The Systematics and Ecology of Boletes with special reference to the Genus Suillus and its Ectomycorrhizal Relationships in Nepal

by

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(ABSTRACT)

The *Suillus* mycota of Nepal was studied. Nine species are recognized and described; five of the nine appear to be new species. Additional species were collected, but material was inadequate to describe them completely. Cultures of eight and synthesized ectomycorrhizae of six of the *Suillus* species are described. Synoptic keys to the basidiocarps and to the cultures are presented. Numerical taxonomic analyses of the cultures generated clusters which paralleled the species concepts developed using basidiocarps and ecology. Each species of *Suillus* from Nepal is host specific based on basidiocarp formation; all hosts are in the Pinaceae. Field associations are *Suillus* cf. *granulatus*, *S.* cf. *placidus*, *S. sibiricus*, and greening-foot *Suillus* with *Pinus wallichiana*; queen's *Suillus* with *P. roxburghii*; waxy *Suillus* with *P. patula*; and *S. laricinus*, himalayan *Suillus*, and orange-pored *Suillus* with *Larix himalaica*. Mycorrhizal syntheses confirmed that the six *Suillus-Pinus* relationships are ectomycorrhizal.

The *Suillus* mycota of western Virginia has 12 known species. Three, of the five which are ectomycorrhizal with *Pinus strobus*, have closely related counterparts in Nepal. These counterparts are ectomycorrhizal with *P. wallichiana*, a five-needled pine closely related to *P. strobus*. The existence of these three pairs of similar fungi, associated with similar pines, suggests the possibility of cladogenic speciation in parallel by the pine lineage and by its ectomycorrhizal fungal associates.

Boletinellus merulioides forms abundant sclerotia in nature throughout its range in eastern North America. Sclerotia collected in the forest germinated to form mycelial colonies that had the same appearance and microscopic characteristics as colonies derived from basidiocarps. Sclerotia which had overwintered in the forest were viable in the spring.

The spatial pattern of *B. merulioides* sclerotia in a forest was compared with basidiocarp frequency recorded over four years. Both estimates of the spatial pattern coincided, but year-to-year basidiocarp frequency varied greatly. Individual *B. merulioides* dikaryons formed large perennial patches. Basidiocarp and sclerotial densities were centered around and declined outward from *Fraxinus americana* trees. *Boletinellus merulioides* and *Fraxinus pennsylvanica* did not form ectomycorrhizae when grown together in growth pouches.

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Cela est bien dit, répondit Candide,

mais il faut cultiver notre jardin.

Voltaire 1759

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CHAPTER 1. SYSTEMATICS AND ECTOMYCORRHIZAL RELATIONSHIPS OF THE BOLETE GENUS SUILLUS IN NEPAL

The bolete genus *Suillus* is ectomycorrhizal almost exclusively with members of the Pinaceae, especially the genera *Pinus* and *Larix* (Miller, 1982; Singer, 1986). Species of *Suillus* have been reported from around the world wherever *Pinus* and *Larix* grow either as native or introduced trees. Therefore, although *Suillus* had not been reported from Nepal, the genus was expected to occur there because of the presence of both *Pinus* and *Larix* (Table 1.1). Nepal has two native pines; *Pinus wallichiana*, a 5-needled pine, and *P. roxburghii*, a 3-needled pine; and two Himalayan endemic native larches; *Larix himalaica* and *L. griffithiana*. Exotic pines, especially *P. patula* (3(-5)-needled), are used in reforestation efforts in Nepal.

The objectives of my research in Nepal were to:

1) Collect and identify the *Suillus* species associated with the conifer genera *Abies, Larix, Picea, Pinus* and *Tsuga*. Attention was also paid to angiospermous forests in case there were *Suillus* species similar to *S. subaureus* (Peck) Snell which is believed to be associated with angiospermous trees in North America (Smith and Thiers, 1964; Chapter 2).
2) Obtain pure cultures of the *Suillus* species.

3) Fully describe and photograph both the basidiocarps and the cultures.
4) Construct keys to the basidiocarps and to the cultures.
5) Develop a list based on field observations of trees associated with the *Suillus* species.
6) Confirm that the relationships between the *Suillus* species and their tree associates are ectomycorrhizal by conducting synthesis experiments.
7) Compare the *Suillus* species from Nepal with species from other areas of the world, especially North America.

My hypotheses that were to be tested by this research were:

1) The genus *Suillus* occurs in Nepal.
2) The *Suillus* species of Nepal are ectomycorrhizal with conifers, specifically with members of the Pinaceae.
3) The *Suillus* species are host specific.
4) *Pinus wallichiana*, like *P. strobus* L., has a rich *Suillus* mycota associated with it as ectomycorrhizal partners.
5) The *Suillus* species associated with *P. wallichiana* have counterparts associated with *P. strobus*.
6) *Suillus* is important in reforestation when pines are used.

This chapter presents the results of my research on the genus *Suillus* and its ectomycorrhizal associations in Nepal. The introduction which follows is divided into the geology, climate and vegetation of Nepal; the

history of mycology in Nepal; boletes of Nepal; and comments on the genus *Suillus*.

The geology, climate and vegetation of Nepal

The country of Nepal is located in the east central part of the Himalaya between India to the east (Sikkim), south and west and Tibet (Xizang, China) to the north (Fig. 1.1). Rectangular in shape, Nepal's longest dimension, 800 km, follows the east-south-east to west-north-west orientation of the Himalaya. Thus western Nepal is slightly further to the north than is eastern Nepal. Nepal's north-to-south dimension ranges from only 100 km to 160 km. Nepal is north of the Tropic of Cancer at the latitudes of mid-Florida, ranging from 26° 27' N in the southeast to 30° 22' in the northwest. In area Nepal is small, 148,000 square kilometres, but in topographical diversity it is large. The altitudinal range is from 55 m in the southeastern corner to 8848 m on top of Mount Everest (Dobremez, 1976). The lowest elevations are in an east to west band along the southern border with India; this flat area is called the terai. The highest elevations, those of the Greater Himalaya, are along or near the northern border with Tibet.

The landmass of Nepal would be a relatively flat coastal area on the north shore of the "continent of India" had the subcontinent of India not collided with Asia about 50 million years ago (Molnar, 1986; Tapponnier, 1986). Nepal is derived, not from Asia, but rather from the subcontinent

of India, pieces of which have been and are being shaved off and piled up to form the hills and mountains of Nepal as the subcontinent of India slips under Asia. This action has resulted in the Greater and Lesser Himalayas. The collision also formed the Tethys Himalaya which lie to the north of the Greater Himalaya. The Tethys Himalaya contain many marine fossils which were formed on the northern continental shelf of the subcontinent of India beneath the Tethys Sea which separated the two landmasses before the collision. The Tethys Himalaya are mostly in Tibet with some parts of Nepal (e.g. Mustang District) extending north into them. The actual suture line between the two landmasses is north of the Tethys Himalaya in Tibet (Molnar, 1986; Tapponnier, 1986). To this day, the hills and mountains of Nepal continue to uplift as the subcontinent of India continues to push into and under the continent of Asia.

In addition to its south to north altitudinal gradient, Nepal has an overall monsoon moisture gradient from the wetter east to the drier west. Nepal's seasons are defined by the monsoon: cool, mostly dry winter from December to February; hot, mostly dry pre-monsoon from March to May; warm-to-hot, wet monsoon from June to September; and warm, mostly dry post-monsoon from October to November. Temperature, naturally, varies with elevation so winter's "cool" is shirt-sleeve weather in the terai while inner Himalayan Langtang Valley is buried in snow. The monsoon which affects Nepal originates in the Bay of Bengal, moves to the north up to the Himalaya, and then to the west along the Himalaya. Thus the monsoon rains reach east Nepal first, usually in early June, and then

several weeks later reach west Nepal. The monsoon rains recede in the reverse direction, ending last in east Nepal, usually in September. In the fall and winter cyclonic disturbances bring some precipitation which is especially important in west Nepal. The pre-monsoon season spawns scattered violent thunderstorms. The monsoon proper, at least at the middle elevations, brings heavy rain but little wind and few or no thunderstorms.

Superimposed on the primary south-north altitudinal and east-west moisture gradients are strong local climatic effects due to the extreme topography. For example, the rain shadow effect of the Greater Himalaya is, of course, substantial and thus the areas to the north of the Himalaya are quite dry, and there are few forested areas. Some of the trans-Himalayan area is in Nepal, e.g. Mustang District; most is part of Tibet. Conversely the south-facing slopes of the Greater Himalaya intercept much moisture, an extreme example being the Pokhara area. Given that the Himalaya are not a simple linear ridge but rather a complex mountain system, a mosaic of differing local conditions results.

The rainfall pattern reflects these local differences, ranging from less than 2.5 cm per year to 600 cm per year (Dobremez, 1976). Some examples are Jomsom, 10-40 cm/yr, (altitude of 2600 m, north central Nepal; no forest); Jumla, 40-90 cm/yr, (2400 m, west Nepal; conifer forest); Biratnagar, 80-250 cm/yr, (80 m, east Nepal; subtropical

angiospermous forest); and Pokhara, 300-400 cm/yr, (900 m, central Nepal; subtropical angiospermous forest) (Stainton, 1972; Dobremez, 1976).

According to Polunin and Stainton (1984), the effect of soil composition on vegetation distribution in the area of the Himalaya, which is strongly influenced by the monsoon, is minimal compared to the overriding effects of altitude, aspect and rainfall.

The flora of Nepal is incredibly diverse with about 6500 flowering plants (Hara *et al.*, 1978) and represents five Provinces of Takhtajan (1986): Upper Gangetic Plain, Bengal, Eastern Himalayan, Western Himalayan, and Tibetian. Virginia, by comparison, is only slightly smaller in size but has fewer than half as many flowering plants. The forests of Nepal range from tropical to boreal, basically on the south to north altitudinal gradient (Stainton, 1972). The affinity between the floras of eastern Asia and eastern North America is present, though weaker, in Nepal. Nepal is on the western side of the eastern Asian area which shows floral affinity to eastern North America.

Members of the Pinaceace are found in many forest types in Nepal over a great range of altitude, 900 m to 4400 m, (Table 1.1) but are not found in the lowland subtropical forests of the terai bordering India. Timberline in Nepal varies with aspect, climate and human activity (Polunin and Stainton, 1984) and also with longitude-latitude from over

4000 m in eastern Nepal down to almost 3000 m in localities in western Nepal.

History of Mycology in Nepal

The earliest mycological collecting in Nepal was by the Englishman J. D. Hooker in 1848 (28 Oct to 15 Dec; Hooker, 1855). Hooker collected in India including Sikkim with this single collecting incursion into far eastern Nepal. All of Hooker's plant and fungus collections from Nepal are most likely from what is now Mechi Zone. He was primarily interested in the vascular plant flora but also collected fungi. Descriptions of 35 of Hooker's Nepali fungi included 17 new species and were published by Berkeley (1854a-d). Most were polypores with some gasteromycetes and miscellaneous other fungi. No agarics or boletes were included in the Nepali collections; however, many of the Sikkim collections were agarics and boletes. Hooker also went a few miles into southeastern Nepal in March 1849 but probably did not collect fungi that day.

Following Hooker, later fungal collecting in Nepal was also often incidental to vascular plant collecting expeditions. Balfour-Browne (1955, 1968) published on the fungi collected by British botanical expeditions of the 1950's and 60's and noted that some specimens were pressed "too enthusiastically" (1968) so that many fleshy fungi were not identifiable. Balfour-Browne's papers reported over 200 species of varied fungi from Nepal mostly in the Aphyllophorales and Uredinales and

included 11 new species, some described by E. J. H. Corner as noted by his initials in the text.

Japanese scientists of many disciplines have been active in Nepal since the late 1950's. The early Japanese botanical expeditions, like the British expeditions, collected fungi incidentally. These fungi, mostly members of the Aphyllophorales, were then worked up by mycologists back in Japan (e.g. Imazeki *et al.*, 1966). Later the Japanese organized expeditions with the primary purpose to study cryptogams including fungi (e.g. Otani, 1982). Such studies have resulted in publications on a wide array of Nepali fungi including ascomycetes (macro- and microscopic), chytrids and myxomycetes.

Mycologists from additional countries have also written papers on fungi from Nepal. Durrieu (1980; 1987) of France published on rusts. Kreisel (1969) of East Germany published a paper describing 15 gasteromycetes, five of which he described as new species. Ryvarden (1977) of Norway and Hjortstam (Hjortstam and Ryvarden, 1984) of Sweden published on fungi in the Aphyllophorales from Nepal describing seven new species in the two papers which list over 100 species.

Mycologists from neighboring India have collected fungi in Nepal. During the 1960's Panjab University in Chandigarh received support from U.S. P.L. 480 funds to collect and describe fungi from the western and central Himalaya "up to Kathmandu in Nepal." A two-month collecting trip

to central Nepal during the monsoon of 1969 provided material which has been deposited at PAN (Panjab Univ.) and at BPI (Waraitch and Thind, 1977b). The results of the P.L. 480-supported work were printed in a series of technical reports from Panjab University dated 1969-1970. Fungal groups covered were operculate discomycetes, inoperculate discomycetes, polypores, thelephores and clavarias. Much of this information plus additional data on Himalayan fungi has also been published in an extensive and continuing array of papers by Thind and co-workers. Some papers have dealt specifically with Nepali fungi, e.g. papers by Waraitch and Thind (1977a, 1977b) on Nepali pyrenomycetes. More often the collections from Nepal have been included in papers on Indian material (e.g. Sharma, 1983). Other Indian mycologists also include material from Nepal in their papers on Indian fungi.

Dibya Deo Bhatt, Shailesh Chandra Singh and their students wrote many of the first papers by Nepali scientists on fungi (see Singh and Joshi, 1977). Bhatt's and Singh's earliest papers were in 1966 and 1967 respectively. Bhatt and his students published on plant parasites and water molds. Although Singh is no longer active in mycology, he and his students published early papers on a wide array of fungi including myxomycetes, plant parasites, aquatic fungi and fleshy fungi. Singh (1973) wrote the first major work by a Nepali mycologist on agarics and boletes. Another early paper was a checklist of fungal plant diseases by Khadka and Shah (1967, later reprinted in part by the FAO, 1968). Current mycological research in Nepal is centered at the Natural History

Museum at Swayambhu, the National Herbarium at Godawari, and the Plant Pathology Division of the Ministry of Agriculture at Khumaltar. Two Nepali mycologists currently working and publishing on agarics and boletes are Mahesh Kumar Adhikari (1984) at the National Herbarium and Hemanta Ram Bhandary (1984) at the Natural History Museum. Lokendra Raj Sharma (1984) compiled a checklist of lichens for Nepal, listing 352 species. Current research at the Plant Pathology Division involves plant pathogenic fungi and the cultivated fungi *Agaricus*, *Lentinus*, *Pleurotus* and *Volvariella*. Since its appearance in 1977, the Journal of Natural History Museum (Kathmandu) has been a central vehicle for publications on Nepali fungi.

Nepali mycologists have had to work without access to much of the world's mycological literature; sadly this includes many papers on fungi from Nepal. Fortunately the amount of mycological literature available in Nepal is slowly increasing. The best collection of mycological literature is in the library of The Plant Pathology Division. Another handicap to mycological research in Nepal is that many collections of Nepali fungi are in the herbaria of Europe, Japan and North America and therefore largely inaccessible to Nepali mycologists. Too often duplicate collections are not deposited in a Nepali herbarium by foreign mycologists studying fungi of Nepal. On the positive side, some have; for example Durrieu (1987) deposited his rust collections at the European herbarium TLA and at the Nepal herbarium KATH.

As this review of the history of mycology in Nepal indicates, the literature on Nepal's fungi is widely dispersed and difficult to assemble. A further complication is that records of Nepali fungi are often buried in works on Indian fungi. A bibliography on Nepal fungi has been published (Singh and Joshi, 1977). Additional references, especially on Nepali lichens, can be found in Dobremez *et al.* (1972), Dobremez (1976) and Sharma (1984). The mycological research in the Himalayan regions of India and Tibet which surround Nepal is often directly applicable to Nepal. Useful bibliographies covering literature on Himalayan fungi are Imazeki *et al.* (1966) and Manjula (1983). Many checklists of Indian fungi have been published starting with Butler and Bisby (1931) and more recently Bilgrami *et al.* (1979). These and other Indian checklists are valuable insights into the Indian mycological literature. In recent years a number of papers have been published on Tibetan fungi. The fungi collected by Chinese expeditions to Tibet from 1966 to 1979 were brought together in the book *Fungi of Xizang* edited by Wang and Zang (1983); 880 species of fungi are covered.

Based on research to date, Nepal's mycota has several components. There is clearly a Himalayan endemic element, because most papers on Himalayan fungi describe new species. *Cavimalum indicum* Doi, Dargan and Thind, a parasite of *Arundinaria*, is a Himalayan endemic (Doi *et al.*, 1977; Cotter and Bhandary, in press), and most of the alpine gasteromycete species appear to be endemic (Kreisel, 1969). A second component of Nepal's mycota is the widespread boreal fungi of which Ryvarden (1977)

lists 12 in the Aphyllophorales. The third group, widespread temperate fungi, is well represented by fungi in all major groups studied. For example, Nepal's temperate gasteromycete mycota consists of such species (Kreisel, 1969), and Ryvarden (1977) notes over 10 widespread temperate species in the Aphyllophorales. Nepal, especially in the south, also has a tropical element in its mycota. Other elements in Nepal's mycota are south Asian and Japanese. Three fungi are known only from Japan and the Himalaya, and two have only been reported from Japan, Formosa and the Himalaya (Imazeki *et al.*, 1966; Ryvarden, 1977). Finally, cosmopolitan fungal species like *Schizophyllum commune* Fr. also occur in Nepal. Durrieu (1975) concluded that the plant pathogenic fungi of Nepal had very diverse geographic origins. With more research the affinities of the Nepal's mycota will become better defined.

As is true in the United States, the older literature on the fungi of Asia needs to be reevaluated. Horak (1980, 1987) reported that one-half of 64 species from China were misdetermined; these were collections and determinations by European mycologists in the early part of this century. Horak (1987) noted that fungal floras and mycogeographic conclusions based on this earlier work need to be reworked.

In summary, fungal research in Nepal, especially by foreign scientists, has focused on plant pathogenic fungi and fungi that preserve easily, such as polypores, ascomycetes, and lichens. Fleshy fungi, however, are largely unexplored. Manjula's (1983) checklist of agarics

and boletes of India and Nepal includes only four species from Nepal; three are in the genera *Lentinus* and *Pleurotus*. Watling (1978) noted, "The study of larger fungi of the Indian subcontinent is at the exploratory stage." This truth about the entire Indian subcontinent is even truer specifically about Nepal. Bhatt (1976) in his presentation on Nepal botany remarked, "Mycology in Nepal is still in an early stage of development." The next section specifically treats what is known about the boletes of Nepal.

Boletes of Nepal

Reports on Nepali boletes are scanty and there were no confirmable reports of *Suillus* before my collecting there. However, Bhandary of the Natural History Museum (Kathmandu) had collected at least two species of *Suillus* and has a paper in preparation which includes a species of *Suillus*.

Although Hooker collected no boletes in Nepal, he did collect 18 bolete species, including three species of *Paxillus*, in adjacent Sikkim and the Darjeeling area of India; all 18 were described as new species by Berkeley (1851a, 1851b, 1852, 1854a). Horak (1980) has done type studies on Hooker's boletes and transferred one to *Suillus*, *S. furfuraceus*. Others of Hooker's Himalayan boletes have also undergone taxonomic revision; for example, the three species of *Paxillus* were reduced to two and transferred to *Phylloporus* (Manjula, 1983). The genus *Strobilomyces* erected by

Berkeley (1851b) from Hooker's material has, however, been accepted. Most of Hooker's bolete collections, including *S. furfuraceus* (Berk.) Horak, are in poor condition (Horak, 1980).

None of the 28 bolete species on Manjula's (1983) checklist for India and Nepal is from Nepal. However, almost all are from the Himalayan states of India and could be expected to occur in Nepal. In fact I did collect some of these (e.g. *Boletellus emodensis* (Berk.) Singer; see Appendix A). Manjula's list includes four *Suillus* species: *S. furfuraceus, S. luteus* (L:Fr.) S. F. Gray, *S. plorans* (Roll.) Kuntze and *S. sibiricus* (Singer) Singer. The latter two were reported from Kashmir under *Pinus wallichiana* (Walting and Gregory, 1980); only a single basidiocarp of *S. plorans* was collected, but *S. sibiricus* was common. *Suillus luteus* occurred on soil in Himachal Pradesh (A. D. Sharma and Munjal, 1977). J. R. Sharma (1980) studied boletes for his Ph.D. degree at Himachal Pradesh University; in his dissertation he reported 69 bolete species from Himachal Pradesh including four *Suillus* species: *S. granulatus* (L.:Fr.) Kuntze, *S. placidus* (Bondorden) Singer, *S. sibiricus* and *S. brunnescens* Smith & Thiers. The former two were specifically noted to be associated with *P. wallichiana*; the latter two in coniferous/angiospermous forest and with pines respectively.

Though he did not cover the large genus *Boletus*, Zang (1985, 1986) still reported over 50 species in the other bolete genera from the eastern

Himalayan region in China; *Suillus sensu lato* was very well represented with 16 species. The Tibetan mycota includes 40 boletes with 9 in *Suillus sensu lato* (Wang and Zang, 1983); one of the *Suillus* species was not found in the eastern Himalaya. The 17 species of *Suillus sensu lato* (*Boletinus, Fuscoboletinus, Suillus*) reported from the greater Himalayan region of China are:

B. cavipes (Opat.) Kalchbr.	eastern Himalaya
B. decipiens (Berk. & Curt.) Peck	eastern Himalaya, Tibet
B. lignicolus Zang	eastern Himalaya, Tibet
B. pictus (Peck) Peck	eastern Himalaya, Tibet
B. pinetorum (Chiu) Teng	eastern Himalaya
F. aeruginascens (Sec.) Pom. & Smith	eastern Himalaya
F. glandulosus (Peck) Pom. & Smith	eastern Himalaya
F. paluster (Peck) Pom.	eastern Himalaya
F. serotinus (Frost) Smith & Thiers	eastern Himalaya
S. bovinus (L.:Fr.) Kuntze	eastern Himalaya, Tibet
S. brevipes (Peck) Kuntze	Tibet
S. flavidus (Fr.:Fr.) Singer	eastern Himalaya
S. granulatus	eastern Himalaya, Tibet
S. luteus	eastern Himalaya, Tibet
S. placidus	eastern Himalaya, Tibet
S. subaureus	eastern Himalaya
S. subluteus (Peck) Snell	eastern Himalaya, Tibet

Only two of the 17 species were originally described from Asia. I suspect that with further study others will prove to be distinct from their counterparts in Europe and North America.

Turning to Nepal itself, three authors have published on boletes. Seven; *Boletellus ananas* (Curt.) Murr., *Boletus dessilens* Corner, *Strobilomyces strobilaceous* (Scop. ex Fr.) Berk., *Paxillus rhodoxanthus* Schw. plus three unidentified *Boletus* species; were listed by Singh and Nishra (1976) in their list of fleshy fungi from the Kathmandu-Dhulikhel area. Pandey's ("1971-76") survey lists 25 bolete collections in the

genera *Boletus, Strobilomyces, Gomphidius* and *Paxillus* but it is uncertain how many species are represented. Identified to species were *Boletus edulis* Bull., *B. piperatus* Fr., *Strobilomyces floccopus* (Fr.) Karsten and *Paxillus panuoides* (Fr.) Fr.. Bhandary (1984), in his review of edible and poisonous Nepali mushrooms, listed four boletes: *Boletus edulis*, *Boletinus cavipes* (Opat.) Kalchbr. and *Strobilomyces floccopus* (Fr.) Karsten as edible and *Boletus satanas* Lenz as poisonous. The citing of *Suillus* (*Boletinus*) *cavipes* (Opat.) Smith & Thiers is significant as it would be the first report of a *Suillus sensu lato* from Nepal. However, unfortunately neither a collection nor a reference for the report was cited. A useful feature of Singh and Nishra's and Bhandary's checklists is the inclusion of Nepali and local names for many of the fungi.

Despite the limited work on boletes of Nepal it is clear from the abundant species in the Fagaceae and Pinaceae in Nepal's flora and from the number of bolete species reported from surrounding countries that Nepal must have a rich bolete mycota.

Comments on the genus Suillus

Suillus is a genus in the family Boletaceae (Singer, 1986; Smith and Thiers, 1964). The Boletaceae are characterized by the production of fleshy, macroscopic, mushroom-shaped basidiocarps with a hymenium of tubes under the pileus; the basidiocarps are not persistent. Many boletes

have been shown to be ectomycorrhizal, and most are believed to be so (Miller, 1982, 1983).

The type species of *Suillus* has long been accepted as *S. luteus* (L.:Fr.) S. F. Gray. However, with the change in starting date accepted at the 1981 International Botanical Congress, Palm and Stewart (1984c) concluded that *S. granulatus* (L.:Fr.) Kuntze, originally described from Europe, is now the proper type species and designated material collected in Sweden as a neotype. Palm and Stewart (1986) have studied the nomenclature of the *Suillus sensu stricto* species of Minnesota; their decisions are followed in this dissertation.

Various authors circumscribe *Suillus* in different ways. For the purposes of my work in Nepal, I adopted a broad definition of the genus *Suillus* including the fungi that Singer (1986) puts into the genera *Suillus* and *Boletinus*. My working definition of *Suillus* thus includes *Fuscoboletinus* of Pomerleau and Smith (1962) but excludes *Paragyrodon*, which Smith and Thiers (1964) include in *Suillus*. (None of my Nepali collections of *Suillus* fits "*Boletinus*" nor "*Paragyrodon*" but several do fit "*Fuscoboletinus*".)

Smith and Thiers (1964) note that the taxonomy of *Suillus* in North America is "confused". The same may be said on a world-wide basis. Both the nature of *Suillus* and human factors, such as not providing good ecological data with descriptions, have contributed to the confusion.

Seven factors, with illustrations, which make definition and identification of *Suillus* species difficult are:

1) Variation in basidiocarps due to environmental influences and aging. The nature of the pileus surface may vary distinctly depending on whether the weather is dry or wet. The size and density of the stipe glandulae may vary greatly even from one side of a stipe to the other. Sunlight and rain strongly affect pileus colors. Many species of *Suillus* in section *Suillus* look very similar when old. In addition, the flesh is often devoured by larvae by the time basidiocarps are mature which destroys the useful taxonomic characters of the flesh.

2) Mixed fruitings leading to mixed collections.
Smith and Thiers (1964) warn that it is easy to make mixed collections of *Suillus* species because different species associated with the same tree fruit at the same time. They note that if *S. granulatus* and "*S. albidipes*" [*S. neoalbidipes* Palm & Stewart] were to fruit together "one would almost certainly consider them to be one species..." Based on my field work in Nepal and in Virginia, I conclude that mixed fruitings of different species of *Suillus* are indeed very common and realize that some of my early collections in both areas could be mixed. Similar appearances when old of species fruiting together may foster such mixed collections.

3) General microscopic similarities among *Suillus* species and thus the frequent need to rely on fresh characters to define and identify species.

Singer (1945a) recognized the American and European fungi called *Suillus granulatus* as separate subspecies stressing the difference in mycorrhizal host relationships and geography and noting that fresh basidiocarps of the two are macroscopically distinct. However, microscopically they are the same except for a small difference in basidiospore size (Singer, 1945a). Palm and Stewart (1984a) compared sizes of basidiospores, basidia and cystidia of the American *S. granulatus, S. neoalbidipes,* and *S. brevipes* (Peck) Kuntze. No statistically significant differences were found. These two examples illustrate the microscopic similarities among *Suillus* species and the difficulty of recognizing species from dried basidiocarps since many of the important fresh characters are no longer available. Thiers (1975) summarizes the microscopic similarity of *Suillus* species as follows, "the spores are very monotonous in their characteristics except for rather slight variations in length and diameter...The relative constancy in shape and general appearance of these cells [cystidia] is rather remarkable." Palm and Stewart (1986) note that "Microscopic differences between *Suillus* species are often insufficient to distinguish macomorphologically distinct taxa."

4) Incomplete and incorrect host data.

Singer (1945b) points out the need for careful field observation when collecting boletes. In Florida all species of *Leccinum* are confined to oak so he was puzzled when he discovered a *Leccinum* in a *Pinus palustris* woods. Close inspection, however, revealed the presence of

two-inch high *Quercus minima* which was probably the mycorrhizal associate of the *Leccinum*.

5) Describing species based on variation which, even if genetically based, is probably better considered intraspecific.

   An example of describing a species on variation better considered intraspecific is *Suillus proximus* Smith and Thiers which was later synonomized with *S. grevillei* (Klotzsch) Singer by Grund and Harrison (1976). Grund and Harrison (1976) found that the greening flesh, a key character in defining *S. proximus*, varied seasonally within populations of *S. grevillei*.

6) The difficulty of germinating spores of *Suillus* which so far has precluded defining biological species based on dikaryon formation. Basidiospores of *Suillus* and of ectomycorrhizal fungi in general are notoriously difficult to germinate in the laboratory. Fries (1976) did succeed in germinating basidiospores of six species of *Suillus* but at low percentages. Some spores germinated spontaneously, but others took over a month. Even then less than one spore in 10,000 (0.01%) germinated. Use of the yeast *Rhodotorula* or self-mycelium increased germination but only to somewhat over one percent. Not reported was whether the single spore germlings grew and developed into maintainable cultures. Cultures of this type would be necessary before crossing experiments could be done to determine sexual mating systems and biological species in *Suillus*. Experimental results have not yet been published on *Suillus* genetics.

7) Nomenclatorial confusion.

In North America, an area of the world where *Suillus* has been relatively well-studied, conflicting species concepts compounded by nomenclatorial confusion exist. Smith and Thiers's (1971) concept of *S. subluteus* is different than Snell and Dick's (1970). The fungus that Smith and Thiers call *S. subluteus* is called *S. pinorigidus* Snell & Dick by Snell and Dick. The fungus that Snell and Dick call *S. subluteus* is divided by Smith and Thiers into three taxa, *S. acidus* (Peck) Singer var. *luteolus* Smith & Thiers, *S. intermedius* Smith & Thiers, and *S. subalutaceus* Smith & Thiers. In this dissertation I will use *S. subluteus* in the sense of Snell and Dick because that sense fits an illustration of the fungus by Peck.

Based on evidence to date, *Suillus* is an ectomycorrhizal genus, usually associated with members of the Pinaceae (Miller, 1982). Trappe (1962) summarized early data from numerous references supporting this contention. Since then additional species of *Suillus* from around the world have been shown to be ectomycorrhizal. Recent examples are Asian *Suillus* species (Miller and Lee, 1987) and North American species (Palm and Stewart, 1984b; Samson, 1986) confirmed to be ectomycorrhizal by synthesis experiments and European species (Treu, 1987) confirmed by direct observation of hyphal connections between basidiocarps and ectomycorrhizae.

Materials and Methods

Field work was carried out in Nepal from April 1985 until April 1986 by Irene Cotter and me. Follow-up cultural work and ectomycorrhizal syntheses were done at Virginia Tech from April 1986 until April 1987.

Field work

The year of field work in Nepal was conducted to collect, culture and describe the *Suillus* species and to define their tree associates by field observation. An apartment in Kathmandu (Tahachal) was kept to serve as a home base and laboratory. Basidiocarps were preserved by drying. Cultures were obtained and maintained on modified Hagem's agar.

Field work in Nepal, especially during the monsoon, involves many unique challenges. As Hooker (1855) phrased it, "[Gigantic nettles] with leeches, mosquitos, peepsas, and ticks sometimes keep the traveller in a constant state of irritation." Pandey ("1971-76") wrote, "One has to face a number of problems... climbing into the slope of the hills and entering into the thick bushes and prickly shrubs. Attacks of leeches and wild animals are always expected. As mushrooms grow mostly in the rainy season, the slimy way, the flooded area along with rains and clouds, etc., hamper the collector..."

Field trips varied from day trips in the Kathmandu Valley to two week treks in the Langtang and in the Kali Gandaki Valleys. During our collecting treks, Irene and I were accompanied by one or two porters who carried gear and acted, beyond their call of duty, as guides. They also served as excellent field assistants spotting many interesting fungi and plants. People who served as our porters were Prem Rai, Ajumbar Rai, Chandra Rai and Hankar Gurung. We relied on local shelter and whenever possible on local food. In contrast, Hooker (1855) travelled with a support staff of 55 when he set off to collect plants in eastern Nepal in 1848. Even so he had to spend the first night "without food or bed" due to problems with his porters.

When collected, fungi were wrapped in wax paper and carried in a basket. At the end of the day we would stop at or return to a tea house where collections were described, photographed and cultured.

Fungi were preserved by drying on stacked, round wire screens from a Sigg dörrex electric food dehydrator (Sigg Limited, 8500 Frauenfeld, Switzerland). Prompt drying of fungal collections is important; given the lack of electricity, we used kerosene to generate heat. The screens were placed on a camp grill held over a Chinese pressurized kerosene lantern which served as a heat source and was surrounded by an aluminum foil "chimney". An upside down doko (a basket carried by a braided rope over the forehead) was often used to support the grill by sticking one set of the grill's folding legs into it; then the grill itself rested on

the top of the lantern. Larger fungi were cut in half to expedite drying. Most fungi dried overnight, but some required several nights of drying. Once dried, fungi were kept in self-sealing, reclosable polyethylene bags and carried within plastic food freezer boxes for protection.

Culturing was done in the tea houses. The lantern was used to sterilize thin forceps which were used to pluck out small pieces of pileus trama from basidiocarps which were broken open. These pieces were placed on modified Hagem's agar containing 0-10 ppm benomyl in test tubes. The formula for my modification of Hagem's agar (Molina and Palmer, 1982) is as follows:

Malt extract	4.0	g
Yeast extract	1.0	g
D-Glucose	5.0	g
NH_4Cl	0.5	g
KH_2PO_4	0.5	g
$MgSO_4 \cdot 7H_2O$	0.5	g
$FeCl_3$	0.5	ml of a 1% aqueous stock solution
Biotin	0.1	ml of a 0.05 g/l aqueous stock solution
Thiamine	0.1	ml of a 1.0 g/l aqueous stock solution
Agar	15	g
Water, distilled	1	l

Amounts of biotin and thiamine in the medium are 5 µg/l and 100 µg/l respectively. After all ingredients had been added, the medium was autoclaved for 15-20 minutes at 15 psi.

Autoclavable polycarbonate centrifuge tubes with polypropylene caps served well as culture tubes; they were small, lightweight and unbreakable. Tubes were tighted capped after the tissue was added and kept so until we were back in Kathmandu; then the caps were loosened.

Otherwise pressure changes with altitude apparently caused contamination problems due to air ingress and egress.

Once back in Kathmandu collections were redried, if necessary, using a Sigg dörrex electric food dehydrator.

Descriptions of basidiocarps

Basidiocarps were described macroscopically in the field. The *Methuen Handbook of Colour* (Kornerup and Wanscher, 1978) was used to describe colors.

Microscopic features were observed from free-hand sections made directly from dried basidiocarps and then mounted in 3% KOH. Further information on methods is in Appendix B on the form used in describing the basidiocarps microscopically.

Cultures and cultural descriptions

Twenty-eight bolete cultures were brought back from Nepal. Each culture was assigned the same number as the basidiocarp collection that was its source (see Appendix A and Table 1.6). All but two of the cultures were *Suillus* species.

Since isolation in 1985, cultures have been maintained on modified Hagem's agar without benomyl with transfers two to three times per year. After colonies have grown for about one month, they are refrigerated until the next transfer. In Nepal, cultures were maintained in test tubes. Once back in the United States, cultures were maintained in Petri dishes because the longevity of *Suillus* cultures under refrigeration appears to be greater in Petri dishes than in test tubes. (U.S. *Suillus* cultures which were refrigerated for the year I spent in Nepal survived better in Petri dishes than in test tubes.)

Nepali cultures which were described and subjected to numerical taxonomic analysis are listed in Table 1.6. *Gyrodon* cf. *lividus* and *Paxillus* cf. *filamentosus* were included in the analyses as outgroups. Like *Suillus*, both are boletes but *Gyrodon* is in a different subfamily and *Paxillus* in a different family (Singer, 1986). Cultures were first described in the summer of 1986 using the character set described in "A manual for the identification of ectomycorrhizal and wood-rotting fungi in pure culture" developed at the Virginia Tech Mycology Laboratory (Miller *et al.*, 1985). The data were entered into a computer and subjected to numerical taxonomic analyses. No meaningful clustering resulted; principal coordinates mapping linked with minimum spanning tree lines was a mess of interwoven lines suggesting that the principal coordinate analysis (PCA) axes greatly distorted the relationships among cultures. Factors contributing to the lack of meaningful clustering were variation within cultures, variation due to observation methodology (how

long and how far does one look before deciding there are no clamps?), and inadequately defined characters. These results are not presented in this dissertation but served as a basis on which to redesign the description procedures to improve their standardization and to make characters more reliable. New characters were developed. Using the new procedures and characters, described below, the cultures were redescribed during the winter of 1987; these results are presented in this dissertation.

Cultural descriptions were based on colonies grown on modified Hagem's agar and on slide cultures (Grand, 1968).

Cultures were grown on modified Hagem's agar (formula above) in 100-mm-diam polystyrene Petri dishes. Four 20-mm-deep dishes were inoculated in the centers with 3.5-mm-diam agar plugs taken from just inside the colony margin of a 2-week old colony growing on double-layered modified Hagem's agar. Only the surface agar layer from the donor dish was taken with the plug which was placed mycelium-side down in the inoculated dishes. A fifth polystyrene Petri dish (15-mm-deep, 4-compartment, modified Hagem's agar) was inoculated with two 3.5-mm-diam plugs in opposite quadrants. The five dishes for a given culture were stacked with the 4-compartment dish on top. Stacks of Petri dishes were placed eight stacks per covered, unsealed polycarbonate box on a laboratory bench. Temperature over the 4 wk incubation period was 20 ± 2 C.

Colonies were observed and measured (two perpendicular diameter measurements) weekly for the four weeks. Particular attention was paid to color patterns, exudation droplets, furrowing, diffusing pigments and any distinctive features. At four weeks comprehensive descriptions were done. No dish had become contaminated. Petri dishes were kept beyond four weeks to check for changes and new characters; no additional useful ones were found.

References especially helpful in designing the description methods and terminology were Miller *et al.* (1985), Grand (1968), Miller *et al,* (1983), Nobles (1965), Pantidou and Groves (1966), Snell and Dick (1971), and Stalpers (1978). Two data forms, one for macrochemical tests and one for morphology and anatomy, were used for the culture descriptions done at four weeks; these forms are included in Appendix B modified to include annotations and changes in procedure which occurred during my observations. Colony odor, height and depth were recorded on both forms and a colony cross-section was drawn on both forms.

Slide cultures were prepared as follows. Ten-cm-diam glass Petri dishes; each containing filter paper, a glass microscope slide, and a 24 x 40 mm coverslip supported by a bent glass rod; were autoclaved. After cooling the filter paper was wetted with sterile water. An 11-mm-diam agar plug was removed from just inside the colony margin of a colony growing on single-layer modified Hagem's agar. The plug was placed upside down on the slide and covered with the coverslip. These slide cultures

were incubated five days in the dark at 22-23 C. Further processing is detailed on the data form used to describe slide cultures (see Appendix B).

