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SYSTEMATICS AND MOLECULAR PHYLOGENY OF *CANTHARELLUS* SPP. IN WESTERN WISCONSIN

A Chapter Style Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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SYSTEMATICS AND MOLECULAR PHYLOGENY OF *CANTHARELLUS* SPP. IN WESTERN WISCONSIN

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ABSTRACT

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Three new species, *Cantharellus phasmatis*, *Cantharellus flavus,* and *Cantharellus spectaculus*, previously considered *Cantharellus cibarius* are described in this study. Morphological differences between these new species and their distinction from other *Cantharellus* species were supported by molecular data from at least two of the three loci included in our analysis (nLSU, ITS, & TEF1). *Cantharellus phasmatis* and *C. flavus* are shown to share a most recent common ancestor with the recently described species from the southern United States, *Cantharellus tenuithrix,* while *C. spectaculus* is in a much more distantly related clade. In addition, we elevate *Cantharellus cibarius* var. *roseocanus* to the species level based on molecular data that suggest its range extends across North America.

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CHAPTER I

INTRODUCTION, LITERATURE, AND PROPOSAL

Introduction

Chanterelle mushrooms are considered choice edibles in many countries around the world because of their delicious taste and odor of apricots. They are important species economically in many countries, with sales grossing over 1.67 billion U.S. dollars annually worldwide (Watling 1997). Chanterelles are ectomycorrhizal, and their abundance in forest ecosystems suggests they play an important role in their habitat. The common yellow-golden chanterelle (*Cantharellus cibarius*) originally described by Fries in 1821 is reported to be found in many parts of the world and is now thought to be a complex of several species that are morphologically similar. Molecular-based approaches using DNA have also shown that *C. cibarius* is a species complex (Feibelman et al. 1994, Dunham et al. 2003, Moncalvo et al. 2006, Arora and Dunham 2008, Buyck and Hofstetter 2011; Bart Buyck, personal communication). No type specimen exists for Fries' collection, and unfortunately, his description encompasses what we now believe are many separate modern taxa.

There has been debate within the scientific community about how to define a species, reflected in the emergence of many different species concepts. Traditionally, fungal species have been identified by morphology. Groups of fungi with distinct morphological characteristics would be considered morphological species. Mating studies

that test biological compatibility have allowed morphologically similar fungi to be either separated from one another or combined into a single species. These organisms would be considered biological species. More recently, fungi have been classified based on DNA comparisons, allowing us to infer evolutionary lineages based on gene phylogeny. Distinct monophyletic lineages based on these analyses are considered phylogenetic species. This terminology has led to conflicts because not all species can fulfill (or can be shown to fulfill) all of the species concepts. Recently, some investigators have addressed these issues and proposed solutions. De Queiroz (2007) points out that all of the "species concepts" have the underlying requirement of being a distinct evolutionary lineage. Morphology, ecology, molecular phylogeny, geographic range, biological compatibility, and other data may provide evidence for an organism being a distinct lineage. Taylor et al. (2000) refer to these separate lines of data as recognition types. The morphological and biological species concepts can be difficult to use to distinguish obligate mycorrhizal fungi such as *Cantharellus* species. However, molecular techniques have allowed us to use the phylogenetic species concept to greatly improve our ability to recognize distinct lineages and more accurately categorize groups according to morphological differences that have phylogenetic utility.

The use of molecular techniques has recently led to the delineation of two new species within the *C. cibarius* complex in the southeastern United States, *Cantharellus tenuithrix* and *Cantharellus altipes* (Buyck and Hofstetter 2011) and two new species in the western United States, *Cantharellus cascadensis* (Dunham et al. 2003) and *Cantharellus californicus* (Arora and Dunham 2008). There are potentially more undescribed species within the *C. cibarius* complex in the eastern and southern U.S.

(Feibelman et al. 1994, Dunham et al. 2003, Moncalvo et al. 2006, Toby Feibelman, personal communication, Bart Buyck, personal communication). The midwestern, northeastern, and northern U.S. varieties of *C. cibarius* could be distinct species, but currently remain under the name *Cantharellus cibarius.*

Presentation of Literature

Morphology

The morphology of cantharelloid fungi in the Eastern United States has been described in detail by a number of mycologists (Peck 1887, Murrill 1910, 1920, Coker 1919, Donk 1964, 1969, Corner 1966, Smith 1968, Petersen 1968, 1969, 1971, 1975, 1976, 1979, 1985, Bigelow 1978, Homola 1993). These works feature extensive description and debate on which morphological characters are important at the species level for separating the genus *Cantharellus* from its sister genus, *Craterellus,* as well as many other genera (some of which no longer exist). Features such as the presence of blunt ridges on the hymenophore, a solid stalk, and clamp connections on the hyphae were typically associated with *Cantharellus.* Having a smooth hymenophore, a hollow stalk, and no clamp connections were typical features of *Craterellus.*

Focusing specifically on the morphology of *Cantharellus cibarius,* E.M. Fries (1821) originally described *C. cibarius* as having "a glabrous, egg-yolk colored pileus that is turned up at the margin…folds swollen, somewhat distant…stipe solid and narrowing toward base…long lived…and having a somewhat compact overall stature" (translation T. Volk). Unfortunately no type specimen exists for Fries' *C. cibarius,* and his description is not sufficient to distinguish this species from modern taxa.

In his extensive monograph of cantharelloid fungi, Corner (1966) identified a

number of varieties of *C. cibarius*. However, Petersen (1969) pointed out that his variety descriptions in the key do not always match the detailed variety descriptions, making it difficult to draw useful conclusions about the morphological characteristics that are important for separating the varieties. For example, in the key to varieties of *C. cibarius, C. cibarius* var. *albus* is found under "Pileus and stem wholly white," but in the short description of each variety that follows the key, it is described as "Wholly white, or tinged pink here and there." Later in the more detailed description it is described again as being "Wholly pure white." The problem with these discrepancies is that many of the varieties included in Corner's work are based on subtle color differences and sometimes have overlapping spore sizes with other varieties. Peck's (1887) early work on chanterelles identified a number of varieties of *C. cibarius,* but upon review of this work, Petersen (1976) found that specimens were not accompanied by adequate notes (such as spore color) to separate them from each other, or from more recently described species.

Smith (1968) described *Cantharellus cibarius* var. *cibarius* from Michigan, which he believed to be the same species as the ones he examined in Europe. Some of the key diagnostic features from this description are the "egg-yellow or paler" hymenium, the "pale-ochraceous" spore print, and the incurved-margin becoming plane-to-wavy and finally, broadly infundibuliform. Smith also described a variety in Michigan with the unique characteristics of a pale stalk and a pale hymenium, which he called *Cantharellus cibarius* var. *pallidofolius* (later reiterated and illustrated by Petersen, 1976, 1979). This variety was also unique in that the spore print was ochraceous-salmon (flushed pink).

Similarly, Petersen (1969) reported finding two varieties in the Appalachian Mountains. He found a cream-spored variety that he considered a close match to the

European specimens, and also a yellow-spored variety. He distinguished the yellow form by its deeper, more brightly-colored gill folds, more crowded and well-developed gill folds, the more everted margin of the pileus, and the more brightly colored and slightly smaller spores. He noted that the cream-spored form matched very closely to the description of *C. cibarius* by Smith & Morse (1947) but that Coker's (1919) description of *C. cibarius* would include both forms. Petersen (1969) also notes that Smith had presented a paper at the meeting of the Mycological Society of America featuring an undescribed chanterelle exhibiting salmon coloration across the entire fruiting body. In Coker's (1919) description of the samples he examined, sample 1168 is described as having "Spores salmon pink, exactly as in *Craterellus cantharellus*… Except for the spore color these plants are exactly *Cantharellus cibarius*."

Different varieties have also been noted in the Western United States. Corner (1966) determined that the most common golden chanterelle in the west was actually *Cantharellus formosus,* not *Cantharellus cibarius*. Petersen (1969) also recognized *C. formosus* in the Western Unites States, as well as what he thought was the cream- and yellow-spored varieties of *C. cibarius* he knew from the East. Despite these works, similar fruiting body morphology and confusion resulted in *C. formosus* being commonly referred to as *C. cibarius* in the Western U.S. until Redhead et al. (1997) published a paper on that species explaining the morphological differences that separate it from other chanterelles. In that paper they also described *C. cibarius* var. *roseocanus*, which will be addressed later.

In the 1970's, based on more extensive personal observations of European specimens, Petersen (1976) came to the conclusion that *C. cibarius* in Europe and *C.*

cibarius in North America were not conspecific. He noted that in both Europe and in the United States, the name *Cantharellus cibarius* was being used ubiquitously to describe what he would consider 8-10 taxa, and probably significantly more (Petersen 1979). These taxa were based on morphological characters such as spore print colors, stipe color, gill-fold anastomosis, and micromorphological characters. Petersen said that in his tentative keys he found at least 3 different taxa in central Sweden, four in southern Germany, and five in the southern Appalachian mountains, all passing under the name *C. cibarius.* In this work he also illustrated *Cantharellus cibarius* var. *pallidofolius*, the variety Smith (1968) had described from Michigan, and recognized it as having one of the largest carpophores in the genus *Cantharellus* (Petersen 1979). Although he described it as common in north America, especially in the east, Petersen noted that in northern and western America, intermediate morphological taxa occur, making the separation very difficult, and that perhaps "several complexes in the genus have yet to evolve sufficiently to show discrete taxa."

In Bigelow's (1978) description of *C. cibarius* in New England, he describes a single variety with a buff-cream spore print. He acknowledged Petersen's varieties as well as Corner's and states that he was not sure which varieties were present in New England, as the focus for this species in his area was primarily on its edible nature. In 1985, Petersen published color illustrations of the yellow-spored and cream-spored varieties to compliment his original descriptions of these varieties (1969), but this time he suggested that the yellow-spored variety was more similar to European specimens from France. Note that this was a different conclusion than his earlier work that suggested the cream-spored variety was most similar to European specimens (Petersen 1969). By 1993,

Homola suggested that spore deposit color and ornamentation were more important for taxonomy than the shape of the fruiting body or the structure of the hymenophore. Homola considered these macroscopic features to be non-diagnostic for systematics, and examples of convergent evolution.

Recently, Buyck and Hofstetter (2011) described two new chanterelles from the southern United States, *Cantharellus tenuithrix* and *Cantharellus altipes*. These authors provide microscopic descriptions of terminal pileus hyphal cells, as well as basidia measurements and spore attributes. They suggest that the length and cell wall thickness of the terminal cell may be an important diagnostic character. Although these characteristics may be diagnostic and useful for species delineation, they admit that these attributes are "extremely delicate" and may be difficult to use for identification without the support of molecular data.

Petersen (1971) summarized some of his conclusions about the morphology of fungi when he wrote "… if anything is clear, it's that gross morphology is deceiving, and that additional characters must be relied on just as heavily in determining probable relationships between groups of organisms." One of the main problems with morphology in *Cantharellus* and *Craterellus* is that using different characters produces different phylogenies, making it difficult to determine which characters are informative for producing a phylogeny in these genera (Feibelman et al. 1997). The use of molecular techniques to provide independent lines of evidence has helped to resolve some of these relationships.

