Phylogenetic analyses of Japanese golden chanterelles and a new species description, *Cantharellus anzutake* sp. nov.

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Text: 28 pages; tables: 3; figures: 5; supplementary figures: 2

Abstract

The Japanese golden chanterelle commonly identified as *Cantharellus cibarius* was sampled in a broad range of forest vegetation. A total of 90 fresh and 11 herbarium specimens were examined microscopically, subjected to sequencing analysis of their nuclear ribosomal RNA (rDNA) and *tef-1* genes, and their characteristics were compared with those of European *C. cibarius*. Based on morphological and ecological characteristics, basidioma samples from Japan were divided into four species. While specimens of *Cantharellus* sp. 4 from Hokkaido Island were included in the European *C. cibarius* clade phylogenetically, the other three species formed three unique clades. Among these, *Cantharellus anzutake* sp. nov. is sister to the clade of *C. cibarius* and was widely sampled from the northern limit of Honshu Island to the southern limit of Kumejima Island in Ryukyu Islands. Although *C. anzutake* was morphologically similar to *C. cibarius*, the two species were phylogenetically distinct. Other morphologically similar but genetically distinct chanterelle species from India exhibited macroscopic and microscopic differences compared with *C. anzutake*.

Keywords

Biogeography, Chanterelle, Cryptic species, Edible ectomycorrhizal mushrooms

1. Introduction

Chanterelles are one of the most important edible ectomycorrhizal mushrooms in the world, with a large market share. Especially, *Cantharellus cibarius* Fr. and several similar species known as yellow chanterelles have high commercial value, likely in excess of a billion dollars annually (Watling 1997; Hall et al. 2003). Therefore, it is important to identify true species of chanterelles for market supply. However, their taxonomy remains confusing. Over 400 species of *Cantharellus* (Hydnaceae, Cantharellales; Hibbett et al. 2014) were described by the end of the 20th century, but only approximately 60 species were considered valid (Eyssartier and Buyck 2000). Over the past 15 y, approximately 70 new *Cantharellus* species have been described (Buyck 2016), and approximately 130 *Cantharellus* species are considered valid (Buyck et al. 2014; Buyck 2016). *Cantharellus cibarius*, the type species of this genus, was first described by Fries (1821), as follows: "vitellinus, pileo carnoso subrepando glabro, plicis tumidis, stipites solido deorsum attenuato." As the description is vague and could include many chanterelle species, a more detailed description of *C. cibarius* by epitypification was adopted recently (Olariaga et al. 2016). Several fleshy yellow and more or less closely related chanterelle species have been described based on staining upon bruising, size, and shape of basidiomata, as well as on the ecology and habitat of the species (Buyck et al. 2016b; De Kesel et al. 2016), all of which approximately match the description by Fries (1821). Recently, molecular methods, such as sequence comparisons of the nuclear ribosomal RNA (nrDNA) or translation elongation factor EF1-alpha (*tef-1*) genes, have been applied to identify *C. cibarius* sensu stricto and its related species (Buyck et al. 2016a, b, c).

Cantharellus cibarius sensu lato was previously believed to have a cosmopolitan distribution not only in the Northern Hemisphere, but also in Central Africa (Pilz et al. 2003). Therefore, many varieties and forms of *C. cibarius* have been described, all essentially from

Europe and North America (Hansen and Knudsen 1997; Pegler et al. 1997; Persson 1997; Eyssartier and Buyck 2000; Pilz et al. 2003). Recent molecular phylogenetic studies of yellow chanterelles revealed their true nature (Buyck et al. 2016b; Olariaga et al. 2016). European golden chanterelle, i.e., *C. cibarius* sensu stricto, is distributed mainly in the northern and central parts of Europe (Buyck et al. 2014; Olariaga et al. 2016). In addition, several species of fleshy yellow chanterelles, e.g., *Cantharellus roseocanus* Redhead, Norvell & Moncalvo, *Cantharellus cascadensis* Dunham, O'Dell & R. Molina, *Cantharellus formosus* Corner, *Cantharellus californicus* Arora & Dunham, and *Cantharellus tenuithrix* Buyck & V. Hofst., have been described from North America over the past two decades, most of which were previously considered as conspecific to European *C. cibarius* (Dunham et al. 2003a, b; Arora and Dunham 2009; Buyck and Hofstetter 2011; Buyck et al. 2011, 2016a, b, c; Foltz et al. 2012; Redhead 2012; Leacock et al. 2016). In Asia, three new species, *Cantharellus applanatus* Deepika, Ram. Upadhyay & Mod.S. Reddy, *Cantharellus elongatipes* Deepika, Ram. Upadhyay & Mod.S. Reddy, and *Cantharellus natarajanii* Ram. Upadhyay & Mod.S. Reddy, were described from India as cryptic species morphologically similar but distinguishable from European *C. cibarius* (Deepika et al. 2014). In addition, two endemic, fleshy, yellow, and ridged hymenium *Cantharellus* species, possibly belonging to the subgenus *Cantharellus*, have been described from China (Shao et al. 2011, 2016a, b; Tian et al. 2012): *Cantharellus yunnanensis* W.F. Chiu (Chiu 1973) and *Cantharellus tuberculosporus* M. Zang (Zang 1980). However, *C. cibarius* s.s. and its closely related species cannot be distinguished from one another based on morphological features alone (Feibelman et al. 1994, 1996; Dunham et al. 2003a, b; Arora and Dunham 2009; Olariaga et al. 2015; Buyck et al. 2016b; Olariaga et al. 2016).

In Japan, *C. cibarius* was first reported by Hennings (1900) without providing any morphological description or specimen designation. The species description was first

conducted by Kawamura (1908) based on a specimen sampled in Jul 1908 under a *Pinus densiflora* Sieb. & Zucc. forest of Mt. Eimeiji-yama, Nagano Prefecture, in the central region of Honshu Island. Unfortunately, the described specimen was lost after the death of Kawamura in 1946. Since then, the Japanese fleshy and yellowish chanterelles sampled in Hokkaido, Honshu, Shikoku, Kyushu Islands, and Ryukyu Islands have been identified consistently as *C. cibarius* (Ito 1955; Kawamura 1955; Imazeki and Hongo 1989; Katsumoto 2010; Tanabe and Ogawa 2015).

It is generally known that the islands of Japan range in latitude between N 25–45° and host varied forest vegetation, from boreal and subalpine in Hokkaido Island to subtropical and tropical in Ryukyu Islands (Peel et al. 2007). Therefore, we hypothesized that the known Japanese *C. cibarius* population includes some cryptic species, as has been noted among North American yellow–golden chanterelles. In fact, the fruiting season of Japanese *C. cibarius* differs according to the geographic region: April and May in Ryukyu Islands (range of maximum temperature: 25–30 °C), Jul to Oct in Nagano (20–30 °C), and Sep to Oct in Hokkaido (15–25 °C). Therefore, it is important to classify Japanese fleshy yellowish chanterelles based on their ecology, morphology, and molecular phylogeny. In the present study, we examined the taxonomy of a probable species complex of known Japanese *C. cibarius* based on macroscopic and microscopic morphological observations and molecular phylogenetic analysis. Here, we present the nature of the Japanese *C. cibarius* species complex and describe a new Japanese golden chanterelle species.

2. Materials and Methods

2.1. Specimens examined

Basidiomata of fleshy yellowish chanterelles were collected from various forest sites in Japan from 2010 to 2014 (Table 1). Fresh Swedish *C. cibarius* basidiomata specimens were gifts from Niclas Bergius. The macroscopic features of these basidiomata, such as the size, shape, color, and texture, were recorded. They were then freeze-dried, oven-dried at 70 °C for one night to inactivate DNase and other oxidative enzymes, and stored in the laboratory as dried specimens. In addition, dried voucher specimens of Japanese *C. cibarius* were obtained from the herbaria of the Rishiri Town Museum (RTMFU), Natural History Museum of Hokkaido University (SAPA), National Museum of Natural Science, Tokyo (TNS), and Tottori Mycological Institute (TMI).

2.2. Microscopic observations of selected specimens

A portion of hymenium tissue was rehydrated in a drop of 1% KOH and then in distilled water for 1 h to prepare basidiospores for microscopy, which were mounted with lactic acid on a slide and observed under a differential interference contrast microscope (AXIO Imager A1, Carl Zeiss Inc., Göttingen) using an immersion ×100 objective lens. Preparation of specimens for microscopic examination and description of hyphal structure followed Largent et al. (1977) and Clémençon (2009, 2012). Fluorescent microscopic analysis was performed to assess the presence/absence of autofluorescence of hyphal structures using UV irradiation (Agerer 1990). Some samples from dried specimens were preliminarily rehydrated in 70% ethanol and subsequently in distilled water to confirm the effects of the KOH solution on the wall structure and some fragile elements on and inside the cells. Fifty spores of each specimen were measured for their length, width, and shape (length/width). Basidia, pileipellis, hymenophoral trama, and stipitipellis were examined microscopically and measured. Numerical data of the microscopic structures were measured using Image-J software after

taking a photograph of the structures. The obtained numerical data were statistically analyzed using one-way ANOVA or Student's *t* test with Kaleida Graph ver. 4.0 (Hulinks, Tokyo). Morphological identification of Japanese *C. cibarius* sensu Kawamura was performed as described by Kawamura (1955) and Imazeki and Hongo (1989). Two voucher specimens, C-84 (TNS-F-61926) and C-85 (TNS-F-61927), were deposited in the herbarium of the National Museum of Nature and Science, Tokyo, Japan.