Numerical taxonomic analysis of cultural data

Cultural data from weekly observations, macrochemical tests, morphology and anatomy, and slide cultures were coded for numerical taxonomic analysis. The inherently inconsistent vegetative incompatiblity data (see below) were not used. Most characters (circa 90%) could be directly coded as binary (0,1). A few characters, e.g. furrows, were coded as additive. Sneath and Sokal (1973) concluded that additive coding is usually "simple and adequate". Continuous quantitative characters were scaled from 0 to 1 by equalizing by Gower ranging (Sneath and Sokal, 1973). Much judgment goes into character selection and coding (Dunn and Everitt, 1982). Growth data (increase in colony diameter) is a good example of my approach. Weekly diameter measurements over four weeks were recorded. Readings for week one were more variable due to greater measurement error given the small colony sizes. Readings for week four were misleading because the faster growing colonies were slowing down due to confinement. Therefore data from weeks one and four were not used. Diameter increases between weeks zero and two and between weeks two and three were selected as the most characteristic of the cultures. Binary coding of the growth data was considered; its appropriateness was tested by an analysis of variance of

mean growth rates. Because the cultures did not segregate into discrete classes (e.g. fast, medium, slow growers) but were for the most part continuously distributed from fast to slow growers, binary coding was rejected. Instead Gower ranging on medians was used to scale the data from 0 to 1 so that its magnitude paralleled the binary characters. Medians were chosen over means to summarize the growth data because sometimes a single colony was radically different in growth rate from its sister colonies (replicates). Such "outliers" affect the mean but do not affect the median.

A similar approach and logic were used to code the other characters. The cultural data for the 28 cultures were summarized as 82 characters (Appendix C).

The cultural data set was analyzed using the NT-SYS (Rohlf *et al.*, 1979) and SAS (SAS Inst. Inc., 1985) computer programs available on the Virginia Tech computing system.

In NT-SYS, clustering was done by both single-linkage (SINGLE) and unweighted pair-group (UPGMA) techniques operating on each of three similarity/distance matrices: Pearson's correlation coefficient (CORR), Euclidian distance (EUCLID), and Manhattan distance = mean character difference (MANH). The clustering from the MANH matrix using the SINGLE technique was chosen as the most satisfactory (see Results and Figs. 1.2 and 1.3). The MANH differences were used to generate minimum spanning

tree linkages. Ordination on MANH differences was performed using two methods: (1) parametric, principal coordinate analysis (PCA), and (2) nonparametric, nonmetric multidimensional scaling. PCA was done using the GOWER and FACTOR routines of NT-SYS. Ordination results were graphed two dimensionally in all possible combination of factors or axes; cultures were then connected into a minimum spanning tree.

In SAS, ordination results were graphed three dimensionally. Discriminate function analysis was run on the clustering to check the placement of each culture.

Vegetative compatibility experiment

An experiment was conducted to test whether the Nepali *Suillus* species have characteristic intra- and interspecific vegetative compatibility reactions. Different genetic individuals of wood-rotting basidiomycete species show strong, easily recognizable reactions when paired in culture (Rayner and Todd, 1982). If *Suillus* species react similarly, then such reactions could be a useful in classifying and identifying cultures. Basically the idea was to let the cultures "recognize" each other. Eight primary testers, (VC 1303, VC 1423, VC 1421, VC 1425, VC 1081a, VC 1185, VC 1450, VC 1449; see Table 1.6), were selected, one for each of eight *Suillus* species defined on basidiocarp morphology and ecology. An additional eight cultures, (VC 1312, VC 1022, VC 1391a, VC 1041, VC 1480, VC 1192, VC 1231, VC 1230), one per species, plus *Paxillus*, VC 1092,

Gyrodon, VC 1416, and VC 1042, a *Suillus* culture of then uncertain affinity, were selected as secondary testers. Each primary tester was tested against each of the 19 testers including itself. VC 1092 and VC 1416 were also paired and each selfed making a total of 155 tests. Plugs (3.5 mm diam) were cut from just inside the margin of actively growing colonies on double-layer modified Hagem's agar. Paired plugs were placed upside down about 1.5 cm apart (center to center) in one compartment of a four-compartment, 100x15-mm polystyrene Petri dish with modified Hagem's agar. Four tests were done per dish. Pairings were set up on 20 Jan 1987 and incubated under room conditions within a covered, unsealed polycarbonate box. Lab temperatures were 20 ± 2 C. Pairings were periodically scored for pigment lines and unusual mycelial development (barrages) where colonies met. Inhibition, under- and overgrowth and changes in colony appearance were also noted. Final scorings were on day 39.

Ectomycorrhizal syntheses

The growth pouch method (Fortin *et al.*, 1980) of ectomycorrhizal synthesis was used to test the nature of the Nepali *Suillus*-conifer associations. Each *Suillus* culture was tested with the pine species with which it was associated in the field. Pine seeds were soaked in water at 4 C for 2.5 days, surface sterilized with 30% hydrogen peroxide for 40 minutes, and then germinated in a sterile environment. The young pine seedlings were placed in growth pouches containing 15 ml of a modified

Melin-Norkrans solution (MMN) (Molina and Palmer, 1982) further modified to contain 1 ml/l of a micronutrient solution Chen *et al.*, 1961), 67 mg/l ferric sodium ethylene diamine di-(o-hydroxyphenylacetate) as the iron source, and 1-10 mg/l benomyl but without malt extract and glucose. The benomyl was used to reduce growth of Deuteromycetes. Pouches were kept in a growth chamber at 20 C with 16 hrs of light (3.2 klux). After secondary root formation began, fungal mycelia were added to the pouches on agar plugs. Each tree-fungus combination was replicated three to four times except the *Pinus patula-Suillus* combinations which were not replicated. Revised versions of the data forms used in describing the ectomycorrhizae and extramatrical phase are included in Appendix B. Ectomycorrhizae were fixed in CRAF fixative (Johansen, 1940) and free-hand sectioned to observe Hartig nets.

Nineteen *Pinus-Suillus* combinations were tested (refer to Table 1.6 for culture numbers):

P. wallichiana-S. cf. *granulatus* (3 cultures);
P. wallichiana-S. cf. *placidus* (3 cultures);
P. wallichiana-S. sibiricus (6 cultures);
P. wallichiana-S. "greening-foot" (3 cultures);
P. roxburghii-S. "queen's" (1 culture);
P. roxburghii-S. sp. (1 culture);
P. patula-S. "waxy" (2 cultures).

Efforts to germinate *Larix himalaica* seeds following Samson and Fortin (1986) failed so the *Larix-Suillus* associations were not tested for ectomycorrhizal formation.

Results

Field work and summary of collections

A list of my fungal collections from Nepal is included as Appendix A. Most fungi were collected in north central Nepal (Bagmati and Dhaulagiri Zones) in temperate and boreal forests. Some fungi were collected in the subtropical terai across the length of southern Nepal. Collection numbers for my collections from Nepal are VC 1001 to VC 1538.

Collections have been deposited in various herbaria (see Appendix A). The *Suillus* collections have been or will be deposited at BPI and KATH with species vouchers at the Natural History Museum (Kathmandu), Shimla and HKAS (Kunming). The usual method of deposition of the Nepali collections is to divide them with part deposited at a Nepali harbarium and part at a United States herbarium. Certain collections, such as plant parasitic fungi, were deposited at Nepali herbaria (KATH, The Division of Plant Pathology) usually without division. Other small collections are being deposited in their entirety at a single herbarium because of the collection's small size.

A total of 495 fungal collections were successfully processed and dried. The major fungal groups represented are Boletales (146 collections, 29%), Agaricales (119 collections, 24%), Aphyllophorales (71

collections, 14%), and Ascomycetes (111 collections, 22%). Table 1.3 lists the major genera of fungi collected.

Irene also collected vascular plant specimens which have been deposited at the Arnold Arboretum Herbarium (AAH).

Definition of Suillus species from Nepal

Eighty-one collections of *Suillus* were made during our year in Nepal. *Suillus* was collected from May through October, primarily during the monsoon. Studying *Suillus* in Nepal was a unique opportunity to collect an unknown group of taxa within a defined genus and to develop species concepts independent of a literature on the taxa. It also offered a test of the hypothesis that *Pinus-Suillus* pairs evolve in parallel.

As collecting progressed, I developed species concepts for the *Suillus* collections and designated them by common names. During the year, these concepts shifted, generally to become broader. For example, initially I defined two "species" within what I now consider one species, *S. sibiricus*; the two initial "species" were based on pileus characters and color reactions of the flesh (± bluing). Subsequent collection convinced me that such differences were environmental and developmental, not genetic.

In this dissertation, nine species of *Suillus* are recognized from Nepal (Table 1.4). Four of them fit existing species well though not completely; the other five appear to be new. These species concepts were developed based on basidiocarp characters, such as morphology (including color) and chemical reactions, and on ecological characters such as host. One purpose of the cultural description work which follows was to test, independently of the basidiocarps, the validity of these concepts.

Numerical taxonomic analysis of cultural data

Twenty-six *Suillus* cultures representing eight of the nine species recognized from Nepal were described (Table 1.6). The orange-pored *Suillus* was collected only once; the first transfer of its culture failed. VC 1480, one of the 26 cultures, represents a tenth species but basidiocarp material was inadequate to describe the species satisfactorily. The use of one culture each of *Gyrodon* cf. *lividus* and *Paxillus* cf. *filamentosus* as outgroups brought the number of cultures analyzed up to 28. The data matrix of 82 characters for the 28 cultures used in the numerical taxonomy analyses is included as Appendix C.

All six phenograms generated by NT-SYS using six combinations of similarity/distance measure (CORR, EUCLID, MANH) and clustering technique (SINGLE, UPGMA) were basically similar with some minor but important differences. The two distance measures, EUCLID and MANH, were better than CORR because their phenograms recognized both outgroups and clustered

VC 1185 with VC 1207. Clusters based on MANH were slightly better defined than those based on EUCLID.

The SINGLE clustering technique was clearly better than UPGMA for all three similarity measures. Reasons were 1) outgroups were better recognized and 2) VC 1423 and VC 1218 each clustered with the appropriate cluster based on basidiocarp species concepts. Furthermore, SINGLE linkage clustering is mathematically better (Dunn and Everitt, 1982).

Therefore the phenogram of choice was based on MANH distances clustered using SINGLE linkage (Fig. 1.2). The outgroups, VC 1092 and VC 1416, are at the bottom separated from all *Suillus* cultures. The clusters of *Suillus* cultures correspond very closely to species concepts based on basidiocarps and ecology. Drawing a straight phenon line fails to recognize all these species clusters because they cluster at different levels. However, a slightly angled phenon line creates 12 clusters which are designated by vertical lines in Fig. 1.2 and are referred to by number top down.

The tightest cluster is #10 which consists of the three greening-foot *Suillus* (Gf) cultures. Their distinctiveness strongly separates them from all other cultures including the *S.* cf. *granulatus* (Gr) cultures. By contrast, the greening-foot *Suillus* and *S.* cf. *granulatus* have similar basidiocarps which can be difficult to tell apart when old if the flesh has been attacked by insect larvae. Other *P. wallichiana* associates also

clustered well, *S.* cf. *placidus* (Pl) in cluster #1, *S. sibiricus* (Si) in #2 and *S.* cf. *granulatus* (Gr) in #7. One *S.* cf. *granulatus* culture clustered by itself in #4. The single cluster of *S. sibiricus* is of note for two reasons: (1) the basidiocarps show much variation and (2) there were many cultures which increases the likelihood of failure to cluster together.

The two *P. roxburghii*-associated cultures clustered near but not close to each other, one (Qu, VC 1081a) in cluster #3 and one (Sp., VC 1480) in cluster #2 with *S. sibiricus*. The basidiocarps of these two collections are different and they appear to be distinct species. Although VC 1480 clustered with the *S. sibiricus* cultures, it is almost certainly not conspecific because the basidiocarps of VC 1480 (Sp.) are distinctly different from those of *S. sibiricus* and their hosts are different.

The three *P. patula*-associated cultures (Wx) did not cluster tightly, one was in cluster #1 with *S. placidus*. The other two were both in cluster #8 but barely clustered together. However, the basidiocarps of the *P. patula* associated collections are similar, and the ecology is the same. Therefore I consider the three to be conspecific (the waxy *Suillus*). In addition to the variability among the three cultures, each culture also showed much intraculture variation.

The *Larix himalaica*-associated cultures basically clustered in accordance with basidiocarp species concepts; the himalayan *Suillus* (Hi) cultures were in cluster #6 and two cultures of *Suillus laricinus* (La) in cluster #5. However, one culture (VC 1438) of *S. laricinus* clustered by itself in cluster #9. The clustering of the *S. laricinus* cultures in the middle of the other *Suillus* cultures supports the retention of the "*Fuscoboletinus*" group within *Suillus*.

In summary, all but three of the 26 *Suillus* cultures clustered in parallel with species concepts based on basidiocarps and ecology. All 26 *Suillus* cultures clustered together before joining the two outgroups, *Gyrodon* and *Paxillus*, which supports the concept that *Suillus* is a good genus.

Host is another character available to a person identifying a fungal culture isolated from an ectomycorrhiza. Accordingly, I reran the clustering of the Nepali cultures with the addition of host data (Fig. 1.3). The addition of host data improved the separation of species clusters (see arrows in Fig. 1.3) and resulted in the clustering of VC 1438 with the other *S. laricinus* (La) cultures.

The discriminate function results were puzzling. Some groups were retained, *S.* cf. *granulatus*, *S.* cf. *placidus*, *S. sibiricus*, and greening-foot *Suillus*. These were the groups with pairs of cultures having the greatest similarity, and all are associates of *Pinus*

wallichiana. The other groups were less tightly clustered and discriminate function analysis completely rearranged them in seemingly meaningless groups. Indeed, I believe they are meaningless because discriminate function analysis requires quantitative variables and assumes that the distribution of each class is multivariant normal (SAS Inst. Inc., 1985). Most of my variables are qualitative (binary), and the normality of each class is untested. However, had the groups been tight and well-separated, such violations of assumptions would probably have not interfered with retention of the groups. Therefore from the discriminate function analysis I conclude that though the clusters of cultures are real, all but the clusters of *P. wallichiana* associates are weak.

The NT-SYS ordinations using parametric and nonparametric methods were similar; the parametric results from principal coordinates analysis (PCA) are presented here.

The percentages of variation among cultures explained by the three PCA factors were 34%, 18% and 14% (or 66% combined). All three two-dimensional mappings of the factors showed much distortion when tested using minimum spanning tree linkage. The linkages criss-crossed abundantly. The three-dimensional representations using all three factors (Figs. 1.4, 1.5) were better and showed less distortion when the cultures were interconnected with the minimum spanning tree linkages (Fig. 1.6). The relatively low percentage of variation explained by the

first three factors, which is reflected in the distortion remaining when even three axes are used, indicates that many dimensions are required to successfully separate the clusters of cultures.

In Figure 1.4, symbols represent basidiocarp species concepts. The outgroups (solid symbols) separated well off in the upper right. Cultures of certain *Suillus* species (greening-foot *Suillus* represented by diamonds) clustered tightly and well apart from other species. All in all, identical symbols were spatially related; thus the PCA analysis agreed with the cluster analysis (Fig. 1.2). The angle of view of the PCA axes can be misleading. In Figure 1.4 the two crosses do not appear to be close to each other, but if the axes are rotated (same data) (Fig. 1.5) their spatial closeness relative to the other cultures becomes apparent.

Vegetative compatibility experiment

The results of the vegetative compatibility experiment showed only weak incompatibility reactions which were not consistent between replicates of the same cross. Mycelial barrages in zones of colony interaction were rare, but pigment production often occurred aerially and in the agar in these zones and was used as the indication of an incompatible reaction. Six of 28 replicated crosses developed pigment in one case but not in the other. As expected all self-crosses lacked pigment production (compatible reaction). Intraspecific crosses of

different cultures were expected to have incompatible reactions but only three of eight did. Interspecific crosses showed both compatible (62 cases) and incompatible (74) reactions. In general, the greening-foot *Suillus* reacted strongly and the himalayan *Suillus* weakly. In conclusion, *Suillus* species do not appear to have a strong vegetative incompatibility system. It is possible that the reactions might be stronger under different environmental conditions. If ectomycorrhizal fungi do not have strong vegetative incompatibility systems, perhaps it is because their key interactions are with living vascular plants rather than with each other.

Ectomycorrhizal syntheses

Nineteen of the Nepali *Suillus* cultures were tested for ability to form ectomycorrhizae with their pine associates in growth pouches. Seventeen (85%) did and represented all six *Suillus* species tested. Ectomycorrhizae formed in 38 (63%) of 60 total pouches and often developed within a week or two after the fungus was added to the pouch. Ectomycorrhizae did not form in any of the nine control pouches with only *P. wallichiana*.

Ectomycorrhizae formed in all six *Pinus-Suillus* combinations (refer to Table 1.6 for culture numbers):

P. wallichiana-S. cf. *granulatus* (2 of 3 cultures);
P. wallichiana-S. cf. *placidus* (2 of 3 cultures);
P. wallichiana-S. sibiricus (6 of 6 cultures);
P. wallichiana-S. "greening-foot" (3 of 3 cultures);
P. roxburghii-S. "queen's" (1 of 1 culture);

P. roxburghii-S. sp.	(1 of 1 culture);
P. patula-S. "waxy"	(1 (VC1192) of 2 cultures).

The three cultures which did not form ectomycorrhizae were VC 1312, VC 1423 and VC 1207. VC 1207 was tested in only one pouch due to a limited number of *P. patula* seedlings. The reason these three cultures failed to form ectomycorrhizae is unknown; it may be related to the length of time in culture which was over a year before the synthesis experiments were done.

The *Suillus* culture was successfully reisolated from ectomycorrhizae in four of eight pouches tried. In none of the other four was an alternative ectomycorrhizal fungus isolated; organisms isolated were bacteria, yeasts and Deuteromycetes.

Descriptions of Nepali Suillus species and their ectomycorrhizae

Color notations (e.g. 7E6) are from the *Methuen handbook of colour* (Kornerup and Wanscher, 1978). Shapes and colors are given for different developmental stages; "when very young convex, hemispherical becoming convex then broadly convex to planar convex" means that the shape changes from hemispherical convex (very young) to convex (mature) and then to broadly convex-to-planar convex (old). Arrows (→) are used for chemical reactions to mean "becoming". "KOH gray → dark gray" means the tissue turns gray when KOH is applied, and then the spot becomes dark gray.

Cultural descriptions are primarily based on the redescriptions of the cultures done during the winter of 1987. Supplemental information is included from the culture notes made in Nepal and from the descriptions done during the summer of 1986. Ectomycorrhizae descriptions are from the ectomycorrhizae synthesis trials in growth pouches.

Suillus cf. granulatus (L.:Fr.) Kuntze
Fig. 1.7a, 1.7b.
Basidiocarp single.
Pileus (2)3.5-10.5(11) cm in diam; when very young convex-hemispherical, becoming convex finally broadly convex to planar convex; surface viscid to glutinous (moist in dry weather with debris stuck to it), glabrous; when very young brown (7E6) becoming yellow brown (5C7) to brown (6D-E7), ± somewhat finely mottled, or light orange yellow (4A4) ground color with radiating brown lines (6D7) of appressed indistinct "fibrils" finally orange yellow to yellow brown ground color (4-5B-C6 or 5D5) with radiating pattern of brown (6D-E8) lines ± grayish yellow (4B5) mixed in ± orange yellow (4A8) at margin or in dry weather a more uniform brown (6D7 to 6E8), ± olive brown (4F3) spots all stages; odor none or slight fungoid; taste mild, pleasant fungoid. **Pellis reactions** $FeSO_4$ no initial reaction ± → faint gray; KOH gray (green gray in VC 1042) → dark gray (green black in VC 1042); NH_4OH orange to red brown → olive gray to gray brown with red orange to red brown halo (halo absent if caps dry).
Inner veil Absent.
Hymenium adnate to short decurrent when mature. **Pores** 0.5(-1) mm in diam; circular to angular; compound, sometimes weakly; not or weakly boletinoid; when very young light yellow (3-4A4) with sparse glandulae (not easily seen) becoming (3-4A5) then gray yellow (4B6) finally curry yellow (4C7-8) to yellow brown (5C7), ± brown spots in age; no color change after bruising; NH_3 red orange to orange. **Tubes** (4)5-7(8) mm long; concolorous with pores or slightly more yellow when very young.
Stipe (2.2)3.5-6 x (0.6)0.8-1.4(1.7) cm; usually more or less equal, ± narrowed apex, ± clavate base, ± enlarging slightly upward, base usually rounded, apex occasionally reticulate; upper half to two-thirds glandulate; when very young white with tiny abundant orange brown to red brown glandulae, usually with yellow white (3A2 soon 3A4) apex, base may be pinkish (8A2-3), stipe becoming yellow, (3A6-8) apex grading down to whitish with light yellow (3A4) hues below, small dots to small smears glandulae remaining brownish, base may be pinkish, brown stains developing, also staining brown from handling at all stages, stipe also becoming brown from basidiospores. **Basal mycelium** whitish often with pink to pink-orange areas, ± yellow, sometimes with strands.
Context Larval tunnels light colored. No browning on exposure. **Pileus** 8-10 mm thick decreasing to margin; white with yellow white (2A2) to light yellow (1-2A4-5) developing above pores and usually under cuticle, center remaining white; unchanging; $FeSO_4$ gray to gray brown ± → pale blue gray → olive gray; KOH pink to red orange to orange → faint purple to metallic purple gray; NH_4OH peach to deep red orange → whitish blue gray to blue with peach to red orange halo. **Stipe** solid; when very young white with base colored light yellow to light orange (3-5A2-4) then whitish center with light yellow (1-2A2-4) developing at apex and along sides, base colored up to yellow (3B8), unchanging or if base 3B8 then base becoming blue-green, if pigment poorly developed base

unchanging, if moderately developed base changing to a diffuse blue (variable!).

Basidiospores 7.5-9 x 3.5-4.5 µm; ellipsoid in face view, ellipsoid to weakly subfusiform in profile with tiny apiculus; surface smooth; faint yellow brown in KOH, not to weakly dextrinoid in Melzers. Spore deposit brown (6D7 to 6E8).

Cystidia Hymenial: 33-57 x 6.5-9.5 µm (pleuro tend on small side), in clusters (pleuro also single), clavate to clavate bulbous, contents yellow brown to orange brown or dark golden brown, densely incrusted with granular brown material around bases. Caulo: 24-40 x 4-6.5 µm, in scattered clusters, near cylindric to clavate, contents brown or clear, densely incrusted around bases with brown material.

Other microscopic features Basidia 21-28.5 x 5.5-7 µm, clavate, four-spored, simple septum at base. Pileipellis surface of interwoven, simple septate, papillate, hyaline hyphae, 2-5 µm in diam, in clear matrix (partially gelatinized); subsurface of periclinally arranged, densely interwoven, simple septate, densely incrusted with brown material, hyphae. Pileus trama of openly interwoven, simple septate hyphae, 3-8 µm in diam. Basal mycelium: single hyphae 2.5-7 µm in diam, partly incrusted with brown material, partly papillate, partly smooth; some golden-yellow-brown oleiferous hyphae; paarige branching present; anastomoses present; strands occasional, thin. Clamp connections absent, all parts.

Culture VC 1042, VC 1303, VC 1312.

Macroscopic Colony felty with sparse aerial cottony mycelium usually giving a frosted appearance; bumpy and wrinkled ± radial furrows; outline often irregular; submerged hyphae in advance of aerial at margin; mosaic of gray brown (9-10E-F3), red brown (8E4), yellow white (4A2) and white or dark brown (9F5) with wide white margin or light brown (6D4) center with rest a mosaic of light brown (6D4), brown gray (11D2) and white. **Reverse** center black to purple black, rest yellow brown white to brown with purple black blotches; hyphal puffs in agar. At 4 weeks (18)20-30 mm in diam, 1-1.25 mm high, 6.5-8 mm deep (to bottom). Syringaldazine negative; gum guaiac negative; KOH no reaction or orange brown to black; NH_4OH no reaction or purple, halo absent to purple.

Microscopic Slide culture: leading hyphae 1.5-3 µm in diam; unclamped; thin strands. Petri dish culture: oleiferous hyphae absent or yellow brown to orange brown to brown; vesicular cells rare to scattered; clear crystals usually present; strands not seen.

Habitat On ground in forests with its ectomycorrhizal host *Pinus wallichiana*.

Ectomycorrhizal synthesis Ectomycorrhizae synthesized between *Pinus wallichiana* and VC 1303 and VC 1042. (VC 1312 did not form ectomycorrhizae.) **Ectomycorrhizae** 1 x 0.5 mm; monopodial to bifurcate, rarely branched dichotomously up to 3 times, branching angle 45-60°; sessile with mantle to parent root to long stipitate (up to 8 mm). Mantle: cottony-felty ± fuzziness, often thin, when young sparse at apex allowing pale yellow brown smooth surface to show through; white

developing purple brown hues then becoming red brown. Hartig net: up to 4 cortical cells deep separating cells by up to 7 µm. **Extramatrical phase** restricted to ectomycorrhizae and roots, not out onto paper; open sometimes dense cottony aerial around ectomycorrhizae; white becoming red brown; very thin strands (may not be discernable to naked eye), 8.5-14.5(25) µm in diam; single hyphae 2-5 µm in diam, without clamp connections, incrusted; KOH red brown or black; NH_4OH red brown halo. **Material studied** Nepal: Dhaulagiri Zone - VC 1042, Ghasa, Mustang District, 2060 m, 29 May 1985; VC 1303, Khobang, Mustang District, 2590 m, 2 Sep 1985; VC 1312, Khobang, Mustang District, 2610 m, 3 Sep 1985. Basidiocarp microscopic data based on VC 1312. **Field diagnosis** *Suillus* cf. *granulatus* is extremely close to *S. granulatus* ssp. *snellii* of eastern North America. They are both associated with 5-needled pines. Both the basidiocarps and cultures are similar. The Nepali fungus differs in sometimes having a blue green to blue reaction on exposure of the flesh of the stipe base and a less mottled cap. Features which distinguish *S.* cf. *granulatus* from other Nepali *Suillus* species are the combination of its pale flesh (usually not strongly turning color), yellow brown to brown cap, and relatively stout stature and association with *P. wallichiana*. In age basidiocarps of this fungus and the greening-foot *Suillus* are indistinguishable if the flesh has been eaten. It can also be confused with *S.* cf. *placidus*.

Suillus cf. placidus (Bonorden) Singer
Fig. 1.8a, 1.8b.
Basidiocarp single to caespitose.
Pileus (1.8)5-9(14) cm in diam; when very young convex with inturned margin soon broadly convex with downturned margin finally planar convex to undulate planar, margin ± uplifted; ± fine free margin when young; surface viscid to glutinous, glabrous, in very dry weather appressed fibrillose; when very young white, rarely yellow white (2A2), sometimes with cracked pattern or diffuse radiating pattern of gray brown (8C2) lines, ground color remaining white or becoming yellow white (1-2A2-4) with light yellow hues often developing near margin or here and there, brown, purple brown or purple areas or blotches often developing, brown usually associated with glutin, purple may be associated with secondary organism (bacterium?), gray brown pattern of lines if present when young retained, if protected from rain appressed brownish "fibrils" may be present; odor none to fungoid; taste none to mild, taste of cuticle none. **Pellis reactions** $FeSO_4$ pale gray to blue gray ± → green gray → blackish; KOH faint violet or blue gray → some shade of brown to deep brown; NH_4OH light purple or light orange or no reaction at first → light purple with light orange halo or purple brown without halo, orange only develop if yellow pigments present.
Inner veil Absent, when very young some mycelial growth along margin, soon disappearing.
Hymenium adnate with decurrent ridges to slightly decurrent. **Pores** 0.5-1 mm in diam; angular; compound; boletinoid half way out from stem; when young pale yellow (4A3) with abundant pink brown glandulae, sometimes with cloudy white to creamy (4A3) droplets becoming light yellow (3A4) finally gray yellow (4B-C5-7) to brown orange (5C6), with brown stains in age; no color change after bruising; glandulae most prominent young and later at margin; hymenium matures outward so midway toward maturity outer pores and inner pores very different. NH_3 red orange to orange to orange brown. **Tubes** 2-6 mm long, concolorous with pores but sometimes paler (1A3) when young.
Stipe 4-7(10) x (0.4)0.6-1.5(2.2) cm; equal or enlarging somewhat downward, very base sometimes tapered, glandulate upper half to third, intensity decreasing downward, apex sometimes reticulate, white mycelium over base; white developing light yellow (1A4) especially at apex (up to 3A5) and here and there at middle, upper half sometimes becoming entirely yellow white (2A2-3), brown stains developing in age and with handling, glandulae (dots and small smears) initally pale pink brown to orange becoming light brown. **Basal mycelium** white, abundant and usually extending out into soil or litter.
Context soft; unchanging; larvae tunnels unchanging at first, later sometimes purple, if tissue riddled then some shade of brown. **Pileus** 6-10 mm decreasing to margin; white developing light yellow (1A3-4) hues above pores and at margin; $FeSO_4$ green gray to gray to brown gray ± → blue green; KOH (± pinkish flash) sordid blue brown or dull purple to dull purple brown; NH_4OH variable depending on amount of yellow pigment present (orange hues and halo depending on its presence), light orange

→ light blue gray with orange halo or orange flash → white or purple gray with faint orange halo or faint purple with no halo. **Stipe** solid, white developing some yellow hues (1A3) with time, base becoming brown to dark brown.

Basidiospores 6.5-9 x 3-3.5 µm; ellipsoid in face view; ellipsoid to weakly subfusiform in profile view with tiny apiculus; surface smooth; faint yellow brown in KOH, most spores slightly dextrinoid in Melzers. Spore deposit light brown (cinnamon) (5-6D4-6) or sometimes gray orange (5B3).

Cystidia Hymenial: 28.5-76 x 6.5-13 µm, in clusters, near cylindric to clavate to clavate bulbous, contents clear or brown, incrusted with brown granular material around bases (pleuro weakly incrusted). Caulo: similar to cheilo except up to 18 µm broad and very densely incrusted around bases, in large clusters.

Other microscopic features Basidia 20-25(28) x 6-6.5 µm, narrow clavate, four spored. Pileipellis surface of repent, interwoven, simple septate, partially gelatinized, papillate hyphae, 4-7.5 µm in diam; subsurface of densely interwoven, incrusted with brown material, simple septate hyphae. Trama of interwoven, simple septate, hyaline hyphae, (4.5)9.5-19 µm in diam. Basal mycelium: single hyphae 2-5 µm in diam with large globular, brown incrustations, simple septate; paarige branching present; strands occasional, thin (hyphal diam to 10 µm). Clamp connections absent, all parts.

Culture VC 1022, VC 1204, VC 1423, VC 1481.

Macroscopic Uniform. Colony fuzzy with cottony aerial mycelium, aerial growth sparser in middle with radial furrows showing through, mound at center, puffs present; outline circular; raised hyphae in advance of submerged at margin; white, ± orange brown to brown tones, usually with circa 5 mm ring of brown (6D-E8 or 7D3) ± brown areas in center. **Reverse** strong radial furrows out from center, look like agaric lamellae; center light yellow brown to orange brown to brown grading through light yellow to yellow white at margin, ± blue black hues in a middle band. At 4 weeks (30)38-40 mm in diam, 4-6 mm high, 1-2(7) mm deep (rarely with pillars to bottom). Syringaldazine negative; gum guaiac negative; KOH red brown to brown, rarely no reaction; NH_4OH red to red purple, halo red purple on white mycelium, green black to dark gray on brown mycelium.

Microscopic Slide culture: leading hyphae 2-3 µm in diam; unclamped; thin strands, 7 µm in diam. Petri dish culture: oleiferous hyphae yellow brown or golden gray brown; vesicular cells absent; strands not seen.

Habitat On ground in forests with its ectomycorrhizal host, *Pinus wallichiana*.

Ectomycorrhizal synthesis Ectomycorrhizae synthesized between *Pinus wallichiana* and VC 1022, VC 1204 and VC 1481. (VC 1423 did not form ectomycorrhizae.) **Ectomycorrhizae** 1.5-3(5) x (0.5)1-3.5(5) mm; sometimes monopodial, usually bifurcate to coralloid (up to 16 tips), branching angle (30)45-90(120)°, sometimes open, antler-like; sessile with mantle to parent root to long stipitate (up to 10 mm). Mantle: cottony-felty, white, thinner at apex allowing brown to show through. Hartig net:

present. **Extramatrical phase** around ectomycorrhizae, roots, and paper between colonized roots; open to dense cottony aerial around ectomycorrhizae; white becoming red brown; thin radiating strands slow to develop, 8.5-15.5(20) µm in diam; single hyphae (1)1.5-4 µm in diam, without clamp connections, incrusted; KOH brown to red brown to purple brown; NH_4OH red brown with red brown halo.
Material studied Nepal: Bagmati Zone - VC 1204, Nagarkot, Bhaktapur District, 1620 m, 28 Jul 1985; VC 1423, Langtang National Park, Rasuwa District, 2070 m, 6 Oct 1985; VC 1481, Kakani, Nuwakot District, 2000 m, 30 Oct 1985. Narayani Zone - VC 1022, Daman, Makawanpur District, 2380 m, 18 May 1985. Basidiocarp microscopic data based on VC 1423.
Field diagnosis When young, *S.* cf. *placidus* can be recognized by its basic white coloration combined with the typical appearance of an unveiled member of section *Suillus*. The pileus may have purple hues. The basidiocarps often arise from a mat of mycelium and may be caespitose. The flesh is unchanging at all stages.

Suillus sibiricus (Singer) Singer
Fig. 1.9a, 1.9b.
Basidiocarp single, stature and size variable, even at one site.
Pileus (1.8)3.5-8 cm in diam; when very young convex to hemispherical convex to subconic, becoming convex to planar, ± knobby, center may be depressed or umbonate, margin may be enrolled, finally broadly convex to planar to concave, ± knobby, ± umbonate, ± undulate; ± free margin when young, soon disappearing; surface (varies greatly depending on weather), in dry weather moist to slightly viscid with radiating, appressed scales and indistinct fibrils, patches of cottony inner veil hanging from margin and on topside of pileus around margin, in wet weather rain may wash off scales, glutinous, glutin clear to light brown (6C6), glabrous, may have radiating pattern of glutinous or indistinct fibrillose appressed scales; when very young light yellow (4A3-5) ground color with brown (7D-E8) overtones (general and radiating), ± reddish hues, in dry weather with yellow white (3A2) patches near margin from veil which continues up around margin onto pileus, later light yellow (3-4A4-5) ground color with abundant to scattered radial, appressed brown (6D-F8) scales (especially near margin), brown may be diffuse over disk and incorporated into general pileus surface, marginal scales though appressed are distinct from ground surface, in wet weather ground color paler (3A3) with clear to light brown (6D6) glutin in a radiating pattern; odor slight fungoid; taste usually mild to pleasant fungoid, cuticle without taste. **Pellis reactions** $FeSO_4$ gray ± blue hues, rarely no reaction; KOH brown → brown black or gray ± olive → dark gray; NH_4OH variable, red orange to red orange brown → variously blue gray to metallic blue green gray or olive or gray black all with halo of red orange to orange brown to brown, or reaction weaker light orange or olive gray → gray to gray brown with light orange halo.
Inner veil Present, cottony rarely with some glutin in wet weather, white to yellow white (4A2) leaving remnants on cap margin ± a whole or partial superior annulus which may become indistinct as basidiocarp matures.
Hymenium adnate to decurrent. **Pores** (0.5-)1 mm in diam; angular; compound; boletinoid; when very young light yellow (3A4) with tiny white glandulae, becoming mustard yellow to orange yellow (3-4B-C6-7) with pale brown to light red brown glandulae, finally yellow (3B8) to orange yellow (4B7) to yellow brown (5C-D8); bruising brown; NH_3 red orange. **Tubes** 4-7 mm long; concolorous with pores or less grayish.
Stipe 3-6(7) x (0.3)0.6-1.4(1.6) cm; equal or tapering downward or middle narrowest or base clavate; tiny to small glandulae over part or all of stipe, if all then scattered at base, glandulae may give frosted appearance; lower half clothed in appressed mycelum; apex may be ridged or reticulate; when young above annulus light yellow (3-4A4-5) with tiny whitish soon pale orange glandulae, below annulus pale yellow (3-4A3) with tiny pale brown to light orange brown glandulae, base whitish ± yellow ± red purple hues, staining brownish, when mature apex light yellow (3-4A5-6 up to 3A8 ± orange tones), rest whitish to yellow white to light yellow (4A-B4-5) ground color, yellow sometimes more prominent

at base, ± red brown to purple brown to gray red tones or blushes (9E-F8 or 10D-E5-6), glandulae orange brown to red brown to dark brown to black. **Basal mycelium** white ± yellow with strands or pale orange to pink orange, may be abundant.

Context firm, larval tunnels brown or sometimes yellow at first. **Pileus** 7-14 mm thick decreasing, usually slowly, to margin; all stages light yellow to gray yellow (3A-B-3-4) changing to light orange to gray orange (5A-C3-4) on exposure, rarely and only when old changing to blue; $FeSO_4$ no reaction or gray ± brown and olive; KOH sometimes an orange flash or purple or red brown → dark gray to gray brown or olive black often finally black; NH_4OH orange → gray blue to gray purple to gray brown or purple black all with orange halo. **Stipe** solid; concolorous at first with cap color and browning on exposure, later color often deepening, e.g. orange yellow (4B-C5) ± brown to red brown (8E7) hues ± green hues, on exposure especially when old usually changes to dull blue to gray or rarely blue (20E3).

Basidiospores 8.5-11 x 3.5-4 µm; ellipsoid to ovoid in face view; subfusiform (± slightly) in profile view with small apiculus; pale yellow brown in KOH, dextrinoid in Melzers; wall thickness medium (circa 0.3 µm). Spore deposit light brown (cinnamon) (5-6D5-6) to brown (6E7-8).

Cystidia Hymenial: 45.5-72 x 5-10.5 µm, in clusters, near cylindric to narrow clavate, contents pale yellow to brown, bases densely incrusted with brown angular material. Caulo: 36-72 x 4.5-9 µm, in clusters, cylindric to narrow clavate, contents light brown, bases densely incrusted with brown material.

Other microscopic features Basidia 24-28 x 6.5-7.5 µm, narrow clavate, four-spored. Pileipellis surface thin, not gelatinized, hyphae slightly papillate with obscure cross striations, hyaline, slightly papillate or with a few incrustations, simple septate, 6.5-9.5 µm in diam; subsurface trama-like but denser. Trama of openly interwoven, hyaline hyphae with tiny light brown incrustations, 4-10.5 µm in diam. Basal mycelium: single hyphae 2.5-4 µm in diam, hyaline, simple septate, with needle-shaped golden brown incrustations, sometimes papillate; golden brown oleiferous hyphae present; anastomoses present; strands occassional, 6 hyphae in diam, central hypha 8 µm in diam. Clamp connections absent, all parts.

Culture VC 1040, VC 1218, VC 1310, VC 1328, VC 1391a, VC 1421.

