Identity and Chemical Composition of an Albino Mutant Chanterelle

R. Greg Thorn (rgthorn@uwo.ca)
Western University
Alicia Banwell
Western University
Thu Hong (Nicole) Pham
Memorial University of Newfoundland
Natalia P. Vidal
Memorial University of Newfoundland
Charles F. Manful
Memorial University of Newfoundland
Muhammad Nadeem
Memorial University of Newfoundland
Alexander G. Ivanov
Western University
Beth Szyszka Mroz
Western University
Michael B. Bonneville
Western University
Norman Peter Andrew Hüner
Western University
Michele D. Piercey-Normore
Memorial University of Newfoundland
Raymond Thomas
Memorial University of Newfoundland

Research Article

Keywords: White chanterelles (Basidiomycota), chemical composition, albino mutant, Cantharellus enelensis

Posted Date: February 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-244471/v1

Identity and chemical composition of an albino mutant chanterelle

R. Greg Thorn[1*], Alicia Banwell[1], Thu Huong (Nicole) Pham[2], Natalia P. Vidal[2], Charles Felix Manful[2], Muhammad Nadeem[2], Alexander G. Ivanov[1,3], Beth Szyszka Mroz[1], Michael B. Bonneville[1], Norman Peter Andrew Hüner[1], Michele D. Piercey-Normore[2], and Raymond Thomas[2].

[1]Department of Biology, University of Western Ontario, 1151 Richmond St. N., London, ON N6A 5B7, Canada

[2]School of Science and the Environment, Grenfell Campus, Memorial University, 20 University Drive, Corner Brook, NL A2H 5G4, Canada

[3]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev str. Bl. 21, 1113 Sofia, Bulgaria

[*]Corresponding author: rgthorn@uwo.ca

Abstract

White chanterelles (Basidiomycota), lacking the orange pigments and apricot-like odour of typical chanterelles, were found recently in the Canadian provinces of Québec (QC) and Newfoundland & Labrador (NL). Phylogenetic analyses confirmed the identification of all white chanterelles from NL and QC as *Cantharellus enelensis*; we name these forma *acolodorus*. We characterized carotenoid pigments, lipids, phenolics, and volatile compounds in these and related chanterelles. White mutants of *C. enelensis* lacked detectable β-carotene, confirmed to be the primary pigment of wild-type, golden-orange individuals, and could also be distinguished by their profiles of fatty acids and phenolic acids, and by the ketone and terpene composition of their volatiles. We detected single base substitutions in the phytoene desaturase (*Al-1*) and phytoene synthase (*Al-2*) genes of the white mutant, which are predicted to result in altered amino acids in their gene products and may be responsible for the loss of β-carotene synthesis in that form.

Introduction

Chanterelles (*Cantharellus*: Basidiomycota) are widely distributed and highly prized edible mushrooms with an estimated annual international export market of over $1.5 billion US[1,2]. Chanterelles are ectomycorrhizal, growing in a mutualistic association with host trees, and thus cannot be cultivated readily for commercial sale but are wild-harvested in the forest by both amateur enthusiasts and commercial mushroom pickers[3–6]. A large part of the culinary appeal of chanterelles is their brilliant golden-orange colour (Fig. 1A), derived from carotenoid pigments[7–8], their apricot-like odour, and firm texture. Chanterelles are famous for their long-lasting fruiting bodies, which can persist in the woods in good condition for weeks or months, often without undergoing decay or being consumed by slugs, fly larvae, or other invertebrates[4,9]. The chemical components that prevent microbial decay or invertebrate consumption are largely unknown, but have been suggested to be associated with the colour and odour[4].

Until recently, most golden-orange chanterelles were referred to as *Cantharellus cibarius* Fr., and this name is still widely used in commerce and by mushroom enthusiasts. Studies using DNA sequence data have shown that *C. cibarius* is restricted to Eurasia, and have delimited multiple species of golden-orange chanterelles around the world[10]. In the Canadian province of Newfoundland and Labrador (NL), the common species of golden-orange chanterelles was recently described as *Cantharellus enelensis*, and differentiated from two other, less common golden species *C. camphoratus* and *C. amethysteus*[11], the latter now separated as a new species *C. betularum*[12]. Soon after that publication, scattered fruitings of pure white chanterelles were reported across the island of Newfoundland (Fig. 1B), often occurring mixed within normally pigmented individuals of *C. enelensis*[13]. Fruiting bodies of the

52 white chanterelles differed not only in colour but in the absence of the apricot-like odour of the
53 typical golden-orange specimens[13]. While investigating the NL white chanterelles, we were sent
54 white chanterelles from Québec (QC), Canada, and specimens of commercially harvested pale
55 chanterelles from Minnesota (MN) in the USA.

56 Golden chanterelles get their colour from carotenoid pigments. Carotenoid analyses
57 have been performed on *C. cibarius*, but not extensively studied because carotenoids can be
58 difficult to analyze since they degrade over time and rapidly with drying[14]. The principal
59 carotenoid in *C. cibarius* is β-carotene, which is responsible for its golden appearance, followed
60 by lycopene, as well as some α-carotene and γ-carotene[8,15–16]. Carotenoid synthesis has been
61 studied in the filamentous ascomycete *Neurospora crassa*, and the genes producing the key
62 enzymes have been named for the albino phenotype of their mutant alleles. The 20-carbon
63 precursor is formed by geranylgeranyl pyrophosphate synthetase, encoded by the gene referred
64 to as albino-3 (*Al-3*). Dimerization to form the 40-carbon colourless phytoene is carried out by
65 the phytoene synthase activity of the dual-function gene product of albino-2 (*Al-2*). Phytoene
66 desaturase (encoded by albino-1, or *Al-1*) converts phytoene to lycopene through a series of
67 cyclic reactions, which is then converted to the coloured product β-carotene by the lycopene
68 cyclase function of *Al-2*[17].

69 The purpose of this study was to determine the species identity of the white and pale
70 chanterelles from NL, QC and MN using phylogenetic analyses of nuclear ribosomal DNA
71 (internal transcribed spacer, or ITS, and large subunit, LSU) and the translation elongation
72 factor gene (*Tef-1*). The presence of a pigmentless chanterelle with altered odour profile raised
73 the questions of the genetic underpinning of the apparent albinism, and of how the chemical

74 composition of these variants compared to typical golden-orange specimens of *C. enelensis, C.*
75 *camphoratus*, and *C. betularum*.
76
77 **Results**
78 New ITS, LSU and *Tef-1* sequences were generated from fifteen specimens in this study
79 (Table 1) and were aligned with sequences downloaded from GenBank. The maximum
80 likelihood tree produced in MEGA X, with node support from 1000x bootstrap replicates and
81 from Baysesian posterior probabilities of an analysis with 5 million trees in MrBayes is shown
82 (Fig. 2A). White chanterelles from NL and QC cluster phylogenetically with the NL golden
83 species, *C. enelensis,* with strong bootstrap support and well separated from *C. roseocanus*, a
84 golden species from the Pacific Coast of North America, *C. cibarius*, a golden species from
85 Europe and *C. cascadensis,* a golden to white species found on the Pacific Coast of North
86 America. We provide a name for this white variant at the rank of "forma".
87 ***Cantharellus enelensis* f. *acolodorus***, Voitk & Thorn, forma nova. Fig. 1B MycoBank
88 MB835379.
89 **Typification**: CANADA. NEWFOUNDLAND AND LABRADOR: Gambo, Mint
90 Forest resource road, in spruce forest mixed with birch, among moss and duff (48°43'02.3" N,
91 54°34'56.5" W; 143 m above sea level), 11 Aug 2017, Eugene Kean, A. Voitk coll. no.
92 17.08.11.av06 (holotype UWO-F730, isotype TU117603). GenBank: ITS = MN206912.
93 **Etymology**: acolodorus is a contracted combination adjective from Latin (a=none), to
94 indicate without colour or odour.
95 **Diagnosis**: A white chanterelle found among golden specimens of *C. enelensis* and
96 resembling them in every regard except for the lack of golden colour and the characteristic

97 "apricot note" to the odour. Briefly described and illustrated in Thorn et al.[13]; known from the
98 Island of Newfoundland and Québec (Table 1).

99 A pale specimen from the US Midwest clustered phylogenetically with the US Midwest
100 golden to whitish species, *C. phasmatis,* with strong bootstrap support (Fig. 2A), and well
101 separated from *C. tenuithrix*, a golden to white chanterelle from the Southeastern US, *C. flavus*,
102 a golden species from the US Midwest, *C. deceptivus*, a golden to white chanterelle from the
103 US Midwest, *C. pallens,* a golden-white species from Europe, *C. subalbidus,* a white
104 chanterelle from the Pacific Coast of North America, and *C. cascadensis,* a golden chanterelle
105 with white hymenophore from the Pacific Coast of North America. Sequences of LSU and *Tef-*
106 *1* of a Chinese specimen identified as *C. cibarius*, for which a draft genome was recently
107 published[18], placed this taxon in the unresolved clade of *C. pallens – C. phasmatis*, i.e, outside
108 the core *C. cibarius* clade (Fig. 2A).

109 Newly designed primers were used to amplify and sequence portions of the phytoene
110 desaturase gene *Al-1* (1381 bases) and dual-function phytoene synthase / lycopene cyclase gene
111 *Al-2* (1752 bases) in the white and gold variants of *C. enelensis*. The *Al-1* gene in the white
112 chanterelle differed from that of the gold one by a 3-base deletion, resulting in the loss of a
113 phenylalanine in the predicted gene product, and 5 single nucleotide substitutions, of which 4
114 were determined to be synonymous, but a fifth was predicted to result in the replacement of
115 valine with phenylalanine in the gene product. The *Al-2* gene of the white variant differed from
116 that of the gold one by two base substitutions, one synonymous and another predicted to alter
117 an arginine residue shared with *Neurospora* to a histidine.

118 Typical chromatograms of high-performance liquid chromatography (HPLC) pigments
119 in acetone extracts from white and golden variants of *C. enelensis* are presented in Fig. 2B.

Comparison of the chromatograms indicates a single distinct peak with a retention time of 10.82 min characteristic for ß-carotene[19] in the golden sample, while the white mutant of *C. enelensis* does not exhibit any pigments peaks detectable by the HPLC method used. Furthermore, the absorption spectrum (Fig. 2C) of the acetone extract from golden *C. enelensis* samples exhibits three peaks at 430, 454, and 481 nm, typical for ß-carotene, which were lacking in the white sample. Thus, both HPLC and spectroscopic analyses allow us to identify the presence of ß-carotene in the golden variant of *C. enelensis*.

Chanterelles presented a complex fatty acid profile with constituents ranging from C8:0 to C24:1n9 (Table 2) with total saturated fatty acids ranging from 8.04 to 12.33 nmole %, monounsaturated fatty acids (MUFA) from 6.41 to 24.11 nmole %, *n*6-polyunsaturated fatty acids (PUFA) from 61.27 to 79.16 nmole % and *n*3-PUFA from 0.71 to 2.66 nmole % (Table 2). Golden variants of *C. camphoratus and C. betularum* were segregated in quadrant 1 of the PCA observation and biplots based on the level of C23, C18:1n9, C20:1n9, C12:0 and C15:1 fatty acids (Fig. 3A–B). Both the white (QC and NL) and golden variants of *C. enelensis* clustered together in quadrant 4 based on the combined levels of C24, C20:3n6 and C22:n6 fatty acids. The ratio of C18:1n7/C18:1n9 was the most effective in discriminating the samples. All NL white *C. enelensis* had similar C18:1n7/C18:1n9 ratio (2.16 to 2.37) compared to significantly higher ratio in QC white *C. enelensis* (5.08) and the golden variants of NL *C. enelensis* (3.49). Conversely, the golden species *C. betularum* and *C. camphoratus* had significantly lower ratios of C18:1n7/C18:1n9 fatty acids (0.47 vs. 0.05) respectively compared to all the other samples evaluated (Table 2).

Intact lipids were more discriminatory than the fatty acids in segregating the NL and QC white *C. enelensis* samples. White NL *C. enelensis* clustered in quadrant 1 based on the levels

of phosphatidic acid (PA), phosphatidylethanolamine (PE), ceramide (Cer), oxidized diacylglycerol (oxDG), oxidized triacylglycerol (OxTG), lysophosphatidylcholine (LPC), stigmasterol ester (StE), and medium-chain triacylglycerol (McTG), while QC white *C. enelensis* clustered in quadrant 2 based on the level of lysophosphatidylethanolamine (LPE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylcholine (PC), campesterol ester (CmE), monoacylglycerol (MG), long-chain triacylglycerol (lcTG), short-chain triacylglycerol (scTG), and Diacylglycerol (DG). The intact lipids were also effective in segregating the golden variants of *C. enelensis, C. betularum* and *C. camphoratus* in quandrants 3 and 4 of the observation and biplots (Fig. 3C–D). Sphingomyelin (SM), hexanoyl ceramide (cerebroside, HexCer) and oxidized phosphatidylcholine (OxPC) clustered in quadrant 4 with the Mac samples and *C. betularum*. The Mac samples are a mixture of golden and white fruiting bodies of *C. enelensis* that were indistinguishable from each other visually once dried. Polar lipid composition was dominated by PA (6.57 to 27.02 nmole %), PC (33.46 to 73.85 nmole %) and PE (12.61 to 27.91 nmole %) in all the samples evaluated. Québec white *C. enelensis* had significantly higher PC (73.85 nmole %), but lower PA (6.57 nmole %) and PE (12.61 nmole %) compared to NL white or golden *C. enelensis*, and golden variants of *C. betularum* and *C. camphoratus* (Table 2). The neutral lipid composition was dominated by HexCer (0.96 to 26.06 nmole %), DG (6.61 to 18.19 nmole %) and lcTG (53.57 to 83.63 nmole %). Golden *C. betularum* and *C. camphoratus* had significantly higher levels of HexCer (25.05 and 13.35 nmole %) and lower levels of DG (6.61 and 7.84 nmole %, respectively) (Table 2).

Principal component analysis demonstrated that the phenolic acids were very effective in segregating the golden variants of *C. enelensis, C. betularum* and *C. camphoratus* from each

other (Fig. 3E). *Cantharellus betularum* clustered in quadrant 4 based on the levels of cinnamic and homovanillic acids, *C. camphoratus* in quadrant 2 with salicylic acids, while *C. enelensis* was located in quadrant 3 with protochatechuic and benzoic acids (Fig. 3E–F). Phenolic acids were the only components that separated the Mac samples; Mac5 sample clustered between the golden *C. enelensis* (NLY) and *C. betularum* (AY), while the other Mac samples clustered with the white *C. enelensis* samples in quadrants 1–2. The phenolic composition of the samples was dominated by phenol (30.54 to 90.51 nmole %) with the golden variant of NL *C. enelensi*s having significantly lower phenol (30.54 nmole %) compared to the other species evaluated (Table 3). Conversely, the levels of protochatechuic (46.10 nmole %) and homovanillic acids (18.26 nmole %) were significantly higher in the golden variant of NL *C. enelensis* compared to the others.