2.3. Molecular phylogenetic analyses

Genomic DNA was extracted from basidioma specimens as described by Gardes and Bruns (1993) with minor modifications. Briefly, a portion of the extracted total DNA was used for PCR amplification of the internal transcribed spacer (ITS) region, the large subunit (LSU) locus of nrDNA, and the *tef-1* locus. The ITS region including 5.8S rDNA was amplified using the previously described primer ITS1F (Gardes and Bruns 1993) and the novel *Cantharellus*-specific primer C28S (5'-cactgacggcctattgtactt-3'). The LSU locus was amplified using the novel *Cantharellus*-specific primers C2F (5'-tgaccgtcataggtgctttg-3') and Tw14 (White et al. 1990). The *tef-1* locus was amplified as described by Morehouse et al. (2003). PCR amplifications were conducted using the GeneAmp PCR System 2700 (Applied Biosystems, Foster). The 25 µL PCR mixture consisted of 2.5 µL 10× DreamTaq buffer, 2.5 µL 0.2 mM dNTP, 2.5 µL 0.5 µM each primer, 0.125 µL 0.625 U DreamTaq DNA polymerase (Thermo Scientific, Carlsbad), and 0.5 µL extracted genomic DNA solution. The PCR reaction consisted of an initial denaturation step at 95 °C for 3 min, 35 amplification cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The PCR products were electrophoresed on 3% agarose gels for 1 h, stained with ethidium bromide, and visualized

under UV light. The targeted amplicons were purified using QIAquick PCR Purification Kits (Qiagen, Hilden). The purified PCR products corresponding to the ITS1 and *tef-1* regions were cloned using the TaKaRa Mighty TA-cloning kit (TaKaRa Bio Inc., Kusatsu) and inserted into JM109 chemically competent *Escherichia coli* cells (TaKaRa Bio Inc.). Up to 10 positive colonies harboring inserts of the expected size were chosen randomly for sequencing. Sequencing was performed using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). For the sequencing reactions, primers ITS1, ITS2, ITS3, and ITS4 (White et al. 1990) were used for the ITS region, Ctb6 and LR5F were used for LSU (White et al. 1990; Tedersoo et al. 2008), and tef-1F and tef-1R were used for *tef-1* (Morehouse et al. 2003). The samples were sequenced using the Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems).

Sequences from forward and reverse primers were assembled, and the resulting consensus sequences in each region were aligned respectively using MUSCLE (Edger 2004) within MEGA 6.06 (Tamura et al. 2013). Alignment gaps were treated as missing data, and ambiguous positions were excluded from the analysis. Datasets of ITS1 (1264 bp), ITS2 (610 bp), LSU (842 bp), and *tef-1* (832 bp) were prepared. Phylogenetic relationships between sequences were inferred using maximum-likelihood (ML) and Bayesian inference (BI) analyses. The best-fit evolutionary model was selected based on the Bayesian information criterion (BIC) scores generated in MEGA. The Kimura 2-parameter model (K2) of nucleotide substitution, with a discrete gamma distribution (+G) was chosen for ML and BI analyses. ML trees were constructed using MEGA 6 with 1000 bootstrap replicates. BI was performed using MrBayes 3.2.1 (Ronquist et al. 2012). Two runs with four chains of Markov chain Monte Carlo iteration were performed for 1,000,000 generations when the average standard deviations of split frequencies were below 0.01 (the first 25% of generations were treated as burn-in). Trees were kept for every 100 generations and the latter 75% of the trees

were used to calculate the 50% majority-rule consensus topology and to determine the posterior probabilities (PP) for individual branches. Also, for the ML and BI analyses, the combined dataset (1438 bp) was partitioned into three partitions following Olariaga et al. (2015): 5.8S (87 bp), ITS2 (458 bp), and LSU (893 bp). The ML analyses of the combined datasets were carried out using MEGA 6.06 with 1000 bootstrap replicates.

3. Results

3.1. Distinction of Japanese fleshy golden chanterelles by morphotype

The majority of fleshy yellowish chanterelle basidiomata sampled in the present study were slightly slender compared with Swedish *C. cibarius* specimen C-122, when using fresh materials. Based on the fruiting season, habitat, discoloration when bruised, and microscopic characteristics, these Japanese yellowish chanterelles were divided into four morphotypes (Table 2): Type 1 (*Cantharellus* sp. 1), Type 2 (*Cantharellus* sp. 2), Type 3 (*Cantharellus* sp. 3), and Type 4 (*Cantharellus* sp. 4). Basidiomata of *Cantharellus* sp. 1 (Fig. 1A) were consistent with the description and illustration of Japanese *C. cibarius* sensu Kawamura (1908, 1955), except for the discoloration to weak reddish brown reaction when bruised and dried, the sometimes smaller size of basidiomata, and the whitish stipe compared with European *C. cibarius*. Basidiomata of *Cantharellus* sp. 2 were ocher-yellow, and the cap margin was more undulate compared with *Cantharellus* sp. 1. Basidiomata of *Cantharellus* sp. 3 were similar to *Cantharellus* sp. 1, except that the tissue surface turned reddish when bruised, similar to European *Cantharellus ferruginascens* P. D. Orton (Orton 1969), and the caps of mature basidiomata were thin and fragile. Another type, *Cantharellus* sp. 4, was externally more massive than *Cantharellus* sp. 1, bright- to orange-yellow, and similar to European *C.*

cibarius, with discoloration to weak reddish brown when bruised (Corner 1966; Pegler et al. 1997). *Cantharellus* sp. 1 specimens were collected from middle to lowland areas of Honshu Island and Ryukyu Islands from May to Aug. The habitats ranged from cool temperate to subtropical forests and included pines, oaks, spruces, firs, and birch vegetation. *Cantharellus* sp. 2 specimens were only collected from subalpine coniferous forests with volcanic rocky and sandy soil in Sep. *Cantharellus* sp. 3 specimens were collected from only three forest sites: cool temperate mixed forests or subalpine fir forests. *Cantharellus* sp. 4 specimens were collected from subalpine fir forests of Rishiri Island.

3.2. Phylogenetic analyses based on rDNA and tef-1 *sequences*

Phylogenetic trees of 5.8S-ITS2-LSU (Fig. 2) and *tef-1* (Fig. 3) showed that all four Japanese *Cantharellus* species were positioned in different clades. The 5.8S-ITS2-LSU tree revealed that *Cantharellus* sp. 1 formed a sister clade to Indian *C. natarajanii* and *C. applanatus*, together with Indian *C. elongatipes*, with moderate support. *Cantharellus* sp. 4 belonged to the clade of European *C. cibarius* with strong support. Specimens of "*C. cibarius"* SAPA-5065 from Hokkaido Island ("*C. cibarius*" aff. sp. 4) were sequenced with respect to the ITS2 region (690 bp), which nested in the *C. cibarius* clade. *Cantharellus* sp. 2 and sp. 3 were isolated from the other species and formed clades with strong support. Specimens of *Cantharellus* sp. 2 and the "*C. cibarius*" specimen SAPA-5067 from Hokkaido Island ("*C. cibarius*" aff. sp. 2) formed a sister clade to *C. formosus*, while the specimens of *Cantharellus* sp. 3 formed a sister clade to *Cantharellus altipes* Buyck & V. Hofst. (Fig. 2; Supplementary Figs. S1, S2). The *tef-1* phylogeny exhibited the same topology as that of the 5.8S-ITS2-LSU phylogeny with respect to *Cantharellus* sp. 3 and sp. 4 (Fig. 3). Although *Cantharellus* sp. 2 and *C. formosus* formed a monophyletic clade with strong support in the *tef-1* phylogeny (Fig.

3), these two species were distinct in other datasets (Fig. 2; Supplementary Figs. S1, S2). *Cantharellus* sp. 1 and "*Cantharellus yunnanensis*" formed a sister clade to the complex of *Cantharellus flavus* Foltz & T.J. Volk, *Cantharellus phasmatis* Foltz & T.J. Volk, *Cantharellus tenuithrix*, *Cantharellus deceptivus* Buyck, Justice & V. Hofst., and *Cantharellus pallens* with strong support. In a phylogenetic tree based on the ITS1 sequence, *Cantharellus* sp. 1 was distinguished from all related Indian species with strong support values (i.e., *C. applanatus*, *C. elongatipes*, and *C. natarajanii*) (Fig. 4). Specimen C-2 of *Cantharellus* sp. 1 from Okinawa formed an intraspecific subclade with moderate support values. *Cantharellus* sp. 4 formed two subclades with strong support values, one of which formed a clade with European *C. cibarius*.