Molecular

In the 1990's, molecular techniques were rapidly advancing and many

investigators were using these approaches to address many questions in mycology, including which morphological characters were most important for distinguishing *Cantharellus* from *Craterellus.* Feibelman et al. (1994) found that the internal transcribed spacer (ITS) region of nuclear ribosomal DNA was abnormally long in *Cantharellus* (1600+ base pairs compared to 600-800 for other basidiomycetes) and that *Craterellus tubaeformis* (at the time *Cantharellus tubaeformis*) had a much shorter ITS region than other chanterelles. This was the first line of molecular evidence showing that *Cantharellus tubaeformis* belonged in the genus *Craterellus.*

Analysis of the ITS region also showed that length of the region was variable within some species, notably *C. cibarius sensu lato* (Feibelman et al. 1994). Samples from several locations across the U.S. were used to account for the morphological variability seen within *C. cibarius*. The length variations suggested that either the ITS region was not a useful region for studying chanterelles at the species level, or that *C. cibarius* could be a species complex (Feibelman et al. 1994). In Europe, Danell (1994) was also using the ITS region for research with *Cantharellus*. He suggested that the American *C. cibarius* could be different from the European *C. cibarius,* and that some of the varieties described by Corner (1966) might be separate species as well.

Feibelman et al. (1996) used ITS length differences and restriction fragment length polymorphism (RFLP) patterns generated from nuclear large subunit (nLSU) sequence digestions to provide molecular support to complement morphological evidence for a new chanterelle from the southern United States, *Cantharellus tabernensis.* In a subsequent study, ITS data and comparisons of sequences of the nLSU locus were used to successfully distinguish members of *Cantharellus* and *Craterellus* at the species level

(Feibelman et al. 1997). The 325 bp fragments of the nLSU that were used in the alignment were sufficient to show differences between genera and species. Maximum parsimony was used to create a phylogenetic tree and morphological traits were mapped on the tree. Data from this study indicated that shape and texture of the basidiomata are more important for separating the genera than clamps, secondary septa, development, or hymenial configuration, although these characters are still informative for relationships between species within a genus (Feibelman et al. 1997). This study also included a second line of molecular evidence (RFLP data from nLSU) showing that *Cr. tubaeformis* belonged in *Craterellus* and not *Cantharellus* (Feibelman et al. 1997). These researchers also suggested that analysis of University of Michigan Herbarium specimens indicated a lack of consistency in the species delineation of *Cr. tubaeformis* and *Cr. infundibuliformis,* and that *Craterellus lutescens* (then *Cantharellus lutescens*) was a separate taxon from the *Cr. tubaeformis* complex. Feibelman et al. (1997) stressed the utility of the 5' end of the nLSU for solving species delineation questions in that complex and for *Cr. lutescens*.

A larger-scale phylogenetic study was done to help determine the relationships of the cantharelloid fungi with other holobasidiomycetes (Pine et al. 1999). Data from the nuclear 18S small subunit gene (nSSU) and the mitochondrial small subunit gene (mSSU) were obtained for several members of *Cantharellus* and *Craterellus*. These data provided additional molecular support for the placement of *Cr. tubaeformis* and *Cr. lutescenes* in *Craterellus,* supporting Feibelman et al. (1994, 1997). Data from this study suggested that stichic meiotic division was an important synapomorphy that distinguished cantharelloid fungi from other holobasidiomycetes that have chiastic nuclear division

(Pine et al. 1999).

Based on success demonstrated by Feibelman et al. (1997), Dahlman et al. (2000) used the nLSU region to perform further investigations into *Craterellus.* A larger segment (~600 bp) was amplified and sequenced. This molecular information allowed these authors to move *C. lutescens, C. ignicolor,* and *C. tubaeformis* to *Craterellus*. Other authors previously indicated that this placement supports Quélet's (1888) placement of *Cr. tubaeformis* in *Craterellus* (Feibelman et al. 1997). Distinct clades separating eastern and western specimens of *Cr. tubaeformis* were also demonstrated. Molecular data have shown that clamp connections are not restricted to *Cantharellus*. Most species of *Craterellus* do tend to have a hollow stipe, so this character may still be useful (Dahlman et al. 2000). This study also synonymized *Cr. cornucopioides* with *Cr. fallax* and *Cr. konradii,* a result that has recently been contested (Matheny et al. 2010).

With more interest developing in chanterelles in the Pacific Northwest, a new species was described from the *C. cibarius* complex, *Cantharellus cascadensis* (Dunham et al. 2003). These investigators used data from RFLP of the ITS and direct sequencing of the ITS and nLSU loci to provide molecular support for *C. cascadensis.* Their study also showed that while closely related to *C. cibarius* var. *cibarius* (from Sweden), *C. cibarius* var. *roseocanus* was monophyletic, and these authors suggest that with more data, this taxon could be elevated to species level (Dunham et al. 2003). This study was also important for showing the relationships of other larger chanterelles like *C. formosus* and *C. subalbidus.* In terms of morphology, microscopic comparisons revealed little useful information. Spore sizes, for example, completely overlapped. Macroscopic morphology was found to be more useful. In general, *C. cascadensis* has a more intensely bright,

pure-yellow pileus, while *C. formosus* shows orange-yellow to brownish-yellow hues. *Cantharellus cascadensis* also tends to have a larger average pileus-width than the others. These color differences are due to unique combinations of carotenoid pigments, as has been shown in other chanterelle species (Mui et al. 1998). Dunham et al. (2003) noted that some *C. cibarius* samples from the eastern United States (North Carolina) were excluded from the study because their sequences were highly divergent from one another and from the samples used in their study, again suggesting that more species exist in the *C. cibarius* complex.

Phylogenetic studies at higher taxonomic levels relying on nSSU/nLSU sequences of cantharelloid fungi have been plagued with alignment difficulties due to an accelerated rate of molecular evolution of the nuclear rDNA genes in these taxa, resulting in their placement on distinctively long branches (Moncalvo et al. 2006). A four-gene phylogeny including nLSU, nSSU, mSSU, and RNA polymerase subunit II (RPB2) was used to provide additional resolution and branch support for many members of this clade. The results of this multi-gene phylogeny were in full agreement with the findings of Dunham et al. (2003) and support the specific status between *C. cascadensis, C. formosus, C. subalbidus, C. persicinus, C. lateritius,* and *C. cibarius* (Moncalvo et al. 2006). Several specimens of *C. cibarius* were used in this study and their phylogeny placed these samples on separate terminal nodes, supporting the hypothesis that several cryptic geographic species exist within *C. cibarius*. This study also indicated that two smaller, slender "yellow chanterelles," *C. appalachiensis* and *C. minor*, are more closely related to the red species of the *C. cinnabarinus* group than they are to the clade of yellow chanterelles (Moncalvo et al. 2006). This was the first study to use RPB2 for molecular

systematics within the cantharelloid clade. The investigators claim it has a more uniform among-taxa rate of evolution and better resolves phylogenetic relationships within the clade, and they recommend this locus for future work (Moncalvo et al. 2006). In addition to RPB2, the TEF1 region was also used for a phylogenetic study that included cantharelloid fungi (Matheny et al. 2007).

Another large yellow chanterelle from the Western United States has recently been described, *Cantharellus californicus* (Arora and Dunham 2008). This species is associated with live oak, and is the largest species in the genus *Cantharellus*. These investigators used RFLP data from ITS sequences to provide molecular support for the distinction of this species from *C. formosus, C. subalbidus,* and *C. cibarius* var. *roseocanus*. The restriction enzymes were those successfully used in a previous chanterelle study (Dunham et al. 2003).

Cantharellus californicus is morphologically described as being most similar to *C. formosus* except for the graying-brown fibrils occasionally seen on the cap of *C. formosus* in dry weather (Redhead et al. 1997). The hymenium is typically paler than the cap but not always. One specimen was found with an orange-yellow hymenium while another had a pale pinkish hymenium (Arora and Dunham 2008). The hymenium is more poroid when mature than in *C. formosus*. *C. cibarius* var. *roseocanus* is typically larger than *C. formosus* but smaller than *C. californicus*. *Cantharellus cibarius* var. *roseocanus* has a brighter and more yellow hymenium and is less likely to develop ochraceous stains when handled than *C. californicus*. These colors can vary, but these authors also found that *C. cibarius* var. *roseocanus* appears to be limited to the northern coast where live oaks are absent while *C. californicus* occurs to the south and/or inland where live oaks

are abundant (Arora and Dunham 2008). Similar to other chanterelle studies, microscopic examination of spores and tissues revealed little information useful for differentiating it from either *C. formosus* or *C. cibarius* var. *roseocanus.* Spore size overlaps with other western chanterelles, and other characters seemed typical of those reported for other specimens resembling *C. cibarius*.

Craterellus fallax was originally described by Smith (1968) and was most easily distinguished from *Craterellus cornucopioides* by its salmon to pink-colored spore print (compared to cream-colored with *Cr. cornucopioides*). This species has also been acknowledged by other chanterelle experts (Petersen 1976, Bigelow 1978). Dahlman et al. (2000) synonymized *Cr. cornucopioides* with *Cr. fallax* and *Cr. konradii* based on nLSU data. Based on ITS sequences, Matheny et al. (2010) argue that *Cr. fallax* collections from the Eastern United States are unique from *Cr. cornucopioides* from Europe. These authors cast doubt upon the use of short nLSU sequences for delimiting the species in this complex, citing the more variable ITS region as superior for barcoding these species (Matheny et al. 2010). While I agree with Matheny et al. that multiple loci should be used whenever possible (even though their study only uses ITS), it is important to point out that the nLSU phylogenetic tree of Dahlman et al (2000) does not show the three taxa occupying a single terminal node. In fact the group is not monophyletic, as it contains a clade that has a Swedish sample labeled *Cr. cornucopioides* (yellow) at one terminal node and *Cr. fallax* from the eastern U.S.A. on a separate terminal node. This clade was supported with 63% bootstrap support, with the *Cr. fallax* sample showing at least 15 character changes on their phylogram. These data were not addressed, suggesting the synonymization of taxa was based on a misinterpretation of data, and was not

necessarily the result of the nLSU locus providing insufficient resolution.

Buyck and Hofstetter (2011) have recently used the TEF1 locus to delineate two new species within the *C. cibarius* complex in the southeastern United States, *Cantharellus tenuithrix* and *Cantharellus altipes.* Although the TEF1 locus does not provide strong basal support in this genus, these authors showed that it can be useful for resolving terminal nodes in the genus. This makes the TEF1 locus particularly useful for delimiting cryptic or closely related taxa.

Ecology

Cantharellus cibarius is a mycorrhizal species, and like many others, difficulty germinating basidiospores and growing mycelia has made cultural studies and mating type analyses impractical (Danell and Fries 1990; Danell 1994; Feibelman et al. 1994). However some investigators have been successful at germinating spores (Fries 1979), and in Europe, *C. cibarius* has been successfully cultured on media and grown as mycorrhizae on tree saplings (Danell 1994). In Sweden, Danell (1994) described *C. cibarius* growing under coniferous trees *Pinus sylvestris* and *Picea albies,* but also under *Betula,* and he mentioned that in other parts of Europe it can be associated with *Quercus*. In lab studies, the strains collected beneath *Picea* were successful in forming mycorrhizae with *Picea* and *Pinus,* but with *Betula pendula* were very slow-growing and not as successful in forming mycorrhizae (Danell 1994). He also compared RFLP profiles from the ITS locus between chanterelles from each host type but did not find any difference. A later investigation showed that direct sequence comparisons of ITS and nLSU loci of Swedish *C. cibarius* from both *Betula* and coniferous hosts had no difference (Dunham et al. 2003). Danell's research in forming mycorrhizae with tree saplings led to the

production of fruiting bodies (Danell and Comacho 1997) and a patent on the technique (Danell 2001). These accomplishments suggest valuable commercial opportunities if fruiting-body production can be achieved consistently.

