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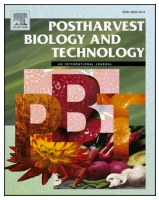

Characterization of fungal species involved in white haze disorder on apples in Northern Italy and description of *Golubevia mali sp. nov.* and *Entyloma mali sp. nov.*

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ABSTRACT

White haze, caused by an extensive fungal colonization of the apple surface, is an emerging postharvest issue in several European apple production areas. It results in compromised quality and decreased marketability of the fruits, leading to economic losses. In this study, the occurrence and the diversity of white haze-related fungi associated with apples was investigated in Northern Italy. Fungal strains were isolated from apple tissues and the species diversity was assessed using molecular and phylogenetic tools. Moreover, the ability of the isolated species to reproduce symptoms on healthy apples artificially inoculated was tested. Seventy-two fungal isolates were collected. Six species belonging to different basidiomycetous genera (*Entyloma*, *Golubevia*, *Tilletiopsis*) were identified, showing high diversity of fungi involved in white haze development in Northern Italy. The strains were identified as *E. belangeri*, *E. randwijkense*, *G. pallescens*, *T. washingtonensis*. Moreover, two new species, *E. mali* sp. nov. and *G. mali* sp. nov. were described. The most frequently isolated strains were inoculated on healthy apple fruit, showing to be able to reproduce symptoms on red-skin apples, fulfilling Koch's postulates. This work provides new insights to increase knowledge about the causal agents of white haze on apple. In addition, the names Golubevia, Golubeviaceae and Golubeviales have been nomenclaturally validated.

1. Introduction

Domesticated apple (*Malus* × *domestica* Borkh.) is the largest fruit crop produced in temperate regions. Global production of apple highly increased in the past 20 years, achieving 93 million tons in 2021 with a total trading value of 79 billion USD (FAOSTAT, 2023). In Italy, apple is mainly cultivated in northern regions with Trentino, South Tyrol, Piedmont and Lombardy as the leading production areas. Among the main cultivars, red skin varieties, represented by the cultivars 'Gala', 'Red Delicious' and 'Fuji', and yellow skin varieties with 'Golden Delicious' are highly produced (ISTAT, 2020).

An emerging postharvest disorder, named white haze, characterized by imperfection of the apple skin has been observed during the last years in the major production areas in Northern Italy and other countries (Spadaro et al., 2019). A thin layer of fungal growth adhering to the cuticle of apples and appearing whitish to pale-grey in colour can be observed already in orchard or after storage with reduction of fruit quality and yields, and causing economic losses (Lindner and Baric, 2006). Microscopical observations of the surface of affected fruit revealed narrow, hyaline fungal hyphae without clamp connections that reproduce asexually with the formation of elongated, sausage-shaped ballistoconidia or elongated to fusoidal blastoconidia, which establish the haze (Boekhout et al., 2006; Weber and Zabel, 2011). White haze disorder was described for the first time in The Netherlands (Boekhout et al., 2006), and later was found also in Northern Italy (Baric et al., 2010), in Northern Germany (Weber and Zabel, 2011) and in Croatia

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(Prencipe et al., 2016). White haze has an erratic incidence over the years, and it has been reported on different apple cultivars, 'Elstar', 'Braeburn', 'Golden Delicious', 'Gala', 'Red Delicious', 'Fuji', and 'Cripps Pink$^{(cov)}$/Pink Lady$^{(cov)}$, among others. Also scab resistant varieties can be susceptible to white haze, including 'Topaz' and 'Inored$^{(cov)}$/Story® (Nari et al., 2022). Initially, white haze was considered as a postharvest disorder by Boekhout et al. (2006), due to the findings on apples stored under Ultra-Low Oxygen (ULO) conditions and rarely on fruits while still in the orchard. However, the disorder was found also in pre-harvest (Baric et al., 2010; Weber and Zabel, 2011; Prencipe et al., 2016). Occasionally, it can also be observed on apples for sale in supermarkets in The Netherlands (T. Boekhout, unpubl. observ.).

Recently, metabarcoding analysis was used to characterize both epiphytic and endophytic microbial communities of 'Opal' and 'Ambrosia' apples, across six time points from early fruit development to the end of shelf life (Garello et al., 2023). Among the genera associated to white haze, *Golubevia* had the highest abundance in epiphytic communities of 'Ambrosia' apples at harvest, after storage and shelf life, the same time points when white haze occurred on the same fruit. All *Golubevia* amplicon sequence variants (ASV) could be assigned to the species *Golubevia pallescens*. *Tilletiopsis* and *Entyloma* were weakly detected as endophytes in both varieties during early summer.

In the previous Dutch investigations, nine species of basidiomycetous fungi within the genus *Tilletiopsis* were identified as the responsible organisms based on comparison of ribosomal DNA (rDNA) regions ITS and D1/D2 domains of the large subunit of ribosomal RNA gene (LSU rRNA). However, only two taxa were assigned to known *Tilletiopsis* species, *T. minor* and *T. pallescens*. All the other taxa were named as *Tilletiopsis* spp. A-G, with *Tilletiopsis* sp. B as dominant species (Boekhout et al., 2006). The same taxon was the cause of white haze also in Northern Germany, Italy and Croatia (Weber and Zabel, 2011; Baric et al., 2010; Prencipe et al., 2016). In Germany, analyses based on ITS sequences revealed that two different species were associated with this disorder on apples of cultivar 'Elstar', namely *Tilletiopsis* sp. B and G (Weber and Zabel, 2011). The study by Baric et al., (2010) was based on apples of different cultivars harvested from different orchards in South Tyrol and in other regions of Northern Italy and revealed six haplotypes (WH1–6) separated into four different lineages identified as *T. pallescens* (WH2 and WH6), *T. washingtonensis* (WH1 and WH5) and *Tilletiopsis* sp. F (WH3) and a new haplotype (WH4) named *Tilletiopsis* sp. H. Fungal strains isolated in Croatia showing white haze were identified as *T. pallescens* based on ITS sequences (Prencipe et al., 2016).

The genus *Tilletiopsis* was proposed for saprotrophic yeast-like fungi within the phylum Basidiomycota, subphylum Ustilaginomycotina, class Exobasidiomycetes, that form clampless, hyaline, narrow hyphae and that reproduce only asexually with so-called ballistoconidia. The genus name *Tilletiopsis* (etymology: *Tilletia* + *-opsis,* similar to *Tilletia*) was introduced by Derx (1930). Nyland (1950) described *T. minor* and *T. washingtonensis* and chose the latter as the neotype for the genus *Tilletiopsis*. Later, Tubaki (1950) described two species and a variety: *T. cremea*, *T. lilacina* and *T. minor* var. *flava*. Gokhale in 1972 added to the genus *T. albescens*, *T. fulvescens* and *T. pallescens* (Gokhale, 1972). Boekhout (1991) reclassified *T. minor* var. *flava* as a separate species named *T. flava* and considered *T. cremea* and *T. lilacina* as synonyms of *T. washingtonensis* due to morphological, physiological, and biochemical similarities (Boekhout, 1991). However, the distinctness of the latter three species was subsequently confirmed by molecular studies and reinstated (Hamamoto et al., 2000). The latest species described were *T. derxii*, *T. oryzicola* and *T. penniseti* (Takashima and Nakase, 2001). Summarizing, the genus *Tilletiopsis* was composed by 11 species described based on morphological and physiological characteristics (Boekhout, 1991; 2011). However, after the introduction of molecular analyses, a taxonomic revision of the genus was initially proposed through the ribosomal RNA (rRNA) gene sequencing, in particular sequencing of D1/D2 domains of the large subunit (LSU) rRNA gene, and phylogenetic analyses demonstrated that the genus *Tilletiopsis* contained species that occur in different orders within the Exobasidiomycetes (Begerow et al., 2000; 2006; Fell et al., 2000; Boekhout, 2011). Wang et al. (2015) based phylogenetic analyses of seven loci, namely three ribosomal rRNA gene regions and four protein-coding genes: the small subunit ribosomal (SSU or 18 S) rRNA gene), the D1/D2 domains of LSU rRNA gene (or 26 S rRNA gene), the ITS 1 and 2 regions, the two RNA polymerase subunits (*RPB1* and *RPB2*), the translation elongation factor 1-α (*TEF1*) and the mitochondrial cytochrome *b*. This study confirmed that the genus *Tilletiopsis* is polyphyletic, thus, most of the accepted species were reclassified into different genera: *T. washingtonensis*, *T. cremea* and *T. lilacina* were retained in *Tilletiopsis*, whilst *T. flava*, *T. fulvescens* and *T. oryzicola* were reclassified as *Phragmotaenium derxii*, *P. flavum*, *P. fulvescens* and *P. oryzicola*, while *T. minor* and *T. penniseti* were recombined as *Gjaerumia minor* and *G. penniseti*. Two new genera, *Robbauera* and *Golubevia* (nomen invalid), were proposed to accommodate *T. albescens* and *T. pallescens*: *T. albescens* was recombined as *Robbauera albescens* and *T. pallescens* as *Golubevia pallescens*. Note that the description of *Golubevia* was not done correctly, as the basionym was not included, hence, this name remains invalid and will be validated in this work. Two new orders were also proposed, as these species could not be assigned to any recognised orders in Exobasidiomycetes: Robbauerales for *R. albescens* and Golubeviales for *G. pallescens* (Wang et al., 2015), and for reasons indicated above the latter was also not validly published. More recently, a study based on multilocus approach provided a replacement of the still undescribed species obtained from white haze (Boekhout et al., 2006) and placed those in the following genera: *Golubevia*, *Jamesdicksonia* and *Entyloma* (Richter et al., 2019). These new species were identified as *Jamesdicksonia mali* (*Tilletiopsis* sp. A), *Golubevia heteromorpha* (also invalid, = *Tilletiopsis* sp. B), *Entyloma davenportii* (*Tilletiopsis* sp. C and E), *Entyloma elstari* (*Tilletiopsis* sp. D), and *Entyloma belangeri* (*Tilletiopsis* sp. F). Isolates belonging to *Tilletiopsis* sp. G, instead, were split into two groups, G1 and G2: G1 provisionally associated to *Entyloma belangeri* and G2 identified as *Entyloma randwijkense* (Richter et al., 2019).

Several factors, such as humidity and low temperatures, could contribute to the extensive overgrowth of white haze-related fungi on apple fruit thus causing the disorder, as reported in the Netherlands where white haze was particularly prominent in years with high rainfall during the harvesting season (Boekhout et al., 2006). Growth of these white haze-related fungal species may be favoured also by cultural practices adopted, such as the use of hail protection nets that increase relative humidity and intensive application of foliar nutrients as observed in South Tyrol, Italy (Baric et al., 2010). During the years 2019–2020, white haze disorder was observed on apple fruit, both in orchards and in storage packinghouses of Piedmont, Trentino, and South Tyrol, three areas of northern Italy.

The current study aims to explore the occurrence and the diversity of white haze-related fungi associated with apple fruit in those regions. Moreover, the ability of the found species to reproduce the disorder was investigated. In particular, the objectives of the study were to: (I) isolate and to collect fungal strains associated with apple tissues; (II) assess the fungal species diversity using molecular and phylogenetic tools; III) establish morphological analyses of the species identified; (IV) test the virulence of the species obtained; and (V) to validate the invalidly published genus name *Golubevia* and higher taxa based on this name.

