Characterization of Agrocybe praecox and its Sibling Species

by

Timothy M. Flynn

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Botany

APPROVED:

Orson K. Miller, Jr., Chairman

Bruce Parker

Khidir Hilu

April, 1986

Blacksburg, Virginia

Characterization of Agrocybe praecox and its Sibling Species

by

Timothy M. Flynn

Orson K. Miller, Jr., Chairman

Botany

(ABSTRACT)

Studies of breeding relationships are integrated with the nomenclature of *Agrocybe praecox*. Type studies of potential synonyms are presented. Nomenclatorial and type studies indicate that *Agrocybe molesta* has been confused with *A. praecox* sensu lato. New field and microscopic characteristics are introduced which differentiate between *A. molesta* and the *A. praecox* group. *A. molesta* is redescribed to reduce confusion and a neotype is designated for this taxon. Four sibling species of *Agrocybe praecox* are identified and neotypes designated for *A. praecox* and *A. gibberosa*. A new species, *Agrocybe montanus* is described.

Acknowledgements

This work was made possible through the guidance, experience, patients, wisdom, and enthusiasm of my thesis advisor, Orson K. Miller, Jr.. I am indebted to his kindness. Committee members, Khidir Hilu and Bruce Parker have made valuable contributions and useful criticisms for this degree and my professional development. Hugo Ferchau, my undergraduate advisor, through his love of nature convinced me that the study of living things, especially plants and fungi, would be a rewarding endeavor, but don't expect to get paid more than Darwin did for his work on the Beagle. The most significant contribution, perhaps even more than my advisors, is the love and support of my parents, Rita and Thomas Flynn. They have nurtured and educated me in many ways. I thank my friends for sharing my joy in the good times and helping me to maintain a proper perspective. John Haines (NYS), Olivier Monthoux (G), James Kimbrough (FLAS), Clark Rogerson (NY), and Ron Peterson (TN) have been most helpful in providing herbarium specimens for this study. Critical specimens were provided by Gerald Bills, Rytas Vilgalys, Van Cotter, Steve Miller, Marie and Leeds Bailey, Hope Miller, Jean Greig, Sylvia Stein, R. Pipenbroek, Steve Rayner, Tom and Rita Flynn, Roy Watling, and Meinhard Moser.

Table of Contents

List of Illustrations

List of Tables

Introduction

The taxon, *Agrocybe praecox* (Pers. : Fries) Fayod (Basidiomycetes, Agaricales, Bolbitiaceae) has several characteristics which prompted this biosystematic investigation. The members of this group are phenotypically polymorphic and degrade a variety of lignicolous substrates. This polymorphism has not been adequately compared within and among populations, thus the potential diagnostic value of variable morphological characteristics was not known. As a result, taxa identified as *A. praecox* have been frequently confused with morphologically distinct taxa such as *A. molesta* (Lasch) Singer and additional taxa have frequently been described from a single collection or single sporocarp. The variable criteria used to recognize taxa have created a nomenclatorial jungle and a confused picture of the known taxa.

This study has three main objectives designed to determine the relationship of morphological variation to interbreeding populations in *A. praecox* and to integrate morphological species concepts with biological species concepts. Firstly, the mating relationships of taxa belonging to the *A. praecox* group representing a large geographic area were determined. Secondly, the range of morphological variation seen within and

among intercompatible populations was evaluated. Thirdly, these findings were related to the nomenclature of *A. praecox* and its satellite taxa. To meet these objectives, an understanding of fungal sexuality is needed to develop species concepts for higher fungi.

Fungal genetics and species concepts

The biological species concept based on mating compatibility has been useful in delimiting clusters of closely related species. As stated by Mayr (1965), a biological species is "a group of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups" and are interpreted by Dobzhansky (1950) as "the largest and most inclusive reproductive community of sexual cross fertilizing individuals which share in a common gene pool". Additional interpretations and the limitations of this concept are summarized by Mayr (1963; 1976) and Dobzhansky (1970). Establishment of fungal heterokaryons is genetically determined and is a requirement for the completion of the sexual life cycle in basidiomycetes. The sexuality of basidiomycete is primarily determined by two separate systems, known as homogenic incompatibility and heterogenic incompatibility. The application of biological species concept to higher fungi is determined by these incompatibility systems.

Homogenic incompatibility

Heterothallism regulated by homogenic incompatibility renders haploid mycelia incompatible if the mating factors are identical. Dikaryons can only be established when the mating types are heterofactorial and the mycelium of a given taxon is either

characterized by clamp connections or simple septa. The homogenic incompatibility system may consist of one, two or three independently segregating, unlinked factors (Raper, 1966; Jurand and Kemp, 1973).

Unifactorial incompatibility system consists of a single factor which can be composed of one or two loci. The alternate factors segregate at meiosis to produce two gamete types. For instance, an individual bearing the genotype AxAy will yield *A*x or *A*y gametes, therefore, selfing gives a bipolar pattern of compatible matings. The bifactorial mating incompatibility system consists of two factors designated as *A* and *B*. The alternate forms of each factor will segregate upon meiosis to propagate four gamete types. An individual with the genotype *A*x*A*y*B*x*B*y will yield gametes bearing *A*x*B*x, *A*x*B*y, *A*y*B*x, and *A*y*B*y genotypes, accordingly, a tetrapolar pattern of compatible matings results from selfing. *Schizophyllum commune* Fries produces characteristic growth patterns from different combinations of the *A* and *B* factors (Papazain, 1950; Raper, 1966). Clamp connections form when both the *A* and *B* are heterofactorial ($A \neq B \neq$). False clamp connections form in heterofactorial *A*, homofactorial *B* heterokaryons ($A \neq B =$).

The *A* and *B* mating factors assume many alternate forms within populations (Whitehouse, 1949; Eggertson, 1953; Raper, 1958). Multiple factors for incompatibility are generated by recombination of the α and β loci of the *A* and *B* mating factors. The structure of the *A* incompatibility factor in *Schizophyllum commune* was first examined by Papazain (1951) and was later characterized by Raper (1960), Koltin (1972), and Stamberg (1969). The *A* factor in this species is composed of an α locus and a β locus. Nine different alleles have been identified at the α locus and 32 at the β locus so a minimum of 288 different *A* factors are possible (Stamberg and Koltin, 1973). The *B*

factor is also composed of α and β loci, but only nine alleles have been identified at each site (Raper, 1958; Koltin, Raper and Simchen, 1969; Koltin, 1969).

Outbreeding potential is the probability that two non-sister haploid mycelia will form a dikaryon. A large outbreeding potential is generated from a relatively limited number of α and β loci. A small number of alleles at these α and β loci, such as 3Aα, 2Aβ, 2Bα, 3Bβ, evenly distributed between the factors will result in an outbreeding potential of 69%. An increase in the number of alleles increases the outbreeding potential, so that natural populations of *S. commune* have outbreeding potentials which exceed 95% (Burnett, 1975).

Ullrich and Raper (1974) estimated that at least 100 to 300 *A* factors exist within each of the five intersterility groups of the heterothallic, unifactorial fungus *Sistotrema brinkmannii.* They have not determined whether the *A* factor has one or two component loci, but there is some evidence suggesting two from recombination data (Burnett, 1983). The *A* factor is composed of α and β loci in *C. lagopus* and from a sample of ten different factors Day (1963) found 4 Aα and 5 to 7 Aβ alleles.

The two locus structure of the incompatibility factors provides a mechanism which regulates the genetic structure of Basidiomycete populations by balancing levels of inbreeding and outbreeding. Estimated average recombination frequencies of the *A* factor in *Schizophyllum* is 9.45% (Raper, 1960) and the *B* factor is 2.3% (Koltin, 1967). These values theoretically increases the inbreeding potential from 25% to 31% because each different combination of α and β alleles yields unique factors (Burnett, 1975). Estimates of recombination frequencies in natural populations of *S. commune* suggest inbreeding potentials closer to 29% (Schaap and Simchen, 1971). Factor recombination

increases inbreeding potential and perhaps most importantly, this recombination creates a dramatic increase in outbreeding potential. Recombination is under the fine control of regulatory genes (Stamberg, 1968; 1969a; 1969b). Through the regulation of recombination, a balance between inbreeding and outbreeding is achieved which determines the genetic constitution of fungal populations. Inbreeding potential may influence local adaptation and new site colonization by maintaining adaptive genotypes and can promote genetic drift in peripheral, isolated populations allowing for allelic fixation and divergence (Dobzhansky, 1970). Outbreeding, on the other hand tends to generate genetic diversity and heterogeneity in populations.

A breeding system which imposes high levels of inbreeding in previously heterothallic fungi is known as secondary homothallism, pseudohomothallism, amphithallism, and homodiaphoromixis (Burnet, 1975; Esser, 1967). This system generally promotes obligate inbreeding by packaging compatible nuclei into a single spore. A single spore will germinate to produce a heterokaryotic mycelium capable of undergoing a complete sexual life cycle. This is commonly seen in two-spored basidiomycetes such as *Coprinus ephemerus* and *Agaricus bisporus* and in some four-spored basidiomycetes such as *Mycocalia denudata.* It also occurs in ascomycetes bearing four-spored asci such as *Neurospora tetrasperma* and *Podospora anserina.* The mating system may be unifactorial as in *C. bisporus* (Langton & Elliot, 1980), or more commonly, bifactorial as in *Coprinus bilanatus* and *C. sassii* (Elliot, 1983). Occasionally, homofactorial nuclei are packaged into the same propagule resulting in a mycelium which is sexually competent. However, for the most part, spores are heterokaryotic for the mating type loci. This nuclear behavior can have adaptive value for individuals or populations which are outside of their normal ecological tolerances by colonizing a new niche, and have reduced contact

with members of the same species. This strategy is used by weedy, annual cleistogamous plants (Dobzhansky, 1970; Stebbins, 1950; Grant, 1958).

Heterogenic incompatibility

A prezygotic isolation barrier which recognize members of the same interbreeding population is known as heterogenic incompatibility, heterokaryon incompatibility, or vegetative incompatibility (see Esser, 1967; Burnett, 1975). These systems have slightly different phenotypic or developmental expression but produce the same result. Heterogenic incompatibility causes the abortion of sporocarp and meiospore production in *Podospora anserina* (Esser, 1967). Vegetative or heterokaryon incompatibility causes the death of heterokaryotic mycelium such as in *Aspergillus nidulans* (Jinks, 1966), and *Heterobasidion annosum* (Chase, 1985). Both systems are similar in that strains are rendered incompatible when the alleles at the ***Het*** loci are heterozygous. These systems recognize, on a genetic basis, self from non-self and ultimately, prevent any possibility of karyogamy and meiosis. Such systems are very effective prezygotic isolation barriers. The development of a visible zone of antagonism is often associated with heterogenic incompatibility when two mycelia with different alleles are paired on agar media. The zone may be a region of sparse growth and lysis or an area of rigorous antagonism characterized by the death of densely interwoven, highly vacoulated, pigmented hyphae. Heterogenic incompatibility occurs in many groups, including Basidiomycetes, and apparently develops in the absence of geographical, ecological, or seasonal isolating mechanisms.

Hoffmann and Esser (1978) demonstrated a heterogenic incompatibility in the group which includes *Polyporus ciliatus*, *P. arcularis*, and *P. brumalis*. These fungi are relatively

easy to distinguish on morphological grounds based on hymenial and sporocarp morphology, but the variation in these characteristics can overlap. For instance, collections received as *P. brumalis*, *P. lepideus* and *P. ciliatus* f. *lepideus* were identified as *P. ciliatus* when they were incorporated in the mating studies which indicates the value of interspecific mating tests. All interspecific combinations of monokaryotic mycelia were sterile and a border line zone is seen when these mycelia are confronted. Some of the intraspecific crosses develop barrage zones, characterized by a broad clear area, lacking pigment production. Hyphae are sparse and with time, lysis is observed. Three genes are found to be responsible for this behavior. Complete barrage formation occurs when at least one strain has the barrage initiation$^+$ allele (bi^+) and both strains are heteroallelic at the barrage formation genes I and II (bfI_1/bfI_2 and $bfII_1/bfII_2$).

Populations of *Heterobasidion annosum* in Finland, form two intersterility groups, each of which occurs in either spruce or pine, and are denoted as ***S*** and ***P***, respectively (Korhonen, 1978). The ***S*** and ***P*** groups have minor morphological differences and are generally restricted to their respective hosts in Europe. Later studies, have determined the genetic system responsible for these mating relationships (Chase and Ullrich, 1985). Five loci have been identified and are termed intersterility genes (***IS***) with alternative alleles designated as + or − for each locus. Compatible interactions occur when the two strains are homozygous + at one of the five loci (***V1***, ***V2***, ***V3***, ***S***, ***P***). These genes function to determine intersterility and act independently from homogenic incompatibility.

In summary, breeding systems in fungi regulate the exchange of genetic information which determines the levels of phenotypic variation seen in populations which influences taxonomic decisions. Homogenic incompatibility determines the levels of outbreeding

and inbreeding which provides the potential for high levels of genetic heterogeneity in fungal populations. Heterogenic incompatibility is an isolation mechanism that recognizes members of the same group and causes positive assortative mating. Hybridization does not present difficulties for fungal biosystematics because it rarely occurs in higher fungi. An exception to this trend is exemplified by laboratory strains of a parasitic Basidiomycete, *Typhula* but hybrids are not found in nature (Christen & Bruehl, 1979). These facts establish mating compatibility as one of the valid criteria used in defining species of Hymenomycetes.

Fungal biological species

In fungi, the biological species concept, based on mating compatibility, has been used to delimit potentially interbreeding populations. Lange (1952) has shown in *Coprinus* that several morphologically distinct but similar species possess genetic isolation mechanisms. Similar findings are seen in *C. congregatus* group (Kemp, 1970). This species concept is especially useful in groups where the phenotypic variation of individuals often equals the variation seen among populations and biological species. Studies such as these have expanded our knowledge of the hymenomycete species and has challenged species concepts based upon morphological criteria alone. Studies of morphological variation found within fungal species forces us to abandon typological or essentialistic thinking for population thinking (Mayr, 1976 and 1963). Examples of these types of investigations are numerous, such as *Armilaria mellea* (Korhonen, 1978; Anderson, 1979), *Collybia dryophilla* (Vilgalys & Miller, 1983), *Pholiota* stirps *adiposa* (Farr et. al., 1977), *Auricularia auricula* (Duncan, 1967), *Tremella mesenterica* (Wong, Wells & Bandoni, 1985) and *Exidiopsis plumbescens* (Wells and Wong, 1985) *Coprinus* section *lanatuli* (Kemp, 1975) *Hirschioporus abietinus* (Macrae, 1967) and others. The

studies cited above show that species with morphological discontinuities are absolutely intersterile and that hybridization rarely occurs under laboratory conditions and hybrids are never seen in nature. Also, many of these taxonomic groups often are composed of several morphologically similar yet intersterile populations. In many of these cases, diagnostic characteristics are identified, but frequently the phenotypic variation can include several biological species.

Breeding studies of the *Hirschioporus abietinus* group have defined morphological criteria useful in circumscribing taxonomic species (Macrae, 1967). Three types of hymenial configurations exist in this group; poroid, irpicoid, and lamellate. Beyond hymenial configuration, the sporocarps of these species are very similar, have broad geographic distribution, and can decompose several substrates. The three morphological groups are intersterile, although partial compatibility is seen in a few interspecific crosses but these results were attributed to experimental error. The poroid group is comprised of two intersterility groups in north America and both American groups show only partial compatibility to the poroid form in Europe. Interspecific confrontations of the lamellate form with the poroid or irpicoid forms generate strong antagonistic interactions. The barrage zones are characterized by dense intertwining hyphae and production of short branches to produce a knot of hyphae with deteriorating cytoplasmic. Similar interactions are observed when the lamellate form is paired with another species, *H. pargamenus*. All other interspecific combinations produce cytoplasmic deterioration at the interaction zone but no hyphal coiling is seen.

The *Sistotrema brinkmannii* group presents interesting problems for biological and biosystematic evaluation. Members of this group exhibit primary homothallism, unifactorial heterothallism, and bifactorial heterothallism (Lemke, 1969). Ullrich (1973)

studied the breeding biology of 96 stock isolates collected from a variety of substrates and distributed among north America, Europe and Australia. Of 96 stock isolates, 16 were homothallic, 74 were bipolar, and 6 were tetrapolar. Five intersterility groups were found with the unifactorial heterothallic forms (*I-1*, *I-2*, *I-3*, *I-4*, and *I-5*) and two bifactorial intersterility groups (*IV-1* and *IV-2*) were identified. Since this sample is not all inclusive, the existence of additional incompatibility groups within the bipolar or tetrapolar species are possible. No obvious relationship between reproductive system and geographic distribution and substrate preference is apparent. Although Ullrich (1973) did not directly address the taxonomic aspects of this group, he was able to relate cultural characteristics to the system of homogenic incompatibility and homothallism. The homothallic forms produce unpigmented, catenate bulbils in culture; the bipolar heterothallic forms do not produce bulbils; and the tetrapolar, heterothallic forms develop brown bulbils aggregated into staphloid clusters, much like clusters of brown grapes. The bulbils of tetrapolar incompatibility group *2* (*IV-2*) are darker than tetrapolar incompatibility group *1* (*IV-1*).

Hallenberg (1984) attempted to develop a taxonomic system which incorporates sporocarp morphology and the mating relationships within the *Sistotrema brinkmamnii* group. The mating relationships and morphological characteristics of 40 living stocks collected from Canada and northern Europe were investigated. The taxonomic system is based primarily on spore morphology, texture and density of the subiculum, hymenial surface characteristics, basidia, hyphal morphology, bulbils associated with the sporocarp. He gave taxonomic status to two different bipolar species, three different tetrapolar species, and four different species with unidentified mating system. He also included two primary homothallic collections into the taxon *S. brinkmannii.*

Hallenberg (1984) criticized Ullrich's work (1973) for the lack of attention given to the taxonomy of the *Sistotrema brinkmannii* group. His most germane criticism pertains to Ullrich's failure to designate voucher collections for his stock cultures. However, Hallenberg's study is far from complete and several voids exist in his taxonomic system. Hallenberg marginally relates sporocarp morphology to the the bipolar and tetrapolar intersterility groups and he does not relate sporocarp morphology to potentially interbreeding populations. Without determining breeding systems, he described *S. adnatum*, *S. atheloides*, *S. porulosum*, and *S. binucleosporum* as new. He included two homothallic collections into the taxon *S. brinkmannii* which is a bipolar species by definition. Representatives of each tetrapolar and bipolar intersterility groups were deposited into American Type Culture collection by Ullrich but Hallenberg did not compare his cultures with these isolates. Intraspecific variation was not recognized by Hallenberg because he described collections with similar appearance as species without determining the mating relationships. Therefore, it is not definitively known to which incompatibility group Hallenberg's taxa belong.

Hallenberg's study could be improved in several ways. The genus *Sistotrema* fruits easily in culture and the lack of voucher collections could be rectified by obtaining sporocarps of Ullrich's incompatibility groups in the laboratory. If phenotypic plasticity is a concern, these fungi could be fruited on natural substrates in conditions reproducing the microclimate when these fungi sporulate. Most importantly, Hallenberg should have related his cultures to the known incompatibility groups and would give Ullrich's cultures voucher collections to refer to. Furthermore, additional incompatibility groups may have been collected which were not present in Ullrich's study.

In some instances, the determination of mating compatibility hinders fungal biosystematics if not evaluated carefully. Ullrich's study is fascinating because it describes a group of morphologically similar species which have very different genetic mechanism of sexual reproduction. The group represents an ideal experimental system for addressing the relationships of homothallism to heterothallism and bipolar to tetrapolar homogenic incompatibility. Hallenberg's study could be more valuable if he related sporocarp morphology to interbreeding populations. Furthermore morphologically distinguishable types could be related to the means of reproduction, levels of variation in populations, and the stable characteristics which correspond to potentially interbreeding populations.

Fungal sibling species

The concept of sibling species, sensu Mayr (1942), is applicable to Basidiomycete systematics. He defines sibling species as "morphologically similar if not identical intersterile populations". Later, Mayr (1963) slightly modified his definition after minor morphological, and cytological differences were discovered in sibling species of fruit flies and mosquitos; these differences were later reinforced by ecological factors. For example, *Drosophila pseudoobscura* and *D. persimilis* are intersterile, yet exceedingly similar species. These two species were found to differ in salivary gland chromosome morphology, male genetalia, sex combs, and relative wing size (Mayr, 1965). *Anopheles*, a malarial mosquito in Europe is composed of six sibling species best distinguished by egg characteristics. Sibling species have been found in many different organisms including other insects, birds, mammals, snakes, salamanders, limpets, nematods, and protozoans (see Mayr, 1965 and 1976; Dobzhansky, 1970). It would

appear, from my studies of *Agrocybe praecox* and the following examples, that sibling species also occur in the Eumycota.

Although the term sibling species has rarely been applied to fungi, *Armillaria mellea*, *Auricularia auricula* and *Heterobasidion annosum* are good examples. The *Armillaria mellea* complex is composed of at least five biological species in Europe (Korhonen, 1978; Anderson, 1979; Watling, 1982). After close analysis, they can be identified with difficulty by minor morphological characteristics, such as specific cultural features, clamped basidia, and substrate difference.

Auricularia auricula forms two intersterility groups in north America, one occurring primarily on hardwoods (*H*), the other on softwoods (*C*) (Duncan, 1967). In Europe, *A. auricula* occurs primarily on *Sambucus nigra* which is not native to north America. The European species is compatible with the north American *H* group, and both are intersterile with the *C* group. Characteristics considered to be of taxonomic importance such as the size and shape of spores, basidia, and sterile hairs are continuous among the three intersterility groups. The European populations however, tend to produce basidia and spores be on the large end of the size range.

Heterobasidion annosum is composed of two biological species in Europe (Korhonen, 1978). Three more mutually exclusive sibling species exist in north America (Chase & Ullrich, 1985) making a total of five. In Europe, the two intersterility groups are generally restricted to either pine or spruce, and only very slight difference can be found when examining the sporocarps and the cultural characteristics. The three intersterility groups identified in Vermont all occur in Red Pine plantations.

Paxillus involutus, a mycorrhizal fungus, forms three intersterility (**I, II and III**) groups in northern Europe (Fries, 1985). Group **I** primarily grows in coniferous forests, groups **II** and **III** grows in gardens and parks associated with birtch. Morphological differences are found between group **I** with **II** and **III**, but all groups produce sporocarps with intermediate characteristics. Therefore, morphological criteria could not consistently differentiate among the three intersterility groups, but group **I** prefers conifers, groups **II** and **III** prefers deciduous trees as mycorrhizal hosts.

The term sibling species simply and accurately describes aggregates of reproductively isolated, morphologically similar species. The abundance of such fungal groups as suggested by the literature merits the adoption of this useful term by mycologists.

Causes for sympatrically distributed sibling species

Four non-exclusive evolutionary processes can account for the abundance of sympatrically distributed fungal sibling species. Firstly, the examples cited above may have recently diverged and have not acquired morphological discontinuities. Secondly, sporocarp morphology may be neutral in these groups where spore production and dissemination is adequate in these sibling species. Variation, adaptation and selection may have have greater impact on the thallus than the sporocarp. Thirdly, these sibling species may have allopatrically speciated with sympatric distributions caused by expanding ranges. Fourthly, sympatric speciation may be mediated by heterogenic incompatibility which is an intrinsic barrier to gene flow.

Maynard-Smith's (1966) model of sympatric speciation is supported by laboratory and field studies of animal populations. According to Maynard-Smith's model of

sympatric speciation (1966), a species can utilize several niches; if disruptive selection is imposed upon populations of a species, positive assortative mating will follow which prevents the propagation of heterozygotes with intermediate characteristics. Disruptive selection removes individuals with intermediate genotypes from the population and can cause genetically similar individuals to mate (positive assortative mating). Later, these populations can evolve and diverge into species of similar appearance but with differing ways of exploiting their environment. For example, the lace wing *Chrysopa carnea* and *C. downesii* are two closely related species which differ only in their pigmentation and period of diapause (Tauber & Tauber, 1977a; 1977b; Tauber et. al., 1977; and Hendrickson, 1978). These phenotypic expressions are controlled by six alleles at three loci. The monophagous fruit flies *Rhogoletis* seems to have speciated sympatrically as new fruits were cultivated in New York (Bush, 1975; Wallace, 1981). Thoday (1958) was able to obtain positive assortative mating and a bimodal distribution of bristle number in laboratory strains of *Drosophila.* The studies of Tauber & Tauber, Bush and Thoday indicate that sympatric speciation is possible in natural and laboratory populations.

The known genetic properties of heterogenic incompatibility in fungi offer a mechanism which is suggested by Maynard-Smith's model of sympatric speciation. Many species of fungi exist in patchy environments and can exploit several niches. Heterogenic incompatibility can isolate ecotypic populations of a species and causes positive assortative mating because gametes with different "**Het**" alleles can not form progeny and forces genetically similar individuals to mate. Disruptive selection may not be necessary because intermediate genotypes are prevented from becoming established. Attempts to demonstrate speciation in higher fungi have not been made by mycologists, but the abundance of fungal sibling species and heterogenic incompatibility suggest exciting lines of investigation for evolutionary mycology.

Species concepts have classically been based on morphological criteria. This is a reasonable assumption because organisms with a similar appearance have common ancestry and share common genes. The literature clearly indicates however, that many species defined by morphological criteria alone may in actuality be several sibling species and conversely several species delimited on minor morphological differences are in fact variant forms of a single biological species. These findings indicate that genetically isolated populations are ***species*** despite their appearance. This opinion may be met with opposition from strict morphologists but species concepts based on the biology of organisms and experimentation has a stronger foundation than species concepts based on subjective judgements alone. Taxonomy and nomenclature are essential to biology by providing a data retrieval system and a stable way of referring to organisms. These disciplines are important, but strict adherence to morphological criteria interferes with the advancement in understanding the nature of species and evolution. Perhaps it is more important to understand how species come about, interact, and recognize each other than what we name them. Modern biosystematics which employs ecology, genetics, biochemistry, and physiology is not the final answer but has nevertheless contributed greatly to our understanding of the nature of species. Experimental taxonomy will often produce findings which are discordant with classical taxonomy, these findings should be embraced, not shunned.

Taxonomic History

The taxon *Agrocybe praecox* (Persoon : Fries) Fayod and other commonly recognized taxa in the section **Agrocybe** (Singer, 1936) such as *Agrocybe dura* (Bolton : Fries) Singer,

A. palusosa (J.E. Lange) Kühner & Romagnesi, *A. sphaleromorpha* (Bulliard : Fries) Fayod and *A. howeana* (Peck) Singer were ill defined since their very conception. Firstly, the epithet *praecox* indicates "early seasonal appearance". This has generated confusion because two annulate species of *Agrocybe* with similar external morphology fruit in the early spring. Secondly, authors have described many phenotypic variants as species. Thirdly, many of these taxa based only on illustrative plates and short descriptions are not supported by authentic specimens. Fourthly, authors who made the generic transfers failed to designate neotypes. Lastly, all descriptions lack critical information such as substrate and partial veil characteristics. Therefore, the lack of critical information has confounded the taxonomy of this group.

Victor Fayod established the genus *Agrocybe* in 1889 to encompass agarics with brown spore prints, relatively robust stature, cellular pileipellis, and saprophytic nutrition. He examined and transferred many species, but did not describe any new taxa. *Agrocybe praecox* was designated by Fayod as the type species for the genus but many have been misidentified and represent collections of *A. molesta* (Horak, 1968).

Agrocybe praecox (Persoon : Fries) Fayod 1889 is based upon Fries's (1821, p.282) recognition of Persoon's original description (1801, p.420). Persoon's protologue depicts a fungus pigmented light yellowish white, with a hemispheric pileus; solid and subtenaceous stipe; fuscus colored lamellae; a fugaceous annulus, and a subgregarious fruitings pattern. Persoon did not formally designate a holotype, but a collection identified by him was preserved and exists at Leiden, Netherlands. Fries (1821) adds to Persoon's diagnosis by saying that the lamellae are adnexed with a decurrent tooth, but did not mention the presence of an annulus in his description. Fries did not preserve a representative of this taxon.

Agrocybe sphaleromorphus (Bulliard : Fries) Fayod is founded upon Bulliard's plate and description (1792, plate 540 fig.2 fasc 166) and no authentic material. This reference depicts sporocarps characterized by hemispheric pilei, equal stipe with a bulbous base, a superior skirt-like and persistent annulus, fruiting on the ground in forests.

Agrocybe gibberosa (Fries) Fayod is based upon Fries' (1838, p.163) description alone. The diagnosis describes sporocarps with lacerate annuli that occur in grassy clearings of montane pine woods in Sweden. The lacerate annulus is not diagnostic because this characteristic is determined by humidity.

Agrocybe molesta (Lasch) Singer is based on the description by Lasch (1828, p.421) which characterizes a fungus occurring in a garden among lush grass and appearing in May through June. The annulus is described to be white and fibrillose. A holotype does not exist for this taxon. *Agrocybe dura* (Bolton : Fries) Singer was established through Bolton's plate and description (1788, plate 67). This taxon erroneously refers to what is now accepted as *A. molesta*, because the illustration and description depicts a *Psathyrella* in the *P. sarcocephala* group (Singer, 1977; Watling, 1982).

Peck collected, preserved, and named many species of *Agrocybe*. All lightly colored, grass associated, annulate species of *Agrocybe* were designated as *Pholoita praecox* (Peck, 1896, p.59) and large luxuriant forms of this species as *Agaricus (Pholiota) vermiflua* Peck (1878, p.34). Both are synonyms of *A. molesta* (Lasch) Singer. To encompass brown pigmented *Agrocybe* species growing on wood or soil in forest or grassy habitats, Peck described several species. *Agrocybe temnophyllus* (Peck) Singer (Peck, 1872, p.90) occurred in grass along a road side and according to Peck this taxon "resembles *A. semiorbicularis* so closely in color, taste, etc, that in the absence of the

annulus it might be taken for a large form of that species". *Agrocybe acericola* (Peck) Singer is derived from *Agaricus acericola* Peck (1873, p.50). This taxon fruited on a moss covered maple log, with a hygrophanous and rugulose pileus. *Agrocybe howeana* (Peck) Singer was noted by Peck (1873, p.53) for its aerolate pileus surface and bitter taste. Singer's (1977) description notes that *Agrocybe howeana* occurs "on the ground in shady places under trees and bushes, among litter, mulch or among grass almost fasiculate or singly, fruiting from May until summer."

