

## Molecular phylogeny and morphology reveal three new species of *Cantharellus* within 20 m of one another in western Wisconsin, USA

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**Abstract:** Three new species, *Cantharellus phasmatis*, *Cantharellus flavus* and *Cantharellus spectaculus*, all previously considered *Cantharellus cibarius*, are described in this study. The circumscription of these three species from *C. cibarius* and other *Cantharellus* species is supported by morphological differences and nuclear DNA sequence data (nLSU, ITS, *TEFI*). All were found under *Quercus* spp. in a small plot in Hixon Forest Park in La Crosse, Wisconsin, emphasizing the need for further taxonomic study of even common and conspicuous genera in North America. In addition, a review of the current state of *C. cibarius* sensu lato systematics is presented, including a review of the recent elevation of *C. cibarius* var. *roseocanus* to the species rank. Taxonomic descriptions and photographs are provided for the newly described species.

**Key words:** Cantharellales, *Cantharellus cibarius*, chanterelle, diversity, systematics

### INTRODUCTION

Chanterelle mushrooms are considered choice edibles in many countries around the world because of their apricot odor and delicious flavor. As choice edible mushrooms they are highly sought after and economically important (Watling 1997), and the taxonomy of this genus recently has undergone much-needed revision (Feibelman et al. 1994, Dunham et al. 2003, Buyck and Hofstetter 2011). The common yellow-golden chanterelle (*Cantharellus cibarius* Fr.) originally was 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” (English translation from Latin by T. Volk). This description is not sufficiently detailed to distinguish this species from taxonomic names in modern use outside Sweden, and no type specimen exists for Fries’ *C. cibarius*. Investigations using

morphological and DNA data have shown that *C. cibarius* in the United States is a species complex that requires further taxonomic attention (Feibelman et al. 1994, Dunham et al. 2003, Moncalvo et al. 2006, Arora and Dunham 2008, Buyck and Hofstetter 2011). In this study we continue efforts to document North American *Cantharellus* diversity.

In the past 50 years, several advancements in taxonomy of *Cantharellus* have been made from morphological data. Smith (1968) described *C. cibarius* var. *cibarius* from Michigan, which he believed to be the same as *C. cibarius* 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 whitish stalk and a pale pink hymenium, which he called *C. cibarius* var. *pallidifolius* A.H. Sm. (later reiterated and illustrated by Petersen [1976, 1979]). This variety also was 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, as well as the more brightly colored and slightly smaller spores. He noted that the cream-spored form closely matched the description of *C. cibarius* by Smith and 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 Mycological Society of America meeting featuring an undescribed chanterelle exhibiting salmon coloration across the entire basidiocarp.

Following extensive observation of European specimens, Petersen (1976) concluded that *C. cibarius* in Europe and *C. cibarius* in North America were not conspecific. He stated that across the two continents

the name *C. cibarius* was being applied to 8–10 taxa and probably many more (Petersen 1979). His concept of these taxa was based on morphological characters such as spore print color, stipe color, gill-fold anastomosis (or lack thereof) 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 *C. cibarius* var. *pallidifolius*, the variety Smith (1968) had described from Michigan, and recognized it as having one of the largest basidiocarps in the genus *Cantharellus* (Petersen 1979). Although he described this chanterelle as common in North America, especially in the east, Petersen stated that in northern and western America, intermediate taxa occur, making species delineation 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 cream-buff spore print. He acknowledged Petersen’s varieties as well as Corner’s and said he was not sure which varieties were present in New England because his focus for the chanterelles was primarily on their edible nature. Homola (1993) suggested that spore deposit color and ornamentation were more important for taxonomy than the shape of the basidiocarp or the structure of the hymenophore. Homola (1993) considered these macroscopic features to be non-diagnostic for systematics and examples of convergent evolution. Feibelman et al. (1997) used molecular data to test the utility of morphological characters, finding 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 still were informative for relationships among species within a genus.

Buyck and Hofstetter (2011) described two new chanterelles from the southern United States, *C. tenuithrix* Buyck and *C. altipes* Buyck. These authors provide microscopic descriptions of terminal hyphal cells from the pileus, as well as basidia measurements and spore attributes. They suggested that the length and cell-wall thickness of the terminal cells might be an important diagnostic character. Although these characteristics may be diagnostic for species delineation or to show subgenus relationships, they report 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.” The use of molecular techniques to provide independent lines of evidence has proved necessary to resolve some of these relationships and delineate species (Feibelman et al. 1994, Feibelman et al. 1997, Buyck and Hofstetter 2011, Tibuhwa et al. 2012).

Phylogenetic studies at genus and family ranks relying on nuclear small subunit (nSSU) and nLSU sequences of cantharelloid fungi have been plagued with alignment difficulties due to an accelerated molecular evolution of the nuclear rDNA genes in these taxa, resulting in their placement on distinctively long branches (Moncalvo et al. 2006). However, a study using nLSU to infer relationships among *Cantharellus* (Feibelman et al. 1997) was supported by a four-gene phylogeny of nLSU, nSSU, mitochondrial small subunit (mSSU) and RNA polymerase subunit II (RPB2) sequences (Moncalvo et al. 2006) and a study of the translation elongation factor 1 $\alpha$  (*TEFI*) region (Buyck and Hofstetter 2011). Species delineation in *Cantharellus* has been assisted by the use of a variety of molecular markers. Arora and Dunham (2008) used RFLP data from ITS sequences to provide molecular support for the distinction of *C. californicus* Arora & Dunham, a large yellow chanterelle from the western United States, from *C. formosus* Corner, *C. subalbidus* A.H. Sm. & Morse and *C. cibarius* var. *roseocanus*. ITS and nLSU were used to distinguish species of *Cantharellus* and *Craterellus* (Feibelman et al. 1997). Dunham et al. (2003) used ITS and nLSU sequences to describe a new species from the *C. cibarius* complex, *C. cascadenis* Dunham, O’Dell, & R. Molina, from the Pacific Northwest. This result was supported by Moncalvo et al. (2006) who also found support for the specific status of *C. cascadenis*, *C. formosus*, *C. subalbidus*, *C. persicinus* R.H. Petersen, *C. lateritius* (Berk.) Singer and European *C. cibarius* (Moncalvo et al. 2006). While these studies successfully delineated species within *Cantharellus*, most found indications of more undescribed diversity.