Macroscopic Variable. Colony felty with sparse cottony aerial mycelium to fuzzy open aerial, rarely dense, if felty ± high aerial areas, margin ± appressed ± frosted; radial furrows usually present; outline circular or irregular; raised and submerged hyphae usually equal at margin; colors sometimes weakly zonate or mottled, light yellow and oranges usually predominate, center pale orange to gray orange (5-6A-B2) ± white, rarely red brown (8E4) with white, middle pale orange to orange gray (5-6A-B-C2-3) to whitish rarely with brown (6E4) band or rarely gray red (7B-C3) with white, margin pale orange to gray orange (5-6A1.5 to 5B-C3-4) to yellow white (3A2) to white. **Reverse** ± indistinct radial furrows; hyphal puffs; center brown, middle orange to orange brown to

red brown ± in mosaic pattern, margin yellow to yellow white or orange gray. At 4 weeks (21.5)25-37(41) mm in diam, 0.5-3(4) mm high, 4.5-7.5 mm deep (to bottom). Syringaldazine positive; gum guaiac positive; KOH no reaction or light gray to brown, rarely reddish; NH_4OH no reaction, rarely slight purple, halo no reaction or light pink to purple ± gray tones, rarely red purple.

Microscopic Slide culture: leading hyphae 1.5-3 µm in diam; unclamped; thin strands sometimes present. Petri dish culture: oleiferous hyphae usually absent or yellow to golden yellow brown; vesicular cells usually a few, sometimes absent; strands absent or present, 7-12 µm in diam.

Habitat On ground in forests and in ornamental settings in association with its ectomycorrhizal host *Pinus wallichiana*; very common.

Ectomycorrhizal synthesis Ectomycorrhizae synthesized between *Pinus wallichiana* and VC 1040, VC 1218, VC 1310, VC 1328, VC 1391a, and VC 1421. **Ectomycorrhizae** 2-5 x 0.5-2.5 mm; usually monopodial, rarely bifurcate to coralloid (up to 11 tips), branching angle 45-80(120)°, sometimes open, antler-like; sessile with mantle to parent root to long stipitate (up to 12 mm). Mantle: felty, thick, may be thin at apex with light brown showing through; white becoming dark brown to purple brown; Hartig net: 3 cell layers deep separating cells up to 5 µm. **Extramatrical phase** around ectomycorrhizae and near roots; usually sparse, sometimes moderate, cottony aerial around ectomycorrhizae, open network of strands, spider-web-like; white becoming light red brown to purple brown; strands scattered, (5)9.5-21(28) µm in diam; single hyphae (1.5)2-5(5) µm in diam, without clamp connections (1 seen), incrusted; KOH no reaction to red brown to brown; NH_4OH brown, ± red or red purple hues, with no halo or red purple to purple brown to red brown halo.

Material studied Nepal: Bagmati Zone - VC 1218, Langtang National Park, Rasuwa District, 2130 m, 3 Aug 1985; VC 1391a, Godawari, Lalitpur District, 1540 m, 23 Sep 1985; VC 1421, Langtang National Park, 2030 m, 6 Oct 1985. Dhaulagiri Zone - VC 1040, Ghasa, Mustang District, 2060 m, 29 May 1985; VC 1310, Khobang, Mustang District, 2610 m, 3 Sep 1985; VC 1328, Larjung, Mustang District, 2650 m, 4 Sep 1985. Basidiocarp microscopic data based on VC 1421.

Field diagnosis Though *S. sibiricus* is variable in appearance, it can be distinguished from the other *Suillus* species associated with *P. wallichiana* by its veil usually leaving an annulus, the scales on the pileus, basic yellow coloration and when old by the often ruddy colors on the stipe and dull blueing reaction of the stipe flesh.

Suillus "greening-foot"
Fig. 1.10a, 1.10b
Basidiocarp single.
Pileus (1.5)3-8(10) cm in diam; when very young convex, becoming broadly convex, finally planar sometimes undulate or concave; surface viscid to glutinous (moist to dry and papery in dry weather), glutin clear to light brown; color somewhat variable, when very young brown (7D-E8), becoming orange to orange brown (6B-D6), then gray orange (4-5B-C5), ground color with pattern of indistinct fine brown lines usually radiating, mature ground color may also be yellower even 3A4 with yellow brown hues (5D5) or browner (6C6), may darken with time, disk may be mottled (5B5 and 6E8), fine lines possibly due to thicker glutin; odor none to fungoid; taste mild, cuticle no taste to slight pleasant acid. **Pellis reactions** $FeSO_4$ slowly faint gray; KOH green gray to olive gray to olive black → green black to black; NH_4OH brownish → green to olive ± blue gray tones with brown to red brown halo.
Inner veil Absent, when very young sometimes with white to pale yellow cottony growth on margin soon disappearing.
Hymenium adnate to usually decurrent. **Pores** 0.5 - 1 mm in diam; angular; compound; boletinoid; when very young light yellow (3-4A5) with dense pallid glandulae ± clear to pale cloudy yellow droplets, becoming yellow (3-4A7) to gray yellow (4B5), finally orange yellow (4B-C7-8) to orange brown (5C7), glandulae now only apparent at margin, bruising slowly brown; NH_3 orange red to red brown. **Tubes** (3)4-6(8) mm long; concolorous with pores or more yellow than pores when hymenium is mature.
Stipe 2.5-5(6) x 0.7-1.5 cm; equal or narrowed at middle or apex; upper half glandulate, often reticulate from extension of hymenium especially if stipe curved from growing on slope, glandulae may extend to base, lower half or sometimes just base covered with appressed mycelium; upper half when very young light yellow to yellow (2-3A4-6) with dense pallid glandulae, base whitish to yellow white (2A3), staining brown on handling, later upper half light yellow (3A5) to bright or deep yellow (3A-B6) with pale-becoming-orange-brown-to-brown tiny glandulae, lower half or just base covered with whitish to yellowish appressed mycelium; ± orange tones. **Basal mycelium** white and yellow with strands.
Context Firm to somewhat soft; larval tunnels brown. **Pileus** 8-15 mm thick tapering gradually to margin; white to pale yellow (2-3A3-2-3) all stages, browning slightly on exposure; $FeSO_4$ gray to green gray; KOH gray to metallic greenish → green gray or gray brown, an initial orange to orange red flash if tissue has not browned yet; NH_4OH orange to orange red → green to olive gray to blue-green-gray with orange to orange red halo, center of drop may become white. **Stipe** solid; upper: light yellow (3A4-6) or marbled gray yellow (2B5), browning some on exposure; base: bright deep yellow (3A-B7-8) usually turning deep green to blue-green on exposure then finally to orange to brown, base sometimes with red brown to dark brown areas.
Basidiospores 7.5-11 x 4-5 µm; ellipsoid to weakly subfusiform in profile with tiny apiculus; surface smooth; light yellow brown in KOH; not or

slightly dextrinoid; wall thickness medium (circa 0.3 µm). Spore deposit light brown to brown (5D5-6 or 6E6 or 6F8).

Cystidia Hymenial: 36-69.5x 5-10 µm (size variable), in clusters, elongate clavate to narrow clavate, contents clear or oily gray brown to golden brown in KOH, granular and globular matrix around bases. Caulo: 41-77 x 5-7.5 µm (size very variable, many small ones), in large clusters, clavate to near cylndric, contents clear or oily gray brown to golden brown in KOH, granular and globular matrix around bases, basically similar to hymenial.

Other microscopic features Basidia 21-24 x 6-7.5 µm, clavate, four-spored. Pileipellis of repent, interwoven, gelatinized hyphae, 3-4 µm in diam; surface layer clear, subsurface layer dense, dark brown, brown due to contents and incrustations, brown decreasing inward until reaching the clear, interwoven tramal hyphae, 6.5-15 µm in diam, incrustations though less dense continue well into trama. Basal mycelium incrusted, papillate; anastomoses common; paarige branching present; strands with large diam core hyphae present. Clamp connections absent, all parts.

Culture VC 1041, VC 1219, VC 1425.

Macroscopic Uniform. Colony high cottony aerial (dense to eye but compresses easily) with surface becoming subfelty; outline circular; raised hyphae in advance of submerged at margin; center yellow brown to brown ((5)6-7E-F6-8), middle pale yellow (3A3), margin white, during active growth bright yellow zone usually present; **Reverse** ± indistinct radial furrows; center orange brown to red brown to brown, middle deep yellow orange and orange with mottled brown band, margin yellow white. At 4 weeks (40.5)62-82 mm in diam, 5-8 mm high, 4-5 mm deep (not to bottom). Syringaldazine negative; gum guaiac negative; KOH no reaction or slowly brown, dissolves brown pigment; NH_4OH no reaction dissolves brown pigment, halo strong green on yellow mycelium, green black to gray black on brown mycelium, none to red purple on white mycelium.

Microscopic Slide culture: leading hyphae (2)3-5.5 µm in diam; clamp connections common; strands present. Petri dish culture: oleiferous hyphae orange brown; vesicular cells absent; strands present, 12± µm in diam.

Habitat On ground, gregarious and locally abundant, in forest with *Pinus wallichiana*.

Ectomycorrhizal synthesis Ectomycorrhizae synthesized between *Pinus wallichiana* and VC 1041, VC 1219, and VC 1425. **Ectomycorrhizae** 1-3 x 0.5-2 mm; usually monopodial to bifurcate, sometimes double bifurcate; branching angle (30)60-120°, sometimes open, antler-like; sessile with mantle to parent root to long stipitate (up to 10 mm). Mantle: cottony becoming felty, may be thin in spots with white or orange brown showing through; white with apex becoming orange brown; Hartig net: 3 cell layers deep separating cells up to 5 µm. **Extramatrical phase** around ectomycorrhizae and roots and locally out onto paper; moderately dense cottony aerial around ectomycorrhizae, sparse with abundant strands away from ectomycorrhizae, cobwebby; white ± yellow becoming yellow brown to orange brown to brown; strands abundant, (6.5)9-28.5(40) µm in diam;

single hyphae unclamped (1)2-3.5 and clamped 3-6 µm in diam, incrusted; KOH usually no reaction or strands red brown; NH_4OH no reaction or red purple to red brown halo.

Material studied Nepal: Bagmati Zone - VC 1219, Langtang National Park, Rasuwa District, 2320 m, 3 Aug 1985; VC 1425, Langtang National Park, Rasuwa District, 2410 m, 6 Oct 1985. Dhaulagiri Zone - VC 1041, Ghasa, Mustang District, 2060 m, 29 May 1985; VC 1301, Khobang, Mustang District, 2590 m, 2 Sep 1985; VC 1326, Larjung, Mustang District, 2620 m, 4 Sep 1985. Basidiocarp microscopic data based mostly on VC 1425.

Field diagnosis The greening-foot *Suillus* is a nonveiled member of section *Suillus* and can be distinguished by the bright yellow flesh of the stipe base which turns blue green on exposure. The stipe is yellow and its apex usually reticulate. In age it can easily be confused with *S.* cf. *granulatus*. The greening-foot *Suillus* is ectomycorrhizal with *P. wallichiana*.

Suillus "queen's"
Fig. 1.11a, 1.11b
Basidiocarp single, rarely caespitose.
Pileus 2.5-4(6) cm in diam; when young convex with inturned margin, becoming broadly convex; up to 1 mm free margin when very young; surface viscid to glutinous (in dry weather sticky without free slime), glutin clear, glabrous; fairly uniform in color, when very young light yellow (1-2A5), becoming (2-3A4), then yellow (1-2A5-6), brown stains in age, disk browning with handling; odor absent to slight sharp fungoid; taste none to mild. **Pellis reactions** $FeSO_4$ gray to gray brown ± deep green gray; KOH purple or light red brown to brown → purple or dark brown black → purple brown or black; NH_4OH light red orange brown or pink brown with pink brown → purple brown with light orange to orange brown halo or black with pink brown halo or mixture or orange and purple ± → clear spot with orange halo.
Inner veil Absent. When very young inturned cap margin has white cottony growth which soon disappears.
Hymenium adnate to very slightly decurrent. **Pores** 0.3-1 mm in diam; round to slightly angular; not compound; not boletinoid except slightly near stipe; when very young whitish to yellow white (1A2) soon light yellow (2-3A3-4), then gray yellow (4B5), finally curry yellow (4C8) to orange brown (5C6); with young covered with milky droplets and abundant pale gray orange (6B3) glandulae; NH_3 rose pink to red orange to brown red. **Tubes** 2-3 mm long, slightly paler than pores when young due to absence of colored glandulae, concolorous when mature, pale yellow (2A4) becoming gray yellow (2B5) then curry yellow (4C8).
Stipe 3.5-5.5 x 0.4-0.8 cm; more or less equal, lower half sometimes tapering (but base clavate when very young); often curved; when young tiny glandulae on upper half and very dense at apex, when mature abundant, tiny to small dot to small smears glandulae over most of length, abundance decreasing downward; white cottony mycelium over very base; when young white to yellow white (1A2), sometimes with yellow at base, yellowing at base if handled, glandulae initially pale pink orange becoming pink brown to light orange brown then dark orange brown (6D8), mature stipe white with light yellow (3A4) hues, base light yellow (3A4), very apex deep yellow (3A8), ± brown stains in age, glandulae dark brown (6-7F8). **Basal mycelium** white with strands, sometimes with yellow hues.
Context On exposure yellow may intensify a little, but no browning. **Pileus** white to yellow white (1A2-3), then white above and yellow (1A6) above pores; larval tunnels yellow becoming brownish. $FeSO_4$ gray to gray brown ± → deep green gray; KOH pink orange flash → purple gray to brown gray → brown black; NH_4OH red orange or pink with pink halo → clear with pink orange halo or purple to purple gray with orange red halo → gray brown to sordid dark violet gray with orange to orange red halo. **Stipe** solid; when young yellow white (1A3) with light yellow (2A5) base, (larval tunnels deeper yellow), becoming light yellow (1A3-5) sometimes light greenish (30A4) with yellow (3A6) base, finally

light yellow (1A4) with yellow (2A-B7-8) base, base sometimes with brown or orange brown areas, old injuried areas may have brown or purple hues.

Basidiospores 6.5-9 x 3-4.5 µm; ellipsoid in face and profile views, sometimes weakly subfusiform in profile; surface smooth; faint to light yellow brown in KOH, slightly dextrinoid in Melzers. Spore deposit brown (6-7E8).

Cystidia Hymenial: 24-60 x (5)6-9.5(11) µm; cheilo in large clusters, pleuro usually single not common; narrow clavate to bulbous clavate; contents golden brown to brown, sometimes grainy, rarely clear; densely incrusted around bases with amorphous brown material, such incrusting material on gill sides not always associated with cystidia.
Caulo: 43-67 x 9.5-16 µm; in large clusters; narrow clavate to bulbous clavate; contents clear to golden brown, grainy or smooth; densely incrusted around bases with amorphous brown material.

Other microscopic features Basidia 21-24 x 5-6.5 µm; narrow clavate; four-spored; simple septum at base. Pileipellis of interwoven, densely papillate, simple septate, light brown hyphae in gelatinous matrix, 2.5-9 µm in diam, contents sometimes becoming dark brown (poor drying?). Pileus trama of interwoven, simple septate, hyaline hyphae with light brown to brown incrustations, sometimes papillate, 4-7.5 µm in diam. Basal mycelium: single hyphae 2-4.5 µm in diam, simple septate with dense brown angular incrustations; anastomoses present; golden brown to brown oleiferous hyphae present; strands common with many over 10 hyphae across, central hyphae 6 µm in diam. Clamp connections absent, all parts.

Culture VC 1081a.

Macroscopic Colony cottony aerial, mixed dense and see-through, radial furrows, margin subparallel cottony with villose margin; outline basically circular; raised and submerged hyphae equal at margin; pale orange to gray orange (4A-B2-3) with white margin, thin areas white over gray brown (9D3); **Reverse** radial furrows; dense hyphal puffs; center brown black starburst pattern, middle purple, margin orange white ± purple hues. At 4 weeks 42.5-48.5 mm in diam, 4-5 mm high, 5-6 mm deep (to bottom). Syringaldazine negative; gum guaiac negative; KOH slight brown; NH_4OH no reaction, halo absent or weak pink brown, (pigments in agar react).

Microscopic Slide culture: leading hyphae 2-4.5 µm in diam; clamp connections common; strands present. Petri dish culture: clamp connections rare; oleiferous hyphae golden yellow; vesicular cells absent; strands not seen.

Habitat Scattered to gregarious on ground in *Pinus roxburghii* forest; fruiting in abundance.

Ectomycorrhizal synthesis Ectomycorrhizae synthesized between *Pinus roxburghii* and VC 1081a. **Ectomycorrhizae** 1 x 1-1.5 mm; monopodial to bifurcate to coralloid (up to 14 tips); tight branching angle 30-45°; sessile with mantle to parent root to short stipitate (up to 0.5 mm). Mantle: cottony below appressed near apex, white ± gray yellow hues. Hartig net: down to endodermis separating cells up to 7.5 µm.

Extramatrical phase very local only around ectomycorrhizae and adjacent

roots; open to dense cottony aerial around ectomycorrhizae, weft-like; white. Strands absent to rare; 4-19 µm in diam; single hyphae 2-3.5 µm in diam, occasional clamp connections, incrusted; KOH no reaction; NH_4OH slight red purple halo.

Material studied Nepal: Bagmati Zone - VC 1081a, Nagarjun Ban, Kathmandu District, 1400 m, 15 Jun 1985; VC 1138, Nagarjun Ban, Kathmandu District, 6 Jul 1985, 1380 m. Basidiocarp microscopic data based on VC 1081 and VC 1138.

Field diagnosis The queen's *Suillus* is hard to distinguish from other non-veiled members of section *Suillus* with unchanging flesh; it has abundant small glandulae, yellow flesh at the stipe base, basic light yellow coloration, milky droplets on the hymenium when young and is ectomycorrhizal with *P. roxburghii*. Its overall macroscopic appearance is very similar to *S. placidus* of North America and of Europe.

Suillus "waxy"
Fig. 1.12.
Basidiocarp single, sometimes caespitose.
Pileus (1.8)2-5(6) cm in diam; when very young convex with inturned margin, becoming convex, finally broadly convex to planar undulate, basically bun-shaped, broad low umbo sometimes present; margin free (up to 1 mm), obvious young and usually discernable when mature; surface viscid to glutinous, glutin brown (6-7D-E7-8) to red brown (8D5), glabrous; when very young pale yellow (3-4A3) ground color covered with brown (6D8) to red brown (8D5) glutin, becoming pale yellow (2-3A2-3) ground color showing through thin areas of brown glutin (6-7D-E7-8) covering, cap appearing color of glutin or somewhat paler (brown orange (6-7C7-8) or gray orange (6B6)), margin often color of ground color, or cap much lighter overall or in blotches (ground color, may be finely mottled with yellow hues); odor fungoid (often slight); taste mild fungoid, cuticle no taste. **Pellis reactions** $FeSO_4$ green gray → green-black; KOH dark gray to black or violet → violet brown → dark brown; NH_4OH no initial reaction to light orange-red-brown → darkening under drop with no halo or faint orange halo or dull orange to violet under drop with similarly colored halo.
Inner veil Absent; when very young some white cottony mycelium along cap margin, soon disappering.
Hymenium adnate ± slightly depressed around stipe, sometimes with short decurrent ridges. **Pores** 0.5(-1) mm in diam, tiny pores near margin; angular; not or slightly compound; slightly boletinoid near stipe only; when very young yellow white (2-3A2) covered with concolorous dense glandulae and cloudy white to yellow droplets, becoming pale yellow (3-4A3-4) with concolorous glandulae and droplets, then gray yellow (4B4) with margin less grayish, finally gray yellow to brown yellow (4-5C5-6) with tiny dark glandulae (handlens), unchanging when bruised; NH_3 dull orange to red orange. **Tubes** (2)3-6 mm long; concolorous with pores.
Stipe (2.5)4-7(9) x 0.7-1.3(1.5) cm; equal or enlarging slightly upward, base rounded or narrowed or rarely clavate; glandulate upper half to two-thirds making surface waxy to touch; rest cottony mycelial fuzzy; when young white to yellow white (2A2) with dense concolorous glandulae ± clear yellow droplets, when mature white to pale yellow (up to 1A4 or 2A3, apex to 2A4) with dense (rarely sparse) concolorous glandulae, glandulae not darkening, developing brown stains in age and with handling. **Basal mycelium** white ± yellow, cottony ± strands.
Context firm, unchanging or sometimes yellow of stipe deepening a little. **Pileus** (7)12-15(20) mm thick decreasing slowly to margin; when very young white to yellow white (1-2A2), becoming yellow white (2A2-3) to light yellow (1A4-6) with disk paler; area above stipe more yellow; $FeSO_4$ green gray usually deep ± → green-black; KOH orange to red orange flash (rarely absent) → pale violet to metallic violet gray with violet fading to sordid blue gray green. NH_4OH orange to red orange → white to very pale violet gray with deep orange halo → white to blue (-violet) gray with orange halo. **Stipe** solid, when young marbled pale yellow

(1-2A3-4), becoming pale yellow (1A3-5) sometimes with some brown at base, finally light yellow (1A4-5), ± brown stains, usually with brown (6D-E8) at base, or green-yellow (1A6) marbled with white and brown.

Basidiospores 6.5-7.5 x 3-3.5 µm; ellipsoid in face view; ellipsoid to weakly subfusiform in profile with tiny apiculus; surface smooth; faint yellow brown in KOH, weakly dextrinoid in Melzers. Spore deposit yellow brown to light brown (5-6C-D5-6) (cinnamon).

Cystidia Hymenial: 36-67 x 5.5-9.5 µm, in clusters (pleuro also single), near cylindric to clavate, contents clear or golden brown, dense brown granular material around bases (pleuro sometimes unincrusted). Caulo 29-72 x 5-7.5(8) µm, in clusters (often large), cylindric to narrow clavate, longer and narrower than hymenial, dense brown angular material around bases.

Other microscopic features Basidia (15)23-24 x (6)6.5-7.5 µm, clavate, four-spored. Pileipellis of interwoven, periclinally arranged, densely papillate to densely finely incrusted hyphae, 2-5 µm in diam, in gelatinous matrix. Trama of interwoven, hyaline hyphae, 2-7.5 µm in diam, often papillate, sometimes with light fine brown incrustations. Basal mycelium: single hyphae 2-6 µm in diam, incrusted (scattered light brown globular, angular and needle-like), simple septate; anastomoses present; golden brown oleiferous hyphae present; strands occasional, thin, central hypha 5 µm in diam. Clamp connections absent, all parts.

Culture VC 1185, VC 1192, VC 1207.

Macroscopic Variable, (among different cultures and among different colonies of the same culture). Colony high cottony aerial or coarsely felty, radial furrowed, bumpy and wrinkled, ± tufts; outline usually irregular; raised in advance or equal to submerged hyphae at margin; center and middle pale orange (4A2) and light brown (6D6) mottled zonate with white margin or center and middle mosaic of white, pale gray orange (5B3), and brown (6E8) or of light brown (6D5-7) ± gray yellow (4C3) with margin with appressed (gray orange (5B6)) and cottony (white) areas. **Reverse** radial furrows; ± hyphal puffs; center black brown with brown white or yellow or center brown with orange hues, middle often yellow, margin brown white to yellow white. At 4 weeks (13.5)31-41.5 mm in diam, 0.5-7 mm high (variable), 5-7 mm deep (to bottom). Syringaldazine negative; gum guaiac negative, rarely positive; KOH slight darkening to black brown to black; NH_4OH no reaction, halo absent or weak purple, rarely red purple, (pigments in agar react).

Microscopic Slide culture: leading hyphae 2-4 µm in diam; unclamped; strands present. Petri dish culture: oleiferous hyphae golden yellow to golden yellow brown or brown; vesicular cells usually present; strands abundant 17-36 µm in diam.

Habitat On ground in nurseries and in plantations in association with its ectomycorrhizal host *Pinus patula*; unknown from natural forests in Nepal.

Ectomycorrhizal synthesis Ectomycorrhizae synthesized between *Pinus patula* and VC 1192. (VC 1207 did not form ectomycorrhizae.)

Ectomycorrhizae 0.5-1 x 0.75-1 mm; monopodial and bifurcate rare, double bifurcate common; tight branching angle; sessile with mantle to parent root, really stipitate. Mantle: felty to cottony, white. Hartig net:

up to 2 cell layers deep. **Extramatrical phase** around ectomycorrhizae and adjacent roots and out onto adjacent paper; dense cottony aerial around ectomycorrhizae, sparse thin strands; white becoming light orange brown. **Material studied** Nepal: Bagmati Zone - VC 1185, Chautara, Sindhupalchok District, 1490 m, 18 Jul 1985; VC 1192, Chautara, Sindhupalchok District, 1600 m, 19 Jul 1985; VC 1207, Nargarkot, Bhaktapur District, 1700 m, 28 Jul 1985. Basidiocarp microscopic data based on VC 1185.
Field diagnosis The most striking character of the waxy *Suillus*, besides being an unveiled member of section *Suillus*, is the abundance of pallid glandulae on the upper stipe making it waxy to the touch. It is a relatively squat mushroom with a bun-shaped pileus. Also, helpful is its association with the introduced *Pinus patula*.

Suillus laricinus (Berk.) Kuntze
= *Fuscoboletinus aeruginascens* (Secr.) Pomerleau & Smith
Fig. 1.13.

Basidiocarp single. Note: Basidiocarps stain paper blue.

Pileus 3-8(9) cm in diam; when young convex ± slight umbo, becoming broadly convex or planar with downturned margin ± broad rounded umbo; surface viscid to glutinous, glutin clear or with brownish cast, glabrous; small sunken spots sometimes developing with time; color darkest at disk grading to lightest color at margin, when young brown to dark brown (6-7E-F8) over pale orange ground color (5A3) which in buttons shows through only at margin, brown sometimes appears as a speckling over the ground color, on expansion margin appears light orange (5A4) with very margin sometimes yellow white (3A2), when mature disk usually brown (6D-E5-6 or 7D-E7) grading to yellow white (4A2) or pale orange (5A3) margin, ground color pale orange (5A3) to gray orange (6B4) over center and paler at margin (yellow white (4A2)), sometimes spotted with brown, when old disk gray orange (5B4) grading to orange white margin (5A2); ± indistinct pattern of radiating dark thin lines; odor sharp fungoid; taste mild to strong fungoid. **Pellis reactions** $FeSO_4$ pale gray ± → light olive gray; KOH olive or bright green → brown; NH_4OH slowly greenish → olive with no halo.

Inner veil Present; up to 3 mm thick "cotton" covered with gluten outside; "cotton" white to yellow white (3-4A2), gluten very pale orange to brown; leaving remnants on pileus margin and annulus. Annulus 0.4-1 cm wide, usually complete (rarely absent, then veil remnants prominent on cap margin), superior, persistant, yellow white (3A2) with very pale brown gluten, becoming brown from basidiospores.

Hymenium decurrent especially when old. **Pores** 1 mm in diam, smaller toward margin, angular, compound, boletinoid, when very young yellow white (1A2) yellow soon deepening and graying (3-4A3 or 3A2 to 3B2) with margin remaining 1A2, when mature brown orange (5C4) to gray brown (5D3-4), when old darker and browner; changing color when bruised, reaction occurring at all stages but weaker when young, color after bruising variable blue gray, gray, blue-gray-brown, gray brown or brown; NH_3 no reaction or pale brown. **Tubes** 5-6 mm long, concolorous with pores except whiter when mature.

Stipe 6-9.5 x 0.7-1.2 cm, base up to 1.4 cm wide; enlarging gradually downward; reticulate above annulus; below annulus surface of matted mycelium or overlaid with thin layer of mycelium ± somewhat fibrillose when old, obscurely reticulate; apex above annulus light yellow (3A3-5) graying and browning with time, also becoming brown from basidiospores; below annulus light yellow (2-3A2-4) ground color with yellow strongest toward base, with sparse to dense brown (6E7-8) to red brown (8F8 or 9F7) tiny dots and lines, dots usually denser toward top; no glandulae. **Basal mycelium** cottony ± strands, white. After drying, whitish with pink brown hues to very pale brown with pink tones.

Context Colorful reactions occur after exposure. **Pileus** when young usually white to yellow white (3A2) or pale orange (5A3) and then orange intensifying on exposure; when mature white to yellow white (1-2A2-3)

usually becoming pale lavender over the tubes on exposure, sometimes yellow deepening and then fading on exposure, sometimes browning on exposure. $FeSO_4$ olive gray brown to olive brown, sometimes first gray brown; KOH light brown or pink orange; NH_4OH light brown with no halo or orange → gray brown with orange halo. **Stipe** solid; when young light yellow (3A3-5) sometimes developing brown hues on exposure, rarely light orange (5A5) when very young; when mature yellow (2A5-8) lower half usually developing green to blue-green hues on exposure, sometimes also developing brown hues, apex may develop lavender hues; all flesh sometimes becoming brown when old, stipe base flesh sometimes mustard yellow when old. Larval tunnels orange brown.

Basidiospores 9-12 x 4-5 µm; ellipsoid in face view, ellipsoid to weakly subfusiform in profile with tiny apiculus; surface smooth; light yellow brown in KOH; dextrinoid in Melzers; wall thickness medium (circa 0.3 µm). Spore deposit brown (6D4 to 6-7E6), usually darker and to red side of cinnamon.

Cystidia Hymenial: 28.5-71 x 5.5-7.5 µm (size variable); single or in small clusters; narrow clavate to cylindric; contents usually clear, sometimes with golden brown coarsely grainy contents; not incrusted. Caulo: similar in size (e.g. 62 x 7 µm) and shape; single; contents clear; not incrusted; restricted to reticulate apex.

Other microscopic features Basidia 25.5-29 x 7.5-9.5 µm; clavate; four-spored; simple septum at base. Caulobasidia common on reticulated apex which is a continuation of the hymenium. Pileipellis of hyaline, interwoven, repent, simple septate, gelatinized hyphae, 4.5-9.5 µm in diam. Below this clear layer is a brown layer of equal thickness and composed of dense, narrow, periclinally arranged hyphae with tiny brown incrustations. Pileus trama of hyaline, broad, interwoven, simple septate hyphae, up to 14.5 µm in diam. Basal mycelium: single hyphae 2.5-5 µm in diam, simple septate with small angular to globose to needle-shaped brown incrustations; gray brown oleiferous hyphae present. Clamp connections absent, all parts.

Culture VC 1231, VC 1438, VC 1450.

Macroscopic Variable. Colony high cottony aerial or wrinkled, bumpy and branny, margin ± silky; outline basically circular; raised in advance of or equal to submerged hyphae at margin; brilliant white ± light brown (5-6D-E5) hues or mottled white, gray yellow (4B-C3-4) and brown orange (5C-D3-4) (± overlaid with white) with white to brown white margin. **Reverse** radial furrows; usually small hyphal puffs; center orange brown to brown to purple brown, sometimes dull yellow, middle brown mottled with orange and yellow or purple or orange white margin white to yellow white ± brown hues. At 4 weeks (13.5)19.5-47 mm in diam, 2-5(6) mm high, (1)2-4.5 mm deep. Syringaldazine positive, sometimes negative; gum guaiac positive; KOH brown to red brown or pink purple; NH_4OH no reaction or pinkish, halo absent or red purple or red brown.

Microscopic Slide culture: leading hyphae 1.5-2.5 µm in diam; unclamped; strands usually present. Petri dish culture: oleiferous hyphae golden yellow to bright yellow brown; vesicular cells usually present; strands present 10-24 µm in diam.

Habitat On ground in boreal conifer forest. Fruiting in abundance. Within root zone of and probably ectomycorrhizal with *Larix himalaica*.
Ectomycorrhizal synthesis Not attempted because *Larix himalaica* seeds did not germinate.
Material studied Nepal: Bagmati Zone - VC 1231, Langtang National Park, Rasuwa District, 3070 m, 6 Aug 1985; VC 1438, Langtang National Park, Rasuwa District, 3020 m, 9 Oct 1985, VC 1450, Langtang National Park, Rasuwa District, 3140 m, 11 Oct 1985. Basidiocarp microscopic data based primarily on VC 1231.
Field diagnosis *Suillus laricinus* of Nepal is distinguished by the bipartate inner veil, cottony inside and glutinous outside; colorful reactions of the context on exposure; hymenium color, often with gray tones and bruising shades of gray, blue, and/or brown; blue staining of paper; and association with *Larix*. *Suillus laricinus* has been reported circumboreally from the Northern Hemisphere in association with *Larix*.

Suillus "himalayan"

Fig. 1.14.

Basidiocarp single, occasionally caespitose. Note: Basidiocarps stain paper yellow.

Pileus 2.5-8.5 cm in diam; when young convex with inturned margin, becoming broadly convex, finally broadly convex to planar, may be undulate, margin may be turned down; conical umbo usually present at all stages; surface glabrous or with brownish scales along margin, viscid to glutinous, glutin clear to pale brown; when very young disk dark brown (6-7F8) paling to margin where the light yellow (2-3A4) ground color predominates, then brown (6D8) with darker (6E8) disk and pale yellow (3-4A3 margin) or brown orange (6C5) with brown (6D6) disk and pale orange (5A3) margin, when mature fairly uniform light to gray orange (5A-B4) or brown (7E8) grading to light orange margin (6A4); odor and taste fungoid, mild to moderate. **Pellis reactions** $FeSO_4$ slowly pale gray; KOH green becoming olive black; NH_4OH slowly yellow olive with narrow pale gray halo.

Inner veil Present; pale yellow (3A3) cottony mycelium inside with clear, becoming brown (6E8) glutin on outside; leaving remnants on cap margin and superior annulus 5-9 mm wide, annulus appressed to stipe and glutinous on outside, may be indistinct when basidiocarp mature.

Hymenium decurrent. **Pores** 1 ± 0.5 mm in diam, smaller toward margin; angular; compound; boletinoid; when very young light yellow (3A4), becoming (2A6 to 3-4A5), finally yellow (3B6-8 to 4B7) with orange brown stains in age, when young bruising brown (6D7); NH_3 brown to red brown. **Tubes** 4-6 mm long; concolorous with pores.

Stipe 3-7 x 0.5-0.9 cm; equal or enlarging downward, base up to 1 cm wide; above annulus: reticulate, yellow (2A8 to 3A6) with orange brown stains in age; below annulus: surface of open cottony mycelium, light yellow (2A4 to 3A5-6) ground color with fine brown (6D-E8) dots forming diffuse reticulate pattern especially upper part or longitudinal fibrous pattern; when mature browish with yellow ground color. **Basal mycelium** abundant mycelium and strands, white to pale yellow.

Context **Pileus** 7-12 mm thick decreasing to margin; light yellow (1-2A3-5) with white under the cuticle becoming yellow (1A6-7) or remaining light yellow, dulling and browning a little on exposure, pale green tones developing if watersoaked. $FeSO_4$ olive gray; KOH greenish → blue green; NH_4OH pale orange with narrow brown halo → blue green. **Stipe** solid, light yellow (2A4) → light yellow to yellow (1A6 to 2A-B4-5) → gray yellow (3B4-5 usually with 3C5 at base), dulling and browning a little on exposure.

Basidiospores 9.5-12.5 x 4-5 µm; ellipsoid in face view, near ellipsoid to subfusiform in profile with small apiculus; surface smooth; light yellow brown in KOH, dextrinoid in Melzers. Spore deposit light brown to brown (5D5 to 6E8).

Cystidia Hymenial: 31-82.5 x 7.5-10 µm; single, sometimes aggregated but not in clusters; cylindrical with narrowed base; contents clear; smooth or incrusted with yellow brown incrustations orientated around cell on middle ± apex. Caulo: not seen.

Other microscopic features Basidia 30.5-38 x 8.5-10.5 µm; clavate; four-spored; simple septum at base. Pileipellis of interwoven, periclinally arranged, simple septate, ligh brown hyphae 2.5-6.5 µm in diam (no incrustations). Pileus trama of somewhat tightly interwoven, periclinally arranged, simple septate, hyaline hyphae 4.5-10.5 µm in diam. Basal mycelium: single hyphae (2)3-6 µm in diam with light brown incrustations (some needle-like), simple septate; paarige branching present; anastomoses present; strands occasional and thin, central hyphae up to 14.5 µm in diam. Clamp connections absent, all parts.

Culture VC 1230, VC 1449.

Macroscopic Variable. Colony felty overlaid with open cottony, bumpy, wrinkled, radial furrows; margin open cottony with villose edge or low cottony fuzzy; outline somewhat irregular; raised and submerged hyphae equal at margin; all white to yellow white (3A-B1.5) ± light brown (5D4) rings or center gray orange (4B3), middle mottled light yellow (3-4A-B2-4) and white, margin white. **Reverse** indistinct radial furrows; center and middle dark orange brown to red brown, margin yellow white to yellow orange. At 4 weeks 17-33 mm in diam, 1-4 mm high, (1)2-4 mm deep. Syringaldazine positive or negative; gum guaiac positive or negative; KOH brown to red brown; NH_4OH no reaction or red purple on margin only, halo absent or weak purple and orange or red purple.

Microscopic Slide culture: leading hyphae 2-3.5(4) µm in diam; unclamped; strands present or absent. Petri dish culture: oleiferous hyphae gray golden to golden brown; vesicular cells present or absent; strands present or absent.

Habitat Gregarious on ground in boreal conifer forest. Within the root zone of and probably ectomycorrhizal with *Larix himalaica*.

Ectomycorrhizal synthesis Not attempted because *Larix himalaica* seeds did not germinate.

Material studied Nepal: Bagmati Zone - VC 1230, Langtang National Park, Rasuwa District, 3070 m, 6 Aug 1985; VC 1449, Langtang National Park, Rasuwa District, 3140 m, 11 Oct 1985. Basidiocarp microscopic data based on VC 1230 and VC 1449.

Field diagnosis The himalayan *Suillus* is distinguished by the bipartate veil, cottony on the inside and glutinous on the outside; conical umbo; yellow hymenium bruising brown; yellow staining of paper; and association with *Larix himalaica*. This fungus is somewhat intermediate between *Suillus sensu stricto* and "*Fuscoboletinus*". Generally the different *Suillus* species associated with *Larix* in Nepal are easy to tell apart, but when young, before the veil breaks, the himalayan *Suillus* and *S. laricinus* are very similar and could be confused.

