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## RESEARCH ARTICLE

## *Monilia* and *Monilinia* – *In silico* genetic analysis of plant pathogenic fungi

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**Abstract** – Species of phytopathogenic fungi *Monilia* [J. Hill, 1751] and *Monilinia* [E.E. Honey, 1936] of the phylum Ascomycota have two host plant families of stone- and pome fruit trees of *Rosaceae*; and bushes of *Ericaceae* (e.g., blueberry, cranberry, heather, etc.). They infect both plants, and human (i.e., moniliasis). Due to the lack of sexual developmental stage (i.e., imperfect fungi; anamorphic fungi) the species of the order Moniliales spread by vegetative spores (i.e., conidia) and not by sexual (i.e., meiotic) ascospores. Here we report *in silico* data mining carried out by analyses of DNA, RNA, and protein sequences to reveal genetic distances in-and-among *Monilia* and *Monilinia* species. Sequences of the (+)ssRNA genomes of mitoviruses, which hyper parasitize the fungi cells, which parasitize the plant cells, during the ecocycles, are also analyzed. Genome size analysis of *Monilinia* were found to be between 30 - 55 x 10<sup>6</sup> bp; the *cytochrome-b* (*cytb*) genes (1543 bp of mtDNA); the transcribed *cytb*-mRNAs; the translated *CYTB* proteins (381 - 391 aa); and the (+)ssRNA genomes of mitoviruses have showed that *Botrytis cinerea* ('grey mold') has the closest molecular similarity to *Monilia* and *Monilinia* clade ('brown rot'). The cladogram of transcribed *cytb*-mRNAs (1175 nt) grouped *Monilinia vaccinii-corymbosi* in a distinct clade, which indicate a far genetic linkage. The application of mitovirus-infected hypovirulent phytopathogenic fungi for plant protection (i.e., virocontrol / biocontrol) is discussed.

**Keywords** – moniliasis (*Monilia* ssp.), aspergillosis (*Aspergillus* ssp.; black mold), mtDNA, (+)ssRNA, mitovirus, brown rot (*Monilia* ssp), white rot (e.g., *Phanerochaete* ssp), grey mold (*Botrytis* ssp).

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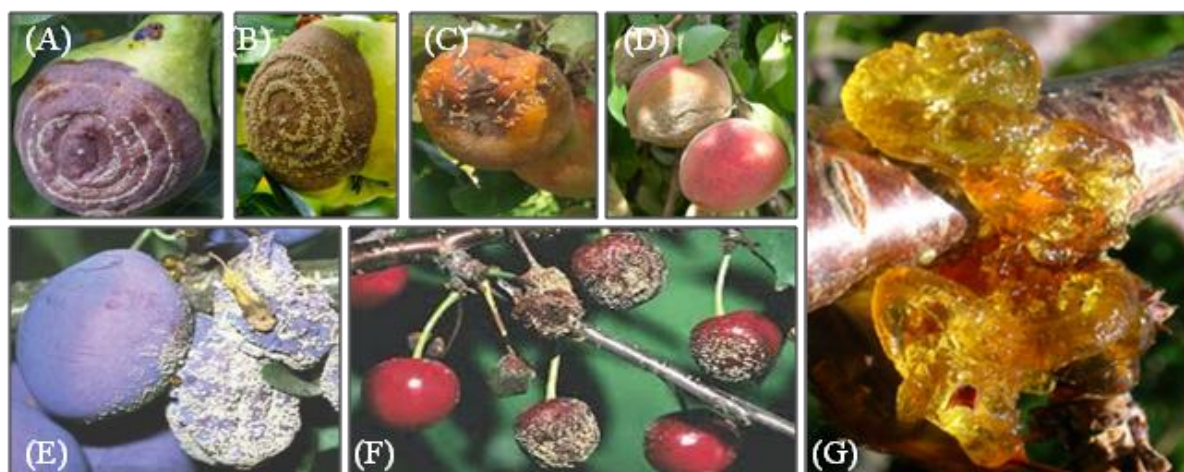
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### 1. INTRODUCTION

FUNGI are eukaryotic organisms 'between plants and animals', and inhabiting every biotope globally, and are found even in fresh- (El-Elimat *et al.*, 2021; Abonyi *et al.*, 2023) and sea waters (Orsi *et al.*, 2013; Jones *et al.*, 2022; Pang *et al.*, 2023). Fungi exist in three different forms of asexual anamorph (*Syn.*, asexual-, mitotic-, imperfect fungi), sexual teleomorph (*Syn.*, sexual-, meiotic-, perfect fungi), and holomorph form (i.e., the higher fungi with fruiting body, either Ascomycota or Basidiomycota). The asexual anamorph fungi propagate by conidia (i.e., budding cells). The sexual teleomorph fungi propagate by spores (e.g., ascospores, basidiospores or zygospores, etc.) (Daniel *et al.*, 2014; Boddy, 2016; Moore *et al.*, 2020; Abarenkov, 2022).