Feibelman et al. (1994) in *C. cibarius* s.l. found variable lengths of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. This variation suggested that *C. cibarius* could be a species complex (Feibelman et al. 1994). Dunham et al. (2003) noted that some *C. cibarius* s.l. samples from North Carolina were excluded from the study because their sequences were highly divergent and non-monophyletic, suggesting that more species exist in the *C. cibarius* complex in North Carolina (eastern United States). Following up on these studies, Buyck and Hofstetter (2011) used the *TEFI* locus to delineate two new species, *C.*

*tenuithrix* and *C. altipes*, within the *C. cibarius* complex in the southeastern United States. However, there are potentially more undescribed species within the *C. cibarius* complex in the eastern, southern, midwestern and northern United States (Feibelman et al. 1994, Dunham et al. 2003, Moncalvo et al. 2006, Buyck and Hofstetter 2011, Toby Feibelman pers comm, Bart Buyck pers comm).

Our study was initiated when morphologically distinct *Cantharellus* specimens were found growing under *Quercus* within 20 m of each other in a well sampled, highly traveled city park (Hixon Forest Park, La Crosse, Wisconsin); the distinctive morphology suggested the existence of more than one species. In addition, the morphology of these chanterelles did not closely match each other or descriptions of *C. cibarius*. The purpose of our study was to (i) identify consistent morphological differences and group chanterelles into morphotypes, (ii) test for differences in nuclear gene sequence between morphotypes and (iii) describe any new species discovered.

#### MATERIALS AND METHODS

*Fieldwork and herbarium materials.*—Chanterelles were collected (MJF and TJV) and grouped into morphotypes using characters such as the color of the pileus, hymenium, stipe, spore print. The associated genus of plant was recorded. Collection sites were georeferenced with the aid of Google Earth (<http://www.google.com/intl/en/earth/index.html>). We also collected chanterelles from Idaho and Colorado to compare with our samples and included specimens from other collectors and from herbaria (TABLE I). Fresh specimens were photographed, and tissue samples (2–3 mm<sup>3</sup> fresh) from the pileus were digested in 50 µL 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 also were taken from the pileus and digested in CLS. Spore prints were taken from freshly collected specimens by setting them on light blue paper and covering them with a beaker 12 h. Fresh basidiocarps were dehydrated at 35 C in a food dehydrator and stored for herbarium accession. Microscopic analyses were performed with dried material reconstituted in 3% KOH. Pileipellis hyphal extremities were examined as in Buyck and Hofstetter (2011). Twenty-four new collections from our study were deposited in the Field Museum of Natural History (F).

*Molecular data collection.*—Tissue samples in 50 µL CLS were ground with sterile plastic pestles fitted into an electric drill. Four hundred fifty microliters CLS was added and tubes were incubated at 65 C for 2 h. Five hundred microliters chloroform/isoamyl alcohol (24:1) was added, and tubes were shaken vigorously for 5 min to form an emulsion. Samples were centrifuged at 13 000 RPM for 12 min. Supernatants were transferred to new tubes, and

the previous two steps were repeated. Six hundred microliters of cold (–20 C) isopropanol were added to each reaction, and tubes were inverted and stored at –20 C for a minimum of 12 h. Reactions were centrifuged at 13 000 RPM for 12 min, and alcohol was decanted. The remaining DNA pellet was rinsed with 1 mL 70% EtOH and centrifuged. Alcohol again was decanted and pellets were allowed to air dry in a fume hood, then resuspended in 50 µL TE buffer (8.0 pH) and 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 ITS4R (White et al. 1990) and LR5 (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 BSA [10×], 1 µL MgCl<sub>2</sub> [25 mM], 0.5 µL each primer [10 µM], 12.5 µL green GoTAQ Master Mix© [2×], 8.5 µL of nuclease-free dH<sub>2</sub>O, and 1 µL DNA [1:50]. For nLSU and ITS, thermal-cycler conditions were: 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 holding at 4 C. For TEF1, conditions were as described by Morehouse et al. (2003). For nLSU and TEF1, PCR cleanups were performed with QIAGEN QIAquick PCR purification kits according to manufacturer protocol. For ITS samples, to limit sequencing to the target locus, gel-extractions were performed followed by the standard QIAGEN QIAquick PCR purification protocol.

Sequencing reactions were performed with BigDye terminator cycle sequencing (ABI Prism). Samples were sequenced in both directions with the same primers used in PCR. Reactions were 10 µL and contained 2.4 µL purified PCR-product sample and 7.6 µL master mix (0.5 µL BigDye, 2.0 µL BigDye Buffer, 0.8 µL primer [2.0 µM] and 4.3 µL dH<sub>2</sub>O). Thermal-cycler conditions consisted of an initial denaturing step at 95 C for 3 min, 35 cycles of denaturing at 95 C for 20 s, annealing at 45 C for 30 s, 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 sterile nuclease-free dH<sub>2</sub>O and then cleaned with QIAGEN DyeEx 2.0 spin columns according to manufacturer protocol. These samples were shipped to the University of Wisconsin Biotechnology Center (Madison) for sequencing.

*Sequence alignment and phylogenetic analyses.*—Sequences were edited manually in Bioedit v.7.0.9 (Hall 2007) by comparison of the ABI chromatographs and alignment with contiguous sequences. Sequences accompanied with sufficient locality information from other published studies from GenBank were included in our analysis (Dunham et al. 2003, Buyck et al. 2011, Buyck and Hofstetter 2011). We also included “*C. cibarius*” sequence DQ200926, which was from the assembling the fungal tree of life (AFTOL) analysis. Fifty sequences (860 bp) were analyzed at the nLSU locus. Thirty-seven sequences were produced in this study (TABLE I; GenBank numbers JX030419–JX030455), and 13 sequences were obtained from GenBank. Fourteen ITS sequences (539 bp) were produced in this study (TABLE I; GenBank numbers JX030456–JX030469) and 15

