

## *Cantharellus chicagoensis* sp. nov. is supported by molecular and morphological analysis as a new yellow chanterelle in midwestern United States

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**Abstract:** Recent molecular systematic studies of *Cantharellus cibarius* sensu lato have revealed previously unknown species in different regions of North America. This study investigates yellow chanterelles in the Midwest using phylogenetic analysis of three DNA regions: nuc rDNA internal transcribed spacer 2 (ITS2) and 28S sequences and translation elongation factor 1 $\alpha$  gene (*EF1 $\alpha$* ). This analysis reveals a locally common taxon *Cantharellus chicagoensis* sp. nov. as distinct from sympatric species present in northeastern Illinois, northwestern Indiana and Wisconsin. This chanterelle features a pileus that often has a greenish yellow margin when immature, a squamulose disk when mature, a yellow spore print and the absence of a fragrant odor. Multiple *Cantharellus* specimens group with *C. flavus* and *C. phasmatis*, expanding their known range, and others with *C. roseocanus*. Our observations highlight the diversity of *Cantharellus* in midwestern USA and further document the need for additional systematic focus on the region's fungi.

**Key words:** Cantharellales, *Cantharellus cibarius*, diversity, phylogeny, taxonomy

### INTRODUCTION

Many new world yellow chanterelles were treated as contaxic with *Cantharellus cibarius* Fr. of Europe before the latter part of the 20th century. Buyck and Hofstetter (2011) and Foltz et al. (2013) cover the history of the *C. cibarius* group and the segregation of species in North America. Systematic analyses based on morphology and molecular data have supported the segregation of numerous North American yellow chanterelle species: one each from Mississippi (Feibleman et al. 1996), Oregon (Dunham et al. 2003) and California (Arora and Dunham 2008), five from Texas (Buyck et al. 2010, Buyck et al. 2011, Buyck and Hofstetter 2011) and three midwestern species from Wisconsin (Foltz et al. 2013). The results of these studies suggest that continued DNA analysis and morphological description of *Cantharellus* may indicate additional cryptic species and provide a greater understanding of their geographic ranges. Buyck et al. (2014) suggests that there may be 100 or more undescribed taxa for the genus *Cantharellus* worldwide.

Twenty years of research on macrofungi in the Chicago region has documented oak-associated chanterelles that were identified as *C. cibarius*, *C. cinnabarinus* (Schwein.) Schwein., *C. lateritius* (Berk.) Singer and *C. minor* Peck. Morphological analysis indicated variation in coloration and stature within the *C. cibarius* group. The most common morphotype sometimes had a paler or scaly pileus disk and often was greenish yellow on the margin when immature. In growing seasons that favored robust development, the pileus seldom exceeded 5 cm diam. This chanterelle, which lacked a fruity odor, did not match the morphology of other described species and also seemed distinct from *C. flavus* Foltz & T.J. Volk, *C. phasmatis* Foltz & T.J. Volk and *C. spectaculus* Foltz & T.J. Volk found 360 km to the northwest in La Crosse, Wisconsin (Foltz et al. 2013).

We report the results of a multigene analysis of yellow chanterelles from midwestern USA. Our analysis yielded a well-supported clade that represents a novel species, described herein as *Cantharellus chicagoensis* sp. nov. Four other regional species were supported including collections of *C. flavus* and its sister species *C. phasmatis*, expanding their known distributions.