2. Materials and methods

2.1. Sampling and isolation

Pure cultures of *Tilletiopsis*-like fungi were collected during 2019 and 2020 in three Italian regions, namely Piedmont, Trentino, and South Tyrol, from asymptomatic and symptomatic *Malus domestica* plant tissues, in both orchard and warehouse environments (Table 1). Several samples showed white haze. Isolations were performed from different plant organs, such as fruit, leaves and wood. The ballistosporous fungi

Table 1
Collection details of *Tilletiopsis*-like fungi isolates collected in this study.

Code	Species	Site	Cultivar	Source	Date	Collector
EM23 = CBS 146952	*Entyloma mali sp. nov.*	Valle Dell'Adige, Trentino, Italy	Modi	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM26 = CBS 146953	*E. mali sp. nov.*	Valle Dell'Adige, Trentino, Italy	Gala	Fruit skin, orchard	Jul-19	Valeria Gualandri
CVG 604 = CBS 146954	*E. belangeri*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
EM1	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Braeburn	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM14	*E. belangeri*	Valsugana, Trentino, Italy	Golden	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM15	*E. belangeri*	Valsugana, Trentino, Italy	Golden	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM16	*E. belangeri*	Valsugana, Trentino, Italy	Golden	Wood bark, orchard	Jul-19	Valeria Gualandri
EM19	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Braeburn	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM20	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Modi	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM22	*E. belangeri*	Valsugana, Trentino, Italy	Golden	Fruit skin, packing house	Jul-19	Valeria Gualandri
EM27	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Golden	Fruit skin, packing house	Jul-19	Valeria Gualandri
EM4	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Braeburn	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM8	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Braeburn	Leaf, orchard	Jul-19	Valeria Gualandri
EM9	*E. belangeri*	Valle Dell'Adige, Trentino, Italy	Fuji	Fruit skin, orchard	Jul-19	Valeria Gualandri
ET07_b_t2_B2_002	*E. belangeri*	Valle Dell'Adige, South Tyrol, Italy	Cripps Pink	Leaf, orchard	Aug-19	Sabine Oettl
ET07_b_t2_F2_002	*E. belangeri*	Valle Dell'Adige, South Tyrol, Italy	Cripps Pink	Fruit skin, orchard	Aug-19	Sabine Oettl
ET07_b_t2_F2_003	*E. belangeri*	Valle Dell'Adige, South Tyrol, Italy	Cripps Pink	Fruit skin, orchard	Aug-19	Sabine Oettl
UL10_b_t3_F1_001	*E. belangeri*	Laives, South Tyrol, Italy	Cripps Pink	Fruit skin, orchard	Aug-19	Sabine Oettl
EM12 = CBS 146955	*E. randwijkense*	Bleggio, Trentino, Italy	Opal	Wood bark, orchard	Jul-19	Valeria Gualandri
EM13	*E. randwijkense*	Bleggio, Trentino, Italy	Golden	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM36 = CBS 146956	*E. randwijkense*	Val di Non, Trentino, Italy	Golden	Fruit skin, packing house	Jul-19	Valeria Gualandri
CVG 411 = CBS 146945	*Golubevia mali sp. nov.*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Apr-19	Vladimiro Guarnaccia
CVG 412 = CBS 146946	*G. mali sp. nov.*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Apr-19	Vladimiro Guarnaccia
CVG 415 = CBS 146947	*G. mali sp. nov.*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Apr-19	Vladimiro Guarnaccia
CVG 414 = CBS 146948	*G. pallescens*	Cuneo, Piedmont, Italy	Jeromine	Fruit skin, packing house	Apr-19	Vladimiro Guarnaccia
CVG 417 = CBS 146949	*G. pallescens*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, packing house	May-19	Vladimiro Guarnaccia
CVG408	*G. pallescens*	Cuneo, Piedmont, Italy	Jeromine	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG409	*G. pallescens*	Cuneo, Piedmont, Italy	Jeromine	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG410	*G. pallescens*	Cuneo, Piedmont, Italy	Jeromine	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG413	*G. pallescens*	Cuneo, Piedmont, Italy	Jeromine	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG416	*G. pallescens*	Cuneo, Piedmont, Italy	Jeromine	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG420	*G. pallescens*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG427	*G. pallescens*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG428	*G. pallescens*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG431	*G. pallescens*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG432	*G. pallescens*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG433	*G. pallescens*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG434	*G. pallescens*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, packing house	Jul-19	Vladimiro Guarnaccia
CVG626	*G. pallescens*	Cuneo, Piedmont, Italy	Gala	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG700	*G. pallescens*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG701	*G. pallescens*	Cuneo, Piedmont, Italy	Red Chief	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG702	*G. pallescens*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG704	*G. pallescens*	Cuneo, Piedmont, Italy	Red Chief	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
EM37	*G. pallescens*	Trento, Trentino, Italy	Opal	Fruit skin, packing house	Jul-19	Valeria Gualandri
BG05_b_t3_B1_003	*Tilletiopsis washingtonensis*	Burggraviato, South Tyrol, Italy	Cripps Pink	Leaf, orchard	Sep-19	Sabine Oettl
BG05_b_t3_F1_001	*T. washingtonensis*	Burggraviato, South Tyrol, Italy	Cripps Pink	Fruit skin, orchard	Sep-19	Sabine Oettl
BG05_b_t3_F2_003	*T. washingtonensis*	Burggraviato, South Tyrol, Italy	Cripps Pink	Fruit skin, orchard	Sep-19	Sabine Oettl
BG05_b_t3_R1_003	*T. washingtonensis*	Burggraviato, South Tyrol, Italy	Cripps Pink	Wood bark, orchard	Sep-19	Sabine Oettl
BG05_b_t3_R1_003	*T. washingtonensis*	Burggraviato, South Tyrol, Italy	Cripps Pink	Leaf, orchard	Sep-19	Sabine Oettl
BG05_b_t3_R2_003	*T. washingtonensis*	Burggraviato, South Tyrol, Italy	Cripps Pink	Wood bark, orchard	Sep-19	Sabine Oettl
CVG 627 = CBS 146951	*T. washingtonensis*	Cuneo, Piedmont, Italy	Red Delicious	Wood bark, orchard	Jul-19	Vladimiro Guarnaccia
CVG602	*T. washingtonensis*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG603	*T. washingtonensis*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG605	*T. washingtonensis*	Cuneo, Piedmont, Italy	Ambrosia	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG606	*T. washingtonensis*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG610 = CBS 146950	*T. washingtonensis*	Cuneo, Piedmont, Italy	Ambrosia	Leaf, orchard	Jul-19	Vladimiro Guarnaccia
CVG613	*T. washingtonensis*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG620	*T. washingtonensis*	Cuneo, Piedmont, Italy	Gala	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG624	*T. washingtonensis*	Cuneo, Piedmont, Italy	Red Delicious	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
CVG625	*T. washingtonensis*	Cuneo, Piedmont, Italy	Gala	Fruit skin, orchard	Jul-19	Vladimiro Guarnaccia
EM17	*T. washingtonensis*	Trento, Trentino, Italy	Opal	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM24	*T. washingtonensis*	Valle Dell'Adige, Trentino, Italy	Granny	Fruit skin, packing house	Jul-19	Valeria Gualandri
EM3	*T. washingtonensis*	Val dei Langhi, Trentino, Italy	Fuji	Fruit skin, orchard	Jul-19	Valeria Gualandri
EM30	*T. washingtonensis*	Valle Dell'Adige, Trentino, Italy	Modi	Fruit skin, packing house	Jul-19	Valeria Gualandri
UE08_b_t2_B1_003	*T. washingtonensis*	Caldaro, South Tyrol, Italy	Cripps Pink	Leaf, orchard	Aug-19	Sabine Oettl
UL10_b_t2_B3_002	*T. washingtonensis*	Laives, South Tyrol, Italy	Cripps Pink	Wood bark, orchard	Aug-19	Sabine Oettl
UL10_b_t2_F2_002	*T. washingtonensis*	Laives, South Tyrol, Italy	Cripps Pink	Fruit skin, orchard	Aug-19	Sabine Oettl
UL10_b_t3_B1_003	*T. washingtonensis*	Laives, South Tyrol, Italy	Cripps Pink	Leaf, orchard	Aug-19	Sabine Oettl
VI01_b_t3_B1_005	*T. washingtonensis*	Val Venosta, South Tyrol, Italy	Bonita	Leaf, orchard	Sep-19	Sabine Oettl
VI01_b_t3_F2_001	*T. washingtonensis*	Val Venosta, South Tyrol, Italy	Bonita	Fruit skin, orchard	Sep-19	Sabine Oettl
VI01_b_t3_R1_004	*T. washingtonensis*	Val Venosta, South Tyrol, Italy	Bonita	Wood bark, orchard	Sep-19	Sabine Oettl

were isolated from discs of apple skin, leaves and bark (10 mm diameter) which were placed on malt extract agar (MEA) plates (modified from Boekhout et al., 2006). A further plate containing MEA was placed upside-down to cover the first plate. Conidia were captured on the upper plate and after 5 days of incubation in the dark at 25 °C, the single spores germinated were observed through a stereoscope and transferred to malt extract agar (MEA) plates to obtain pure cultures. The isolate CR1 isolated from apple collected in Croatia in a previous study by Prencipe et al. (2015) was also included in this study to gain a more complete overview of the diversity of these fungi. Isolates used in this study are deposited in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, in the working collection of Agroinnova, University of Torino (Torino, Italy), Foundation Edmund Mach (San Michele all'Adige, Italy) and Laimburg Research Centre (Auer/Ora, Italy).

2.2. DNA extraction, PCR amplification and sequencing, and molecular species recognition

Genomic DNA was extracted using the E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-Tek) following the manufacturer's instructions. Partial regions of four loci were amplified. Primers ITS5 and ITS4 (White et al., 1990) were used to amplify the internal transcribed spacer regions (ITS1 and 2) of the nuclear ribosomal RNA operon, including the 3′ end of the 18 S nrRNA gene, the first internal transcribed spacer region, the 5.8 S nrRNA gene; the second internal transcribed spacer region and the 5′ end of the 28 S nrRNA gene. The primer NL1 and RLR3R were used to amplify the D1/D2 domains of the LSU rRNA gene (Boekhout et al., 2006); RPB2–6 F and RPB2–7R primers were used to amplify *RPB2* (Liu et al., 1999), and EF1–983 F and EF1–2218R to amplify *TEF1* (Matheny et al., 2007). The PCR amplification mixtures and cycling conditions adopted for all three loci were followed as described in each of the cited references above. A total of 5 μL of PCR product of each PCR reaction was examined by electrophoresis at 100 V on 1% agarose (VWR Life Science AMRESCO® biochemicals) gels stained with GelRedTM. PCR products were sequenced in both directions by Eurofins Genomics Service (Ebersberg, Germany). The DNA sequences generated were analyzed and consensus sequences were computed using the program Geneious v. 11.1.5 (Auckland, New Zealand).

