GENERIC CONCEPTS IN THE CREPIDOTACEAE AS INFERRED FROM NUCLEAR LARGE SUBUNIT RIBOSOMAL DNA SEQUENCES, MORPHOLOGY, AND BASIDIOSPORE DORMANCY PATTERNS

by

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(ABSTRACT)

The Crepidotaceae (Imai) Singer (Basidiomycetes: Agaricales) represents a proposed family of saprophytic fungi containing five agaricoid (*Crepidotus, Tubaria, Melanomphalia, Simocybe, Pleurotellus*) and four cyphelloid (*Episphaeria, Phaeosolenia, Pellidiscus, Chromocyphella*) genera. Several contemporary classification systems exist that delegate some or all of these genera to other agaric families. Phylogenetic relationships for the most prevalent genera in the Crepidotaceae were investigated using nuclear large subunit ribosomal DNA (LSU rDNA) sequences. Parsimony analysis of the molecular data supports the Singer classification of *Crepidotus, Melanomphalia,* and *Simocybe* as a single monophyletic unit within the Agaricales. The affinities of the genus *Tubaria* remain uncertain.

Crepidotus (Fr.) Staude is the largest and most phenotypically variable genus in the Crepidotaceae. Sequencing of the LSU rDNA region for a cross-section of morphologically diverse species suggests that *Crepidotus* is not monophyletic. Analysis of morphological characters for 23 *Crepidotus* taxa shows that characters traditionally

applied for infrageneric classification of *Crepidotus* are homoplasic, but that less commonly emphasized characters, such as spore shape and ultrastructure of spore wall ornamentation, may be indicative of monophyletic clades for this complex.

A unique pattern of basidiospore dormancy and germination, unknown in any other species of agaric, is reported for 11 species of *Crepidotus*. Similar patterns were also encountered in species of *Simocybe* and *Melanomphalia*. In these species an endogenous period of spore dormancy of four to six months is followed by an activation period where the factors necessary for subsequent germination appear to involve a minimal nutritional component, water, and exposure to light.

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INTRODUCTION

Phylogenetic relationships within the Crepidotaceae (Imai) Singer (Basidiomycetes: Agaricales) have never been satisfactorily demonstrated, nor unanimously agreed upon. What is certain is that the limits of systematic resolution by morphology alone have been reached with these fungi. Even within the large type genus, *Crepidotus* (Fr.) Staude, no single sub-classification system has been suggested that can adequately address, in terms of probable evolutionary relationships, the great phenotypical variance expressed within this genus.

Advances in molecular systematics offer an alternative to morphology-based phylogenetics. A phylogenetic hypothesis based on DNA sequences of the nuclear large ribosomal subunit has been derived for these fungi with the following objectives. (1) The existence of a monophyletic clade of genera roughly corresponding to the traditional family classification of the Crepidotaceae *sensu* Singer is established. Genera forming the core of this family are *Crepidotus* (Fr.) Staude, *Simocybe* Karsten, and *Melanomphalia* Christiansen. (2) Infrageneric relationships for *Crepidotus* are examined through parsimony analysis of both molecular and morphological characters. Monophyletic groups indicated by these analyses are compared to the pre-existing sections proposed by Singer and Hesler and Smith. (3) Subsequent examination of morphological characters, within the context of these new data, allows inference about the limits and validity of the use of certain traditional morphological characters in describing evolution in these fungi.

Hypotheses about the relationship of genera and species that were not sampled in this study can be made, based on morphological characteristics that are now believed to be useful in delimiting the members of the Crepidotaceae.

In the course of investigating these fungi, an unusual form of basidiospore dormancy in *Crepidotus* and related genera, that appears to be unique in the Agaricales, was recorded. Harvesting of single spore isolates (SSIs) was attempted for 41 collections of *Crepidotus*, *Simocybe*, and *Melanomphalia*, and was successful for 17 of these. In all instances where SSIs were recoverable, basidiospores first underwent a period of endogenous dormancy, lasting a minimum of two months, prior to germination. The results of a preliminary investigation into spore dormancy and germination in these fungi is presented.

The taxonomy and identification of the Crepidotaceae, and of *Crepidotus* species in particular, is notoriously difficult and few modern investigations therefore concern these fungi. Hence, the overall objective of this study is to provide a foundation, based on natural phylogenetic relationships, (i) as a framework for future systematic studies, and (ii) to stimulate further research into the biology of the Crepidotaceae.

BACKGROUND AND LITERATURE REVIEW

The Crepidotaceae (Imai) Singer (1951)

In 1938, Sanshi Imai elevated a little studied genus, *Crepidotus* (Fr.) Quélet, to tribal status within the Agaricaceae. The tribe, Crepidoteae, contained eight species of *Crepidotus* (most of which have since been placed in other, unrelated genera), with *Agaricus mollis* Schaeff. ex Fr. designated as the type. Imai described the Crepidoteae simply as those species of agarics with eccentric, lateral, or absent stipes, subdecurrent lamellae, and ochreous or ferruginous spores (Imai 1938).

In the ensuing years Rolf Singer, in his works on the Agaricales, gradually came to include a total of nine genera within his family Crepidotaceae (Singer 1951, Singer 1962, Singer 1967, Singer 1973a). The last edition of *The Agaricales in Modern Taxonomy* (Singer 1986) lists the agaricoid genera *Tubaria* (W.G. Smith) Gillet, *Melanomphalia* Christiansen, *Simocybe* Karsten, *Crepidotus* Kummer, and *Pleurotellus* Fayod, as well as four cyphelloid genera, as the closely related members of this family. *Crepidotus* (Schaeff. ex Fr.) Kummer is designated as the type genus.

Singer's (1986) definition of the Crepidotaceae includes genera with pip-shaped, ellipsoid, or globose basidiospores usually without a germ pore, and with pale yellow to dark brown spore prints. The inamyloid spores may be either smooth walled or ornamented. Hyphae may or may not form clamp connections; cheilocystidia are nearly always present but pleurocystidia are rare. Species may be pleurotoid, collybioid, omphalioid, or cyphelloid in habit, and are decomposers, found on various types of

organic debris and wood. Development is not known for all species, but believed to be gymnocarpic or hemiangiocarpic (Singer 1986). Species have little or no known economic importance. As a family, Singer believes these fungi to be related to the agaric families Cortinariaceae Roze, Entolomataceae Kotl. and Pouzar, and Paxillaceae R. Maire apud Maire, Dumée and Lutz.

Alternative classification schemes place some or all of these genera within other families, mainly the Cortinariaceae and the Strophariaceae Singer and Smith. For example, *Tubaria* is often allied within the Strophariaceae (Moser 1978, Breitenbach and Kränzlin 1995). Watling and Gregory (1989) prefer a close relationship between *Tubaria* and the Cortinariaceae, while deferring to Singer's concepts. Hawksworth et al. (1995) place *Simocybe* within the Cortinariaceae. *Melanomphalia*, *Simocybe*, and *Tubaria* are transferred to the Cortinariaceae by Jülich (1981). All genera of the Crepidotaceae *sensu* Singer are placed within the Cortinariaceae in the *Flora Agaricina Neerlandica* (Bas 1988). Nor does Kühner (1980) accept the Crepidotaceae, instead placing *Crepidotus*, *Tubaria*, and *Simocybe* (he does not treat *Melanomphalia* or *Pleurotellus*) within the Strophariaceae, and this position is supported by Nordstein (1990).

Tubaria *(W.G. Smith) Gillet (1876).* There exist about 15 widely recognized species of *Tubaria* (Hawksworth et al. 1995, Singer 1986). The type species for the genus is *T. furfuraceae* (Pers. ex Fr.) Gillet. The main character that distinguishes members of

this genus from the other Crepidotaceae taxa is the monostratous[1] wall (Largent et al. 1977, Singer 1986) of the lightly pigmented and unornamented ellipsoid spores. The fruiting bodies contain clamp connections. Known *Tubaria* species are centrally stipitate, fruiting on substrates such as wood mulch and dead leaves in temperate regions. All species probably have hemiangiocarpous development (Singer 1986), a few showing remnants of a partial veil around the stipe at maturity.

The only monographs for *Tubaria* describe a total of nine species (Romagnesi 1940, Romagnesi 1943). Species of *Tubaria* are known from North America, Europe and Asia.

Melanomphalia *M.P. Christiansen (1936).* In 1936, *Melanomphalia* was erected as a monotypic genus; *M. nigrescens* was described as having an umbilicate pileus, lacking a veil, with adnate-decurrent lamellae, without cystidia, and possessing broadly ovoid spores with dark pigmented walls (Christiansen 1936). Christiansen believed his new genus to be closely related to *Gomphidius* Fries (Gomphidiaceae Maire ex Jülich). Singer (1955a) re-examined the type, concluding that *Melanomphalia* shared more affinities with the Cortinariaceae, especially the genus *Descolea* Singer. However, while compiling the first monograph for *Melanomphalia,* Singer reassigned the genus to the Crepidotaceae, believing it to be intermediate between *Tubaria* and *Crepidotus* (Singer 1967). The most current monograph describes 17 species (Singer 1971). There now exist 20 to 24 known species of *Melanomphalia* (Singer 1986, Hawksworth et al. 1995), inhabiting Europe and North America.

[1] I.e. thin spore walls lacking an endosporial layer.

Many species of *Melanomphalia* possesses pleurocystidia, which are lacking in the rest of the family (with the exception of a few *Crepidoti*). The basidiospores are large, robust, darkly pigmented, and normally possess punctate ornamentation. Clamp connections are present. Fruiting bodies are centrally stipitate and are found on plant debris, dead woody matter, or among mosses. A partial veil, indicative of hemiangiocarpic development, is sometimes present (Singer 1986).

Simocybe *P. Karsten (1879).* Nomenclatural problems abound within the complex consisting of *Naucoria sensu lato*, *Ramicola* Velen., *Alnicola* Kühner, and *Simocybe* (see Singer 1973a, Horak and Miller 1997). The genus *Simocybe*, as typified by *S. centunculus* (Fr.) Karst. was approved as a *nomen conservandum* (Greuter 1994 cited in Horak and Miller 1997). Those European treatments using the now voided generic name of *Ramicola* (e.g. Hansen and Knudson 1992, Watling and Gregory 1989) in general concern those fungi that are related to *S. centunculus*. Although the *Index of Fungi* shows 63 validly published species under the generic name of *Simocybe*, only between 25 (Hawksworth et al. 1995) and 38 (Singer 1986) of these species truly belong in the genus *Simocybe* as it is now understood.

Simocybe is one of the centrally stipitate agaricoid members of the Crepidotaceae, usually found on wood or other organic debris, with clamp connections in the fruiting body, and a partial veil reportedly evident in a few species (Singer 1986). *Simocybe* can be distinguished from the other stipitate Crepidotaceae mainly by its microscopic characters. The pigmented basidiospores are thick walled, smooth, and although they lack a true germ pore, there is often an apical thinning of the wall at the distal end. Basidiospores are

wedge-shaped, ovoid, or ellipsoid, and often reniform in profile. Cystidia are very distinctive in this genus. Pileo-, caulo-, and cheilocystidia are long, hyaline, subcapitate, and abundant. Often the cystidia can be viewed with a hand-lens, imparting a whitish bloom to the cap and stipe, and forming a fine white edge to the otherwise dark lamellae. Fruiting bodies, while normally some shade of brown, often possess characteristic olive tones.

Regional monographs for *Simocybe* have been published by Singer (1973a) for 18 Neotropical species, and Horak (1979a, 1979b, 1980) for a total of 12 species from New Guinea and New Zealand. The species of *Simocybe* are globally widespread.

Crepidotus *(Fr.) Staude (1857).* *Crepidotus* is the largest genus in the family, with at least 150 species known worldwide (Hawksworth et al. 1995). In 1821, Fries gave tribal status to eight species of *Agaricus* that did not possess a stipe. Thirty-six years later, Staude elevated Tribus *Crepidotus* to generic rank[2], with *C. mollis* as the monotypic representative. *Crepidotus* now represents those astipitate agarics that possess pigmented spores lacking a germ pore, and usually lacking pleurocystidia. Many brown-spored astipitate genera have been historically confused with *Crepidotus*, but the presence of a germ pore and/or pleurocystidia in these genera (for example, *Melanotus* Pat.) readily distinguish them as allies of other families. The remaining defining characters of *Crepidotus* include a heterogeneous group of fungi.

[2] There is some doubt as to whether Staude intended to elevate many Friesian tribes to genera, or whether he simply misunderstood the taxonomic difference between the two ranks (see Singer 1955b). This has caused historical debate between accepting Staude as generic authority (as, for example, Donk 1962), or designating as authority those authors who made their intentions clear in later-dated works. Hence, Singer's insistence on *Crepidotus* Kummer (1871), and not *Crepidotus* (Fr.) Staude (1857).

Basidiospore shape ranges from subfusiform to globose, and spore walls may be smooth or may exhibit punctate or rugose ornamentation. Clamp connections may or may not be formed by the mature fruiting body. Hyphae of the pileipellis may contain a gelatinous layer or not, may have encrusting pigments or not, or may have intercellular pigments, or may remain completely hyaline. Substrate preferences vary from decorticated and well decomposed logs or the bark of freshly shed twigs and sticks, to herbaceous litter and stems. *Crepidotus* is a cosmopolitan genus.

The first monograph for the genus *Crepidotus* (Singer 1947) was rapidly succeeded by the works of Pilát (1948, 1950) treating a total of 69 European species, Hesler and Smith (1965) for 125 North American species and varieties, many of them new, and Singer (1973b) treating 62 Neotropical species. No subsequent comprehensive treatment of this group has been compiled.

Pleurotellus *Fayod (1889).* *Pleurotellus* is a small genus, with only two widely accepted species (Singer 1986, Hawksworth *et al*. 1995). The type is *Pleurotus hypnophilus* (Berk.) Sacc., and the species are found in temperate zones throughout the world. As now defined, *Pleurotellus* contains those agarics that macroscopically resemble *Crepidotus*, but differ microscopically in that clamp connections are never present in the fruiting body, and in the nearly pip-shaped spores that are hyaline but may possess slightly yellow or pinkish tints in mass. There are no monographs and very little published data on species of *Pleurotellus*.

Cyphelloid Genera. There remain four genera, containing a total of nine species, whose members are very reduced in form, lacking both a stipe and the gills normally

associated with the Agaricales. They are: *Episphaeria* Donk apud Singer ex Donk (type species *Cyphella fraxinicola* Berk. and Br., monotypic); *Phaeosolenia* Spegazzini (type species *P. platensis* Speg., five species); *Pellidiscus* Donk (type species *Cyphella pallida* Berk. and Br., monotypic); and *Chromocyphella* de Toni and Levi (type species *Cymbella crouanii* Pat. and Doassan apud Pat., two species) (Singer 1986). All species are found mainly in temperate regions on wood, mosses, and herbaceous matter. These four genera were originally classified in the Cyphellaceae Lotsy, and were later transferred to the Crepidotaceae (Singer 1962).

The species possess clamp connections in the fruiting body, are inamyloid, lack cheilo- and pleurocystidia, and have pigmented spores with no germ pore. The characters that delimit the different genera in this group are spore shape and ornamentation. *Episphaeria* has smooth-walled basidiospores that are ellipsoid to ovoid in shape. In *Phaeosolenia* the spores are also smooth, but lemon-shaped. The spores of *Pellidiscus* are elongate but punctate, while the punctate spores of *Chromocyphella* are subglobose in shape.

Nuclear Large Subunit Ribosomal DNA

Taxonomy in mycology has always been implicitly based on the reflection of phylogenetic relationships (Lutzoni and Vilgalys 1994, Hibbett and Donoghue 1998). Difficulties in mycological systematics arise due to the limited number of truly synapomorphic characters at the level of species, genus and even family, and because, in most cases, the plesiomorphous versus apomorphous character states are not known

(Kuyper 1994). Differences in character interpretation have ultimately led to very different taxonomic classifications in the Agaricales (for example, Kühner 1980, Jülich 1981, Singer 1986, Bas 1988). Some characters, such as the absence of clamp connections or an astipitate habit, are often considered synapomorphic but are just as likely to have arisen independently in different fungal lineages as a result of convergent evolution. The Crepidotaceae, and *Crepidotus* in particular, were chosen for this study because diagnosis and classification of these taxa relies upon such potentially homoplasious characters.

The development of the technology that led to discovery of the polymerase chain reaction (PCR) has completely revolutionized modern systematics (Erlich et al. 1991, Erlich and Arnheim 1992). Creating phylogenetic hypotheses for organisms not previously comparable is now possible by analyzing sequences of PCR-amplified portions of their DNA (Hillis and Dixon 1991). Application of these techniques has allowed for the confirmation or rejection of phylogenetic hypotheses drawn by other methods such as morphology, and for the creation of new phylogenetic hypotheses for a wide variety of organisms (Hillis et al. 1996). Sequence analysis has become especially important as a means of defining phylogenetic trees in taxa, such as fungi, for which the fossil record is grossly incomplete or in cases where morphology alone can not provide all the data needed to make a sound phylogeny (for example, Cantrell and Hanlin 1997).

Phylogenetic analysis by molecular methods has become rapidly assimilated by mycologists (Hibbett 1992, Foster et al. 1993, Hibbett and Donoghue 1998). Analysis of DNA coding for the nuclear large ribosomal subunit (LSU rDNA) has been a useful tool for examining phylogenies of related genera for many groups of organisms (De Rijk et al.

1995, Hillis and Dixon 1991), and LSU rDNA sequences are increasingly being utilized for constructing fungal phylogenetic hypotheses at the generic and subgeneric levels (for example, Moncalvo et al. 1995, Vilgalys et al. 1996, Moncalvo et al. 1999). Finally, LSU rDNA sequences have been instrumental in confirming sometimes controversial phylogenetic hypotheses, such as elucidating the relationship between lichenized and non-lichenized basidiomycetes (Lutzoni 1997); between anamorphic and teleomorphic ascomycetes (Kuldau et al. 1997); between gastroid and non-gastroid ascomycetes (O'Donnell et al. 1997); and between the dark and light spored agarics *Lepiota sensu lato*, *Agaricus*, and *Coprinus comatus* (Johnson and Vilgalys 1998).

Fungal Spore Dormancy

Dormancy in fungal spores is traditionally placed into two categories: exogenous and endogenous (or constitutional). In spores that display exogenous dormancy, germination of the spore is inhibited by either the presence or absence of specific environmental factors. Such factors may consist of nutritional requirements, temperature constraints, the presence or absence of light or chemical agents, water and oxygen thresholds, and inhibitory or stimulatory fungal growth factors (Sussman 1966, Griffin 1994).

Endogenous dormancy is less well characterized, but would encompass those spores that undergo a required period of maturation following release from the sporocarp before germination is possible, and is an innate property of the dormant spore (Sussman

1966). In other words, endogenous dormancy involves constraints that are not overcome even after supplying all the external conditions suitable for germination.

***Exogenous dormancy*.** There are numerous reports of exogenous dormancy in basidiospores and the factors necessary to break this form of dormancy *in vitro*. Optimal temperatures for spore germination vary from species to species, although the majority studied have optima of about 26° C, and there is a sharp decrease in germination with deviation from this optimum (Gottlieb 1978). The presence of light has been shown to be both necessary for sporangia germination in the chytrid *Physoderma maydis*, and inhibitory to spore germination in the rust *Tilletia tritici* (Sussman 1966). Because growth in most fungi is an aerobic process, the presence of water and oxygen are almost universally necessary for successful spore germination (Šubíková and Šubík 1974, d'Enfert 1977, Gottlieb 1978).

Spore activation is the application of the environmental stimuli necessary for spore germination (Sussman 1966). Activation factor is a term often applied to cases where extreme temperature or chemical treatments are necessary to induce germination (Sussman 1969). The application of activation factors usually mimics the conditions encountered in an unusual natural environment for which a particular species has become evolutionarily adapted.[3] Heat shocking for 30 minutes at between 50° C and 60° C induces germination in the ascospores of *Neurospora tetrasperma* (Sussman 1969). Mimicking the environment of an herbivore gut, with chemical and heat application may induce the

germination of some coprophilous fungi (Sussman 1966, Griffin 1994). Cold treating spores at temperatures of 20° C or lower for an extended period of time, followed by exposure to normal or elevated temperatures, will activate the germination of some fungal spores, including those of many rusts (Bromfield 1964, Booth 1971).

Externally produced biotic factors also play a role in the germination of some basidiomycetes. Spores, mycelia, or sporocarps may exude biochemical compounds that influence germination of nearby spores, either of the same or different species (Macko et al. 1974, Nguyen et al. 1987). These biotic factors are poorly characterized, but at least one is known to act as both an inhibitor and a stimulator to germination, depending on concentration of the compound and the species of the influenced spores (Ghachtouli et al. 1996). Nils Fries and others have worked to elucidate the effects of either supplying or removing these growth factors on spore germination. Excitatory factors may be supplied through the inoculation of living mycelia of the same species (Fries 1979) or another species such as the yeast *Rhodotorula* (Fries 1977, Fries 1983). Inhibitory factors may be removed by the application of activated charcoal (Fries 1977), or by decreasing the concentration of basidiospores per plate (Bulmer and Beneke 1964). Often, removal of some inhibitory factors while simultaneously supplying excitatory factors is necessary for optimal basidiospore germination (Fries 1978, Fries 1983).

[3]Examples of such are the colonization of burn sites by some *Neurospora* species (Dennis 1968); the passage through an animal gut by certain species of *Coprinus* (Ingold 1971), and the overwintering function of the specialized asexual spores of some rusts (Alexopoulos and Mims 1979).

Endogenous dormancy. All of the above are examples of exogenous spore dormancy[4]. Exogenous dormancy is broken only by the application or removal of the appropriate external factors. Thus, germination occurs only when favorable environmental conditions are met, and importantly, germination has never been reported in any controls for these studies where the required conditions were not met. In contrast, endogenously dormant spores, as the term is defined here in this paper, remain dormant for what appears to be a genetically determined and enforced length of time, without regard to environmental conditions (so long as they meet the minimum necessary for subsequent germination and growth), before germination is initiated.

Different fungal species require different time lapses between maturation (as measured by time of spore release) and germination (as measured by the first appearance of the germ tube), although these times have only been quantified for durations of between 45 minutes and 12 hours for many species (Gottlieb 1978). Dormancy in the zoospores of the chytrid *Blastocladiella emersonii* apparently can not be explained by environmental factors, and seems to be under the control of a protein synthesis inhibitor attached internally to the spore ribosomes (Griffen 1994). Endogenous dormancy has been found in

[4] Those species with spores requiring "activation factors" *in vitro* for germination might still be considered to possess a form of exogenous dormancy, as the need for favorable conditions must be met before germination can occur. Other researchers (Sussman 1966, Griffen 1994) would disagree, limiting the definition of exogenous dormancy to include only those species whose minimal needs for growth alone must be met. In this view, the term endogenous dormancy would then encompass those species with special requirements (activation factors) for germination (as differentiated from the requirements needed simply for growth). Still others (Gottlieb 1978) adopt a much more restricted terminology, considering true dormancy in fungal spores to be an innate state that does not allow germination even though the requirements for germination have been supplied (i.e., what is termed "endogenous dormancy" in this paper). In this last view, all cases termed "exogenous dormancy" are not, in fact, true dormancy at all.

some VAM (zygomycete) spores when the spores are formed during the non-growing season of the host (Gemma and Koske 1988).

In basidiomycetes, the teliospores of some rust fungi are known to exhibit what appears to be endogenously controlled spore dormancy. Teliospores are thick-walled, asexually produced, resting propagules that are produced in the fall, overwinter on the ground, and germinate in the spring to produce the sexual basidiospores (Alexopoulos and Mims 1979). In *Tilletia caries*, germination of the teliospores does not appear to be regulated by the same external mechanisms that regulate germination of the urediniospores of the same species, but rather, germination in these spores is apparently under endogenous control (Macko et al. 1974). Endogenous dormancy has not been demonstrated in agarics, although it has been implicated in the work of Miller et al. (1993) as one factor for explaining vital-staining and viability patterns in some basidiospores.

***Basidiospore germination in the Crepidotaceae*.** The basidiospores of saprophytes, in general, germinate readily in culture.[5] A few saprophytic basidiomycetes, however, have never been successfully germinated *in vitro*, for example, some species of the Tremellales (Kenneth Wells, personal communication). Others have only rarely been observed to germinate in culture, and only in very low frequencies, for example, species of *Pluteus* Fries (Banerjee and Sundberg 1993) and *Hygrophorus* Fries (Orson K. Miller, personal communication). The mechanism behind failure to germinate *in vitro* in these instances has never been characterized.

[5] Compendiums of just a small portion of the saprophytic Agaricales that germinate in culture can be found in the works of Lamoure (1989) and Petersen (1997).

In the Crepidotaceae, only the spores of species of *Tubaria* have been observed germinating *in vitro* (Ingold 1983). Reports of single spore isolate recovery or mating intercompatability tests have not been reported for any species of *Crepidotus, Melanomphalia*, or *Simocybe*. The only data on cultural growth in any of these genera is provided by Senn-Irlet (1994) on nine species of *Crepidotus sensu lato* collected from Switzerland.

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MATERIALS AND METHODS

Materials

Collections processing. Field collections were assigned a unique collection number; data on substrate and ecology was made in the field; spore prints were set up while in the field or immediately upon return to the laboratory; detailed macroscopic notes were made upon return to the laboratory, and collections were photographed and/or sketched in the fresh condition. Collections were dried on a convection air drier at low heat. Microscopic notes were made upon return to the laboratory and/or after drying with subsequent rehydration by applying a drop of EtOH on a wedge of dried material followed by soaking in dH_2O. Sectioned material was mounted and examined in 1% NH_4OH. In addition, one or more of the following reagents were often used for examination: dH_2O, 3% KOH, Melzer's solution, Congo red, Cotton blue, and/or Sudan black. Appendix A contains data for all original collections cited in this work.

Identification and housing. For consistency, all species identifications of *Crepidotus* were made according to the keys in Hesler and Smith (1965) for temperate species, and Singer (1973) for Neotropical species. If a reasonable species identification was not attainable based on one of these two monographs, identifications followed the primary literature. Species identifications within the other genera follow the primary literature. All collections are housed in the Virginia Tech Mycology Herbarium (VPI), and

assigned a unique VTMH number in addition to their individual collection numbers. See appendix B for list of assigned VTMH numbers.

Molecular Data

DNA Extractions. A 2-3 mm wedge of pileus tissue was suspended in 300 µL of 2X CTAB extraction buffer (Hillis et al. 1996) in a 1.5-mL eppendorf tube after drying (1996 field season) or immediately upon return to the lab (subsequent collections). Material was macerated with a plastic tissue grinder in the eppendorf and heated in a water bath at 65 C for approximately 30 min. An equal volume of chloroform/isoamyl alcohol (24:1) was added to the eppendorf and the tube was vigorously agitated for several min. The sample was centrifuged at 13,000 *g* for 5 min, and the supernatant pipetted into a clean labeled eppendorf. A second extraction was performed with an amount of chloroform/isoamyl alcohol equal to the amount of supernatant, and the sample was again agitated and centrifuged.

Supernatant was then pipetted into a clean labeled eppendorf and an equal volume of isopropyl alcohol was added to the tube. The tube was inverted several times and then placed in a freezer at 4 C for 30 min to 2 h. The eppendorf was then centrifuged at 13,000 *g* for 5 min. Once the precipitate had pelletized, the alcohol was discarded. Pellets were washed in 100 µL of cold 80% EtOH for several min. The alcohol was discarded and the eppendorfs with DNA pellet were allowed to completely dry, either by air, vacuum

centrifugation, or over a convection air drier. Pellet was then suspended in 25 μL of TE buffer and stored at -4 C.

Dried herbarium material was extracted in the same manner as the fresh material, but heated for 1 h in the water bath prior to extraction, and one additional chloroform/isoamyl alcohol extraction was performed. Culture material was extracted in the same manner as the fresh material, except that the first extraction was done in phenol/chloroform/isoamyl alcohol (25:24:1), followed by 2 chloroform/isoamyl extractions.

DNA dilutions. The amount of extracted DNA was checked electrophoretically on a 1% agarose gel by loading 2 μL of each extraction mixed with loading dye into separate lanes, and 2 μL of a DNA sample of known molecular weight in the last lane. Gels were stained with ethidium bromide and photographed under UV light. Optimal dilution amount was gauged by eye and original extractions were then diluted. DNA from fresh material was usually diluted 1/100. Dilutions were made by pipetting 1 μL of extracted DNA into a clean labeled eppendorf containing the appropriate amount of ddH_2O.

