FUSE
Fungal Systematics and Evolution
VOLUME 17
JUNE 2026
PAGES 58–68

doi.org/10.15761/fuse.2026.17.04

Redefining *Mollisia cinerea*

M.M. Nauta[1], J.Z. Groenewald[1], P.W. Crous[1,2,3]

[1]Westerdijk Fungal Biodiversity Institute, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.
[2]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.
[3]Microbiology, Department of Biology, Utrecht University, Padualaan 8, Utrecht, 3584 CH, The Netherlands.

*Corresponding author e-mail: m.nauta@wi.knaw.nl

Keywords:
Diplococcium
epitype
new taxa
Phialocephala
systematics

Abstract: No type material of the type species of *Mollisia* (Fr.) P. Karst. has been retained, and the taxonomy of the genus is presently unclear. It is therefore urgent to redescribe and redefine the type species *M. cinerea* (Batsch) P. Karst. by designating the plate of Batsch as lectotype for its basionym *Peziza cinerea* Batsch, and by designating a corresponding epitype. In accordance with the consensus of morphology of the sexual and asexual morph, together with molecular characters of three genes, a voucher specimen is herewith selected, with an accompanying ex-epitype culture. Furthermore, *Phialocephala* is reduced to synonymy under the older name, *Mollisia*, along with several new combinations in *Mollisia.*

Citation: Nauta MM, Groenewald JZ, Crous PW (2026). Redefining *Mollisia cinerea*. *Fungal Systematics and Evolution* **17**: 58–68. doi: 10.15761/fuse.2026.17.04
Received: 4 November 2025; **Accepted:** 17 February 2026; **Effectively published online:** 13 March 2026
Corresponding editor: M. Arzanlou

Introduction

Mollisia (*Mollisiaceae, Helotiales, Leotiomycetes*) is a widely distributed and common non-lichenized genus of *Ascomycetes* with about 150 mostly saprotrophic species. It is known for its small, several millimetres wide apothecial ascomata mostly occurring on dead wood or bark of trees, or herbaceous or *Poaceae* tissues. It plays a significant role in decomposition of organic material in various ecosystems. The confusing taxonomy of the genus *Mollisia* and related genera has traditionally been based on morphology of the apothecia. The lack of clear characters has not hampered publication of new species however, and presently up to 600 names exist in *Mollisia* (http://www.mycobank.org/), of which the identity is often uncertain. As a genus, *Mollisia* is paraphyletic (Grunig *et al*. 2009, Day *et al*. 2012), or even polyphyletic (Baschien *et al*. 2013).

Understanding a genus starts with the type and closely related species, which is even more reason to study and redefine the type species of *Mollisia*, *M. cinerea*. Phylogenies of related *Ascomycetes* often use DNA sequences of *M. cinerea*, but the identity of these sequences is not clear or coherent. Original or type material is not available for morphological or phylogenetic study since the herbarium of Batsch at Jena (Germany) was destroyed during WWII (Stafleu & Cowan 1976). Furthermore, no material of Karsten is available (S. Huhtinen, pers. comm.). Therefore, designating an epitype is necessary, comprising a voucher specimen with apothecia and an accompanying culture.

The name *Mollisia* was first mentioned by Fries as a subdivision of *Peziza* (Fries 1823: 116) and comprised several species among which *P. cinerea* (Fries 1823: 142). In the description of *P. cinerea* a reference is made to *P. cinerea* Batsch who published the first description and a colour illustration (Batsch 1787: p. 196, f. 137). Karsten erected the genus *Mollisia* (Karsten 1871: 187) with 24 species among which *M. cinerea*. *Mollisia cinerea* was selected as type species of the genus by Nannfeldt (1932: 122).

Since molecular data alone did not clearly demarcate *Mollisia cinerea* and the genus *Mollisia* we have chosen to define *Mollisia cinerea* based on a sound consensus of this species concerning its morphology. We studied the morphology of the sexual and asexual morphs, following closely the morphological descriptions and consensus interpretations of *M. cinerea* (Batsch) P. Karst. and the elaborate descriptions of Nannfeldt (1932: 124–126) and LeGal & Mangenot (1958: 31–46). The figures 13a and 13b (Nannfeldt loc. cit.), combined with the description are characteristic according to Nannfeldt. Neither Batsch (1787) nor Karsten (1871) published an unambiguous description and although the image of Batsch (plate 137a and b) is clear, it can refer to several species of *Mollisia*. Nevertheless, since this is the only plate which refers to original material, the plate and description of Batsch is herewith designated as lectotype.

Apothecia from *Mollisia* species and morphologically related genera growing on wood and herbs were collected in The Netherlands and abroad, and used for morphological studies. Many of these were grown in pure culture by the first author mainly in the period of 1985–2000 during the project “Studies on Mollisioid fungi”.

For this phylogenetic study 300 cultures of Mollisioid fungi were available which were isolated by the first author, supplemented with relevant molecular data available on GenBank (www.ncbi.nlm.nih.gov/genbank/; Benson *et al*. 2013). Voucher specimens are deposited at the National Fungarium Leiden, the corresponding cultures at CBS Culture Collection at the Westerdijk Fungal Biodiversity Institute (WI) in Utrecht. Data were supplemented with relevant strains from

Articles are licensed under Creative Commons Attribution 4.0 International (CC BY 4.0).

the CBS Culture Collection (CBS). Furthermore, additional sequences were used which were deposited later in the DNA databank of the Westerdijk Fungal Biodiversity Institute (DNA Barcoding project).

To get a more accurate understanding of *Mollisia cinerea* and its relationship within the genus a single gene phylogeny of three loci was compiled in addition to a morphological study. Besides the ribosomal RNA ITS and LSU genes, the single copy protein coding gene *RPB2* was used. Protein coding markers are employed in phylogenetic analyses because of their greater levels of phylogenetic informativeness compared to ribosomal RNA genes and have proven a useful region for ascomycete phylogenetics at inter-generic level (Hansen *et al*. 2005, Schoch *et al.* 2009, Větrovský *et al*. 2016). Also sequences of the *RPB1-TOP1* region from GenBank and Tanney & Seifert (2020) were used to carry out a concatenated multiple gene phylogeny.

In recent phylogenetic studies strains or specimens with the name *M. cinerea* were often used, sometimes from CBS, and also from GenBank. In the CBS there are several strains which are named *Mollisia cinerea* but appear not to be conspecific (Table 1). The first author added several additional cultures of *M. cinerea* to the CBS, based on morphological characters of the sexual morph.

In recent years studies have been carried out on endophytes in the genus *Phialocephala*, which have proven to be related or sometimes identical to *Mollisia* species (Tanney 2017, Tanney & Seifert 2020). As far as these strains concern the group related to *M. cinerea* they have been taken into consideration. Furthermore, the work of Itagaki & Hosoya (2023) who deposited many sequences on GenBank of Mollisioid fungi has been important for this study (http://www.ncbi.nlm.nih.gov/).

MATERIALS AND METHODS

Isolates

Pure cultures from freshly collected sexual material (apothecia) were routinely made on 2 % malt extract agar (MEA), judged on their reliability by microscopic investigation and transferred to other MEA Petri dishes or tubes. These cultures were added to CBS. Although some of the strains produced conidia or even apothecia, they lost this ability with subculturing. Also, several of the strains remained sterile, and could at first not be assigned to Mollisioid fungi with certainty based on morphology. Molecular data confirmed them to belong to this group.