2.3. Phylogenetic analyses

New sequences obtained in this study were blasted against the NCBI's GenBank nucleotide database to determine the closest relatives to place the studied isolates in a taxonomic and phylogenetic framework. Alignments of different gene regions, including sequences obtained from this study and sequences downloaded from GenBank, were initially performed with the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley, 2013), and then manually adjusted in MEGA v. 7 (Kumar et al., 2016). An initial phylogenetic analysis was conducted using 72 ITS sequences of isolates collected in this study and 33 reference strains deposited in GenBank (Table 1), to give an overview of genus identification. The analysis included sequences from 195 isolates spanning nine genera selected on the preliminary results provided by BLAST analysis and one outgroup taxon (i.e., *Robbauera albescens* CBS 608.83). To distinguish the isolates at species level, a subset of 16 representative isolates was selected based on the results of the overview ITS analysis and was processed through different phylogenetic analyses conducted individually for each locus of ITS, D1D2, *TEF1* and *RPB2* (data not shown) and as multilocus sequence analyses. Moreover, 33 reference strains deposited in GenBank spanning 10 genera and one outgroup taxon (i.e., *Mycosarcoma maydis* CBS 504.76) were used. The phylogenies were based on Bayesian Inference (BI) and Maximum Parsimony (MP) for the multilocus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander, 2004) and incorporated into the analyses. MrBayes v. 3.2.5 (Ronquist et al., 2012) was used to generate phylogenetic trees under optimal criteria per partition. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The heating parameter was set at 0.2 and trees were sampled every 1000 generations. Analyses stopped when the average standard deviation of split frequencies was below 0.01. The MP analyses were performed using Phylogenetic Analysis Using Parsimony (PAUP) v. 4.0b10 (Swofford, 2003). Phylogenetic relationships were estimated by heuristic searches with 100 random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on 'best trees', with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony, and the bootstrap analyses (Hillis and Bull, 1993) were based on 1000 replications. Sequences generated in this study were deposited in GenBank (Table 2).

2.4. Biodiversity and taxonomy

Isolates of putative new species were studied phenotypically as follows. Colony and microscopic morphology was studied after 1–2 weeks of growth at 10 and 25 °C, using line inoculations on Yeast Morphology agar (Difco, YMoA), Malt extract agar (MEA), Glucose Yeast extract Peptone Glucose agar (GYPA), Yeast extract Maltose agar (YMA), Potato dextrose agar (PDA), DG18 medium (recipes at https://wi.knaw.nl/page/Growth_media_table) and in liquid yeast nitrogen base (Difco) fermentation medium with glucose (Difco). Scanning electron microscopy was done as described in Visagie et al. (2023) using cultures growing on MEA. The nutritional requirements, i.e., fermentation, carbon assimilation, growth on different nitrogen compounds, growth at different temperatures, and some further standard biochemical tests were done according to standard practices used in yeast taxonomy according to Kurtzman et al. (2011).

2.5. Reproduction of white haze on apple fruit

Three fungal strains (*Tilletiopsis washingtonensis* FR23, *Entyloma belangeri* FR22 and *Golubevia pallescens* FR4B) were grown on PDA supplemented with 0.0025 g streptomycin (Merck, Germany) for 21 days at 25 °C. Conidia were collected by adding 1% solution of Tween-20 and using a Drigalsky spatula. A suspension of 10^6 conidia/mL was obtained by counting with a Burker's chamber under microscope. Apples cultivars 'Ambrosia' and 'Inored$^{(cov)}$'/Story®, previously washed with 1% NaClO and air dried, were inoculated by immersion in the conidial suspension for 2 min. Sixty fruits per fungal strain were used. Apples were stored at 20 °C for 30 days. The incidence of white haze was evaluated as percentage of fruits showing white haze on their skin.

3. Results

3.1. Sampling and isolation

Symptoms of white haze caused by *Tilletiopsis*-like spp. were frequently observed on apple fruit in the three Italian regions investigated during the 2019 summer and autumn. Affected fruits sampled from orchards and cold storage were characterized by a grey hazy appearance of the epidermis due to the presence of a thin, whitish fungal layer clearly visible over the red-pigmented apple surface (Fig. 1). No symptoms were observed on leaves, bark trunk and branches. Under storage conditions (around 0–1 °C, 95–98% relative humidity, normal atmosphere), abundant masses of spores, i.e., ballistoconidia, and hyphae appeared on the fruit skin. A total of 72 monosporic isolates resembling *Tilletiopsis*-like were collected. The strains were recovered from samples originating from 18 orchards: 7 located in Piedmont, 6 in Trentino and 5 in South Tyrol. A total of 59 strains were collected from symptomatic fruits, 7 from asymptomatic leaves and 7 from

Table 2
GenBank accession numbers of sequences used for the phylogenetic analyses included in this study.

Species	Culture No.[a]	GenBank Accession Nos.[b]			
		ITS	D1/D2	*TEF1*	*RPB2*
Entyloma arnoseridis	CBS 203.36	DQ911609	DQ645528	DQ645531	DQ645530
Entyloma belangeri	CVG604 = CBS 146954	*OR030418*	*OR164873*	*OR116411*	*OR116394*
Entyloma belangeri	EM1	*OR030417*	*OR164875*	*OR116413*	*OR116396*
Entyloma belangeri	EM14	*OR030416*	-	-	-
Entyloma belangeri	EM15	*OR030415*	-	-	-
Entyloma belangeri	EM16	*OR030414*	-	-	-
Entyloma belangeri	EM19	*OR030413*	-	-	-
Entyloma belangeri	EM20	*OR030412*	-	-	-
Entyloma belangeri	EM22	*OR030411*	-	-	-
Entyloma belangeri	EM27	*OR030410*	-	-	-
Entyloma belangeri	EM4	*OR030409*	-	-	-
Entyloma belangeri	EM8	*OR030408*	-	-	-
Entyloma belangeri	EM9	*OR030407*	-	-	-
Entyloma belangeri	ET07_B_T2_B2_002	*OR030406*	-	-	-
Entyloma belangeri	ET07_B_T2_F2_002	*OR030405*	-	-	-
Entyloma belangeri	ET07_B_T2_F2_003	*OR030404*	*OR164874*	*OR116412*	*OR116395*
Entyloma belangeri	UL10_B_T3_F1_001	*OR030403*	-	-	-
Entyloma belangeri	CBS 111599	AY259075	AY272020	LT614992	LT614966
Entyloma belangeri	CBS 111600	AY259074	AY272019	LT614993	LT614967
Entyloma calendulae	CBS 746.85	DQ663689	DQ663687	KP323124	DQ663690
Entyloma davenportii	CBS 111603	AY259054	AY272026	LT614986	LT614960
Entyloma davenportii	CBS 111604	AY259064	AY272010	LT614987	LT614961
Entyloma davenportii	CBS 111607	AY259051	AY272024	LT614985	LT614959
Entyloma elstari	CBS 111593	AY259048	AY272021	DQ028593	DQ234552
Entyloma ficariae	CBS 480.91	JQ586199	AJ235295	KP323125	KP323102
Entyloma mali	EM23 = CBS 146952	*OR030380*	*OR164879*	*OR116417*	*OR116400*
Entyloma mali	EM26 = CBS 146953	*OR030379*	*OR164880*	*OR116418*	*OR116401*
Entyloma mali	WH4_Lb-Fr2A2_06	GQ281314	–	-	-
Entyloma randwijkense	EM12 = CBS 146955	*OR029682*	*OR164877*	*OR116415*	*OR116398*
Entyloma randwijkense	EM13	*OR029681*	*OR164878*	*OR116416*	*OR116399*
Entyloma randwijkense	EM36 = CBS 146956	*OR029680*	*OR164876*	*OR116414*	*OR116397*
Entyloma randwijkense	CBS 111606	AY259080	AY272033	LT614988	LT614962
Erratomyces patelii	CBS 669.70	DQ846894	DQ094784	DQ846898	DQ846896
Gjaerumia minor	CBS 543.50	KP322989	AJ235287	KP323114	KP323097
Gjaerumia minor	CBS 111629	AY259066	AY272012	-	-
Gjaerumia minor	CBS 111631	AY259081	AY272034	-	-
Golubevia heteromorpha	CBS 111610	AY259058	AY272003	LT614999	LT614973
Golubevia heteromorpha	CBS 111616	AY259049	AY272022	LT615004	LT614978
Golubevia heteromorpha	CBS 111617	AY259050	AY272023	LT615005	LT614979
Golubevia mali	CVG411 = CBS 146945	*OR030902*	*OR164884*	*OR116422*	*OR116405*
Golubevia mali	CVG412 = CBS 146946	*OR030901*	*OR164885*	*OR116423*	*OR116406*
Golubevia mali	CVG415 = CBS 146947	*OR030900*	*OR164886*	*OR116424*	*OR116407*
Golubevia pallescens	CVG408	*OR030401*	-	-	-
Golubevia pallescens	CVG409	*OR030400*	-	-	-
Golubevia pallescens	CVG410	*OR030399*	-	-	-
Golubevia pallescens	CVG413	*OR030398*	-	-	-
Golubevia pallescens	CVG414 = CBS 146948	*OR030397*	*OR164889*	*OR116427*	*OR116410*
Golubevia pallescens	CVG416	*OR030396*	-	-	-
Golubevia pallescens	CVG417 = CBS 146949	*OR030395*	*OR164887*	*OR116425*	*OR116408*
Golubevia pallescens	CVG420	*OR030394*	-	-	-
Golubevia pallescens	CVG427	*OR030393*	-	-	-
Golubevia pallescens	CVG428	*OR030392*	-	-	-
Golubevia pallescens	CVG431	*OR030391*	-	-	-
Golubevia pallescens	CVG432	*OR030390*	-	-	-
Golubevia pallescens	CVG433	*OR030389*	-	-	-
Golubevia pallescens	CVG434	*OR030388*	-	-	-
Golubevia pallescens	CVG626	*OR030387*	-	-	-
Golubevia pallescens	CVG700	*OR030386*	-	-	-
Golubevia pallescens	CVG701	*OR030385*	-	-	-
Golubevia pallescens	CVG702	*OR030384*	-	-	-
Golubevia pallescens	CVG704	*OR030383*	-	-	-
Golubevia pallescens	EM37	*OR030382*	*OR164888*	*OR116426*	*OR116409*
Golubevia pallescens	CBS 364.85	DQ317636	AJ235292	KP323123	KP323101
Golubevia pallescens	CBS 111622	AY259059	AY272004	-	-
Golubevia pallescens	CBS 111624	AY879278	AY272036	-	-
Jamesdicksonia mali	CBS 111625	AY879279	AY879274	LT615009	LT614983
Jamesdicksonia mali	CBS 111628	AY879281	AY272007	LT615010	LT614984
Mycosarcoma maydis	CBS 504.76*	AY854090	AF453938	KP323130	KP323090
Phragmotaenium derxii	CBS 110078	AB045707	AB052823	KP323138	KP323086
Phragmotaenium flavum	CBS 401.84	KP322987	AJ235285	KP323126	-
Phragmotaenium fulvescens	CBS 607.83	KP322988	AJ235282	KF706483	KF706530
Phragmotaenium oryzicola	CBS 110079	AB045708	AB052824	-	-
Robbauera albescens	CBS 608.83	KP322986	AJ235289	KP323127	KP323095

(*continued on next page*)

Table 2 (*continued*)