DNA amplification. A portion of the nuclear large ribosomal subunit DNA, and adjacent ITS2 and 5.8S regions, were first amplified using primers 5.8S-R (5'-TCGATGAAGAACGCAGCG) and LR7 (5'-TACTACCACCAAGATCT) (Vilgalys and Hester 1990). The basic 15 μL PCR cocktail contained 2.5 μL of 10X PCR buffer with Mg^{++}, 4 μL of dNTP mix (containing 2 m*M* each of dATP, dCTP, dGTP, and dTTP), 1.25 μL each of the 10 μ*M* primers, and 0.1μl μL of Taq polymerase in 5.9 μL of ddH_20

per reaction. This cocktail was later modified to include 2.5 μL of bovine serum albumin (Stommel et al. 1997) with the concomitant reduction of ddH_20 to 3.4 μL per reaction.

Diluted DNA (10 μL) and 15 μL of PCR cocktail were pipetted into a clean, labeled, mini-eppendorf for each reaction, and capped with a drop of mineral oil. A Perkin Elmer DNA Thermal Cycler #480 was used for PCR amplification at: one cycle of 94 C for 3 min; 35 cycles of 94 C for 1 min, 55 C for 30 sec, and 72 C for 2 min; and one cycle of 72 C for 5 min.

PCR purification. Products were checked by electrophoresing on a 1% agarose gel and then purified with QUAquick PCR purification kit (Qiagen) following the manufacturer's protocol, or by Millipore filters, following the manufacturer's protocol. Purified PCR products were then checked by electrophoresing on a 1% agarose gel, and the necessity for further dilution/concentration of product was gauged by eye to give about 13-25 ng/μL for each sample.

Sequencing reactions. Primers LR0R (5'-ACCCGCTGAACTTAAGC), LR3R (5'-GTCTTGAAACACGGACC), LR5 (5'-TCCTGAGGGAAACTTCG), and LR16 (5'-TTCCACCCAAACACTCG) (Rytas Vilgalys, personal communication) were used to amplify overlapping sections of a portion of the 5' end (approximately 1200 bases) of the large subunit coding region. For each reaction, 4 μL of manufacturer's FS cocktail containing polymerase, dNTPs, and fluorescently-labeled ddNTPs, (Perkin/Elmer ABI), 2 μL of a 1 μ*M* primer, and 4 μL of purified DNA, were pipetted into a mini-eppendorf tube. Thus, four reactions (one for each primer) were conducted for each DNA sample.

Samples were topped with a drop of mineral oil and PCR carried out on a Perkin Elmer DNA Thermal Cycler #480. Reaction conditions were set at: one cycle of 96 C for 2 min; and 25 cycles of 96 C for 30 sec, 50 C for 15 sec, and 60 C for 4 min.

The PCR products were purified in sephadex columns. Sephadex was prepared fresh by decanting 10 g in 200 mL of dH_20, and then pipetted (600 µL) into prepared columns and centrifuged at 2,600 *g* for 2 min. Columns were subsequently placed in clean, labeled eppendorfs, and the PCR product was pipetted from under the oil drop and onto the sephadex. Samples were centrifuged at 2,600 *g* for 2 min to collect filtered product. Product was thoroughly dried by placing eppendorfs in a vacuum centrifuge for approximately 20 min. Dried samples were stored at -20 C until sequenced.

Dried sequencing preps were suspended in 3 µL of sequencing buffer, vortexed briefly, and gently heated in a water bath to denature the DNA. Samples were loaded and phoresed by a technician on 8% polyacrylamide gels on an automated sequencer (ABI373 or ABI377).

Sequence Analysis

Contiguous sequences were assembled from the four primers using Sequencher 2.0 (GeneCodes Corp.) to obtain a sequence of approximately 1000 bases. Sequences were manually aligned in PAUP 3.0 or PAUP 3.1.1 (Swofford 1993), and regions with ambiguous alignment were excluded from analysis. Gaps were treated as a fifth character; all characters were given equal weight.

A heuristic search for the most parsimonious trees using a tree-bisection-reconnection (TBR) branch swapping algorithm was conducted in PAUP 3.1.1 or test version 40d63 of PAUP* (written by D.L. Swofford) with MULPARS in effect, and addition sequences set to random with ten replicates. Consensus trees were derived from all most parsimonious trees described. Support for the clades found by parsimony analysis was assessed by bootstrapping replications (Hillis and Bull 1993), jackknifing replications (Lanyon 1985), and calculation of decay indices (Bremer 1988). Bootstrapping and jackknifing analyses were performed using TBR branch swapping with 100 replicates for each. Starting trees were obtained by ten random addition sequence replicates with MULPARS in effect. Decay values were calculated up to five steps longer than the most parsimonious tree. Consensus trees were then obtained for all trees of each saved length, and compared by eye. Taxon sampling methods will be discussed individually within the applicable chapters.

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THE CREPIDOTACEAE (IMAI) SINGER: NEW EVIDENCE BASED ON NUCLEAR LARGE SUBUNIT rDNA SEQUENCES

Abstract

The Crepidotaceae (Basidiomycetes: Agaricales) as conceived by Singer consists of five agaric and four cyphelloid genera, although many alternate classification schemes for these fungi exist. Phylogenetic relationships were investigated for the four most prevalent genera of the Crepidotaceae (*Crepidotus*, *Simocybe*, *Melanomphalia*, and *Tubaria*) in conjunction with representatives from each of the other families of dark-spored agarics, by DNA sequencing. Parsimony analysis of sequences from the nuclear DNA encoding approximately 1,000 bases from the 5' end of the large ribosomal subunit (LSU rDNA) gene supports the Singer family of *Crepidotus*, *Simocybe*, and *Melanomphalia* as a monophyletic clade within the Agaricales. Affinities of the genus *Tubaria* to the other members of the Crepidotaceae are uncertain from these data. The genus *Crepidotus* does not appear monophyletic within this family. The Crepidotaceae appears more closely related to the Bolbitiaceae and the genus *Panaeolus* (Coprinaceae) than to the Cortinariaceae or the Strophariaceae as traditionally hypothesized.

Introduction

The Crepidotaceae, as conceived by Singer, consists of the agaric genera *Crepidotus* (Fr.) Staude, *Tubaria* (W.G. Smith) Gillet, *Melanomphalia* Christiansen,

Simocybe Karsten, and *Pleurotellus* Fayod, and the cyphelloid genera *Episphaeria* Donk apud Singer ex Donk, *Phaeosolenia* Spegazzini, *Pellidiscus* Donk, and *Chromocyphella* de Toni and Levi (Singer 1951, Singer 1962, Singer 1967, Singer 1973b). All genera are saprophytic, possess dark spores with the exception of *Pleurotellus*, and lack a true apical germ pore. The family includes both astipitate (*Crepidotus*, *Pleurotellus*) and stipitate (*Simocybe*, *Melanomphalia*, *Tubaria*) agaricoid members. These fungi are worldwide in distribution. At present there exist approximately 150 known species of *Crepidotus*, 38 species of *Simocybe*, 24 species of *Melanomphalia*, 15 species of *Tubaria*, two species of *Pleurotellus*, and nine species in the cyphelloid genera (Singer 1986, Hawksworth et al. 1995). A worldwide monograph to any of these genera is not available, however several regional studies have been compiled for the most prevalent of these, i.e. *Crepidotus* (Singer 1947, Pilát 1948, Pilát 1950, Hesler and Smith 1965, Singer 1973a), *Tubaria* (Romagnesi 1940, Romagnesi 1943), *Melanomphalia* (Singer 1971), and *Simocybe* (Singer 1973b, Horak 1979, Horak 1980).

Alternative classification schemes place some or all of these genera within other families, mainly the Cortinariaceae and the Strophariaceae Singer and Smith. For example, *Tubaria* is often allied within the Strophariaceae (Moser 1978, Breitenbach and Kränzlin 1995). Watling and Gregory (1989) prefer a close relationship between *Tubaria* and the Cortinariaceae, while deferring to Singer's concepts. Hawksworth et al. (1995) place *Simocybe* within the Cortinariaceae. *Melanomphalia*, *Simocybe*, and *Tubaria* are transferred to the Cortinariaceae by Jülich (1981). All genera of the Crepidotaceae *sensu* Singer are placed within the Cortinariaceae in the *Flora Agaricina Neerlandica* (Bas

1988). Nor does Kühner (1980) accept the Crepidotaceae, instead placing *Crepidotus*, *Tubaria*, and *Simocybe* (he does not treat *Melanomphalia* or *Pleurotellus*) within the Strophariaceae, and this position is supported by Nordstein (1990).

Crepidotus is a large and morphologically diverse genus. Basidiospore form is particularly variable between the different species. Spore shapes range from globose to subfusoid and spore walls may be smooth, rugose, punctate, or echinulate. Basidiospore morphology is one of the keystones of agaric taxonomy (Jülich 1981, Singer 1986), and such a wide range in basidiospore morphology is rarely encountered within other agaric genera. The only researchers to deal extensively with infrageneric classification within this genus are Singer and Hesler and Smith. Singer (1986) divided the genus into two sections and four subsections based primarily on the presence or absence of spore ornamentation and secondarily on the presence or absence of clamp connections in the mature fruiting body. Hesler and Smith (1965) divided *Crepidotus* into three subgenera based primarily on the presence or absence of clamp connections and secondarily on spore shape.

The following questions were addressed in this study: (i) Is there a monophyletic group of genera roughly corresponding to the Crepidotaceae *sensu* Singer, within the context of the other dark-spored agaric families? (ii) Is the genus *Crepidotus* monophyletic? (iii) What other fungi are closely related to this group? The classification of Singer (1986) was used as a foundation from which representative genera of other dark-spored agaric families were sampled. Four species of *Crepidotus* were selected to include one representative from each of the four Singer subsections. Traditional classification of these fungi has been based solely on morphology, with varying results. A molecular

approach enabled the testing of traditionally-derived phylogenetic hypotheses by a non-morphologically based source of characters.

Materials and Methods

Materials. Methods for obtaining and identifying original collections were described on page 22. Collection data for all materials studied are given in Appendix A. Virginia Tech Mycology Herbarium (VPI) assignments are listed in Appendix B.

Molecular data. Methods for DNA extraction, amplification, purification, sequencing, and analysis were described on pages 23-27. Sequencing data for the non-Crepidotaceae fungi were obtained from the data bases of the Mycology Laboratory of the Department of Botany, Duke University.

Taxon sampling. The 25 taxa were sampled are listed in Table 1.1. Of the Crepidotaceae, as recognized by Singer, at least one representative was chosen from each genus with more than five recognized species. These were *Tubaria*, *Melanomphalia*, *Simocybe*, and *Crepidotus*. Four species of *Crepidotus* were sampled to include one species from each of the four Singer subsections while simultaneously including at least one representative of each of the Hesler and Smith subgenera, in an effort to cover the wide range of phenotypical variance within this genus. A broad based sampling technique was employed to represent the other dark-spored agaric families as

Table 1.1. Taxa selected for analysis of the Crepidotaceae within the Agaricales
Taxa listed following the classification of Singer (1986)

Taxon	Collection #	Locality	Source/ GenBank #
Agaricaceae			
Agaricus bisporus (Lange) Singer	SAR 88/411	Washington, USA	U11911
Agaricus pocillator Murrill	J 173	North Carolina, USA	AF041542
Leucocoprinus cepaestipes (Sow.: Fr.) Patouillard	EFM 548	Colombia	U85286
Coprinaceae			
Coprinus atramentarius (Bull.: Fr.) Fries	C 114 = VT 1131	Virginia, USA	AF041484
Coprinus nudiceps P.D. Orton	C 159 = KEMP 737/1	unknown	AF041517
Panaeolus acuminatus (Schaeff.: Secr.) Quélet	J 129	Scotland	AF041535
Psathyrella candolleana (Fr.) Maire	J 181	Kew, England	AF041531
Bolbitiaceae			
Bolbitius vitellinus (Pers.) Fries	SAR 84/100	Washington, USA	U11913
Conocybe rickenii (Schaeff.) Kühner	J 183	North Carolina, USA	AF041546
Strophariaceae			
Hypholoma subviride (Berk. & Curt.) Dennis	JJ 69	North Carolina, USA	AF042570
Pholiota squarrosoides Peck	JJ 7	North Carolina, USA	AF042568
Psilocybe silvatica (Peck) Singer & Smith	RV 5-7-1989	North Carolina, USA	AF042618
Stropharia rugosoannulata Farlow ex Murrill	D 258	Maryland, USA	AF041544
Cortinariaceae			
Cortinarius iodes Berkeley & Curtis	JM 96/23	Virginia, USA	AF042613
Cortinarius sp.	JM 96/40	North Carolina, USA	AF042614
Dermocybe marylandensis Ammirati & Smith	JM 96/24	Virginia, USA	AF042615
Crepidotaceae			
Crepidotus applanatus (Pers. ex Fr.) Kummer	MCA 188	Virginia, USA	this work
Crepidotus cinnabarinus Peck	MCA 387	New York, USA	this work
Crepidotus fraxinicola Murrill	OKM 26739	Washington, USA	this work
Crepidotus sp.	OKM 27303	Dominican Republic	this work
Melanomphalia sp.	OKM 27270	Puerto Rico, USA	this work
Simocybe sumptuosa (Orton) Singer	OKM 27046	Virginia, USA	this work
Tubaria conspersa (Pers. ex Fr.) Fayod	MCA 388	Virginia, USA	this work
Tubaria hiemalis Romagnesi ex Bon	MCA 385	Virginia, USA	this work
Outgroup			
Ganoderma australe group	JM RSH-0705	Toayuan, Taiwan	X78780

recognized by Singer. At least two generic representatives were selected from each family (Coprinaceae, Bolbitiaceae, Strophariaceae, Cortinariaceae, Agaricaceae). The Gomphidiaceae and Paxillaceae were excluded from analysis since they are most commonly regarded as allies to the boletes rather than the agarics (Singer 1986, Hawksworth et al. 1995) . *Ganoderma* (Basidiomycetes: Aphyllophorales) was chosen as the outgroup since the Aphyllophorales are considered as ancestral to the Agaricales by most modern researchers.

Results

The 25 analyzed taxa are shown in Table 1.1. Sequence alignments with excluded variably aligned regions indicated are shown in Appendix C. Of the 933 characters aligned, 79 characters were excluded from analysis, and 107 characters were parsimony-informative.

Figure 1.1 shows the strict consensus of the two equally most parsimonious trees that were obtained with *Ganoderma australe* designated as the outgroup (length = 446, CI = 0.511, RI = 0.536). Branches with bootstrapping support of 60% or greater are noted as the first number given above the supported branch; jackknifing support of >60% are noted as the second number given above the supported branch. Decay values of >1 are noted below the supported branches.

Four distinct nodes with support can be defined that represent the traditional agaric families Agaricaceae (represented by *Agaricus pocillator, A. bisporus,*

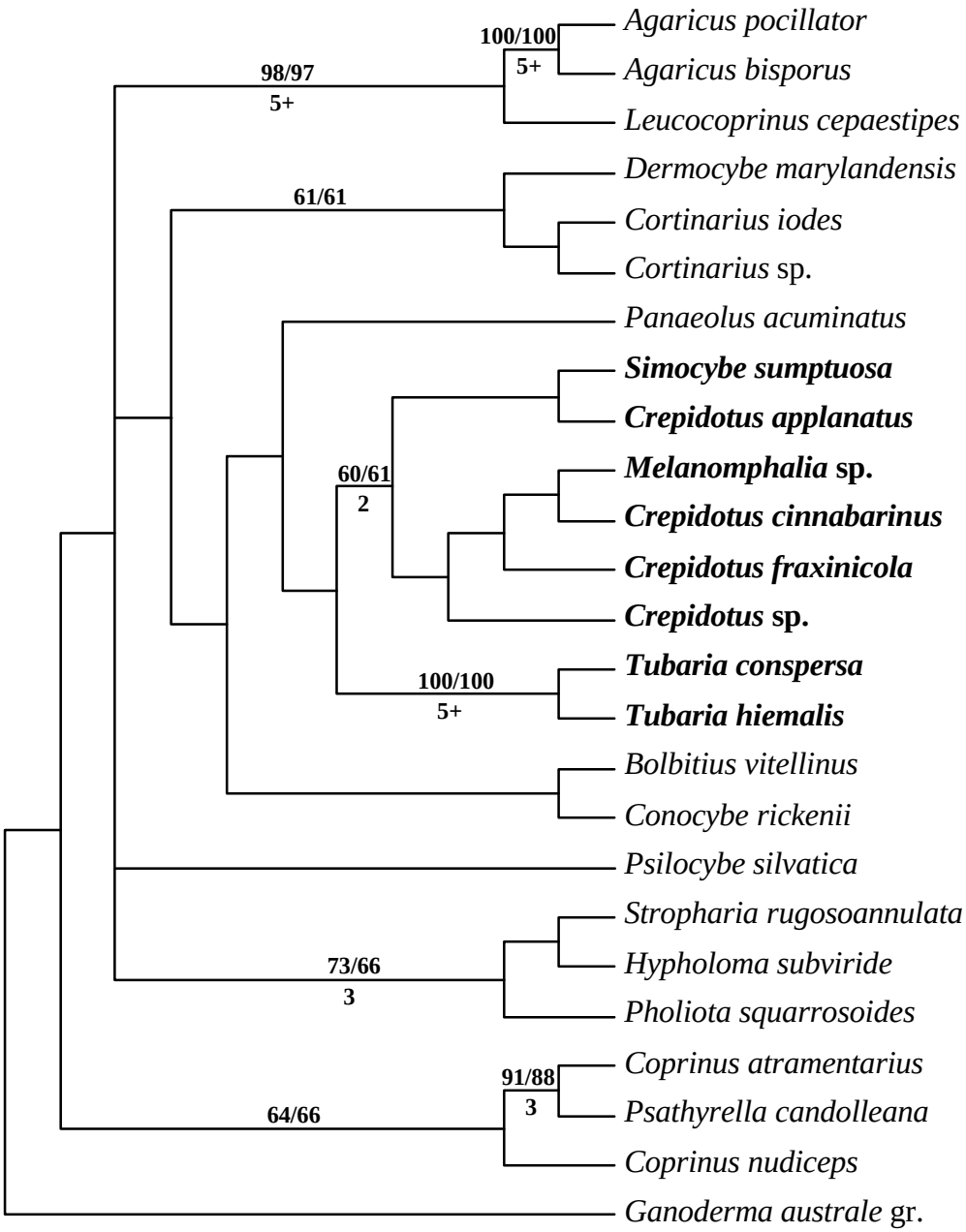

Figure 1.1. Crepidotaceae within the Agaricales: strict consensus of the two equally most parsimonious trees (length = 446, CI = 0.511 and RI = 0.536) obtained with LSU rDNA sequencing data. Bootstrap values 60% or greater from 100 replicates shown as first number above supported branch; jackknife values >60% from 100 replicates shown as second number above supported branch. Decay values shown below supported branch. Names in bold are new sequences of Crepidotaceae *sensu* Singer reported in this study.

Leucocoprinus cepaestipes), Cortinariaceae (represented by *Dermocybe marylandensis*, *Cortinarius* sp., *C. iodes*), Strophariaceae (represented by *Stropharia rugosoannulata*, *Hypholoma subviride*, *Pholiota squarrosoides*), and Coprinaceae (represented by *Coprinus atramentarius*, *C. nudiceps*, *Psathyrella candolleana*). The node representing the Bolbitiaceae (*Bolbitius vitellinus*, *Conocybe rickenii*) is unsupported with these data. Two remaining supported nodes represent members of the Crepidotaceae. One node defines the core Crepidotaceae (*Crepidotus*, *Simocybe*, *Melanomphalia*) while the other represents the genus *Tubaria*. There is no support at the node joining these two clades of the Crepidotaceae *sensu* Singer.

Discussion

Molecular data from the LSU rDNA gene region support the morphologically - based conclusions of Singer in recognizing a monophyletic group of dark-spored agarics as the family Crepidotaceae. The core members of this family include the genera *Crepidotus*, *Melanomphalia*, and *Simocybe*, although the inclusion of *Tubaria* within this family is ambiguously supported by the sequencing data. There is evidence that the genus *Crepidotus*, represented by four morphologically divergent species in this study, is not monophyletic. These results also indicate that the closest relatives of the Crepidotaceae may be found among the Bolbitiaceae or segregates of the Coprinaceae, although relationships between the dark-spored agaric families remain poorly resolved from these data. A detailed discussion follows.

The Crepidotaceae. Since the establishment of the Crepidotaceae (Singer 1951), the existence of this family, and its generic components, have been historically debated. These data are evidence for recognizing the genera *Crepidotus*, *Simocybe*, and *Melanomphalia* as forming a monophyletic clade of fungi consistent with Singer's Crepidotaceae. Morphologically these genera form a cohesive and diagnosable core of fungi that are saprophytic on woody and herbaceous plant debris, have brown pigmented basidiospores with thick spore walls that lack both a true germ pore and a suprahilar plage, and that never form a cellular cuticle. Most species develop gymnocarpically.

While parsimony analysis suggests that the genus *Tubaria* is a sister taxon to the core Crepidotaceae, there is no support from bootstrapping, jackknifing, or decay indices for this node. Some *Tubaria* have recently been segregated from *Phaeomarasmius* Scherff. (Cortinariaceae) (Harmaja 1978), and *Tubaria* is frequently transferred from the Crepidotaceae to the Cortinariaceae (Jülich 1981) or the Strophariaceae (Moser 1978, Breitenback and Kränzlin 1995). Singer (1962) believes *Tubaria* to be the ancestor of the astipitate *Crepidoti*. Although species of *Tubaria* possess many characters in common with the core Crepidotaceae, they also possess characters not otherwise found in this family, i.e. thin, lightly-pigmented, monostratous spore walls (Pegler and Young 1971, Largent et al. 1977), and hemiangiocarpic development often manifesting as a cortinous partial veil (Singer 1986). Until other genera with similar characters, most notably *Phaeomarasmius*, are sampled, the relationship between *Tubaria* and the Crepidotaceae remains uncertain.

Pleurotellus consists of two species traditionally placed within the Crepidotaceae. Macroscopically these species resemble *Crepidoti*. Microscopically these species differ from the other Crepidotaceae in spore pigmentation and spore form, being hyaline and pip-shaped. Historically, *Pleurotellus* has been allied with *Crepidotus* based on macro-morphology (Singer 1951), and on microscopic similarities between *P. hypnophilus* (Pers. ex Berk.) Fayod and *C. herbarum* (Peck) Sacc. (Hesler and Smith 1965), although *C. herbarum* has since been designated as a synonym for *P. hypnophilus* (Singer 1986). From the present study it can be inferred that microscopic (particularly those of basidiospore morphology), rather than macroscopic characters are most useful for delimiting the members of the Crepidotaceae. However, sequence sampling of *Pleurotellus* is indicated to further assess the relationship between this genus and the Crepidotaceae.

Although not empirically tested in this study, the cyphelloid taxa *Episphaeria, Phaeosolenia, Pellidiscus,* and *Chromocyphella* possess a suite of morphological and ecological characters that are entirely consistent with those found in the core Crepidotaceae. Even though these species lack the gills normally associated with the Agaricales, some cyphelloid basidiomycetes may represent reduced forms derived from agaric ancestors (Singer 1963, Singer 1966, Miller and Watling 1987). Recently published studies using DNA sequencing evidence support other theoretical associations between form-reduced taxa and their agaric ancestors (Kretzer and Bruns 1997, Johnson and Vilgalys 1998). Sampling from these four cyphelloid genera could ascertain whether these represent gill-less members of the Crepidotaceae.

Crepidotus sensu lato. Parsimony analysis of the LSU rDNA region suggests that the genus *Crepidotus* is not monophyletic within the Crepidotaceae although none of the nodes representing polyphyletic clades are supported by bootstrapping, jackknifing, or decay indices with these data. The potential polyphyly of *Crepidotus* was further evaluated by analyzing the same data matrix with the addition of several other *Crepidotus* taxa, and bootstrapping support of the nodes that contain species of *Simocybe* and *Crepidotus*, and *Melanomphalia* and *Crepidotus* was found to be >50% (results not shown). This question will be addressed in depth in Chapter 2 of this work.

Related taxa. Singer has hypothesized the Crepidotaceae to be intermediate between the Cortinariaceae and the Entolomataceae and also as being closely related to the Paxillaceae (Singer 1972, Singer 1986). Other researchers, most notably Kühner (1980) believe the genera of the Crepidotaceae to be components of the Strophariaceae. The evidence from these data suggest that the Crepidotaceae are unrelated to the mycorrhizal representatives of the Cortinariaceae sampled, and unrelated to the Strophariaceae. Since the Entolomataceae, characterized by pink pigmented, angular spores, have been shown to be unrelated to the dark-spored agarics in the work of Moncalvo et al. (1999), they were not sampled in this study. Likewise, the Paxillaceae are now considered to be members of the Boletales (Hawksworth et al. 1995), which form a sister to the Agaricales (Moncalvo et al. 1999) and therefore were not sampled in this work. Although relationships between families remain largely unresolved with the broad based sampling technique employed here, the LSU rDNA data do suggest that a search for sister taxa to the Crepidotaceae be conducted more rigorously from within the

Bolbitiaceae, and from *Panaeolus* and its relatives. Additional analysis should also include the saprophytic segregates of the Cortinariaceae, such as *Gymnopilus* and *Phaeomarasmius,* that were not sampled for this study.

In summary, the existence of a core clade of dark-spored agarics, composed of the genera *Crepidotus*, *Melanomphalia*, and *Simocybe*, is indicated by DNA sequencing analysis. The goal of any modern systematic study should be the delimitation of monophyletic phylogenetic clades (Kuyper 1994, Hibbett and Donoghue 1998). Therefore, given our present morphological knowledge and the molecular evidence presented here, it is recommended that the Crepidotaceae, as conceived by Singer, be maintained as a family of Agaricales. No generic exclusions are indicated pending further molecular investigation.

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SEGREGATES OF *CREPIDOTUS* (FR.) STAUDE AS INFERRED FROM MOLECULAR AND MORPHOLOGICAL DATA

Introduction

The genus *Crepidotus* (Fr.) Staude (Agaricales: Crepidotaceae) consists of saprophytic fungi, mainly pleurotoid in habit, that are world-wide in distribution. At least 233 species (*Index of Fungi*) have been described, although current generic concepts have been refined to leave closer to 150 species (Hawksworth et al. 1995) in the genus. Species identification in this group is notoriously difficult, and infrageneric classification remains controversial. Analysis of the nuclear genes coding for a portion of the large ribosomal subunit (LSU rDNA) support a universal origin of this genus within the Crepidotaceae (Imai) Singer, but suggest that the genus itself is not monophyletic within the family (Chapter 1).

Species of *Crepidotus* are dark-spored and lack an apical germ pore. Basidiospore form, however, is particularly variable between the different species. Spore shapes range from globose to subfusoid and spore walls may be smooth, rugose, punctate, or echinulate. Such range in basidiospore morphology is rarely encountered within other agaric genera. Two classification systems have been proposed for *Crepidotus* (summarized in Table 2.1). Singer (1986) divides *Crepidotus* into two sections and a total of four subsections based primarily on the presence or absence of spore ornamentation and secondarily on the presence or absence of clamp connections in

Table 2.1. Infrageneric Classification of *Crepidotus* (Fr.) Staude
as proposed by Hesler and Smith (1965) and Singer (1986)

HESLER AND SMITH Subgenera and Sections	**defining characters**
I. Subgenus *Crepidotus*	- clamp connections
1. Section *Cinnabarini* Hes. & Sm.	+ dissolved red pigments
2. Section *Tubariopsis* Hes. & Sm.	fusoid to subfusoid spores
3. Section *Stratosi* Hes. & Sm.	pileus structure a duplex
4. Section *Parvuli* Hes. & Sm.	spores ornamented and globose
5. Section *Crepidotus*	+ gelatinization
6. Section *Versuti* Hes. & Sm.	spores ellipsoid
II. Subgenus *Sphaerula* Hes. & Sm.	+ clamp connections, spores globose
7. Section *Nyssicolae* (Sing.) Hes. & Sm.	fruiting body stipitate
8. Section *Sphaerula*	fruiting body pleurotoid
a. Subsection *Sphaerula*	pileus and fibrils white
b. Subsection *Colorantes* Hes. & Sm.	pileus colored, fibrils white
c. Subsection *Fulvofibrillosi* Hes. & Sm.	fibrils colored
III. Subgenus *Dochmiopus* (Pat.) Pilát	+ clamp connections, not globose
9. Section *Cystidiosi* Hes. & Sm.	+ pleurocystidia
10. Section *Fulvidi* Hes. & Sm.	pileus and/or fibrils colored
11. Section *Phaseoli* Hes. & Sm.	spores phaseoliform
12. Section *Fusisporae* Hes. & Sm.	spores fusoid
13. Section *Betulae* Hes. & Sm.	spores ellipsoid, smooth
14. Section *Dochmiopus* (Pat.) Pilát	spores ornamented, small
15. Section *Crepidotellae* Hes. & Sm.	spores ornamented, large
SINGER Sections and Subsections	**defining characters**
I. Section *Echinospori* Pilát	ornamented spores
1. Subsection *Porpophorini* Singer	+ clamp connections
2. Subsection *Aporpini* Singer	- clamp connections
II. Section *Crepidotus*	smooth spores
3. Subsection *Fibulatini* Singer	+ clamp connections
4. Subsection *Crepidotus* (=*Defibulatini* Singer 1947)	- clamp connections

the sporocarp. Hesler and Smith (1965) divide the genus into three subgenera and a total of 15 sections based primarily on the presence or absence of clamp connections and secondarily on spore shape. A worldwide monograph is not available for *Crepidotus*, although several regional monographs (Singer 1947, Pilát 1948, Pilát 1950, Hesler and Smith 1965, Singer 1973) and studies (Imai 1938, Orton 1960, Hesler 1975, Horak 1977, Ortega and Buendia 1989, Stangl et al. 1991, Senn-Irlet and De Meijer 1998) continue to further this goal.