Agrocybe praecox var. *cutifracta* (J.E. Lange) Singer was introduced by the illustrative plate and description of J.E. Lange (1921, plate 106, f. D), and no holotype was designated. Singer (1977) goes through great lengths to differentiate this taxon from *A. howeana*, primarily on spore dimensions and taste. The typical aerolate pileus surface of *A. howeana* and *A. praecox* var. *cutifracta* is a generic characteristic. Anticlinally arranged cells lack tensile strength and are therefore, easily separated as the pileus expands. *Agrocybe paludosa* (J.E. Lange) Kühner & Romagnesi was illustrated by J.E. Lange (1921 plate 106, f. E and E^1) and is not supported with authentic material. The illustration depicts a delicately statured annulate *Agrocybe* that was collected in a marshy meadow.

Agrocybe praecox var. *britzermayrii* (S. Schulzer) Singer was described by S. Schulzer (1898, p.171) and is not supported with authentic material. The description speaks of extremely large and luxuriant annulate *Agrocybe* sporocarps. *Agrocybe praecox* var. *sylvestris* Peck (1896, p.60) is a taxon delineated by its occurrence in forests. *Agrocybe highlandensis* (Peck) Singer is not an *Agrocybe* but is a *Pholiota highlandensis* (Peck) Smith & Hesler determined from my type studies discussed below.

Contemporary mycologists who have inherited these confusing species concepts have contributed substantially to the rectification of this taxonomic problem. Overholts (1927) in his monograph of *Pholiota* has treated many of the species above and recognized that many taxa may be phenotypic variants. Watling (1981) compiled an excellent list of all published taxa of the Bolbitiaceae and published excellent keys and descriptions of taxa occurring in Europe (Watling, 1982).

Materials and Methods

Spore prints were made on 25% cotton rag paper, placed into manilla coin envelopes and stored at 2°C for later use. Fungi were routinely isolated and maintained on modified Melin-Norkrans (MMN) agar (Molina & Palmer, 1982) using Sequestrine® iron chelate to substitute for $FeCl_3$ which was amended with 1g/l of yeast extract. Concentrated 20x stock solution of the phosphate salts and a separate stock for the other salts were diluted to the final concentration. This procedure saved time, increased accuracy, and reduced salt precipitation. All crossing experiments used 0.5% malt extract, 1.0% agar medium. A glass rod, approximately 20cm x .4-.5cm was bent to 130° into the shape of a hockey stick, with a 4cm long foot. Micro-spatulas were constructed with flat-tipped steel straight pins embedded in 3mm diam. glass tubing. The pins were flattened with vice-grip pliers and the glass tubing was drawn to a point and cooled. The glass tip was broken to fit the pin which was then melted into place. Cross matrices of 45 confrontation or less used 14 x 2cm round glass Petri dishes which allowed 12.5mm radius per confrontation (Vilgalys & Miller, 1982). A round sheet of paper with grid-lines and confrontation addresses was taped face up to the Petri dish underside. Large cross matrices, up to 190 confrontations allowed a 10mm radius per

confrontation on 38 x 25 x 2.5cm stainless steel trays with fitted galvanized sheet metal lids. The trays contained water saturated 25% cotton rag paper labeled with grid-line and confrontation addresses were autoclaved at 121°C at 1Kg/cm^2 for 30 minutes. The trays were reautoclaved with the cross medium. Safranin-o solution (Bandoni, 1979) was modified by dissolving 1g of Na-acetate in 50ml H_20, adding 1g of Safranin-O [Sigma chemical practical grade (Cl basic red; Cl 50240) no. S-2255 or Eastman Kodak certification no. C 1753] and bringing the final volume to 100ml with 95% ethanol.

Single spore isolation

Several methods for isolation of single spore colonies were attempted, including the serial dilution method (Korhonen, 1980; Watling, 1981). These methods were unsatisfactory because they failed to separate clumps of spores. The "hockey stick" method borrowed from bacteriology proved best because spore clusters were mechanically broken and well separated. Spores were inoculated onto the isolation medium with a flame sterilized bacterial transfer loop. If too many spores were inoculated, they were removed with a flamed loop and either transferred to another Petri dish to facilitate further dilution, or flame killed. If a spore print was not available, a small piece of lamella (1-2mm^2) was placed onto the isolation medium. The transferred spores were then smeared and separated with the cooled, alcohol flame sterilized hockey stick. A Petri dish turntable was useful when spreading spores. Spore dilutions were incubated at 20°C and checked daily for signs of germination; most isolates germinated within two to ten days. Young colonies were magnified with a dissecting microscope using transmitted light and were transferred onto MMN agar using a micro-spatula. Five single spore isolates, four peripheral and one central, were placed on a Petri dish to give maximum colonial diameter. These isolates were incubated at 20° C. and were

checked for the presence of clamp connections when they reached 15mm to 20mm in diameter. These original single spore isolates were used to determine the mating system and incubated at 2°C so that representatives of each mating type could be placed in a stock culture collection after the matrix was evaluated.

Mating systems

Matrices of ten single spore isolates per collection were combined on the large Petri dishes containing the cross medium to determine the mating system. Small cubes of agar containing mycelium, $2mm^3$ to $3mm^3$, were made by making perpendicular cuts with a flamed scalpel. These cubes were placed side by side in direct contact in all unique combinations. The cross matrices were incubated at 20° C for seven days. Subcultures taken from the junction line were transferred to a daughter matrix and incubated for seven days at 20°C (Jurand, 1983; Chase, 1983); the parent matrix was stored at 2°C. After seven days of incubation, the subculture matrix was either evaluated for the presence of clamp connections or incubated at 2°C until evaluation. Blocks of agar, taken from the colonial margin were stained by placing a drop Safranin-O on the block, followed by a drop of 3% KOH. The stained confrontations were examined for the presence of clamp connections, pseudoclamps or no reaction. Representatives of each identified mating type were placed in a stock culture collection using the original single spore isolates that were stored at 2°C. Single spore isolates were coded with the herbarium number followed by a decimal number. For example, **GB750.4** was collected by **GB**, collection number **750**, single spore isolate number **4**. Compatible single spore isolates are separated with a slash, for instance **GB750 .2,.6,/.4,.7,.8 : .3/.1,.5,.9,.10** indicates that single spore isolate **2, 6** is compatible with **4, 7, 8** and single spore isolate **3** is compatible with **1, 5, 9, 10.**

Mating relationships

To determine the mating relationships of different collections, confrontation matrices were constructed using two compatible single spore isolates representing each collection. This adds an element of control by decreasing the chances of misinterpretation of negative interactions. Matrices of 20 x 20 unique combinations used ($2mm^3$-$3mm^3$) small mycelial cubes placed in direct contact on the stainless steel trays containing the cross medium and matrix grid. Confrontations were incubated for seven days at 20°C and junction line subcultures were transferred to new trays and incubated under identical conditions. Parental cross matrices were saved and incubated at 2°C for macroscopic evaluation of brown pigmented barrage zones. Subculture cross matrices were evaluated macroscopically for barrage zones and microscopically using Safranin-o for clamp connections, false clamps and no reaction. These were stored at 2°C to avoid excessive growth during the days of microscopic evaluation.

Nuclear migration and Di-mon crosses

Nuclear migration and di-mon matings were done using the method of Korhonen (1983). Strips 30mm x 3mm of homokaryotic isolates **SAR84/27 .1/.6; .2/.5: GB750 .1/.3; .6/.8** and **TMF80 .4/.7; .1/.8** were inoculated end to end leaving a 20mm gap. A matrix was constructed using 100mm x 15mm standard plastic Petri dishes containing 0.5% malt, 1.0% agar for each confrontation. These were incubated for fourteen days at 20°C and the margins were evaluated for clamp connections at 5mm intervals along the colony length.

Di-mon crosses used 5mm diam. mycelium plugs of tissue isolate of TMF80 confronted with 40mm x 3mm strips of homokaryons of **SAR84/27 .2/.5; .1/.6: GB750 .1/.3; .6/.8: TMF530 .7/.8; .4/.9** and **TMF80 .4/.7; .1/.8** with a 20mm gap between the strip end and plug. Separate Petri dishes were used for each confrontation and were incubated at 20°C for fourteen days. Samples of the mycelia were taken at 5mm intervals along the length of the colony examined for clamp connections.

Anatomical studies

Sections of dried specimens were revived in 70% ethanol, mounted in 3% NH_4OH and highlighted with Congo Red when desired. In several cases, type specimens would not revive in 70% ethanol; cross sections of these specimens were placed in 3% KOH and gently warmed over a spirit lamp for ten to twenty seconds. As the solution evaporated, it was replaced with distilled water. The components of the hymenium became inflated and spore walls became thicker but spore size did not increase and the wall deflated to normal compared with ethanol revived spores. Line drawings were made at 1000x with the aid of a drawing tube. A minimum of ten discharged spores taken from spore prints, the annulus, stipe or pileus cuticle were measured to the nearest 0.5µm for each collection. Cystidia, basidia, elements of the pileus cuticle and annulus were measured to the nearest 1µm. Spore and pleurocystidial dimensions are reported as the mean ± one standard deviation. Color designations in the macroscopic descriptions use the Methuen system (Kornerup & Wanscher, 1978).

Results

All collections analyzed in the selfing experiments showed bifactorial homogenic incompatibility. A sample intrastock matrix is given in Figure 2 on page 37.

Experiment designed to examine nuclear migration and the Buller phenomenon failed to demonstrate these nuclear behaviors in the sibling species of *A. praecox*. These findings were confirmed when experiments were repeated using the same isolates and design. Nuclear migration and dikaryotization of preexisting haploid mycelium with a heterokaryotic doner does not occur in the *A. praecox* group. Dikaryotization only occurs at the junction line where newly formed dikaryons out-grow their parental haploids.

Ten matrices of unique composition were inoculated to identify the mating relationships of several individuals representing populations from the United States and Europe. The reliability of the experimental system was tested by repeating the first 5 cross matrices. These repetitions confirmed the previous findings and provided confidence for this experimental design. In all subsequent mating experiments only

questionable interactions were repeated. Each experiment combined new isolates and tester isolates identified to mating group from the preceding experiments. To simplify the figures, collections are given the official two letter postal abbreviation for states of the United States or the two letter European country abbreviation found on license plates. The numbers following the state abbreviation indicate collections found in that region. Sibling species are given a number, 1 through 4 and precedes the state abbreviation. Thus, **4DK4** represents sibling species 4 collected in Denmark, and is the fourth collection made from that country. The abbreviations for each collection and herbarium number are grouped by sibling species and geographic region in Table 1 on page 33 and Table 2 on page 34. The interstock cross matrices are labeled with the collection abbreviation on the outermost axes (**a**). Two single spore isolates representing each collection were used and are labeled on the inner most axes (**b**). Collections are delimited by thick lines and single spore isolates by thin lines. This system is used consistently throughout this study and has the advantages of concisely indicating which population the specimen represents. The scatter plots of spore and pleurocystidia dimensions include collections of *A. molesta* and holotypes. Collections of *Agrocybe molesta* are abbreviated as **M** followed by where they were collected. Holotypes and authentic specimens are given a three letter abbreviation representing their specific epithet.

The validity of interspecific sterility between morphologically distinguishable taxa was demonstrated by combining haploids of *A. praecox* taken from different geographic locations with *A. molesta*, *A. smithii* and *A. pediades* (Figure 3 on page 38). This experiment shows that morphologically distinct taxa are completely intersterility with all others. Isolates of *A. praecox* collected from the eastern and western United States showed complete and absolute sterility despite of their morphological similarities.

This first experiment established that at least two sibling species of *A. praecox* exist. This reproductive isolation could be attributed to geographic divergence, but this matrix shows two intersterile sibling species which are sympatric to members of sibling species 1 in Virginia and Montana. Figure 4 on page 39 shows a matrix composed of isolates collected exclusively along the eastern seaboard of the United States. Two sibling species (**1e** and **3e**) were recovered from this experiment indicating that these reproductively isolated groups occur sympatrically.

Figure 5 on page 40 shows a cross matrix composed of isolates representing populations in the western United States. An additional species, *Agrocybe putaminum* (Maire) Singer, was included in this experiment before it was morphologically evaluated by me. This matrix show three intersterility groups with one collection determined as *A. putaminum* by morphological and breeding criteria and two sibling species of *A. praecox* (**1w** and **2w**) defined by breeding criteria alone. Barrage zones characterized by brown pigmented, densely interwoven, cytoplasmically damaged hyphae were first recognized at this time. This behavior was expressed in many confrontations of different sibling species and is designated **B** in the following figures.

Three intersterility groups were identified with in the cross matrix composed of individuals representing populations of *A. praecox* in western Europe Figure 6 on page 41. Sibling species 1 is the most abundant group in this sample. The collection from Switzerland was reproductively isolated from all other sibling species and two collections from Denmark were intersterile and sympatric with species 1.

Two sibling species were identified using isolates representing the eastern and western United States which included one European collection from Scotland (see Figure 7 on page 42).

Tester isolates representing each of the four sibling species in Europe and north America were compared with each other (Figure 8 on page 43). These results are consistent with my previous findings and support the conclusion that at least four reproductively isolated species with similar external appearance are distributed throughout the northern hemisphere. Sibling species **1** is found throughout North America and Europe and occurs sympatrically with sibling species **2** of the Rocky Mountains, **3** in Virginia and **4** in Demark.

From these experiments, four breeding groups have been found with one collection from Switzerland showing mating compatibility with sibling species **2**. Sibling species **1** is the most common species in eastern north America and Europe and is occasionally found in the western United States. Sibling species **2** is dominant in the mountainous regions of the western United states (the Rocky mountains, Cascades and the Coastal Ranges of northern California) but is sympatric with sibling species **1** in Montana. Sibling species **3** is sympatric with sibling species **1** occurring in Virginia. Sibling species **4** is sympatric with sibling species **1** in Denmark. These conclusions are derived from the mating relationships of 16, 28, and 16 collections respectively represent eastern and western portions of North America and Europe to total 60 collections. This sample is not all inclusive so that more intersterility groups may exist.

To determine whether these sibling species are ephemeral aberrations or can exist through time at a given locality, new individuals were collected adjacent to previously

collected specimens. The results shown in Figure 9 on page 44 indicate that for a period of at least two to three years, intersterility groups exist through time. This experiment also expanded the range of sibling species **2** into western Canada and recovered the rarely collected sibling species 3 in Virginia. These results are incorporated into Table 1 on page 33and Table 2 on page 34.

Morphology

In light of the mating results, an attempt was made to find characteristics which could be used to distinguish among the potential breeding groups. Spore and pleurocystidial dimensions have been used to delimit species of *Agrocybe* by previous authors. A scatter plot of mean spore length by width of the sibling species of *A. praecox*, type collections and *A. molesta* is shown in Figure 10 on page 45. This result shows two distinct size classes, the smaller occupied by the sibling species of *A. praecox* and the larger by *A. molesta.* No pattern of clustering is seen with the sibling species of *A. praecox* and indicates the levels of overlap for this characteristic. The holotypes, *A. praecox*, *A. alachuana*, *A. howeana*, and *A. acericola* occupy different portions of the distribution and represent only a portion of the total variation seen when populations are sampled. Unequal variances (see Table 3 on page 36) for all collections prevented the use of ANOVA tests, and the extreme levels of overlap indicates that spore dimensions are not diagnostic for distinguishing among sibling species of *A. praecox.* *Agrocybe molesta* however, can be distinguished from all sibling species of *Agrocybe praecox* based on spore dimensions, ecology, stature and anatomy of the partial veil as seen in Figure 1 on page 35

The dimension and shape of pleurocystidia are highly variable in *A. praecox* and *A. molesta.* Figure 11 on page 46, shows a scatter plot of mean length by width of the four sibling species, *A. molesta* and holotypes. The distribution indicates tremendous overlap among all species analyzed but *A. molesta* generally produces pleurocystidia which are short and broad whereas the sibling species of *A. praecox* tend to develop longer pleurocystidia.

Observations of morphology

Agrocybe praecox and *A. molesta* have anatomical similarity which indicates relationship but both groups can nevertheless be distinguished. The terminal cells of the hymenium, partial veil and pileipellis are homologous. The orientation, arrangement, and branching pattern of these cells consist of catenulate chains of spherical, pyriform, globose, vesiculose elements giving an inflated ramose structure. True cheilocystidia derived from the lamellar trama are not seen in *A. praecox*, but in many instances elements derived from the partial veil will adhere to the lamellar edge to give the appearance of cheilocystidia. If pleurocystidia are abundant, then true leptocystidia derived from the hymenium will be seen on the lamellar edge, which are similar to the pleurocystidia. If pleurocystidia are not abundant, then true cheilocystidia will be rarely seen. *Agrocybe molesta*, however, does have true cheilocystidia which are derived from the hymenium and are often abundant. These cells are clavate, utriform to globose which arise from ramose subtending cells. The pleurocystidia of *A. praecox* are extremely variable, often within a single sporocarp, but generally extend well above the hymenium and range from ventricose to ventricose-rostrate. The pleurocystidia of *A. molesta* are variable as well, but tend to be deeply imbedded in the hymenium and range from broad clavate, vesiculose, globose to broadly utriform. The pleurocystidia of *A.*

molesta can often be easily dislodged with slight percussion in fresh material, whereas the cystidia of *A. praecox* are more tenuous. The abundance of pleurocystidia can range from almost none to being extremely abundant in both species. Caulocystidia are not observed in either species except for remnants of the partial veil and the cells have the exact same aspect. Otherwise, microscopic evaluation of both species indicates that terminal, taxonomically useful cells are very similar in shape and organization in both species. To generalize, the taxonomically significant cells of *A. molesta* are consistently larger than the cells of *A. praecox*.

Table 1. The sibling species of Agrocybe praecox.

Isolate code	Accession number	Location	Mo.Yr	Substrate/ Habitat	Mating types recovered
SIBLING SPECIES 1					
eastern North America					
QB1	CG84/27	Quebec, Canada	5.84	mulch	3
NJ1	GB750	New Jersey	5.84	mulch	4
NJ2	GB754	New Jersey	5.84	mulch	4
NJ3	GB751	New Jersey	5.84	mulch	3
NY1	TMF546	New York	5.84	mulch	3
NJ5	GB747	New York	5.84	mulch	nd
NJ4	GB749	New Jersey	5.84	mulch	nd
NY3	GB755	New York	5.84	mulch	3
NY4	CHAM1795	New York	5.84	mulch	3
VA1	TMF530	Virginia	5.84	mulch	3
VA2	TMF532	Virginia	5.84	mulch	nd
VA5	TMF780	Virginia	5.85	mulch	nd
VA6	TMF795	Virginia	5.85	mulch	
western Europe					
D1	TMF877	BDR	6.85	spruce; larch	nd
DK1	RV84/161	Denmark	6.84	deciduous	3
DK2	RV84/163	Denmark	6.84	deciduous	3
DK3	RV84/164	Denmark	6.84	deciduous	4
F1	RV84/66	France	6.84	sequoia	3
GB1	TMF554	Scotland	6.84	grass	4
NL1	RV84/112	Netherlands	6.84	deciduous	nd
NL2	RV84/121	Netherlands	6.84	deciduous	nd
NL3	RV84/110	Netherlands	6.84	deciduous	nd
NL4	RV84/113	Netherlands	6.84	deciduous	nd
NL6	PIP1476	Netherlands	6.85	deciduous	nd
NL5	PIP1475	Netherlands	6.85	deciduous	nd
NL7	JVD6/5	Netherlands	6.85	deciduous	nd
western North America					
MT1	TMF80	NW Montana	7.83	conifer/trail	4
MT2	TMF114	NW Montana	7.83	conifer/trail	3
MT3	TMF817	NW Montana	6.85	conifer/trail	nd
MT4	TMF818	NW Montana	6.85	*Betula*	nd
WA1	SAR84/17	Seattle, WA	5.84	mulch	4
ID11	OKM20133	NC Idaho	6.84	conifer	4

Legend: nd = not determined. Regions - NC = north central, NW = north western, SW = south western.

Table 2. The sibling species of Agrocybe praecox continued.

Isolate code	Accession number	Location	Mo.Yr	Substrate/ Habitat	Mating types recovered
SIBLING SPECIES 2					
western North America					
CA1	TMF488	N California	3.84	soil; shrubs	4
CA2	OKM20987	N California	6.84	conifer/corral	nd
CO1	TMF674	Colorado	8.84	aspen	3
CO2	TMF697	Colorado	8.84	aspen, spruce	4
AL2	TMF837	SW Alberta	7.85	conifer	nd
AL3	TMF839	SW Alberta	7.85	*Salix*	nd
AL4	TMF847	SW Alberta	7.85	conifer/trail	nd
ID1	OKM20119	NC Idaho	6.84	conifer	4
ID2	OKM20120	NC Idaho	6.84	conifer	4
ID3	OKM20123	NC Idaho	6.84	conifer	3
ID4	TMF799	NC Idaho	6.85	conifer	nd
ID5	TMF800	NC Idaho	6.85	conifer	nd
ID6	TMF802	NC Idaho	6.85	conifer	nd
ID7	TMF813	NC Idaho	6.85	conifer	nd
ID8	TMF814	NC Idaho	6.85	conifer	nd
ID9	TMF815	NC Idaho	6.85	conifer	nd
ID10	TMF816	NC Idaho	6.85	conifer	nd
MT5	TMF819	NW Montana	6.85	conifer	nd
MT6	JLG43	NW Montana	6.85	conifer/trail	nd
OR1	OKM20113	Oregon	5.84	conifer/corral	3
western Europe					
CH1	OKM21272	Switzerland	8.84	conifer/moss	3
SIBLING SPECIES 3					
eastern North America					
VA3	TMF576	SW Virginia	7.84	maple	3
VA4	TMF596	SW Virginia	7.84	maple, oak	3
VA7	TMF798	SW Virginia	5.85	maple, sycamore	nd
SIBLING SPECIES 4					
western Europe					
DK4	RV84/165	Denmark	6.84	deciduous	3
DK5	RV84/166	Denmark	6.84	deciduous	4

Legend: nd = not determined. Regions - NC = north central, NW = north western, SW = south western.

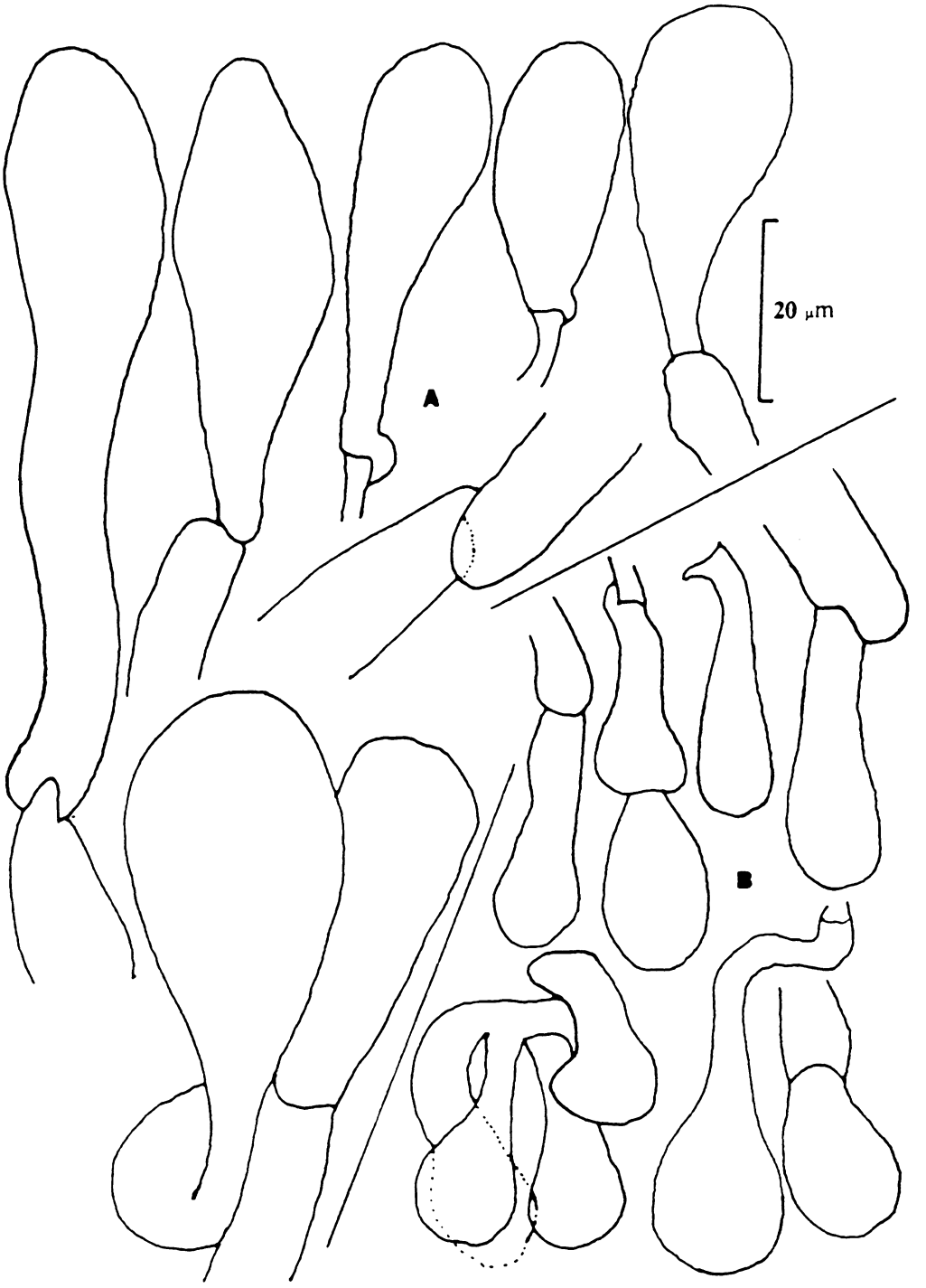


Figure 1. Partial veil of A. praecox and A. molesta.: a = *Agrocybe molesta*, b = *Agrocybe praecox*.

Table 3. Spore and pleurocystidia dimensions.

species	N	Q	Mean Length	L-SD	Mean Width	W-SD
Spores						
sibsp 1	224	1.54	9.4	.78	6.8	.46
sibsp 2	90	1.50	9.5	.79	6.5	.61
sibsp 3	20	1.57	9.4	.47	6.0	.44
sibsp 4	20	1.61	9.6	.91	6.0	.54
sibsp 5	10	1.40	11.3	.67	8.1	.46
PRX	14	1.56	10.2	1.27	6.8	.59
ACER	11	1.29	9.8	.52	6.7	.42
ALAC	12	1.91	10.9	.63	5.8	.40
BROD	10	1.58	15.3	.54	9.7	.58
HOW	11	1.51	10.6	.49	7.1	.42
MOL	116	1.65	13.4	1.02	8.3	.63
TEMN	12	1.43	11.8	.87	8.3	.50
VERM	11	1.53	12.8	.88	8.4	.32
Pleurocystidia						
sibsp 1	129		49	10.52	21	4.47
sibsp 2	41		53	7.15	20	3.16
sibsp 3	9		52	3.84	21	1.90
sibsp 4	10		59	6.94	24	3.14
sibsp 5	5		56	5.95	21	4.09
ACER	8		49	7.93	19	1.55
ALAC	13		36	3.73	25	3.13
BROD	5		37	10.38	26	5.66
HOW	14		52	9.36	23	2.99
MOL	68		43	10.20	25	5.29
PRX	13		39	7.30	21	2.29
TEMN	4		54	8.74	21	1.50
VERM	8		34	6.32	22	3.48

Legend: Q = length/width; L-SD = length standard deviation, W-SD = length standard deviation.
Populations - sibsp 1-4 = sibling species 1 through 4, 2eur = 2 in Europe,
MOL = *A. molesta*. Holotypes and authentic specimens -
ACER = *A. acericola*, ALAC = *A. alachuana*, BROD = *A. broadwayii*,
HOW = *A. howeana*, PRX = *A. praecox*, TEMN = *A. temnophylla*, VERM = *A. vermiflua*.

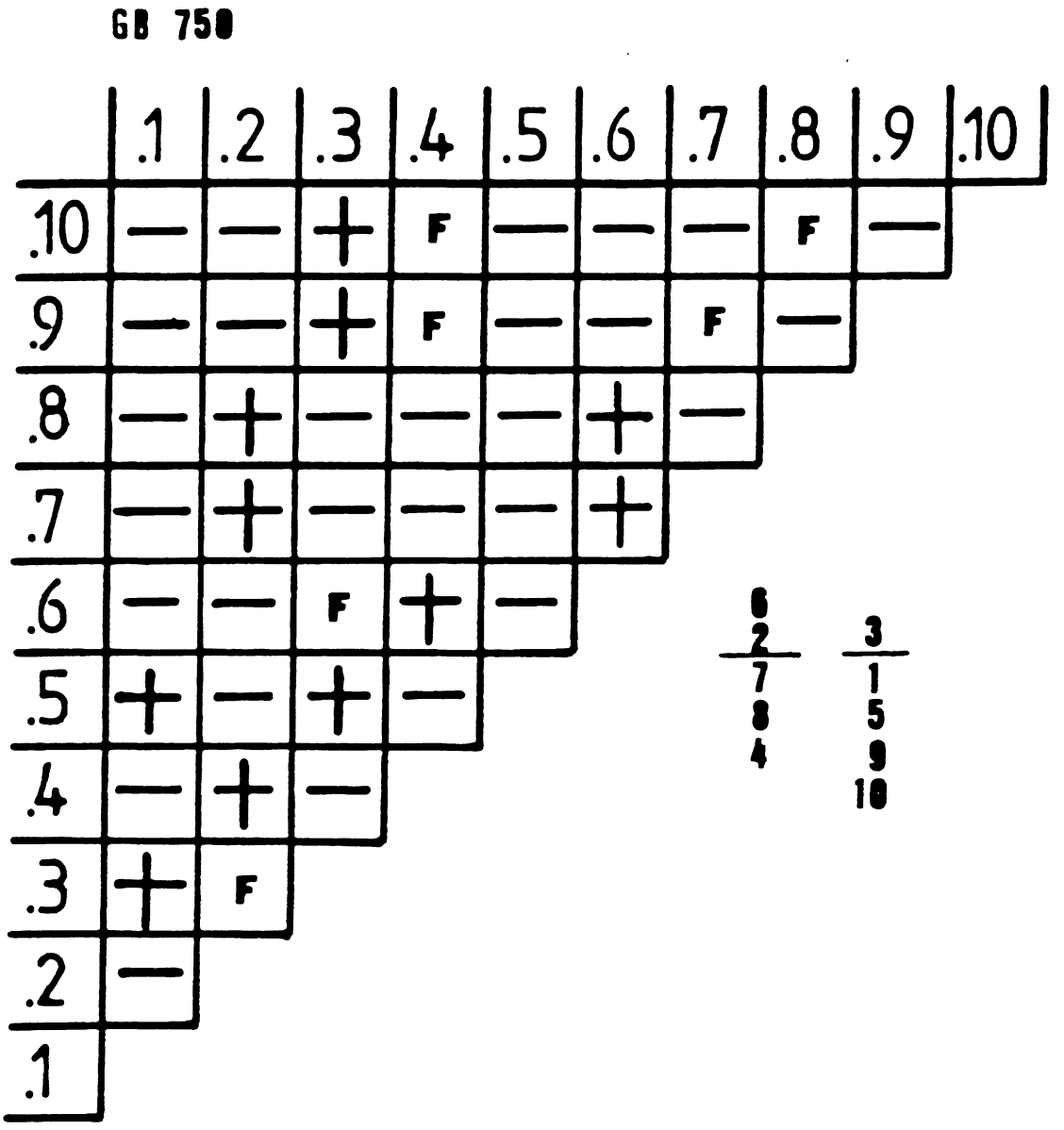


Figure 2. Intrastock cross, Agrocybe praecox (GB750).: symbols used - + = clamp connections, F = false clamp connections, — = no reaction.