In the United States, host associations may not be clear at this point due to the confusion associated with the *C. cibarius* species complex. Some of the host-associations that have been published from studies in North America are reviewed here for comparison. From New York, Peck (1887) described the American *C. cibarius* growing under *Tsuga*, but also in deciduous woods, especially in damp weather. Although Coker (1919) did not specify a host preference in his description of *C. cibarius*, in his descriptions of examined collections from North Carolina, he mentions pine, oak, and upland mixed-woods. From Michigan, Smith (1968) described *C. cibarius* var. *cibarius* growing in deciduous woods, especially along old roads, and *C. cibarius* var. *pallidofolius* growing in forests dominated by beech and maple.

In a molecular study of the ITS locus, Feibelman et al. (1994) included 7 samples identified as *C. cibarius* from many geographic areas and host-types in the United States, including a sample from Louisiana under *Quercus virginiana*, a sample from Mississippi from *Pinus elliotii*, a sample from pine/hardwood forest from Michigan, a sample from old growth conifer forest of Washington, as well as samples from *California* (host not specified). While variation was found in the length of the ITS in these samples, there was no apparent correlation between length and geographical origin or host.

Redhead et al. (1997) described *C. cibarius* var. *roseocanus* as growing under second growth *Picea sitchensis* without other tree associates, or under *Picea* spp. mixed with *Tsuga* spp. and/or *Abies* species. In a molecular study, Dunham et al. (2003) found

C. cibarius var. *roseocanus* samples from Oregon and Washington associated with *Picea* species. Arora and Dunham (2008) also found samples of *C. cibarius* var. *roseocanus* from California associated with *Pinus muricata* and mixed-hardwood forests containing *P. muricata.* In addition to these *C. cibarius* varieties, *C. californicus* was described as being strongly associated to *Quercus agrifolia* (Arora and Dunham 2008), and *C. cascadensis* was described associated with *Pseudotsuga menziesii* and *Tsuga heterophylla* (Dunham et al. 2003).

In addition to the mutualism between cantharelloid fungi and trees, a symbiotic relationship was also found between *Pseudomonas* bacteria and *C. cibarius* strains in Sweden (Danell 1994). The fluorescent bacteria were found growing on external hyphal surfaces even within the fruiting bodies, apparently causing no harm to the fungi. Danell suggested these bacteria are being incidentally incorporated into the fruiting bodies as they grow. These bacteria were also found associated with cultivated fruiting bodies, suggesting this symbiosis is inevitable (Danell 1999). This phenomenon was studied further and ^{13}C-NMR analyses indicated *C. cibarius* hyphae exude trehalose and mannitol, which may explain the apparently commensalistic bacterial association (Ragnel-Castro et al. 2002).

The commercial value of chanterelles and lack of reliable commercial cultivation makes the harvesting of these mushrooms from natural populations the only way of obtaining them. A decline in chanterelle production in Europe has sparked further interest into the ecology of these mushrooms and the effects of pollution on fungi in general (Jansen and Van Dobben 1987). In the Pacific Northwest, chanterelles (as well as mushrooms in general) are much more popular than they are in the midwestern United

States. Several ecological studies have addressed western chanterelle species, particularly *C. formosus*. Starting in 1986, The Oregon Mycological Society has been conducting a long-term study on the effects of harvesting mushrooms on continued production (Pilz et al. 2003). Chanterelles in the Pacific Northwest are seen as an important forest product. The U.S. Forest service is helping to monitor and maintain their populations (Pilz et al. 1998). Pilz et al. (2003) give an excellent overview of how chanterelles fit into the Northwest Forest Plan and which species are being monitored and surveyed.

Fungal ecologists have used molecular tools to determine the size of individual genets of *C. formosus*, as well as providing information about the approximate size of populations and the extent of genetic variation at both the population scale and at the watershed scale (Dunham et al. 2003, Collins 2005, Dunham et al. 2006). Although *C. formosus* is somewhat similar to *C. cibarius*, all molecular studies mentioned in this manuscript indicate that *C. formosus* shares a less recent common ancestor with Swedish *C. cibarius* than any of the other *C. cibarius*-like species including *C. subalbidus*; therefore, the results of the *C. formosus* ecological studies may not be indicative of ecological properties of all the *C. cibarius* varieties.

Proposal

The primary goal of this project was to identify the *Cantharellus cibarius*-like chanterelles growing in association with *Quercus* spp. in western Wisconsin. Morphological variation of *Cantharellus* in Hixon Forest in La Crosse, Wisconsin suggested that cryptic species may exist. Fruiting bodies were collected from forests near La Crosse and compared to those from various other regions in the country, including specimens from herbaria. When consistent macro- and micro-morphological differences

were found, phylogenetic analysis of relatively conserved DNA regions was used to test for novel monophyletic groups with the intent of describing any new species found.

The nuclear large subunit (nLSU) region was the primary target locus in this study. This region was chosen because previous investigators have shown that it can be useful for distinguishing between species of *Cantharellus* (Feibelman et al. 1997, Dunham et al. 2003, Moncalvo et al. 2006). Secondary loci that were considered and tested in this investigation included the nuclear small subunit (nSSU) and mitochondrial small subunit (mSSU) (Pine et al. 1999), internal transcribed spacer (ITS) (Danell 1994, Feibelman et al. 1994, Dunham et al. 2003, Arora and Dunham 2008), and translation elongation factor 1α (TEF1) regions (Moncalvo et al. 2006, Matheny et al. 2007, Buyck and Hofstetter 2011). Although investigators have expressed concern for using the nLSU region for comparisons between *Cantharellus* and more distant genera due to evolutionary rate heterogeneity (Moncalvo et al. 2006, Matheny et al. 2007), this region still shows promise at the species level in *Cantharellus*. Phylogenetic analysis of multiple gene regions can be used to help determine how species of *Cantharellus* are related. One of the major advantages of using complementary data sets is to gain additional support at critical internal nodes (Lutzoni 1997). Multi-locus concordance can provide additional confidence when using molecular data to delineate species (Taylor et al. 2000).

CHAPTER II

PAPER FOR SUBMISSION TO MYCOLOGIA

Short title: Cantharellus systematics

Systematics and molecular phylogeny of *Cantharellus* spp. in western Wisconsin

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Abstract: Three new species, *Cantharellus phasmatis, Cantharellus flavus,* and *Cantharellus spectaculus,* previously considered *Cantharellus cibarius* are described in this study. Morphological differences between these new species and their distinction from other *Cantharellus* species were supported by molecular data from at least two of the three loci included in our analysis (nLSU, ITS, and TEF1). *Cantharellus phasmatis* and *C. flavus* are shown to share a most recent common ancestor with the recently described species from the southern United States, *Cantharellus tenuithrix,* while *C. spectaculus* is in a much more distantly related clade. In addition, we elevate *Cantharellus cibarius* var. *roseocanus* to species level based on molecular data that suggest its range extends across North America.

Key words: Cantharelloid, *Cantharellus cibarius,* chanterelle, cryptic species

INTRODUCTION

Chanterelle mushrooms are considered choice edibles in many countries around the world because of their delicious taste and odor of apricots. The common yellow-golden chanterelle (*Cantharellus cibarius*) was originally described by Fries in 1821 as having "a glabrous, egg-yolk colored pileus that is turned up at the margin…folds swollen,

somewhat distant…stipe solid and narrowing toward base…long lived…and having an overall stature somewhat compact" (Translation T. Volk). Unfortunately no type specimen exists for Fries' *C. cibarius,* and his description is not sufficient to distinguish this species from modern taxa. In addition, modern molecular-based investigations using DNA have shown that in the western, southern, and eastern United States, *C. cibarius* is a species complex (Feibelman et al. 1994, Dunham et al. 2003, Moncalvo et al. 2006, Arora and Dunham 2008, Buyck and Hofstetter 2011).

In the last 50 years, several advancements have been made with *Cantharellus* systematics from morphological data. Smith (1968) described *Cantharellus cibarius* var. *cibarius* from Michigan, which he believed to be the same as *C. cibarius* he examined from Europe. Some of the key diagnostic features from this description are the "egg-yellow or paler" hymenium, the "pale-ochraceous" spore print, and the incurved-margin becoming plane-to-wavy and finally broadly infundibuliform. Smith also described a variety in Michigan with the unique characteristics of a pale stalk and a pale hymenium, which he called *Cantharellus cibarius* var. *pallidofolius* (later reiterated and illustrated by Petersen (1976, 1979)). This variety was also unusual in that the spore print was ochraceous-salmon (flushed pink). Another pink-spored chanterelle was mentioned in Coker's (1919) description of the samples he examined. Sample 1168 was described as having "Spores salmon pink, exactly as in *Craterellus cantharellus*… Except for the spore color these plants are exactly *Cantharellus cibarius*."

Petersen (1969) initially reported finding two varieties in the Appalachian Mountains. He found a cream-spored variety that he considered a close match to the European specimens, and also a yellow-spored variety. He distinguished the yellow form

by its deeper, more brightly-colored gill folds, more crowded and well-developed gill folds, the more everted margin of the pileus, and the more brightly colored and slightly smaller spores. He noted that the cream-spored form matched very closely to the description of *C. cibarius* by Smith & Morse (1947) but that Coker's (1919) description of *C. cibarius* would include both forms. Petersen (1969) also noted that in 1967, Smith had presented a paper at the meeting of the Mycological Society of America featuring an undescribed chanterelle exhibiting salmon coloration across the entire fruiting body.

In the 1970's, based on more extensive personal observations of European specimens, Petersen (1976) came to the conclusion that *C. cibarius* in Europe and *C. cibarius* in North America were not conspecific. He noted that in both Europe and in the United States, the name *Cantharellus cibarius* was being used ubiquitously to describe what he would consider 8-10 taxa, and probably significantly more (Petersen 1979). These taxa were based on morphological characters such as spore print colors, stipe color, gill-fold anastomosis, and micromorphological characters. Petersen said that in his tentative keys he found at least three different taxa in central Sweden, four in southern Germany, and five in the southern Appalachian Mountains, all passing under the name *C. cibarius.* In this work he also illustrated *Cantharellus cibarius* var. *pallidofolius*, the variety Smith (1968) had described from Michigan, and recognized it as having one of the largest carpophores in the genus *Cantharellus* (Petersen 1979). Although he described this chanterelle as common in north America, especially in the east, Petersen noted that in northern and western America, intermediate taxa occur, making the separation very difficult, and that perhaps "several complexes in the genus have yet to evolve sufficiently to show discrete taxa."

In Bigelow's (1978) description of *C. cibarius* in New England, he described a single variety with a buff-cream spore print. He acknowledged Petersen's varieties as well as Corner's and stated that he was not sure which varieties were present in New England, as his focus for this species in this area was primarily on their edible nature. By 1993, Homola suggested that spore deposit color and ornamentation were more important for taxonomy than the shape of the fruiting body or the structure of the hymenophore. Homola considered these macroscopic features to be non-diagnostic for systematics and examples of convergent evolution.

