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# *Cantharellus alborufescens* and *C. ferruginascens* (Cantharellaceae, Basidiomycota) new to Iran

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**Abstract. -** Two *Cantharellus* species collected from different forest types in northern Iran are reported as new to the country. Phylogenetic analyses of DNA sequences from the internal transcribed spacer 2 (ITS2), nuclear ribosomal large subunit (nLSU), RNA polymerase II (*RPB2*) and translation elongation factor 1-a gene (*TEF1*) are presented. *Cantharellus alborufescens* was collected in slightly acidic soils and *C. ferruginascens* in moderate acidic soils. Illustrations from fresh material are provided for both species.

**Cantharellaceae / edible mushroom / Iran / multigene phylogeny**

## INTRODUCTION

The Hyrcanian forests are one of the last post-glacial remnants of old-growth deciduous forests in the Northern Hemisphere, and are recognized as an important genetic reservoir (Sagheb-Talebi *et al.*, 2005). Yet, these areas decreased in recent decades from 3.6 million hectares to 1.8 million hectares by deforestation, grazing, illegal logging, wood smuggling etc. (Marvi-Mohajer, 2007). This problem will be intensified with the shortage of information about essential components of

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the ecosystem, particularly the understory species including macroscopic fungi that have important roles in sustainable forest management. The genus *Cantharellus* Adans.: Fr. is one of the important ectomycorrhizal (ECM) genera of wild edible mushrooms found in all major continents of the world (Kumari *et al.*, 2011; Buyck, 2016; Buyck *et al.*, 2013; Buyck *et al.*, 2014; Shao *et al.*, 2014; De Kesel *et al.*, 2016; Leacock *et al.*, 2016; Thorn *et al.*, 2017).

Despite the economic and ecological importance of *Cantharellus* in the world, there is no precise information about the occurrence of this genus in Iran, and only sporadic reports have been made, summarized by Ershad (2009) and Ghobad–Nejhad & Hallenberg (2012), reporting only *C. cibarius* from Iran.

In this paper, we report two species of *Cantharellus* from northern Iran as new to the country on the basis of multigene phylogenetic analyses.

## MATERIAL AND METHODS

### *Sampling*

The specimens of *Cantharellus* were collected from northern forests of Iran, in the three provinces Gilan (Lakan), Mazandaran (Tonekabon, Nowshahr, Noor, Neka and Sari), and Golestan (Loveh and Shast-Kalateh) during September-December 2015, after a first rainy week in September. The average rainfall and temperature varies from 2000 mm and 15 °C in the western part of the Hyrcanian region, to 530 mm and 17.5 °C in the east, (Sagheb-Talebi *et al.,* 2005). Brown soils (under *Querco-Carpinetum* and especially *Fagetum* communities) and Rendzina soils (under *Parrotio-Carpinetum* and *Tilio-Buxetum* communities) are the most important soil types in these areas (Habibi Kasseb, 1992; Zarrinkafsh, 2002). The ectomycorrhizal and non-ectomycorrhizal host tree species surrounding the collecting areas of *Cantharellus* were noted following the list of Bahram *et al.* (2012) in some Hyrcanian regions along an altitudinal gradient. *Fagus orientalis* and *Quercus castaneifolia* (Fagaceae) and *Carpinus betulus*, *Alnus subcordata* and *Alnus glutinosa* (Betulaceae) are the main candidate ectomycorrhizal hosts for *Cantharellus* species in these regions. *Parrotia persica* (Hamamelidaceae), *Buxus hyrcanus* (Buxaceae) and *Acer velutinum* (Sapindaceae) are the main non-ectomycorrhizal tree species found in the area.

Each site was regularly visited during the rainy season, and fresh basidiocarps were collected. Specimens were air-dried at room temperature. For microscopic observation, dried specimens were revived with Congo-red ammonia solution after a short pretreatment in 3% aqueous potassium hydroxide solution (KOH) to be studied using a Nikon 80i at 1000× magnification. The specimens are preserved at Department of Forestry, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University (Noor, Iran), and duplicates of most of the collected specimens were deposited at the ICH herbarium (Tehran, Iran).

### *DNA extraction, PCR, and sequencing*

Total genomic DNA was extracted from the inner part of the basidiomes according to proteinase K protocol in a lysis buffer (0.8 M Tris-HCl, 0.2 M

(NH4)2SO4, 0.2% w/v Tween-20; Solis BioDyne, Tartu, Estonia) from dried basidiocarps. Specimens were incubated in 100 ml of lysis buffer and 2.5 ml of proteinase K at 56°C for 24 h and at 98°C for 15 min., and centrifuged for 2 min at 8000 rpm.

