Mushrooms: Beauty, diversity, relevance

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The literature on mushrooms is scattered, vast and rich. Current knowledge of mushrooms is essentially a synthesis of mushroom legends and myths, cults and folklore, re-inforced by mushroom biology and science. Mushrooms, indeed, are beautiful: beauty derives not merely from elegance and variety of form and colour, but structure and architecture superbly linked to function and life-style. Of ancient lineage, as decomposers, symbionts, and root-infecting pathogens, mushrooms have evolved with plant life through the ages. They are thus a unique component of the biota. Their diversity is a reflection of their lineage and their evolution. Mushrooms have merit, and meaning: species range from being delicious to edible to hallucinogenic to poisonous, even of potential value and use in medicine. Their enumeration, taxonomy, distribution, biology, chemistry, cultivation and conservation, naturally, are of great relevance and will remain of perennial interest and fascination for us.

THE theme of this paper reflects man's perennial fascination for mushrooms from times immemorial. Mushrooms are of ancient lineage, omnipresent, remarkably beautiful and diverse in their form, in their interaction with other biota and in all that they do. The suddenness and immensity of their presence in the wake of thunder and lightning, and their transience and fleetingness had been known since antiquity imparting a sense of mystery about the mushroom in man: amazed, he holds the thunderbolt, the thunder God responsible! Legend apart, the observation is in place. Impelled by curiosity, he notes that, of the many around him, some may be eaten and, with experience, he soon finds that some can be poisonous and so cannot be eaten. It seems plausible he also knew how to distinguish the edible from the poisonous, an exercise in taxonomy that calls for reliability, absolute reliability, ensuring safety. He does not stop there: the mushroom must be domesticated, like plants, must be grown, cultivated. Indeed, Dioscorides showed the way: the ancient method of sprinkling mushroom spores on to moist wood and waiting for the fruiting bodies to appear is apparently crude, but yet is the forerunner of modern methods of mushroom cultivation; it also marks the beginning of our insight into the biology of the mushroom. Experience with a diversity of mushrooms eventually leads to the recognition, in some, of inebriant qualities which, in time, we see, are built into religious practices or cults in many parts of the world. The use of mushrooms by primitive peoples is now well documented and the rise of the subdisciplines, ethnomycology and ethnopharmacology, and the corpus of data they present on the chemistry, metabo-

the 'mycelium') often forming 'rhizomorphs', capable of free and extensive spread in litter and in soil. The distribution and development of 'mycelial cord systems' of decomposer mushrooms are often conspicuous in soil and in litter of deciduous woodlands. Many of these are what we may call true inhabitants of the soil though the routine soil dilution plate technique widely used by soil microbi-

lism, and biological activity of hallucinogens and other bioactive compounds is clear evidence of the remarkable intuition and ingenuity of primitive man in discovering the true properties of these mushrooms. Indeed, legend, myth and science all now become part of a totality that heightens the meaning and fascination of mushrooms for man.

One would imagine it was easy for early man to collect mushrooms in the wild to satisfy his needs. The practice of collecting mushrooms in the wild persists to this day in many parts of the world. Man's early concern with the natural occurrence of his favourite mushrooms, the habitats where they occur, the periodicity or the seasonal character of their fruiting, their abundance, and so on, indeed, marks the beginnings of mushroom sociology or ecology.

Mushroom biology

The occurrence of mushrooms on such familiar substrates as wood, litter and soil, implies a role for them in these microhabitats. Forest litter and forest soils are often literally permeated by fungal threads or tubes (collectively, ologists to isolate fungi from soils may not reveal their presence in soil. These fungi may obtain food directly from the substrate which then serves as a food base for the mycelium of the fungus spreading radially therefrom.

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They have the enzyme systems required to break down substrates, especially lignin, hemicellulose and cellulose which are the main components of litter and of wood in the forest floor. In fact, degradation of lignin and wood is primarily a function of the great fungal group, the Basidiomycotina, to which all mushrooms belong, besides the pore fungi or polypores ('bracket fungi') and a host of others, more modest only in their general appearance and profile. Collectively, then, they are key scavengers of so much of the organic material in forest floors and soils, of agricultural and other wastes, all of which must be recycled for the sustenance of the biota. In short, the basidiomycetes are a key component in re-cycling of nutrients in many terrestrial ecosystems. Their biomass in forest soils and in forest litter is not easily quantified, but must be considerable. What is more, many mushroom species, and also other specific mycota, are symbionts of tree species, often obligately so, so that the symbionts in this remarkable partnership depend on each other for development and sustenance. That is a further indication of their relevance in forestry and afforestation programmes. Symbiosis leads to the development of mycorrhizas (literally, 'fungus roots') and there are innumerable such mycorrhizal associations ranging from the loosely specific to the not so specific. From the occurrence and characteristic distribution of their fruit bodies one might discern associations with particular tree species, reflecting a possible mycorrhizal relationship. Mycorrhizal fungi mostly are species of genera such as Amanita, Boletus, Lactarius, Russula and Tricholoma. The well-known fly-agaric, Amanita muscaria forms mycorrhizae with Betula, Pinus and possibly with *Carpinus*. Production of fruit bodies by mycorrhizas depends upon supplies of host-assimilates, and on host genotype, age of the host tree, soil type and soil amendments, and on weather and climate. The diversity of mycorrhizal fungi on a given tree species may increase with ageing of the tree species, so that there would be a succession of mycorrhizal associates on a given tree, a reflection of the low specificity of the mushrooms involved in such succession. Some mushroom species such as Laccaria laccata, and species of Boletus and Tricholoma present a dual behaviour, being mycorrhizal symbionts or being saprotrophs capable of decomposing leaf litter.

There is a succession also on dying and dead wood and other substrates. Some species in some genera preferentially colonize specific substrates: many wood-rotters belong to the group, the Aphyllophorales. Those involved in the decomposition of leaf litter and non-woody litter largely belong to the genera Collybia, Clitocybe, Marasmius and Mycena.

The mycelium of some species in litter and wood shows bioluminiscence. In fact, the fruit bodies also are luminiscent in some species, e.g. jack-o-lantern (Clitocybe illudens), the poisonous moonlight mushroom (Lampteromyces japonicus). In the case of Mycena rorida v. lamprospora the spores are luminous.