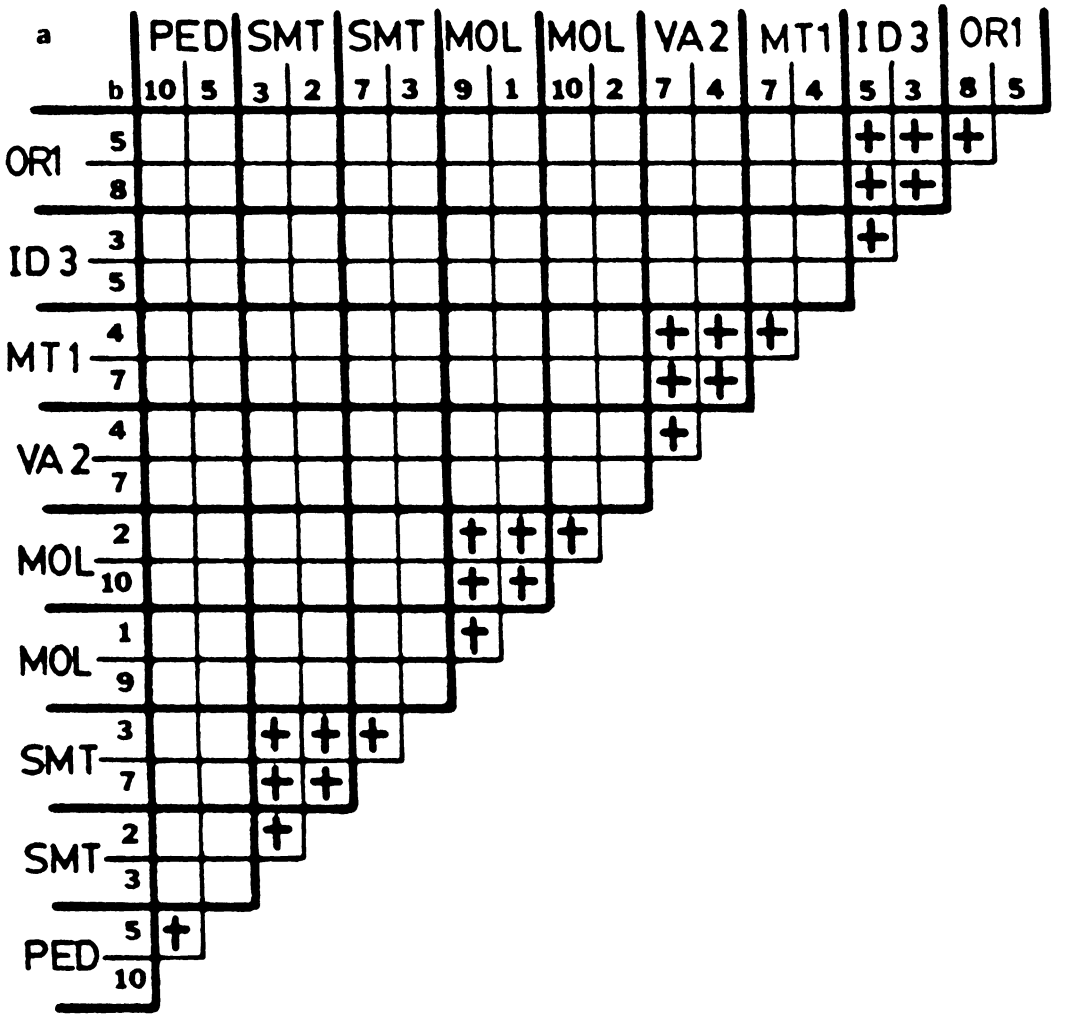


Figure 3. Sibling species 1e, 2w and taxonomic species: + = clamp connections, □ = no reaction; a axis = collections, b = single spore isolates; ID = Idaho, MT = Montana, MOL = *A. molesta*, MT = Montana, VA = Virginia, PED = *A. pediades*, SMT = *A. smithii*.

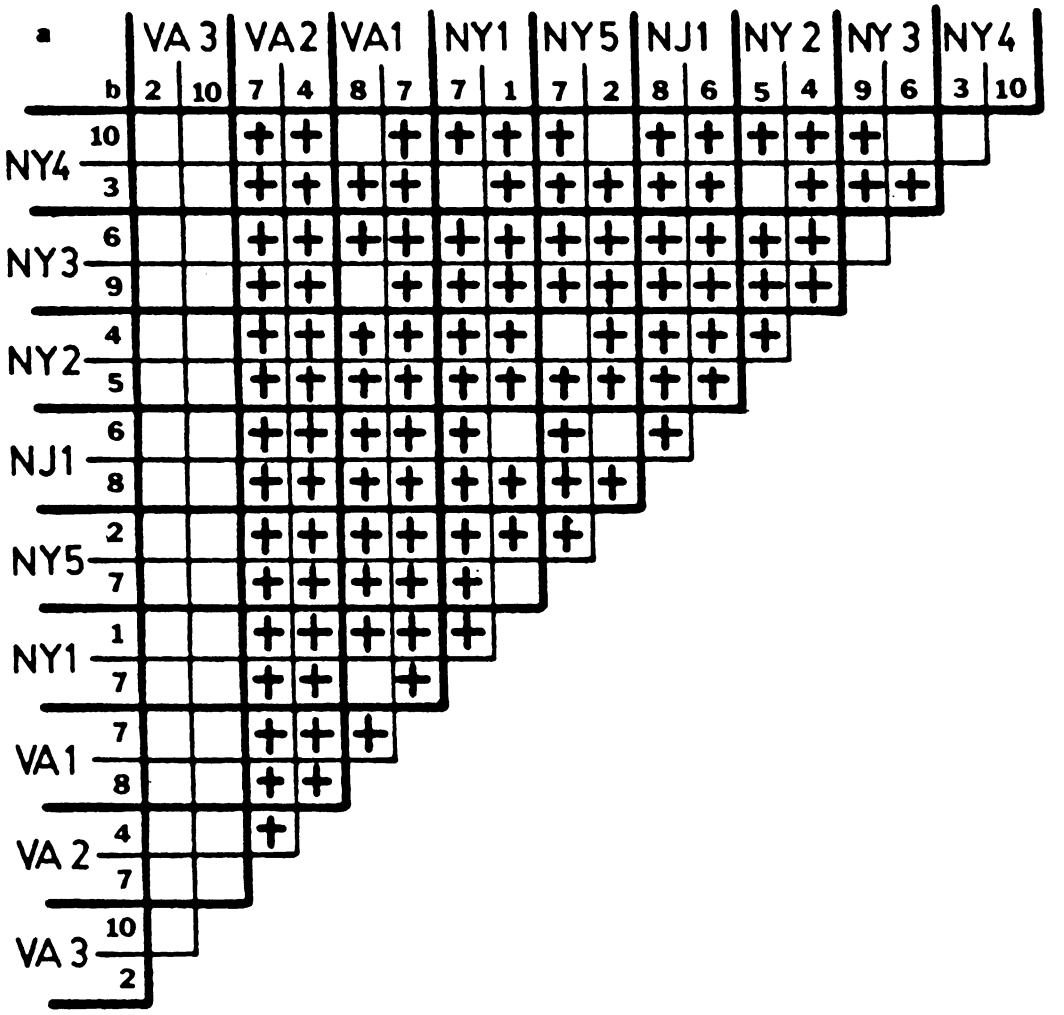


Figure 4. Sibling species 1e and 3e: + = clamp connections, □ = no reaction; a axis = collections, b axis = single spore isolates; NJ = New Jersey, NY = New York, VA = Virginia.

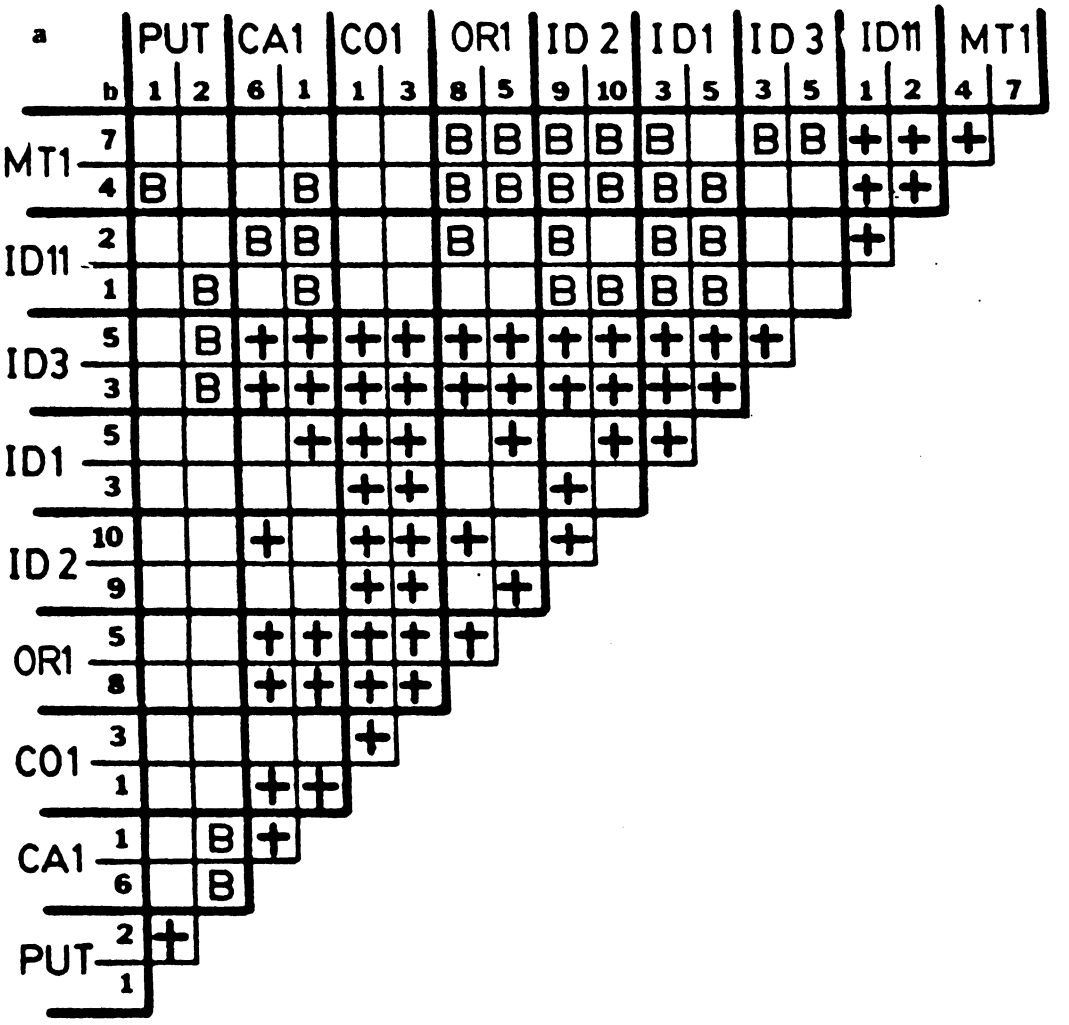


Figure 5. Sibling species 1w and 2w with A. putaminum: + = clamp connections, B = barrage, □ = no reaction; a axis = collections, b axis = single spore isolates; CA = California, CO = Colorado, ID = Idaho, MT = Montana, PUT = *Agrocybe putaminum*.

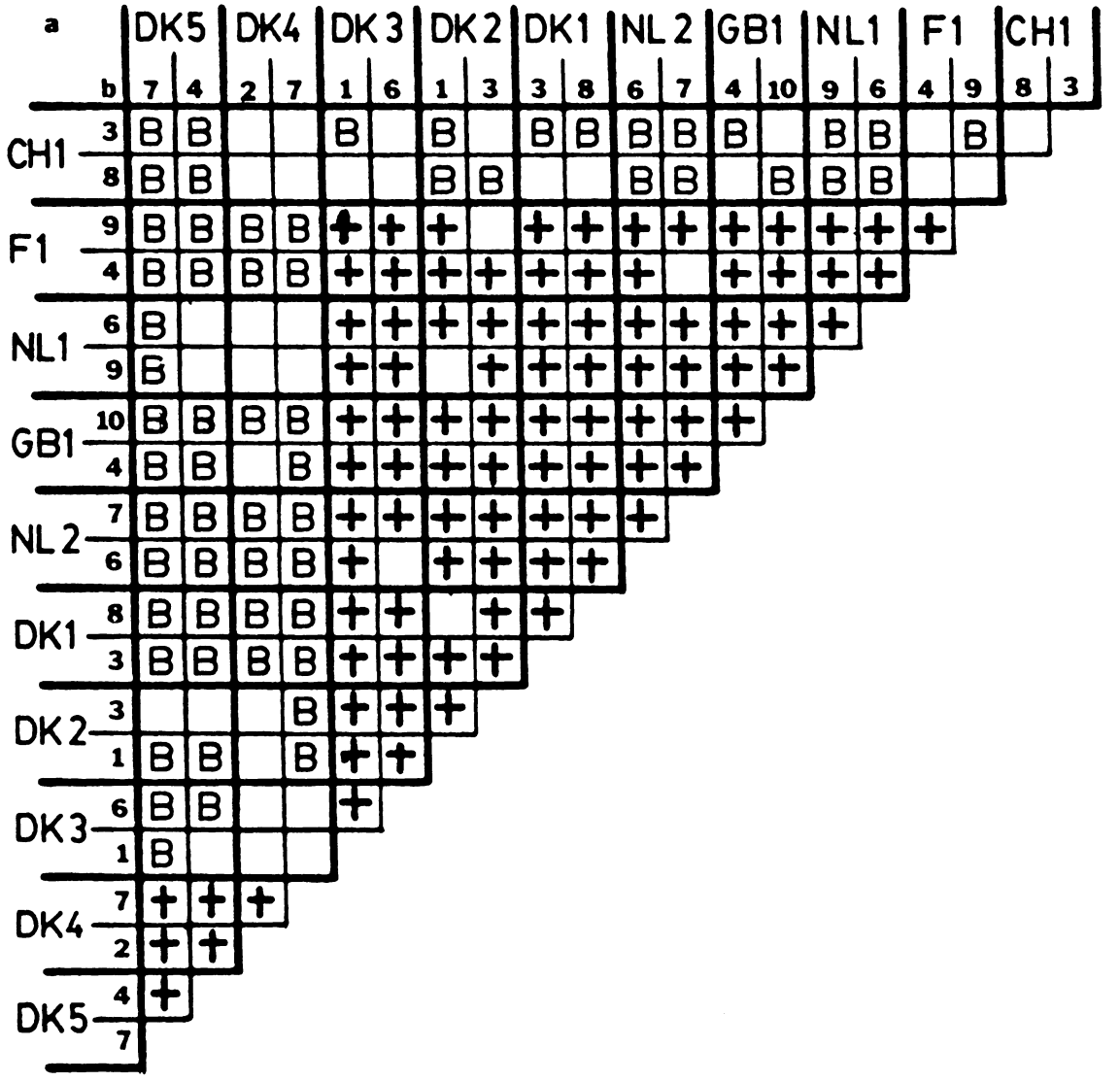


Figure 6. Sibling species 1eur and 4eur.: + = clamp connections, B = barrage, □ = no reaction; a-axis = collections, b-axis = single spore isolates; CH = Switzerland, DK = Denmark, F = France, GB = Great Britain, NL = Netherlands.

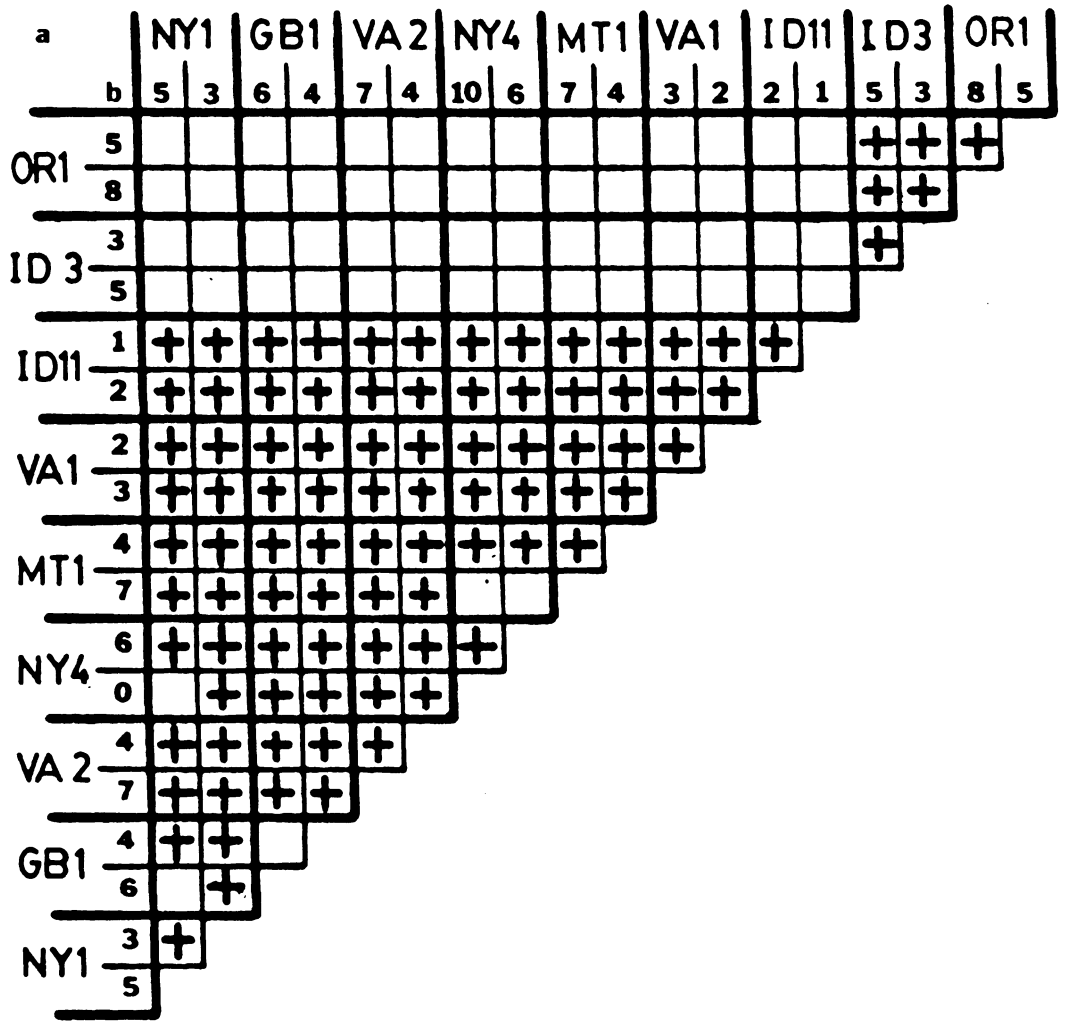


Figure 7. Sibling species 1eur and 2w.: + = clamp connections, B = barrage, □ = no reaction; a axis = collections, b = single spore isolates; GB = Great Britain, ID = Idaho, MT = Montana, NY = New York, OR = Oregon, VA = Virginia.

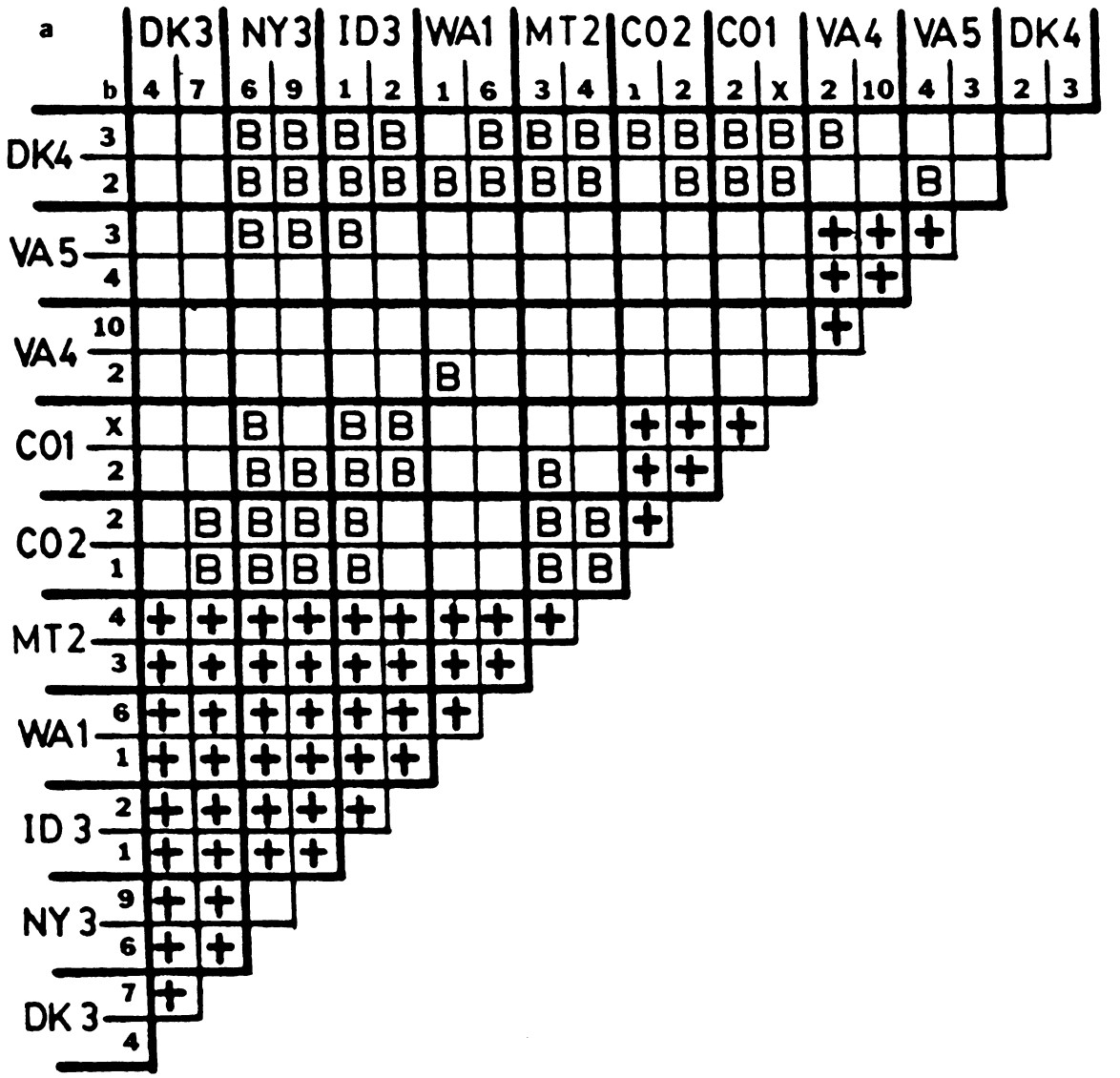


Figure 8. Sibling species 1e, 1w, 1eur, 2w, 3e, and 4eur: + = clamp connections, B = barrage, □ = no reaction; a-axis = collections, b-axis = single spore isolates; DK = Denmark, CO = Colorado, ID = Idaho, NY = New York, VA = Virginia

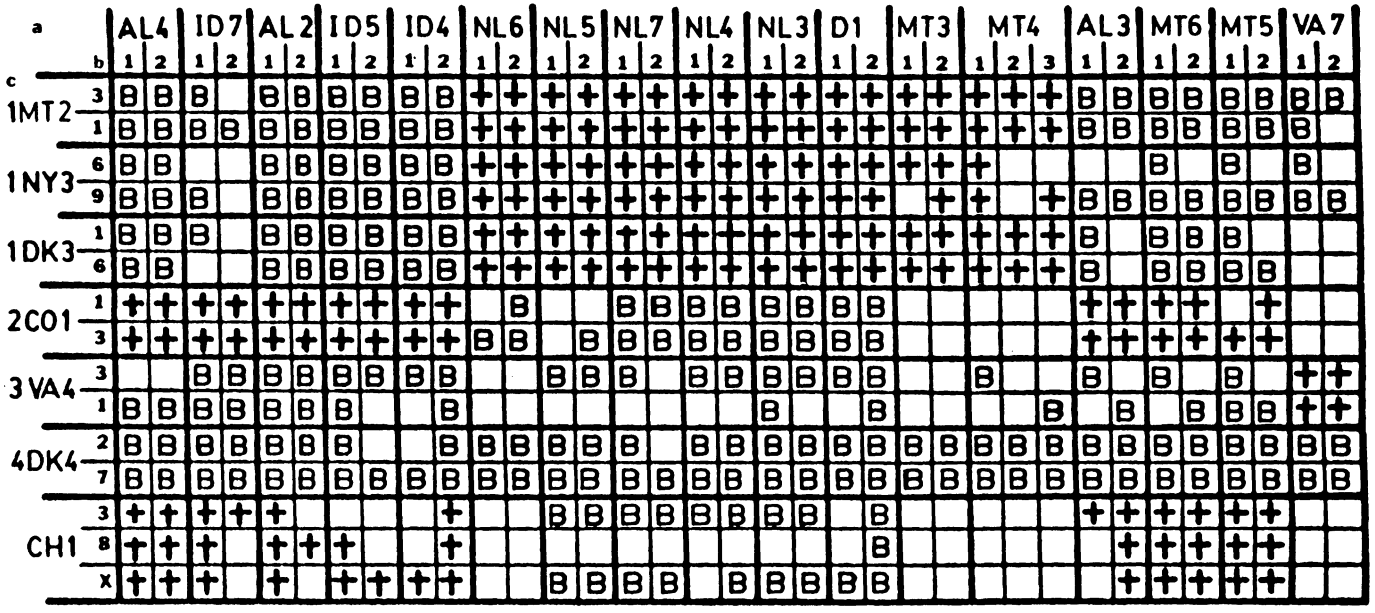


Figure 9. Sibling species and time: + = clamp connections, B = barrage, □ = no reaction; a axis = collections, b axis = single spore isolates; AL = Alberta, Canada; CH = Switzerland, CO = Colorado, D = West Germany, DK = Denmark, ID = Idaho, MT = Montana, NL = Netherlands, VA = Virginia.

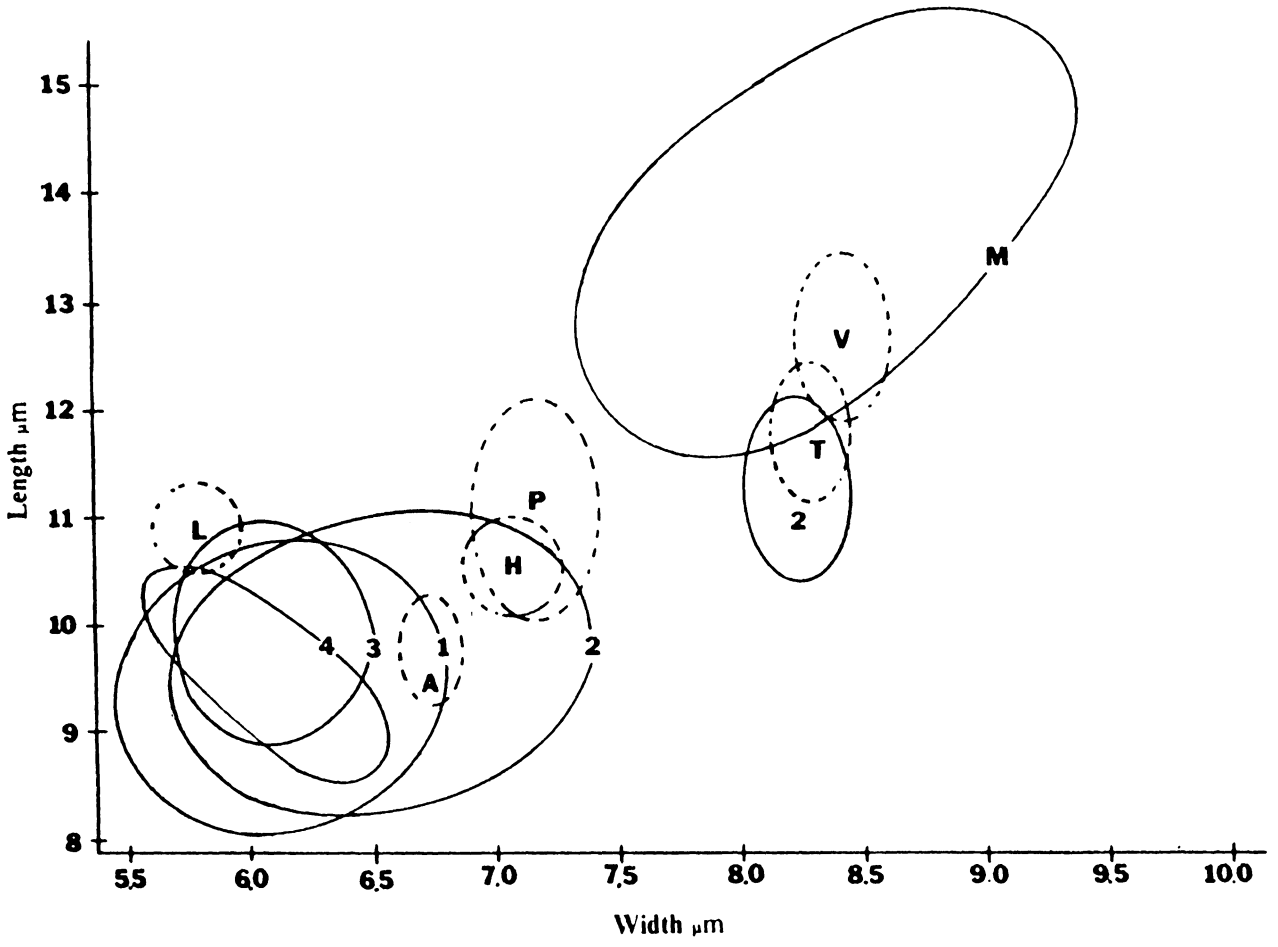


Figure 10. **Outlined scatter plot of mean spore length by width:** Species variation is outlined with the peripheral samples plus one standard deviation. Solid lines represent the variation in the populations of sibling species 1, 2, 3, and 4 and *A. molesta*. Dotted lines represent the variation measured for the holotypes abbreviated as - ACR = *A. acericola*; ALC = *A. alachuana*; BRD = *A. broadwayii*; HOW = *A. howeana*; PRX = *A. praecox*; TMN = *A. temnophylla*; VRM = *A. vermiflua*.