TABLE I. *Cantharellus* specimens sequenced for this study

Taxon	Location	Hosts	Collector ID	Field Museum accession Nos.	GenBank accession Nos.		
					ITS2	28S	EF1 $\alpha$
<i>C. chicagoensis</i>	IL, Cook Co.	<i>Quercus</i>	GMM 7801	C0075013F		KP639226	KP639232
<i>C. chicagoensis</i>	IL, Cook Co.	<i>Quercus</i>	KPL 32	C0075011F		KP639215	
<i>C. chicagoensis</i>	IL, Cook Co.	<i>Quercus</i>	PRL 3910	C0171593F	KP639198	KP639217	
<i>C. chicagoensis</i>	IL, Cook Co.	<i>Quercus</i>	PRL 5450	C0210207F		KP639212	
<b><i>C. chicagoensis</i></b>	<b>IL, Cook Co.</b>	<b><i>Quercus</i></b>	<b>PRL 8332</b>	<b>C0201027F</b>	<b>KP639200</b>	<b>KP639214</b>	<b>KP639233</b>
<i>C. chicagoensis</i>	IL, Cook Co.	<i>Quercus</i>	PRL 8916	C0171594F	KP639201	KP639218	KP639230
<i>C. chicagoensis</i>	IL, DuPage Co.	Unknown	CLM 50	C0171595F	KP639197	KP639227	KP639231
<i>C. chicagoensis</i>	IN, Porter Co.	<i>Quercus</i>	PRL 7686	C0201001F	KP639199	KP639213	
<i>C. chicagoensis</i>	WI, Vernon Co.	<i>Quercus</i>	NAMA 2005-435	C0075012F		KP639216	
<i>C. flavus</i>	IN, Lake Co.	<i>Quercus velutina</i>	PGA 08-044	C0075017F	KP639203	KP639209	
<i>C. flavus/phasmatis</i>	IL, McHenry Co.	<i>Quercus</i>	PRL 9171	C0075016F	KP639206	KP639210	
<i>C. flavus/phasmatis</i>	WI, La Crosse Co.	Unknown	NAMA 2005-123	C0075019F		KP639211	
<i>C. aff. phasmatis</i>	WI, Florence Co.	<i>Quercus rubra</i>	PRL 10157	C0075020F		KP639208	KP639235
<i>C. aff. phasmatis</i>	WI, Florence Co.	<i>Quercus rubra</i>	PRL 10158	C0075021F		KP639207	
<i>C. roseocanus</i>	ID, Valley Co.	<i>Picea</i>	NAMA 2008-034	C0204715F	KP639205	KP639224	
<i>C. roseocanus</i>	CO, Grand Co.	<i>Pinus contorta</i>	NAMA 2010-015	C0075018F		KP639225	
<i>C. sp.</i>	IL, Cook Co.	<i>Quercus</i>	CDW 549	C0075014F		KP639223	
<i>C. sp.</i>	IL, Cook Co.	Unknown	CDW 686	C0075015F	KP639202	KP639222	KP639229
<i>C. sp.</i>	MI, Marquette Co.	<i>Pinus strobus</i>	JR 4	C0075022F		KP639219	KP639234
<i>C. sp.</i>	MI, Marquette Co.	<i>Pinus strobus</i>	JR 7	C0075023F	KP639204	KP639220	KP639228
<i>C. sp.</i>	MI, Marquette Co.	<i>Tsuga canadensis</i>	JR 12	C0075024F		KP639221	

Holotype is in boldface.

#### MATERIALS AND METHODS

**Collections.**—Specimens were collected Jul–Sep in 2000–2014 in Illinois, Indiana, Michigan and Wisconsin and from three forays of the North American Mycological Association in Colorado, Idaho and Wisconsin. Latitude and longitude were recorded for each specimen with a handheld GPS (Garmin eTrex Vista H) or iTouchMap.com (<http://itouchmap.com/latlong.html>). Fresh specimens were photographed and morphological descriptions were recorded. Spore prints were taken on white paper or aluminum foil. All colors were described subjectively and with color guides. Color names follow ISCC-NBS color-name charts (Kelly 1965), and codes in parentheses after color names follow the Methuen Handbook of Colour (Kornerup and Wanscher 1978). Dried specimen material was mounted in 3% KOH and examined with an Olympus BH-2 light microscope at 625 $\times$  or 1562.5 $\times$ . For each specimen examined 20–50 basidiospores were measured and features of basidia and pileipellis hyphae recorded. Specimens (TABLE I) are deposited in the Field Museum of Natural History (F) with photos and collection data online (<http://www.fieldmuseum.org/science/research/area/focus-fungi-and-lichens>).

**DNA extraction, amplification and sequencing.**—DNA was extracted from 21 specimens, 20 from dried herbarium material and one from a piece of a recent collection preserved in 2 $\times$  CTAB-EDTA buffer (100 mM Tris, 20 mM EDTA, 1.4 M NaCl, 2% CTAB) (TABLE I). DNA was isolated with QIAGEN DNeasy Plant Mini Kit (QIAGEN USA, Valencia, California), following the manufacturer's protocol, with an extended incubation of 1 h for dried herbarium samples. Polymerase chain reactions (PCR) were performed to obtain sequences from three regions with the following primers: the nuc rDNA ITS1-5.8S-ITS2 (ITS barcode) was amplified with the primer pair ITS1F/ITS4 (White et al. 1990, Gardes and Bruns 1993); the nuc rDNA 28S large subunit (28S), spanning domains D1 and D2, was amplified with primer pair LR0R/LR5 (Vilgalys and Hester 1990); and part of the gene coding for translation elongation factor 1 $\alpha$  (EF1 $\alpha$ ) was amplified with a combination of primer pairs TEF1F/TEF1R (Morehouse et al. 2003) and 983F/2212R (Rehner and Buckley 2005). Amplification of the entire ITS barcode region with the ITS1F and ITS4 primer combination was difficult. To work around this, secondary amplification of ITS2 product was performed with primers ITS3/ITS4 (White et al. 1990, Feibelman et al. 1994). PCR amplification of ITS2 and