There are also mushroom species such as Armillaria mellea that infect living trees, especially root systems, and cause root disease. Root infection may also be due to polypores such as *Fomes annosus*. When the tree has been weakened, colonization by other mushroom species or by polypores and other basidiomycetes may follow. The mushroom group, the Marasmieae, especially the Marasmiineae, comprising species of genera such as Mycena, Crinipellis, etc. are common on tropical wood and litter, but some are pathogens. Crinipellis perniciosa is pernicious, being parasitic on cocoa trees (Theobroma cacao) and on T. bicolor and the wild species, T. speciosum: the fruit bodies are formed only on witches broom deformations of the diseased host. Originally known only in S. America, the disease now occurs wherever cocoa is grown, from Ecuador to the West Indies. Crinipellis perniciosa is unique in having a distinct parasitic and a saprophytic phase. The parasitic mycelium in the tissues of the brooms is intercellular without clamp connections; the saprotrophic mycelium arises from the parasitic, following death of broom tissues and has clamp connections: it can then be isolated and grown on media. Marasmius viegasii causes a root rot of coffee in Brazil: rhizomorphs on roots of affected trees are conspicuous. Curiously, some mushroom species that are parasitic on trees such as, for example, Armillaria mellea, are also symbionts of large orchids. Some achlorophyllous achlorophyllous 'saprophytic' angiosperms, including the Orchidaceae, obtain their carbon compounds through fungi that are. parasitic or mycorrhizal on green plants.

The life of the mushroom

The life of the mushroom from the germination of the spore through development of a hyphal system to the production of the characteristic fruit body is a continuum punctuated by events of import. First, nutrients and water are transported from the vegetative mycelium which draws its nutrition from the substrate or from a host. The elongation of the stipe is a result of divisions as well as elongation of individual cells. The fruit body itself is short-lived, and is organized for the production and dissemination of spores. The variations in colour, consistency, configuration, construction and dimensions of the fruit body, and the range in shape, colour and size of spores and other features all contribute to the diversity of mushrooms we see around us. The fruit body size of the most common mushrooms falls within a normal range, but some may be unusually small and others unusually large. The largest fruit body of a mushroom is perhaps that of Boletus colossus from the Malagasi Republic: it has a stipe 30 cm tall and 22 cm thick, and a pileus 60 cm wide and 4-6 cm thick, and weighs about 6 kg. Some bracketfungi are many times larger and weigh much more.

Highly synchronized basidia are typical of the agaries and the polypores; in the latter the hymenium is protected



Figure 1. Termitomices microcarpus 'mushrooms' like this



Figure 3. Pleurotus cystidiosus Pleurotus spp. are edible and are cultivated. Note gill architecture.

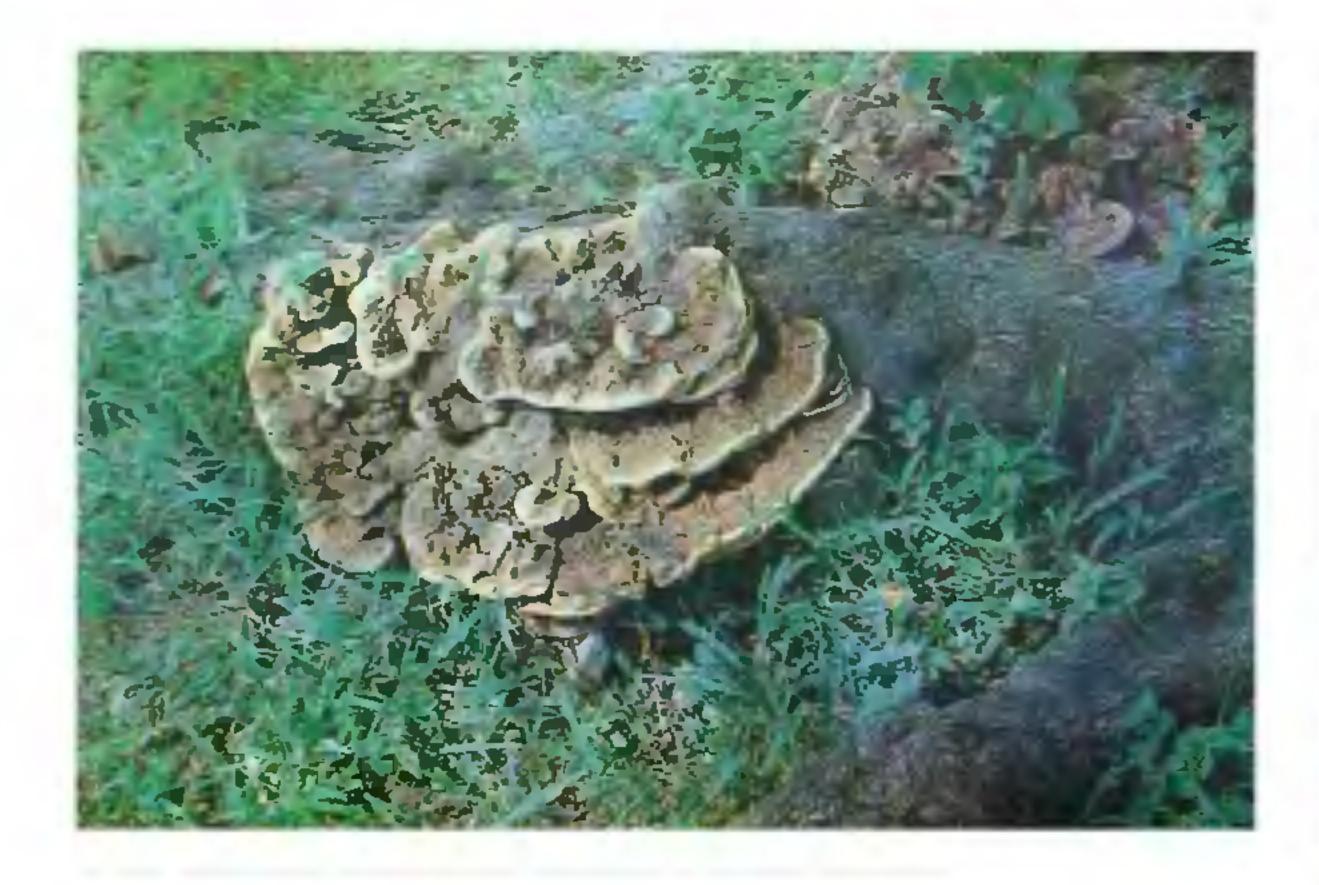


Figure 2. Polypore on wood Polypores are great decomposers of wood and play a major role in recycling of nutrients in the forest floor



Figure 4. Auricularia polytricha, the Chinese wood-ear (mu erh) fungus. Edible, cultivated Hong Kong is the principal exporter of this species.

by elaborate trimitic fruit bodies adapted to spore discharge under adverse conditions; in the former well-organized hymenia are formed on relatively fragile fruit bodies that can expand rapidly to discharge the spores during favourable environmental time-frames. The jelly-fungi (Tremellales) are unique in having relatively primitive hymenia, and in their ability to remain viable through time-frames of repeated wetting and drying.