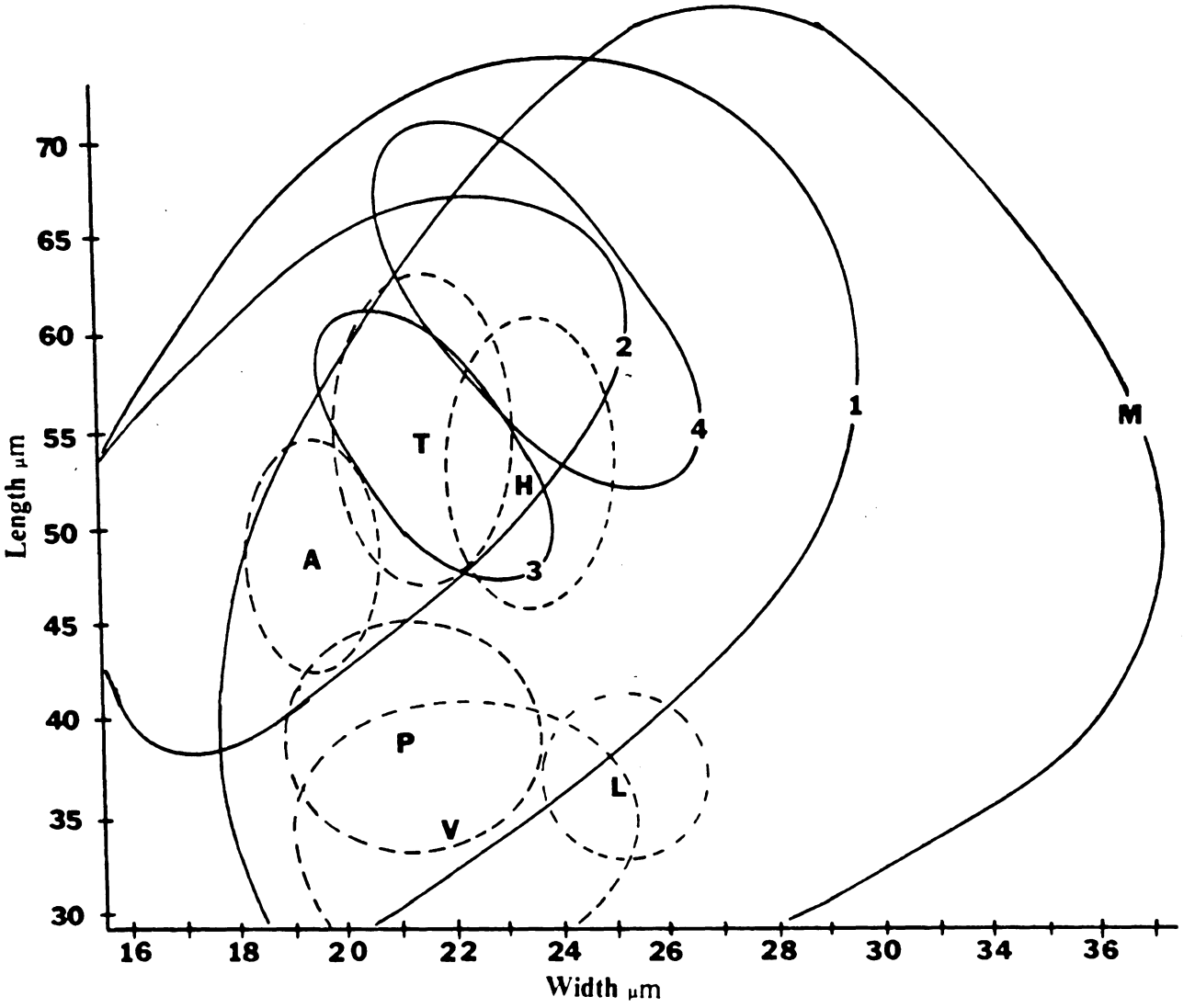


Figure 11. Outlined scatter plot of mean pleurocystidial length by width: Species variation is outlined with the peripheral samples plus one standard deviation. Solid lines represent the variation in the populations of sibling species 1, 2, 3, and 4 and *A. molesta*. Dotted lines represent the variation measured for the holotypes abbreviated as - ACR = *A. acericola*; ALC = *A. alachuana*; BRD = *A. broadwayii*; HOW = *A. howeana*; PRX = *A. praecox*; TMN = *A. temnophylla*; VRM = *A. vermiflua*.

Type studies

Holotypes or authentic specimens of potential synonyms of *A. praecox* were examined. A description of the microscopic anatomy, ecological data where known and an evaluation of the condition of type specimens are provided. These descriptions include the holotype only, but additional material was examined when available to ascertain species concepts for the taxa described by each author. Judgements on the status are not made here, but lists of synonymy can be found under the descriptions of accepted taxa.

Agrocybe praecox (Pers. : Fries) Fayod (1889). *Annales des Sciences Naturelles Botanique* p.358.

≡ *Agaricus praecox* Persoon (1801). *Syn. Meth. Fung.*. p.420.

Figure 12 on page 57

Spores are variable in size, (8-) 8.9-11.4 (-12) µm x (6-) 6.2-7.4 (-8)µm, Q= 1.558, ovate to narrow ovate elliptic in face view, elliptic in profile, thick walled (1 µ), apex truncate and open, large spores deformed and irregular, smaller spores almost tear shaped. Basidia 23-28 x 7-8 µm, cylindro-clavate, mostly four-spored but difficult to

determine, some may be one, two or three spored. Pleurocystidia are not abundant and deeply imbedded, (30-) 32-47 (-58) x (18-) 19-23 (-27) µm., ventricose, vesiculose, to ventricose short rostrate. Cheilocystidia could not be found. Gill trama subparallel. Pileipellis a hymenoderm composed of broad clavate, vesiculose elements, 20-40 µm in diam.

Observations: This specimen was labeled "type or authentic" by "R.S." but was not designated so by Persoon. A single poorly preserved sporocarp was glued to a herbarium sheet and appears to have been partially decayed then dried and mounted to give highly collapsed and compressed tissues. No evidence of a partial veil could be found and the little amount of organic substrate adhering to the stipe base could be identified. A partial veil is described by Persoon but was not mentioned by Fries. Fries did not describe the habitat or substrate until he treated variant forms which occur in fields fruiting commonly in grass during summer. He also refers to *Agrocybe dura* (Bolton : Fries) Singer at this point which is now believed to be a *Psathyrella.* Fries apparently considered these taxa to be variant forms of *A. praecox* and failed to provide information on the nature of the partial veil and substrate which are the two most critical field characteristics. Fries (1828) described the habitat of *Agrocybe praecox* to be grass and the annulus was glabrous and white. The information found in the descriptions and authentic collection make it impossible to determine with complete certainty which currently recognized taxon, *A. praecox* or *A. molesta* Persoon and Fries described.

Material examined: One authentic specimen preserved at (L) Netherlands.

Agrocybe highlandensis (Peck) Singer (1936). *Beih. Bot. Centralblatt* abt. B, 56:167.
≡ *Agaricus highlandensis* Peck (1872) *Ann. Rep. N.Y. State Mus. Nat. Hist.* 24:67.

= *Pholiota highlandensis* (Peck) Smith & Hesler (1968). *The N.Am. Species of Pholiota* p.287-289, text figs. 330, 332-335, pl. 67a, 70b, 72.

Spores (8-) 8.5 (-9) x 5 - 5.5 µm, elliptic in face view, inequilateral to elliptic in profile, no apical germ pore, wall thick, hilum narrowly open. Pleurocystidia abundant, 60 - 70 x 13 - 15 µm, ventricose-rostrate, rostrum 5-6 µm broad, thick walled, with golden brown contents in 3% KOH. Cheilocystidia equivalent to pleurocystidia. Pileipellis an ixomixocutis, epicutis composed of sparse clamped hyphae 2-6 µm in diam., interspersed in a gelatinous matrix, subcutis composed of golden brown, thin-walled, clamped hyphae, 5-10 µm diam..

Observations: Singer (1936) fide Watling (1981) transferred this taxon to the genus *Agrocybe*. Anatomical studies of Peck's holotype show that this fungus is a *Pholiota* with all collections showing evidence of a charcoal substrate. The macroscopic appearance and microscopic features of material designated as *Agaricus (Flammula) highlandensis* by Peck agree with his holotype and should be referred to as *Pholiota highlandensis* (Peck) Smith and Hesler.

Material examined: **New York** - Highland Falls, on charcoal, June 1871 (**Holotype: NYS**); West Albany, August,; West Albany, no date; Blossvale, Oneida, Co., June; Green Island, June; **Pennsylvania** - leg. Wm. Herbst, May; **Vermont** - leg. S. Dunmore and E. A. Burt, Sept. 9, 1898; **Washington** - Carinas, no date; (all at NYS).

Agrocybe howeana (Peck) Singer (1951). *Lilloa* 22:492.

≡ *Agaricus (Stropharia) howeanus* Peck (1873). *Bulletin of the Buffalo Society of Natural Science*. p.53.

Figure 13 on page 58

Spores (10-) 10.2-11 x (6.5-) 6.6-7.5 (-8) µm, ovate, ovate-rhomboidal, to ovate-elliptic in face view, elliptic in profile, thick walled, apex truncate and open, hilum open. Basidia four-spored, 25-30 x 8-10 µm, cylindro-clavate, often flexous. Pleurocystidia very

abundant, (32-) 43-62 (-70) x 20-26 (-28) µm, rostrum narrowing to 4-7 µm wide, ventricose rostrate, ventricose papillate, conic, ampulaceous. Cheilocystidia not seen. Gill trama subparallel. Pileipellis a polycystoderm about two cell layers deep.

Observations: These specimens were well preserved, a skirt-like partial veil is seen and rhizomorphs are abundant. The stipe base was inserted in soil and no organic matter could be seen.

Material examined: New York Center, Albany Co., no date (Holotype: NYS); North River, 29 Sept. 1911, (this collection is a *Galerina*); Day, July; Claryville, Aug; Bolton Landing, 29 July 1905; Indiand Lake, July; Massachusetts - leg. Simon Davis, 1 Sept. 1911, solitary on road side at edge of swamp (all at NYS).

Agrocybe acericola (Peck) Singer (1951). *Lilloa* 22:492.

≡ *Agaricus acericola* Peck (1873). *Bull. Buff. Soc. Nat. Sci.* p.50.

Figure 14 on page 59

Spores (9-) 9.3-10.3 (-11) x (6-) 6.3-7.1 µm, ovate-rhomboidal in face view, elliptic in profile, thick walled, apex truncate and open, hilum open and occasionally apiculate. Basidia four-spored, 25-30 x 7-8 µm, cylindro-clavate. Pleurocystidia abundant, (35-) 41-56 (-60) x (17-) 17.6-21 (-22) µm, rostrum narrowing to 8-13 µm broad, ventricose-rostrate, ventricose-capitate, to conical overall. Cheilocystidia not seen. Gill trama subparallel. Pileipellis a polycystoderm composed of sphaeropedunculate elements.

Observations: This specimen is well preserved, but the cap had darkened from partial decay before Peck dried it. A well developed annulus is still present which was illustrated by Peck, rhizomorphs are present at the stipe base. Peck described this taxon for its ruggulose pileus and the distinctive moss covered maple log substrate. The pileus surface texture is not diagnostic because this characteristic develops in rain soaked pilei but the substrate and habitat may be important.

Material examined: New York North elba, August, on Acer log East Bern, Albany Co. (Holotype: NYS); Howe's Cave, Schoharie Co., July; East Worchester, Oleigo, Co., July; Crogan, Lewis Co., Sept.; Edmonds Ponds, Essex Co., July; (all at NYS).

Agrocybe vermiflua (Peck) Watling (1977). *Kew Bulletin* 31:592.

≡ *Pholiota vermiflua* Peck (1878). *Annual Report to the New York State Museum* 31:34.

Figure 15 on page 60

Spores (11-) 11.9-13.6 (-14) x 8-8.7 (-9) µm, elliptic, broadly elliptic, to slightly ovate in face view, elliptic in profile, very thick walled, apex truncate and open, hilum open. Basidia four-spored. Pleurocystidia deeply imbedded, (25-) 28-41 x (18-) 18.4-25 (-27) µm, inflated ventricose. Cheilocystidia 28-30 x 12-15, abundant, utriform. Gill trama subparallel. Pileipellis cellular.

Observations: These specimens are very well preserved. The partial veil is intact and has a cottony-wooly texture. This is simply a collection of large *Agrocybe molesta* as stated by Watling (1977). One collection is a member of the *A. praecox* group.

Material examined: New York Ticonderoga, no date (Holotype: NYS); Rochester, Aug.; Menands, Albany Co., 19 July 1911; Menands, Albany Co., July 1908, slender form; Menands, Albany Co., 16 June 1912, large luxuriant fruiting; D.C.- leg. F.J. Braendle, no date, on deciduous leaf mold, this is *A. praecox* (all at NYS).

Agrocybe molesta (Lasch) Singer (1977). *Sydowia* 30:197.

≡ *Agaricus molestus* Lasch (1828). *Linnea* p.421.

Observations: Authentic material was not preserved by Lasch. The protologue describes lightly colored sporocarps fruiting in grass and fibrillose annulus. These characteristics recognized by Lasch are good field characteristics which correspond to natural populations of *A. molesta* (Lasch) Singer.

Agrocybe temnophylla (Peck) Singer (1950). *Acta Instituti botanici Nomine V.L. Komarovi Academiae Scientiarum Unionis rerum Publicarum Soveticarum Socialisticarum* series 2(6):448.

≡ *Agaricus (Pholiota) temnophyllus* Peck (1872). *Annual Report to the New York State Cabinet* 23:90.

Figure 16 on page 61

Spores (10.5-) 10.9-12.7 (-13) x (7.5-) 7.8-8.8 (-9.0) µm, ovate-rhomboidal to rhomboidal in face view, elliptic in profile, thick walled, apex truncate and open, hilum open. Basidia four-spored, 28-30 x 8 µm. Pleurocystidia not abundant, 45-62 x 20-23 µm, ventricose rostrate. Cheilocystidia derived from partial veil, ventricose to utriform. Gill trama subparallel. Pileipellis cellular.

Observations: This fungus is well preserved but is glued to cards. The partial veil has left an obvious skirt-like annulus and rhizomorphs are abundant.

Material examined: New York - Sandlake, June 1871, on grassy ground (Holotype: NYS).

Agrocybe alachuana (Murrill) Singer (1962) *Agaricales in Modern Taxonomy*, p.530.

≡ *Pholiota alachuana* Murrill (1943). *Mycologia* 35(5):533.

Figure 17 on page 62

Spores (10-) 10.3-11.6 (-12) x (5-) 5.4-6.1 µm, narrow ovate to elliptic in face view, elliptic in profile, apex open but not truncate, hilum narrowly open, thick walled. Basidia four-spored. Pleurocystidia not abundant, (30-) 33-40 (-42) x (18-) 22-28 (-29) µm, deeply imbedded, vesiculose. Cheilocystidia not found. Gill trama subparallel. Pileipellis cellular.

Observations: This specimen has been studied because only one fourth of a pileus and one stipe represented this taxon but anatomical studies have not been published. Unpublished notes by L. R. Hesler states that pleurocystidia are not present, the

hymeniform pileus cuticle supports pileocystidia and the spores measure 9-12 x 4.5-5.5 µm. My spore measurements agree with Hesler but disagree on pleurocystidia and pileocystidia. The partial veil as mentioned by Murrill was not preserved. This fungus appears to be a member of the *A. praecox* group.

Material examined: Rotten hardwood log, Planera Hammock 16 July 1938 **(Holotype: FLAS).**

Agrocybe sphaleromorpha (Bulliard : Fries) Fayod (1889). *Ann. Sci. Nat. Bot.* 7(9):358.
≡ *Agaricus sphaleromorpha* Bulliard : Fries (1821). *Syst. Mycol.* vol. 1, p.283. based on Bulliard (1791-1812). *Hist. Champ. Fr.* vol. 2, p.629, t.540 f.2.

Observations: This taxon is based wholly on a description and illustrative. The plate illustrates sporocarps with hybrid stature of *A. praeocox* and *A. molesta* as if Bulliard incorporated both species into his paintings. The annulus is described and illustrated as membranous and says these sporocarps are found in the forest.

Agrocybe praecox var. ***britzelmayri*** (S. Schulzer) Singer (1950). *Acta Inst. Bot. Komarov. Acad. Sci. USSR* series 2(6):454.
≡ *Agaricus britzelmayri* S. Schulzer (1898). *Bot. Centralblatt* 75:171.

Observations: This taxon is based only on Schulzer's description. The description pictures luxuriant fruiting of *A. praecox* occurring in a garden after rain. These characteristics are not diagnostic because many fungi will respond favorably under such conditions.

Agrocybe praecox var. ***cutifracta*** (J.E. Lange) Singer (1950). *Acta. Inst. Bot. Komarov. Acad. Sci. USSR* series 2(6):454.
≡ *Pholiota cutifracta* J.E. Lange (1921). *Dansk. Bot. Arkiv* 2(11):7.

Observations: This taxon is not supported with authentic material. The aerolate pileipellis is not a diagnostic characteristic at the species level but is a characteristic of the genus. The genus *Agrocybe* is partially defined by the presence of a cellular pileipellis. Anticlinally arranged cells have no tensile strength and can be easily separated when the pileus expands at a greater rate than the pileipellis. This often occurs at times of low humidity and an aerolate pileipellis is a field characteristic which applies to the entire genus.

Agrocybe paludosa (J.E. Lange) Kühner & Romagnesi (1953). *Flore Analytique des Champignons Supérieus de France* p.341.

≡ *Pholiota praecox* var. *paludosa* (1921) *Bot. Dansk Arkiv* 2(11):7.

Observations: This stature type and habitat can be seen in many of the sibling species, therefore this taxon is most likely based on a phenotypic habit frequently encountered in several biological species. I have collected specimens on marshy ground in Virginia and Alberta, Canada both with the distinctive stature. The Canadian collection belongs to biological species **2** and the Virginian collections belong to either **1** or **3**.

Agrocybe praecox var. ***sylvestris*** (Peck) Watling (1981). *Bib. Mycol.* 82:52.

≡ *Pholiota praecox* var. *sylvestris* Peck (1896). *Annual Report to the New York State Museum* 49:60.

Observations: The holotype represents typical morphology of the *A praecox*. The description is a small note describing rusty-brown capped sporocarps occurring in thin woods.

Material examined: New York Caroga, no date (Holotype: NYS); Chestnut Hill, Mass., 6 June 1907, this collection is mixed with a *Hypholoma* species (NYS).

Pholiota praecox - sensu Peck (1896). *Annual Report to the New York State Museum* 49:59-60.

Observations: Seventeen collections were examined, 16 belong to the taxon *A. molesta* and one was correctly identified. Peck's description is not clearly diagnostic for either species but his reference to lightly colored sporocarps occurring in grassy ground and fruiting from May to July indicates characteristics of *A. molesta.* These collections represent a large majority of the United States and indicates the potential range of this species.

Material examined: All of the following collections represent specimens of *A. molesta.* **New York** - Rochester, leg. Weaver, July, in grass; Westport, Oct., grass; Fulton Chain, Herkemer Co., 3 Oct. 1907, grass; Albany, leg. F.F.(E.?) Hastings, June, grass; Goeymans, leg. A.M. Baker, June 1902, soil; Menands, 16 June 1911, grass; Menands, 16 June 1911, dung fertilized ground; Menands, var. *squarrosa*, June; **New Hampshire** - leg. Mrs. Hadley, June, grass; **Kanasas** - Rooks Co., leg. E. Bartholomew (#1863), 26 July 1895, cultivated ground; Rooks Co., leg. E. Bartholomew (#2143), 25 May 1896, among short grass, **Idaho** - leg. L.F. Henderson (#5315). **Illinois** - Chicago, leg. W.S. Moffat, June 1905, in lawn, is *A. molesta.* **California** - var. *minor* Pasadena, leg. A.F. McClatchie (#762), grass; Woodside, leg E.B. Copeland, 7 Dec. 1902. **D.C.**- var. *minor*, leg. Mrs. Fuller. (All at NYS). One collection from New York represents *A. praecox*; Luzerne, Warren Co., pine needles (NYS).

Agrocybe gibberosa (Fries) Fayod (1889). *Annales des Sciences Naturelles Botanique* 7(9):358.

≡ *Agaricus gibberosus* Fries (1836-1838). *Epicrisis*, p.163.

Spores 11-13 x 8-9 µm, ovate face and elliptic profile. Basidia four-spored. Pleurocystidia ventricose capitate in face view of the lamellae.

Observations: A holotype was not designated by Fayod. The collection examined (GK 11200) was collected in Strassbourg, 1882 was labeled by Fayod as *Ag. gibberosus* Fr., *Ag. aestivalis* (?) and he notes "Tris variable!!!". The herbarium at Geneva labels this collection as *A. praecox.* Fayod's notes and diagrams were provided by G and they

are excellent. Fayod is doubtful as to the correct determination of the specimens he studied, for instance, the illustration of GK 946 of macroscopic and microscopic features shows *Agrocybe pediades* (Fries) Fayod. Fayod initially determined this collection (GK 946) as *A. semiorbicularis* and later scratched that determination in favor of *A. praecox*. Pret 1137/3 is a clear illustration of *A. molesta* which was determined by Fayod as *A. praecox*. Pret 1137/4 illustrates *A. molesta* on the left and *A. praecox* on the right, both were interpreted as *A. praecox*. This illustrates how incomplete descriptions have confused a very competent mycologist such as Victor Fayod.

Material examined: **France** Strassbourg, no date, leg. det. V. Fayod GK 11200 (G) Switzerland.

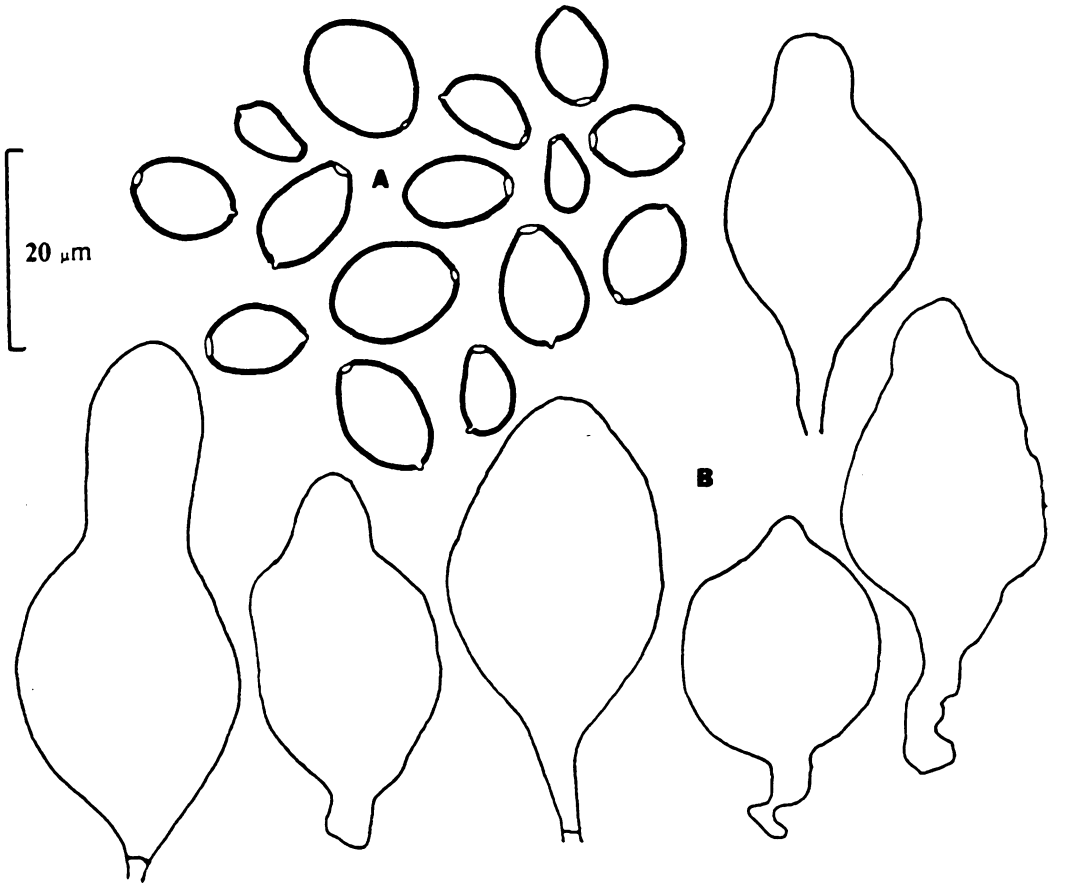


Figure 12. Authentic specimen, Agrocybe praecox: A = spores; B = pleurocystidia.

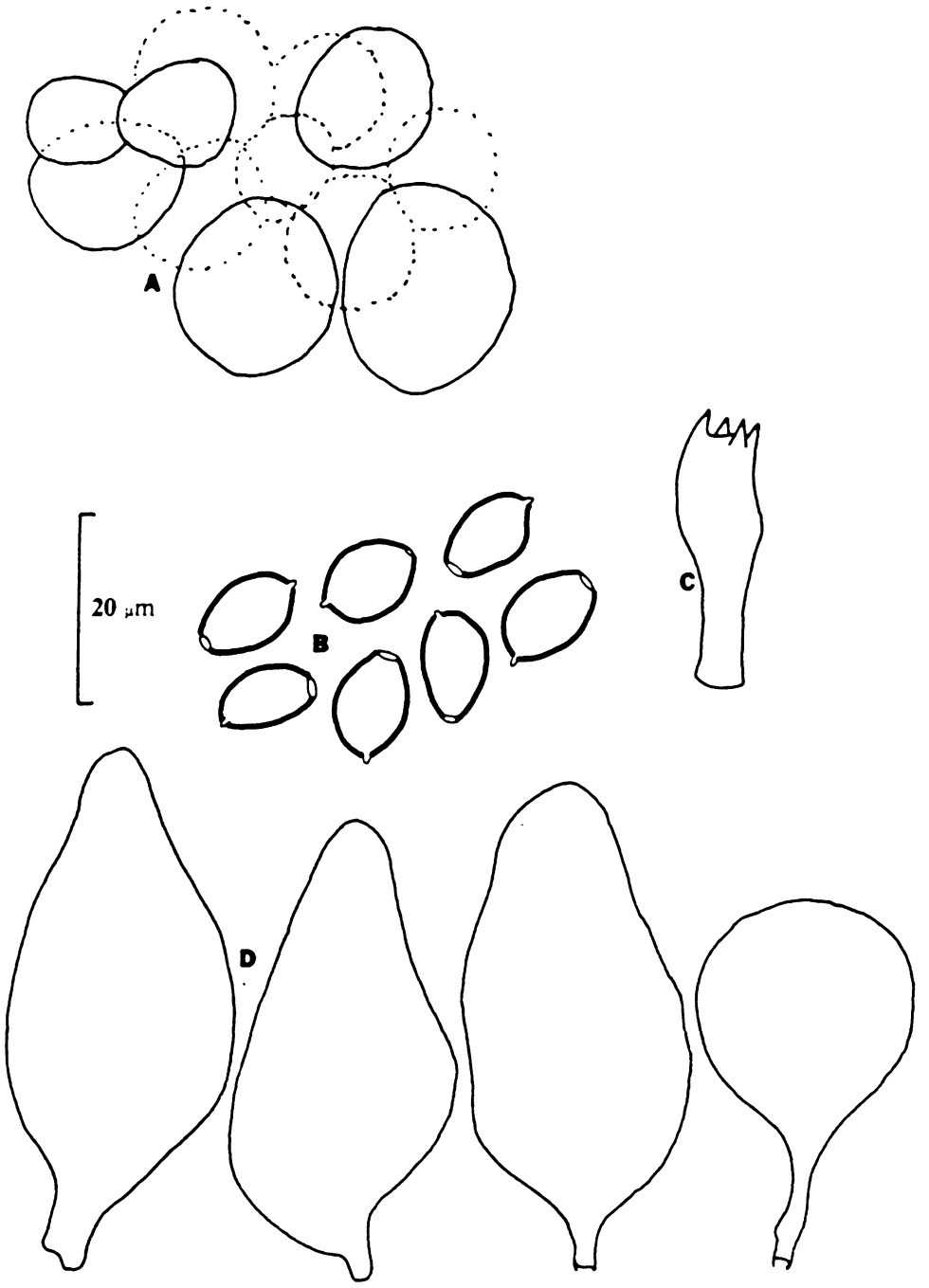


Figure 13. Holotype - Agrocybe howeana: A = paradermal section of pilcipellis; B = spores; C = basidium; D = Pleurocystidia.