The following questions were addressed in this study: (i) Is the genus *Crepidotus* monophyletic within the Crepidotaceae? (ii) What are the monophyletic groups of *Crepidotus sensu lato*, and do they correspond to either the subclassification of Singer (1986) or Hesler and Smith (1965)? (iii) What are the morphological characters that might reliably diagnose the segregates of *Crepidotus sensu lato*? Taxa were chosen to cover the wide phenotypic range in this genus by including at least one representative from each of the Singer subsections and the Hesler and Smith subgenera. First, phylogenetic relationships were evaluated using a portion of the nuclear large subunit ribosomal DNA (LSU rDNA) gene region. Since traditional classification of these fungi has been based solely on morphology, a molecular approach was employed to enable the testing of traditionally-derived phylogenetic hypotheses by a non-morphologically based source of characters. A data set of 25 morphological characters was then compiled and analyzed separately and in conjunction with the molecular characters to further define the segregates of *Crepidotus.*

Materials and Methods

Materials. Methods for obtaining and identifying collections were described on page 22. Collection data for all materials studied are given in Appendix A. Virginia Tech Mycology Herbarium (VPI) assignments are listed in Appendix B.

Molecular data. Methods for DNA extraction, amplification, purification, and sequencing were described on pages 23-26. Sequencing data for the non-Crepidotaceae fungi was obtained from the databases of the Mycology Laboratory of the Department of Botany, Duke University.

Taxon sampling. A total of 31 taxa were sampled (Table 2.2). Sampling included a morphologically diverse set of 23 *Crepidotus* taxa, chosen to include at least two members from each of Singer's four subsections, and representatives from all three of Hesler and Smith's subgenera, encompassing 10 of their 15 sections. Two to three taxa from each of the allied genera *Melanomphalia*, *Simocybe*, and *Tubaria* (Crepidotaceae), were evaluated as additional ingroup constituents, with *Panaeolus acuminatus* (Coprinaceae) designated as the outgroup. Previous analysis in Chapter 1 has shown *P. acuminatus* to be the nearest relative of the Crepidotaceae from among those taxa sampled, while the monophyly of *Tubaria* with the core Crepidotaceae (*Crepidotus*, *Melanomphalia*, and *Simocybe*) remains uncertain. Sequence alignments with variable regions excluded from analysis indicated are given in Appendix C. Methods for phylogenetic analysis were described on pages 26-27.

Table 2.2. Taxa selected for analysis of *Crepidotus* within the Crepidotaceae
Taxa listed following the classification of Singer (1986)

Taxon	H&S[i]	Collection #	Locality	Source
Crepidotus* section *Echinospori* subsection *Porpophorini				
C. applanatus (Pers.) Kummer	8	MCA 188	Virginia, USA	this work
C. aureus Horak	8[ii]	OKM 27300	Puerto Rico, USA	this work
C. cystidiosus Hesler & Smith	8	OKM 27048	Virginia, USA	this work
C. distortus Hesler & Smith[iii]	8	MCA 386	North Carolina, USA	this work
C. cf. *fusisporus* Hesler & Smith[iv]	12	MCA 258	Virginia, USA	this work
C. lanuginosus Hesler & Smith[v]	15	OKM 26976	Oregon, USA	this work
C. malachius (Berk. & Curt.) Saccardo[vi]	8	MCA 343	Virginia, USA	this work
C. nephrodes (Berk. & Curt.) Saccardo	8	MCA 189	Virginia, USA	this work
C. nephrodes (Berk. & Curt.) Saccardo	8	OKM 25896	Virginia, USA	this work
C. nyssicola (Murr.) Singer	7	TJB 8699	Maine, USA	this work
C. sphaerosporus (Pat.) Lange	15	OKM 27013	Colorado, USA	this work
C. sp.	12	OKM 26899	Thailand	this work
C. sp.	8	MCA 170	Virginia, USA	this work
Crepidotus* section *Echinospori* subsection *Aporpini				
C. cinnabarinus Peck	1	MCA 387	New York, USA	this work
C. versutus (Peck) Saccardo	6	MCA 250	Virginia, USA	this work
C. versutus (Peck) Saccardo	6	MCA 381	North Carolina, USA	this work
Crepidotus* section *Crepidotus* subsection *Fibulatini				
C. antillarum (Pat. apud Duss) Singer	13	OKM 26827	Dominican Republic	this work
C. betulae Murrill	13	MCA 384	North Carolina, USA	this work
C. inhonestus Karsten	13	OKM 26740	Washington, USA	this work
C. sp.	13	OKM 27303	Puerto Rico, USA	this work
Crepidotus* section *Crepidotus* subsection *Crepidotus				
C. fraxinicola Murrill	5	OKM 26739	Washington, USA	this work
C. fraxinicola Murrill	5	OKM 26748	Washington, USA	this work
C. mollis (Fr.) Staude	5	OKM 26279	Montana, USA	this work

[i] Hesler and Smith (1965) infrageneric classification of *Crepidotus*; number corresponds to section # given in Table 2.1.
[ii] *Crepidotus aureus* is not monographed by Hesler and Smith (1965). Morphology would place it in this section.
[iii] Singer does not include *C. distortus* as a species in the *Agaricales in Modern Taxonomy* (1986). Morphology would place it in this subsection.
[iv] Singer does not include *C. fusisporus* as a species in the *Agaricales in Modern Taxonomy* (1986). Morphology would place it in this subsection.
[v] Singer (1986) does not recognize *C. lanuginosus* as a species in the *Agaricales in Modern Taxonomy* (1986). Morphology would place it in this subsection.
[vi] Singer (1986) considers *C. malachius* (Berk. & Curt.) Sacc. as a synonym for *C. nephrodes* (Berk. & Curt.) Sacc.

Table 2.2. Continued

Taxon	H&S	Collection #	Locality	Source
***Simocybe* P. Karsten**				
Simocybe sumptuosa (Orton) Singer		OKM 27046	Virginia, USA	this work
Simocybe sp.		MCA 294	Virginia, USA	this work
Simocybe sp.		MCA 424	Virginia, USA	this work
***Melanomphalia* M.P. Christiansen**				
Melanomphalia sp.		TJB 8496	Puerto Rico, USA	this work
Melanomphalia sp.		OKM 27270	Puerto Rico, USA	this work
***Tubaria* (W.G. Smith) Gillet**				
Tubaria conspersa (Pers. ex. Fr.) Fayod		MCA 388	Virginia, USA	this work
Tubaria hiemalis Romagnesi ex. Bon		MCA 385	North Carolina, USA	this work
Outgroup				
Panaeolus acuminatus (Schaeff.: Secr.) Quélet		J 129	Scotland	AF041535[i]

[i] GenBank accession number.

Morphological characters and analysis. Morphological studies were performed on all 23 *Crepidotus* taxa sampled, and a data set of 25 morphological characters constructed. Characters evaluated are shown in Table 2.3; the derived data matrix is given in Table 2.4. Characters chosen for analysis include both those traditionally applied in *Crepidotus* taxonomy (such as character 8), those sometimes applied (such as character number 14), and some that are rarely or never applied, but may have taxonomic value (for example, characters 15 and 17). An explanation for the derivation of character values follows; values in parentheses correspond to character number (Table 2.3).

Substrate and development—Individual *Crepidotus* collections are normally observed inhabiting just one of the following substrate types: herbaceous litter, decorticated wood, or bark (1); and are found on either hardwoods or conifers (2). Values were established from fresh field notes or by direct observation of preserved material. Development of the sporocarp is a character that may have strong taxonomic value (Reijinders 1986). Various stages of stipe development can be found in different species of *Crepidotus* (3). A stipe may completely fail to develop, whereby the mature pileus is directly attached to the substrate. Primordia may exhibit a centrally attached stipe, which fails to continue developing at an early stage as the pileus expands, and results in the mature pileus being attached to the substrate by a lateral plug that originated as the primordial stipe. The stipe may continue to develop beyond the primordia, but either halts before maturity, or develops at a much slower rate than the

Table 2.3. Characters and character states

1. Found on herbaceous litter (1); on bark (2); on decorticated wood (3).
2. Found on hardwoods (1); on conifers (2).
3. Mature sporocarps astipitate, attached to substrate by pileus surface (1); aborted stipe, mature sporocarps attached by sterile, lateral plug (2); underdeveloped stipe, mature sporocarps attached by lateral, recurved stipe (3); fully stipitate (4).
4. Average mature cap diameter <5 mm (1); between 5 and 15 mm (2); >15 mm (3).
5. Color of dried sporocarps pure white or cream (1); ochre or yellow (2); brown (3); red (4).
6. Lamellae length narrow (1); medium (2); broad (3).
7. Lamellae spacing close or crowded (1); medium (2); distant (3).
8. Clamp connections numerous in the sporocarp (1); clamp connections rare (2); clamp connections absent (3).
9. Epicuticular hyphae with encrusting pigments (1); without encrusting pigments (2).
10. Pigments dissolving in alkali mounting media and DNA extraction buffer (1); no dissolving pigments (2).
11. Pileus fibrillose (1); pileus glabrous or hygrophanous (2); pileus squamulose (3).
12. Cuticle <1/3rd of overall cap thickness (1); cuticle about 1/3rd of overall cap thickness (2); cuticle >1/3rd of overall cap thickness (3).
13. Epicuticular hyphae not differentiated (1); forming scattered pileocystidia (2); forming a loosely tangled layer (3); forming a dense turf (4).
14. No hyphal gelatinization (1); some hyphae of the pileus and/or hymenium gelatinized (2).
15. Oleiferous hyphae present in the pileus and/or hymenium (1); no oleiferous hyphae present (2).
16. Pleurocystidia present on the lamellae sides (1); no pleurocystidia present (2).
17. Cheilocystidia Q value <4 (1); between 4 and 7 (2); between 7 and 10 (3); >10 (4).
18. Cheilocystidia more narrow at apex than base (1); cylindrical (2); wider at apex than base (3); >50% forked at the apex (4).
19. Basidia 4-spored only (1); 2- and 4-spored basidia present (2); 1-, 2-, 3-, and 4-spored basidia present (3).
20. Spore Q value between 1.0 and 1.25 (1); between 1.25 and 1.5 (2); between 1.5 and 1.75 (3); between 1.75 and 2.0 (4).
21. No collapsed spores present after rehydration (1); collapsed spores rare, <10% (2); about 50% of spores collapsed (3); >50% of spores collapsed (4).
22. Spore walls smooth, no ornamentation (1); ornamentation rugose (2); ornamentation finely punctate, barely visible under oil-immersion (3); ornamentation coarsely punctate, consisting of broadly rounded projections (4); ornamentation strongly punctate, consisting of truncate columns (5); ornamentation strongly echinulate, consisting of spiny projections (6).
23. Spore shape tapered toward the apiculus (1); not tapered toward the apiculus, equal at both ends (2); shape broader at the apiculus (3).
24. Spore shape inequilateral in profile (1); equilateral in profile (2).
25. Apical thinning of the spore wall present (1); apical thinning absent (2).

Table 2.4. Character matrix

	Character numbers																								
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
C. antillarum OKM 26827	3	1	2	3	3	3	3	2	2	2	2	3	1	2	2	1	1	3	3	3	1	1	2	1	2
C. applanatus MCA 188	3	1	2	3	1	1	2	1	2	2	2	2	1	1	1	2	2	3	1	1	2	5	2	2	2
C. aureus OKM 27300	2	1	3	2	2	1	3	1	1	1	1	3	3	1	2	2	1	1	1	1	4	6	2	2	2
C. betulae MCA 384	3	1	2	2	1	3	2	1	2	2	1	3	1	2	2	2	3	2	2	3	2	1	2	1	2
C. cinnabarinus MCA 387	3	1	1	2	4	2	2	3	2	1	1	1	4	1	2	2	1	1	1	2	2	4	1	2	2
C. cystidiosus OKM 27048	3	1	2	3	1	1	2	1	2	2	1	1	2	1	1	1	2	3	1	1	2	5	2	2	2
C. distortus MCA 386	3	1	2	3	3	3	1	1	1	2	2	3	2	1	1	1	2	1	1	1	2	5	2	2	2
C. fraxinicola OKM 26739	3	2	2	3	1	3	2	3	2	2	2	2	1	2	2	2	4	2	1	3	2	1	1	1	1
C. fraxinicola OKM 26748	3	2	2	3	1	3	2	3	2	2	2	2	1	2	2	2	4	2	2	3	1	1	1	1	1
C. cf. *fusisporus* MCA 258	2	2	2	2	1	2	1	1	2	2	1	1	3	1	2	2	2	3	1	4	2	3	1	1	2
C. inhonestus OKM 26740	3	2	2	3	1	2	2	1	2	2	2	2	3	1	2	2	3	2	2	2	1	2	1	1	1
C. lanuginosus OKM 26976	2	2	2	2	1	1	3	1	2	2	1	1	3	1	2	2	1	4	1	2	3	4	2	1	2
C. malachius MCA 343	3	2	2	3	1	3	1	1	2	2	2	2	1	1	1	2	2	3	1	1	2	5	2	2	2
C. mollis OKM 26279	3	1	2	3	2	3	1	3	1	2	3	2	1	2	2	1	1	1	2	3	1	1	1	1	1
C. nephrodes MCA 189	3	1	2	3	2	1	1	1	1	2	2	2	1	1	1	2	2	3	1	1	2	5	2	2	2
C. nephrodes OKM 25896	3	1	2	3	2	2	2	1	1	2	3	3	2	1	1	2	2	3	1	1	1	5	2	2	2
C. nyssicola TJB 8699	3	1	4	3	2	1	1	2	2	2	1	1	4	1	1	2	1	3	1	1	2	3	1	2	2
C. sphaerosporus OKM 27013	2	1	2	3	1	3	3	1	2	2	1	2	4	1	2	2	1	4	2	2	3	6	2	2	2
C. versutus MCA 381	3	1	1	2	1	2	2	3	2	2	1	1	3	1	2	2	4	2	2	4	1	2	3	1	1
C. versutus MCA 250	1	1	1	2	1	1	2	3	2	2	1	1	3	1	2	2	4	2	3	4	1	2	3	1	1
C. sp. MCA 170	3	1	3	3	2	1	1	2	2	2	2	3	1	1	1	2	1	3	2	1	2	3	1	2	2
C. sp. OKM 26899	2	1	1	1	1	2	2	1	2	2	1	2	3	1	2	2	2	2	1	3	2	2	1	1	2
C. sp. OKM 27303	3	1	2	3	1	3	3	1	2	2	1	3	1	2	1	1	2	3	2	4	1	1	2	1	2
Tubaria conspersa MCA 388	1	1	3	3	3	2	2	1	1	2	1	1	3	1	2	2	1	2	1	3	3	1	1	1	2

pileus, resulting in a pileus attached by a short (< 50% in length of the mature pileus), recurved stipe. The stipe may develop completely and at maturity reach a length at least equal to the width of the pileus. This character was keyed by examination of all specimens in a collection.

Macroscopic characters—Those characters traditionally applied in *Crepidotus* taxonomy are those of cap size (4), cuticle condition (11), color (5), and lamellae length (6) and breadth (7). Cap size is measured as the average size of all mature sporocarps in a collection (or five to ten specimens in very large collections), taken while in fresh condition. Cuticular condition, and lamellae length and breadth, are taken from fresh field notes. Cap color was scored from dried material; color in fresh material often varies within a collection due to spore deposition on caps, and amount of moisture absorbed by the sporocarp, but is fairly consistent between members of a dried collection. The thickness of the cuticle (12) is an inexact measure of relative cuticle thickness in relation to overall pileus (cuticle and lamellae) thickness, as viewed in cross section under oil-immersion.

Hyphal characters—Dissolving pigments (10) were noted when a piece of fresh or rehydrated pileus tissue placed in an alkali mounting medium and CTAB extraction buffer exuded pigment into the surrounding fluid. Other hyphal characters were gauged from sectioning of a mature sporocarp, viewed under oil-immersion. The presence or absence of clamp connections is a key character in *Crepidotus* taxonomy (8) (see Table 2.1), and the presence of gelatinized hyphae (14) in the pileus and/or hymenium is diagnostic for section *Crepidotus* (Hesler and Smith 1965). The presence of encrusted pigments on the epicuticular hyphae (9), the condition of the epicuticular hyphae (13), and the presence of

oleiferous hyphae in the pileus and/or hymenium (15) are not traditionally emphasized, but may be of diagnostic value.

Cystidia and basidia—All values were taken from observation of at least two sections of mature pileus for each collection, viewed under oil-immersion. Pleurocystidia (16) are rare in this genus, but their presence is diagnostic for some species. Cheilocystidia measurements are standard in species descriptions. However, measurements can vary even on one gill section for some *Crepidotus* species. Cheilocystidia shape, however, appears to be fairly consistent for most observed species and, therefore, Q values as a measurement of shape were employed (17). Cheilocystidia from at least two gill sections were photographed with a Sony Videographic Printer (model UP-890MD) attached to a Leitz compound microscope that had been previously calibrated. Approximately ten cheilocystidia were measured from the photographs for each collection and Q values calculated as the average length divided by width per collection. As a further measure of cheilocystidia shape, it was noted whether the majority of those photographed were wider at the base, at the apex, cylindrical, or mainly forked and contorted at the apex (18). Basidia sizes are also normally reported, but somewhat variable within a given collection. The number of sterigmata per basidia, however, appears to be distinctive and consistent for a given collection, and these measurements were likewise taken from direct observation of at least two gill sections per collection (19).

Spore characters—Basidiospore morphology has been of utmost importance in the diagnosis of Agaricales since the time of Fries (Jülich 1981, Singer 1986). In the taxonomy of *Crepidotus*, much emphasis has been placed on whether or not there is some

form of exosporial ornamentation exhibited, and some emphasis on spore shape, but these classifications still result in species with very different spore morphologies in related segregates. Several additional characters of spore morphology were analyzed in this study. Basidiospores were photographed with a Sony Videographic Printer (model UP-890MD) attached to a Leitz compound microscope, and measurements made from the photos of approximately 30 randomly selected mature spores. Spore sizes have not been used since they have been shown to be variable within a species (Nordstein 1990) these were not used. Spore Q values, as a measure of overall spore shape, however, appear consistent for a species and were calculated for each collection as the average spore length divided by width (20). Additional peculiarities of spore shape were noted by shape at the apicular end (23), and shape in profile (24). Collapsed spores (21) may be indicative of structural differences in the spore wall components, such as in the monostratous walls of species of *Tubaria* (Largent et al. 1977). Although a true germ pore is absent in these species, several posses a thinning of the spore wall at the apical end (25). Finally, current SEM studies are illustrating the wide variety in type of ornamentation (22) exhibited among the species of *Crepidotus* (Pegler and Young 1971, Pegler and Young 1972, Clémençon 1977, Bigelow 1980, Luther and Redhead 1981, Ortega and Buendia 1989, Senn-Irlet 1993). Ornamentation type was scored from these published accounts; where no published SEMs exist for a sampled taxon, ornamentation type was scored from observation under oil-immersion.

Characters were unordered and unweighted. Characters for *Tubaria conspersa* were also keyed and this taxon was designated as the outgroup. Parsimony analysis in

PAUP 3.1 (Swofford 1993) was conducted as described on page 27. A final data matrix was constructed, combining the molecular and morphological data, and analyzed as described on pages 26-27.

Results

Molecular data. Sequence alignments for the 31 taxa analyzed (Table 2.2) with excluded variably aligned regions indicated are shown in Appendix C. Of the 1046 molecular characters aligned, 21 were excluded, and 134 of the remainder were parsimony-informative.

Figure 2.1 shows the strict consensus of the 20 equally most parsimonious trees that were obtained with *Panaeolus acuminatus* designated as the outgroup (length = 419, consistency index = 0.513, retention index = 0.715). Branches with bootstrapping support of >60% are noted as the first number given above the supported branch; branches with jackknifing support of >60% are noted as the second number given above the supported branch. Decay values of >1 are noted below the supported branches.

A distinct node with high support (98% bootstrapping, 98% jackknifing, 5+ decay index) can be defined that represents the core Crepidotaceae (*Crepidotus sensu lato*, *Melanomphalia*, and *Simocybe*). There is no support from these data for including

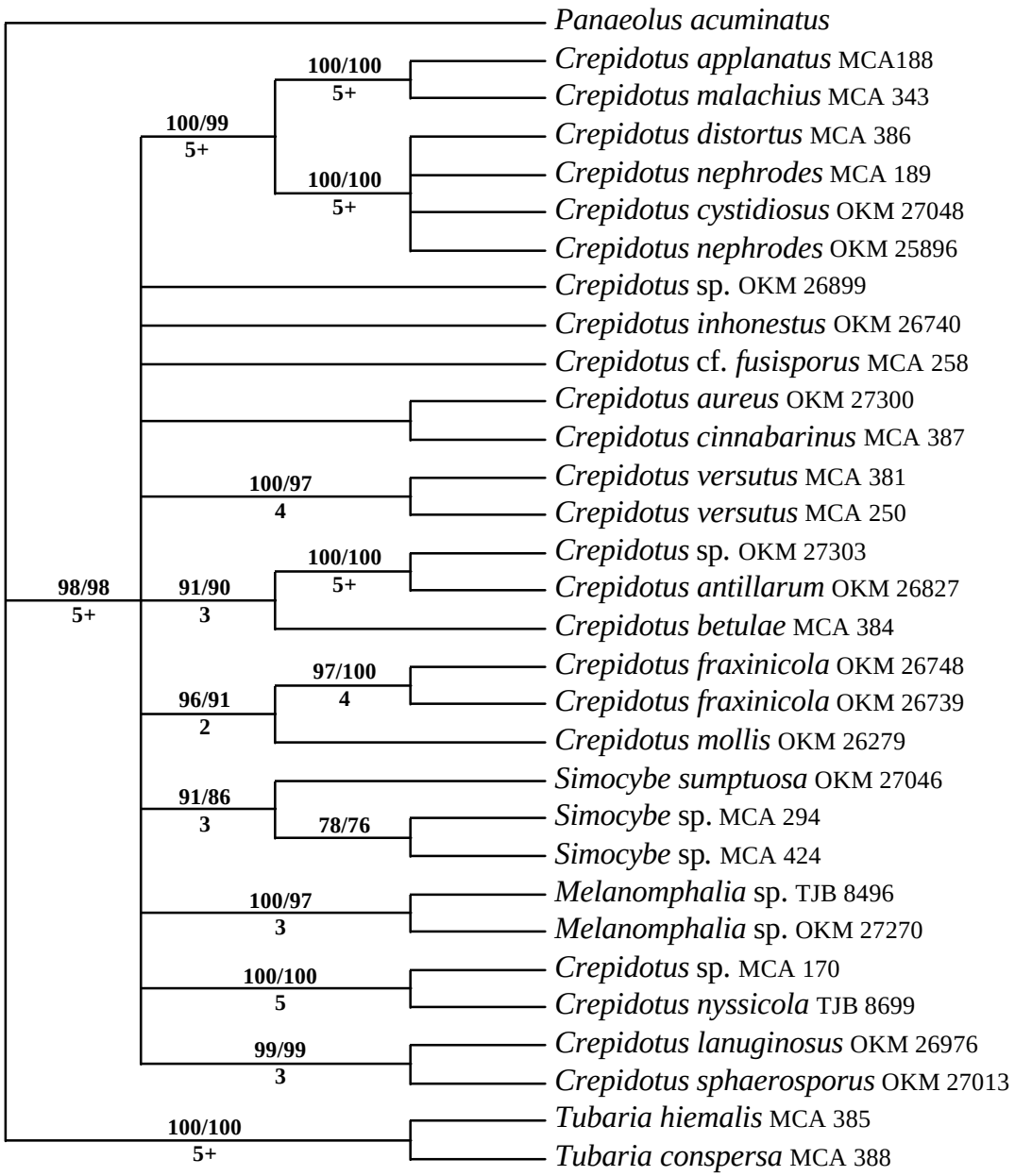

Figure 2.1. The Crepidotaceae: strict consensus of 20 equally most parsimonious trees obtained from sequences of the nuclear DNA encoding a portion of the large ribosomal subunit (length = 419, CI = 0.513, RI = 0.715). Bootstrapping values >60% from 100 bootstrapping replicates shown as first number above supported branch; jackknifing values >60% of 100 jackknifing replicates shown as second number above supported branch; decay values >1 shown below supported branch.

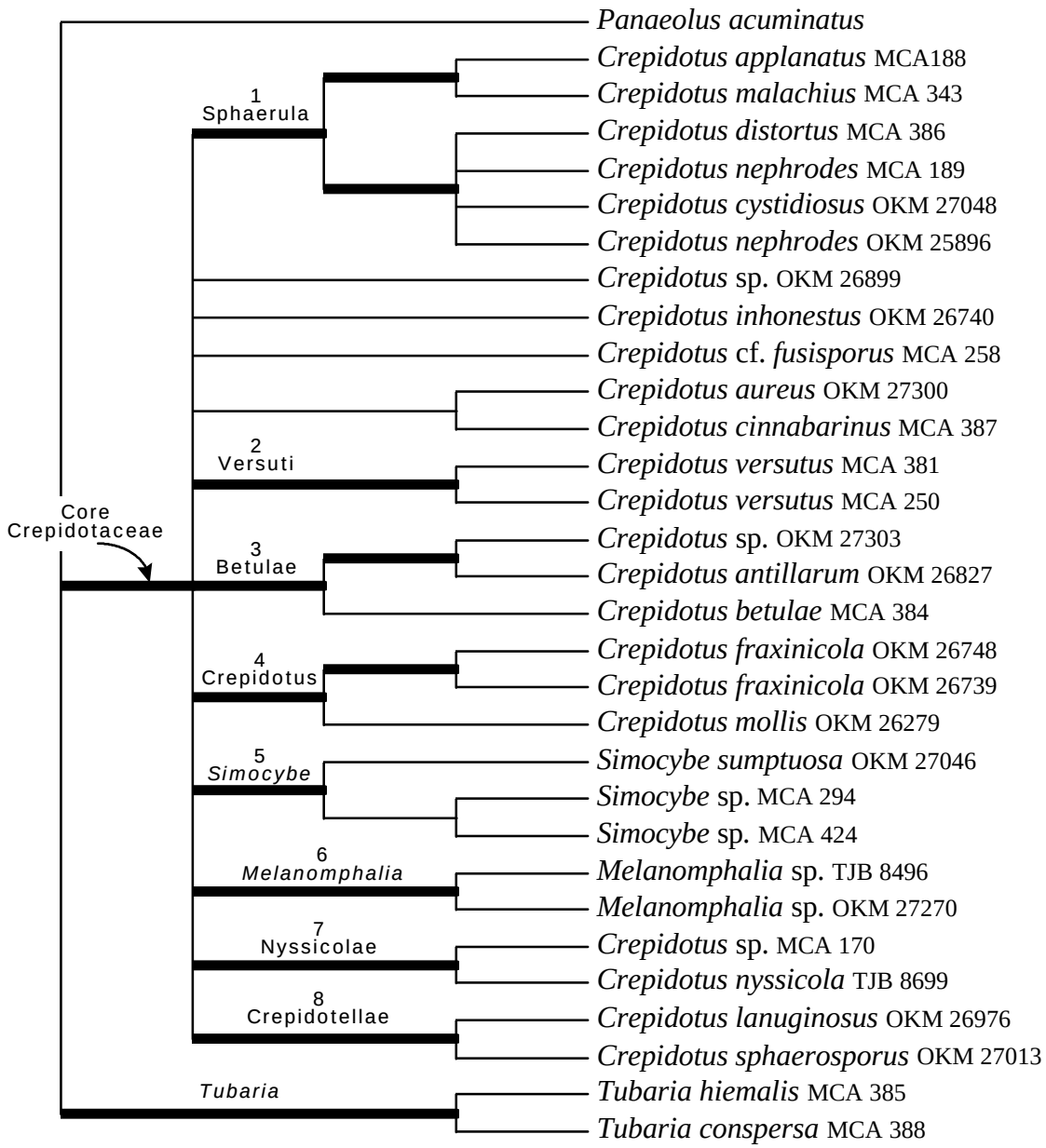

Figure 2.2. Monophyletic clades in the Crepidotaceae: strict consensus of 20 equally most parsimonious trees obtained from sequences of the nuclear DNA encoding a portion of the large ribosomal subunit (length = 419, CI = 0.513, RI = 0.715). Nodes supported by bootstrapping, jackknifing, and decay indices (Figure 2.1) shown with thick lines. Names and numbers (as referred to in text) of supported clades shown above the branches.

the genus *Tubaria* within this clade. Eight highly supported nodes represent the genus *Simocybe* as a monophyletic clade, the genus *Melanomphalia* as a monophyletic clade, and six clades representing the genus *Crepidotus sensu lato*. Figure 2.2 shows this same data, with nominal numbers assigned for the supported clades. Five of the *Crepidotus* species [*C. inhonestus*, *C.* cf. *fusisporus*, *C. aureus*, *C. cinnabarinus*, and *C.* sp. (OKM 26899)] do not form supported alliances with any of the other sampled taxa. The genus *Crepidotus* itself appears polyphyletic within this family.