There have been many penetrating studies of the development of the fruit body itself and its morphogenesis, the relevance of which must be obvious to anyone interested in the induction of fruit bodies and increasing their production at will experimentally. Once dikaryotization is accomplished, the dikaryotic mycelium is virtually perennial and persistent and often long-lived, and can pro-

duce fruit bodies year after year, season after season. Radial spread and fruiting often produce 'fairy rings', especially noticeable in the case of *Marasmius oreades* and *Chlorophyllum molybdites*. Normally, dikaryotization is a pre-requisite for fruiting, but monokaryotic mycelia may produce fruit bodies under special conditions. Morphogenesis begins with initiation and, following a pause, is consummated by sudden and rapid growth, expansion and maturation. The fruit body appears all on a sudden, and vanishes quickly too. Size and form of fruit body are closely related: smaller fruit bodies usually have slender stipes and larger ones stout stipes. The stipe serves to hold the pileus in position for release and dissemination of spores; it is held erect due to the turgor pressure of its cells. The positive role the pileus plays in the elongation

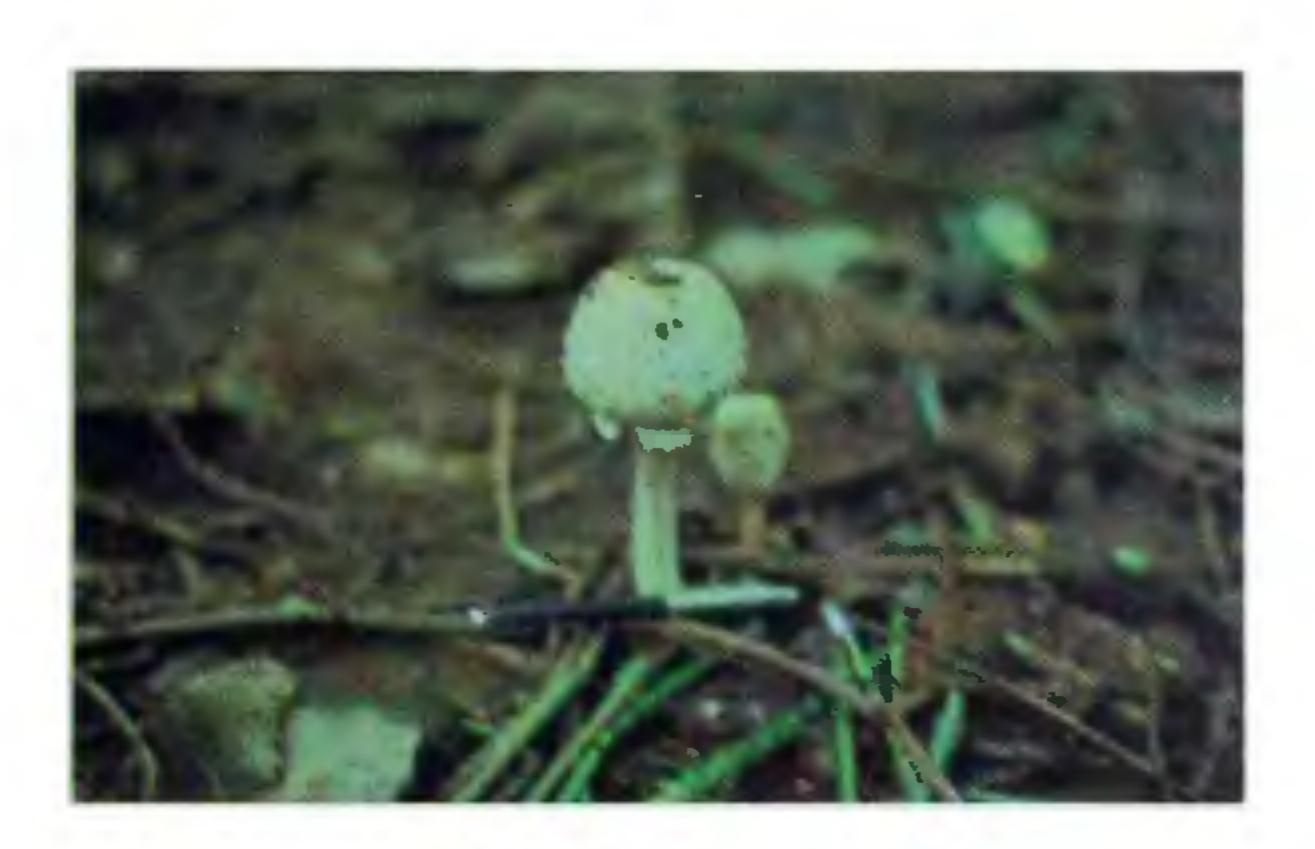


Figure 5. Ternutomyces heumu. Tropical, associated with termite nests. Edible, collected in the wild and eaten



Figure 7. Chlorophyllum molybdites Poisonous Often forms fairy rings in lawns



Figure 6. I ermitomyces Associated with termite nests in the tropics. The stipe arises from subterranean termite nest. Edible.



Figure 8. Gymnopillus spectabilis.

of the stipe first postulated by Schmitz in 1842, is now recognized and has been demonstrated experimentally, for example, in *Agaricus bisporus*, *Coprinus radiatus* and *Flammulina velutipes*: growth control resides in the pileus, specifically in the lamellae.

The way in which the fruit body as a whole, and the configuration and arrangement of the gills and the basidia in the pileus, are functionally adapted to life in a habitat is remarkable. There is a regularity in gill development and gill spacing. The architecture is such that a basidiospore discharged violently falls freely without striking the adjacent gill.

How is this accomplished? As described by the great Buller, to whom we owe much of our knowledge of the biology of the mushroom, the spore trajectory is a spo-

rabola: the horizontal flight is completed in a fraction of a second and is followed by a vertical fall that is gravitational and slow. The system of gills is such that, while they converge from the circumference of the pileus to wards the stipe, they all cannot be of the same length as that would make them far apart towards the periphery, if they are satisfactorily apart near the stipe. The solution to this situation is seen in the insertion of shorter gills be tween longer ones. A spore fall rate of the order of about half-a-million a minute is common for a normal-sized mushroom. Naturally, a spore cloud is the result. By removing the cap from the stipe and placing the cap naturally oriented on, say a sheet of glass, one can have a



Figure 9. The Monument to Lewis Carrol in Central Park, New York. The sequelae to nibbling the mushroom by Alice, described by Lewis, are well known Lewis got the idea from the reference to hallucinogenic mushrooms in M. C. Cooke's text-book on fungi.



Figure 10. Cortolus versicolor is highly valued in Chinese medicine. Causes white rot in wood; sometimes a weak parasite, it forms mycorrhizas with Galeola foliata, an achlorophyllous liane.

spore print on the glass in a matter of a few hours. The colour of spores is diagnostic.