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

Field museum accession no.	Collection no.	Morphological ID	Origin	Host	Target locus	GenBank accession no.	Collector
C0171587F	C057	<i>C. phasmatis</i>	WI	<i>Quercus</i>	mLSU, ITS, TEF1	JX030431, JX030464, JX030417	M. Foltz
C0171587F	C059	<i>C. phasmatis</i>	WI	<i>Quercus</i>	ITS	JX030463	M. Foltz
C0171588F	C073	<i>C. phasmatis</i>	WI	<i>Quercus</i>	nLSU	JX030426	M. Foltz
C0171588F	C074	<i>C. phasmatis</i>	WI	<i>Quercus</i>	mLSU, TEF1	JX030438, JX030418	M. Foltz
C0171588F	C075	<i>C. phasmatis</i>	WI	<i>Quercus</i>	ITS	JX030465	M. Foltz
C0171588F	C076	<i>C. phasmatis</i>	WI	<i>Quercus</i>	nLSU, ITS	JX030425, JX030466	M. Foltz
C0171589F	Ch2	<i>C. phasmatis</i>	WI	<i>Quercus</i>	nLSU, ITS	JX030428, JX030458	T. Volk
C0171589F	Ch3	<i>C. phasmatis</i>	WI	<i>Quercus</i>	nLSU, ITS	JX030429, JX030459	T. Volk
C0171591F	Ch4	<i>C. phasmatis</i>	WI	<i>Quercus</i>	ITS	JX030460	T. Volk
C0171589F	Ch6	<i>C. phasmatis</i>	WI	<i>Quercus</i>	ITS	JX030461	T. Volk
C0171592F	Ch7	<i>C. phasmatis</i>	WI	<i>Quercus</i>	ITS	JX030462	D.Lindner
C0171585F	C065	<i>C. flavus</i>	WI	<i>Quercus</i>	nLSU	JX030432	M. Foltz
C0171585F	C066	<i>C. flavus</i>	WI	<i>Quercus</i>	nLSU	JX030437	M. Foltz
C0171585F	C067	<i>C. flavus</i>	WI	<i>Quercus</i>	ITS, TEF1	JX030416, JX030467	M. Foltz
C0171585F	C068	<i>C. flavus</i>	WI	<i>Quercus</i>	nLSU, ITS	JX030436, JX030468	M. Foltz
C0171586F	Ch1	<i>C. flavus</i>	WI	<i>Quercus</i>	nLSU, ITS	JX030427, JX030456	T. Volk
C0171586F	Ch5	<i>C. flavus</i>	WI	<i>Quercus</i>	nLSU	JX030430, JX030457	T. Volk
C0171590F	C081	<i>C. spectaculus</i>	WI	<i>Quercus</i>	nLSU, TEF1	JX030421, JX030414	M. Foltz
C0171590F	C082	<i>C. spectaculus</i>	WI	<i>Quercus</i>	nLSU	JX030422	M. Foltz
C0171590F	C084	<i>C. spectaculus</i>	WI	<i>Quercus</i>	nLSU	JX030423	M. Foltz
C0074994F	CC03	<i>C. roseocanus</i>	MI	<i>Tsuga</i>	nLSU	JX030444	A.&L.Baines
C0074995F	CC29	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU, ITS, TEF1	JX030445, JX030469, JX030415	T. Volk
C0074995F	CC31	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU	JX030446	T. Volk
C0074995F	CC33	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU	JX030447	T. Volk
C0074995F	CC34	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU	JX030448	T. Volk
C0074995F	CC35	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU	JX030449	T. Volk
C0074996F	CC36	<i>C. roseocanus</i>	MA	<i>Tsuga</i>	nLSU	JX030452	M. Binder
C0074997F	CC38	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU	JX030450	T. Volk
C0074998F	CC40	<i>C. roseocanus</i>	CO	<i>Picea</i>	nLSU	JX030451	T. Volk
C0074999F	IC01	<i>C. roseocanus</i>	ID	*	nLSU	JX030453	T. Volk
C0074985F	CC05	" <i>C. cibarius</i> "	MO	<i>Quercus</i>	nLSU	JX030420	M. Rogers
—	CC13	<i>C. cibarius</i>	SWE	<i>Picea</i>	nLSU	JX030442	OSC
—	CC15	<i>C. cibarius</i>	SWE	<i>Betula</i>	nLSU	JX030441	OSC
—	CC17	<i>C. cibarius</i>	SWE	<i>Betula</i>	nLSU	JX030443	OSC
C0074986F	CC23	<i>Cantharellus</i> sp.	WI	<i>Quercus</i>	nLSU	JX030433	S. Nelson
C0074987F	CC27	" <i>C. cibarius</i> "	CT	<i>Quercus</i>	nLSU	JX030434	B. Yule

TABLE I. Continued

Field museum accession no.	Collection no.	Morphological ID	Origin	Host	Target locus	GenBank accession no.	Collector
C0074990F	CC25	<i>Cantharellus</i> sp.	GA	<i>Quercus</i>	nLSU	JX030454	C. Matherly
C0074989F	CA01	<i>Cantharellus</i> sp.	IL	<i>Quercus</i>	nLSU	JX030419	J. McFarland
C0074993F	CAF1	<i>C. formosus</i>	ID	*	nLSU	JX030424	T. Volk
C0074988F	CC42	“ <i>C. cibarius</i> ”	VA	<i>Quercus</i>	nLSU	JX030435	T. Melin
C0075000F	CAS1	<i>C. subalbidus</i>	ID	*	nLSU	JX030439	T. Volk
C0074991F	ICW1	<i>C. cascadenis</i>	ID	*	nLSU	JX030440	T. Volk
C0074992F	CN09	<i>C. cinnabarinus</i>	VA	<i>Quercus</i>	nLSU	JX030455	T. Melin

sequences were obtained from GenBank. Sequences could be obtained only in one direction (with the ITS4 primer) and were truncated before alignment. Fifty-seven sequences (1021 bp) were analyzed for the *TEF1* locus. Five sequences were produced in this study (TABLE I; GenBank numbers JX030414–JX030418), and 52 sequences were obtained from GenBank (Buyck et al. 2011, Buyck and Hofstetter 2011). There was insufficient overlap among sequenced individuals among any of the loci to perform a combined analysis. Therefore, sequence alignments for each locus were analyzed separately. Any *Cantharellus* sequences from North American species available on GenBank were included in each analysis to provide context and outgroups. Sequences were aligned with MUSCLE (executed by EMBL-EBI, <http://www.ebi.ac.uk/>). The nLSU sequence alignment contained gaps in a region of ~ 100 bp near the middle of the alignment in the outgroup taxa. The ingroup taxa were consistently aligned in that region. We analyzed the nLSU alignment both with and without that region and the tree topologies and support values were similar. Because that region adds information for the ingroup, it was retained in the analysis. Evolutionary models were determined with jModelTest (Guindon and Gascuel 2003, Posada 2008). These analyses indicated a GTR + I + G model was the most appropriate for our data. Maximum likelihood (ML) analyses were performed with Garli 2.0 (Zwickl 2006) for 64-bit operating systems. Garli also was used to perform 1000 ML bootstrap replicates, and bootstrap values were obtained with PAUP (Swofford 1993) via PaupUp (Calendini and Martin 2007). To view trees, TreeView (Page 1996) was used. Mesquite 2.75 (Maddison and Maddison 2011) was used to view and edit trees and prepare data for TreeBASE. Taxa used for rooting trees were those consistently supported by Feibelman et al. (1997), Dunham et al. (2003), Moncalvo et al. (2006), Buyck and Hofstetter (2011). Taxonomic determination of new species was made by designating the least inclusive monophyletic groups that were supported by distinct morphological characteristics.