Species	Culture No.[a]	GenBank Accession Nos.[b]			
		ITS	**D1/D2**	***TEF1***	***RPB2***
Tilletia goloskokovii	LMC321	DQ832248	AY818998	DQ832251	DQ832249
Tilletiaria anomala	CBS 436.72	DQ234558	AJ235284	DQ835991	AY803750
Tilletiopsis cremea	CBS 605.83	AB025690	AJ235279	KP323129	KP323108
Tilletiopsis lilacina	CBS 435.92	KP322984	KP322984	KP323112	KP323110
Tilletiopsis washingtonensis	BG05_B_T3_B1_003	*OR029717*	-	-	-
Tilletiopsis washingtonensis	BG05_B_T3_F1_001	*OR029716*	*OR164883*	*OR116421*	*OR116404*
Tilletiopsis washingtonensis	BG05_B_T3_F2_003	*OR029715*	-	-	-
Tilletiopsis washingtonensis	BG05_B_T3_R1_003	*OR029714*	-	-	-
Tilletiopsis washingtonensis	BG05_B_T3_R2_003	*OR029713*	-	-	-
Tilletiopsis washingtonensis	CVG602	*OR029712*	-	-	-
Tilletiopsis washingtonensis	CVG603	*OR029711*	-	-	-
Tilletiopsis washingtonensis	CVG605	*OR029710*	-	-	-
Tilletiopsis washingtonensis	CVG606	*OR029709*	-	-	-
Tilletiopsis washingtonensis	CVG610 = CBS 146950	*OR029708*	*OR164881*	*OR116419*	*OR116402*
Tilletiopsis washingtonensis	CVG613	*OR029707*	-	-	-
Tilletiopsis washingtonensis	CVG620	*OR029706*	-	-	-
Tilletiopsis washingtonensis	CVG624	*OR029705*	-	-	-
Tilletiopsis washingtonensis	CVG625	*OR029704*	-	-	-
Tilletiopsis washingtonensis	CVG627 = CBS 146951	*OR029703*	*OR164882*	*OR116420*	*OR116403*
Tilletiopsis washingtonensis	EM17	*OR029702*	-	-	-
Tilletiopsis washingtonensis	EM24	*OR029701*	-	-	-
Tilletiopsis washingtonensis	EM3	*OR029700*	-	-	-
Tilletiopsis washingtonensis	EM30	*OR029699*	-	-	-
Tilletiopsis washingtonensis	UE08_B_T2_B1_003	*OR029698*	-	-	-
Tilletiopsis washingtonensis	UE08_B_T2_B3_002	*OR029697*	-	-	-
Tilletiopsis washingtonensis	UL10_B_T2_B1_001	*OR029696*	-	-	-
Tilletiopsis washingtonensis	UL10_B_T2_F2_002	*OR029695*	-	-	-
Tilletiopsis washingtonensis	UL10_B_T2_R3_002	*OR029694*	-	-	-
Tilletiopsis washingtonensis	VI01_B_T3_B1_005	*OR029693*	-	-	-
Tilletiopsis washingtonensis	VI01_B_T3_F1_007	*OR029692*	-	-	-
Tilletiopsis washingtonensis	VI01_B_T3_F2_001	*OR029691*	-	-	-
Tilletiopsis washingtonensis	VI01_B_T3_R1_004	*OR029690*	-	-	-
Tilletiopsis washingtonensis	CBS 544.50	DQ835994	AJ235278	DQ835996	DQ835995

[a] CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.

[b] ITS: internal transcribed spacers 1 and 2 together with 5.8 S nrDNA; D1D2: D1/D2 domains of the LSU; *TEF1*: translation elongation factor 1-α gene; *RPB2*: second largest subunit of RNA polymerase II. Sequences generated in this study indicated in italics.

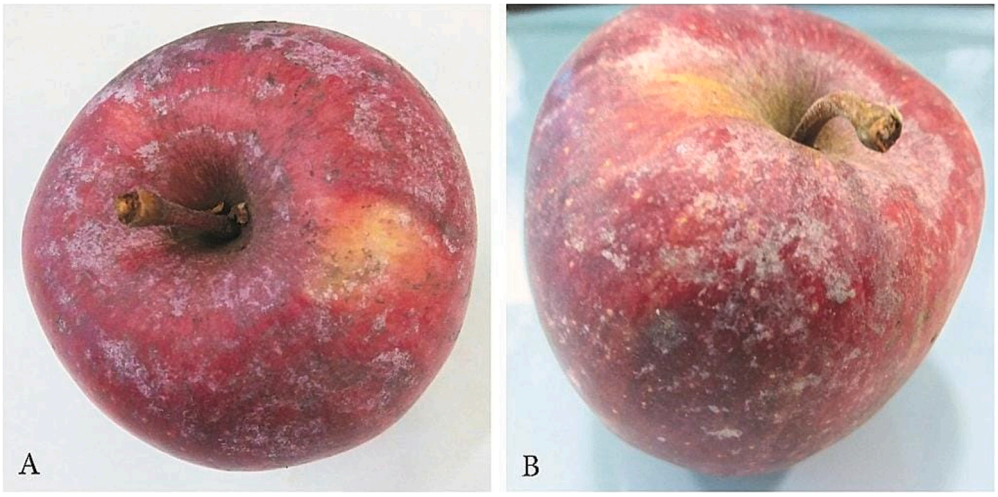

Fig. 1. Appearance of white haze on 'Red Chief' (A) and on 'Red Delicious' (B) fruit skin.

asymptomatic trunk bark. Moreover, *Tilletiopsis*-like strains were obtained from 13 cultivars: 'Ambrosia', 'Bonita', 'Braeburn', 'Cripps Pink$^{(cov)}$/Pink Lady$^{(cov)}$', 'Fuji', 'Gala', 'Golden Delicious', 'Granny Smith', 'Jeromine$^{(cov)}$', 'Civ198$^{(cov)}$/Modì', 'UEB32642′/Opal®, 'Campsur'/Red Chief®, 'Red Delicious', 'Inored$^{(cov)}$/Story®.

From white haze-infested apple trees, six fungal species were isolated from various regions and time points in Northern Italy (Table 1). *Golubevia pallescens* (nom. invalid, see below) was isolated from apple skin with white haze from Passatore di Cuneo and Savigliano, both Cuneo, Piedmont, in April and May 2019, respectively. *Entyloma belangerii* was isolated apple skin with white haze from Manta, Cuneo, Piedmont and Valle Dell'Adige, Trentino-Alto Adige, both in July 2019. *Tilletiopsis washingtonensis* was obtained from an apple leaf in Manta, Cuneo, and a trunk, Cuneo, Piedmont, Italy in July 2019. *Entyloma randwijkense* was isolated from a branch and an apple fruit with white haze, in Bleggio, Trentino-Alto Adige and Val di Non, Trentino-Alto Adige, respectively. Two putative new species, *Golubevia* spec. 1 and *Entyloma* spec. 1, were found on apples with white haze in Lagnasco, Cuneo, Piedmont, April

2019, and Valle Dell'Adige, Trentino-Alto Adige, July 2019, respectively, and will be described below.

3.2. Reproduction of white haze on fruit

The three species most frequently isolated, *G. pallescens*, *E. belangeri* and *T. washingtonensis*, were able to cause white haze on the skin of apples in the inoculation experiments. The presence of white haze was easily visible on apples of the cv. 'Inored$^{(cov)}$'/Story®, characterized by a red skin, whereas it was present with a very low incidence on the skin of apples of 'Ambrosia' that are characterized by a yellow to reddish-pink skin. By considering the three species used, *G. pallescens* and *E. belangeri* produced the highest incidence of white haze, particularly on apples 'Story Inored' (Table 3). *T. washingtonensis* was able to colonize a relatively low percentage of apples 'Inored$^{(cov)}$'/Story® and white haze was not observed on apples 'Ambrosia' inoculated with this species. Thus, the formation of white haze seems related to fungal species and apple variety.

3.3. Phylogenetic analyses

The phylogenetic analysis based on ITS sequences of all 72 isolates obtained from apple samples showed that 23 isolates belonged to the genus *Golubevia*, 28 isolates clustered in a group with known species of the genus *Tilletiopsis*, and 21 formed a distinct, well-supported lineage with eight reference species of *Entyloma* (Fig. 2). This phylogeny consisted of 105 sequences and a total of 762 characters. A maximum of 1000 equally most parsimonious trees were saved (tree length = 2261, CI = 0.622, RI = 0.934 and RC = 0.581). Bootstrap support values from the parsimony analysis are plotted on the Bayesian phylogenies in Fig. 2. For the Bayesian analyses, MrModeltest recommended GTR+I+G. In the Bayesian analysis, the ITS partition had 603 unique site patterns, and the analysis ran for 1545,000 generations, resulting in 3092 trees of which 2320 trees were used to calculate the posterior probabilities. The combined species phylogeny consisted of 51 sequences, including the outgroup sequences of *Mycosarcoma* (= *Ustilago*) *maydis* CBS 504.76. A total of 4655 characters (ITS: 1– 895, D1D2: 902 –2501, *TEF1*: 2508–3926, *RPB2*: 3932–4655) were included in the combined phylogenetic analyses, of which 2624 characters were parsimony-informative, 469 were variable and parsimony-uninformative and 1545 characters were constant. A maximum of 1000 equally most parsimonious trees were saved (Tree length = 8379, CI = 0.627, RI = 0.860 and RC = 0.540). Bootstrap support values from the parsimony analysis were plotted on the Bayesian phylogenies presented in Fig. 3. For the Bayesian analyses, MrModeltest suggested that all partitions should be analysed with Dirichlet state frequency distributions. The following models were recommended by MrModeltest and used in the Bayesian analysis: HKY+I+G for ITS, GTR+G for D1D2, GTR+I+G for *TEF1* and *RPB2*. The ITS partition had 663 unique site patterns, the D1D2 partition 579, the *TEF1* partition 614, the *RPB2* partition 498, and the analysis ran for 355,000 generations, resulting in 712 trees of which 534 trees were used to calculate the posterior probabilities.

Table 3
Incidence of white haze on apple cultivars 'Ambrosia' and 'Story Inored' inoculated with *Tilletiopsis washingtonensis* FR23, *Entyloma belangeri* FR22 and *Golubevia pallescens* FR4B and stored at 20 °C for 30 days. Fruits were considered affected when the surface covered by white haze was higher than 20%.

Fungal strain	'Story Inored'		'Ambrosia'	
Tilletiopsis washingtonensis FR23	20%	ab*	0%	a*
Entyloma belangeri FR22	50%	bc	3%	a
Golubevia pallescens FR4B	53%	c	7%	a
Control	0%	a	0%	a

* Values in the same column with the same letter are not significantly different according to Tukey's test ($P < 0.05$).

In the multilocus phylogenetic analysis three isolates clustered with three reference strains of *G. pallescens*, whilst three isolates clustered with a reference strains of *T. washingtoniensis*, three with *E. belangeri* and three with *E. randwijkense*. Moreover, three isolates were identified as *G. mali* and a further two as *E. mali*, forming two highly supported subclades (1.00/100).