Collected sexual morphs were studied in fresh condition and described, using characters of subiculum, presence of crystals, inner and outer excipulum, medullary excipulum, paraphyses, spores and asci. Macroscopical and microscopical photographs were made using a Nikon DS-Ri2 camera attached to a Nikon eclipse 80i microscope. Transverse sections of apothecia were made with a freezing microtome, the apothecia were embedded either in a watery Arabic gum solution or in Tissue-Tek O.C.T. Compound.

DNA extraction, PCR amplification

Strains stored in liquid nitrogen at the CBS were transferred to MEA directly from the liquid nitrogen -185 °C storage. DNA was isolated from Petri dishes with cultures grown on MEA after 7–14 d, using a Mobio kit according to the manufacturer's instructions. Occasionally sexual morphs were used for DNA extraction using a Forensic DNA kit from voucher specimens and compared with the results obtained from cultures.

The nuclear rDNA region from the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene (LSU) were amplified with primers V9g (De Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990).

The primers ITS1 and ITS4 (White *et al.* 1990) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. If the sequences were not of good quality additional primers were used as LR3 (Vilgalys & Hester 1990), and LR0R (Rehner & Samuels 1994).

In addition to the ITS and LSU genes, the protein coding gene *RPB2*, encoding for the second largest part of RNA polymerase II was sequenced. As primers RPB2-5F2 and RPB2-7CR were used (Liu *et al.* 1999).

Table 1. Isolates identified as *Mollisia cinerea* deposited in the CBS Culture Collection.

CBS number	Collector	Isolated	Locality	Substrate	Identity
CBS 216.38	J.G. ten Houten	J.G. ten Houten	Netherlands (Locality unknown)	Gymnosperm wood	Unknown, no molecular data
CBS 288.59	F. Mangenot	F. Mangenot	France (Locality unknown)	Unknown	*M. cinerea* complex
CBS 412.81	Unknown	O. Petrini, No. Jn co7	Switzerland Ticino, Cademario	*Juniperus communis*	*Mollisiaceae*,no *Mollisia, melaleuca lineage*
CBS 413.81	Unknown	O. Petrini, No. Ju co4	Switzerland Ticino, Cademario	*Juniperus communis*	*Mollisiaceae*, no *Mollisia*
CBS 434.81	Unknown	O. Petrini, No. Ju co11	Switzerland Graubünden, Grüsch	*Juniperus communis*	*Mollisiaceae*, no *Mollisia*, *melaleuca lineage*
CBS 121284	Unknown	A.M. Stchigel	Spain Tarragona, Vimbodi, La Vall de Poblet	Dead tree's branch of *Quercus ilex*	*Mollisiaceae*, no *Mollisia lividofusca lineage*
CBS 122029 = AFTOL ID 76	Unknown	C. Schoch	USA Oregon, Alsea Falls	Fallen log	*Mollisiaceae*, no *Mollisia monilioides lineage*
CBS 128349	D.M. Walker	D.M. Walker	France Foret Hermitain, Melle	Dead canes of *Rubus* sp.	*Mollisiaceae*, no *Mollisia melaleuca lineage*

Phylogeny

Three datasets were used in this study. The first dataset consisted mainly of novel nucleotide sequences of the RNA gene *RPB2* generated in this study, supplemented with related sequences obtained through blast searches against the NCBI GenBank nucleotide database (Zhang *et al*. 2000). (http://www.ncbi.nlm.nih.gov/). Secondly, the multilocus dataset of Tanney *et al.* (2020) of ITS, LSU, *RPB1* and *TOP1* of *Phialocephala* and related species was used to slot in the new sequences of the rDNA genes 28S and ITS obtained in the present study. The third dataset consisted of ITS sequences that were initially selected from GenBank based on organism name and/or sequence similarity to the novel ITS sequences, together with the new ITS sequences generated in this study.

The sequences of the ITS and *RPB2* datasets were aligned separately using the online version of MAFFT v. 7 (http://mafft.cbrc.jp/alignment /server/index.html; Katoh *et al*. 2019) with default settings and, if necessary, manually improved using MEGA v. 11: Molecular Evolutionary Genetics Analysis v. 11 (Tamura *et al.* 2021). Leading and trailing gaps were removed as far as possible without removing too much potential phylogenetic signal.

Initial guide trees of the alignments were constructed with IQ-TREE v. 2.4.0 (Minh *et al.* 2020b), after which the sequences in the alignment were sorted according to the tree topology using Mesquite v. 3.70 (Maddison & Maddison 2023), and the local alignment of adjacent sequences improved by eye where necessary using Geneious Prime v. 2025.2.2 (https://www.geneious.com). After the ITS and *RPB2* datasets were trimmed by removing incorrect or poor-quality sequences, the alignments were subjected to final phylogenetic analyses as described below. The Tanney *et al.* (2020) alignment was not trimmed but subject to final analyses after the novel sequences were added.

Maximum-likelihood (ML) phylogenetic trees were constructed using IQ-TREE v. 2.4.0 (Minh *et al.* 2020b) and branch support values were calculated with 1000 non-parametric bootstrap replicates, concordance factors [Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT)], Minh *et al*. 2020a) and optimal modelfinding per locus using the TESTNEW option of ModelFinder (Kalyaanamoorthy *et al.* 2017) as implemented in IQ-TREE. The final Bayesian posterior probability analysis was performed using MrBayes v. 3.2.7a (Ronquist *et al.* 2012), using the parameter settings of two parallel runs of four chains each, run for 100 M generations but with the stop value set at 0.01, the temperature set at 0.20 and the sample frequency every 100th generation. The models identified in Tanney et al. (2020) as the best models for the different partitions were also used in this study. The 50 % majority rule consensus tree was created after the first 25 % of saved trees were discarded as burn-in. Characteristics of alignments and models used for phylogenetic analyses are summarized in Table S1 (Suppl. Table S1).

All resulting trees were printed with Geneious Prime v. 2025.2.2 and layout of the tree was done Microsoft PowerPoint. Novel sequences derived in this study are deposited in GenBank (Suppl. Table S2), the alignment and phylogenetic trees in figshare.com (doi: 10.6084/m9.figshare.31353622) and the taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al*. 2004). The phylogenetic trees shown were inferred from ML in IQ-TREE, since the Bayesian trees did not conflict with these. All strains included the phylogenetic analyses with their origin, GenBank accession numbers and other details are given in Table S3 (Suppl. Table S3).

RESULTS AND DISCUSSION

Mollisia "cinerea"

To delimit and redefine *M. cinerea* several related isolates were taken into consideration, also including many isolates called *M.* (cf.) *cinerea*. In the CBS collection several isolates are available which are named *M. cinerea*, but which are not all conspecific. An overview is given in Table 1.

Apart from the above-mentioned cultures at CBS (Table 1), our own cultures identified as *M. cinerea* were used and other species which were relevant to place *M. cinerea* in the right perspective. These are all at the CBS and have where available voucher specimens deposited at National Fungarium of The Netherlands, Leiden, the Netherlands (NHN). Furthermore, sequences were also taken into consideration which are labeled *M. cinerea* in GenBank (Clark *et al.* 2016) and other sequences which were likely to be related after using BLAST (Camacho *et al*. 2009). Suppl. Table S3 gives an overview of all strains and sequences used. Relevant sequences of taxa belonging to *Mollisiaceae* of the sequence data set of Tanney & Seifert (2020) and Itagaki & Hosoya (2023) were also included in this study.

