The mycobiome of symptomatic wood of *Prunus* trees in Germany

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Summary

Diseases on woody plant hosts caused by fungal pathogens have far-reaching ecological and economic consequences. In spite of their importance, the knowledge about the fungal diversity associated with symptomatic wood, even in economically important crops like stone fruits (*Prunus* spp.) is meagre. Furthermore, the taxonomic basis for the species identification of many pathogenic fungal taxa is insufficient, which impedes quick and targeted control measures in cases of sudden disease outbreaks. In order to fill this knowledge gap, an extensive assessment was conducted in orchards of *Prunus avium*, *P. cerasus* and *P. domestica* in three important fruit production areas in Germany. The fungi were isolated from symptomatic woody tissue and examined by means of morphological and molecular data. Selected genera were analysed in more depth; some additional strains from other origins were included as well.

Fungi with reduced conidiogenous cells and a yeast-like phase were frequently isolated from all three *Prunus* species and in all sampling areas in Germany. Initial molecular identifications placed these fungi and ten similar strains from spore traps in a vineyard in Rhineland-Palatinate in the genus *Collophorina* (*Leotiomycetes*, *Tympanidaceae*). A family-wide phylogenetic analysis of the *Tympanidaceae* revealed the genus *Collophorina* to be polyphyletic. Consequently, *C. paarla* and *C. euphorbiae* were transferred to the newly described genera *Pallidophorina* and *Ramoconidiophora*, respectively. The fungi from wood of *Prunus* belonged to five species, namely *Pa. paarla*, *C. africana* and three new *Collophorina* species, described as *C. badensis*, *C. germanica* and *C. neorubra*. The strains from spore traps belonged to seven new species, including *C. badensis*, in five genera, of which three were newly described as well, namely *Capturomyces*, *Variabilispora* and *Vexillomyces*. The results of this study provide far-reaching insights into this group of understudied fungi that revealed an unknown diversity and high abundance in *Prunus* trees in Germany.

One strain isolated from *P. domestica* resembled *Collophorina* as well. However, a preliminary molecular analysis indicated a relationship to the *Hyaloscyphaceae* (*Leotiomycetes*). A family-wide molecular and morphological analysis did not reveal a close relationship or resemblance, respectively, to any known species or genus, and consequently the new genus *Arboricolonus* and the new species *A. simplex* were described. The morphology reminiscent of *Collophorina* was attributed to convergent evolution, since both genera occupy the same habitat.

Species of the genus *Cadophora* (*Leotiomycetes*) are known from different habitats like plants, fruits and deadwood, and were frequently isolated from *Prunus* wood in this study. An analysis of the genus comprised 29 strains from *Prunus* in Germany and three additional strains from *Prunus* in South Africa. In total, five species of *Cadophora* were differentiated, including two known species, *Ca. luteo-olivacea* and *Ca. novi-eboraci* from Germany and three new species, namely *Ca. prunicola* from

Germany and South Africa, *Ca. africana* from South Africa, and *Ca. ramosa* from Germany. Moreover, *Margarinomyces bubakii* was combined in *Cadophora*, while *Ca. obscura* was resurrected.
Based on DNA sequence data, eight strains from *Prunus* forming asexual morphs were assigned to the genus *Proliferodiscus* (*Leotiomycetes*). In the past, species of this genus were almost exclusively described based on the morphology of their sexual morphs, while few sequence data and data on asexual morphs were available. Our study resulted in the first comprehensive phylogenetic analysis of this genus. The new species *Pr. ingens* was described, and detailed descriptions of the asexual morphs of this species and a second species referred to as *Proliferodiscus* sp. were provided.
Two species of the genus *Minutiella* (*Eurotiomycetes*) were isolated, one of which was newly described as *M. pruni-avium*.
Within the general assessment of the fungal diversity, a total of 1,018 isolates from wood of the three *Prunus* species in Germany were obtained, morphologically observed, and their ITS and LSU sequences generated. A combination of blastn searches with strong focus on reliable reference sequences and thorough phylogenetic analyses revealed a diversity of 172 species of *Ascomycota*, *Basidiomycota* and *Mucoromycota* with the majority of strains belonging to the *Ascomycota* classes *Sordariomycetes*, *Leotiomcetes* and *Dothideomycetes*. Based on the number of nucleotide differences to reference sequences, species identifications were divided into categories. According to this scheme, 82 species were identified with high and 20 species with low certainty. By far the most dominant species detected were the coelomycetous species *Aposphaeria corallinolutea* (*Dothideomycetes*) and the collophorina-like species *Pallidophorina paarla*, both isolated from all three host species and in all sampling regions. The remaining 70 taxa could not be assigned to any species; most of them were therefore regarded as potentially new species. On this basis, *Prunus* wood, even in a well-explored region like Germany, was recognised as a habitat harbouring high numbers of potentially new species. Culturing and preservation of all strains isolated from *Prunus* wood in this study allow further follow-up studies of their taxonomy, physiology, ecology, pathogenicity and bio-chemistry.