PHYLUM ASCOMYCOTA is the largest and most species-rich group of fungi with over 100,000 species (Kirk *et al.*, 2008;

Gyulai *et al.*, 2018; Shen *et al.*, 2020). The taxonomy has divided the phylum in to three subphyla of [1] Saccharomycota ('budding yeasts') with over 1000 species including, e.g., the bread yeast *Saccharomyces cerevisiae* [(Desm.) Meyen, F.J.F. and Hansen, E.C. (1838)], [MB#492348; [www.gbif.org](http://www.gbif.org)], and human pathogens (Zörgö, *et al.*, 2013), e.g., *Candida albicans* and *C. auris*; [2] Pezizomycota ('filamentous fungi'; with 82,000 species including *Monilinia* ('brown rot') (with about 300 species/isolates) (Table 1, 2), *Aspergillus* ssp. ('black mold') (with 592 species/isolates) etc., and the major plant pathogen genus *Fusarium* (with over 300 species/isolates); and [3] Taphrinomycota, a small group of fission yeasts with over 150 species including, e.g., *Schizosaccharomyces pombe*; and *Taphrina deformans* ('leaf curl') [Berkeley, M.J. and Tulasne, E., 1866] that 'new fungi' – which reached Hungary fifty-sixty years ago [S. Gyulai, 1923-2000, at Budatétény, H., personal communications] (Varga *et al.*, 2010; Shen *et al.*, 2020; [web1](#); [mycobank.org](#)).



**Figure 1.** Samples of the infection types of *Monilia* and *Monilinia* fungi on *Rosaceae* fruit trees. (A) pear (photo: Yuru Herasimenka; Authors' ID# 229600280); (B) quince (photo: Claudia Schmidt, Authors' ID# 240218379); (C) apple (source: [web3](#)); (D) peach (source: [web4](#)); (E) plum (photo: Fernando Torres P., CAB Int.); (F) cherry (photo: Fernando Torres P., CAB Int.); and (G) cherry gummosis (photo: Rosser, 1954, Chapelton, Ayrshire, Scotland).

ORDER MONILIALES of Ascomycota fungi (Honey, 1936; Moesz, 1939; Shen *et al.*, 2020; Bokor *et al.*, 2021) comprises hundreds of species/isolates with the difficulties of double/triple description names and synonyms. *E.g.*, synonyms of *Monilia* include *Candida*, *Neurospora*, *Ambrosiella*, or *Aspergillus* ('black mold') (Varga *et al.*, 2010) (Table 1, 2). Series of synonyms also show how the current name *Monilinia* have developed to date: *Monilia* [Hill, 1751] → *Torula fructigena* [Persoon, 1796] → *Monilia fructigena* [Persoon, 1796] → *Ocidum fructigenum* [Kunze and Schmidt, 1817] → *Oospora fructigena* [Wallroth, 1833] → *Monilia cinerea* [Bonorden, 1851] → *Sclerotinia cinerea* [(Bonorden) Schröter, 1893] → *Sclerotinia fructigena* [Aderhold and Ruhland, 1905] → *Monilinia* (Honey, 1936) (<http://taxonomicon.taxonomy.nl>; Van Leeuwen *et al.*, 2000). The main reason of numerous synonyms is that most of the phytopathogenic fungi are pleomorphic as the asexual and sexual morphs differ during the life cycles (Boddy, 2016; Crous *et al.*, 2021).

FAMILY MONILIACEAE comprises mainly saprobe fungi (*Syn.*, necrotrophs), however, many species are plant- (Batra, 1991) and animal parasites (*i.e.*, biotrophs), and human pathogens (Gruby, 1843). *Monilinia* ssp. infects human body (*i.e.*, moniliasis) (Mendelblatt, 1953). Human pulmonary aspergillosis the CAPA (Covid-19 Associated Pulmonary Aspergillosis), ABPA and AAS infects human lung (Arastehfar *et al.*, 2020). The MD and researcher Gruby, D. [1810-1898] was the first identifier (Gyulai *et al.*, 2018) of fungi which were capable to live in/on human body as pathogen.

GENUS MONILINIA has 54 species/isolates, registered at [www.mycobank.org](http://www.mycobank.org), and four of them *M. fructicola* ('brown rot'), *M. fructigena* ('brown rot'), *M. laxa* ('blossom blight'), and *M. linhartiana* are the major fungal plant pathogens of prune crops (Fig. 1) causing blossom blight of flowers, brown rot on fruits, canker and gummosis of tree trunk (Yildiz and Ozkilinc, 2021). *M. linhartiana* [Saccardo, P.A., 1883] (Table 2) was reported to infect mainly the quince (*Cydonia oblonga*) (Moral *et al.*, 2011; Martini *et al.*,

2014; Lantos and Petróczy, 2016) (Fig. 1.B). In China, there are four more pathogenic *Monilinia* ssp. of *M. mumecola* (after the name of *Prunus mume* – Japanese apricot), *M. polystroma*, and *M. yunnanensis* (Harada *et al.*, 2004; Zhang *et al.*, 2023). The numerous common names of *Monilia* [Hill, 1751] and *Monilinia* (Honey, 1936) reflect the devastating symptoms of diseases such as spur blight, twig canker, wither tip, blossom blight/wilt, brown rot of fruit, and European brown rot of stone fruit.

SPECIES of MONILIA and MONILINIA fungi with hyper infection by bacterial canker (*Pseudomonas syringae*), fungal dieback (caused by, *e.g.*, *Trichothecium roseum*, *Neoscytalidium*, *Botryosphaeria*, *etc.*), and viral Little Cherry Disease (LCD, caused by Little Cherry Virus-2; LChV-2, a +ssRNA virus, *e.g.*, NCBI ID#: MW249041) (Hulo *et al.*, 2011) results in gum exudates (*i.e.*, gummosis) through lenticels of the trunk and twigs of trees (Fig. 1.G) and the fruits (*Hung.*, macskaméz / 'cat honey') (Lantos and Petróczy, 2016; Mgbечи-Ezeri, *et al.*, 2017). *Monilinia* species were identified in the last centuries by structure and color of spores and conidia by using light microscopes, and by visual observations of the types of fungal colony. *E.g.*, conidial pustules of *M. laxa* and *M. fructicola* show grey color compared to light brown colored conidia of *M. fructigena*, *etc.* (OEPP / EPPO, 2020).