RPB2 phylogeny

Phylogenetic analysis based on the *RPB2* gene of 90 strains comprises 712 nucleotides. As outgroup *Dermea acerina* (*Dermateaceae, Helotiales*) was chosen (Fig. 1).

The phylogram incorporates species belonging to the *Chlorociboriaceae* (*Helotiales, Leotiomycetes*) to place *Mollisiaceae* in *Leotiomycetes*. Two clades can be distinguished within *Chlorociboriaceae*, viz. *Chlorociboria* and *Brahmaculus*. As a group this is not well-supported, and this should be further investigated. Furthermore, Johnston *et al*. (2021) already stated that the group of species formerly known as *Chlorociboria* is probably paraphyletic, which was the reason for them to establish the new genus *Brahmaculus* based on *B. moonlighticus*. *Brahmaculus* forms a sister group to *Chlorociboria* and is well-supported.

Mollisiaceae is strongly supported here. Within the *Mollisiaceae* several clades can be recognized which are all strongly supported but of which the mutual relationships are not always clear. *Mollisia cinerea* AFTOL-ID 76 (https://lutzonilab.org/wp-content/uploads/aftol-voucher.xlsx) is often used in publications, but is not *M. cinerea*, and probably not even *Mollisia*. Unfortunately the identity of this strain could not be established.

Clade A2 comprises the type species of *Phaeomollisia*, *P. piceae* (Grünig *et al*. 2009), with two other *Mollisia* strains. Placing this species in *Mollisia* competes with the older homonym *Mollisia piceae* (1884) which is not a *Mollisia*. This was the reason for Gminder *et al.* (2022) to rename it as *Mollisia albogrisea*. It appears likely from

our phylogeny that this is not a *Mollisia*, in which case the combination *Phaeomollisia piceae* T.N. Sieber & Grünig is more appropriate.

Clade B can be recognized as *Mollisia* in a strict sense and is strongly supported. It has as sister groups *Phialocephala dimorphospora*, the type of *Phialocephala* (Kendrick 1961), clade B1 *Mollisia cinerea,* and clade B2 *Phialocephala mallochii.* Tanney already noticed that *P. dimorphospora* is congeneric with *Mollisia* (Tanney *et al*. 2020). The genus name *Mollisia* (Fr.) P. Karst. (1871) is much older than *Phialocephala* W.B. Kendrick (1961). Clade B1 contains the species which are morphologically identified as *Mollisia cinerea* s.s. (type species of *Mollisia*) and which morphologically corresponds to the concept of Nannfeld (1932) and LeGal & Mangenot (1958). Clade B1 also includes isolates identified as *Diplococcium spicatum* Grove (type species of *Diplococcium* Grove, 1885), suggesting a synonymy with *Mollisia.* However, the type specimen of the genus is not available nor sequenced. Therefore, we presently refrain from synonymizing this genus with *Mollisia*.

Within *Mollisiaceae* another well-supported Clade C can be recognized, representing an unnamed genus. It comprises several well-supported groups at species level of which the name is not yet clear since no type material is available. Clade C comprises taxa which are morphologically identified as "*Mollisia" ligni* and "*M." discolor.* Furthermore, the relatively new genera are found here as *Neobelonopsis* (Itagaki & Hosoya 2023), *Neomollisia* and *Pulvinata* (Ekanayaka *et al*. 2019) of which the types are sequenced. Also, the type of *Loramyces macrosporus* is found here, which is not the type of the genus. We strongly recommend further taxonomic investigation before naming this clade or proposing any name changes.

The availability of sequences of the *RPB2* gene is limited, and therefore the conclusions are limited. The single gene *RPB2* does not give enough unambiguous results to propose name changes, thus necessitating additions of other genes and a multigene approach. For the purpose of this article, redefining *Mollisia* and redescribing *M. cinerea*, the *RPB2* gene has been very helpful.

Multigene phylogeny

Following the example of Tanney & Seifert (2020), concatenated sequences of the ITS-LSU-*RPB1*-*TOP1* genes were used to carry out phylogenetic analyses to compare to the single gene phylogenies. Missing data were represented by N. Sequences of the nuclear rDNA gene of the LSU region alone are less valuable at species level than *RPB2*, and usually give information at a higher taxonomic level (Lumbsch *et al.* 2005).

The phylogenetic tree comprises 116 members of the *Mollisiaceae* with as outgroup "*Mollisia" dextrinospora*, which is well-known as not a *Mollisia* (Fig. 2). From this tree it is hard to delimit *Mollisiaceae*, especially as to whether *Vibrissea* Fr. (1822) belongs to this family or should be placed in a family of its own (*Vibrisseaceae* Korf 1990). According to Johnston *et al.* (2019), *Vibrissea* does not belong to *Mollisiaceae*, which was confirmed by Crous *et al*. (2021). Although two strains are sequenced with the name *V. truncorum,* which is the type species of the genus, the type specimen has not been sequenced.

Within the group three more or less sister groups can be distinguished apart from *Vibrissea*, which are all monophyletic and well-supported.

Clade A comprises several species and types of species of *Phialocephala.* Several well-supported clades can be recognized, of which the names remain unresolved. Further molecular and morphological studies are required to investigate which genus name should be used for this clade. Clade A1 includes the type strain of the type species of *Acephala*, *A. applanata*. Although this clade is well-supported the relationships and taxonomical rank of the groups within this clade are not clear. Clade A2 comprises *Phialocephala helenae* and the well-supported group of *Phaeomollisia piceae.* The placement of this group in a clade which is sister to *Mollisia* emphasizes the conclusion from the *RPB2* sequences, i.e. the conclusion that *Phaeomollisia piceae* is not a real *Mollisia*.

Clade B is monophyletic and recognized as *Mollisia* in a strict sense with several types belonging to species of *Phialocephala.* Clade B1 is identified as *Mollisia cinerea* with strong support, comprising the epitype of *Mollisia cinerea*. Also strains identified as *Phialocephala oblonga* belong here. Several strains identified as *Phialocephala* or *Mollisia* form well-supported groups, but their relationships are not clear.

Clade C is another monophyletic and well-supported sister group to *Mollisia.* Although with many well-supported subclades, it is premature to name these subclades. Since the purpose of this article is merely to establish the outlines of *Mollisia* and starts with the type of *Mollisia*, we will not propose name changes at this stage. The risk of renaming species without a thorough investigation is that the combinations will prove to be superfluous and unnecessary at a later stage.

ITS phylogeny

In addition to *RPB2*, the ITS region was used to construct a phylogram, as it was available for a larger sampling of taxa. The ITS dataset of species of *Mollisiaceae* and its allies (Tanney *et al*. 2016) was downloaded from GenBank with additional sequences of our own material and mBlast results from GenBank. Only unambiguous results which are (partly) supported by other genes were used to clarify the taxonomy and introduce name changes. Since the purpose of this article is a redefinition of *Mollisia cinerea*, not all GenBank sequences of *Mollisiaceae* were used. A future article will focus on these aspects in more detail.

In total 539 strains were used with 830 nucleotides. As outgroup *Chloridium humidicola* was chosen. The phylogram is attached as an appendix (Suppl. Fig. S1).

