

The golden chanterelles of Newfoundland and Labrador: a new species, a new record for North America, and a lost species rediscovered

R. Greg Thorn, Jee In Kim, Renée Lebeuf, and Andrus Voitk

Abstract: Three species of golden chanterelles were found in Newfoundland and Labrador and were compared with other *Cantharellus* species by macromorphology, microscopy, and multilocus phylogenetic studies. The commonest species is a member of the *C. cibarius* group, usually found with *Picea*, and is differentiated from European *C. cibarius* by its pinkish-orange rather than yellow hymenium, and from both *C. cibarius* and *C. roseocanus* of the Pacific Northwest by its ITS and TEF1 sequences. We describe it as a new species, *Cantharellus enelensis*; published sequences extend its range to Michigan and Illinois. An uncommon species with reduced, merulioid hymenophore, found growing only with *Betula*, has rDNA and TEF1 sequences nearly identical to *C. amethysteus*, but only occasionally shows the amethyst scales on its cap characterizing that species in Europe. Ours is the first report of this species from North America. A third species was recognized by its sequences as *C. camphoratus*, but our collections, found with *Abies balsamea*, lack the odour of camphor for which this species was named and have longer and more slender spores than in the original description. This species has not been reported since its description from a single collection in Nova Scotia. All three species are edible.

Key words: basidiomycetes, wild-harvested mushrooms, TEF1, efla, ITS, nuclear ribosomal large subunit, phylogeny.

Résumé : Trois espèces de chanterelles dorées ont été trouvées à Terre-Neuve et Labrador et comparées à d'autres espèces de *Cantharellus* par des études en macromorphologie, en microscopie et en phylogénie multi-locus. L'espèce la plus commune est un membre du groupe *C. cibarius*, habituellement trouvé avec *Picea*, et qui se différencie de *C. cibarius* européen par son hyménium rose-orangé plutôt que jaune, et de *C. cibarius* et *C. roseocanus* du Pacific Northwest par les séquences de son ITS et de TEF1. Les auteurs la décrivent comme une nouvelle espèce, *Cantharellus enelensis*; les séquences publiées étendent sa distribution au Michigan et en Illinois. Une espèce peu commune comportant un hyménophore mérulioïde réduit, qui croit seulement avec *Betula*, possède des séquences d'ADNr et de TEF1 quasi identiques à celles de *C. amethysteus* mais ne présente qu'occasionnellement seulement, des écailles améthystes sur son chapeau, caractéristiques de cette espèce en Europe. Il s'agit du premier signalement de cette espèce en Amérique du Nord. Une troisième espèce a été reconnue par sa séquence comme étant *C. camphoratus*, mais les collections des auteurs, trouvées avec *Abies balsamea*, sont dépourvues de l'odeur camphrée d'après laquelle cette espèce a été nommée et possèdent des spores plus longues et plus minces que celles décrites à l'origine. Cette espèce n'a pas été rapportée depuis sa description à partir d'une seule collection en Nouvelle-Écosse. Les trois espèces sont comestibles. [Traduit par la Rédaction]

Mots-clés : basidiomycètes, champignons sauvages, TEF1, efla, ITS, grande sous-unité ribosomique nucléaire, phylogénie.

Introduction

Most large golden chanterelles in North America were at first thought to be *Cantharellus cibarius* Fr., the prized edible chanterelle of Europe, which was first described by Linnaeus (1753) as *Agaricus chantarellus* L. Fries (1821) recognized a broad geographic distribution when he re-described the species under its current name: “In pinetis,

etiam Americae, ubique” (In coniferous woods, even in America, everywhere). However, a number of new North American species have been segregated from *C. cibarius*, including species described from the Pacific coast (Smith and Morse 1947; Corner 1966; Redhead et al. 1997; Dunham et al. 2003; Arora and Dunham 2008), the southern United States (Feibelman et al. 1994, 1996, 1997; Buyck

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and Hofstetter 2011; Buyck et al. 2011), and the north-eastern United States and Atlantic Canada (Smith 1968; Bigelow 1978; Petersen 1979). Further research in the last decade has shown *Cantharellus* to consist of several regional species across North America, all differing from known European species (Buyck and Hofstetter 2011; Buyck et al. 2011; Foltz et al. 2013; Leacock et al. 2016; Buyck et al. 2016a, 2016c). Indeed, the global pattern of species of golden chanterelles is of continental or broad regional endemism, with morphologically similar but genetically distinct species around the globe (Buyck et al. 2014). Buyck et al. (2016c) recognize 29 species of *Cantharellus* from North America, none of them occurring in or first described from Europe.

Our own research into the chanterelles in the Canadian province of Newfoundland and Labrador (NL), showed that the common chanterelle in NL fell in a clade together with *C. roseocanus* (Redhead, Norvell & Danell) Redhead, Norvell & Moncalvo from the Pacific northwest coast, based on partial sequences of the nuclear large ribosomal subunit (LSU; Vilneff and Thorn 2011), although this gene region is known to be insufficiently informative to distinguish closely allied species in *Cantharellus* (Moncalvo et al. 2007; Buyck et al. 2011). Further study identified a second chanterelle morphospecies associated with birch (Thorn and Voitk 2011). Addition of sequence data from the ribosomal internal transcribed spacer (ITS) region and from the transcription elongation factor 1 alpha gene (TEF1; nomenclature following <http://www.uniprot.org/uniprot/P02994>) revealed that the common species in the province differs from, but is close to both the European *C. cibarius* and Western *C. roseocanus*; further collections allowed us to identify a third chanterelle morphospecies in the province. This study was undertaken to investigate these three chanterelle species in NL, using a combination of morphological and multilocus phylogenetic analysis.

Materials and methods

Morphology

Specimens were photographed in situ; macroscopic characters were recorded from fresh specimens; specimens were air-dried with low heat (30–35 °C). All collections have been deposited in the Canadian National Mycological Herbarium in Ottawa (DAOM), with duplicates of many at UWO (herbarium acronyms following Thiers 2017). Descriptions of macroscopic and microscopic features of the fruiting bodies follow Largent (1986) and Vellinga (1988), with colour codes in parentheses from Kornerup and Wanscher (1978). Microscopic observations (Zeiss 392560 with Apo 100/1.25) reported here were conducted at 1000× magnification (oil immersion), using 2% KOH for both fresh and dried material, except for spore measurements and pileipellis observations. Spores were measured to a precision of 0.5 μm; measurements deviating from 0.5 μm increments are due to calculation of a lens correction factor. Pileipellis

elements were observed in scalp sections mounted in ammoniacal Congo Red (Largent et al. 1977) viewed at 630× magnification (planapo 63/1.4, oil immersion) using a Zeiss Photoscope. Additional specimens of taxa from Western North America were requested from various herbaria; these are listed under “Additional specimens examined”, below.

Molecular phylogeny

Genomic DNA was isolated from dried collections (in a few cases, from tissue preserved in the field in 2× CTAB buffer; Gardes and Bruns 1993) using the GeneJET Plant Genomic DNA Purification Kit (Fermentas Life Sciences). Samples (5–10 mg) of the dried fruiting bodies were physically lysed using garnet beads in 350 μL of lysis buffer using a Fast Prep FP 120 homogenizer (Thermo Savant), followed by the remainder of the kit protocol. Extracts of DNA were quantified using a Nanodrop 2000 (Thermo Fisher) and stored at –20 °C. Four PCR reactions of 30 μL with different quantities of DNA extract (0.5, 1, 2, 4 μL) were conducted per sample. The PCR reactions contained 3.0 μL 10× PCR buffer, 25 mmol/L MgCl₂, 100 mmol/L dNTPs, 5 μmol/L of each primer, 1 U GenScript Taq DNA polymerase, and molecular H₂O to 30 μL. Amplifications of the nuclear ribosomal internal transcribed spacer (ITS) and 5′ ~1000 bases of the large subunit (LSU) were performed using primers ITS1 and LR5 (White et al. 1990; Vilgalys and Hester 1990). A portion of the nuclear-encoded transcription elongation factor 1-alpha gene (TEF1) was amplified using primers Canth-ef1a983-F (5′-GCY CCY GGV CAY CGY GAY TTY AT-3′) and Canth-ef1a-1567-R (5′-GGG ACR GTR CCR ATR CCV CCR AT-3′), with bases in bold modified from Rehner and Buckley (2005) for better performance with *Cantharellus*. The PCR products were checked using gel electrophoresis in 1.5% agar in 1× TAE buffer at 100 V for 60 min and were cleaned using EZ-10 Spin Column PCR Products Purification Kit (Bio Basic) prior to submission for sequencing. When products of TEF1 primers yielded two strong bands, the band of expected size was excised from the gel and cleaned using a Gel Extraction kit (Bio Basic) prior to submission for sequencing. Cleaned PCR products were submitted for sequencing at the sequencing facility of Robarts Institute of Western University with primers ITS1, LS1R, 5.8S, 5.8S-R, LS1, LR3, LR3R, and LR5 (White et al. 1990; Hausner et al. 1993; Vilgalys and Hester 1990) and the new internal primer Canth ITS1 Internal-R (5′-CGG ACA CTG AGG GTG ACA-3′) to obtain the sequences of the ITS and LSU regions, and Canth-ef1a983-F and Canth-ef1a1567-R for a portion of TEF1. Sequences were cleaned and assembled using SeqEd version 1.03 (ABI Software). New sequences were deposited in GenBank as accession numbers KX592684–KX592729, KX519731–KX519768, and KX610318–KX610319. BLAST analyses (Altschul et al. 1997) and preliminary phylogenetic analyses were used to select related sequences for further study, and LSU sequences were used to place NL collections to species-groups. Sequences