To test the monophyly of the genus *Crepidotus*, an additional analysis was performed using the same data matrix, but constraining all *Crepidotus* taxa as monophyletic. Figure 2.3 shows the strict consensus of the six equally most parsimonious constraint trees, which were six steps longer (length = 425, consistency index = 0.506, retention index = 0.707) than the unconstrained tree.

Morphological data. The twenty-three *Crepidotus* taxa and *Tubaria conspersa* (Table 2.4) were scored for 25 morphological characters (Table 2.3). Figure 2.4 shows the strict consensus of the 77 equally most parsimonious trees (length = 134, CI = 0.388, RI = 0.596) obtained with *T. conspersa* designated as outgroup. Bootstrapping values from 100 replicates are noted above the supported branch. Clades that are supported from the molecular data alone (Fig. 2.2) are indicated by a bold line in Figure 2.4.

There is less resolution within the morphological than the molecular data. Clades supported in the morphologically-derived tree (Fig. 2.4) are also supported in the molecularly-derived tree (Fig. 2.2), however, the strongly supported Clades 1 and 4 of the molecular tree are collapsed in the morphological tree.

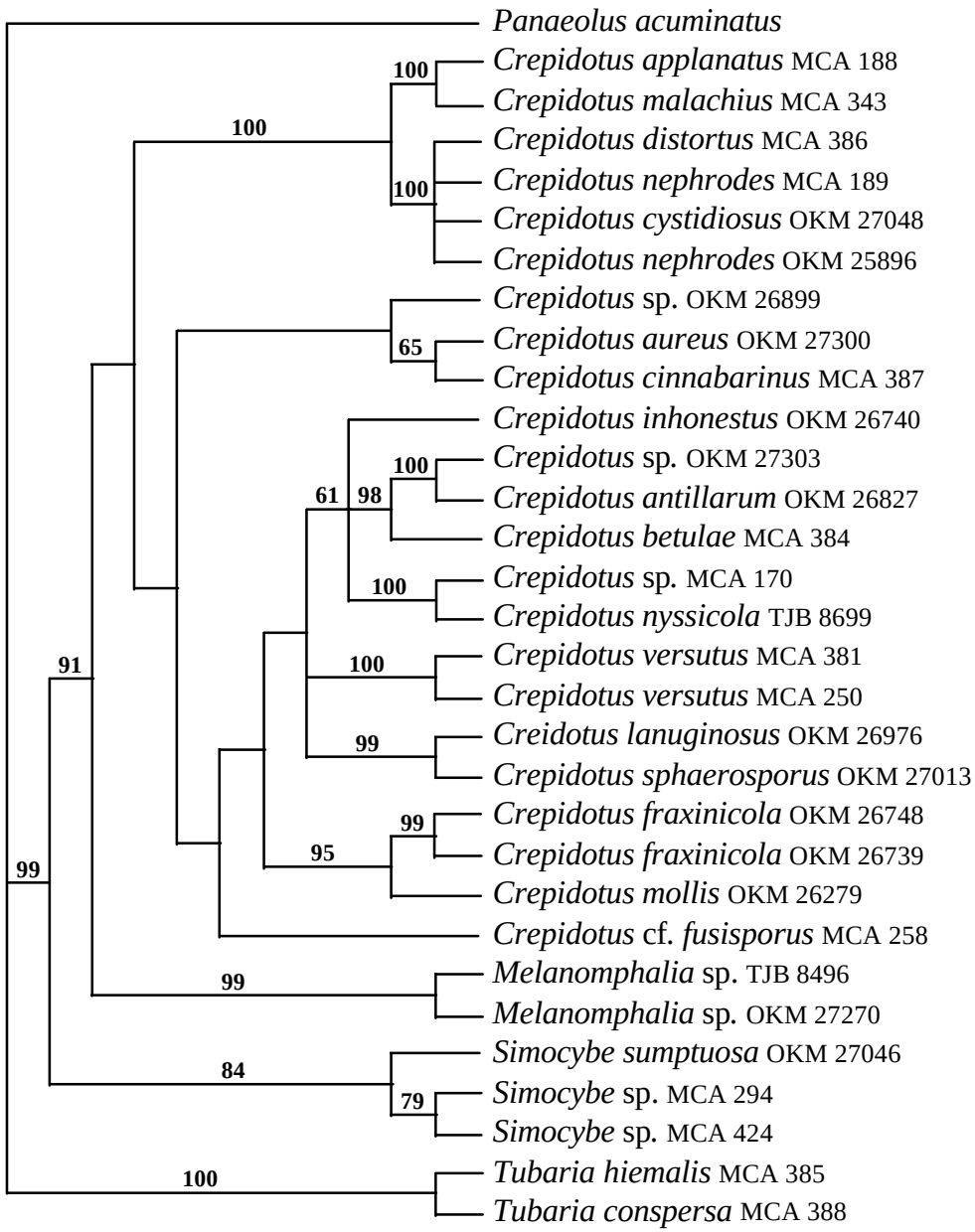

Figure 2.3. Constraint tree: strict consensus of six equally most parsimonious trees obtained from LSU rDNA sequencing data, with all *Crepidotus* taxa constrained as monophyletic (length = 425, CI = 0.506, and RI = 0.707). Bootstrapping values >60% from 100 replicates shown above supported branch.

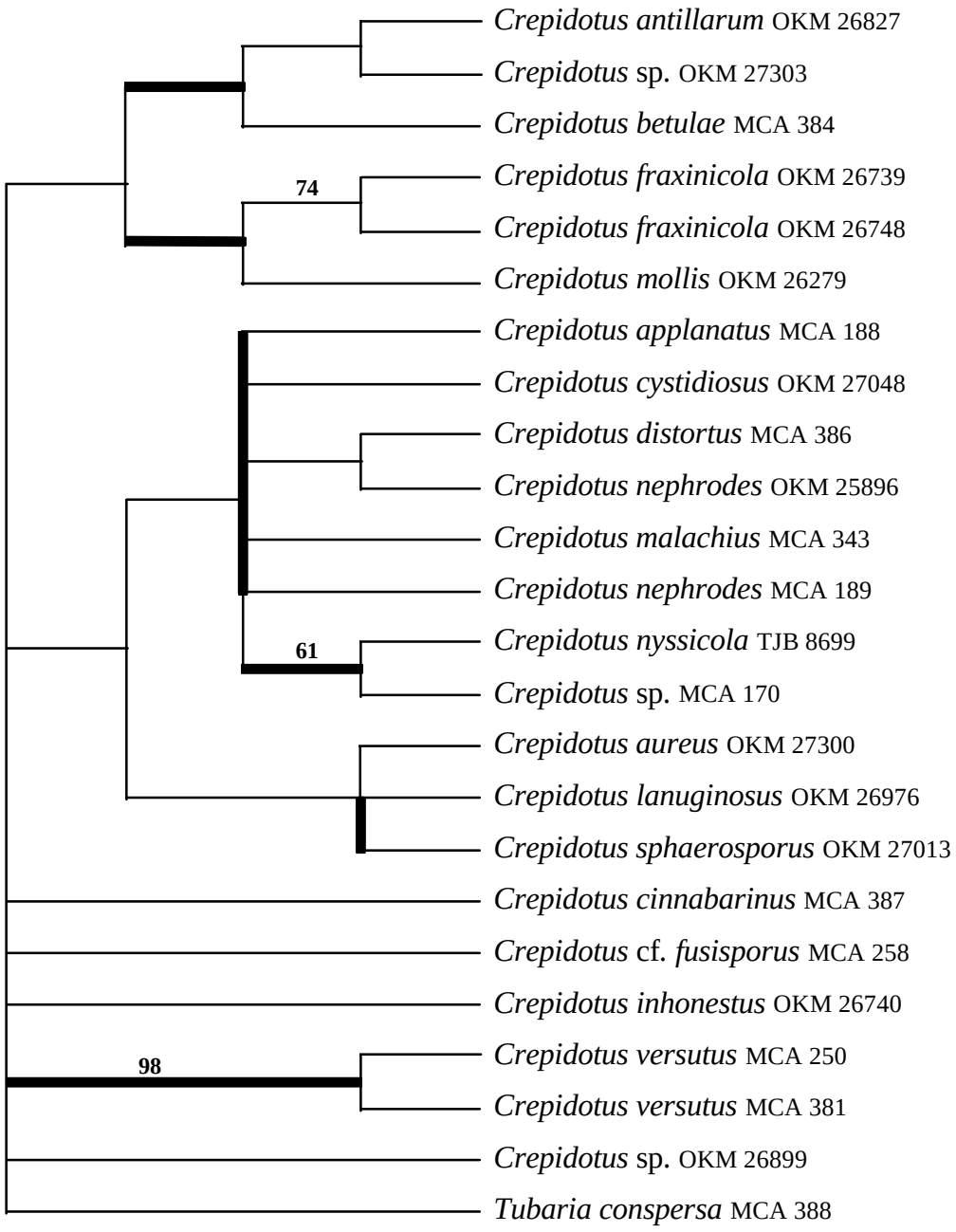

Figure 2.4. ***Crepidotus****:* strict consensus of 77 equally most parsimonious trees (length = 134, CI = 0.388, RI = 0.596) obtained when morphological data are analyzed. Bootstrapping values >60% form 100 bootstrapping replicates shown above supported branch. Thickened lines indicate a clade independently supported by LSU rDNA sequencing data (Figure 2.1).

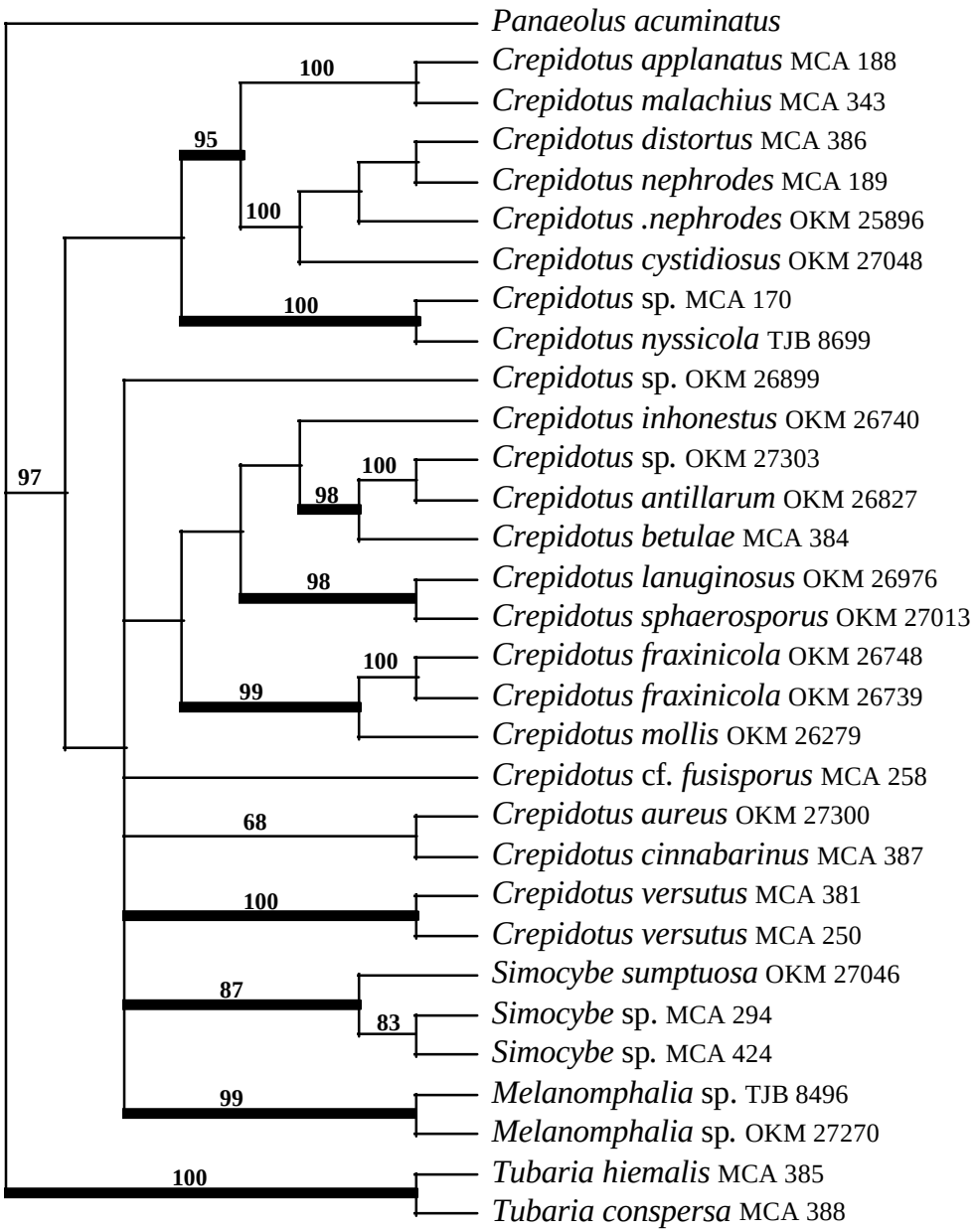

Figure 2.5. The Crepidotaceae: strict consensus of eight equally most parsimonious trees (length = 569, CI = 0.469, RI = 0.672) obtained from combining morphological data and LSU rDNA sequencing data. Bootstrapping values >60% from 100 bootstrapping replicates shown above supported branch. Thick lines indicate clades supported by LSU rDNA sequencing data alone (Figure 2.1).

Combining molecular and morphological data. The matrices from the LSU rDNA sequencing data and the morphological data were combined into one matrix with 1071 aligned characters. Figure 2.5 shows the strict consensus of eight equally most parsimonious trees (length = 569, CI = 0.469, RI = 0.672) obtained with *Panaeolus acuminatus* designated as outgroup. Bootstrapping values are noted above the supported branch. Nodes that represent a supported clade from the molecular data alone (Fig. 2.2) are indicated by a bold line leading to that node.

Clades supported by analysis of the sequencing data alone are also supported by the combined data. Topologically the two derived consensus trees (Fig. 2.2 and Fig. 2.5) differ in the following. (i) The combined data splits the Crepidotaceae into two clades. One clade consists of those species of *Crepidotus* with globose, punctate spores. The other clade consists of all *Crepidotus* species with elongate (Q>1.01) spores and the genera *Melanomphalia* and *Simocybe*, which also posses elongate spores. There is no bootstrapping support for either of these associations. (ii) The node representing *C. aureus* and *C. cinnabarinus* is more robust in the combined analysis, with bootstrapping support of 68%, where similar support is lacking in the molecular tree alone.

Discussion

Sequence data from the nuclear DNA encoding the large ribosomal subunit (LSU rDNA) have been demonstrated as reliable for the construction of fungal phylogenetic hypotheses at the generic and subgeneric levels (Moncalvo et al. 1995, Vilgalys et al. 1996, Moncalvo et al. 1999). Sequence data from a portion of the 5' end of the LSU

rDNA molecule suggest that the genus *Crepidotus* is not monophyletic. Results of parsimony analysis of the sequencing data, morphological data, and from combining both data sets, indicate that the Crepidotaceae consists of several monophyletic clades representing the genera *Simocybe*, *Melanomphalia*, and several segregates of *Crepidotus sensu lato*, although there is limited resolution of relationships between these clades. Neither the classification for *Crepidotus* proposed by Singer (1986), nor that of Hesler and Smith (1965), completely match the six monophyletic *Crepidotus* groupings supported by these data. However, the sections proposed by Hesler and Smith are fairly descriptive for most. Characters traditionally applied in *Crepidotus* taxonomy, such as clamp connections, or the presence of spore ornamentation, are inadequate for accurate prediction of phylogenetic relationships in this genus. These topics will be discussed in depth below.

Comparing and combining data sets. Mid-range consistency and retention indices (0.513 and 0.715, respectively, Fig. 2.1) obtained with parsimony analysis of the first data set (sequencing data only), indicate that some homoplasy is necessary to explain these data (Bremer 1988). Many more parallelisms and reversions are needed to explain the tree derived from the morphological data alone (CI = 0.388, RI = 0.596, Fig. 2.4), and this tree is the least resolved of the three data sets analyzed.

The utility of, and methodology for, combining data sets from different sources for deriving phylogenetic hypotheses is debatable (Rodrigo 1993, Lutzoni and Vilgalys 1994, Lutzoni 1997). However, the combination of morphological data and molecular data in this instance did not result in a tree much different from that derived by sequencing data

alone, excepting that the relationship between *C. aureus* and *C. cinnabarinus* is more strongly supported by the former (Fig. 2.5).

Analysis of all three data sets strongly support the taxon groupings represented by Clades 7 and 2 (illustrated in Fig. 2.2). The other *Crepidotus* groupings, represented by Clades 1, 3, 4, and 8, are strongly supported in both the first and third data sets, and are indicated in the morphological data with parsimony analysis. There remain five *Crepidotus* taxa, *C.* sp. (OKM 26899), *C. inhonestus, C.* cf. *fusisporus, C. aureus,* and *C. cinnabarinus*, whose affinities are unresolved with these data.

***Polyphyly of* Crepidotus sensu lato.** Historically, there is little basis for the supposition that *Crepidotus* is not a monophyletic genus. The genus *Dochmiopus* Pat. would encompass all those species of *Crepidotus sensu lato* possessing any type of spore ornamentation (Singer 1973), but this name was placed in synonymy with *Crepidotus* in 1946 (Hawksworth et al. 1995), and there is no indication from this study that all ornamented spored *Crepidotus* taxa (Clades 1, 2, 7, and 8) form a monophyletic group (Fig. 2.2).

These data indicate that "*Crepidotus*" does, however, represent a non-monophyletic group. Constraining all *Crepidotus* taxa as monophyletic within the Crepidotaceae resulted in a most parsimonious solution (Fig. 2.3) that was six steps longer than the unconstrained tree (Fig. 2.1). While relationships among clades are unresolved in the strict consensus tree of the LSU rDNA sequencing data, individual parsimony trees (not shown) all illustrate topologies inconsistent with a monophyletic origin for the *Crepidotus* taxa. Finally, combining both morphological and molecular data results in a

most parsimonious solution that does not maintain the monophyly of the genus *Crepidotus*.

Infrageneric classification. The classification for *Crepidotus* proposed by Singer (1986) does not reflect the phylogenetic groupings found in this study. For example, Singer's subsection *Porpophorini* includes all those taxa in Clades 1, 7, and 8, as well as *C. aureus*, *C.* sp. (OKM 26899), and *C.* cf. *fusisporus* (see Fig. 2.2). The classification proposed by Hesler and Smith (1965) fails to describe monophyletic groupings, as found in this study, at the subgeneric level, for example, subgenus *Crepidotus* includes all taxa of Clades 2 and 4, as well as *C. cinnabarinus*. However, the classification of Hesler and Smith at the section level is broadly useful for diagnosing the phylogenetic groupings of *Crepidotus* taxa found in this study.

A detailed discussion of the sections of *Crepidotus sensu lato* as inferred from these data follows. Clade numbers refer to the clades, illustrated in Figure 2.2, that are supported by all three data sets (molecular, morphological, and combined molecular and morphological).

Clade 1—The "Sphaerula" clade is a well-sampled clade that describes only a subset of Singer's subsection *Porpophorini* (typified by *C. applanatus* (Pers. ex. Fr.) Kummer), but is fairly well described by Hesler and Smith's section *Sphaerula* (typified by *C. applanatus* (Fr.) Kummer). These collections are all temperate in origin; fruiting bodies are hygrophanous with either white or brown cuticular fibrils; fruiting bodies and cultures form abundant clamp connections; and the spores are globose (Q=1) with column-like punctations that are truncate on the apex of the column as shown in SEM studies (Pegler

and Young 1972, Clemençon 1977). Oleiferous hyphae are common and abundant in these collections, and the basidia are normally 4-spored. The "Sphaerula" clade is represented here by *Crepidotus applanatus, C. cystidiosus, C. distortus, C. malachius,* and *C. nephrodes*.

Species with published descriptions that would place them in Clade 1 include: *Crepidotus subapplanatus* Hesler and Smith, *C. crocophyllus* (Berk.) Sacc. *C. maculans* Hesler and Smith, *C. conchatus* Hesler and Smith, *C. tahquamenonensis* Hesler and Smith, *C. avellaneus* Hesler and Smith, *C. angustifolius* Hesler and Smith, *C. obfuscens* Hesler and Smith, *C. sinuosus* Hesler and Smith, *C. varicolor* Hesler and Smith, *C. appalachianensis* Hesler and Smith, *C. subaureifolius* Hesler and Smith, and *C. aureifolius* Hesler and Smith.

Clade 2—The "Versuti" clade, represented by two collections of *Crepidotus versutus* (a normal 4-spored collection, and a 2-spored variety) coincides with Hesler and Smith's section *Versuti* (typified by *C. versutus* (Pk.) Sacc.). This clade is distinguishable from all others in that the species lack clamp connections, the cuticle is dry and fibrillose, and the spores are lightly pigmented and elliptical in shape with ornamentation that has been described as minutely punctate. Published SEM studies show the ornamentation of *C. versutus* to actually consist of a shallow, interwoven network of ridges (Senn-Irlet 1993). There are no other species of *Crepidotus* with this particular combination of characters, and *C. versutus* may be the only member of this evolutionary lineage. Hesler and Smith included three other species in this section: *Crepidotus herbarum* (Pk.) Sacc., now believed to be a synonym for *Pleurotellus hypnophilus* (Singer 1986); *C. bicolor* Murr.,

which is not considered a species by Singer (1986); and *C. coloradensis*, also not considered valid by Singer (1986).

Clade 3—The "Betulae" clade, represented by *Crepidotus betulae*, *C.* sp. (OKM 27303), and *C. antillarum*, coincide moderately well with Hesler and Smith's section *Betulae* (typified by *C. betulae* Murr.). In these collections, clamp connections are present in the fruiting body and the basidiospores are smooth under the light microscope and ellipsoid to elongate (Q=1.5-1.75), with a slight thinning on the apical end. In two of the collections a very definite gelatinous layer is visible in the subhymenium (MCA 384 and OKM 26827). The subhymenium in collection OKM 27303 appears composed of lysed cells within a thin gelatinous matrix with numerous oleiferous hyphae throughout the trama. Two of these collections are subtropical in origin (OKM 27303 and OKM 26827). One additional feature of these collections is that the basidia are predominantly 2-spored, interspersed with 1-, 3-, and 4-spored basidia.

Based on published descriptions, species with similar morphology to those in this group include *Crepidotus maximus* Hesler and Smith, and *C. serotinus* Singer. Several described species exist that share most of these features with Clade 3, but lack a gelatinous layer. They include: *Crepidotus amarus* Murr., *C. rhizomorphus* Burt. Ann., *C. albidus* E. and E., *C. albissimus* Murr., *C. acanthosyrinus* Singer, and *C. parlatorei* Singer.

Clade 4—The "Crepidotus" clade includes the type, *Crepidotus mollis*, and two collections of *C . fraxinicola*. It is defined both by Singer's subsection *Crepidotus* and by Hesler and Smith's section *Crepidotus*. The unique combination of characters that delimit

this group are the fruiting bodies that do not form clamp connections and the completely smooth, ellipsoid spores. In addition, these fungi have a thin gelatinous layer of hyphae in the pileus which can extend to the subhymenium.

Other described species that share this particular suite of characters include: *Crepidotus ochraceus* Hesler and Smith, *C. alabamensis* Murr., *C. uber* (B. and C.) Sacc., *C. sububer* Hesler and Smith, *C. tuxtlae* Singer, *C. molliformis* Singer, *C. levisporus* Singer, *C. yungicola* Singer, *C. sublevisporus* Singer, *C. citri* Pat., *C. subaffinis* Pilát, *C. parasiticus* Mass. ex Pilát, *C. calolepidoides* Murr., *C. alveolus* (Lasch) Kummer, *C. xanthophaeus* Singer, *C. brasiliensis* Rick, *C. melleus* (Berk. and Br.) Petch, *C. ampullicystis* Singer, *C. epigloeus* Singer, *C. eucalypticola* Singer, *C. variisporus* Singer, *C. calolepis* (Fr.) Karst, *C. spathulata* Bres., and *C. geophilus* (Murr.) Redhead.

Clade 7—The "Nyssicola" clade, represented by *Crepidotus nyssicola* and *C.* sp. (MCA 170), contain representatives from Hesler and Smith's section *Nyssicolae* (typified by *C. nyssicola*) and Hesler and Smith's *Sphaerula*. *Crepidotus nyssicola* is one of only two known stipitate species of *Crepidotus*, and although collection MCA 170 is an astipitate specimen, it does posses an elongate, attenuated sterile base. The spores in these two collections are subglobose (Q = 1.01) and punctate with a prominent, hyaline hilar appendage; oleiferous hyphae are numerous.

Clamp connections in the fruiting body of these two collections are never abundant, and not always evident in different mounts from a single collection.

Furthermore, collection MCA 170[6] does not produce clamps in dikaryotic culture whereas all dikaryotic cultures obtained for species from the "Sphaerula" clade produced numerous clamps.

Other species described as rarely forming clamps and having subglobose spores with punctate ornamentation are: *Crepidotus parvulus* Murr., *C. hygrophanus* Murr., *C. quitensis* Pat., *C. latifolius* Pk, and possibly *C. putrigenus* (Berk. and Curt.) Sacc.

Clades 1 and 7 apparently represent two lineages whose members have similar phenotypes but distinct evolutionary histories. For many *Crepidotus* species, alliance with either Clade 1 or Clade 7 cannot be determined from published descriptions. These include: *Crepidotus harperi* Singer, *C. praelatifolius* Murr., *C. confertus* Hesler and Smith, *C. flexuosus* Hesler and Smith, *C. constans* Hesler and Smith, *C. sublatifolius* Hesler and Smith, *C. pallidobrunneus* Hesler and Smith, *C. aquosus* Murr., *C. cuneiformis* Pat., *C. contortus* Hesler and Smith, *C. roseus* Singer, *C. montanus* Hesler and Smith, *C. campylus* Hesler and Smith, *C. subnidulans* (Overholtz) Hesler and Smith, and *C. subfibrillosus* Hesler and Smith.