Suillus "orange-pored"
Fig. 1.15.
Basidiocarp single to caespitose.
Pileus 1.7-5.7 cm in diam; convex, becoming broadly convex to planar-undulate, margin even or scalloped; surface viscid with glutin sometimes drying into ridges, glabrous ± sparse indistinct radiating fibrils, bumpy with shallow pits; gray yellow (4B-C5), becoming pale gray yellow (4B-C4) or light yellow (3-4A4) with darker stains and brown stains, stains sometimes with olive hues; basically drab in color with drying glutin adding brownish drab tones; odor fungoid, somewhat fragrant; taste pleasant fungoid, pellis mild. **Pellis reactions** $FeSO_4$ none; KOH light gray; NH_4OH no reaction under drop, halo light red brown or orangish.
Inner veil None. Young basidiocarps have some white mycelium along pileus margin.
Hymenium decurrent. **Pores** 0.5-1 mm in diam; angular to rounded angular; compound; boletinoid, sometimes weakly; when young deep orange (5A7) with tiny white glandulae (handlens) remaining orange (4-5A7) then fading to orange yellow (4B6-7); bruising light orange brown; NH_3 red orange. **Tubes** 3-5 mm long; concolorous with pores. Dried tubes peppered with dark tiny glandulae (handlens).
Stipe 2.5-5 x 0.6-1.2 cm; surface covered with cottony mycelium which mats with time, sometimes furfuraceous; when young very apex yellow (4A5-6) paling downward to light yellow (3A5 to 3A-B4), mature apex amber yellow (4B6) grading downward to gray yellow (3B-C4) with olive (3C-F4 to 3F8) stains, stains olive (3C-F4) from handling (especially base); glandulae tiny and restricted to apex, when young white becoming brown black, some appearing as tiny short-triangular spines. **Basal mycelium** white with strands, inside becoming mustard yellow. After drying, brown to olive brown.
Context firm, colors generally drab but pileus context may appear bright orange yellow. **Pileus** 6-9 mm thick tapering to margin, all stages light yellow to gray yellow (4B4, 3-4A4 or 4A3) ± oranger on exposure; $FeSO_4$ dull brown; KOH gray → gray brown; NH_4OH dull orange → gray brown with dull orange halo. **Upper stipe** solid; young colored like pileus, becoming amber yellow (4B6) then brown (5F5), when amber yellow becoming darker and oranger (4C6 or 5B-C6) on exposure. **Lower stipe** solid; olive brown (4F8) becoming brown (5E-F5).
Basidiospores 7.5-9 x 3-3.5 µm; ellipsoid in face view, ellipsoid to weakly subfusiform in profile with small apiculus, shape somewhat variable; surface smooth; pale yellow brown in KOH, slightly dextrinoid in Melzers; wall thickness medium (circa 0.3 µm). Spore deposit gray brown to brown orange (a little darker than 5B4).
Cystidia Hymenial: 28.5-60 x 5.5-7.5 µm (size variable), in small clusters, clavate to near cylindric, apex sometimes broadly mucronate, contents clear or gray brown in KOH, amorphous brown incrustations around bases. Caulo: 66-86 x 4.5-7.5 µm (size variable, overall longer than hymenophoral), in small clusters, clavate to near cylindric,

sometimes wavy, sometimes with a septum towards apex, contents clear or gray brown in KOH, dense brown amorphous incrustations around bases.

Other microscopic features Basidia 24-28.5 x 5-6.5 µm, clavate, four-spored, simple septum at base. Pileipellis of repent, interwoven, simple septate, weakly gelatinized hyphae, 4.5-10.5 µm in diam, with very pale yellow brown contents in KOH. Pileus trama of hyaline, interwoven, simple septate hyphae. Basal mycelium: single hyphae simple septate, with small brown angular incrustations. Clamp connections absent, all parts.

Culture VC 1239.

Macroscopic Only a small colony developed due to presence of stray contaminent fungal colonies. Transfers failed so culture lost.

Microscopic Hyphae 2.5-4 µm in diam, simple septate with globular brown incrustations.

Habitat Gregarious on ground in boreal forest of *Larix himalaica, Tsuga dumosa*, and *Rhododenron barbatum*. *Abies spectabilis* also in general area. Only collected once; appears to be rare.

Ectomycorrhizal synthesis Not attempted because culture died and *Larix himalaica* seeds did not germinate.

Material studied Nepal: Bagmati Zone - VC 1239, Langtang National Park, Rasuwa District, 3000 m, 7 Aug 1985.

Field diagnosis The orange-pored *Suillus* is distinguished by the bright orange color of the young pores with contrasting white glandulae (tiny, need handlens), olive hues and stains on the stipe and sometimes on the cap especially when older, the olive hues in the lower stipe context, the tiny spiny glandulae at the stipe apex, and the boreal conifer habitat. The orange-pored *Suillus* is most likely ectomycorrhizal with *Larix*; if so, then its affinities are with *S. tridentinus* (Bres.) Singer. *Suillus tridentinus* differs in having an inner veil and larger basidiospores.

Synoptic keys to Suillus species of Nepal

Synoptic keys based on basidiocarps and on cultures follow a discussion of cultural identification. It should be possible to identify most collections of *Suillus* basidiocarps from Nepal using the synoptic key presented. One case that might present problems would be an old, larval-ridden collection in section *Suillus*; such collections are often not identifiable.

The large intraspecific and intracultural variation in *Suillus* cultures make a dichotomous key for their identification untenable. Definitive identification of ectomycorrhizal fungal species from cultures may itself be untenable for *Suillus*, but with a synoptic key it should be possible to make reasonable approximate identifications. This is especially true when noncultural data like host is also available. Korf (1972) describes the construction and use of synoptic keys. Advantages of synoptic keys include the ability to enter the key using any character and then to continue using any combination of characters. New taxa and new characters can easily be incorporated into synoptic keys. Korf (1972) lists further advantages and notes that the main disadvantage is that synoptic keys become cumbersome to use if over 30 taxa are treated. Use of the following synoptic key to cultures of *Suillus* from Nepal requires growing the unknown culture(s) on modified Hagem's agar for four weeks as described in the Materials and Methods; slide cultures of the unknown(s) must also be prepared. Of course, to use the key one must

first know that the unknown culture is a *Suillus*. This would be true if isolated from the basidiocarp and the cultural identification could serve as a check on the identification based on the basidiocarps. The following combination of characters is strong evidence that the culture from Nepal is a *Suillus*: isolated from or near ectomycorrhizae of *Pinus* or *Larix*, incrustations (some shade of brown) on the hyphal walls, paarige branching, oleiferous hyphae (color varies), only vegetative structures (no spores of any type), clamp connections absent or restricted to leading hyphae, thin plate of fungal tissue at the surface of the agar, hyphal strands and ability to grow on media like Hagem's agar. The expression of these characters depends on environment. For example, in agar culture a given *Suillus* culture may form few or no strands but in an ectomycorrhizae synthesis pouch may form abundant strands.

Synoptic key to basidiocarps of *Suillus* from Nepal.

Key to species codes:

Gr = *Suillus* cf. *granulatus*
Pl = *Suillus* cf. *placidus*
Si = *Suillus sibiricus*
Gf = *Suillus* "greening-foot"
Qu = *Suillus* "queen's"
Wx = *Suillus* "waxy"
La = *Suillus laricinus*
Hi = *Suillus* "himalayan"
Op = *Suillus* "orange-pored"

* indicates that the species ot the character is variable. Chemical reactions are complex and variable; if a fungus is listed for a color, say green, it means that that color usually occurs; many other colors may also occur.

1. Ecological character.

1.1. Ectomycorrhizal host.

a. *Larix himalaica*	La,Hi,Op
b. *Pinus patula*	Wx
c. *Pinus roxburghii*	Qu
d. *Pinus wallichiana*	Gr,Pl,Si,Gf

2. Pileus characters (including inner veil).

2.1. Pileus size (must have large collection).

a. All pilei 6 cm or less in diam	Qu,Wx,Op
b. Some pilei greater than 6 cm in diam	Gr,Pl,Si,Gf,La,Hi

2.2. Pileus umbo.

a. Absent	Gr,Pl,Gf,Si*,Qu,Wx,La*
b. Present	Si*,La*,Hi

2.3. Scales on pileus surface (may wash off in rain).

a. Absent	Gr,Pl,Gf,Qu,Wx,La,Hi,Op
b. Present	Si

2.4. Predominant pileus color (young basidiocarps).

a. White to yellowish white	Pl
b. Light yellow to yellow	Gr*,Gf*,Si,Qu,Wx*,La*,Hi*
c. Gray yellow	Op
d. Brown (all shades)	Gr*,Gf*,Wx*,La*,Hi*

2.5. KOH reaction of pileus surface.*

a. Gray to black	Gr,Pl,Si,Gf,Hi,Op
b. Green to olive hues	Gr,Gf,La,Hi
c. Blue to purple hues	Pl,Su,Wx
d. Brown hues (all shades)	Pl,Gf,Si,Su,Wx,La

2.6. NH_4OH reaction of pileus surface, color of halo.*

a. Absent	Pl,Wx,La
b. Gray	Hi
c. Orange, red orange, browns	Gr,Pl,Gf,Si,Qu,Wx,Op

2.7. Inner veil.

a. Absent	Gr,Pl,Gf,Qu,Wx,Op
b. Present, cottony	Si
c. Present, cottony and glutinous layers	La,Hi

3. Hymenium characters.

3.1. Pore size.

a. All pores 1 mm or less in diam	Gr,Pl,Si,Gf,Qu,Wx,Op
b. Some pores more than 1 mm in diam	La,Hi

3.2. Pore arrangement.

a. Not or weakly boletinoid	Gr,Qu,Wx
b. Somewhat to strongly boletinoid	Pl,Si,Gf,La,Hi,Op

3.3. Pore color (young basidiocarp).

a. Light yellow	Pl,Qu*,Wx*,La*
b. Yellow	Gf*,Hi
c. Gray yellow	Gr,Si*,Gf*,Qu*,Wx*,La*
d. Orange yellow	Si*
e. Orange	Op

3.4. Discoloration of pores after bruising.

a. Absent	Gr,Pl,Qu,Wx
b. Orange brown to brown	Si,Gf(slowly),La*,Hi,Op
c. Gray to blue gray	La*

3.5. NH_3 reaction of pores.

a. Absent to weak	La
b. Orange to orange red	Gr,Pl*,Si,Gf*,Qu*,Wx,Op
c. Brown (all shades)	Pl*,Gf*,Qu*,Hi

4. Stipe characters.

4.1. Predominant stipe color (young basidiocarp).

a. White	Gr,Pl,Si*,Gf*,Qu*,Wx*.
b. Light yellow to yellow	Si*,Gf*,Qu*,Wx*
c. Gray yellow	Op

d. Light yellow overlaid with brown Hi
e. Light yellow overlaid with red brown La

4.2. Glandulae.
a. Absent La,Hi
b. Present Gr,Pl,Si,Gf,Qu,Wx,Op

4.3. Color of middle-aged glandulae.
a Absent La,Hi
b. Pallid Gf*,Wx,Op*
c. Brown (all shades) Gr,Pl,Si,Gf*,Qu,Op*

4.4. Color of mycelium attached to base.
a. White Gr*,Pl,Si*,Gf*,Qu*,Wx*,La,Hi*,Op*
b. Yellow Si*,Gf*,Qu*,Wx*,Hi*,Op*
c. Pink to orange Gr*,Si*

5. Context characters.

5.1. Predominant color of pileus context (young basidiocarp).
a. White Gr,Pl,Gf,Qu,Wx*,La,Hi*
b. Yellow to gray yellow Si,Wx*,Hi*,Op

5.2. Discoloration of mature pileus context after exposure.
a. Unchanging Gr,Pl,Qu,Wx
b. Duller to brown Si*,Gf(slowly),La*,Hi
c. Oranger Si*,Op
d. Blue to gray Si*
e. Lavender La*

5.3. Discoloration of mature lower stipe context after exposure.
a. Unchanging Gr*,Pl,Qu,Wx
b. Duller to brown Si*,Gf*(slowly),La*,Hi,Op*
c. Oranger Si*,Op*
d. Blue to gray Si*
e. Blue green Gr*,Gf*,La*

5.4. Initial KOH reaction of pileus context (young basidiocarp).*
a. Gray hues Gf,Qu,Op
b. Blue to purple hues Pl,Si,Wx,La,Hi
c. Green hues Si*,Hi
d. Red to orange hues Gr,Si,Gf,Qu,Wx
e. Brown hues Pl,Si,Qu,Wx,La

5.5. NH_3 reaction of pileus context, halo color (young basidiocarp).*
a. Absent Pl,La,Hi
b. Orange to red orange Gr,Pl,Si,Gf,Qu,Wx,La,Op
c. Brown Hi

Synoptic key to cultures of *Suillus* from Nepal.

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Wx = *Suillus* "waxy"
La = *Suillus laricinus*
Hi = *Suillus* "himalayan"

* indicates that the species is variable for that character.

1. Macroscopic colony characters.

1.1. Colony diameter after four weeks growth.

a. less than 30 mm (slow growing)	Gr,Si*,Wx*,La*,Hi
b. 30-50 mm	Pl,Si*,Qu,Wx*,La*
c. more than 50 mm (fast growing)	Gf

1.2. Colony height after four weeks growth.

a. 4 mm or less (low growing)	Gr,Si,Wx,La*,Hi
b. more than 4 mm	Pl,Gf,Qu,La*

1.3. Colony depth into agar after four weeks growth.

a. 5 mm or less	Pl,Gf,La,Hi
b. more than 5 mm (deep growing)	Gr,Si,Qu,Wx

1.4. Color of colony surface, any portion of colony surface.
(many colors are not listed)

a. Yellow at week 2	Gf,Wx*
b. Brown at week 2	Gr,Gf,Wx*,La*
c. Purple at week 4	Gr*

1.5. Color of colony reverse, any portion of colony reverse.

a. Black at week 4	Gr*,Qu,Wx*
b. Purple at week 4	Gr

1.6. Diffusion of pigment into agar, consider all four weeks.

a. Absent	Gr*,Pl*,Si*,Wx*,La*
b. Slight to moderate	Gr*,Pl*,Si*,Gf,Qu,Wx*,La*
c. Intense	Hi

1.7. Radial furrows at week 4.
Furrows can be observed from surface or reverse.

a. Absent to weakly developed	Gr*,Si*,Gf,Wx*,Hi*
b. Well developed	Gr*,Pl,Si*,Qu,Wx*,La,Hi*

1.8. Irregular wrinkling of colony surface at week 4.

a. Absent	Gr,Wx*,La*,Hi*
b. Present	Pl,Si,Gf,Qu,Wx*,La*,Hi*

1.9. Colony margin at week 4.

a. Aerial growth in advance of submerged	Pl,Gf,Wx*,La*
b. Aerial and submerged growth equal	Si*,Qu,Wx*,La*,Hi
c. Submerged growth in advance of aerial	Gr,Si*

1.10. Enzyme spot tests with gum guaiac and syringaldazine at week 4.

a. Both positive	Si,La*,Hi*
b. Gum guaiac only positive	Wx*,La*
c. Both negative	Gr,Pl,Gf,Qu,Wx*,Hi*

1.11. Halo of NH_4OH spot test at week 4.

a. Absent or weak	Gr,Si,Qu,Wx*,La,Hi
b. Immediately strong (any color)	Pl,Gf,Wx*

2. Microscopic slide culture characters.

2.1. Septae of leading hyphae.

a. Regularly clamped	Gf,Qu
b. Simple (unclamped)	Gr,Pl,Si,Wx,La,Hi

2.2. Branches arising from middle of cells.

a. Absent	Gr*,Pl,Si*,Gf,Qu,Wx*,La*,Hi
b. Present	Gr*,Si*,Wx*,La*

3. Ecological character.

3.1. Ectomycorrhizal host.

a. *Larix himalaica*	La,Hi
b. *Pinus patula*	Wx
c. *Pinus roxburghii*	Qu
d. *Pinus wallichiana*	Gr,Pl,Si,Gf

Discussion

The primary conclusion is that *Suillus* does indeed occur in Nepal. Furthermore the basidiocarps and separately the cultures of different populations display nodal variation which can be utilized to define species. The variation is not continuous across the genus.

The genus *Suillus* was a common component of the conifer forests of Nepal and also occurred in young pine plantations suggesting an importance in reforestation. Eighty-one collections of *Suillus* were made, most during the monsoon, with 26 also successfully maintained in culture. Nine species of *Suillus*, five of which appear to be new, are recognized from Nepal (Table 1.4). The collected material for these nine covered all stages of basidiocarp development; cultures were described for eight of the nine. The 81 collections of *Suillus* from Nepal also include additional species but the material is inadequate for full descriptions. Sharma (1980) and Watling and Gregory (1980) have collected species of *Suillus* in NW India with *Pinus wallichiana*; two of these differ from my *Suillus* species from Nepal. These additional *Suillus* species collected in India no doubt occur in Nepal, at least in the western part. *Suillus* species in the *Boletinus* group have been reported from Tibet (Wang and Zang, 1983) and most likely occur across the border in Nepal. Thus the total number of *Suillus* species in Nepal is probably from 15 to 20 which is similar to the *Suillus* diversity found in an area of similar size and floristic composition in Europe or North America. Although Nepal has only

four native species of trees in genera which are typical hosts for *Suillus*, those four represent three divergent taxonomic groups: the hard pines, the soft pines and the larches. The number of *Suillus* species in areas with trees in these three conifer groups are 21 for Europe (Moser, 1983), 20 for Nova Scotia (Grund and Harrison, 1976), 21 for Quebec (Pomerleau, 1980), and 29 for Michigan (Smith and Thiers, 1971). By contrast, Virginia which has no larch has only about 12 species of *Suillus* (Chapter 2). North Carolina also has 12 (Coker and Beers, 1943; Grand, 1970, 1981). Further south, Florida lacking both larch and five-needle pines has only four species of *Suillus* (Singer, 1945b).

Based on basidiocarp formation, all of Nepal's *Suillus* species are host specific within Nepal. Field associates for the nine species are *Pinus wallichiana* (4 *Suillus* spp.), *P. roxburghii* (1 sp.), *P. patula* (1 sp.), and *Larix himalaica* (3 spp.). Laboratory syntheses confirmed that the six *Suillus-Pinus* associations are ectomycorrhizal demonstrating that the ecological role of *Suillus* in Nepal is the same as elsewhere in the world. The three *Suillus-Larix* associations could not be tested because the *L. himalaica* seed did not germinate. The host specificity of *Suillus* has an important consequence in forestry practice in that if *Suillus* is used, then the correct host-specific species should probably be chosen for each tree. Using ectomycorrhizal inoculum soil from a stand of the tree of interest is one way to achieve this. This practice, of course, has the undesirable potential of spreading pathogens and pests as well as the

mycorrhizal inoculum. Even so, it is the practice of choice in Nepal because of its simplicity and effectiveness.

As hypothesized, *Pinus wallichiana* has a rich *Suillus* mycota associated with it just as does the closely related *P. strobus* of eastern North America. In fact there are three pairs of related suilli in which one taxon of each pair is associated with *P. wallichiana* and one with *P. strobus* (Table 1.5). These pairs are *S. sibiricus* - *S. americanus*, *S.* cf. *granulatus* - *S. granulatus* (ssp. *snellii*), and *S.* cf. *placidus* - *S. placidus*. The existence of these pairs of similar fungi suggests the possibility of cladogenetic speciation in parallel by the pine lineage and its fungal associates. Within the respective ranges of these two pines, each associated *Suillus* species is host specific only being found with the five-needled pine.

Suillus brunnescens is another species reported from the Himalaya (Sharma, 1980) and may be associated with *P. wallichiana*; it was first described from North America (Smith and Thiers, 1964) as an associate of *P. lambertiana* Dougl. which is another closely related five-needled pine.

In addition to the pairs of related suilli, *P. wallichiana* and *P. strobus* each has unique species associated with it for which there is no counterpart associated with the other pine (Table 1.5). These unique species are the greening-foot *Suillus* with *P. wallichiana* and

S. subluteus sensu Snell and *S. spraguei* with *P. strobus*. Although *S. subluteus* is not known to have a counterpart with *P. wallichiana*, it does with *P. monticola* as *S. subolivaceous* Smith & Thiers.

Adequate material of only one species of *Suillus*, the queen's *Suillus*, was collected with *P. roxburghii*, the other native pine of Nepal. However, at least one other species was collected with *P. roxburghii*, e.g. VC 1480. *P. roxburghii* is more of a dry site tree than is *P. wallichiana*. The drier site conditions may account, at least in part, for the relatively infrequent collection of *Suillus* with *P. roxburghii*. The closest relative of *P. roxburghii* is *P. canariensis* C. Smith, an endemic pine of the Canary Islands. As far as I know nothing has been published on the *Suillus* mycota of the Canary Islands.

One of the three *Suillus* species collected with *Larix himalaica* fits *S. laricinus*, which is variable enough around the world to be considered a species complex. *Suillus laricinus* is associated with *Larix* throughout the Northern Hemisphere. The other two species with *L. himalaica* appear to be new species and may be Himalayan endemics. I did not do field work in far northeastern Nepal where the other native larch, *L. griffithiana*, grows but expect that *S. laricinus* would be found there. The other two species or closely related forms might also be present.

The Himalayan region as represented by Nepal appears to have many indigenous species of *Suillus*. Examples are the greening-foot *Suillus*

with *P. wallichiana* and the orange-pored *Suillus* with *L. himalaica*. The four species of *Suillus* associated with *P. wallichiana* are widespread in native forests and are no doubt themselves native. The three species associated with *L. himalaica* were found in a high inner Himalayan valley and are also surely native. The queen's *Suillus* with *P. roxburghii* is probably native as well. Non-queen's *Suillus* collections associated with *P. roxburghii* including VC 1480 were all collected at ornamental, not forest, sites; thus their status as native or introduced requires further work either linking them to *Suillus* species known elsewhere in the world or to populations in native Himalayan forests.

The waxy *Suillus* with *P. patula* is intriguing. *Pinus patula* is a native of Mexico and is widely planted as an exotic around the world. The association of the waxy *Suillus* with an exotic pine and the fact that it was not collected in Nepal outside plantations of the exotic pine strongly suggest that the waxy *Suillus* is also introduced to Nepal. *Suillus chiapasensis* Singer, the only species of *Suillus* described from Mexico in association with *P. patula* (Singer, 1973), does not appear to be the same species. Four species of *Suillus* have been reported from *P. patula* plantations in India (Natarajan and Raman, 1983). I considered it likely that one of these would match the waxy *Suillus*, but none of the descriptions in the article does fit.

One surprise was not finding any fungi of section *Boletinus* of *Suillus*. I expected to find at least *S. spraguei* or a counterpart.

S. spraguei has been reported from Tibet (Wang and Zang, 1983), but, given the amount of time spent collecting around *P. wallichiana*, I conclude that if such a fungus does occur in central Nepal, it is uncommon. Western Nepal would be a logical region to explore for such a fungus as well as other additional species of *Suillus* in association with Nepal's pines.

One purpose of the cultural descriptions was an independent check on my species concepts based on basidiocarps and ecology. Such an independent check of a proposed classification system is an example of operational taxonomy (Sneath and Sokal, 1973). Using numerical taxonomic analyses meant that the check was relatively more "objective" and also more complete because numerical taxonomy integrates all of the available data. Initial descriptions and analyses were disappointing as clusters did not correspond to species concepts based on basidiocarps and ecology. The principle coordinate analysis and minimum spanning tree disagreed. Furthermore, the outgroup used also did not cluster apart from the *Suillus* cultures. Reevaluation of the methods resulted in redoing the descriptions using revised and new characters and more "controlled" methods with the objective of reducing the random variation by making the data acquisition more consistent.

The second data set based on these revised methods and characters clustered the *Suillus* cultures in parallel with basidiocarp species concepts thus reinforcing those concepts. There was no incongruence based

on different life history stages. Moreover the two outgroups clustered apart from the *Suillus* cultures.

One key conclusion is that Nepali *Suillus* species in culture are distinct enough to be classified using the 82 characters. However, identification of cultures may not be practical because no small set of characters is sufficient to place an isolate into a cluster (species). The first three principal coordinate analysis axes only explained 66% of the variation in the culture description data set. In culture, these *Suillus* species are true polythetic groups where no one character is necessarily shared by all members of species.

Changes in the culture description procedures which seem to have been noteworthy improvements are two. First, drawing cross-sections of the colony above and below the agar coupled with measuring the depth of growth down into the agar gave new useful characters. The characters related to growth below agar appear to be more stable because submerged growth of a colony is subject to a less variable environment than is aerial growth. The submerged growth is also less subject to disturbance when viewed; aerial growth often collapses as soon as the Petri dish lid is removed. The second improvement was using slide cultures. The slide cultures provided a means to standardize observation of characters like cell size, presence of clamp connections and paarige branching. Below agar characters (e.g. depth of growth) and slide culture characters (e.g.

clamp connections on leading hyphae) contributed to the better results from the second description attempt.

Visually clustering the 28 Nepali cultures resulted in clusters incredibly close to the numerical taxonomic clusters. The numerical taxonomic clusters were more refined probably because they were based on a broader data base including anatomical and macrochemical characters.

My cultures of *S.* cf. *granulatus* from Nepal compare well with cultures of *S. granulatus* described from eastern North America by Pantidou and Groves (1966) and Miller *et al.* (1983). The Nepali cultures shared the slow growth rate (2-3 cm/mo), low furry furrowed colony mat, and clampless hyphae characteristic of *S. granulatus* cultures from North America. Colors were reasonably similar. Pantidou and Groves (1966) reported that North American *S. granulatus* cultures were negative with NH_4OH whereas the Nepali cultures had a very weak red purple reaction. Pantidou and Groves (1966) reported no reaction with gum guaiac which was also true of the Nepali cultures; Miller *et al.* (1983) however reported their isolate was gum guaiac positive.

The Nepali *S.* cf. *placidus* cultures differed somewhat from North American *S. placidus* as described by Pantidou and Groves (1966) and by Miller *et al.* (1983); the North American descriptions in turn differed between themselves.

Suillus sibiricus cultures from Nepal were quite similar to those from western North America (Grand, 1968) sharing a similar growth rate, short cells, common anastomoses, and positive gum guaiac reaction. One major difference, however, was that the North American cultures had clamp connections. *Suillus sibiricus* was distinct from *S. americanus* of eastern North America (Pantidou and Groves, 1966), although both are gum guaiac positive.

Suillus laricinus from Nepal agreed in many respects with Pantidou and Groves's (1966) description of North American cultures (*Fuscoboletinus aeruginascens*). The changing growth rate over time in culture noted by Pantidou and Groves was also true of the Nepali cultures.

Cultures of *Suillus* from Nepal agree at the generic level with cultures of *Suillus* described from North American and Europe. Only vegetative structures were produced in culture. Papillate and incrusted hyphae and oleiferous hyphae were present in all species. Clamp connections were either absent, or present only on leading hyphae.

At odds with the concept that *Suillus* only produces vegetative structures in culture is Miller *et al.*'s (1983) report of arthrospores for one and chlamydospores for four of five *Suillus* species studied. Other workers (e.g. Pantidou and Groves, 1966; Laut, 1966; Grand, 1968) have not reported arthrospores or chlamydospores in *Suillus* cultures nor did I see any in the cultures from Nepal. Miller *et al.*'s figures of

"arthrospores" (Figs. 19 and 26) are probably not arthrospores. The chlamydospores they reported may have been interpreted as vesicular cells (thin-walled swollen cells) by other workers including myself. As yet I have not seen thick-walled, swollen cells (chlamydospores) in cultures of *Suillus*.

My Nepali *Suillus* cultures showed only weak reaction zones when paired in culture. They were inconsistent in replication and did not serve to demonstrate different species or different individuals of the same species. Fries (1987) conducted preliminary vegetative incompatibility experiments with six other ectomycorrhizal genera and failed to see any reaction zones. He speculated that, unlike decomposer basidiomycetes, ectomycorrhizal fungi may not exhibit strong individual mycelia. However, without anatomical study it is impossible to know whether the lack of strong reactions is indeed due to a reduced maintenance of individual mycelia or if individual mycelia are maintained without obvious visual reactions.

The taxonomy of *Suillus* has not yet reached a stable, useable state. Reasons for the confusion in *Suillus* taxonomy are outlined in the introduction.

I hope my work on *Suillus* in Nepal will serve as a solid base on which further work on the genus *Suillus* in the Himalaya can proceed and that stressing ecology and using cultural characters as an independent check

on species concepts based on basidiocarps has resulted in reasonable *Suillus* species concepts for the Himalayan area. Tying the Nepali species concepts into the world-wide *Suillus* mycota will require much further work, ideally on the level of a world monograph. The queen's *Suillus* serves as a good illustration of this challenge. Mycologists from both North America and Europe sight identified this fungus as *S. placidus*. Yet *S. placidus* is a five-needled pine associate, and the queen's *Suillus* is associated with the three-needled *P. roxburghii*. In culture the queen's *Suillus* is quite distinct from *S. placidus* as described from North America (Pantidou and Groves, 1966; Miller *et al.*, 1983) and from Nepal. Thus similar basidiocarp features result in two distinct *Suillus* species appearing conspecific.

European and North American names for *Suillus* species have been used throughout the Northern Hemisphere. For example, Japanese and Chinese mycologists have often used these names for their *Suillus* mycota. Comparative studies are needed to determine if these taxa are indeed conspecific or if they are different but closely related. The question of whether a *Suillus* species is native or introduced is a major one. *Suillus* species are easy to introduce into new countries; examples are Australia and Chile which had no native *Suillus* species and *S. luteus* in North America.

How can a stable, useable system for *Suillus* taxonomy on a world basis be developed? First, comparative work with fresh basidiocarps and

cultures from around the world is needed to determine whether taxa like *S. granulatus* are truly cosmopolitan species with wide host ranges. My sense is otherwise; I suspect that such taxa are clusters of different species and that populations with hard pines will prove to be clearly distinct from populations with soft pines.

Possibly fruitful future avenues of investigation in *Suillus* systematics are:

1) Further numerical taxonomic analyses. Basidiocarp and ectomycorrhiza data for Nepali *Suillus* species could be combined with the cultural data and analyzed to confirm species concepts. Secondly, similar broad-based data for North American *Suillus* species could be added to test the hypothesis of related pairs of *Suillus* species from the two continents.
2) Pigment analysis especially from dried basidiocarps. There is a need to find additional taxonomic characters in dried basidiocarps since they serve as the type specimens. Location of pigments relative to the basidiocarp and relative to the hypha may be useful characters.
3) Mating studies. Work on spore germination may eventually lead to a reliable method of germinating *Suillus* spores. Once this is achieved, mating studies to define biological species can be carried out. Some species of *Suillus* form clamp connections which would be a convenient marker for successful crosses. In other species nuclear staining would probably be necessary. Ultimately, fruiting *Suillus* in the laboratory would open endless doors of systematic and ecological investigation.

4) Hybridization. Investigation of possible *Suillus* hybrids could provide insight into the interactions of different *Suillus* species. *Suillus* species often co-occur and overlap in basidiocarp phenology; it may be a reasonable that the phenologies of dikaryon formation also overlap. This situation suggests that hybridization is a possibility. Furthermore basidiocarps with intermediate characteristics occur in North America where *S. granulatus* and *S. americanus* fruit together and in Nepal where *S.* cf. *granulatus* and the greening-foot *Suillus* fruit together. Singer (1945a) noted intermediate forms between *S. granulatus* ssp. *leptopus* (Pers.) Singer and *S. granulatus* ssp. *granulatus* (ssp. *typicus*) in mixed stands of *P. pinea* L. and *P. sylvestris* L., their respective hosts.

5) Ecology. I believe that Snell and Dick's (1970) and Singer's (1945a, 1986) stress on ecology and host relationships is appropriate and necessary to understand *Suillus*.

6) Molecular studies. Studies of DNA (e.g. Bruns and Palmer, 1986) offer great hope in helping to understand higher-level relationships among *Suillus* species and to unravel "species" like *S. granulatus*. Immunology may offer some potential to recognize species from basidiocarps or vegetative mycelium.

7) Replication. Whatever character is being studied, adequate replication is essential to understanding intraspecific variation. Therefore I think one should be circumspect before erecting new taxa based on material from only one collection.

The results of my numerical taxonomic analysis of Nepali *Suillus* cultures point to a sticky problem regarding identification. Though the whole suite of characters could be used to classify the cultures into clusters which did correlate with species concepts based on basidiocarps and ecology, no small set of characters alone could be used to definitely identify unknown cultures. This same situation may also be true of basidiocarps.

First, a *Suillus* classification system needs to be refined on a world-wide basis using all available characters (basidiocarps, cultures, molecules like DNA, ecology). Then, identification techniques need to be worked out for basidiocarps and ideally for cultures. The second task of making the classification system workable allowing reliable, repeatable identification of *Suillus* species may even be more difficult than the first task of working toward a stable classification system for the genus.

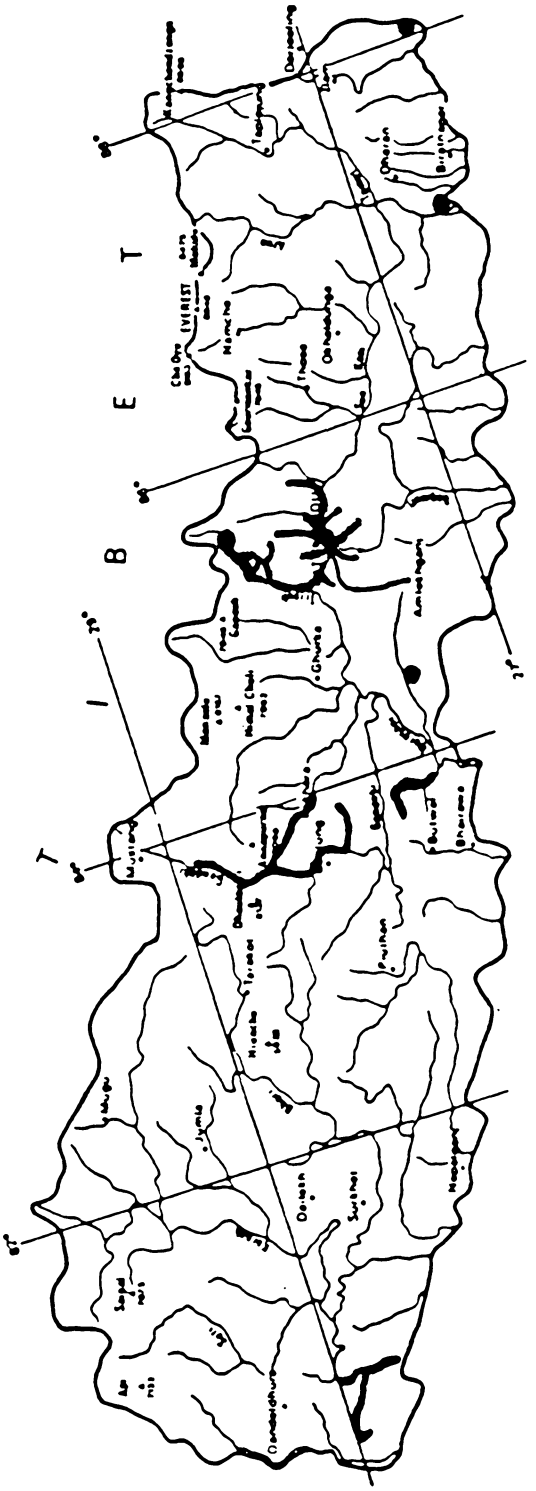

Fig. 1.1. Van and Irene Cotter's collecting routes in Nepal marked with heavy lines and dots.

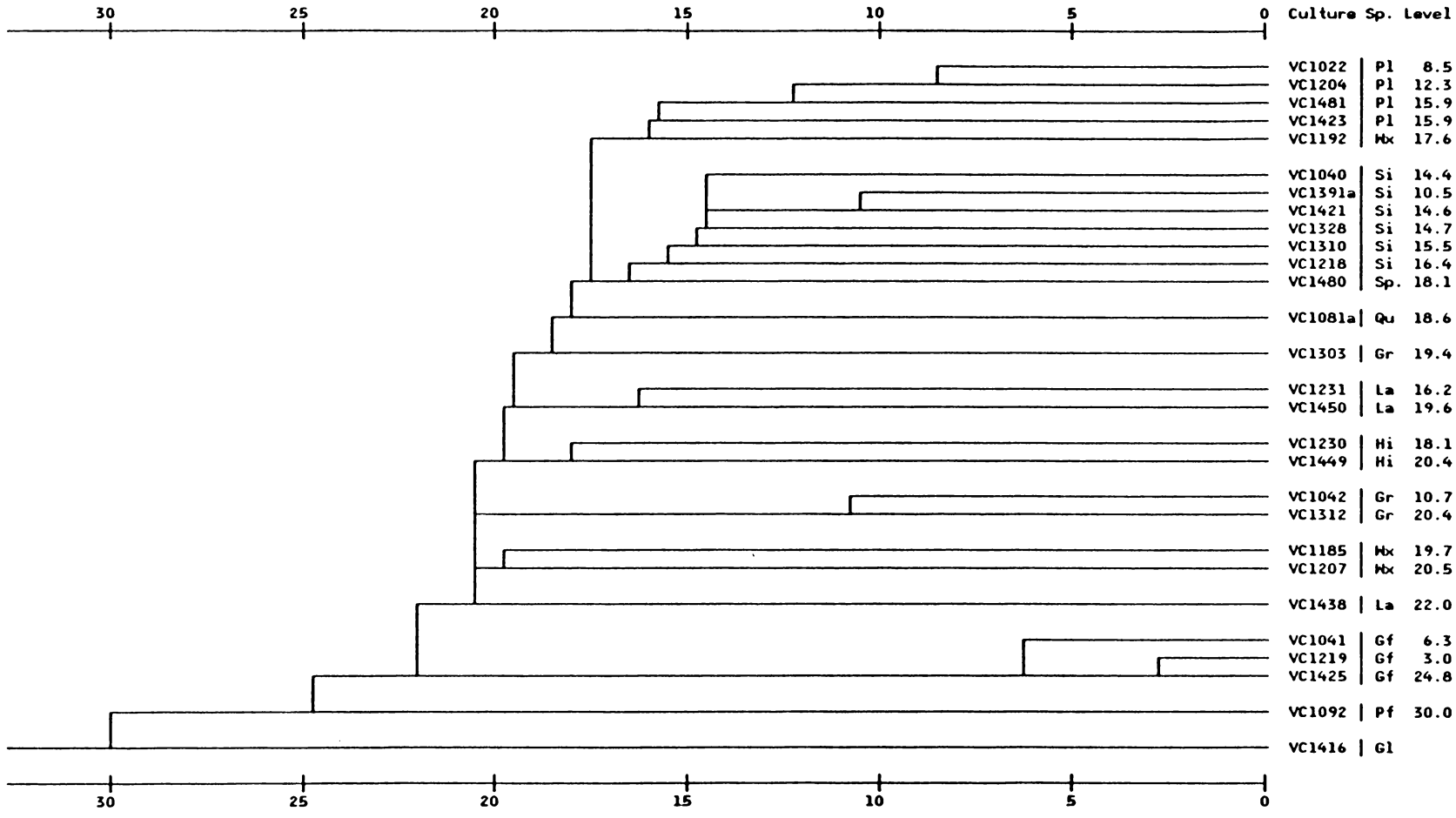

Fig. 1.2. Dendrogram of Nepali Suillus cultures based on Manhattan Distances using single linkage clustering; 28 cultures (OTU's) and 82 cultural characters with no host characters. Cultures connected by a vertical line form a cluster; clusters are numbered top down. Thus the S. cf. placidus cultures plus VC1192
form cluster #1. Outgroups = Gyrodon cf. lividus (Gl) and Paxillus cf. filamentosus (Pf).