## RESULTS

*Morphology.*—Chanterelles resembling *C. cibarius* found in La Crosse were categorized into three groups based on morphology. The first group, with a white morphotype, was defined by having white lamellae that turn pink as they mature, an orange-yellow pileus with an incurved margin, a thick white stalk and a light pink spore print (FIG. 1A, B). The second group, with a yellow morphotype, was distinguished by yellow lamellae, a yellow stalk, a yellow pileus with the margin often everted at maturity, a yellow spore print and a more slender and slightly smaller stature than the white morphotypes (FIG. 1C, D, E). The third group, with a salmon morphotype, features salmon lamellae, the pileus is a shade of orange/pink/salmon with a margin that is often curled down when young, becoming plane and wavy with age, and an orange stalk that is whitish at the base (FIG. 2A, B, C). The stature is smaller and



FIG. 1. A, B. *Cantharellus phasmatis* sp. nov. type collection (C0171588F). C, D, E. *Cantharellus flavus* sp. nov. type collection (C0171585F). Both were found in Hixon Forest Park in La Crosse, Wisconsin, July 2010. Scale bars are ~1 cm.



FIG. 2. A, B, C. *Cantharellus spectaculus* sp. nov. type collection (C0171590F) found in Hixon Forest Park in La Crosse, Wisconsin, Jul 2010. D, E. *Cantharellus roseocanus* found in Colorado. Scale bars are ~1 cm.

more slender than the other morphotypes, and the spore print is salmon-pink. None of our species had similar morphology to those from Idaho and Colorado, which were much smaller, more squat and grew associated with conifers (FIG. 2D, E).

*nLSU analysis.*—Maximum likelihood analysis of the nLSU locus resulted in a single tree with  $-\ln L = -1989.6209$  (FIG. 3). Alignments, trees and analyses are deposited in TreeBASE at the following URL: <http://purl.org/phylo/treebase/phylovs/study/TB2:S12738>. The salmon-morphotype chanterelles were separated with strong support from other *C. cibarius*-like chanterelles, with relatives from southern Illinois and Missouri (FIG. 3). Although indistinguishable from each other at the nLSU locus, the white and yellow morphotypes formed a strongly supported clade that was distinct from *C. cibarius* from Sweden, as well as from *C. roseocanus*. All of the samples in the *C. roseocanus* clade were found beneath coniferous trees (mainly *Tsuga* and *Picea*), whereas the white, yellow and salmon morphotype samples were found beneath species of *Quercus*.

*ITS analysis.*—Maximum likelihood analysis of the ITS region resulted in a single tree with  $-\ln L = -965.5421$  (FIG. 4). The white and yellow morphotypes were separate clades from *C. cibarius* and *C. roseocanus*, however the relationships among these taxa are unresolved. The white and yellow morphotypes also formed mutually exclusive monophyletic clades (FIG. 4). They formed clades distinct from *C. cibarius* and *C. roseocanus*, which group together 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. 5). 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 infers the salmon morphotype sister to *C. amethysteus* (Qué.) Sacc. from Europe (FIG. 5). Our sample of *C. roseocanus* from Colorado is sister to *C. cibarius* from Europe (100% bootstrap). The white and yellow morphotypes are sister taxa (64% bootstrap) that share a most recent common ancestor with two specimens of the newly described *C. tenuithrix* from the southern United States (100% bootstrap). The yellow morphotype sample forms a monophyletic group with one collection of *C. tenuithrix* described in Buyck and Hofstetter (2011).

#### TAXONOMY

***Cantharellus phasmatis*** M.J. Foltz & T.J.Volk, sp. nov. FIG. 1A, B, SUPPLEMENTARY FIGS. 1–3

MycoBank MB800425, C0171588F.

Pileus yellow; hymenium pale cream to white, becoming pink; stalk pale yellowish white, becoming yellow; tissues staining ochraceous brown when bruised; spore print pink. Molecular data from ITS and *TEF1* loci distinguish this species from all other *Cantharellus* species (FIGS. 4, 5).

*Etymology.*: Named *phasmatis* meaning “ghostly” for the distinctive ghostly white hymenium of young specimens of this chanterelle.

*Holotypus.*: UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest Park, along the path running parallel to Bliss Road, associated with oak in oak-hickory mixed deciduous forest. N43.8157° W91.2091°, 18-VII-2010, here designated *Foltz C073*, Collection CP002, C0171588F.

Pileus egg-yolk yellow or paler (especially in sunlight or dry conditions), 6–12 cm diam, plano-convex, becoming broadly convex to depressed, often mottled at maturity, partly due to spore deposit, surface dry and covered in a thin layer of fibrils, staining ochraceous brown when bruised; margin incurved when young, regular to irregular, sometimes lobed, often wavy with age, context thick, white, firm; lamellae deeply decurrent, white when young, becoming pinkish buff with age, often yellowish near the margin, often forking and anastomosing, sometimes almost poroid in some specimens near the margin, bruising ochraceous brown; stipe white and solid, yellowing and peeling with maturity, bruising ochraceous brown, 4–8 cm long, 1–3 cm thick, context white; spore deposit salmon-pink, (7)7.5–10(11) × 4–6(7) μm, subglobose to ovate when immature, 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 cells, 95–105 × 4.5–5.5 μm, sometimes with thickened walls; clamps found in all tissues; odor strong and pleasant, like apricots; flavor mild at first, becoming peppery; KOH intensifying color of all tissues. Habitat, habit and distribution: gregarious to scattered; associated with *Quercus* (Fagaceae) and *Carya* (Juglandaceae) (oak-hickory); often found growing in lines along roots; common Jul–Aug in La Crosse, Wisconsin, presumably more widespread. Edibility: choice; these are the most delectable chanterelles we have eaten.