Recently, Buyck and Hofstetter (2011) described two new chanterelles from the southern United States, *Cantharellus tenuithrix* and *Cantharellus altipes*. These authors provide microscopic descriptions of terminal pileus hyphal cells, as well as basidia measurements and spore attributes. They suggested that the length and cell wall thickness of these terminal cells may be an important diagnostic character. Although these characteristics may be diagnostic for species delineation, they admit that these attributes are "extremely delicate" and may be difficult to use for identification without the support of molecular data.

In the United States, host associations may not be clear at this point due to the confusion associated with the *C. cibarius* species complex. Some of the host-associations published from studies in North America are reviewed here for comparison. From New York, Peck described the American *C. cibarius* growing under *Tsuga*, but also in deciduous woods (Peck 1887). Coker's (1919) descriptions of examined collections from North Carolina mention pine, oak, and upland mixed-woods. From Michigan, Smith (1968) described *C. cibarius* var. *cibarius* growing in deciduous woods, and *C. cibarius*

var. *pallidofolius* growing in forests dominated by beech and maple. Feibelman et al. (1994) included *C. cibarius* from many geographic areas and host-types in the United States, including *Quercus virginiana, Pinus elliotii,* a sample from pine/hardwood forest from Michigan, and a sample from old growth conifer forest of Washington. Redhead et al. (1997) described *C. cibarius* var. *roseocanus* as growing under second growth *Picea sitchensis* without other tree associates, or under *Picea* spp. with *Tsuga* spp. and/or *Abies* species. Dunham et al. (2003) found *C. cibarius* var. *roseocanus* samples from Oregon and Washington associated with *Picea* species. Arora and Dunham (2008) also found samples of *C. cibarius* var. *roseocanus* from California associated with *Pinus muricata* and mixed-hardwood forests containing *P. muricata.* In addition to these *C. cibarius* varieties, *C. californicus* was described as being strongly associated to *Quercus agrifolia* (Arora and Dunham 2008), and *C. cascadensis* was described associated with *Pseudotsuga menziesii* and *Tsuga heterophylla* (Dunham et al. 2003). Both *C. californicus* and *C. cascadensis* were formerly known as *C. cibarius.*

Petersen (1971) summarized some of his conclusions about the morphology of fungi when he wrote "… if anything is clear, it's that gross morphology is deceiving, and that additional characters must be relied on just as heavily in determining probable relationships between groups of organisms." One of the main problems with morphology in *Cantharellus* and its sister genus *Craterellus* is that using different characters produces different phylogenies, making it difficult to determine which characters are informative for a phylogeny in these genera (Feibelman et al. 1997). The use of molecular techniques to provide independent lines of evidence has helped to resolve some of these relationships.

Feibelman et al. (1994) found that the internal transcribed spacer (ITS) region of nuclear ribosomal DNA was abnormally long in *Cantharellus* (1600+ base pairs compared to 600-800 for other basidiomycetes) and that the length of the region was variable within some groups, notably *C. cibarius sensu lato.* Length variations in the ITS region of samples from several locations across the U.S. suggested that *C. cibarius* could be a species complex (Feibelman et al. 1994). In a subsequent study, ITS data and comparisons of sequences of the nuclear large subunit (nLSU) locus were used to successfully distinguish members of *Cantharellus* and *Craterellus* at the species level (Feibelman et al. 1997). Data from this study indicated that the shape and texture of the basidiomata was more important for separating the genera than clamps, secondary septa, development, or hymenial configuration, although these characters were still informative for relationships between species within a genus (Feibelman et al. 1997). Four-gene phylogenies of *Cantharellus* (Moncalvo et al. 2006) and the most recent international phylogeny of the translation elongation factor 1α (TEF1) region in *Cantharellus* spp. (Buyck and Hofstetter 2011) show the same topologies Feibelman et al. (1997) showed with 325 bp of the nLSU.

Dunham et al. (2003) used the ITS and nLSU regions to describe a new species from the *C. cibarius* complex, *Cantharellus cascadensis,* from the Pacific Northwest. These investigators also noted that some *C. cibarius* samples from North Carolina were excluded from the study because their sequences were highly divergent from one another and from the samples used in their study, suggesting more species exist in the *C. cibarius* complex in the eastern United States. Arora and Dunham (2008) used RFLP data from ITS sequences to provide molecular support for the distinction of a large yellow

chanterelle from the western United States, *Cantharellus californicus*, from *C. formosus, C. subalbidus,* and *C. cibarius* var. *roseocanus.*

Phylogenetic studies at higher taxonomic levels relying on nuclear small subunit (nSSU) and nLSU sequences of cantharelloid fungi have been plagued with alignment difficulties due to an accelerated rate of molecular evolution of the nuclear rDNA genes in these taxa, resulting in their placement on distinctively long branches (Moncalvo et al. 2006). A four-gene phylogeny including nLSU, nSSU, mitochondrial small subunit (mSSU), and RNA polymerase subunit II (RPB2) was used to provide additional resolution and branch support for many members of this clade. The results of this multi-gene phylogeny were in agreement with the findings of Dunham et al. (2003) and support the specific status of *C. cascadensis, C. formosus, C. subalbidus, C. persicinus, C. lateritius*, and European *C. cibarius* (Moncalvo et al. 2006). Several specimens of *C. cibarius sensu lato* were used in this study and these did not form a monophyletic group, supporting the hypothesis that several cryptic geographic species exist within *C. cibarius*. This was the first study to use RPB2 for molecular systematics within the cantharelloid clade. The investigators claim it has a more uniform among-taxa rate of evolution and better resolves phylogenetic relationships within the clade, and they recommend this locus for future work (Moncalvo et al. 2006).

In addition to RPB2, the TEF1 region was also used for a phylogenetic study that included cantharelloid fungi (Matheny et al. 2007). These researchers showed the TEF1 locus was useful for determining phylogenetic relationships of these fungi when comparing them to other groups, but the study did not contain many species from *Cantharellus*. Buyck and Hofstetter (2011) used the TEF1 locus to delineate two new

species within the *C. cibarius* complex in the southeastern United States, *Cantharellus tenuithrix* and *Cantharellus altipes.* They showed that this locus can be useful for resolving this genus at the species level.

There are potentially more undescribed species within the *C. cibarius* complex in the eastern, southern, and northern United States (Feibelman et al. 1994, Dunham et al. 2003, Moncalvo et al. 2006, Buyck and Hofstetter 2011, Toby Feibelman, personal communication, Bart Buyck, personal communication). The midwestern and northern U.S. varieties of *C. cibarius* could be distinct species, but currently remain under the name *Cantharellus cibarius.*

This study was initiated when *Cantharellus* species in La Crosse, Wisconsin were found growing under *Quercus* species. These specimens were extremely variable in morphology, suggesting the existence of more than one species. In addition, the morphology of these chanterelles did not closely match each other or descriptions of true *C. cibarius.* The purpose of this study was to (i) identify consistent morphological differences and group chanterelles into morphotype groups, (ii) test for molecular differences between morphotype groups, and (iii) describe any new species discovered.

MATERIALS AND METHODS

Chanterelles were collected from La Crosse, Wisconsin and from other parts of the United States and grouped into morphotypes using characters such as the color of the pileus, hymenium, stipe, and spores, and the type of host. Chanterelles from Colorado were collected during the NAMA 2010 foray and from Idaho in the fall of 2010. In addition to these, chanterelle samples were sent to us from other regions of the country from collectors and from herbaria (TABLE I). Fresh specimens we collected were

photographed, and tissue samples (2-3mm^3 fresh) from the context of the pileus were digested in 50 μL of filter-sterilized cell lysis solution (CLS; Lindner and Banik 2009) containing 1.4 M NaCl, 0.1 M Tris-HCl, 20 mM EDTA, and 2% hexadecyltrimethylammonium bromide (CTAB) and frozen at -20 C. Tissue samples from dried specimens were also taken from the context of the pileus and digested in CLS. Fresh fruiting bodies were dehydrated at 35 C in a food dehydrator and stored for eventual herbarium accession. Microscopic analyses were performed using dried material reconstituted in 3% KOH. All specimens were deposited in the Field Museum of Natural History (F).

DNA extraction and PCR.—Tissue samples in 50 μL CLS were ground using sterile plastic pestles fitted into an electric drill. Four-hundred fifty μL CLS was added and tubes were incubated at 65 C for 2 hr. Five hundred μL of chloroform/isoamyl alcohol (24:1) were added and tubes were shaken vigorously for 5 min to form an emulsion. Samples were centrifuged at 13 000 RPM for 12 min. Supernatents were transferred to new tubes, and the previous 2 steps were repeated. Six hundred μL of cold (–20 C) isopropanol were added to each reaction and tubes were inverted and stored at –20 C for a minimum of 12 hours. Reactions were centrifuged at 13 000 RPM for 12 min and alcohol was decanted. The remaining DNA pellet was rinsed with 1 mL of 70% EtOH and then centrifuged. Alcohol was again decanted and pellets were allowed to air dry in a fume hood, then resuspended in 50 μL TE buffer (8.0 pH). Samples were stored at –20 C. Electrophoresis was used to visualize DNA products on 1.5% agarose gels in TAE buffer at 100 V for 45 min.

PCR primers used for the nLSU locus were ITS4F (White et al. 1990) and LR5

TABLE I**.** New sequences produced in this study. Species designations include species described in this work. An asterisk indicates the information is not available. OSCH = Oregon State University Herbarium.

Number	Morphological ID	Origin	Host	Target Locus	Collector
C057	*C. phasmatis*	WI	*Quercus*	nLSU, ITS, TEF1	M. Foltz
C059	*C. phasmatis*	WI	*Quercus*	ITS	M. Foltz
C065	*C. flavus*	WI	*Quercus*	nLSU	M. Foltz
C066	*C. flavus*	WI	*Quercus*	nLSU	M. Foltz
C067	*C. flavus*	WI	*Quercus*	ITS, TEF1	M. Foltz
C068	*C. flavus*	WI	*Quercus*	nLSU, ITS	M. Foltz
C073	*C. phasmatis*	WI	*Quercus*	nLSU	M. Foltz
C074	*C. phasmatis*	WI	*Quercus*	nLSU, TEF1	M. Foltz
C075	*C. phasmatis*	WI	*Quercus*	ITS	M. Foltz
C076	*C. phasmatis*	WI	*Quercus*	nLSU, ITS	M. Foltz
C081	*C. spectaculus*.	WI	*Quercus*	nLSU, TEF1	M. Foltz
C082	*C. spectaculus*	WI	*Quercus*	nLSU	M. Foltz
C084	*C. spectaculus*	WI	*Quercus*	nLSU	M. Foltz
CC03	*C, roseocannus*	MI	*Tsuga*	nLSU	A&L.Baines
CC05	*"C. cibarius"*	MO	*Quercus*	nLSU	M. Rogers
CC13	*C. cibarius*	SWE	*Picea*	nLSU	OSCH
CC15	*C. cibarius*	SWE	*Betula*	nLSU	OSCH
CC17	*C. cibarius*	SWE	*Betula*	nLSU	OSCH
CC23	*Cantharellus* sp.	WI	*Quercus*	nLSU	S. Nelson