28S follows the thermal-cycler protocol: 96 C for 2 min; 35 cycles of 96 C for 30 s, 55 C for 30 s and 72 C for 90 s; 72 C for 10 min; ending with an infinite 14 C step (Wilson et al. 2012). PCR amplification of *EF1 $\alpha$*  follows the thermal-cycler protocol: 94 C for 2 min; nine cycles of 94 C for 30 s, 66 C for 30 s, 72 C for 60 s; 35 cycles of 94 C for 30 s, 56 C for 30 s and 72°C for 60 sec; 72 C for 10 min; ending with an infinite 14 C step. All cycle sequencing used the following thermal-cycler protocol: 96 C for 60 s; 25 cycles of 96 C for 10 s, 50 C for 5 s and 60 C for 4 min; ending with an infinite 14 C step (Rehner and Buckley 2005, Foltz et al. 2013).

*Phylogenetic analysis.*—Raw sequence data were processed and assembled with CodonCode Aligner 5.0.1 (CodonCode Corp, Dedham, Massachusetts). Assembled or single-strand nucleotide sequences for all genes were compared to related sequences with a BLAST query of the GenBank database (<http://www.ncbi.nlm.nih.gov>). A similar query was performed for only the ITS sequences using the UNITE database (Köljalg et al. 2005). These queries were used to assess the possibility of contamination and to confirm sequence identity to genus. Individual datasets of ITS2, 28S and *EF1 $\alpha$*  were created to compare our specimens with other *Cantharellus* species. A combination of novel sequences and GenBank sequences was aligned in Mesquite 3.0 with MUSCLE and MAFFT nucleotide sequence alignment functions (Kato et al. 2002, Edgar 2004, Maddison and Maddison 2014). *Cantharellus guyanensis* Mont. was used as outgroup in the ITS and 28S datasets, which follows its placement basal to the rest of *Cantharellus* in Wilson et al. (2012). *Craterellus tubaeformis* (Fr.) Quél. was used as outgroup in the *EF1 $\alpha$*  dataset. Manual adjustments on alignment and format were applied to each dataset. Maximum likelihood and Bayesian analyses were performed on the CIPRES web portal (<http://www.phylo.org/portal2/>) (Miller et al. 2010). Maximum likelihood (ML) and ML bootstrap analysis was performed with RAxML 8.0.24 (Stamatakis 2006) with 1000 bootstrap replicates. Bayesian phylogenetic analysis was performed with MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003, Ronquist et al. 2012) with default parameters of Nst = 6, with two runs, four chains per run, each run searching 1 000 000 generations sampling every 1000th generation. The first 10% of the posteriorly sampled Bayesian trees (10 000 trees) was discarded as the burn-in.

## RESULTS

Sequence length varied from 196 bp (KP639199) to 423 bp (KP639202) for our 10 new ITS2 sequences and from 712 bp (KP639217) to 718 bp (KP639223) for 21 28S sequences. The eight *EF1 $\alpha$*  sequences varied from 593 bp (KP639233) to 894 bp (KP639230). The wide range is due to the sequence length produced from primer pairs TEF1F/TEF1R and 983F/2212R, respectively. The ITS2 dataset is 502 characters long after being trimmed with 40 sequences; 10 of these are new, of which five represent the proposed taxon *Cantharellus chicagoensis*. The 28S dataset is 790 nucleotides, trimmed, with 66 sequences; 21 are new with

nine of these representing *C. chicagoensis*. The *EF1 $\alpha$*  dataset has 1001 characters with 57 sequences, eight of which are new with four representing *C. chicagoensis*, and this dataset used the entire region sequenced, including introns, to help identify conspecific sequences.