Zusammenfassung

Durch pilzliche Erreger verursachte Krankheiten an Bäumen haben weitreichende ökologische und ökonomische Folgen. Trotz ihrer Bedeutung bestehen große Wissenslücken bezüglich der Pilzgemeinschaften in symptomatischem Holz. Dies betrifft auch ökonomisch bedeutende Pflanzengruppen wie Steinobstgewächse (*Prunus* spp.). Darüber hinaus ist die taxonomische Grundlage zur Artidentifikation vieler pathogener Pilze unklar, was zügige und gezielte Bekämpfungsmaßnahmen im Falle plötzlicher Krankheitsausbrüche erschwert. Um diese Wissenslücken zu schließen, wurde eine umfassende Untersuchung in Plantagen von *Prunus avium*, *P. cerasus* und *P. domestica* in drei wichtigen Obstanbaugebieten in Deutschland durchgeführt. Aus symptomatischem Holzgewebe isolierte und auf Nährböden kultivierte Pilze wurden anhand ihrer Morphologie und molekularen Daten untersucht. Ausgewählte Gattungen wurden intensiv untersucht, z. T. unter Einbeziehung zusätzlicher Isolate aus anderen Studien.

Pilze mit reduzierten conidiogenen Zellen und einem Hefe-artigen Stadium wurden zahlreich von allen drei *Prunus*-Arten und in allen Beprobungsregionen in Deutschland isoliert. In ersten molekularen Untersuchungen wurden diese Pilze sowie zehn ähnliche Stämme, aus an Weinstöcken befestigten Sporenfallen in Rheinland-Pfalz, der Gattung *Collophorina* (*Leotiomycetes*, *Tympanidaceae*) zugeordnet. Durch die phylogenetische Analyse der Familie *Tympanidaceae* wurde die Polyphylie der Gattung *Collophorina* aufgedeckt. Daher wurden *C. paarla* und *C. euphorbiae* in zwei neu beschriebene Gattungen, *Pallidophorina* und *Ramoconidiophor*a, überführt. Die Pilze, die aus Holz von *Prunus* isoliert wurden, gehörten den Arten, *Pa. paarla*, *C. africana* und drei neuen Arten der Gattung *Collophorina* an, die als *C. badensis*, *C. germanica* und *C. neorubra* beschrieben wurden. Die Stämme aus Sporenfallen gehörten sieben Arten in fünf Gattungen an, darunter *C. badensis*. Drei dieser Gattungen, *Capturomyces, Variabilispora* und *Vexillomyces*, wurden neu beschrieben. Die Ergebnisse dieser Studie erlauben weitreichende Einblicke in diese wenig untersuchte Gruppe von Pilzen und offenbaren ihre bislang unbekannte Vielfalt und Häufigkeit im Holz von *Prunus*-Bäumen in Deutschland.

Ein Stamm aus dem Holz von *P. domestica* wies ebenfalls typische collophorina-artige Merkmale auf. Erste molekulare Untersuchungen deuteten jedoch auf eine Zugehörigkeit zur Familie *Hyaloscyphaceae* (*Leotiomycetes*). Eine familienweite molekulare und morphologische Analyse ergab keine Zugehörigkeit zu einer bekannten Art oder Gattung. Als Konsequenz wurden die neue Gattung *Arboricolonus* mit der neuen Art *A. simplex* beschrieben. Die morphologische Ähnlichkeit zur Gattung *Collophorina* wurde mit der Anpassung an das identische Habitat und somit konvergenter Evolution erklärt.

Arten der Gattung *Cadophora* (*Leotiomycetes*) sind von verschiedenen Habitaten wie Pflanzen, Früchten oder Totholz bekannt und wurden in dieser Studie vermehrt von *Prunus*-Holz isoliert. Eine Analyse der Gattung umfasste 29 Stämme, die aus

Prunus-Holz in Deutschland isoliert wurden, sowie drei zusätzliche Stämme aus *Prunus*-Holz in Südafrika. Insgesamt wurden fünf Arten differenziert. Mehrere Stämme aus Deutschland wurden *Ca. luteo-olivacea* und *Ca. novi-eboraci* zugeordnet. Drei Arten wurden neu beschrieben. Dabei handelte es sich um *Ca. prunicola*, das aus *Prunus*-Holz sowohl in Deutschland als auch Südafrika isoliert wurde, *Ca. africana* ausschließlich aus Südafrika, und *Ca. ramosa*, ausschließlich aus Deutschland. Darüber hinaus wurde *Margarinomyces bubakii* in die Gattung *Cadophora* kombiniert und von *Ca. obscura* abgetrennt, das als Art wieder anerkannt wurde.

Basierend auf DNA-Daten wurden acht von *Prunus* isolierte Stämme mit asexuellen Stadien der Gattung *Proliferodiscus* (*Leotiomycetes*) zugeordnet. In der Vergangenheit wurden Arten dieser Gattung fast ausschließlich anhand der Morphologie der sexuellen Stadien beschrieben. Sequenzdaten sowie Daten zu asexuellen Stadien waren kaum vorhanden. Diese Studie stellt die erste umfassende phylogenetische Analyse der Gattung *Proliferodiscus* dar. Die neue Art *Pr. ingens* wurde beschrieben und detaillierte Beschreibungen der asexuellen Morphen dieser Art, und einer weiteren, als *Proliferodiscus* sp. bezeichneten Art, vorgelegt.

Zwei Arten der Gattung *Minutiella* (*Eurotiomycetes*) wurden isoliert; davon wurde eine als *M. pruni-avium* neu beschrieben.