of the ITS, LSU, and TEF1 regions were separately aligned using MAFFT version 7 (Kato and Standley 2013) with the “leave gappy regions” option invoked, then the rough ends of alignments trimmed using Mega 7.0 (Kumar et al. 2016) and the regions compiled into one matrix of 30 terminals, 3243 bases long including alignment gaps. Maximum parsimony (MP) and neighbor-joining (NJ) analyses were conducted in PAUP* 4.10b (Swofford 2003), maximum likelihood (ML) analyses in Mega 7.0, and Bayesian analyses in MrBayes 3.2.6 (MB; Ronquist et al. 2012) with 5 000 000 generations, 4 chains, and a burn-in of 25% (when the average standard deviation of split frequencies between chains had stabilized below 0.001). Node support was determined as posterior probabilities in MrBayes, and as bootstrap support in ML and MP analyses using 100 replicates (and, in MP, with 100 random-order additions of taxa). The alignments and trees have been deposited to TreeBase as S19665.

Results

Molecular phylogeny

Eighty-five new sequences were obtained from 51 collections of *Cantharellus*: 8 NL collections of *C. amethysteus* (Qué.) Sacc., 12 of our new species *C. enelensis*, and 15 of *C. camphoratus* R.H. Petersen, plus two collections of *C. cibarius* from Europe, and 6 of *C. formosus* Corner (one of which proved to be *C. cascadiensis* Dunham, O’Dell & R. Molina), 5 of *C. roseocanus*, 2 of *C. subalbidus* A.H. Sm. & Morse from western North America, and one of *Cantharellus* cf. *phasmatis* Foltz & T.J. Volk from Ontario (Table 1). Sequences obtained of the internal transcribed spacer regions, and to a lesser extent the 5’ end of the LSU and the TEF1 gene, frequently contained one or two-base indels or base substitutions between haplotype copies in the dikaryotic genome. These sometimes made it impractical to obtain the sequence of the 5’ or 3’ end of the PCR products; alignments were trimmed as necessary, and partial sequences used only to place collections to species. Results from separate analyses of LSU, ITS, and TEF1 data each supported the existence of three taxa of chanterelles from NL, allied to *Cantharellus cibarius* sensu stricto and *C. roseocanus*, *C. amethysteus*, or *C. formosus*. Our single collection of “*Cantharellus cibarius*” from Ontario clustered within the species complex of *Cantharellus phasmatis* (Buyck et al. 2016a) by both LSU and TEF1 sequences (data not shown) and remains unidentified; it was excluded from further analyses. Combined data of a smaller taxon sample yielded strong support in MB, ML, and MP analyses for each of the clusters bearing the same species identification (Fig. 1a). Branch-tips of the LSU analyses are shown to place the majority of collections cited, in the “amethysteus clade”, core “cibarius clade”, or “formosus clade” (Fig. 1b).

Multilocus analyses (Fig. 1a) provide strong support for the placement of the NL taxon we identify as *C. amethysteus* with the neotype and one other European collection identi-

fied by that name (Buyck et al. 2016c). There appears to be considerable sequence distance between the NL and European representatives, suggesting the possibility that the NL taxon is a separate, undescribed species, but the differences seen in any one of the loci were small and inconsistent. The most common NL chanterelle, which we identify as the new species, *Cantharellus enelensis*, was recovered in all multilocus analyses, and receives strong support in MP and MB analyses but only weak support in ML analysis. Two NL collections cluster together with two collections from under conifers in Michigan and Illinois, recently published as *Cantharellus* sp. (Leacock et al. 2016), suggesting that *C. enelensis* is widespread in temperate to boreal eastern North America. Unfortunately, the ITS sequences of specimens from Quebec reported as *C. cibarius* var. *roseocanus* (Rochon et al. 2011) are not available in GenBank or from the authors (C. Rochon and A. Fortin, personal communication, 2016). Our data suggest that the name *C. roseocanus* should be restricted to collections from west of the Rocky Mountains. Two collections from Colorado and Idaho identified as *C. roseocanus* (Foltz et al. 2013; Leacock et al. 2016) could not be placed based on their available sequences, and may represent a separate taxon, but morphological studies and additional sequence data will be required to identify them with confidence. The recent publication of sequence data from the type specimen of *C. camphoratus* (Buyck et al. 2016c) provided a match for our NL collections that we had previously identified as *C. formosus*; although no TEF1 sequence of the type is available, the partial ITS and LSU sequences are a nearly perfect match for those from our collections. As mentioned by Buyck et al. (2016c), *C. camphoratus* is the east coast counterpart to the west coast *C. formosus* in North America. An undescribed species from Japan plus the recently described *C. chicagoensis* Leacock, J. Riddell, Rui Zhang & G.M. Muell. (Leacock et al. 2016) and *C. velutinus* Buyck & V. Hofst. (Buyck et al. 2016b) are other members of this clade (Fig. 1a).

Morphology

Three species were identified (Fig. 2), as described below. The commonest, *C. enelensis*, found throughout NL, resembles *C. cibarius*, but differs from it by a more orange hymenium. The other two, much less common, are readily distinguished from *C. enelensis* by their flatter, lighter coloured, more sinuous hymenium, and from each other by cap colour and spore morphology, both of which showed considerable variation. However, average spore size readily separated the longer spores of *C. amethysteus* from *C. camphoratus*, which it resembles macroscopically, as well as *C. enelensis* (Figs. 2B, 2E, and 2H).

Taxonomy

Cantharellus amethysteus (Qué.) Sacc. Figs. 2A–2C

MACROMORPHOLOGY: Pileus: 20–70 mm diameter, edges evenly round and inrolled, opening up to become plane, then

Table 1. *Cantharellus* collections and sequences used in phylogenetic analyses; full collection data provided in the text under the section heading “Specimens examined.”