MOLECULAR STUDIES (Shen *et al.*, 2020; Marcet-Houben, *et al.*, 2021; Yildiz and Ozkilinc, 2021; Poniatowska *et al.*, 2021; De Miccolis Angelini *et al.*, 2022a,b; Arvas *et al.*, 2023) have precisely identified *Monilia* and *Monilinia* ssp. genetically. *E.g.*, an ITS1-5.8S-ITS2 analysis (Moral *et al.*, 2011) indicated a strict host plant preference and plant - pathogen interactions (Gullner and Kömives, 2001; Szabó *et al.*, 2023).

The study of Moral *et al.*, (2011) discriminated *Monilia mumecola* and all the *Monilinia* species in to two groups of infected plant families based on the conidia structures, described by Honey (1936) of the *JUNCTORIAE*: *Monilinia fructicola*\*, *M. fructigena*\*, *M. laxa*\*, *M. polystroma*\*

*Sclerotinia sclerotiorum*\*, *Ovulinia azaleae*\*; and the DISJUNCTORIAE: *M. amelanchieris*\*, *M. aucupariae*\*, *M. azaleae*, *M. baccarum*, *M. cassiopes*, *M. gaylussaciae*, *M. jezoensis*, *M. johnsonii*\*, *M. linhartiana*\*, *M. mali*\*, *M. megalospora*, *M. oxycocci*, *M. padi*\*, *M. polycodii*, *M. saeverii*, *M. vaccinii-corymbosi*, and *M. urnula*. Species indicated with asterisk\* infect *Rosaceae* trees, and species without asterisk label parasitize *Ericaceae* bushes (Moral *et al.*, 2011).

*Monilinia* disease spreads globally, and had reached the East edge of Eurasia in China by 1920 (Zhang *et al.*, 2023). Relatively new *Monilinia* species had been described, e.g., *Monilia mumecola* (see above) [Harada, Y., Sasaki, Y., Sano, T., 1982] in Japan (Harada *et al.*, 2004) [<http://taxonomicon.taxonomy.nl/>].

MITOVIRUSES (Polashock and Hillman, 1994; Li *et al.*, 2023; Ezawa *et al.*, 2023) were discovered in hypovirulent strains of plant pathogenic Ascomycota fungus *Cryphonectria parasitica*, which causes chestnut (*Castanea dentata*) blight (Polashock and Hillman, 1994). Later, mitovirus infections were also identified in plant mitochondria (Nibert *et al.*, 2018) (e.g., NCBI#: BK010425.1, in *Solanum chacoense*). The mitoviral genomes (e.g., *Cryphonectria mitovirus*, *Ophiostoma mitovirus*, etc.) are non-segmented, linear, and short (+)ssRNA (with about 2973 nt length), which usually occur in dsRNA replicative form (web2), and encode only for a single gene of RdRp (RNA-directed RNA polymerase), and have no capsid or envelop (i.e., naked viruses).

Mitoviruses of *NaRNAviridae* / *Mitoviridae* family (<https://ictv.global/taxonomy>; with 109 entries to date) replicate inside the mitochondria of pathogenic fungi cells (hence the name 'mito'-) (Ghabrial and Suzuki, 2009; Wu *et al.*, 2010), and they are presumably unaffected by antiviral RNA silencing due probably to the double mitochondrial membrane protection (Shahi *et al.*, 2019). Mitoviruses have given a new technology of virocontrol for plant protection.

STONE- and POME FRUIT TREES ('prune crops') are extremely susceptible to *Monilinia* infections (Fig. 1) with extreme

### 3. RESULTS

#### 3.1. *Monilia* [Hill, 1751] or *Monilinia* (Honey, 1936)?

yield loss of both the pre-harvest and post-harvest losses, which levels may reach e.g., 27.2% to 41.6% in apple (Holb, 2004).

In the Netherlands, *M. fructigena* caused 4.2% - 4.3% yield loss in apple plantations (Van Leeuwen *et al.*, 2000). All PRUNE and APPLE CROPS belong to *Rosaceae* plant family (Simoncsics, 2017). These are the sour- and sweet cherry (*Prunus cerasus*, and *P. avium*), peach (*P. persica*), apricot (*P. armeniaca*), plum (*P. domestica*), almond (*P. dulcis*); and the POME FRUIT TREES of apple (*Malus domestica*), pear (*Pyrus communis*), and quince (*Cydonia oblonga*), etc. This indicate a strict molecular interactions between *Rosaceae* - *Ericaceae* species and pathogens (Gullner and Kőmives, 2001).

BREEDING of sour/cherry (Lippai, J. [1606-1666]; Maliga, P. [1913-1987]; Nyujtó, F. [1922-1999]; Brózik, S. [1925-2001]; Apostol, J. [1941-]; and Szabó, T. [1943-]) for *Monilia/Monilinia* resistance (Faust and Surányi, 2011; Mgbechi-Ezeri *et al.*, 2017) has resulted in several disease resistant clones, e.g., sour cherry cv. 'Balaton' (registered 1984, by Amy F. Iezzoni, Michigan State University, East Lansing, Michigan, USA) (Apostol *et al.*, 1995; Szügyi and Sárdi, 2017). *Monilia/Monilinia* resistant natural clones (e.g., a sour cherry cv. 'Feketicisi') were selected recently (web5).