Edible mushrooms: Occurrence, diversity

That many mushrooms are edible, and some are delicious, has been known, from ancient times. There are Roman mosaics depicting mushrooms. There is a range or diversity of mushrooms in the wild that are edible and, in countries where they are popular, where there are mycophiles, for example, in eastern Europe, collecting in the wild is common. The common wild species include Can-



Figure 11. Pleurotus ostreatus. Edible, cultivated.



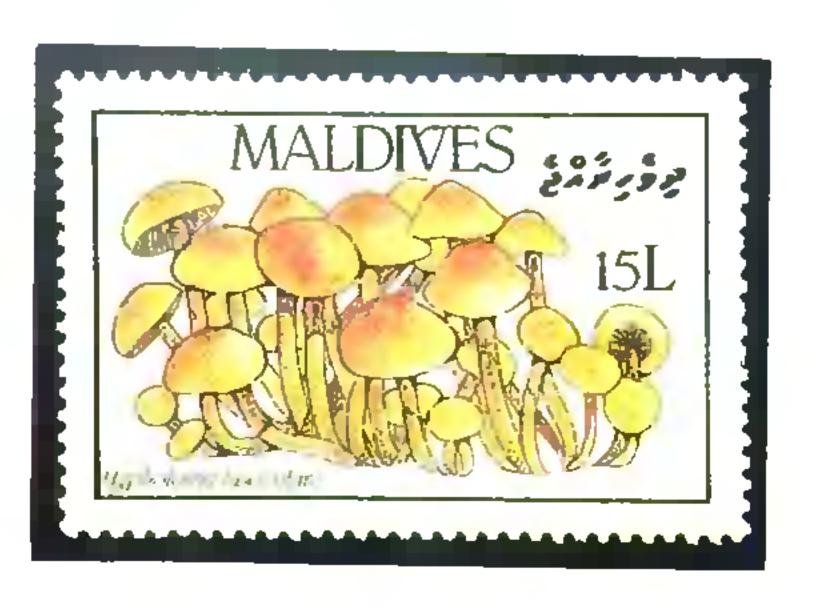
Figure 12. Ganoderma lucidum (ling-cht in Chinese, linjue in Thai; also known as the Reishi mushroom). Cultivated extensively in China, Japan, Korea, Thailand. The World Reishi Mushroom Institute in New York and the Arunyik Mushroom Centre in Thailand are engaged in standardizing the quality of the mushroom and its medicinal properties and use.

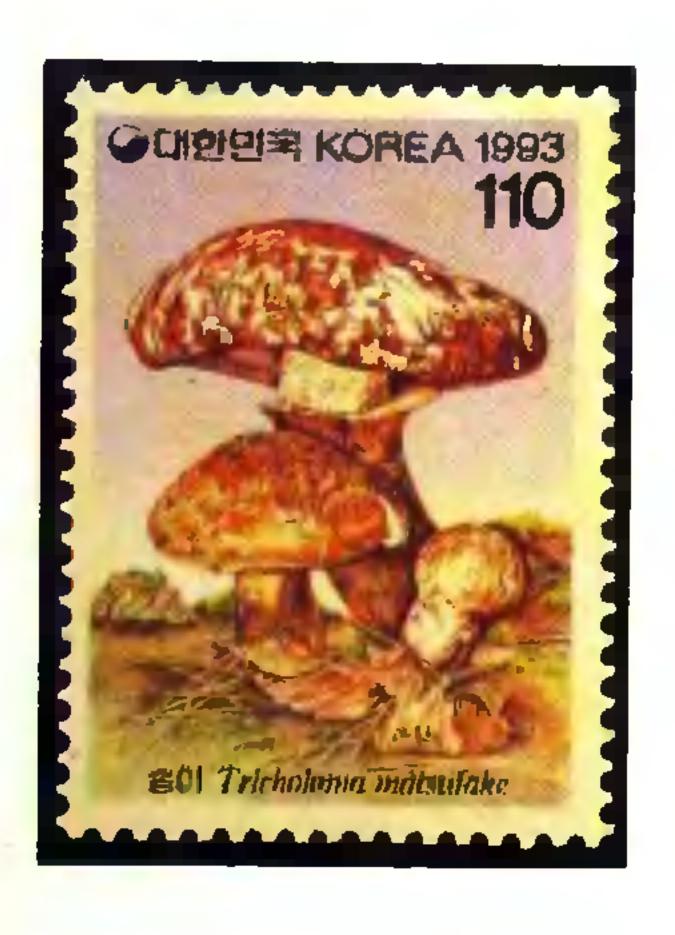
therellus cinnabarinus (Chanterells), C. cibarius, the boletes, Boletus edulis, Suillus luteus, S. granulatus, Leccinium aurantiacum, L. testaceoscabrum and the Amanitas such as A. caesarea. Many of these are not easily cultivated since they are mycorrhizal and fruit bodies are normally produced only in mycorrhizal association. It is then quite reasonable to speculate on the possibility of having, in afforestation programmes, tree species with mycorrhizal associates from among mushroom species that are edible. The introduction into France and Central Europe of the White Pine (Pinus strobus) from the United States in the middle of the last century is an example. The new French pine woods showed an abundance of fruit bodies of the delicious Suillus placidus: one cannot be certain if the fungus came with the pine from the US or

Mushrooms on postage stamps



















was already present in French soil and now had the host of its choice to colonize. The introduction of the white pine, a blessing in this way, was otherwise, for quite another reason: its high susceptibility to white pine blister rust. However, one can cite other examples of tree introductions with a high measure of success in providing the tree with an effective or suitable symbiont and, in the process; establishing a mycorrhizal relationship productive of a crop of fruit bodies of a prized mushroom. Success in such a venture implies judicious choice of a mushroom that is edible, and knowledge of its taxonomy and biology, especially its behaviour in symbiosis and in fruiting. The diversity from which a choice can be made includes many species such as Tricholoma matsutakae, Amanita caesarea, Suillus granulatus, Boletus edulis, Leccinium aurantiacum, Lactarius deliciosus, Cantharellus cibarius and C. cinnabarinus.

There are also many mushrooms that are primarily saprophytic and so can be grown in culture and cultivated to yield fruit bodies that can then be harvested. The button mushroom, Agaricus bisporus is the most popular, accounting for slightly over one half of the total of world mushroom production. 'Speciality mushrooms' such as Shiitake (Lentinus edodes), paddy straw mushroom (Volvariella volvacea), oyster mushroom (Pleurotus, especially P. ostreatus: also P. eryngii, P. floridanus, P. sojor-caju, P. cystidiosus) and enokitake (Flammulina velutipes) are becoming increasingly popular. Shiitake which is the mushroom of choice in Japan, Korea and China, has a long tradition of cultivation: it is cultivated on wood, virtually on logs of wood cut from hardwood trees such as oak, chestnut, etc. - species of Quercus, Castanea, Carpinus, Alnus, Acer. Shiia stands for Pasania and the name Shiitake stems from the fact of its frequent occurrence on shiia and the fact that it can be cultivated on shiia wood. Shiitake occurs naturally as a saprophyte on wood of deciduous trees such as oak, chestnut and beech and on shiia in eastern Asia from China and Japan to Vietnam and Cambodia. Volvariella and Flammulina are sold fresh. The button mushroom and shiitake are sold both in the fresh and the dried forms.