The results of phylogenetic analysis of ITS2, 28S and *EF1 $\alpha$*  datasets are summarized (FIG. 1A–C, respectively). Each phylogeny represents the best tree produced from the RAxML analysis. Maximum likelihood bootstrap (MLB) percentages  $\geq 70\%$  are represented above branches, in boldface, before the forward slash or singly. Bayesian posterior probabilities (BPP)  $\geq 0.98$  are represented on branches after the forward slash or singly. The GenBank sequence accession numbers are provided on the tip labels (FIG. 1) along with the appropriate species names and geographic origins as obtained from the relevant publications. The novel sequences in this study are identified in boldface (FIG. 1), their GenBank accession numbers provided (TABLE 1). Datasets and tree files for this study are deposited in TreeBase (<http://purl.org/phylo/treebase/phylo/study/TB2:S18405>).

Statistical support for specimens that represent *C. chicagoensis* was lowest in the 28S dataset (FIG. 1, MLB 94%, BPP 0.99). This in large part is due to a lesser amount of sequence variation in 28S for *Cantharellus*. Conversely statistical support for *C. chicagoensis* in both ITS2 and *EF1 $\alpha$*  datasets was maximized (MLB 100% and BPP 1.0).

*Cantharellus chicagoensis* was found in northeastern Illinois, northwestern Indiana and in Vernon County, Wisconsin (FIG. 1 inset). Several other specimens were found to group with the clade containing *C. flavus* and *C. phasmatis* of La Crosse County, Wisconsin. No new collections of *C. spectaculus* were identified by the current study. Several specimens grouped with the clade containing *C. cibarius* and *C. roseocanus* (Redhead, Norvell & Danell) Redhead, Norvell & Moncalvo. One collection from Illinois was found to be near a sequence of *C. persicinus* R.H. Petersen from South Carolina. In total five separate species were uncovered from the *C. cibarius* group for the Chicago region.

## TAXONOMY

***Cantharellus chicagoensis*** Leacock, J. Riddell, Rui Zhang & G.M. Muell., sp. nov.

FIGS. 2, 3; SUPPLEMENTARY FIGS. 1–4  
MycoBank MB812608

*Typification:* UNITED STATES. ILLINOIS: Cook County, Westchester, Bemis Woods North, *Quercus* dominated woodland, approx. N41.829° W87.904°, 11 Jul 2010, P.R. Leacock 8332 (HOLOTYPE F C0201027F). GenBank accessions: ITS = KP639200; 28S = KP639214; *EF1 $\alpha$*  = KP639233.