3.4. Taxonomy and Nomenclature

Hyaline filamentous basidiomycetous fungi of *Exobasidiomycetes* Begerow, Stoll & R. Bauer that reproduce asexually with ballistoconidia have been classified in the genus *Tilletiopsis* Derx ex Derx (Derx, 1930; Nyland, 1950; Tubaki, 1952; Boekhout, 1991; 2011). Molecular phylogenetic analyses, however, demonstrated that these fungi contained an assembly of phylogenetically different fungi that were recently reclassified in the following genera *Gjaerumia* R. Bauer, M. Lutz & Oberw., *Phragmotaenium* R. Bauer, Begerow, A. Nagler & Oberw., and two newly described monotypic genera, viz. *Golubevia* Q.M. Wang, F.Y. Bai, Begerow & Boekhout and *Robbauera* Boekhout, Begerow, Q.M. Wang & F.Y. Bai (Wang et al., 2015). *Tilletiopsis washingtonensis* Nyland, *Tilletiopsis lilacina* Tubaki, and *Tilletiopsis cremea* Tubaki were maintained in the genus *Tilletiopsis* (Wang et al., 2015). Unfortunately, the name *Golubevia* (MB812694) has not been validly published according to the current nomenclatural rules (Turland et al., 2018) as the citation of the basionym of the type species was not provided. This makes not only the genus name invalidly described, but also the species recognized in the genus and the higher taxa based on that genus name, namely *Golubeviaceae* Q.M. Wang, F.Y. Bai, Begerow & Boekhout (MB812692) and *Golubeviales* Q.M. Wang, F.Y. Bai, Begerow & Boekhout (MB812082) (Wang et al., 2015).

One of the most abundant species isolated from white haze on apples was described as *Golubevia heteromorpha* Boekhout, Richter & Yurkov (MB823152) (Richter et al., 2019). Interestingly, this species also showed an antagonistic interaction against the powdery mildew *Blumeria graminis* (DC.) Speer f.sp. *tritici* (Köhl et al., 2019). Unfortunately, this species has not been described validly, and this will be corrected below.

Richter and co-workers (2019), however, made an attempt to validate the name *Golubevia*. Here, the basionym, *Tilletiopsis pallescens* Gokhale and its citation, were correctly listed under *Golubevia pallescens* (Gokhale) Q.M. Wang, F.Y. Bai, Begerow & Boekhout, the type species of the genus *Golubevia*, but as no new identifier was made in Mycobank, Index Fungorum, or Fungal Names, the name *Golubevia* and those that are based on this name, remained invalid. Moreover, no attempt was made to validate the names at family and ordinal levels. Below we will validate the Golubevia-related names.

3.5. Validation of names

***Golubevia* Q.M. Wang, F.Y. Bai, Begerow & Boekhout, gen. nov.**

MycoBank MB 847646 [originally described as: *Golubevia* Q.M. Wang, F.Y. Bai, Begerow & Boekhout, Stud. Mycol. 81: 78 (2015) [MB 812694], nom. inval., Art. 40.1, see Arts 6.3, 12.1 and 40.3 (Shenzhen)].

For a detailed description see Wang et al., Studies in Mycology 81: 78 (2015).

Type species: *Golubevia pallescens* (Gokhale) Q.M. Wang, F.Y. Bai, Begerow & Boekhout (see below).

***Golubevia pallescens* (Gokhale) Q.M. Wang, F.Y. Bai, Begerow & Boekhout, comb. nov.**

MycoBank MB 847647.

Basionym: *Tilletiopsis pallescens* Gokhale, Nova Hedwigia 23: 805 (1972). MB 324632.

Holotype: UBC 8007, preserved in the Mycology Herbarium University of British Colombia, Vancouver 8, B.C., Canada (Gokhale, 1972). Ex-type cultures: CBS 606.83, CBS collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands and ATCC 24345,

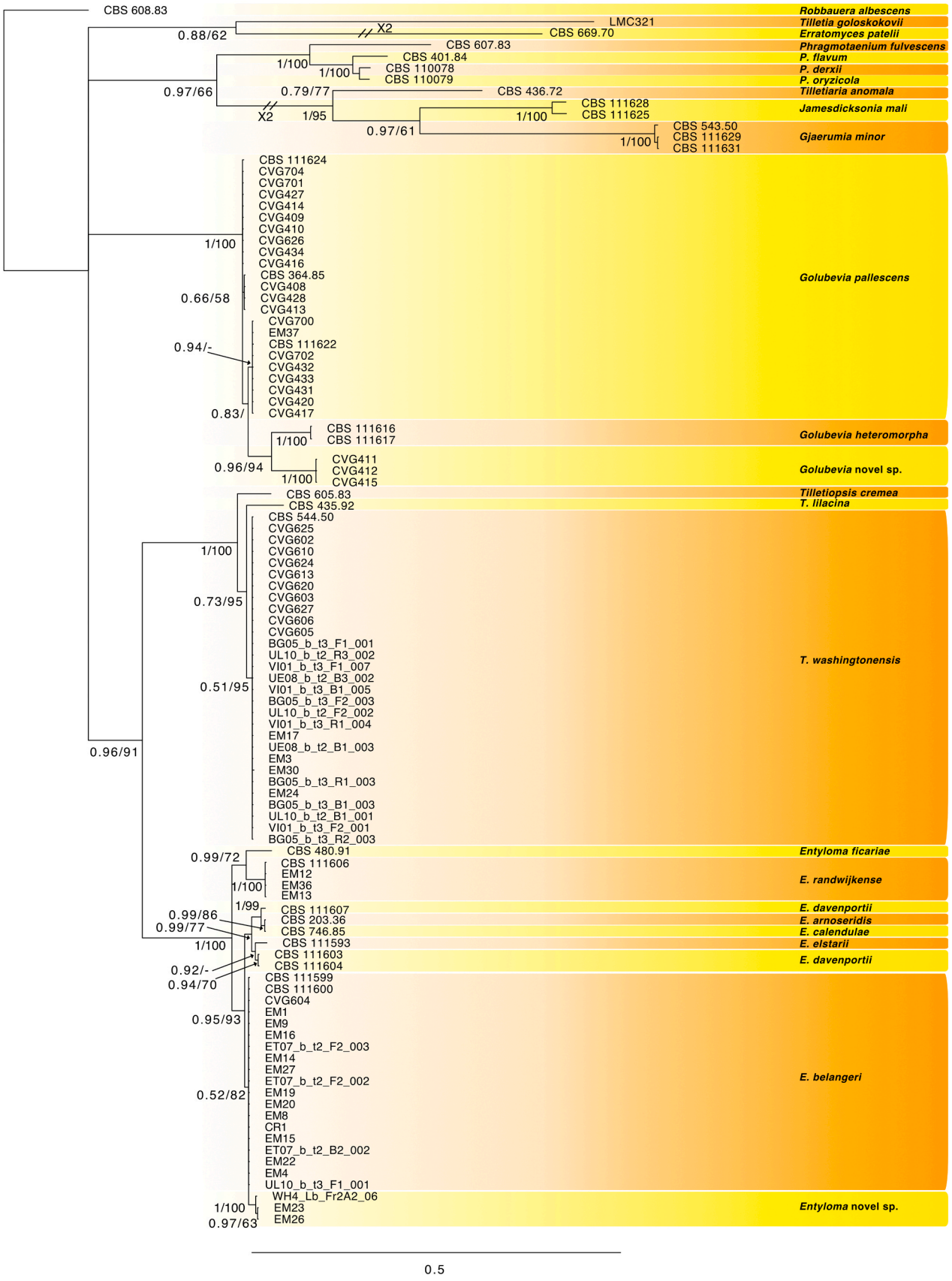

Fig. 2. Consensus phylogram resulting from a Bayesian analysis of the ITS sequence alignments. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The tree was rooted to *Robbauera albescens* (CBS 608.83).

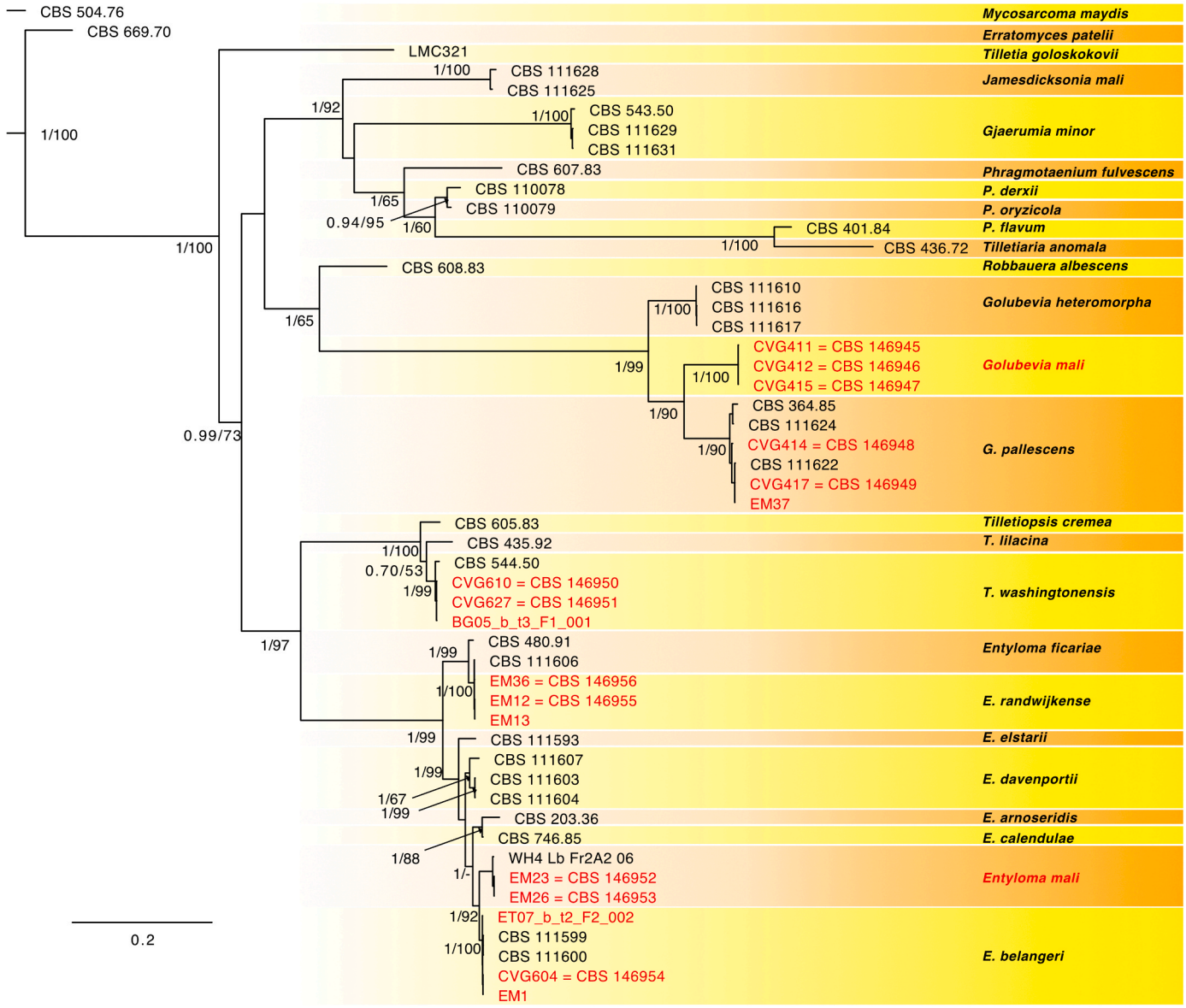

Fig. 3. Consensus phylogram resulting from a Bayesian analysis of the combined ITS, D1D2, *TEF1*, and *RPB2* sequence alignments. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The strains collected in this study and the new described species are in red. The tree was rooted to *Mycosarcoma maydis* (CBS 504.76).

American Type Culture Collection, Manassas, VI, USA (Richter et al. 2019).

Synonyms:

Golubevia pallescens (Gokhale) Q.M. Wang, F.Y. Bai, Begerow & Boekhout, Stud. Mycol. 81: 78 (2015) [MB 812695], nom. inval., Art. 41.5, Note 1 (Shenzhen).