Auricularia polytricha, the ear fungus, and A. fuscosuccinea – not strictly mushrooms, but both basidiomycetes – are quite popular in many parts of southeast Asia
(China, Japan, Thailand, Taiwan and some islands in the
Pacific): they are cultivated on logs of wood or sawdust.
There are also several species, not yet intensively cultivated but with potential such as Grifola frondosa, Hypsizigus marmoreus, Hericium erinaceum, Oudemansiella
mucida, and several species of Termitomyces. Termitomyces, as the very name suggests, is associated with termite nests in the tropics and is collected in the wild and
eaten.

The nutritive value of some of the quality mushrooms very nearly equals that of milk. Mushrooms contain high levels of amino acids such as lysine and leucine, carbohydrates, vitamins, and unsaturated fatty acids, with a min-

eral content exceeding that in fish and meat, and twice that in most vegetables.

Recent studies culminating in the discovery of thermostable components, cytotoxic substances, and antibiotics in mushrooms (see Subramanian, Curr. Sci., 1991, 60, 290 for Steglich's report of antibiotics in mushrooms) reinforces the need for caution in using raw mushrooms in the dietary, in salads, for example. The occurrence of toxic metabolites such as the thermolabile audiotoxic volvatoxin in the paddy straw mushroom and flammutoxin in enokitake call for a reassessment of the wisdom of their use as food. Hydrocyanic acid is produced in quantity by some species (e.g. Marasmius oreades, the fairy fungus), but from its volatility little of it would remain after cooking.

The genus Agaricus to which the common edible button mushroom (A. bisporus) belongs, comprises two groups of species, viz. (a) those turning somewhat yellow on bruising (Flavescentes) and (b) those staining red (Rubescentes). The yellow staining species such as the edible A. arvensis and A. bisporus are known to accumulate considerable amounts of the heavy metals, cadmium and mercury. Other heavy metals known to accumulate in mushrooms include lead, caesium, iron, cobalt, copper, manganese, molybdenum, nickel, selenium, silver, strontium, thallium, uranium and zinc. Accumulation of cadmium and selenium and other heavy metals in shiitake would render it unsafe for human consumption.

The occurrence of the hydrazine derivative agaritine, and the diazonium derivative from it, in Agaricus, and the fact that the p-hydroxymethyl-phenyldiazonium ion and decomposition products of agaritine can be carcinogenic pinpoint the need for caution in the use of these mushrooms. The agaritine content of fresh and processed cultivated mushroom has been studied: the average agaritine content is around 0.088% based on fresh weight. We certainly need more data on the agaritine content in mushroom species and their possible biological effects.

The corpus of data on methods of cultivation, strain improvement, and biotechnology of mushroom production is enormous and cannot even be touched on here.

Poisonous mushrooms: Syndromes, toxins

Poisoning from eating mushrooms has been known since antiquity. As one would expect, there is a variety of syndromes, and a variety of mushrooms involved.

The most deadly and the most notorious is the Death Cap, Amanita phalloides, presumably also the mushroom ingeniously used by Agrippina to do away with her husband, the Roman emperor, Claudius Caesar, in her bid to ensure succession to the throne by her son, Nero—ingenious in the sense that the essential properties of A. phalloides had been known to the Romans in advance of chemical studies of the toxins which came centuries later. Claudius, in fact, was poisoned with a dish of a mixture of

the edible A. caesarea (so named because Caesar was very fond of this mushroom!) and the deadly A. phalloides so that the poisoning itself went unsuspected. The Romans, then, knew the incubation period required by the poison to act, as arousal of suspicion had necessarily to be avoided in administering the poison to the Emperor. Knowledge, then, of the poisonous nature of mushrooms and of the precise symptoms of poisoning naturally preceded critical chemical data on the toxins and the modus operandi of the toxins. As far back as 1759, the great German naturalist Jacob Christian Schaeffer (of Regensburg: see Subramanian, Curr. Sci., 1991, 66, 289) underlined the need for chemical studies of the fungi. In Germany, fungal chemistry flourished under the leadership of Zopf, Zellner and Kogl. And more recently, knowledge of the chemistry of the metabolites and toxins of poisonous mushrooms has been strengthened by the work of several scientists, notably Wieland and Faulstich.

The most potent toxic substances in mushrooms are the cyclic tryptophan-containing oligopeptides that characteristically occur in species of the genus Amanita. The genus Amanita is unique in having species that are edible, some that are poisonous, and some that are hallucinogenic. The 'Phalloides syndrome' refers to poisoning by A. phalloides: it is characterized by a latent period of about 10 h (so that the victim remains unaware of the poisoning and, by the time he becomes aware, it may be too late to seek a remedy!) and a two-phase sequel in which gastrointestinal disturbances (severe abdominal pain and colic, nausea, diarrhoea, vomiting) are followed by progressive liver dysfunction. Accelerated pulse, dehydration, cramps in the legs, and a lowering of blood pressure may accompany the primary symptoms. Two groups of bicyclic peptide toxins have been identified: (a) the amatoxins that are octapeptides with an indole (R)-sulphoxide bridge, and (b) the phallotoxins, that are heptapeptides with an indole-thio-ether bridge. Nine amatoxins and seven phallotoxins have so far been identified. Compared to the amatoxins, the toxicity of phallotoxins to liver cells is quite low. It is now generally agreed that phallotoxins are not involved in the syndrome; on the other hand, most of the effects are ascribed to amatoxins. The damage to the liver is primarily on nuclei of liver cells, specifically, inhibition of RNA-polymerase B. Amatoxins are known to inhibit RNA-polymerase B in all eucaryotes except amatoxin-accumulating fungi and a few Drosophila species, some of which inhabit these fungi. Because of their specisic inhibition of RNA polymerase B, amatoxins have become an important tool in biochemical studies. About 5-7 mg of amatoxin would be a lethal dose, depending on the body weight of the victim. Ingestion of the equivalent of 50 g of fresh weight of the fungus or a single fiuit body, then, can be fatal.