However, ITS phylograms are not really suitable to delimit families. We have therefore focused on clade B (as in the other two phylograms), which is identified as *Mollisia* and is well-supported. *Mollisia cinerea* AFTOL-ID 76 belongs to the "*Mollisia"monilioides* lineage, which is not a *Mollisia*.

Within clade B several clades can be recognized, based on sequences from type material. *Mollisia cinerea* forms a well-supported group, and forms a well-supported clade with sequences of *Mollisia oblonga*, which may well be a synonym of *M. cinerea*, but no type sequence is available for *M. oblonga*. The synnematous asexual morph (Wang &

Sutton 1984) is aberrant from the asexual morph of the type of *M. cinerea*, and the spores are also slightly larger (Tanney *et al*. 2016).

Phialocephala catenospora, *P. lignicola* and *Mollisia nodosa* form a clade which is not well-supported but includes the types of these species and is part of the *Mollisia* clade.

Phialocephala mallochii includes the type and forms a well-supported clade within *Mollisia*.

Phialocephala lagerbergii and the type of *P. botulispora* form a well-supported clade. *Phialocephala botulispora* is here considered a synonym of *P. lagerbergii* which belongs to the *Mollisia* s.s. clade.

Another well-supported clade is formed by several *Phialocephala* types and strains named as such. The mutual relationships are not clear, but they all belong to *Mollisia* s.s. These are (the types of) *P. meliteae*, *P. aylmerensis*, *P. cladophialophoroides*, *P. biguttulata*, *P. dimorphospora*, *P. collarifera* and *P. repens*. These all belong to *Mollisia* s.s.

Phialocephala glacialis and *P. vermiculata* form a well-supported clade including the types of the two species, but it is not clear whether this clade also belongs to *Mollisia*.

Taxonomy

Mollisia cinerea (Batsch) P. Karst., *Bidr. Känn. Finl. Nat. Folk* **19**: 189. 1871. Fig. 3.

Basionym: *Peziza cinerea* Batsch, *Elench. fung., cont. prim*. (Halle): 197. 1786.

Apothecia gregarious, later scattered to confluent, at first urn-shaped to cup-shaped, later spreading to convex or even concave, and flattened and attached to the surface of the host, 1–1.5(–2) mm wide, 100–250 µm thick, towards margin thinner to ca 50 µm thick; later shortly stipitate; at first regular, later with rather irregular outline. *Margin* clearly visible, especially when older, paler than rest of hymenium, fimbriate. *Receptaculum* dark brown, smooth to granulate. *Subiculum* present around stipe, limited. *Hymenium* more or less translucent when moist, pale grey to bluish grey, drying yellowish, 60–70 µm thick, composed of 8-spored asci and numerous filiform paraphyses, 1–2 µm wide with rounded top, exceeding asci with ca 5–8 µm, crystals absent. *Paraphyses* 50–70 × 1–2 µm, sometimes septate, hyaline, slightly enlarged at top up to 3 µm wide, rounded to truncate, with oil-filled elongated vacuolar body when fresh. *Asci* J+ in Lugol and Melzers reagens, with truncate top, cylindrical to conical, with clamps at base (croziers), 54–65 × 5–6 µm. *Ascospores* biseriate, cylindrical to fusiform, slightly curved, in the end occasionally septate, (6–)7–8(–10) × 2 µm. *Subhymenial layer* ca 10 µm thick, composed of entangled erect elongated hyphae with colourless elements of 3 × 1–1.5 µm. *Inner excipulum* (15–)20(–30) µm thick, composed regular horizontal hyphae with rectangular slightly brown-walled elements of 15 × 2–3 µm, forming a textura prismatica. *Outer excipulum* 20–40 µm thick at base, towards margin slightly thicker, composed of hyphae curved outwards composed of rectangular relatively thick- and brown-walled elements of 10–15 × 6(–8) µm. Hyphae ending at margin with elongated pyriform paler walled elements of up to 10 × 4 µm, forming textura angularis to prismatica or textura globulosa. *Outer excipulum* towards base of stipe more compressed and composed of small dark brown- and thick-walled elements of 3–4 × 2 µm, forming a textura angularis. *Subicular hyphae* moderately present, especially around stipe, thick and brown-walled, with encrusting pigment, 3–4 µm wide. (Fig. 3).

Cultures (Fig. 4): Colonies on MEA with abundant pale grey to grey aerial mycelium, lanose. Reverse blackish with paler centre. Margin entire, blackish. Marginal growth zone narrow, 1–2 mm, wax-like, hyaline. Growth 15–16 mm diam. / 7 d. Reverse blackish, at centre paler yellowish/black with 3 mm wide yellowish waxy zone. Young colony convex, covered with pale grey aerial mycelium, lanose.

Microscopy: Aerial mycelium on MEA abundant, composed of loosely interwoven rather thick-, and brown-walled, hyphae of 2.0–2.5 µm wide, with encrustations on the walls. Paler hyphae present, 1.5–2 µm wide. Substrate hyphae darker, more condensed. Nodulations abundant, mostly formed in aerial mycelium, (18–)20–25 µm wide. *Asexual morph* not observed.

Typus: **Lectotype** designated here, Batsch, A.J.G.C. (1787). Elenchus fungorum. Continuatio prima: 197-199. Plate 137 a and b (MBT 10030705). **Netherlands**, Heemstede, Woestduin, on rotten tree trunk of *Quercus*, 10 Oct. 1987, *M. Nauta* 3584 (**epitype** designated here MBT 10030704), ex-epitype culture CBS 110.88 (preserved in a metabolically inactive state).

Additional materials examined: **Netherlands**, Heemstede, Leyduin, on tree trunk of *Fagus sylvatica*, 10 Oct. 1987, *M. Nauta* 3585 (CBS 111.88); Baarn, on wood of *Betula pubescens*, 14 May 1986, *M. Nauta* (CBS 646.88).; De Bult near Steenwijk, on trunk of *Fagus sylvatica*, 4 Jul. 1987, *M. Nauta* 3392 (CBS 765.87). **Sweden**, Småland, Åminne, on *Fagus sylvatica* trunk, 13 Jul. 1987, *W. Gams* (*M. Nauta* 3509; CBS 888.87).

Notes: The diagnostic characters are, apart from the thin, flat apothecia with wavy margin, the short stipe which is mostly sunken in the substrate and lined with dark brown, 3–5 um wide, densely septate, tortuous hyphae which are also to a limited extent present on the wood. Towards the margin 1–2 layers of paler "hairs" are present, macroscopically making the margin fimbriate (Nannfeldt 1932). Le Gal & Mangenot mentioned in their description of *M. cinerea* two types of conidia, viz. dark brown thalloconidia and small, hyaline microconidia, 0.5–1 × 1–2 µm in groups on clear brown phialidic conidiogenous cells (Le Gal & Mangenot 1956). The thalloconidia very much look like the nodulations mentioned above. In the CBS collection one strain exists that was deposited by Mangenot as *Mollisia cinerea* (CBS 288.59). Unfortunately, none of the *M. cinerea* cultures sporulate anymore.

Substrates: In their description of the apothecia of *M. cinerea* Le Gal & Mangenot mentioned the occurrence on decorticated, rotten wood of several deciduous species: *Betula*, *Alnus*, *Salix*, *Quercus* (Le Gal & Mangenot 1958).