Here, we aimed to find new molecular characteristics (Tóth *et al.*, 2007; Gyulai *et al.*, 2023) in-and-among *Monilia* and *Monilinia* species by *in silico* data mining of genomes, gene and protein of mtDNAs, and (+)ssRNAs of mitoviruses.

### 2. METHODOLOGY

Sequences of fungal genomes (Fig. 2), mtDNA gene (*cytb*) and protein (*CYTB*), and viral (+)ssRNAs were downloaded from NCBI server (web6), aligned by computer program BioEdit (Hall, 1999), and the molecular cladograms were edited by MEGA7 computer program (Kumar *et al.*, 2016).

The analytical (Jo *et al.*, 2022) and statistical (Csárdi and Nepusz, 2006; Gyulai *et al.*, 2015) approaches of the gums of gummosis (Fig. 1.G) will be excluded.

The web site of Species Fungorum (web1) lists 241 *Monilia* entries (e.g., Table 1); and 39 *Monilinia* records. Most of the *Monilinia* records are grouped in to family *Sclerotiniaceae* (e.g., Table 2).

**Table 1.** Samples of *Monilia* [Hill, 1751] species, strains and synonyms. Source: *Species Fungorum* (web1).

Scientific names	Synonyms
<i>Monilia alba</i> [Castell. & Chalm., 1919]	<i>Candida albicans</i> (Saccharomycetales)
<i>Monilia aurantiaca</i> [Lév. ex Mont.; Herter, 1933]	<i>Neurospora sitophila</i> (Sordariaceae)
<i>Monilia azaleae</i> [L.R. Batra, 1991]	<i>Monilinia azalea</i> (Sclerotiniaceae)
<i>Monilia candida</i> [R. Hartig, 1844]	<i>Ambrosiella hartigii</i> (Ceratocystidaceae)
<i>Monilia cinerea</i> [Bonord., 1851]	<i>Monilinia laxa</i> (Sclerotiniaceae)
<i>Monilia crataegi</i> [Died., 1904]	<i>Monilinia johnsonii</i> (Sclerotiniaceae)
<i>Monilia glauca</i> [(L.) Pers., 1794]	<i>Aspergillus glaucus</i> (Aspergillaceae)



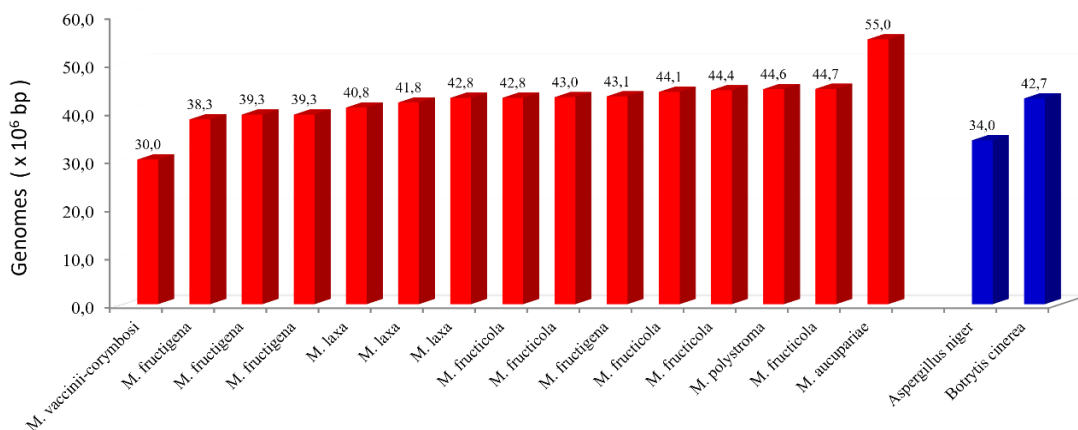
**Table 2.** Samples of *Monilinia* (Honey, 1936) species, strains and synonyms. Source: Species Fungorum ([web1](#)).

- Monilinia alpina* [L.R. Batra, 1991], Sclerotiniaceae
- Monilinia corni* [J.M. Reade; E. E. Honey, 1936], Sclerotiniaceae
- Monilinia cydoniae* [Schellenb.;Whetzel, 1945], Sclerotiniaceae
- Monilinia fructicola* [G. Winter; E. E. Honey, 1928], Sclerotiniaceae
- Monilinia laxa* [Aderh. & Ruhland; E. E. Honey, 1945], Sclerotiniaceae
- Monilinia mali* [Takah.; Whetzel, 1945], Sclerotiniaceae
- Monilinia linhartiana*\* [Saccardo, 1883; Prill. & Delacr.,1893; Dennis, 1949], Syn.: *Peziza l.*
- Monilinia mespili* [Whetzel, 1945], Sclerotiniaceae
- Monilinia mume* [Hara; W. Yamam, 1959], Sclerotiniaceae
- Monilinia padi* [Woronin; E.E. Honey, 1936], Sclerotiniaceae
- Monilinia vaccinii-corymbosi* [J.M. Reade; E.E. Honey, 1936], Sclerotiniaceae

\*named after the Hungarian mycologist György Linhart (1844-1925) (Paál, 1926; Lantos and Petróczy, 2016).

### 3.2. Genomes of *Monilinia* ssp.

Genome sizes of *Monilinia* species - recently fifteen are available at the NCBI server ([web6](#)), vary from 30 - 55 x 10<sup>6</sup> bp (Fig. 2).