3.3. Sequence analysis of the ITS1 region and morphological comparison of Cantharellus *sp.1, Indian relatives, and European* C. cibarius

Since *Cantharellus* sp. 1 formed a clade with several Indian *Cantharellus* species based on the phylogenetic analysis of the 5.8S-ITS2-LSU region (Fig. 2), a detailed sequence analysis of the ITS1 region was conducted among these species. For *Cantharellus* sp. 1, 5–30 cloned sequences were obtained from selected specimens; there were variations among sequences in terms of the number of tandem repeats or single base substitutions, and the mutation sites differed between specimens or within a specimen. All cloned ITS1 sequences of *Cantharellus* sp. 1, amplified using primers ITS1 and ITS2, were approximately 100 bp shorter than those of the Indian relatives and 145 bp shorter than that of European *C. cibarius*. Based on European *C. cibarius* sequence DQ200926, the differences in length were primarily caused by the deletion of a common region spanning bases 474–601 (Fig. 5). The Indian relatives had a

37-bp partial deletion, while *Cantharellus* sp. 1 had a complete deletion of this region. This region was excluded from the phylogenetic tree analysis (Fig. 4). Therefore, the *Cantharellus* sp. 1 population was considered to have unique features compared with its Indian relatives at the species level.

Japanese *Cantharellus* sp. 1 and the phylogenetically related Indian species were distinct from one another based on their macroscopic and microscopic characteristics (Table 3). Basidiomata of the three Indian species, *C. applanatus*, *C. natarajanii*, and *C. elongatipes*, are smaller and slender in shape, and stronger in color compared with basidiomata of *Cantharellus* sp. 1. In addition, the discoloration when bruised differs. Of the microscopic characteristics, the state of the pileipellis differs; in *Cantharellus* sp. 1 it is moderately thick-walled in most cases, compared with the Indian species in which it is usually thick-walled, ~1–1.5 µm in thickness. In addition, *C. elongatipes* is distinguished from *Cantharellus* sp. 1 by its small spores. Based on these results, *Cantharellus* sp. 1 is described as a new species.

4. Taxonomy

Cantharellus anzutake W. Ogawa, N. Endo, M. Fukuda & A. Yamada, sp. nov.

Fig. 1A–E, H.

MycoBank no.: MB 813057

Diagnosis: *Cantharellus anzutake* can be distinguished from phylogenetically related following species: *C. applanatus*, *C. natarajanii*, *C. elongatipes* and *C. flavus* in both macroscopic and microscopic characteristics, *C. tenuithrix* in spore shape, *C. pallens* and *C. subalbidus* in their external color or discoloration.

Type: JAPAN, Nagano, Chino, Mt. Eimeiji-yama, alt. 1040 m in a forest of Japanese red

pine, *Pinus densiflora* Sieb. & Zucc., 9 Aug 2013, W. Ogawa & N. Endo (holotype, TNS-F-61925)

Gene sequences ex-holotype: LC 085359 (ITS), LC 085415 (LSU), and LC179800 (*tef-1*)

Etymology: Anzutake, the common name of this fungus.

Description: *Basidiomata* 5–7 cm in height (Fig. 1A). *Pileus* 3–8(–12) cm in width, fleshy, pulvinate to plano-convex when young, becoming depressed or funnel-shaped to broadly trumpet-shaped, irregular; surface egg-yellow or pale yellow in drier weather conditions to bright- to orange-yellow in wet conditions, glabrous, smooth but cottony-felty under hand lens; margin incurved at first but soon uplifted with undulations and becoming irregularly lobed or wavy; slowly become brown or reddish brown from drying or damage within hours or within a day; flesh 1–2 mm in width at the margin and 9–17 mm in width at the center, firm, yellowish at the surface layer until 2–3 mm depth, white in the inner depths. *Hymenium* with ridges 1–2 mm in height, close and narrow, decurrent, variously forked or anastomosed, pale yellow, beige, or rather white. *Stipe* 2.5–3.5 cm in height and 7–12 mm in width, central, equal or thickened at the base, white or beige or pale yellow, solid, rhizomorphs present at the base; flesh white. Gregarious or solitary. Basidiospore color cream. *Basidiospores* 5.8–9.2(–9.4) × 4–6.3 µm, 7.3 × 5.3 µm on average; Q = 1.1–1.8 (Fig. 1D, E). *Basidia* 4–6 spored, 55–74 × 7–10 µm, cylindric to clavate, sinuous, (Fig. 1B, C); sterigmata straight to curved, 4.1–6.5 µm in length, 1.7–2.6 µm in basal width. *Cystidia* none. Hymenial trama with compactly arranged hyphae with pseudoparenchymatous tissue at the base of basidia, 3–9.6 µm in width, interwoven arrangement of generative hyphae in the center, straight or sinuous, 2–4.3 µm in width. Pileipellis of a cutis mostly cylindric, sinuous, 4–16.5 µm in width, thin to moderately thick-walled, 0.5–0.7 µm and rarely 0.9 µm in wall thickness (Fig. 1H), pileus tramal hyphae mostly thin-walled, cylindric and parallel, or pseudoparenchymatous tissue, 2.6–9.1 µm in width. Stipitipellis of a cutis cylindric, sinuous, 2.1–9.7 µm in width, terminal

cells sometimes clavate, cylindro-clavate, or obclavate shaped, mostly thin-walled but sometimes thick-walled, up to 0.7 µm in thickness; stipe tramal hyphae cylindric, parallel, and sometimes fused with neighboring hyphae, 3.6–16.2 µm in width, or sinuous and entangled as exploiting hyphae or secretory hyphae. Autofluorescence of hyphae against UV irradiation moderate, whitish green-blue. Clamp connections abundant in all hyphae and tissues.

Ecology: Under various ectomycorrhizal tree species, i.e., those of the genera *Pinus*, *Picea*, *Tsuga*, *Quercus*, *Betula*, and *Carpinus*, with a moderate to weakly developed litter layer on the forest floor. Secondary and young forests have productive basidiomata, in contrast to deep and climax forest conditions. The habitat ranges from ~0 m in altitude in the coastal pine forests to 1360 m at the boundary between cool temperate and subalpine forests. Fruiting occurs during the warm and humid seasons: Apr–May in Ryukyu Islands and Jul–Aug in Honshu Island. Yellowish ectomycorrhizal root tips can be observed underneath basidiomata in the soil A-layer (unpublished data), which sometimes can be traced from the rhizomorphs of basidiomata stipes.

Materials examined: See Table 1.

Remarks: *Cantharellus anzutake* can be distinguished from phylogenetically related species based on a single ~100 bp deletion in the ITS1 region. *Cantharellus anzutake* is different from *C. applanatus*, *C. natarajanii*, and *C. elongatipes* in both macroscopic and microscopic characteristics (Table 3). Furthermore, *C. tenuithrix* differs from *C. anzutake* in spore size and shape. *Cantharellus flavus* was distinguished by the yellowish stipe color and longer spores. *Cantharellus tenuithrix* has narrower spores. *Cantharellus pallens* and *Cantharellus subalbidus* differ from *C. anzutake* in their external color or discoloration.

5. Discussion

The Japanese golden chanterelle, *C. anzutake*, described here as a new species, was first reported as *C. cibarius* by Kawamura (1908). Since then, most Japanese fleshy and yellowish chanterelles have been identified as *C. cibarius* (Ito 1955; Kawamura 1955; Imazeki and Hongo 1957, 1989; Katsumoto 2010; Tanabe and Ogawa 2015). Since species delimitation of *C. cibarius* is difficult based on its morphology alone, and since this species name has been applied to its cryptic species in various geographic areas (Dunham et al. 2003a, b; Pilz et al. 2003), it is possible that *C. cibarius* sensu Kawamura has been applied to the four Japanese species revealed in the present study. Of these, only one species, *Cantharellus* sp. 4, was nested in the clade *C. cibarius*, as epitypified by Olariaga et al. (2016), and the other three species were possibly undescribed. In this study, we described one of the latter three, *Cantharellus* sp. 1, as the new species *C. anzutake*, based on a single 100-bp deletion in ITS1. *Cantharellus anzutake* specimens C-84 and C-85 were collected from the forest site where Kawamura (1908) first collected Japanese *C. cibarius* and named it after the Japanese common name "Anzutake." The only difference in basidiomata between *C. anzutake* and *C. cibarius* sensu Kawamura is the weak but distinct discoloration of *C. anzutake* when bruised and dried. This discoloration progresses slowly, i.e., over several h. At first, we did not regard such discoloration of *C. anzutake* as a distinguishing characteristic. However, repeated sampling of these fungal basidiomata, especially under drier weather conditions, showed such discoloration. This was also the case with *Cantharellus* sp. 4, as well as a European *C. cibarius* population (Olariaga et al. 2016). This type of discoloration in *C. anzutake* is sometimes prominent at the points where mycophagous insects have fed, which can be seen as small reddish brown spots on the basidioma surface. The taxonomic affinities of the other two probably undescribed *Cantharellus* species will be explored in a future study.