*Additional specimens examined.*: UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest Park, along the path parallel to Bliss Road, associated with *Quercus* (oak) in oak-hickory mixed deciduous forest. 18-VII-2010, CP001, CP002, CP003.

*Comments.*: This species can be distinguished from other chanterelles by its white hymenium that becomes pink with age, and by its pink spore print. This species is

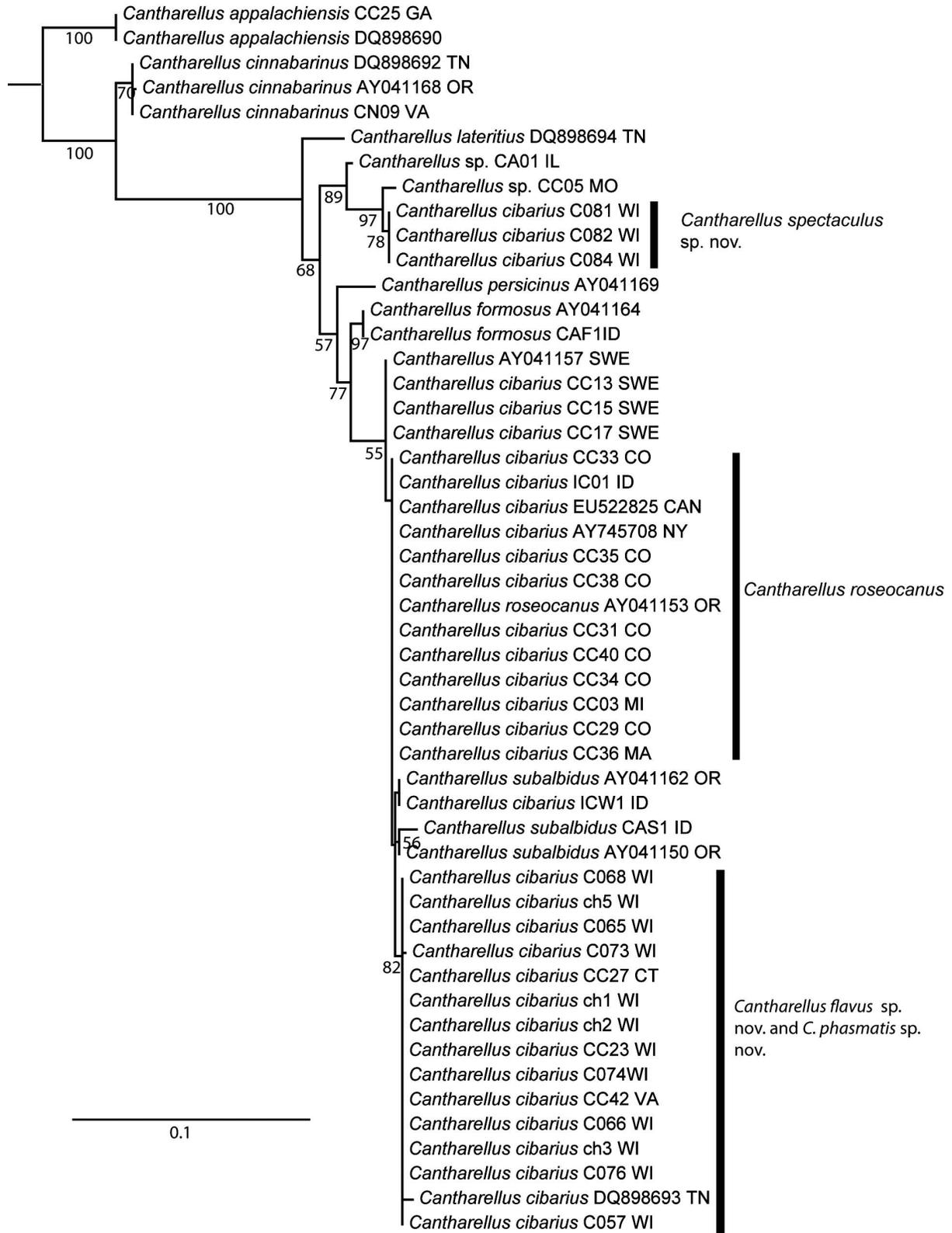


FIG. 3. Molecular phylogeny of *Cantharellus*. Maximum likelihood phylogram of 50 nLSU sequences. Maximum likelihood bootstrap values from 1000 replicates are near branches. ML  $-\ln L = -1989.6209$ . GenBank number or collection numbers are indicated after the name of the collection, followed by an abbreviation of the collection locality if available.