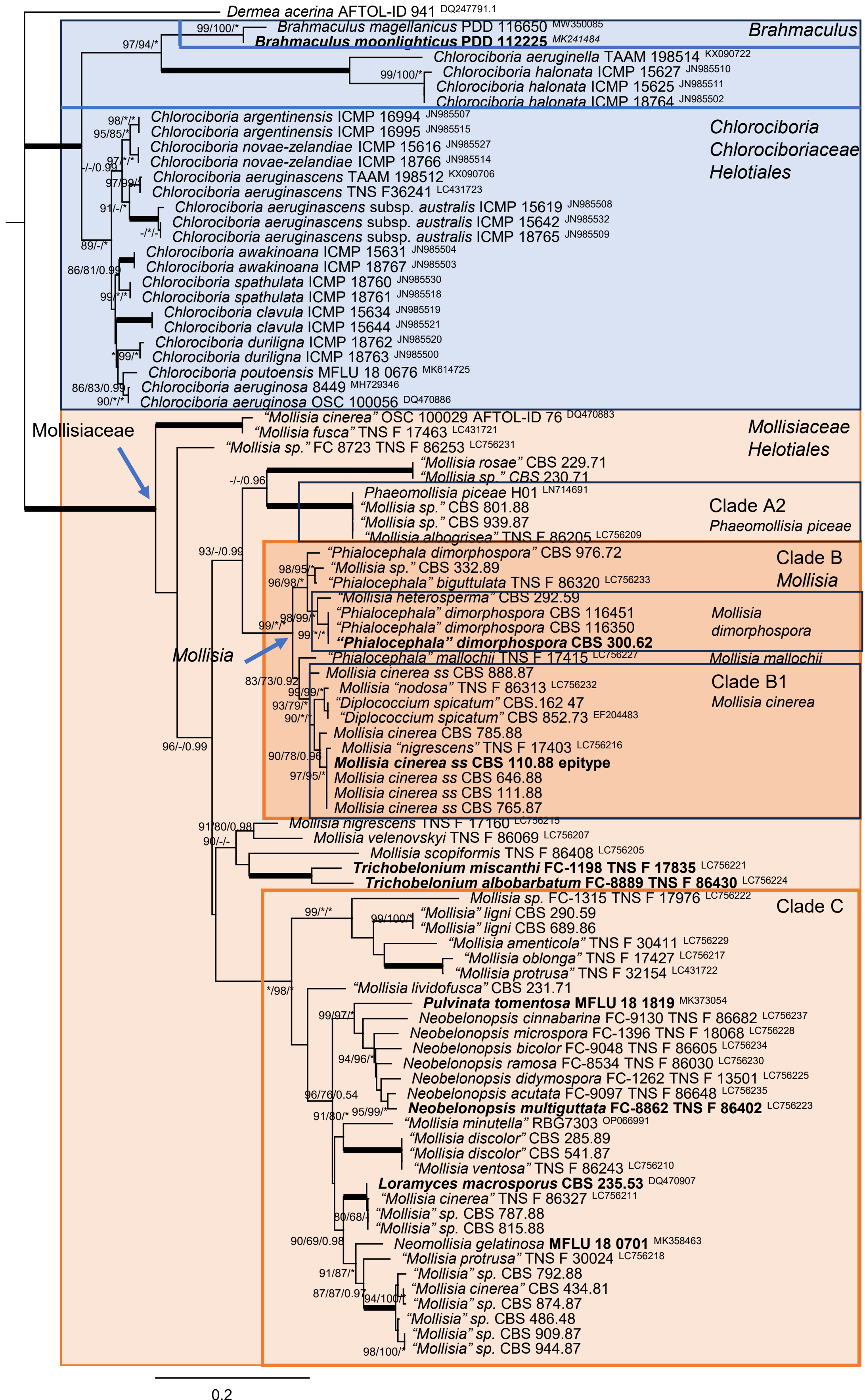

Fig. 1. Maximum likelihood tree inferred from *RPB2* sequences. Significant branch support by SH-aLRT (> 80 %)/non parametric Bootstrap replications (> 50 %)/Posterior Probability (> 0.95) are indicated, with lower supports indicated by an en dash (–) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Thick lines are 100/100/1.0. Types of species and genera are indicated in bold. The tree is rooted with *Dermea acerina*.