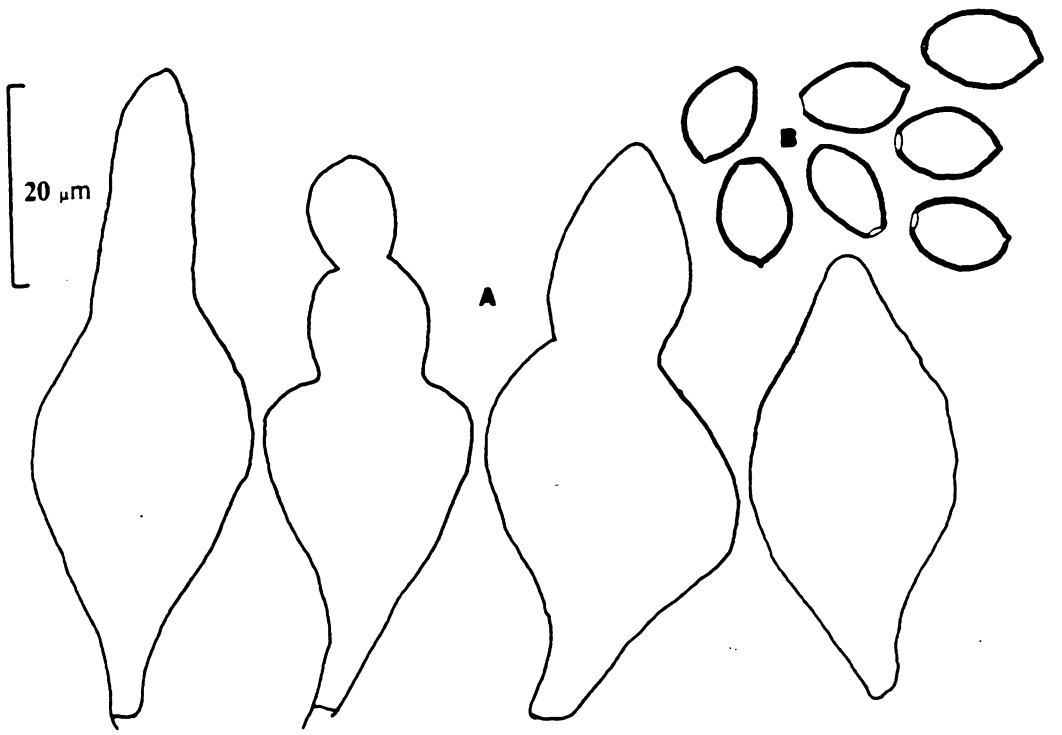


Figure 14. Holotype - Agrocybe acericola: A = spores; B = pleurocystidia.

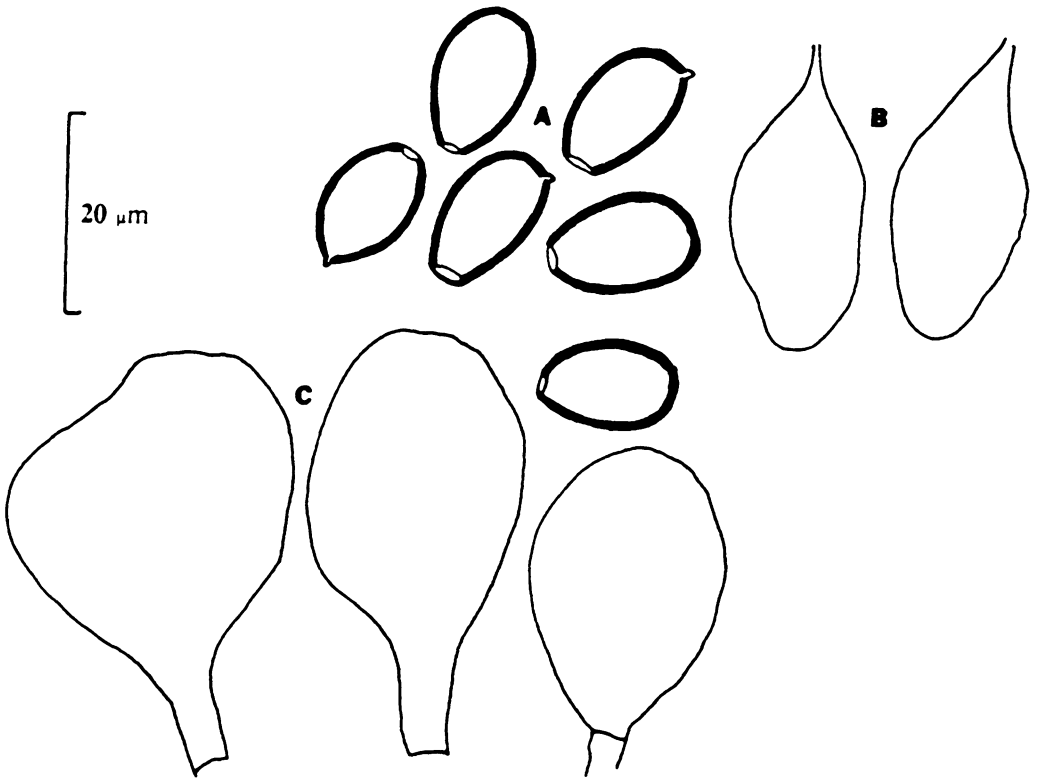


Figure 15. Holotype - Agrocybe vermiflua: A = spores; B = cheilocystidia; C = pleurocystidia.

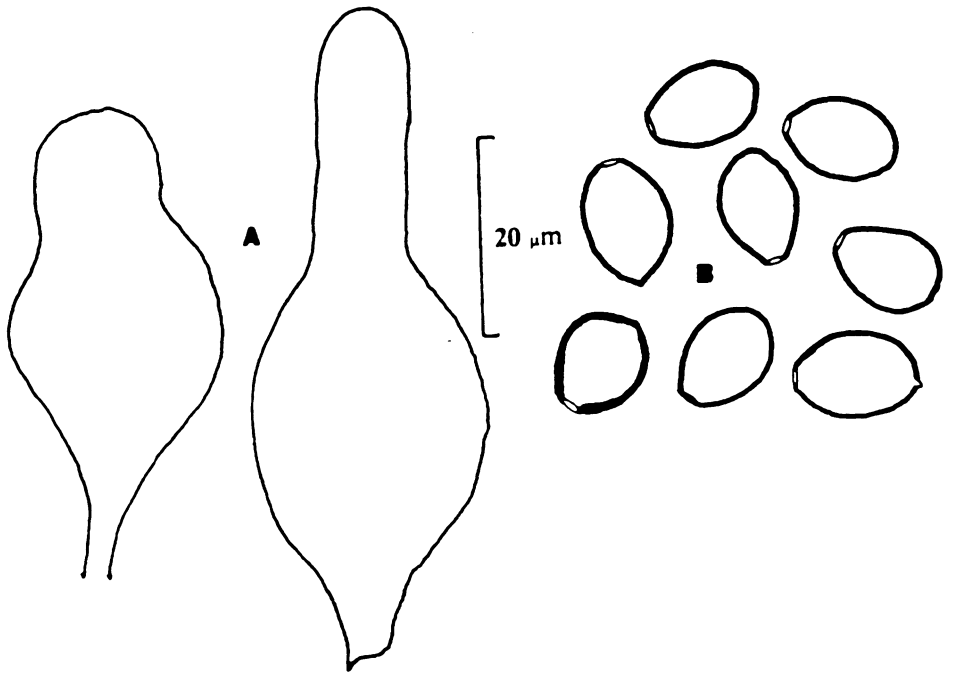


Figure 16. Holotype - Agrocybe temnophylla: A = spores; B = pleurocystidia.

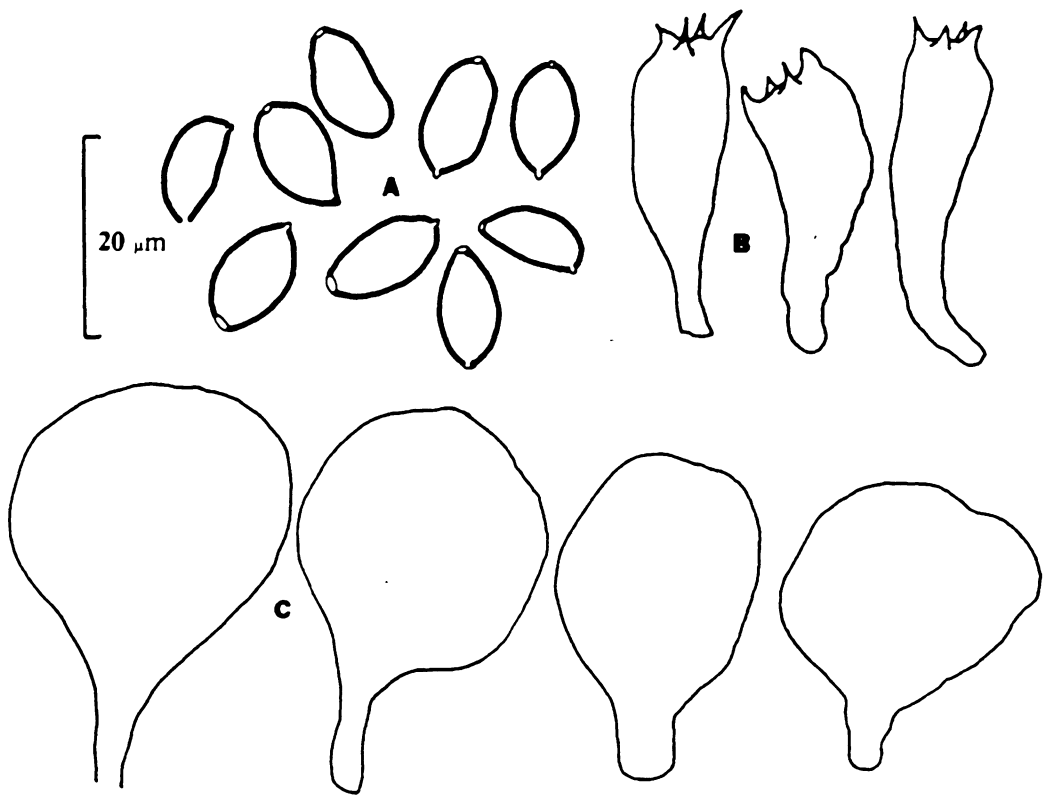


Figure 17. Holotype - Agrocybe alachuana: A = spores; B = basidia; C = pleurocystidia.

Descriptions

Agrocybe praecox

Agrocybe praecox (Persoon : Fries) Fayod (1889, p.358)

Figure 18 on page 69, Figure 19 on page 70, Figure 20 on page 71.

≡ *Agaricus praecox* Persoon (1801, p.420)
≡ *Agaricus (Pratella) praecox* Persoon : Fries (1821, p.282)
= *Agaricus (Pratella) praecox* β *sphaleromorphus* Bulliard : Fries (1821, p.283)
≡ *Agrocybe sphaleromorphus* (Bulliard : Fries) Fayod (1889, p.358)
= *Pholiota praecox* var. *paludosa* J.E. Lange (1921, 2(11):7)
≡ *Agrocybe paludosa* (J.E. Lange) Kühner & Romagnesi (1953, p.341)
= *Pholiota praecox* var. *britzelmayri* S. Schulzer (1889, p.171)
≡ *Agrocybe praecox* var. *britzelmayri* (S. Schulzer) Singer (1950, p.454)
= *Pholiota praecox* var. *cutifracta* J.E. Lange (1921, 2(11):7)
≡ *Agrocybe praecox* var. *cutifracta* (J.E. Lange) Singer (1950, p.454)
= *Agaricus (Pholiota) temnophyllus* Peck (1872, 23:90)
≡ *Agrocybe temnophylla* (Peck) Singer (1950, p.448)
= *Agaricus (Stropharia) howeanus* Peck (1873, p.53)
≡ *Pholiota howeana* Peck (1908, p.147)
≡ *Agrocybe howeana* (Peck) Singer (1951, p.492)

Macroscopic

Pileus (15-) 30-60 (-110) mm broad, convex, parabolic, convex-subumbonate becoming pulvinate, plano-convex or or recurved, often with a broad umbo and at times

with an undulating aspect; margin inrolled to incurved when young, becoming decurved to planar with age, edge sterile and acute, translucent-striate when moist, often with small or large appendiculate remains of ruptured partial veil; surface dull and hygrophanous when moist, shiny and coreacious when dry, glabrous, smooth, sometimes ruggulose reticulate after very wet weather, almost always developing rimose to lacerate cracks from the margin inwards in dry weather; buttons colored dark brown (7F6) to light brown (5D-E7) often with a vinaceous tinge, fading in age to brownish orange (6C6) and greyish yellow (5C4, 4B4-6), disk usually retaining the darker colors of the button stage; flesh firm to spongy, tough pliant or brittle, 2-5 mm thick at disk, creamy white; odor farinaceous, sweetly farinaceous or lacking; taste farinaceous or lacking. Partial veil thin to robustly membranous in consistency, hyphae compactly interwoven, surface glabrous **not cottony**, creamy white and sometimes with darker brown radial streaks of pigmentation; leaving either a fugaceous lacerate pendant annulus, or a fairly persistent, pendant, skirt-like annulus.

Lamellae adnexed to emarginate with a decurrent tooth composed of sterile tissue derived from the partial veil, often seceding, close to crowded, thin and pliant, 3-10 mm broad at mid radius, with 1-3 tiers of lamellulae, margin often fimbriate from adhering cells of the partial veil, colored grayish yellow (4B3) and often with violaceous tinge when young, becoming brownish orange (5C4), brownish yellow (5E7), finally deep brown (6E5) when mature.

Stipe (25-) 60-90 (-130) mm long, 5-15 mm wide, equal to subequal or equal with clavate to slightly flared to bulbose base, or tapered with a flared costate apex to acute base, straight or flexous, usually terete, at times compressed; surface dry, vertically or helically translucent striate, silky and polished, glabrous or with scanty superficial

fibrillose fibers (10x mag.); colored yellowish white (3A2, 4A3) above the annulus, greyish yellow (4B2-4) to olive brown (4C-D6-7) below, some specimens with vertical streaks of darker olive brown pigmentation imbedded in the ground color; context fleshy-fibrous, stuffed then hollow; basal insertion velutinous or fealty, usually attached to abundant, highly branched white rhizomorphs reaching diameters up to 1.5 mm.

Microscopic

Spore deposit brown. Spores (8-) 8.6-10.1 (-12) x (5-) 5.6-6.6 (-7.5) µm, Q= 1.54, obovoid to ovate in face view, broad elliptical in profile view, thick walled (0.5 um), apex truncate or with a large pore, base often short apiculate, hilum narrowly open. Basidia 22-33 x 7-9 µm, four-spored, clavate to cylindro-clavate, often flexous and irregular in outline, thin walled, hyaline, occasionally with refractive cytoplasmic contents. Pleurocystidia (35-) 28-41 (-80) x 18-25 (-27) µm, apex either obtuse or narrowing to 7-12 µm diam., ventricose-rostrate, lageniform, broad clavate, ventricose , ventricose-capitate or ventricose-papillate in outline, thin walled, hyaline, often with refractive cytoplasmic contents. Cheilocystidia are either simple clavate to utriform 12-20 x 7-8 µm similar to the elements of the partial veil, or are ventricose-papillate to ventricose-rostrate similar to pleurocystidia 45-60 x 20-25 µm, cells are thin walled, hyaline and often contain refractive cytoplasmic contents. Lamellar trama composed of an interwoven medial stratum and parallel lateral stratum of unbranched, isodiametric, hyaline, clamped, thin walled hyphae, ranging from 15-3 µm diam; subhymenium inflated-ramose organization. Pileipellis a polycystoderm of ovoid to globose, sphaeropedunculate cells, 18-45 x 11-25 µ, with the terminal cells usually forming an irregular hymenoderm arising from catenulate chains of cells, thin walled, hyaline, occasionally with refractive cytoplasmic contents, subpellis pigmented, a muscilaginous layer is present in specimens with

collapsed and lysed cells. Partial veil composed of highly branched, densely interwoven hyphae, 4-8 µm diam, with large clamps, terminal cells in catenulate chains of clavate, sphaeropedunculate, to ovoid cells, 19-28 x 8-11 µm. Stipitipellis a layer of vertically oriented hyphae, thick-walled, resinous in appearance, unbranched, angular in cross section; caulocystidia absent. Clamps connections present in all tissues.

Genetics heterothallic bifactorial. *A. praecox* is sibling species 1.

Habit, habitat, distribution: Typically subcaespitose, gregarious often fruiting prolifically in fragmented, wood-chip mulch beds, or nearby the margins of grass, also in gardens with buried, fragmented wood; in any case, always attached to or near fragmented wood, or in areas disturbed by man's activities or natural catastrophe. Appearing in early spring, late May to early June. Distributed along the eastern United States and known from Knoxville, TN north to Quebec, Canada, found throughout Europe and collected in the western United States.

NEOTYPE: New Jersey- Bills 749, margin of grass and wood-chip mulch, Montvale, 14 May 1984 (BPI).

Material examined: *EUROPE*: **Denmark-** Vilgalys 84/161, 84/163, 84/164, forested trail, Allendemagleskov, 15 June 1984 (VPI). **France;** Vilgalys 84/66, grassy path under *Sequoia*, Forte de Ferries, 6 June 1984 (VPI). **West Germany-** Flynn 877, leg. M. Moser, June 1985 (VPI). **Scotland-** Flynn 554, leg. R. Watling, May 1984 (VPI). **Netherlands-** Vilgalys 84/112, Leiden; 84/121, Harmeben; 84/110, Castle at Doorworth; 84/113, North Holland; all in grassy areas in forest on deciduous litter, 11-13 June 1984 (VPI); Pipenbroek 1475, clear felled deciduous forest replanted with oak and spruce, south west of Doesburg, Netherlands, May 1985 (VPI); Pipenbroek 1476, clear felled deciduous forest replanted with oak and spruce, south west of Doesburg, 5 May 1985 (VPI); J.V. der Veer 6/5, grass near road side under Poplar, Leiden, 6 May 1985 (VPI). *NORTH AMERICA*: **Canada-** Quebec- Godbout 84/27, wood chip mulch, Laval University, May 1984 (VPI). **USA- Massachusetts** - Peck, leg. Simon Davis, *A. howeana*, 1 Sept. 1911, solitary on road side at edge of swamp (NYS). **Montana-** Flynn 114, leg. M.&L. Bailey, conifer wood fragments in soil near trail, U. Montana Biological Station (UMBS), Lake Co., 6 July 1983 (VPI); Flynn 80,

conifer wood fragments near trail, Glacier Nat. Park 1 July 1983 (VPI); Flynn 817, fragmented conifer wood in soil, UMBS, Lake Co., 17 June 1985 (VPI); Flynn 818, fragmented *Betula* imbedded in grass, Ferndale, Lake Co., 18 June 1985 (VPI). **Idaho**- Miller 20133, buried fragmented wood along dirt road, Cascade Co., 17 June 1984 (VPI). **New Jersey**- Bills 750, wood chip mulch, Park Ridge, 16 May 1984 (L); Bills 751, wood chip mulch, Wood Cliff Lake, New Jersey, 18 May 1984 (VPI); Bills 754, wood chip mulch, Wood Cliff Lake, 23 May 1984. **New York**- Peck, *A. temnophylla* **Holotype** Sandlake, June 1871, on grassy ground (NYS). Peck, *A. howeana* **Holotype**, Center, Albany Co., no date (NYS); Day, July #; Claryville, Aug #; Bolton Landing, 29 July 1905 #; Indiand Lake, July #; Flynn 546, leg. S. Stein, wood chip mulch, Armonk, 25 May 1984 (VPI); Bills 755, wood-chip mulch, Watson Bldg., New York Botanical Gardens, Bronx, 23 May 1984 (VPI); Chamuris 1795, wood-chip mulch, Syracuse campus, Syracuse, May 1984 (VPI). **Virginia**- Flynn 530, wood-chip mulch, Montgomery Co., 27 May 1984 (VPI); Flynn 532, wood-chip mulch, VPI & SU campus, Blacksburg, 29 May 1984 (VPI); Flynn 780, wood-chip mulch, Grissom Lane, Blacksburg, 6 May 1985 (VPI); Flynn 795, wood-chip mulch in grass, Primrose Ave., Blacksburg, Virginia, 27 May 1985 (VPI). Flynn 884, wood-chip mulch, VPI & SU campus, Blacksburg, same thallus as Flynn 532, 20 May 1986 (VPI). **Washington**- Rainer 84/17, wood-chip mulch, U. of Washington, Seattle, 15 May 1984. (All at VPI) **Tennessee**- Hesler 9082, trail, Indian gap to Chimneys, GSMNP, 26 July 1936 (TN); Hesler 12699 saw dust in lawn, Gatlinburg, 15 May 1966 (TN); Hesler 13372 soil under elm, Knoxville, 4 April 1941 (TN); Hesler 12527 soil, lawn, Fountain City, 5 June 1940 (TN); Hesler 21350, humus, Whittles near LeConte, GSMNP, 27 April 1954 (TN); Hesler 16725, soil, Island Home, Knowville, 4 April 1945 (TN); Hesler 21327, deep leaf-mold, oak woods, New Hopewell, 9 May 1954 (TN).

Observations: This species is most easily determined with mating compatibility tests. European and western populations of sibling species 1 are very similar in macroscopic feature except that the sporocarps tend to be smaller and fruitings are less prolific with scattered, as opposed to gregarious or subcaespitose fruitings. They are found along foot trails and road sides where fragmented wood is present but not as abundant as in the eastern U.S.. One collection was taken from a University of Washington mulch bed. It would appear that the abundance of suitable substrate determines the size and habit of sporocarps in this species. Microscopically, the populations from Europe and the western United States of sibling species 1 fall into the range of this species. As a general

observation there is an apparent homology in fungal tissue elements and organization of partial veil, pileipellis, and hymenium. The gill edge will often have clavate cells derived from the partial veil, pleurocystidia like cells may or may not be present depending on the density of the pleurocystidia, All of the cells are subject to weathering and can be lost in very dry weather.

The neotype is designated because the specimen at Leiden was not clearly designated as a type specimen. Furthermore, this specimen is poorly preserved and it is impossible to determine with certainty whether this is *A. praecox* or *A. molesta* even if Fries could make that distinction (Table 1 on page 33).

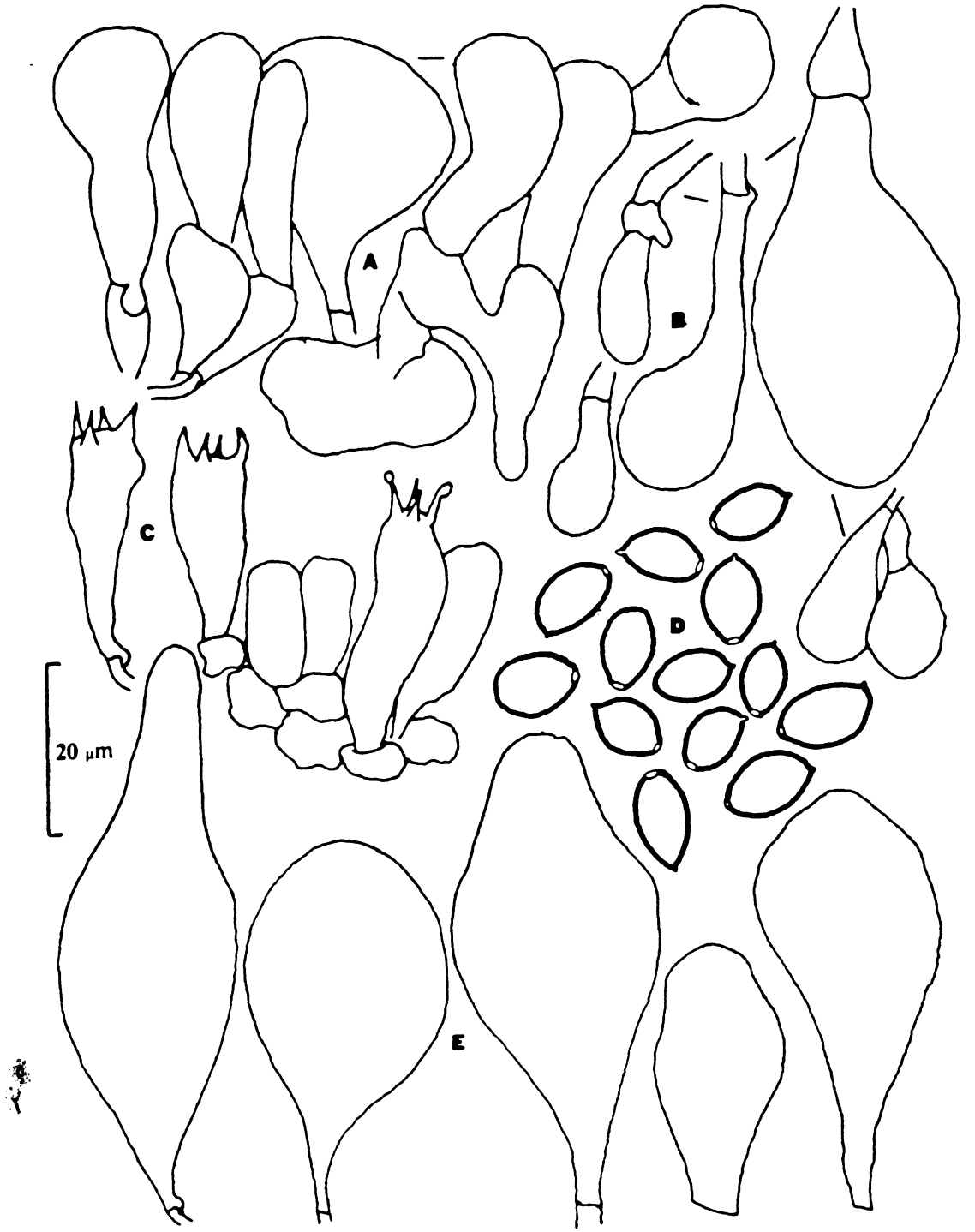


Figure 18. **Agrocybe praecox in eastern north America:** a = pileipellis, b = cheilocystidia, c = basidia, d = spores, e = pleurocystidia.

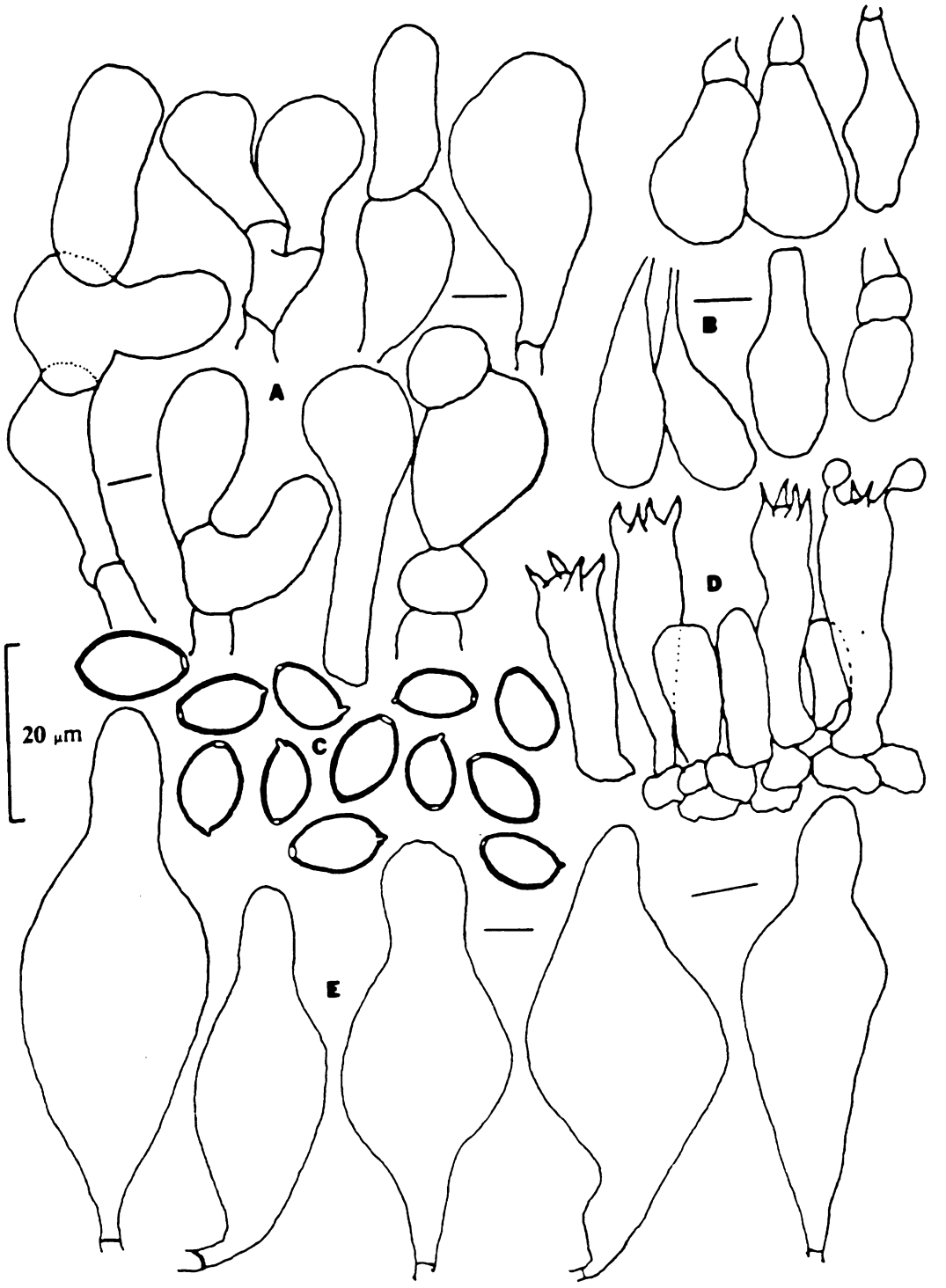


Figure 19. Agrocybe praecox in western north America: a = pileipellis, b = cheilocystidia, c = spores, d = basidia, e = pleurocystidia.