The nuclear ribosomal 5.8S-ITS2 and part of the nuclear large subunit (nucLSU) regions were amplified with the primers ITS3CF and LR5R (Vilgalys & Hester 1990; Tibuhwa *et al.,* 2012). The RNA polymerase II region (*RPB2*) was amplified using the specific primers for *Cantharellus* RPB2-6F-Cth, RPB2-7R-Cth2 (Olariaga *et al.*, 2017). The transcription elongation factor 1-alpha (*TEF1*) region was amplified with TEF-1Fcanth (Buyck *et al.,* 2014) and TEF-1R primers (Morehouse *et al.,* 2003). The PCR reactions were performed using 5 x HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia) with a 25 µl reaction volume. For 5.8S-ITS2 and LSU regions, the amplification conditions followed Olariaga *et al.* (2015). For amplification of the *TEF1* and *RPB2* regions, we followed Morehouse *et al.* (2003) and O'Donnell *et al.* (2011), respectively.

### *Phylogenetic analyses*

Newly obtained sequences were assembled and edited in DNABaser v. 4. The 5.8S-ITS2-LSU amplicon was partitioned via ITSx (Bengtsson-Palme *et al.,* 2013). Four datasets were constructed for the four gene regions. Additional taxa for phylogenetic analyses were added to the datasets mainly based on Olariaga *et al.* (2017), with special attention to the type materials, geographic locations, and taxonomy.

Sequences for each dataset were aligned via MUSCLE (Edgar, 2004). Ambiguous and poorly aligned regions were trimmed with Gblocks v. 0.91b (Castresana, 2000). The introns were not excluded from the analyses. The concatenated dataset (cf. Olariaga *et al.,* 2017) was analysed using MrBayes v. 3.2.6 (Ronquist *et al.,* 2012), implementing the best-fit model of nucleotide evolution for each dataset as inferred from MrModeltest 2.3 (Nylander, 2004). *Cantharellus platyphyllus* Heinem was selected as an outgroup following Olariaga *et al.* (2017).

The dataset was analyzed using two independent runs, with eight MCMCMC chains. The analysis was set to run for 20 M generations, and the trees and parameters were sampled every 5000 generations. Burn-in was set to discard 50 % of samples. The majority-rule consensus tree was assembled from post-burn-in trees.

## RESULTS

Altogether, 136 new sequences were obtained in this study, and were submitted to GenBank (Table 1). The best-fit models of nucleotide evolution for each dataset as suggested by MrModeltest were as follows: HKY+I+G (for ITS2), GTR+I+G (for LSU), SYM+I+G (for *RPB2* and *TEF1*). The number of characters for each partition included: 388 ITS2, 795 LSU, 1087 *RPB2*, and 682 *TEF1*. The concatenated dataset covered 92 taxa and 2952 characters, with 1992 constant and 649 informative positions.

The Bayesian tree based on combined sequences of ITS2, LSU, *RPB2* and *TEF1* is presented in Fig 1, showing that the Iranian *Cantharellus* samples collected

**Table 1.** Taxa used in the phylogenetic analyses and their GenBank accession numbers. Newly generated sequences are marked in bold. HT, PT, NT and ET refer to holo-, para-, neo- and epitype respectively.