Name	Herbarium No. (other number) ^a	GenBank No.			Provenance (type status)
		TEF1	ITS	LSU	
<i>C. amethysteus</i>	DAOM 721713 (09.08.24.av04)			KX592684, KX592685	Canada: NL
<i>C. amethysteus</i>	DAOM 734021 (10.08.25.av02)			KX592686, KX592687	Canada: NL
<i>C. amethysteus</i>	DAOM 734022 (11.08.12.av01)			KX610318, KX610319	Canada: NL
<i>C. amethysteus</i>	DAOM 734023 (11.08.30.av02)			KX592688, KX592689	Canada: NL
<i>C. amethysteus</i>	UWO 12.09.02.av11	KX592690		KX592691, KX592692	Canada: NL
<i>C. amethysteus</i>	DAOM 734025 (12.10.03.av01)	KX592693	KX592696, KX592697	KX592694, KX592695	Canada: NL
<i>C. amethysteus</i>	DAOM 734026 (13.08.21.av01)			KX592698, KX592699	Canada: NL
<i>C. amethysteus</i>	DAOM 734027 (13.09.14.av01)			KX592700, KX592701	Canada: NL
<i>C. camphoratus</i>	DAOM 734049 (10.08.13.av01)			KX592728	Canada: NL
<i>C. camphoratus</i>	DAOM 734050 (11.08.05.av01)		KX592729	KX592729	Canada: NL
<i>C. camphoratus</i>	DAOM 695785 (12.07.10.av01)			KX592731	Canada: NL
<i>C. camphoratus</i>	UWO 12.08.04.av01			KX592732	Canada: NL
<i>C. camphoratus</i>	UWO 12.09.03.av17			KX592733	Canada: NL
<i>C. camphoratus</i>	DAOM 695788 (12.09.06.av01)	KX592735	KX592736	KX592734	Canada: NL
<i>C. camphoratus</i>	DAOM 737291 (12.09.22.av02)	KX592738, KX592739	KX592737	KX592737	Canada: NL
<i>C. camphoratus</i>	DAOM 737292 (13.08.26.av01)			KX592740	Canada: NL
<i>C. camphoratus</i>	DAOM 737293 (13.08.26.av04)			KX592741	Canada: NL
<i>C. camphoratus</i>	DAOM 734047 (13.09.12.av01)			KX592742	Canada: NL
<i>C. camphoratus</i>	DAOM 737294 (13.09.13.av01)			KX592743	Canada: NL
<i>C. camphoratus</i>	DAOM 737295 (13.09.15.av01)			KX592744	Canada: NL
<i>C. camphoratus</i>	DAOM 737296 (13.09.27.av04)			KX592745	Canada: NL
<i>C. camphoratus</i>	DAOM 737297 (13.09.27.av05)			KX592746	Canada: NL
<i>C. camphoratus</i>	DAOM 737298 (MR3-057)		KX592747	KX592747	Canada: NL
<i>C. cascadenis</i>	UBC F23801 (as <i>C. formosus</i>)	KX592702	KX592703, KX592704, KX592705		Canada: BC
<i>C. cibarius</i>	UWO 12.07.24.av01	KX592707		KX592706	Estonia
<i>C. cibarius</i>	DAOM 734029 (13.08.14.av01)			KX592708, KX592709	Norway
<i>C. enelensis</i>	DAOM 734040 (11.08.16.av03)	KX592711		KX592710	Canada: NL
<i>C. enelensis</i>	DAOM 734041 (13.08.21.av02)			KX592712	Canada: NL (isotype)
<i>C. enelensis</i>	DAOM 734042 (13.08.26.av02)			KX592713	Canada: NL
<i>C. enelensis</i>	DAOM 734043 (13.08.26.av03)			KX592714	Canada: NL
<i>C. enelensis</i>	DAOM 721714 (13.09.01.mb)			KX592715	Canada: NL
<i>C. enelensis</i>	DAOM 721712 (13.09.25.av01)			KX592716	Canada: NL
<i>C. enelensis</i>	DAOM 721701 (C. Vilneff E3)		KX592717, KX592718	KX592717, KX592718	Canada: NL
<i>C. enelensis</i>	UWO C. Vilneff E5	KX592720	KX592719	KX592719	Canada: NL
<i>C. enelensis</i>	DAOM 721703 (C. Vilneff W3)		KX592721, KX592722	KX592721, KX592722	Canada: NL
<i>C. enelensis</i>	UWO C. Vilneff W5	KX592724	KX592723	KX592723	Canada: NL
<i>C. enelensis</i>	DAOM 734044 (MR3-020)	KX592726	KX592725	KX592725	Canada: NL
<i>C. enelensis</i>	DAOM 721711 (MR3-021)		KX592727	KX592727	Canada: NL
<i>C. formosus</i>	DAVP 28141	KX592749		KX592748	Canada: BC
<i>C. formosus</i>	DAVP 28291	KX592750			Canada: BC

Table 1 (concluded).

Name	Herbarium No. (other number) ^a	TEFI	ITS	LSU	Provenance (type status)
<i>C. formosus</i>	SAT-13-298-20	KX592751	KX592752		USA: OR
<i>C. formosus</i>	UBC F15948	KX592753			Canada: BC
<i>C. formosus</i>	UBC F16607	KX592754			Canada: BC
<i>C. roseocamus</i>	DAVP 28001			KX592755	Canada: BC
<i>C. roseocamus</i>	SAT-12-299-06	KX592756	KX592757		USA: OR
<i>C. roseocamus</i>	UBC F16825	KX592758			Canada: BC
<i>C. roseocamus</i>	UBC F16989	KX592759			Canada: BC
<i>C. roseocamus</i>	UBC F23802	KX592761			Canada: BC
<i>Cantharellus</i> cf. <i>phasmatris</i>	DAOM 721705 (RG1130716/01)	KX592764, KX592765		KX592760, KX592762	Canada: ON
<i>C. subalbidus</i>	DAVP 28155	KX592766			Canada: BC
<i>C. subalbidus</i>	DAVP 28283	KX592768			Canada: BC

Note: Contiguous ITS-LSU sequences share the same accession number but are shown in both columns. NL, Newfoundland and Labrador; BC, British Columbia; ON, Ontario; OR, Oregon.

^aCollection acronyms following Thiers (2017); SAT, the herbarium of S.A. Trudell.

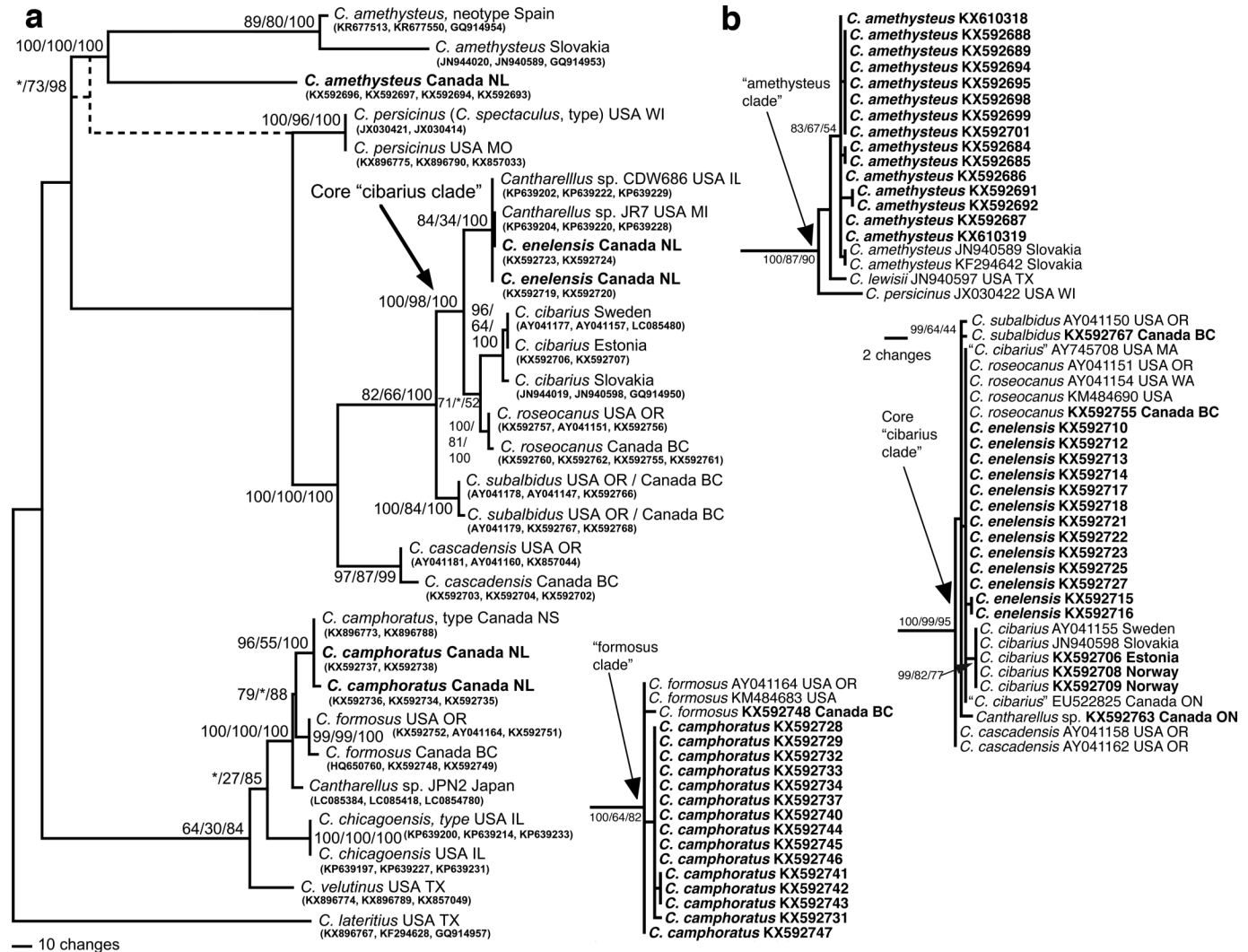
funnel shaped, with an irregularly wavy margin; opaque, not hygrophanous; variously covered with thin coating that breaks up into small scales, which often disappear; pale yellow (3A2, 3A3) to pale orange (5A3), scales amethyst (greyish red, 9AB1½ to 12C3), turning violet-brown, then brown; amethyst colour not always present. Hymenium: flattened, blunt, wide folds, close, sinuous, deeply decurrent, forked, cross-veined and anastomosing, may be almost absent; light yellow to almost white (3A2½, 4A2½, 3½A1½). Stipe: 5–25 mm × 30–65 mm, enlarging upwards from narrow base, solid; pale yellow (3½A2, 3½A3) to pale orange (5A3). Context: whitish yellow (3½A1½), immediately violaceous (12–15D4) with FeCl₃, then slowly turning grey; odour sweet and fruity, resembling apricots. All tissues stain reddish-brown with injury or prolonged exposure. Fruiting bodies relatively immune to invertebrate parasites, and remain over a month in the field in good condition. Development is “normal” when the hymenium is protected from drying by moss or a high layer of leaf litter. Habitus in exposed sites (no moss or a low layer of leaf litter) varies from solitary pegs with greatly reduced hymenophore ridges, to connate, cespitose or fused fruiting bodies.