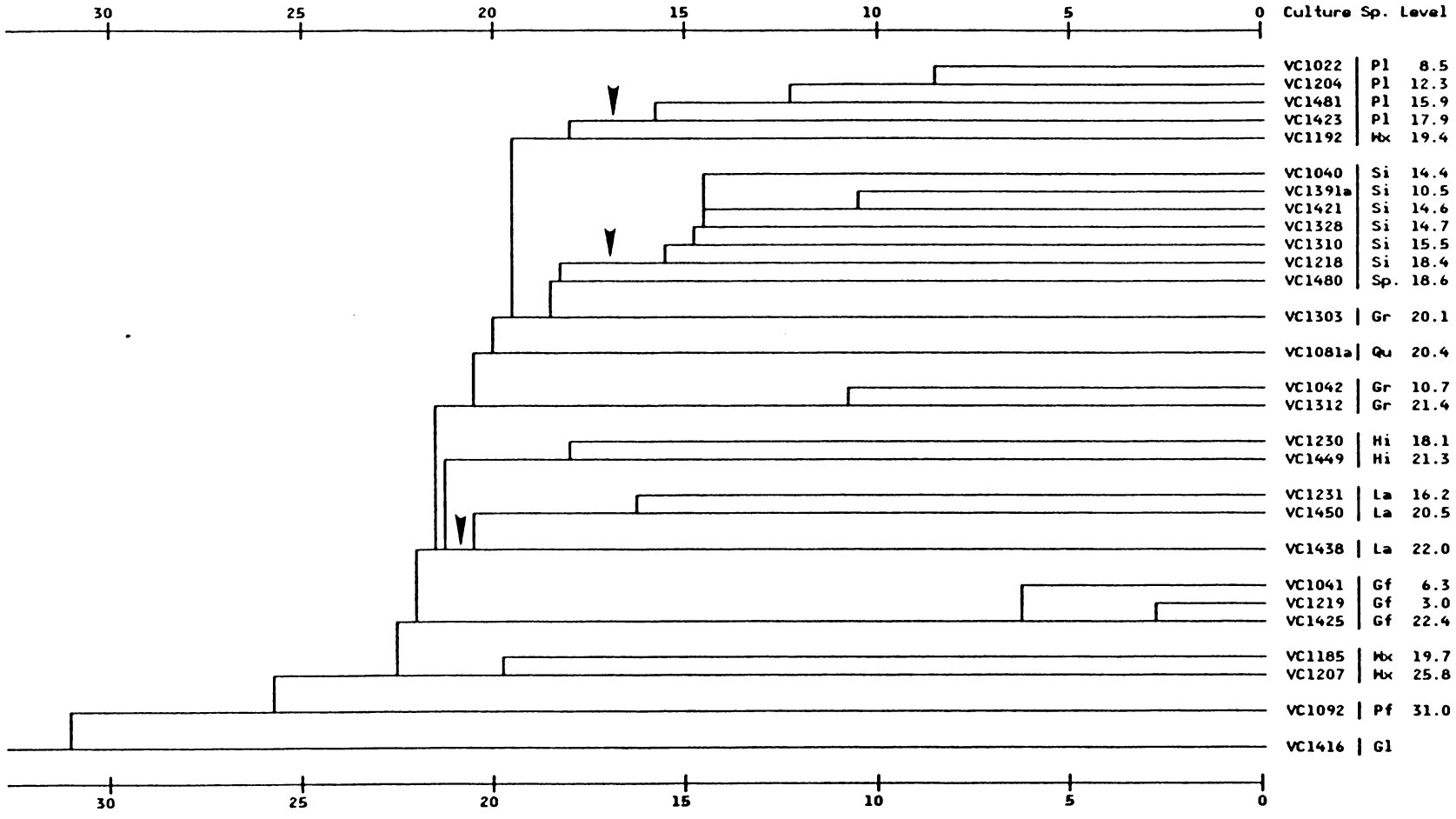

Fig. 1.3. Dendrogram of Nepali Suillus cultures based on Manhattan Distances using single linkage clustering; 28 cultures (OTU's) and 86 characters; the same 82 cultural characters used in Fig. 1.2 plus 4 host characters. Cultures connected by a vertical line form a cluster; clusters are numbered top down. Thus the S. cf. placidus cultures plus VC1192 form cluster #1. Outgroups = Gyrodon cf. lividus (Gl) and Paxillus cf. filamentosus (Pf).

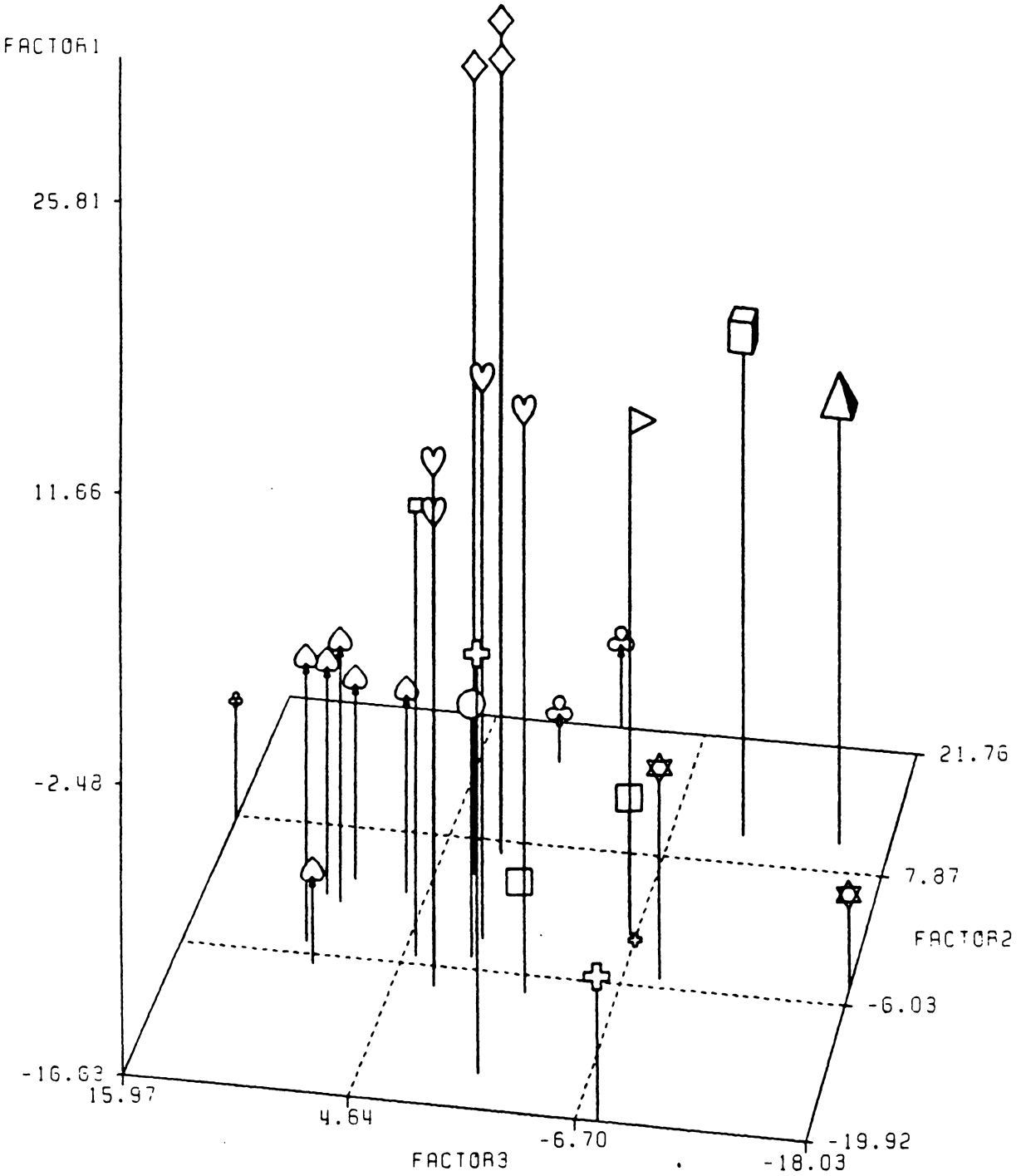

Fig. 1.4. Principal Coordinates Analysis of Nepali *Suillus* culture data; three-dimensional graph of the first three factors.
Symbol codes:
club = *S.* cf. *granulatus*, flag = *S.* "queen's", circle = *S. sp.*,
heart = *S.* cf. *placidus*, square = *S.* "waxy", pyramid = *G.* cf. *lividus*,
spade = *S. sibiricus*, cross = *S. laricinus*, cube = *P.* cf. *filmentosus*,
diamond = *S.* "greening-foot", star = *S.* "himalayan".

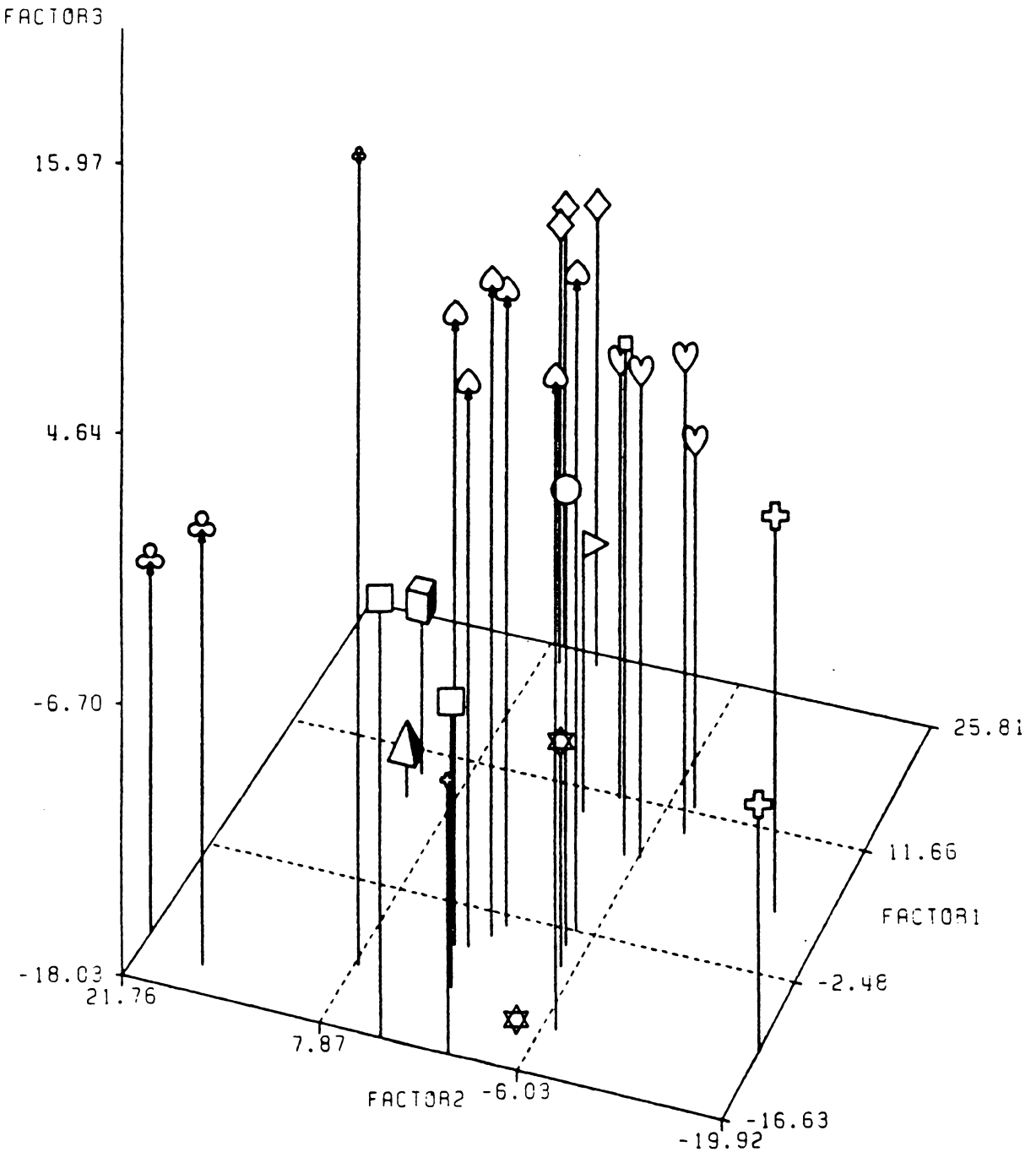

Fig. 1.5. Same Principal Coordinates Analysis of Nepali *Suillus* cultures as presented in Fig. 1.4 but axes in different orientation.
Symbol codes:
club = *S.* cf. *granulatus*, flag = *S.* "queen's", circle = *S. sp.*,
heart = *S.* cf. *placidus*, square = *S.* "waxy", pyramid = *G.* cf. *lividus*,
spade = *S. sibiricus*, cross = *S. laricinus*, cube = *P.* cf. *filmentosus*,
diamond = *S.* "greening-foot", star = *S.* "himalayan".

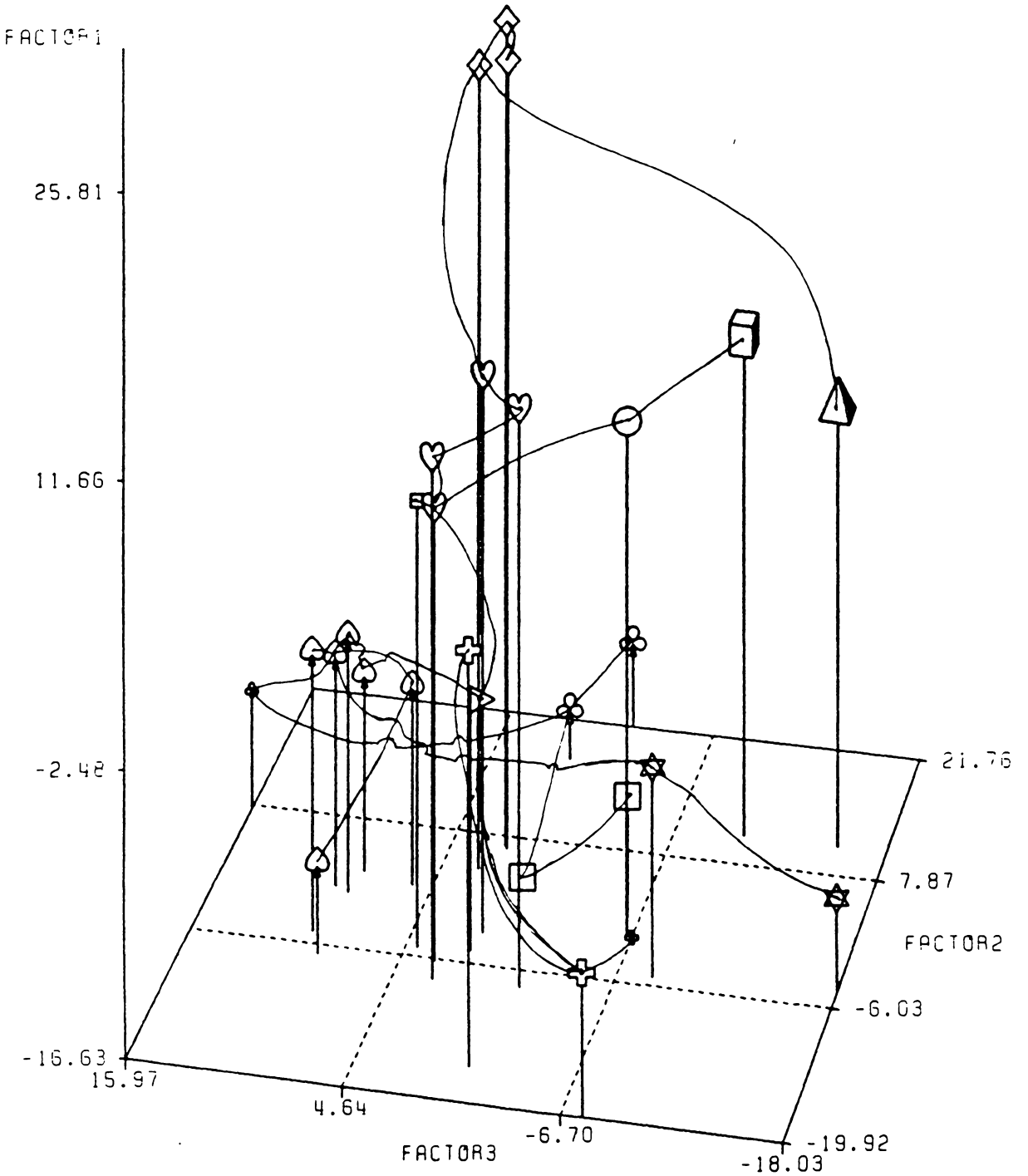

Fig. 1.6. Principal Coordinates Analysis of Nepali *Suillus* cultures; same orientation as Fig. 1.4 but with cultures interconnected by minimum spanning tree linkages.
Symbol codes:
club = *S.* cf. *granulatus*, flag = *S.* "queen's", circle = *S. sp.*,
heart = *S.* cf. *placidus*, square = *S.* "waxy", pyramid = *G.* cf. *lividus*,
spade = *S. sibiricus*, cross = *S. laricinus*, cube = *P.* cf. *filmentosus*,
diamond = *S.* "greening-foot", star = *S.* "himalayan".

Fig. 1.7a. *Suillus* cf. *granulatus* (VC 1303).

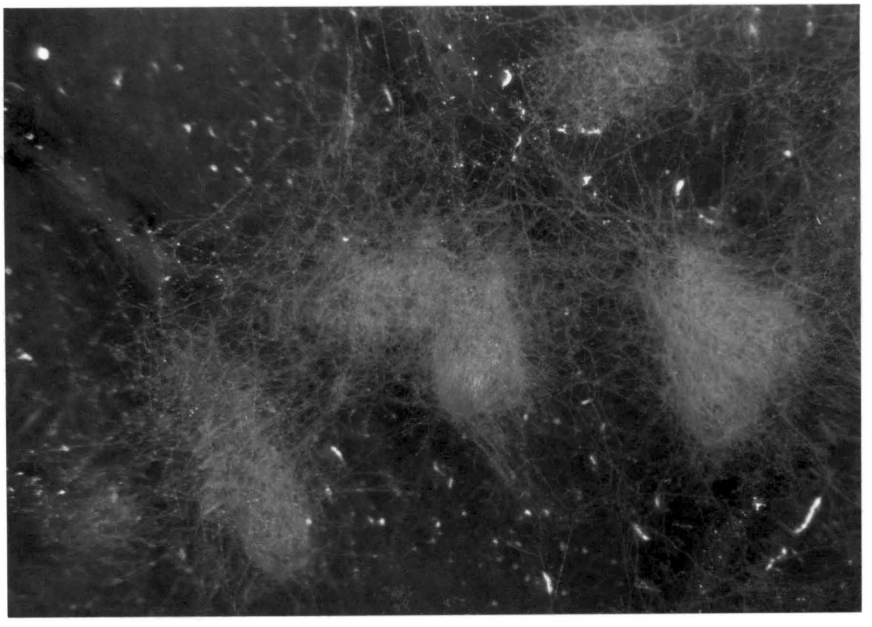

Fig. 1.7b. Synthesized ectomycorrhizae between *Pinus wallichiana* and *Suillus* cf. *granulatus* (VC 1303).

Fig. 1.8a. *Suillus* cf. *placidus* (VC 1022).

Fig. 1.8b. Synthesized ectomycorrhizae between *Pinus wallichiana* and *Suillus* cf. *placidus* (VC 1022).

Fig. 1.9a. *Suillus sibiricus* (VC 1421).

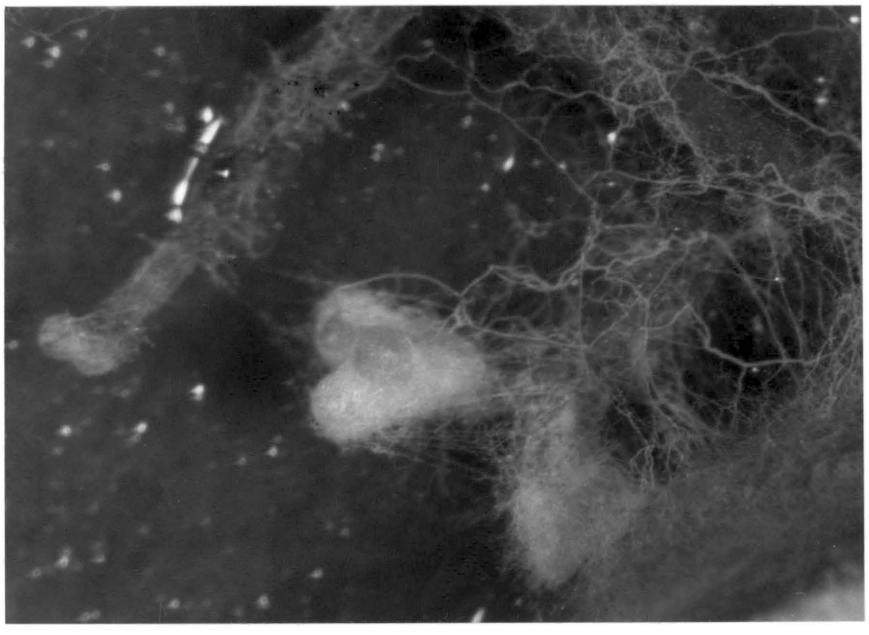

Fig. 1.9b. Synthesized ectomycorrhizae between *Pinus wallichiana* and *Suillus sibiricus* (VC 1218).

Fig. 1.10a. *Suillus* "greening-foot" (VC 1301).

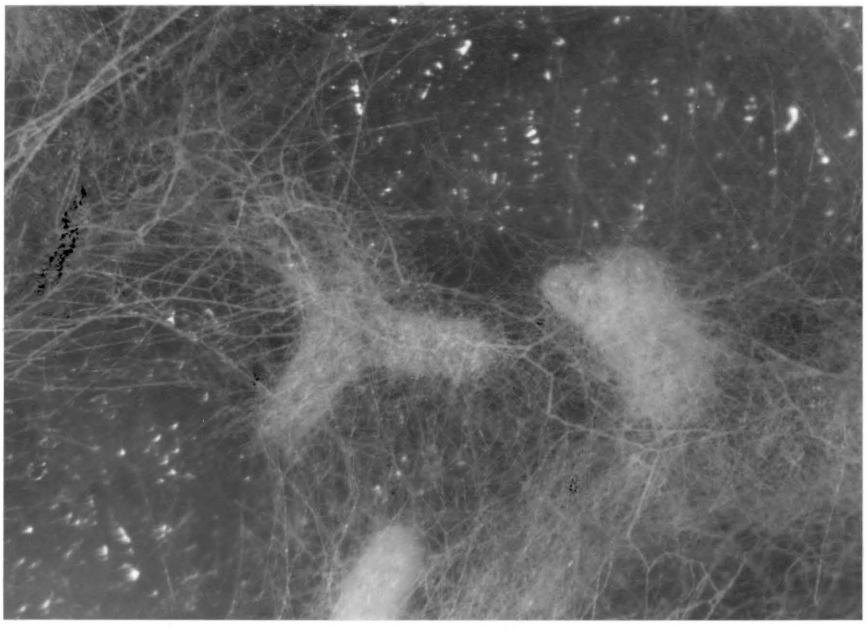

Fig. 1.10b. Synthesized ectomycorrhizae between *Pinus wallichiana* and greening-foot *Suillus* (VC 1219).

Fig. 1.11a. *Suillus* "queen's" (VC 1081a).

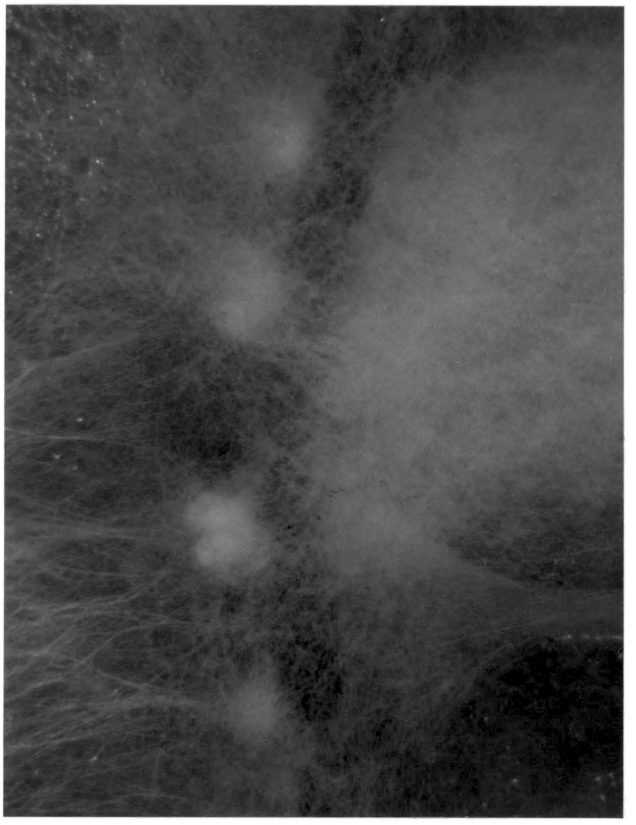

Fig. 1.11b. Synthesized ectomycorrhizae between *Pinus roxburghii* and queen's *Suillus* (VC 1081a).

Fig. 1.12. *Suillus* "waxy" (VC 1185).

Fig. 1.13. *Suillus laricinus* (VC 1438).

Fig. 1.14. *Suillus* "himalayan" (VC 1230).

Fig. 1.15. *Suillus* "orange-pored" (VC 1239).

Table 1.1. Summary of the members of the Pinaceae which occur in Nepal.[1]

Species[2] Comments in reference to Nepal	Geographic and Altitudinal Range in Nepal	
Native species:		
Abies densa W. Griff. ex Parker Shrestha (1984) considers Nepal collections of *A. densa* to be *A. spectabilis*.	East	3000-4000 m
Abies pindrow Royle Some synonomize with *A. spectabilis*; damp site; associated with *Picea* or as a minor component in an otherwise angiosperm forest.	West	2100-2500 m
**Abies spectabilis* (D. Don) Mirbel In magnificant pure stands with *Rhododendron* in the understory; major component of the boreal forest above the *Quercus* and *Tsuga* forests; associated with *Betula* and locally with *Larix*, at lower elevations with *Quercus* and *Tsuga*.	East-Central-West	2400-4400 m
Cedrus deodara (Roxb. ex D. Don) G. Don Cultivated in west and central; in pure stands and associated with *Picea* and *Pinus wallichiana*; now occurring naturally only in a few places, may have been more widespread previously.	West	2000-2500 m
Larix griffithiana Carrière Restricted to the far northeast; associated with *Abies, Betula* and *Rhododendron*.	East	2500-3900 m
**Larix himalaica* Cheng & L. K. Fu Restricted to inner Himalayan valleys; colonizes landslides; boreal forest in association with *Abies, Betula* and *Rhododendron*, at lower elevations with *Tsuga*.	Central	2400-3600 m
Picea smithiana (Wall.) Boiss. Dry site; occurs in pure stands and also associated with *Pinus wallichiana* and *Cedrus*, *Abies*, and also with various angiosperm trees.	West	2300-3600 m
**Pinus roxburghii* Sargent 3-needled; dry site; colonizer; valuable in reforestation; occurs above *Shorea robusta* and below *Pinus wallichiana* forests in altitude; occurs in pure stands maintained by fire; associated with a variety of angiosperm trees.	East-Central-West	900-2100 m

Table 1.1. Concluded.

Species[2] Comments in reference to Nepal	Geographic and Altitudinal Range in Nepal
***Pinus wallichiana** A. B. Jackson 5-needled; dry site but often moister than *P. roxburghii*; colonizer, commonly colonizing moister areas where the natural *Quercus* forest is dieing due to human activity; widely distributed; associates vary and include *Cedrus* and *Picea* or *Quercus*, also grows in pure stands.	East-Central-West 1800-3300 m
***Tsuga dumosa** (D. Don) Eichler Associated with *Rhododendron* and various angiosperm trees; in forests forming an altitudinal belt between boreal forest above and *Quercus-Rhododendron* forest below.	East-Central-West 2100-3600 m
An exotic species:	
***Pinus patula** Schlecht. & Cham. 3-(5)-needled; planted widely in reforestation efforts; excellent growth rate but fire sensitive; regenerating naturally.	Central (planted) 1000-2600 m

[1]Sources that this table is based on are Campbell (1980), Hara *et al.* (1978), Shrestha (1984), Stainton (1972) and personal observations.
[2]Fungal collecting was done under those tree species marked with an asterisk.

Table 1.2. Outline of Van and Irene Cotter's collecting activity in Nepal.

Date(s)	Location (Zone is listed last)	Collection numbers
1985		
13 Apr - 15 May	Kathmandu environs[1], Bagmati	VC 1001-1020
18-19 May	Daman, Makawanpur District, Narayani	VC 1021-1033
24 May - 7 Jun	Trip to the Kali Gandaki Valley	
27-28 May	Myagdi District, Dhaulagiri	VC 1035-1038
29 May - 2 Jun	Mustang District, Dhaulagiri	VC 1039-1060
3-4 Jun	Myagdi District, Dhaulagiri	VC 1061-1063
4-6 Jun	Kaski District, Gandaki	VC 1064-1075
8-17 Jun	Kathmandu environs[1], Bagmati	VC 1076-1084
18-28 Jun	Trip to the Langtang Valley Langtang National Park, Rasuwa District, Bagmati	VC 1085-1135
29 Jun - 6 Jul	Kathmandu environs, Bagmati	VC 1136-1141
9-13 Jul	Trip to Helambu	
9 Jul	Kathmandu District, Bagmati	VC 1142-1148
10-13 Jul	Nuwakot District (?), Bagmati	VC 1149-1170 & 1174
13 Jul	Kathmandu District, Bagmati	VC 1171-1173
16 Jul	Kathmandu environs, Bagmati	VC 1175-1176
17-19 Jul	Chautara, Sindhupalchok Dist., Bagmati	VC 1177-1196
22 Jul - 1 Aug	Kathmandu environs, Bagmati	VC 1197-1210 & 1215
2-14 Aug	Trip to Langtang Valley Langtang National Park, Rasuwa District, Bagmati	VC 1211-1214 & 1216-1280
22-25 Aug	Kathmandu environs, Bagmati	VC 1281-1291
28 Aug - 12 Sep	Trip to Kali Gandaki Valley	
30 Aug	Kaski District, Gandaki	VC 1292-1294
1-7 Sep	Mustang District, Dhaulagiri	VC 1295-1346
8 Sep	Myagdi, Dhaulagiri	VC 1347-1349
11 Sep	Kaski District, Gandaki	VC 1351
14 Sep - 3 Oct	Kathmandu environs[1], Bagmati	VC 1350 & 1352-1420
5-18 Oct	Trip to Langtang Valley Langtang National Park, Rasuwa District, Bagmati	VC 1421-1476
21-30 Oct	Kathmandu environs, Bagmati	VC 1477-1486
5 Nov	Suklaphanta Wildlife Reserve, Kanchanpur District, Mahakali	VC 1488-1491
12 Nov	Kathmandu environs, Bagmati	VC 1492-1502

Table 1.2. Concluded.

Date(s)	Location (Zone is listed last)	Collection numbers
1985 continued		
19-21 Nov	Trip to Lumbini Zone	
19 Nov	Nawalparasi District, Lumbini	VC 1503
20 Nov	Palpa District, Lumbini	VC 1504-1511
21 Nov	Nawalparasi District, Lumbini	VC 1512-1513
9 Dec	Royal Chitwan National Park, Chitwan District, Narayani	VC 1514-1524
29 Dec	Kathmandu environs[1], Bagmati	VC 1525-1526
1986		
15 Jan	Koshi Tappu Wildlife Reserve, Sunsari District, Koshi	VC 1527-1528
17 Jan	Hetauda, Makawanpur District, Narayani	VC 1529
17-21 Jan	Kathmandu environs[1], Bagmati	VC 1530-1534
23 Feb	Prithivinagar, Jhapa District, Mechi	VC 1535
Mar	Kathmandu environs[1], Bagmati	VC 1536-1538

[1]Some of these fungi were obtained in Kathmandu but were actually collected elsewhere.

Table 1.3. Summary by genus of the fungal collections from Nepal.[1]

Major taxonomic group Genus	Number of Collections	
Boletales (Ectomycorrhizal[2])		146 collections
Boletellus	4	
Boletus	26	
Chroogomphus	3	
Gomphidius	2	
Gyrodon	2	
Gyroporus	4	
Leccinum	4	
Paxillus	4	
Phylloporus	3	
Rhizopogon	3	
Strobilomyces	5	
Suillus	81	
Tylopilus	4	
Gastroid bolete	1	
Agaricales (Ectomycorrhizal)		46 collections
Amanita	8	
Hebeloma	1	
Hygrophorus	4	
Inocybe	3	
Laccaria	1	
Lactarius	13	
Russula	15	
Tricholoma	1	
Agaricales (Saprophytic, parasitic)		73 collections
Agaricus	1	
Agrocybe	2	
Armellaria	2	
Asterophora	2	
Bolbitius	1	
Collybia	5	
Coprinus	2	
Hygrocybe	1	
Leucocoprinus	1	
Omphalotus	1	
Oudemansiella	14	
Panaeolus	4	
Pholiota	1	
Tricholomopsis	1	
Unidentified to genus	34	

Table 1.3. Continued.

Major taxonomic group Genus	Number of Collections	
Aphyllophorales (All ecological groups)		71 collections
Albatrellus	1	
Auriscalpium	2	
Cantharellus	2	
Craterellus	1	
Coltricia	1	
Dentinum	1	
Fomes	1	
Ganoderma	2	
Gomphus	2	
Hymenochaete	1	
Laetiporus	3	
Lentinellus	1	
Lentinus	6	
Lenzites	1	
Panus	3	
Pleurotus	3	
Polyporus	4	
Pycnoporus	1	
Ramaria	2	
Schizophyllum	2	
Thelephora	1	
Trametes	1	
Unidentified to genus	29	
Gasteromycetes (All ecological groups)		16 collections
Calostoma	1	
Hymenogaster	1	
Lycoperdon	1	
Rhizopogon - see Boletales		
Scleroderma	9	
Sphaerobolus	1	
Unidentified to genus	3	
Uredinales (Plant parasites)		9 collections
Cronartium	1	
Unidentified to genus	8	
Ustilaginales (Plant parasites)		3 collections
Sphacelotheca	1	
Ustilago	1	
Unidentified to genus	1	

Table 1.3. Concluded.

Major taxonomic group Genus	Number of Collections
Ascomycetes (All ecological groups)	111 collections
including Deuteromycetes with Ascomycete affinities	
Aleuria	2
Cavimalum	3
Cheilomania	1
Claviceps	1
Cordyceps + *Isaria*	5 + 3
Entonaema	1
Geoglossum, Trichoglossum	6
Helvella	17
Humaria	2
Hypocrea	2
Hypomyces + *Mycogone* + *Sepedonium* + *Stephonoma*	5 + 6 + 4 + 1
Lasiosphaeria	1
Leotia	1
Morchella	2
Nectria + *Tubercularia*	0 + 1
Otidea	3
Peziza	3
Plectania	1
Sarcoscypha	1
Scutellinia	3
Spathularia	2
Sporocadus	1
Tarzetta	1
Tuber	1
Wynnea	1
Xylaria + *Xylocoremium*	8 + 1
Unidentified to genus	21
Miscellaneous fungi (All ecological groups)	3 collections
Exobasidium	1
Pseudohydnum	1
Synchytrium	1
Unidentified fungi	17 collections
Grand total	495 collections

[1]This table lists only those collections which were successfully preserved and have been or will be deposited in herbaria.
[2]Ecological groups are putative.

Table 1.4. Species of *Suillus* in Nepal and their ectomycorrhizal partners.

Suillus species[1]	Conifer associate[2]	Confirmed[3]
S. cf. *granulatus*	*Pinus wallichiana*	Yes
S. cf. *placidus*	*Pinus wallichiana*	Yes
S. sibiricus	*Pinus wallichiana*	Yes
S. "greening-foot"	*Pinus wallichiana*	Yes
S. "queen's"	*Pinus roxburghii*	Yes
S. "waxy"	*Pinus patula*	Yes
S. laricinus[4]	*Larix himalaica*	No
S. "himalayan"	*Larix himalaica*	No
S. "orange-pored"	*Larix himalaica*	No

[1]English specific epithets refer to species which appear to be new and will later be described and given latin binomials.
[2]Field work was not done in far NE Nepal where *L. griffithiana* grows.
[3]Yes = ectomycorrhizal synthesis was successful.
No = synthesis has not yet been attempted.
[4]*S. laricinus* = *S. aeruginascens*.

Table 1.5. Comparison of *Suillus* species associated with *Pinus strobus* of North America and *Pinus wallichiana* of the Himalaya.

Pinus wallichiana	*Pinus strobus*
S. cf. *granulatus*	*S. granulatus*
S. cf. *placidus*	*S. placidus*
S. sibiricus	*S. americanus*
S. "greening-foot"	No counterpart known
No counterpart known	*S. subluteus* sensu Snell
No counterpart known	*S. spraguei* (=*S. pictus*)

Table 1.6. *Suillus* cultures from Nepal. Cultures were isolated during 1985, usually in the field on the same day the fungus was collected.

Taxon	Number[1]	Collection Date and Location	Associate[2]
Suillus:			
cf. *granulatus*:	VC1042	29 May Ghasa	Pw
	VC1303	2 Sep Larjung	Pw
	VC1312	3 Sep Larjung	Pw
cf. *placidus*:	VC1022	18 May Daman	Pw
	VC1204	28 Jul Nagarkot	Pw
	VC1423	6 Oct Syabru	Pw
	VC1481	30 Oct Kakani	Pw
sibiricus:	VC1040	29 May Ghasa	Pw
	VC1218	3 Aug Syabru	Pw
	VC1310	3 Sep Larjung	Pw
	VC1328	4 Sep Larjung	Pw
	VC1391a	23 Sep Godawari	Pw
	VC1421	6 Oct Syabru	Pw
"greening-foot":	VC1041	29 May Ghasa	Pw
	VC1219	3 Aug Syabru	Pw
	VC1425	6 Oct Syabru	Pw
"queen's"	VC1081a	15 Jun Nagarjun	Pr
undefined species	VC1480	29 Oct Kirtipur	Pr
"waxy":	VC1185	18 Jul Chautara	Pp
	VC1192	19 Jul Chautara	Pp
	VC1207	28 Jul Nagarkot	Pp
laricinus:	VC1231	6 Aug Ghora Tabela	Lh
	VC1438	10 Oct Ghora Tabela	Lh
	VC1450	11 Oct Ghora Tabela	Lh
"himalayan":	VC1230	6 Aug Ghora Tabela	Lh
	VC1449	11 Oct Ghora Tabela	Lh
Gyrodon cf. *lividus*	VC1416	3 Oct Nargarjun	An
Paxillus cf. *filamentosus*	VC1092	20 Jun Lama Hotel	An

[1]Culture numbers match collection numbers.
[2]Associated ectomycorrhizal host based on field observation and confirmed by synthesis:
Pp = *Pinus patula* (exotic to Nepal; all other hosts are native).
Pr = *Pinus roxburghii*. Pw = *Pinus wallichiana*.
Associated mycorrhizal host based on field observation only:
An = *Alnus nepalensis*. Lh = *Larix himalaica*.

CHAPTER 2. THE GENUS SUILLUS IN WESTERN VIRGINIA: CHECKLIST AND HOST RELATIONSHIPS.

Comments on the genus Suillus

Please see Chapter 1. As mentioned there, despite the large amount of work done on *Suillus* in North America, species concepts and nomenclatorial usage are in disagreement for certain species. Two fundamental schools exist regarding eastern North American *Suillus* taxonomy: Smith and Thiers (1964, 1971) as contrasted to Singer (1945b and later publications) and Snell and Dick (1970). In this overview of Virginia *Suillus* species I have chosen basically to follow the Singer, Snell and Dick "school" for two reasons: their greater stress on ecology as an important character and their species concepts which are more clear-cut and thus easier to apply. Palm and Stewart (1986) have studied the nomenclature of some North American *Suillus* species; the names used in this chapter follow their decisions.

Materials and Methods

Specimens of *Suillus* were collected in the western part of Virginia during 1982-86. Basidiocarps were preserved by drying. Collections will be deposited at VPI (Virginia Tech Herbarium).

The holdings of VPI were examined for collections of *Suillus* from Virginia.