Clade 8—The "Crepidotellae" clade, as represented by *Crepidotus lanuginosus* and *C. sphaerosporus,* coincides well with Hesler and Smith's section *Crepidotellae*. Members of this clade possess clamp connections on the fruiting body, and have broadly ellipsoid (Q=1.2-1.3) spores that are punctate and frequently (about 25%) collapsed. Published SEM studies show the ornamentation of *C. sphaerosporus* to be composed of pointed, conical columns (Clemençon 1977). The spores of *C. lanuginosus* have not been

[6]Dikaryotic cultures of *C. nyssicola* were unavailable for comparison.

examined by electron microscopy, but also appear echinulate under oil-immersion. Cheilocystidia in both these collections are often forked or knobbed at the apices; basidia are 2- or 4-spored. Described species with similar characters include: *Crepidotus ellipsoideus* Hesler and Smith, *C. regularis* Hesler and Smith, *C. villosus* Hesler and Smith, *C. vulgaris* Hesler and Smith, *C. submollis* Murrill, *C. cesati* (Rob.) Sacc., *C. circinatus* Hesler and Smith, and *C. milleri* Hesler and Smith.

Of the 23 *Crepidotus* taxa sampled, five show no supported affiliations with any of the above described clades. *Crepidotus inhonestus* would appear to belong to Clade 3 based on the presence of clamp connections and non-punctate elliptical spores, and both Hesler and Smith (1965) and Singer (1986) have postulated this association. The spores in the *C. inhonestus* collection though are ellipsoid (Q=1.4), inequilateral in profile, distinctly attenuated on the distal end, and are finely rugose in SEM studies (Pegler and Young 1972). Basidia are mainly 4-spored, but a few 2-spored exist. There is no gelatinous layer. Other described species that share these characters are: *Crepidotus lundellii* Pilát, *C. occidentalis* Hesler and Smith, and *C. pecten* (B. and C.) Sacc.

Crepidotus aureus is a subtropical collection not covered in the work of Hesler and Smith (1965). Morphologically this species would be placed in their section *Sphaerula*, based on the presence of clamps and the globose ornamented spores. Phenotypically, however, this collection possesses characters not found in the other members of Clade 1: yellow encrusting pigments on some of the cuticular hyphae that rapidly dissolve in 2X CTAB DNA extraction buffer and in KOH and NH_4OH mounting reagents; and encrusted metuloid cheilocystidia. Furthermore, although the spores of *C.*

aureus have not been examined by electron microscopy, under oil-immersion the ornamentation appears echinulate, like the ornamentation in *C. sphaerula*, not punctate, and 80^{+}% of the spores collapse when rehydrated. Other described species of *Crepidotus* with metuloid cheilocystidia and spiny globose spores are: *C. episphaeria* (Berk.) Saccardo, *C. hirsutellus* Horak, *C. nanicus* Horak, and *C. parietalis* Horak. There is no historically proposed segregate of *Crepidotus* that would accommodate these species.

Crepidotus cinnabarinus Peck represents a monotypic Hesler and Smith section, *Cinnabarini*. The spores of this species are robust, broadly ellipsoid (Q=1.27), punctate, and closely resemble the spores of some species of *Melanomphalia*. Published SEMs show the ornamentation composed of crowded, raised nodules, not columns (Luther and Redhead1981). Unlike *Melanomphalia*, *C. cinnabarinus* lacks clamp connections on the fruiting body. And like *C. aureus*, it contains pigments, red in this case, that dissolve in CTAB extraction buffer and alkaloid mounting reagents. Unlike *C. aureus*, however, the pigments of *C. cinnabarinus* are intracellular in origin, not encrusting.

Crepidotus cf. *fusisporus* (MCA 258) represents the monotypic section *Fusisporae* Hesler and Smith. There is a great deal of morphological variability in the species descriptions for *C. fusisporus*, and Hesler and Smith (1965) proposed several varieties of *C. fusisporus*, distinguished by spore size and form of the epicuticular hyphae, to deal with some of this heterogeneity. In this collection the spores are distinctive in that they are subfusoid (Q=1.99) in shape, attenuated at both ends, obscurely punctate, and remain clinging together even after discharge from the basidia. Published SEM studies (Senn-Irlet 1991) show that ornamentation in *C. fusisporus* is not columnar, but actually composed of

scattered, embedded nodules. Clamp connections are present on the fruiting body. The epicuticular hyphae in this collection is composed of scattered, erect, hyaline hyphae with frequent short lateral branches and diverticulate termini.

Collection OKM 26899, from Thailand, is morphologically similar to *C. fusisporus*, except that the spores are larger and broader (Q=1.66) and the epicuticular hyphae in this collection is formed of long hyaline ropes of clamped, parallel hyphae. Fruiting bodies of OKM 26899 are small (attaining a maximum of about 1mm in diameter) and gregarious. This collection is similar microscopically to published descriptions of *C. amygdalosporus* Kühner.

Morphology. These data indicate that the characters most widely employed for identification of *Crepidotus* taxa do not reflect phylogenetic relationships. Taxa inferred to be closely related on the basis of these molecular data do share the traditionally applied characters, but the same characters can also be found in other monophyletic clades. For example, clamp connections can be found in all the taxa of Clades 1, 3,5,6,7, and 8, and spore ornamentation is present in all taxa of Clades 1, 2, 6,7, and 8 (Fig. 2.2). The nature of basidiospore ornamentation, however, is a distinctive feature for each clade, and may be one of the key characters for diagnosing species of *Crepidotus sensu lato* to section.

The diagnostic value of clamp connections in agaric taxonomy has been questioned (Smith 1965), and their relevance to *Crepidotus* taxonomy debated (Horak 1964, Singer 1973). From these data, the presence or absence, and relative abundance, of clamp connections does appear diagnostic for a given clade, although their loss seems to have occurred more than once within the evolutionary history of these fungi.

The presence or absence of spore ornamentation is one of the key traditional characters used in diagnosing segregates of *Crepidotus sensu lato*. These data indicate that the type of ornamentation formed is of primary diagnostic value in these fungi, and that exosporial ornamentation has arisen independently several times in this family. Other characters that may be valuable diagnostically are: presence of gelatinous hyphae; sterigmata number; spore shape in profile and as measured by Q values; pigment characteristics; presence of oleiferous hyphae; and nature of the epicutis.

In summary, although this study indicates that the genus *Crepidotus* is not monophyletic, it is recommended that further studies be conducted before a taxonomic revision of these taxa is undertaken. Future research should concentrate on establishing the monophyletic groupings of *Crepidotus sensu lato*, their important diagnostic features, and phylogenetic relationships within the Crepidotaceae.

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OBSERVATIONS ON BASIDIOSPORE GERMINATION IN *CREPIDOTUS* (FR.) STAUDE AND RELATED GENERA

Abstract

Recovery of single spore isolates (SSI) is the fundamental first step for investigating fungal relationships via mating studies. The basidiospores of some Agaricales, however, are notoriously difficult to germinate in culture. Reported here for the first time is the recovery of SSIs from 11 species of *Crepidotus sensu lato*, one species of *Simocybe*, and one species of *Melanomphalia*. The identity of the derived SSI cultures was confirmed using DNA sequencing. In these species, basidiospores remained dormant on agar plates for an average of four to six months after collection. The dormant period remained consistent for a given collection, regardless of whether spores were plated immediately after harvesting, or stored for one to several months prior to plating. The factors frequently cited for breaking dormancy in basidiospores, i.e. cold treatments, and inhibitory and excitatory biotic growth factors, and regular exposure to light, had no effect on the length of dormancy in these species. Varying the media type and nutritional supplements likewise had no effect toward breaking the dormant period. Together, these results suggest that the basidiospores of these species undergo a period of endogenous spore dormancy, previously undemonstrated in the Agaricales. Post-dormancy germination appears optimized by providing a minimal carbon source, adequate water, and exposure to light.

Introduction

The Crepidotaceae (Basidiomycetes: Agaricales) are a dark-spored, saprophytic family, cosmopolitan in distribution, and most frequently inhabiting woody substrates (Singer 1986). Of the nine genera in the family proposed by Singer, the majority of species belong to the genus *Crepidotus* (Fr.) Staude, with approximately 150 described species (Hawksworth et al. 1995). These species are a common component of forest ecosystems. The other Crepidotaceae genera with more than five described species each are *Simocybe* P. Karsten, *Melanomphalia* M.P. Christiansen, and *Tubaria* (W.G. Smith) Gillet. Recent evidence from nuclear DNA large subunit ribosomal (LSU rDNA) sequencing data suggest that *Crepidotus*, *Melanomphalia*, and *Simocybe*, form a monophyletic core clade in the family (Chapters 1 and 2).

Obtaining single spore isolates from germinating basidiospores is the first step in performing mating studies in basidiomycetes (Raper 1966). Most saprophytic basidiomycetes germinate readily in culture (for example see Lamoure 1989, and Petersen 1997), although a few documented exceptions exist, most notably for species of *Pluteus* Fries (Agaricales: Pluteaceae) (Banerjee and Sundberg 1993). The mechanism behind failure to germinate *in vitro* in these instances has never been understood. Of the genera in the Crepidotaceae as proposed by Singer, only the basidiospores of *Tubaria* have been observed germinating in culture (Ingold 1983). Successful mating studies with any species of *Crepidotus*, *Simocybe*, or *Melanomphalia* remain unreported.

Accomplishing spore germination *in vitro* of mycorrhizal and parasitic basidiomycetes is more difficult, in general, than for saprophytes, and much research has

been conducted toward understanding spore dormancy in these cases. Fries and others have worked to elucidate the effects of either supplying or removing external growth factors, produced by basidiospores or growing hyphae, on the basidiospore germination of mycorrhizal agarics (Bulmer and Beneke 1964, Fries 1977, Fries 1978, Fries 1979, Fries 1983, see also Macko et al. 1974, and Nguyen et al. 1987). Other researchers have found that cold regimens will break dormancy in the spores of some basidiomycetes (Bromfield 1964, Booth 1971, Gemma and Koske 1988, Griffen 1994). In addition, spore germination in some parasitic basidiomycetes as well as a few saprophytic phycomycetes appears to be activated by exposure to light (Sussman 1966). Nutritional supplements such as thiamin, biotin, and nitrogen have also been shown to be important for germination and subsequent growth of recalcitrant basidiomycetes (Palmer 1969).

In an attempt to recover SSIs from species of *Crepidotus*, it was discovered that those basidiospores that germinated did so only after incubation on malt agar plates for approximately five months. Sequencing of the LSU rDNA region of randomly selected basidiocarps and derived SSI cultures confirmed the identity of these cultures. Further experimentation was designed in an attempt to break this period of spore dormancy by cold treating, varying spore concentration per plate, altering the supplied nutritional component, varying the time interval between harvesting and plating spores, and by varying the amount of light exposure received by spores. Similar experiments were also conducted on the basidiospores of the related genera *Simocybe* and *Melanomphalia*. Herein are reported the results from a preliminary investigation into the phenomena of spore dormancy in these fungi.

Materials and Methods

Materials. Methods for obtaining and identifying collections are described on page 22. Collection data for all materials studied are given in Appendix A. Virginia Tech Mycology Herbarium (VPI) and Virginia Tech Culture Collection (VTCC) assignments are listed in Appendix B.

Basidiospore harvesting. Fresh basidiocarps were placed on sterile white bond paper and covered with a glass Petri dish until spores were cast. The resulting spore prints were labeled, kept moisture free, and stored in individual manila envelopes at room temperature. Spores from some basidiocarps were also cast directly onto Petri plates containing nutritive agar media and stored in a dark incubator at 26 C. Spore prints were harvested on the day of collection, therefore, spore release date (the assumed time of spore maturation) is identical to collection date.

Single spore isolate (SSI) harvesting. The methods for recovering SSIs have been frequently described (for example, Booth 1971). In brief, basidiospores from a spore print were aseptically transferred into a test tube of sterile de-ionized water and agitated. Approximately three drops of this suspension were spread evenly onto a Petri dish containing agar media, labeled with the collection number, date, and dilution letter “A”. Approximately 10 mL from this first suspension was transferred to a new test tube of sterile water, agitated, plated, and marked as dilution “B”. This process was repeated with successive serial dilutions marked “C” and “D”. Plates were incubated and checked approximately twice a week for germinations. The germination date was recorded as the time when mycelial growth was first visible to the unaided eye.

As soon as it was observed, the mycelia from a germinating spore was aseptically transferred to a 60-mm Petri plate containing malt agar and marked with the collection number, isolate number (serially ascribed, beginning with .1), and date. A maximum of approximately 20 germinations were isolated for a single collection. Isolates were allowed to grow out at 26 C in the dark. Once sufficient growth was achieved, a squash mount of a piece of the growing mycelia was stained in congo red and examined under an oil-immersion objective to confirm that the growth was consistent with growth of a basidiomycete and not a contaminant.

SSI screening. Basidiomycete cultures were next screened to ascertain their nuclear status. Two methods were employed. First, in order to know if a given culture will produce clamps in the dikaryotic state, the mycelium of a multisporous culture, produced by allowing many of the unselected spores on an isolation plate to grow together, were screened for clamps. A small piece of the actively growing mycelium was aseptically transferred onto a slide, stained with congo red, and viewed under oil immersion. If a culture produced clamps in the dikaryotic state, SSIs were checked by screening for the absence of clamps in the same manner. If no clamps were found, after screening for approximately 10 minutes, the isolate was assumed to be monokaryotic. Cultures for which the dikaryotic mycelium did not produce clamps were screened by nuclear staining. A small piece of the growing mycelium was placed on a microscope slide and stained with DAPI (4,6-diamidino-s-phenylindole) (Williamson and Trezzi 1979). The nuclei were then viewed by fluorescence microscopy on the highest objective. The absence of two nuclei per hyphal cell would indicate a monokaryotic isolate, or SSI.

Standard conditions. Standard experimental control conditions consisted of basidiospore dilutions plated on malt agar (15 g malt extract, 15 g agar, 1 L dH_20) on 100-mm Petri plates and incubated in the dark at 26 C for the duration of the experiments. Plates were wrapped tightly in parafilm and re-wrapped whenever the original parafilm appeared to be loosing its integrity. Controls were exposed to only brief intervals of light during regular screenings. Signs of growth on any plate were immediately checked under the dissecting or compound light microscope. Plates with contaminating bacterial or filamentous fungal colonies were destroyed.

Nutritional experiments. In addition to platings on malt agar, the same basidiospore suspensions from many collections were also plated on one or more of the following media: water agar (15 g agar in 1 L dH_20); yeast extract agar (15 g yeast extract, 15 g agar in 1 L dH_20); potato dextrose agar (39 g DIFCO potato dextrose agar in 1 L dH_20); nitrogen-enriched agar (15 g malt extract, 1.5 g ammonium hydroxide, 15 g agar in 1 L dH_20); biotin- and thiamin-enriched agar (15 g malt extract, 5 µg biotin, 100 µg thiamin, 15 g agar in 1 L dH_20). Plates were subsequently treated as controls.

Light experiments. To test the effects of light exposure, duplicate dilution series plates for selected collections were made from the same dilution tubes onto malt agar plates. One series of plates was incubated under standard conditions, the other series was placed in a growth chamber and exposed to a continual cycle of 12 h of light and 12 h of dark at 26 C for the duration of the study.

Cold treatments. Duplicate dilution series plates were made from the same serial dilution tubes onto malt agar. One series was incubated under standard conditions, and the other series was first incubated at 4 C for two weeks and then incubated at 26 C for the remainder of the study.

Repeated platings. Those collections with sufficiently strong spore deposits were also subjected to additional dilution platings, made from the original spore prints, at monthly intervals. These plates were treated under the same incubation conditions as all standard control plates.

Gauging of inhibitory and excitatory factors. The use of serial dilutions and spore drop cultures for each collection was used to gauge any inhibitory or excitatory effects due to spore concentration. Plates with *Rhodotorula* cultures were allowed to continue incubating in sealed plastic containers within the growth chambers.

DNA sequencing and analysis. Methods for DNA extraction, amplification, purification, sequencing, and sequencing analysis were described on pages 22-27. Three SSI cultures were randomly selected from the first (1996-1997) growing season and the LSU rDNA region was sequenced. A total of 29 sequences were combined in this data set, consisting of 16 species of dark-spored agarics, and eight species from the Crepidotaceae *sensu* Singer, with *Ganoderma australe* (Basidiomycetes: Aphyllophorales) chosen as the outgroup. The eight *Crepidotus* sequences comprising four species were obtained from the sporocarps of the germinating collections, from the SSI cultures, and from two other *Crepidotus* taxa. Taxa analyzed are shown in Table 1.1 (Chapter 1, p.33) with the following additions: *C. fraxinicola* OKM 26748, collected from Washington, USA, first

cited in this work; *C. fraxinicola* OKM 26739.5, single spore isolate from culture, first cited in this work; *C. fraxinicola* OKM 26748.2, single spore isolate from culture, first cited in this work; *C. applanatus* MCA 188.8, single spore isolate from culture, first cited in this work. Sequence alignments are given in Appendix C.

Results

A total of 41 collections of the agaricoid taxa of the Crepidotaceae consisting of 35 collections of *Crepidotus sensu lato*, one collection of *Melanomphalia*, and five collections of *Simocybe*, were plated during a two-year interval in an attempt to obtain single spore isolates (Table 3.1). Of the 386 total plates made, more than half were eventually destroyed due to contamination or drying, and basidiospore germination was recorded on only 51 of the remainder. On those plates where more than one germination occurred, subsequent germinations were scattered, sporadic but steady, gradually tapering in frequency for several months. For species of *Crepidotus*, *Melanomphalia*, and *Simocybe*, a minimum of 85 days and a maximum of 277 days elapsed on average, before germination, with the bulk of plates requiring between four and six months incubation time. Overall statistics are given in Table 3.1. Collection dates and data are provided in Appendix A.

Table 3.1. List of *Crepidotus, Melanomphalia,* and *Simocybe* collections plated

Species	Coll. #	# plates made[i]	# plates germinated[ii]	avg. days to germ.[iii]	germination time frame[iv]	% germ.[v]	#SSIs[vi]
Crepidotus* section *Versuti:							
C. versutus	MCA 250	5	0			0	
C. versutus	MCA 267	5	0			0	
C. versutus	MCA 382	5	0			0	
C. versutus	MCA 383	2	0			0	
Crepidotus* section *Betulae							
C. antillarum	OKM 26827	9	0			0	
C. antillarum	OKM 27248	7	0			0	
C. betulae	MCA 384	7	1	116	Feb-Mar	14.3	20+, psi
Crepidotus* section *Sphaerula:							
C. appalachianensis	MCA 322	3	0			0	
C. appalachianensis	MCA 373	34	9	178	Jan-May	26.5	38+, psi
C. applanatus	MCA 188	7	1	156	Feb-Apr	14.3	19+, psi
C. applanatus	MCA 317	24	0			0	
C. applanatus	MCA 348	10	0			0	
C. applanatus	MCA 351	13	2	209	Jan-May	15.4	9
C. applanatus	MCA 366	20	0			0	
C. cystidiosus	OKM 27048	11	0			0	
C. distortus	MCA 386	9	7	153	Feb-May	77.8	28+, psi
C. latifolius	OKM 27051	9	0			0	
C. malachius	MCA 335	3	0			0	
C. malachius	MCA 343	19	4	188	Feb-June	21.1	33+, psi
C. nephrodes	MCA 189	11	5	199	Feb-May	45.5	20+, psi
C. subapplanatus	MCA 331	12	0			0	
Crepidotus* section *Nyssicolae:							
C. nyssicola	TJB 8699	7	0			0	
C. sp.	MCA 170	8	1	213	Apr	12.5	psi
Crepidotus* section *Crepidotellae:							
C. lanuginosus	OKM 26976	11	0			0	
C. sphaerosporus	OKM 27013	14	1	146	Jan	7.1	2
Crepidotus* section *Crepidotus:							
C. fraxinicola	OKM 26739	7	5	151	Jan-Sep	71.4	7
C. fraxinicola	OKM 26741	7	2	112	Jan-Apr	28.6	2
C. fraxinicola	OKM 26748	4	2	277	July-Oct	50	2
C. mollis	MCA 289	12	0			0	
C. mollis	MCA 394	3	1	125	June	33.3	1

Table 3.1. Continued

Species	Coll. #	# plates made[i]	# plates germinated[ii]	avg. days to germ.[iii]	germination time frame[iv]	% germ.[v]	#SSIs[vi]
Crepidotus* section *Fusisporae:							
C. cf. *fusisporus*	MCA 258	11	3	199	Feb-July	27.3	19+, psi
***Crepidotus sensu lato* (unassigned section):**							
C. aureus	OKM 27300	17	5	85	Mar-May	29.4	4, psi
C. cf. *levisporus*	OKM 26826	3	0			0	
C. cf. *lundellii*	MCA 163	5	0			0	
C. inhonestus	OKM 26740	4	0				
Melanomphalia:							
M. sp.	OKM 27270	7	1	~180	July	14.3	psi
Simocybe:							
S. centuncula	MCA 393	3	0				
S. sumptuosa	OKM 27046	14	0				
S. sumptuosa	OKM 27047	14	0				
S. sp.	MCA 294	7	1	104	Oct-Nov	14.3	4
S. sp.	MCA 424	3	0				

[i] The total of all experimental and standard control plates made for the given collection.
[ii] The total number, out of all plates made, that showed at least one germinating basidiospore for the collection.
[iii] The number of days to germination for a single plate is measured as the number of days between the date of collection and the date a germinating basidiospore was first recorded for that plate. The average for a collection is then obtained by dividing the sum of all plate averages for the collection by the total of number of plates with germinating spores for the collection.
[iv] The month in which the first record of a basidiospore germination was recorded on any plate for the collection, and the last month in which a newly germinating basidiospore was recorded for the collection.
[v] The number of plates with at least one basidiospore germination recorded divided by the total number of plates made for the collection.
[vi] The number of SSIs recovered for the collection. A "+" indicates that spores were still germinating, but not recovered. "psi" indicates a polysporous isolate (dikaryon) was recovered in lieu of or in addition to the SSIs recovered.

Dikaryotic cultural characters on malt agar were in general agreement with Senn-Irlet (1994). Monokaryotic hyphae was always multinucleate in those cultures examined. Stephanocysts (Burdsall 1969, Senn-Irlet and Scheidegger 1994) were observed on dikaryotic cultures of *C. appalachianensis* (MCA 373) and *C. nephrodes* (MCA 189). Collections that did not form clamps in the fruiting bodies never formed clamps in dikaryotic culture. Collections that form clamps in the fruiting bodies formed them in dikaryotic culture, with the exception of *Crepidotus* sp. (MCA 170) which forms clamps in the cuticular hyphae of the fruiting body, but not in culture.

DNA sequencing. The LSU rDNA gene region was sequenced for a total of 29 taxa representing three randomly selected *Crepidotus* SSI cultures, sporocarp tissue from five *Crepidotus* collections, and 20 other agaric taxa with *Ganoderma australe* as an outgroup. Sequence alignments with excluded variably aligned regions indicated are shown in Appendix C. Of the 933 characters aligned, 79 characters were excluded from analysis, and 112 of the remaining characters were parsimony-informative.

Two equally most parsimonious trees were obtained (length = 448, CI = 0.5123, RI = 0.6324). Figure 3.1 shows the bootstrapping consensus tree. Bootstrapping values >50% from 100 replicates are shown as the first number above the supported branch. Jackknifing values >50% from 100 replicates are shown as the second number above the supported branch. Decay values of >1 are noted below the supported branches. Sequences from SSI cultures and the associated sporocarp tissue are indicated in bold type. In each case, the culture sequences are indistinguishable from the associated sporocarp tissue sequences. In fact, sequences from monokaryotic SSI cultures are

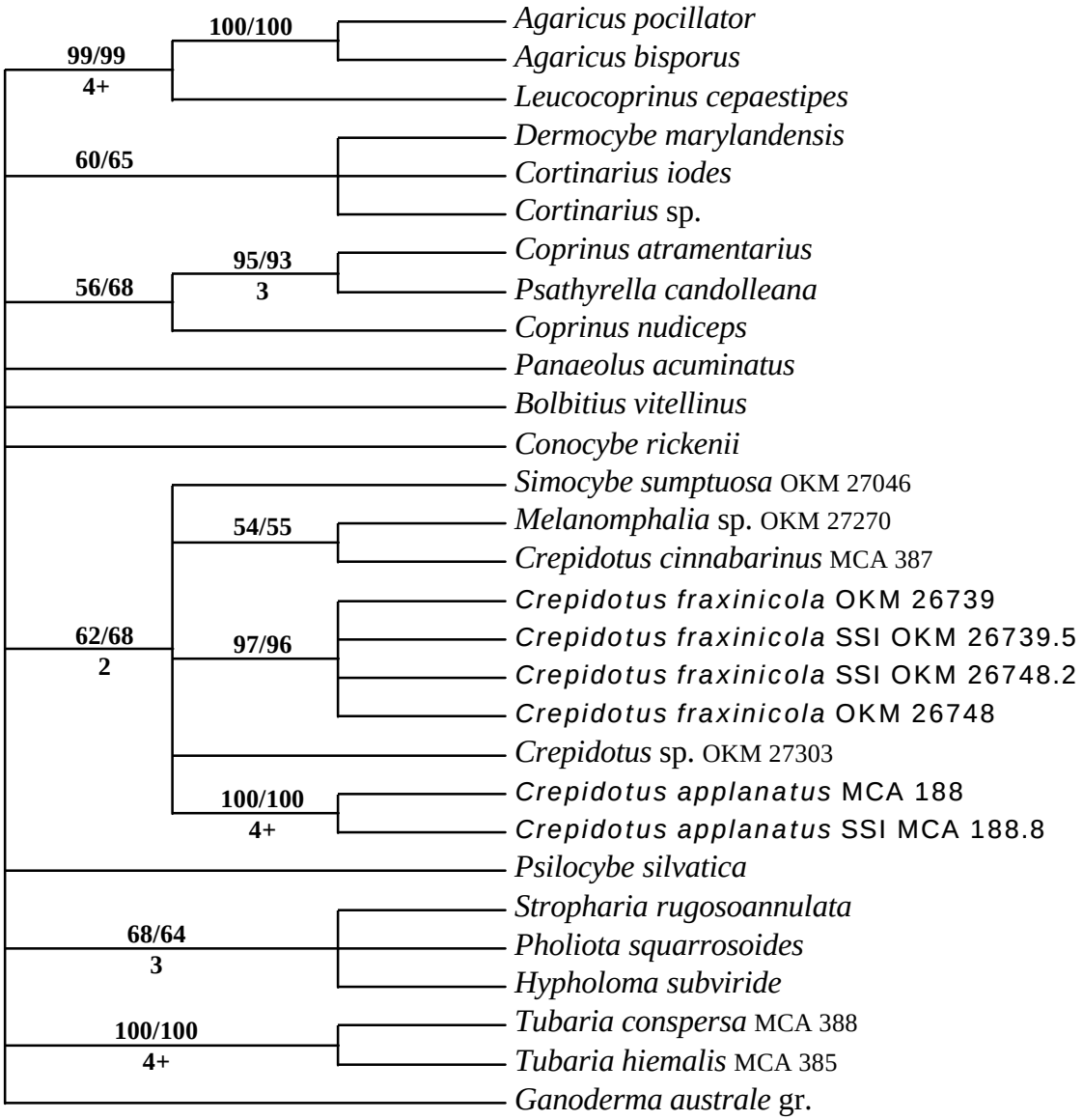

Figure 3.1. Identity of SSI cultures: bootstrapping 50% majority rule consensus tree from 100 bootstrapping replicates. Two equally most parsimonious trees were found (length = 448, CI = 0.5123, RI = 0.6324). Bootstrap values shown as first number above supported branch; jackknife values from 100 replicates shown as second number above supported branch. Decay values shown below supported branch. Names in bold are sequence pairs taken from cap material and culture material from single spore isolates (SSI).

nearly identical to sequences from the associated dikaryotic cap tissue (Appendix C), the only difference being a one-base insert at position 691 in the sporocarp sequence of collection MCA 188 that is lacking in the SSI sequence for culture MCA 188.8. The parsimony-derived clades of *Crepidotus fraxinicola* (sporocarps OKM 26739 and OKM 26748, and SSIs OKM 26739.5 and OKM 26748.2) and *C. applanatus* (sporocarp MCA 188 and SSI MCA 188.8) are strongly supported by both bootstrapping (97% and 100% respectively) and jackknifing (96% and 100% respectively). What is interesting, and inexplicable, is that although the four sequences of *C. fraxinicola* are identical (Appendix C), this node collapses in the consensus of all trees only one step greater (449) than the most parsimonious tree (448). The node supporting the *C. applanatus* clade, however, remains stable for trees at least four steps greater than the most parsimonious trees.