Headspace SPME detected 104 volatile organic compounds from the mushroom samples, including aldehydes, acids and esters, alcohols, ketones, furan derivatives, terpenes, and unidentified compounds (Table 4). Among all samples, *C. betularum* was the richest in volatiles, but combined analysis of all analytes did not yield consistent separation of white from golden samples of *C. enelensis* (Fig. S1 and Table 4). In particular, *C. betularum* was rich in substituted aldehydes such as 2-butyl-2-octenal, 2-ethyl-2-hexenal, and 2-propyl-2-heptenal, which were much less abundant in *C. camphoratus* and both white and golden *C. enelensis* (Fig. 3G–H and Table 4). A biplot of the ketones detected separated all white samples of *C. enelensis*, in the upper half, from all golden samples of *C. betularum, C. camphoratus*, and *C. enelensis*, in the bottom half, the latter characterized by greater quantities of 4-nonanone (versus 3-nonanone and 3-nonen-2-one), 4-octanone (versus 3-octen-2-one and 2,5-octendione), and cyclopentanone (Fig. 3I–J, Table 4). Similarly, a biplot of the terpenes detected separated

189 all white samples of *C. enelensis* in the left quadrants, from all golden samples of *C. betularum,*
190 *C. camphoratus*, and *C. enelensis* in the right quadrants, the latter characterized by greater
191 quantities of trans-alpha-ionone, alpha-ionon-5,6-epoxy-cubenol, and beta-cyclocitral (Fig. 3K–
192 L, Table 4).
193
194 **Discussion**
195 In Europe, occasional white chanterelles have been recognized as albino forms of
196 *Cantharellus amethysteus, C. cibarius, C. ferruginascens,* and *C. romagnesianus*, and the
197 varieties named for their white colouration, *C. cibarius* var. *inodorus* and *C. cibarius* var.
198 *gallaecicus*, have been reduced to synonymy of *C. cibarius* and *C. romagnesianus*,
199 respectively[14]. The white chanterelles of Newfoundland and Québec are clearly conspecific
200 with *C. enelensis* but, if precision is required, they may be referred to as *C. enelensis* f.
201 *acolodorus*. The presence of typical and albino forms stands in contrast to some other species
202 of chanterelles that are normally pallid, such as *C. subalbidus, C. pallens*, and *C. phasmatis*[14,
203 20–21], although in the latter two species, some specimens are particularly pale, as in the
204 specimen of *C. phasmatis* sent to us from Minnesota or white individuals of *C. pallens* reported
205 by Olariaga et al.[14].
206 *Neurospora crassa* (Ascomycota) forms rapidly growing pinkish-orange cultures, with
207 white variants that have been studied extensively due to the ease of culture of this species and
208 the early availability of its genome sequence[22]. In albino variants of this species, a mutation of
209 one of three genes, *Al-1, Al-2,* or *Al-3*, that encode for phytoene desaturase[23], phytoene
210 synthase[24], and geranylgeranyl pyrophosphate synthetase[25], respectively, causes the lack of
211 carotenoid pigments through the loss of function of one of these enzymes required in the

212 carotenoid biosynthetic pathway. We were able to detect sequence variants in the *Al-1* and *Al-2*
213 genes of the white variant of the NL chanterelles but, because we do not have it in culture, we
214 were unable to follow up with functional analyses.

215 In albino and wild-type variants studied in lab culture, the presence of carotenoid
216 pigments may exhibit a benefit under certain environmental or physiological stress conditions,
217 such as oxidative stress or high light exposure[26]. Under oxidative stress, free radicals react with
218 the structural polyene chain of carotenoids, deflecting potential damage[27]. Beta-carotene has
219 been shown to protect the photosensitized oxidation of phospholipid bilayers[28], which has been
220 observed in other fungi, including the ascomycete *Arthobotrys ferox*[29]. In *N. crassa* under high
221 light exposure, albino mutants have lower respiration rates of hyphal suspensions[30]. Given that
222 the NL golden chanterelles, *C. enelensis*, are much more common than the white mutants, it is
223 likely that they possess some ecophysiological advantage over the albinos, possibly conferred
224 by their carotenoid pigments.

225 The white and golden variants of *C. enelensis* differ in far more than just carotenoid
226 pigmentation, or the lack of it. Their chemical composition differs in lipids and fatty acids,
227 phenolic acids, and multiple classes of volatile compounds, and these differences may affect
228 their palatability to both human and invertebrate consumers. Among the lipids, the fatty acid
229 composition of NL white and golden *C. enelensis* was dominated by C18:2n6, consistent with
230 the composition of other species of edible mushrooms reported in the literature[31]. The ratio of
231 C18:1n7/C18:1n9 fatty acids appears to be a particularly useful chemotaxonomy biomarker for
232 differentiating C. *betularum, C. camphoratus*, and *C. enelensis* (Table 2 and Fig. 3A–B), as
233 well as distinguishing the QC and NL white mutants (Table 2). The C18:1n7/C18:1n9 ratios
234 have similarly been shown to be very effective in the chemotaxonomic classification of 12

Brassica species[32]. The intact lipids reported in this paper represented both membrane and storage lipids. From a chemotaxonomy perspective, *C. betularum, C. camphoratus*, and golden individuals of *C. enelensis* appear to have a similar composition of intact membrane and storage lipids, placing them together in a PCA biplot, separated from three out of four samples of the white mutants of *C. enelensis,* which had more variety of lipids, from phosphatidic acid (PA) to lysophosphatidylethanolamine (LPE) (Fig. 3C–D). Hexanoyl ceramide (HexCER) is a sterol present in the fungal membrane[33,34]. Golden variants of *C. enelensis* (and one white sample, NLW2), plus *C. betularum* and *C. camphoratus* have similar levels of HexCER, whereas the other three samples of white *C. enelensis* have less. From a compositional perspective, phosphatidylcholine (PC) was the predominant membrane lipid and various forms of triacyglycerols are the major storage lipids of *C. betularum, C. enelensis* and *C. camphoratus,* consistent with other reports demonstrating these as the major membrane and storage lipids in edible mushrooms[31,35].

Phenolic compounds are important in the detection and perception of organisms as well as in their response to biotic and abiotic stressors in their environment. As such, they have been a common choice of secondary compounds used as biomarkers in chemotaxonomic classification of plants, lichens and increasingly in non-lichenized fungi[36,37]. We found the phenolic acids subclass of phenolic compounds to be effective in differentiating golden *C. enelensis* (with more homovanillic and protochatechuic acids) from their white mutants (with less, and with more phenol and salicylic acid), as well as from the golden species *C. betularum* (with more cinnamic acid) and *C. camphoratus* (with less cinnamic acid and more benzoic acid). These results suggest that phenolic acids may be useful chemotaxonomic markers to differentiate different species of chanterelles in commerce. To the best of our knowledge this is

the first study demonstrating the application of phenolic acids as chemotaxonomic markers in chanterelles.

Chanterelles are known to have very distinctive colours, flavours and fruity aromas that vary between species, although their perception also varies with their human assessors. In plants, fungi, and the fruit and fruiting bodies they produce, these characteristics are determined in part by the composition of aromatic or aliphatic volatile compounds present in individuals, often as a result of complex mixtures[38,39]. In this study, we observed over 100 volatile compounds in both golden and white chanterelles (Table 4) and of these compounds, the aldehydes, ketones and terpenes appear to be the most effective as chemotaxonomic biomarkers to differentiate species and colour variants of chanterelles. The terpenes, particularly α-ionone, cubenol and β-cyclocitral, characterize and differentiate the golden chanterelles *C. enelensis, C. betularum,* and *C. camphoratus,* whereas all samples of *C. enelensis* f. *acolodorus* were characterized by a lower concentration of each of these compounds (Fig 3K–L). A reduction of ionones in the albino mutant is unsurprising since these and related "rose ketones" are derived from the breakdown of carotenoids[40]; this absence may partly explain the perceived lack of an "apricot note" in the odour of the albino mushrooms[13]. Among the ketones, principal component 1 (PC1, roughly parallel to the ratio of hexanone : heptanone and undecanone) separates the three golden chanterelle species included, and PC2 separates white from all golden chanterelles (Fig. 3I–J). In contrast, two white mutant samples (QW and NLW2) and *C. betularum* are separated by the aldehydes hexanal, 2-octenal and ethyl-pentenal, respectively, leaving golden *C. enelensis, C. camphoratus*, and two other white samples of *C. enelensis* in a central cluster (Fig. 3G–H). Our work suggests that volatile aldehydes, ketones and terpenes can be used as chemotaxonomic markers to separate chanterelles based on species and colour.

This knowledge could be useful to distinguish white from golden chanterelles after drying, which is often used to prolong shelf life or for convenience during food formulation, during which they become the same dull brownish orange colour.

Collectively, the output of the chemical analyses presented in this paper demonstrates for the first time the applications of metabolomics to separate chanterelles based on species, colour, aroma, and geography of production. The fatty acids, membrane and storage lipids, phenolic acids, volatile terpenes, aldehydes and ketones are presented as chemotaxonomic biomarkers that are useful in differentiating the recently discovered white mutant of *C. enelensis* from its golden relatives. In addition to its chemotaxonomic potential, this work raises questions as to the functional significance of these compounds in nature.

Materials and methods

Specimens of fresh, field-collected mushroom fruiting bodies were either air-dried at a temperature of 30–35 °C or frozen at -80 °C until processing (Table 1).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from air-dried specimens following Thorn et al.[11]. Primers ITS1 and ITS6R were used to amplify the ITS region, LS1 and LR3 to amplify ~650 bases of the 5'-LSU region and Canth-ef1a983-F and Canth-ef1a-1567-R to amplify *Tef-1*[11,41–44]. The PCR products were checked using gel electrophoresis and successful products were cleaned using Bio Basic EZ-10 Spin Column PCR Products Purification Kit. Cleaned PCR products were submitted to the sequencing facility of Robarts Institute (University of Western Ontario) to obtain sequences through Sanger sequencing with amplification primers, and internal sequencing primers CanthITS1_Internal-R, 5.8S-R-Canth, and ITS86R-Canth for the ITS

304 region[11]. New sequences produced for this study were deposited in GenBank as accessions
305 MN181445–MN181461 and MN206911–MN206945.
306 Phylogenetic analyses
307 Sequences of the ITS, LSU and *Tef-1* regions were cleaned and assembled with SeqEd
308 v.1.03, then, together with sequences of related species downloaded from GenBank, each
309 region was aligned separately with MAFFT v.7[45] under the G-INS-i strategy, with "leave gappy
310 regions" selected. The draft *Cantharellus cibarius* genome (QOWL00000000.1)[18] was searched
311 by BLASTn for ribosomal and *Tef-1* sequences. The full-length match to our *Tef-1* sequences
312 was found in a single scaffold (QOWL01010594_RC), but no ITS sequences and only partial
313 LSU sequences were found (in QOWL01010930_RC, QOWL01012415,
314 QOWL01007530_RC, and QOWL01005980_RC). Alignments were imported into MEGA
315 X[46,47], trimmed and concatenated into a single ITS-LSU-*Tef-1* dataset, then optimized
316 manually. Phylogenetic trees were constructed with maximum likelihood (ML), with 1000
317 bootstrapping replicates in MEGA X. Analyses were repeated with Bayesian inference using
318 MrBayes 3.2.6 with 4 chains and 5 million generations, discarding the first 25% of trees, when
319 the average standard deviation of split frequencies had stabilized below 0.01[48]. Tree topologies
320 were compared, and Bayesian prior probabilities transferred to the ML bootstrap tree in Adobe
321 Acrobat.
322 Genetic analysis of carotenoid synthesis genes
323 In order to design primers to amplify portions of the *Al-1* and *Al-2* genes in white and
324 golden chanterelles, these genes were first located in three published *Cantharellus* genome
325 sequences[18] using tBLASTn[49] to query the genomes with protein sequences from *N. crassa*
326 (PRJNA132; *Al-1* XM_959620.2 and *Al-2* XM_960632.3). Candidate gene sequences from

Cantharellus appalachiensis (QLPK00000000.1; *Al-1* Scaffold 4647: QLPK01003932.1 and *Al-2* Scaffold 1419: QLPK01001208.1), *C. cibarius* (QOWL00000000.1; *Al-1* Scaffold 15338: QOWL01009792.1 and *Al-2* Scaffold 1560: QOWL01001169.1), and *C. cinnabarinus* (QLPJ00000000.1; *Al-1* Scaffold 1057: QLPJ01000880.1 and *Al-2* Scaffold 2474: QLPJ01001998.1) were annotated in Geneious using a discontiguous megablast against GenBank to search for homologous motifs[50]. Based on these alignments, putative ORFs were annotated and aligned, and an overlapping set of PCR primers for *AL-1* and *AL-2* were designed in Geneious (Table 5). Designed primers were tested for specificity using the BLAST algorithm against the three *Cantharellus* genomes[18]. PCR amplified products were assessed for quality, cleaned, sent for sequencing, and assembled as above. Assembled sequences of white and gold samples were compared, along with their putative amino acid products determined using ExPASy[51], guided by the translations of the *Neurospora crassa Al-1* (XM_959620.2) and *Al-2* (XM_960632.3) genes. Partial sequences of the *Al-1* and *Al-2* genes of gold and white variants have been deposited in GenBank as MW442833–MW442836.

Pigment analysis

Field-collected mushroom fruiting bodies were weighed while fresh, wrapped in aluminum foil, and frozen at -80 °C for pigment analyses, and other samples were weighed fresh and then dried to obtain a conversion for fresh to dry weight. Pigments were extracted with ice-cold 100% acetone at 4 °C and dim light. The supernatant was filtered through a 0.22 µm syringe filter and samples were stored at -80 °C until analysed. Pigments were separated and quantified by high-performance liquid chromatography (HPLC) as described previously[19], with some modifications. The system consisted of a Beckman System Gold programmable solvent module 126, diode array detector module 168 (Beckman Instruments, San Ramon, California, USA),

CSC-Spherisorb ODS-1 reverse-phase column (5 mm particle size, 25 × 0.46 cm I.D.) with an Upchurch Perisorb A guard column (both columns from Chromatographic Specialties Inc., Concord, Ontario, Canada). Samples were injected using a Beckman 210A sample injection valve with a 20 µL sample loop. Pigments were eluted isocratically for 6 min with a solvent system of acetonitrile:methanol:0.1 M Tris-HCl (pH 8.0), (72:8:3.5, v/v/v), followed by a 2 min linear gradient to 100% methanol:hexane (75:25, v/v) which continued isocratically for 4 min. Total run time was 12 min. Flow rate was 2 mL min^{-1}. Absorbance was detected at 440 nm and peak areas were integrated by Beckman System Gold software. Retention times and response factors of Chl *a*, Chl *b*, lutein and ß-carotene were determined by injection of known amounts of pure standards purchased from Sigma (St. Louis, MO, USA). The retention times of zeaxanthin, antheraxanthin, violaxanthin and neoxanthin were determined by using pigments purified by thin-layer chromatography as described by Diaz et al.[52].