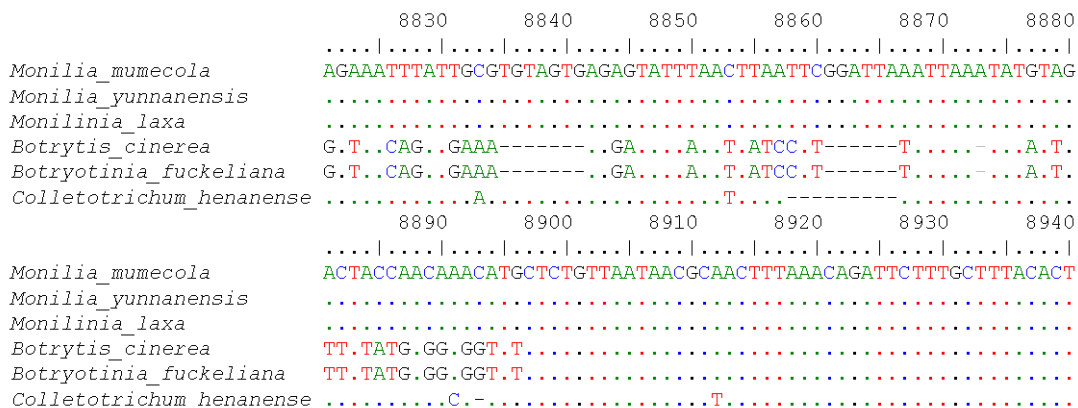


**Figure 2.** Genome sizes (x10<sup>6</sup> bp) of the fifteen available *Monilinia* species downloaded from NCBI/Genome server (e.g., NCBI ID#: [GCA\\_017357885.1](#)). Genomes of two species of *Aspergillus n.* ('black mold') and *Botrytis c.* ('grey mold') are included for genomic control.

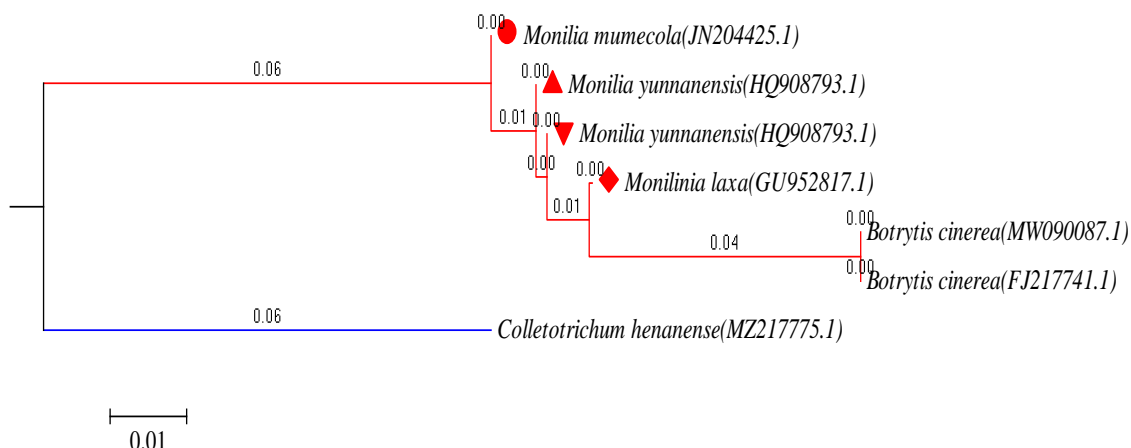
### 3.3. Sequence analysis

Samples of mitochondrial (mt) DNA (mtDNA) sequences of *cytochrome-b* (*cytb*) genes (complete cds; 1543 bp) from six Ascomycota fungi revealed consensus regions between *Monilia* and *Monilinia* species, however, with

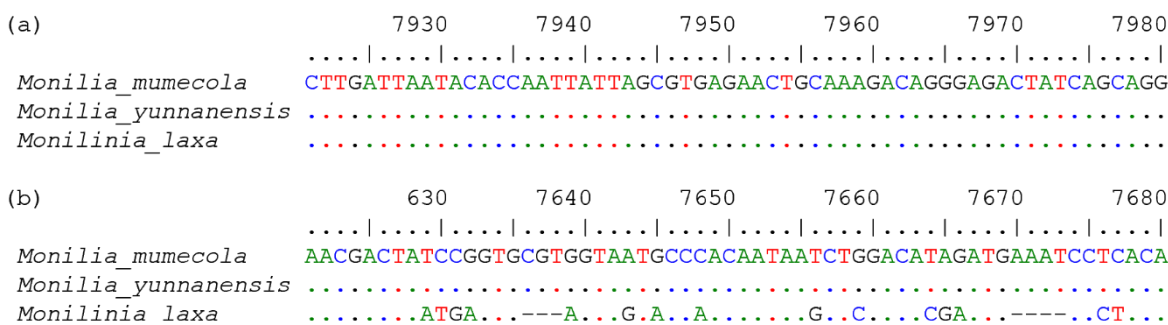
significant DNA nt variations (Fig. 3). The genetic distance analysis of *cytochrome-b* (*cytb*) genes (Hily *et al.*, 2011) showed *Botrytis* ('grey mold') to be the closest, and *Colletotrichum* to be the most distant from the cluster of *Monilia* and *Monilinia* clade (Fig. 4).