MICROMORPHOLOGY: Basidiospores (5 collections, 7 sporocarps, 131 spores) (7.7–)8.7–14.3 μm × (3.9–)4.6–7.1(–7.7) μm (average 10.4 × 5.6), average Q = 1.9; elliptical to oblong, usually narrower at the apex, often with an asymmetrically placed constriction, frequently slightly bent; content homogeneous. Basidia 65–90 μm × 7.7–11.6 μm; 4–6-spored; clavate. No cystidia. Pileipellis a cutis of collapsing, thin-walled, tubular hyphae with vacuolar and cytoplasmic orange pigments, 3.4–6.7 μm broad, a few with walls 0.5–0.7 μm thick, apices tubular and rounded; underlain by progressively hyaline and inflated, thin-walled hyphae (4.5) 6.7–20.0 μm broad. Clamp connections in all tissues, sometimes looping. Wide range in micromorphology between collections and individual sporocarps within a single collection.

ECOLOGY AND NL DISTRIBUTION: Grows in colonies among leaf litter with *Betula*. Uncommon; least common of the three species; large fruitings unknown. Found from the end of August to the beginning of October, most plentiful in September. So far documented from three sites in the Bay of Islands region of western Newfoundland (Fig. 3).

SPECIMENS EXAMINED: CANADA, Newfoundland and Labrador, Humber Village, trail to Barry's Lookout, 48.988°N, 57.792°W, elevation 60 m a.s.l., in leaf litter under *Betula papyrifera* and *B. alleghaniensis*, 24 August 2009, Andrus Voitk 09.08.24.av04 (DAOM 721713), 25 August 2010, A. Voitk 10.08.25.av02 (DAOM 734021), 12 August 2011, A. Voitk 11.08.12.av01 (DAOM 734022), 10 August 2012, A. Voitk 12.08.10.av01 (DAOM 734016), 02 September 2012, A. Voitk 12.09.02.av11 (DAOM 734024, portion at UWO), 03 October 2012, A. Voitk 12.10.03.av01 (DAOM 734025), 11 August 2013, A. Voitk 13.08.11.av01 (DAOM 734017), 14 September 2013, A. Voitk 13.09.14.av01 (DAOM 721702 and 734027), 30 September 2013, A. Voitk, 13.09.30.av04 (DAOM

Fig. 1. (a) Phylogenetic tree based on the concatenated dataset of ITS–LSU and TEF1 sequences. One of 117 equally parsimonious trees ($L = 907$ steps, 396 parsimony-informative characters, $CI = 0.86$, $RC = 0.77$). Node support from $100 \times$ MP bootstrap / $100 \times$ ML bootstrap / Bayesian posterior probabilities; nodes that collapsed in one method shown by an asterisk (*). A sister relation of *Cantharellus amethysteus* and *C. persicinus* was moderately supported (ML) or strongly supported (MB) but not recovered in MP, and is shown as a broken line. GenBank accession numbers of all sequences used are shown beneath the names, and collecting localities after the names (with two-letter codes of states and provinces for USA and Canada). (b) Branch-tips from a phylogenetic tree based on separate LSU sequences, clustering all NL *Cantharellus* collections studied in one of three taxa. Branch support shown at nodes is from Bayesian posterior probabilities / NJ bootstrap (1000 \times) / $100 \times$ ML bootstrap, with each terminal identified by its GenBank accession number.



734018), 01 October 2013, A. Voitk 13.10.01.av02 (DAOM 734019), Foot of Blowmedown Mountain, Elaine Humber property, 49.058°N 58.209°W, 39 m a.s.l., on soil in deciduous woods of birch, 01 September 2014, E. Humber 14.09.01.av01 (DAOM 734020), Humber Village, Weldon's Road, 48.994°N 57.749°W, 29 m, in moss in coniferous woods of balsam fir, spruce and birch, 30 August 2011, A. Voitk 11.08.30.av02 (DAOM 734023), same location, among balsam fir, speckled alder, birch quite far, 21 August 2013, Maria Voitk 13.08.21.av01 (DAOM 734026).

COMMENT: In NL, whitish and flattened hymenial folds and frequent aberrant forms distinguish this species from the common *C. enelensis*. Amethyst scales, if present, associa-

tion with birch, and markedly longer spores separate it from both *C. enelensis* and *C. camphoratus*. In fact, this species shares with *C. persicinus* R.H. Petersen (= *C. spectaculus* Foltz & T.J. Volk) the largest basidiospores among North American species of *Cantharellus* (Buyck et al. 2016a). Other members of the *C. amethysteus* clade include *C. lewisii* Buyck & V. Hofst. and *C. persicinus* from the southern USA, *C. subamethysteus* Eyssart. & Stubbe from Malaysia (Eyssartier et al. 2009), and *C. pseudoformosus* D. Kumari, Ram. Upadhyay & Mod.S. Reddy from the western Himalaya region of India (Kumari et al. 2010).

In Europe, *Cantharellus amethysteus* has been well described and illustrated (Dähnke 1993; Pegler et al. 1997;

Fig. 2. Fruit bodies (A, D, and G), spores (B, E, and H) and “aberrant variations” (C, F, and I) of the three species of chanterelle from Newfoundland and Labrador. (A, B, and C): *Cantharellus amethysteus*. (A) Amethyst scales on the cap, as here, are seldom seen, DAOM 734017 (13.08.11.av01). (B) Basidiospores, from multiple collections. (C) Clockwise, from top left, a multiheaded specimen, DAOM 734022 (11.08.12.av01), an unusually orange specimen, DAOM 721713 (09.08.24.av04), another fused, multiheaded specimen, DAOM 734021 (10.08.25.av02), and a dried, peg-like fruiting body, DAOM 734017 (13.08.11.av01). (D, E, and F) *Cantharellus enelensis* sp. nov. Commonest, biggest and most deeply yellow of our species. (D) Typical form, among *Pleurozium* under *Picea glauca*, DAOM 734038 (13.09.28.av01). (E) Basidiospores, from multiple collections. (F) Somewhat distorted specimens, all with exposed hymenium, without moss or lichen “protection”: clockwise, from top left, a multiheaded specimen, DAOM 721714 (13.09.01.mb-1), specimen with hypertrophic hymenium, DAOM 721714 (13.09.01.mb-2), dry specimen, DAOM 721714 (13.09.01.mb-3), and a fruiting body with a markedly contorted cap and hypertrophic hymenium, DAOM 734039 (14.08.28.av01). (G, H, and I): *Cantharellus camphoratus*. (G) Brownish scales on the cap, as seen here, present only some of the time, DAOM 695786 (12.08.04.av01). (H) Basidiospores, from multiple collections. (I) Clockwise, from top left, peg-like fruiting bodies with reduced hymenophore, DAOM 695785 (12.07.10.av01), thick, fused fruiting bodies, DAOM 734050 (11.08.05.av01), peg-like and dried orange fruiting bodies, DAOM 734050 (11.08.05.av01), and more completely fused sporocarps, DAOM 734049 (10.08.13.av01). Scale bars for fruit bodies = 5 cm; line for spores drawn at 10 μ m.