Extraction and analysis of chanterelle lipids

Samples of each chanterelle species were homogenized to fine powder in a cryomill (Reitch, Germany) and 100 mg of the homogenized powder mixed with 1 mL methanol (MeOH), 1 mL chloroform ($CHCl_3$) and 0.8 mL water following Pham et al.[53]. The sample mixture was thoroughly vortexed, then centrifuged (Sorvall Legend XT/XF centrifuge; ThermoFisher Scientific, Mississauga, Ontario) at 2500 rpm for 15 min. The organic layer was transferred to new vials, dried under nitrogen and then reconstituted in 1 mL chloroform:methanol (1:1 v/v). Aliquots were then used for either gas chromatography with mass spectrometric and flame ionization detection (GC-MS/FID) or ultra-high-performance liquid chromatography with heated electrospray ionization high resolution accurate mass

372 tandem mass spectrometric analysis (UHPLC-HESI-HRAM/MS-MS) for fatty acids and intact
373 lipids analysis, respectively.
374 For GC-MS/FID analysis, chanterelle fatty acids were converted to fatty acid methyl
375 esters (FAMEs) as follows: To 300 µL aliquot of the lipid extract, 50 µL of C18:0 alkane (1 mg
376 mL^{-1} in chloroform: methanol 1:1 v/v) was added as internal standards and the samples dried
377 under nitrogen and the fatty acids esterified by adding 400 µL methanolic HCl (1.5N). The
378 samples were then incubated in a pre-heated oven at 60 ºC for 30 min. After incubation, 0.8 mL
379 of distilled water was added to the cooled samples and the FAMEs extracted with 3 aliquots
380 each of 500 µL of hexane. The fractions were combined, dried under N_2, re-suspended in 50 µL
381 hexane, and the FAMEs analyzed using a Trace 1300 gas chromatograph coupled to a Flame
382 Ionization Detector and TSQ 8000 mass spectrometer (Thermo Fisher Scientific). The FAMEs
383 were separated on a BPX70 high-resolution column (10 m × 0.1 mm ID × 0.2 µm, Canadian
384 Life Science, Peterborough, Ontario) using helium as the carrier gas at a flow rate of 1 mL min^{-1}.
385 One µL of each sample was injected in split mode (1:15) using a Tri-plus auto-sampler
386 (Thermo Fisher Scientific). The operation conditions were as follows: initial oven temperature
387 set at 50 °C for 0.75 min, increased to 155 °C at 4 °C min^{-1}, ramped to 210 °C at 6 °C min^{-1},
388 then 240 °C at 15 °C min^{-1} and final temperature held for 2 min. Methylated fatty acids were
389 determined by comparison with retention times and mass spectra obtained from commercial
390 standards (Supelco 37 component mix, Supelco PUFA No. 3, Sigma Aldrich, Oakville,
391 Ontario) and the NIST database (Thermo Fisher Scientific). Standard curves were employed to
392 determine the amount of individual fatty acids, and values are presented as nmole %.
393 For the UHPLC-HESI-HRAM/MS-MS analysis, a Q-Exactive Orbitrap mass
394 spectrometer (Thermo Fisher Scientific) coupled to an automated Dionex UltiMate 3000

UHPLC system was used to analyze the intact chanterelle lipids according to our previously published method[53]. Briefly, the intact lipids were resolved using an Accucore C30 column (150 mm × 2 mm I.D., particle size: 2.6 µm, pore diameter: 150 Å) and the following solvent systems: (i) Solvent A consisted of acetonitrile:water (60:40 v/v) containing 10 mM ammonium formate and 0.1% formic acid and (ii) Solvent B consisted of isopropanol:acetonitrile:water (90:10:1 v/v/v) with 10 mM ammonium formate and 0.1% formic acid. The conditions used for separation were 30 °C (column oven temperature), flow rate of 0.2 mL min^{-1}, and 10 µL of sample injected. The gradient system used was as follow: solvent B increased to 30% in 3 min; 43% in 5 min, 50% in 1 min, 90% in 9 min, 99% in 8 min, and finally maintained at 99% for 4 min. The column was re-equilibrated for 5 min before each new injection. Full scans and tandem MS acquisitions were performed in both negative and positive modes using the following parameters: sheath gas: 40, auxiliary gas: 2, ion spray voltage: 3.2 kV, capillary temperature: 300 °C; S-lens RF: 30 V; mass range: 200–2000 m/z; full scan at 70,000 m/z resolution; top-20 data-dependent MS/MS resolution at 35,000 m/z, collision energy of 35 (arbitrary unit); injection time of 35 min for C30RP chromatography; isolation window: 1 m/z; automatic gain control target: 1e5 with dynamic exclusion setting of 5.0 s. The instrument was externally calibrated to 1 ppm using electrospray ionization (ESI); negative and positive calibration solutions (Thermo Fisher Scientific) were used to calibrate the instrument at 1 ppm. Tune parameters were optimized using PC 18:1(9Z)/18:1(9Z), Cer d18:1/18:1(9Z), PG 18:1(9Z)/18:1(9Z), sulfoquinovosyl diacylglycerols [SQDG] 18:3(9Z,12Z,15Z)/16:0, monogalactosyl diglyceride [MGDG] 18:3(9Z,12Z,15Z)/16:3(7Z,10Z,13Z), and digalactosyldiacylglycerol [DGDG] 18:3(9Z,12Z,15Z)/18:3(9Z,12Z,15Z) lipid standards (Avanti Polar Lipids, Alabaster, AL, USA) in both negative and positive ion modes. The data

418 were processed using either X-Calibur 4.0 (Thermo Fisher Scientific) or LipidSearch version
419 4.1 (Mitsui Knowledge Industry, Tokyo, Japan) software packages.
420 Phenolics analysis by GC-MS
421 Reagent grade phenolic acid standards including benzoic acids, p- hydroxybenzoic acid,
422 vanillic acid, gallic acid, 3,4-dihydroxybenzoic acid, syringic acid, gentisic acid, veratric acid,
423 salicylic acid, cinammic acid, o-coumaric acid, m-coumaric acid, p-coumaric acid, ferulic acid,
424 sinapic acid, caffeic acid, sodium hydroxide, N,O-Bis(trimethylsilyl)trifluoroacetamide
425 (BSTFA-TCMS) were purchased from Sigma Aldrich. Methanol, ethyl acetate, and
426 hydrochloric acid (36% w/v) were purchased from VWR (Mississauga, Ontario, Canada). For
427 alkaline hydrolysis of powdered chanterelles, 100 µL of aqueous 3,4-dihydroxybenzoic acid
428 solution (0.2 mg mL^{-1}) was added to a mixture containing 4 g of sample in 8 mL 1M sodium
429 hydroxide. The resultant mixture was incubated in the dark for 24 h at 25 °C on an orbital
430 shaker (50 rpm). The pH of the reaction mixture was adjusted to 2.0-2.5 using concentrated
431 HCl then vortexed. The organic components were extracted four times with 4 mL methanol:
432 ethyl acetate (1:3 ratio) into pre-weighed vials. The solvent was evaporated under nitrogen at
433 35 °C to determine the crude extraction yield. The extracts were resuspended in 1 mL ethyl
434 acetate, vortexed, then 300 µL of extract transferred into a pre-weighed vial, dried under
435 nitrogen, and 50 uL of BSTFA-TCMS and 50 µL of pyridine added. The resultant mixture was
436 incubated at 70 °C in darkness for 30 min then transferred to GC vials for GC-MS analysis.
437 Standard solutions were derivatized in a similar manner.
438 A Thermo Scientific Trace 1300 gas chromatograph coupled to a Triple Quad mass
439 spectrometer (Thermo Fisher Scientific) was used for the analysis and the compounds resolved
440 on a ZB-5MS non-polar stationary phase column (30m × 0.25 mm I.D., 0.25 µm film thickness,

441 Phenomenex, Torrance, CA, USA) with helium as the carrier gas (flow rate of 0.6 mL min^{-1}).
442 One microliter of the standard or sample was injected in basic mode (15:0) using a Tri-plus
443 auto-sampler. The oven temperature program was as follows: the initial oven temperature was
444 70 °C (held for 1 min), was increased at 12 °C min^{-1} to 220 °C (held for 3 min), 15 °C min^{-1} to
445 reach 250 °C and held for 1 min.. Identification of the phenolic acids (as trimethylsilyl ether,
446 TMS) was based on the comparison of their retention times and mass spectra with that of the
447 NIST library and commercial standards, with quantities calculated and expressed as nmole %.
448 Analysis of the volatile profile of chanterelles by SPME-GC/MS
449 Volatile metabolites were extracted and analysed by Solid-Phase Microextraction and
450 Gas Chromatography/Mass Spectrometry (SPME-GC/MS) following Vidal et al.[54]. Briefly, 100
451 mg of sample powder obtained after cryo homogenization was placed in 10 mL headspace glass
452 vials and kept at 50 °C for 5 min (sample equilibration) before volatile metabolites extraction
453 and analysis began. A divinylbenzene/carboxen/polydimethylsyloxane (DVB/CAR/ PDMS)
454 coated fibre (1 cm long, 50/30 μm film thickness; Supelco, Sigma-Aldrich), was inserted into
455 the headspace of the sample vial and held there for 60 min[55,56]. Chanterelle volatile composition
456 was analyzed using a Trace 1300 gas chromatograph coupled to a TSQ 8000 Triple Quadrupole
457 mass spectrometer (Thermo Fisher Scientific). The extracted volatile compounds were
458 separated using a ZB-5MS non-polar stationary phase column (30m × 0.25mm I.D., 0.25 μm
459 film thickness; Phenomenex) with He used as the carrier at a flow rate of 1 mL min^{-1}. After
460 extraction the fibre was desorbed for 10 min in the injection port and the instrument operated as
461 follows: splitless mode with a purge time of 5 min, initial oven temperature set at 50 °C (5 min
462 hold) and increased to 290 °C at 4 °C min^{-1} (2 min hold). Ion source and quadrupole mass
463 analyzer temperatures were set at 230 and 150 °C respectively, injector and detector

464 temperatures held at 250 and 290 °C respectively, mass spectra ionization energy set at 70 eV,
465 and data acquisition done in scan mode. After each sample desorption, the fiber was cleaned for
466 10 min at 250 °C in the conditioning station. Volatile compounds were identified by matching
467 the obtained mass spectra with those of available standards, and mass spectra from commercial
468 libraries NIST/EPA/NIH (version 2.2, Thermo Fisher Scientific) or the scientific literature[55,56].
469 Volatile compounds in the chanterelle samples were semi-quantified based on the area counts ×
470 10^{-6} of the base peak. Compounds with lower abundances than 10^{-6} area counts were
471 considered as traces. Although the chromatographic response factor of each compound is
472 different, the area counts determined are useful for comparison of the relative abundance of
473 each compound in the different samples analysed[55,56].
474 Statistics and reproducibility
475 Results of analyses of lipids and phenolics are presented as means and standard errors of
476 4 replicates and those of head-space analyses of volatiles are based on 2 replicates (Tables 2-4).
477 One-way analysis of variance (ANOVA) was used to determine if there were significant
478 differences between the chemical constituents in chanterelle samples. Where differences were
479 significant, the means were compared with Fisher's Least Significant Difference (LSD), α =
480 0.05. Principal component analysis was conducted using XLSTAT Premium version
481 (Addinsoft, Paris, France) to discern similarities or differences between the variants. Figures
482 were prepared using XLSTAT Premium version and SigmaPlot 13.0 software programs (Systat
483 Software Inc., San Jose, CA).
484 Data availability
485 All newly determined DNA sequences have been deposited in GenBank, including
486 ribosomal ITS and LSU, *Tef1*, *Al-1* and *Al-2*.

References

1. Watling, R. The business of fructification. *Nature*. **385,** 299–300 (1997).

2. Hall, I. R. & Wang, Y. Edible mushrooms as secondary crops in forests. *Quart. J. For*. **94,** 299-304 (2000).

3. Redhead, S. A., Norvell, L. L. & Danell, E. *Cantharellus formosus* and the Pacific golden chanterelle harvest in Western North America. *Mycotaxon*. **65,** 285–322 (1997).

4. Pilz, D., Norvell, L., Danell, E. & Molina, R. *Ecology and management of commercially harvested chanterelle mushrooms*. PNW-GTR-576, 1–83 (USDA, 2003).

5. Endicott, W. My descent into the dark side of foraging. *Omphalina*. **4**(9)**,** 3–4 (2013).

6. Endicott, W. Commercial picking 101. *Omphalina* **4**(9)**,** 5–8 (2013).

7. Arpin, N. & Fiasson, J. L. The pigments of Basidiomycetes: their chemotaxonomic interest. In *Evolution in the higher Basidiomycetes* (ed. Petersen, R. H.) 63–98 (Univ TN Press, 1971).

8. Mui, D., Feibelman, T. & Bennett, W. A preliminary study of the carotenoids of some North American species of *Cantharellus*. *Int. J. Plant Sci.* **159,** 244–248 (1998).

9. Vilneff, C. & Thorn, R. G. Newfoundland golden chanterelles: examining their identity and regional levels of damage by slugs and larvae. *Omphalina* **2**(4)**,** 13–17 (2011).

10. Buyck, B., Kauff, F., Eyssartier, G., Couloux, A. & Hofstetter, V. A multilocus phylogeny for worldwide *Cantharellus* (Cantharellales, Agaricomycetidae). *Fungal Divers*. **64,** 101–121 (2014).

11. Thorn, R. G., Kim, J. I., Lebeuf, R. & Voitk, A. The golden chanterelles of Newfoundland and Labrador: a new species, a new record for North America, and a lost species rediscovered. *Botany* **95,** 547–560 (2017).

12. Thorn, R. G., Banwell, A., Kim, J. I., Lebeuf, R. & Voitk, A. *Cantharellus betularum* Voitk & Thorn, sp. nov. *Persoonia* **45,** 330–331 (2020).

13. Thorn, G., Bryden, B. & Voitk, A. White chanterelles of NL & Labrador: preliminary report. *Omphalina* **9**(7)**,** 7–11 (2018).