Im Zuge der Erfassung der pilzlichen Diversität im Holz der drei *Prunus*-Arten in Deutschland wurden 1018 Stämme isoliert, z. T. morphologisch untersucht, sowie deren ITS- und LSU-Sequenzen generiert. Durch die Kombination von Datenbank-Vergleichen mit einem starken Fokus auf verlässliche Referenzsequenzen und sorgfältigen phylogenetischen Analysen wurden 172 Arten der *Ascomycota*, *Basidiomycota* und *Mucoromycota* differenziert. Die Mehrzahl der Stämme gehörte den *Ascomycota*-Klassen *Sordariomycetes*, *Leotiomycetes* und *Dothideomycetes* an. Artidentifikationen wurden basierend auf der Anzahl der Nukleotidunterschiede zu Referenzsequenzen in Kategorien eingeteilt. Nach diesem Schema wurden 82 Arten mit hoher Sicherheit und 20 Arten mit geringer Sicherheit identifiziert. Die mit Abstand am häufigsten und im Holz von allen drei Wirtsarten und in allen Beprobungsregionen nachgewiesenen Arten waren die coelomycetische Art *Aposphaeria corallinolutea* (*Dothideomycetes*) und die collophorina-artige Art *Pallidophorina paarla*. Weitere 70 Taxa konnten keiner bekannten Art zugeordnet werden, und wurden größtenteils als potentiell neue Arten eingestuft. Daher wird Holz von *Prunus*-Bäumen in Deutschland als ein Habitat mit einer großen Anzahl potentiell neuer Arten angesehen. Die Kultivierung und Aufbewahrung der in dieser Studie isolierten Pilze erlaubt vielfältige weitere Untersuchungen z. B. bezüglich deren Taxonomie, Physiologie, Ökologie und Biochemie.

Abbreviations

BSR	Biological Species Recognition
EF-1α	translation elongation factor 1α
ESR	Ecological Species Recognition
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GBOL	German Barcoding of Life project
GCPSR	Genealogical Concordance Phylogenetic Species Recognition
ha	hectare
ITS	5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS1 and ITS2
LSU	28S large ribosomal subunit
MSR	Morphological Species Recognition
PSC	Phylogenetic Species Concept
TUB	β-tubulin gene
USDA	United States Department of Agriculture

1 General introduction

1.1 Importance of fungal diseases of wood and the knowledge about the associated fungal diversity

Plant diseases have far reaching consequences for agriculture, horticulture and forestry. In particular, overall yield loss in agriculture due to pathogens, pests and weeds varies between 20 and 40% of the total global productivity (Oerke 2006). The highest damage is caused by fungal pathogens with estimated global losses of more than 200 billion US dollars each year (Birren et al. 2002). Yield loss assessments have so far only been conducted for a few major annual crops that are crucial for food security (Oerke & Dehne 2004, Oerke 2006). However, for most pathogen-crop combinations, especially those including perennial crops, like trees and other woody hosts, no exact data are available (Heaton et al. 1981).

Trees have a major effect on biodiversity (e.g. organisms living inside of them) as well as on ecosystem services as they dominate in many natural and agricultural landscapes (Freer-Smith & Webber 2015). Ecosystem services of forests (tropical and temperate/boreal) are estimated to globally comprise a value of 16.2 billion US dollars on average per year (Costanza et al. 2014). Thus, loss of particular tree species due to fungal diseases can have a fundamental effect on the ecosystem services provided by tree dominated landscapes (Freer-Smith & Webber 2015), including those used for fruit production. Woody hosts are frequently affected by outbreaks of fungal diseases as examples on olives in Italy and the USA (Úrbez-Torres et al. 2013, Carlucci et al. 2015), pistacia in Iran (Mohammadi et al. 2015) and pome fruit trees in South Africa (Smit et al. 1996, Cloete et al. 2011) demonstrate. In agricultural plantations, diseases appear suddenly and spread quickly, promoted by the highly homogenous environment and dense plant populations (Stukenbrock & McDonald 2008, Ratnadass et al. 2012, Hantsch et al. 2014, Setiawan et al. 2014).

The global trade increases the frequency of plant disease emergence (Boyd et al. 2013, Santini et al. 2013, Pace & Gephart 2017). Imported plants or plant products can be infested with exotic pathogens, for which the local flora had no time yet to develop defense and resistance mechanisms by means of evolutionary adaptation (Brasier 2008, Wingfield et al. 2010, Burgess et al. 2016). Pathogens with low aggressiveness in their natural distribution area might therefore have devastating effects on an unprepared flora. As a result, many countries have implemented quarantine measures in order to prevent entry of exotic pathogens, which would be able to damage the local natural and agricultural ecosystems. Quarantine measures can span from regular inspections of plants and plant products directly at the producer, the nursery or during trade actions up to strict trade restrictions (Grünwald et al. 2008, Roy et al. 2014). Furthermore, many exotic plants are introduced and planted for production of food or other goods or as ornamental plants that have no defense mechanisms against the local pathogen diversity (Strange & Scott 2005).

In addition, the risk of disease emergence will further increase in the foreseeable future due to climate change (Fisher et al. 2012). An increase of global temperature leads to significant geographical shifts of crop cultivation, widens the habitable area of pathogens adapted to warmer climates, and increases the chance of severe droughts, which weaken host plants due to abiotic stress (Newton et al. 2011, Bebber et al. 2013, Boyd et al. 2013, Chappelka & Grulke 2016).