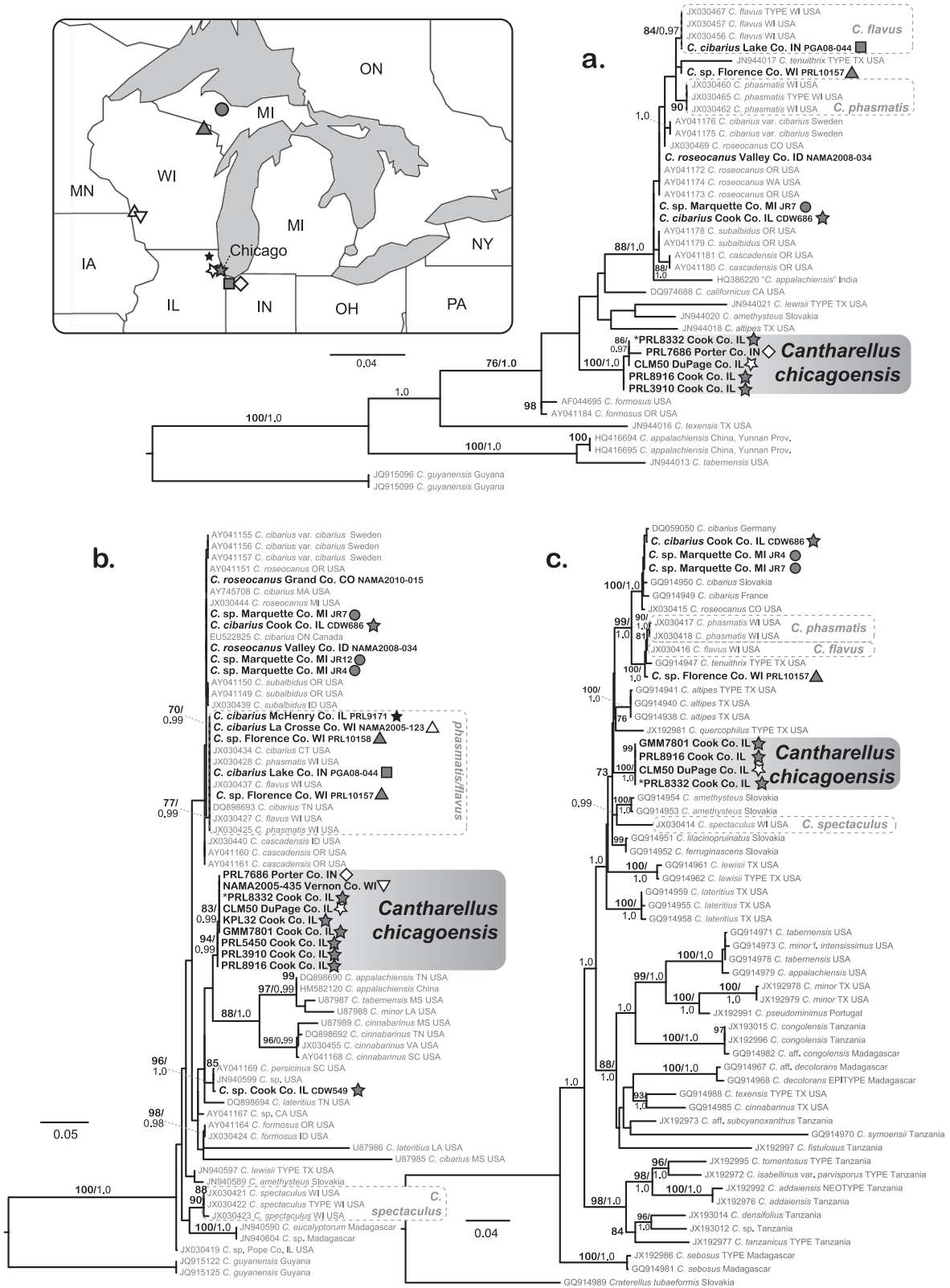


FIG. 1. Single-gene phylogenies for *Cantharellus*. Trees A–C represent individual gene trees corresponding to the molecular markers ITS2 (A), 28S (B) and *EF1α* (C). GenBank accession numbers precede each specimen's taxon. Our sequences are indicated in boldface with collection numbers. GenBank numbers are given (TABLE I). Numbers above branches indicate maximum likelihood bootstrap percentages followed by Bayesian posterior probabilities. The map inset uses symbols to indicate approximate collecting locations for select specimens. Bars refer to the mean number of nucleotide substitutions per site for the adjacent phylogeny. The HOLOTYPE collection for *C. chicaoensis* (PRL8332) is indicated with an asterisk in the phylogenies.



FIG. 2. *Cantharellus chicagoensis* sp. nov. collection P.R. Leacock 8332 (HOLOTYPE F C0201027F) from Cook County, Illinois.

*Etymology*: Named after the Chicago metropolitan region. This species is the yellow chanterelle commonly found in forest preserves in Cook and adjacent counties.

*Diagnosis*: Pileus yellow, margin when immature often greenish yellow; surface dry, glabrous to squamulose when mature. Hymenium orange yellow, decurrent; ridges with regular to interconnected forking. Stipe yellow. Staining brownish orange. Context of pileus and stipe white, solid. Odor and flavor mild. Spore print pale yellow. Basidiospores  $6\text{--}9 \times 4\text{--}5.5 \mu\text{m}$ . Molecular sequence data from ITS2 and *EF1 $\alpha$*  distinguish this species from other *Cantharellus* species (FIG. 1).

Basidioma 25–65 mm tall. Pileus 20–75 mm diam, plano-convex to shallowly depressed, uplifted with age; margin inrolled when immature then decurved to recurved when mature and way to lobed or folded; color of margin when immature light to moderate greenish yellow (2A7, 2B7–2B5) or a greenish hue of moderate yellow (4C6, 3B7) otherwise light yellow (3A4) to moderate yellow (4B5–4B7) or brighter (4A4–4A7); disk when immature moderate yellow to grayish yellow (4B5–4B4), when mature same or light to moderate orange yellow (4A4–4A6, 5C5) or grayish yellow (4B3–4A3) to yellowish gray (4B2); staining none or very slowly (30 min) brown (e.g. 5B4–5B6); surface dry, dull, matt, glabrous to squamulose when mature. Hymenium decurrent with well-developed ridges, thin, shallow, spacing close (10/cm), with forking regular dichotomous to interconnected, anastomosing more developed in older basidiomata, sometimes with ladder-like cross veins; color when immature pale orange-yellow (5A3) to light orange yellow (5A4–5A5/4A6), when mature more yellowish (4A3–4A5), staining absent or slowly brownish orange (6D8) or rarely moderate reddish brown (8D6). Stipe 20–37 mm long (below hymenium), 8–24 mm diam at apex, equal or tapering to base, 5–13 mm diam; light yellow (3A7–3A5) or moderate yellow (4B7) toward