CC27	*"C. cibarius"*	CT	*Quercus*	nLSU	B. Yule
CC29	*C. roseocanus*	CO	*Picea*	nLSU, ITS, TEF1	T. Volk
CC31	*C. roseocanus*	CO	*Picea*	nLSU	T. Volk
CC33	*C. roseocanus*	CO	*Picea*	nLSU	T. Volk
CC34	*C. roseocanus*	CO	*Picea*	nLSU	T. Volk
CC35	*C. roseocanus*	CO	*Picea*	nLSU	T. Volk
CC36	*C. roseocanus*	MA	*Tsuga*	nLSU	M. Binder
CC38	*C. roseocanus*	CO	*Picea*	nLSU	T. Volk
CC40	*C. roseocanus*	CO	*Picea*	nLSU	T. Volk
CAF1	*C. formosus*	ID	*	nLSU	T. Volk
IC01	*C. roseocanus*	ID	*	nLSU	T. Volk
Ch1	*C. flavus*	WI	*Quercus*	nLSU, ITS	T. Volk
Ch2	*C. phasmatis*	WI	*Quercus*	nLSU, ITS	T. Volk
Ch3	*C. phasmatis*	WI	*Quercus*	nLSU, ITS	T. Volk
Ch4	*C. phasmatis*	WI	*Quercus*	ITS	T. Volk
Ch5	*C. flavus*	WI	*Quercus*	nLSU	T. Volk
Ch6	*C. phasmatis*	WI	*Quercus*	ITS	T. Volk
Ch7	*C. phasmatis*	WI	*Quercus*	ITS	D.Lindner
CC42	*"C. cibarius"*	VA	*Quercus*	nLSU	T. Melin
CAS1	*C. subalbidus*	ID	*	nLSU	T. Volk
ICW1	*C. cascadensis*	ID	*	nLSU	T. Volk
CC25	*Cantharellus* sp.	GA	*Quercus*	nLSU	C. Matherly
CA01	*Cantharellus* sp.	IL	*Quercus*	nLSU	J. McFarland

CN09	*C. cinnabarinus*	VA	*Quercus*	nLSU	T. Melin

(Vilgalys and Hester 1990). For the ITS region we used ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). For the TEF1 locus we used TEF1F and TEF1R (Morehouse et al. 2003). PCR reactions (25 μL) contained 1 μL of BSA [10x], 1 μL of $MgCl_2$ [25 mM], 0.5 μL of each primer [10 μM], 12.5 μL of green GoTAQ Master Mix © [2x], 8.5 μL of nuclease-free dH_2O, and 1 μL DNA [1:50]. For nLSU and ITS, thermal-cycler conditions were as follows: denature at 94 C for 30 min, 30 cycles of denature at 94 C for 1 min, anneal at 53 C for 1 min, and extend at 72 C for 3 min, a final extension at 72 C for 10 min and a 4 C holding temperature. For TEF1, conditions were followed as described by Morehouse et al. (2003). For nLSU and TEF1, PCR cleanups were performed using QIAGEN© QIAquick PCR purification kits according to manufacturer protocol. For ITS samples, gel-extractions were performed followed by the standard QIAGEN© QIAquick PCR purification protocol.

Sequencing.—Sequencing reactions were performed using BigDye terminator cycle sequencing (ABI Prism). Samples were sequenced in both directions using the same primers used in PCR except for the ITS locus, which only sequenced in one direction with ITS4. Reactions were 10 μL and contained 2.4 μL of purified PCR-product sample and 7.6 μL of master mix (0.5 μL of BigDye, 2.0 μL of BigDye Buffer, 0.8 μL of primer [2.0 μM] and 4.3 μL of dH_2O). Thermal-cycler conditions consisted of an initial denaturing step at 95 C for 3 min, 35 cycles of denaturing at 95 C for 20 sec, annealing at 45 C for 30 sec, and extending at 60 C for 4 min, with a final extension at 72 C for 7 min

and a 4 C holding temperature. Reactions were brought to 20 μL with nuclease-free dH_2O and then cleaned using QIAGEN© DyeEx 2.0 spin columns according to manufacturer protocol. These samples were shipped to the University of Wisconsin Biotechnology Center (Madison, Wisconsin) for sequencing.

Sequences were edited manually in Bioedit v.7.0.9 (Hall 2007) by comparison with the ABI chromatographs and alignment with contiguous sequences. Sequences from other published studies which contained sufficient locality information were downloaded from GenBank and used in our analysis (TABLE II). Sequences were aligned using MUSCLE (executed by EMBL-EBI, http://www.ebi.ac.uk/). Evolutionary models were determined using jModelTest (Guindon and Gascuel 2003, Posada 2008). These analyses indicated GTR+I +G models were the most appropriate for our data. Maximum Likelihood (ML) analyses were performed using Garli (Zwickl 2006) version 2.0 for 64-bit operating systems. Garli was also used to perform 1000 ML bootstrap replicates, and bootstrap values were obtained using PAUP (Swofford 1993) via PaupUp (Calendini and Martin 2007). To view and edit the trees, TreeView (Page 1996) was used. Taxa used for rooting trees were those consistently supported by previous studies (Feibelman et al. 1997, Dunham et al. 2003, Moncalvo et al. 2006, Buyck and Hofstetter 2011).

RESULTS

Morphology.—Chanterelles resembling *C. cibarius* found in La Crosse were categorized into three groups based on morphology. The first group, the pale morphotype, was defined by having pale lamellae that turn more pink as they mature, an incurled margin, pileus orange-yellow, a thick white stalk, and a light-pink colored spore print (FIG. 1a). The second group, the yellow morphotype, was distinguished by yellow lamellae, a

TABLE II. Sequences from GenBank used in my molecular analyses. All GenBank sequences used for the ITS analysis were published in Dunham et al. (2003) with the exception of DQ200926 (from AFTOL). Sequences obtained from GenBank that were used in the TEF1 analysis were published by Buyck and Hofstetter (2011) and Buyck et al. (2011).

GenBank #	Morphological ID	Location	Target	Study
DQ898690	*C. appalachiensis*	TN, USA	nLSU	1
AY041168	*C. cinnabarinus*	OR, USA	nLSU	2
DQ898692	*C. cinnabarinus*	TN, USA	nLSU	1
DQ898694	*C. lateritius*	TN, USA	nLSU	1
AY041167	*Cantharellus sp.*	OR, USA	nLSU	2
AY041169	*C. persicinus*	SC, USA	nLSU	2
AY041164	*C. formosus*	OR, USA	nLSU	2
AY041162	*C. cascadensis*	OR, USA	nLSU	2
AY041150	*C. subalbidus*	OR, USA	nLSU	2
AY041153	*C. cibarius* var. *roseocanus*	OR, USA	nLSU	2
AY745708	*C. cibarius*	NY, USA	nLSU	4
AY041157	*C. cibarius* var. *cibarius*	Sweden	nLSU	2
EU522825	*C. cibarius*	Canada	nLSU	3
DQ898693	*C. cibarius*	TN, USA	nLSU	1

1 – Moncalvo et al. 2006

2 – Dunham et al. 2003

3 – Porter et al. 2008

4 – AFTOL

yellow stalk, a margin often everted at maturity, pileus yellow, a yellow spore print, and a more slender and slightly smaller stature than the pale morphotypes (FIG. 1b). The third group, the salmon morphotype, features salmon-colored lamellae, a margin that is often curled down when young, becoming plane and wavy with age; the pileus is a shade of orange/pink/salmon, and the stalk is orange, becoming pale at the base (FIG. 1c). The stature is smaller and more slender than the other morphotypes, and the spore print is salmon-pink colored.

nLSU analysis.— Maximum Likelihood analysis of the nLSU locus resulted in a single tree with $-\ln L = -1989.6209$ (FIG. 2). Fifty sequences (860 bp) were analyzed at the nLSU locus. Thirty-seven sequences were produced in this study (TABLE I) and 13 sequences were obtained from GenBank (TABLE II). An ambiguously aligned region near the middle of the alignment (approx. 100 bp) was removed to test for changes in topology or support. No difference in topology or support was found so this region was included in the ML analysis.

The salmon-morphotype chanterelles were separated with strong support from other *C. cibarius*-like chanterelles, with basal relatives from southern Illinois and Missouri (FIG. 2). Although indistinguishable from each other at the nLSU locus, the pale and yellow morphotypes formed a strongly supported clade that was distinct from *C. cibarius* from Sweden, as well as from *C. cibarius* var. *roseocanus*. All of the samples in the *C. cibarius* var. *roseocanus* clade were found beneath coniferous trees (mainly *Tsuga* and *Picea*), whereas the pale, yellow, and salmon morphotype samples were all found beneath species of *Quercus*.

FIG. 1. Chanterelles from La Crosse, Wisconsin. A. Pale morphotype B. Yellow morphotype C. Salmon morphotype.

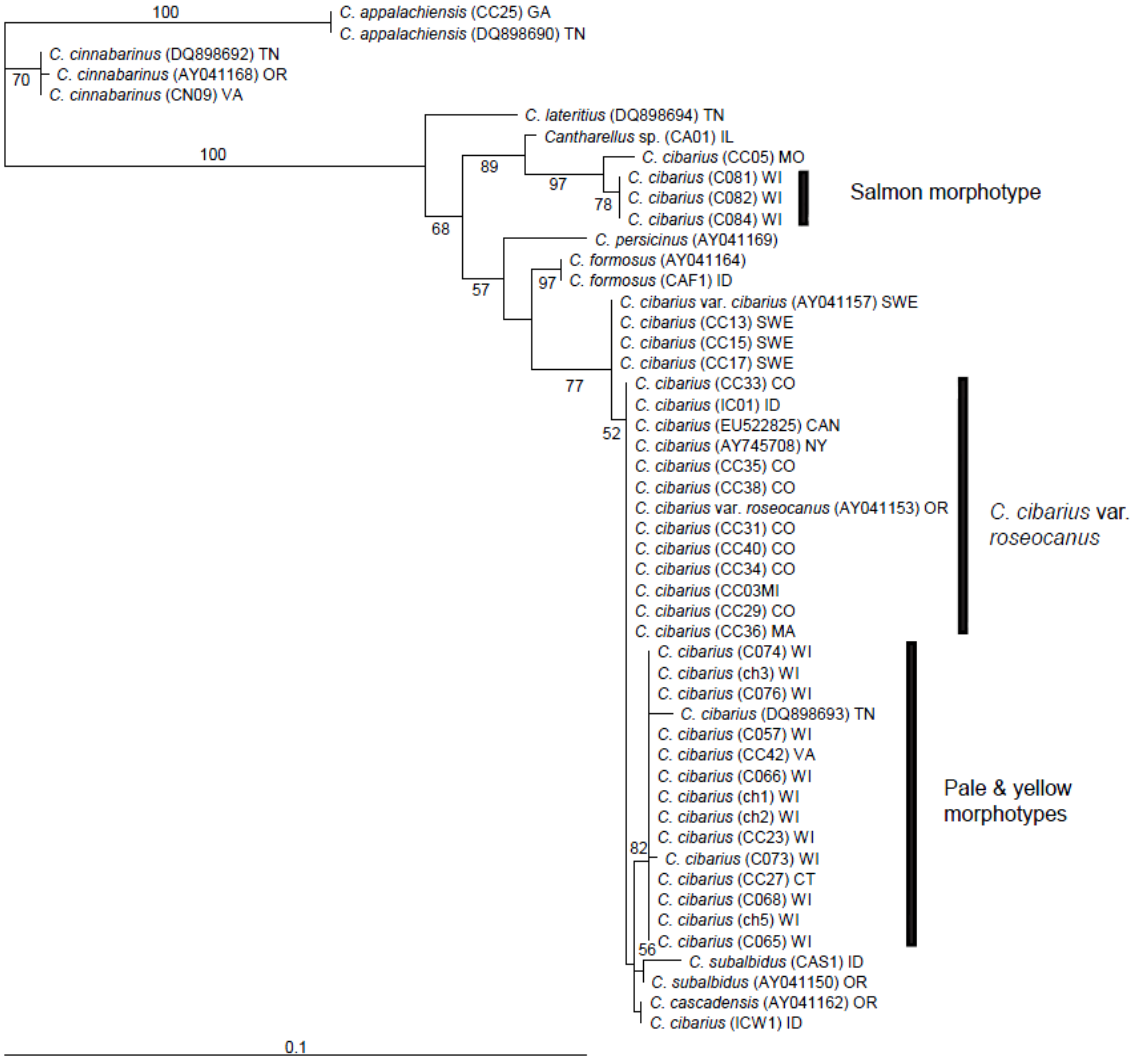

FIG. 2. Majority rule consensus tree of 50 nLSU sequences. Maximum likelihood bootstrap values from 1000 replicates are shown near branches. ML $-\ln L = -1989.6209$. GenBank/collection numbers are indicated in parenthesis after the name of the collection.