Knowledge on the fungal species diversity associated with symptomatic wood, especially of important agricultural crops, and a reliable and fast identification of these fungi is crucial for the development of counteractions against old and newly emerging fungal threats and for choosing appropriate control strategies, such as the correct selection of fungicides (Bertsch et al. 2013, Gramaje et al. 2018). Furthermore, a reliable diagnosis and knowledge of the fungal biology is necessary to prepare phytosanitary regulations against the introduction and spread of plant pathogens. Monitoring is the basis for risk analyses and targeted precaution measures, and needs to be repeated regularly (Rossman 2009, Cai et al. 2011).

Additionally, knowledge of the species composition of a specific habitat directly influences conservation policies, since appropriate conservation strategies can only be developed based on the known biodiversity (Raxworthy et al. 2008, Conroy et al. 2011, Werner & Umberto 2016). In this context, the fungal community living inside plant tissue is of particular importance, as it remains completely unnoticed, if not scientifically surveyed. Several authors have highlighted the need to include pathogens into every biodiversity conservation effort as they have crucial functions within ecosystem networks and are threatened by extinction events (Windsor 1997, Whiteman & Parker 2005, Strona 2015).

However, apart from a few major pathogens of few commercial tree species, little knowledge is available about the diversity of fungi associated with symptomatic woody tissue (Boyd et al. 2013). For many fungal pathogens of important cultivated trees, information like host-specificity and distribution is lacking (Schmit & Mueller 2007). Studies of plant diseases and fungi inhabiting woody plants are furthermore hampered by the little knowledge of the underlying taxonomy of the respective fungi. Hence, one of the most striking difficulties faced by plant pathologists is the lack of a reliable basis for fungal species identification (Strange & Scott 2005).

1.2 Host-fungus interactions in wood and wood diseases

The fungal communities inhabiting wood of trees and shrubs comprise species with several life styles, which affect the physiology of the plants in various ways. Fungal pathogens cause diseases that weaken or kill the plant host. Saprophytic fungi contribute to the nutrient cycle of the ecosystem by degradation of dead organic substances and may occur in diseased wood as secondary invaders following pathogenic organisms. Endophytic fungi, which live inside plant tissue without causing visible symptoms, may even have beneficial effects on their hosts, like an increased nutrient uptake, inhibition of pathogen growth, reduction of disease

severity, and enhancing tolerance to environmental stresses (Mei & Flinn 2010). The direct ecological functions and physiological actions of endophytic fungi inhabiting woody plants are, however, poorly understood and need to be further explored (Hoff et al. 2004, Oses et al. 2008). Though, it has been hypothesised that they change either to a saprotrophic life style once senescence has occurred, or to a pathogenic life style once the plant host is weakened (Boddy & Griffith 1989, Whalley 1996, Davis et al. 2003, Sieber 2007). Such transitions between different life styles have been demonstrated for different fungal species (Redman et al. 2001, Álvarez-Loayza et al. 2011, Eaton et al. 2011, Kuo et al. 2014). Hence, a strict separation of the different life strategies is often not possible.

Fungal pathogens are further divided into several groups according to their strategy to infect and exhaust their host (Doehlemann et al. 2017). Biotrophic pathogens persist in and utilize living tissue of the host plant. In contrast, necrotrophic pathogens kill the host tissue and extract its nutrients from the dead tissue. In severe cases species from both groups are able to kill whole plants. Hemibiotrophic pathogens, for example several *Colletotrichum* species, infect their hosts and utilise living tissue like biotrophic pathogens, however, later switch to a necrotrophic life style (O'Connell et al. 2012).

The development and severity of plant diseases caused by biotic agents is generally explained by the disease triangle, which is a concept to illustrate the main factors involved in disease causation (Keane & Kerr 1997, Francl 2001). The requirements for the development of a plant disease are a susceptible host, a virulent pathogen and an environment favourable for disease development. Especially in agricultural systems humans influence the three factors by cultivation and planting practices (e.g. homogenous monocultures), genetic manipulation of plant hosts (e.g. breeding) or environmental manipulations (e.g. irrigation; Francl 2001).

Disease development depends on molecular mechanisms, which determine the susceptibility of the host and the virulence of the pathogen. Plant species are resistant to most potential pathogens due to non-host resistance (Nürnberger & Lipka 2005). In this type of resistance, the establishment of infection structures may initially be stopped by preformed physical or chemical barriers on the plant surface (wax layers, rigid cell walls, antimicrobial secondary metabolites). If a pathogen is able to breach these inital barriers it becomes subject to recognition at the plasma membrane of the plant cells, where pathogen-associated molecular patterns (PAMPs) trigger receptor-mediated defence responses, such as the hypersensitive response (see below; Nürnberger & Lipka 2005, Pieterse & Dicke 2007).

In contrast, in compatible host-pathogen interactions the pathogen is able to overcome these barriers either by production of specialised proteins, which suppress recognition or response, or by secretion of toxic proteins or metabolites that kill host cells (Barrett et al. 2009).