FIG. 3. *Cantharellus chicagoensis* sp. nov. collection K.P. Lauer 32 (F C0075011F) from Cook County, Illinois.

light or moderate orange yellow (4A4–4A7, 5A3–5A5), staining or bruising light orange (5A5–5A6) or brownish orange (6C7, 6D8); surface dry, dull, matt, glabrous, in age may develop shaggy fibrils as surface breaks up. Context of pileus not thin; context of pileus and stipe continuous, solid, firm to brittle-fibrous, white (under pellis tinted with surface color), staining none or slowly pale yellow (3A3), invertebrate damage may hollow the stipe or cause yellow to orange stains. Basal tomentum scant, yellowish white. KOH reaction negative on hymenium and context, intensifies color of pileipellis;  $\text{FeSO}_4$  reaction light grayish yellowish brown (6C2) on hymenium and context;  $\text{NH}_4\text{OH}$  negative on hymenium (bleaching) and context. Odor and flavor mild. Spore print pale yellow.

Rare pale forms have the following variations for all parts or just the hymenium (SUPPLEMENTARY FIGS. 3, 4). Pileus pallid, pale tints of orange yellow and yellowish brown (5A2–5B2) or toward light grayish yellowish brown (5B3–5C3, 5B4); margin with or without tint of grayish greenish yellow (3B2–3C2). Hymenium pallid (5A2–4A3 or 5A3/5B3). Stipe pale yellow (3A3) or pallid (4A2–5A2/5B3).

Basidiospores  $(5.5)\text{--}9(10) \times (3.5)\text{--}4\text{--}5.5 \mu\text{m}$  (mean =  $7.5 \times 4.6 \mu\text{m}$ ;  $n = 250/9$ ),  $Q$  range = 1.3–2.2,  $Q$  mean = 1.6; ellipsoid, smooth, thin-walled, hyaline in KOH, inamyloid, with minutely granular contents. Basidia  $55\text{--}65 \times 7\text{--}9.5 \mu\text{m}$ , subclavate, hyaline in KOH; sterigmata (3)4–6. Basidioles numerous, cylindrical. Cystidia none. Pileipellis a cutis of periclinal inflated hyphae, pale yellowish in KOH, scarcely differentiated from trama; terminal elements  $24\text{--}74 \times 5\text{--}9.5 \mu\text{m}$ , smooth, with slightly thickened walls; trama pale yellowish in KOH. Stipitipellis a cutis of periclinal hyphae, pale yellowish in KOH; cells  $3\text{--}6 \mu\text{m}$  diam, parallel and tightly packed. Basal tomentum hyphae smooth, thin-walled, slightly tortuous,  $3\text{--}5 \mu\text{m}$  diam. Clamp connections present in all tissues.

*Habit, habitat and distribution:* Solitary to gregarious or rarely caespitose, on soil under oak, *Quercus alba*, *Quercus rubra*, in oak-dominated woodland or hardwood forest; Jul–Sep. Known from northeastern Illinois, northwestern Indiana and Vernon County, Wisconsin.