Single-locus phylogenetic analyses of the LSU, ITS2, and ITS1 regions, including

European, North American, and Asian chanterelle specimens (Fig. 4; Supplementary Figs. S1, S2), indicated sequence conservation and variation among the specimens: extensive conservation was detected in the LSU region, moderate conservation in the ITS2 region, and extensive variability in the ITS1 region. This hierarchical relationship has been reported in diverse fungal taxa (Porras-Alfaro et al. 2014). However, in the Indian species presented in this study, two phylogenetic clades (the *C. applanatus–C. elongatipes* clade and the *C. natarajanii* clade) distinguished by our ITS2 sequence analysis (Supplementary Fig. S2) were grouped into a single clade by phylogenetic analysis of the ITS1 sequence (Fig. 4). We suggest that these Indian sequence data are the result of contamination of the DNA sequencing reactions or errors in deposited DNA sequences, as suggested previously by Das et al. (2015). In our obtained sequence data used for phylogenetic and sequence analyses, we confirmed complementary sequences in all DNA regions. In addition, our ITS1 sequence data were obtained based on replicated cloning procedures and showed many point mutations in the tandem repeat of the rDNA cluster. *Cantharellus anzutake* described in this study and its Indian relatives mentioned above were clearly distinguished by our sequence comparison of the ITS1 region. Therefore, it is clear that *C. anzutake* is a different species from its relatives, such as *C. applanatus* and *C. natarajanii*.

Regarding spore characteristics, spore print colors such as white, cream, and pale yellow are used to indicate a difference in the spore wall structure or lipid storage. However, such a slight difference is sometimes not applicable as a distinct identification characteristic because a spore print is only observed in a fully mature, fresh basidioma. Although we repeatedly sampled *C. anzutake* basidiomata and found that the fungus showed a cream-colored spore print, Kawamura (1908) described the spore print as white in Japanese *C. cibarius* (= *C. anzutake*). Japanese *C. cibarius* has generally been described as having a white or cream spore print (Ito 1955; Kawamura 1955; Imazeki and Hongo 1957, 1989). Deepika et

al. (2014) described Indian fleshy yellowish chanterelles with different spore print colors in each species and described a white spore print in the cases of *C. applanatus* and *C. natarajanii*. However, as these two Indian species exhibited golden yellow hymenium (Table 3), their spore print may show a cream color. Therefore, if a limited number of specimens were observed, spore prints might not function as a diagnostic tool due to a lack of precision.

Although we characterized four Japanese fleshy yellowish chanterelles in the present study, additional cryptic species of fleshy yellowish chanterelles are thought to be present in Japan. In fact, we sampled a further ~50 fresh specimens of yellowish chanterelles but excluded them from the microscopic and complete phylogenetic analyses, because they showed trends of slightly smaller size of basidiomata and distinctly paler, pale olive, or distinctly orange colors on the yellowish cap. Some showed phylogenetic similarities with *C. pseudoformosus* Deepika, Upadhyay & Reddy, *C. sikkimensis* K. Das, Buyck, D. Chakr., A. Baghela, S.K. Singh & V. Hofstetter, *C. cinnabarinus* Schwein., or *C. tabernensis* Feib. & Cibula in our preliminary study (data not shown). Therefore, we selected distinctly yellowish, fleshy, and moderate-to-large sized chanterelles, i.e., probable 1c clade in the subgenus *Cantharellus* (Buyck et al. 2014, 2016b), in the present study to specify the most commonly found golden chanterelle in Japan.

It is interesting to note that *C. anzutake* was found from the northern limit of Honshu Island to the southern limit of Ryukyu Islands, in contrast to *Cantharellus* sp. 4, probably conspecific with *C. cibarius* s.s. (a local population of *C. cibarius*), which was found only in the far northern Hokkaido Island. These geographic patterns suggested that *C. anzutake* has adapted from a cool temperate to subtropical climate, in contrast to *Cantharellus* sp. 4 found in the cool temperate to boreal areas. The geographic isolation of these two golden chanterelles among the islands of Japan should be examined in a future study to determine the primary factor of this allopatric pattern. In this respect, it is inevitably necessary to clarify the

geographic distribution (presence/absence) of these two chanterelle species in the area surrounding the Japanese islands. It is worth noting that the Chinese "*C. yunnanensis*" specimen XieXD174 showed a high degree of sequence identity to *C. anzutake* in both the LSU (KU720333) and *tef*-1 (KU720337) regions, suggesting that they may be conspecific. However, the description of *C. yunnanensis* (Chiu 1973) shows distinct differences from that of *C. anzutake*: pileus 1.5–2.5 cm diam, stipe 3–5 cm in height, 5–10 mm in width, and spores pale olive in color, ellipsoidal, 4–5 × 2–3.5 µm, suggesting that *C. yunnanensis* lies within the subgenus *Parvocantharellus* (Buyck et al. 2014, 2016a, b, c). In addition, several Chinese specimens of "*C. tuberculosporus*" show high *tef*-1 sequence identity with "*C. yunnanensis*" XieXD174 and *C. anzutake*. The description of *C. tuberculosporus* (Zang 1980) suggests its taxonomic position in the Gomphaceae because of the tuberculate spores and shape of its basidioma. Therefore, further studies are required to perform a comprehensive comparison between the "*C. yunnanensis*" XieXD174 specimen and *C. anzutake*. It will be valuable to clarify the relationships within the *C. flavus*/*C. phasmatis*/*C. deceptive*/*C. pallens* complex positioned as a sister clade of *C. anzutake* by *tef-1* phylogeny.

Disclosure

The authors declare no conflicts of interest. All of the experiments in this study were performed in compliance with the current laws of Japan.

Acknowledgements

We thank Masahiko Sato, Yoshie Terashima, Niclas Bergius, and Toshiaki Tanabe for their

support in the sampling of basidiomata specimens, and Takahito Kobayashi, Eiji Nagasawa, and Kentaro Hosaka for loaning the herbarium specimens. We also thank the staff of the Research Center for Human Environmental Sciences, Shinshu University for the DNA sequencing. This study was supported in part by a Grant-in-Aid for Scientific Research (15H01751) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and a general research grant from the Institute for Fermentation, Osaka.

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Figure legends

Fig. 1 – Morphological characteristics of *Cantharellus anzutake* (A–E, H: holotype) and *C. cibarius* (F, G, I: C-122). A: External morphology of the basidiomata. B: Basidium with four sterigmata at the apex. C: Basidium with a clamp connection at the base. D, E: Basidiospores of *C. anzutake*. F, G: Basidiospores of *C. cibarius*. H: Thin-walled pileipellis hyphae of *C. anzutake*. I: Thick-walled pileipellis hyphae of *C. cibarius*. Micrograph B was constructed from four micrographs that were taken in the same field but differed in focus by a depth of ~1 μm using the synthetic function of Adobe Photoshop CC (Adobe Systems Inc., San Jose) to show the apex and base of a mature basidium (right) in a single field. In addition, micrograph I was constructed from four micrographs. *Bars*: A 2 cm; B, C, H, I 20 μm; D–G 10 μm.

Fig. 2 – ML phylogenetic tree of 5.8S-ITS2-LSU. Four Japanese yellow chanterelles were analyzed alongside several known yellow chanterelle species. Bootstrap (BS) values > 50% from ML (left) and Bayesian posterior probabilities (PP) > 0.50 (right) are shown near the nodes. Thick nodes indicate significant support by both BS and PP values. Taxon names shown in bold indicate the specimens examined in this study.

Fig. 3 – ML phylogenetic tree of *tef-1*. Four Japanese yellow chanterelles were analyzed with several known yellow chanterelle species. Bootstrap (BS) values > 50% from ML (left) and Bayesian posterior probabilities (PP) > 0.50 (right) are shown near the nodes. Thick nodes indicate significant support by both BS and PP values. Taxon names shown in bold indicate the specimens examined in this study.

Fig. 4 – ML phylogenetic tree of ITS1. *Cantharellus* sp. 1 (= *C. anzutake*) was analyzed alongside several related species. *Cantharellus* sp. 3 was adopted as the outgroup. Bootstrap (BS) values > 50% from ML (left) and Bayesian posterior probabilities (PP) > 0.50 (right) are shown near the nodes. Thick nodes indicate significant support by both BS and PP values. Taxon names shown in bold indicate the specimens examined in this study.