Melanomphalia. A dikaryotic culture derived from a polysporus deposit was recovered for a species of *Melanomphalia*. This collection, OKM 27270, was plated two weeks after collection on malt agar, and incubated under standard conditions for an approximately six months before germination was recorded (Table 3.1).

Simocybe. Five collections of *Simocybe* were plated. Of these, only one plate, from collection MCA 294, contained any germinating basidiospores. This species was plated seven weeks after collection on malt agar, and incubated under standard conditions. The first germinating spore appeared 51 days after plating and the remaining three spores germinated within the following week. A total of three and a half months (104-111 days) elapsed between time of spore release and time of spore germination (Table 3.1).

Crepidotus sensu lato. Single spore isolates were recovered for 15 collections of *Crepidotus sensu lato*, representing 11 different species (Table 3.1). For those collections of *Crepidotus sensu lato* that germinated, the spores remained dormant on average for 167 days (from time of collection) until germination. Patterns of basidiospore germination in these 15 collections of *Crepidotus sensu lato* will be discussed in the remainder of this section.

Variation by section. The sections of *Crepidotus sensu lato* were described in the previous chapter. Enough data are available to compare germination patterns for three of these: *Sphaerula*, *Crepidotus,* and *Versuti*. Species in section *Crepidotus* took an average of 166 days to germinate. Basidiospores began germinating as soon as two months after collection, and continued germinating for nearly a year after collection. Germination events in this section were scattered and few. Those plates with germination contained only one to two germinating spores each.

Spores on germinating plates in Section *Sphaerula* took an average of 181 days to germinate. Spores germinated between four and seven months after collection, with the majority of spores germinating four to five months after collection. Germination events in this section were steady after the initial germination, usually with a minimum of ten germinations occurring per plate. No spore germinations were recorded for a collection of *Crepidotus* in the section *Versuti*.

Table 3.2. Germination by media type in *Crepidotus sensu lato*

Agar media	# plates	# germinating	expected	% germinating
Malt extract	261	45	45	17.2
Water	40	0	6.7	0
Potato dextrose	21	1	3.5	4.8
Yeast extract	6	0	1	0
Biotin-thiamin enriched	4	0	0.7	0
Nitrogen enriched	6	2	1	33.3

The number of plates expected to germinate is based on the control (malt) plates. Approximately one of every six controls plated showed germination.

Table 3.3. Effects of cold treatment and light exposure on germination in *Crepidotus appalachianensis* (MCA 373)

	coll. date	plate date	germ. date	#SSIs	days to germination: from coll.	days to germination: from plating
Control	9/17/98	2/24/98	5/4/98	15	229	69
Cold treated	9/17/98	2/24/98	4/21/98	14	216	56
Light exposure	9/17/98	2/24/98	4/2898	~312	223	63

The number of SSIs for the light exposed plate was estimated by counting the number of spores in a quadrant and multiplying by four. All three plates are of malt agar and were plated from the same spore suspension. "coll." = collection; "germ." = germination.

Table 3.4. Time of plating and germination in *Crepidotus sensu lato*

Collection	coll. date	plate date	germ. date	#SSIs	days to germination: from coll.	from plating
C. appalachianensis (MCA 373)	9/17/97	10/18/97	1/6/98	5	111	80
"	"	11/15/97	2/5/98	8	141	82
"	"	"	2/8/98	4	144	85
"	"	"*	2/24/98	9	160	101
"	"	1/13/98	3/5/98	12	169	51
"	"	"	4/10/98	3***	205	87
"	"	2/24/98	5/4/98	15***	229	69
C. applanatus (MCA 351)	8/28/97	9/8/97	5/19/98	1	264	253
"	"	11/15/97*	1/29/98	8	154	75
C. distortus (MCA 386)	10/31/97	11/15/97	2/24/98	17	116	101
"	"	"	3/13/98	1	133	118
"	"	"	3/26/98	1	146	131
"	"	1/13/98	4/10/98	5	161	87
"	"	"**	2/24/98	4	116	42
C. malachius (MCA 343)	8/21/97	9/8/97	3/5/98	6	196	178
"	"	10/18/97	2/2/98	18	165	107
"	"	"	3/1/98	3	192	134
"	"	1/13/98	3/9/98	6	200	55
C. nephrodes (MCA 189)	9/14/96	9/17/96	4/10/97	5***	208	205
"	"	"	2/17/97	4	156	153
"	"	"	3/4/97	6	171	168
"	"	2/27/97	4/10/97	10	208	42
"	"	"	5/25/97	1***	253	87

*All plates incubated under standard conditions on malt agar except *incubated on nitrogen-enriched agar and **incubated on potato dextrose agar. Number of SSIs indicates number of isolates recovered for a plate except ***indicates approximate number of germinations on a plate that were not recovered (due to already large number of existing isolates for that collection). Data shown is for all dilution (A-D) plates. "coll." = collection; "germ." = germination.*

Table 3.5. Plate dilution and germination in *Crepidotus sensu lato*

Collection	coll. date	plate date	germ. date	#SSIs	days to germ. from coll.	dilution
C. aureus (OKM 27300)	1/19/98	1/19/98	5/19/98	1	120	A
“	“	“	3/20/98	1	60	A
“	“	“	3/17/98	1	57	B
C. appalachianensis (MCA 373)	9/17/97	11/15/97	2/5/98	8	141	A
“	“	“	2/8/98	4	144	B
“	“	1/13/98	3/5/98	12	169	A
“	“	“	4/10/98	3**	205	B
C. distortus (MCA 386)	10/31/97	11/15/97	2/24/98	17	116	A
“	“	“	3/13/98	1	133	B
“	“	“	3/26/98	1	146	C
“	“	1/13/98	4/10/98	5	161	A
“	“	“*	2/24/98	4	116	B
C. fraxinicola (OKM 26739)	11/16/96	11/25/96	9/3/97	1	291	A
“	“	“	5/7/97	1	172	B
“	“	“	1/6/97	1	51	C
“	“	“	1/6/97	2	51	D
C. fraxinicola (OKM 26748)	11/16/96	11/25/96	7/25/97	1	221	B
“	“	“	10/14/97	1	332	D
C. cf. *fusisporus* (MCA 258)	10/10/96	2/27/97	4/28/97	12	200	A
“	“	“	6/25/97	2	258	B
C. malachius (MCA 343)	8/21/97	10/18/97	2/2/98	18	165	A
“	“	“	3/1/98	3	192	B
C. nephrodes (MCA 189)	9/14/96	9/17/96	4/10/97	5**	208	B
”	“	“	2/17/97	4	156	C
“	“	“	3/4/97	6	171	D

*All plates incubated under standard conditions on malt agar except *incubated on potato dextrose agar. Number of SSIs indicates number of isolates recovered for a plate except **indicates approximate number of germinations on a plate that were not recovered (due to already large number of existing isolates for that collection). Dilutions are relative measures within one single series, and do not denote comparable spore concentrations between series. Within a single series, concentrations range from A (highest) to D (lowest spore concentration), with an approximate 5-fold decrease in spore concentration at each dilution. “coll.” = collection; “germ.” = germination.*

Variation by locale. All germinating collections of *Crepidotus sensu lato* were from temperate North America with the exception of *C. aureus* (OKM 27300). The individual plates of *C. aureus* averaged between 57 and 120 days to germinate, with an overall average of 85 days. The temperate collections took, on average, between 112 and 277 days to germinate.

Nutrition. Basidiospores were plated on a total of six different media types (malt extract, potato dextrose, yeast extract, biotin-thiamin enriched, nitrogen enriched, and water agar). A breakdown of the plates made for collections of *Crepidotus sensu lato* by medium is given in Table 3.2. The alternate carbon source plates (potato dextrose and yeast) show no increase in number of germinated plates, and deviate little from the expected number of germinating plates. Likewise, germination on the nutritionally supplemented plates (biotin-thiamin and nitrogen) does not vary much from the expected. Length of dormancy and numbers of germinating spores were no higher for any of these alternate nutritional media. However, of 40 plates made on water agar, all failed to germinate.

Cold treating and light. The effects of treatment at cold temperatures on basidiospore germination in *Crepidotus sensu lato* was tested on seven collections. Both experimental and control plates failed to germinate for six of these trials. The effects of regular, periodic light exposure were also tested on seven collections; these plates were especially prone to contamination, and six of the trials were destroyed. Results from the successful trial are shown in Table 3.3.

Multiple platings. The spore prints from 23 of the collections of *Crepidotus sensu lato* were subjected to multiple monthly platings. Plates from five of these collections showed germination in more than one trial. Results from these successful trials are shown in Table 3.4.

Inhibitory and excitatory factors. Nine plates with *Rhodotorula* cultures (Fries 1977, Fries 1983) in addition to spore suspensions were incubated for up to one year after plating, but never showed basidiospore germination. The effects of spore concentration were gauged by making several different dilutions per collections. Those collections that showed germination on more than one different dilution plate from the same plating date are shown in Table 3.5. The concentration of spores on a given plate had little effect on whether spores germinated on that plate or not; spores germinated during the same time period for the same collection regardless of dilution for many collections (Table 3.5). Overall, more SSIs were recovered from low dilution (A-B) plates, where the spore concentration is highest, than on successively higher (C-D) dilution plates.

Discussion

Molecular data from a 933 base region of the 5' end of the LSU rDNA gene confirm the identity of three randomly selected SSI cultures as species of *Crepidotus* (Fig. 3.1), and not a late growing contaminant. All other cultures were consistent in morphology and growth patterns with the confirmed *Crepidotus* cultures and with the studies of Senn-Irlet (1994).

Germinating collections of *Crepidotus* did so on average from 85 to 277 days after spore discharge (Table 3.1); the germination range for individual spores was 49-277 days after discharge. This latent period was observed in all germinating species of *Crepidotus* examined. Patterns of dormancy and germination varied somewhat between sections of *Crepidotus sensu lato*. Species from at least six of the previously described sections of *Crepidotus* (Chapter 2) showed some basidiospore germination, but collections from section *Versuti*, collected and plated over a two year period, completely failed to germinate. The species in section *Sphaerula* fruited in late summer or fall; those collections that germinated began to do so sporadically in mid-winter, with the bulk of spores germinating in late winter in relatively high numbers, and then tapering off into late spring. Species from section *Crepidotus*, in contrast, were collected during the winter, and germinated from a few months to nearly a year after collection, but in extremely low frequencies. The other sections of *Crepidotus* contained only one germinating collection each, and therefore cannot be meaningfully compared. *Crepidotus aureus* (OKM 27300), a sub-tropical species, showed a latent period of between 57 and 120 days which is shorter than for all the other *Crepidotus* species studied.

An attempt was made to recover SSIs for the other agaricoid members of the Crepidotaceae. Only four SSIs germinated for a species of *Simocybe*, and only one dikaryon was recovered for a species of *Melanomphalia*. These numbers are too small to draw conclusions, however, the lengthy interval between plating and germination for these two collections (104 and 124 days respectively) is consistent with that found in germinating collections of *Crepidotus sensu lato*.

Two different types of dormancy have been widely described in fungal spores (Sussman 1966, Griffin 1994). Exogenous dormancy is the condition of delayed germination due to the lack of favorable environmental conditions. Exogenous dormancy is broken with the addition or subtraction of the necessary external factors, such as provision of specific nutritional requirements, temperature constraints, and the presence or absence of light, and of excitatory or inhibitory biotic growth factors. Endogenous (sometimes termed constitutional) dormancy cannot be broken by environmental factors, but rather, is an innate property of the spore. These spores remain dormant for a required period of time, regardless of external conditions. Examples of endogenous spore dormancy have not been demonstrated in the Agaricales, but many mycorrhizal and parasitic basidiomycetes are known to exhibit some form of exogenous spore dormancy.

None of the treatments most commonly proscribed for breaking exogenously dormant spores were effective in shortening the latent period for the species of *Crepidotus* examined. Cold temperatures and light exposure are frequently employed to activate germination of parasitic and mycorrhizal basidiomycete spores (Bromfield 1964, Sussman 1966, Bloom 1971, Safir et al. 1990). Cold treating showed no affect on either length of the dormant period or frequency of germinations as compared to controls for the same collection, nor did regular exposure to light alter the latent period for this same collection (Table 3.3).

Differences in spore concentration from the same dilution series was on the order of 10,000:1 between an A and D plate. In successful dilution trials, spores germinated within the same time frame on several different dilution platings (Table 3.5). Thus,

inhibitory or excitatory factors due to relative spore concentration do not appear to have a role in dormancy length for these species. Excitatory factors supplied through inoculation of *Rhodotorula* cultures likewise failed to break spore dormancy. Finally, the various nutritional media employed here had no effect on breaking spore dormancy.

These preliminary data are suggestive of an innate latent period in the spores of some *Crepidotus* species that is not broken by external factors. For example (Table 3.4), basidiospores from a single spore print of *C. malachius* (MCA 343) were diluted and plated two weeks after collection, two months after collection, and five months after collection, and three plates (one from each different interval) all began germinating within 10 days of each other, six months after the original collection was made. No environmental factors have been discovered thus far that are capable of shortening or breaking this endogenous dormant period.

However, the low numbers of recovered germinating spores per plate, and the low overall number of germinating plates in this study, would suggest that once endogenous dormancy has ended, some other requirement for growth is not being satisfactorily met. Some researchers have implicated more complex patterns of dormancy in other fungi, where both exogenous and endogenous dormancy may be exhibited by spores of the same species (Gemma and Koske 1988, Miller et al. 1993, d'Enfert 1997). In the Crepidotaceae taxa examined here, it is possible that a threshold period of maturation, during which the spore is impervious to environmental influence, is followed by an environmentally activated stage.

The factors required for activation at the end of the maturation period would certainly include water and oxygen (Šubíková and Šubík 1974, d'Enfert 1977, Gottlieb 1978), and as the water content and humidity on these plates is gradually depleted over the long incubation time, this may account, in part, for the relatively low number of germinations. And there definitely appears to be a minimal nutritional requirement, as no germinations were ever encountered on non-nutritive water agar (Table 3.2).

Interestingly, exposure to light may be a secondary requirement for germination. Although all three plates shown in Table 3.3 were made from the same spore suspension, and all germinated within two weeks of each other, the plate that was regularly exposed to light showed about a 20-fold increase in the number of germinating spores compared to the control plate. Exposure to light however, is not a requirement for subsequent culture growth, and it does not shorten the endogenously dormant stage.

All plates were exposed to daylight during the regular weekly screenings, and if this requirement is minimal, some spores may have accumulated enough light exposure to germinate. This activation by light exposure would explain why there was a delay in germination when spores from a spore print were plated after spores from the same collection were already germinating on plates made at an earlier time.

In summary, these experiments demonstrate that obtaining single spore isolates from species of *Crepidotus* and its relatives *Simocybe* and *Melanomphalia* appear dependent on several factors. First, in those species sampled, basidiospore dormancy appears to be under endogenous control, and is not affected by such external factors as light, nutrition, temperature, or spore concentration. Second, once the endogenous period

has expired, subsequent germination of the basidiospores appears to be optimized by supplying a minimal nutritive source such as malt extract, and most likely by maintaining moisture content and by exposure to light. However, more work is needed to further elucidate this phenomenon of basidiospore dormancy and germination in these fungi.

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CONCLUSIONS

The Crepidotaceae (Imai) Singer is a morphologically diverse family of saprophytic agarics with members that have repeatedly undergone reductions in form leading to an astipitate or cyphelloid habit in a large portion of the species. Phylogenetic inference, based on strongly supported LSU rDNA sequencing data, is that *Crepidotus* (Fr.) Staude, *Simocybe* P. Karsten, and *Melanomphalia* M.P. Christiansen form a monophyletic core clade of fungi in the Agaricales (Fig. 1.1), recognizable as the Crepidotaceae. Together, these genera represent about 215 of the approximately 240 species believed to belong in the Crepidotaceae *sensu* Singer. No support is found for other classifications that ally these genera within either the Cortinariaceae or the Strophariaceae.

The genus *Crepidotus* may actually consist of several different evolutionary lineages that do not appear to be monophyletic in origin based on LSU rDNA sequencing data (Figs. 2.1 and 2.3). Traditional morphological methods for dividing this heterogeneous group into evolutionarily meaningful taxonomic sections do not adequately reflect phylogenetic histories between the natural segregates. The data in Chapter 2 show that a few characters not taxonomically applied in this group, for example, the nature of observable spore ornamentation, and spore quotients as a measure of spore shape, may be synapomorphies, but that traditionally applied characters such as clamp connection formation are homoplasic in origin.

Several species of *Crepidotus sensu lato*, and potentially also species of *Simocybe* and *Melanomphalia*, display a pattern of basidiospore dormancy and germination that has

not been previously described in other species of Agaricales. Extended dormant periods were observed in all germinating taxa, from seven of the eight evolutionary clades described in Figure 2.2. (Table 3.1). This phenomena, too, may be further evidence for the unique evolutionary history shared by these fungi.

APPENDIX A. EXAMINED COLLECTIONS CITED IN THIS WORK

Taxon	Collection #	Date	Locale	Collector	Determination
Crepidotus antillarum (Pat. apud Duss) Sing.	OKM 26827	1/14/97	Dominican Republic	O.K. Miller & S. Cantrell	M.C. Aime
Crepidotus antillarum (Pat. apud Duss) Sing.	OKM 27248	1/13/98	Puerto Rico, USA	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus appalachianensis Hes. & Sm.	MCA 322	7/23/97	North Carolina, USA	S. Miller	M.C. Aime
Crepidotus appalachianensis Hes. & Sm.	MCA 373	9/17/97	North Carolina, USA	A. Stanley	M.C. Aime
Crepidotus applanatus (Pers.) Kummer	MCA 188	9/14/96	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus applanatus (Pers.) Kummer	MCA 317	7/22/97	North Carolina, USA	M.C. Aime	M.C. Aime
Crepidotus applanatus (Pers.) Kummer	MCA 348	7/31/97	Vermont, USA	S. Miller	M.C. Aime
Crepidotus applanatus (Pers.) Kummer	MCA 351	8/28/97	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus applanatus (Pers.) Kummer	MCA 366	9/18/97	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus aureus Horak	OKM 27300	1/19/98	Puerto Rico, USA	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus betulae Murr.	MCA 384	10/31/97	North Carolina, USA	R. Vilgalys & S. Miller	M.C. Aime
Crepidotus cinnabarinus[i] Pk.	MCA 387	7/29/96	New York, USA	A. Bessette	A. Bessette
Crepidotus cystidiosus Hes. & Sm.	OKM 27048	9/19/97	Virginia, USA	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus distortus Hes. & Sm.	MCA 386	10/31/97	North Carolina, USA	R. Vilgalys & S. Miller	M.C. Aime
Crepidotus fraxinicola Murr.	OKM 26739	11/16/96	Washington, USA	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus fraxinicola Murr.	OKM 26741	11/16/96	Washington, USA	B. Woo	M.C. Aime
Crepidotus fraxinicola Murr.	OKM 26748	11/16/96	Washington, USA	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus cf. *fusisporus* Hes. & Sm.	MCA 258	10/10/96	Virginia, USA	O.K. Miller	M.C. Aime
Crepidotus inhonestus Karst.	OKM 26740	11/16/96	Washington, USA	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus lanuginosus Hes. & Sm.	OKM 26976	7/2/97	Oregon, USA	M. Bailey	O.K. Miller
Crepidotus latifolius Pk.	OKM 27051	9/20/97	Virginia, USA	O.K. Miller	M.C. Aime
Crepidotus cf. *levisporus* Sing.	OKM 26826	1/14/97	Dominican Republic	O.K. Miller	M.C. Aime
Crepidotus cf. *lundellii* Pilát	MCA 163	9/5/96	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus malachius (B. & C.) Sacc.	MCA 335	9/23/95	Virginia, USA	J. Walker	M.C. Aime
Crepidotus malachius (B. & C.) Sacc.	MCA 343	8/21/97	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus mollis (Schaeff. ex Fr.) Kummer	MCA 289	4/27/97	Virginia, USA	J. Walker	M.C. Aime
Crepidotus mollis (Schaeff. ex Fr.) Kummer	MCA 394	2/15/98	California, USA	S. Miller	M.C. Aime
Crepidotus mollis (Schaeff. ex Fr.) Kummer	OKM 26279	6/13/95	Montana, USA	L. Bailey	L. Bailey
Crepidotus nephrodes (B. & C.) Sacc.	MCA 189	9/14/96	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus nephrodes (B. & C.) Sacc.	OKM 25896	9/26/93	Virginia, USA	O.K. Miller	O.K. Miller
Crepidotus nyssicola[ii] (Murr.) Sing.	TJB 8699	1/16/98	Maine, USA	S. Ristich	T. Baroni
Crepidotus sphaerosporus (Pat.) Lange	OKM 27013	8/14/97	Colorado, USA	J. Murphy	M.C. Aime
Crepidotus subapplanatus Hes. & Sm.	MCA 331	8/10/97	Virginia, USA	S. Miller	M.C. Aime

APPENDIX A. CONTINUED

Taxon	Collection #	Date	Locale	Collector	Determination
Crepidotus versutus (Pk.) Sacc.	MCA 250	10/10/96	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus versutus (Pk.) Sacc.	MCA 267	10/12/96	Virginia, USA	M.C. Aime	M.C. Aime
Crepidotus versutus (Pk.) Sacc.	MCA 381	10/18/97	North Carolina, USA	foray member	M.C. Aime
Crepidotus versutus (Pk.) Sacc.	MCA 382	10/18/97	North Carolina, USA	foray member	M.C. Aime
Crepidotus versutus (Pk.) Sacc.	MCA 383	10/18/97	North Carolina, USA	foray member	M.C. Aime
Crepidotus sp.	MCA 170	9/11/96	Virginia, USA	O.K. Miller	M.C. Aime
Crepidotus sp.	OKM 26899	5/29/97	Thailand	O.K. Miller & H.H. Miller	M.C. Aime
Crepidotus sp.	OKM 27303	1/19/98	Puerto Rico, USA	O.K. Miller & H.H. Miller	M.C. Aime
Melanomphalia sp.	OKM 27270	1/15/98	Puerto Rico, USA	O.K. Miller & H.H. Miller	M.C. Aime
Melanomphalia sp.[iii]	TJB 8496	6/7/97	Puerto Rico, USA	T. Baroni & R. Vilgalys	T. Baroni
Simocybe centuncula (Fr.) Karst.	MCA 393	2/15/98	California, USA	foray member	M.C. Aime
Simocybe sumptuosa (Orton) Sing.	OKM 27046	9/18/97	Virginia, USA	O.K. Miller	M.C. Aime
Simocybe sumptuosa (Orton) Sing.	OKM 27047	9/18/97	Virginia, USA	O.K. Miller	M.C. Aime
Simocybe sp.	MCA 294	7/17/97	Virginia, USA	R. Vilgalys & M.C. Aime	R. Vilgalys & M.C. Aime
Simocybe sp.	MCA 424	4/26/98	Virginia, USA	J. Johnson	M.C. Aime
Tubaria conspersa (Pers. ex Fr.) Fayod	MCA 388	11/11/97	Virginia, USA	M.C. Aime	M.C. Aime
Tubaria hiemalis Romagnesi ex Bon	MCA 385	10/31/97	North Carolina, USA	R. Vilgalys & S. Miller	M.C. Aime

[i] MCA 387, *C. cinnabarinus* denotes a split collection, donated in part by Dr. Alan Bessette; Bessette coll. #10645. This collection is illustrated on page 283 of *Mushrooms of Eastern North America* (Bessette, A. E., A.R. Bessette, and D.W. Fischer. 1998. Syracuse University Press, NY. 583 pp.).
[ii] TJB 8699, *C. nyssicola*, was examined on loan from Dr. Tim Baroni, SUNY-Cortland (CORT).
[iii] TJB 8496, *Melanomphalia* sp., was examined on loan from Dr. Tim Baroni, SUNY-Cortland (CORT).

APPENDIX B. HERBARIA AND CULTURE COLLECTION ASSIGNMENTS

Virginia Tech Herbarium (VPI) and Virginia Tech Culture Collection numbers for collections cited in this work

Taxon	Collection #	Herbarium #	Culture Collection #[i]
Crepidotus antillarum	OKM 26827	VTMH 3956	
Crepidotus antillarum	OKM 27248	VTMH 4662	
Crepidotus appalachianensis	MCA 322	VTMH 5294	
Crepidotus appalachianensis	MCA 373	VTMH 5281	VTCC 3600
Crepidotus applanatus	MCA 188	VTMH 5206	VTCC 3587
Crepidotus applanatus	MCA 188.8		VTCC 3587.8
Crepidotus applanatus	MCA 317	VTMH 5282	
Crepidotus applanatus	MCA 348	VTMH 5295	
Crepidotus applanatus	MCA 351	VTMH 5283	VTCC 3601
Crepidotus applanatus	MCA 366	VTMH 5285	
Crepidotus aureus	OKM 27300	VTMH 4655	VTCC 3602
Crepidotus betulae	MCA 384	VTMH 5208	VTCC 3595
Crepidotus cinnabarinus	MCA 387 (AB10645)	VTMH 5209	
Crepidotus cystidiosus	OKM 27048	VTMH 4456	
Crepidotus distortus	MCA 386	VTMH 5211	VTCC 3603
Crepidotus fraxinicola	OKM 26739	VTMH 3795	VTCC 3586
Crepidotus fraxinicola	OKM 26739.5		VTCC 3586.5
Crepidotus fraxinicola	OKM 26741	VTMH 3796	VTCC 3584
Crepidotus fraxinicola	OKM 26748	VTMH 3797	VTCC 3583
Crepidotus fraxinicola	OKM 26748.2		VTCC 3583.2
Crepidotus cf. *fusisporus*	MCA 258	VTMH 5201	VTCC 3604
Crepidotus inhonestus	OKM 26740	VTMH 3798	
Crepidotus lanuginosus	OKM 26976	VTMH 5212	
Crepidotus latifolius	OKM 27051	VTMH 4455	
Crepidotus cf. *levisporus*	OKM 26826	VTMH 3984	
Crepidotus cf. *lundellii*	MCA 163	VTMH 5251	
Crepidotus malachius	MCA 335	VTMH 5286	
Crepidotus malachius	MCA 343	VTMH 5203	VTCC 3605
Crepidotus mollis	MCA 289	VTMH 5293	
Crepidotus mollis	MCA 394	VTMH 5287	VTCC 3606
Crepidotus mollis	OKM 26279	VTMH 3078	
Crepidotus nephrodes	MCA 189	VTMH 5207	VTCC 3588
Crepidotus nephrodes	OKM 25896	VTMH 1624	
Crepidotus sphaerosporus	OKM 27013	VTMH 5214	VTCC 3607
Crepidotus subapplanatus	MCA 331	VTMH 5284	
Crepidotus versutus	MCA 250	VTMH 5289	
Crepidotus versutus	MCA 267	VTMH 5291	
Crepidotus versutus	MCA 381	VTMH 5288	
Crepidotus versutus	MCA 382	VTMH 5292	
Crepidotus versutus	MCA 383	VTMH 5290	

APPENDIX B. CONTINUED

Virginia Tech Herbarium (VPI) and Virginia Tech Culture Collection numbers for collections cited in this work

Taxon	Collection #	Herbarium #	Culture Collection #[i]
Crepidotus sp.	MCA 170	VTMH 5210	VTCC 3608
Crepidotus sp.	OKM 26899	VTMH 4026	
Crepidotus sp.	OKM 27303	VTMH 4661	
Melanomphalia sp.	OKM 27270	VTMH 4656	VTCC 3615
Simocybe centuncula	MCA 393	VTMH 5308	
Simocybe sumptuosa	OKM 27046	VTMH 4457	
Simocybe sumptuosa	OKM 27047	VTMH 4458	
Simocybe sp.	MCA 294	VTMH 5213	VTCC 3616
Simocybe sp.	MCA 424	VTMH 5204	
Tubaria conspersa	MCA 388	VTMH 5202	VTCC 3610
Tubaria hiemalis	MCA 385	VTMH 5205	VTCC 3613

[i] Individual VTCC assignments are not given for each SSI culture, unless the isolate was specifically cited in this work. Culture SSIs are given the same VTCC root number, followed by a decimal and the SSI number.