The primary infection path of fungal pathogens of wood usually happens through open wounds on trunks or branches, produced by grafting, pruning or injury (Mostert et al. 2006b, Rolshausen et al. 2010, Gramaje et al. 2012). Spores can be transferred

to open wounds by wind circulation or rain splash. Furthermore, insects can act as vectors transferring spores of potential pathogens after contact with spore masses of an inoculum source (Moyo et al. 2014).
After infection, the fungi distribute within the plant body by growth of mycelium or by production of sexual or asexual spores, which are distributed in the vascular system, both xylem and phloem, via water flow (Mostert et al. 2006a). Further distribution of fungal pathogens within the plant body can be prevented by structural and biochemical mechanisms of the plant (Schlösser 1997, Garcion et al. 2014). In case of wood infestation by fungi, plants produce tyloses and/or gum as structural defense mechanism. Tyloses are protoplasts of parenchymatic cells outgrowing into adjacent xylem cells, which are subsequently blocked. Gums are rubber-like compounds consisting of polysaccharides, which are deposited in intercellular spaces as well as in vascular bundles around lesions of infected or injured spots. In both ways, an impenetrable barrier is built enclosing and isolating the pathogen including its spores. A biochemical mechanism provoked by fungal infestation is the hypersensitive response, in which toxic compounds released by the plant kill the infected and surrounding cells, enclosing and possibly killing the pathogen (Heath 2000, Greenberg & Yao 2004).
The movement of water and inorganic nutrients in the xylem and of organic compounds in the phloem gets disrupted, if the vessel system is blocked or if cell walls of the vessels are destructed by cell-wall-degrading substances (Agrios 2005, Horbach et al. 2011). The mechanisms explained above result in typical symptoms observed in or on wood of infected fruit trees. These might be internal wedge shaped necroses and black streaking, gummosis, cankers, or swellings, disordered flower and fruit production, wilting and dieback of branches and whole trees (Ogawa et al. 1995, Gramaje et al. 2012, Sessa et al. 2016).

1.3 The genus *Prunus*

The plant genus *Prunus* is classified in the subfamily *Amygdaloideae* of the *Rosaceae* and consists of over 200 species of shrubs, deciduous and evergreen trees, which grow in temperate regions of the Northern Hemisphere and in subtropical and tropical regions (Lee & Wen 2001, Shi et al. 2013). The members of the *Amygdaloideae* differ from species of other rosaceous subfamilies by having a drupe, a fleshy fruit with a stony endocarp or stone and are hence known as drupaceous plants (Lee & Wen 2001).
The genus *Prunus* is assumed to originate from Central Asia (Badenes & Parfitt 1995, Rungjindamai et al. 2014). About 20 wild and cultivated species of *Prunus* are to be found in Europe (Hanelt 1997). Sweet (*P. avium*) and sour (*P. cerasus*) cherries are native to the Middle East and have been cultivated throughout Europe for millennia (Das et al. 2011). The European plum (*P. domestica*) is assumed to be a relatively young species with an origin in Southern Europe or Western Asia (Das et al. 2011). Many *Prunus* species are of considerable horticultural importance as they

are known and cultivated for their edible fruits and seeds or as ornamentals, such as plums (e.g. *P. americana*, *P. cerasifera*, *P. domestica*, *P. salicina*), cherries (e.g. *P. avium*, *P. cerasus*, *P. pseudocerasus*, *P. serrulata*), peaches (*P. persica*), apricots (*P. armeniaca*) or almonds (*P. dulcis*). Moreover, the wood especially of *P. avium* and *P. domestica* is used in joinery, turnery and marquetry (Poonam et al. 2011).

Furthermore, a myriad of secondary plant compounds has been discovered in *Prunus* species. Several of these compounds, such as different flavonoids produced by *P. avium* and *P. cerasus*, have been found to have promising effects in heart disease and cancer treatment (Poonam et al. 2011).

In the last decade, plums and sloes were produced on approximately 2.5 million ha worldwide. The annual production averaged out at approximately 4.5 tonnes of fruits per hectare. At the same time, the production of sweet and sour cherries averaged out at approximately 11.5 tonnes per hectare, produced on approximately 630,000 ha (FAO 2020).

In Germany, the production of cherries and plums is the most important production of tree fruits after apples regarding cultivated area and yield (Garming et al. 2018). Within the last decade, approximately 100,000 tonnes of cherries and 50,000 tonnes of plums were produced per year on an area of 7,768 ha and 4,128 ha, respectively. Fluctuations in yield are the result of the early blossoming of stone fruit trees and the risk of late frost during this time.

The most important fruit production areas in Germany are the 'Rhein Ebene' (Ortenau district) in Baden-Württemberg and the 'Alte Land' (Stade district) in Lower Saxony. Both federal states comprise more than 50% of all German areas used for fruit production (Garming et al. 2018). One of the core areas of sour cherry production is located in the Free State of Saxony.

In many regions, fruit production is a landscape-characteristic element and part of the local culture and, therefore, important for tourism. In the Black Forest region in Baden-Württemberg, Southern Germany, the cultivation of stone fruits and the processing of cherries and plums to liqueurs in small business distilleries is considered a cultural heritage.

1.4 Diseases and fungal communities of *Prunus* wood

Several fungi are well-known as pathogens of stone fruit trees causing major economic losses either for local fruit production or for the fruit production worldwide.