Results

Twelve species of *Suillus* were collected in western Virginia (Table 2.1). Twelve are also known from North Carolina (Coker and Beers, 1943; Grand, 1970, 1981) with nine of the 12 in common with the western Virginia list presented here. The other three species reported from North Carolina are *S. cothurnatus* Singer, *S. decipiens* (Berk. & Curt.) Kuntze, and *S. placidus* (Bonorden) Singer. All three certainly also occur in Virginia, the former two in the tidewater region and the latter one in western Virginia. A survey of herbaria might turn up such records for Virginia. Other potential additions to Virginia's *Suillus* mycota are introduced species associated with *Larix* which is planted as an ornamental in the state. Although I have found *S. grevillei* (Klotzsch) Singer with *Larix* in West Virginia outside the natural range of *Larix*, I have not yet found this species in Virginia. The three species known from Virginia but not North Carolina are *S. flavoluteus, S. salmonicolor*, and *S. tomentosus*.

A nomenclatorial note comparing the North Carolina reports with the names used in this chapter is necessary. The species herein called *S. spraguei* and *S. subluteus* are called *S. pictus* (Peck) Smith & Thiers and *S. subalutaceus* Smith & Thiers respectively in the papers on *Suillus*

in North Carolina. The reports of *S. salmonicolor* (Virginia) and *S. cothurnatus* (North Carolina) may also prove to be conspecific.

Host relationships

One set of Virginia's *Suillus* mycota is consistently associated with *Pinus strobus*. These *Suillus* species are *S. americanus, S. granulatus* ssp. *snellii, S. spraguei,* and *S. subluteus*. A second and larger set is associated with two- and three-needled pines: *S. brevipes, S. hirtellus, S. luteus, S. salmonicolor* and *S. tomentosus*. A third set, *S. flavoluteus* and *S. subaureus*, appears to be associated with angiosperms which is very unusual in the genus *Suillus*. *Suillus punctipes* is associated with pines in Virginia and is found in sites with both soft and hard pines. Grand (1970) reports *S. punctipes* is a two- and three-needle pine associate in North Carolina; Snell and Dick (1970) describe the fungus as a five-needle pine (*P. strobus*) associate. Based on host patterns in the genus *Suillus* it is doubtful that *S. punctipes* is both.

Checklist of Virginia species of Suillus

Suillus americanus (Peck) Snell

Habitat: On ground, ectomycorrhizal[1] with *Pinus strobus*; common in ornamental settings, less common in forests.
Material studied from Virginia: Bath Co. - VC 248, Cow Pasture River Farm, 30 Oct 1982. Giles Co. - VC 567, Jefferson National Forest, Kelly Flats, 2 Sep 1983. Montgomery Co. - VC 234, Blacksburg, Plaza One, 23 Oct 1982; VC 626, Christiansburg, 4 Oct 1983; VC 650, Blacksburg, Plaza One, 9 Oct 1983; VC 651, Blacksburg, VPI & SU PPWS Glade Road Research Complex, 9 Oct 1983; VC 653, Blacksburg, Marriott Motel, 9 Oct 1983; VC 734, Poverty Creek Hollow, 26 Jun 1984; VC 885, Blacksburg, VPI & SU PPWS Glade Road Research Complex, 20 Oct 1984; VC 886, Blacksburg, Capri Twin Theatre, 20 Oct 1984; VC 1600, Blacksburg, Carroll Dr., 7 Jun 1986. Pulaski Co. - OKM 15180, Claytor Lake State Park, 5 Oct 1975. Roanoke Co. - OKM 9049, Salem, 6 Oct 1971.

Suillus brevipes (Peck) Kuntze

Habitat: On ground, ectomycorrhizal[1] with hard pines, common at ornamental and disturbed sites.
Material studied from Virginia: Montgomery Co. - VC 233, Blacksburg, Plaza One, 23 Oct 1982; VC 622, Blacksburg Airport, 25 Oct 1983; VC 628, Blacksburg, Plaza One, 5 Oct 1983; VC 654, VPI & SU Horticulture Farm off US 460, 13 Oct 1983; VC 687, Blacksburg, Estes Farm, 21 Nov 1983; VC 889, Blacksburg Airport, 20 Oct 1984.

Suillus flavoluteus (Snell) Singer

Habitat: On ground associated with angiospermous trees, rare.
Material studied from Virginia: Augusta Co. - VC 870, George Washington National Forest, 28 Aug 1984.

Suillus granulatus (L.:Fr.) Kuntze ssp. *snellii* Singer

Habitat: On ground, ectomycorrhizal[1] with *Pinus strobus*, common in forests and ornamental plantings.
Material studied from Virginia: Bath Co. - VC 247, Cow Pasture River Farm, 30 Oct 1982. Giles Co. - VC 1611, Jefferson National Forest, Interior, 3 Aug 1986; VC 1629, Rte. 613 Stony Creek North Fork crossing, 18 Sep 1986. Montgomery Co. - VC 235, Blacksburg, Plaza One, 23 Oct 1982; VC 624, Blacksburg, Palmer Drive, 2 Oct 1983; VC 632, Jefferson National Forest, Poverty Creek, 7 Oct 1983; VC 652, Blacksburg, Capri Twin Theatre, 9 Oct 1983; VC 735, Poverty Creek Hollow, 26 Jun 1984; VC 887, Blacksburg, Capri Twin Theatre, 20 Oct 1984; VC 1601, Blacksburg, Plaza One, 8 Jun 1986; VC 1602, Blacksburg, Capri Twin

Theatre, 8 Jun 1986. Pulaski Co. - OKM 15182, Claytor Lake State Park, 5 Oct 1975. Roanoke Co. - VC 837, Poor Mountain along Laurel Creek & Rte. 631, 5 Aug 1984. Smyth Co. - VC 611, Clinch Mountain Wildlife Management Area, Ray Bottoms, 17 Sep 1983.

Suillus hirtellus (Peck) Kuntze

Habitat: On ground, associated with hard pines in forests.
Material studied from Virginia: Appomattox Co. - VC 660, 23 Oct 1983; VC 661, 23 Oct 1983. Franklin Co. - VC 1634, Booker T. Washington National Monument, Hardy, 11 Oct 1986. Montgomery Co. - VC 827, Brush Mountain, Preston Forest, 31 July 1984.

Suillus luteus (L.:Fr.) S. F. Gray

Habitat: On ground, ectomycorrhizal[1] with introduced hard pines in ornamental settings. *Suillus luteus* itself must have been an introduction.
Material studied from Virginia: Montgomery Co. - VC 232, Blacksburg, Gables Shopping Center, 23 Oct 1982; VC 630, Blacksburg, Plaza One, 5 Oct 1983; VC 700, Blacksburg, Ambler-Johnson Hall, 7 Dec 1983.

Suillus punctipes (Peck) Singer

Habitat: On ground, associated with pines in forests, especially low areas.
Material studied from Virginia: Giles Co. - VC 620, Jefferson National Forest, Kelly Flats, 27 Sep 1983; VC 1628, Rte. 613 Stony Creek North Fork crossing, 18 Sep 1986.

Suillus salmonicolor (Frost) Halling
=*S. pinorigidus* Snell & Dick

Habitat: On ground, associated with hard pines in forests.
Material studied from Virginia: Augusta Co. - VC 871, George Washington National Forest, 28 Aug 1984.

Suillus spraguei (Berk. & Curt.) Kuntze
= *S. pictus* (Peck) Smith & Thiers

Habitat: On ground, ectomycorrhizal[1] with *Pinus strobus* in forests, not seen in ornamental plantings.
Material studied from Virginia: Bath Co. - VC 246, Cow Pasture River Farm, 30 Oct 1982. Giles Co. - VC 778, Rte. 714 Laurel Creek crossing, 20 Jul 1984; VC 1610, Interior, 3 Aug 1986. Pulaski Co. - MB/PD 85, Foster Mine Site, 2 Aug 1985. Roanoke Co. - VC 840, Rtes. 631 & 637 intersection, 5 Aug 1984.

Suillus subaureus (Peck) Snell

Habitat: On ground, associated with angiospermous trees in forests, rare.
Material studied from Virginia: Augusta Co. - VC 869, George Washington National Forest, 28 Aug 1984.

Suillus subluteus (Peck) Snell sensu Snell and Dick (1970)

Habitat: On ground, associated with *Pinus strobus* in forests, not seen in ornamental plantings.
Material studied from Virginia: Albemarle Co. - OKM 15895, Charlottesville, 15 Oct 1976; Floyd Co. - MB 111, Beaver Creek, 6 Nov 1984. Giles Co. - VC 1627, Rte. 613 Stony Creek North Fork crossing, 18 Sep 1986. Montgomery Co. - VC 902, Jefferson National Forest, Pandapas Pond, 1 Nov 1984.

Suillus tomentosus Singer, Snell & Dick

Habitat: On ground, associated with hard pines in forests. Also collected in angiospermous forest where pines were not seen.
Material studied from Virginia: Augusta Co. - VC 868, George Washington National Forest, 28 Aug 1984. Franklin Co. - VC 1633, Booker T. Washington National Monument, Hardy, 11 Oct 1986. Montgomery Co. - VC 686, Blacksburg, Estes Farm, 19 Nov 1983; VC 804, Brush Mountain, Rte. 780 near Mission Chapel, 25 Jul 1984.

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[1]Ectomycorrhizal relationship confirmed (Palm and Stewart, 1984b).

Discussion

Species of *Suillus* fruit commonly and abundantly in western Virginia, usually in association with pines. Their abundance suggests that they are important ectomycorrhizal partners for the pines, both in forests and in ornamental plantings. Some species appear to be restricted to forests; others are more commonly found in ornamental plantings. Some, like *S. granulatus*, occur commonly in both.

The species of *Suillus* in Virginia occur generally in eastern North America. Many, if not most, of them may be restricted to eastern North American in their natural distribution although they often have close relatives around the world. One species, *S. luteus*, is introduced to Virginia. Although eastern North American *Suillus* species like *S. spraguei* have been reported from around the Northern Hemisphere, comparative studies are needed to determine if they are truly conspecific or closely related taxa. If conspecific, studies are needed to determine where they are native and where they are introduced. Experience in South America and Australia shows how easy *Suillus* species are to introduce, presumably when soil or ectomycorrhizae are moved. In southern Illinois which has only one native pine (Critchfield and Little, 1966) and no native larches, and thus probably has a limited indigenous *Suillus* mycota, the introduction of new pines has also resulted in the introduction of additional species of *Suillus* (Sundberg, 1981).

Table 2.1. Species of *Suillus* in western Virginia.

Suillus species	Associated trees	Counties of Virginia[1]
S. americanus	*Pinus strobus*	Bath, Giles, Montgomery, Pulaski, Roanoke.
S. brevipes	*P. virginiana*, hard pines	Montgomery.
S. flavoluteus	Angiosperms	Augusta.
S. granulatus ssp. *snellii*	*P. strobus*	Bath, Giles, Montgomery Pulaski, Roanoke, Smyth.
S. hirtellus	Hard pines	Appomattox, Franklin, Montgomery.
S. luteus	*P. nigra*	Montgomery.
S. punctipes	Pines	Giles.
S. salmonicolor	*P. rigida*, *P. pungens*	Augusta.
S. spraguei	*P. strobus*	Bath, Giles, Pulaski, Roanoke.
S. subaureus	Angiosperms	Augusta.
S. subluteus	*P. strobus*	Albemarle, Floyd, Giles, Montgomery.
S. tomentosus	*P. pungens*, *P. virginiana*	Augusta, Franklin, Montgomery.

[1]Only counties with voucher specimens are listed.

CHAPTER 3. SCLEROTIAL PRODUCTION AND ECOLOGY OF BOLETINELLUS MERULIOIDES

Small black spheres discovered in West Virginia in association with basidiocarps of *Boletinellus merulioides* (Schwein.) Murrill turned out to be fungal sclerotia produced by *B. merulioides* and proved useful in studying the ecology of this fungus. Confirmation that the sclerotia were indeed formed by *B. merulioides* (Cotter and Miller, 1985) and studies on the ecology of *B. merulioides* (Cotter and Bills, 1985) are presented in sections 3.1 and 3.2 of this chapter respectively. Gerald Bills closely collaborated on the ecological work.

3.1 SCLEROTIA OF BOLETINELLUS MERULIOIDES IN NATURE

Boletinellus merulioides forms sclerotia in culture (Pantidou, 1961), but the sclerotia have not been reported from nature. *Boletus porosporus* (Imler) Watling produced sclerotia in mycorrhizal synthesis trials (Giltrap, 1979). We know of no other reports of sclerotial formation by boletes. *Paxillus involutus* (Batsch:Fr.)Fr. forms sclerotia in culture and in nature (Laiho, 1970) and is in an agaric genus considered closely related to the boletes (Singer, 1975).

The purposes of this study were to substantiate that the sclerotia associated with basidiocarps of *Boletinellus merulioides* were formed by this fungus, to determine the geographic range of the sclerotia, and to test the viability of the sclerotia after overwintering.

Materials and Methods

Field collections

Basidiocarps of *Boletinellus merulioides* and sclerotia were collected at five sites. Sclerotia were also collected at a sixth site (VC 881) where *B. merulioides* was not fruiting and had not been collected before. *Fraxinus americana* L. occurred at all sites in combination with mixed angiosperm trees or mixed angiosperm and conifer trees. Collections were made as follows: Michigan: Grand Traverse Co., 18 Aug 1983, VC 559 (basidiocarps) and from the same site 4 Sep 1984, VC 877 (sclerotia); New Jersey: Hunterdon Co., Califon, 7 Oct 1984, VC 881 (sclerotia); New Jersey: Hunterdon Co., Vorhees State Park, 7 Oct 1984, VC 882; Vermont: Bennington Co., 10 Sep 1984, VC 878; Virginia: Montgomery Co., 5 Aug 1984, VC 838; and West Virginia: Pocahontas Co., 7 Sep 1983, VC 587. Additional current-year sclerotia (28 Oct 1984, VC 892-895) and overwintered sclerotia (23 Apr 1984, VC 729, and 13 Jun 1984, VC 730) were collected at the West Virginia site. Collections will be deposited at BPI. Cultures VC 587.f (basidiocarp-derived) and VC 587.s1 (sclerotium-derived) have been deposited in the Virginia Tech mycology culture collection as VT 1782 and VT 1783 respectively and also in the American Type Culture Collection as ATCC 62612 and ATCC 62613 respectively.

Laboratory cultures

Cultures were obtained from basidiocarps by transferring pieces of pileus trama into 100x15-mm polystyrene Petri dishes with Hagem's agar (Molina and Palmer, 1982) modified to contain 4 g/l malt extract, 1 g/l yeast extract, 100 µg/l thiamine, 5 µg/l biotin, and 0-10 mg/l benomyl. Cultures were obtained from sclerotia by agitating the sclerotia in sterile distilled water to remove adhering soil and organic matter, surface-sterilizing with 30% hydrogen peroxide for 1-60 min or with 1% NaOCl (1 part household bleach : 4 parts water) for 5-10 min and then placing them with or without rinsing on the modified Hagem's agar. Highest germination rates were achieved by surface sterilization for 5 min in 30% hydrogen peroxide without rinsing or for 5-10 min in 1% NaOCl with rinsing plus placement on Hagem's agar with 10 mg/l benomyl. Sclerotial isolations were done within five days of collection except those from VC 729 which were done 6 months after collection. The latter sclerotia were stored at 4 C and exposed to two periods over 30 C when the refrigerator malfunctioned during the fifth month of storage. Incubation of all isolation Petri dishes was in the dark at 24 ± 2 C.

Herbarium collections

All collections of *B. merulioides* in ACAD, BPI, NY, TENN and VPI were examined for sclerotia and those with *B. merulioides* sclerotia were annotated.

Results

Description and identification of the naturally occurring sclerotia

Sclerotia associated with basidiocarps of *Boletinellus merulioides* in the forest had the same morphology (Fig. 3.1) and anatomy as those formed in culture and described by Pantidou (1961).

Macroscopic features of natural sclerotia 0.1-0.3(0.6) cm x 0.1-0.3(0.35) cm; globose (usually 0.2-0.3 cm in diam) to ellipsoidal (e.g. 0.25 x 0.2 or 0.3 x 0.25 cm), flat-sided, lobed, or both when densely aggregated, irregularly shaped when fused; outside surface smooth, shiny when moist, dull and usually wrinkled or dimpled when dry, black to dark brown, sometimes with a reddish-brown tinge; scattered surface hyphae giving young sclerotia a golden-brown cast; rind dark; medulla white or light-colored, darkening in age to pale brown, brownish black, grayish black or black, sometimes developing an olive cast; center hollow, sometimes lined with orange-brown hyphae; inner surface often becoming black due to formation of a rind-like layer; fresh sclerotia exuding fluid when cut; young sclerotia connected by branched, golden-brown, orange-brown to reddish brown hyphal strands.

Microscopic features of natural sclerotia Rind very dark, pseudoparenchymatous, 5-7 cells thick; medulla pseudoparenchymatous, cell walls hyaline in young sclerotia, darkening in age to yellow-brown or

orange-brown, intersections of more than 2 cell walls often dark brown in old sclerotia; inner rind-like layer, if present, partially or completely lining interior and consisting of very dark, amorphous material usually enveloping innermost layer of pseudoparenchymatous cells of medulla, cylindrical hyphae sometimes present within inner rind-like layer; clamp connections present.

The cultures obtained from basidiocarps of *B. merulioides* and from sclerotia were alike (Fig. 3.3) and matched the description of *B. merulioides* in culture (Pantidou, 1961). Paarige branching with three clamps (see Pantidou, 1961), sclerotia (Fig. 3.3), brownish exudate droplets from developing sclerotia, and colored aerial hyphae make *B. merulioides* distinctive in culture.

Based on the morphological, anatomical, and cultural evidence presented above, we conclude that *B. merulioides* formed the sclerotia collected at the six forest sites.

Occurrence and distribution

Sclerotia of *B. merulioides* (Fig. 3.2; Coker and Beers, 1943: pl. 58, upper left basidiocarp) were present at all five sites (representing five U.S. states) where basidiocarps of the fungus were collected. Sclerotia most commonly occurred between the mineral and organic layers of the soil and at one site with sandy soil within the mineral layer as well. They

were often attached to basidiocarp bases and also on, within, and under rotten wood. Sclerotia occurred singly and in aggregations that sometimes exceeded 100 sclerotia.

One hundred and one of the 234 collections (43%) of *B. merulioides* examined contained sclerotia of the type formed by this fungus. Sclerotia were found only in collections having complete stipe bases with adhering soil or rotten wood. Considering only the 52 collections with five or more basidiocarps with stipe bases and some substrate, 38 (73%) had sclerotia. The 101 collections with sclerotia had 1-195 sclerotia and came from throughout the range of the fungus in eastern North America from Nova Scotia west to Minnesota and Missouri and south to Florida (Fig. 3.4). *Boletinellus merulioides* also occurs in Mexico but is rare there (Guzman, 1973); the single collection from Mexico (BPI) examined had no sclerotia. *Boletinellus merulioides* has also been reported from Japan (Hongo, 1982) but may have been introduced there. The collections on which a report of *B. merulioides* from China was based (Zang, 1986) were examined in Kunming and found to be a species of *Suillus*. Sclerotia occurred in collections over the range of basidiocarp collection dates (19 June to 24 November). Most (83%) collections were made from 15 July to 30 September.

Most of the herbarium labels (56%) said nothing about the vegetation associated with *B. merulioides*. *Fraxinus*, as *Fraxinus* or ash, occurred on 79% (56 of 71) of the labels which mentioned vascular plant genera.

The only other vascular plant genera listed on more than one label were *Tsuga*, *Acer* and *Betula* which occurred on six, five and three labels respectively. Often when these trees occurred on a label *Fraxinus* was also listed. In my collecting experience, *Fraxinus* trees have always been nearby wherever I have collected *B. merulioides*. The distribution of *B. merulioides* parallels the distribution of the eastern North American specis of *Fraxinus*, especially *F. americana*.

Germination and overwintering

The sclerotia of *B. merulioides* germinated only into mycelium. Mycelium emerged from multiple locations on each sclerotium.

Germination rates for fresh *B. merulioides* sclerotia from the forest were usually over 90% and often 100%. Forty-two of the 109 overwintered sclerotia collected in June (VC 730) and surface-sterilized in various ways germinated. The highest germination level for a treatment was 71% indicating that most sclerotia were still viable after overwintering. One-third of the 52 overwintering sclerotia collected in April (VC 729) and tested six months later germinated.

Discussion

The sclerotia formed by *Boletinellus merulioides* in nature are persistent, resistant, compact vegetative structures differentiated into

a rind and a medulla. Thus they satisfactorily fit the term "sclerotium" (Snell and Dick, 1971; Willetts, 1978).

Sclerotia are probably significant in the life cycle of *B. merulioides* because they are formed abundantly throughout its geographic range. The sclerotia may be universally present wherever the fungus grows because all isolates readily form sclerotia (our data; Pantidou, 1961). The persistent sclerotia allow *B. merulioides* to survive certain adverse conditions such as cold. The *B. merulioides* sclerotia should have a high inoculum potential (Garrett, 1956) enabling the fungus to colonize nearby substrates rapidly when favorable conditions return.

The sclerotia of *B. merulioides* are easily recognized in the forest by their size, color, hollowness (Fig. 3.1), and when young by the brownish hyphal strands which interconnect them. Because the sclerotia are present year-round and easily recognized, the presence of *B. merulioides* can be determined in a forest without relying on the ephemeral basidiocarps as was illustrated by the Califon, New Jersey, site. This provides an opportunity for ecological studies not possible with other boletes.

3.2 COMPARISON OF SPATIAL PATTERNS OF SEXUAL AND VEGETATIVE STATES OF BOLETINELLUS MERULIOIDES

Quantitative studies of macrofungal communities (Lange, 1948; Hering, 1966; Petersen, 1977; Arnolds, 1981; Fogel, 1982) have often relied on basidiocarp numbers or basidiocarp biomass in sample areas of fixed dimensions. A fundamental assumption of these studies is that the relative production of basidiocarps among fungal species in some way reflects their dominance or mycelial biomass. Only some limited evidence indicates this is true (Laiho, 1970; Newell, 1984). A second assumption - that the absence of basidiocarps at a location over several years reliably indicates that the vegetative state is absent - has rarely been directly tested.

As shown above, *B. merulioides* (Schwein.) Murrill produces abundant, persistent sclerotia in nature which can be easily found and recognized in the field. Mapping sclerotia and comparing their spatial pattern with that of basidiocarps offered a unique opportunity to test the hypothesis that repeated observations of basidiocarps over several years can accurately estimate the spatial pattern of a fungus. In addition, the question of whether density of basidiocarp occurrence is related to the density of the occurrence of vegetative mycelium as indicated by sclerotia is addressed. The spatial pattern of *B. merulioides* is described in relation to its associate, *Fraxinus americana*. Finally, the nature of the

relationship between *B. merulioides* and *Fraxinus* is tested using growth pouches.

Materials and Methods

The study area was a stand of 66-year-old hardwood trees on the crest of Kennison Mt (elev. 1207 m) in the Monongahela National Forest, Pocahontas Co., WV, U.S.A. (38° 11' N lat., 80° 17' W long.). The soil at the site was a shallow, rocky, well-drained clay-loam with a pH of 3.8 ± 0.3 S.D. (n=8) and organic matter content of 8.5 ± 3.7 % S.D. (n=8). The canopy was dominated by *Prunus serotina* Ehrh., *Acer saccharum* Marsh., *Fagus grandifolia* Ehrh., *Fraxinus americana* and *Betula alleghaniensis* Britt. Other *Acer* spp. were abundant in the understory. Two permanent plots, 5 and 6, were established as representative samples of a mixed hardwood forest. Each plot was 16 x 16 m in size and was further subdivided into 64 2 x 2 m subplots for sampling; each subplot was identified by a column letter and row number (Fig. 3.6). Three *Fraxinus* trees were located within plot 6 (Fig. 3.6), one in subplot d7 (49.5 cm DBH (diam at breast height)), another in subplot h4 (27 cm DBH), and the third in subplot h5 (18.5 DBH). A large *Fraxinus* tree (68.5 cm DBH) was also located 2.6 m outside the western corner of plot 6. Plot 5 had one *Fraxinus* tree (26.5 cm DBH). These plots were part of a fungal ecology study by Bills (Bills *et al.*, 1986).

Both plots were visited eight to ten times at 7- to 17-day intervals during each of the four growing seasons of 1981-4 and the locations of basidiocarps mapped. Basidiocarps occurred from mid-July to September. In October 1984 the litter layer was raked off the plots row by row and the surface of the mineral soil examined for sclerotia. Those of *B. merulioides* were identified based on their size, shape, color and internal characteristics; their identity was confirmed by culturing. Sclerotial density within each subplot was rated on a scale from 0 to 4: 0 = absent; 0.5 = rare; 1 = low (only a few aggregations of sclerotia); 2 = medium (scattered, not numerous); 3 = high (numerous throughout subplot); 4 = very high (dense aggregations throughout subplot).

Sclerotia were collected and cultured to confirm their identity and test their viability. Ten sclerotia from each of subplots d5, d7, g6 and h5 were agitated in sterile water to remove dirt and then surface sterilized in 30% hydrogen peroxide for five minutes. Without rinsing, sclerotia were placed, five per 100x15-mm polystyrene Petri dish, on Hagem's agar (Molina and Palmer, 1982) modified to contain 10 ppm benomyl, 100 µg/l thiamine, 5 µg/l biotin, 1 g/l yeast extract and 4 g/l malt extract, and incubated at 24 C ± 2 C in the dark for 26 days. Isolations from all sclerotia from subplots d7, g6 and h5 and from one of ten sclerotia from subplot d5 yielded colonies of *B. merulioides*. The overall isolation frequency was 77.5%. The reason sclerotia from subplot d5 germinated at such a low rate is not known. No other fungus was isolated more than once except a hyaline fungus, perhaps in the Pythiaceae, which

was isolated from five of the subplot d5 sclerotia. The high isolation frequency of *B. merulioides* confirmed that we were observing sclerotia of *B. merulioides*.

The vegetative compatibilities of sclerotium-derived isolates from the four subplots were tested using the method of Rayner and Todd (1982) to determine how many individuals of *B. merulioides* were represented. Two sclerotium-derived isolates each from subplots d7, g6 and h5 and one from subplot d5 were used. A sclerotium-derived isolate from New Jersey (VC 882.s2) served as a genetically different control. All isolates were dikaryons based on the presence of clamp connections. Isolates were paired in all possible combinations including against themselves; the pairings were replicated. For each pairing 7-mm-diam agar plugs from colony margins were placed upside down and 1 cm apart on modified Hagem's agar in 100x15-mm polystyrene Petri dishes. Three pairings were put into each dish. Interactions were rated at days 16, 24 and 32. Evidences for antagonism were 1) a distinct dark pigment line where the colonies of the two isolates met and 2) puffy aerial mycelium where colonies met. Compatible interactions lacked pigment lines and aerial puffs along the line where the colonies met; in compatible interactions the two colonies often knitted together. An antagonistic interaction indicates that the two isolates are genetically distinct dikaryons and a compatible interaction suggests that the two isolates are genetically identical (the same 'individual' or clone) (Rayner and Todd, 1982). Stenlid (1985) compared vegetative compatibility patterns, sexual compatibility patterns

and malate dehydrogenase isoenzyme patterns for isolates of *Heterobasidion annosum* (Fr.) Bref. and found that vegetative incompatibility was the easiest and most reliable method for identification of clones.

The growth pouch method of Fortin's laboratory (Fortin *et al.*, 1980; Godbout and Fortin, 1983) modified as follows was used to test the nature of the relationship between *B. merulioides* and *Fraxinus*. About four-month old seedlings of *F. pennsylvanica* Marsh., which had been grown in unfertilized sand flats in the greenhouse, were obtained from Carter Johnson. They were 1.5 to 6.5 cm tall with 1-3 primary leaf pairs. Each of 20 growth pouches received 15 ml of a modified Melin-Norkrans solution (MMN) (Molina and Palmer, 1982) further modified to contain 1 ml/l of a micronutrient solution (Chen *et al.*, 1961), 67 mg/l ferric sodium ethylene diamine di-(o-hydroxyphenylacetate) as the iron source, and 1 mg/l benomyl but without NaCl, malt extract, and glucose. One *Fraxinus* seedling was then added to each pouch. Growth pouches were hung on glass rods and suspended in buckets which were kept in a growth chamber with 14-16 hrs of light per day. Pouches were setup on 30 Jul 1984. Due to the growth chamber failing, the pouches were kept on a laboratory bench near a window for the last two months of the four-month experiment. Pouches were watered with double distilled water as needed. Eight days after the seedlings were put into the pouches, *B. merulioides* inoculum as five sclerotia or five 7-mm-diam agar plugs per pouch was added. The plugs had been previously cut from the margin of colonies and allowed to

fuzz out for one week. The four treatments, each with five replications, were no fungus, sclerotia from cultures of VC 587f, bleach-treated field sclerotia of VC 838, and agar plugs of VC 559. At this time foam spacers and 10 ml of an aqueous 5 ppm benomyl solution were added to each of the 20 pouches. The MMN nutrient solution was replaced at four and eight weeks. At eight weeks 5 ml of a 1.5 g/l aqueous KNO_3 solution were added to each pouch. Pouches were periodically observed over the four months and notes on the growth and vigour of the seedlings and fungi taken. Roots were free-hand sectioned to look for fungal penetration.

Agar plugs of *B. merulioides* were added to similarly handled growth pouches of *Pinus strobus*. The fungus grew for one week, and then the chamber overheated killing the fungus.

Results

The vertical profile of *B. merulioides* sclerotia within plot 6 is depicted in Fig. 3.5. Sclerotia were located between the organic and mineral layers of the soil, beneath, within and on rotten wood, or attached to the bases of basidiocarps. Basidiocarps (136 over 4 years) were recorded for 23 (36%) subplots and sclerotia recorded for 34 (53%) subplots. Thus *B. merulioides* basidiocarp frequency was 68% of the sclerotial frequency in plot 6. All 23 subplots positive for basidiocarps were also positive for sclerotia (Fig. 3.6).

Spatial patterns of basidiocarps and of sclerotia were very similar; both showed two main patches of occurrence (Fig. 3.6). Subplots with sclerotia but without basidiocarps occurred at the periphery of the patches. Basidiocarp frequency varied greatly from year-to-year (Fig. 3.7). The year with the most rainfall during the growing season, 1982, had fewer basidiocarps than 1981 yet basidiocarp frequency for 1982 gave the best estimate of sclerotial frequency as measured in 1984. Basidiocarp frequency for the driest year, 1983, gave a poor estimate. Bills *et al.* (1986) present data on the phenology of the basidiocarps.

Basidiocarp density summed over the 4 years and sclerotial density in 1984 were positively correlated (Spearman's $\rho = 0.70$, $P = 0.0001$). Most basidiocarps (82%) occurred within subplots with sclerotial densities of 2 or higher. The remaining basidiocarps (18%) occurred in subplots with a sclerotial density of 1.

We conclude that the fungus is perennial and that the spatial pattern of its mycelium changed little during 1981-4. Basidiocarps fruited across the full extent of plot 6 in 1981, 1982 and 1984. Spot sampling in April 1984 of sclerotia which had overwintered from 1983 revealed that they were generally present in the subplots surrounding the *Fraxinus* tree in subplot d7 even though basidiocarps rarely occurred here during 1983. The only indication of change was a possible receding in subplot h4, where basidiocarps were abundant in 1981, but only one occurred in 1982 and none

in 1983 and 1984. In 1984 sclerotia were restricted to one corner of the subplot.

Each of the two patches of *B. merulioides* in plot 6 surrounded *Fraxinus* trees (Fig. 3.6). The densities of sclerotia and of basidiocarps were highest near the trees and decreased outward (sclerotia: Spearman's ρ = -0.35, P = 0.014; basidiocarps: Spearman's ρ = -0.39, P = 0.006). The extent of sclerotial occurrence corresponded to the crown coverages of the *Fraxinus* trees and probably was confined to the root zones of these trees. Sclerotia were continuously and densely present between plot 6 and the *Fraxinus* tree outside the plot. This tree no doubt contributed to the density of *B. merulioides* in that corner of plot 6. Plot 5 had one *Fraxinus* tree, but neither basidiocarps nor sclerotia occurred in that plot. Sclerotia were also associated with four of six additional *Fraxinus* trees examined near plots 5 and 6.

The vegetative compatibility tests confirmed that each of the two large patches of sclerotia represented a single dikaryotic individual and that the two patches were different individuals (Table 3.1). The New Jersey isolate, as expected, represented another genetically distinct dikaryon. All 16 self pairings and all 18 pairings of different isolates from the same patch were compatible. All 24 pairings between patches and all 14 between New Jersey and West Virginia isolates were incompatible with antagonistic interactions.

The intensity of the antagonistic vegetative interaction varied greatly, even between replicates of the same pairing. The New Jersey isolate probably was genetically most different among the isolates tested, but the intensity of the antagonistic interactions between New Jersey and West Virginia isolates was not different ($\alpha = 0.05$) from the intensity of the antagonistic reactions between isolates from different West Virginia patches. In other basidiomycetes, such as *Fomitopsis cajanderi* (Karst.) Kotl. and Pouz., *Heterobasidion annosum* and *Coriolus versicolor* (L.:Fr.) Quél., the intensity of the antagonistic interaction increases as isolates are more distantly related (Rayner and Todd, 1982; Stenlid, 1985).

Both the roots of the *Fraxinus pennsylvanica* seedlings and the mycelium of *B. merulioides* derived from basidiocarps and from sclerotia grew well in all growth pouches but the two organisms grew independently. No mycorrhizae formed nor did the fungus cause lesions on the roots. Hyphae grew over roots but did not form a mantle and did not penetrate the roots. *Boletinellus merulioides* formed new sclerotia in most of the fifteen pouches into which it was inoculated.

Boletinellus merulioides grew well in the pouches with *Pinus strobus* but no mycorrhizae formed before the chamber overheated after one week. With *Suillus*, ectomycorrhizae can form within a week of inoculating growth pouches.

Discussion

Basidiocarp density and frequency were probably underestimated in this study. Richardson (1970) estimated that with weekly or two-weekly sampling intervals one-fourth to one-half of fungal basidiocarps would not be observed. Bolete basidiocarps are relatively short lived, so if our sampling intervals had been shorter, basidiocarp frequency might have provided an even better estimate of sclerotial frequency.

Advantages of mapping the spatial pattern of *B. merulioides* by observing sclerotia are that their occurrence should closely match the occurrence of the vegetative mycelium within the forest, that they are present year-round and occur in quantity even in dry years, and that repeated sampling is not necessary as with basidiocarps because a single observation of sclerotia reveals the spatial pattern of the fungus. Another possible advantage is that it may permit recognition of individuals in the forest.

Each of the two patches of *B. merulioides* (Fig. 3.6) consisted of a large area over which sclerotia were continuously present, and each probably represents a single individual. This is supported by the vegetative compatibility tests; all isolates from within a patch were compatible, and all confrontations between isolates from different patches were incompatible.

Our results demonstrate that the absence of basidiocarps of *B. merulioides* over a prolonged period indicates either absence of the fungus (plot 5) or its presence at low levels (plot 6) and that frequency of basidiocarps in contiguous subplots is a more appropriate measure of mycelial spatial pattern than basidiocarp numbers (or basidiocarp biomass). *B. merulioides* was most abundant in 1981 based on basidiocarps; but 1982 basidiocarps provided a better estimate of the mycelial spatial pattern because they occurred in more subplots. The resolution achieved by frequency can be increased or decreased by varying the subplot size and shape (Grieg-Smith, 1983).

Boletinellus merulioides has long been known to fruit in association with *Fraxinus* (Singer, 1945b; Snell and Dick, 1970). Our results confirm their association and furthermore demonstrate that the vegetative state of the fungus is closely associated with *Fraxinus* and occurs only within the limits of its root zone. Although *B. merulioides* is obligately associated with *Fraxinus*, the reverse is not true because sclerotia were not associated with all *Fraxinus* trees in the stand. The continuous spatial pattern of *B. merulioides* over the root zone of *Fraxinus* represents a very different pattern than the rings of ectomycorrhizal fungi surrounding trees described by Last *et al.* (1983).

Claims by Singer *et al.* (1983) and others that *B. merulioides* is ectomycorrhizal with *Fraxinus* have never been substantiated. In my growth pouch experiment, *B. merulioides* did not form ectomycorrhizae with

Fraxinus pennsylvanica. Nor did *B. merulioides* act as a direct parasite and attack the *Fraxinus* seedlings. Pierre Dery of our laboratory repeated this experiment with the same results. *B. merulioides* did not form mycorrhizae with *Quercus rubra* L. (Beckjord and McIntosh, 1983), with *Populus tremuloides* Michx. (Godbout and Fortin, 1985), or with *Alnus crispa* (Ait.)Pursh. or *A. rugosa* var. *americana* (Regel)Fern. (Godbout and Fortin, 1983). However Hatch and Hatch (1933) reported that they synthesized ectomycorrhizae between *B. merulioides* and *Pinus strobus* L. My limited attempt to repeat this in growth pouches did not result in formation of mycorrhizae. *Boletinellus merulioides* is not known to fruit in association with species of *Quercus*, *Populus*, *Alnus* or *Pinus*. Ruling out a mycorrhizal or a directly parasitic role for *B. merulioides* leaves its ecological role as an enigma. However, Brundrett and Kendrick (1987) have recently proposed that *B. merulioides* is indirectly parasitic on *Fraxinus* by feeding on honeydew excreted by the aphid *Meliarhizophagus fraxinifolii* (Riley) which feeds on *Fraxinus* roots (and leaves). Brundrett and Kendrick further proposed that the fungus's hollow sclerotia serve as protective shelters for the aphid, and therefore the relationship between *B. merulioides* and the aphid is mutually beneficial but detrimental to *Fraxinus*.

The generic placement of *B. merulioides* is controversial. Smith and Thiers (1971) recognize the monotypic genus *Boletinellus* whereas Singer (1986) synonymizes *Boletinellus* with the genus *Gyrodon*. I have followed Smith and Thiers because of these unique characteristics of

B. merulioides: 1) production of sclerotia, 2) association with the endomycorrhizal genus *Fraxinus*, and 3) possible association with an aphid. *Gyrodon lividus* (Bull. ex. Fr.)Sacc., the type species of *Gyrodon*, does not produce sclerotia, is associated with the facultatively ectomycorrhizal genus *Alnus*, and is not known to be associated with an insect. If *Gyrodon lividus* turns out to be non-mycorrhizal and to have the same ecological role as *B. merulioides* then I might agree with Singer because, excepting the sclerotia, the morphology and anatomy of the two fungi are similar.