Fig. 5 – Sequence alignment of the partial ITS1 regions of *Cantharellus* sp. 1 (= *C. anzutake*), *C. natarajanii*, *C. applanatus*, *C. elongatipes*, and *C. cibarius*. Sequence names are shown to the left of each sequence. On the right, the position of the last nucleotide in the line is shown, aligned based on the *C. cibarius* DQ2000926 sequence.

Table 1

Table 1 – Basidiomata samples of fleshy yellow chanterelles collected in Japan.

Species	Specimen ID[a]	Sampling				Accession number of DNA sequence[e]			
		Site	Canopy tree[c]	Date	Sample status[d]	ITS1	ITS2	LSU	*tef*-1
Cantharellus sp. 1	S-117	Dosho-ji Temple, Shichigahama, Miyagi	Pt, Pd	2010/8/15	F	LC085349	LC085349	LC085411	LC085468 – 9
Cantharellus sp. 1	C-124	Shima Park, Koriyama, Fukushima	Pd, Qs	2011/7/16	D	P	LC085350		
Cantharellus sp. 1	S-10	Lake side of Koshibu-ko, Nakagawa, Nagano	Qs, Qa	2009/7/20	F	P	P	LC085412	
Cantharellus sp. 1	S-89	Oshiba Park, Minami-minowa, Nagano	Pa	2010/7/18	F	LC085351	LC085351	LC085413	P
Cantharellus sp. 1	S-111	Oshiba Park, Minami-minowa, Nagano	Pa, Bp	2010/7/25	F	LC085352	LC085352	LC085414	
Cantharellus sp. 1	C-23	Norikura Highland, Matsumoto, Nagano	Qc, Bp	2011/8/5	F	LC085353 – 6	LC085357	P	P
Cantharellus sp. 1	C-26	Yachiho Highland, Sakuho, Nagano	Qc, Bp, Pd	2011/8/7	F	P	LC085358		
Cantharellus sp. 1	C-84 (TNS-F-61925)	Mt. Eimeiji-yama, Chino, Nagano	Pd	2013/8/9	F	LC085359	LC085359	LC085415	LC179800–3
Cantharellus sp. 1	C-85 (TNS-F-61926)	Mt. Eimeiji-yama, Chino, Nagano	Pd	2013/8/9	F	LC085360	LC085360		
Cantharellus sp. 1	C-12	Kayano Highland, Minowa, Nagano	Pd, Qs	2011/8/3	F	P	LC085361		
Cantharellus sp. 1	S-244	Oshiba Park, Minami-minowa, Nagano	Qs, Be	2011/7/30	F	LC085362	LC085362		
Cantharellus sp. 1	C-17	Chusen-ji Temple, Ina, Nagano	Pd	2011/8/5	F	P	LC085363		
Cantharellus sp. 1	C-18	Chusen-ji Temple, Ina, Nagano	Pd	2011/8/5	F	LC085364 – 8	LC085369		
Cantharellus sp. 1	C-19	Chusen-ji Temple, Ina, Nagano	Pd	2011/8/5	F	P	LC085370		
Cantharellus sp. 1	C-130	Mt. Kurama-yama, Kyoto	ND	2014/8/21	F	P	LC085371		

Cantharellus sp. 1	C-126	Central Park, Nago, Okinawa	Pl, Qm	2014/5/19	F	P	LC085372		
Cantharellus sp. 1	C-2	Mt. Daruma-yama, Kume-jima Island, Okinawa	Pl, Qm	2011/5/24	F	LC085373 – 7	LC085373	LC085416	LC085470, 1
"C. cibarius" aff. sp. 1	TNS-F-208576	Aichi	ND	1914/10/26	D	-	-	-	-
"C. cibarius" aff. sp. 1	TNS-F-195140	Gunma	ND	1945/9/17	D	-	-	-	-
"C. cibarius" aff. sp. 1	TNS-F-50844	Nagano	ND	1975/8/12	D	-	-	-	-
Cantharellus sp. 2	C-114	Mt. Shirane-san, Nikko, Tochigi	Av, Be	2013/9/27	F		LC085378	LC085417	
Cantharellus sp. 2	C-97	Mt. Kurofu-yama, Komoro, Nagano	Av, Am	2013/9/12	F		LC085379		
Cantharellus sp. 2	C-58	Mt. Tateshina-yama, Saku, Nagano	Td	2011/9/27	F	P	LC085380		
Cantharellus sp. 2	C-88	Mt. Tateshina-yama, Saku, Nagano	Td	2013/9/6	F		LC085381	P	LC085472
Cantharellus sp. 2	S-128	Mugikusa Pass, Sakuho, Nagano	Td	2010/9/24	F		LC085382		
Cantharellus sp. 2	C-15	Mugikusa Pass, Sakuho, Nagano	Td	2011/8/4	F	P	LC085383		
Cantharellus sp. 2	C-106	Mt. Ontake-san, Gero, Gifu	Td, Pk	2013/9/25	F		LC085384	LC085418	LC085473
Cantharellus sp. 2	C-100	Karei Highland, Ina, Nagano	Av	2013/9/14	F		LC085385		
Cantharellus sp. 2	C-117	Shirabiso Highland, Iida, Nagano	Av	2013/9/29	F		LC085386	LC085419	
"C. cibarius" aff. sp. 2	TMI-5066	Mt. Meakan-dake, Ashoro, Hokkaido	ND	1927/9/13	D			P	LC085478
"C. cibarius" aff. sp. 2	SAPA-5069a	Mt. Meakan-dake, Ashoro, Hokkaido	ND	1933/9/15	D	-	-	-	-
"C. cibarius" aff. sp. 2	SAPA-5069b	Mt. Meakan-dake, Ashoro, Hokkaido	ND	1933/9/15	D		LC085400		
"C. cibarius" aff. sp. 2	SAPA-5070	Mt. Meakan-dake, Ashoro, Hokkaido	ND	1934/9/21	D	-	-	-	-
"C. cibarius" aff. sp. 2	TMI-5068	Nopporo Forest Park, Ebetsu, Hokkaido	ND	1927/8/29	D		P		

"C. cibarius" aff. sp. 2	SAPA-5067	Lake side of Shikaribetsu-ko, Shikaoi, Hokkaido	ND	1929/9/30	D		LC085401		
Cantharellus sp. 3	C-101	Togakushi Shrine, Nagano, Nagano	Qc, Ah	2013/9/15	F		LC085387	LC085420	LC085474
Cantharellus sp. 3	C-118	Mt. Nagakabe-yama, Matsumoto, Nagano	Td, Av	2013/10/3	F		LC085388		
Cantharellus sp. 3	C-21	Norikura Highland, Matsumoto, Nagano	Av	2011/8/5	F		LC085389		
Cantharellus sp. 3	C-53	Norikura Highland, Matsumoto, Nagano	Av	2011/9/25	F	LC085390 – 4	LC085395	LC085421	LC085475
Cantharellus sp. 4	C-141	Kutsugata, Rishiri-tou Island, Hokkaido	As	2014/9/25	F	LC085396	LC085396	LC085422	LC085476
Cantharellus sp. 4	C-142	Kutsugata, Rishiri-tou Island, Hokkaido	As	2014/9/25	F	LC085397 – 8	LC085399	LC085423	LC085477
"C. cibarius" aff. sp. 4	RTMFU-174	Kutsugata, Rishiri-tou Island, Hokkaido	As	1996/10/5	D		LC085402		
"C. cibarius" aff. sp. 4	SAPA-5065	Mt. Teine-yama, Sapporo, Hokkaido	ND	1925/9/27	D		LC085403		
C. cibarius	SweN[b]	Northern Scandinavian Peninsula, Sweden	ND	2011	D	LC085404	LC085405		LC085479
C. cibarius	SweC[b]	Central Scandinavian Peninsula, Sweden	ND	2011	D	LC085406	LC085407		
C. cibarius	C-122[b]	Uppsala, Sweden	ND	2013/10/5	F	P	LC085408	LC085424	LC085480
C. roseocanus	2030820-1[b]	Vancouver Island, Victoria, Canada	ND	2003/8/20	D	LC085409 – 10			

[a] Specimen ID that is not initially indicated RTMFU, SAPA, TNS, or TMI is held in the Applied Mycology Laboratory, Faculty of Agriculture, Shinshu University.

[b] Specimens were sampled outside Japan.