Leucostoma canker (also known as Cytospora, Valsa or perennial canker), is of high importance as disease of *Prunus* trees, especially of *P. persica*, *P. persica* var. *nucipersica*, and *P. avium* in fruit growing areas with cold winters, most notably in the northern United States and Canada (Biggs & Grove 2005). Infections of other *Prunus* species and further woody hosts like *Malus*, *Alnus* and *Populus* are known from North and South America, Europe and Asia (Biggs 1989, McIntyre et al. 1996, Worrall et al. 2010, Mehrabi et al. 2011). The causal agents of the diseases, *Cytospora leucostoma* and *Valsaria insitiva*, are able to invade all above ground

woody parts through wounds or dead tissue, kill leaves, twigs and branches and overall reduce yield and lifetime of the infected trees (Biggs 1989, Biggs & Grove 2005).

Eutypa dieback, caused by *Eutypa lata* is a disease, able to affect a broad range of different woody hosts worldwide, however, its severity is host dependent (Ogawa et al. 1995). The disease is of special importance for the grapevine industry, because it can spread quickly across entire vineyards. Without removal of infected branches, the fungus reaches the trunk via xylem vessels, eventually killing the plant (Munkvold et al. 1994). The disease was first reported on *P. armeniaca* in the 1930's (Petzoldt et al. 1981), on which it shows a similar destructive behaviour as in grapevine (Munkvold 2001). Pathogenicity of *E. lata* has been confirmed for the above-mentioned species, for additional *Prunus* species (viz. *P. avium*, *P. cerasus*, *P. domestica*, *P. dulcis, P. virginiana* var. *demissa*), and for other fruit and ornamental tree species (Munkvold et al. 2001, Moyo et al. 2018, 2019).

Further notable wood diseases are silver leaf disease of stone fruit trees caused by *Chondrostereum purpureum*, constriction canker of *P. persica* caused by *Diaporthe amygdali*, black knot of wild and cultivated plum and cherry trees caused by *Apiosporina morbosa*, Verticillium wilt of several *Prunus* species (including *P. avium*, *P. cerasus* and *P. domestica*) and Ceratocystis canker of several *Prunus* species (especially *P. dulcis* and *P. domestica*) caused by *Ceratocystis fimbriata* (Smith et al. 1970, Lamey & Stack 1991, Ogawa et al. 1995, Lalancette & Polk 2000, Lalancette & Robison 2002).

Most of these diseases are caused by a single pathogen; however, as several pathogens are usually isolated from old cankers, trunk diseases are generally regarded as complexes of pathogens (Larignon & Dubos 1997, Rolshausen et al. 2004, Úrbez-Torres et al. 2006). Few studies aimed to investigate the whole fungal community of *Prunus* wood. Most studies focused on particular taxonomic groups, and observations on taxa beyond that respective group were rarely reported.

Searches for fungi associated with the three host plant species *P. avium*, *P. domestica* and *P. avium* in the USDA database (Farr & Rossman 2020) revealed 249, 317 and 152 fungal species (including synonyms and taxa unidentified on species level), respectively. However, as the search cannot be restricted to the tissue type or to the nature of the plant-fungal association, many of the listed species probably originate from tissues other than living wood. Due to the easier access, fungal species detected on the surface or in the inner tissue of leaves or fruits are more often reported. The following compilation of fungal species reported from symptomatic or healthy above ground wood of *Prunus* trees must be regarded as far from being complete.

The most extensive studies on the fungal diversity associated with symptomatic wood of *Prunus* trees have been undertaken in South Africa and in California, USA.

Isolation of fungi from six different *Prunus* species (*P. armeniaca*, *P. avium*, *P. domestica*, *P. dulcis*, *P. persica*, *P. salicina*) in fruit production areas in climatically different parts of South Africa resulted in several publications focusing on specific

genera or higher taxonomic groups, encompassing 46 species including 22 novel descriptions. The highest fungal species diversity was detected in the genus *Phaeoacremonium* (14 species; Damm et al. 2008b) and in the order *Botryosphaeriales* (10 species; Damm et al. 2007a, b, Slippers et al. 2007). Furthermore, species of the *Phaeomoniellales*, *Calosphaeriaceae*, *Diatrypaceae*, *Tympanidaceae*, *Coniochaeta* and *Paraconiothyrium* were reported (in total 22 species, Damm et al. 2008a, c, 2010, Moyo et al. 2018); however, the whole fungal diversity isolated was not disclosed.

California is the most important production area for fruit and nut crops in North America (Trouillas et al. 2012, Lawrence et al. 2018). Inderbitzin et al. (2010) studied *Botryosphaeria dothidea* and allied genera infecting almond grown in the area. A total of 45 isolates was obtained from symptomatic branches of *P. dulcis* and seven species of *Botryosphaeriaceae* were identified by morphological and molecular analysis, including *B. dothidea*, *Diplodia seriata* and a new species, *Neofusicoccum nonquaesitum*. All isolated species were positively tested to be pathogenic on *P. dulcis*.

Trouillas et al. (2012) sampled branches of *P. avium* exhibiting dieback symptoms, external lesions and cankers from 20 orchards in California, as well as from orchards in Adelaide, South Australia. Isolates were analysed morphologically and molecularly. *Calosphaeria pulchella* was the most commonly isolated species from 95% of all orchards sampled in California, followed by *Eutypa lata* and *Leucostoma persoonii*. Further fungi isolated, but only mentioned were species of *Botryosphaeriaceae*, *Phaeoacremonium* and collophorina-like fungi, *Cryptovalsa ampelina*, and few basidiomycetous fungi. In Australia, *Calosphaeria pulchella* was isolated from two of three orchards. Further fungi isolated in this region were *Leucostoma persoonii*, *Trametes versicolor* and unidentified species of *Chaetomium* and *Chondrostereum*. The authors conducted pathogenicity tests with the three most commonly isolated species in California obtaining positive results for all of them.