Amatoxins occur in Amanita verna and A. virosa, besides A. phalloides, all of which are mycorrhizal. Amatoxins are also known to occur in several species of the genus Galerina which are deadly poisonous: G. marginata,

G. autumnaris, G. badipes, G. beinrothii, G. unicolor, G. venenata and G. sulciceps. G. marginata is common on deciduous wood, particularly on conifers. G. sulciceps is tropical and fatal cases of poisoning from this fungus were reported from Java in Indonesia many years ago. Poisoning from species of Lepiota several of which contain amatoxins and are deadly poisonous (e.g. L. helveola) represents another example of the Phalloides syndrome.

Protein conjugates of amatoxins are ten times as toxic as natural amanitins and yet, the fact that these can be used for raising amatoxin-specific antibodies would now facilitate use of polyclonal antibodies for amanitin immunoassays. A monoclonal antibody, then, could be a potential antidote in the immunotherapy of human poisoning by the deadly Amanitas.

A syndrome with a long latent period and a two-phase course similar to the Phalloides syndrome is often due, not to Amanita, nor even a true mushroom, but a species of a fungus belonging to the Ascomycotina. Gyromitra esculenta has long been considered edible, as the specific epithet itself suggests. But there have been many cases of poisoning in some parts of Europe: the demonstration of the occurrence of a toxic substance, gyromitrin with high toxicity to the liver, confirms that the species is poisonous. On the other hand, gyromitrin is not stable, and most poisonings are apparently from eating of raw or insufficiently cooked fungus. In pure culture, even the mycelium has been shown to have the toxin. It is also likely that there are strains of the fungus that are not poisonous in some geographical regions in Europe, which perhaps serves to explain the conflicting reports about the edibility or otherwise of this fungus.

Another syndrome which has an extended and long latent period and in which impairment of kidney function is the primary damage is from poisoning by species of the genus Cortinarius. Cortinarius orellanus is a European species and poisoning has been reported from several countries in Europe. The key poison has been identified as orellanin for which a bipyridial structure has been proposed; it has also been synthesized. It is a typical nephrotoxin. In acute cases, kidney damage has even precipitated the need for kidney transplants.

A syndrome with intense bouts of perspiration coupled with salivation and lachrymation is characteristic of *Inocybe* poisoning. These effects are ascribed to *muscarine* which has been detected in several species of *Inocybe*, e.g. I. patouillardii. Small amounts of muscarine are known to occur in a number of mushrooms, but normally, very large quantities of these would have to be ingested for them to be able to cause any poisoning.

There are then mushrooms that are not poisonous and are edible on their own, but known to produce toxic effects when alcohol is taken after eating them. The common ink cap, Coprinus atramentarius is a good example. A sense of being hot and a metallic taste in the mouth accompany a reddening of the face, neck and chest: there may also be a tingling sensation in the legs and the arms,

and palpitation. The active component, coprine, has been isolated. Coprine is an inhibitor of the enzyme, acetaldehyde-dehydrogenase in the liver: it inhibits oxidation of the acetaldehyde formed during the metabolism of alcohol to acetate. The resulting heightened content of the aldehyde in the blood shows up in the symptoms. The normal yield of coprine is about 160 mg kg⁻¹ of fresh fungus. Clitocybe clavipes is another mushroom species that is incompatible with alcohol.

The most widely occurring syndrome from mushroom poisoning is the gastro-intestinal syndrome. According to one report 40% of all notified poisonings in Switzerland during a 40-year period were from gastro-intestinal cases. It should be noted, however, that the syndrome is heterogeneous, being due not to a single toxic species. In general, irritation of the intestinal and gastric mucosae is typical. Several species are suspect in this category: Chlorophyllum molybdites, Hypholoma fasciculare, Megacol-Ivbia platyphylla, Tricholoma pardinum, and species of Hebeloma, Hygrocybe, Leucocoprinus, and Macrolepiota. Toxic principles have been identified or isolated in some species. Thus, Dermocybe sanguinea contains anthroquinones such as dermocybin and dermorubin which are considered responsible. The sesquiterpene, illudin S. (alunamycin, lampterol) is one of the toxic principles isolated from Omphalotus olearius, O. subilludens and Lampteromyces japonicus. The pungency and bitter taste of species of Lactarius (Milk Caps) and of Russula (Brittle Gills) are well known. Pungency derives from sesquiterpenes, stearyvelutinal or necatorone. Some at least of these species may be rendered edible by cooking, but those tasting bitter and pungent (e.g. Lactarius piperatus, Russula emetica) are best avoided. And yet, we must note that in many parts of eastern Europe Lactarius piperatus is considered edible.

Poisoning is often due to ingestion of mushrooms, fresh and raw. These include, especially, the following: Amanita rubescens, A spissa, A. strobiliformis, A. vaginata, Armillaria mellea, Boletus erythropus, B. luridus, Entoloma clypeatum, Lepiota nebularis, Lepista nuda, Russula olivacea. These need to be cooked before being eaten. Edibility even after cooking is questionable for some species such as Paxillus involutus and Sarcosphaera crassa.

Hallucinogenic mushrooms

The fly-agaric

The fly-agaric – Amanita muscaria – is a mushroom that has perennially arrested our attention by its beauty and its unmistakable identity. It is quite natural that it is popularly illustrated in many a children's book. The fly-agaric is so called from its long-known property of repelling-flies, a property now known to be linked to a chemical, the isoxazole, ibotenic acid, first isolated from the mush-

room by Japanese scientists. The other properties of this mushroom, quite remarkable, in producing hallucinations on ingestion, have been known for a long time, for example, to the Koryaks and the Chuckchis in Siberia. Their use of this mushroom and the characteristic hallucinogenic effects which are part of the Siberian experience are remarkably well documented by travellers, historians and anthropologists: the accuracy of these accounts is matched by current knowledge of the chemistry of hallucinogenic substances in the mushroom, their metabolism in the human, and their effects on the human nervous system. Wasson reviewed knowledge of the fly-agaric with precision and elegance and even forcefully argued the case for his belief that the fly-agaric is indeed the 'soma' of the Vedas. The first bit of chemistry of the flyagaric came from the pioneering work of Schmiedeberg and Koppe who isolated from it muscarine in 1869. Concentrations of muscarine in the fungus are, however, low and so, for muscarine poisoning to take effect, one would need to ingest unusually large quantities of fruit bodies of the mushroom. Ingestion of the fly-agaric, nevertheless, produces a characteristic syndrome in which the primary effects are neurological. The effects have been well described by many, notably in the documentation of the use and effects of the fly-agaric by the Koryaks and the Chukchis in Siberia. From its unmistakable features, wide distribution and abundance - being mycorrhizal, one may find it under pines, beech, and birch – it is one of the most widely used psychotropic mushrooms in many parts of the world, but especially in the USA. Symptoms include disturbances of vision, motor excitation, confusion that may be reflected in laughter, dancing, and singing, alteration in perception of personality, and of space and time, of colour, hallucinations, a feeling of illusion and even euphoria. Beginning within about 30 min following ingestion, all is over in about three hours. In the Siberian experience, the Koryaks in their wisdom dry the fly-agaric and it is in the dried form that the mushroom seems most effective. The active principles that are responsible for the effects are now known: apart from ibotenic acid, already mentioned, two other derivatives, muscimol and muscazone, have been discovered. Only ibotenic acid occurs as such in the fungus; the other two are secondary products that appear, for example, quite interestingly, when the fungus is dried. We must therefore assume that the heightened hallucinogenic properties of the mushroom in its dried form must be due to these secondary products. The predominant effect is inhibition of motor functions: muscimol is five to ten times more potent than ibotenic acid. Muscimol is stereochemically y-aminobutyric acid (GABA) and so its effect on the nervous system stems from stimulation of GABA-receptors of the cells of the central nervous system. A further point of interest is, besides ibotenic acid, muscimol too has insecticidal properties.