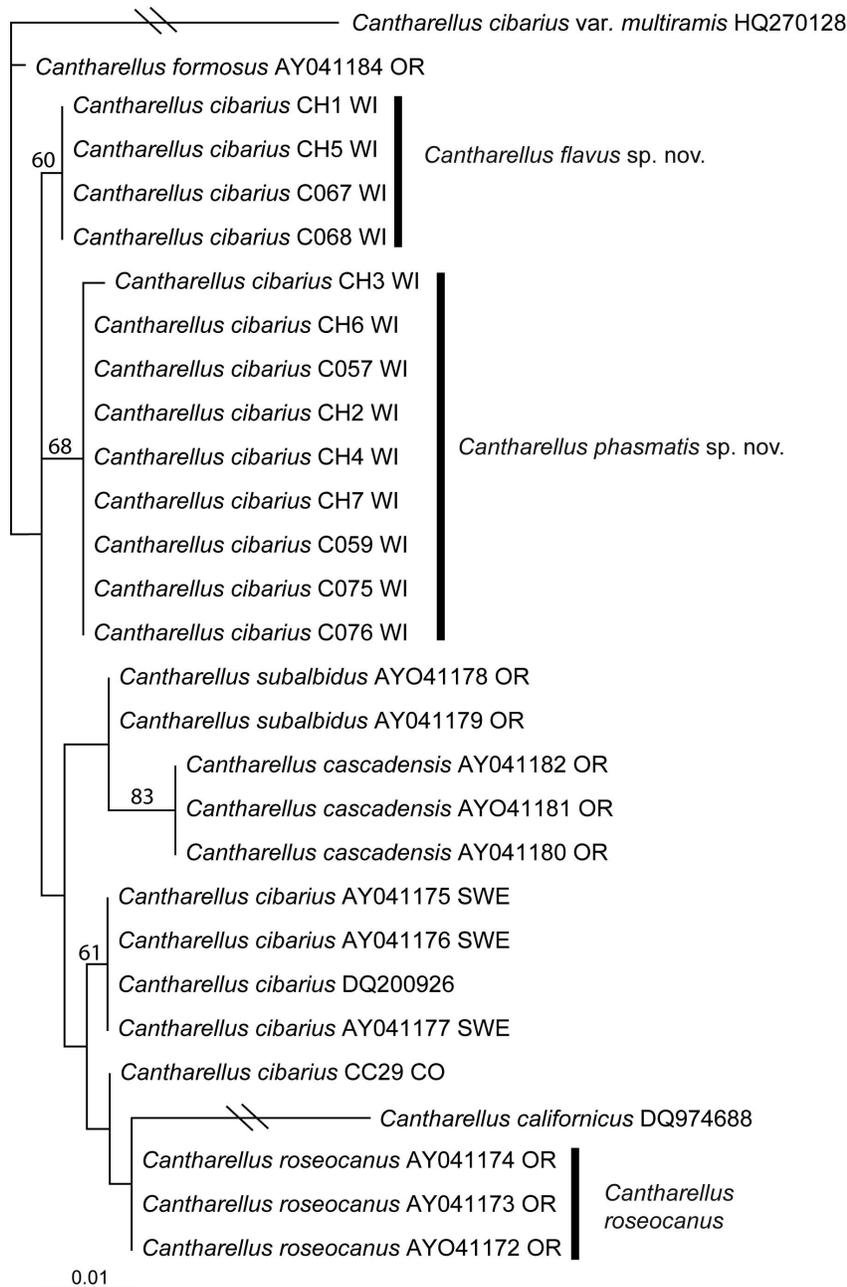


FIG. 4. Molecular phylogeny of *Cantharellus*. Maximum likelihood phylogram of 29 ITS sequences. Maximum likelihood bootstrap values from 1000 replicates are above branches. ML  $-\ln L = -965.5421$ . GenBank number or collection numbers are indicated after the name of the collection, followed by an abbreviation of the collection locality if available. Double line indicates branch was shortened.

probably the same as *C. cibarius* var. *pallidifolius* described by Smith in 1968 (see DISCUSSION). We suggest the common name “ghost chanterelle” for this species.

***Cantharellus flavus*** M.J. Foltz & T.J.Volk, sp. nov.  
 FIG. 1C, D, E, SUPPLEMENTARY FIG. 4  
 Mycobank MB800426, C0171585F.

Pileus yellow; hymenium yellow; stalk yellow; spore print yellow. Molecular data from ITS and *TEFI* loci distinguish this species from all other *Cantharellus* (FIGS. 4, 5).

*Etymology*: named *flavus* for the yellow stipe, hymenium, pileus and spore print.

*Holotypus*: UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest Park, along the path

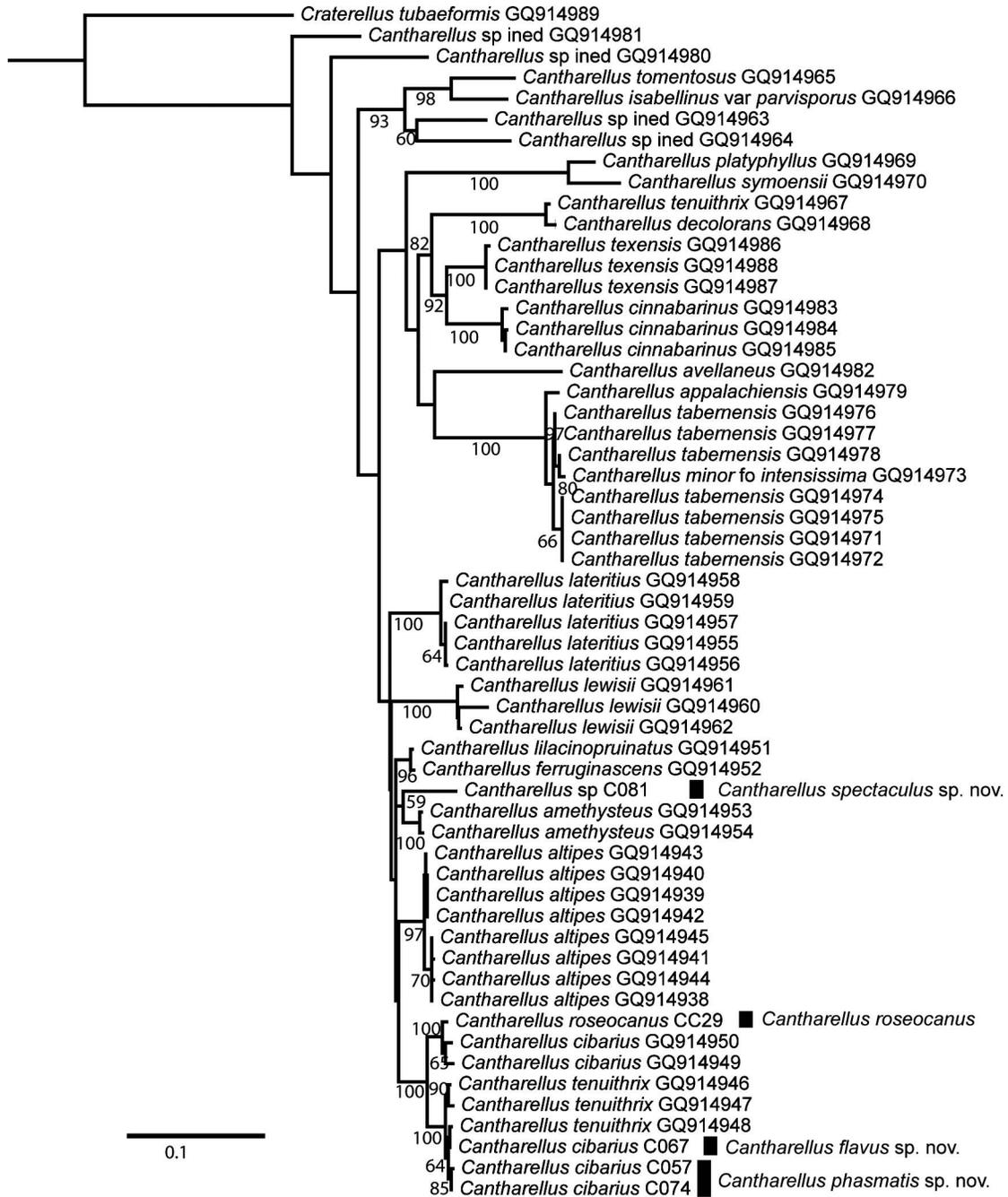


FIG. 5. Molecular phylogeny of *Cantharellus*. Maximum likelihood phylogram of 57 *TEF1* sequences. Maximum likelihood bootstrap values from 1000 replicates are near branches. ML  $-\ln L = -7127.1283$ . GenBank number or collection numbers are indicated after the name of the collection.