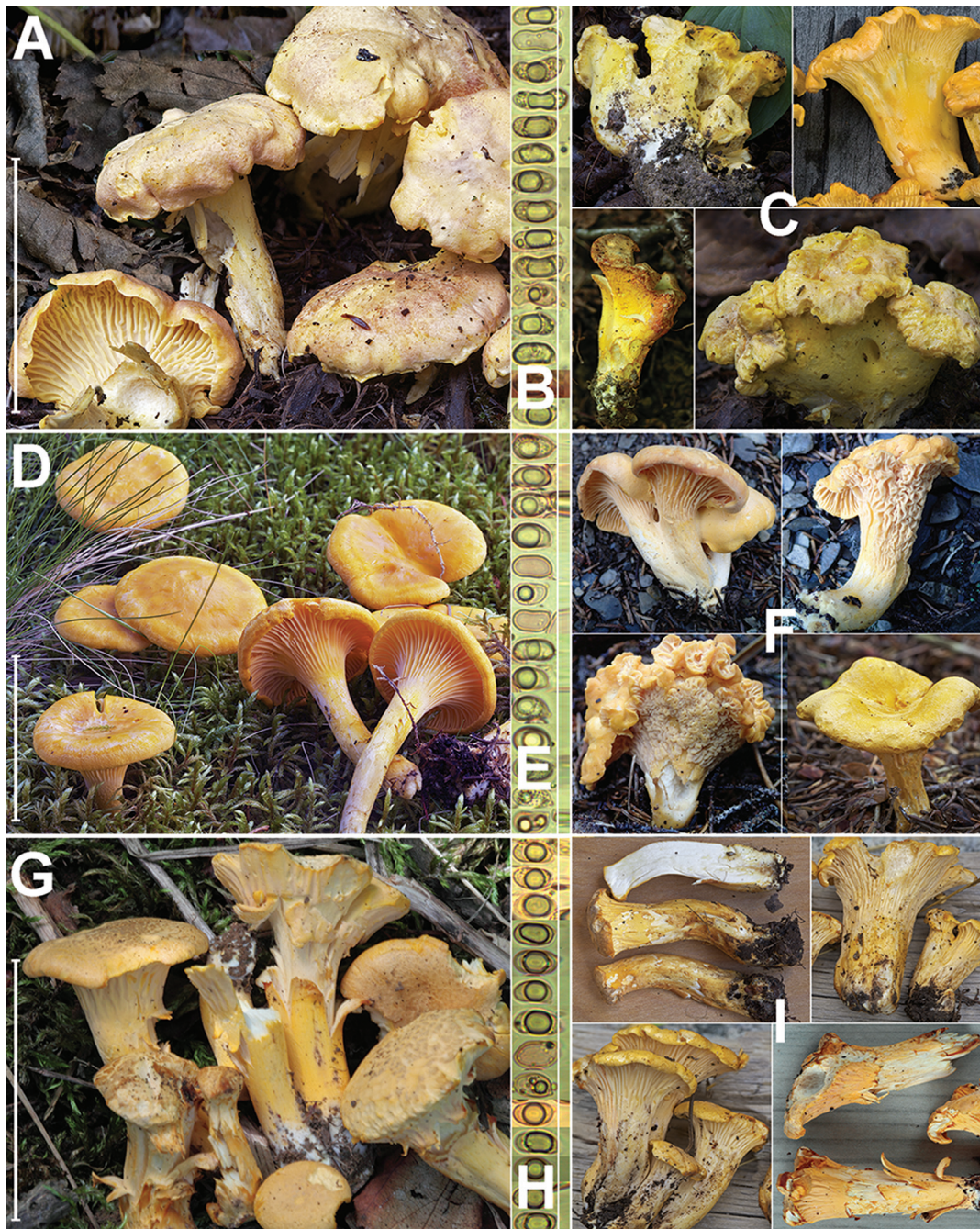
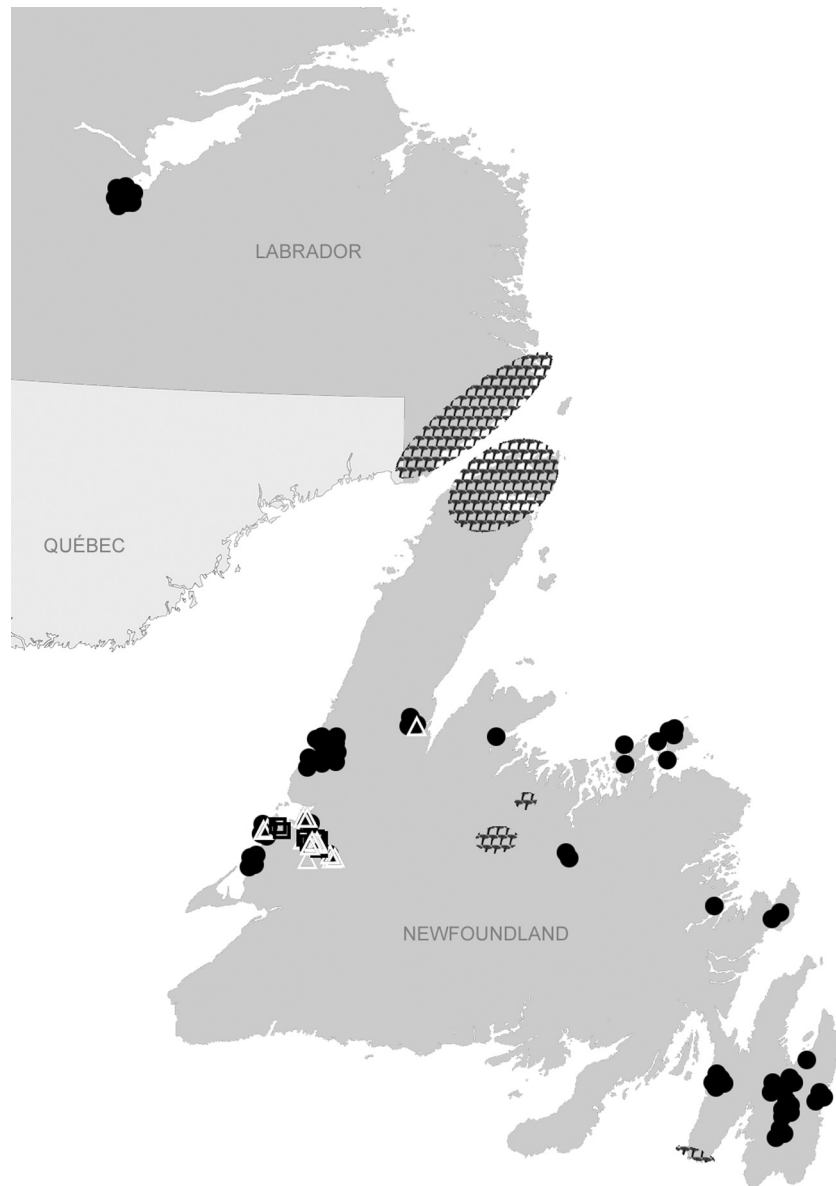


Fig. 3. Distribution of chanterelles in Newfoundland and Labrador. Hatching shows areas surveyed where chanterelles were not found. Absence of ectomycorrhizal mushrooms in nonforested barrens and ericaceous heaths, as at Cape Saint Mary's, the tip of the Great Northern Peninsula, or the shores of the Labrador Straits from Blanc Sablon to Battle Harbour, is not surprising. More surprising was not finding them in two of our few red pine forests (smaller hatched areas in central NL), particularly because elsewhere they are known as pine partners; in Goose Bay, where *Cantharellus enelensis* is abundant, no chanterelles were found under jack pine. Collecting sites of *C. amethysteus* are marked with squares, and *C. camphoratus* with triangles. So far, both are known primarily from the Bay of Islands region. One of the authors lives and collects in that area, which likely accounts for the discovery of these uncommon species there, and not elsewhere. Collection sites for *C. enelensis* are indicated by black circles. This species is found throughout the forested areas of the Island. In Labrador, one of the most productive places is on the Canadian Forces Base in Goose Bay, under white spruce. We did not find any chanterelles in northern Labrador at Konrad Brook (56.219°N 62.777°W; not shown), although the valleys there were abundantly forested with black and white spruce on sandy soil. Base map adapted by A.V., with permission, from an ecoregion map (http://www.ecc.gov.nl.ca/parks/maps/ecoregions_nf_lab.pdf) produced by Parks and Natural Areas Division, Department of Environment and Conservation, Government of Newfoundland and Labrador.



Eyssartier and Buyck 2000; Eyssartier and Roux 2011). In North America, the taxon has been reported previously from the Gulf Coast region of the southern United States (Feibelman et al. 1997; Pilz et al. 2003, as *Cantharellus cibarius* var. *amethysteus* Quél.), but Buyck and Hofstetter

(2011) have shown those reports to be based on a distinct species, *C. lewisii*, differentiated by its sequences and short basidiospores.