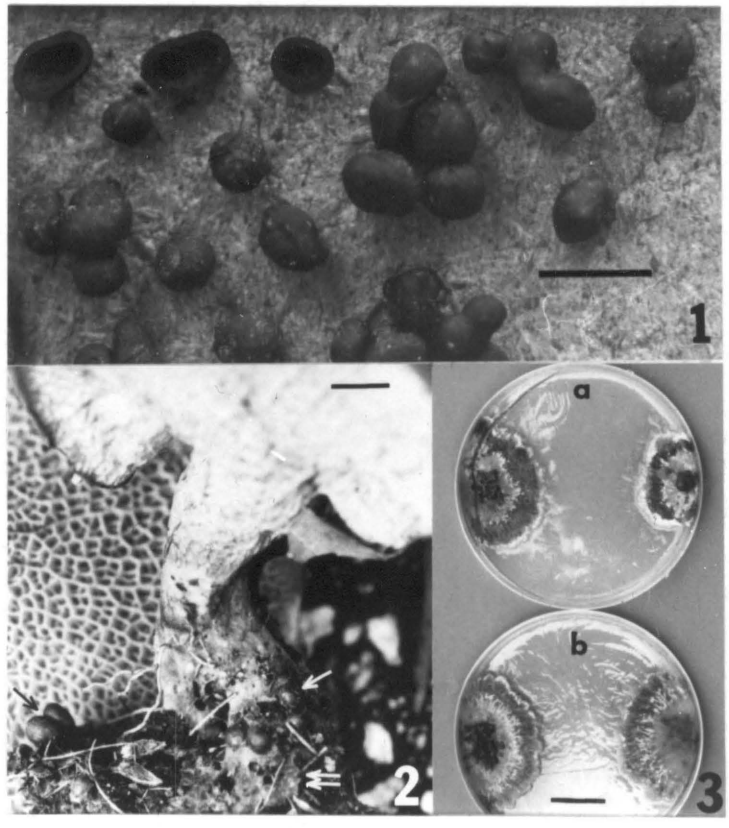

Fig. 3.1-3.3. *Boletinellus merulioides*. 3.1. Sclerotia; those in the upper left cut to show the hollow interior (VC 893). Bar = 0.5 cm. 3.2. Sclerotia (single arrows) around base of basidiocarp (mycelium designated by double arrows) (VC 878). Bar = 0.5 cm. 3.3. Cultures (of VC 587) derived from a sclerotium (a) and a basidiocarp (b) on modified Hagem's agar. Bar = 2 cm.

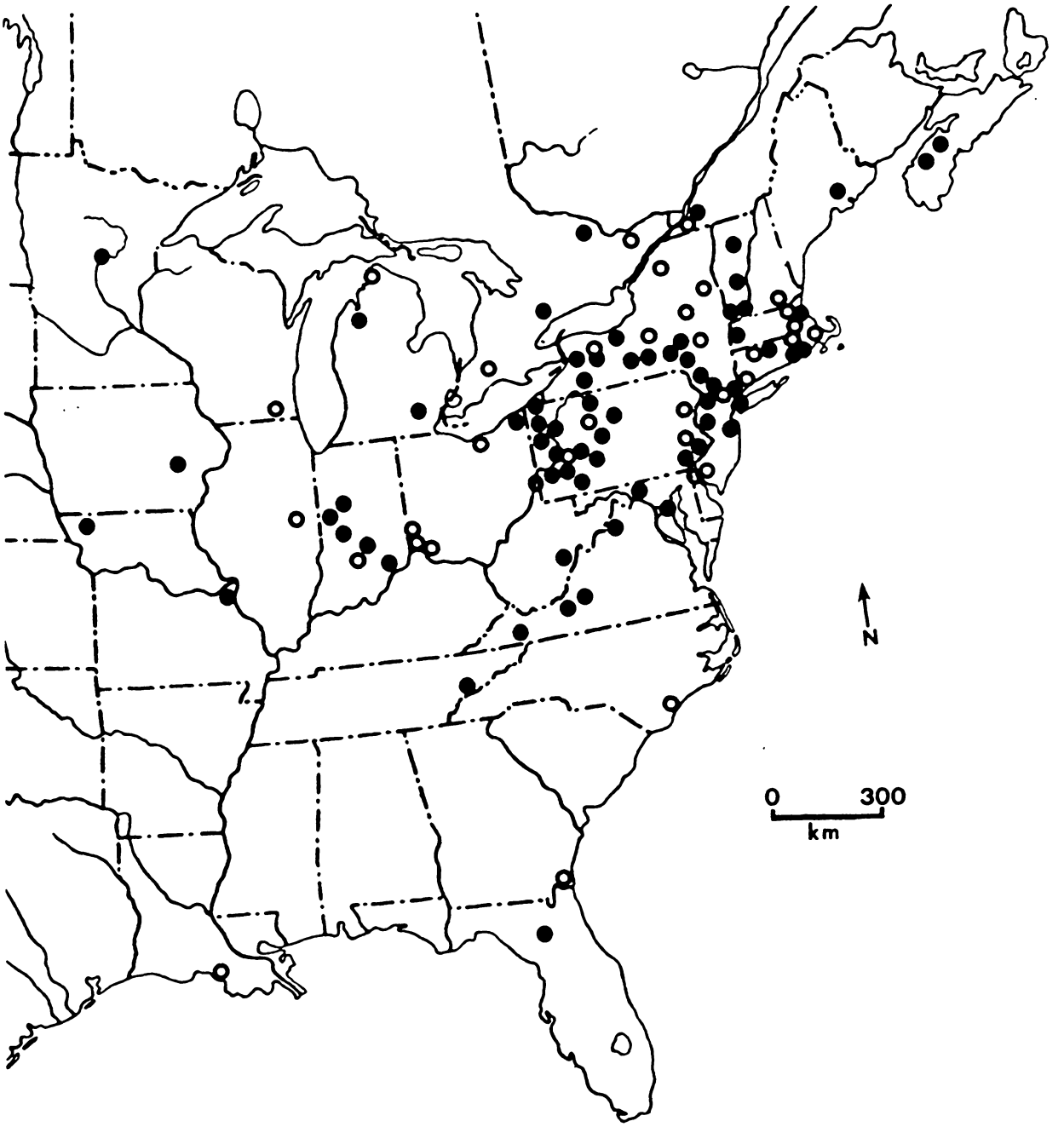

Fig. 3.4. Range of *Boletinellus merulioides* basidiocarps and sclerotia based on herbarium and personal collections. Dots and circles represent counties from which collections of *B. merulioides* basidiocarps were studied. Solid black dots indicate that at least one collection from that county had sclerotia; open circles indicate that no collection from that county had sclerotia.

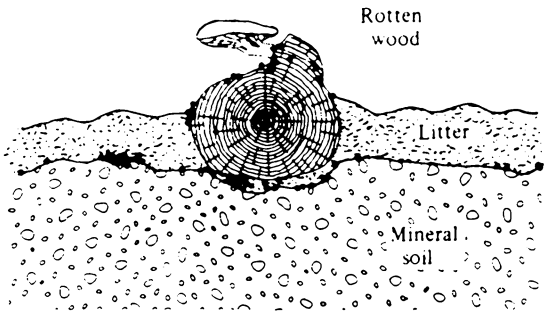

Fig. 3.5. Vertical profile of sclerotia of *Boletinellus merulioides*. Larger black dots represent sclerotia. Basidiocarps occur on the ground as well as on rotten wood.

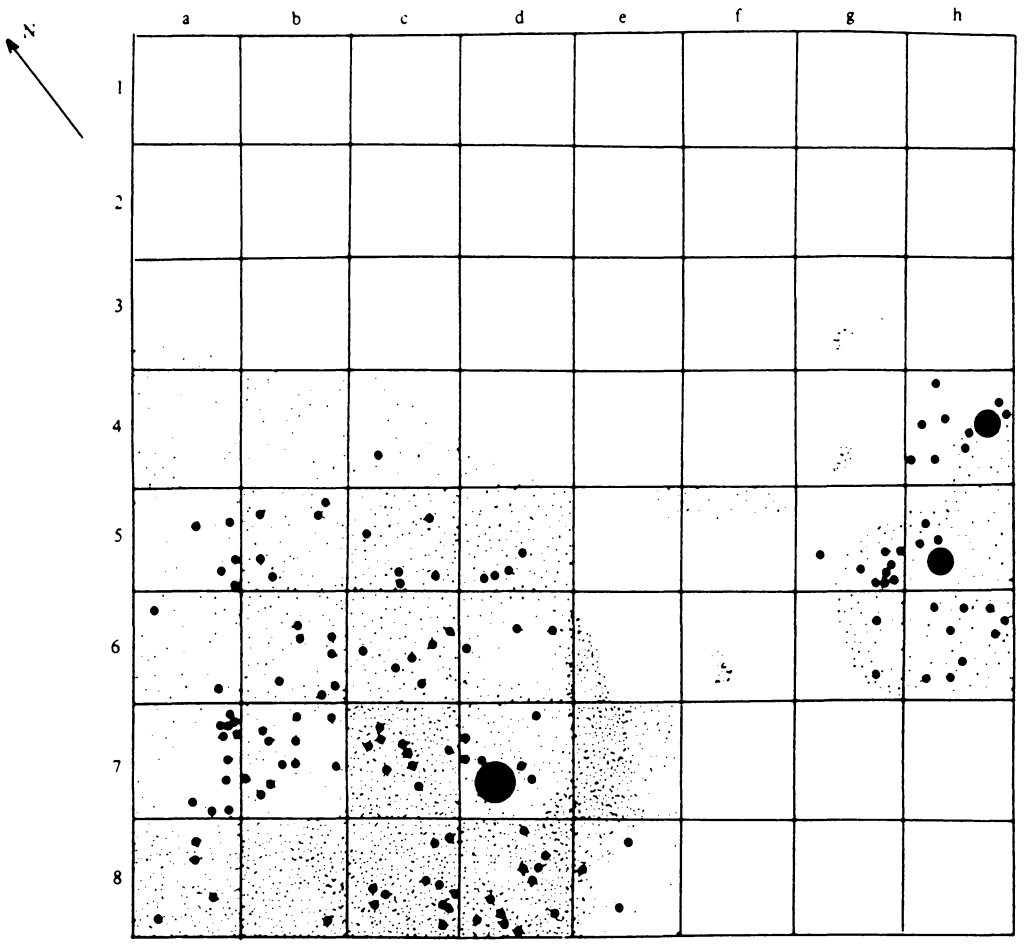

Fig. 3.6. Spatial pattern of *Boletinellus merulioides* in plot 6. Sclerotial density in 1984 is represented by the density of stippling. Small black dots are locations of basidiocarps 1981-4. Larger black dots are locations of *Fraxinus americana* trees.

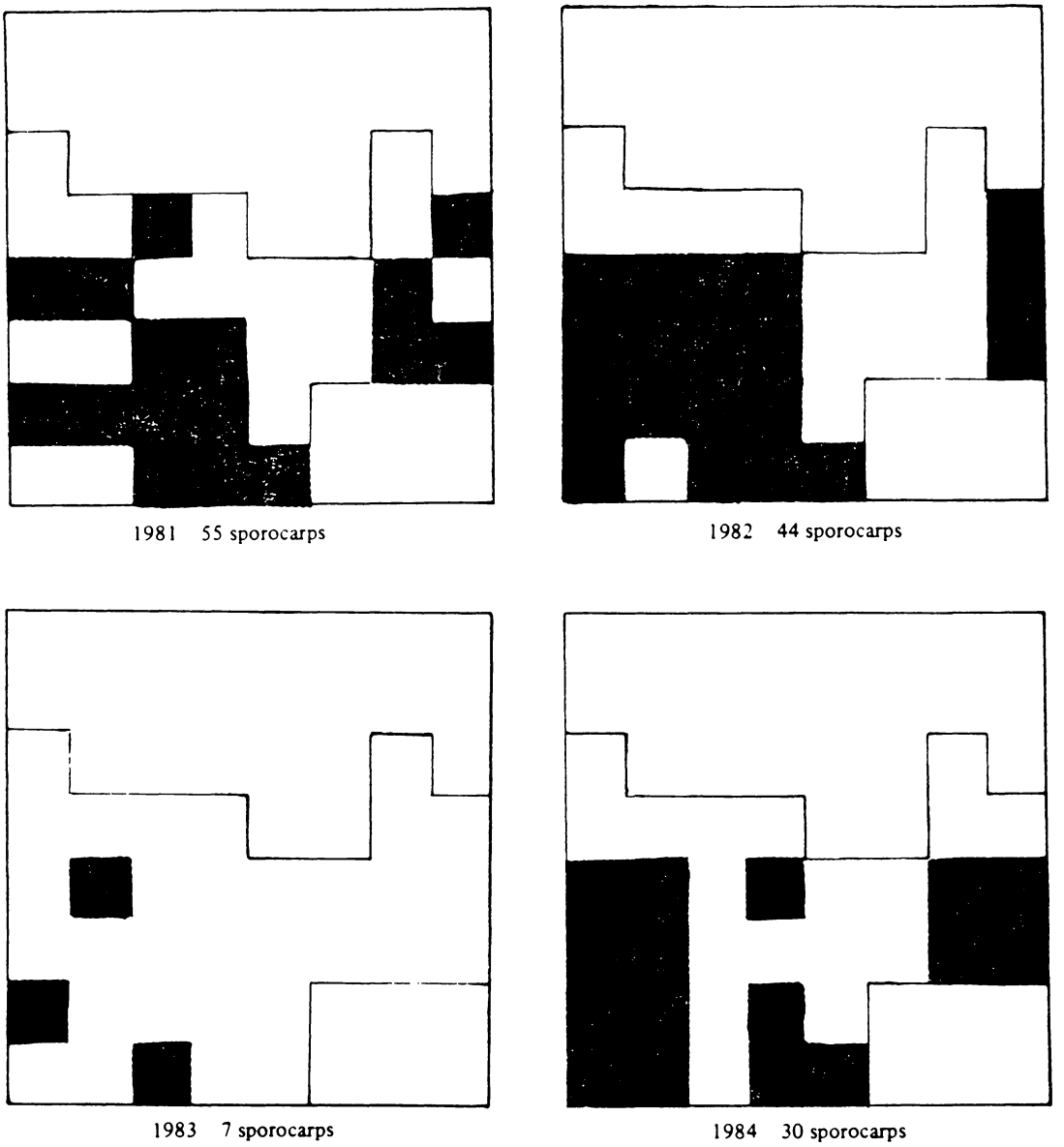

Fig. 3.7. Basidiocarp frequency in plot 6 year by year. Blackened subplots are those in which basidiocarps were observed during the year. Sclerotial frequency in 1984 is outlined. Sclerotia were absent from the upper part and lower right corner.

Table 3.1. Vegetative compatibility among isolates of *Boletinellus merulioides* from West Virginia subplots d5, d7, g6 and h5 and isolate VC 882.s2 from New Jersey.

Isolate	Isolate[1]							
	WV Patch			WV Patch				NJ
	d5.s1	d7.s1	d7.s2	g6.s1	g6.s2	h5.s1	h5.s2	VC 882.s2
VC 882.s2	A	A	A	A	A	A	A	=
h5.s2	A	A	A	=	=	=	=	
h5.s1	A	A	A	=	=	=		
g6.s2	A	A	A	=	=			
g6.s1	A	A	A	=				
d7.s2	=	=	=					
d7.s1	=	=						
d5.s1	=							

[1] A indicates antagonistic interaction; isolates are genetically different dikaryons (different individuals).
= indicates compatible interaction; isolates are genetically identical dikaryons (same individual).

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APPENDIX A. LIST OF FUNGAL COLLECTIONS FROM NEPAL 1985-1986

This list, prepared in May 1987, is of the fungi collected by H. Van T. and Irene S. Cotter (Biology Department, Virginia Tech, Blacksburg, VA 24061) in Nepal during 1985-86. Numbers VC 1001-1526 were collected in 1985 and numbers VC 1527-1538 in 1986.

Key to the information and codes:

Photo, column 1. P = color slide.
If blank, then not photographed.

Culture, columns 3-6. C = culture attempted.
+ or - = successful or not successful.
d = died (isolation successful but culture lost).
If blank, then no culture attempted.

Location where collections are deposited, columns 8-16.
These codes indicate herbaria where collections have been deposited. Capital letters indicate official herbarium codes; lower case letters are used to indicate herbaria without official codes. Most of the collections of Suillus will be deposited at BPI. Some collections are on loan to or in the personal herbaria of various mycologists.

- BPI National Fungus Collections. U. S. Dept. of Agr. Beltsville, MD. U. S.
- KATH National Herbarium of Nepal. Godawari.
- nhm Natural History Museum of Nepal. Swayambhu. Kathmandu.
- NY New York Botanical Garden. Bronx, NY. U. S.
- pp Division of Plant Pathology. Khumaltar. Nepal.
- VPI Virginia Tech. Blacksburg, VA. U. S.
- WSP Washington State Univ. Pullman, WA. U. S.
- \- Collection discarded.

If blank, then the collection is still retained in Cotter's personal herbarium.

Photo	Culture	Location deposited	Coll. Number	Taxon	Collection Date and Location
			VC1001	discomycete	13 Apr Godawari
			VC1002	agaric	18 Apr Solu market
			VC1003	chanterelle?	18 Apr Solu market
			VC1004	polypore	18 Apr Solu market
			VC1005	Pleurotus "sajor-caju"	12 Jun Bhoddha, cultivated
		KATH,pp	VC1006	leafspot of Gaultheria	28 Apr Shivapuri
		KATH	VC1007	polypore	28 Apr Shivapuri
P		KATH,nhm	VC1008	Ganoderma	28 Apr Shivapuri
			VC1009	pyrenomycete	28 Apr Shivapuri
			VC1010	cyphallaceous	28 Apr Shivapuri
		KATH	VC1011	polypore	28 Apr Shivapuri
		KATH	VC1012	Lenzites	28 Apr Shivapuri
	C+		VC1013	Lentinus "mirge chyau"	28 Apr Shivapuri
		KATH	VC1014	Fomes fomentarius	28 Apr Shivapuri
			VC1015	Exobasidium on camellia	28 Apr Shivapuri
			VC1016	Agrocybe	12 May Chobar
			VC1017	agaric	14 May Kathmandu market
			VC1018	polypore	14 May Kathmandu market
			VC1019	agaric	14 May Kathmandu market
P		nhm	VC1020	Agaricus	15 May Godawari
		KATH,pp	VC1021	Coleosporium barclayense Bagchee	18 May Daman
P	C+		VC1022	Suillus cf. placidus	18 May Daman
			VC1023	agaric	18 May Daman
			VC1024	agaric	18 May Daman
		KATH	VC1025	polypore	18 May Daman
P	C+d		VC1026	Suillus cf. placidus	19 May Daman
P			VC1027	Collybia	19 May Daman
	C+d		VC1028	Lentinus "mirge chyau"	19 May Daman
P	C+		VC1029	Lentinus "salla chyau"	19 May Daman
			VC1030	agaric	19 May Daman
			VC1031	Suillus cf. placidus	19 May Daman
P	C+d		VC1032	Suillus cf. placidus	19 May Daman
			VC1033	Suillus cf. placidus	19 May Daman
		-	VC1034	Psathyrella	22 May Kathmandu
			VC1035		27 May Beni

Appendix A. Continued.

		VC1036		27 May	Beni
		VC1037		27 May	Beni
		VC1038	mixture (inc. Lactarius?)	28 May	Tatopani
P	-	VC1039	Calvatia (eaten)	29 May	Ghasa
P	C+	VC1040	Suillus sibiricus	29 May	Ghasa
P	C+d	VC1041	Suillus greening-foot	29 May	Ghasa
P	C+d	VC1042	Suillus cf. granulatus	29 May	Ghasa
P	C+d pp	VC1043	Suillus sp.	29 May	Ghasa
P		VC1044	Rhizopogon	29 May	Ghasa
P	KATH	VC1045	Lentinus	29 May	Ghasa
P		VC1046	Suillus sibiricus	30 May	Lete
	KATH	VC1047	Berberis rust	30 May	Kalopani
P		VC1048	Suillus sibiricus	30 May	Kalopani
P	nhm	VC1049	Armellaria mellea	30 May	Kalopani
		VC1050	Lactarius?	30 May	Kalopani house, dried
P		VC1051	Suillus cf. p? or g?	31 May	Larjung
P		VC1052	Suillus greening-foot	01 Jun	Lete across river
P		VC1053	Suillus sibiricus	01 Jun	Lete across river
P		VC1054	Suillus sibiricus	01 Jun	Lete across river
P		VC1055	Suillus sibiricus	02 Jun	Ghasa
P		VC1056	Suillus greening-foot	02 Jun	Ghasa
P		VC1057	Suillus sp. (mixed?)	02 Jun	Ghasa
	nhm	VC1058	Collybia	02 Jun	Ghasa
	nhm	VC1059	Hebeloma	02 Jun	Ghasa
		VC1060	Mycogone	02 Jun	Ghasa
	nhm	VC1061	Paneolus	03 Jun	Tatopani
	nhm	VC1062	Lycoperdon?	03 Jun	Ghara
		VC1063	Oudmansiella radicata	04 Jun	Chitre-Ghorapani
	nhm	VC1064	Collybia "velutipes"	04 Jun	Ghorapani-Ulleri
	nhm	VC1065	Oudmansiella	04 Jun	Ghorapani-Ulleri
		VC1066	Oudmansiella yellow	04 Jun	Ghorapani-Ulleri
		VC1067	Oudmansiella Acer	04 Jun	Ghorapani-Ulleri
	NY	VC1068	Sepedonium chrysospermum (Bull.) Link on Boletus	05 Jun	Hille
		VC1069	Hypomyces	06 Jun	Nagdanda-Suikhet
	nhm	VC1070	Scleroderma	06 Jun	Nagdanda-Suikhet
P		VC1071	Boletus "chrysenteron"	06 Jun	Nagdanda-Suikhet
P		VC1072	Phylloporus	06 Jun	Nagdanda-Suikhet
P		VC1073	Boletus	06 Jun	Nagdanda-Suikhet
P		VC1074	Boletus	06 Jun	Nagdanda-Suikhet
P		VC1075	Boletus	06 Jun	Nagdanda-Suikhet
		VC1076	Pleurotus "sajor-caju"	08 Jun	Bouddha, cultivated
P	nhm	VC1077	agaric	13 Jun	Kathmandu basket
P	nhm	VC1078	agaric	13 Jun	Kathmandu basket
		VC1079	Scleroderma	15 Jun	Chobar
		VC1080	puffball	15 Jun	Chobar
P	C+	VC1081a	Suillus queen's	15 Jun	Nagarjun (Sep C+)
	NY	VC1082	Sepedonium chrysospermum on Suillus	15 Jun	Nagarjun
P		VC1083a	Russula	15 Jun	Nagarjun
P		VC1083b	Russula	15 Jun	Nagarjun
	nhm	VC1084	coral	17 Jun	Kathmandu market
		VC1085	discomycete	19 Jun	Bharku-Syabru
		VC1086	Lasiosphaeria nepalensis sp. nov.	19 Jun	Bharku-Syabru
		VC1087	Oudmansiella	19 Jun	Bharku-Syabru
P	C- KATH	VC1088	Suillus cf. placidus.	19 Jun	Bharku-Syabru
P	pp	VC1089	Sphacelotheca reiliana (Kuhn) Clint.	20 Jun	Syabru
P		VC1090	leafspot	20 Jun	Syabru-Lama Hotel
P		VC1091	Boletus	20 Jun	Syabru-Lama Hotel
P	C+	VC1092	Paxillus cf. filamentosus	20 Jun	near Lama Hotel
	KATH,pp	VC1093	Berberis rust	?	Lama Hotel-Ghora Tabela
P	nhm	VC1094	Xylaria	21 Jun	Lama Hotel-Ghora Tabela
P	nhm,WSP	VC1095	Xylocoremium flabelliforme (Schw.:Fr.) JD Rogers	21 Jun	Lama Hotel-Ghora Tabela
P	nhm	VC1096	Xylaria	21 Jun	Lama Hotel-Ghora Tabela
P		VC1097	Boletus	21 Jun	Lama Hotel-Ghora Tabela
P		VC1098	Oudmansiella	21 Jun	Lama Hotel-Ghora Tabela
P		VC1099	Oudmansiella	21 Jun	Lama Hotel-Ghora Tabela
P		VC1100	Peziza	21 Jun	Lama Hotel-Ghora Tabela
P	-	VC1101	Collybia	21 Jun	Lama Hotel-Ghora Tabela

Appendix A. Continued.

P		VC1102	Boletus	21 Jun	Lama Hotel-Ghora Tabela
P	-	VC1103	Paneolus	21 Jun	Lama Hotel-Ghora Tabela
P		VC1104	chanterelle	21 Jun	Lama Hotel-Ghora Tabela
P	-	VC1105	Coprinus	22 Jun	Ghora Tabela
P		VC1106	Tylopilus	22 Jun	Ghora Tabela
P	-	VC1107	Pleurotus	22 Jun	Ghora Tabela
		VC1108	Lentinus	22 Jun	Ghora Tabela
P	-	VC1109	witches butter	22 Jun	Ghora Tabela
P	-	VC1110	Naematoloma	22 Jun	Ghora Tabela
P	-	VC1111	slime mold	22 Jun	Ghora Tabela
P	nhm	VC1112	Pholiota	22 Jun	Ghora Tabela
P		VC1113	Strobilomyces	22 Jun	Ghora Tabela
P	-	VC1114	Collybia	22 Jun	Ghora Tabela
P C+d		VC1115	Suillus himalayan Sepedonium also isolated.	22 Jun	Ghora Tabela
P	-	VC1116	Lactarius	22 Jun	Ghora Tabela
P	-	VC1117	Oudmansiella	22 Jun	Ghora Tabela
P	-	VC1118	Cortinarius	23 Jun	Ghora Tabela
P		VC1119	Lentinellus	23 Jun	Ghora Tabela
P		VC1120	Albetrellus?	23 Jun	Ghora Tabela
P	-	VC1121	Strobilomyces	23 Jun	Ghora Tabela
P	-	VC1122	Scuttelinia	23 Jun	Ghora Tabela
P		VC1123	Boletus	24 Jun	Ghora Tabela-Lama Hotel
P		VC1124	Ramaria	24 Jun	Lama Hotel
P	-	VC1125	Clitocybe	24 Jun	Ghora Tabela-Lama Hotel
P	-	VC1126	Pluteus	24 Jun	Ghora Tabela-Lama Hotel
P		VC1127	Oudmansiella?	24 Jun	east of Syarpagaon
		VC1128	yeast (alcohol)	24 Jun	Syarpagaon
P C-		VC1129	Suillus cf. placidus	25 Jun	w. of Syarpagaon
P C-		VC1130	Suillus sibiricus	25 Jun	w. of Syarpagaon
P C+d		VC1131	Suillus sibiricus	25 Jun	w. of Syarpagaon
	KATH	VC1132	Cronartium himalayense Bagchee	25 Jun	above Syabrubensi
P		VC1133	agaric	26 Jun	Syabrubensi
P	KATH	VC1134	polypore	26 Jun	Syabrubensi
	nhm	VC1135	Aleuria	27 Jun	road to Dhunche
P	nhm	VC1136	Agrocybe pediades	29 Jun	Phora Durbar
P	nhm	VC1137	Leucocoprinus luteus	02 Jul	Tahachal
P C+d		VC1138	Suillus queen's	06 Jul	Nagarjun young Pr stand
P		VC1139	Oudmansiella	06 Jul	Nagarjun
P		VC1140	Amanita	06 Jul	Nagarjun
P		VC1141	earthstar	06 Jul	Nagarjun
P		VC1142	Boletus	09 Jul	Sundarijal-Mulkharka
		VC1143	agaric	09 Jul	above Mulkharka
		VC1144	Geoglossum	09 Jul	Sundarijal-Mulkharka
		VC1145	Paxillus cf. filamentosus	09 Jul	Sundarijal-Mulkharka
P		VC1146	Oudmansiella radicata	09 Jul	above Mulkharka
P	-	VC1147	Russula	09 Jul	above Mulkharka
P		VC1148	Strobilomyces	09 Jul	above Chaubas
P	-	VC1149	Russula	10 Jul	n. of Chipling
P		VC1150	Boletus	10 Jul	n. of Chipling
P		VC1151	Calostoma junghuhnii (Schlechtendal & K Muller) Masse	10 Jul	n. of Chipling
	pp	VC1152	Syntrichium on potato	10 Jul	s. of Gul Bhanjyang
P		VC1153	Tricholomopsis	11 Jul	n. of Kutumsang
P	nhm	VC1154	Armellaria mellea	11 Jul	n. of Kutumsang
P		VC1155	Russula	11 Jul	n. of Kutumsang
P		VC1156	Boletus	11 Jul	n. of Kutumsang
P	nhm	VC1157	Tricholoma	11 Jul	n. of Kutumsang
P		VC1158	Russula	11 Jul	n. of Kutumsang
P		VC1159	Oudmansiella (platyphylla?)	11 Jul	n. of Kutumsang
P		VC1160	Boletus	11 Jul	n. of Kutumsang
		VC1161	Polyporus	11 Jul	Kutumsang
P		VC1162	parasite of Armellaria	12 Jul	n. of Chipling
		VC1163	Hygrocybe	12 Jul	n. of Chipling
P		VC1164	Boletus	12 Jul	n. of Chipling
P		VC1165	Leccinum	12 Jul	n. of Chipling
P	KATH	VC1166	Ganoderma	12 Jul	n. of Chipling
P	nhm	VC1167	agaric	12 Jul	Chipling
P		VC1168	Oudmansiella radicata	13 Jul	Pati Bhanjyang

Appendix A. Continued.

P		VC1169	Peziza?	13 Jul	s. of Pati Bhanjyang
P		VC1170	discomycete	13 Jul	s. of Pati Bhanjyang
P		VC1171	Lactarius	13 Jul	n. of Chaubas
P		VC1172	Phylloporus	13 Jul	n. of Chaubas
P		VC1173	Boletus	13 Jul	Chaubas-Mulkharka
	KATH	VC1174	leafspot of Arisaema	10 Jul	Gul Bhanjyang-Kutamsang
	nhm	VC1175	agaric	16 Jul	Swayambhu
P C+	KATH	VC1176	Amanita "caesarea"	16 Jul	near Swayambhu
	nhm	VC1177	clavarioid	17 Jul	along road to Chautara
P	nhm	VC1178	Scleroderma "citrinum"	17 Jul	along road to Chautara
P		VC1179	Russula	18 Jul	above Chautara
P	nhm	VC1180	Asterophora lycoperdoides	18 Jul	above Chautara
P C+d		VC1181	Suillus waxy	18 Jul	above Chautara
P		VC1182	Amanita	18 Jul	above Chautara
P	nhm	VC1183	Laccaria	18 Jul	above Chautara
P C-		VC1184	Suillus cf. placidus	18 Jul	above Chautara
P C+		VC1185	Suillus waxy	18 Jul	above Chautara
P	nhm	VC1186	Oudmansiella radicata	18 Jul	above Chautara
P	nhm	VC1187	Amanita "caesarea"	18 Jul	above Chautara
P	nhm	VC1188	agaric	19 Jul	Chautara
P		VC1189	Suillus waxy	19 Jul	road to Chautara
P	nhm	VC1190	Inocybe	19 Jul	road to Chautara
P		VC1191	hydnum	19 Jul	road to Chautara
P C+		VC1192	Suillus waxy	19 Jul	road to Chautara
P C-		VC1193	Suillus cf. placidus	19 Jul	road to Chautara
P		VC1194	Inocybe	19 Jul	road to Chautara
P		VC1195	Inocybe	19 Jul	road to Chautara
		VC1196	Scleroderma "citrinum"	19 Jul	Chautara
P		VC1197	Russula	26 Jul	TU Kirtipur
C-		VC1198	Suillus sp.	26 Jul	TU Kirtipur
		VC1199	Helvella	26 Jul	Chobar
P		VC1200	Lactarius	26 Jul	Chobar
P		VC1201	Boletellus emodensis	27 Jul	Bajrajogini
P		VC1202	Russula	28 Jul	Nagarkot
P		VC1203	Tylopilus	28 Jul	Nagarkot
P C+		VC1204	Suillus cf. placidus	28 Jul	Nagarkot
C-		VC1205	Suillus cf. placidus	28 Jul	Nagarkot
P C+d		VC1206	Suillus waxy	28 Jul	Nagarkot
C+d		VC1207	Suillus waxy	28 Jul	Nagarkot
P C+d	nhm	VC1208	Suillus cf. placidus	28 Jul	Nagarkot
	KATH	VC1209	Suillus sibiricus	01 Aug	Godawari
		VC1210	Coltricia	01 Aug	Godawari
P		VC1211	Aleuria	03 Aug	n. of Dhunche
	nhm	VC1212	earthstar	03 Aug	Bharku-Syabru
	-	VC1213	Amanita	03 Aug	Bharku-Syabru
P	nhm	VC1214	Scleroderma	03 Aug	Bharku-Syabru
		VC1215	Xylaria	22 Jul	Kathmandu
	-	VC1216		03 Aug	Bharku-Syabru
P	-	VC1217	agaric	03 Aug	Bharku-Syabru
C+		VC1218	Suillus sibiricus	03 Aug	Bharku-Syabru
P C+		VC1219	Suillus greening-foot	03 Aug	Bharku-Syabru
P	nhm	VC1220	Boletus "edulis"	04 Aug	Syabru-Lama Hotel
		VC1221	Xylaria	04 Aug	Syabru-Lama Hotel
		VC1222	Xylaria	04 Aug	Syabru-Lama Hotel
	WSP	VC1223a	Xylaria	04 Aug	Syabru-Lama Hotel
	WSP	VC1223b	Xylaria dichotoma (Mont.)Fr.	04 Aug	Syabru-Lama Hotel
P		VC1224	Gyroporus	04 Aug	Syabru-Lama Hotel
	KATH	VC1225	polypore	05 Aug	Lama Hotel-Ghora Tabela
P		VC1226	Boletus	06 Aug	Ghora Tabela
P		VC1227	earthstar	06 Aug	Ghora Tabela
P		VC1228	Boletus	06 Aug	Ghora Tabela
P	nhm	VC1229	Sphaerobolis "stellatus"	06 Aug	Ghora Tabela
P C+		VC1230	Suillus himalayan	06 Aug	Ghora Tabela
P C+		VC1231	Suillus laricinus	06 Aug	Ghora Tabela
P	nhm	VC1232	Gomphus	06 Aug	Ghora Tabela
P		VC1233	Spathularia	06 Aug	Ghora Tabela
	-?	VC1234	Gomphidius	06 Aug	Ghora Tabela
P	nhm	VC1235	Tarzetta	06 Aug	Ghora Tabela
P		VC1236	Otidea	06 Aug	Ghora Tabela

Appendix A. Continued.

P		VC1237	Boletus	06 Aug	Ghora Tabela
P		VC1238	Boletus	06 Aug	Ghora Tabela
P C+d		VC1239	Suillus orange-pored	07 Aug	Ghora Tabela
P		VC1240	Chroogomphus tomentosus (Murr.)OK Miller	07 Aug	Ghora Tabela
P C+		VC1241	Suillus laricinus	07 Aug	Ghora Tabela
P C+d		VC1242	Suillus himalayan	07 Aug	Ghora Tabela
P		VC1243	Gomphidius	07 Aug	Ghora Tabela
P C-		VC1244	Suillus laricinus	07 Aug	Ghora Tabela
P	nhm	VC1245	agaric	07 Aug	Ghora Tabela
P	NY	VC1246	Sepedonium chrysospermum on bolete	07 Aug	Ghora Tabela
P		VC1247	Boletus	07 Aug	Ghora Tabela
P		VC1248	Suillus himalayan	08 Aug	Langtang-Ghora Tabela
P	KATH	VC1249	Pseudohydnum	07 Aug	Ghora Tabela
P		VC1250	Helvella	08 Aug	Langtang-Ghora Tabela
P		VC1251	Leccinum	08 Aug	Langtang-Ghora Tabela
P		VC1252	Gomphus	08 Aug	Langtang-Ghora Tabela
P		VC1253	Peziza	08 Aug	Langtang-Ghora Tabela
P		VC1254	discomycete	08 Aug	Langtang-Ghora Tabela
P	nhm	VC1255	Spathularia	08 Aug	Langtang-Ghora Tabela
P	nhm	VC1256		08 Aug	Langtang-Ghora Tabela
P		VC1257	Leccinum	08 Aug	Langtang-Ghora Tabela
P		VC1258	Boletus	08 Aug	Langtang-Ghora Tabela
P		VC1259	Helvella	08 Aug	Langtang-Ghora Tabela
P		VC1260	Suillus laricinus	08 Aug	Langtang-Ghora Tabela
P	nhm	VC1261	Helvella	09 Aug	Ghora Tabela
P		VC1262	Boletus	09 Aug	Ghora Tabela
P		VC1263	Marasmiellus?	09 Aug	Ghora Tabela-Lama Hotel
P	nhm	VC1264	Panus	09 Aug	Ghora Tabela-Lama Hotel
P	KATH,NY	VC1265	Cavimalum indicum Doi, Dargan & Thind	09 Aug	Ghora Tabela-Lama Hotel
P	nhm,NY	VC1266	Cavimalum indicum Doi, Dargan & Thind	10 Aug	Ghora Tabela-Lama Hotel
P		VC1267	Suillus sibiricus	10 Aug	w. of Syarpagaon
P		VC1268	parasite of bamboo	10 Aug	w. of Syarpagaon
		VC1269	agaric	10 Aug	Syabrubensi
	-	VC1270	rust	11 Aug	Syabrubensi
P	KATH	VC1271	Hydnum "dentinum"	11 Aug	Syabrubensi-Syabru
P C-		VC1272	Suillus sibiricus	11 Aug	Syabrubensi-Syabru
P		VC1273	Suillus cf. placidus	11 Aug	Syabrubensi-Syabru
P	KATH	VC1274	Auriscalpium vulgare	11 Aug	Syabrubensi-Syabru
P		VC1275	Russula	11 Aug	n. of Syabru
P	-	VC1276	Boletus	12 Aug	Syabru-Chandrabari
P		VC1277	Wynnea	12 Aug	Syabru-Chandrabari
	-	VC1278	Suillus sp.	12 Aug	Syabru-Chandrabari
P		VC1279	Hygrophorus	12 Aug	Syabru-Chandrabari
P	-	VC1280	Tylopilus "gracilis"	12 Aug	Syabru-Chandrabari
		VC1281		22 Aug	Bisakhu Narayan
		VC1282	Scleroderma "citrinum"	22 Aug	Bisakhu Narayan
P C+d		VC1283	Suillus waxy	24 Aug	Nagarkot
		VC1284	Suillus (cf. placidus?)	24 Aug	Nagarkot
P	-	VC1285	Suillus sp.	24 Aug	Nagarkot
		VC1286	agaric	24 Aug	Nagarkot
P	nhm	VC1287	chanterelle	25 Aug	Chaimale
P		VC1288	Suillus sp.	25 Aug	Chaimale
P		VC1289	coral	25 Aug	Chaimale
P		VC1290	Suillus sibiricus	25 Aug	Chaimale
		VC1291	parasite of Suillus	25 Aug	Chaimale
P		VC1292	Scleroderma "citrinum"	30 Aug	Pokhara
P	-	VC1293	Lycoperdon	30 Aug	Pokhara
P	nhm	VC1294	earthstar	30 Aug	Pokhara
P		VC1295	Suillus (greening-foot?)	01 Sep	Larjung
P		VC1296	Suillus sibiricus	01 Sep	Larjung
	-	VC1297	Suillus sp.	01 Sep	Larjung
	-	VC1298	Suillus sp.	02 Sep	Larjung
	-	VC1299	Suillus sp.	02 Sep	Larjung
	-	VC1300	Rhizopogon	02 Sep	Larjung
P C+d		VC1301	Suillus greening-foot	02 Sep	Larjung
		VC1302	number not used		
P C+	KATH	VC1303	Suillus cf. granulatus	02 Sep	Larjung

Appendix A. Continued.