ITS analysis.—Maximum Likelihood analysis of the ITS region resulted in a single tree with $-\ln L = -918.6856$ (FIG. 3). Fourteen sequences (539 bp) were produced in this study (TABLE I) and 13 sequences were obtained from GenBank (TABLE II). Sequences could only be obtained in one direction (using the ITS4 primer), and were truncated before alignment.

Cantherellus formosus was used to root the ITS tree. The pale and yellow morphotypes were basal to *C. cibarius* and *C. roseocanus*. The pale and yellow morphotypes were also reciprocally monophyletic (FIG. 3). They also formed clades distinct from *C. cibarius* and *C. cibarius* var. *roseocanus*, which are shown as sister species in this phylogeny, although with weak support.

TEF1 analysis.—Maximum Likelihood analysis of the TEF1 locus resulted in a single tree with $-\ln L = -7127.1283$ (FIG. 4). Fifty-seven sequences (1021 bp) were analyzed for the TEF1 locus. Five sequences were produced in this study (TABLE I) and 52 sequences were obtained from GenBank (Buyck and Hofstetter 2011, Buyck et al. 2011).

The topology of our TEF1 phylogeny of *Cantharellus* is identical to the topology from Buyck and Hofstetter (2011) with the addition of the five samples from this study. The TEF1 phylogeny finds the salmon morphotype sister to *C. amethysteus* from Europe (FIG. 4). Our sample of *Cantharellus cibarius* var. *roseocanus* from Colorado is sister to *C. cibarius* from Europe with moderate support (65% bootstrap). The pale and yellow morphotypes are sister taxa that share a most recent common ancestor with two specimens of the newly described *C. tenuithrix* from the southern United States. These relationships are highly supported (85 and 90% bootstrap support respectively). The yellow morphotype sample forms a monophyletic group with one collection of *C. tenuithrix* described in Buyck and Hofstetter (2011).

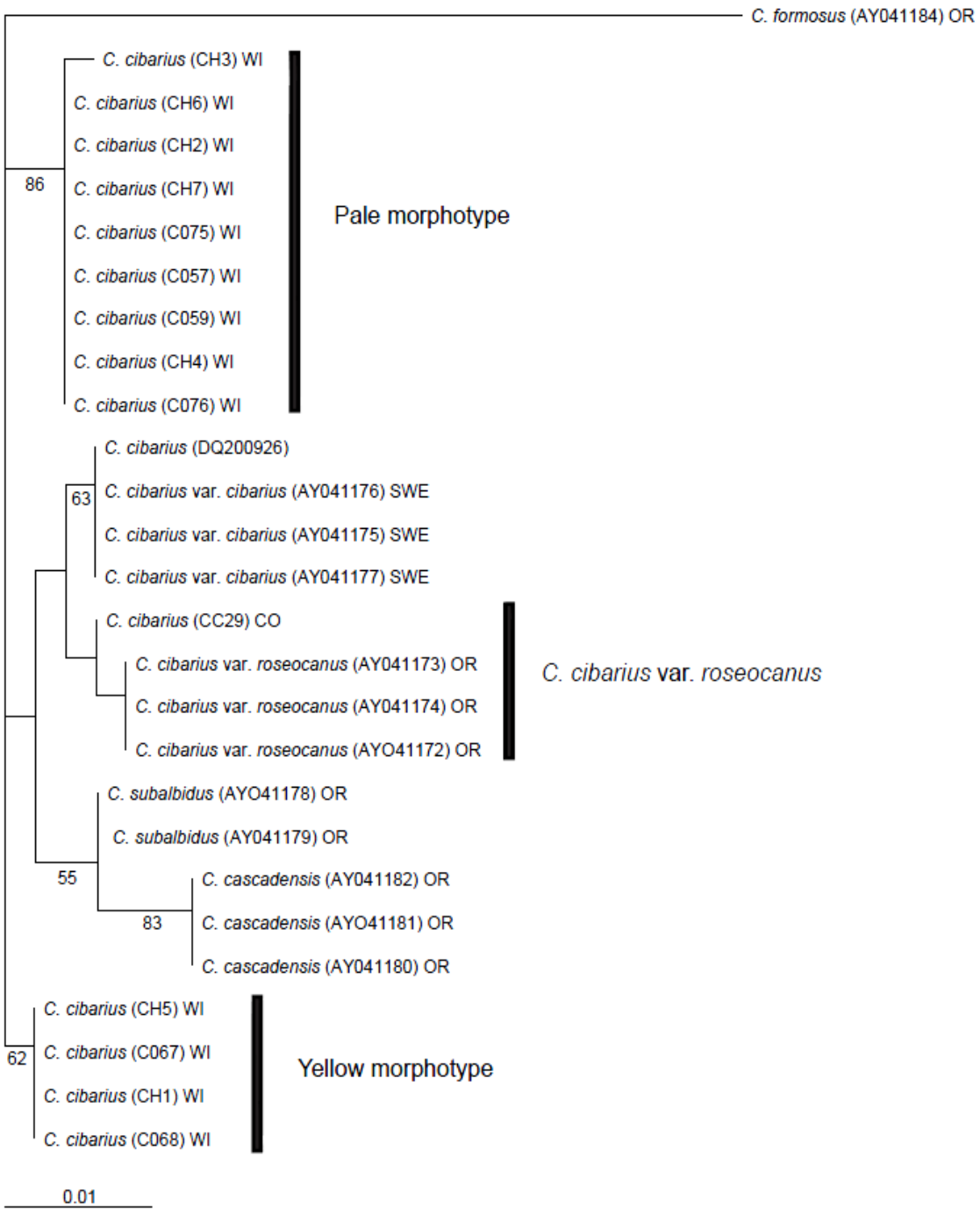

FIG. 3. Majority rule consensus tree of 27 ITS sequences. Maximum likelihood bootstrap values from 1000 replicates are shown below branches. ML $-\ln L = -918.6856$. GenBank/collection numbers are indicated in parenthesis after the name of the collection.

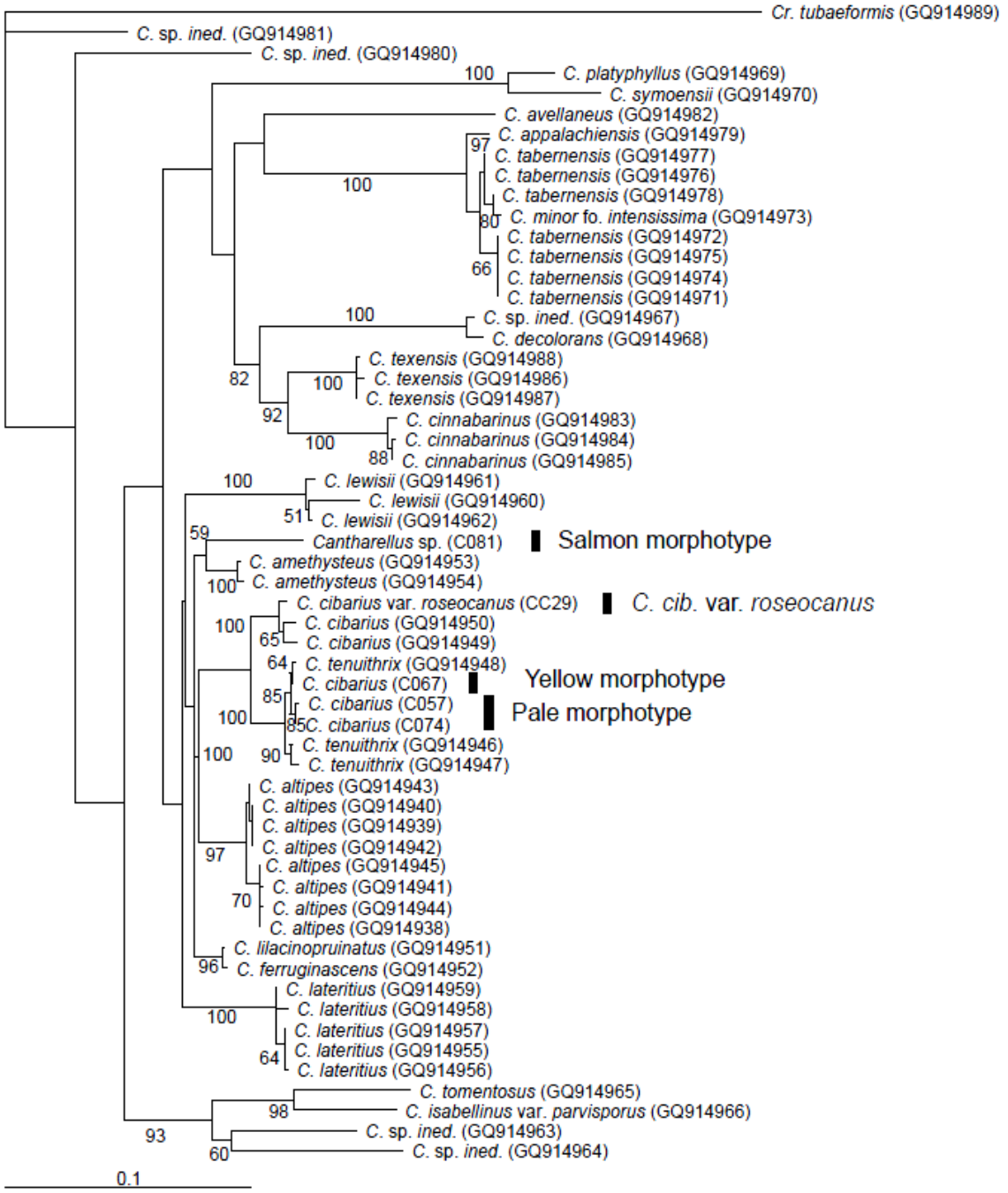

FIG. 4. Majority rule consensus tree of 57 TEF1 sequences. Maximum likelihood bootstrap values from 1000 replicates are shown near branches. ML $-\ln L = -7127.1283$. GenBank/collection numbers are indicated in parenthesis after the name of the collection.

TAXONOMY

Cantharellus phasmatis Foltz & Volk **sp. nov.**

FIGS. 1a, 5. SUPPLEMENTARY FIGS. 1-3

[Accession #]

[Latin diagnosis] Hymenium pale becoming pink, stalk pale becoming yellow, tissues staining ochraceous brown when bruised, spores pink. Molecular data from ITS and TEF1 loci distinguish this species from all other *Cantharellus* species.