parallel to Bliss Road, approximately halfway up the path when heading uphill, on right side, associated with *Quercus* (oak) in oak-hickory mixed deciduous forest, N43.8157° W91.2091°, 18-VII-2010, here designated *Foltz C066*, Collection *CF001*, C0171585F.

Pileus egg-yellow or paler with age or exposure to light, 6–9 cm diam, plano-convex when immature, becoming plane to wavy and depressed to broadly

infundibuliform; margin incurved, regular to irregular, usually everted when mature, sometimes lobed or sinuate on one side, context yellowish, thin, somewhat spongy and watery in texture; lamellae deeply decurrent, egg-yellow, often forking and anastomosing, not readily staining when bruised, bruises appear ochraceous brownish yellow in dried specimens; stipe yellow and solid, sometimes patched with white and mottled in age,

3–8 cm long, 0.5–2 cm thick, context yellowish white; spore deposit yellow, (7.5)8–10(11) × (4)4.5–6 μm, subglobose to obovate when young, becoming oblong with maturity (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; odor fragrant like apricots; flavor slightly peppery; KOH intensifying color of all tissues. Habitat, habit and distribution: caespitose to gregarious; associated with *Quercus* (Fagaceae) and *Carya* (Juglandaceae) (oak-hickory); on well drained soil, especially on hillsides; common Jul–Aug in western Wisconsin, presumably more widespread. Edibility: choice.

*Additional specimens examined:* UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest Park, along the path running parallel to Bliss Road, associated with oak in oak-hickory mixed deciduous forest, 18-VII-2010, *CF001*; UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest Park, on hillside, behind the National Weather Service Station on County Highway FA, 18-VIII-2009, *CF002*. C0171586F.

*Comments:* This species is most easily distinguished from other chanterelles by its yellow hymenium, stalk, and spore print. We suggest the common name “Midwestern yellow chanterelle” for this species.

***Cantharellus spectacularis*** M.J. Foltz & T.J. Volk, sp. nov.  
FIG. 2A, B, C, SUPPLEMENTARY FIG. 5  
Mycobank MB800427, C0171590F.

Pileus orange-salmon; hymenium salmon, sometimes with a purple hue; spore print salmon-pink and larger than *C. phasmatis*. Molecular data from nLSU and *TEF1* loci distinguish this species from all other *Cantharellus* (FIGS. 3, 5).

*Etymology:* named *spectaculus* for the unusual salmon-pinkish, sometimes purple hymenium and orange stalk, a showy combination.

*Holotypus:* UNITED STATES. WISCONSIN: La Crosse County, Hixon Forest Park, along the path parallel to Bliss Road, approximately halfway up the path on right side, associated with oak in oak-hickory mixed deciduous forest, N43.8157° W91.2091°, 18-VII-2010, here designated *Foltz C081*, *Collection CS001*, C0171590F.

Pileus orange/salmon, 4–8 cm diam when mature, usually circular but sometimes elongated, umbraculiform when young, becoming plano-convex to depressed/infundibuliform with maturity, pileus surface textured or subtomentose under a hand lens, context thin, similar in color to pileus but more pale; margin incurled, becoming wavy with age, usually regular; lamellae salmon/pink, sometimes with purple hues under certain light, often forking and sometimes anastomosing; stipe orange and solid, white at the

base, slender and usually as long or longer than the diameter of the pileus, context whitish, with cortex tissue similar in color to the surface; spore deposit salmon-pink, 10–12(14) × 5–7 μm, oblong-elliptical when mature (n = 30); basidia (104)110–120(125) × (9)10–11 μm, mostly 4(5)-sterigmate, 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, habit and distribution: caespitose to gregarious; associated with *Quercus* (Fagaceae) and *Carya* (Juglandaceae) (oak-hickory); Jul–Aug, rare in the type locality in Wisconsin. Edibility: choice.

*Additional specimens examined:* None found beyond the type collection.

*Comments:* This chanterelle can be distinguished from other chanterelles by its salmon hymenium, orange stalk, lack of egg-yellow pileus, salmon-pink spore print and larger spore. We suggest the common name “Spectacular chanterelle” for this species based on its unusual color combination.

## DISCUSSION

Three new species, *Cantharellus phasmatis*, *Cantharellus flavus* and *Cantharellus spectacularis*, are proposed based on phylogenetic analysis of molecular characteristics and comparison of morphological features that distinguish them from other *Cantharellus* species.

*Utility of morphological characters in Cantharellus taxonomy.*—The morphological characteristics that were useful to delineate species included the hymenium and spore print colors, as well as the larger spores of *C. spectacularis*. Geographic distribution and mycorrhizal host-type associations are useful for separating the newly proposed species from *C. cibarius* sensu stricto, which is known only from Europe (Buyck and Hofstetter 2011), and from *C. roseocanus*, which has been reported only under coniferous trees (Redhead et al. 1997, Dunham et al. 2003, Arora and Dunham 2008, this study). Buyck and Hofstetter (2011) noted that *C. tenuithrix* had characteristically long terminal hyphal cells on the surface of the pileus. We found that both *C. phasmatis* and *C. flavus* share this characteristic with *C. tenuithrix*.

Spore print color has been used to distinguish species in the Cantharellales. In this study, ITS supports the spore-color discrimination hypothesis of Matheny et al. (2010) of differences between *Cr. fallax* A.H. Sm. and *Cr. cornucopioides* (L.) Pers., two species that were difficult to resolve with nLSU and had been combined by Dalhman et al. (2000). Our study provides additional support for spore-print color as an important diagnostic feature at species

rank and the use of the ITS locus to delineate *Cantharellus* species.