In order to investigate the diversity of fungi of the genus *Cytospora* causing diseases like canker, branch dieback, leaf wilting, wood discoloration, gummosis and cracked bark on fruit and nut trees in California, Lawrence et al. (2018) collected samples of several tree species, including *P. armeniaca*, *P. avium*, *P. domestica*, *P. dulcis* and *P. persica* in the Central Valley region. Of the 15 *Cytospora* species identified, six were found on *Prunus* hosts, namely *Cy. amygdali*, *Cy. californica*, *Cy. eucalypti*, *Cy. longispora*, *Cy. plurivora* and *Cy. sorbicola*.

The diversity of species of *Botryosphaeriaceae* on infected twigs and branches of pome and stone fruit trees in Uruguay was investigated by Sessa et al. (2016). Between 2013 and 2014, 96 trees including 41 *P. persica* trees, showing symptoms like cankers, gummosis, dead shoots or dieback were sampled in five fruit production areas in the country. In total, 247 strains encompassing eight species were isolated. Four species were found on *Prunus*, namely *Diplodia pseudoseriata*, *D. seriata*, *Neofusicoccum parvum* and *Pseudofusicoccum* sp. All three taxa identified to species level showed positive results in a pathogenicity trial.

In some cases, investigations were conducted following a sudden and severe disease outbreak in orchards of a certain fruit production region. For example, in 2008 a severe decline was noticed in several *P. dulcis* orchards on the island of Mallorca, Spain. The symptoms included rapid collapse of branches, chlorosis of leaves as well as bud and shoot dieback. Some infected trees died a few weeks after showing first symptoms. Gramaje et al. (2012) isolated 10 fungal species from branches with necrotic xylem tissue, most of which known to be pathogenic or potentially pathogenic. Five of these species belonged to the *Botryosphaeriaceae*, namely *Botryosphaeria dothidea*, *Diplodia olivarum*, *D. seriata*, *Neofusicoccum australe* and *N. parvum* as well as two new species, *Collophorina hispanica* and *Phaeoacremonium amygdalinum*.
Olmo et al. (2014) investigated a severe disease outbreak in a *P. armeniaca* orchard, also on Mallorca island in 2011 with approximately 10% of trees showing collapse of branches, chlorosis of leaves or shoot dieback. The xylem tissue of the sampled branches was necrotic. From more than half of the branches *Phaeoacremonium venezuelense* was isolated. Subsequent pathogenicity testing revealed a positive result for this species.
A severe outbreak of dieback and canker symptoms on *P. persica* was observed in the Hamadan province, Iran in 2016, with approximately 58% of commercially grown trees infected. Following an extensive sampling, Bagherabadi et al. (2017) identified *Cytospora chrysosperma* as the causal agent of the disease, which was confirmed by pathogenicity testing; they reported this pathogen for the first time on this host species. It was assumed that expanded damages on the trees, caused by a severe hail incidence earlier in the year, favoured the disease outbreak.
After an outbreak of peach shoot blight in Yangshan, China, Tian et al. (2018) isolated fungi from infected twigs of *P. persica* in three orchards. Of the 358 isolates obtained, 202 isolates were identified as *Diaporthe amygdali* and 156 isolates as *Botryosphaeria dothidea*, by morphological and molecular analysis. Pathogenicity testing confirmed both species as causal agents of the disease.
Many assessments are focusing on endophytic fungi, wood inhabitants, which do not cause any disease symptoms. For example, Haddadderafshi et al. (2011) investigated the endophytic fungal community in twigs, roots and leaves of 110 *P. avium* trees from an orchard in Hungary. In total 3,053 isolates were obtained from symptomless twigs and differentiated based on ITS sequences. They found 13 different taxa in this tissue, six of which identified to species level, namely *Alternaria alternata*, *Neocosmospora solani*, *Epicoccum nigrum*, *Macrophomonia phaseolina*, *Colletotrichum acutatum* (s. lat.) and *Botryotinia fuckeliana*. The unidentified species belonged to the genera *Alternaria*, *Embellisia*, *Fusarium*, *Chaetomium* and *Diaporthe*. By far the highest abundance was shown by the two species of *Alternaria*. They found distinct differences of fungal communities in the three tissues; of the 26 taxa isolated in total, only three were found in all three tissues. Hortová & Novotný (2011) investigated the endophytic fungal community of 80 symptomless branches of *Prunus cerasus* from orchards in the Czech Republic by isolation and morphological

inspection. Fifteen taxa were differentiated, including *Alternaria alternata*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Epicoccum nigrum*, *Pezicula* sp. and *Sarcinomyces* sp. as well as seven unidentified taxa.

Additionally, focusing on endophytic fungal communities, Abdollahi Aghdam & Fotouhifar (2016) examined healthy twigs of *P. avium* and *P. cerasus* from different regions of Iran. After inspection, isolation and molecular analysis of 60 isolates from *P. avium* and 56 isolates from *P. cerasus*, four fungal species were identified from both host plants, namely *Microsphaeropsis olivacea*, *Sarocladium strictum*, *Pallidophorina paarla* (as *Collophora paarla*) and *Quambalaria cyanescens*. The latter two had been reported for the first time in Iran.