Etymology: Named *phasmatis* meaning "ghost" for the distinctive ghostly-pale hymenium of young specimens of this chanterelle.

Holotypus: UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest, along the path running parallel to Bliss road, approximately halfway up the path when heading uphill, on the right side, under oak in oak-hickory mixed deciduous forest. 18-VII-2010, *Foltz C073* in [herbarium info].

Pileus egg-yolk yellow or paler (especially in sunlight or dry conditions), often mottled in appearance upon maturity, partly due to spore deposit, 6 – 12 cm in diameter, plano-convex with an incurved margin when young, becoming broadly convex to depressed, margin regular to irregular, sometimes lobed, often wavy with age, surface dry and covered in a thin layer of fibrils, staining ochraceous-brown when bruised. Context thick, white, firm. Odor of apricots is strong and pleasant. Taste mild at first, becoming peppery. KOH intensifying color. Lamellae deeply decurrent, pale when young becoming pinkish-buff with age and often yellowish near the margin, often forking and anastomosing, sometimes almost poroid in some specimens, bruising ochraceous brown. Stipe white and solid, yellowing and peeling with maturity, bruising ochraceous brown, 4

FIG. 5. *Cantharellus phasmatis* sp. nov. found in La Crosse, Wisconsin, July 2010

– 8 cm long, 1 – 3 cm thick, solid, context white. Spore deposit salmon-pink; (7) 7.5 – 10 (11) × 4 – 6 (7) μm, sub-globose to ovate when small, more mature spores are obovate to oblong, sometimes reniform (n = 30). Basidia (55) 60 – 70 (75) × (7) 7.5 – 11 (13) μm, 4

– 6 sterigmate, clavate, often undulate. Pileus hyphae with long terminal cell, 95 – 105 × 4.5 – 5.5 μm, sometimes with thickened walls. Clamps found in all tissues. Habitat: Gregarious to scattered under oak-hickory, July-August in La Crosse, WI, presumably more widspread. Often growing in lines along roots. Common. Edibility: choice, these are the most delectable chanterelles we have eaten.

Specimens examined. UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest, along the path running parallel to Bliss road, under oak in oak-hickory mixed deciduous forest. 18-VII-2010, *Foltz C073* (*holotypus*), *C074, C075, C076, C077, C078, C079, C080* in [herbarium info].

Comments: Distinguished from other chanterelles by its pale hymenium and pink spore print. This species is almost certainly the same as *C. cibarius* var. *pallidofolius* as described by Smith in 1968 (see discussion for details). We suggest "ghost chanterelle" as a common name for this species.

Cantharellus flavus Foltz & Volk **sp. nov.**

FIGS. 1b, 6. SUPPLEMENTARY FIG. 4

[Accession info]

[Latin diagnosis] Hymenium yellow, stalk yellow, spores yellow. Molecular data from ITS and TEF1 loci distinguish this species from all other *Cantharellus.*

Holotypus: UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest, along the path running parallel to Bliss road, approximately halfway up the path when heading uphill, on right side, under oak in oak-hickory mixed deciduous forest. 18-VII-2010, *Foltz C066* in [herbarium info]. *Etymology:* named *flavus* for the yellow color of the stipe, hymenium, pileus, and spores.

FIG. 6. *Cantharellus flavus* sp. nov. found in La Crosse, Wisconsin, July 2010.

Pileus egg-yellow or paler with age or exposure to light, 4 – 9 cm in diameter, plano-convex when young, margin incurved, regular to irregular, becoming plane to wavy and depressed to broadly infundibuliform with a margin that is usually everted when mature, sometimes lobed or sinuate on one side, fragrant odor, taste slightly peppery. Lamellae

deeply decurrent, egg-yellow in color, not readily staining when bruised but sometimes bruises show up ochraceous brownish-yellow in dried specimens. Stalk yellow in color, sometimes patched with white appearing mottled in age, 3 – 8 cm long, 0.5 – 2 cm thick. Spore print yellow. Spores (7.5) 8 – 10 (11) × (4) 4.5 – 6 μm , sub-globose to obovate when young, becoming oblong (n = 30). Basidia (63) 75 – 80 (84) × 7 – 9 (10) μm, 4 – 6 sterigmate, clavate, often undulate. Pileus hyphae with a long terminal cell (78) 85 – 95 (100) × 4.5 – 5.5 (6) μm, sometimes with thickened walls. Clamps found in all tissues. Habitat: cespitose to gregarious under oak-hickory, on well drained soil, especially on hillsides. Common in July – August in western Wisconsin, presumably more widespread. Edibility: choice.

Specimens examined. UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest, along the path running parallel to Bliss road, under oak in oak-hickory mixed deciduous forest. 18-VII-2010, *Foltz C066* (*holotypus*), *C065, C067, C068, C069, C070, C071, C072* in [herbarium info].

Comments: Most easily distinguished from other chanterelles by its yellow coloration of the hymenium, stalk, and spores. We suggest the common name "Eastern yellow chanterelle" for this species.

Cantharellus spectaculus Foltz & Volk **sp. nov.**

FIGS. 1c, 7. SUPPLEMENTARY FIG. 5

[Accession info]

[Latin diagnosis] Pileus orange-salmon, hymenium salmon-purple, spores salmon-pink and larger in size than in *C. phasmatis.* Molecular data from nLSU and TEF1 loci distinguish this species from all other *Cantharellus.*

Holotypus: UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest, along the path running parallel to Bliss road, approximately ¾ of the up the path on right side, under oak in oak-hickory mixed deciduous forest. 18-VII-2010, *Foltz C081* in [herbarium info]. *Etymology:* named *spectaculus* for the unusual salmon-pinkish purple color of the hymenium and orange colored stalk, a showy combination.

Pileus orange/salmon colored and 6 – 9 cm in diameter when mature, usually circular but sometimes elongated, umbraculaform when young becoming plano-convex to depressed with maturity, with the outer margin incurled and becoming wavy and more plane to infundibuliform with age. The pileus surface is somewhat textured or subtomentose under a hand lens. Lamellae salmon/pink colored with almost purplish hues under certain light, with gill folds that often fork and sometimes anastomose. Stipe orange in color, white at the base, slender and usually as long or longer than the diameter of the pileus. The context tissue is whitish, with cortex tissue similar in color to the surface. Spore print salmon-pink. Spores 12 μm × 6.5 μm, oblong-elliptical. Basidia (104) 110 – 120 (125) × (9) 10 – 11 μm, mostly 4-sterigmate (5), clavulate, sometimes with clamps at the base that stem new basidia directly from the clamp. Pileus hyphae terminal cell (52) 60 – 65 × 4.5 – 5.5 μm. Clamps found in all tissues. Habitat: cespitose to gregarious under oak-hickory, especially on hillsides. Rare in the type locality in Wisconsin, presumably more widespread. July-August. Edibility: choice.

Specimens examined. UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest, along the path running parallel to Bliss road, approximately ¾ of the up the path when heading uphill, on right side, under oak in oak-hickory mixed deciduous forest. 18-VII-2010, *Foltz C081* (*holotypus*), *C082, C083, C084, C085, C086, C087, C088* in

FIG. 7. *Cantharellus spectaculus* sp. nov. found in La Crosse, Wisconsin, July 2010.

[herbarium info].

Comments: Distinguished from other chanterelles by its salmon-colored hymenium, orange stalk, lack of egg-yellow coloration of the pileus, salmon-pink spores, and larger spore size. We suggest the common name “Spectacular chanterelle” for this species based on its unusual color combination.

Cantharellus roseocanus (Redhead, Norvell, & Danell) Foltz & Volk, **comb. nov.**

Basionym: *Cantharellus cibarius* var. *roseocanus* Redhead, Norvell, & Danell, Mycotaxon 65: 285 (1997)

DISCUSSION

Three new species, *Cantharellus phasmatis* (the pale morphotype), *Cantharellus flavus* (the yellow morphotype), and *Cantharellus spectaculus* (the salmon morphotype) are proposed based on morphological and molecular characteristics that distinguish them from related species. In addition, *C. cibarius* var. *roseocanus* is elevated to species status.

The morphological characteristics that were phylogenetically informative included the hymenium color and spore color, as well as the larger spore size of *C. spectaculus.* Geographic distribution and mycorrhizal host-type associations are useful for separating the newly proposed species from *Cantharellus cibarius sensu stricto,* which is only known from Europe (Buyck and Hofstetter 2011), and from *C. cibarius* var. *roseocanus* which has only been reported under coniferous trees (Redhead et al. 1997, Dunham et al. 2003, Arora and Dunham 2008, this study).

Spore print color has been used previously to distinguish species in the Cantharellales. Matheny et al. (2010) found that ITS data supported spore color differences between *Craterellus fallax* and *Craterellus cornucopioides*, two species that were difficult to resolve using nLSU and were previously combined (Dalhman et al. 2000). Our study provides additional support for spore color as an important diagnostic feature at the species level, as well as the use of the ITS locus to delineate closely related species.

Cantharellus phasmatis is almost certainly *C. cibarius* var. *pallidofolius* as

described by Smith (1968) based on morphological description. We propose to recognize this taxon at the taxonomic level of species. This distinction is supported by its placement in a monophyletic clade separate from *C. cibarius* in three separate gene phylogenies (FIGS. 2, 3, 4). Buyck and Hofstetter (2011) stated that Petersen and Eyssartier had both concluded upon re-examination of Peck and Smith's type collections that they were not accompanied by sufficient descriptions, illustrations, and molecular data to be useful. However, it is clear that Petersen recognized Smith's *C. cibarius* var. *pallidofolius* as a valid taxon (1976) and he even illustrated it himself (1979). In addition, we found Smith's original description (1968) to be quite thorough, including adequate microscopic descriptions as well as a photograph. Arora and Dunham (2008) attempted to sequence the type specimen of *C. cibarius* var. *pallidofolius,* but reported that DNA degredation in the type collection prevented them from producing a sequence. Given this uncertainty, we have decided to describe this species using a new epithet, *Cantharellus phasmatis.*

Cantharellus flavus is morphologically very similar to Smith's (1968) description of *C. cibarius* var. *cibarius* with the one major exception of spore color. Smith described the spores as cream-buff, but our samples produced bright yellow spores. Petersen's (1969, 1985) description of a yellow-spored chanterelle is quite fitting and could be the same taxon we have described here. Molecular data from the nLSU locus suggest closely related chanterelles exist in the Appalachian region, supporting this hypothesis.

Cantharellus phasmatis and *C. flavus* are sister species that share their most recent common ancestor with *C. tenuithrix.* One of the main morphological features we used to distinguish between *C. phasmatis* and *C. flavus* was the hymenium color. Unfortunately the description of *C. tenuithrix* does not include the color of the

hymenium. However, Buyck's photograph (http://www.mtsn.tn.it/cantharellus-news/tx_photos.asp?index=20008) clearly shows a yellow hymenium. One of the specimens of *C. tenuithrix* was a very close sequence match to *C. flavus,* a chanterelle that shares the same color hymenium. The other two samples of *C. tenuithrix* (including the holotype) were monophyletic with strong support. Our other main distinguishing morphological character was spore print color. The pink spores of *C. phasmatis* and yellow spores of *C. flavus* are distinct from the cream colored spores of *C. tenuithrix.* The full geographic range of the new species described here and those described by Buyck and Hofstetter (2011) are not yet clear, but these data suggest that the southern range of *C. flavus* extends to the Gulf coast of the United States.