Golubevia pallescens (Gokhale) Q.M. Wang, F.Y. Bai, Begerow & Boekhout, Frontiers in Microbiology 10 (no. 2544): 11 (2019), nom. inval., Art. F.5.1 (Shenzhen).

***Golubevia heteromorpha* Boekhout, C. Richt. & Yurkov, sp. nov.**

MycoBank MB 847648.

For a detailed description see Richter et al., Frontiers in Microbiology 10 (no. 2544): 8 (2019).

Holotype: The Netherlands, Wageningen, isolated from an apple of the cultivar Elstar, CBS 111610, preserved in a metabolically inactive state in the CBS collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands. Ex-type culture is deposited at the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (DSM 100176).

Synonym: *Golubevia heteromorpha* Boekhout, C. Richt. & Yurkov, Frontiers in Microbiology 10 (no. 2544): 8 (2019) [MB 823152], nom. inval., Art. 35.1 (Shenzhen).

Golubeviales Q.M. Wang, F.Y. Bai, Begerow & Boekhout, ord. nov.

MycoBank MB 847650.

[originally described as: *Golubeviales* Q.M. Wang, F.Y. Bai, Begerow & Boekhout, Stud. Mycol. 81: 78 (2015) [MB 812083], nom. inval., Art. 32.1(c), see Art. 16.1(a) (Shenzhen)].

Short diagnosis: Member of Exobasidiomycetes represented by clade including genus *Golubevia*, see Wang et al., Studies in Mycology 81: 78 (2015). Fruitbodies unknown, sexual state unknown; narrow hyaline hyphae present, reproduction with sausage-shaped ballistoconidia.

Type family: Golubeviaceae Q.M. Wang, F.Y. Bai, Begerow & Boekhout MB 847649.

Golubeviaceae Q.M. Wang, F.Y. Bai, Begerow & Boekhout, fam. nov.

MycoBank MB847649.

[originally described as: *Golubeviaceae* Q.M. Wang, F.Y. Bai, Begerow

& Boekhout, Stud. Mycol. 81: 78 (2015) [MB 812692], nom. inval., Art. 32.1(c), see Art. 18.1 (Shenzhen)].

Short diagnosis: Similar, but subordinate to diagnosis of Golubeviales, see above.

Type genus: Golubevia Q.M. Wang, F.Y. Bai, Begerow & Boekhout.

3.6. New species descriptions

Based on the above observations on the Italian white haze isolates, we propose to describe two species new to science based on their phylogenetic support as demonstrated in the multilocus phylogeny conducted in this study, namely one species in the genus *Golubevia* and the other in *Entyloma*.

***Entyloma mali* Spadaro, Guarnaccia & Boekhout sp. nov. (**Figs. 4, 5**).**

Etymology: Refers to *Malus*, the host genus from which this fungus was isolated.

Mycobank number: MB 847652.

Morphology

Growth after 7 days at 25 °C in glucose yeast extract broth with a thick film; in 5% glucose in yeast nitrogen base broth with suspension or flocks.

Colony after 5 days at 25 °C on malt extract agar, 10–15 mm width, flat to somewhat elevated in central part, whitish, dull, smooth to somewhat irregular, warty to furrowed, tough; surface velutinous to arachnoid, covered with hairs and hairy synnemata; margin of colony straight but eroded; reverse pale yellowish brown. On yeast morphology agar and glucose-yeast extract-peptone agar with pale yellowish exudate in agar, on the latter also with hyaline droplets on surface; on potato dextrose agar with somewhat reddish pigment; on DG18 agar with lateral smooth sectors. After 6 weeks of growth on corn extract - malt extract agar 80 mm width, with branched white flocks on surface; Dalmau culture on MEA with septate hyphae.

In glucose fermentation broth hyphae 1.0–2.0 μm width, with slender sterigma-like conidiophores, 8.0–30 μm long, with apically formed fusiform blastoconidia, 4.0–7.0 × 2.0–2.5 μm; yeast-like cells subglobose, with multilateral budding, 3.0–4.5 × 2.0–4.5 μm; hyphae on MEA branched, thin-walled, hyaline, 1.0–2.0 μm width, closely adhering in synnemata, with lateral tapered to somewhat ventricose conidiophores, with or without basal septum, 15–30 × 2.0–3.5 μm, on which fusiform blastoconidia are formed, 5.0–9.0 × 1.8–2.0 μm; in glucose fermentation broth long cylindrical blastoconidia present, up to 40 × 2.0 μm. [note in CVG 415 after 2months on GYPA somewhat thick-walled, inflate, (sub)globose cells present, 4.5–8.0 μm in diameter]. Data on nutritional growth patterns, growth at different temperatures and other tests are presented as Suppl. Table 1.

Holotype: CBS H-24918, preserved in a metabolically inactive state in CBS fungarium of Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands; ex-type strain CBS 146953, preserved in CBS collection of WI, and EM26, preserved in collection of Department of Agricultural, Forestry and Food Sciences (DISAFA), AGROINNOVA, University of Torino, 10095 Grugliasco (TO), Italy.

Origin of isolates

CBS 146952 = EM23 and CBS 146953 = EM26, both from white haze on apples (*Malus domestica* variety 'Civ198$^{(cov)}$'/Modì ® and 'Gala', respectively), Valle Dell'Adige, Trentino-Alto Adige, Italy, July 2019, isolated by Valeria Gualandri. CBS 146952 and CBS 146953 preserved in CBS collection of WI; EM 23 and EM26 preserved in collection of Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, 10095 Grugliasco (TO), Italy, and also in Diagnostic Laboratory, Technology Transfer Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige (TN), Italy.

Notes: *Entyloma mali* is phylogenetically distinct from all seven species of the genus. This species was isolated from skin of 'Civ198$^{(cov)}$'/Modì ® and 'Gala' apple fruit collected in Trentino region. Only two strains of this species were isolated, thus, likely, its role in apple white haze seems marginal.

***Golubevia mali* Guarnaccia, Spadaro & Boekhout sp. nov.** (Figs. 6 and 7).

Etymology: Refers to *Malus*, the host genus from which this fungus was isolated.

Mycobank number: MB 847651.

Morphology

Growth in 5% glucose in yeast nitrogen broth with a film and some sediment; colony after 5 days at 25 °C on malt extract agar 10–15 mm width, flat to somewhat raised in central part, cream-brown (beige/isabella) to whitish, with whitish spots, dull, smooth to somewhat irregular, warty to furrowed, tough, with velutinous surface that locally may be hairy to synnematous, or glabrous; synnemata with thin lateral hyphae; margin eroded; reverse pale yellowish brown; ballistoconidia visible on lid of Petri dish; satellite colonies may be present; on glucose-yeast extract-peptone agar, potato dextrose agar and yeast extract-malt extract agar hyaline droplets may be present at surface; on potato dextrose agar with somewhat reddish pigment; on DG18 agar with lateral smooth sectors, on YMA surface strongly ridged; Dalmau culture on MEA with extensive true hyphae.

In 5% glucose in yeast nitrogen broth hyphae occur, thin-walled, hyaline, 1.0–2.5 μm width, without clamp connections, with cytoplasm retracted and with many vestigial septa; ballistoconidia sausage-shaped to cylindrical, 7.0–15.0 × 2.0–2.5 μm, formed on sterigmata;

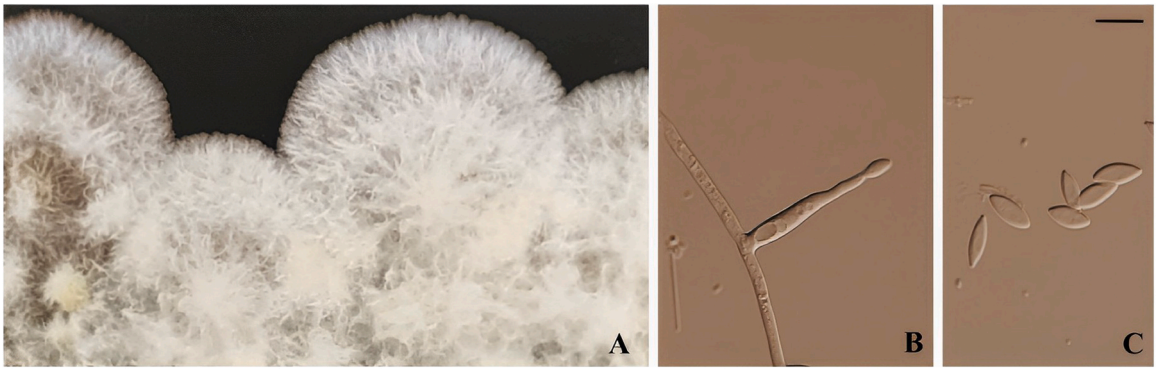

Fig. 4. Morphology of *Entyloma mali* CBS146952. A: culture on MEA after 1 week; B: hypha with conidiogenous cell and blastoconidium; C: fusoidal blastoconidia. Scale bar = 5 μm.