14. Olariaga, I. et al. Assessing the taxonomic identity of white and orange specimens of *Cantharellus*: occasional colour variants or independent species? *Cryptogam. Mycol*. **36,** 287–300 (2015).

15. Fiasson, J. L., Petersen, R. H., Bouchez, M. P. & Arpin, N. Contribution biochimique à la connaissance taxinomique de certains Champignons cantharelloïdes et clavarioïdes. *Rev. Mycol.* **34,** 357-364 (1970).

16. Barros, L., Venturini, B. A., Baptista, P., Estevinho, L. M. & Ferreira, C. F. R. Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. *J. Agric. Food Chem.* **56,** 3856-3862 (2008).

17. Hanson, J. R. *The Chemistry of Fungi*. (Roy. Soc. Chem., 2008).

18. Li, H. et al. The genome sequences of 90 mushrooms. *Sci. Rep.* **8,** 9982 (2018).

19. Ivanov, A. G., Krol, M., Maxwell, D. & Hüner, N. P. A. Abscisic acid induced protection against photoinhibition of PSII correlates with enhanced activity of the xanthophyll cycle. *FEBS Lett.* **371,** 61–64 (1995).

20. Smith, A.H. & Morse, E. E. The genus *Cantharellus* in the western United States. *Mycologia*. **39,** 497–534 (1947).

21. Foltz, M. J., Perez, K. E. & Volk, T. J. Molecular phylogeny and morphology reveal three new species of *Cantharellus* within 20 m of one another in western Wisconsin, USA. *Mycologia*. **105,** 447–461 (2013).

22. Galagan, J. E. et al. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*. **422,** 859–868 (2003).

23. Schmidhauser, T .J., Lauter, F. R., Russo, V. E. & Yanofsky, C. Cloning, sequence, and photoregulation of *al-1*, a carotenoid biosynthetic gene of *Neurospora crassa*. *Mol. Cell. Biol. 10,* 5064–5070 (1990).

24. Schmidhauser, T. J., et al. Characterization of *al-2*, the phytoene synthase gene of *Neurospora crassa*. Cloning, sequence analysis, and photoregulation. *J. Biol. Chem.* **269,** 12060–12066 (1994).

25. Nelson, M. A., Morelli, G., Carattoli, A., Romano, N. & Macino, G. Molecular cloning of a *Neurospora crassa* carotenoid biosynthetic gene (albino-3) regulated by blue light and the products of the white collar genes. *Molec. Cell. Biol.* **9,** 1271–1276 (1989).

26. Avalos, J. & Limón, M. C. Biological roles of fungal carotenoids. *Curr. Genet.* **61,** 309–324 (2015).

27. Britton, G. Structure and properties of carotenoids in relation to function. *FASEB J.* **9,** 1551–1558 (1995).

28. Oshima, S., Ojima, F., Sakamoto, H., Ishiguro, Y. & Terao, J. Inhibitory effect of β-carotene and astaxanthin on photosensitized oxidation of phospholipid bilayers. *J. Nutr. Sci. Vitaminol.* 39(6): 607–615 (1993).

552 29. Arcangeli, C. & Cannistraro, S. In situ Raman microspectroscopic identification and
553 localization of carotenoids: Approach to monitoring of UVB irradiation stress on
554 Antarctic fungus. *Biopolymers.* **57,** 179–186 (2000).
555 30. Ramadan-Talib, Z. & Prebble, J. Photosensitivity of respiration in *Neurospora*
556 mitochondria. A protective role for carotenoid. *Biochem. J.* **176,** 767–775 (1978).
557 31. Sande, D. et al. Edible mushrooms as a ubiquitous source of essential fatty acids. *Food Res.*
558 *Intl.* **125,** 108524 (2019).
559 32. Barthet, V. J. (n-7) and (n-9) cis-Monounsaturated fatty acid contents of 12 *Brassica*
560 species. *Phytochemistry*. **69,** 411–417 (2008).
561 33. Barreto-Bergter, E., Sassaki, G. L. & de Souza, L. M. Structural analysis of fungal
562 cerebrosides. *Front. Microbiol.* **2,** 239 (2011).
563 34. Guimarães, L. L., Toledo, M. S., Ferreira, F. A. S., Straus, A. H. & Takahashi, H. K.
564 Structural diversity and biological significance of glycosphingolipids in pathogenic and
565 opportunistic fungi. *Front. Cell. Infect. Microbiol*. **4,** 138 (2014).
566 35. Sinanoglou, V. J. et al. Lipid and fatty acid profile of the edible fungus *Laetiporus*
567 *sulphureus*. Antifungal and antibacterial properties. *J. Food Sci. Technol.* **52,** 3264–
568 3272 (2015).
569 36. Lumbsch, H. T. Analysis of phenolic products in lichens for identification and taxonomy. In
570 *Protocols in Lichenology* (eds. Kranner, I., Beckett, R. & Varma, A.) 281–295
571 (Springer, 2002).
572 37. Mešić, A., Šamec, D., Jadan, M., Bahun, V. & Tkalčec, Z. Integrated morphological with
573 molecular identification and bioactive compounds of 23 Croatian wild mushrooms
574 samples. *Food Biosci*. **37,** 100720 (2020).

38. El Hadi, M. A. M., Zhang, F. J., Wu, F. F., Zhou, C. H. & Tao, J. Advances in fruit aroma volatile research. *Molecules.* **18,** 8200-8229 (2013).

39. Zhou, J., Feng, T. & Ye, R. Differentiation of eight commercial mushrooms by electronic nose and gas chromatography-mass spectrometry. *J. Sensors* **2015,** 374013 (2015).

40. Aloum, L., Alefishat, E., Adem, A., & Petroianu, G. Ionone is more than a violet's fragrance: a review. *Molecules*. **25,** 5822 (2020).

41. Vilgalys, R. & Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol*. **172,** 4238–4246 (1990).

42. White, T. J., Bruns, T., Lee, S. B. & Taylor, J. W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a guide to methods and applications* (eds. Innis, M. A., Gelfand, D. H., Shinsky, J. J. & White, T. J.) 315–322 (Academic, 1990).

43. Hausner, G., Reid, J. & Klassen, G. R. On the subdivision of *Ceratocystis* s.l., based on partial ribosomal DNA sequences. *Can. J. Bot*. **71,** 52–63 (1993).

44. Dentinger, B. T. M., Margaritescu, S. & Moncalvo, J.-M. Rapid and reliable high throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. *Molec. Ecol. Resour.* **10,** 628–633 (2010).

45. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molec. Biol. Evol*. **30,** 772–780 (2013).

46. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molec. Biol. Evol*. **35,** 1547–1549 (2018).

47. Stecher, G., Tamura, K. & Kumar, S. Molecular evolutionary genetics analysis (MEGA) for macOS. *Molec. Biol. Evol*. **37,** 1237–1239 (2020).

48. Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol*. **61,** 539–542 (2012).

49. Altschul, S.F. et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids Res*. **25,** 3389–3402 (1997).

50. Kearse, M. et al. Geneious Prime Version 2020.0.5. (2012).

51. Gasteiger, E. et al. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucl. Acids Res*. **31,** 3784–3788 (2003).

52. Diaz, M., Ball, E. & Luttge, U. Stress-induced accumulation of the xanthophyll rhodoxanthin in leaves of *Aloe vera*. *Plant Physiol. Biochem*. **28,** 679–682 (1990).

53. Pham, T. H. et al. Targeting modified lipids during routine lipidomics analysis using HILIC and C30 reverse phase liquid chromatography coupled to mass spectrometry. *Sci. Rep*. **9**, 5048 (2019).

54. Vidal, N. P. et al. Novel unfiltered beer-based marinades to improve the nutritional quality, safety, and sensory perception of grilled ruminant meats. *Food Chem.* **302,** 125326 (2020).

55. Goicoechea, E. & Guillén, M. D. Volatile compounds generated in corn oil stored at room temperature. Presence of toxic compounds. *Eur. J. Lipid Sci. Technol*. **116,** 395–406 (2014).

56. Vidal, N. P., Manzanos, M. J., Goicoechea, E. & Guillén, M. D. 2016. Farmed and wild sea bass (*Dicentrarchus labrax*) volatile metabolites: a comparative study by SPME-GC/MS. *J. Sci. Food Agric.* **96,** 1181–1193 (2016).

Acknowledgements

We extend our thanks to the collectors, Bill Bryden, Shawn Dawson, Eugene Kean, Renée Lebeuf, Henry Mann, Mac Pitcher, David Sparks, Maria Voitk, and others, and to Andrus Voitk for photographs and helpful comments on the manuscript. This project was supported by funding from the NSERC Discovery Grant program to NPAH, NDPN and RT, the Canada Foundation for Innovation to NPAH and RGT, the Canada Research Chairs programme to NPAH, and an ACOA Collaborative Research Grant to RT; RGT thanks Foray Newfoundland and Labrador for support and hospitality.

Author contributions

RGT and RT contributed to the conception and design of the study; RGT, NPAH, MDPN and RT provided funding and research facilities; AB and MBB performed DNA sequence analysis; AB and RGT performed phylogenetic analysis; BSM and AGI performed carotenoid analysis; THP performed lipid analysis; NPV analyzed volatile metabolites; CFM and MN performed phenolics analysis; AGI, RGT, THP, NPV, CFM and MN constructed the figures; AB and RGT wrote the manuscript with input from AGI, MBB, THP, NPV, CFM and MN; and all authors read and approved the final version.

Competing interests

The authors declare no competing interests.

Figure legends

644 Figure 1. Typical and albino forms of *Cantharellus enelensis*. A. Typical form, with golden-
645 orange colouration (henceforth referred to as gold or golden). B. The albino form, *C. enelensis*
646 f. *acolodorus* (17.08.15.av01; NLW3 in chemical analyses), showing buffy yellow staining in
647 age or on handling. When dried, the two forms are morphologically indistinguishable. Photos:
648 Andrus Voitk.
649
650 Figure 2. White chanterelles from Newfoundland and Québec are members of the species
651 *Cantharellus enelensis* and are distinguished by their lack of β-carotene. A. Maximum
652 likelihood phylogeny of white and golden representatives of *Cantharellus enelensis*, related
653 species of the core *C. cibarius* clade (arrow) and its sister group, the clade including *C. pallens*
654 through *C. phasmatis*, rooted with C. chicagoensis. The tree is based on sequences from nuclear
655 ribosomal internal transcribed spacer (ITS), large subunit (LSU) and translation elongation
656 factor 1-alpha (Tef1) regions. All sequences are identified by the GenBank accession numbers
657 and the name they were deposited under, and new sequences obtained in this study are
658 indicated in bold font. Sequences from type specimens are indicated with HT (holotype), NT
659 (neotype) or ET (epitype). Table 1 provides collection details for samples used in chemical
660 analyses; their specimen codes used in Fig. 3 are included in bold following the species name in
661 this tree. Node support values (%) are provided from a Bayesian inference analysis (posterior
662 probabilities, above nodes) and a 1000x maximum likelihood bootstrap analysis (below nodes).
663 Nodes with less than 50% support are shown by dashes, and a single node that collapsed in
664 Bayesian analysis is shown by asterisks. B–C. Representative HPLC chromatograms and
665 absorbance spectra of pigments extracted from white and golden variants of *Cantharellus*

enelensis. B. Representative HPLC chromatograms, the upper traces showing an enlargement of the area of interest. C. Absorbance spectra of acetone extracts.

Figure 3. Principal components analysis (PCA) of chemical constituents of white and golden variants of Newfoundland chanterelles, showing the observations (sample clustering) and biplots showing loadings of chemical variables. A–B, fatty acids; C–D, intact lipids; E–F, phenolics. G–L, volatile compounds detected by headspace solid phase microextraction tandem mass spectrometry (HS-SPME-MS/MS). G–H, aldehydes; I–J, ketones (ellipse highlighting the golden chanterelles); K–L, terpenes (ellipses highlighting the white and golden chanterelles). Sample codes AY: *Cantharellus betularum* (golden); CY: *C. camphoratus* (golden); Mac1–Mac5: individuals from a mixture of golden and white NL chanterelles; NLW1–3: *C. enelensis* (Newfoundland, white 1–3); NLY: *C. enelensis* (Newfoundland, golden); QW: *C. enelensis* (Québec, white). For identities of fatty acids and intact lipids, see Table 2.

Supporting information

S1 Fig. Principal components analysis (PCA) of volatile compounds detected by headspace solid phase microextraction tandem mass spectrometry (HS-SPME-MS/MS) in white and golden variants of Newfoundland chanterelles. A, observations (sample clustering; ellipse highlighting the position of three of four samples of white chanterelles); and B, biplots showing loadings of chemical variables. Sample codes AY: *Cantharellus betularum* (golden); CY: *C. camphoratus* (golden); NLW1–3: *C. enelensis* (Newfoundland, white 1–3); NLY: *C. enelensis* (Newfoundland, golden); QW: *C. enelensis* (Québec, white). Numbers in panel B represent volatile compounds as listed in Table 4.

689 **Table 1.** *Cantharellus* specimens used for chemical analyses, their colour, source, preservation conditions, and GenBank accession

690 number of reference ITS sequence. Specimens labelled white/golden are white or golden individuals from a mixed collection of both

691 white and golden chanterelles that were indistinguishable after desiccation. NL, Newfoundland & Labrador; QC, Québec.

Identification	Colour, code	Source, collection no. (Herbarium)	Preservation	GenBank No.
C. betularum (formerly identified as *C. amethysteus*)	Golden, AY	Humber Village, NL, M. Voitk, 17.09.30.av01 (UWO)	Dried	MN206940
C. camphoratus	Golden, CY	Deer Lake, NL, H. Mann, 17.10.22.av02	Dried	ND[a]
C. enelensis	Golden, NLY	Gambo, NL, A. Voitk, 17.08.15.av02 (UWO)	Dried	MN206930
C. enelensis	Golden (pigment analysis)	Avalon Peninsula, NL, S. Dawson, RGT 190913/02 (UWO)	Frozen	ND
C. enelensis	White (pigment analysis)	Avalon Peninsula, NL, S. Dawson, RGT 190913/01 (UWO, DAOM)	Frozen	ND
C. enelensis	White, NLW1	Gambo, NL, E. Kean, 17.08.11.av06 (UWO)	Dried	MN206912
C. enelensis	White, NLW2	St. John's, NL, D. Sparks, 17.08.13.av01 (UWO)	Dried	MN206917
C. enelensis	White, NLW3	Gambo, NL, B. Bryden, 17.08.15.av01 (UWO)	Dried	MN206913
C. enelensis	White, QW	St. Alban, QC, R. Lebeuf, HRL 2585 (UWO)	Dried	MN206931
C. enelensis	White/golden, Mac1	Brigus Junction, NL, M. Pitcher 1 (UWO)	Dried	MN206919
C. enelensis	White/golden, Mac2	Brigus Junction, NL, M. Pitcher 2 (UWO)	Dried	MN206921
C. enelensis	White/golden, Mac3	Brigus Junction, NL, M. Pitcher 3 (UWO)	Dried	MN206923
C. enelensis	White/golden, Mac4	Brigus Junction, NL, M. Pitcher 4 (UWO)	Dried	MN206925
C. enelensis	White/golden, Mac5	Brigus Junction, NL, M. Pitcher 5 (UWO)	Dried	MN206927

692

[a] 18.09.13.av07, from same population, MN206937

693 **Table 2.** Lipid composition (nanomole percent ± standard error) observed in white and golden variants of Newfoundland chanterelles.