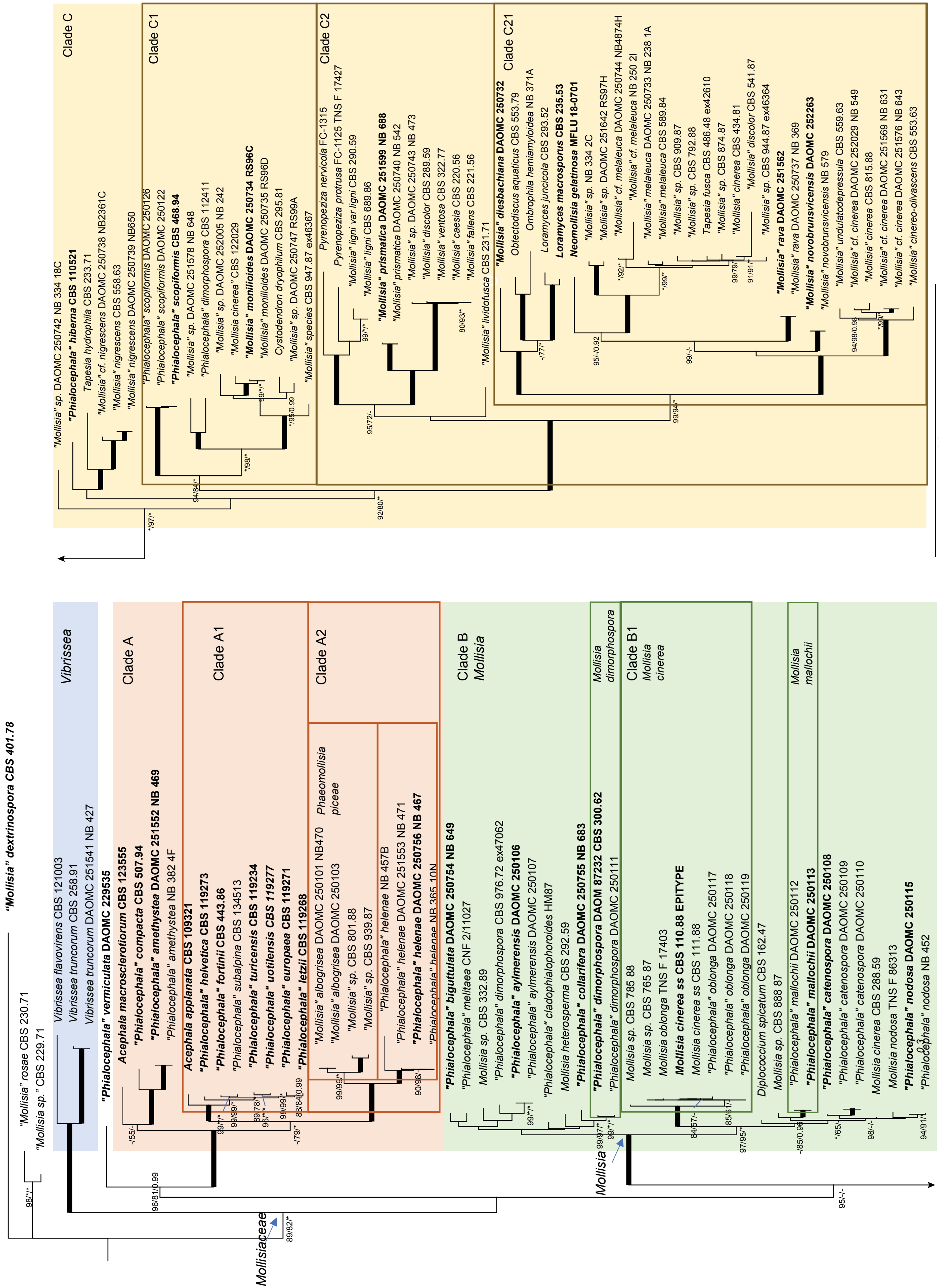

Fig. 2. Maximum likelihood tree inferred from concatenated sequences of ITS, LSU, *RPB1, TOP1*. Significant branch support by SH-aLRT (> 80 %)/ non parametric Bootstrap replications (> 50 %)/Posterior Probability (> 0.95) are indicated, with lower supports indicated by an en dash (–) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Thick lines are 100/100/1.0. Types of species and genera are indicated in bold. The tree is rooted with *"Mollisia" dextrinospora*.

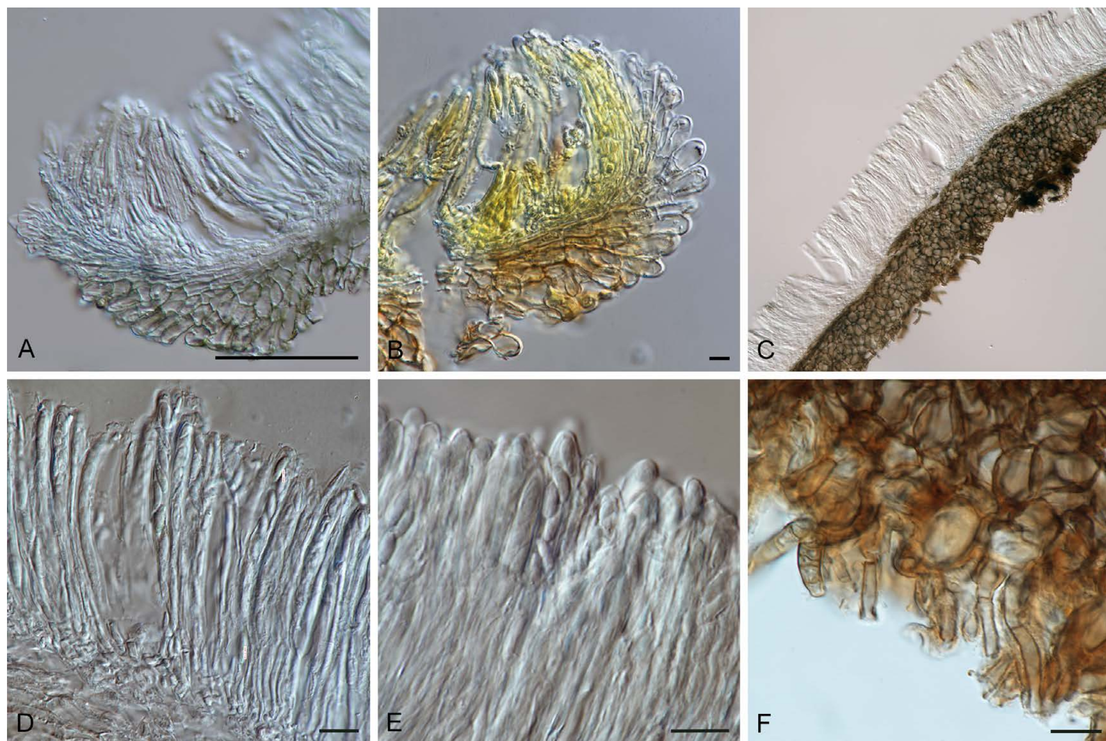

Fig. 3. *Mollisia cinerea* (MN 3584). **A.** Vertical section of margin of apothecium. **B.** Idem with asci and paraphyses. **C.** MN 3585. Vertical section of apothecium. **D.** MN 3584. Asci and paraphyses. **E.** MN 3585. Asci and paraphyses. **F.** Detail of anchoring hyphae. Scale bars: A = 50 µm, B = 5 µm, D–F = 10 µm.

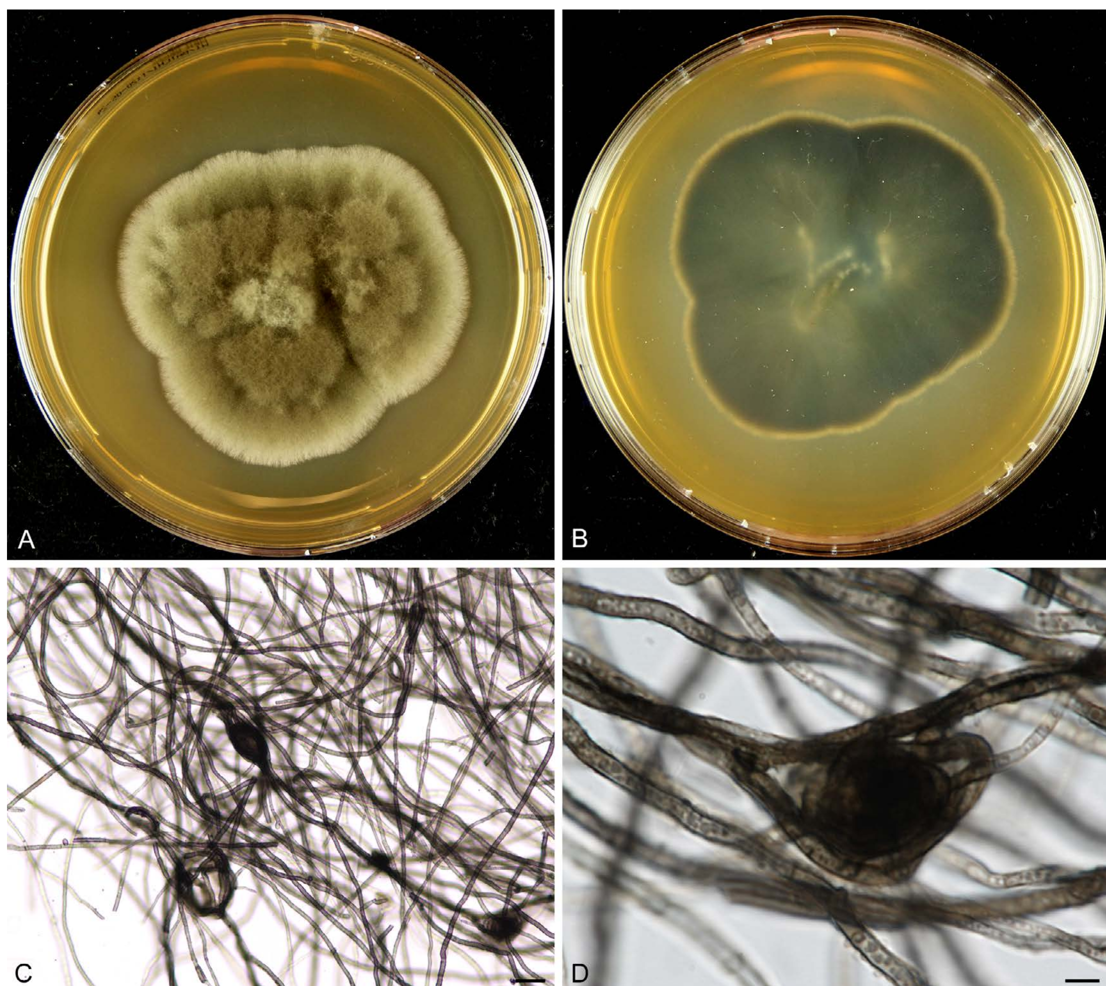

Fig. 4. *Mollisia cinerea* CBS 110.88 Culture. **A.** Colony of 3 weeks old. **B.** Reverse. **C.** Hyphae of aerial mycelium with nodulations. **D.** Idem. Scale bars: C = 10 µm, D = 20 µm.