| Taxon | Voucher | Country | GenBank no. | | | |
|---|---|---|---|---|---|---|
| | | | ITS2 | LSU | *RPB2* | *TEF1* |
| *C. afrocibarius* HT | 496/BB 96.235 | Zambia | – | KF294668 | KF294746 | JX192993 |
| *C. albidolutescens* HT | 457/BB 08.070 | Madagascar | – | KF294646 | KF294723 | JX192982 |
| *C. alborufescens* HT | MPU027371 | Morocco | KX828764 | – | – | – |
| *C. alborufescens* (1) | JLS880 (AH) | Spain | KR677493 | KR677531 | KX828735 | KX828816 |
| *C. alborufescens* (2) | 1108/BB 12.075 | Italy | KX907209 | KX929161 | KX907232 | KX907243 |
| *C. alborufescens* (5) | AH44783 | France | KR677492 | KR677530 | KX828736 | KX828817 |
| *C. alborufescens* (6) | BIO-Fungi 11687 | Spain | KX828765 | KX828793 | KX828737 | KX828818 |
| *C. altipes* (1) | 337/BB 07.115 | USA | JN944018 | JN940599 | JN993602 | – |
| *C. alborufescens* | ICH431F | Iran | **MH463257** | **MH463258** | **MH463259** | **MH463260** |
| *C. alborufescens* | ICH432F | Iran | **MH463265** | **MH463266** | **MH463267** | **MH463268** |
| *C. alborufescens* | ICH433F | Iran | **MH463269** | **MH463270** | – | **MH463271** |
| *C. alborufescens* | ICH434F | Iran | **MH463280** | **MH463281** | **MH463282** | **MH463283** |
| *C. alborufescens* | ICH435F | Iran | **MH463284** | **MH463285** | – | **MH463286** |
| *C. alborufescens* | ICH436F | Iran | **MH463287** | **MH463288** | – | **MH463289** |
| *C. alborufescens* | ICH437F | Iran | **MH463290** | **MH463291** | **MH463292** | **MH463293** |
| *C. alborufescens* | ICH438F | Iran | **MH463261** | **MH463262** | **MH463263** | **MH463264** |
| *C. alborufescens* | ICH439F | Iran | **MH463272** | **MH463273** | **MH463274** | **MH463275** |
| *C. alborufescens* | ICH440F | Iran | **MH463276** | **MH463277** | **MH463278** | **MH463279** |
| *C. altipes* (2) PT | 344/BB 07162 | USA | – | KF294636 | KF294713 | GQ914945 |
| *C. amethysteus* NT | AH44796 | Spain | KR677512 | KR677550 | KX828738 | KX828819 |
| *C. amethysteus* (1) | 994/Estades 10.454 | France | KX907205 | KX907214 | KX907226 | KX907237 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. amethysteus* (2) | 349/BB 07.284 | Slovakia | JN944020 | KF294639 | KF294716 | GQ914953 |
| *C. californicus* HT | OSC 122878 | USA | KX828768 | KX828795 | KX828739 | KX828820 |
| *C. cibarius* ET | BIO-Fungi 10986 | Sweden | KR677501 | KR677539 | KX828742 | KX828823 |
| *C. cibarius* (1) | AH44780 | Spain | KR677508 | KR677546 | KX828740 | KX828821 |
| *C. cibarius* (2) | BIO-Fungi 10780 | France | KR677503 | KR677541 | KX828741 | KX828822 |
| *C. cibarius* (4) | BIO-Fungi 12684 | Switzerland | KR677505 | KR677543 | KX828744 | KX828825 |
| *C. cibarius* var. albidus NT (= *C. pallens*) | BIO-Fungi 11150 | Spain | KR677494 | KR677532 | KX828745 | – |
| *C. cinnabarinus* NT | 312/BB 07.001 | USA | – | KF294624 | KF294698 | GQ914985 |
| *C. decolorans* ET | 469/BB 08.278 (PC) | Madagascar | KX907203 | KF294654 | KF294731 | GQ914968 |
| *C. ferruginascens* HT | E00204187 | United Kingdom | KX828773 | – | – | – |
| *C. ferruginascens* (3) | AH44782 | France | KR677488 | KR677526 | KX828747 | KX828826 |
| *C. ferruginascens* (2) | AH44794 | Spain | "KR677485" | KR677523 | KX828748 | KX828827 |
| *C. ferruginascens* (4) | AH44795 | Spain | KX828775 | KX828800 | KX828749 | KX828828 |
| *C. ferruginascens* (5) | BIO-Fungi 11700 | Spain | KR677486 | KR677524 | KX828750 | KX828829 |
| *C. ferruginascens* | ICH441F | Iran | **MH463294** | **MH463295** | **MH463296** | **MH463297** |
| *C. ferruginascens* | ICH442F | Iran | **MH463298** | **MH463299** | **MH463300** | **MH463301** |
| *C. ferruginascens* | ICH443F | Iran | **MH463302** | **MH463303** | **MH463304** | **MH463305** |
| *C. ferruginascens* | ICH444F | Iran | **MH463367** | **MH463368** | **MH463369** | **MH463370** |
| *C. ferruginascens* | ICH445F | Iran | – | – | – | **MH463306** |
| *C. ferruginascens* | ICH446F | Iran | **MH463307** | **MH463308** | **MH463309** | **MH463310** |
| *C. ferruginascens* | ICH447F | Iran | **MH463311** | **MH463312** | **MH463313** | **MH463314** |
| *C. ferruginascens* | ICH448F | Iran | **MH463315** | **MH463316** | – | **MH463317** |
| *C. ferruginascens* | ICH449F | Iran | **MH463318** | **MH463319** | **MH463320** | **MH463321** |
| *C. ferruginascens* | ICH450F | Iran | – | – | – | **MH463388** |

**Table 1.** Taxa used in the phylogenetic analyses and their GenBank accession numbers. Newly generated sequences are marked in bold. HT, PT, NT and ET refer to holo-, para-, neo- and epitype respectively. (suite)