Polar Lipids	NLW1	NLW2	NLW3	QW	Mac	NLY	AY	CY
LPC	19.85±2.50^{a}	1.64±0.11^{c}	10.11±1.32^{b}	0.20±0.01^{c}	0.67±0.07^{c}	1.00± 0.10^{c}	0.87± 0.05^{c}	0.55±0.03^{c}
LPE	0.09±0.05^{abc}	0.21± 0.01^{a}	0.0026±0.0008^{c}	0.16±0.09^{ab}	0.05±0.02^{bc}	0.19± 0.06^{ab}	0.11 ±0.04^{abc}	0.11±0.03^{abc}
PA	18.50±0.67^{e}	20.58±1.83^{de}	26.17±0.51^{ab}	6.53±0.48^{g}	27.44±1.18^{a}	23.73±0.19^{bc}	22.41± 1.45^{cd}	14.33±0.47^{f}
PC	36.95±1.08^{fg}	51.59±3.33^{bc}	33.46±0.60^{g}	73.86±0.85^{a}	40.75±2.65^{ef}	45.19±0.60^{de}	48.96± 2.61^{cd}	55.74± 1.09^{b}
OxPC	0.17± 0.01^{cd}	0.42±0.09^{a}	0.12± 0.03^{cd}	0.03±0.01^{d}	0.024±0.008^{d}	0.25±0.02^{bc}	0.484± 0.130^{a}	0.35±0.02^{ab}
PE	21.08±0.85^{c}	22.48±1.70^{c}	27.91±0.51^{a}	12.61±0.52^{d}	27.03±1.49^{a}	25.88±0.36^{ab}	23.17± 1.55^{bc}	20.41±0.46^{c}
PG	0.03±0.02^{d}	0.19±0.04^{b}	0.012±0.002^{d}	0.42±0.07^{a}	0.08±0.03^{cd}	0.15±0.04^{bc}	0.004±0.002^{d}	0.027±0.005^{d}
PI	0.052± 0.006^{c}	0.082±0.007^{b}	0.064±0.001^{c}	0.086±0.004^{b}	0.094±0.009^{ab}	0.105±0.007^{a}	0.007± 0.004^{d}	0.0010±0.0007^{d}
PS	2.79±0.06^{c}	2.80±0.21^{c}	1.781±0.035^{d}	6.07±0.09^{b}	3.07±0.15^{c}	2.95±0.07^{c}	2.98±0.19^{c}	7.82±0.19^{a}
SM	0.49±0.14^{bc}	0.008±0.005^{d}	0.362±0.096^{c}	0.035±0.004^{d}	0.80±0.24^{ab}	0.554± 0.008^{bc}	0.98± 0.07^{a}	0.66±0.03^{bc}
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Neutral Lipids	**NLW1**	**NLW2**	**NLW3**	**QW**	**Mac**	**NLY**	**AY**	**CY**
Cer	0.100±0.004^{c}	0.140± 0.004^{bc}	0.262±0.018^{ab}	0.043± 0.001^{c}	0.37±0.13 ^{a}	0.114±0.008^{c}	0.12±0.01^{c}	0.12±0.01^{c}
HexCer	4.72±0.24^{c}	1.55±0.04^{c}	5.95±0.18^{bc}	0.965±0.012^{c}	26.06±7.71 ^{a}	2.12±0.06^{c}	25.05±0.22^{a}	13.35±0.55^{b}
CmE	0.12±0.01^{bc}	0.17±0.02^{ab}	0.182± 0.009^{ab}	0.216± 0.007^{ab}	0.20±0.09^{ab}	0.062±0.004^{c}	0.124±0.003^{bc}	0.24±0.02^{a}
StE	0.024±0.001^{b}	0.035± 0.001^{a}	0.023±0.001^{b}	0.012± 0.003^{c}	0.04±0.01^{a}	0.0162±0.0003^{bc}	0.0182±0.0003^{bc}	0.0104±0.0003^{c}
MG	1.47±0.09^{ab}	2.51±1.25^{a}	1.41±0.44^{ab}	1.83± 0.20^{ab}	0.45±0.28^{b}	0.87±0.24^{b}	0.53±0.03^{b}	0.49±0.15^{b}
DG	10.91±0.39^{cd}	18.19±0.31^{a}	15.84±0.32^{b}	11.91± 0.10^{c}	15.32±0.98^{b}	10.53±0.27^{d}	6.61±0.05^{e}	7.84±0.36^{e}
oxDG	0.067±0.009^{b}	0.074±0.008^{b}	0.20±0.03^{a}	0.003± 0.001^{c}	0.07±0.01^{b}	0.028±0.008^{c}	0.014±0.002^{c}	0.0017±0.0005^{c}
scTG	0.162±0.002^{e}	0.182±0.005^{cd}	0.225±0.008^{b}	0.201± 0.002^{c}	0.07±0.01^{f}	0.159±0.002^{e}	0.053±0.004^{f}	0.327±0.008^{a}
mcTG	0.519±0.023^{d}	0.79±0.02^{c}	1.39±0.02^{a}	0.58± 0.02^{d}	1.08±0.17^{b}	0.28±0.02^{e}	0.214±0.003^{e}	0.193±0.008^{e}
lcTG	78.33±1.06^{abc}	72.38±0.89^{cd}	66.75±0.67^{d}	82.54± 0.22^{ab}	53.58±7.59^{e}	83.63±0.39^{a}	64.98±0.32^{d}	75.46±1.06^{bc}
oxTG	3.58± 0.47^{bc}	3.98± 0.13^{b}	7.77±0.49^{a}	1.70± 0.10^{e}	2.76±0.35^{cd}	2.19± 0.07^{de}	2.29±0.07^{de}	1.96±0.11^{cde}
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Fatty acids	NLW1	NLW2	NLW3	QW	Mac	NLY	AY	CY
8:0	0.110±0.006^{bc}	0.085±0.003^{de}	0.130±0.006^{b}	0.065±0.004^{e}	0.13±0.01^{b}	0.094±0.001^{cd}	0.134±0.009^{b}	0.18±0.02^{a}
10:0	0.083±0.005^{b}	0.06±0.01^{bc}	0.08±0.01^{b}	0.028±0.004^{c}	0.118±0.009^{a}	0.06±0.01^{b}	0.12±0.02^{a}	0.14±0.02^{a}
11:0	0.301±0.008^{c}	0.232±0.008^{d}	0.354±0.008^{c}	0.178±0.008^{d}	0.44±0.04^{b}	0.30±0.02^{c}	0.49±0.03^{ab}	0.54±0.03^{a}
12:0	0.060±0.004^{e}	0.051±0.004^{e}	0.074±0.007^{bd}	0.062±0.003^{e}	0.079±0.007^{b}	0.052±0.002^{de}	0.094±0.006^{ab}	0.11±0.02^{a}
14:0	0.05±0.01^{c}	0.073±0.008^{b}	0.094±0.004^{a}	0.059±0.005^{c}	0.09±0.02^{ab}	0.062±0.006^{b}	0.074±0.009^{ab}	0.063±0.007^{b}
15:0	0.096±0.004^{b}	0.034±0.003^{de}	0.12±0.03^{b}	0.032±0.006^{de}	0.19±0.03^{a}	0.070±0.005^{d}	0.103±0.004^{b}	*ND*
16:0	6.06± 0.05^{c}	5.68±0.05^{c}	8.54±0.02^{a}	3.99±0.05^{d}	8.34±0.91^{a}	6.26±0.05^{c}	7.77± 0.04^{ab}	7.29±0.05^{b}
18:0	1.73±0.01^{b}	1.37±0.02^{d}	2.43±0.01^{a}	1.57±0.03^{c}	0.79±0.13^{e}	1.84±0.02^{b}	1.36± 0.03^{d}	1.84±0.02^{b}
23:0	0.167±0.008^{d}	0.191±0.004^{b}	0.09±0.01^{f}	0.219± 0.009^{ab}	0.13±0.01^{e}	0.197±0.007^{bc}	0.16± 0.01^{de}	0.25±0.01^{a}
24:0	0.27±0.01^{c}	0.259±0.007^{c}	0.419±0.009^{a}	0.394±0.004^{a}	0.18±0.03^{d}	0.34±0.01^{b}	0.24±0.01^{c}	0.37±0.03^{ab}
14:1	*ND*	0.035±0.003^{a}	0.02±0.01^{ab}	0.033±0.005^{a}	0. 04±0.02^{a}	*ND*	*ND*	*ND*
15:1	0.09±0.01^{de}	0.065±0.002^{ef}	0.097±0.003^{d}	0.046±0.004^{f}	0.11±0.01^{c}	0.09±0.01^{de}	0.14±0.01^{b}	0.161±0.004^{a}
16:1*n*9	0.156±0.006^{d}	0.321±0.009^{b}	0.280±0.002^{c}	0.355±0.007^{b}	0.58±0.05^{a}	0.175±0.007^{d}	0.187±0.008^{d}	0.164±0.005^{d}
16:1*n*7	0.637±0.008^{d}	0.735±0.01^{d}	1.383±0.003^{a}	1.218±0.02^{b}	1.16±0.06^{b}	1.06±0.01^{c}	0.05±0.01^{e}	0.10±0.05^{e}
16:1*n*5	0.319±0.003^{d}	0.279±0.004^{d}	1.31±0.02^{a}	0.108±0.008^{e}	0.53±0.10^{c}	0.35±0.01^{d}	0.67±0.03^{b}	0.26±0.02^{d}
18:1*n*9	2.91±0.02^{c}	2.421±0.008^{d}	5.66±0.02^{b}	2.10±0.09^{e}	1.77±0.11^{f}	2.59±0.05^{d}	2.12±0.02^{e}	13.05±0.09^{a}
18:1*n*7	6.29±0.02^{e}	5.74±0.03^{e}	12.67±0.02^{a}	10.62±0.10^{b}	7.88±0.55^{d}	9.04±0.03^{c}	1.01±0.02^{f}	0.753±0.004^{f}
20:1*n*9	*ND*	*ND*	0.141±0.006^{b}	*ND*	*ND*	*ND*	0.128±0.005^{b}	0.42±0.02^{a}
22:1*n*9	0.13±0.01^{b}	0.137±0.006^{b}	0.183±0.002^{a}	*ND*	0.14±0.03^{b}	0.11±0.01^{cd}	0.17±0.02^{ab}	0.086±0.008^{d}
24:1*n*9	2.02±0.02^{b}	2.23±0.04^{ab}	2.369±0.007^{a}	0.311±0.005^{f}	1.42±0.27^{d}	1.62±0.02^{d}	1.94±0.02^{c}	0.67±0.01^{e}
18:2*n*6	31.27±0.04^{d}	26.92±0.07^{e}	32.47±0.04^{d}	29.67±0.11^{de}	45.10±3.91^{a}	33.86±0.08^{c}	29.76± 0.10^{de}	39.02±0.11^{b}
20:3*n*6	43.91±0.03^{b}	49.68±0.22^{a}	28.80±0.07^{d}	45.59±0.16^{ab}	27.80±4.96^{d}	38.15±0.13^{c}	49.40±0.09^{a}	29.77±0.08^{d}
22:2*n*9	1.30±0.05^{c}	1.01±0.03^{d}	1.59±0.03^{b}	0.71±0.02^{e}	1.74±0.14^{b}	1.25±0.05^{c}	2.00± 0.07^{a}	2.11±0.07^{a}
22:6*n*3	2.05±0.01^{c}	2.38±0.01^{b}	0.71±0.02^{f}	2.66±0.05^{a}	1.24±0.13^{e}	2.42±0.03^{b}	1.88±0.01^{d}	2.63±0.05^{a}
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
SFA	8.92±0.04^{d}	8.04±0.05^{d}	12.33±0.04^{a}	6.60±0.07^{e}	10.49±1.14^{b}	9.28±0.05^{c}	10.55±0.09^{b}	10.80±0.04^{b}
MUFA	12.54±0.04^{e}	11.97±0.10^{e}	24.11±0.05^{a}	14.78±0.10^{c}	13.62±0.70^{d}	15.03±0.05^{b}	6.41±0.03^{f}	15.66±0.09^{b}

***n*6-PUFA**	75.18±0.05[b]	76.60±0.15[b]	61.27±0.04[e]	75.25±0.08[b]	72.90±1.65[c]	72.02±0.10[c]	79.16±0.16[a]	68.80±0.06[d]
***n*3-PUFA**	2.05±0.01[c]	2.38±0.01[b]	0.71±0.02[f]	2.66±0.05[a]	1.24±0.13[e]	2.42±0.03[b]	1.88±0.02[d]	2.63±0.049[a]
18:1*n*7/*n*9	2.17±0.02[d]	2.37±0.02[d]	2.237±0.007[d]	5.08±0.24[a]	4.46±0.21[b]	3.49±0.08[c]	0.475±0.005[e]	0.058±0.001[f]
16:1*n*7/*n*9	4.11±0.12[c]	2.26±0.02[e]	4.94±0.04[b]	3.40±0.02[d]	2.04±0.19[e]	6.12±0.27[a]	0.26±0.09[f]	0.61±0.29[f]
16:1*n*5/*n*7	0.50±0.007[b]	0.39±0.006[b]	0.94±0.01[b]	0.09±0.006[b]	0.47±0.10[b]	0.33±0.007[b]	9.94±3.01[a]	2.39±0.74[b]

Values (nanomole percent by weight composition) represent means ± standard errors for four replicates. Means in the same row accompanied by different superscripts are significantly different among chanterelles at α = 0.05. *ND*: not detected. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. *n* position: position of the first double bond counted from methyl end group of unsaturated fatty acid. Sample codes AY: *Cantharellus betularum* (golden); CY: *C. camphoratus* (golden); Mac: average of Mac1–Mac5 from a mixture of golden and white NL chanterelles; NLW1–3: *C. enelensis* (Newfoundland, white 1–3); NLY: *C. enelensis* (Newfoundland, golden); QW: *C. enelensis* (Québec, white). **Lipid acronyms:** Cer: Ceramide, CmE: Campesterol ester, DG: Diacylglycerol, HexCer: Hexanoyl ceramide (cerebroside), lcTG: long-chain triacylglycerol, LPC: Lysophosphatidylcholine, LPE: Lysophosphatidylethanolamine, mcTG: medium-chain triacylglycerol, MG: Monoacylglycerol, oxDG: Oxidized diacylglycerol, OxPC: Oxidized phosphatidylcholine, oxTG: Oxidized triacylglycerol, PA: Phosphatidic acid, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, PG: Phosphatidylglycerol, PI: Phosphatidylinositol, PS: Phosphatidylserine, scTG: short-chain triacylglycerol, SM: Sphingomyelin, StE: Stigmasterol ester.