Cantharellus spectaculus is in a separate and more distant clade and represents the first representative in this clade in North America. Its closest relative from the TEF1 dataset is *Cantharellus amethysteus* from Europe. In 1967, Smith presented a paper at a meeting of the Mycological Society of America featuring an undescribed chanterelle exhibiting salmon coloration across the entire fruiting body (Petersen 1969). This salmon-colored taxon could be the one described here. We sequenced the nLSU region of two *C. cibarius*-like chanterelles from southern Illinois and Missouri that represent two undescribed basal lineages that form a clade with *C. spectaculus* in our nLSU gene phylogeny. These taxa will require further investigation.

Cantharellus cibarius var. *roseocanus* was distinct from *C. cibarius* in all three of the gene phylogenies in this study. In addition, nLSU data suggest this taxon is the most widespread chanterelle in North America, with a range spread across Oregon, Idaho, Colorado, Michigan, New York, Massachusetts, and Newfoundland (Greg Thorn,

personal communication, July 2011). Our study is not the first to find this result, but it is the first to report it. Moncalvo et al. (2006) used a sample labeled *C. cibarius* from the eastern United States that was a 100% sequence match to a sample of *C. cibarius* var. *roseocanus* from Oregon that was included in their study. The relationship between these two taxa was not clear in the tree they published, which is perhaps why the result was not mentioned in their discussion or any other chanterelle paper since. This taxon seems to be ecologically separated from the newly described species in this study in that it has only been found associated with coniferous trees. It may have gone previously unnoticed because of morphological differences associated with geographic location. For example, the samples we found in Colorado were only about half the size of the original description (Redhead et al. 1997), and the samples from Michigan were even smaller. We propose to raise this taxon to species level based on molecular support, ecological association, and geographic separation from *C. cibarius*, which is only known from Europe. Further analysis of ITS and TEF1 regions may show that this clade is composed of more than one geographically isolated species. However, based on our data, we see no support for continuing to describe members of this clade as a variety of *C. cibarius.*

Of the three loci analyzed in our study, we found that the ITS and TEF1 loci had more resolution than the nLSU region at terminal nodes, but that the nLSU region was still useful and provided additional confidence in defining relationships. The nLSU was also by far the least problematic locus to work with. Initially we also tried to amplify the RPB2 locus, as suggested by Moncalvo et al. (2006), but we could not get consistent amplification. We suspect cloning is necessary as other researchers have suggested (Bart Buyck, personal communication).

The genus *Cantharellus* is proving to be very diverse, and potentially harbors many other undescribed lineages within North America. Several commonly accepted species may represent species complexes (unpublished data). Large-scaled geographic sampling is needed to address these issues and clarify the *Cantharellus* species in North America.

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LITERATURE CITED

Arora D, Dunham S. 2008. A new, commercially valuable chanterelle species, *Cantharellus californicus* sp. nov., associated with live oak in California, USA. Economic Botany. 62(3): 376-391.

Bigelow H. 1978. The Cantharelloid fungi of New England and adjacent areas. Mycologia. 70(4): 707-756.

Buyck B, Hofstetter V. 2011. The contribution of *tef-1* sequences to species delimitation in the *Cantharellus cibarius* species complex in the southeastern USA. Fungal Diversity 49: 35-46.

———, Cruaud C, Couloux A, Hofstetter V. 2011. *Cantharellus texensis* sp. nov. from Texas, a southern lookalike of *C. cinnabarinus* revealed by *tef-1* sequence data. Mycologia. *In press.*

Coker WC. 1919. *Craterellus, Cantharellus*, and related genera in North Carolina; with a key to the genera of gilled fungi. Journal of the Elisha Mitchell Scientific Society. 35: 24-48.

Collins K. 2005. Ph.D. Dissertation: Spatial and Temporal Population Dynamics of *Cantharellus formosus*, the Pacific Golden Chanterelle, in Oregon. Oregon State University. Oregon. Pp 136.

Corner E. 1966. A monograph of Cantharelloid fungi. Oxford University Press: London.

Dahlman M, Danell E, Spatafora J. 2000. Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. Mycological Research. 104(4): 388-394.

Danell E. 1994. Thesis: *Cantharellus cibarius*: mycorrhiza formation and ecology. Uppsala University. Sweden. Pp 63.

———, Camacho, F. 1997. Successful cultivation of the golden chanterelle. Nature. 385: 303.

———. 1999. *Cantharellus*. In: Cairney JWG, Chambers SM, eds. Ectomycorrhizal Fungi: Key Genera in Profile. Berlin: Springer Verlag: 253-267.

———. 2001. Chanterelle mycelium. U.S. Patent 6,173,525.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research. 36 (Web Server Issue):W465-9.

De Queiroz K. 2007. Species Concepts and Species Delimitation. Systematic Biology. 56(6): 879-886.

Donk MA. 1964. Conspectus of the families of Aphyllophorales. Persoonia. 3(2): 199-324.

———. 1969. Notes on *Cantharellus* sect. *Leptocantharellus*. Persoonia. 5(3): 265-284.

Dunham S, O'Dell T, Molina R. 2003. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov.

from the American Pacific Northwest. Mycological Research. 107(10): 1163-1177.

———, O'Dell T, Molina R. 2006. Spatial analysis of within-population microsatellite variability reveals restricted gene flow in the Pacific golden chanterelle (*Cantharellus formosus*). Mycologia. 98(2): 250-259.

Feibelman T, Bayman P, Cibula W. 1994. Length variation in the internal transcribed spacer of ribosomal DNA in Chanterelles. Mycological Research. 98(6): 614-618.

———, Bennett J, Cibula W. 1996. *Cantharellus tabernensis*: A new species from the Southeastern United States. Mycologia. 88(2): 295-301.

———, Doudrick R, Cibula W, Bennett J. 1997. Phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of the nuclear large subunit rDNA. Mycological Research. 101(12): 1423-1430.

Fries EM. 1821. *Cantharellus*. Systema Mycologicum I. 318.

Fries N. 1979. Germination of spores of *Cantharellus cibarius*. Mycologia. 71: 216-219.

Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes – applications to the indentification of mycorrhizae and rusts. Molecular Ecology. 2: 113-118.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology. 52: 696-704.

Homola RL. 1993. Cantharelloid fungi of Maine. Maine Naturalist. 1(2): 5-12.

Jansen E, van Dobben HF. 1987. Is decline of *Cantharellus cibarius* in the Netherlands due to air pollution? Ambio. 16(4): 211-213.

Lindner D, Banik M. 2009. Effects of cloning root-tip size on observations of fungal ITS sequences from *Picea glauca* roots. Mycologia. 101(1): 157-165.

Lutzoni FM. 1997. Phylogeny of lichen- and non-lichen forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Systematic Biology. 46(3): 373-406.

Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Froslev T, Ge Z, Kerrigan RW, Slot JC, Yang Z, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS. 2007. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution. 43: 430-451.

———, Austin EA, Birkebak JM, Wolfenbarger AD. 2010. *Craterellus fallax*, a Black

Trumpet mushroom from eastern North America with a broad host range. Mycorrhiza. 20: 569-575.

Moncalvo J, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, Porter TM, Margaritescu S, Garnica MWS, Danell E, Langer G, Langer E, Larsson E, Larsson K, Vilgalys R. 2006. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia 98(6): 937-948.

Morehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy PJ, Longcore JE. 2003. Multilocus sequence typing suggests the chytid pathogen of amphibians is a recently emerged clone. Molecular Ecology. 12: 395-403.

Mui D, Feibelman T, Bennett JW. 1998. A preliminary study of the carotenoids of some North American species of *Cantharellus*. International Journal of Plant Sciences. 159(2): 244-248.

Murrill WA. 1910. Family 7: Agaricaceae. North American Flora. 9: 163-172.

———. 1920. Notes and Brief Articles. Mycologia. 12(2): 104-111.

Page RDM. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences. 12: 357-358.

Peck C. 1887. New York species of *Cantharellus*. Bulletin of the New York State Museum of Natural History. 1: 34-43.

Petersen RH. 1968. Notes on Cantharelloid fungi. I. Gomphus S. F. Gray, and some clues to the origin of Ramarioid Fungi. Journal of the Elisha Mitchell Scientific Society. 84(3): 373-381.

———. 1969. Notes on Cantharelloid fungi. II. Some new taxa, and notes on *Pseudocraterellus*. Persoonia. 5(3): 211-223.

———. 1971. Interfamilial relationships in the Clavarioid and Cantharelloid fungi. In Evolution in the Higher Basidiomycetes (ed. R.H. Petersen), pp. 345-374. University of Tennessee Press: Knoxville, TN, USA.

———. 1975. Cantharelloid fungi. V. A new species of *Cantharellus.* Beihefte zur Nova Hedwigia. 51: 183-189.

———. 1976. Notes on Cantharelloid fungi. VII. The taxa described by Charles H. Peck. Mycologia. 68: 304-326.

———. 1979. Notes on Cantharelloid fungi. IX. Illustrations of new or poorly understood taxa. Nova Hedwigia. 31: 1-23.

———. 1985. Notes on Clavarioid Fungi. XIX. Colored illustrations of selected taxa, with comments on *Cantharellus.* Nova Hedwigia. 42: 151-160.

Pine E, Hibbett D, Donoghue M. 1999. Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mitochondrial and rDNA sequences. Mycologia. 91(6): 944-963.

Pilz D, Molina R, Liegel L. 1998. Biological productivity of Chanterelle Mushrooms in and near the Olympic Peninsula Biosphere Reserve. Ambio. 9: 8-13.

———, Norvell L, Danell E, Molina R. 2003. Ecology and management of commercially harvested chanterelle mushrooms. USDA Forest Service PNW-GTR-576. 90 Pp.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution. 25: 1253-1256.

Quélet L. 1888. Flore Mycologique de la France et des Pays Limitrophes. Otave Doin: Paris.

Rangel-Castro JI, Danell E, Pfeffer PE. 2002. A 13C-NMR study of exudation and storage of carbohydrates and amino acids in the ectomycorrhizal edible mushroom *Cantharellus cibarius.* Mycologia. 94(2): 190-199.

Redhead S, Norvell L, Danell E. 1997. *Cantharellus formosus* and the Pacific golden chanterelle harvest in western North America. Mycotaxon. 65: 285-322.

Smith AH, Morse EE. 1947. The genus *Cantharellus* in the western United States. Mycologia. 39(5): 497-534.

———. 1968. The Cantharellaceae of Michigan. The Michigan Botanist. 7: 143-167.

Swofford, DL. 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1. Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D.C., USA.

Taylor J, Jacobson D, Kroken S, Kasuga T, Geiser D, Hibbett D, Fisher M. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology. 31: 21-32.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology. 172: 4238-4246.

Watling R. 1997. The business of fructification. Nature. 385: 299-300.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White

T, eds. PCR protocols: A Guide to Methods and Applications. Academic Press: New York, USA. 315-322.

Zwickl DJ. 2006. Garli: Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.

APPENDIX

SUPPLEMENTARY FIG. 1. *Cantharellus phasmatis* sp. nov.

SUPPLEMENTARY FIG. 2. *Cantharellus phasmatis* sp. nov.

SUPPLEMENTARY FIG. 3. *Cantharellus phasmatis* sp. nov.

SUPPLEMENTARY FIG. 4. *Cantharellus flavus* sp. nov.

SUPPLEMENTARY FIG. 5. *Cantharellus spectaculus* sp. nov.