| Taxon | Voucher | Country | GenBank no. | | | |
|---|---|---|---|---|---|---|
| | | | ITS2 | LSU | *RPB2* | *TEF1* |
| *C. ferruginascens* | ICH453F | Iran | **MH463325** | **MH463326** | **MH463327** | **MH463328** |
| *C. ferruginascens* | ICH454F | Iran | **MH463329** | **MH463330** | **MH463331** | **MH463332** |
| *C. ferruginascens* | ICH455F | Iran | **MH463333** | **MH463334** | **MH463335** | **MH463336** |
| *C. ferruginascens* | ICH456F | Iran | **MH463337** | **MH463338** | **MH463339** | **MH463340** |
| *C. ferruginascens* | ICH457F | Iran | – | – | – | **MH463390** |
| *C. ferruginascens* | ICH458F | Iran | **MH463341** | **MH463342** | – | **MH463343** |
| *C. ferruginascens* | ICH459F | Iran | **MH463344** | **MH463345** | **MH463346** | **MH463347** |
| *C. ferruginascens* | ICH460F | Iran | **MH463348** | **MH463349** | – | **MH463350** |
| *C. ferruginascens* | ICH461F | Iran | – | – | – | **MH463391** |
| *C. ferruginascens* | ICH462F | Iran | **MH463351** | **MH463352** | **MH463353** | **MH463354** |
| *C. ferruginascens* | ICH463F | Iran | **MH463355** | **MH463356** | **MH463357** | **MH463358** |
| *C. ferruginascens* | ICH464F | Iran | **MH463359** | **MH463360** | **MH463361** | **MH463362** |
| *C. ferruginascens* | ICH465F | Iran | **MH463363** | **MH463364** | **MH463365** | **MH463366** |
| *C. ferruginascens* | ICH466F | Iran | **MH463371** | **MH463372** | **MH463373** | **MH463374** |
| *C. ferruginascens* | ICH467F | Iran | – | – | – | **MH463392** |
| *C. ferruginascens* | ICH468F | Iran | **MH463375** | **MH463376** | – | **MH463377** |
| *C. ferruginascens* | ICH469F | Iran | **MH463378** | **MH463379** | **MH463380** | **MH463381** |
| *C. ferruginascens* | ICH470F | Iran | **MH463382** | **MH463383** | – | **MH463384** |
| *C. ferruginascens* | ICH471F | Iran | **MH463385** | **MH463386** | – | **MH463387** |
| *C. formosus* | SAR220712 (DAOM) | Canada | KR677515 | KR677553 | KX828751 | KX828830 |
| *C. friesii* (1) | 1004/EC 09.43 | Italy | – | KX857084 | KX856988 | KX857016 |
| *C. friesii* (2) | AH44798 | Spain | KR677484 | KR677522 | KX828752 | KX828831 |

| | | | | | | |
|---|---|---|---|---|---|---|
| C. ilicis HT (= *C. alborufescens*) | BIO-Fungi 11689 | Spain | KX828778 | KX828802 | KX828753 | KX828832 |
| *C. lateritius* ET | 320/BB 07.025 | USA | – | KF294633 | KF294708 | GQ914959 |
| *C. lewisii* HT | 314/BB 07.003 | USA | JN944021 | JN940597 | JN993612 | GQ914962 |
| *C. lourizanianus* HT (= *C. romagnesianus*) | LOU-Fungi 19494 | Spain | KX828780 | KX828803 | – | – |
| *C. minor* (1) | 313/BB 07.002 | USA | – | KF294625 | KF294699 | JX192978 |
| *C. nigrescens* | 66/BB 06.176 | Madagascar | – | KF294606 | KF294680 | JX192967 |
| *C. pallens* (1) | AH44799 | Spain | KR677499 | KR677537 | KX828754 | KX828833 |
| *C. pallens* (2) | AH39124 | Morocco | KX828781 | KX828804 | KX828755 | KX828834 |
| *C. pallens* (3) | 996/BB 09.409 | Italy | KX929162 | KX907215 | KX929160 | KX857014 |
| *C. phasmatis* | C057 | USA | JX030464 | JX030431 | – | JX030417 |
| *C. platyphyllus* ET | 262/BB 98.126 | Tanzania | – | KF294620 | KF294694 | JX192975 |
| *C. quercophilus* HT | 455/BB 07.097 | USA | – | KF294644 | KF294721 | JX192981 |
| *C. romagnesianus* HT | PC0085043 | France | KX828783 | KX828806 | – | – |
| *C. romagnesianus* (1) | AH44218 | Spain | KX828784 | KX828807 | KX828757 | KX828836 |
| *C. romagnesianus* (2) | PC0142170 | France | KX828785 | KX828808 | – | – |
| *C. roseocanus* HT | DAOM220723 | Canada | KX828787 | KX828810 | KX828758 | KX828837 |
| *C. roseofagetorum* HT | AH44789 | Georgia | KX828789 | KX828812 | KX828760 | KX828839 |
| *C. roseofagetorum* | AH44786 | Georgia | KX828790 | KX828813 | KX828761 | KX828840 |
| *C. subalbidus* | OSC81782 | USA | KX828791 | KX828814 | KX828762 | KX828841 |
| *C. tabernensis* (1) | 333/BB 07064 | USA | JN944012 | JN940608 | JN993600 | GQ914975 |
| *C. tabernensis* (2) | 323/BB 07040 | USA | JN944013 | JN940609 | JN993599 | GQ914977 |
| *C. tenuithrix* HT | 343/BB 07.125 | USA | JN944017 | JN940600 | JN993596 | – |
| *C. texensis* HT | 317/BB 07.018 | USA | – | KF294626 | KF294701 | GQ914988 |
| *C. tomentosus* HT | 500/BB 98.060 | Tanzania | – | KF294672 | KF294750 | JX192995 |