CONCLUSIONS

As inferred from the various phylograms based on *RPB2*, LSU, and ITS clade B1 contains all strains which are morphologically identified as *Mollisia cinerea* in the sense of Nannfeldt (1932). It seems plausible to choose an epitype from that group. Furthermore, the morphology of the apothecia, and the cultural characters should be considered. Therefore, the plate and description of Batsch is hereby selected as lectotype, and strain CBS 110.88 is hereby described as epitype with the corresponding voucher specimen.

Since *Mollisia cinerea* has been redefined molecularly and morphologically and therefore also *Mollisia* s.s. as a monophyletic clade, some new combinations have become necessary. The type of *Phialocephala*, *P. dimorphospora* W.B. Kendr. (1961) belongs to *Mollisia* and may even be a synonym of *M. cinerea.* However, not all *Phialocephala* species belong to *Mollisia*.

Phialocephala lignicola is a *Mollisia*, and since the basionym *Fuscosclera lignicola* Hernández-Restrepo *et al.* (2017) is the type of the genus *Fuscosclera* the latter is considered synonymous to *Mollisia*.

New combinations and synonymies

Mollisia (Fr.) P. Karst., *Bidr. Känn. Finl. Nat. Folk* **19**: 189. 1871.
Synonym: *Phialocephala* W.B. Kendr., *Canad. J. Bot.* **39**(5): 1079. 1961.

Mollisia aylmerensis (Tanney & B. Douglas) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861684.
Basionym: *Phialocephala aylmerensis* Tanney & B. Douglas, *Mycologia* **108**(2): 271. 2016. MB 811721.

Mollisia biguttulata (Tanney & Seifert) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861683.
Basionym: *Phialocephala biguttulata* Tanney & Seifert, *Stud. Mycol*. **95**: 345. 2020. MB 833625.

Mollisia catenospora (Tanney & B. Douglas) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861685.
Basionym: *Phialocephala catenospora* Tanney & B. Douglas, *Mycologia* **108**(2): 264. 2016. MB 811719.

Mollisia cladophialophoroides **(**Madrid *et al.*) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861686.
Basionym: *Phialocephala cladophialophoroides* Madrid *et al.*, *Persoonia* **38**: 359. 2017. MB 820647.

Mollisia collarifera (Tanney & Seifert) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861687.
Basionym: *Phialocephala collarifera* Tanney & Seifert, *Stud. Mycol*. **95**: 345. 2020. MB 833626.

Mollisia dimorphospora **(**W.B. Kendr.) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861688.
Basionym: *Phialocephala dimorphospora* W.B. Kendr., *Canad. J. Bot*. **39(5)**: 1080. 1961. MB 336287.

Mollisia lagerbergii **(**Melin & Nannf.) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861689.
Basionym: Cadophora lagerbergii Melin & Nannf., *Svenska Skogsvårdsför. Tidskrift* **32**: 415. 1934. MB 268867.
Synonyms: *Phialocephala lagerbergii* (Melin & Nannf.) Grünig & T.N. Sieber, *Mycol. Res.* **113**: 217. 2009. MB 512378.
Phialocephala botulispora (Cole & W.B. Kendr.) Grünig & T.N. Sieber, *Mycol. Res.* **113**: 217. 2009. MB 512379.
Phialophora botulispora Cole & W.B. Kendr., *Mycologia* **65**: 678. 1973. MB 319834.

Mollisia mallochii (Tanney & B. Douglas) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861690.
Basionym: *Phialocephala mallochii* Tanney & B. Douglas, *Mycologia* **108**(2): 269. 2016. MB 811720.

Mollisia melitaea **(**Matočec *et al.*) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861692.
Basionym: *Phialocephala melitaea* Matočec *et al.*, *Persoonia* **45**: 375. 2020. MB 836891.

Mollisia neolignicola Nauta, J.Z. Groenew. & Crous, ***nom. nov.*** MB 861693. non *Mollisia lignicola* W. Phillips, *Grevillea* **5**(35): 113. 1877. MB 149099.
Basionym: Fuscosclera lignicola Hern.-Restr. *et al.*, *Stud. Mycol*. **86**: 82. 2017. MB 820287.
Synonym: *Phialocephala lignicola* (Hern.-Restr. *et al.*) Tanney & Seifert, *Stud. Mycol*. **95**: 351. 2020. MB 833759.

Mollisia repens (Cooke & Ellis) Nauta, J.Z. Groenew. & Crous, ***comb. nov.*** MB 861694.
Basionym: *Penicillium repens* Cooke & Ellis, *Grevillea* **7**(41): 6. 1878. MB 208583.
Synonym: *Phialocephala repens* (Cooke & Ellis) W.B. Kendr., *Canad. J. Bot*. **41**: 574. 1963. MB 336290.

ACKNOWLEDGEMENTS

We acknowledge the Dutch NWO Roadmap grant agreement No. 2020/ENW/00901156, project "Netherlands Infrastructure for Ecosystem and Biodiversity Analysis – Authoritative and Rapid Identification System for Essential biodiversity information" (acronym NIEBA-ARISE).

Declaration on conflict of interest The authors include members of the Editorial Board of Fungal Systematics and Evolution. They were not involved in the journal's review of, or decisions related to, this manuscript.

REFERENCES

Baschien C, Tsui CK, Gulis V, *et al*. (2013). The molecular phylogeny of aquatic hyphomycetes with affinity to the Leotiomycetes. *Fungal Biology* **117**: 660–672. https://doi.org/10.1016/j.funbio.2013.07.004

Batsch AJGC (1787). *Elenchus fungorum. Continuatio prima.*

Benson DA, Cavanaugh M, Clark K, *et al*. (2013). GenBank. *Nucleic Acids Research* **41**(D1): D36–D42. https://doi.org/10.1093/nar/gks1195

Camacho C, Coulouris G, Avagyan V, *et al*. (2009). BLAST+: architecture and applications. *BMC Bioinformatics* **10**: 421. https://doi.org/10.1186/1471-2105-10-421

Clark K, Karsch-Mizrachi I, Lipman DJ, *et al.* (2016). GenBank. *Nucleic Acids Research* **44**(D1): D67–72. https://doi.org/10.1093/nar/gkv1276

Crous PW, Gams W, Stalpers JA, *et al.* (2004). MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50***:* 19–22. [Google Scholar]

Crous PW, Cowan DA, Maggs-Kölling G, *et al*. (2021). Fungal Planet description sheets: 1182–1283. *Persoonia* **46**: 313–528. https://doi.org/10.3767/persoonia.2021.46.11

Day MJ, Hall JC, Currah RS (2012). Phialide arrangement and character evolution in the helotialean anamorph genera *Cadophora* and *Phialocephala*. *Mycologia* **104**: 371–381. https://doi.org/10.3852/11-059

De Hoog GS, Gerrits van den Ende AHG (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41**: 183–189. https://doi.org/10.1111/j.1439-0507.1998.tb00321.x

Felsenstein J (1985). Phylogenies and the comparative method. *The American Naturalist* **125**: 1–15. http://www.jstor.org/stable/2461605

Fries EM (1823). *Systema Mycologicum* **2**. Gryphiswaldiae : Sumtibus Ernesti Mauritii, 1822-1823

Gminder A, Wagner I, Prell F, *et al.* (2022). *Index Fungorum* **509**: 1

Grove WB (1885). New or noteworthy fungi:- Part II. *Journal of Botany, British and Foreign* **23**: 161–169.