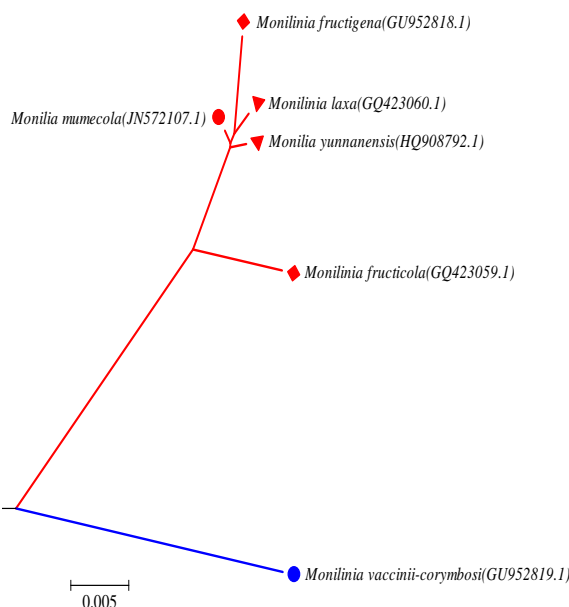
**Figure 3.** Samples of mtDNA sequence variations (2 x 60 nt stretches) of *cytochrome-b* (*cytb*) genes (complete cds; 1543 bp) of six Ascomycota fungi. The scale line with nt numbers refer the genomic loci of mitochondrial DNAs. Consensus nucleotides (dotted lines), different DNA nts (block color letters), and DNA nt deletions (-) are indicated. Sequence ID#s are indicated on Fig. 4.



**Figure 4.** Distance tree of mtDNA sequences of six *Ascomycota* fungi at *cytochrome-b* (*cytb*) gene locus (complete cds; 1543 bp) (NJ; Max Seq Difference: 0.85; Distance: Kimura). *Monilia yunnanensis* is represented with two sequences. Branch lengths and sequence ID#s are indicated. The unit of genetic distance of scale bar (0.01) is indicated which gives the numbers of DNA nucleotide substitutions along a 100 bp mtDNA nt stretch. *Monilia* and *Monilinia* species are labeled.



**Figure 5.** Samples of DNA sequence variations of consensus (a), and variable (b) stretches of mitochondrial mtDNAs (60 bp stretch each) of *cytochrome-b* (*cytb*) genes (complete cds; 1543 bp) of *Monilia* and *Monilinia* fungi species. The nt numbers on the scale lines refer to the loci of mtDNA genomes. Consensus nucleotides (dotted lines), different DNA nt (block color letters), and DNA nt deletions (–) are indicated. Sequence ID#s are indicated on Fig. 4.

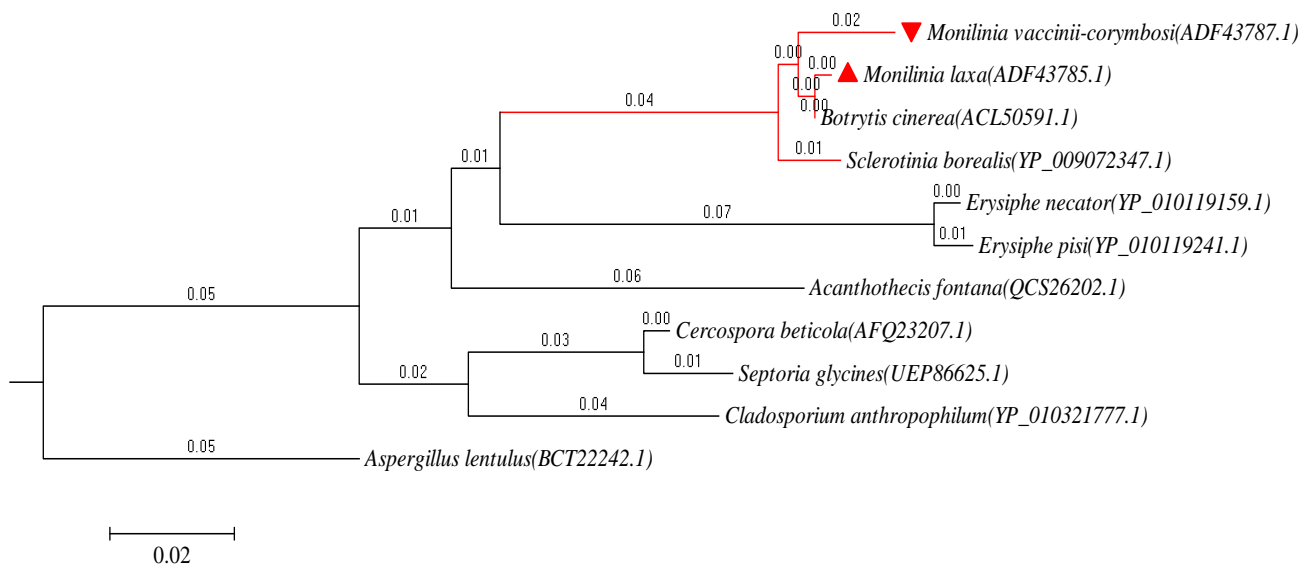


**Figure 6.** Radial cladogram of *cytb*-mRNAs (1175 nt) transcribed from *cytochrome-b* (*cytb* DNA) genes of *Monilia* and *Monilinia* species. The ID#s are given. The unit of genetic distance of scale bar (0.005) is indicated which number gives the mRNA nucleotide substitutions along a 100 bp mRNA stretch

To estimate genetic divergence between *Monilia* [Hill, 1751] and *Monilinia* (Honey, 1936) fungal species, consensus and diverse stretches of *cytochrome-b* (*cytb*) genes of mtDNAs were observed (Fig. 5).

When sequences of the transcribed *cytb*-mRNA were analyzed the cladogram (Fig. 6) grouped *Monilinia vaccinii-corymbosi* in a far distant clade.