H ₃ C CHR ²	
HN-CH-CO-NH-CH ₂ -CO H ₂ C NH	
	-CH2-CH3
OC-CH-NH-CO-CH-NH-CO-CH ₂ -NH H ₂ C-COR ³	

	R ¹	R ²	R ³	R ⁴	R ⁵
α-Amanitin	CH ₂ OH	ОН	NH ₂	ОН	ОН
β-Amanitin	CH ₂ OH	ОН	OH	ОН	ОН
γ-Amanıtin	CH ₃	ОН	NH_2	OH	НО
ε-Amanitin	CH ₃	ОН	ОН	OH	ОН
Amanin	CH ₂ OH	ОН	ОН	H	ОН
Amaninamide	CH ₂ OH	ОН	NH_2	Н	ОН
Amanullin	CH ₃	Н	NH_2	ОН	ОН
Amanullinic acid	CH ₃	Н	OH	OH	ОН
Proamanullin	CH_3	H	NH_2	OH	H

Amatoxins

			CH ₂ R ¹
H3C-CHCONI	н со ин-	-ÇнСН2	-C-CH2R2
I NH	H ₂ C \	ု ငှံဝ	о́н
CO		J NH	
Ĭ H	2C H		
N - CO -	- CH	CH—R ³ †	
H 	NH-CO-CH-NH	– ċo	
គ្គី5	CH — OH		
	, .		

Phallotoxins

	R ¹	R ²	R ³	R ⁴	R ⁵
Phalloidın	OН	Н	CH ₃	CH ₃	ОН
Phalloin	·H	Н	CH_3	CH_3	OH
Prophalloin	H	H	CH_3	CH_3	H
Phallisin	OH	OH	CH_3	CH_3	ЮΗ
Phallacin	H	H	$CH(CH_3)_2$	COOH	OH
Phallacidin	OH	H	$CH(CH_3)_2$	COOH	OH
Phallisacin	OH	OH	$CH(CH_3)_2$	COOH	ОН

Chemical structure of mushroom toxins and hallucinogens

Coprine

Other Amanitas that produce somewhat similar effects on ingestion are: A. regalis, considered by some to be only a brown variety of the fly-agaric, and A. pantherina, and A. cothurnata, all of which are known to contain ibotenic acid and muscimol.

The Psilocybes

The use of the fly-agaric by Siberian tribes finds a parallel in the ritual use of small mushrooms of the genus Psilocybe in Mexico. In pre-Spanish Mexico, they have long been in use to induce visions and have been called 'teonanacatl', literally, 'flesh of the Gods'. The human response to ritualistic administration of these hallucinogenic mushrooms by curanderos in Mexico is well described by Gordon Wasson from his own experience. To Aldous Huxley, the experience is: 'a state of affairs in which verbalizing and conceptualizing are in some sort by-passed. One can talk about the experience – but always with the knowledge that "the rest is silence". The latent period is short (15 to 120 min). Alteration of perception of time and space, a sense of elation and joy – or bliss, or alternatively (for others) anxiety and depression have been reported. The finale is a deep unconsciousness. These effects are comparable to those due to LSD (lysergic acid diethylamide) and have been amply described. Little had been known about the Mexican mushrooms until the ethnobotanical expeditions to Mexico by R. E. Schultes of Harvard University in the forties, and then followed, with the mushrooms which Schultes brought with him as the base, the pioneering and exciting studies of Gordon Wasson, mycologist Roger Heim of the Museum Cryptogamie in Paris, and Hoffman, biochemist with Sandoz in Basel on the ritual use, taxonomy and chemistry of these mushrooms, leading in essence to a remarkable synthesis of data. Roger Heim's invaluable monograph of these fungi is not only encyclopaedic but authoritative. Guzman's monograph on *Psilocybe* updates information. About 140 species of *Psilocybe* are known of which about 80 are hallucinogenic.

The largest number of hallucinogenic species are from Mexico. P. aztecorum var. aztecorum is believed to be the species used by the Aztecs. The species most popular or common in current use include P. aztecorum var. aztecorum, P. zapotecorum, P. caerulescens vat. caerulescens, P. cubensis and P. mexicana. P. cubensis is coprophilous and comes up on cow dung: it is believed to have been introduced when the Spaniards brought in the cow from Africa. Several of the species can be grown in pure culture and can be cultivated. The active principle in several species is psilocybin, first isolated and characterized from P. mexicana, and also synthesized, by Hofmann. Psilocybin has the structure N, N-dimethyl-Hphosphoryloxytryptaminė. The resemblance in structure to lysergic acid serves to explain the similarity in human response to psilocybin and LSD, already mentioned.

In nature, the unstable but related psilocin often occurs in small amounts along with psilocybin. In some species such as *P. baeocystis* and *P. semilanceata*, two other substances, also hallucinogenic, are known to occur: baeocystin and nor-baeocystin. Primarily, Psilocybe hallucinogens act as serotonin-antagonists: therefore they are used in psychotherapy and neurophysiological research. Psilocybin is active in man in very low doses: 4 mg taken orally causes wild intoxication, but 6-20 mg can elicit marked psychotropic response. Overdose can be dangerous in causing psychoses, leading to suicide mania.

Mushrooms in medicine – bioactive products

Apart from antibiotics, there are a number of substances that have been isolated from mushrooms and other basidiomycotina – substances that are variously labelled 'host defence potentiators' (HDPS), 'protein-bound polysaccharides' or 'polysaccharide-protein complexes' (PSPCs). The function of these is believed to lie in the toning up of defence mechanisms in the human system against a number of ailments. A great many of these are from polypores (Aphyllophorales) or other Basidiomycotina. At least about sixty species are traditionally used in Chinese medicine. These include species of Ganoderma, besides Wolfiporia cocos, Polyporus mylittae, Fomitopsis officinalis, Coriolus versicolor, Fomes fomentarius and Bjerkandera fumosa. Tremella fuciformis (Basidiomycotina, Tremellales) and Coriolus versicolor (Aphyllophorales) are used in Chinese folk medicine for their nutritive and tonic properties in the treatment of debility and exhaustion.