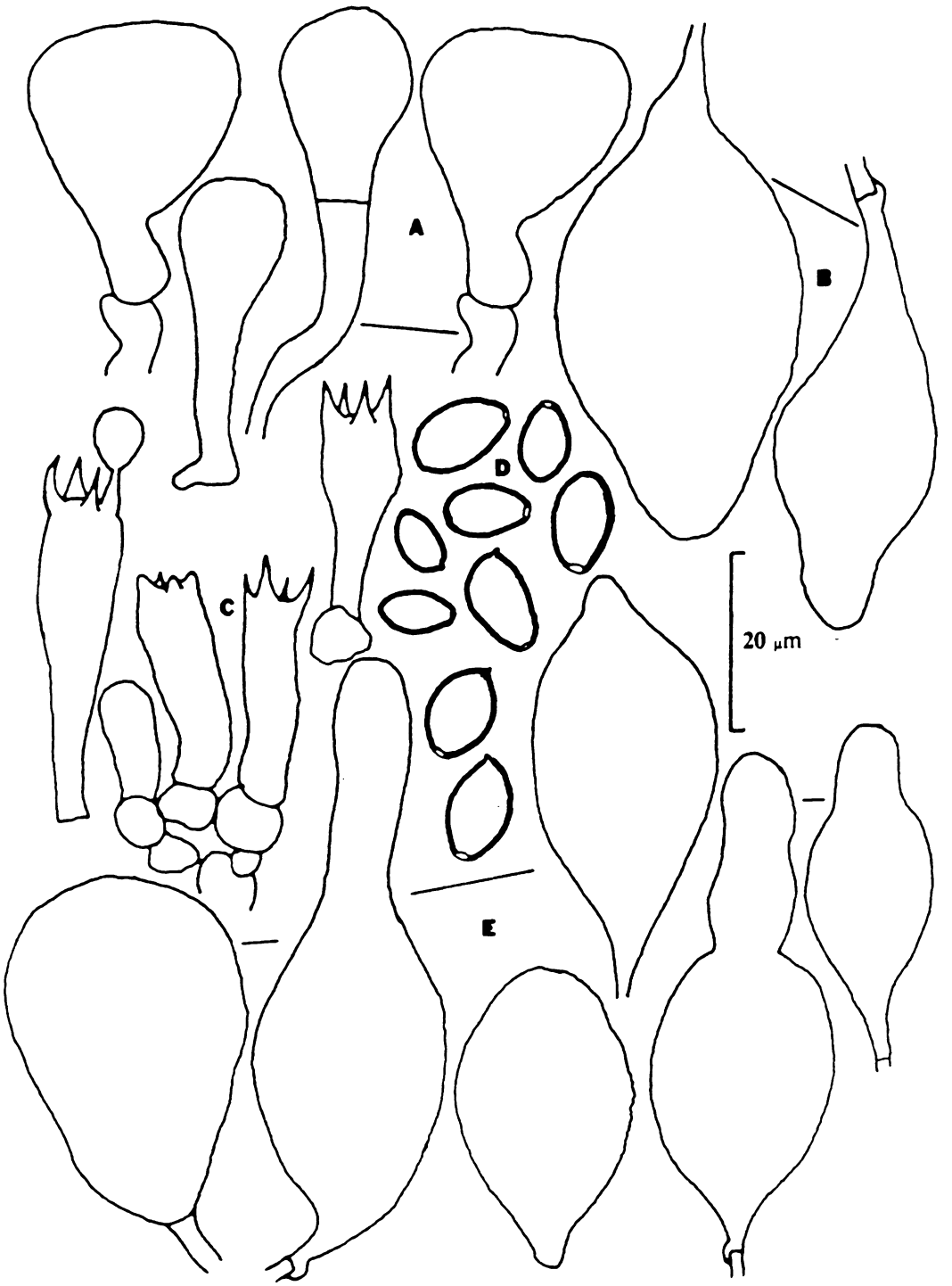


Figure 20. Agrocybe praecox in Europe: a = pileipellis, b = cheilocystidia, c = basidia, d = spores, e = pleurocystidia.

Agrocybe montana

Agrocybe montana nom. prov.

Figure 21 on page 78 and Figure 22 on page 79

Macroscopic

Pileus (25-) 30-60 (-95) mm broad, hemispheric, conic to convex at first, becoming convex, pulvinate, to applanate and often subumbonate, at times undulating; margin inrolled to incurved when young, decurved then plane in moist weather, remaining decurved in dry weather, edge acute, translucent striate when moist, usually with appendiculate remains of partial veil; surface dull hygrophanous when moist, shiny coriaceous when dry, lubricus at times, glabrous, smooth or scrobiculate pitted, rugulose to rugose in dry weather much like wrinkled skin, often developing rimose cracks; buttons colored light yellow, grayish yellow, brownish yellow or light brown (4A4-5, 4B5, 5C7, 6D7-8) or dull yellow (3B4) over all, darkening to reddish yellow, brownish yellow (4B7, 5C6) to brown (5D4) over all, disk often darker than ground color, brownish yellow (5C8), brown (6D-E7), to dark blond (5D4); flesh variable, firm to spongy, tough pliant or brittle, 3-6 mm thick at disk, creamy white. Odor farinaceous or lacking; taste sweetly farinaceous. Partial veil membranous-fibrous in consistency, densely interwoven, underside glabrous, not tomentose or cottony, rupturing early or persistent with radial lacerations, upper side costate from gill impressions, apically attached, often adhering to the pileus margin as lacinate appendiculate remains on pileus margin; annulus persistent to ephemeral, booted or pendant, often extremely lacerate at times fugaceous almost disappearing completely, but can be detected with (10x)

magnification; ground color cream, pale yellow to grayish yellow, at times absorbing streaked pigmentation of the stipe producing dark streaks and swirls imbedded in a lighter ground color.

Lamellae adnexed to emarginate, with sterile decurrent tooth, often seceding in age, ventricose or equal in profile, close to crowded with 2-3 tier of lamellulae, thin and pliant, (1.5-) 4-6 (-10) mm broad at mid radius, margin acute, entire, crenulate, toothy, eroded, or white fimbriate from adhering partial veil fibers derived; face evenly colored or mottled in moist weather, colored grayish yellow (4B-C3) with lilac, or tinged reddish-lilac when young, becoming brown (6E6-8) and dark brown (5D5, 6F7, 7F7) in age.

Stipe (30-) 40-65 (-110) mm tall, 5-10 (-20) wide at mid length, subequal with slightly clavate bulbous base, apex often flaired, usually flexous, occasionally straight, terete or strongly compressed; context fleshy-fibrous, stuffed then hollow; surface smooth or vertically costate-striate, dull or polished, apex at times strongly ribbed from sterile decurrent tooth; colored yellowish white (3-4A2, 3A2-3) or grayish yellow (4B3-4, 4A-B3, 5C5) grayish orange (6E-F7-8) light brown (6D-E6-7) orange brown (5D5) and brownish orange (5C4), apex usually lighter in color than lower portions, some individuals have distinctive vertical streaks of pigmentation darker than the ground color, olive brown (4C-D5-6) to yellowish brown (5D-F5-7). Rhizomorphs absent or present depending on moisture, usually sparse and up to 0.5 mm thick, not highly branched, either thick and fleshy or thin and wiry.

Microscopic

Spore deposit brown. Spores (8-) 9-10.5 (-12) x (5-) 5.6-6.6 (-8) μm, Q= 1.5, ovate to obovoid in face view, broad to narrow elliptic in profile view, apex truncate or with large pore, often apiculate, hilum narrowly open, wall smooth and thick, interior with several to zero oil guttules, colored olive brown in NH_4OH. Basidia 22-32 x 7-10 μm, four-spored, cylindric-clavate often flexous and irregular in outline, hyaline, sterigma often robust. Pleurocystidia extending well beyond the hymenium, (40-) 46-61 (-78) μm long x (13-) 16-23 (-29) μm wide, apex narrowing to (6-) 9 (-14) um wide, mostly ventricose-rostrate, lageniform, capitate-ventricose, some populations with digitate protrusions at the apex, thin walled, hyaline, sometimes with refractive cellular contents. Cheilocystidia are either 15-55 x 7-16 μm, simple clavate to utriform, similar to and derived from the terminal elements of the partial veil, or are 30-34 μm long x 15-20 μm wide, apex narrowing to 5-7 μm wide, ventricose-capitate, ventricose-papillate to sphaeropedunculate, similar to the pleurocystidia and arising from the hymenium, pleurocystidia-like cells may or may not be present depending on pleurocystidial density, in any case, all cells thin walled, hyaline and sometimes with refractive cellular contents. Lamellar trama regular with slightly interwoven mediostratum, medial hyphae 10-22 μm diam., lateral elements becoming progressively narrower in proximity to the hymenium, 7-4 μm diam., clamped, thin walled, sparingly branched, mostly isodiametric or barrel shaped with constricted septa; subhymenium inflated ramose in organization. Pileipellis a polycystoderm composed of clavate, sphaeropedunculate, pyriform, globose cells 15-65 x 8-30 μm, terminal cells often organized in an inflated ramose structure or born in catenulate chains forming an irregular hymenoderm, subterminal cells similar to and supporting the apical cells much like brachybasidioles; elements hyaline, thin walled, sometimes overlayed with a muscilaginous layer from lysed cells or completely collapsing into a mucid golden brown layer. Stipitipellis of vertically oriented thick walled, isodiametric hyphae, wall resinous in appearance, caulocystidia absent. Partial veil

composed of highly branched, thin walled, hyaline hyphae bearing clamped septa and catenulate chains of clavate to sphaeropedunculate terminal cells; hyphae 5-8 µm diam., terminal cells 20-45 x 10-17 µm. Clamp connections are seen in all tissues.

Genetics heterothallic bifactorial. ***A. montana*** is sibling species **2.**

Habit, habitat and distribution Sporocarps occur either singly, scattered or in dense sub-caespitose clusters depending on the richness and abundance of the substrate. Specimens are found in deep forests away from human impact or more frequently occur along dirt road sides, trails, picnic areas, and highly disturbed logged forests where abundant fragmented slash becomes buried from heavy machinery. This fungus is found in a variety of forest communities and substrates such as *Populus tremuloides*, *Pinus ponderosa*, *P. contorta*, *Pseudotsuga*, *Abies grandis*, *A. lasiocarpa*, *Picea engelmanii*, *Salix glauca* and others. The material used in this study is distributed from the southern Rocky Mountains in Colorado north to SW Alberta, Canada; west to the Coastal Range of Northern California and the Cascade range of Oregon. Fruiting occurs from March through June in California and June through August in the Rocky Mountains with later fruitings in Colorado and Alberta, Canada.

HOLOTYPE: Idaho- Flynn 799 debris of *Psuedotsuga*, *Abies*, *Picea* and *Pinus* from logging operation partially buried, Brundage Mtn., Cascade Co., 12 June 1985 (BPI).

Material examined: *CANADA*: **Alberta-** Flynn 837 *Picea* litter and grass, Livingston falls CG, Alberta, grass, 5,650 ft., 14 July 1985 (VPI) Flynn 839, leg. Ardean Watts, moist alpine bog, *Salix glauca* grasses and sedges, Highwood Pass, Alberta, 7,500 ft., 15 July 1985 (VPI). Flynn 847, leg. Bart Clennon, gaps in flag stones, picnic area at Moraine Lake/Ten Peaks area, Banff Nat. Park, Alberta, 5,400 ft., 17 July 1985 (VPI). **USA: California-** Flynn 488, leg. Roy Watling, soil under *Arctostaphlos aranzanita*, Willow Creek, Calf., 22 March 1984 (VPI); Miller 20987, buried woody debris, Siskyou Co., Calf., 18 June 1984 (VPI); **Colorado-** Flynn 674, buried *Populus tremuloides* debris by dirt road, Niwot Ridge, Rocky Mountain research Station, Boulder Co., Colorado, 2 August 1984 (VPI). Flynn 697, fragments of aspen and spruce in aspen leaf litter, Chicago Creek, 12 August 1984 (VPI). **Idaho-** Flynn 802 slash from *Pinus contorta* and *Abies*, Brundage Mtn., Valley Co., 13 June 1985 (VPI). Miller 20119, conifer debris near ashes, Bear Basin,

Valley Co., 14 June 1984 (L); Miller 20120, conifer debris from logging operation, Bear Basin, Valley Co., 14 June 1984 (VPI); Miller 20123, conifer debris, Warm lake Summit, Valley Co., 17 June 1984 (VPI); Miller 20136, conifer litter, Valley Co., 17 June 1984 (VPI); Flynn 799 and Flynn 800, debris of *Psuedotsuga, Abies, Picea* and *Pinus* from logging operation partially buried, Brundage Mtn., Valley Co., 12 June 1985 (VPI); Flynn 813, Flynn 814, and Flynn 815, conifer and aspen debris near dirt road side, Lick Creek, Valley Co., 15 June 1985 (VPI); Flynn 816, fir needles and moss, Lick Creek, Valley Co., 15 June 1985 (VPI). Montana- JLG43, conifer litter, *Thuja, Populus, Pinus,* and *Larix,* Mission Falls, Flathead Indian Reservation, T18N R19W S12, 8 July 1985 (VPI); Flynn 819, mesic to xeric *Pinus ponderosa, Abies grandis* and *Pseudotsuga* litter, Swan Valley, Flathead Co., T26N R18W S1, 18 June 1985 (VPI); Oregon: Miller 20113, manured pine litter near horse corral, Lilly Glen Co., 21 May 1984 (VPI).

Observations: These populations have the ability to decompose gymnospermous and angiospermous woody substrates. This species is opportunistic as is sibling species 1 *A. praecox* and can be predictably found in areas of disturbance. Morphologically, this species can not be distinguished from the other sibling species of *A. praecox.* However, the habitat, ecological characteristics and distribution, makes this species distinctive. One collection from Colorado (Flynn 697) is interesting because of its fairly unique pleurocystidia with digitate apices similar to those found in *A. arvalis* (Fries) Singer. Overholtz (1927) collected *Agrocybe* in the same region and documented this anatomy. This anatomy is not taxonomically important, but it does indicate that populations may have certain distinctive features which are maintained through a period of time. A similar phenomenon is seen in the Idaho populations (Miller 20119, Flynn 802 and 799) where many specimens have streaks of darker pigmentation on the stipe and annulus. This expression is not unique to Idaho, but is striking in some instances and has been seen independently by different collectors several years in succession.

Agrocybe montana Swiss representative

Figure 22 on page 79

Spores (10.5-) 10.6-12 (-12.5) x (7.5-) 7.6-8.6 (-9) µm, Q= 1.396, broad ovate to obovoid in face view almost globose and occasionally constricted at the apex, broad elliptic in profile view, apex truncate and occasionally constricted, hilum broadly open, apiculus stubby if present, wall thick (± 1-1.5 µm), brown, empty or with oily refractive contents. Basidia 27-29 x 8-9 µm, clavate to cylindro-clavate, often irregular in outline, four-spored, sterigmata robust, hyaline, thin walled, refractive contents present or absent. Pleurocystidia 46-62 x 12-28 µm, rostrum 6-12 µm wide, ventricose rostrate, often with papillate, digitate protrusions at the apex, thin walled, hyaline, often with refractive contents, half imbedded, fairly abundant. Cheilocystidia 11-18 x 6 µm, clavate. Pileipellis a polycystoderm composed of clavate, sphaeropedunculate inflated cells 25-70 x 15-34 µm with terminal cells arising from catenulate chains.

Habit, habitat and distribution: Fruiting singly on a moss covered stream bank. A single specimen collected in August represents this Swiss *Agrocybe*

Material examined: Europe: Switzerland- OKM21272, mossy stream bank in conifers, Graubunden, 27 August 1984 (VPI).

Observations: This species was delicately statured much like the concept of *A. paludosa.* Single spore isolates grew slowly, were intersterile with all other isolates during the first year of cultivation. I first thought this fungus to belong to its own intersterility group but later experiments indicated intercompatibility with members of *Agrocybe montana.* I tentatively identify this single collection as *A. montana* until more isolates are taken from Switzerland and mountainous areas of western Europe.

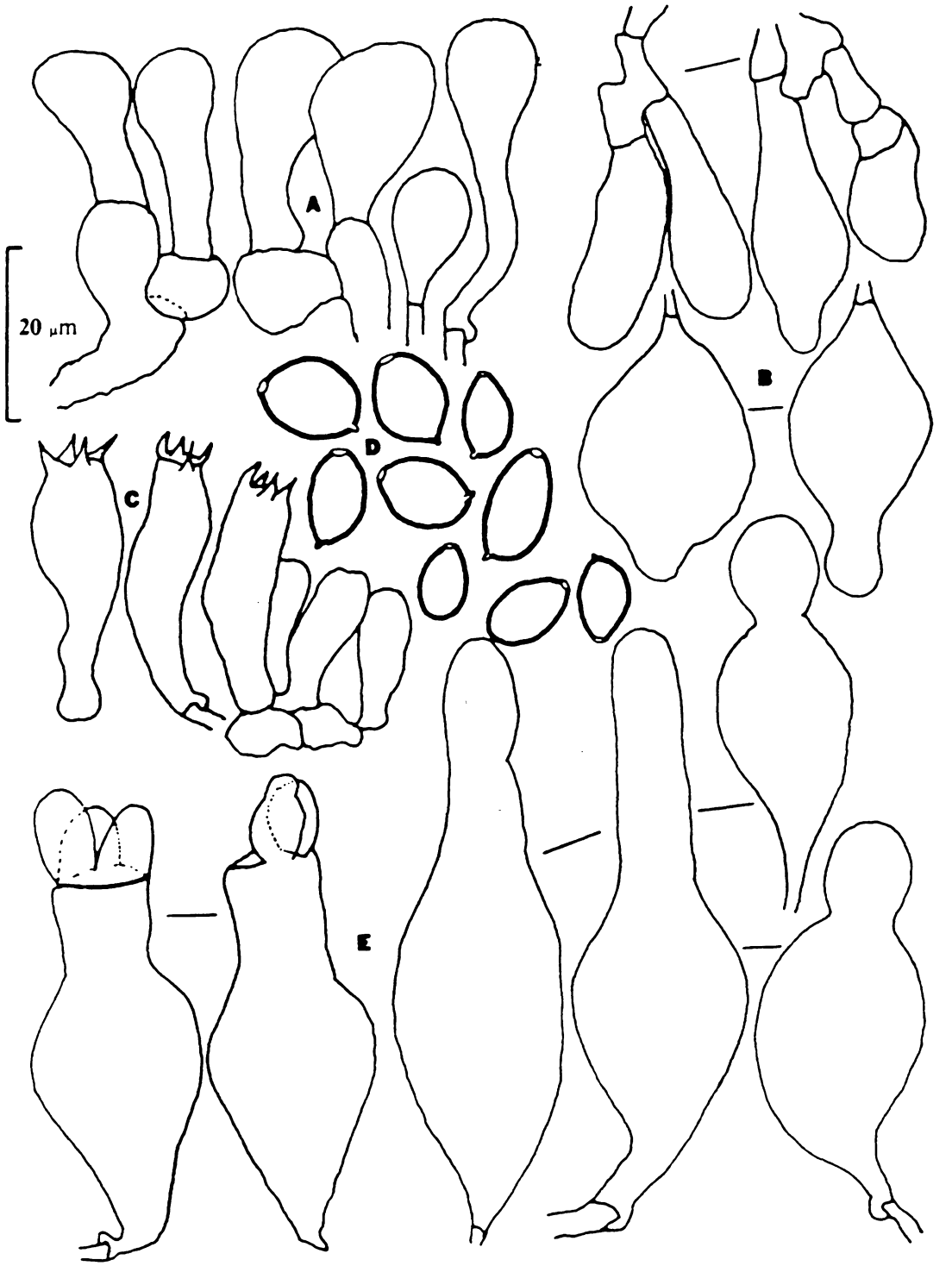


Figure 21. **Agrocybe montana:** a = pileipellis, b = cheilocystidia, c = spores, d = basidia, e = pleurocystidia.

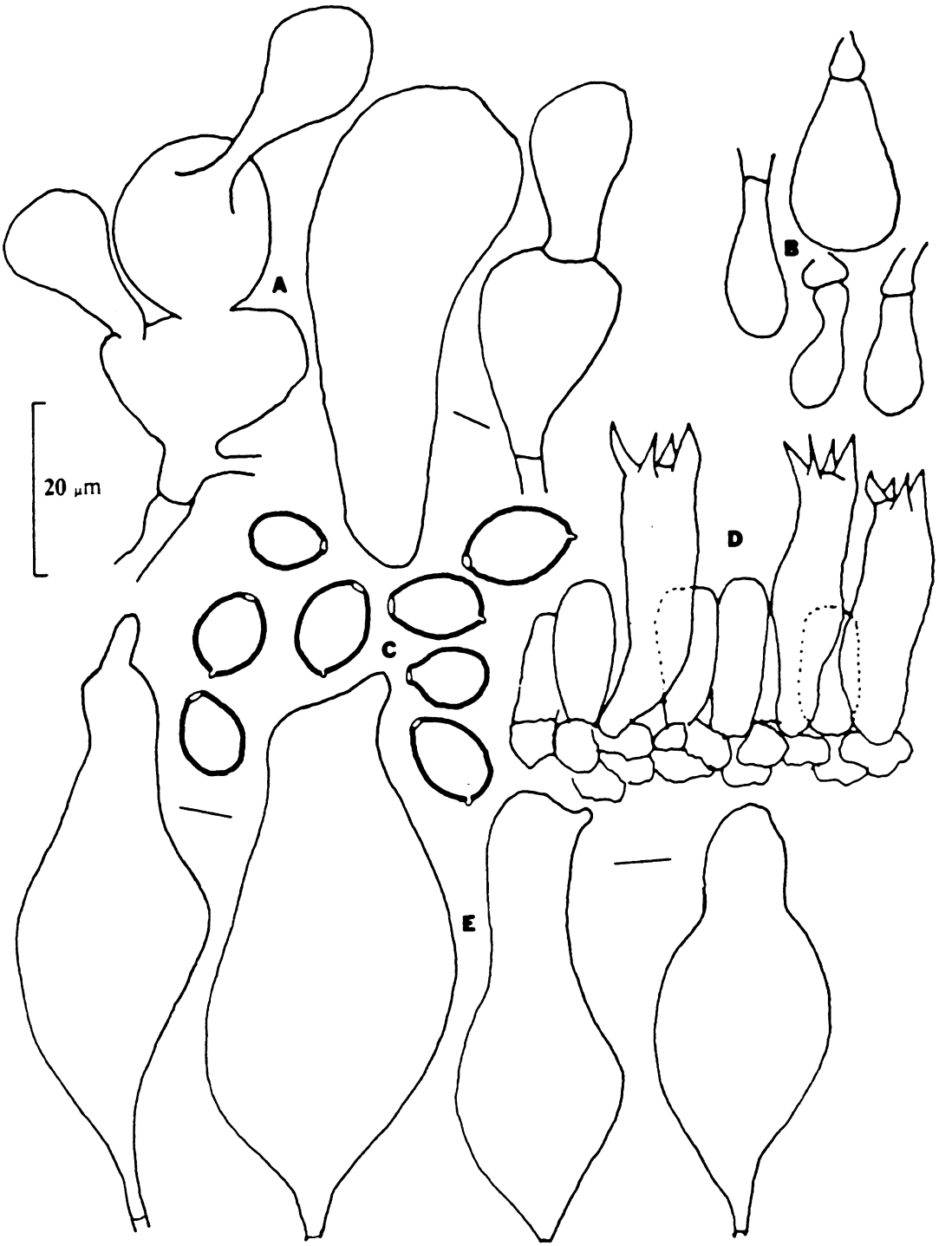


Figure 22. Agrocybe montana, Swiss collection: a = pileipellis, b = cheilocystidia, c = spores, d = basidia, e = pleurocystidia.

Agrocybe acericola

Agrocybe acericola (Peck) Singer (1950, p.448)

see Figure 23 on page 84.

≡ *Agaricus acericola* Peck (1873, p.50)
= *Pholiota praecox* var. *Sylvestris* Peck (1896, p.74)
= *Pholiota alachuana* Murrill (1943, p.533)
≡ *Agrocybe alachuana* (Murrill) Singer (1962, p.530)

Macroscopic

Pileus 20-50 mm broad, convex then broad convex often with obtuse umbo, sometimes with an undulating aspect; margin incurved the decurved then becoming straight, sometimes with appendiculate remains of partial veil; surface dull when moist, coriaceous in dry weather, marginal area translucent striate when moist, rugulose or pitted as pileus dries or smooth, glabrous, aerolate in dry weather; colored yellowish brown (5D-E8) to pale yellow (4A3-4) then grayish yellow (4B5-7) grayish orange (5B6) light orange (5A5) brownish orange (5C6-7) umbo usually remaining darker than surrounding areas brownish orange to yellowish brown (5C6-7). Flesh 3-5 mm thick at mid radius, creamy white, pliant to brittle; taste farinaceous, odor farinaceous to lacking. Partial veil thin membranous, with considerable tensile strength, densely interwoven, not cottony cottony or cortinous, leaving a thin, long lived or fugaceous lacerate annulus.

Lamellae adnexed with a sterile decurrent tooth, seceding in age, close to crowded, 2-3 tiers of lamellulae, thin and tough pliant, 3-4 mm broad, narrow ventricose to equal in face view; margin crenulate to fimbriate from adhering remains of the partial veil;

colored light grey with a lilac tint at first (6B2) becoming light brown (5D4) brownish orange (6C4) and finally brown (6E7).

Stipe 35-90 mm long, 4-9 mm broad at mid length, sub-equal to equal, base often clavate or inflated, straight or flexous in outline, terete, consistency fleshy-fibrous, stuffed or hollow, surface vertically striate, polished, base with a felty or velvety tomentium; colored pale yellow (3A3) grayish white to grayish yellow (4B3-4) or pale orange to orangish gray (5A-B3), usually darkening with age. Rhizomorphs abundant to merely present, thin to thick, highly branched, off white in color.

Microscopic

Spore deposit brown. Spores (8.5-) 9.0-9.9 (-10) x (5.5-) 5.6-6.5 (-7.5) µm, Q= 1.57, obovoid to ovate in face view, broad elliptic in profile, thick walled (± 0.5µm), apex truncate with large pore, base often apiculate, hilum narrowly open; unusual spores 14-17 x 6-7.5 µm, fusiform with papillate apex in face view, fusiform-elliptic in profile. Basidia 23-27 (-55) x 7-9 µm, primarily four-spored, two and three-spored basidia produce fusiform spores on very long sterigmata up to 20µm long, clavate to cylindro-clavate, regular or flexous outline, thin walled, hyaline. Pleurocystidia 48-56 (-59) x 19-23 (-25) µm, apex either obtuse or narrowing to 7-11 µm, ventricose-rostrate sometimes with two rostra or branching, ventricose-papillate, or conic-ventricose in three tiers, thin walled, hyaline, often with refractive contents. Cheilocystidia abundant or absent, 15-30 x 7-16 µm, clavate, utriform, to narrow ventricose, similar to the terminal cells of the partial veil. Lamellar trama parallel to subparallel, composed of isodiametric unbranched septate hyphae with clamp connections, cells thin walled, hyaline, ranging from 4-14 µm diam.; subhymenium of inflated-ramose cells. Pileipellis

a polycystoderm composed of clavate, sphaeropedunculate, globose cells, 21-26 x 8-17 µm, with terminal cells forming an irregular hymenoderm arising from catenulate chains, cells thin walled, hyaline, sometimes with refractive contents. Partial veil composed of highly branched, densely interwoven hyphae 4-8 µm diam, with large clamp connections, terminal elements in catenulate chains of clavate to sphaeropedunculate cells 18-30 x 7-12 µm. Stipitipellis a layer of vertically orientated, resinous appearing thick walled cells, unbranched, angular in cross section; caulocystidia absent. Clamp connections present in all tissues.

Genetics heterothallic bifactorial, *A. acericola* is sibling species 3.

Habit, habitat, and distribution: Found singly in deciduous leaf litter and one subcaespitose cluster fruiting from bark at the base of a healthy *Acer nigra*. Basically indistinguishable from species 1. This species fruits from May through July, two out of the three specimens fruited during cool weather in July.

Material examined: Virginia- Flynn 576, bark at base of *Acer nigra*, VPI&SU campus, Blacksburg, 6 July 1984 (L); Flynn 596, leg. R. Vilgalys, forest litter under maple and oak, VPI&SU campus, Blacksburg, 8 July 1984 (BPI); TMF 798, leg. G. Bills, forest litter under maple and sycamore, Plum Creek near Christiansburg, Montgomery Co, 31 May 1985 (VPI). New York- (holotype) Peck, moss covered maple log, North Elba, August, NYS; Peck, Howe's caves, Schohane Co.?, July NYS; Peck, East Worchester, Oliego Co.?, July NYS; Peck, Croghan, Lewis Co., September NYS;

Observations: This taxon was formerly recognized based on its distinctive habitat and rugulose pileus, a characteristic which can occur in many taxa. As defined in this study, *A. acericola* is best identified in the field by its habitat. The definitive test is mating compatibility. Macroscopically, this species tends to be delicately statured and fruits singly. Microscopically, a Virginian collection (TMF798) produced one, two and three spored basidia and oddly shaped fusiform spores similar to the basidia and spores of *A.*

erebia (Fries) Kühner. This observation coupled with the external morphological similarity of *A. erebia* and the *A. praecox* group, especially *A. acericola* (Peck) Singer as defined here may indicate some relationship which merits further study. I recognize this taxon by its occurrence in deciduous leaf litter of mesic hardwood forest, but the only definitive method is to perform the mating experiments.

A neotype could not be designated for this study because adequate material was preserved by Peck. Peck defined this species with characteristics which are found in many species of *Agrocybe*, but he did recognize the distinctive habitat of this fungus.

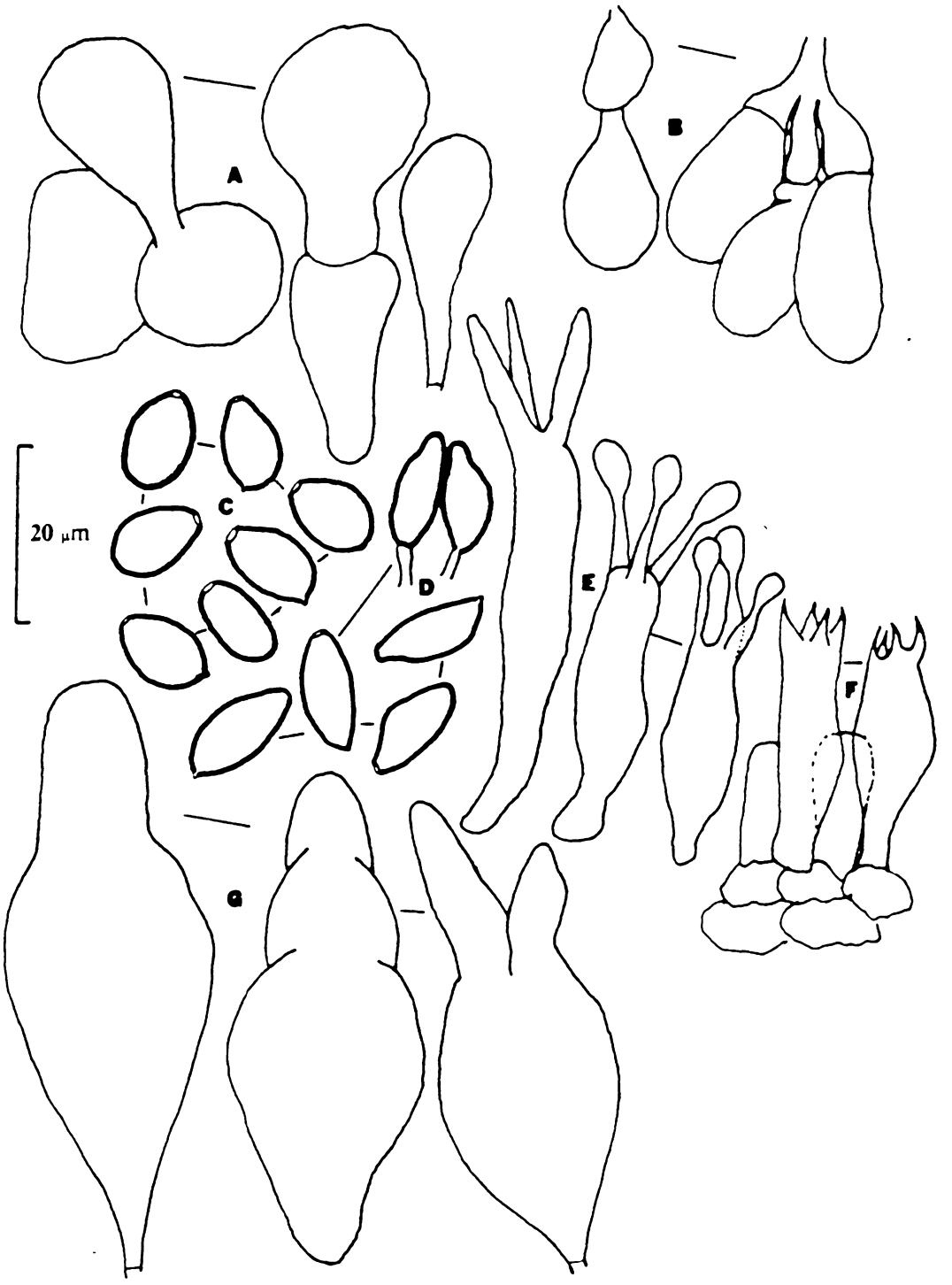


Figure 23. **Agrocybe acericola:** a = pileipellis, b = cheilocystidia, c = spores, d = basidia, e = pleurocystidia.