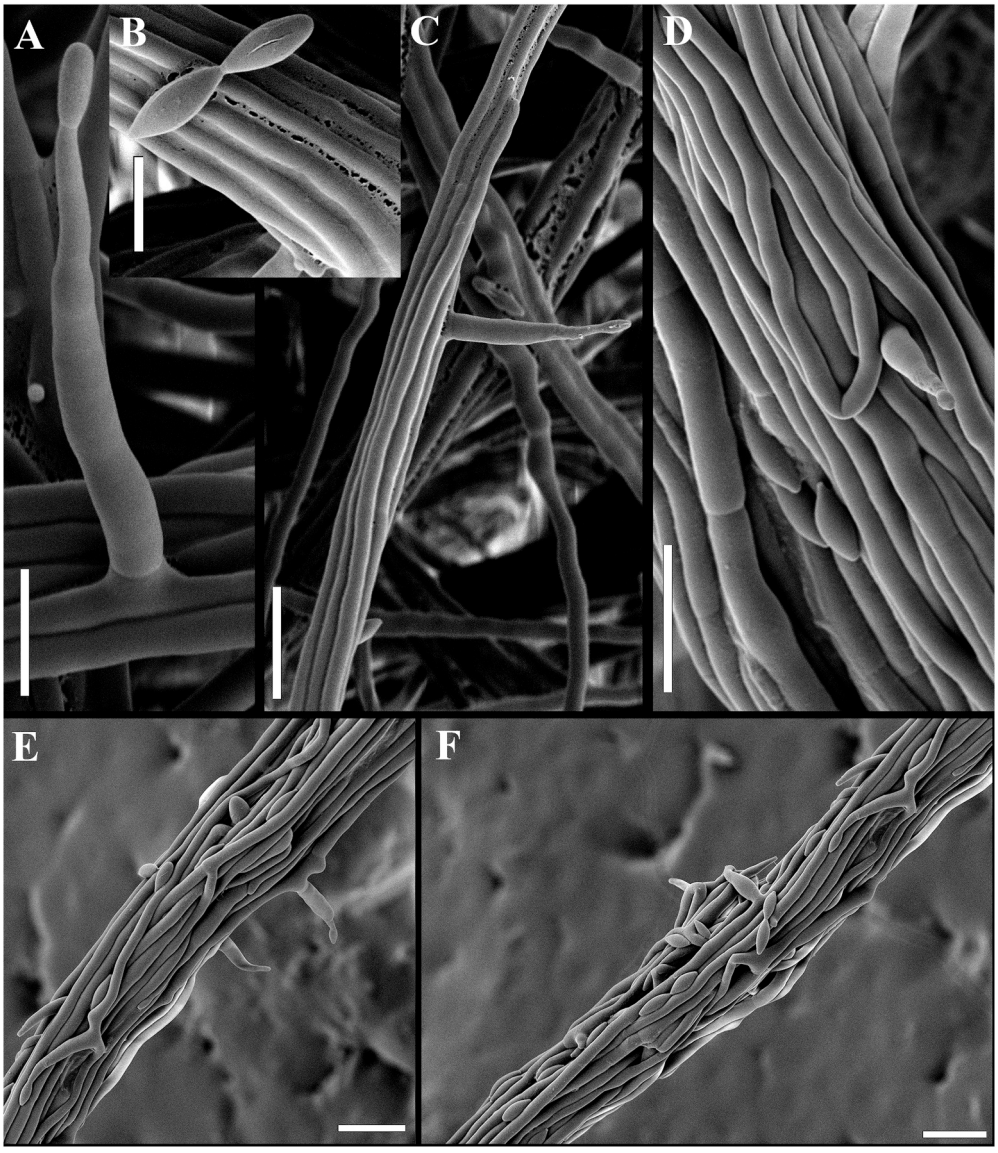

Fig. 5. Scanning electron microscopic images of *Entyloma mali* CBS 146953 on MEA after 10 days. A.) Long phialide with fusiform conidium. A clear septum is visible at the base of this structure. B.) Two fusiform conidia with a small area of contact. C.) Aerial bundle with one phialide. One of the aerial hyphae seems to grow along the bundle. D.) Bundle of hyphae exhibiting differences in diameter of hyphae and a remarkable bend in one of the hyphae. Fusiform conidia are visible with tapered end. Several septa are visible. E.) and F.) Two areas of the same bundle with phialides and conidia. Scale bars are 5 (A, B) or 10 μm (C-F).

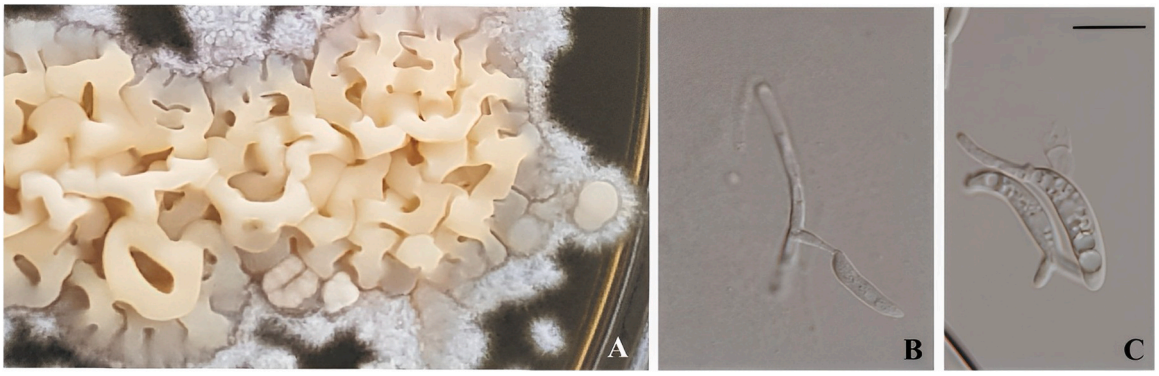

Fig. 6. Morphology of *Golubevia mali* CBS146945. A: culture on MEA after 1 week; B: hypha with sterigma and ballistoconidium; C: germinating ballistoconidia. Scale bar = 5 μm.

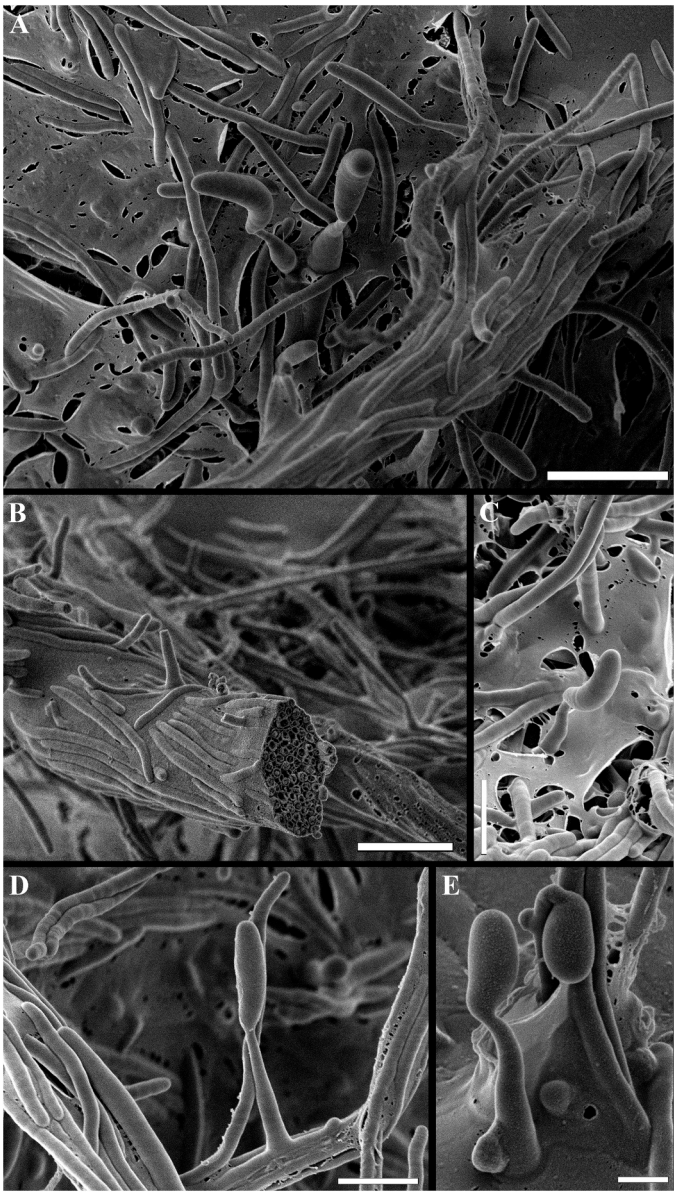

Fig. 7. Scanning electron microscopic images of *Golubevia mali* CBS 146945 on MEA after 10 days. A.) Ballistoconidia amidst hyphal cells and next to a bundle of hyphae. To the right (next to the scale bar) an early stage of ballistoconidia formation is visible. B.) Fractured bundle of aerial hyphae consists out of over 50 hyphae. Note the thin diameter of these hyphae as the scale bar is 10 μm. C-E.) Various views of ballistoconidia. Bars are 2 (E), 5 (C, D) and 10 μm (A, B).

ballistoconidia germinate by hyphae or secondary ballistoconidia; also slender lanceolate to filiform blastoconidia present, 10–21 × 1.5–2.0 μm; on MEA hyphae, ballistoconidia and blastoconida similar to those formed on liquid medium; inflate cells, 12–26 × 3.0–4.0 μm present on MEA and PDA. Data on nutritional growth patterns, growth at different temperatures and other tests are presented as Suppl. Table 2.

Holotype: CBS H-24917, preserved in a metabolically inactive state in CBS fungarium of Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands; ex-type strain CBS 146945, preserved in CBS collection of WI, and CVG411, preserved in collection of Department of Agricultural, Forestry and Food Sciences (DISAFA), AGROINNOVA, University of Torino, 0095 Grugliasco (TO), Italy.

Origin of isolates

CBS 146945 = CVG 411 and CBS 146946 = CVG 412, both from white haze on apples (*Malus domestica* variety 'Ambrosia'), Lagnasco, Cuneo, Piedmont, Italy, April 2019, isolated by Vladimiro Guarnaccia. CBS 146945 and CBS 146946 preserved in CBS collection of WI; CVG 411 and CVG 412, preserved in collection of Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, 10095 Grugliasco (TO), Italy.

Notes: *Golubevia mali* is phylogenetically distinct from the other known species of the genus, *G. heteromorpha* and *G. pallescens*. This species was isolated from skin of 'Ambrosia' fruit collected in Piedmont region. Three strains of this species were sporadically isolated, thus, its role in the apple white haze occurring is marginal.

4. Discussion

White haze, an emerging postharvest disorder occurring on apples, is caused by extensive fungal growth on the fruit surface, resulting in a compromised quality of marketable fruit. Biotic factors represented by several fungal species cause this disorder, however these microorganisms are not causing infection in the apple tissues. Moreover, this disorder is distinguishable from the scarf skin which is known to appear as a more whitish or opalescent sheen caused by the presence of subepidermal air spaces (Weber and Zabel, 2011). In this study, the occurrence and the diversity of fungal species associated with white haze in Northern Italy was investigated. Seventy-two *Tilletiopsis*-like fungi were isolated from samples collected in Piedmont, Trentino and South Tyrol, the major Italian apple production areas. Fungal strains were isolated from symptomatic apples, coming from orchards and from packinghouses, and from asymptomatic fruits, leaves and wood collected during the growing season. As they were detected and isolated also early during the cropping season, from all asymptomatic plant tissues, it was confirmed that *Tilletiopsis*-like fungi are common inhabitants of the phylloplane and are present on apple skin, as previously reported (Boekhout et al., 2006; Boekhout, 2011; Shen et al., 2018). Environmental conditions lead to the development of the disorder on fruits, both in orchard and in postharvest, however further studies are needed to understand their role in the disorder. In our study, symptoms were detected both at harvest time and on cold-stored apples. This suggest that white haze cannot be considered only as a postharvest disorder, in agreement with Baric et al., (2010) and Weber and Zabel (2011). Moreover, a metabarcoding analysis on epiphytic and endophytic microbial communities of apples showed that *Golubevia* was the most abundant genus in epiphytic communities of 'Ambrosia' apples affected by white haze both at harvest and after storage (Garello et al., 2023). In this work white haze-related fungi were isolated from samples belonging to 13 apple cultivars, showing the widespread occurrence of the fungi in apple cultivars.

Identification of the collected isolates was conducted by comparison of informative genomic regions as reported in previous studies on basidiomycetous yeasts (Begerow et al., 2000; Boekhout et al., 2006, Wang et al., 2015). Six species belonging to three different basidiomycetous genera (i.e., *Entyloma*, *Golubevia*, *Tilletiopsis*) were identified, demonstrating the high diversity of fungi involved in white haze development in Northern Italy. High variability was also found in the Netherlands, where eight different species were isolated and identified (Boekhout et al., 2006; Richter et al., 2019). Less diversity was observed in Northern Germany (Weber and Zabel, 2011) and in Croatia (Prencipe et al., 2016), where two and one species were associated with the disorder, respectively.