in different forest types are clustered in two distinct clades. One group was clustered with *C. alborufescens* in a clade with BBP = 0.73. These samples grew in slightly acidic soil (average pH = 6.9) under mixed and pure forest of *Q. castaneifolia*, *A. glutinosa* and *C. betulus* as ectomycorrhizal trees, with other non- ectomycorrhizal species such as *B. hyrcana*, *A. velutinum* and *P. persica* trees. Another group of Iranian *Cantharellus* samples clustered with European collections of *C. ferruginascens*, forming a polytomy (BBP = 0.58). Our samples in this clade grew in moderately acidic soils under *Fagus orientalis* (average pH=5.7) and *C. betulus* (average pH=6.3) as the main dominant ectomycorrhizal species

*Examined collections:* ***Cantharellus alborufescens***. IRAN: **Mazandaran Prov.,** near Noor city, on slightly acidic soil under *Q. castaneifolia*, ca. −10 m elev., 39s N36°34′45.02″; E52°2′48.34″, 20 Sep. 2015, Parad 11 (ICH431F); ibidem, ca. −12 m elev., N36°34′45.84″; E52°2′46.66″, 20 Sep. 2015, Parad 21 (ICH432F); ibidem, under *C. betulus* and *Q. castaneifolia*, -9 m elev., N36°34′44.43; E52°2′ 45.07″, 20 Sep. 2015, Parad 31 (ICH433F); ibidem, under *Q. castaneifolia*, ca. −12 m elev., N36°34′48.02; E52°2′46.53″, 20 Sep. 2015, Parad 51 (ICH434F); ibidem, N36°34′48.66; E52°2′47.11″, 20 Sep. 2015, Parad

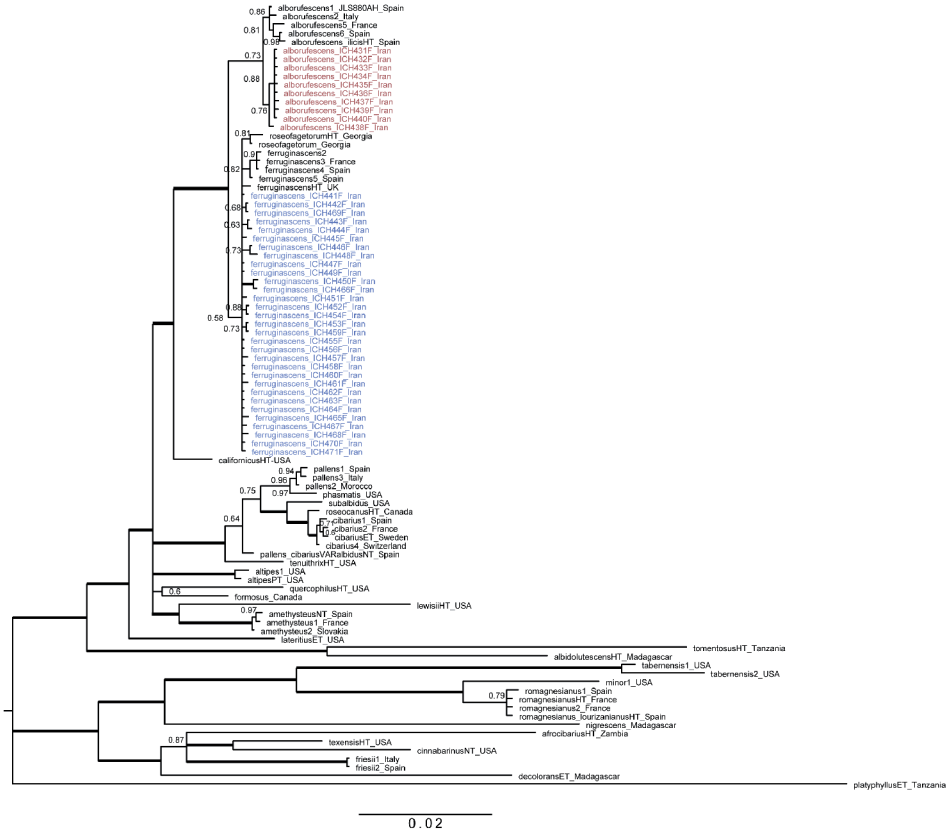


**Fig. 1.** Phylogenetic tree of the European *Cantharellus* species obtained from the Bayesian analysis of combined dataset of ITS2-LSU-*RPB2-TEF1* sequences, representing the position of Iranian samples of *C. alborufescens* (red) and *C. ferruginascens* (blue). Branches in bold have BPP ≥ 0.99.