706 **Table 3.** Phenolic acid compounds (nanomole percent ± standard error) observed in white and golden variants of Newfoundland chanterelles.
707

Chanterelles	Benzoic acid	Salicylic acid	Phenol	Cinnamic acid	Protocatechoic acid	Homovanillic acid
Mac1	5.84±0.09 ^{a}	4.93±0.14 ^{bcd}	81.79±1.43 ^{ef}	-	5.58±0.06 ^{ef}	3.60±0.14 ^{f}
Mac2	1.23±0.06 ^{f}	5.23±0.11 ^{ab}	85.03±0.28 ^{cd}	-	4.49±0.13 ^{fg}	4.01±0.13 ^{f}
Mac3	0.63±0.06 ^{g}	5.11±0.07 ^{abc}	82.80±0.32 ^{de}	-	6.97±0.24 ^{de}	4.49±0.05 ^{ef}
Mac4	1.32±0.03 ^{f}	4.97±0.09^{abcd}	86.12±0.12 ^{bc}	0.01±0.00 ^{c}	5.25±0.16 ^{f}	2.33±0.18 ^{g}
Mac5	2.91±0.10 ^{e}	4.20±0.15 ^{fg}	68.54±0.57 ^{h}	0.02±0.00 ^{b}	10.89±0.35 ^{c}	14.03±0.50 ^{b}
AY	2.58±0.20 ^{e}	4.40±0.14 ^{ef}	73.74±0.77 ^{g}	0.04±0.00 ^{a}	9.62±0.32 ^{c}	9.63±0.59 ^{c}
QW	1.07±0.08 ^{fg}	5.03±0.07^{abcd}	87.69±1.31 ^{b}	0.01±0.00 ^{d}	3.03±0.01^{gh}	5.28±0.18 ^{de}
CY	2.67±0.14 ^{e}	4.74±0.02 ^{de}	81.54±1.53 ^{ef}	-	7.80±0.26 ^{d}	6.04±0.61 ^{d}
NLY	3.78±0.27 ^{d}	1.72±0.07 ^{h}	30.54±1.16 ^{i}	-	46.10±1.70 ^{a}	18.26±0.44 ^{a}
NLW1	4.98±0.12 ^{c}	4.01±0.20 ^{g}	66.72±0.67 ^{h}	-	22.97±0.71 ^{b}	1.32±0.14 ^{h}
NLW2	1.34±0.01 ^{f}	5.31±0.08 ^{a}	90.51±0.17 ^{a}	-	2.60±0.14 ^{h}	-
NLW3	6.56±0.33 ^{a}	4.88±0.16 ^{cd}	79.71±0.60 ^{f}	-	5.66±0.40 ^{ef}	3.56±0.25 ^{f}

708
709 Values (nanomole percent by weight composition) represent means ± standard errors for four replicates. Means in the
710 same column accompanied by different superscripts are significantly different among chanterelles at $\alpha = 0.05$. Sample codes AY: *Cantharellus*
711 *betularum* (golden); CY: *C. camphoratus* (golden); Mac1–Mac5: individuals from a mixture of golden and white NL chanterelles; NLW1–3:
712 *C. enelensis* (Newfoundland, white 1–3); NLY: *C. enelensis* (Newfoundland, golden); QW: *C. enelensis* (Québec, white).
713

Table 4. Abundances of the volatile metabolites, expressed as area counts of the mass spectra base peak (bp) of each compound x10^{-6}, from the headspace of white (QW, NLW1-3) and golden (CY, NLY, AY) variants of Newfoundland chanterelles extracted by SPME and separated, identified and semi-quantified by GC/MS.

No	Compounds (MW)	Bp	QW	NLW1	NLW2	NLW3	CY	NLY	AY
	Aldehydes								
3	3-Methyl-butanal (86)	44	15.1±0.8^{abc}	12.3±2.02^{bc}	21.4±1.9^{ab}	5.3±0.2^{c}	24.2±7.3^{a}	12.9±0.5^{bc}	17.7±1.7^{ab}
6	Pentanal (86)	44	150.5±23.4^{a}	85.5±2.85^{b}	60.9±2.8^{bc}	44.6±1.9^{c}	56.0±0.4^{bc}	66.8±5.7^{bc}	53.2±1.7^{c}
10	Hexanal (100)	56	1107.5±148.8^{a}	620.9±38.9^{bc}	471.9±7.5^{cd}	502.0±2.9^{bcd}	476.8±84.5^{cd}	737.7±73.9^{b}	315.9±3.4^{d}
20	Heptanal (114)	70	105.4±18.4^{a}	37.8±4.6^{bcd}	31.6±0.9^{cd}	56.6±0.7^{bc}	24.8±8.1^{d}	62.62±8.30^{b}	17.8±0.8^{d}
22	2-Ethyl-2-pentenal (112)	55	5.0±1.1^{b}	7.6±0.6^{b}	7.0±2.2^{b}	5.6±1.3^{b}	4.1±0.4^{b}	16.59±2.89^{b}	47.8±9.3^{a}
26	2-Ethylhexanal (128)	57	0.8±0.2^{c}	6.3±0.1^{b}	1.1±0.6^{c}	0.4±0.1^{c}	2.5±1.0^{bc}	1.92±0.4^{c}	18.0±2.8^{a}
27	(*Z*)-2-Heptenal (112)	41	34.9±5.5^{b}	30.3±3.7^{b}	71.3±5.4^{a}	23.1±0.4^{b}	39.6±9.8^{b}	39.52±3.0^{b}	28.9±2.1^{b}
28	Benzaldehyde (106)	105	106.0±17.1^{bc}	57.6±9.9^{c}	181.0±25.9^{a}	58.9±9.8^{c}	59.4±24.9^{c}	68.5±16.7^{c}	150.9±12.8^{ab}
35	2-Ethyl-2-hexenal (126)	55	16.6±4.5^{d}	331.5±104.9^{bc}	112.2±40.3^{cd}	79.5±10.2^{cd}	28.1±12.9^{cd}	508.0±134.2^{b}	1420.7±166.0^{a}
49	(*E*)-2-Octenal (126)	70	23.9±4.0^{b}	20.2±1.50^{b}	72.6±13.77^{a}	34.5±0.3^{b}	19.8±4.3^{b}	26.6±1.4^{b}	21.2±2.0^{b}
72	(*E*)-2-nonenal (140)	41	5.8±0.5^{e}	10.9±0.5^{bc}	19.1±0.7^{a}	11.9±0.5^{b}	9.01±0.6^{cd}	8.7±0.8^{d}	6.1±0.1^{e}
75	2-Propyl-2-heptenal (154)	55	43.0±4.1^{e}	462.1±54.7^{bc}	102.5±23.4^{de}	269.3±20.5^{cd}	67.1±16.7^{de}	509.2±55.2^{b}	1331.0±144.8^{a}
77	2-Propyl-2-heptenal (isomer) (154)	55	5.5±0.4^{c}	18.4±1.9^{b}	5.0±1.4^{c}	21.2±2.2^{b}	17.4±3.5^{b}	16.7±1.9^{b}	29.5±3.2^{a}
78	2-Propyl-2-heptenal (isomer) (154)	55	35.4±3.5^{e}	286.1±30.3^{bc}	68.7±11.9^{de}	173.3±11.9^{cd}	40.5±8.8^{e}	313.1±29.1^{b}	892.8±90.5^{a}
111	2-Butyl-2-octenal (182)	55	124.4±6.5^{de}	159.6±7.0^{c}	109.9±6.9^{e}	437.8±7.8^{a}	145.2±7.0^{cd}	246.1±8.9^{b}	463.4±12.3^{a}
	Ketones								
4	2-Pentanone (86)	43	47.4±3.9^{ab}	48.1±2.3^{a}	53.6±8.5^{a}	9.3±0.2^{c}	13.7±1.2^{c}	35.6±0.0^{b}	41.2±0.8^{ab}
7	2-Hexanone (100)	43	50.2±3.7^{a}	14.8±0.3^{cd}	46.3±5.5^{a}	8.4±1.0^{d}	26.3±3.1^{b}	13.2±0.1^{cd}	20.8±0.1^{bc}
16	2-Heptanone (114)	43	73.5±18.6^{ab}	71.1±17.5^{ab}	58.1±15.1^{b}	107.7±10.9^{ab}	54.6±24.7^{b}	126.5±30.1^{a}	115.2±15.7^{ab}
24	3-Hepten-2-one (112)	55	8.4±1.2^{c}	39.2±7.9^{bc}	62.7±14.7^{ab}	82.4±12.8^{ab}	8.2±3.1^{c}	82.1±21.3^{ab}	100.7±18.4^{a}
29	4-Octanone (128)	57	4.0±0.0^{b}	10.8±3.5^{b}	6.0±2.6^{b}	9.7±3.0^{b}	0.1±0.0^{b}	31.4±9.4^{a}	37.9±7.9^{a}
31	1-Octen-3-one (126)	55	23.9±1.2^{cd}	31.7±1.2^{cd}	115.6±17.4^{a}	73.5±9.6^{b}	7.1±0.7^{d}	36.9±2.3^{c}	11.3±0.6^{d}

33	Methyl-5-hepten-2-one (isomer) (126)	43	107.8±29.3^{b}	52.3±16.3^{b}	67.6±31.9^{b}	479.7±130.0^{a}	51.3±25.5^{b}	121.2±35.5^{b}	45.1±7.9^{b}
42	3-Octen-2-one (126)	55	625.2±114.5^{b}	299.7±58.5^{cd}	318.2±77.5^{bcd}	974.8±114.2^{a}	210.3±87.4^{d}	489.6±88.4^{bcd}	544.6±103.1^{bc}
44	2-Nonanone (142)	43	10.7±1.6^{b}	13.8±3.0^{b}	8.86±2.30^{b}	24.9±1.1^{ab}	51.9±24.7^{a}	24.7±4.8^{ab}	38.4±5.9^{ab}
54	4-Nonanone (142)	43	5.8±1.0^{d}	55.9±8.1^{bcd}	28.7±10.8^{cd}	70.4±15.9^{bc}	6.8±3.4^{d}	88. 7±10.7^{b}	149.7±33.4^{a}
55	2-Methyl-3,1methyethyl-cyclopentanone (isomer) (140)	55	9.7±0.3^{c}	64.1±13.9^{bc}	16.6±5.9^{c}	42.5±5.8^{bc}	8.2±3.9^{c}	85.3±18.9^{b}	250.8±43.2^{a}
57	3-Nonanone (142)	72	31.3±2.8^{b}	16.4±0.3^{cd}	10.5±2.8^{d}	58.8±4.6^{a}	12.6±1.9^{d}	18.6±1.7^{cd}	24.2±3.8^{bc}
63	2,5-Octanedione (142)	43	2.5±0.2^{de}	18.3±1.2^{b}	7.1±0.2^{cd}	25.3±3.0^{a}	1.6±0.4^{e}	7.6±2.6^{cd}	12.4±0.7^{c}
69	3-Nonen-2-one (140)	55	8.5±0.8^{e}	23.6±1.5^{cd}	18.9±4.1^{de}	62.3±3.8^{a}	17.3±4.3^{de}	32.6±3.5^{c}	45.7±1.9^{b}
74	5-Decanone (156)	43	10.0±0.6^{b}	20.6±2.0^{b}	17.6±4.0^{b}	50.4±7.1^{a}	8.4±2.2^{b}	41.6±5.0^{a}	38.7±0.6^{a}
95	Cyclodecanone (182)	98	6.9±0.3^{f}	8.9±0.4^{ef}	11.7±1.1^{d}	29.5±1.1^{a}	9.3±0.3^{e}	14.8±0.3^{c}	23.4±0.2^{b}
102	2-Undecanone (170)	58	57.2±2.9^{f}	208.3±10.9^{d}	157.1±8.6^{e}	398.8±13.5^{a}	136.5±12.8^{e}	289.4±11.5^{c}	341.8±16.3^{b}
106	5-Undecen-4-one (*)(204)	55	0.1±0.1^{g}	49.6±2.9^{e}	31.5±2.9^{f}	84.7±1.2^{c}	191.8±0.3^{b}	70.2±1.0^{d}	223.1±7.4^{a}
	Terpenes								
52	(*Z*)-Linalool oxide (170)	59	8.5±0.8^{cd}	12.8±1.4^{bc}	4.9±0.7^{d}	19.8±0.1^{a}	8.9±2.6^{cd}	12.0±1.2^{bc}	14.9±1.7^{b}
80	*β*-Cyclocitral (152)	81	8.5±1.2^{d}	11.1±1.0^{cd}	20.7±1.2^{bc}	11.7±1.9^{cd}	52.2±7.5^{a}	28.0±1.5^{b}	19.5±0.6^{bc}
99	γ-Diosphenol (168)	55	25.9±1.5^{de}	36.3±2.5^{d}	19.2±2.1^{e}	85.8±3.1^{b}	34.6±4.2^{d}	57.3±3.8^{c}	128.3±9.4^{a}
118	(*E*)-α-Ionone (192)	121	1.7±0.2^{d}	0.3±0.0^{d}	1.0±0.1^{d}	0.4±0.0^{d}	223.3±5.1^{a}	122.3±0.8^{b}	58.9±1.8^{c}
119	α-Ionone epoxide (208)	121	1.3±0.1^{c}	0.7±0.0^{c}	2.9±0.2^{c}	1.5±0.0^{c}	222.2±5.6^{a}	213.1±3.9^{a}	147.2±4.5^{b}
121	3-Hydroxy-α-ionene (208)	165	0.2±0.0^{f}	1.8±0.1^{d}	1.7±0.1^{d}	4.0±0.1^{b}	0.8±0.1^{e}	2.9±0.1^{c}	6.8±0.1^{a}
124	Di-epi-1,10-cubenol (222)	105	0.7±0.1^{e}	1.3±0.2^{de}	17.5±0.8^{a}	3.2±0.7^{b}	2.8±0.2^{bc}	1.8±0.1^{cde}	2.6±0.2^{bcd}
125	Cubenol (222)	161	0.1±0.0^{d}	0.3±0.1^{cd}	0.7±0.1^{b}	0.3±0.0^{d}	1.4±0.1^{a}	0.5±0.0^{c}	0.9±0.1^{b}

Values (means ± standard errors; n = 2) represent the abundances, expressed as area counts of their mass spectra base peak (Bp) divided by 10^6. Rows with different letters show significant differences between treatments at α = 0.05. (*) Tentatively identified; Bp: base peak; MW: molecular weight; Sample codes AY: Cantharellus betularum (golden); CY: C. camphoratus (golden); NLW1–3: C. enelensis (Newfoundland, white 1–3); NLY: C. enelensis (Newfoundland, golden); QW: C. enelensis (Québec, white).