P C-		VC1304	Suillus sibiricus	02 Sep	Larjung
	-	VC1305	Suillus sp.	02 Sep	Larjung
	-	VC1306	Suillus sp.	02 Sep	Larjung
	K-	VC1307	Ustilago maydis (DC) Cda.	03 Sep	Larjung
	K-	VC1308	leafspot of corn	03 Sep	Larjung
	K KATH	VC1309	rust	03 Sep	Larjung
P C+		VC1310	Suillus sibiricus	03 Sep	Larjung
	-	VC1311	Ramaria	03 Sep	Larjung
P C+	nhm	VC1312	Suillus cf. granulatus	03 Sep	Larjung
P		VC1313	Lactarius	03 Sep	Larjung
P	nhm	VC1314	Rhizopogon	03 Sep	Larjung
P		VC1315	Helvella with parasite	03 Sep	Larjung
P	nhm	VC1316h	Humaria hemisphaerica (Wiggers:Fr.)Fuckel	03 Sep	Larjung
P	nhm,NY	VC1316p	Stephanoma strigosum on Humaria hemisphaerica	03 Sep	Larjung
P	nhm	VC1317	chanterelle	03 Sep	Larjung
		VC1318	Helvella	03 Sep	Larjung
		VC1318p	parasite of Helvella	03 Sep	Larjung
P		VC1319	Helvella	03 Sep	Larjung
P		VC1320	Helvella	03 Sep	Larjung
P		VC1321	Helvella	03 Sep	Larjung
P		VC1322	Helvella	03 Sep	Larjung
P		VC1323	Helvella	03 Sep	Larjung
P		VC1323p	parasite of Helvella	03 Sep	Larjung
P		VC1324	Helvella	03 Sep	Larjung
P		VC1324p	parasite of Helvella	03 Sep	Larjung
P	nhm	VC1325	Collybia	03 Sep	Larjung
P C-		VC1326	Suillus greening-foot	04 Sep	Larjung
P	-	VC1327	Collybia	04 Sep	Larjung
P C+		VC1328	Suillus sibiricus	04 Sep	Larjung
P		VC1329	Helvella	04 Sep	Larjung
P		VC1330	Russula	04 Sep	Larjung
P		VC1331	Morchella	04 Sep	Larjung
		VC1332	agaric	04 Sep	Larjung
	nhm	VC1333	Pholiota?	05 Sep	Larjung
		VC1334	Crepidotus?	05 Sep	Larjung
	KATH	VC1335	rust	05 Sep	s. of Larjung
		VC1336	parasite of Helvella	05 Sep	Larjung
	nhm	VC1337	earthstar	06 Sep	Kalopani
		VC1338	parasite of Helvella	06 Sep	Kalopani
	nhm	VC1339	'Marasmiellus'	06 Sep	Lete
		VC1340	Helvella	06 Sep	Lete
		VC1341	Otidea	06 Sep	Lete
P	KATH,pp	VC1342	leafspot of Cannabis	06 Sep	Lete-Ghasa
P		VC1343	Russula	07 Sep	Ghasa
P C-		VC1344	Suillus cf. granulatus	07 Sep	Ghasa
P	nhm	VC1345	Ramaria	07 Sep	Ghasa
P		VC1346	Paxillus cf. filamentosus	07 Sep	Ghasa-Rukse Chara
		VC1347	Paxillus cf. filamentosus	08 Sep	Tiplyan-Galeswar
P		VC1348	Morchella	08 Sep	Tiplyan-Galeswar
	nhm	VC1349	Leotia	08 Sep	Tiplyan-Galeswar
		VC1350	cyphellaceous	15 Sep	Nagarjun
	-	VC1351	molds on ipil-ipil seeds	11 Sep	Pokhara
	NY	VC1352	Hypomyces chrysospermus (Bull.) Tul.	14 Sep	Nagarjun
P		VC1353	Boletellus emodensis	14 Sep	Godawari
P		VC1354a	Trichoglossum	15 Sep	Nagarjun
P		VC1354b	Trichoglossum	15 Sep	Nagarjun
P		VC1354c	Trichoglossum	15 Sep	Nagarjun
		VC1355	Amanita	14 Sep	Godawari
		VC1356	Tylopilus	14 Sep	Godawari
P		VC1357	Thelephora	14 Sep	Godawari
P		VC1358	Strobilomyces	15 Sep	Nagarjun
		VC1359	Strobilomyces	15 Sep	Nagarjun
		VC1360	Hypocrea	15 Sep	Nagarjun
P		VC1361	Gyrodon cf. lividus	15 Sep	Nagarjun
P		VC1362	Boletus	15 Sep	Nagarjun
P		VC1363	Gyroporus	15 Sep	Nagarjun
P		VC1364	Isaria	15 Sep	Nagarjun
P	KATH	VC1365	Laetiporus sulphureus	13 Sep	Thamel vendor

Appendix A. Continued.

	KATH	VC1366	Polyporus s.s.	13 Sep	Thamel vendor
	nhm	VC1367	agaric	13 Sep	Thamel vendor
		VC1368	Pleurotus	13 Sep	Thamel vendor
P		VC1369	Lactarius indigo (Schw.) Fr.	15 Sep	Nagarjun
P		VC1370	Phylloporus	15 Sep	Nagarjun
		VC1371	Hypomyces	15 Sep	Nagarjun
		VC1372	agaric	15 Sep	Nagarjun
P		VC1373	Amanita	15 Sep	Nagarjun
P		VC1374	hydnum	15 Sep	Nagarjun
		VC1375	Cantharellus	16 Sep	Kathmandu
		VC1376	Craterellus	16 Sep	Kathmandu
		VC1377	Amanita	21 Sep	Godawari-Phulchoki
P		VC1378	Boletus	21 Sep	Godawari-Phulchoki
P	nhm	VC1379	Boletellus emodensis	21 Sep	Godawari-Phulchoki
P		VC1380	Helvella	21 Sep	Godawari-Phulchoki
P		VC1381	Gyroporus	21 Sep	Godawari-Phulchoki
P		VC1382	Tylopilus	21 Sep	Godawari-Phulchoki
P		VC1383	Boletellus emodensis yellow form	21 Sep	Godawari-Phulchoki
P		VC1384	agaric	21 Sep	Godawari-Phulchoki
P		VC1385	discomycete	21 Sep	Godawari-Phulchoki
P		VC1386	Scleroderma	21 Sep	Godawari-Phulchoki
P		VC1387	Helvella	21 Sep	Godawari-Phulchoki
		VC1388	discomycete	21 Sep	Godawari-Phulchoki
P		VC1389	Strobilomyces	21 Sep	Godawari-Phulchoki
P		VC1390	Lactarius	23 Sep	Badegaon
P	C+	VC1391a	Suillus sibiricus	23 Sep	Godawari
			Sepedonium also cultured from VC1391 but it died.		
P	C-	VC1392	Suillus sibiricus	23 Sep	Godawari
P		VC1393	Russula	26 Sep	Nagarjun
P		VC1394	Cordyceps nutans Pat.	26 Sep	Nagarjun
P		VC1395	Tuber	26 Sep	Nagarjun
P		VC1396	Inocybe?	26 Sep	Nagarjun
P		VC1397	Cordyceps	26 Sep	Nagarjun
P		VC1398	Trichoglossum	26 Sep	Nagarjun
P		VC1399h	Trametes hirsuta (Fr.)Pilat	26 Sep	Nagarjun
P	NY	VC1399p	Hypomyces aurantius (Pers.)Fr.	26 Sep	Nagarjun
P	nhm	VC1400	Omphalotus	26 Sep	Nagarjun
P		VC1401	chanterelle	26 Sep	Nagarjun
P		VC1402	Lactarius	26 Sep	Nagarjun
P	-	VC1403	Russula	26 Sep	Nagarjun
P	-	VC1404	Russula	26 Sep	Nagarjun
		VC1405	coral	03 Oct	Nagarjun
P		VC1406	agaric	03 Oct	Nagarjun
P		VC1407	Oudmansiella	03 Oct	Nagarjun
P		VC1408	Boletus	03 Oct	Nagarjun
	-	VC1409	Boletus	03 Oct	Nagarjun
P		VC1410	hydnum	03 Oct	Nagarjun
P	-	VC1411	chanterelle	03 Oct	Nagarjun
P		VC1412	Isaria	03 Oct	Nagarjun
P		VC1413	Auriscalpium vulgare	03 Oct	Nagarjun
	KATH,nhm	VC1414	Claviceps	03 Oct	Nagarjun
		VC1415	Oudmansiella radicata	03 Oct	Nagarjun
P C+		VC1416	Gyrodon cf. lividus	03 Oct	Nagarjun
P		VC1417	Cordyceps	03 Oct	Nagarjun
P	KATH	VC1418	Laetiporus sulphureus	03 Oct	market
		VC1419	Asterophora	26 Sep	Nagarjun
		VC1420	Suillus sp.	03 Oct	Phora Durbar
P C+	nhm	VC1421	Suillus sibiricus	06 Oct	Bharku-Syabru
P		VC1422	Lactarius	06 Oct	Bharku-Syabru
P C+		VC1423	Suillus cf. placidus	06 Oct	Bharku-Syabru
P	-	VC1424	Suillus sp.	06 Oct	Bharku-Syabru
C+		VC1425	Suillus greening-foot	06 Oct	Bharku-Syabru
P		VC1426	Scleroderma verrucosum Pers.	07 Oct	Syabru-Lama Hotel
P	WSP	VC1427	Entonaema cinnabarina (Cooke & Masse) Lloyd	07 Oct	Syabru-Lama Hotel
P		VC1428	Lactarius	08 Oct	Lama Hotel-Ghora Tabela
P		VC1429	Lactarius	08 Oct	Lama Hotel-Ghora Tabela
P		VC1430	Lactarius	08 Oct	Lama Hotel-Ghora Tabela
P		VC1431	Leccinum	08 Oct	Lama Hotel-Ghora Tabela
P	-	VC1432	Amanita	08 Oct	Lama Hotel-Ghora Tabela

Appendix A. Continued.

P		VC1433	Lactarius	09 Oct	Ghora Tabela
P		VC1434	Gomphidius	09 Oct	Ghora Tabela
P		VC1435	Hymenogaster	09 Oct	Ghora Tabela
P		VC1436	Hygrophorus	09 Oct	Ghora Tabela
P		VC1437	Boletus	09 Oct	Ghora Tabela
P C+		VC1438	Suillus laricinus	09 Oct	Ghora Tabela
		VC1439	Suillus himalayan	10 Oct	Ghora Tabela
		VC1440	Suillus laricinus	10 Oct	Ghora Tabela
		VC1441	Tubercularia vulgaris Tode	10 Oct	Ghora Tabela
		VC1442	Hygrophorus	10 Oct	Ghora Tabela
P		VC1443	Suillus himalayan	11 Oct	Langtang-Ghora Tabela
		VC1444	goober	11 Oct	Langtang-Ghora Tabela
P		VC1445	Scuttelinia	11 Oct	Langtang-Ghora Tabela
P		VC1446	Plectania	11 Oct	Langtang-Ghora Tabela
	KATH	VC1447	Humaria hemisphaera	11 Oct	Langtang-Ghora Tabela
P	nhm	VC1448	Otidia	11 Oct	Langtang-Ghora Tabela
P C+	KATH	VC1449	Suillus himalayan	11 Oct	Langtang-Ghora Tabela
P C+		VC1450	Suillus laricinus	11 Oct	Langtang-Ghora Tabela
P		VC1451	Larix himalaica seed	11 Oct	Langtang-Ghora Tabela
P	nhm	VC1452	Trichoglossum	11 Oct	Langtang-Ghora Tabela
P		VC1453	Lactarius	11 Oct	Langtang-Ghora Tabela
P		VC1454	Helvella	11 Oct	Langtang-Ghora Tabela
P	KATH	VC1455	Polyporus s.s.	11 Oct	Langtang-Ghora Tabela
P	KATH	VC1456	coral	11 Oct	Langtang-Ghora Tabela
P		VC1457	Cordyceps ophioglossoides	11 Oct	Langtang-Ghora Tabela
		VC1458	parasite of Amanita	12 Oct	Ghora Tabela-Lama Hotel
	-	VC1459	yeast (alcohol)	12 Oct	Syarpagaon
	NY	VC1460	Cavimalum indicum Doi, Dargan & Thind	12 Oct	w. of Syarpagaon
P		VC1461	Gyroporus	12 Oct	Khangjung-Syabrubensi
P		VC1462h	Rhizopogon	12 Oct	Khangjung-Syabrubensi
P	NY	VC1462p	Sepedonium chrysospermum on Rhizopogon	12 Oct	Khangjung-Syabrubensi
P	-	VC1463	yeast (alcohol)	13 Oct	Syabrubensi
P	-	VC1464	Suillus sp.	13 Oct	Syabrubensi-Syabru
P		VC1465	Hypocreaceae (immature)	06 Oct	Syabru-Bharku
		VC1466	Chroogomphus	12 Oct	Khangjung-Syabrubensi
	-	VC1467	yeast (alcohol)	14 Oct	Syabru
P		VC1468	Collybia	14 Oct	Syabru-Chandrabari
P		VC1469	gastroid bolete	14 Oct	Syabru-Chandrabari
P		VC1470	Suillus laricinus	14 Oct	Syabru-Chandrabari
P		VC1471	Lactarius	14 Oct	Syabru-Chandrabari
	KATH	VC1472	Laetioporus sulphureus	14 Oct	Chandrabari
P		VC1473	Scutellinia	15 Oct	Chandrabari
P	KATH	VC1474	Hymenochaete cruenta (Pers.:Fr.) Donk	15 Oct	Chandrabari
P		VC1475	Amanita	16 Oct	Chandrabari-Dhunche
P		VC1476	Hygrophorus	16 Oct	Chandrabari-Dhunche
	-	VC1477	yeast (alcohol)	21 Oct	Bode
	KATH	VC1478	polypore	29 Oct	Chobar
		VC1479	Chroogomphus	29 Oct	Chobar
P C+		VC1480	Suillus sp.	29 Oct	Kirtipur
P C+		VC1481	Suillus cf. placidus	30 Oct	Kakani
		VC1482	Sarcoscypha coccinea (s.l.)	30 Oct	Kakani
		VC1483	Cheilymania stercorea	30 Oct	Kakani
		VC1484	tarspot	30 Oct	Kakani
P		VC1485	Suillus sibiricus	30 Oct	Kakani
		VC1486	yellow fuzz balls	30 Oct	Kakani
	-	VC1487	Lycoperdon?	04 Nov	Dhangadhi
	-	VC1488	Lycoperdon?	05 Nov	Suklaphanta Reserve
	KATH	VC1489	Sporocadus or Coryneum leafspot	05 Nov	Suklaphanta Reserve
		VC1490	bolete?	05 Nov	Suklaphanta Reserve
	-	VC1491	Laccaria	05 Nov	Suklaphanta Reserve
P	KATH	VC1492	Schizophyllum	12 Nov	Phulchoki
		VC1493	tar spot on Rubia	12 Nov	Phulchoki
P	KATH	VC1494	Polyporus xanthopus	12 Nov	Phulchoki
P		VC1495	agaric	12 Nov	Phulchoki
P		VC1496	Scuttelinia	12 Nov	Phulchoki
P	nhm	VC1497	Paneolus	12 Nov	Phulchoki
	-	VC1498	Hygrophorus?	12 Nov	Phulchoki

Appendix A. Concluded.

		VC1499	Russula	05 Nov	Shivapuri
		VC1500	Russula	05 Nov	Shivapuri
		VC1501	Russula	05 Nov	Shivapuri
		VC1502	Russula	05 Nov	Shivapuri
		VC1503	clavarioid	19 Nov	Batasya
	nhm	VC1504	chanterelle	20 Nov	Rumsing Bhanjyang
P		VC1505	Isaria	20 Nov	Rumsing Bhanjyang
P		VC1506	discomycete	20 Nov	Rumsing Bhanjyang
		VC1507	Deuteromycete	20 Nov	Rumsing Bhanjyang
P	WSP	VC1508	Xylaria probably sp. nov.	20 Nov	Rumsing Bhanjyang
P		VC1509	yellow mycorrhizae	20 Nov	Rumsing Bhanjyang
P	KATH	VC1510	leafspot of Quercus	20 Nov	Rumsing Bhanjyang
		VC1511	parasite of agaric	20 Nov	Rumsing Bhanjyang
		VC1512	polypore	21 Nov	Arung Khola X Murti Khola
		VC1513	Panus	21 Nov	Batasya-Murli Khola
	nhm	VC1514	Stropharia	09 Dec	Royal Chitwan Nat'l Park
	nhm	VC1515	agaric	09 Dec	Royal Chitwan Nat'l Park
		VC1516	Deuteromycete	09 Dec	Royal Chitwan Nat'l Park
P	nhm	VC1517	Paneolus	09 Dec	Royal Chitwan Nat'l Park
	nhm	VC1518	agaric	09 Dec	Royal Chitwan Nat'l Park
		VC1519	Bolbitius sp. nov.	09 Dec	Royal Chitwan Nat'l Park
		VC1520	Coprinus	09 Dec	Royal Chitwan Nat'l Park
	nhm	VC1521	Coprinus	09 Dec	Royal Chitwan Nat'l Park
	KATH,nhm	VC1522	smut	09 Dec	Royal Chitwan Nat'l Park
		VC1523	agaric	09 Dec	Royal Chitwan Nat'l Park
		VC1524	chanterelle	09 Dec	Royal Chitwan Nat'l Park
	pp	VC1525	rust on pea	29 Dec	Kathmandu market
	KATH	VC1526	Schizophyllum	29 Dec	Phora Durbar
	-	VC1527	agaric	15 Jan	Koshi Tapppu
		VC1528	rust	15 Jan	Koshi Tappu
		VC1529	leafspot of Shorea	17 Jan	Piple, fish farm
P		VC1530	Lentinus edodes	17 Jan	Bhutan, imported into Nepal
P	KATH	VC1531	Pycnoporus cinnabarinus	17 Jan	Jogboda
	KATH	VC1532	polypore	17 Jan	Jogboda
	KATH	VC1533	polypore	17 Jan	Jogboda
	KATH,pp	VC1534	rust on Oxalis	21 Jan	Kathmandu
		VC1535	fungus on Bombax twig	23 Feb	Prithivinagar
		VC1536	Cordyceps sinensis (Berk.)Sacc.	Mar	
P		VC1537	Panus	01 Mar	Suklaphanta
		VC1538		01 Mar	Suklaphanta

APPENDIX B. FORMS USED IN DESCRIBING CULTURES, BASIDIOCARPS AND ECTOMYCORRHIZAE.

DESCRIPTION OF CULTURES, MACROCHEMICAL TESTS, ODOR, HEIGHT

V. Cotter. May 1987. Date Observed _____ Isolate _____

More than one Petri dish may be needed to complete these tests. Odor is recorded first. Next fluorescence is tested with long-wave ultraviolet light. Then height and depth are measured and a cross-section of the colony drawn. The first eight macrochemical tests (water through formalin) are conducted in a ceramic spot plate. Eight 11-mm plugs are cut to include a portion of the colony margin and placed face up in the spot plate depressions. The appropriate reagent(s) is added and reactions are recorded at 30 minutes. The final three macrochemical reagents, NH_4OH, KOH, and $FeSO_4$, are tested directly in the Petri dish. These reagents are put on the colony midway from disk to margin. are watched over time and Initial reactions and changes in the reactions are recorded. Final notes are taken at 10 minutes. Ammonium hydroxide must be tested last or separately. The halo with ammonium hydroxide is important to watch.

ODOR:

FLUORESCENCE:

COLONY GROWTH Draw cross-sectional pattern of aerial and submerged hyphae. Cut a radial sliver from the colony with a razor blade and observe under the dissecting microscope with transmitted light. Since the colony mats down as soon as the lid is removed, its height should be measured through the lid if possible.

- Submerged
- Appressed
- Raised (aerial)
- X.S. aerial pattern (convex, plateau, concave)
  - Height (mm)
- X.S. submerged pattern (mare's tails, parallel hyphae, waves)
  - Depth (mm)

Marginal hyphae. Use previously cut cross-sectional sliver.

- Submerged
- Appressed
- Raised
- Equal (submerged and (appressed or raised))

MACROCHEMICAL TESTS

- Water
- 95% ethanol
- 2% aqueous phenol
- tyrosine
- syringaldazine
  - with 0.3% hydrogen peroxide
- gum guaiac
- formalin
- 10% KOH
- 10% $FeSO_4$
- NH_4OH

OTHER OBSERVATIONS:

DESCRIPTION OF CULTURES, MORPHOLOGY AND ANATOMY

V. Cotter. May 1987. Observation Date _____ Isolate _____

Odor is recorded first. Microscopic features are observed last. Refer to "A manual for the identification of ectomycorrhizal and wood-rotting fungi in culture".

ODOR:

COLONY MORPHOLOGY Use dissecting scope as needed to confirm, but colony outline, surface and margin characteristics, and texture are described as they appear to the naked eye.

Colony outline
- Circular
- Lobed
- Irregular (trace)

Colony surface characteristics
- Droplets (note size to naked eye)
  - Clear
  - Colored (note color)
- Smooth
- Pitted
- Bumpy
- Tufted
- Funiculose
- Wrinkled
- Radial furrows
  - Surface
  - Reverse
- Zonate furrows
- Sectoring

Colony surface textures. Observe from the top and from the side. Hyphae mat down as soon as the Petri dish lid is removed so observe through the lid first.
- Zonate pattern
- Cottony
- Woolly
- Felty
- Velvety
- Silky
- Downy
- Villose
- Pruinose
- Furfuraceous

Margin characteristics
- Distinct
- Erose
- Ciliate
- Feathered
- Deltoid

Colour - Surface
- Mottled
- Zonate Number of zones ___
- Bruises
  (wound by tapping with edge of glass slide)

Reverse
- Mottled
- Zonate Number of zones ___

Colony Description in words, surface and reverse, note colors and distinctive features:

DESCRIPTION OF CULTURES, MORPHOLOGY AND ANATOMY PAGE 2. ISOLATE _____

COLONY CONSISTENCY. Probe colony with dissecting needle just out from original plug.
- Crustose
- Fleshy Thickness _____ mm
- Leathery
- Mat free

COLONY GROWTH. Draw cross-sectional pattern of aerial and submerged hyphae. Cut a radial sliver from the colony with a razor blade and observe under the dissecting microscope with transmitted light. Since the colony mats down as soon as the lid is removed, its height should be measured through the lid if possible.
- Submerged
- Appressed
- Raised (aerial)
- X.S. aerial pattern (convex, plateau, concave)
  - Height (mm)
- X.S. submerged pattern (mare's tails, parallel hyphae, waves)
  - Depth (mm)

Marginal hyphae. Use previously cut cross-sectional sliver.
- Submerged
- Appressed
- Raised
- Equal (submerged and (appressed or raised))

MICROSCOPIC FEATURES. Make three slides: two of aerial and one of surface hyphae, include a little agar with the latter. Take material from the middle of the colony, slightly closer to center than margin. Mount one aerial in water and the other in 3% KOH; the surface hyphae is mounted in 3% KOH. A=Aerial, S=Surface.

KOH reaction
- Contents
- Incrustations

Free crystals
- Shape
- Color

Pseudoparenchyma
Prosynchyma
Knots
Strands (record hyphal organization)
Hyphae
- Fluid
- Sinuous
- Contorted
- Spiral
- Clavate
- Cystidia

Hyphal diameter varies
- Among hyphae
- Along single hypha

Vesicular cells
- Frequency
- Catenulate

Anastomoses
Paarige branching
Clamps
- Frequency
- Which hyphae?

Hyphal walls
- Pigmented
- Thick
- Incrustations (note color)
  - Globular
  - Granular

Conidia Chlamydospores Sclerotia

WALL CLIMBING. _____ number of walls (maximum possible is four) isolate grew over in 4-compartment Petri dish. _____ number of additional walls scaled but no growth beyond.

OTHER OBSERVATIONS:

DESCRIPTION OF HYPHAE FROM SLIDE CULTURES, MICROSCOPIC FEATURES Isolate _____
V. Cotter and C. Lanphear-Cook. May 1987. Date slide prepared _____ Date observed _____

Slide cultures are carefully disassembled by lifting off (straight up) the coverslip and then, if the plug did not come off the slide with the coverslip, lifting off (straight up) the plug. Condensation away from the hyphae is wiped up. A drop of lactophenol blue is added near the hyphae and then drawn across the hyphae as a coverslip is lowered over top. The slide is gently warmed. These lactophenol mounts can be examined when convenient. All observations on this page are intended to be made from the lactophenol blue mounts. If the fungus also grew out onto the coverslip it can be used to make a second slide with a different mountant.

DRY OBJECTIVES. Scan and observe the following using 4x, 10x and 40x.

- Cell wall colour
- Clamps (note type)
  - Leading hyphae Lateral hyphae
- Anastomoses abundance
- Branching angle
- Fluid hyphae colour
- Surface ornamentation which hyphae?
  - Abundance
  - Colour
  - Globular
  - Granular
  - Papillate hyphae
- Hyphal strands
  - Cell wall colour
  - Surface ornamentation same as single hyphae
  - Rim (following hyphae) diam. of rim hyphae < = > parent hyphae
    - Number of hyphae thick
    - Parallel, subparallel (wavy parallel), interwoven
  - Core (parent hyphae) diam. of core hyphae < = > leading hyphae
    - Number of hyphae thick
    - Thin, medium, broad
    - Shape of septal area

100x OIL OBJECTIVE. Observe 5 leading hyphae (the main, initial, radiating hyphae), more if characters variable. Measure diameter (max.) of subterminal cell. Observe contents of subterminal and terminal cells, try to locate and count nucleoli. Each horizontal rule represents a leading hypha and each tick a septum. Draw the branches carefully noting position, if short mark apex as A. Arc across septum = clamp. Note anastomoses. Use 4x lens to confirm leading hyphae are being viewed by 100x lens. Record cell lengths on rule as micrometre units (30 = 28.6 µm) in pencil; later replace with µm in pen. Hyphal apices are to the left.

Contents
- 'Texture'
- Nucleoli

Diam. (µm)

OTHER FEATURES AND OBSERVATIONS:

DESCRIPTION OF SUILLUS BASIDIOCARPS, MICROSCOPIC FEATURES Collection _____
V. Cotter. Apr. 1987. Date collected _____ Date observed _____
Observations are made from dried collections. One typical, young (but with basidiospores) basidiocarp is chosen. Sections are made with a sharp razor directly from the dried basidiocarp. Material is mounted in 3% KOH to parallel most previous work with Suillus. Three areas are sectioned: 1) radial XS's of pileus including pellis and some trama taken at disk and midway from centre to margin, 2) longitudinal section of the tubes, and 3) XS of the stipe from just above middle including caulocystidia if present. Two additional mounts are made: 1) basal mycelium and 2) spores from a spore print. Five items are measured for each measurement, more if variable.

DISSECTING SCOPE OBSERVATIONS
Glandulae (abundance, distribution pattern, size, colour)
  Pores
  Stipe
Basal mycelium
  Amount and kind
  Colour
Other observations:

COMPOUND MICROSCOPE OBSERVATIONS
Pileipellis (draw XS)
  cutis / derm ixo + / - tricho
    Hyphal diam µm
    Clamps
  trama
    Hyphal diam µm
    Clamps

Hymenophore
  spores
  Basidia
    shape
    # sterigmata
    size µm
  Pleurocystidia, abundance single/clustered
    shape
    size µm
    contents
    incrusted
  Cheilocystidia, abundance single/clustered
    shape
    size µm
    contents
    incrusted
  Trama bilateral

Stipe
  Basidia
  Clamps
  Caulocystidia, abundance single/clustered
    shape
    size µm
    contents
    incrusted

Spores from spore print
  shape and surface
  colour in 3% KOH Dextrinoid
  size µm

Basal mycelium
  strands
  clamps

OTHER FEATURES AND OBSERVATIONS:

DESCRIPTION OF ECTOMYCORRHIZAE FROM SYNTHESIS TRIALS IN GROWTH POUCHES,
MACROSCOPIC FEATURES
V. Cotter and C. Cook. May 1987. Date: _____ Isolate x tree: _____
with thanks to Dr. Palmer.

Vigor ratings: 0 = no growth; 1 = poor, stagnant, 2 = slight growth; 3 = okay, good; 4 = excellent; 5 = superior.

OBSERVATIONS BEFORE DISASSEMBLING:
Date tree into pouch _____ fungus into pouch _____
Other pouch notes:

Tree shoot vigor
Secondary shoots
Tree root vigor
Fungal growth and vigor
Contamination

Cut plastic front of pouch down each side so that front can be rolled down to reveal the root system. The front should be kept over the root system as much as possible during observations to reduce drying out and collapse of the extramatrical mycelium. Later if desired the front can be rolled back over the roots and taped along the sides; in this form the pouch can be returned to the growth chamber under a frequent watering regime. If the root system and colonization pattern are traced directly on the plastic front of the pouch, the front can be kept as a record.

ECTOMYCORRHIZAE:

Number of nonectomycorrhizal short roots

Number of ectomycorrhizae by form (draw examples)

monopodial

bifurcate

double bifurcate

coralloid (count tips)

pinnate

Size (mm) L of mantled part x W at widest
Branching closeness and angle
Apex: pointed, rounded

Mantle color
Bruising
Mantle texture

Mantle position relative to parent root
Sessile, down to parent root
Stipitate, length of stipe
Branching position relative to parent root
Sessile, at parent root
Stipitate, length of stipe

EXTRAMATRICAL PHASE:

Color
Amount
Texture
Strands
Thin, thick
Color

Other observations:

DESCRIPTION OF EXTRAMATRICAL PHASE FROM ECTOMYCORRHIZAL SYNTHESIS TRIALS IN GROWTH POUCHES, MICROSCOPIC FEATURES

V. Cotter and C. Cook. May 1987. Date: _____ Isolate x tree: _____

Observations are made on fresh material freshly mounted in 3% KOH. Note colour changes that occur when the material is mounted. Then all measurements are made by mounting in lactophenol cotton blue. These lactophenol mounts are semipermanent and can be observed when convenient. Mount aerial hyphae and strands. Try to obtain XS of a strand. Choose material near ectomycorrhizae, but do not include the mantle itself.

3% KOH:

Single hyphae
  Wall colour
  Contents, texture and colour

  Surface ornamentation
    Abundance
    Colour
    Globular
    Granular
    Papillate

  Branching
    Simple (single lateral branches arise below septa)
    Paarige
    Paarige with 3 clamps
    Other

  Clamps
    Abundance
    Type

  Anastomose abundance
  Other features:

Hyphal strands
  Cell wall colour
  Surface ornamentation same as single hyphae
  Rim (following hyphae) diam of rim hyphae < = > parent hyphae
    Number of hyphae thick
    Parallel, subparallel (wavy parallel), interwoven
  Core (parent hyphae) diam of core hyphae < = > leading hyphae
    Number of hyphae thick
    Thin, medium, broad
    Shape of septal area

LACTOPHENOL BLUE MEASUREMENTS:
  Measure 5 for each measurement, more if variable.

Single hyphae - diam (µm)
Strands - diam (µm)
  Rim hyphae - diam (µm)
  Core hyphae - diam (µm)

Lactophenol blue observations:

APPENDIX C. DATA SET OF 82 CHARACTERS FOR 28 NEPALI BOLETE CULTURES USED IN NUMERICAL TAXONOMIC ANALYSES.

ISOLATE	RADOTO2	RAD2TO3	ZONATE	RVZONATE	HEIGHT	DEPTH	WALLCLMB	DIAMETER	BR3SEP	BR3O	SEPTOBR	LENGTH	WHITE1	WHITE2	YELLOW2	BROWN2	WHITE4	BROWN4	PURPLE4	GRAY4	YELLOW4	RVBLACK	RVPURPLE	RVYELLOW	DEFUSE1	DEFUSE2	FURROW1	FURROW2	FURROW3	IRRMAR	BUMPY	TUFTED
VC1022	0.36	0.24	0.55	0.44	0.74	0.00	1.00	0.50	1.00	0.55	0.0	0.38	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	1	0	1	1
VC1040	0.16	0.17	0.36	0.44	0.07	0.83	0.50	0.00	0.25	0.91	0.2	0.00	1	0	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	1	0	
VC1041	0.72	0.51	0.55	1.00	1.00	0.58	1.00	0.50	0.25	0.36	0.0	0.44	1	0	1	1	1	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0
VC1042	0.14	0.06	0.00	0.00	0.11	0.88	0.00	0.50	0.62	1.00	0.0	0.02	1	0	0	1	0	1	1	1	0	1	1	0	1	0	0	0	0	1	1	0
VC1081A	0.41	0.41	0.55	0.67	0.67	0.67	1.00	0.50	1.00	0.36	0.0	0.68	1	0	0	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0
VC1092	0.17	0.12	0.00	0.22	0.44	0.21	0.00	0.50	0.75	0.36	0.0	0.38	0	0	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	1	1	0
VC1185	0.23	0.32	0.18	0.22	0.15	0.75	0.12	0.50	0.25	0.18	0.4	0.17	1	0	1	1	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	1
VC1192	0.38	0.25	0.18	0.44	0.93	0.71	1.00	0.00	0.00	0.18	0.6	0.05	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0
VC1204	0.40	0.22	0.18	0.44	0.59	0.08	1.00	0.25	0.75	0.55	0.2	0.21	1	1	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1
VC1207	0.00	0.05	0.18	0.44	0.00	0.88	0.12	0.00	0.25	0.36	0.4	0.14	1	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	0	1	1	1
VC1218	0.30	0.38	0.18	0.67	0.44	1.00	0.12	0.00	0.00	0.00	1.0	0.10	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0
VC1219	0.81	1.00	0.36	0.89	0.67	0.42	1.00	1.00	0.50	0.18	0.2	0.65	1	0	1	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0
VC1230	0.17	0.00	0.00	0.00	0.52	0.08	0.62	0.50	0.75	0.18	0.2	0.43	1	1	0	0	1	1	0	1	0	0	0	1	1	1	1	0	0	1	1	1
VC1231	0.48	0.29	0.36	0.67	0.67	0.17	1.00	0.00	0.75	0.55	0.2	0.25	1	1	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	1
VC1303	0.14	0.18	0.36	0.44	0.07	0.88	0.38	0.00	0.25	0.18	0.2	0.43	1	0	0	1	1	1	1	1	1	0	1	1	0	0	1	1	1	0	1	0
VC1310	0.23	0.31	0.45	0.67	0.11	0.58	0.00	0.00	0.25	0.18	0.2	0.13	1	0	0	0	1	1	0	1	1	0	0	1	0	0	1	1	1	0	0	0
VC1312	0.15	0.14	0.00	0.44	0.07	0.96	0.12	0.50	0.75	0.55	0.0	0.35	1	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	0
VC1328	0.31	0.20	0.00	0.22	0.37	0.79	1.00	0.00	0.25	0.18	0.4	0.10	1	1	0	0	1	1	0	1	0	0	0	1	0	0	1	1	1	0	1	0
VC1391A	0.28	0.27	0.55	0.44	0.04	0.75	0.25	0.50	0.25	0.18	0.4	0.24	1	0	0	0	1	1	0	1	0	0	0	1	1	0	1	1	1	0	0	0
VC1416	0.74	0.68	1.00	0.89	0.22	1.00	1.00	1.00	0.50	0.36	0.2	0.59	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1
VC1421	0.20	0.16	0.18	0.22	0.22	0.92	0.25	0.00	0.00	0.00	0.8	0.16	1	0	0	0	1	1	0	1	0	0	0	1	1	0	1	1	1	1	0	0
VC1423	0.35	0.27	0.18	0.44	0.74	0.46	0.75	0.25	0.25	0.18	0.2	0.32	1	1	0	0	1	1	0	0	0	0	0	1	1	0	1	1	1	0	0	0
VC1425	1.00	0.93	0.36	0.89	0.81	0.50	1.00	0.50	0.75	0.36	0.0	1.00	1	0	1	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0
VC1438	0.02	0.04	0.36	0.67	0.22	0.46	0.12	0.00	0.25	0.55	0.2	0.16	1	0	0	1	1	1	0	0	0	0	0	1	0	1	1	0	0	0	1	0
VC1449	0.20	0.21	0.18	0.44	0.22	0.42	0.62	0.50	0.62	0.36	0.2	0.71	1	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0
VC1450	0.20	0.07	0.18	0.67	0.74	0.33	0.38	0.00	0.00	0.00	0.4	0.40	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1
VC1480	0.25	0.14	0.18	0.44	0.59	0.75	0.12	0.25	0.25	0.18	0.2	0.05	1	0	0	0	1	1	0	0	0	1	0	1	1	0	1	1	1	1	1	0
VC1481	0.29	0.14	0.55	0.44	0.59	0.88	0.50	0.50	0.25	0.18	0.2	0.56	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0

Appendix C. Concluded.

ISOLATE	FUNICULE	WRINKLED	SECTORED	ACTINOM	ZONATETX	FELTYS	FELTYA	SILKY	VILLOSE	PRUINOSE	EROSE	CILIATE	FEATHERD	MOTTLED	BRUISES	RVMOTTLD	FLESH	APPRESSD	RVPILLAR	RVWAVE	SUBMERGD	RAISED	CRYSTALS	PSEUDOP	STRANDS	FLUIDHYP	FLUIDHG	FLUIDHYB	FLUIDHOB	IRRHYP	VESICULE	INCRUST	INCRUSTL	ODOR	TYROSINE	SYRING	GUMG	KOHRP	FESO4	NH4OH	NH4OHRP	NH4OHGRN	CLAMPS	CLAMPSM	CLAMPSL	ANASTOMO	PAPILATE	MDCELLBR	PAARIGE	GENICULT
VC1022	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	1	1	1	0	0	0	0	1	1	1	1	0	0	0	1	1	0	1	0
VC1040	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	1	0	1	0	1	1	0	0	0	1	0	0	0	0	1	0	1	0	0
VC1041	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	0	0	1	0	0	0
VC1042	0	1	0	1	1	1	0	0	0	1	1	0	0	1	0	1	1	1	0	0	1	0	1	0	0	1	0	0	1	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
VC1081A	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0
VC1092	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	1	1	1	1	0	0	1	0
VC1185	0	1	1	0	0	1	0	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
VC1192	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1
VC1204	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	1	0
VC1207	0	1	1	1	0	1	0	0	0	1	1	0	0	1	0	1	0	1	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	1
VC1218	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	1	0	0	0	0	1	0	0	0	1
VC1219	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	0	1	0
VC1230	0	1	0	0	1	1	0	0	1	0	1	1	0	1	1	0	1	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1
VC1231	0	0	0	1	0	0	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0	1	0	1	0	0	1	0	0	1	1
VC1303	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1
VC1310	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	1	1	1
VC1312	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	1	1	1	0	0	1	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1
VC1328	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	1	0	1
VC1391A	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1
VC1416	1	0	0	1	1	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	1	0	1	1	1	0	0	1	1	0
VC1421	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0
VC1423	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	1	1	0	0	1	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
VC1425	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	0	0	1	0	1	0
VC1438	0	1	0	0	1	1	0	1	1	1	1	1	0	1	0	1	1	0	0	0	1	1	0	1	0	1	1	0	0	1	1	1	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	1	0	1
VC1449	0	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	1	0	1	1	0	0	1	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
VC1450	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0	1	1	0	0	0	0	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	0	0	1	0	1	0	0	1	0	0	0	1
VC1480	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	1	0	1	1	1	1	0	0	0	1	1	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1
VC1481	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0

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