[c] Ah: *Abies homolepis* Sieb. & Zucc., Am: *A. mariesii* Mast., As: *A. sachalinensis* (Fr.Schmidt) Masters, Av: *A. veitchii* Lindl., Be: *Betula ermanii* Cham., Bp: *B. platyphylla* var. *japonica* (Miq.) H. Hara, Cc: *Carpinus tschonoskii* Maxim., Pj: *Picea jezoensis* (Sieb. et Zucc.) Carrière, Ph: *P. jezoensis* var. *hondoensis* (Mayr)

Rehde, Pd: *Pinus densiflora* Sieb. et Zucc., Pk：*P. koraiensis* Sieb & Zucc., Pl: *P. luchuensis* Mayr, Pt: *P. thunbergii* Parl., Qa: *Quercus acutissima* Carruth., Qc: *Q. crispula* Blume, Qm: *Q. miyagii* Koidz., Qs: *Q. serrata* Murray, Td: *Tsuga diversifolia* (Maxim.) Mast., Ts: *T. sieboldii* Carrière, ND: not determined.

[d] F: Fresh basidiomata, D: Dried basidiomata.

[e] P: Partially sequenced but not obtained the accession number.

Table 2

Table 2 – Basidiomata comparisons between four morphological groups of the Japanese fleshy yellow chanterelles.

	Cantharellus sp. 1 (=*C. cibarius* sensu Kawamura)	*Cantharellus* sp. 2	*Cantharellus* sp. 3	*Cantharellus* sp. 4	Swedish *C. cibarius*
Color and shape of pileus	Pale- to orange-yellow, circular or undulated	Ocher-yellow, undulated	Lemon to bright yellow, circular or undulated, thin and fragile in the margin when matured	Bright- to orange-yellow, circular or undulated	Bright- to orange-yellow, circular or undulated
Color of stipe	White to pale yellow	Ocher-yellow	White to pale yellow	Bright yellow	Bright yellow or pale yellow
Discoloration of basidioma surface	Weak reddish brown when bruised and dried	Absent	Reddish when bruised	Weak reddish when bruised and dried	Weak reddish brown when bruised and dried
Color of hymenium	White to lemon yellow	Bright yellow with sometimes pinkish color	White to lemon yellow	Bright yellow	Bright yellow
Shape of the decurrent hymenium	Straight, thinner, dense, ridges are common on the acropetally area but few on the base petal area	Often undulated when matured, thicker and sparse when young, ridges are common even on the base petal area	Often undulated when matured, ridges are common even on the base petal area	Straight, ridges are common on the acropetally area but few on the base petal area	Straight, ridges are common on the acropetally area but few on the base petal area
Wall thickness of pileipellis	0.5–0.7(–0.9) µm	0.5–1 µm	0.8–1 µm	0.8–1 µm	0.9–1 µm
Spore size	5.8–9.2 × 4–6.3 µm, Q = 1.1–1.8	7.6–9.9 × 4.6–6.2 µm, Q = 1.3–1.9	8.2–10.8 × 5.3–6.9 µm, Q = 1.3–1.9	7.2–8.9 × 4.3–5.8 µm, Q = 1.3–1.9	7.6–10.6 × 4.6–6.5 µm, Q = 1.3–1.9
Geographic distribution in Japan	Ryukyu Islands to Honshu Island	Central mountain areas of Honshu Island and Hokkaido Island	Central mountain areas of Honshu Island	Hokkaido Island	
Climate of sampled area	Subtropical to cool temperate	Subalpine	Cool temperate to subalpine	Cool temperate to subalpine	
Canopy vegetation	Various coniferous, oaks, and white birch	Hemlock and fir	Fir	Fir	

Fruiting season	Late rainy season to mid-summer	Mainly early to mid-autumn	Late rainy season, and mid-summer to mid-autumn	Mid-autumn

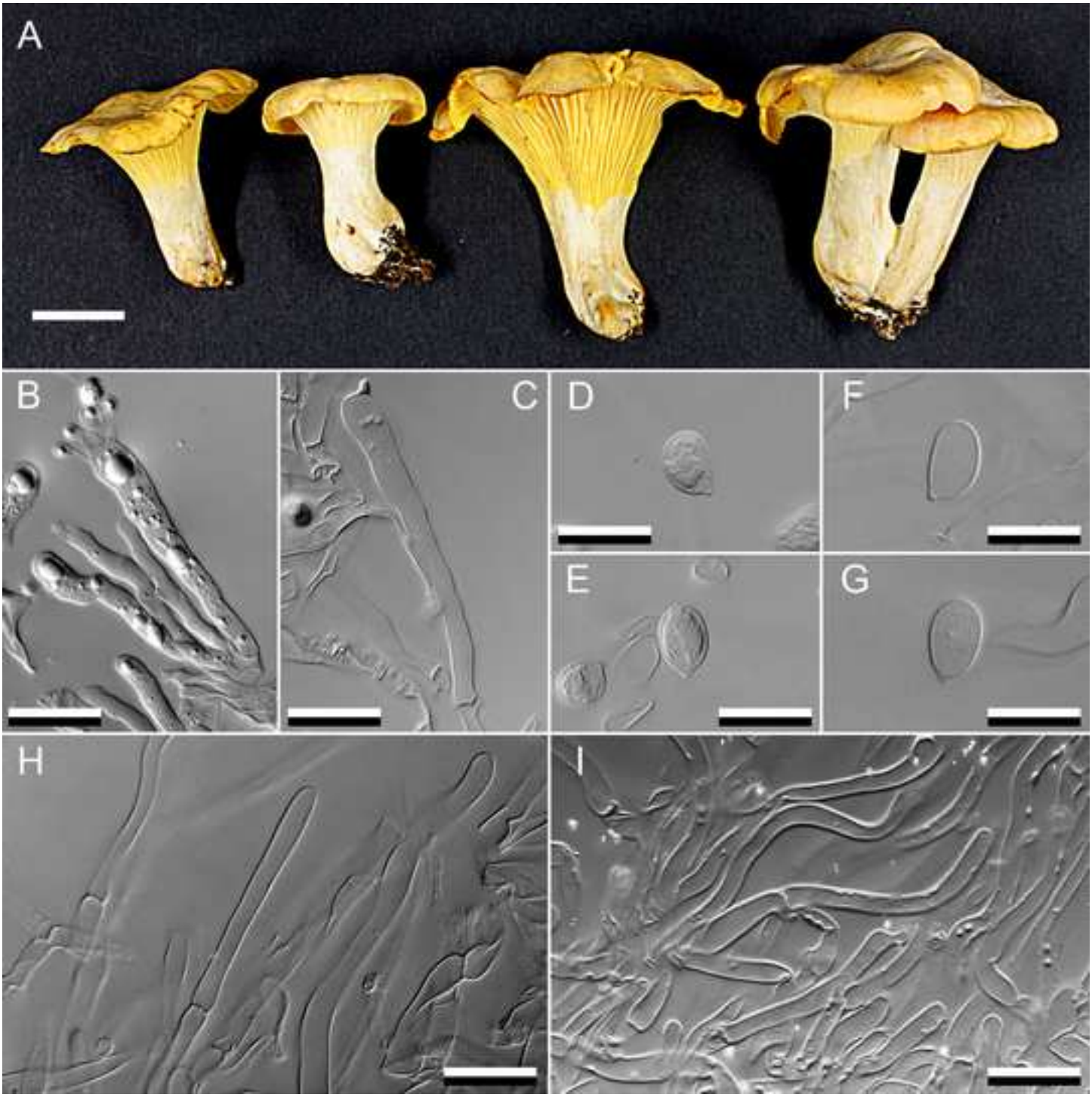
Table 3

Table 3 – Basidiomata comparisons between *Cantharellus* sp.1 and Indian relatives

	Cantharellus sp.1 (=*C. cibarius* sensu Kawamura)	*C. natarajanii*	*C. applanatus*	*C. elongatipes*	*Indian *C. cibarius*
Color and shape of pileus	Pale to orange yellow, 3–8(–12) cm in width, circular or undulated, planoconvex when young, depressed to broadly infundibuliformis when matured, context 9–17 mm in thickness at the center	Golden yellow to Chinese yellow, 5–10 cm in width, hemispherical, planoconvex to finally depressed, context 3–5 mm in thickness	Golden yellow, 3–6.5 cm in width, applanate to shallow depressed, context 3–7 mm in thickness	Orange yellow, upto 1.5 cm in width, convex to planoconvex with slightly depressed in the center, context thin	Ochraceous brown to yellowish brown, over 7 cm in width, surface with matted, fibrils or glabrous, depressed
Color and shape of stipe	White to pale yellow 2.5–3.5 cm in length and 0.7–1.2 cm in width	Pinckish yellow to citron yellow, stipe surface fibrous, 3.5–6 cm in length and 0.4–1 cm in width	Yellowish, surface glabrous to thin hairy, 2.5–5 cm in length and 0.4–0.7 cm in width	Dirty orange, 3–3.5 cm in length and 0.4–0.7 cm in width	Ochraceous brown to yellowish brown
Discoloration of basidiomata surface	Weak reddish brown when bruised and dried	No discoloration of pileus, not indicated in the stipe	No discoloration of pileus, not indicated in the stipe	Not indicated	Not indicated
Color of hymenium	White to lemon yellow	Golden yellow	Golden yellow	Orange	Ochraceous brown to yellowish brown
Pileipellis	Mostly cylindric, 4–16.5 µm in diam, thin- to moderately thick-walled up to 0.5–0.7(–0.9) µm in the thickness	End-cells distinctly subclavate to subventricose, 3–10 µm in diam, thin- to thick-walled, up to 1.5 µm in the thickness**	Cylindric to filamentous, 3–8 µm in diam, thin- to thick-walled, up to 1.5 µm in the thickness**	Cylindric, 2–3.5 µm in diam, end-cells are clavete to subclavate or cylindric, 9–18 µm in diam	
Stipitipellis	Cylindric, sinuous, 2.1–9.7 µm in width, sometimes terminal cells show clavate, clindro-clavete, or obclavate shape, mostly thin-walled but sometimes thick-walled up to 0.7 µm in the thickness	End-cells are cylindric to filamentous, 2.5–10 µm in diam	Irregulary to interwoven, branched, 3–7.5 µm in diam	Cylindric, 2.5–6 µm in diam	