Table 5. PCR primers designed to amplify portions of the phytoene desaturase gene (*Al-1*) and phytoene synthase gene (*Al-2*) from *Cantharellus* species, based on genomic sequences from *Cantharellus appalachiensis*, *C. cibarius*, and *C. cinnabarinus*[18], listed in Methods.

Gene	Primer Name	Sequence	Product Size
Phytoene desaturase (*Al-1*)	Al-1-F1	CACCGARAAGASTCACAGAARCCC	838
Phytoene desaturase (*Al-1*)	Al-1-R1	TGTSGTGTTGGTGCCTGTTGG	bp
Phytoene desaturase (*Al-1*)	Al-1-F2	GCACCACGRTCGAGGTTGAAC	996
Phytoene desaturase (*Al-1*)	Al-1-R2	CGTTYACATTCGCCTCSATGTAC	bp
Phytoene desaturase (*Al-1*)	Al-1-F3	TGGCAAAACCWCCGATAGGGTACC	1169
Phytoene desaturase (*Al-1*)	Al-1-R3	AGTCGCCATGATATCTGCGG	bp
Phytoene synthase (*Al-2*)	Al-2-F1	GTAACGAGGGTAGACCAGGC	1071
Phytoene synthase (*Al-2*)	Al-2-R1	CCTAGGTATGCCTTTTGCCG	bp
Phytoene synthase (*Al-2*)	Al-2-F2	AAATGCGAGCCTTCCTGTCC	919
Phytoene synthase (*Al-2*)	Al-2-R2	GAACAGGTAGCGGTGCATGG	bp
Phytoene synthase (*Al-2*)	Al-2-F3	ACGACGCACTCKAYGTCGAGATG	862
Phytoene synthase (*Al-2*)	Al-2-R3	RGATTACGATTTGGTGTASGTGACATG	bp

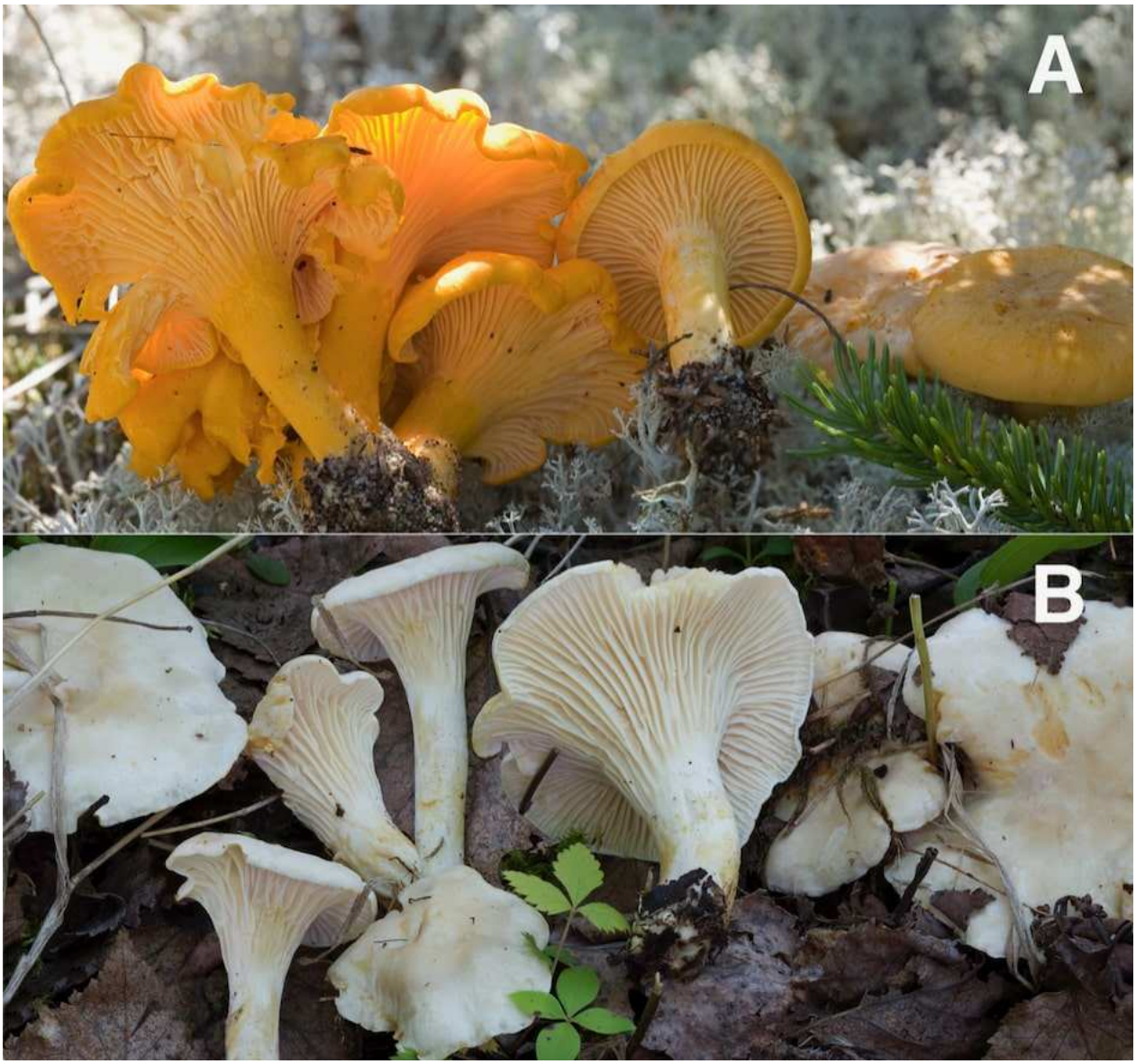
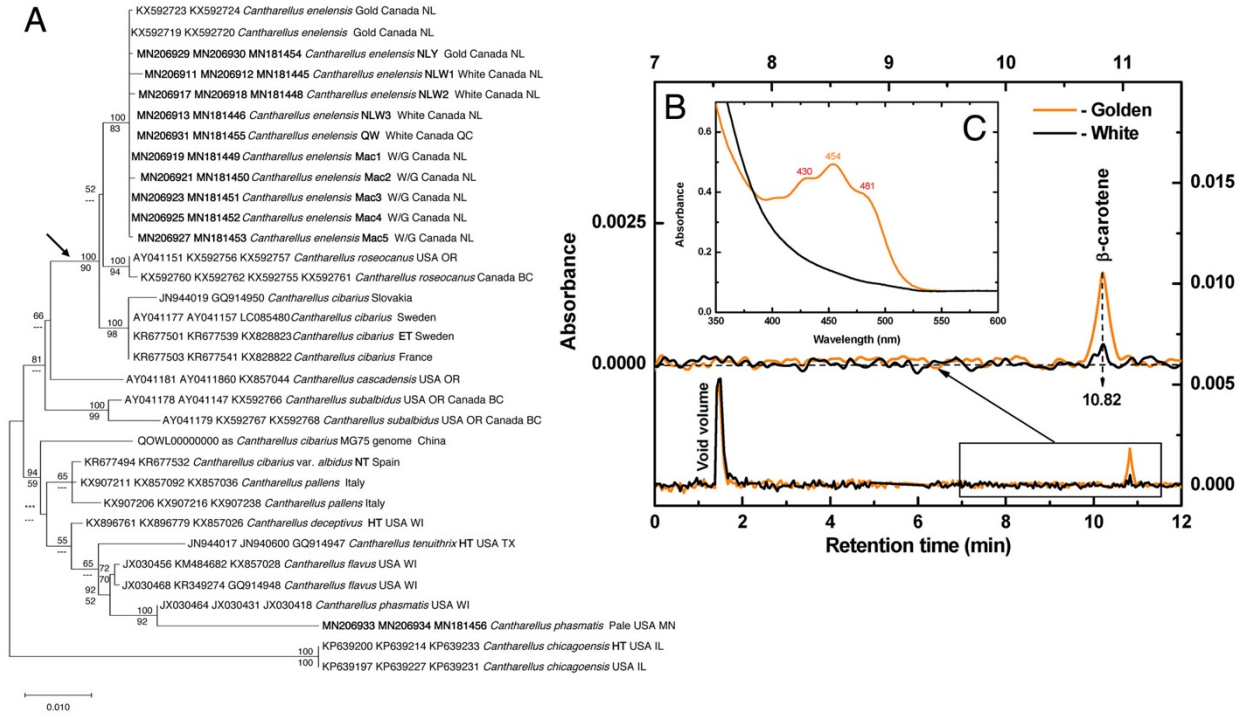


Figure 1.



728

729 Figure 2.

730

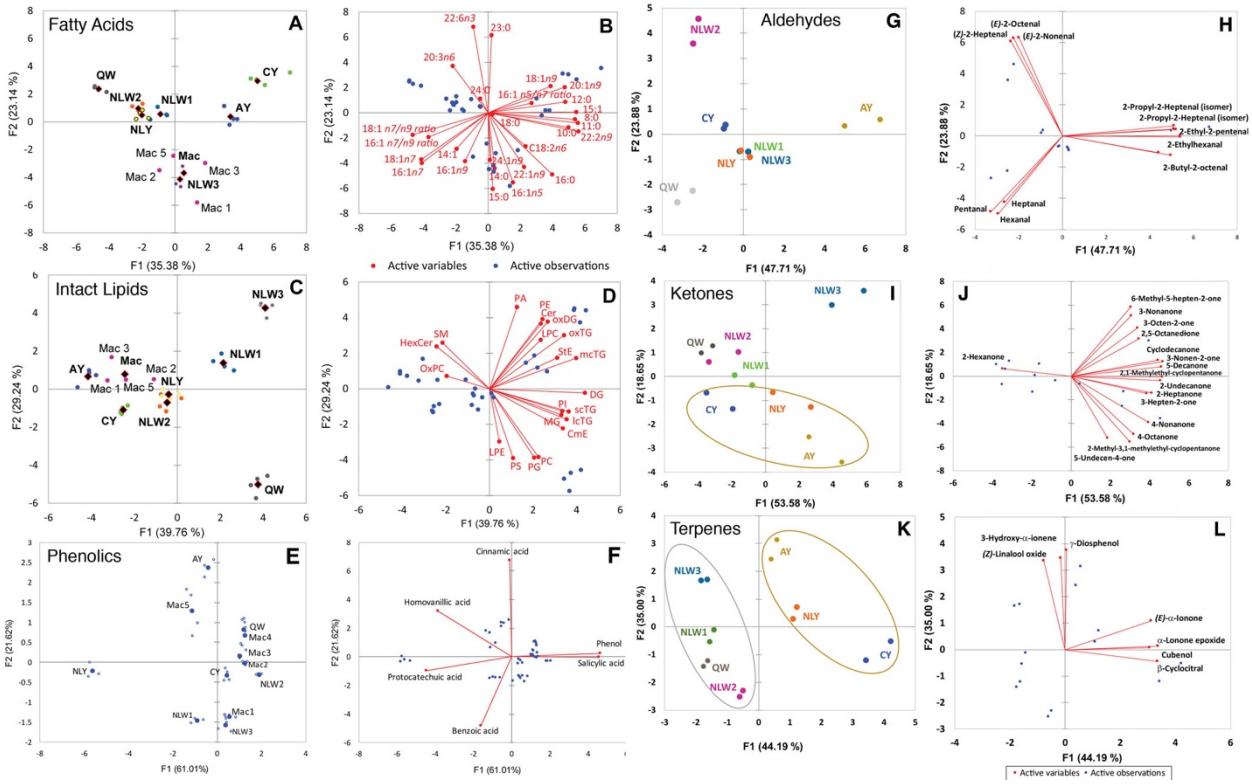

Fatty Acids
A
QW
CY
NLW2
NLW1
AY
NLY
Mac 5
Mac
Mac 3
Mac 2
NLW3
Mac 1
F1 (35.38 %)
F2 (23.14 %)
B
22:6n3
23:0
20:3n6
18:1n9
20:1n9
24:0
16:1 n5/n7 ratio
12:0
15:1
8:0
18:0
11:0
10:0
22:2n9
18:1 n7/n9 ratio
16:1 n7/n9 ratio
C18:2n6
18:1n7
14:1
24:1n9
16:1n9
14:0
22:1n9
16:0
15:0
16:1n5
Active variables
Active observations
Aldehydes
G
NLW2
CY
AY
NLW1
NLY
NLW3
QW
F1 (47.71 %)
F2 (23.88 %)
H
(E)-2-Octenal
(Z)-2-Heptenal
(E)-2-Nonenal
2-Propyl-2-Heptenal (isomer)
2-Propyl-2-Heptenal (isomer)
2-Ethyl-2-pentenal
2-Ethylhexanal
2-Butyl-2-octenal
Pentanal
Heptanal
Hexanal
Intact Lipids
C
NLW3
Mac 3
Mac
Mac 2
AY
NLW1
Mac 1
Mac 5
NLY
CY
NLW2
QW
F1 (39.76 %)
F2 (29.24 %)
D
PA
PE
Cer
oxDG
LPC
oxTG
SM
HexCer
StE
mcTG
OxPC
DG
PI
scTG
MG
lcTG
CmE
LPE
PS
PG
PC
F1 (39.76 %)
F2 (29.24 %)
Ketones
I
NLW3
QW
NLW2
NLW1
CY
NLY
AY
F1 (53.58 %)
F2 (18.65 %)
J
6-Methyl-5-hepten-2-one
3-Nonanone
3-Octen-2-one
2,5-Octanedione
Cyclodecanone
3-Nonen-2-one
5-Decanone
2,1-Methylethyl-cyclopentanone
2-Undecanone
2-Heptanone
3-Hepten-2-one
2-Hexanone
4-Nonanone
4-Octanone
2-Methyl-3,1-methylethyl-cyclopentanone
5-Undecen-4-one
F1 (53.58 %)
F2 (18.65 %)
Phenolics
E
AY
Mac5
QW
Mac4
Mac3
Mac2
NLY
CY
NLW2
NLW1
Mac1
NLW3
F1 (61.01%)
F2 (21.62%)
F
Cinnamic acid
Homovanillic acid
Phenol
Salicylic acid
Protocatechuic acid
Benzoic acid
F1 (61.01%)
F2 (21.62%)
Terpenes
K
NLW3
AY
NLY
NLW1
QW
CY
NLW2
F1 (44.19 %)
F2 (35.00 %)
L
3-Hydroxy-α-ionene
γ-Diosphenol
(Z)-Linalool oxide
(E)-α-Ionone
α-Lonone epoxide
Cubenol
β-Cyclocitral
F1 (44.19 %)
F2 (35.00 %)
Active variables
Active observations

Figure 3.