Grünig CR, Queloz V, Duò A, *et al.* (2009). Phylogeny of *Phaeomollisia piceae gen. sp. nov*.: a dark, septate, conifer-needle endophyte and its relationships to *Phialocephala* and *Acephala*. *Mycological Research* **113**: 207–221. https://doi.org/10.1016/j.mycres.2008.10.005

Hansen K, LoBuglio KF, Pfister DH (2005). Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: *RPB2*, β-tubulin, and LSU rDNA. *Molecular Phylogenetics and Evolution* **36**: 1–23. https://doi.org/10.1016/j.ympev.2005.03.010.

Hernández-Restrepo M, Gené J, Castañeda-Ruiz RF, *et al*. (2017). Phylogeny of saprobic microfungi from Southern Europe. *Studies in Mycology* **86**: 53–97. https://doi.org/10.1016/j.simyco.2017.05.002

Itagaki H, Hosoya T (2023). A new genus *Neobelonopsis* and two new species of *Trichobelonium* (*Helotiales, Ascomycota*) discovered mainly from poaceous grasses native to Asia in Japan. *MycoKeys* **99**: 45–85. https://doi.org/10.3897/mycokeys.99.90117

Johnston PR, Quijada L, Smith CA, *et al*. (2019). A multigene phylogeny toward a new phylogenetic classification of *Leotiomycetes. IMA Fungus* **10**: 1. https://doi.org/10.1186/s43008-019-0002-x

Kalyaanamoorthy S, Minh BQ, Wong TKF, *et al*. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589. https://doi.org/10.1038/nmeth.4285

Karsten PA (1871). Mycologia fennica I. Discomycetes. *Bidrag till kännedom af Finlands natur och folk* **19**: 1–263.

Katoh K, Rozewicki J, Yamada KD (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kendrick WB (1961). The *Leptographium* complex. *Phialocephala gen. nov*. *Canadian Journal of Botany* **39**(5): 1079–1085.

Le Gal M, Mangenot F (1956). Contribution à étude des Mollisioïdées I. Note préliminaire: les formes conidiennes. *Revue de Mycologique* **21**: 3–13.

Le Gal M, Mangenot F (1958). Contribution à l'étude des Mollisioïdées. II (1re série). *Revue de Mycologie* **23**: 28–86.

Liu YJ, Whelen S, Hall BD (1999). Phylogenetic relationships among Ascomycetes: Evidence from an RNA Polymerse II Subunit *Molecular Biology and Evolution* **16**(12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Lumbsch HT, Schmitt I, Lindemuth R, *et al*. (2005). Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (*Leotiomyceta*). *Molecular Phylogenetics and Evolution* **34**: 512–524. https://doi.org/10.1016/j.ympev.2004.11.007

Maddison WP, Maddison DR. (2023). Mesquite: a modular system for evolutionary analysis. Version 3.70. *http://www.mesquiteproject.org.*

Minh BQ, Hahn MW, Lanfear R (2020a) New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution* **37**: 2727–2733. https://doi.org/10.1093/molbev/msaa106

Minh BQ, Schmidt HA, Chernomor O, et al. (2020b). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**: 1530–1534. https://doi.org/10.1101/849372

Nannfeldt JA (1932). Studien über die morphologie und systematik der nicht-lichenisierten Inoperculaten Discomyceten. *Nova Acta Regiae Societatis Scientiarum Upsaliensis* ser. IV **8**(2): 1–368.

Nguyen T-T, Schmidt HA, von Haeseler A, *et al*. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274. https://doi.org/10.1093/molbev/msu300

Quélet L (1884). *Fungi Tridentini*. 1.

Ronquist F, Teslenko M, van der Mark P, et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology 61: 539–542.* [DOI] [PMC free article] [PubMed] [Google Scholar]

Schoch CL, Sung G-H, López-Giráldez F, *et al.* (2009). The Ascomycota Tree of Life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits *Systematic Biology* **58**: 224–239. https://doi.org/10.1093/sysbio/syp020

Stafleu FA, Cowan RS (1976). *Taxonomic literature. A selective guide to botanical publications and collections with dates, commentaries and types*. Second edition, Volume I: A-G. Regnum vegetabile 94, Bohn, Scheltema & Holkema, Utrecht: [I]-XL, 1-1136.

Tamura K, Stecher G, Kumar S (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **38**: 3022–3027. https://doi.org/10.1093/molbev/msab120

Tanney JB, Douglas B, Seifert KA (2016). Sexual and asexual morphs of some endophytic *Phialocephala* species of *Picea*. *Mycologia* **108**: 255–280. https://doi.org/10.3852/15-136

Tanney JB, Seifert KA (2020). *Mollisiaceae*: An overlooked lineage of diverse endophytes. *Studies in Mycology* **95**: 293–380. https://doi.org/10.1016/j.simyco.2020.02.005

Větrovský T, Kolařík M, Žifčáková L, *et al*. (2016). The *rpb2* gene represents a viable alternative molecular marker for the analysis of environmental fungal communities. *Molecular Ecological Resources* **16**: 388–401. https://doi.org/10.1111/1755-0998.12456

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wang CJK, Sutton B (1984). Two new synnematous genera: *Chalarodendron* and *Paradidymobotryum*. *Mycologia* **76**: 569–572. https://doi.org/10.2307/3793345

White TJ, Bruns T, Lee SJWT, *et al*. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (Innis MA, Gelfand DH, Sninsky JJ, *et al.*, eds). Academic Press, USA: 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang Z, Schwartz S, Wagner L et al. (2000). A greedy algorithm for aligning DNA sequences. Journal of Computational Biology **7**: 203–214. https://doi.org/10.1089/10665270050081478

Supplementary Material: http://fuse-journal.org/

Fig. S1. Maximum likelihood tree inferred from sequences of ITS. Significant branch support by SH-aLRT (> 80 %)/ non parametric Bootstrap replications (> 50 %)/Posterior Probability (> 0.95) are indicated, with lower supports indicated by an en dash (–) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Thick lines are 100/100/1.0. Types of species and genera are indicated in bold. The tree is rooted with *Chloridium humicola*. Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. The clade of *Neobelonopsis* has a different layout for technical reasons.

Table S1. Summary of phylogenetic information for the different analyses in this study.

Table S2. Novel sequences included in the phylogenetic analyses with their origin, GenBank accession numbers and other details.

Table S3. Strains included the phylogenetic analyses with their GenBank accession numbers. Types are in bold.