Sequences of the translated mt protein *CYTOCHROME-b* (*CYTB*) (381-391 aa) of eleven available Ascomycota species revealed the most distant, e.g., *Aspergillus* ('black mold'), and the closest relatives of *Botrytis cinerea* ('grey mold') and *Sclerotinia borealis* to *Monilinia* clade (Fig. 7).

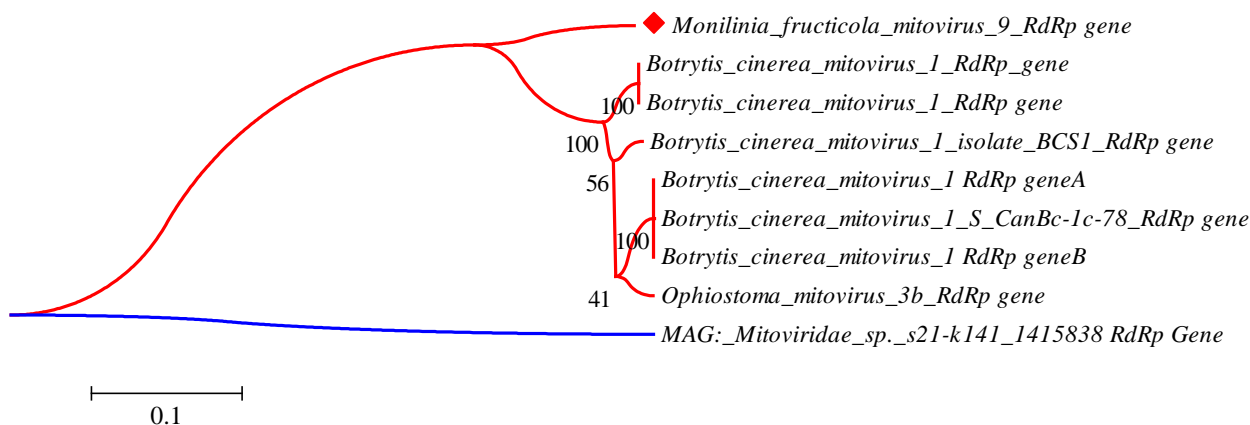


**Figure 7.** Protein dendrogram (tree method: NJ; Max Seq Difference: 0.85; Distance: Kimura) of mitochondrial protein *CYTOCHROME-b* (*cytb*) (381-391 aa) of eleven available Ascomycota fungi species (*Monilinia* species are labeled). Branch lengths, and NCBI ID#s are indicated. The unit of genetic distance of scale bar (0.02) which gives the numbers of aa substitutions along a 100 amino acid (aa) stretches of the proteins are indicated.

### 3.4. Mitoviruses – Virocontrol

Molecular distance tree of mitoviruses (+ssRNA) which parasitize *Monilinia fructicola* fungus cells showed close

molecular similarity in one clade, however with distinct differences compared to other mitovirus genomes analyzed (Fig. 8).



**Figure 8.** Molecular distance tree (NJ) of mitovirus genomes (+ssRNA), which hyper parasitize the mitochondria of phytopathogenic fungi cells of *Monilinia*, *Botrytis* ('grey mold'), and *Ophiostoma*. Sequences were downloaded from NCBI server. Statistical bootstrap (x 1000 repetitions) analysis (41 - 100), and the unit of genetic distance of scale bar (0.1) is indicated which gives the numbers of (+)ssRNA nucleotide substitutions along a 100 bp RNA stretch. Sequences were aligned to *Monilinia fructicola* mitovirus 9 (*Mfrc*MV9; NCBI/Nucleotide ID# ON038383.1; length: 2438 nt).

### 4. CONCLUSION

Genome sizes of *Monilinia* species was found to vary between 30 - 55 x 10<sup>6</sup> bp (Fig. 2). To compare, genomes of green plants, e.g., *Arabidopsis thaliana* is about three times

larger: 140 x 10<sup>6</sup> bp (NCBI ID#: GCA\_946413285.1). However, genome sizes of Ascomycota mushrooms (i.e., higher fungi) are also about in this range; e.g., genome of *Tuber melanosporum* (*Eng./Hung.*: black truffle / feketete

szarvasgomba) is  $125 \times 10^6$  bp (Gyulai *et al.*, 2018; [www.mycobank.org](http://www.mycobank.org)).

Genomes of three *Monilinia* species of *M. fructicola*, *M. fructigena* and *M. laxa* were analyzed recently (De Miccolis Angelini *et al.*, 2022a). The study revealed that *M. laxa* shows more close genetic distance to *M. fructigena* than *M. fructicola*. The result suggested that during speciation (Taylor and Taylor, 1993), *M. laxa* may have been the most ancient *Monilinia* species of the three species studied (De Miccolis Angelini *et al.*, 2022a). However, *M. laxa* and *M. fructicola* show closer similarity by the shared grey colored conidial pustules, compared to that of light brown colored of *M. fructigena* (web9). Similar to the results presented here, *Botrytis cinerea* ('grey mold') showed the closest genetic distance to *Monilinia* species (De Miccolis Angelini *et al.*, 2022a) (Fig. 4, 6, 7).