*Additional specimens examined:* UNITED STATES. ILLINOIS: Cook County, Chicago, Edgebrook Woods, N41.991° W87.765°, 16 Aug 2014, *P.R. Leacock 11818* (F C0075032F); Schiller Woods South, 5 Aug 2011, coll. B.T. Reed, *P.R. Leacock 8916* (F C0171594F); 5 Jul 2014, *P.R. Leacock 11685* (F C0075029F); N41.95046° W87.85275°, 31 Aug 2014, *P. R. Leacock 11838* (F C0075036F); N41.950° W87.852°, 31 Aug 2014, *P. R. Leacock 11839* (F C0075037F); N41.95045° W87.85244°, 31 Aug 2014, *P. R. Leacock 11840* (F C0075038F); N41.95044° W87.85242°, 31 Aug 2014, *P. R. Leacock 11841* (F C0075039F); N41.950° W87.852°, 31 Aug 2014, *P. R. Leacock 11842* (F C0075040F); N41.95052° W87.85225°, 31 Aug 2014, *P. R. Leacock 11843* (F C0075041F); N41.94871° W87.85147°, 31 Aug 2014, *P. R. Leacock 11844* (F C0075042F); Elk Grove Village, Busse Forest, 12 Jul 2003, *P.R. Leacock 5086* (F C0075027F); Glencoe, Chicago Botanic Garden, Mary Mix McDonald Woods, 12 Aug 2013, *G.M. Mueller 7801* (F C0075013F); Palatine, Deer Grove West, 2 Sep 2000, *P.R. Leacock 3910* (F C0171593F); Palos Park, Swallow Cliff Woods, N41.676° W87.864°, 6 Jul 2006, *K.P. Lauer 32* (F C0075011F); Tinley Park, St. Mihiel Woods, N41.5861° W87.7542°, 31 Jul 2010, *P.R. Leacock 8383* (F C0075028F); Yankee Woods, N41.5924° W87.7563°, 9 Aug 2003, *P.R. Leacock 5450* (F C0210207F); Westchester, Bemis Woods North, N41.8292° W87.9035°, 31 Jul 2014, *P.R. Leacock 11743* (F C0075031F); Wheeling, Potawatomi Woods, N42.1428° W87.8994°, 21 Aug 2014, *P.R. Leacock 11830* (F C0075033F); DuPage County, Wheaton, St. James Farm Forest Preserve, 29 Jul 2013, *C.L. McAllister 50* (F C0171595F); INDIANA: Porter County, Porter, Indiana Dunes National Lakeshore, Bailly-Chellberg Unit, 23 Aug 2008, *P.R. Leacock 7686* (F C0201001F); WISCONSIN: Vernon County, Wildcat Mountain State Park, 23 Jul 2005, coll. Benjamin Burghardt, *NAMA 2005-435* (F C0075012F).

*Commentary:* The yellow coloration, ridged hymenium, lack of lilac-purple color and the phylogenetic position place this species in subgenus *Cantharellus*, section *Cantharellus* as delimited by Buyck et al. (2014). The pileus margin often appears to have a greenish hue in contrast with the more orange-yellow hymenium. When present this color distinguishes this species from other midwestern chanterelles. The lower incurved margin may retain a more intense color. The pileus disk can appear drab or grayish compared to the margin and become scaly with age. The size of basidiospores is somewhat smaller than *C. flavus*, *C. phasmatis* and *C. spectaculus*. The odor is mild, not apricot or fruity as with *C. flavus*, *C. phasmatis* and other species. We suggest the common name “Chicago chanterelle”.

Paler forms of this taxon were found near typical forms at two locations in Cook County (St Mihiel Woods 2010, Schiller Woods South 2011 and 2014, SUPPLEMENTARY FIGS. 2–4). Some or all of the pileus, hymenium and stipe are notably paler. These pale forms have the same morphology, shape, texture, surface features and brownish orange staining as the yellow form. Some of these forms have tints of the normal colors, including a pale greenish margin.

Morphological distinctions among yellow chanterelles can be evident in fresh specimens, but identification using color is not possible from dried herbarium specimens. Even *C. cinnabarinus* after drying fades to dull yellow over time.

#### DISCUSSION

The incorporation of molecular phylogenetics has greatly benefited systematic and taxonomic studies of fleshy fungi. The old reliance on European names is insufficient to appropriately describe North American diversity. There has been a significant number of new species of *Cantharellus* described in North America, specifically from the southern state of Texas (Buyck et al. 2010, Buyck et al. 2011, Buyck and Hofstetter 2011) and the midwestern state of Wisconsin (Foltz et al. 2013).

Early understanding of North American *Cantharellus* biodiversity was limited because morphological characters often are difficult to interpret. Coloration and surface features may change during basidioma development or in response to moisture. Molecular analysis suggests that five species from the *C. cibarius* group occur in the Chicago region. One represents a new oak-associated species with a pileus that often has a greenish yellow margin when immature and a squamulose disk when mature, a yellow spore print and the absence of a fragrant odor. *Cantharellus chicagoensis* is proposed based on morphology and its phylogenetic placement as a well-supported clade in relation to known *Cantharellus* species. The phylogenetic analysis of ITS2, 28S and *EF1 $\alpha$*  (FIG. 1a–b) identify *C. chicagoensis* as a separate species from the recently described oak-associated species *C. flavus*, *C. phasmatis* and *C. spectaculus* from nearby Wisconsin (Foltz et al. 2013). The molecular analysis failed to provide resolution and statistical support to indicate the sister taxon.