71 (ICH435F); ibidem, ca. −5 m elev., N36°34′44.77; E52°2′51.24″, 20 Sep. 2015, Parad 81 (ICH436F); ibidem, under *C. betulus*, −8 m elev., N36°34′41.76; E52°2′46.13″, 20 Sep. 2015, Parad 91 (ICH437F); ibidem, near Sari city, on under *C. betulus* and *Q. castaneifolia*, 503 m elev., N36°23′37.42; E53°8′7.26″, 1 Oct. 2015, Parad 111 (ICH438F). **Golestan Prov.,** near Minudasht, Loveh, under *C. betulus* and *Q. castaneifolia*, 721 m elev., N37°20′33.92; E55°40′37.47″, 7 Nov. 2015, Parad 431 (ICH439F); ibidem, near Gorgan, Shastkalateh, on slightly acidic soil under *Q. castaneifolia* and *C. betulus*, 159 m elev., N37°21′14.45″; E55°39′37.71″, 8 Nov. 2015, Parad 481 (ICH440F).

***Cantharellus ferruginascens*.** IRAN: **Mazandaran Prov.,** Sari, on moderate acidic soil under *C. betulus*, 459 m elev., N36°23′33.59; E53°8′3.5″, 1 Oct. 2015, Parad 121 (ICH441F); ibidem, on slightly acidic soil under pure stand of *F. orientalis*, 624 m elev., N36°22′46.65; E53°7′37.66″, 1 Oct. 2015, Parad 131 (ICH442F); ibidem, 629 m elev., N36°22′55.17; E53°7′24.05″, 1 Oct. 2015, Parad Sp3 (ICH469F); ibidem, under *F. orientalis* and *C. betulus*, 730 m elev., N36°22′38.68″; E53°7′38.77″, 1 Oct. 2015, Parad Sp4 (ICH470F); ibidem, 658 m elev., N36°22′49.04; E53°7′21.55″, 1 Oct. 2015, Parad Sp6 (ICH471F); Neka, Mollakheil forests, on slightly acidic soil under pure *F. orientalis* stand, 839 m elev.,


**Fig. 2. A-B** *Cantharellus alborufescens*. **A**. Fruiting body with reddish-brown pileus and orange hymenophore becoming yellow near margin (Parad 81). **B**. Young fruiting bodies with a reddish-brown coating and mature fruiting body with a yellow coating (Parad 71). **C-D**. *Cantharellus ferruginascens*. **C**. Pale specimen with a pinkish-white coating on the pileus (Parad 131). **D.** Citrine-yellow fruiting bodies at different stages of development (Parad 131). Photographs by G. Parad.