The recent spate of new species described and reported from North America (Foltz et al. 2013; Leacock

et al. 2016; Buyck et al. 2016a, 2016b, 2016c) has not turned up *C. amethysteus*. Ours is the first report of sequence-confirmed *C. amethysteus* from North America, so far only known from three locations in the Bay of Islands region of western Newfoundland. All sites are in wooded areas, away from adjacent habitation, and the furthest is about 50 km from the other two, suggesting that human introduction is unlikely. That it is the first, and so far only, European species of chanterelle in North America, raises the possibility that the two may not be truly conspecific, but this will require studies of multiple loci in individuals of each population; our experience suggests that many published short, partial LSU, ITS and TEF1 sequences are inadequate for critical identification and phylogenetic analyses.

Cantharellus enelensis Voitk, Thorn, Lebeuf, J.I. Kim, sp. nov. Figs. 2D–2F

MYCOBANK: MB 817851.

TYPELOCALITY: CANADA, Newfoundland and Labrador, Sandy Point, 48.568°N, 58.517°W, elevation 0.4 m a.s.l., in moss or fruticose lichens on moist sandy soil under *Picea glauca*, 21 August 2013, Urve Voitk, 13.08.21.av02 (holotype DAOM 721704; isotype DAOM 734041).

ETYMOLOGY: *Enelensis* is a Latinized reference to its presence in NL (i.e., en = N, el = L), the Canadian province of Newfoundland and Labrador, where it is the commonest species of chanterelle.

DIAGNOSIS: A golden chanterelle with well-formed hymenophoral ridges, differing from *C. cibarius* of Europe by its more orange hymenium, and from both *C. cibarius* and *C. roseocanus* by its distinctive ITS and TEF1 sequences.

MACROMORPHOLOGY: Pileus: 25–110 mm diameter, edges initially evenly round and inrolled, opening up to become plane, then funnel shaped, with an irregularly wavy margin; opaque, not hygrophanous; very finely downy with a whitish or occasionally pinkish bloom, becoming smooth; deep orange-yellow (4½4, 5A5, 5B4). Hymenium of sharp, straight, deep folds resembling gills, close, strongly decurrent, forked, joined by low cross-veins in age, deep orange-yellow, often with a pinkish tinge (5A2, 5A3, 6A2). Stipe: 5–25 mm × 30–75 mm, enlarging upwards from narrow base, solid, solitary; deep orange-yellow (4A2 to 5A3). Context: white or light yellow (3½A1½), immediately violaceous (12–15D4) with FeCl₃, then slowly turning greyish; smell fruity, pungent, resembling apricots. All tissues stain somewhat brownish with injury. Fruiting bodies are relatively immune to invertebrate parasites, and remain over a month in the field in good condition, particularly in the more humid western parts of Newfoundland. If the hymenium is exposed (no moss or fruticose lichen) fruiting bodies may become contorted, and the hymenial folds become low, undulating, and often hypertrophied with multiple anastomoses. Not as likely as the other two species to form connate or cespitose forms in exposed situations.

MICROMORPHOLOGY: Basidiospores (5 collections, 8 sporocarps, 127 spores) 6.7–11.6 µm × 4.3–6.6 µm (average 8.2 × 5.1), average Q = 1.6; elliptical to oblong, usually narrower at the apex, content homogeneous. Basidia 60–100 µm × 7.7–10.6 µm; 4–6-spored, uncommonly 8, clavate. No cystidia. Pileipellis is a cutis composed of thin-walled, readily collapsing, tubular hyphae 4.5–9.0 µm broad, with both vacuolar and cytoplasmic orange pigments, underlain by a layer of progressively broader, hyaline hyphae with thicker walls and clavate tips commonly 7–10 (–18 µm) broad and with walls 0.9–1.7 µm thick; tramal hyphae hyaline, tubular to inflated, to 20 µm broad. Clamp connections in all tissues, sometimes looping. Wide range in micromorphology between collections and individual sporocarps within a single collection.

ECOLOGY: Grows in small groups to very large colonies on sandy, well-drained but moist soil, among moss or fruticose lichen; conifer associated, more common under *Picea* than *Abies*. Common in the province, with several areas of annually recurrent massive fruitings. Found from the end of July to the end of September, but most plentiful in August. This and other species of *Cantharellus* have been sought with no success under the limited stands of red pine (*Pinus resinosa*) in central Newfoundland and jack pine (*P. banksiana*) in Labrador.

DISTRIBUTION: So far, documented from Labrador and across the Island of Newfoundland, by far the most common and abundant chanterelle in Newfoundland and Labrador (Fig. 3). This species may also be one of the common golden chanterelles of Ontario (e.g., GenBank accession EU522825) and Massachusetts (GenBank accession AY745708), but these LSU sequences do not discriminate among members of the *C. cibarius* complex (Fig. 1b). Recently released ITS and TEF1 sequences of collections from Michigan (under *Pinus strobus*) and Illinois (tree associates unknown) as *Cantharellus* sp. (Leacock et al. 2016) can now be identified as *C. enelensis* (Fig. 1a).

SPECIMENS EXAMINED: CANADA, Newfoundland and Labrador, Gros Morne National Park, Green Gardens, 49.507°N 58.054°W, elevation 22 m a.s.l., in mixed woods of balsam fir and birch, 15 August 2004, Andrus Voitk 04.08.15.av06 (DAOM 734030), near Harbour Grace, 47.68°N 53.21°W, fruiting in moss under *Abies* and white birch, 15 August 2009, 15 August 2009 Cassia Vilneff [E1] and Ralph Jarvis (DAOM 721706), same location, 15 August 2009 *C. Vilneff* [E2] and R. Jarvis (DAOM 721707), same location, 15 August 2009, *C. Vilneff* [E3] and R. Jarvis (DAOM 721701), same location, 15 August 2009, *C. Vilneff* [E4] and R. Jarvis (DAOM 721708), same location, 15 August 2009, *C. Vilneff* [E5] and R. Jarvis (UWO), Sandy Point, near Stephenville, 48.449°N 58.522°W, 1 m, under balsam fir (*Abies balsamea*) in mossy coastal forest, 15 August 2009, A. Voitk et al. [*C. Vilneff* W1] (DAOM 721709), same location, 15 August 2009, A. Voitk et al. [*C. Vilneff* W2] (DAOM 721710), same location, 15 August 2009, A. Voitk et al. [*C. Vilneff* W3] (DAOM 721703), same location, 15 August 2009, A. Voitk

et al. [*C. Vilneff W4*] (UWO), same location, 15 August 2009, A. Voitk et al. [*C. Vilneff W5*] (UWO), same location, 14 August 2010, A. Voitk 10.08.14.av02 (DAOM 734031), same location, 21 August 2010, A. Voitk 10.08.21.av02 (DAOM 734032), same location, 28 September 2013, A. Voitk 13.09.28.av01 (DAOM 734038), Burnt Hill, Norris Point, 49.520°N 57.872°W, 51 m a.s.l., in coniferous woods of balsam fir and spruce, 16 July 2011, A. Voitk 11.07.16.av01 (DAOM 734033), same location, 09 September 2012, A. Voitk 12.09.09.av04 (DAOM 734037), same location, under *Betula*, *Abies*, *Picea*, 01 September 2013, Michael Burzynski 13.09.01.mb (DAOM 721714), Cedar Cove Trail, 49.102°N 58.425°W, 134 m a.s.l., on soil in coniferous woods of balsam fir, 25 July 2011, A. Voitk 11.07.25.av03 (DAOM 734034), same location, 26 August 2013, Maria Voitk 13.08.26.av02 (DAOM 734042) and A. Voitk 13.08.26.av03 (DAOM 734043), Goose Bay Base, 53.293°N 60.496°W, 65 m a.s.l., on sandy soil, among reindeer lichens in mixed woods of balsam fir, *Betula glandulosa*, *Abies*, *Picea*, 16 August 2011, Aare Voitk 11.08.16.av03 (DAOM 734040), same location, in moss and lichen in sand field, open forest of spruce and *Betula glandulosa*, 24 August 2012, Andrus Voitk 12.08.24.av02 (DAOM 734035), Lower Main River, trail 3, 49.782°N 56.954°W, 38 m a.s.l., in moss under *Abies*, *Picea*, *Betula* in mixed woods, 06 September 2011, Aare Voitk MR3-020 (DAOM 734044), Lower Main River, Steep Unit, 49.81° 57.11°W, in moss under *Abies*, *Picea*, *Betula*, 06 September 2011, Aare Voitk MR3-021 (DAOM 721711), Pasadena Ski trails, 48.996°N 57.586°W, 90 m a.s.l., on soil in mixed forest of balsam fir, birch, spruce, 03 September 2012, A. Voitk 12.09.03.av19 (DAOM 734036), near Gillams, 49.02°N 58.06°W, fruiting under *Picea* in coniferous woods, 25 September 2013, A. Voitk 13.09.25.av01 (DAOM 721712), Gros Morne National Park, Lobster Cove Head Lighthouse, 49.604°N 57.954°W, 22 m a.s.l., on soil and duff in coniferous woods of balsam fir and spruce, 28 August 2014, A. Voitk 14.08.28.av01 (DAOM 734039).