Most specimens of *C. chicagoensis* were collected in Illinois; two specimens were collected from the adjoining states of Indiana and Wisconsin. The Vernon County, Wisconsin, specimen, which had a yellow-green tint to the pileus and brownish orange staining, was collected approximately 320 km northwest of Chicago and 50 km east of the type locality for the three *Cantharellus* species described by Foltz et al. (2013).

Although sequences from the nuclear ribosomal 28S region did not provide strong support for most *Cantharellus* species included in this analysis, they resolved many of the species. The 28S by far is the easiest marker to amplify of the three markers we tested. Sequences of the more informative ITS region generally are difficult to obtain for *Cantharellus* specimens due to high interspecies heterogeneity in the ITS1 region, which is likely the cause of weak amplification during PCR. Given the challenges in obtaining data for the ITS region, the *EF1 $\alpha$*  gene has been used as an alternative for *Cantharellus* phylogenetic analysis (Buyck et al. 2011, Buyck and Hofstetter 2011, Buyck et al. 2013).

Our study expands the known range for the *C. flavus* and *C. phasmatis* clade. A specimen collected in northwestern Indiana was determined to be *C. flavus* using ITS2 sequence data (FIG. 1a, specimen PGA 08-044). Other specimens of this clade were collected by us from Illinois and Wisconsin (FIG. 1b: NAMA 2005-123, PRL 9171, PRL 10158). These specimens are clearly not *C. cibarius*, but unfortunately 28S is insufficient for species identification, and because PCR failed to amplify both the ITS2 and *EF1 $\alpha$*  regions their identities as either *C. flavus* or *C. phasmatis* cannot be determined. Sequence data for PRL 10157 were obtained for all three regions (FIG. 1). However, its placement in ITS2 and *EF1 $\alpha$*  phylogenies fell just outside both *C. flavus* and *C. phasmatis*. This specimen resembled *C. phasmatis* in color and stature but produced a pale yellow spore print rather than the salmon-pink described for *C. phasmatis*. The Texas species *Cantharellus tenuithrix* Buyck & V. Hofstetter occurs in the *C. phasmatis-flavus* complex based on the ITS2 and *EF1 $\alpha$*  datasets (FIG. 1a, c). Species limits need to be resolved for this group of putative oak-associated taxa.

Further study is needed to clarify the largely conifer-associated taxa *C. roseocanus*, European *C. cibarius* and related specimens in the Midwest (FIG. 1). Foltz et al. (2013) raised the possibility that the western, northern Midwest and northeastern members of “*C. roseocanus*” may represent more than one species. Morphological evidence for this is provided by the specimens in our study that lack the pinkish coating of the pileus that defines *C. roseocanus* (Redhead et al. 1997). Furthermore, western *C. roseocanus* and the midwestern specimens under this name are not resolved as a clade in the ITS2 dataset (FIG. 1a).

The combination of molecular and morphological analysis has facilitated documentation of chanterelle diversity in North America (Feibelman et al. 1996, Dunham et al. 2003, Arora and Dunham 2008, Buyck et al. 2010, Buyck et al. 2011, Buyck and Hofstetter 2011, Foltz et al. 2013). However, distributions for some of these species remain poorly known. The presence of unnamed *Cantharellus* in recently published

phylogenies suggests more American species await documentation and descriptive taxonomy to clarify their identities.

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SUPPLEMENTARY FIG. 1. *Cantharellus chicagoensis* sp. nov. PRL 5450 (F C0210207F), Cook County, Illinois.



SUPPLEMENTARY FIG. 2. *Cantharellus chicagoensis* sp. nov. PRL 11841 (F C0075039F), Cook County, Illinois.



SUPPLEMENTARY FIG. 3. *Cantharellus chicagoensis* sp. nov. PRL 11839 (F C0075037F), Cook County, Illinois.



SUPPLEMENTARY FIG. 4. *Cantharellus chicagoensis* sp. nov. PRL 11843 (F C0075041F), Cook County, Illinois.