Agrocybe gibberosa

Agrocybe gibberosa (Fries) Fayod (1889, p.358)

Figure 24 on page 87

≡ *Agaricus gibberosus* Fries (1838, p.163)

Microcscopic

Spore deposit brown. Spores (8-) 8.7-10.5 (-11) x (5-) 5.5-6.5 (-7) µm, Q= 1.605, ovate almost rhomboidal in face view, elliptical in profile view, apex truncate, hilum narrowly open, apiculus stubby or absent, wall thick (± 0.5 µm). Basidia 22-32 x 7-13 µm, irregular to flexous cylindro-clavate, four-spored, thin walled, hyaline. Pleurocystidia (50-) 52-66 (-71) x (19-) 21-27 (-30) µm, apex obtuse or narrowing rostrum 5-12 µm diam., lageniform to ventricose rostrate, rostrum occasionally branched. Cheilocystidia clavate. Lamellar trama parallel with interwoven mediostratum, medial hyphae generally wider than lateral hyphae, cells isodiametric with constricted septa bearing clamp connections, sparingly branched, thin walled, hyaline, olive brown oleiferous hyphae present. Stipitipellis typical, caulocystidia absent. Partial veil typical. Clamps seen in all tissues.

Genetics- heterothallic tetrapolar, this species belongs to sibling species 4.

Habit, habitat and distribution: Sporocarps are found singly in mixed deciduous and coniferous forests. This mating group is known only from Denmark fruiting in June.

NEOTYPE: Denmark- RV84/166, litter of mixed coniferous and deciduous forest, Allendemagleskov, 15 June 1984 (BPI).

Material examined: Denmark- RV84/165, litter of mixed conifer and deciduous forest, Allendemagleskov, 15 June 1984 (L).

Observations: This species belongs to sibling species 4 and is most reliably identified with mating compatibility tests. Fries described this species based on the lacerate partial veil and fruiting in coniferous forests. These characteristic are not diagnostic. This species is indistinguishable from the other sibling species of *A. praecox*.

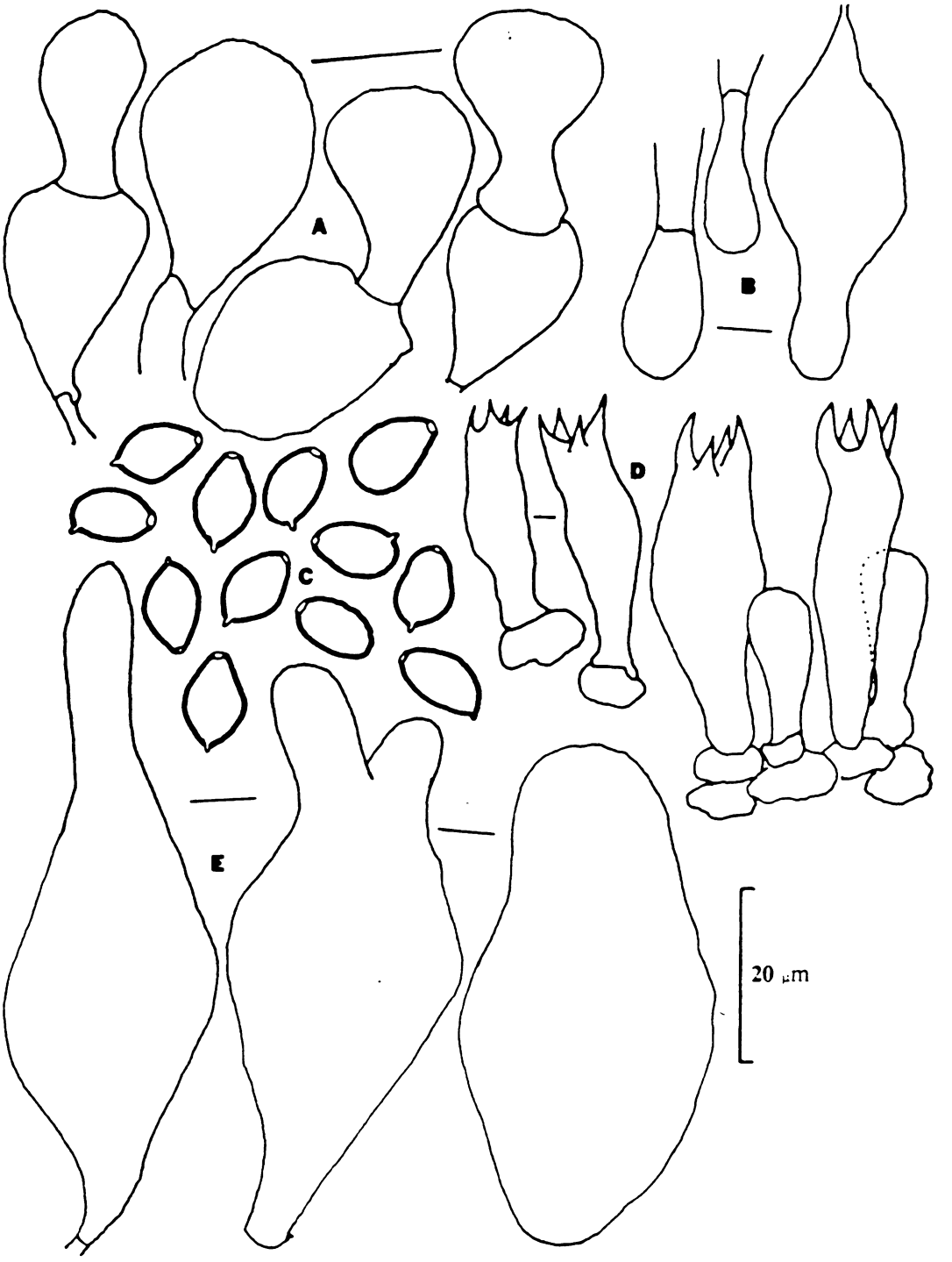


Figure 24. Agrocybe gibberosa: a = pileipellis, b = Cheilocystidia, c = spores, d = basidia, e = pleurocystidia.

Agrocybe molesta

Agrocybe molesta (Lasch) Singer (1977, p.197)

Figure 25 on page 92

≡ *Agaricus molestus* Lasch (1828, p.421)
= *Agaricus vermiflua* Peck (1879, p.34)
≡ *Agrocybe vermiflua* (Peck) Watling (1976, p.592)
= *Pholiota praecox* sensu Fayod (1889, p.358)
= *Pholiota praecox* sensu Peck (1896, p.60)

Macroscopic

Pileus (20-) 35-50 (-60) mm broad, hemispheric to convex becoming pulvinate with incurved margin then plano-convex, margin decurved, entire, often with cortinous remains of the partial veil; surface lubricus in moist weather or dull to shiny coriaceous when dry, often developing aerolate cracks as pileus expands, cuticular layers thin; colored light yellow, grayish yellow to cream (4A-B2-3, 4A2-3, 4A4, 3A2-3, 4B4, 4A2 margin 3A2-3 4A2, disk 4A5); trama 6-20 mm thick at disk, firm, white, odor and taste pleasant, that of *Agaricus bisporus.* Partial veil creamy white, loosely interwoven giving a fealty, cottony or wooly appearance to the surface, rupturing early, leaving cortinous remains on the pileus margin and thin cortinous annulus which at times is only discernible by the presence of adhering spores.

Lamellae adnexed to adnate then seceding, close when young becoming subdistant with age, 4-10 mm broad, thin or thick pliant, margin entire to more or less crenulate, often fimbriate from adhering remains of the partial veil; colored grayish yellow then yellowish brown to light brown (4B3 then 5D5-6, 6D5, 7F6-7).

Stipe 50-100 mm tall, (2-) 5-10 mm wide at apex, equal, straight; context fleshy-fibrous and brittle; surface smooth and with helical vertical optical striations, hollow, costate above annulus, shiny often with loose superficial cortinous fibers, concolorus with pileus. Rhizomorphs thick and fleshy (± 0.5-1 mm) not branched, sporocarps arising from a single thick rhizomorph. Rhizomorphs scanty, sparse but obvious, usually a single large, fleshy, thick (up to 2 mm diam.), at times not seen in drying specimens or ones with damaged bases.

Microscopic

Spore deposit brown. Spores (12-) 12.4-14.4 (-17) x (7-) 7.6-8.8 (-10) µm Q= 1.645, narrow obovate to ovate elliptical in face view, elliptical in profile, thick walled, with large, truncating apical pore, hilum open and often apiculate. Basidia 28-38 x 8-11 µm, four-spored, cylindric-clavate to clavate, flexous, extending well beyond the hymenium, wall thin, hyaline, often with refractive oily contents. Pleurocystidia (26-) 32-53 (-73) x (17-) 20-31 (-46) µm, inflated clavate, ventricose, utriform, often deeply imbedded in the hymenium but easily dislodged in fresh specimens with percussion, thin walled, hyaline, empty or with refractive guttules. Cheilocystidia abundant, derived from the hymenium, 30-60 x 13-23 µm, similar to pleurocystidia. Gill trama subparallel to interwoven, hyphal elements thin walled, isodiametric, sparingly branched, septa constricted and bearing clamps, cells 6-17 µ diam.. Pileipellis a cystoderm composed of inflated ramose branched elements giving rise to broad clavate, sphaeropedunculate, pyriform, terminal cells, 25-35 x 12-20 µm thin walled, hyaline; organization similar to *A. praecox* but less densely compacted; subpellis interwoven filamentous, light yellowish brown in NH_4OH, suprapellis at time collapsing and leaving a muscilaginous layer. Partial veil composed of branched, isodiametric hyphae with constricted septa bearing clamps 7-11 µm diam.,

terminal cells elongate clavate, hyphal like, to inflated clavate, pyriform, 45-85 x 10-28 µm hyaline, thin walled, empty.

Genetics heterotahllic bifactorial.

Habit, habitat and distribution: Sporocarps occur singly, scattered or densely gregarious but are never caespitose. This species occurs in the grassy habitats of prairies, pastures, and most abundantly in fertilized and irrigated lawns and the mycelium never occupies fragmented wood. Distributed from Hawaii, California and Colorado to the eastern United States. Fruiting bodies occur from May through July during cool weather.

NEOTYPE: Virginia- TMF536, lawn, Harding ave., Blacksburg, 29 May 1984 (BPI).

Material examined: NORTH AMERICA; Colorado-TMF683, riparian grass under cottonwood, Casha la Pudre Canyon, 4 August 1984 (VPI). Virginia-TMF405, lawn, VPI&SU campus, Blackburg, 21 September 1983 (VPI); TMF528, lawn, route 696, Montgomery Co., 20 May 1984 (VPI); TMF529, disturbed lawn, route 696, Montgomery Co., VA, 27 May 1984 (VPI); TMF559, scattered in soil and grass, Holden Hall, VPI & SU, Blacksburg, VA, 29 June 1984 (VPI); TMF565, lawn, VPI&SU, 2 July 1984 (VPI); TMF566, lawn, Blacksburg, 2 July 1984 (VPI); TMF567, lawn, E. Roanoak & Harding Ave., Blacksburg, 2 July 1984 (VPI); TMF569, lawn, route 696, Montgomery Co., 6 July 1984 (VPI); TMF583, lawn, Shenandoah Nat. Park, 7 July 1984 (VPI); TMF640, lawn, S.W. Burrus Hall, VPI&SU, 23 July 1984 (VPI); TMF791, leg. Steve Miller, fertilized lawn, Radford, VA, 24 May 1985 (L). Hawaii-OKM21914, lawn, Hilo, 27 October 1984 (VPI); Washington-SAR84/102, lawn, 3600 Univ. Way, Seattle (VPI); Quebec-CG84/28, grass, University of Laval, spring 1984 (VPI); New York-TMF547, grass, Westmoreland nature sanctuary, 20 May 1984 (VPI);

Observations: This fungus is easily recognized by occurring singly in troops, fairy rings or scattered in maintained turf but never fruit in subcaespitose clusters. The mycelium of the species degrades grass and is rarely attached to fragmented wood. The pileus is lightly pigmented ranging from yellowish white to cream. The partial veil is very

distinctive by being loosely organized into fealty-wooly texture which leaves a cortinous annulus. Cheilocystidia are derived from the hymenia trama. The neotype was designated to give stability to the taxon *A. molesta* since no authentic material was ever deposited in a herbarium.

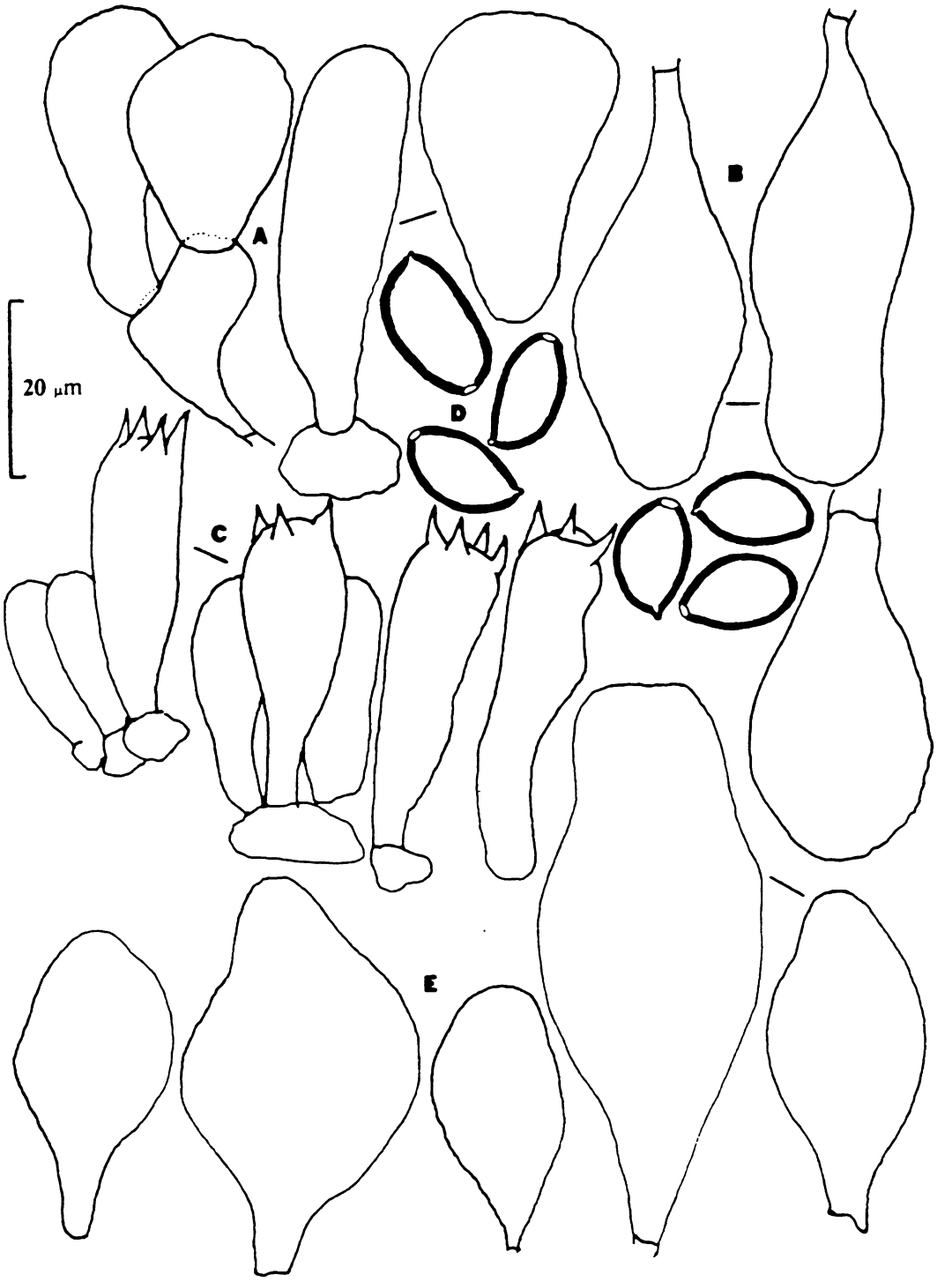


Figure 25. Agrocybe molesta: a = pileipellis, b = cheilocystidia, c = spores, d = basidia, e = pleurocystidia.

Discussion

Victor Fayod and Charles Peck have had historical impact on on the concept of *A. praecox* even without being directly involved with the nomenclature of these species. The prologues of Persoon and Fries have mislead preceding and contemporary mycologists alike. Type studies indicate that Fayod and Peck both considered the earliest fruiting *Agrocybe* to be *A. praecox*. Even today, as with Persoon and Fries, the difference between habitat and substrate is still not specified (Watling, 1979) which compounds the problem further. It is now apparent that the mycelium of *A. praecox* degrades fragmented wood, whereas *A. molesta* degrades graminicolous substrates, both species fruit in either grass dominated or silvan communities. The problem is further compounded by the biological relationships of the four sibling species of *A. praecox* where the levels of intraspecific variation of each sibling species equals the interspecific variation as demonstrated in the scatter plots (Figure 10 on page 45 and Figure 11 on page 46) and by comparing the anatomical characteristics seen in the descriptions. Sporocarp morphology is subject to variables such as temperature, light, humidity, and substrate quality (Taber, 1966) and is shown experimentally in the Bolbitaiceae (Watling, 1975) and *Rhodotus palmatus* (Miller et. al., 1980). It must follow that the morphology

of sporocarps fruiting in nature is influenced by a setting as heterogeneous as the natural environment. Many of the described taxa of *A. praecox* such as *A. praecox* var. *cutifracta* (J.E. Lange) Singer and *A. gibberosa* sensu Fries are fruiting bodies subjected to low humidity and are altered because of it.

Review of taxa

Agrocybe molesta is reproductively isolated from the sibling species of *A. praecox* and is easily distinguished by morphological and ecological criteria (see description). These criteria nevertheless do suggest evolutionary relationship with *A. praecox* because both groups are white-rotters and are morphologically similar in the terminal elements of the pileipellis, hymenium and partial veil. Investigations at the molecular level may provide additional evidence to defined the extent of these relationships between both taxa.

Agrocybe praecox (**sibling species 1**) is the most commonly collected *Agrocybe* in North America and Europe. It fruits abundantly and is a robust taxon in wood chip mulch beds but has a more diminutive stature when it fruits along trails or paths and is always attached to fragmented wood even in grassy areas. Extremely large sporocarps occurring in caespitose clusters are produced when the weather is favorable and the substrate is abundant. The stature of European Collections tend to be slightly less robust than American material. This species is ruderal and opportunistic and is commonly found near man's activities.

The three collections of *A. acericola* (**sibling species 3**) in Virginia are sympatric with populations of *A. praecox*. *Agrocybe acericola* is not found in abundance. One collection, VA3, occurred on the base of a senescent black maple on the Virginia Tech

campus whereas and the other collections, VA4 and VA5, were found in forest situations dominated by maple, sycamore and oak. The morphology of these sporocarps is virtually identical to *Agrocybe praecox*. However, VA5 which was collected and identified one year following the earlier specimens, produced 1,2,3 and 4-spored basidia. This morphology suggests the possibility of obligate inbreeding through secondary homothallism, which in turn promotes allelic fixation for local adaptation. Also, the unusual spores and basidia of VA5 are similar to the secondarily homothallic fungus, *Agrocybe erebia* (Fries) Kühner. This may suggest a relationship between *A. acericola* and *A. erebia*. It could also indicate a genetic potential exists in the *A. praecox* can develop into a species such as *A. erebia*. Strains of *A. acericola* produced pigmented barrage zones when confronted with members of other breeding groups which indicates vigorous heterokaryotic incompatibility.

In Denmark a similar situation exists where two sibling species of *A. praecox* are sympatric. The phenotypic plasticity of *Agrocybe gibberosa* (**sibling species 4**) is included by the variation all sibling species of *A. praecox*. These collections were made along with representatives of *A. praecox* all in one afternoon at Allendemagelskov, Denmark. Once again, strongly pigmented barrage zones are produced when homokaryotic strains are confronted with the other sibling species.

Agrocybe montana (**sibling species 2**) is distinct in its habitat preferences and capacity for the utilization of diverse substrates such as aspen, spruce, cottonwood and fir. This species is also virtually indistinguishable from its sibling species, *A. praecox, A. acericola* and *A. giberosa*. Its known range is north in the Canadian Rockies from Banff Nat. Park south through Montana, Idaho, and Colorado and west to the coastal ranges of California, Oregon and Washington. North central Idaho populations commonly fruit

from the buried slash of logging operations or in disturbed conifer forest. The slash is very similar in physical properties to the wood chip mulch in the eastern U.S. and is a comparable concentrated source of fragmented wood. Populations in the Front Range of Colorado exist in mixed successional forests composed of mature aspen and young *Picea* and *Abies*. One collection, CO2, produced unique pleurocystidia similar to the ventricose-digitate pleurocystidia of *A. arvalis*. Overholts (1927) documented this anatomy in his collections which were made about 40 miles north of my collections also in the Front Range. This suggests that the very distinctive pleurocystidia are not useful taxonomic characteristics because this collection is compatible with populations which have less ornate pleurocystidia. It is likely that the same genetic potential may exist among the sibling species and within populations of *A. montanus* through time. This observation is strengthened by the production of digitate protuberances at the pleurocystidial apex by European collections of *A. praecox*.

Sympatry in N.W. Montana is interesting because the sibling species *A. praecox* and *A. montanus* are found adjacent to one another. *Agrocybe praecox* fruits along paths and the borders of dirt roads at Glacier National Park and at the University of Montana Biological Station (UMBS) (MT1 and MT2) beside Flathead Lake. Representatives of *A. praecox* were collected two years later at the UMBS (MT3) and in a birch stand near Bigfork, Montana (MT4). A third collection, MT5 was made the same day in in a conifer stand about 3 miles from MT4 and 20 miles from MT3. The conifer isolate is *A. montanus* and the path and road side collections are *A. praecox*. The following week, *A. montanus* (MT6) was collected under conifer forest at Mission Falls near Saint Ignatius, Montana. This indicates that the birch and path side collections of *A. praecox*, inhabit areas frequented by humans and *A. montanus* exists in more or less undisturbed plant communities. This situation is not comparable to Idaho populations

of *A. montanus* where fragmented wood is generated by logging operations and in Colorado where CO1 fruited from aspen wood buried along a dirt road side at the Rocky Mountain Biological Research Station at Niwot Ridge.

A single collection from Switzerland (CH1) is distinctive from other European collections based on spore dimensions, habitat, stature and cultural features. This individual was difficult to culture in the first year because the single spore isolates grew very slowly. Inbreeding experiments showed self-incompatibility and all interstock crosses were negative as well. After a year of cultivation, this fungus began to grow normally. Later intrastock crosses with new single spore isolates resulted in the recovery of three mating types. The interstock confrontation test for mating relationships through time showed vigorous compatibility with all members of *A. montanus.* More collections from Switzerland and the mountainous regions of Europe are needed to investigate this problem. This fungus is identified as *A. montanus* at this time.

Evolution of the A. praecox group

The habitats, distributions and mating relationships of *A. praecox* with its sibling species suggests several hypothesis. Firstly, all sibling species fruit in disturbed areas which have either hard-wood or soft-wood substrates. The forest inhabiting species, *A. acericola*, *A. gibberosa* or *A. montanus* may have given rise to *A. praecox* if one assumes that forest and natural disturbances existed before man's influence. The practice of wood chip mulching in eastern north America provided a niche for pre-adapted organisms such as one of the sibling species of *A. praecox.* This conclusion does not suggest which species is the ancestor of *A. praecox* nor is it safe to assume that the less abundant species, *A. acericola* and *A. gibberosa* are derived from *A. praecox* (sibling

species 1). Heterogenic incompatibility has not been demonstrated in the sibling species of *A. praecox* but the barrage zones produced in mating tests suggest a genetic mechanism similar to *Heterobasidion annosum* (Chase, 1985) and *Polyporus ciliatus* (Hoffmann, 1978). Secondly, if a heterogenic incompatibility system does exist within these species, then it can account for the common occurrence of sympatric sibling species but does not provide a selective advantage for instant and immediate reproductive isolation except to establish and maintain stable polymorphisms at the physiological level. Heterogenic incompatibility does not suggest the direction of evolution or which sibling species is the modern representatives of the ancestral species. The current distributions of the sibling species can also be explained by allopatric speciation followed by expansion, redistribution and colonization to give the appearance of sympatric speciation. This may account for the sympatry of *A. praecox* and *A. montanus* but it is more difficult to explain the sympatry of *A. praecox* with *A. acericola* in north America and *A. praecox* with *A. gibberosa* in Europe.

Conclusion

This study has examined controversial species concepts and indicates that fungal species can recognize each other but difficulties arise when only microscopic and morphological characteristics are used to distinguish taxa. Many characteristics, both breeding and phenetic must be examined and evaluated to differentiate and recognize fungal species. Many times, biological characteristics such as mating tests are the best means to distinguish among closely related taxa but these tests are not convenient for routine taxonomy. This does not diminish the fact that morphologically similar but genetically isolated populations are distinct species and implies that it is more important to understand what a taxon is than to simply seek a name with no biological basis.

Nomenclatorial studies show that *A. molesta* has been confused with the *A. praecox* complex and it is impossible to know with complete certainty which taxon Persoon and Fries were referring to when they described *A. praecox*. The chances are high that the taxon described here as *A. praecox* represents the Fresian concept based on the distribution and high frequency of *A. praecox* (sibling species 1) in Europe. Mating compatibility studies show that *A. praecox* sensu lato is composed of at least four reproductively isolated species. The term sibling species is adopted to communicate the morphological similarity of these reproductively isolated species. Neotypes are designated for *A. molesta*, *A. praecox* and *A. gibberosa*. A new taxon, *A. montanus* is described and a holotype is preserved.

Bibliography

Anderson, J.B. and Ullrich, R.C. 1979. Biological species of *Armillaria mellea* in north America. *Mycologia* 71(2):402-414.

Bolton, J. 1788-91. ***An History of Fungusses growing about Halifax*** (*Hist. fung.*) vol. 1. Halifax and Huddersfield, etc.

Bulliard, J.B.F. *P.* 1791-1812 ***Historie des Champignon de la France*** (*Hist. champ. Fr.*) vol. 2. Paris.

Burnett, J.H. 1955. Mating systems of fungi I. ***New Phytol.*** 54:50-90.

_____. 1975. ***Mycogenetics***. John Wiley & Sons. London.

_____. 1983. Speciation in fungi. ***Trans. Br. Mycol. Soc.*** 81(1):1-14.

Bush, G.L. 1975. Modes of animal speciation. ***Ann. Rev. Ecol. Syst.*** 6:339-364.

Chase, T.E. and Ullrich, R.C. 1983. Sexuality, distribution, and dispersal of *Heterobasidion annosum* in pine plantations in Vermont. ***Mycologia*** 75(5):825-831.

_____ and _____. 1985. Genetics of intersterility in *Heterobasidion annosum.* ***Mycological society of America Newsletter*** 36(1):20.

Christen, A.A. and Bruehl, G.W. 1979. Hybridization of *Typhula ishikariensis* and *T. idahoensis.* ***Phytopathology*** 69(3):263-266.

Dobzhansky, 1950. Mendelian populations and their evolution. ***Amer. Nat.*** 84: 401-418.

_____, 1970. ***Genetics of the Evolutionary Process*** Columbia University Press, New York.

Day, P.R. 1963. Mutation of the A mating type factor in *Coprinus lagopus*. ***Genet. Res., Camb.*** 4:55-64.

Duncan, E.G. and Macdonald, J.A. 1967. Micro-evolution in *Auricularia auricula*. ***Mycologia*** 59(5): 803-818.

Eggertson, E. 1953. An estimate of the number of alleles at the loci for heterothallism in a local concentration of *Polyporus obtusus* Berk.. ***Can. J. Bot.*** 31: 750-759.

Elliott, T.J. and Challen, M.P. 1983. Genetic ratios in secondarily homothallic Basidiomycetes. ***Expimental Mycology*** 7: 170-174.

Grant, V. 1981. ***Plant Speciation.*** Columbia University Press, New York.

Farr, E.R., Miller, O.K., Jr. and Farr, D.F. 1977. Biosystematic studies in the genus *Pholiota* stirps *adiposa*. ***Can. J. Bot.*** 55(9): 1167-1180.

Fayod, V. 1889. ***Annales des Sciences Naturelles Botanique*** 7(9):358.

Fries, E.M. 1821 ***Systema mycologicum*** (*Syst. mycol.*) vol. 1. Lund and Griefswald.

_____.1836-1838 ***Epicrisis systematis mycologici.*** (*Epicrisis*). Uppsala and Lund.

Fries, N. 1985. Intersterility groups in *Paxillus involutus*. ***Mycotaxon*** 24: 403-409.