State University College of New York-Cortland Herbarium (CORT) numbers for collections cited in this work

Taxon	Collection #
Crepidotus nyssicola	TJB 8699
Melanomphalia sp.	TJB 8496

Appendix C. DNA Sequence Alignments/Chapter 1 and Chapter 3

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        5                 1 5                 2 5                 3 5                 4 5                 5 5
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G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C G G T C T T J 173
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C G G T C T T SAR 88/411
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A G A G C T C A A A T T T A A A A T C T G N C G G T C T C EFM 548
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T JM 96/24
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G T C A G T C T T JM 96/23
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T JM 96/40
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G T G G T C T T C 114
G A T T C C C C T A G T A A C T G C G A G T G A A G A G G G A A A A G C T C A A A T T T A A A A T C T G G C A G C C T T C 159
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G T G G T C T C J 181
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T J 129
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T SAR 84/100
G A T T C C C C T A G T A A C T G C N A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T J 183
N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N OKM 27046
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C G G T C T T RV 5-7-1989
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G A C A G T C T T D 258
G A T T C C C C T A G T A A C T G C G A G T G A A N C G G G A A A A G C T C A A A T T T A A A A T C T G G T G G T C T T JJ 7
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T JJ 69
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T MCA 388
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T MCA 385
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T G A A A T C T G G C A G T C T T OKM 27270
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T A A A A T C T G G C A G T C T T OKM 26739
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T G A A A T C T G G C A G T C T T OKM 27303
N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N MCA 387
G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T G A A A T C T G G C G G T C T T MCA 188
G A T T C C C C T A G T A A C T G C G A G T G A A N N G G G A A A A G N N N A A A T T T A A A A T C T G G C G G T C T T JM RSH-0705

G A T T C C C C T A G T A A C T G C G A G T G A A G C G G G A A A A G C T C A A A T T T G A A A T C T G G C A G T C T T MCA 188.8
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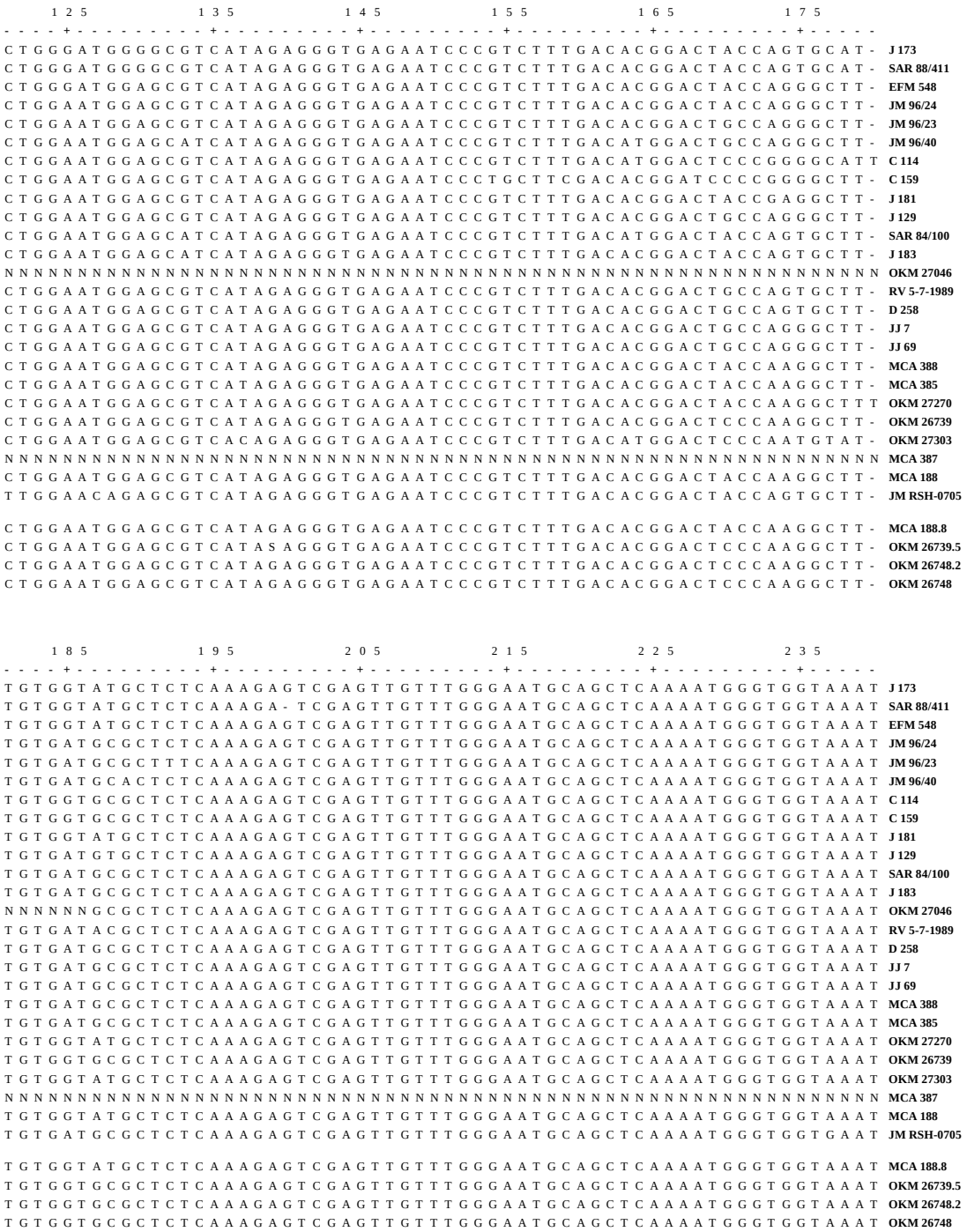
First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.

*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapters 1 & 3



First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.
*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapters 1 & 3

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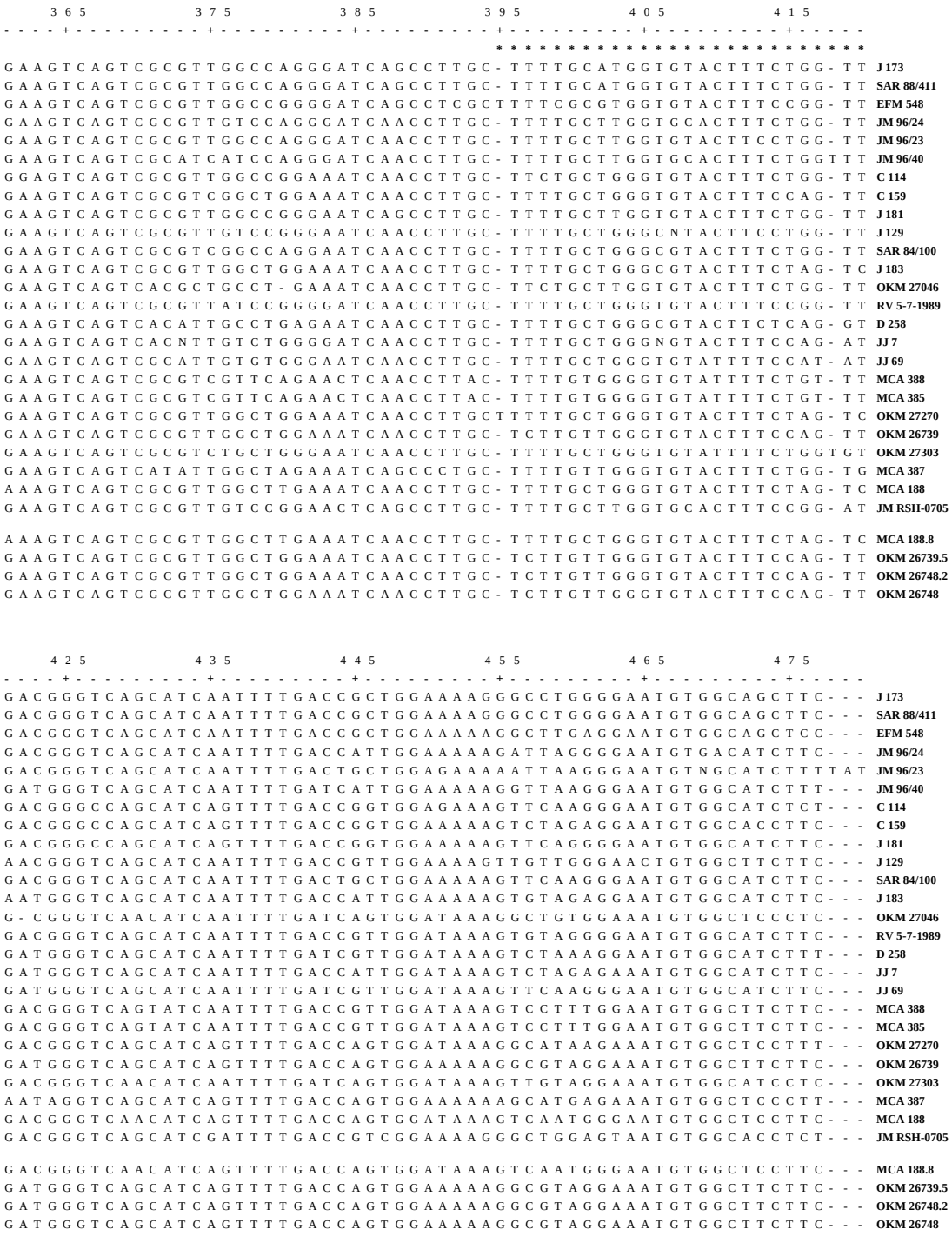
First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.
*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapters 1 & 3



First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.
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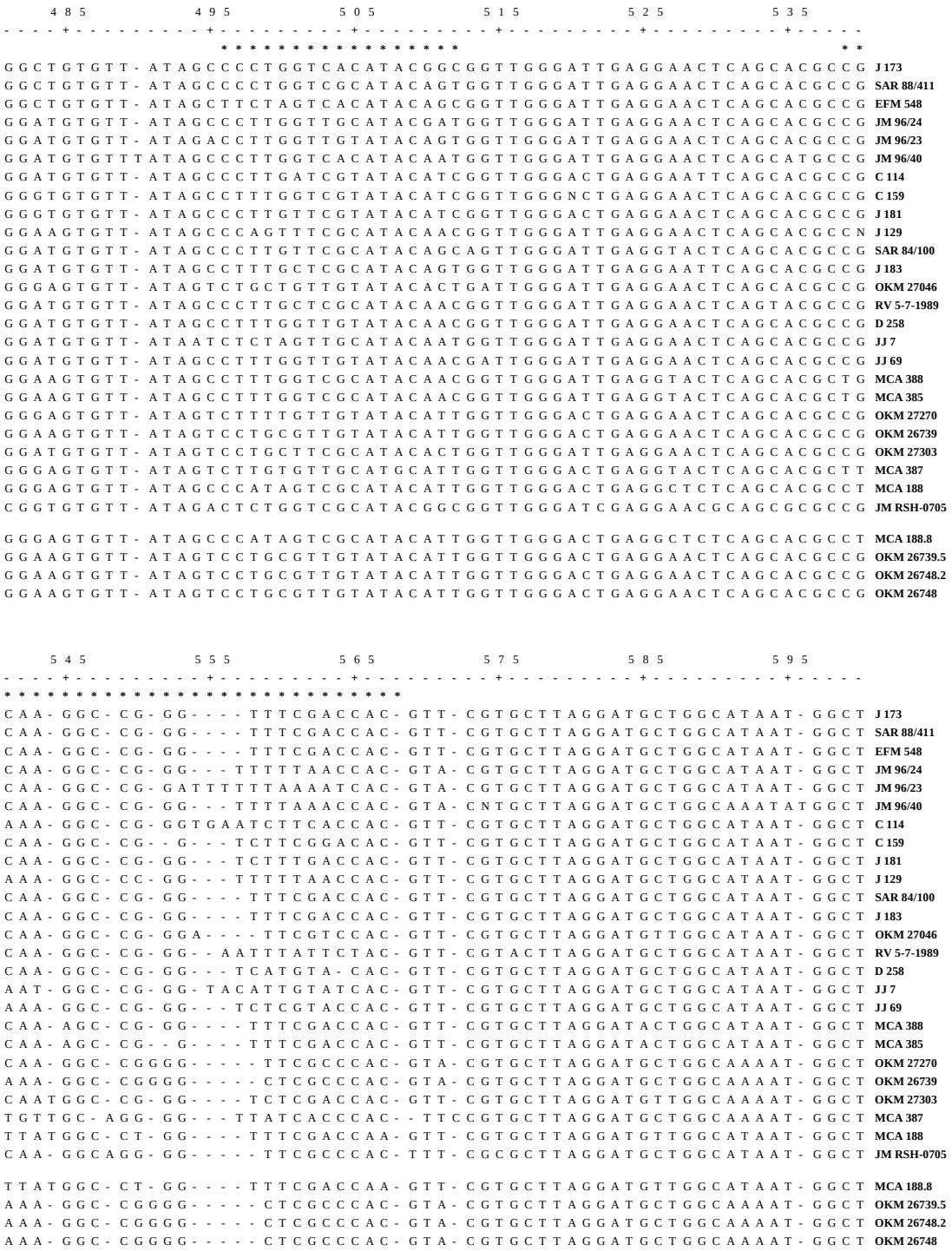
Appendix C. Continued: Sequence alignments for Chapters 1 & 3



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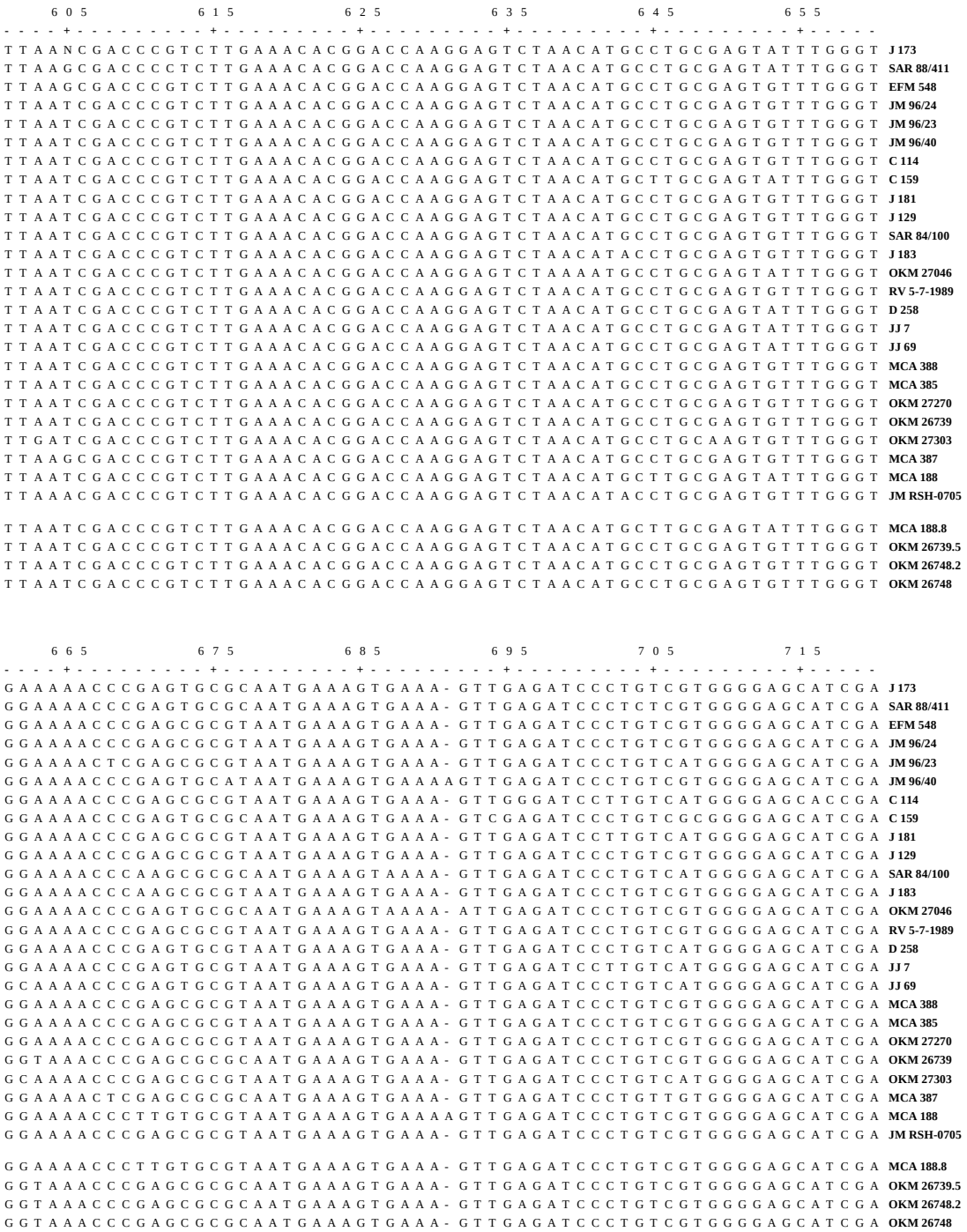
Appendix C. Continued: Sequence alignments for Chapters 1 & 3



First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.
*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapters 1 & 3

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C G C C C G G A C C A G A A C T T T T G G G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A  EFM 548
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G A T G G T G A A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C  MCA 387
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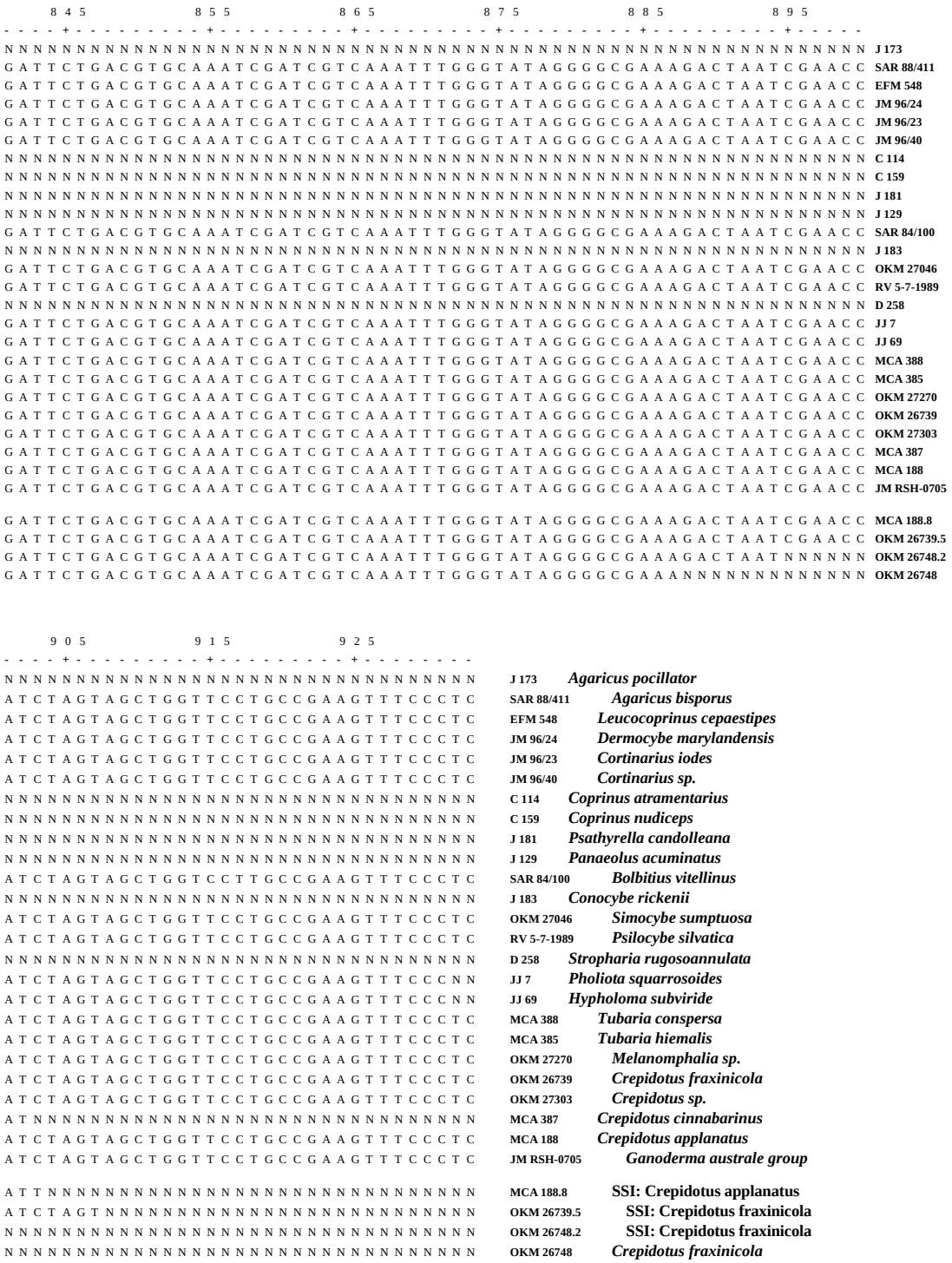
First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.
*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapters 1 & 3



First 25 sequences analyzed in Chapter 1. All 29 sequences analyzed in Chapter 3.
*indicates ambiguously aligned region excluded from analysis.

Appendix C. DNA Sequence Alignments/Chapter 2

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      5              15              25              35              45              55
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      65              75              85              95             105             115
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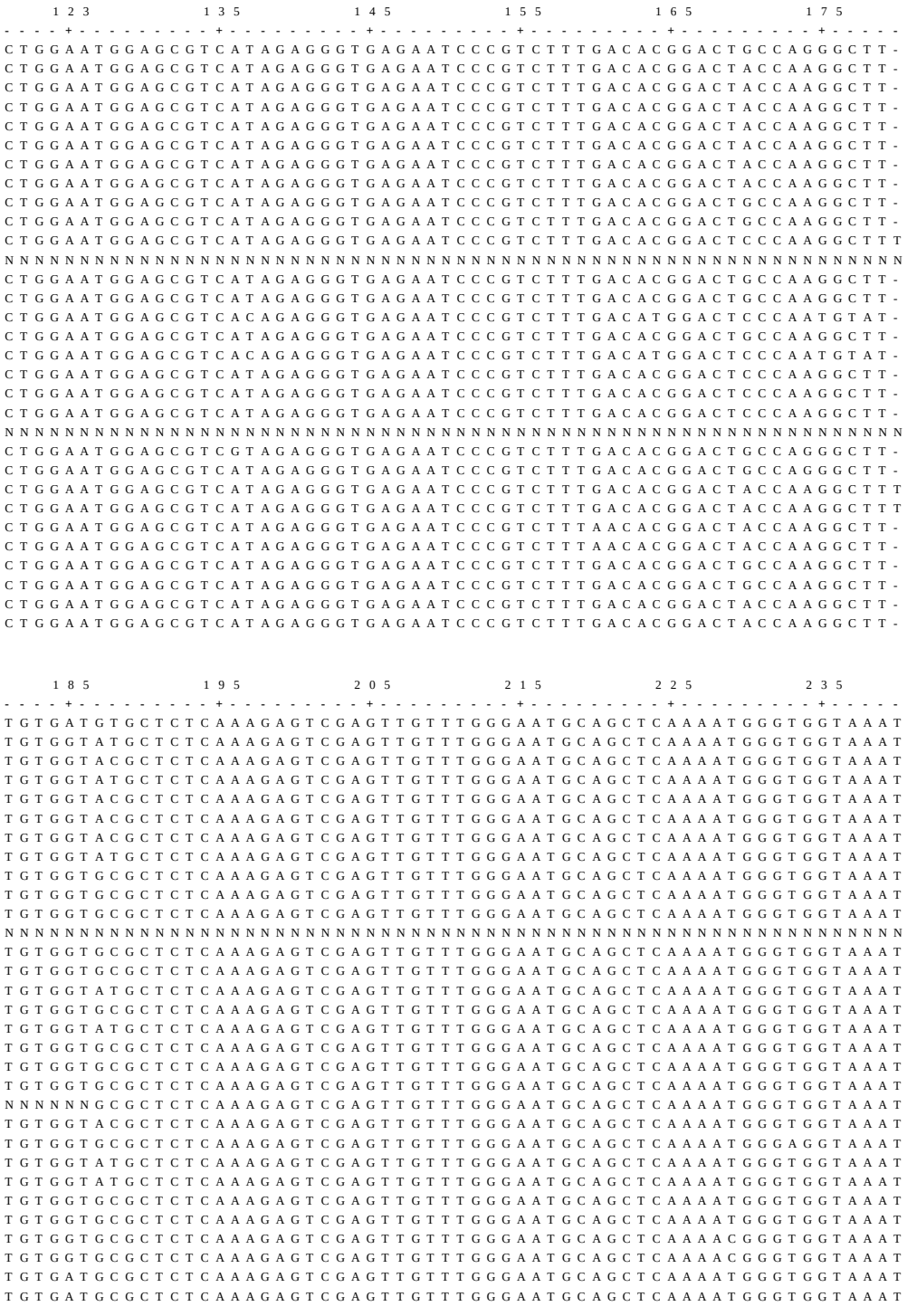
*indicates ambiguiously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2



*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2

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G C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 189
G C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 27048
G C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 25896
G C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26899
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26740
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 258
G C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 27300
N N N N N N N N N N N N N N N N N N N N N N N N N N G A G A C G G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 387
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 381
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 250
G C C A T C T A A A G C T A A A T A T A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 27303
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 384
G C C A T C T A A A G C T A A A T A T A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26827
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26748
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26739
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26279
G C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 27046
G C C A T C T A A A G C T A A A T A T A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 294
G C C T T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 424
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   TJB 8496
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 27270
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 170
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   TJB 8699
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 26976
G C C A T C T A A A G C T A A A T A C A G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   OKM 27013
T C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 385
T C C A T C T A A A G C T A A A T A T T G G C G A G A G A C C G A T A G C G A A C A A G T A C C G T G A G G G A A A G A   MCA 388


      305             315             325             335             345             355
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - -
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G N N A A A T T G C C G A A A G G G A A A C G C T T   J 129
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 188
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 386
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 343
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 189
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 27048
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 25896
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 26899
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 26740
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 258
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 27300
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 387
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 381
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 250
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 27303
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C A C T T   OKM 26827
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 26748
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 26739
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 26279
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 27046
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 294
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 424
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   TJB 8496
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 27270
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 170
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   TJB 8699
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 26976
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   OKM 27013
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 385
T G A A A A G A A C T T T G G A A A G A G A G T T A A A C A G T A C G T G A A A T T G C T G A A A G G G A A A C G C T T   MCA 388
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*indicates ambiguiously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2