Ganoderma lucidum, ling-chi in Chinese folk medicine, is highly valued in China and in Japan for its medicinal properties and its ingredients, considered both a tonic and a panacea for a variety of ailments neither specific nor specifiable. An almost magical effect on intelligence, enhancing memory, and hearing, vision and smelling, has been attributed to this fungus. In practice, only the fruit bodies of G. lucidum and G. japonicum are used, the spores are discarded. During the past two decades there have been several studies on the chemistry of bioactive substances in ling-chi and the human response to these substances, but more specially their potential use in the control of a number of specific diseases such as cancer, diabetes and hepatitis. The spectrum of bioactive substances in G. lucidum includes organic germanium, polysaccharides (B-D-glucans), triterpenoids and adenosine.

Host-mediated anti-tumour polysaccharides of G. lucidum have been patented as immunopotentiators in the treatment of cancer. Besides, G. lucidum, G. tsugae and G. boninense are included in Japanese and US patents. Also, over one hundred novel, highly oxygenated, lanosatype triterpenoids have been reported from fruit-bodies and mycelium of G. lucidum: their chemical structure has been determined. These include: ganoderic acids, gan-

oderemic acids, ganodermic acids, ganoderal, lucidernic acids and lucidone and ganoderols. It is tempting to speculate if these diverse triterpenes could explain the multiple beneficial effects for which *ling-chi* is reputed.

A freeze-dried powder containing mycelium of G. lucidum has been shown to bring down blood sugar levels in experimental diabetic rats. Three hypoglycaemic principles, ganoderans A, B and C have been isolated from fruit-bodes: these are characterized as peptidoglycans. Of these, ganoderan B is considered to be the major one.

Extracts of the fungus have also been shown to be hepato-protective: apart from liver regeneration, beneficial effects against hepatic necrosis and hepatitis have been noted. Ganodosterone from G. lucidum is a liver-protectant and stimulates liver function. Similarly, ganoderic acids T, S and R, from the mycelium of the fungus are hepato-protective. Hepato-protective triterpenoids have also been isolated from G. tsugae: lucidone A, lucidenol, ganoderic acid B, and ganoderic acid C2.

Ganoderma is cosmopolitan; some species cause serious decay in wood; some are root-disease pathogens of a variety of plantation crops and are economically important. Some species have the ability to selectively degrade lignin and so are a potential source of lignin-degrading enzymes useful in the bioconversion of lignin in agricultural and other wastes. The new wave of interest in Ganoderma has prompted studies on taxonomy, distribution, and even speciation. The low nucleotide variation observed in the 25 S rDNA and the very high transition bias in the region suggest that the genus is young and is still evolving.

Lentinan from the edible mushroom, Lentinus edodes has been shown to have distinct anti-tumour properties and has also potential use in enhancing host resistance to a variety of infections, including HIV-1 or AIDS. Lentinus edodes is remarkably effective in lowering levels of lipids in the blood: the hypolipidemic effects are due to a secondary metabolite identified as 2(R), 3(R)-dihydroxy-4-(I-adenyl)-butyric acid which has the trivial name eritadenine (=lentysine, lentinacin). A lowering of serum cholesterol levels in man has also been reported.

Mushrooms: antiquity, speciation

The oldest known fossil mushroom species is perhaps Coprinites dominicana described as trapped in amber about 40 Mya. Mushrooms are believed to have radiated in the Cretaceous about 130 Ma, after the great rise of the angiosperm land flora. Ectomycorrhizal fossil mushrooms date back to the same time.

In the case of *Pleurotus* (the oyster mushroom) compatibility tests have shown the existence of several distinct intersterility groups (biological species). Recent work on speciation, from phylogenetic analysis of ribosomal DNA sequences among these intersterility groups, indicates that several species lineages evolved quite early: species lineages of more recent origin appear to be re-

Stricted in distribution, viz. to the northern hemisphere. Older lineages, on the other hand, have a distribution world-wide. The explanation for this pattern of distribution may well be that the old lineages were extant before the break-up of 'continents' from drift, and new lineages arose in the northern hemisphere following continental drift. On the other hand, one might also postulate dispersal as the primary factor governing the current distribution pattern. Whichever of these two postulates we accept, it is clear that older lineages have a wider distribution and more recent lineages have relatively narrower distribution, some thing akin to Willis's Age and Area hypothesis. Genetic divergence appears to be linked to allopatric speciation within populations from different continents in the northern hemisphere.

This contribution is but a feeble attempt to capture and present for the reader something of the beauty, diversity, and relevance of mushrooms. The literature on the subject is enormous and an account like this cannot be anything but incomplete. The list of selected references appended here should, however, help the reader to delve deeper into the subject. And, it would give me great joy if what I have written and presented provokes curiosity, further probing into 'mushroom science' and, most important, evokes excitement, fascination and wonder.

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Note:

Authorities for Latin binomials are not given for ease of reading These will be found in the monographs cited. Photo credits: Figures 1, 2, 5 courtesy K. Natarajan; 7, 10, 11 courtesy Chen, Hsinchu.

RESEARCH ARTICLE

Role of fluorescence microscopy in the assessment of Indian Gondwana coals

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When a light of short wavelength excites organic matter, light of relatively longer wavelength is emitted from it and this phenomenon is known as autofluorescence. The coal maceral analysis under fluorescence mode (blue light/UV light excitation), therefore, has been found to be best suited to properly identify, characterize and quantify hydrogen-rich macerals. Utilizing this technique, macerals like bituminite, fluorinite and exsudatinite were recognized for the first time. Certain

other macerals – alginite and liptodetrinite, normally mistaken for mineral matter under routine petrographic analysis, were also identified. Fluorescence microscopy, thus, not only added to the overall tally of liptinite group of macerals in Indian Gondwana coals, but also to their quantity. In addition to this, recognition of fluorescing vitrinite (perhydrous vitrinite) significantly contributed to the abundance of hydrogenrich microconstituents for these coals.

FLUORESCENCE microscopic study of solid fossil fuels (coals/lignites) and kerogen (in sedimentary rocks) has been found quite successful particularly in identifica-

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tion, characterization and quantitative assessment of hydrogen-rich macerals, especially the liptinite (or exinite) group of macerals. It is a useful technique for determination of rank or maturity and even better suited in cases where vitrinite/huminite macerals are not in sufficient quantities or not suited for reflectivity measure-