literature. However, Yu-Chen Dai (*in litt*.) informs me that he used the 10 % figure because "many species are complex, and in fact more species inside a single name".

There has been an accelerating interest in fungal systematics in China since the 1980s, largely stimulated by the vigorous Systematic Mycology and Lichenology Laboratory of the Institute of Microbiology of Academia Sinica in Beijing. Nevertheless, it is evident that huge additional resources need to be devoted to inventorying fungi in China to raise the level of knowledge to a level approaching that in European countries. While such investment would be unthinkable in western countries today, perhaps that need not be dismissed out of hand so readily in China, considering the enormous resources now being placed on molecular sequencing in the country through the BGI genomics centre in Beijing (<genomics.cn>) – established in 1999 and set to become the world leader (Cyranoski 2010). Dai & Zhuang's synopsis of the numbers of fungi known in China deserves to prompt and stimulate discussions within China as to how the description of the mycobiota of the country can be accelerated.

I am indebted to Yu-Cheng Dai and Jiang-chun Wei for the supplementary information they kindly supplied while this note was being prepared.

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Cyranoski D (2010) The sequence factory. *Nature* **464**: 22–24.
Dai Y-C, Zhuang J-Y (2010) Numbers of fungal species hitherto known in China. *Mycosystema* **29**: 625–628.
Hawksworth DL (1991) The fungal dimension of biodiversity: magnitude, significance and conservation. *Mycological Research* **95**: 641–655.
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Lai M-J (2000) *Lichen Checklist of Taiwan*. Taichung: M-J Lai.
Wei J-C (1991) *An Enumeration of Lichens in China*. Beijing: International Academic Publishers.