Basidium	55–74 × 7–10 µm, cylindric to clavate, sinuous; sterigma 4.1–6.5 × 1.7–2.6 µm in size, 4–6 per basidium	57–85 × 6.5–10.5 µm, clavate; sterigma 4.5–8 × 1.2–2.5 µm in size, 4–5 per basidium	55–78 × 6–7.5 µm, clavate; sterigma 2.5–4 µm in length, 4–5 per basidium	52–70 × 7–10 µm, narrowly clavate to clavate; sterigma 2–4.5 µm in length, 4–5 per basidium	
Spore size	5.8–9.2 × 4–6.3 µm, Q = 1.1–1.8	6.5–9 × 5–6.5 µm, Q = 1.34	7–8.5(–9) × 4.5–5.5 Q = 1.54	6–7.5 × 4.5–5.5 µm, Q = 1.35	8.5–10.5(–11) × 4.5–6 µm, Q = 1.54

*This species is not phylogenetically related but most similar to *Cantharellus* sp. 1 in the macroscopic characteristics as described by Deepika et al. (2014).
**Hyhapl wall thickness was measured on the drawing figures of Deepika et al. (2014).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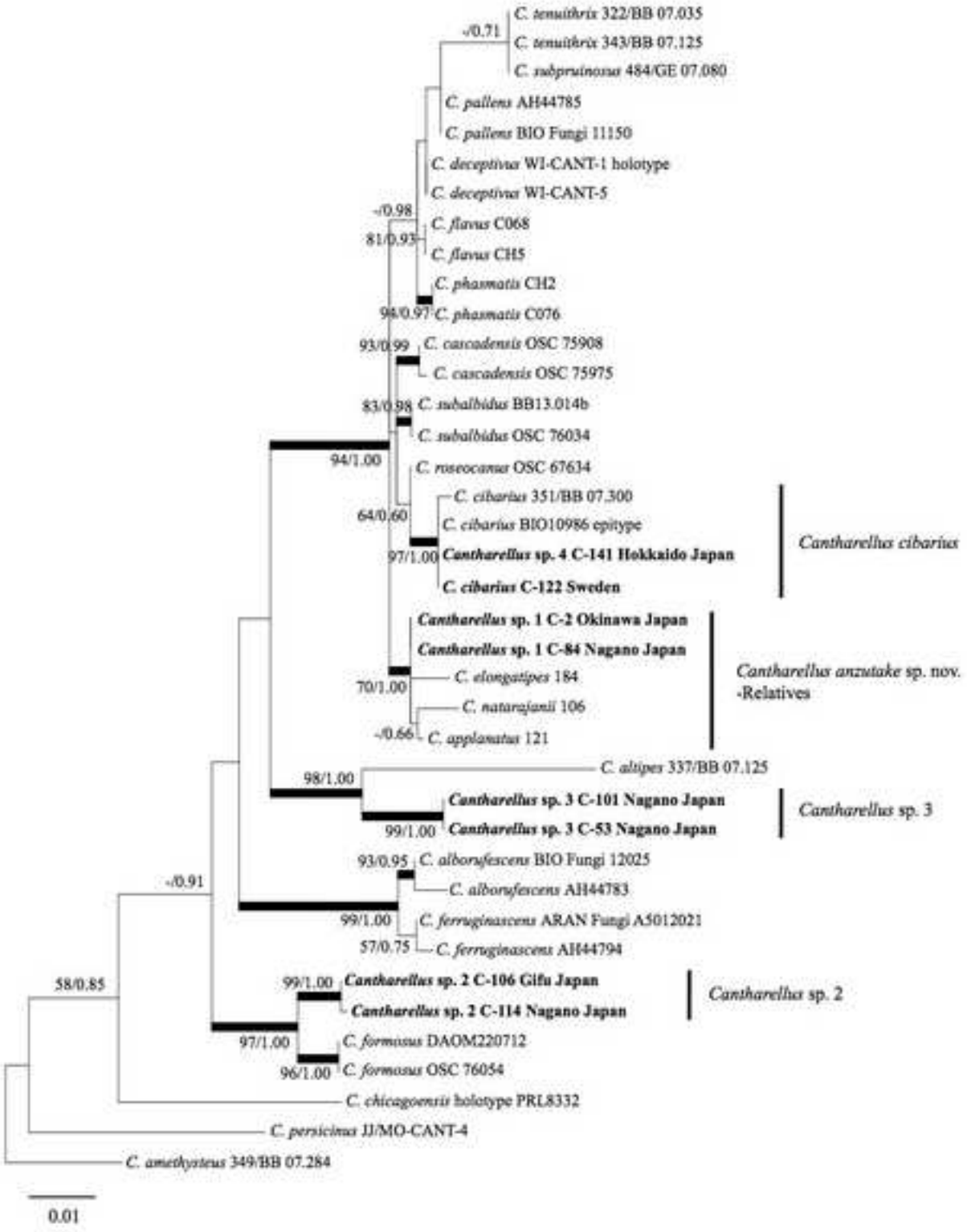
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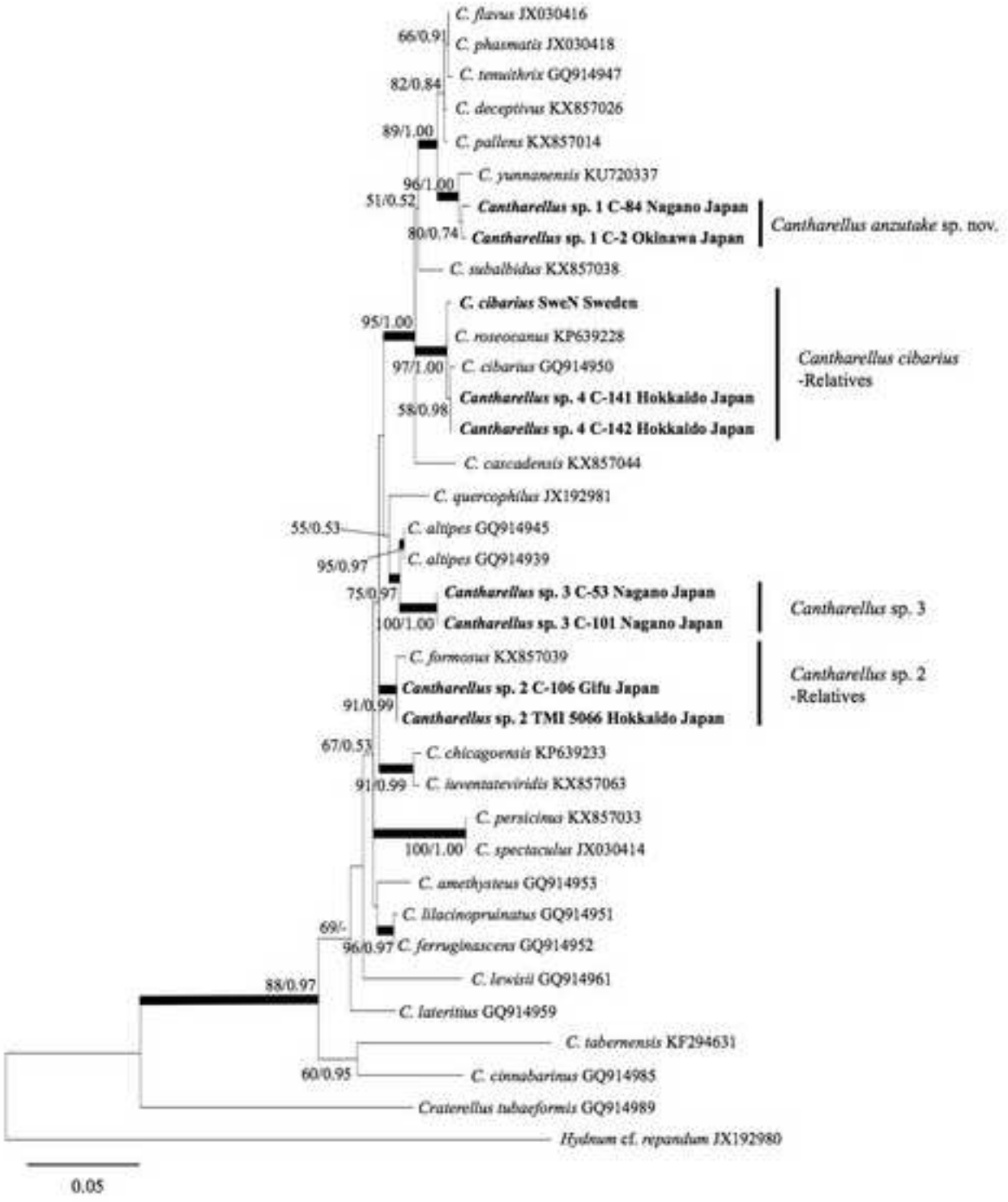
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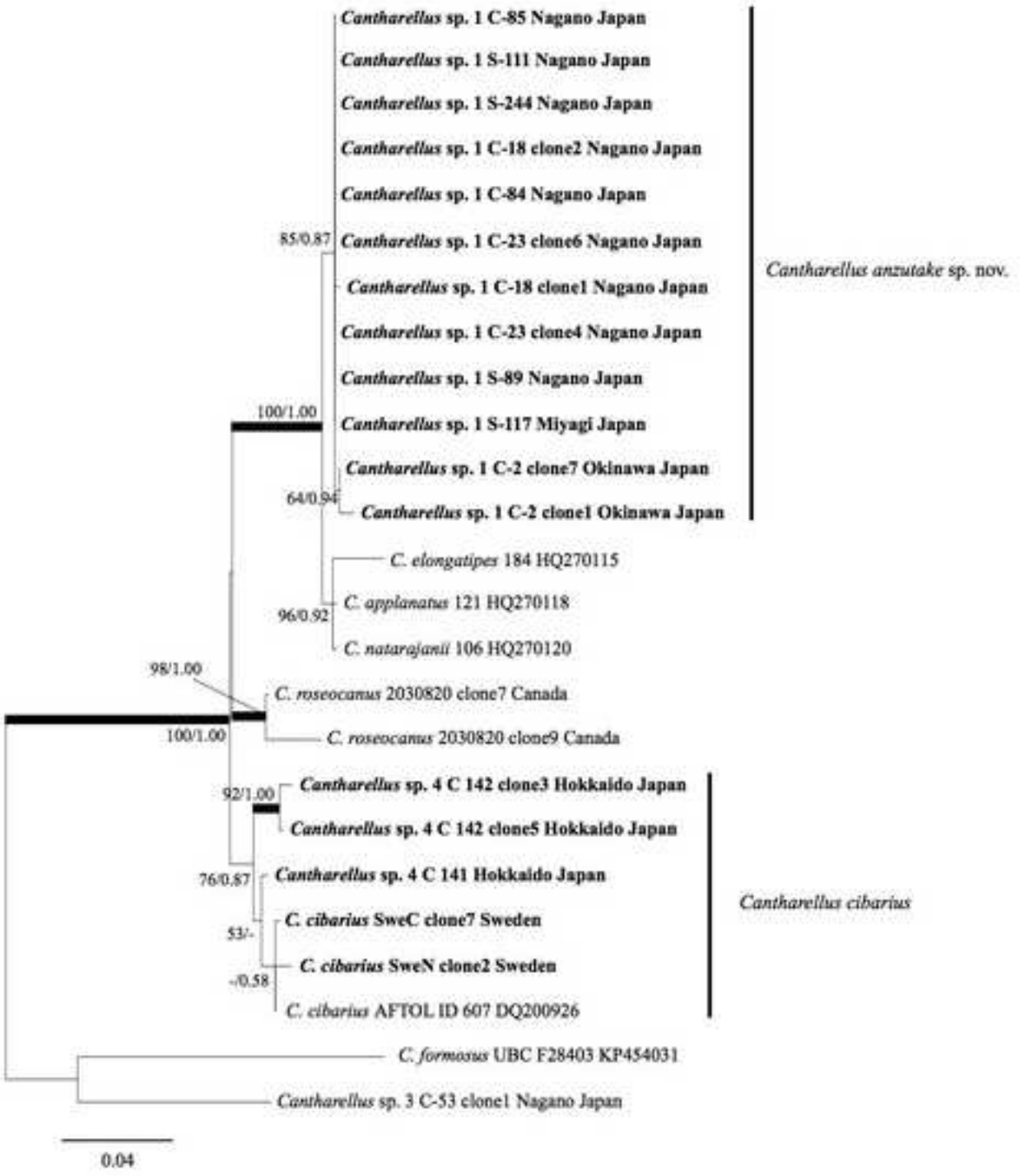
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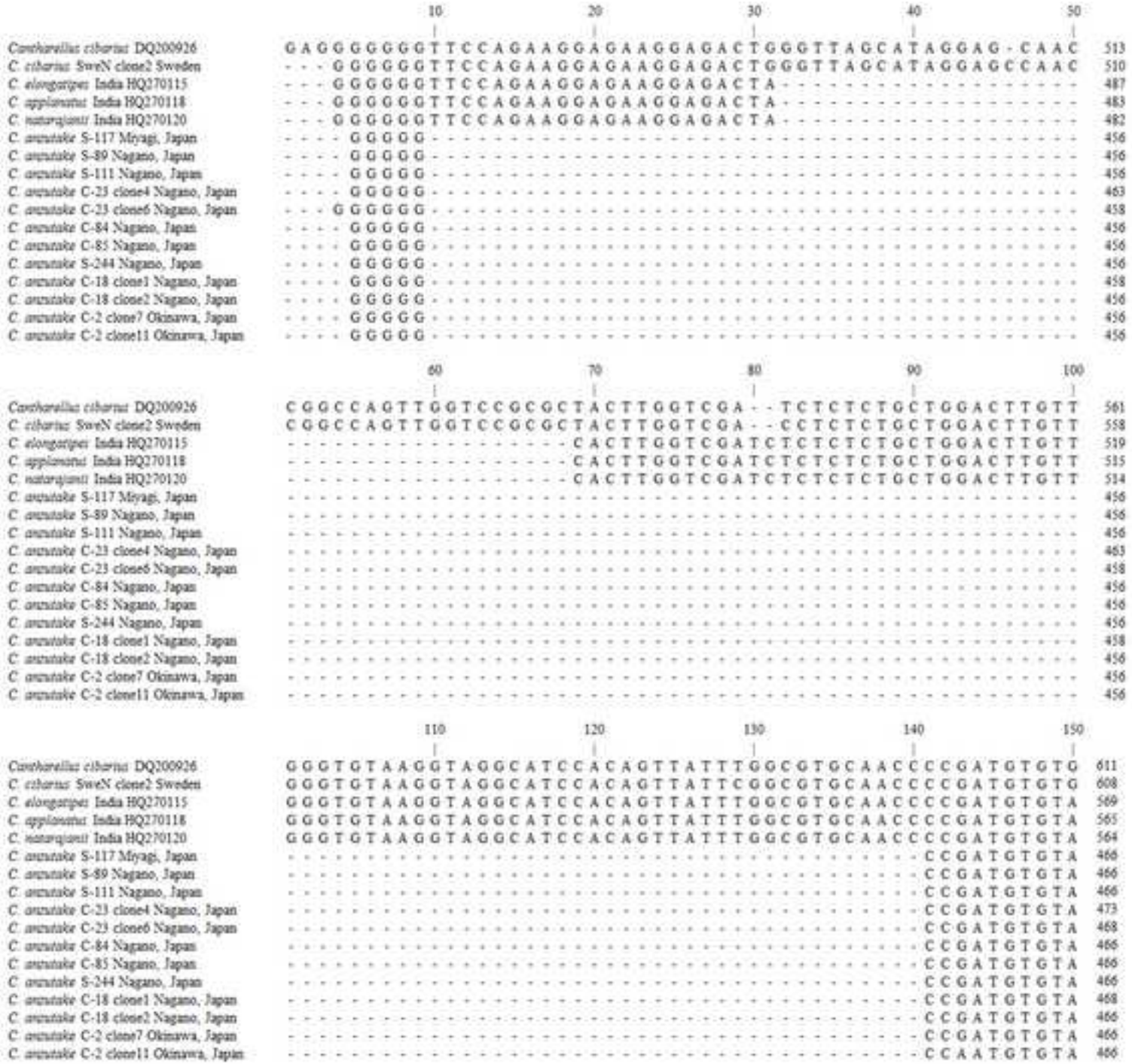
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