N36°31´8.71; E53°26´56.62″, 12 Oct. 2015, Parad 171 (ICH443F); ibidem, under *Pure F. orientalis* stand, 784 m elev., N36°31´9.67; E53°27´17.27″, 12 Oct. 2015, Parad 191 (ICH446F); ibidem, under *F. orientalis*, 806 m elev., N36°30´31.03; E53°27´14.24″, 12 Oct. 2015, Parad 251 (ICH449F); ibidem, on moderate acidic soil under *C. betulus,* 635 m elev., N36°31´8.23; E53°27´13.24″, 12 Oct. 2015, Parad 181 (ICH445F); ibidem, under *C. betulus,* 811 m elev., N36°30´43.87; E53°27´22.64″, 12 Oct. 2015, Parad 201 (ICH447F); under *Pure C. betulus* stand, 781 m elev., N36°30´31.01; E53°27´35.82″, 12 Oct. 2015, Parad 231 (ICH448F); ibidem, on slightly acidic soil under *F. orientalis*, 806 m elev., N36°30´31.03; E53°27´14.24″, 12 Oct. 2015, Parad 251 (ICH449F); Chalous, on moderate acidic soil under *Pure C. betulus* stand, 201 m elev., N36°37´34.002; E51°26´5.11″, 17 Oct. 2015, Parad 291 (ICH450F); ibidem, under *C. betulus*, 220 m elev., N36°37´ 27.95; E51°25´59.4″, 12 Oct. 2015, Parad 301 (ICH451F); ibidem, 195 m elev., N36°37´0.27; E51°26´9.66″, 12 Oct. 2015, Parad 311 (ICH452F); ibidem, 228 m elev., N36°37´ 28.33; E51°26´3.18″, 17 Oct. 2015, Parad Ch4 (ICH466F); Nowshahr, on moderate acidic soil under pure *F. orientalis* stand, 1025 m elev., N36°30´0.36; E51°48´20.23″, 25 Oct. 2015, Parad 331 (ICH453F); ibidem, under *F. orientalis* and *C. betulus*, 613 m elev., N36°30´15.75; E51°49´21.13″, 25 Oct. 2015, Parad 341 (ICH454F); ibidem, under *F. orientalis* and *C. betulus*, 624 m elev., N36°.30´15.59; E51°49´17.27″, 25 Oct. 2015, Parad 351 (ICH455F); ibidem, under *F. orientalis* and *C. betulus*, 624 m elev., N36°30´59.84; E51°46´58.91″, 25 Oct. 2015, Parad D4 (ICH468F); Tonekabon, under pure *F. orientalis* stand, 625 m elev., N36°44´32.69; E50°45´21.08″, 30 Oct. 2015, Parad 501 (ICH444F). **Gilan Prov.,** near Rasht, Lakan Forest, on moderate acidic soil under *C. betulus* stand, 99 m elev., N37°7´57.49; E49°34´12.29″, 29 Oct. 2015, Parad 361 (ICH456F); ibidem, under *C. betulus* and *F. orientalis* stands, 135 m elev., N37°7´53.53; E49°34´33.96″, 29 Oct. 2015, Parad 371 (ICH457F); ibidem, on under *C. betulus* and *F. orientalis* stands, 167 m elev., N37°7´40.41; E49°35´8.57″, 29 Oct. 2015, Parad 391 (ICH458F); ibidem, 178 m elev., N37°7´37.65; E49°34´57.24″, 29 Oct. 2015, Parad 401 (ICH459F); ibidem, 162 m elev., N37°7´37.9; E49°34´56.66″, 29 Oct. 2015, Parad 411 (ICH460F). **Golestan Prov.,** Gorgan, Shastkalateh forest, on moderate acidic soil under *C. betulus* and *F. orientalis*, 262 m elev., N37°21´30.37; E55°39´36.24″, 10 Nov. 2015, Parad 441 (ICH461F); ibidem, 542 m elev., N36°46´23.69; E54°23´25.11″, 10 Nov. 2015, Parad 451 (ICH462F); ibidem, 658 m elev., N36°44´47.71; E54°24´17.82″, 10 Nov. 2015, Parad 461 (ICH463F); ibidem, under *C. betulus* and *F. orientalis*, 542 m elev., N36°46´20.72; E54°23´33.27″, 10 Nov. 2015, Parad 491 (ICH465F); ibidem, under *F. orientalis* and *C. betulus*, 769 m elev., N36°44´43.73; E54°24´30.57″, 10 Nov. 2015, Parad Gorgan2 (ICH467F); ibidem, on slightly acidic soil under *C. betulus*, 159 m elev., N37°21´26.625; E55°39´32.44″, 8 Nov. 2015, Parad 471 (ICH464F).

## DISCUSSION

The results from the multigene phylogenetic analyses in our study (Fig. 1) confirm the recognition of the two species *C. alborufescens* and *C. ferruginascens* in Iran. Across the Hyrcanian forests, *Cantharellus* is considered as a source of food and seasonal income. Local communities in the west of Hyrcanian forests refer to *Cantharellus* as "Zarde leng" (meaning "yellow foot") while in the east (Mazandaran and Golestan province) it is called "Zarde Kija" meaning "yellow girl". To elucidate the precise distribution of Cantharellus species in Iran, the remaining forests in the country need to be investigated. Moreover, previous reports of C. cibarius needs molecular confirmation based on new collections.. The name *Cantharellus cibarius* has been a collective name mis-applied to almost any "yellow" *Cantharellus* and alike genera (Buyck *et al.*, 2013, 2014; Olariaga *et al.*, 2017).

According to Olariaga *et al.* (2017) the European *C. alborufescens* grows in calcareous soils and is mostly associated with Mediterranean evergreen oaks, while *C. ferruginascens* generally grows in calcareous to moderately acidic soil and extends also in more temperate areas. Both species are associated with angiosperm hosts, especially Fagaceae (*Castanea*, *Fagus*, *Quercus*) and Betulaceae (*Carpinus*). In northern Iran, *C. alborufescens* and *C. ferruginascens* have largely the same ecological preferences given by Olariaga *et al.* (2017): *C. alborufescens* grows on slightly acidic soils under *Q. castaneifolia* and *C. betulus*, and *C. ferruginascens* inhabits moderate to slightly acidic soils under *F. orientalis* (pH=5.7) and *C. betulus* (pH=6.3).

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## REFERENCES

BAHRAM M., PÕLME S., KõLJALG U., ZARRE S. & TEDERSOO L., 2012 — Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist* 193 (2): 465-473.

BENGTSSON-PALME J., RYBERG M., HARTMANN M., BRANCO S., WANG Z., GODHE A., WIT P., SÁNCHEZ-GARCíA M., EBERSBERGER I. SOUSA F., et al., 2013 — Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* 4 (10): 914-919.

BUYCK B., 2016 — Editorial Special Issue: *Cantharellus*. Towards completing the world inventory of Cantharellus. *Cryptogamie Mycologie* 37(3):255–258

BUYCK B., KAUFF F., CRUAUD C. & HOFSTETTER V., 2013 — Molecular evidence for novel *Cantharellus* (Cantharellales, Basidiomycota) from tropical African miombo woodland and a key to all tropical African chanterelles. *Fungal Diversity* 58 (1): 281-298.