In this work, the isolated strains were identified as *E. belangeri*, *E. randwijkense*, *G. pallescens, T. washingtonensis*. Moreover, two new species, *E. mali* and *G. mali*, were described. Most of the fungal strains from Piedmont were identified as *G. pallescens*. In addition, a new species, *Golubevia mali* was isolated and described, becoming the third

species of the genus together with *G. pallescens* (Wang et al., 2015) and *G. heteromorpha* (Richter et al., 2019). Other isolates were identified as *T. washingtonensis* and only one as *E. belangeri*. In contrast, in Trentino and in South Tyrol, the most prominent isolated species belonged to the Entylomatales. In Trentino and South Tyrol most isolates were identified as *E. belangeri*, while others belonged to *E. randwijkense*. Two strains clustered with a strain previously reported as *Tilletiopsis* WH4 (Baric et al., 2010), within the order Entylomatales and these are described here as *E. mali*. Few isolates were identified as *T. washingtonensis* and one as *G. pallescens*, showing that in this area, Golubeviaceae are less represented than in Piedmont. In South Tyrol, *T. washingtonensis* was found as the most common species, as reported in a previous survey conducted in this area (Baric et al., 2010). Other isolates were identified as *E. belangeri*. Contrarily to Baric et al., (2010), no isolates were identified as *G. pallescens* and *E. mali* (previously reported as *Tilletiopsis* WH4). In short, our results showed that several species belonging to various genera are involved in white haze development in Northern Italy, depending on the geographical areas where apples are cultivated.

Results revealed also different species involvement in white haze between Northern Italy and other European areas. Together with the newly described species *E. mali* and *G. mali*, *T. washingtonensis* was never associated with white haze in other European regions. However, this species was isolated from samples coming from all areas surveyed in Italy. *T. washingtonensis* was previously found to be able to assimilate volatiles produced by ripe apples (Vishniac et al., 1997), which may be a factor leading to the overgrowth of white haze-related fungi on the apple surface.

In contrast to the findings from northern Europe, no Italian isolates were identified as *E. davenportii*, *E. elstari*, *Gjaerumia minor, Golubevia heteromorpha* and *Jamesdiscksonia mali*. *Golubevia heteromorpha* was reported as the most spread species involved in the disorder in the Netherlands (Boekhout et al., 2006) and in northern Germany (Weber and Zabel, 2011). To date, the Netherlands are the only country where representatives of the order Georgefischerales, *G. minor* and *J. mali*, were found in association with white haze. In addition, a greater diversity of *Entyloma* species was found in the Netherlands compared to northern Italy (Boekhout et al., 2006).

White haze symptoms were artificially reproduced on 'Inored$^{(cov)}$/Story® and 'Ambrosia' apples placed at 20 °C. A whitish fungal mycelium adhering to the fruit skin was observed on inoculated fruits. Symptoms were more easily visible on 'Inored$^{(cov)}$/Story® apples, characterized by red skin, whereas the incidence was lower on 'Ambrosia' apples, characterized by yellow skin. *G. pallescens* produced the highest incidence of white haze for both cultivars, compared to the other two tested species. *T. washingtonensis* developed a low incidence of white haze on 'Inored$^{(cov)}$/Story® apples and was not able to reproduce the disorder on 'Ambrosia' fruits. Artificial reproduction of white haze was previously reported by Prencipe et al. (2016), who reproduced white haze symptoms on 'Cripps Pink$^{(cov)}$/Pink Lady$^{(cov)}$ apples inoculated with *G. pallescens*. With these inoculation experiments, Koch's postulates were unequivocally fulfilled, demonstrating that these fungal species are the causal agents of white haze. More awareness by apple growers and packing houses, but also by phytosanitary experts might contribute to understand the real diffusion of white haze in Europe and elsewhere. Also, the use of real time PCR diagnostics might help to answer this question.

5. Conclusion

The present study provides an overview of the diversity of *Tilletiopsis*-like fungi associated with white haze in Northern Italy. Six species were found associated with white haze, showing high diversity of the fungal species involved. Moreover, two new species associated with white haze were described, namely *E. mali* and *G. mali*. Considering the economic relevance of apple in this area, further research is required to understand the ecology and the epidemiology of white haze-related species, such as their dispersal and growth at different temperatures, and factors leading to the overgrowth of *Tilletiopsis*-like fungi on the apple skin in orchard and during storage. Future studies could focus on the comprehension of the effect of climate change on the incidence of white haze, as rainfall, relative humidity and minimum and maximum temperature during day- and night times may significantly affect the white haze fungal agents.

Declaration of Competing Interest

No conflict of interest exists. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2023.112678.

References

Baric, S., Lindner, L., Marschall, K., Dalla Via, J., 2010. Haplotype diversity of *Tilletiopsis* spp. causing white haze in apple orchards in Northern Italy. Plant Pathol. 59, 535–541. https://doi.org/10.1111/j.1365-3059.2009.02217.x.

Begerow, D., Bauer, R., Boekhout, T., 2000. Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. Mycol. Res 104, 53–60. https://doi.org/10.1017/S0953756299001161.

Boekhout, T., 1991. A revision of ballistoconidia-forming yeasts and fungi. Stud. Mycol. 33, 1–192.

Boekhout, T., 2011. Tilletiopsis Derx ex Derx (1930), in. The Yeasts. Elsevier, pp. 2003–2014. https://doi.org/10.1016/B978-0-444-52149-1.00160-9.

Boekhout, T., Gildemacher, P., Theelen, B., Muller, W.H., Heijne, B., Lutz, M., 2006. Extensive colonization of apples by smut anamorphs causes a new postharvest disorder. FEMS Yeast Res. 6, 63–76. https://doi.org/10.1111/j.1567-1364.2005.00002.x.

Derx, H.C., 1930. Étude sur les Sporobolomycètes. Ann. Mycol, 28, 1–23.

Fell, J.W., Boekhout, T., Fonseca, A., Scorzetti, G., Statzell-Tallman, A., 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int J. Syst. Evol. Microbiol 50, 1351–1371. https://doi.org/10.1099/00207713-50-3-1351.

Garello, M., Piombo, E., Prencipe, S., Schiavon, G., Berra, L., Wisniewski, M., Droby, S., Spadaro, D., 2023. Fruit microbiome: a powerful tool to study the epidemiology of dry lenticel rot and white haze – emerging postharvest diseases of apple. Postharvest Biol. Tec. 196, 112–163. https://doi.org/10.1016/j.postharvbio.2022.112163.

Gokhale, A.A., 1972. Studies on the genus *Tilletiopsis*. Nova Hedwig. 23, 795–809.

Hamamoto, M., Tamura, M., Nakase, T., 2000. Emended descriptions of *Tilletiopsis washingtonensis*, *Tilletiopsis cremea* and *Tilletiopsis lilacina*. Int J. Syst. Evol. Microbiol 50, 925–930. https://doi.org/10.1099/00207713-50-2-925.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing Confidence in phylogenetic analysis. Syst. Biol. 42, 182–192. https://doi.org/10.1093/sysbio/42.2.182.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Köhl, J., de Geijn, H.G., Haas, L.G., Henken, B., Hauschild, R., Hilscher, U., der Plas, C.L., van den Bosch, T., Wikström, M., 2019. Stepwise screening of candidate antagonists for biological control of *Blumeria graminis* f. sp. tritici. Biol. Control 136, 104008. https://doi.org/10.1016/j.biocontrol.2019.104008.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Kurtzman, C.P., Fell, J.W., Boekhout, T., Robert, V., 2011. Methods for isolation, phenotypic characterization and maintenance of yeasts. in: The Yeasts. Elsevier, pp. 87–110. https://doi.org/10.1016/B978-0-444-52149-1.00007-0.

Lindner, L., Baric, S., 2006. La "patina bianca'': una nuova alterazione che danneggia l'epidermide della mela. Inf. Fitopatol. 56, 60–63.

Liu, Y.J., Whelen, S., Hall, B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol. Biol. Evol. 16, 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092.

Matheny, P., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Henrik Nilsson, R., Hughes, K. W., Hofstetter, V., Ammirati, J.F., Schoch, C.L., 2007. Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol. Phylogenet Evol. 43, 430–451. https://doi.org/10.1016/j.ympev.2006.08.024.

Nari, L., Nari, D., Berra, L., Spadaro, D., Tavella, L., 2022. In Piemonte aumentano le superfici di mele resistenti a ticchiolatura. Rivista di. Frutticoltura 84, 28–34.

Nyland, G., 1950. The Genus *Tilletiopsis*. Mycologia 42, 487–496. https://doi.org/10.1080/00275514.1950.12017853.

Nylander, J.A.A., 2004. MrModeltest v. 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Upps., Swed.

Prencipe, S., Spadaro, D., Fruk, G., Jemric, T., 2016. First report of *Tilletiopsis pallescens* causing white haze on apple in croatia, 225-225 Plant Dis. 100. https://doi.org/10.1094/PDIS-05-15-0505-PDN.

Richter, C., Yurkov, A.M., Boekhout, T., Stadler, M., 2019. Diversity of *Tilletiopsis*-Like Fungi in Exobasidiomycetes (Ustilaginomycotina) and Description of Six Novel Species. Front Microbiol 10. https://doi.org/10.3389/fmicb.2019.02544.

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. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029.

Shen, Y., Nie, J., Li, Z., Li, H., Wu, Y., Dong, Y., Zhang, J., 2018. Differentiated surface fungal communities at point of harvest on apple fruits from rural and peri-urban orchards. Sci. Rep. 8 https://doi.org/10.1038/s41598-017-17436-5.

Spadaro, D., Torres, R., Errampalli, D., Everett, K., Ramos, L., Mari, M., 2019. Postharvest diseases of pome fruit. In: Palou, L., Smilanick, J.L. (Eds.), Postharvest pathology of fresh horticultural produce. CRC Press, Boca Raton, FL, USA, pp. 55–109.

Swofford, D.L., 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods), v. 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Takashima, M., Nakase, T., 2001. *Tilletiopsis derxii*, *Tilletiopsis oryzicola* and *Tilletiopsis penniseti*, three new species of the ustilagionomycetous anamorphic genus *Tilletiopsis* isolated from leaves in Thailand. Antonie Van. Leeuwenhoek 80, 43–56. https://doi.org/10.1023/A:1012218108640.

Tubaki, K., 1952. Studies on Sporobolomycetaceae in Japan: I. On *Tilletiopsis*. Nagaoa 1, 26–31.

Turland, N., Wiersema, J., Barrie, F., Greuter, W., Hawksworth, D., Herendeen, P., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T., McNeill, J., Monro, A., Prado, J., Price, M., Smith, G. (Eds.), 2018. International Code of Nomenclature for algae, fungi, and plants. Koeltz Botanical Books. https://doi.org/10.12705/Code.2018.

Visagie, C.M., Boekhout, T., Theelen, B., Dijksterhuis, J., Yilmaz, N., Seifert, K.A., 2023. Da Vinci's yeast: *Blastobotrys davincii* f.a., sp. nov. Yeast 40, 7–31. https://doi.org/10.1002/yea.3816.

Vishniac, H.S., Anderson, J.A., Filonow, A.B., 1997. Assimilation of volatiles from ripe apples by *Sporidiobolus salmonicolor* and *Tilletiopsis washingtonensis*. Antonie Van. Leeuwenhoek 72, 201–207. https://doi.org/10.1023/A:1000363510580.

Wang, Q.M., Begerow, D., Groenewald, M., Liu, X.Z., Theelen, B., Bai, F.Y., Boekhout, T., 2015. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. Stud. Mycol. 81, 55–83. https://doi.org/10.1016/j.simyco.2015.10.004.

Weber, R.W.S., Zabel, D., 2011. White haze and scarf skin, two little-known cosmetic defects of apples in Northern Germany. Eur. J. Hort. Sci. 76, 45–50.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. in: PCR Protocols. Elsevier, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.