*Taxonomic details of the three new species.*—*Cantharellus phasmatis* is probably *C. cibarius* var. *pallidifolius* as described by Smith (1968) based on morphological description. We propose to recognize this taxon at the rank of species. This distinction is supported by its placement in a monophyletic group, separate from *C. cibarius* in three gene phylogenies (FIGS. 3–5). Buyck and Hofstetter (2011) said that Petersen and Eyssartier had both concluded upon re-examination of Peck's and Smith's type collections that they were not accompanied by sufficient descriptions, illustrations or molecular data to be useful. Petersen recognized Smith's *C. cibarius* var. *pallidifolius* as a valid taxon (1976) and he illustrated it himself (1979). In addition, we found Smith's original description (1968) to be thorough, including adequate microscopic descriptions and a photograph. Arora and Dunham (2008) attempted to sequence the type specimen of *C. cibarius* var. *pallidifolius* but reported that DNA degradation in the type collection prevented them from producing a sequence. Given this uncertainty, we have decided to describe this species with a new epithet, *C. phasmatis*.

*Cantharellus flavus* is morphologically very similar to Smith's (1968) description of *C. cibarius* var. *cibarius* with the one major exception of spore-print color. Smith described the spores as cream-buff, whereas our samples produced a bright yellow spore print. However, Petersen's (1969, 1985) discussions of a "yellow-spored chanterelle" are fitting and could be the same taxon we have described here.

Among the species we examined, *C. phasmatis* and *C. flavus* are sister taxa sharing a close relationship with *C. tenuithrix*. One of the primary morphological features we used to distinguish between *C. phasmatis* and *C. flavus* was the hymenium color. 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](http://www.mtsn.tn.it/cantharellus-news/tx_photos.asp?index=20008)) shows an orange hymenium. One of the specimens of *C. tenuithrix* was a close sequence match (*TEFI*) to *C. flavus*. The other two samples of *C. tenuithrix*, including the holotype, were monophyletic with strong support. This suggests that one of the individuals called "*C. tenuithrix*" in Buyck and Hofstetter (2011) is not that species but instead is *C. flavus*. Our other main distinguishing morphological character was spore-print color. The pink spore print of *C. phasmatis* and yellow spore print of *C. flavus* are distinct from the cream spore prints 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 range of *C. flavus* extends to the southern United States.

*Cantharellus spectaculus* is in a more distant clade and represents the first representative in this clade in North America. Its closest relative from the *TEFI* dataset is *C. 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 basidiocarp (Petersen 1969), although Smith never formally described this species. This salmon taxon could be the one described here. In addition, we sequenced the nLSU region of two *C. cibarius*-like chanterelles from southern Illinois and Missouri that represent two undescribed lineages that are closely related to *C. spectaculus* in our nLSU gene phylogeny. These taxa require further investigation.

It is noteworthy that the three new *Cantharellus* species described here were found in a small forest plot within 20 m of one another. It is sometimes erroneously assumed that most North American species of mushrooms have been described, especially in conspicuous genera like *Cantharellus*. That is not the case. This is a pattern being uncovered in North America with many common genera, such as *Armillaria* (Anderson and Ullrich 1979), *Laetiporus* (Banik et al. 1998) and *Morchella* (Kuo et al. 2012), where many species are found to be masquerading under one scientific name. Intraspecific variation in morphology has been attributed to widespread genetic variability and phenotypic plasticity. Molecular tools have given mycologists the framework and the confidence to discern morphological differences that separate the species. Sometimes these differences turn out to be striking (What were we thinking?), but often they are more subtle. Clearly much more systematic work needs to be done on North American fungi, even the charismatic megamycota, to elucidate these species.

*Cantharellus roseocanus*.—The combination *Cantharellus roseocanus* (Redhead, Norvell & Danell) Redhead, Norvell & Moncalvo was made (Redhead 2012), but there was no explanation given for this online elevation to species rank. In all three gene phylogenies in this study *C. roseocanus* was distinct from *C. cibarius* and other *Cantharellus* species, supporting the new combination of Redhead (2012). Our nLSU data suggest this taxon may be the most widespread chanterelle in North America, with a known range across Washington, Oregon (Dunham et al. 2003), Idaho, Colorado, northern Michigan, Massachusetts (this study), New York (AFTOL), and Newfoundland, Canada (Greg Thorn pers comm Jul 2011). Our data indicate that *C. roseocanus* might be found associated with the northern tier of conifers across the northern

United States and into Canada. *Cantharellus roseocanus* appears to be ecologically separated from the newly described species in this study in that it has been found associated only with coniferous trees. This might have gone unnoticed because of morphological differences associated with geographic location. For example, the specimens we found in Colorado were only about half the size of the original description (Redhead et al. 1997), and the specimens from Michigan were even smaller (pileus about 20 mm diam), although they may have been immature. Our data support Redhead's (2012) recognition of *C. roseocanus* at the species rank based on molecular phylogeny, ecological association and geographic separation from *C. cibarius*, which is known only from Europe. Further analysis of ITS and TEF1 regions eventually may show that *C. roseocanus* is composed of more than one geographically isolated species. However, we see no support in our data for continuing to describe members of this clade as a variety of *C. cibarius*.

The genus *Cantharellus* is proving to be diverse, and, in addition to the three new species described here, our sequence data (some not shown) indicates the potential for many other undescribed lineages and species complexes within North America. Extensive geographic sampling, ecological, morphological and molecular data will be needed to fully describe the diversity of the North American *Cantharellus*.

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SUPPLEMENTARY FIG. 1. *Cantharellus phasmatis* sp. nov. found in Hixon Forest Park in La Crosse, Wisconsin, July 2010. Scale bars are ~1 cm.

SUPPLEMENTARY FIG. 2. *Cantharellus phasmatis* sp. nov. found in Hixon Forest Park in La Crosse, Wisconsin, July 2010. Scale bars are ~1 cm.

SUPPLEMENTARY FIG. 3. *Cantharellus phasmatis* sp. nov. found in Hixon Forest Park in La Crosse, Wisconsin, July 2010. Scale bars are ~1 cm.

SUPPLEMENTARY FIG. 4. *Cantharellus flavus* sp. nov. found in Hixon Forest Park in La Crosse, Wisconsin, July 2010. Scale bars are ~1 cm.

SUPPLEMENTARY FIG. 5. *Cantharellus spectaculus* sp. nov. found in Hixon Forest Park in La Crosse, Wisconsin, July 2010. Scale bars are ~1 cm.