Figures

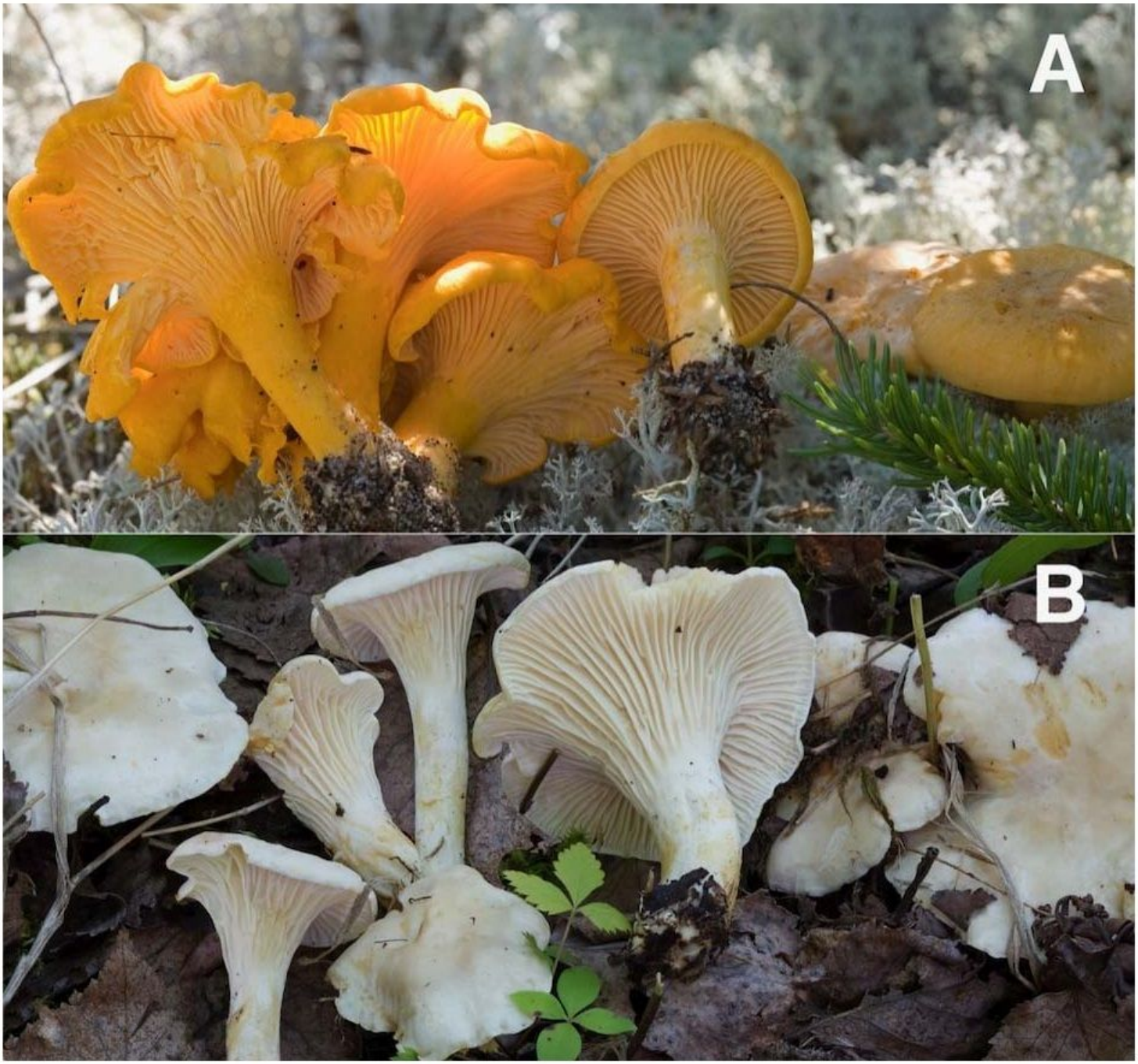


Figure 1

Typical and albino forms of Cantharellus enelensis. A. Typical form, with golden-orange colouration (henceforth referred to as gold or golden). B. The albino form, C. enelensis f. acolodorus (17.08.15.av01; NLW3 in chemical analyses), showing buffy yellow staining in age or on handling. When dried, the two forms are morphologically indistinguishable. Photos: Andrus Voitk.

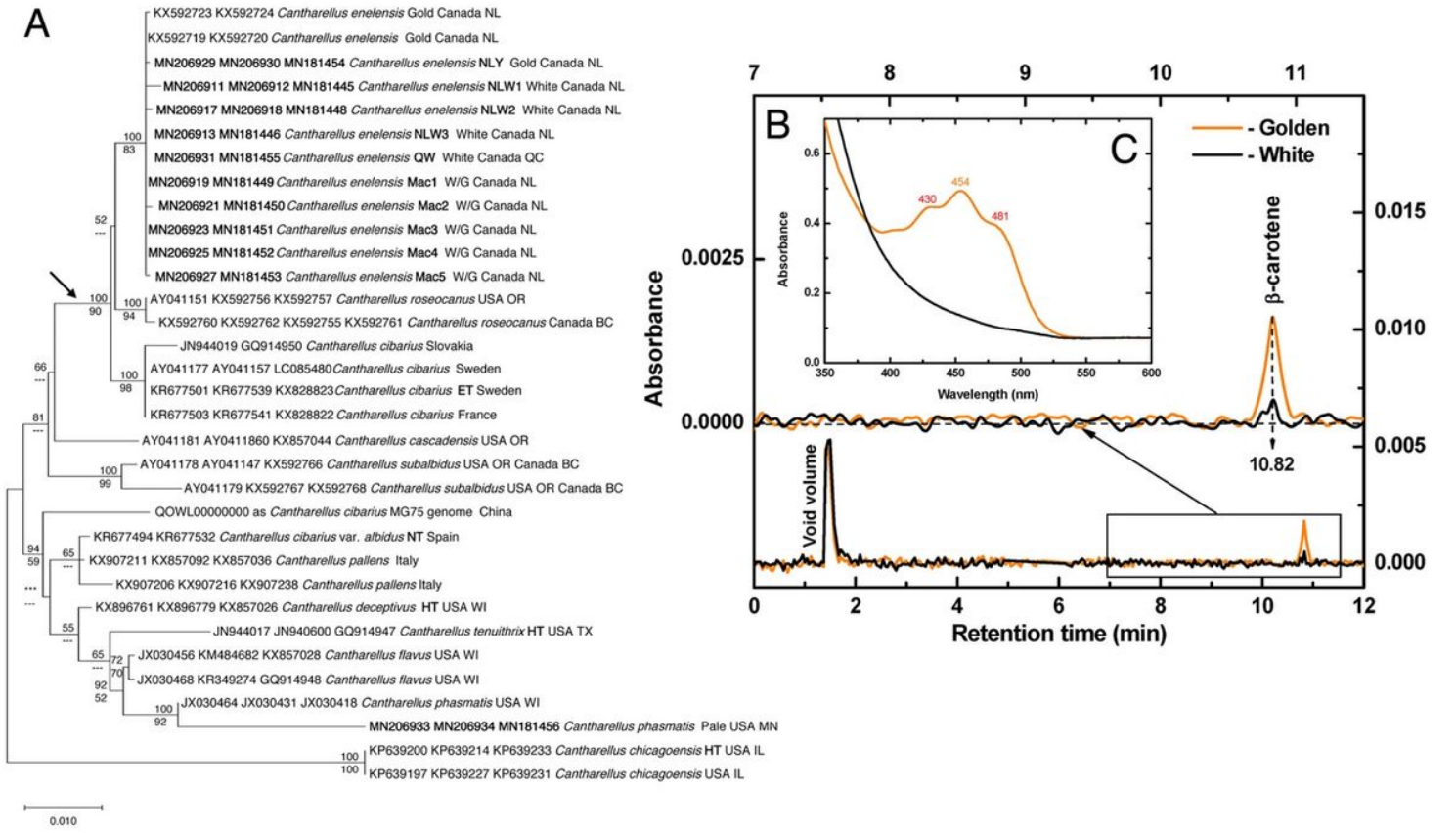


Figure 2

White chanterelles from Newfoundland and Québec are members of the species Cantharellus enelensis and are distinguished by their lack of β-carotene. A. Maximum likelihood phylogeny of white and golden representatives of Cantharellus enelensis, related species of the core C. cibarius clade (arrow) and its sister group, the clade including C. pallens through C. phasmatis, rooted with C. chicagoensis. The tree is based on sequences from nuclear ribosomal internal transcribed spacer (ITS), large subunit (LSU) and translation elongation factor 1-alpha (Tef1) regions. All sequences are identified by the GenBank accession numbers and the name they were deposited under, and new sequences obtained in this study are indicated in bold font. Sequences from type specimens are indicated with HT (holotype), NT (neotype) or ET (epitype). Table 1 provides collection details for samples used in chemical analyses; their specimen codes used in Fig. 3 are included in bold following the species name in this tree. Node support values (%) are provided from a Bayesian inference analysis (posterior probabilities, above nodes) and a 1000x maximum likelihood bootstrap analysis (below nodes). Nodes with less than 50% support are shown by dashes, and a single node that collapsed in Bayesian analysis is shown by asterisks. B–C. Representative HPLC chromatograms and absorbance spectra of pigments extracted from white and golden variants of Cantharellus enelensis. B. Representative HPLC chromatograms, the upper traces showing an enlargement of the area of interest. C. Absorbance spectra of acetone extracts.

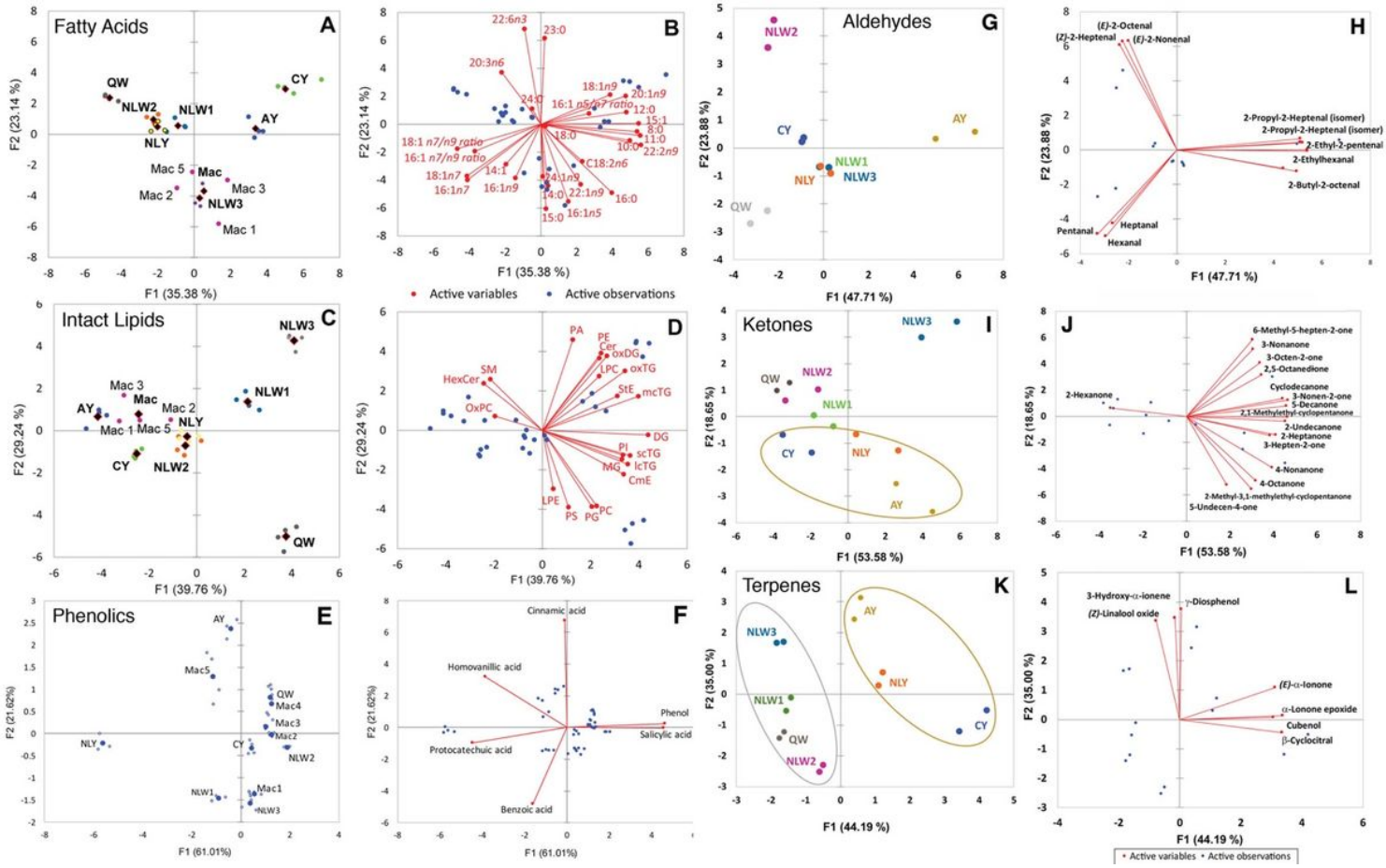


Figure 3

Principal components analysis (PCA) of chemical constituents of white and golden variants of Newfoundland chanterelles, showing the observations (sample clustering) and biplots showing loadings of chemical variables. A–B, fatty acids; C–D, intact lipids; E–F, phenolics. G–L, volatile compounds detected by headspace solid phase microextraction tandem mass spectrometry (HS-SPME-MS/MS). G–H, aldehydes; I–J, ketones (ellipse highlighting the golden chanterelles); K–L, terpenes (ellipses highlighting the white and golden chanterelles). Sample codes AY: Cantharellus betularum (golden); CY: C. camphoratus (golden); Mac1– Mac5: individuals from a mixture of golden and white NL chanterelles; NLW1–3: C. enelensis (Newfoundland, white 1–3); NLY: C. enelensis (Newfoundland, golden); QW: C. enelensis (Québec, white). For identities of fatty acids and intact lipids, see Table 2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigS1new.pdf