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      365            375            385            395            405            415
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - -
                                                                                        * *
G A A G T C A G T C G C G T T G T C C G G G A A T C A A C C T T - G C T T T T G C T G - G G C N T A C T T C C T G G - T   J 129
A A A G T C A G T C G C G T T G G C T T G A A A T C A A C C T T - G C T T T T G C T G - G G T G T A C T T T C T A G - T   MCA 188
A A A G T C A G T C G C G T C G G C T T G G A A T C A A C C T T - G C T T T T G C T G - G G T G T A C T T C C T T G - T   MCA 386
A A A G T C A G T C G C G T T G G C T T G A A A T C A A C C T T - G C T T T T G C T G - G G T G T A C T T T C T A G - T   MCA 343
A A A G T C A G T C G C G T C G G C T T G G A A T C A A C C T T - G C T T T T G C T G - G G T G T A C T T C C T T G - T   MCA 189
A A A G T C A G T C G C G T C G G C T T G G A A T C A A C C T T - G C T T T T G C T G - G G T G T A C T T C C T T G - T   OKM 27048
A A A G T C A G T C G C G T C G G C T T G G A A T C A A C C T T - G C T T T T G C T G - G G T G T A C T T C C T T G - T   OKM 25896
G A A G T C A G T C A C G T T G G C T G G A A A T C A A C C T T - G C T T T T G C T T - G G T G T A C T T T C T A G - T   OKM 26899
G A A G T C A G T C T C G T T G G C T G G G A A T C A A C C T T - G C T T T T G T T G - G G T G T A C T T T C C A G - T   OKM 26740
G A A G T C A G T C G C G T T G G C T G G A A A T C A A C - T T - A C T T T T G T T G - G G T G T A C T T T C T A G - T   MCA 258
G A A G T C A G T C T C G T C G G C C T G G A A T C A G C C T T - G C T T T T G C T G - G G T G T A C T T T C T G G - T   OKM 27300
G A A G T C A G T C A T A T T G G C T A G A A A T C A G C C C T - G C T T T T G T T G - G G T G T A C T T T C T G G - T   MCA 387
G A A G T C A G T C G C G T T G G C T G G G A A T C A A C C T T - G C T T T T G C T T - G G T G T A C T T T C T A G - T   MCA 381
G A A G T C A G T C G C G T T G G C T G G G A A T C A A C C T T - G C T T T T G C T T - G G T G T A C T T T C T A G - T   MCA 250
G A A G T C A G T C G C G T C T G C T G G G A A T C A A C C T T - G C T T T T G C T G - G G T G T A T T T T C T G G T G   OKM 27303
G A A G T C A G T C G C G T C T T C C A G G A A T C A A C C T T - G C T T A T G C T G - G G T G T A T T T T C T G G T T   MCA 384
G A A G T C A G T C G C G T C T G C C G G G A A T C A A C C T T - G C T T T T G C T G - G G T G T A T T T T C T G G T G   OKM 26827
G A A G T C A G T C G C G T T G G C T G G A A A T C A A C C T T - G C T C T T G T T G - G G T G T A C T T T C C A G - T   OKM 26748
G A A G T C A G T C G C G T T G G C T G G A A A T C A A C C T T - G C T C T T G T T G - G G T G T A C T T T C C A G - T   OKM 26739
G A A G T C A G T C G C G T T G G C T G G A A A T C A A C C T T - G C T T T T G T T G - G G T G T A C T T T C C A G - T   OKM 26279
G A A G T C A G T C A C G C T G C C T - G A A A T C A A C C T T - G C T T C T G C T T - G G T G T A C T T T C T G G - T   OKM 27046
G A A G T C A G T C A C G C T G C G A - G A A A T C A A C C T T - G C T T T T G C T T - G G T G T A C T T T C T T G - T   MCA 294
G A A G T C A G T C A C G C T G C T A - G A A A T C A A C C T T - G C T T T T G C C T - G G T G T A C T T T C T T G - T   MCA 424
G A A G T C A G T C G C G T T G G C T G G A A A T C A A C C T T T G C T T T T G C T G - G G T G T A C T T T C T A G - T   TJB 8496
G A A G T C A G T C G C G T T G G C T G G A A A T C A A C C T T T G C T T T T G C T G - G G T G T A C T T T C T A G - T   OKM 27270
G A A G T C A G T C G C G T T A G C T G G G A A T C A A C C T T - G C T T T T G C T T T G G T G T A C T T T C C A G - T   MCA 170
G A A G T C A G T C G C G T T G G C T G G G A A T C A A C C T T - G C T T T T G C T T T G G T G T A C T T T C C A G - T   TJB 8699
G A A G T C A G T C G C G T C G G C C G G G A A T C A A C C T T - A C T T T T G T T G - G G T G T A C T T T C C G G T T   OKM 26976
G A A G T C A G T C G C G T C G G C C G G G A A T C A A C C T T - A C T T T T G T T G - G G T G T A C T T T C C G G T T   OKM 27013
G A A G T C A G T C G C G T C G T T C A G A A C T C A A C C T T - A C T T T T G T G G - G G T G T A T T T T C T G T - T   MCA 385
G A A G T C A G T C G C G T C G T T C A G A A C T C A A C C T T - A C T T T T G T G G - G G T G T A T T T T C T G T - T   MCA 388

      425            435            445            455            465            475
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - -
* *
T A A C G G G T C A G C A T C A A T T T T G A C C G T T G G A A A A G T T G T T G G G A A C T G T G G C T T C T T C G G   J 129
C G A C G G G T C A A C A T C A G T T T T G A C C A G T G G A T A A A G T C A A T G G G A A T G T G G C T C C T T C G G   MCA 188
C G A T G G G T C A A C A T C A G T T T T G A C C G G T G G A T A A A G T C A G T C G G A A T G T G G C T C C C T C G G   MCA 386
C G A C G G G T C A A C A T C A G T T T T G A C C A G T G G A T A A A G T C A A T G G G A A T G T G G C T C C T T C G G   MCA 343
C G A T G G G T C A A C A T C A G T T T T G A C C G G T G G A T A A A G T C A G T C G G A A T G T G G C T C C C T C G G   MCA 189
C G A T G G G T C A A C A T C A G T T T T G A C C G G T G G A T A A A G T C A G T C G G A A T G T G G C T C C C T C G G   OKM 27048
C G A T G G G T C A A C A T C A G T T T T G A C C G G T G G A T A A A G T C A G T C G G A A T G T G G C T C C C T C G G   OKM 25896
T T A C G G G T C A G C A T C A G T T T T G A C C A G T G G A A A A A G G C A T G A G A A A T G T G G C T C C C T C G G   OKM 26899
T G A T G G G T C A G C A T C A A T T T T G A C C A G T G G A T A A A G T T G T A G G G A A T G T G G C A T C C T C G G   OKM 26740
C G A C G G G T C A A C A T C A G T T T T G A C C A G T G G A A A A A G G T G T A G G A A A T G T G G C T C C C T C G G   MCA 258
C G A T G G G T C A G C A T C A G T T T T G G C C A G T G G A T A A A G G C G T G G G A A A T G T G G C T C C C T C G G   OKM 27300
G A A T A G G T C A G C A T C A G T T T T G A C C A G T G G A A A A A A G C A T G A G A A A T G T G G C T C C C T T G G   MCA 387
T A A C G G G T C A G C A T C A G T T T T G A T C A G T G G A T A A A G G C A T G A G A A A T G T G G C T T C C T C G G   MCA 381
T A A C G G G T C A G C A T C A G T T T T G A T C A G T G G A T A A A G G C A T G A G A A A T G T G G C T T C C T C G G   MCA 250
T G A C G G G T C A A C A T C A A T T T T G A T C A G T G G A T A A A G T T G T A G G A A A T G T G G C A T C C T C G G   OKM 27303
T G A T G G G T C A A C A T C A A T T T T G A T C A G T G G A C A A A G T T G T G G G A A A T G T G G C A T C C T T G G   MCA 384
T G A C G G G T C A A C A T C A A T T T T G A T C A G T G G A T A A A G T T G T A G G A A A T G T G G C A T C C T C G G   OKM 26827
T G A T G G G T C A G C A T C A G T T T T G A C C A G T G G A A A A A G G C G T A G G A A A T G T G G C T T C T T C G G   OKM 26748
T G A T G G G T C A G C A T C A G T T T T G A C C A G T G G A A A A A G G C G T A G G A A A T G T G G C T T C C T C G G   OKM 26739
T G A C G G G T C A G C A T C A G T T T T G A C C A G T G G A A A A A G G C G T A G G A A A T G T G G C T T C C T C G G   OKM 26279
T G - C G G G T C A A C A T C A A T T T T G A T C A G T G G A T A A A G G C T G T G G A A A T G T G G C T C C C T C G G   OKM 27046
T T G C G G G T C A G C A T C A G T T T T G A T C G T T G G A T A A A G A T T G T G G A A A T G T G G C A C C T T C G G   MCA 294
T T G C G G G T C A G C A T C A G T T T C G A T C A G T G G A T A A A G G C G G C A G A A A G G T G G C A C C C T C G G   MCA 424
C G A C G G G T C A G C A T C A G T T T T G A C C A G T G G A T A A A G G C A T A A G A A A T G T G G C T C C T T T G G   TJB 8496
C G A C G G G T C A G C A T C A G T T T T G A C C A G T G G A T A A A G G C A T A A G A A A T G T G G C T C C T T T G G   OKM 27270
T G A C G G G T C A A C A T C A A T T T T G A T C G G T G G A T A A G G T C A T G A G G A A T G T G G C A T C T T C G G   MCA 170
T G A C G G G T C A A C A T C A A T T T T G A T C G G T G G A T A A G G T C A T G A G G A A T G T G G C A T C T T T G G   TJB 8699
C G A C G G G T C A G C A T C A G T T T C G A C C A G T G G A T A A A G G C G T A G G A A A T G T G G C A T C C T C G G   OKM 26976
C G A C G G G T C A G C A T C A G T T T C G A C C A G T G G A T A A A G G C G T A G G A A A T G T G G C A T C C T C G G   OKM 27013
T G A C G G G T C A G T A T C A A T T T T G A C C G T T G G A T A A A G T C C T T T G G A A T G T G G C T T C T T C G G   MCA 385
T G A C G G G T C A G T A T C A A T T T T G A C C G T T G G A T A A A G T C C T T T G G A A T G T G G C T T C T T C G G   MCA 388
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*indicates ambiguiously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2

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      485            495            505            515            525            535
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - -
                                                                                      * *
A A G T G T T A T A G C C C A G T T T C G C A T A C A A C - G G T T G G G A T T G A G G A A C T C A G C A C G C C N A A   J 129
G A G T G T T A T A G C C C A T A G T C G C A T A C A T T - G G T T G G G A C T G A G G C T C T C A G C A C G C C T T T   MCA 188
G A G T G T T A T A G C C T T C T G T C G C A T G C A T C - G G T T G G G A C T G A G G A C C G C A G C A C G C C G T T   MCA 386
G A G T G T T A T A G C C C A T A G T C G C A T A C A T T - G G T T G G G A C T G A G G C T C T C A G C A C G C C T T T   MCA 343
G A G T G T T A T A G C C T T C T G T C G C A T G C A T C - G G T T G G G A C T G A G G A C C G C A G C A C G C C G T T   MCA 189
G A G T G T T A T A G C C T T C T G T C G C A T G C A T C - G G T T G G G A C T G A G G A C C G C A G C A C G C C G T T   OKM 27048
G A G T G T T A T A G C C T T C T G T C G C A T G C A T C - G G T T G G G A C T G A G G A C C G C A G C A C G C C G T T   OKM 25896
G A G T G T T A T A G T C Y T G T G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C T G C A   OKM 26899
A T G T G T T A T A G C C T T G C T T C G T A T A C A T T - G G T T G G G A T T G A G G A A C T C A G C A C G C C G A A   OKM 26740
G A G T G T T A T A G T C C T G C A T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   MCA 258
G A G T G T T A T A G T C C T G C G T T G C A T A C A C T G G G C T G G G A C T G A G G A A C T C A G C A C G C T T G T   OKM 27300
G A G T G T T A T A G T C T T G T G T T G C A T G C A T T - G G T T G G G A C T G A G G T A C T C A G C A C G C T T T G   MCA 387
A A G T G T T A T A G T C T T G T G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   MCA 381
A A G T G T T A T A G T C T T G T G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   MCA 250
A T G T G T T A T A G T C C T G C T T C G C A T A C A C T - G G T T G G G A T T G A G G A A C T C A G C A C G C C G A A   OKM 27303
A T G T G T T A T A G T C C T G C T T C G T A T A C A T T - G G T T G G G A T T G A G G A A C T C A G C A T G C C G C A   MCA 384
A T G T G T T A T A G T C C T G C T T C G C A T A C A C T - G G T T G G G A T T G A G G A A C T C A G C A C G C C G C A   OKM 26827
A A G T G T T A T A G T C C T G C G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G A A   OKM 26748
A A G T G T T A T A G T C C T G C G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G A A   OKM 26739
A A G T G T T A T A G T C C T G C G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   OKM 26279
G A G T G T T A T A G T C T G C T G T T G T A T A C A C T - G A T T G G G A T T G A G G A A C T C A G C A C G C C G C A   OKM 27046
G T G T G T T A T A G T C T G C A G T T G C A T A C A A C - G G T T G G G A C T G A G G C A C T C A G C G C G C C G C A   MCA 294
G T G T G T T A T A G T C T G T T G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   MCA 424
G A G T G T T A T A G T C T T T T G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   TJB 8496
G A G T G T T A T A G T C T T T T G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G C A   OKM 27270
A T G T G T T A T A G C C T T G T G T C A T A T A C A T T - G A T T G G G A T T G A G G A A C T C A G C A C G C C G A A   MCA 170
A T G T G T T A T A G C C T T G T G T C A T A T A C A T T - G G T T G G G A T T G A G G A A C T C A G C A C G C C G A A   TJB 8699
A T G T G T T A T A G T C C T G C G T T G T A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G A A   OKM 26976
A T G T G T T A T A G T C C T G C G T T G C A T A C A T T - G G T T G G G A C T G A G G A A C T C A G C A C G C C G A A   OKM 27013
A A G T G T T A T A G C C T T T G G T C G C A T A C A A C - G G T T G G G A T T G A G G T A C T C A G C A C G C T G C A   MCA 385
A A G T G T T A T A G C C T T T G G T C G C A T A C A A C - G G T T G G G A T T G A G G T A C T C A G C A C G C T G C A   MCA 388

      545            555            565            575            585            595
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - -
* * * * * * * * * * * * * * *
A G G C C C G G - - T T T T T A A C C A C G T T C G T G C T T A G G A T G C T G G C A T A A T G G C T T T A A T C G A C   J 129
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A A G G C C T G G - - T T T C G A C C A A G T T C G T G C T T A G G A T G T T G G C A T A A T G G C T T T A A T C G A C   OKM 27048
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A A G C A G G G G - - - T T C G C C C A C T T T C G T G C T T A G G A T G C T G G C A T A A T G G C T T T A A T C G A C   OKM 26899
A G G C C A G G - - - T T T C G A C C A T G T T C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   OKM 26740
A G G C C G G G G - - - T T C G C C C A C G T T C G T G C T C A G G A T G T T G G C A T A A T G G C T T T A A T C G A C   MCA 258
A A A A - G C C G G G G T T C G C C C A C G T C C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   OKM 27300
T T G C A G G G - - T T A T C A C C C A C T T C C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A G C G A C   MCA 387
A G G C C G G G G - - - T T C G C C C A C G T T C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   MCA 381
A G G C C G G G G - - - T T C G C C C A C G T T C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   MCA 250
A T G G C C G G G - - T C T C G A C C A C G T T C G T G C T T A G G A T G T T G G C A A A A T G G C T T T G A T C G A C   OKM 27303
A G G C C G G G T - - C T C T G A C C A C G T T C A T G C T T A G G A T G T T G G C A A A A T G G C T T T A A T C G A C   MCA 384
A T G G C C G G G - - T C T C G A C C A C G T T C G T G C T T A G G A T G T T G G C A A A A T G G C T T T A A T T G A C   OKM 26827
A G G C C G G G G - - - C T C G C C C A C G T A C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   OKM 26748
A G G C C G G G G - - - C T C G C C C A C G T A C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   OKM 26739
A G G C C G G G G - - - T T C G C C C A C G T A C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   OKM 26279
A G G C C G G G A - - - T T C G T C C A C G T T C G T G C T T A G G A T G T T G G C A T A A T G G C T T T A A T C G A C   OKM 27046
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A G G C C G G G G - - - T T C G C C C A C G T T C G T G C T T A G G A T G C T G G C A T A A T G G C T T T A A T C G A C   MCA 424
A G G C C G G G G - - - T T C G C C C A C G T A C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   TJB 8496
A G G C C G G G G - - - T T C G C C C A C G T T C G T G C T T A G G A T G C T G G C A A A A T G G C T T T A A T C G A C   OKM 27270
A G C C A G G T T - - - - T C G A C C A T G T T C G T G C T T A G G A T G T T G G C A A A A T G G C T T T A A T C G A C   MCA 170
A G G C C A G G T - - - T T C G A C C A T G T T C G T G C T T A G G A T G T T G G C A A A A T G G C T T T A A T C G A C   TJB 8699
A G G C C G G G G - - - T T C G C C C A C G T T C G T G C T T A G G A T G C T G G C A T A A T G G C T T T A A T C G A C   OKM 26976
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A A G C C G G T T - - - - T C G A C C A C G T T C G T G C T T A G G A T A C T G G C A T A A T G G C T T T A A T C G A C   MCA 385
A A G C C G G G T - - - T T C G A C C A C G T T C G T G C T T A G G A T A C T G G C A T A A T G G C T T T A A T C G A C   MCA 388
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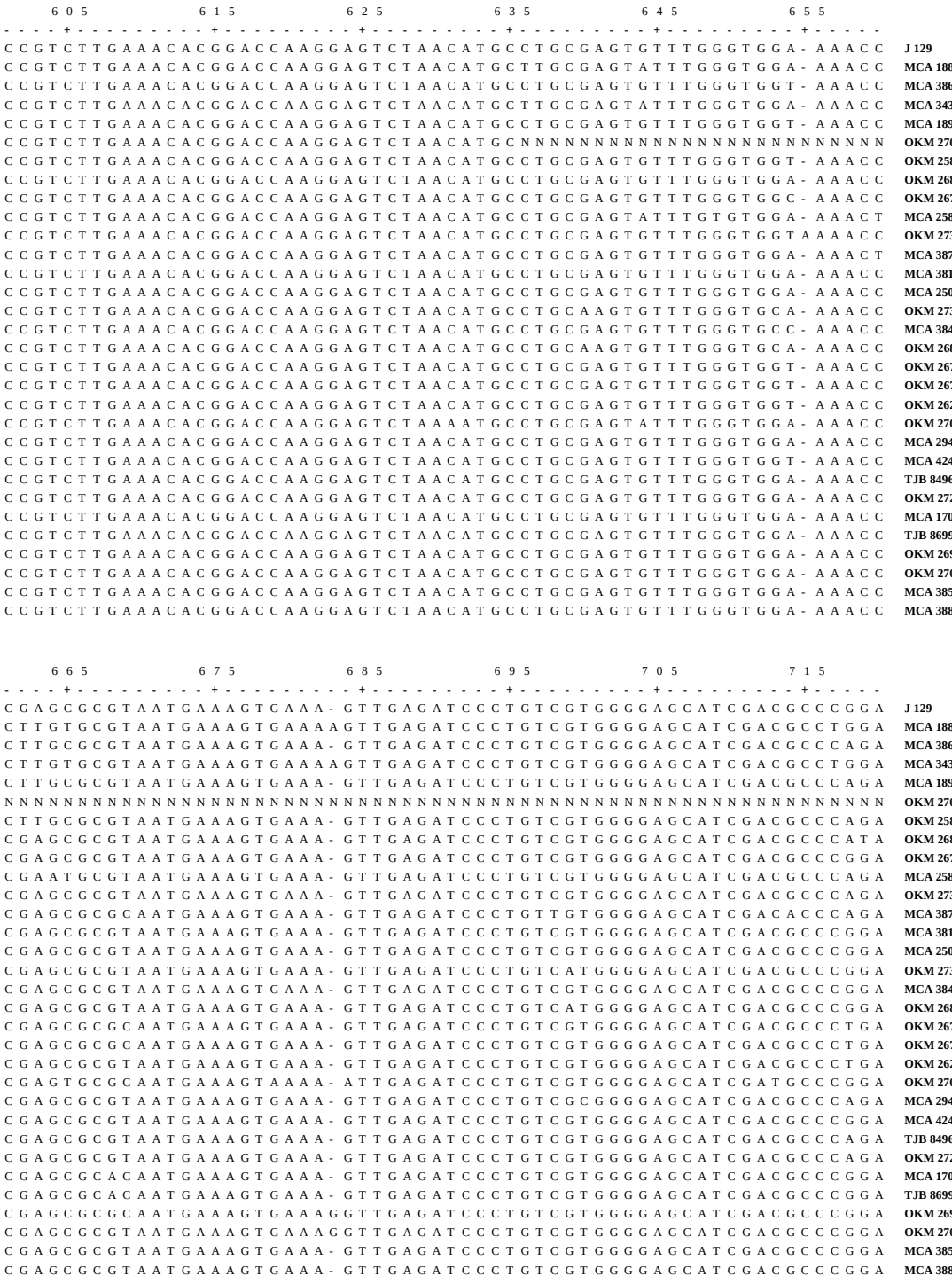
*indicates ambiguiously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2



*indicates ambiguiously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2

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      725            735            745            755            765            775
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - -
C T T G A A C T T C T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   J 129
C C A G A G C T T T T G T G A C G G A T C C A C G G T A G A G C A C G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 188
C C A G A G C T T T T G T G A C G G A T C T G C G G T A G A G C A T G T G T G T T G G G A C C C G A A A G A T G G T G A   MCA 386
C C A G A G C T T T T G T G A C G G A T C C A C G G T A G A G C A C G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 343
C C A G A G C T T T T G T G A C G G A T C T G C G G T A G A G C A T G T G T G T T G G G A C C C G A A A G A T G G T G A   MCA 189
N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N   OKM 27048
C C A G A G C T T T T G T G A C G G A T C T G C G G T A G A G C A T G T G T G T T G G G A C C C G A A A G A T G G T G A   OKM 25896
C C T G A T G T T T A C T G A C G G A T A T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 26899
C C A G A T G T T T A C T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 26740
C C A G A G C T T T T G T G A C G G A T C T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 258
C C T G A A G T T T T C T G A C G G A C C T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 27300
C C A G A C C T T N T G T G A T G G A T C T G C G G T T G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 387
C C A G A C C T T C T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 381
C C A G A C C T T C T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 250
C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 27303
C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 384
C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 26827
C C A G A C C T T T T G T G A C G G A T C A G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 26748
C C A G A C C T T T T G T G A C G G A T C A G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 26739
C C A G A C C T T C T G T G A C G G A T C T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 26279
C C A G A T G T T T A C T G A C G G A T C C G C G G T T G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 27046
C C A G A C C T T C T G T G A C G G A T C T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 294
C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 424
C C A G A C C T T T T G T G A C G G A T C T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   TJB 8496
C C A G A C C T T T T G T G A C G G A T C T G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 27270
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C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   OKM 27013
C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 385
C C A G A C C T T T T G T G A C G G A T C C G C G G T A G A G C A T G T A T G T T G G G A C C C G A A A G A T G G T G A   MCA 388
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      785            795            805            815            825            835
- - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - - - - + - - - - -
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C N N N N N N N N   J 129
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 188
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A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 343
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A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 26899
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A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 384
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 26827
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 26748
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 26739
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 26279
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 27046
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 294
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 424
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   TJB 8496
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 27270
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 170
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   TJB 8699
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 26976
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   OKM 27013
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 385
A C T A T G C C T G A A T A G G G T G A A G C C A G A G G A A A C T C T G G T G G A G G C T C G T A G C G A T T C T G A   MCA 388
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*indicates ambiguously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2

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  845       855       865       875       885       895
----+---------+---------+---------+---------+---------+-----
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  J 129
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 188
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 386
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CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTNNNN  MCA 189
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  OKM 27048
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 25896
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 26899
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 26740
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 258
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 27300
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATNNNNNN  MCA 387
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 381
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 250
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 27303
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 384
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 26827
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAANNNNNNNNNNNNNNNNNNNNN  OKM 26748
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 26739
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 26279
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 27046
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CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 424
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  TJB 8496
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 27270
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CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  TJB 8699
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 26976
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  OKM 27013
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 385
CGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTA  MCA 388
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  905       915       925       935       945       955
----+---------+---------+---------+---------+---------+-----
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  J 129
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 188
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 386
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 343
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  MCA 189
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  OKM 27048
GCTGGTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  OKM 25896
GCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  OKM 26899
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 26740
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 258
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 27300
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  MCA 387
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 381
GCTGGTTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  MCA 250
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 27303
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 384
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 26827
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  OKM 26748
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 26739
GCTGGTTCCTGCCGAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  OKM 26279
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 27046
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 294
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  MCA 424
GCTGGTTCCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  TJB 8496
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 27270
GCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  MCA 170
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  TJB 8699
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 26976
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATATCAGATTTATGTGGTA  OKM 27013
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATTTCAGATTTATGTGGTA  MCA 385
GCTGGTTCCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCATTTCAGATTTATGTGGTA  MCA 388
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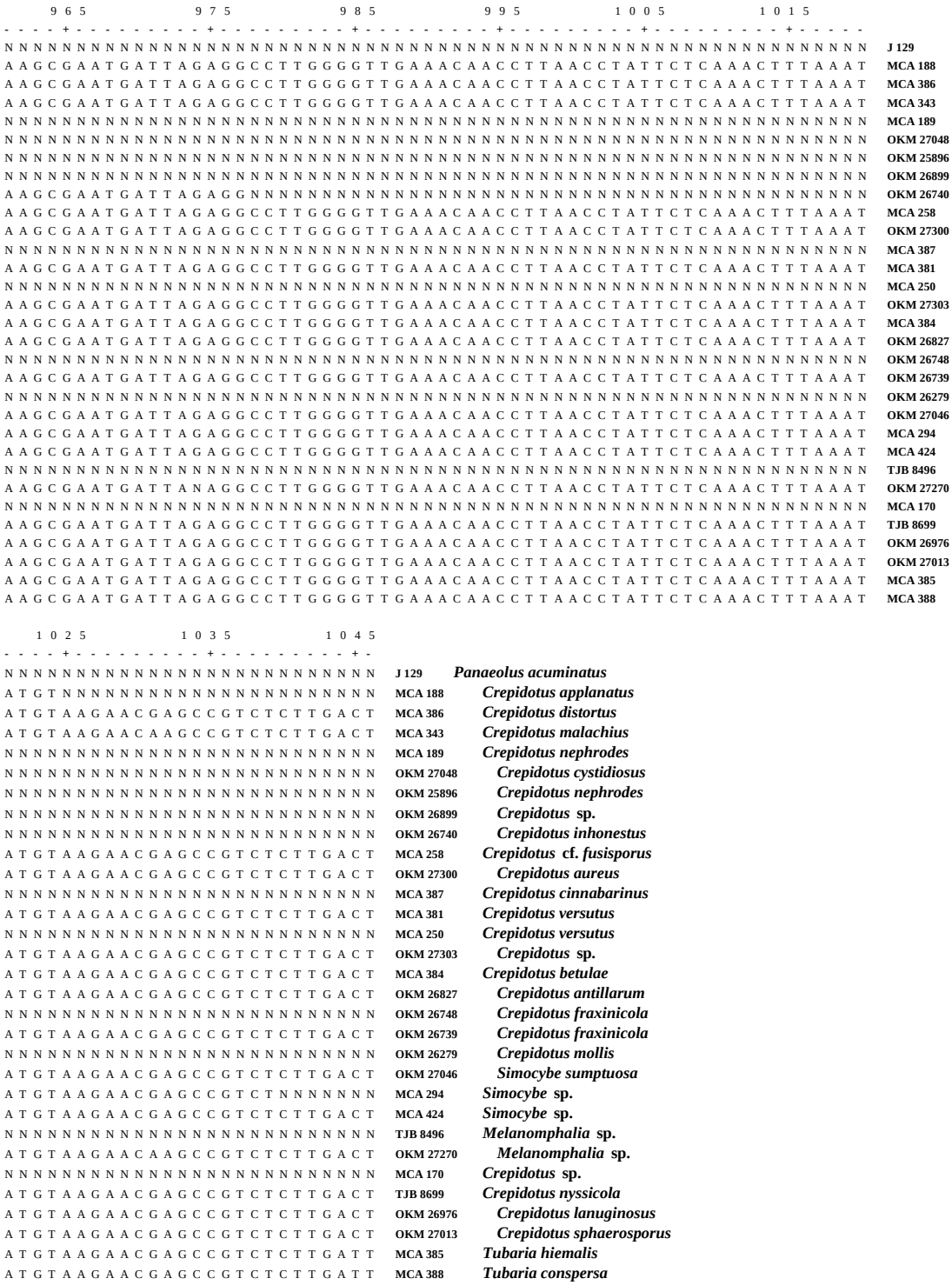
*indicates ambiguiously aligned region excluded from analysis.

Appendix C. Continued: Sequence alignments for Chapter 2



*indicates ambiguously aligned region excluded from analysis.

VITA

Mary Catherine Aime was born May 2, 1965 to David and Carolyn Aime in Winchester, Virginia. She was raised in Springfield, Virginia, where she graduated from Robert E. Lee High School in 1983.

Cathie began work towards a B.S. in Biology at Virginia Tech in 1983, leaving in 1986, and returning to complete her degree in 1995. During the interval, she lived, worked, and played music in Louisiana, California, Italy, Virginia, and Washington, DC. Cathie is a classical pianist, and plays bass and acoustic guitars. She has been employed as a graphic artist, scientific meetings coordinator, journal reporter, exhibition manager, substitute teacher of special education, collections curator, and managing editor, among other things, and is an amateur entomologist.

While completing her undergraduate degree, Cathie had the fortune of enrolling in the Introductory Mycology class taught at Virginia Tech by Orson K. Miller Jr. She began graduate studies in his laboratory during the Fall of 1996. She is a member of the Mycological Society of America, the North American Mycological Association, the British Mycological Society, the Entomological Society of America, the Virginia Academy of Sciences, and the Honor Society of Phi Kappa Phi.