BUYCK B., KAUFF F., EYSSARTIER G., COULOUX A. & HOFSTETTER V., 2014 — A multilocus phylogeny for worldwide *Cantharellus* (Cantharellales, Agaricomycetidae). *Fungal Diversity* 64 (1): 101-121.

CASTRESANA J., 2000 — Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular biology and evolution* 17 (4): 540-552.

DE KESEL A., AMALFI M., NGOY B.K.W., YOROU N.S., RASPE O., DEGREEF J. & BUYCK B., 2016 — New and interesting *Cantharellus* from tropical Africa. *Cryptogamie, Mycologie* 37 (3): 283- 327.

EDGAR R.C., 2004 — MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 32 (5): 1792-1797.

ERSHAD J., 2009 — *Fungi of Iran*. 3rd ed. Iranian Research Institute of Plant Protection, Tehran, Iran, 531p.

GHOBAD-NEJHAD M. & HALLENBERG N., 2012 — Checklist of Iranian non-gilled/non-gasteroid hymenomycetes (Agaricomycotina). *Mycotaxon* 119 (494): 1-41.

HABIBI KASSEB H., 1992 — *Fundaments of forest soil science*. Tehran University Press, Tehran, No. 2118, 428p.

KUMARI D., UPADHYAY R.C. & REDDY M.S., 2011 — *Cantharellus pseudoformosus*, a new species associated with *Cedrus deodara* from India. *Mycoscience* 52 (2): 147-151.

LEACOCK P.R., RIDDELL J., WILSON A.W., ZHANG R., NING C. & MUELLER G.M., 2016 — *Cantharellus chicagoensis* sp. nov. is supported by molecular and morphological analysis as a new yellow chanterelle in midwestern United States. *Mycologia* 108 (4): 765-772.

MARVI-MOHAJER M.R., 2007 — *Silviculture*. Tehran University Press, Tehran, 388p.

MOREHOUSE E.A., JAMES T.Y., GANLEY A.R., VILGALYS R., BERGER L., MURPHY P.J. & LONGCORE J.E., 2003 — Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology* 12 (2): 395-403.

NYLANDER J.A.A., 2004. *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Center, Uppsala University, Sweden.

O'DONNELL K., ROONEY A.P., MILLS G.L., KUO M., WEBER N.S. & REHNER S.A., 2011 — Phylogeny and historical biogeography of true morels (*Morchella*) reveals an early Cretaceous origin and high continental endemism and provincialism in the Holarctic. *Fungal Genetics and Biology* 48 (3): 252-265.

OLARIAGA I., BUYCK B., ESTEVE-RAVENTóS F., HOFSTETTER V., MANJóN J.L., MORENO G. & SALCEDO I., 2015 — Assessing the taxonomic identity of white and orange specimens of *Cantharellus*: occasional colour variants or independent species? *Cryptogamie*, *Mycologie* 36 (3): 287-300.

OLARIAGA I., MORENO G., MANJóN J.L., SALCEDO I., HOFSTETTER V., RODRíGUEZ D. & BUYCK B., 2017 — *Cantharellus* (Cantharellales, Basidiomycota) revisited in Europe through a multigene phylogeny. *Fungal Diversity* 83 (1): 263-292.

RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D.L., DARLING A., HöHNA S., LARGET B., LIU L., SUCHARD M.A. & HUELSENBECK J.P., 2012 — MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539-542.

SAGHEB-TALEBI K.H., SAJEDI T. & YAZDIAN F., 2005 — *Forests of Iran*. Research Institute of Forest and Rangeland, Tehran, 152p.

SHAO S.C., BUYCK B., HOFSTETTER V., TIAN X.F., GENG Y.H., FU Q.Y. & LIU P.G., 2014 — *Cantharellus hygrophorus*, a new species in subgenus *Afrocantharellus* from tropical southwestern China. *Cryptogamie, Mycologie* 35 (3): 283-291.

THORN R.G., KIM J.I., LEBEUF R. & VOITK A., 2017 — The golden chanterelles of Newfoundland and Labrador: a new species, a new record for North America, and a lost species rediscovered. *Botany* 95 (6): 547-560.

TIBUHWA D.D., SAVIĆ S., TIBELL L. & KIVAISI A.K., 2012 — *Afrocantharellus* gen. stat. nov. is part of a rich diversity of African Cantharellaceae. *IMA fungus* 3 (1): 25-38.

VILGALYS R. & HESTER M., 1990 — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 (8): 4238-4246.

ZARRINKAFSH M.K., 2002 — *Forestry soil, interaction of soil and plants regarding ecological factors of forest ecosystems*. Publication of Research Institute of Forests and Rangelands, Tehran, No. 292, 361p.