Evolutionary analyses of fungi genomes (Ragan, 1997) have opened a new field of knowledge (Ács-Szabó *et al.*, 2021; Bokor *et al.*, 2021; De Miccolis Angelini *et al.*, 2022a). The (no)evolution theories started by C. Linnaeus' [1707-1778] work of *Systema Naturae* (1735), followed by the theories of the inheritance of acquired characters by J.-B. Lamarck [1744-1829], 1802, and G. Cuvier [1769-1832] (*in*: Burkhardt, 2013); followed by the morphological observations (*e.g.*, the 'overestimated' bird diversity of finches in Galápagos Islands) coupled with the theories of natural selection and adaptation of Ch. Darwin [1809-1882] and A.R. Russel [1823-1913], 1858 (*in*: Burkhardt, 2013); and by the embryological E.H.Ph.A. Haeckel [1834-1919] (1868); and paleontological theories of F. Nopcsa [1817-1933] (1929); and A.S. Romer [1894-1973] (1933) (*in*: Weishampel and Kerscher, 2013); and ended up in the neutral selection theory including the molecular clock steps of M. Kimura [1924-1994], (1968).

When reasons were studied here to find common evolutionary lineages between the two host plant families to *Monilinia* ssp., *Rosaceae* and *Ericaceae* were found in two distinct lineages of Rosales→Fabales and Dilleniales→Ericales clades (Dioscorides, 40-90 A.D., *in*: Janick and Hummer, 2012; Bauhin, 1623; Linnaeus, 1753; Jávorka and Csapody, 1934; Greguss, 1964; Soó, 1965; Borhidi, 1998; Debreczy and Rácz, 2011; Cornwell *et al.*, 2014).

The mtDNA and cpDNA (which is lacking in fungi) of plant cell organelles were reported to be highly conserved by species (Dudits *et al.*, 1980; Medgyesy *et al.*, 1986; Maliga, 2003; Gyulai *et al.*, 2012; Ali *et al.*, 2014). However, as a results of the next-generation sequencing (NGS) technologies (*e.g.*, by Illumina), high levels of mtDNA genomes variability were detected in fungi including SNPs (single nucleotide polymorphism), gain or loss of introns and repetitive sequences, due mainly to the activity of transposable elements (McClintock, 1950; Kalendar and Schulman, 2006; Hane and Oliver, 2008; Schulman and Wicker, 2013; Alzohairy *et al.*, 2014, 2015; De Miccolis Angelini *et al.*, 2022a; Arvas *et al.*, 2023; web7; web8).

The fungal mtDNA variability was found to be increased by recombination of mitochondrial plasmids – with independent replications and recombinations from the nuclear genome, which generate continuous new mtDNA rearrangements. This molecular mechanism has also been detected in mushrooms (*i.e.*, higher fungi) including Basidiomycota *Agaricus* (*e.g.*, NCBI ID#: KY357500.1); and Ascomycota *Neurospora* (Sandor *et al.*, 2018).

The variability of mtDNA of fungal mt-genomes (*i.e.*, mitom or mitogenomes) were also found to be resulted from changes in size. An analysis of 16 *Monilinia* isolates revealed that the mtDNA sizes vary from 158,607 to 179,780 DNA nt (Yildiz and Ozkilinc, 2021).

To compare, fungal mtDNAs are ten times greater than that found for human mtDNA (16,569 bp) (NCBI ID#: NC\_012920), but much smaller compared to that of plants, especially of *Cucurbita* species (*Cucumis sativus* and *C. melo*) with the largest mtDNA sizes to date which have mt-genome sizes > milliard ( $10^9$ ) bp (Gyulai *et al.*, 2012; Ali *et al.*, 2014).

For practical use, during mitovirus infection of pathogenic fungi cells the fungal virulence reduces (*i.e.*, hypovirulence), and by this way the damage caused by fungi also reduces on the infected plants (Wu *et al.*, 2010; De Miccolis Angelini *et al.*, 2022b). *E.g.*, *Monilinia fructicola* has 1-12 mitoviruses (*Mfrc*MV1-12) (Fig. 8). *Erysiphe necator* ('grape powdery mildew') has 34 mitovirus entries (web10; web11). The observation of fungal hypovirulence caused by mitoviral hyper infection of mitochondria of the fungal cell (Polashock and Hillman, 1994) gave a new tool of virocontrol of biocontrol for plant protection, which is in use already in chestnut (*Castanea* ssp.) white-rot fungi (*Gnomoniopsis*, *Diaporthe*, *etc.*) (Chiba *et al.*, 2011), and for *Botrytis cinerea* ('grey mold') (Wu *et al.*, 2010). The technology of virocontrol seems to have more potentials than the former biocontrol technologies which tried to use antagonistic bacteria (*e.g.*, *Bacillus amyloliquefaciens*) or fungi (*e.g.*, *Epicoccum nigrum*, *Aspergillus flavus*, *Penicillium frequentans* and *P. purpurogenum*, *Trichoderma viride*, and *Sordaria fimicola*) for *Monilia* / *Monilinia* control (Szódi and Turóczy, 2014; Jiang *et al.*, 2023).

No virocontrol has been available to date for mitoviruses to develop hypovirulent *Monilia* / *Monilinia* fungi to protect PRUNE- and APPLE CROPS (web13).

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- web1: [www.speciesfungorum.org](http://www.speciesfungorum.org)
- web2: <https://ictv.global/taxonomy>
- web3: [Wikimedia.org/Wikipedia/commons/0/0f/Brown\\_Rot\\_on\\_Apple.jpg](https://commons.wikimedia.org/wiki/File:Brown_Rot_on_Apple.jpg)
- web4: <https://wiki.bugwood.org>
- web5: <https://biokiskert.hu/bio/feketicsi>
- web6: [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)
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- web10: <https://ftp.ncbi.nlm.nih.gov/genomes>
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