Hallenberg, N. 1984. A taxonomic analysis of the *Sistotrema brinkmannii* complex (Corticiaceae, Basidiomycetes). ***Mycotaxon*** 21: 389-411.

Hendrickson, H.T., Tauber, C.A. and Tauber, M.J. 1978. Sympatric speciation: Evidence? ***Science*** 200:345-346.

Hoffmann, P. and Esser, K. 1978. Genetics of speciation in the Basidiomycete genus *Polyporus*. ***Theor. Appl. Genet.*** 53: 273-282.

Horak, E. 1968. Synopsis generum agaricalum. ***Beit. Kryptogamenflora Schweiz..*** 13:1-741.

Jurand, M.K. and Kemp, R.F.O. 1973. An incompatibility system determined by three factors in a spedies of *Psathyrella* (Basidiomycetes). ***Genet. Res., Camb.*** 22: 125-134.

Kemp, R.F.O. 1970. Inter-specific sterility in *Coprinus bisporus, C. congregatus* and other Basidiomycetes. ***Trans. Br. Mycol. Soc.*** 54(3):488-489.

_____. 1975. Breeding biology of *Coprinus* species in the section *Lanatuli*. ***Trans. Br. Mycol. Soc.*** 65(3): 375-388.

Koltin, Y. and Raper, J.R. 1966. *Schizophyllum commune*: New mutations in the B incompatibility factor. ***Science*** 154: 510-511.

Koltin, Y. and Raper, J.R. 1967. The genetic structure of the incompatibility factors of *Schizophyllum commune*: Three functionally distinct classes of B factors. ***Genetics*** 58: 1220-1226.

Koltin, Y., Stamberg, J. and Lemke, P. A. 1972. Genetic struture and evolution of the incompatibility factors in higher fungi. ***Bacteriological Reviews*** 36(2): 156-171.

Korhonen, K. 1978a. Interfertility and clonal size in the *Armillariella mellea* complex. ***Karstenia*** 18: 31-42.

_____.1978b. Intersterility groups of *Heterobasidion annosum*. ***Seloste Juurikäävän risteytymissuhteet. Commun. Inst. For. Fenn.*** 94(6): 1-25.

_____ 1983. Observations on nuclear migratin and heterokaryotization in *Armillaria*. ***Cryptog., Mycol.*** 4: 79-85.

Korhonen, K. and Hintika, V. 1973. Cytological evidence for somatic diploidization in dikaryotic cells of *Armillariella mellea*. ***Arch. Microbiol.*** 95: 187-192.

_____ and _____ 1980. Simple isolation and inoculation methods for fungal cultures. ***Karstenia*** 20: 19-20.

Kornerup, A. and Wanscher, J.H. 1978. ***Methuen Handbook of Colour*** Ed.3. Eyre Methuen Ltd., 11 New Fetter Lane, London, EC4P 4EE, Great Britain.

Lange, J.E. 1935-40. ***Flora agaricina Danica*** (*Fl. agar. Dan.*) vol. 3. Copenhagen.

Lange, M. 1952. The species concept in the genus *Coprinus*. ***Dansk. Botanisker Arkiv*** 14: 1-164.

Langton, F.A. and Elliott, T.J. 1980. Genetics of secondarily homothallic Basidiomycetes. ***Heredity*** 45(1): 99-106.

Lasch, W.G. 1828. Enumeratio agaricorum marchiae Brandenburgicae nondum in floris nostratibus nomatorum, cum observationibus in cognitos et novorum descriptionibus. ***Linnea*** 3:421-422.

Macrae, R. 1967. Pairing incompatibility and other distinctions among *Hirschioporus (Polyporus) abietinus*, *H. fusco-violaceus*, and *H. laricinus*. ***Can. J. Bot.*** 45: 1371-1398.

Mather, K. 1942. Heterothally as an outbreeding mechanism in fungi. ***Nature*** 149: 54-56.

Matuo, T. and Snyder, W. C. 1973. Use of morphology and Mating populations in the identification of formae speciales in *Fusarium solani*. ***Phytopathology*** 63: 562-565.

Maynard-Smith, J. 1966. Sympatric speciation ***The American Naturalist*** 100(916):637-650.

Mayr, E. 1965. Classification and phylogeny. ***Am. Zoologist*** 5: 165-174.

Mayr, E. 1965. ***Animal species and evolution.*** The Belknap Press of Harvard University Press, Cambridge, Massachusetts.

_____. 1976. ***Evolution and the Diversity of Life Selected Essays.*** The Belknap Press of harvard University Press, Cambridge, Massachusetts.

Meinhardt, F., Epp, B.D. and Esser, K. 1980. Equivalence of the A and B mating type factors in the tetrapolar Basidiomycete *Agrocybe aegerita.* ***Curr. Genet.*** 1: 199-202.

Miller, O.K., Jr., Palmer, J.G. and Gillman, L.S. 1980. The fruiting and development of *Rhodotus palmatus* in culture. ***Mycotaxon*** 11(2):409-419.

Molina R. and Palmer J.G. 1982. Isolation, maintenance and pure culture manipulation of ectomycorrhizal fingi. ***Methods and principles of mycorrhizal research*** Schenck, N.C. [ed.], The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, Minnesota, 55121.

Murrill, W.A. 1913. Agaricaceae of tropical north America ***Mycologia*** 5(1):32.

_____ 1943. Additions to Florida fungi. ***Mycologia*** 35(5):533.

Olive, L.S. 1958. On the evolution of heterothallism in fungi. ***The Am. Nat.*** 92(865): 233-251.

Overholts, L.O. 1927. A monograph of the genuus *Pholiota* in the United States. ***Annals of the Missouri Botanical Garden*** 14(2):87-177.

Papazian, H.P. 1950. Physiology of the incompatibility factors in *Schizophyllum commune.* ***Bot. Gazette*** 112(2): 143-163.

_____. 1951. The incompatibility factors and a related gene in *Schizophyllum commune.* ***Genetics*** 36: 441-459.

Peck, C.H. 1872 ***Annual report to the New York State Cabinet*** 23:90.

_____ 1872 ***Annual report to the New York State Museum*** 24:67.

_____ 1873 ***Bulletin of the Buffalo Society of Natural Science*** 1:50, 53.

_____ 1878 ***Annual report to the New York State Museum*** 31:34.

_____ 1896 ***Annual report to the New York State Museum*** 49:59-60.

_____ 1899 ***Bulletin of the New York State Museum*** 28:209.

Persoon, D.C.H. 1801 ***Synopsis Methodica Fungorum*** (*Syn. Meth. Fung.*). Göttingen.

Raper, J.R. 1966. ***The genetics of sexuality in higher fungi.*** The Ronald Press Coompany, New York. 283 pp.

Raper, J.R., Krongelb, G.S. and Baxter, M.G. 1958. The number and distribution of incompatibility factors in *Schizophyllum.* ***The American Naturalist*** 92(865): 221-232.

Raper, J.R., Baxter, M.G. and Ellingboe, A.H. 1960. The genetic structure of the incompatibility factors of *Schizophyllum commune*: the A-factor. ***Proc. N. A. S.)*** 46:833-842.

Raper, J.R. and Hoffman, R.M. ? *Schizophyllum commune* in ***Handbook of Genetics*** vol. 1. Plenum Press, New York, pp. 597-626.

Sass, J.E. 1929. The cytological basis for homothallism and heterothallism in the Agaricaceae. ***Am. J. bot.*** 16:663-701.

Schulzer, S. 1898. Revision der diagnosen zu den von M. Britzermayr auf gestellten Hymenomyceten-Arten. ***Botanisches Centralblatt*** 75:171.

Singer, R. 1950. *Naucoria* Fries and related species in the USSR. ***Acta Instituti Botanici Nomine V.L. Komarovi Academiae Scientiarum, Unionis Rerum Publicarum Soveticarum Socialsticarum*** series 2(6):445-462.

_____ 1951. Agaricales in modern taxonomy. ***Lilloa*** 22:1-853.

_____ 1977. Keys for the identification of species of Agaricales I. ***Sydowia, Annales Mycologici Ser. II*** 30(1-6):194-206.

Simchen, G. 1967. Independent evolution of a polygenic system in isolated populatons of the fungus *Schizophyllum commune.* ***Evolution*** 21: 310-315.

Simchen, G. and Stamberg, J. 1969. Genetic control of recombination in *Schizophyllum commune*: specific and independent regulation of adjacent and non-adjacent chromosomal regions. ***Heredity*** 24: 369-381.

Snider, P.J. and Raper, J.R. 1958. Nuclear migration in the basidiomycete *Schizophyllum commune.* ***Amer. J. Bot.*** 45: 538-546.

Stamberg, J. 1969b. Genetic control of recombination in *Schizophyllum commune*: The natural occurence and significance of natural variation. ***Heredity*** 24: 361-368.

_____ 1969a. Genetic control of recombination in *Schizophyllum commune*: Separation of the controlled and controlling loci. ***Heredity*** 24: 306-309.

_____ and Koltin, Y. 1973. Genetic control of recombination in *Schizophyllum commune*: Evidence for a new type of regulatory site. ***Genet. Res., Camb.*** 22: 101-111.

Stebbins, G.L. 1950. ***Variation and Evolution in Plants***, Columbia University Press, New York.

Taber, W.A. 1966. Morphogeneis in basidiomycetes. in ***The Fungi, an advanced tretise*** Vol. 2, Ainsworth, G.C., Sparrow, F.K. and Sussman, A.S. [eds.]. Academic Press, New York, pp. 387-412.

Tauber, C.A. and Tauber, M.J. 1977a. A genetic model for sympatric speciation through habitat diversification an seasonal isolation. *Nature* 268:702-705.

_____ 1977b. Sympatric speciation based on allelic changes at three loci: Evidence from natural populations in two habitats. *Science* 197:1298-1299.

Tauber, C.A., Tauber, M.J. and Nechols, J.R. 1977. Two genes control seasonal isolation in sibling species. *Science* 197:592-593.

Thoday, J.M. 1958. Effects of disruptive selection: The experimental production of a polymorphic population. *Nature* 181:1124-1125.

_____ and Gibson, J.B. 1962. Isolation by disruptive selection. *Nature* 193:1164-1165.

Ullrich, R.C. 1973. Sexuality, incompatibility, and intersterility in the biology of the *Sistotrema brinkmanii* aggregate. *Mycologia* 65: 1234-1249.

_____ and Raper, J.C. 1974. Number and distribution of bipolar incompatibility factors in *Sistotrema brinkmanii*. ***The American Naturalist*** 108(962): 507-518.

Vilgalys, R. and Miller, O.K., Jr. 1983. Biological species in the *Collybia dryophila* group in north America. *Mycologia* 75(4): 707-722.

Wallace, B. 1981. ***Basis Population Genetics***, Columbia University Press, New York.

Watling, R. 1975. Studies in fruit-body development in the Bolbitiaceae and the implications of such work. ***Beiheft 51 zur Nova Hedwigia*** Royal octavo. VI. J. Cramer, A.R. Ganter Verlag K.G., FL-9490 Vaduz, Germany. pp.319-346, 8 plates.

Watling, R. and Gregory, N.M. 1981. Census catalogue of world members of the Bolbitiaceae. ***Bibliotheca mycologica*** band 82:1-224. Strauss & Cramer GmbH, 6945 Hirschberg 2, Germany.

Watling, R. 1982. ***British fungus flora Agarics and Boleti 3 Bolbitiaceae: Agrocybe, Bolbitius & Conocybe.*** Henderson, D.M., Orton, P.D. and Watling, R. [eds.]. Her Majesty's Stationary Office, Edinburgh, Great Britain.

Watling, R., Kile, G.A. and Gregory, N.M. 1982. The genus *Armillaria*- nomenclature, typification, the identity of *Armillaria mellea* and species differentiation. ***Trans. Br. Mycol. Soc.*** 78(2): 271-285.

Whitehouse, H.L.K. 1949. Multiple-allelomorph heterothallism in the fungi. ***New Phytol.*** 48: 212-244.

Wells, K. and Wong, G. 1985. Interfertility and comparative morphological studies of *Exidiopsis plumbescens* from the west coast. *Mycologia* 77(2): 285-299.

Wong, G.J., Wells, K. and Bandoni, R. J. 1985. Interfertility and comparative morphological studies of *Tremella mesenterica*. *Mycologia* 77(1): 36-49.

Appendix A. Selfing experiments

Results of selfing experiments used to determine the mating system are given below. Compatible single spore isolates are summarized (see materials and methods). Sibling species are abbreviated with a number followed with the region. Responses are given with a + meaning clamp connections, – a negative response, and f indicates false clamp connections. Representative testers of each recovered mating type are deposited in the Virginia Tech culture collection.

SAR84/17
Agrocybe praecox 1w
3,6/1,4,7,9,10 : 2/5,8

```
10
 9 —
 8  f f
 7 — — —
 6 +  + — +
 5  f f  — — —
 4 — — — —  + —
 3 +  + —  + — —  +
 2 — —  + — f  + — —
 1 — — f  —  + — —  + —
   10 9  8  7  6  5  4  3  2  1
```

CG84/27
Agrocybe praecox 1e
1,9/2,3,7,8 : 10/....

```
10
 9 —
 8 —  +
 7 —  + —
 5 — —  + —
 3 —  + — — —
 2 —  + — — — —
 1 — —  +  + —  +  +
   10 9  8  7  5  3  2  1
```

CG84/28
Agrocybe molesta
2,7/1,3,6 : 10,4/5,8,9

```
10
 9 +
 8 + —
 7 — — —
 6 — — —  +
 5 + — — — —
 4 —  +  + — —  +
 3 — — —  + — — —
 2 — — — —  + — —  +
 1 — — —  + — — — —  +
   10 9  8  7  6  5  4  3  2  1
```

RV84/66
Agrocybe praecox 1eur
9/3,4 : 5,7,10/....

```
10
 9 +
 7 — —
 5 — — —
 4 —  + — —
 3 —  + — — —
 2 — — — — — —
   10 9  7  5  4  3  2
```

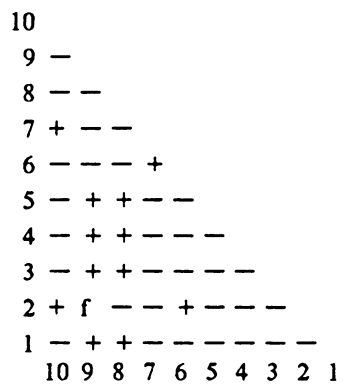
TMF80
Agrocybe praecox 1w
1,10/3,6,8 : 2,7/4,5

```
10
 9 —
 8 +  —
 7 — — —
 6 +  — — —
 5 — — —  + —
 4 — — —  + — —
 3 +  — — — — — —
 2 — — — — —  +  + —
 1 — —  + —  +  — —  + —
   10 9  8  7  6  5  4  3  2  1
```

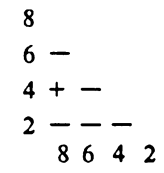
TMF114
Agrocybe praecox 1w
5/1,2,4 : 3/....

```
 5
 4 +
 3 — f
 2 +  — —
 1 +  — — —
    5 4  3  2  1
```

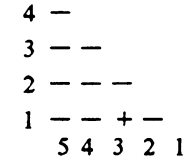
RV84/121
Agrocybe praecox 1eur
8,9/1,2,4,5 : 7,2/6,10



RV84/161
Agrocybe praecox 1eur
4/8 : 2,6/....



RV84/163
Agrocybe praecox 1eur
1/3 : 2,4,5/....



RV84/164
Agrocybe praecox 1eur
2/8,6,5 : 7/3,4

RV84/165
Agrocybe praecox 4eur
2/7,10 : 1,3,4,5,6,8,9/....

RV84/166
Agrocybe praecox 4eur
7/6,4 : 5/1,2,3

TMF473
Agrocybe smithii
2,3,6/9,10 : 1,5/4,8

```
10
 9 —
 8 — —
 7 — f  f
 6 +  + — —
 5 — —  + — —
 4 — f  — — —  +
 3 +  + — — — — —
 2 +  + — — — — — —
 1 — —  + — — —  + — —
   10 9  8  7  6  5  4  3  2  1
```

TMF476
Agrocybe smithii
1,9/2,3,8,10 : 4,5/6,7

```
 9
 8 +
 7 — —
 6 —  + —
 5 — —  +  +
 4 — —  +  + —
 3 +  — — —  + —
 2 +  — — — — — —
 1 —  +  — — — —  +  +
    9  8  7  6  5  4  3  2  1
```

TMF479
Agrocybe smithii
2/3 : 1,4,11/5,7,9,12

```
12
11 +
 9 —  +
 8 — — —
 7 —  + —  +
 5 —  + —  + —
 4 +  —  +  — +  +
 3 — — — — — — —
 2 — — — — — — —  +
 1 +  —  +  —  +  +  — — —
   12 11 9  8  7  5  4  3  2  1
```

TMF484
Agrocybe praecox
4/5 : 1,2,3,6,7,8,10/....

```
10
 9 —
 8 — —
 7 — — —
 6 — — — —
 5 — — — — —
 4 — — — — —  +
 3 — — — — — — —
 2 — — — — — — — —
 1 — — — — — — — — —
```

TMF488
Agrocybe praecox 2w
1,7/6 : 5/2,3,4,10

```
10
 9 —
 8 — —
 7 — — —
 6 — — —  +
 5 +  — — —  +
 4 — — — — —  +
 3 — — — — —  + —
 2 — — — — —  + — —
 1 — — — —  +  — — — —
   10 9  8  7  6  5  4  3  2  1
```

TMF504
Agrocybe pediades
2/3,7 : 1/10,8,6,4

```
10
 9 —
 8 — —
 7 —  +  +
 6 — — — —
 5 +  — —  + —
 4 — — — — — —
 3 — —  +  —  +  — —
 2 — — —  +  — — —  +
 1 +  —  +  —  +  —  +  — —
   10 9  8  7  6  5  4  3  2  1
```

TMF527
Agrocybe smithii
8/1,2,5,10 : 7/3,4,9

```
10
 9 —
 8 + —
 7 — + —
 6 — — — —
 5 — — + — —
 4 — — — + — —
 3 — — — + — — —
 2 — — + — — — — —
 1 — — + — — — — — —
   10 9 8 7 6 5 4 3 2 1
```

TMF530
Agrocybe praecox 1e
2,9/3,4,6 : 1,5,7,8,9,10/....

```
10
 9 —
 8  f —
 7 — — —
 6 — + — —
 5 — — — — —
 4 — + — — — —
 3 — + — f — — —
 2 — — — — + — + +
 1 — — — — — — — — —
   10 9 8 7 6 5 4 3 2 1
```

TMF532
Agrocybe praecox 1e
: ..../....

```
 9
 8 —
 7 — —
 6 — — —
 5 — — — —
 4 — — — — —
 3 — — — — — —
 2 — — — — — — —
 1 — — — — — — — —
   9 8 7 6 5 4 3 2 1
```

TMF536
Agrocybe molesta
9,2/1,3,4 : 5,6,7/....

```
 9
 8  f
 7 — —
 6 — — —
 5 — — — —
 4 + f — — —
 3 + — — — — —
 2 — f — — — + +
 1 + — — — — — — +
   9 8 7 6 5 4 3 2 1
```

TMF546
Agrocybe 1NY
3/5 : 1,2,4,6,7,8,9,10/....

```
10
 9 —
 8 — —
 7 — — —
 6 — f — —
 5 — — — — —
 4 — — f  f  — f
 3 — — — — — + —
 2 — — — — — — — f
 1 — — f  + — — — — —
   10 9 8 7 6 5 4 3 2 1
```

TMF554
Agrocybe praecox 1eur
5/1,2 : 3,4/10,6

```
10
 6 — —
 5 — — f
 4 + — + —
 3 + — + — —
 2 — — — + — —
 1 — — — + — — —
   10 7 6 5 4 3 2 1
```

TMF559
Agrocybe praecox
2/9,10 : 1,7/4,6

```
10
 9 —
 7 — —
 6 — — +
 4 — — + —
 2 +  + — — —
 1  f — — + + —
   10 9 7 6 4 2 1
```

TMF576
Agrocybe praecox 3e
10/2,3,9 : 4,6/....

```
10
 9 +
 8 — +
 6 — — —
 4 — — — —
 3 + — — f  —
 2 + — — — — —
   10 9 8 6 4 3 2
```

TMF596
Agrocybe praecox 3e
1,4/3,5,6 : ..../....

```
 6
 5 —
 4 +  +
 3 — — +
 1 +  + — +
    6 5 4 3 1
```

TMF604
Agrocybe pusilla
1,5,6,9/8 : 2,4,7,10/....

```
10
 9 —
 8  f +
 7 — — —
 6 — f  + —
 5 — — + — —
 4 — — — — — —
 2 — — — — — — —
 1 — — + — — — — —
```

TMF640
Agrocybe molesta
2,4,6,9/3,5,7,10 : 8/....

```
10
 9 +
 8 — —
 7 — + f
 6 + — — +
 5 — + — — +
 4 + — — + — —
 3 — + — — + — +
 2 — — — + — — — —
 1 — — — — — — — — +
   10 9 8 7 6 5 4 3 2 1
```

TMF674
Agrocybe praecox 2w
6,1/3 : 9,4/5,10

```
10
 9 +
 8  f —
 6 — — —
 5 — — — —
 4 — — — — +
 3 — — — + — —
 2 — — + — — — —
 1 — f  — — f  — + —
   10 9 8 6 5 4 3 2 1
```

TMF683
Agrocybe molesta
1/3,6,10 : 2,4/5,7,8,9

```
10
 9 —
 8 — —
 7 — — —
 6 — — f  —
 5 — — — — —
 4 — + + + — +
 3 — — — — — — —
 2 — + + + — + — —
 1 + — — — + — — + —
   10 9 8 7 6 5 4 3 2 1
```

TMF697
Agrocybe praecox 2w
2/5 : 7/1,3,4

```
 7
 6 —
 5 — —
 4 + — —
 3 + — — —
 2 — — + — —
 1 + — — — — —
   7 6 5 4 3 2 1
```

SLM737
Agrocybe praecox
3/4,5,9 : 1,6,7,8,10/....

```
10
 9 —
 8 — —
 7 — — —
 6 — — — —
 5 — — — — —
 4 — — — — — —
 3  f + — — — + +
 1 — — — — — + — —
   10 9 8 7 6 5 4 3 1
```

SLM739
Agrocybe praecox
1,5,6/4,8 : 7/10

```
10
 9 —
 8 — +
 7 + — f
 6 — — + —
 5 — — + — —
 4  f f — — + +
 1  f — + — — — +
   10 9 8 7 6 5 4 1
```

GB748
Agrocybe smithii
4/4,6,10 : 8/2,6,7,9

```
10
 9 —
 8 — +
 7 — — +
 6 — — + —
 5 — — — — —
 4 — — — — — —
 3 + — — — + — +
 2 — — + — — — + —
 1 — — — — — — f —
   10 9 8 7 6 5 4 3 2 1
```

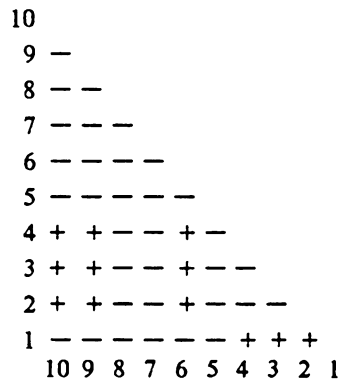
GB749
Agrocybe praecox 1e
1,2/7,8 : 10/3,4,5,6

```
10
 8 —
 7 — —
 6 + — —
 5 + — — —
 4 + — — — —
 3 + — — — — —
 2 — + + — — — +
 1 — + + — + — — —
   10 8 7 6 5 4 3 2 1
```

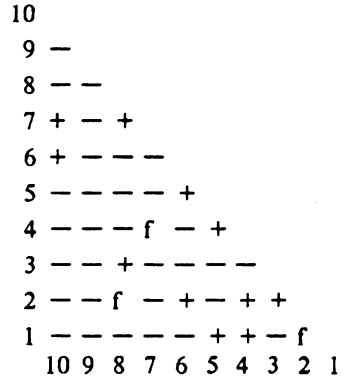
GB750
Agrocybe praecox 1e
2,6/4,7,8 : 3/1,5,9,10

```
10
 9 —
 8  f —
 7 — f —
 6 — — f  +
 5 — — — — —
 4  f f — — f —
 3 +  + — — f  + —
 2 — — + + — — + f
 1 — f — — f  + — + —
   10 9 8 7 6 5 4 3 2 1
```

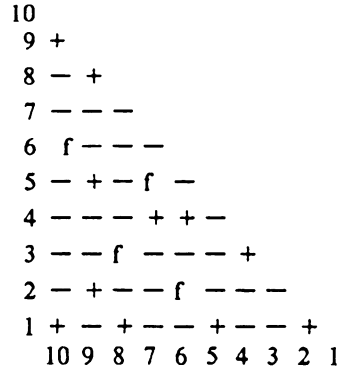
GB751
Agrocybe praecox 1e
2,3,4/1,6,9,10 : 5,7,8/....



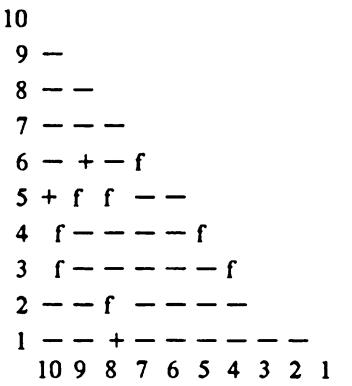
GB754
Agrocybe praecox 1e
5/1,4 : 3,6,7/2,8,10



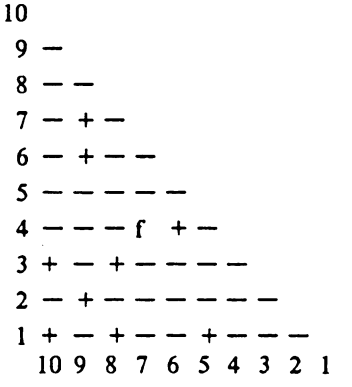
TMF755
Agrocybe putaminum
1,9/2,5,8,10 : 4/3,6,7



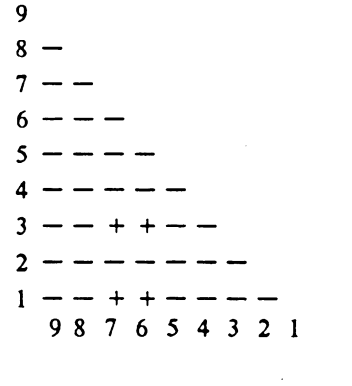
GB755
Agrocybe praecox 1e
?/? : ?/?



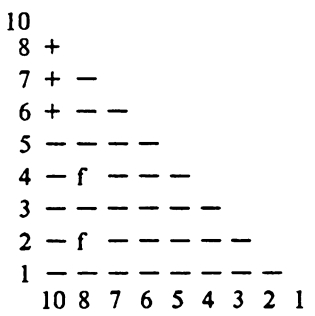
TMF756
Agrocybe putaminum
1,3/10,8,5 : 6,7,2/4,9



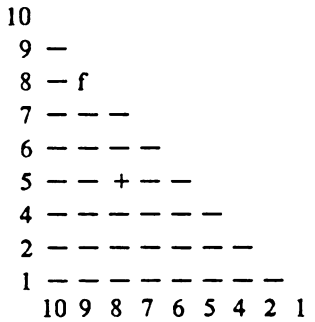
TMF758
Agrocybe firma
1,3/6,7 : 2,4,5,8,9/....



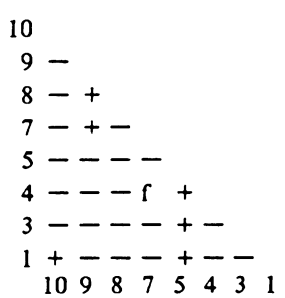
CHAM1795
Agrocybe praecox 1e
10/6,7,8 : 1,2,3,4,5/....



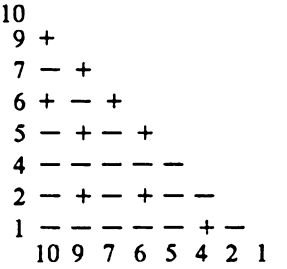
OKM20113
Agrocybe praecox 2w
5/8 : 1,2,4,5,6,7,9,10/....



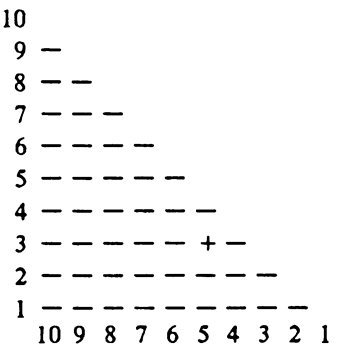
OKM20119
Agrocybe praecox 2w
5,10/1,3,4 : 7,8/9



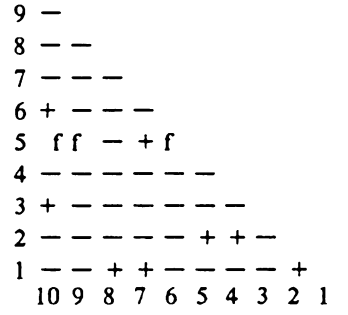
OKM20120
Agrocybe praecox 2w
2,5,7,10/6,9 : 1/4



OKM20123
Agrocybe praecox 2w
3/5 : 1,2,4,6,7,8,10/....



OKM20133
Agrocybe praecox 1w
1,4,5/2,7,8 : 10/3,6



OKM20987
Agrocybe praecox 2w
2/1,6 : 3,7/4,8,9

```
 9
 8 —
 7 +  +
 6  f — —
 5  f + — —
 4 — — + f  —
 3 +  + — — —  +
 2 — — f  + — — —
 1 — — — — — — — +
    9 8 7 6 5 4 3 2 1
```

OKM21272
Agrocybe praecox 2eur
3/8 : 1,2,4,5,6,7,9,10/....

```
10
 9 —
 8 — —
 7 — — —
 6 — — — —
 5 — — — — —
 4 — — — — — —
 3 — — + — — — —
 2 — — — — — — — —
 1 — — — — — — — — —
   10 9 8 7 6 5 4 3 2 1
```

OKM21914
Agrocybe molesta
1,6/9,10 : 2,3,4,5,7,8/....

```
10
 9 —
 8 — —
 7 — — —
 6 +  + — +
 5 — — — — f
 4 — — — — — —
 3 — — — — — — —
 2 — — — — — — — —
 1 +  + — — —  + — — —
   10 9 8 7 6 5 4 3 2 1
```

The vita has been removed from the scanned document