COMMENT: Larger size, more regular shape, smooth cap, deep orange-yellow colour on all surfaces, deep and sharp gill-like hymenial folds, preference for tall moss or fruticose lichens in moist sandy soil and association with spruce separate it from the other two chanterelles of our area. Known locally as “the NL chanterelle”, or “the Newfoundland chanterelle.” Collected privately, and commercially mostly for local restaurant consumption, with limited export.

This species is the eastern counterpart to *C. roseocanus* in the core “cibarius clade” of golden chanterelles. Multi-locus analyses show it to be equally distinct from *C. cibarius* as *C. roseocanus* is, and that *C. enelensis* and *C. roseocanus* are well-delimited at the sequence level (Fig. 1a). Colour of the hymenophore separates *C. enelensis* from typical members of *C. cibarius* of Europe, with pinkish orange colours in the former and a yolk yellow colour in the latter (Thorn and Voitk 2011), but we have not

found a consistent means of separation of *C. enelensis* from *C. roseocanus* using macro- or micromorphology. However, the ITS sequences of western *C. roseocanus* and eastern *C. enelensis* are quite distinctive. In the approximately 422 bases (over 1200 bases in the multiple species alignment) between the forward and reverse primers RS1A and RS1R proposed to be specific to the ITS of *C. cibarius* var. *roseocanus* (Rochon et al. 2011), there are 11–12 base substitutions and 22–26 bases of indels differentiating western *C. roseocanus* and eastern *C. enelensis*; a difference of approximately 8%. Primers RS1A and RS1R have a perfect match to *C. roseocanus*, *C. subalbidus*, *C. cascadiensis*, and *C. enelensis*, except for a C (instead of a second T) at the fifth-last position in RS1A in all but western *C. roseocanus* (data not shown), and thus should amplify these species plus *C. cascadiensis* and European *C. cibarius*. Fruiting body pigmentation frequently correlates poorly with taxonomy and molecular data in *Cantharellus* (Olariaga et al. 2015; Buyck et al. 2016b), but these authors recommend the acceptance of morphologically cryptic taxa that are well-separated in ecology, geographic distribution, and molecular data. This species may be conspecific with some collections identified as *C. cibarius* from Ontario or Massachusetts (unpublished LSU sequences in GenBank), as *C. cibarius* var. *roseocanus* from Quebec (Rochon et al. 2011), and is conspecific with collections reported as *Cantharellus* sp. from Michigan and Illinois (Leacock et al. 2016; Fig. 1a). The few reports of what appears to be this taxon from the Midwest, where *Cantharellus* is more diverse (Foltz et al. 2013; Leacock et al. 2016; Buyck et al. 2016a) suggests that *C. enelensis* may be more abundant in NL and the more boreal areas of northeastern North America, where potential hosts in the Pinaceae are more dominant on the landscape.

Cantharellus camphoratus R.H. Petersen, Figs. 2G–2I

MACROMORPHOLOGY: Pileus: 20–75 mm diameter, edges evenly round and inrolled, opening up to become plane, then funnel shaped, with an irregularly wavy margin; opaque, not hygrophanous; variously covered with thin layer of flat scales, which often disappear; deep yellow (4A3 to 4½AB3), scales brownish (to 6CD½). Hymenium: folds flat to moderately deep with blunt edges, close, somewhat sinuous, strongly decurrent, forked and becoming cross-veined; light yellow, cream to whitish (4A1½ to 5A2). Stipe: 5–25 mm × 30–65 mm, enlarging upwards from narrow base, solid; deep yellow to greyish orange (4A3, 5A3 to 5B3). Context: white or whitish yellow (4½A1½), immediately violaceous (12–15D4) with FeCl₃, then slowly turning greyish; odour fruity, resembling apricots. All tissues stain reddish-brown with injury or prolonged exposure. Fruiting bodies are relatively immune to invertebrate parasites, and remain over a month in the field in good condition. If the hymenium is exposed (no or low moss or a low layer of duff), fruiting bodies may fuse to make a polycephalic or single massive fruiting body, cap development may be arrested beyond

the stipe to produce peg-like fruiting bodies, and the hymenial folds become underdeveloped as flat, short, undulating ridges with multiple anastomoses, even absent. Habitus then varies from solitary pegs, to connate, cespitose or fused fruiting bodies.

MICROMORPHOLOGY: Basidiospores (10 collections, 14 sporocarps, 227 spores) 7.7–11.2(–12.1) μm \times 3.9–6.7 μm (average 9.1 \times 5.0), average $Q = 1.8$; elliptical to oblong, usually narrower at the apex, content homogeneous. Basidia 75–100 μm \times 8.2–11.6 μm ; 4–6-spored, rare 2-spored; clavate. No cystidia. Pileipellis a cutis of tubular, clamped hyphae (3.4–)4.5–8 μm broad, a few finely spiral-encrusted, most with vacuolar and cytoplasmic orange pigment, hyphal tips slightly clavate, basidiole-like, 25–40 μm \times 5.5–9 μm , with walls 1.1–1.7 μm thick. Tramal hyphae hyaline, tubular to inflated, (6–)10–16 μm broad. Clamp connections in all tissues, sometimes looping. Wide range in micromorphology between collections and individual sporocarps within a single collection.

ECOLOGY: Grows in small colonies, often in relatively open places with low moss near conifers, more often with *Abies* than *Picea*. Relatively uncommon, but seems to be widespread; large fruitings unknown. Found from mid-July to mid-September, most plentiful in August.

DISTRIBUTION: In Newfoundland, documented from six sites in the Bay of Islands and one in the Main River area (Fig. 3); previously recorded from Kentville, Nova Scotia, where the habitat was not reported (Petersen 1979).

SPECIMENS EXAMINED: CANADA, Newfoundland and Labrador, Humber Village, Mt. Harrison, 48.976°N 57.764°W, 263 m a.s.l., on soil under birch, balsam fir, and spruce in mixed woods, 13 August 2010, *Andrus Voitk 10.08.13.av01* (DAOM 734049), Cedar Cove Trail, near Lark Harbour, 49.102°N 58.425°W, 134 m a.s.l., fruiting in moss under birch, balsam fir, and spruce in mixed woods, 05 August 2011, *Jaan Tepp 11.08.05.av01* (DAOM 734050), same location, in moss in mixed woods of mountain alder, birch, and balsam fir, 04 August 2012, *Maria Voitk 12.08.04.av01* (DAOM 695786, portion at UWO), same location, on duff in coniferous woods of balsam fir, 26 August 2013, *A. Voitk 13.08.26.av01* (DAOM 737292), same location, on duff in coniferous woods of balsam fir, but under mountain ash, 26 August 2013, *A. Voitk 13.08.26.av04* (DAOM 737293), same location, 27 September 2013, *A. Voitk 13.09.27.av04* (DAOM 737296), Lower Main River, trail 2, 49.783°N 56.947°W, 42 m, fruiting in moss under *Abies*, *Picea*, and *Betula* in coniferous woods with alder, 06 September 2011, *Nathan Wareham MR3-057* (DAOM 737298), Humber Village, Trail to Weldon's, 48.994°N 57.749°W, 29 m a.s.l., on soil in mixed forest of balsam fir, birch, spruce, 10 July 2012, *A. Voitk 12.07.10.av01* (DAOM 695785), same location, on soil in mixed woods of alder, balsam fir, birch, maple, spruce, 31 August 2012, *A. Voitk 12.08.31.av04* (DAOM 734045), same location, on soil in mixed woods of alder, balsam fir, birch, maple and spruce, 31 August 2012, *A. Voitk 12.08.31.av05* (DAOM 734046), same location,

22 September 2012, *Gro Gulden 12.09.22.av02* (DAOM 737291), same location, in open low moss in mixed forest of alder, balsam fir, birch, 13 September 2013, *A. Voitk 13.09.13.av01* (DAOM 737294), Humber Village, Jenniex property, 48.986°N 57.763°W, 16 m a.s.l., on soil among low moss under pin cherry in mixed forest with balsam fir, 15 September 2013, *A. Voitk 13.09.15.av01* (DAOM 737295), Pasadena Ski trails, 48.996°N 57.586°W, 90 m a.s.l., on soil in mixed woods of alder, balsam fir, birch, larch, spruce, 03 September 2012, *Henry Mann 12.09.03.av17* (DAOM 695787, portion at UWO), same location, *H. Mann 12.09.06.av01* (DAOM 695788), same location, 12 September 2013, *H. Mann 13.09.12.av01* (DAOM 734047), Gillams trail, 49.019°N 58.064°W, 9 m a.s.l., on soil in coniferous woods of spruce, 27 September 2013, *Urve Voitk 13.09.27.av05* (DAOM 737297), Humber Village, Rubber Road, 48.990°N 57.788°W, 162 m a.s.l., on soil in mixed woods of balsam fir, birch, spruce, 02 September 2014, *M. Voitk 14.09.02.av01* (DAOM 734048).

COMMENT: Our collections were not immediately recognized as *C. camphoratus*, described from Nova Scotia, because of the emphasis in the description of that species on the characteristic camphor odour (Petersen 1979). In contrast, the odour of our specimens when fresh was the typical fruity-apricot odour associated with *C. enelensis* and *C. amethysteus*, although perhaps not as strong as in these species. In addition, the spores of our collections were consistently longer and narrower than reported for the type (8.8–10.4 μm \times 5.2–6.7 μm , $Q = 1.60$; Petersen 1979), but in the redescription of the type by Eyssartier (2001), the average spore size and ratio of length to width is almost identical to our observations (9.01 μm \times 5.18 μm , $Q = 1.76$). Sequences of the ITS and LSU rDNA from the type (Buyck et al. 2016c) are identical to our collections, clinching the identification. This species has not been reported since the single collection described nearly 40 years ago (Petersen 1979). Within NL, lighter and flatter hymenial folds and frequent aberrant forms distinguish this species from *C. enelensis*. This species greatly resembles *C. amethysteus* in macromorphology, including the variable presence of brown scales (described as grey to vinaceous by Petersen 1979), but is distinguished by its shorter spores and association with balsam fir, not birch. *Cantharellus formosus* from the Pacific Northwest may also develop brownish scales on the cap (Redhead et al. 1997) or develop brownish stains in age or when bruised, and also has a pallid hymenial surface and similar basidiospores. However, *C. formosus* has better-developed hymenial ridges (both deeper and sharper; Corner 1966), and has not been described as having frequent aberrant fruiting bodies (Redhead et al. 1997; Pilz et al. 2003; Trudell and Ammirati 2009). In our analyses (Fig. 1a) and those of Buyck et al. (2016c), *C. camphoratus* and *C. formosus* are clearly differentiated by their ITS, LSU, and TEF1 sequences.

Additional specimens examined***Cantharellus cascadensis***

CANADA, British Columbia, Vancouver Island, Observatory Hill, Saanich, 48.520°N 123.419°W, in coniferous forest under *Pseudotsuga*, 28 September 2011, *Oluna Ceska* (UBC F23801, as *C. formosus*).

Cantharellus cibarius

ESTONIA, Hiiumaa Korbi mäed, Kõpu poolsaar, 58.897°N 22.197°E, 37 m a.s.l., in moss in coniferous woods of pine, 24 July 2012, *Jaanika Kuusik 12.07.24.av01* (DAOM 734028, portion at UW), NORWAY, Ålesund, 62.470°N 6.386°E, 69 m a.s.l., in coniferous woods of spruce, 14 August 2013, *Eemil Voitk 13.08.14.av01* (DAOM 734029).

Cantharellus formosus

CANADA, British Columbia, Vancouver Island, Jeune Landing, 50.44°N 127.50°W, fruiting in coniferous forest under *Pseudotsuga*, 14 October 2009, *J.M. Kranabetter* (DAVP 28141), Quadra Island, 50.20°N 125.25°W, fruiting in coniferous forest under *Pseudotsuga* and *Tsuga heterophylla*, 01 October 2011, *M. & P. Weigold* (DAVP 28291), Moresby Island, Quadra Rocks Point, 52.09°N 131.06°W, 05 September 2005, *Paul Kroeger et al.* (UBC F15948), Bischoff Island, Haida Gwaii, 52.58°N 131.57°W, 09 September 2008, *P. Kroeger et al.* (UBC F16607), USA, Oregon, Lane Co., H.J. Andrews Experimental Forest, 44.21°N 122.25°W, fruiting on mossy soil and needle litter under Douglas-fir, western hemlock and western red cedar, 25 October 2013, *Steve Trudell SAT-13-298-20*.

Cantharellus roseocanus

CANADA, British Columbia, Vancouver Island, Mount Cain, near Woss, 50.22°N 126.36°W, in coniferous forest under *Tsuga mertensiana*, 12 October 2003, *T. Ehlers et al.* (DAVP 28001), Vancouver Island, Pacific Rim National Park, Wickanninnish Beach Trail, 49.12°N 125.90°W, in mossy conifer forest, 10 October 2008, *Vancouver Mycological Society* (UBC F16989), Vancouver Island, Tauca Lea Resort, near Ucluelet, 48.95°N 125.56°W, in coniferous forest under *Tsuga heterophylla* and *Picea sitchensis*, 08 July 2007, *O. Ceska* (UBC F23802), Burnaby Island, Haida Gwaii, Section Cove, 52.25°N 131.22°W, in mossy conifer forest, 14 September 2008, *P. Kroeger et al.* (UBC F16825), USA, Oregon, Honeyman St. Park campground, near Florence, 43.93°N 124.11°W, on soil in mixed conifer forest with Sitka spruce, Douglas-fir, western hemlock, western red cedar, and red alder, 25 October 2012, *S.A. Trudell SAT-12-299-06*.

Cantharellus cf. phasmatis

CANADA, Ontario, London, Medway Valley Forest, 43.005°N 81.290°W, under *Fagus grandifolia* in deciduous forest, 16 July 2013, *R.G. Thorn 130716/01* (DAOM 721705, portion at UW).

Cantharellus subalbidus

CANADA, British Columbia, Pemberton, 50.32°N 122.82°W, in coniferous forest under *Pseudotsuga*, 06 October 2009,

J.M. Kranabetter (DAVP 28155), Quadra Island, 50.20°N 125.25°W, in coniferous forest under *Pseudotsuga* and *Tsuga heterophylla*, 01 October 2011, *K. Wong*, (DAVP 28283).

Discussion

The North American species of *Cantharellus* have recently been reviewed by [Buyck et al. \(2016c\)](#), who accepted 29 species, while admitting that possible synonymy of multiple names proposed within the species complex of *C. phasmatis* ([Buyck et al. 2016a](#)) will have to be sorted out with additional sequence and morphological data. Recounting, and excluding *C. cibarius*, which despite being the most frequently reported species of *Cantharellus* from North America has not been documented to occur here, and accounting for the synonymy of *C. spectaculus* with *C. persicinus* ([Buyck et al. 2016a](#)), our report brings the number of North American *Cantharellus* species back to 29 by the addition of *C. amethysteus*, which is new to North America, and the new species *C. enelensis*. Across North America, local species richness of *Cantharellus* is highest in regions with a diversity of ectomycorrhizal host associates from both the Fagaceae and Pinaceae ([Buyck and Hofstetter 2011](#); [Leacock et al. 2016](#)). The forests of Newfoundland and Labrador have no beech or oaks (*Fagus* and *Quercus*, Fagaceae), and the three *Cantharellus* species here are associated with Pinaceae (*Abies* and *Picea*) or Betulaceae (*Betula*).

In *Cantharellus* and in other genera of macrofungi such as *Amanita* ([Zhang et al. 2010](#); [Sánchez-Ramírez et al. 2015](#)), *Armillaria* ([Anderson and Ullrich 1979](#); [Tsykun et al. 2013](#)) and *Morchella* ([Kuo et al. 2011](#)), the previously held view of common, widespread, and ecologically ubiquitous species has been replaced with multiple species, often difficult to resolve with morphology, with limited distributions that make better sense in terms of their ecology, phylogeography, and probability of interbreeding. Clearly, more species of *Cantharellus* await discovery in North America, including in our back yards of Ontario (R.G.T., J.I.K.) and Quebec (R.L.).

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