A list of fungal species isolated from symptomatic or healthy above ground woody tissue of different *Prunus* species around the world is compiled in table 1. Comparing fungal communities of *Prunus* wood from different surveys irrespective of infected or uninfected tissue, it becomes apparent that the fungi detected (or at least reported) almost exclusively belong to the phylum *Ascomycota*.

Basidiomycota are rarely reported from studies investigating wood inhabiting fungal communities. In their investigation of pathogenic fungi of cherry trees, Trouillas et al. (2012) reported three species of *Basidiomycota* from symptomatic branches of *P. avium* in Australia and California, USA, namely *Trametes versicolor* (Australia, USA), *Schizophyllum commune* (USA) and *Chondrostereum* sp. (Australia). Gierl & Fischer (2017) isolated nine species of *Ascomycota* from symptomatic branches of *Prunus* spp. as well as *Stereum hirsutum* from symptomatic branches of *P. dulcis* in Germany.

Species of *Basidiomycota* are more commonly reported from studies focusing on wood decay fungi, in which the respective fungi are identified based on their fruiting bodies that already developed on the bark. Schmidt et al. (2012) sampled fruiting bodies from different pruned or felled trees in German urban areas and identified *Trametes hirsuta* from *P. avium* and *Phellinus pomaceus* from a *Prunus* sp. in the city of Hamburg. Adaskaveg & Ogawa (1990) conducted an extensive survey of wood decay fungi producing fruiting bodies on different living or dead fruit and nut trees in California, Oregon and Washington, USA. In total 39 species from *Basidiomycota* were differentiated growing on different living *Prunus* hosts.

Only few geographical areas have been investigated extensively in terms of the fungal diversity of above ground woody parts of *Prunus* hosts. Despite the economic importance of this host genus for fruit production, only few data are available from Germany.

Table 1 List of fungal species isolated from symptomatic or healthy above ground woody tissue of *Prunus*.

Fungal species	***Prunus* host species**	**Substrat**[1]	**Country**[1]	**Source**
Ascomycota				
Alternaria chartarum	*P. avium*, *P. cerasus*	twigs	Lithuania	Valiuškaitė 2002
Alternaria sp.	*P. avium*	healthy twigs	Hungary	Haddadderafshi et al. 2011
Apiosporina morbosa	*P. pensylvanica*, *P. virginiana*	symp. branches	Canada, USA	Zhang et al. 2005
Aplosporella prunicola	*P. persica* var. *nucipersica*	symp. branches	South Africa	Damm et al. 2007b
Aquabiliella dura	*P. salicina*	symp. branches	South Africa	Damm et al. 2010
Aquabiliella effusa	*P. salicina*	symp. branches	South Africa	Damm et al. 2010
Ascochyta chlorospora	*P. avium*, *P. cerasus*, *P. domestica*	twigs	Lithuania	Valiuškaitė 2002
Aureobasidium pullulans	*P. armeniaca*, *P. avium*, *P. cerasus*, *P. dulcis*, *P. persica*	healthy and symp. branches	Czech Republic, Iran	Hortová & Novotný 2011, Arzanlou 2014
Botryosphaeria dothidea	*P. dulcis*, *P. persica*	symp. twigs and branches	China, USA, Spain	Inderbitzin et al. 2010, Gramaje et al. 2012, Tian et al. 2018
Botryotinia fuckeliana	*P. avium*	healthy twigs	Hungary	Haddadderafshi et al. 2011
Botrytis cinerea	*P. cerasus*	healthy branches	Czech Republic	Hortová & Novotný 2011
Brachysporium bloxami	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Brachysporium masonii	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Brachysporium obovatum	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Calosphaeria africana	*P. armeniaca*	symp. branches	South Africa	Damm et al. 2008a
Calosphaeria pulchella	*P. avium*, *P. dulcis*, *P. mahaleb*, *P. persica*	symp. branches and trunks	Australia, France, Iran, Italy, Spain, USA	Trouillas et al. 2012, Arzanlou & Dokhanchi 2013, Berbegal et al. 2014
Capronia nigerrima	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Celerioriella prunicola	*P. salicina*	symp. branches	South Africa	Damm et al. 2010
Ceratocystis fimbriata	*P. armeniaca*, *P. domestica*, *P. persica*	symp. branches and trunks	USA	DeVay et al. 1962, 1965
Chaetomium spp.	*P. avium*	healthy twigs and symp. branches	Australia, Hungary	Haddadderafshi et al. 2011, Trouillas et al. 2012
Cladosporium cladosporioides	*P. cerasus*	healthy branches	Czech Republic	Hortová & Novotný 2011
Cladosporium herbarum	*P. cerasus*	healthy branches	Czech Republic	Hortová & Novotný 2011
coelomyceteous species	*P. cerasus*	healthy branches	Czech Republic	Hortová & Novotný 2011
Colletotrichum acutatum	*P. avium*	healthy twigs	Hungary	Haddadderafshi et al. 2011
Collophorina africana	*P. dulcis*, *P. salicina*	symp. branches	Germany, South Africa	Damm et al. 2010, Gierl & Fischer 2017

Collophorina hispanica	*P. armeniaca*, *P. dulcis*	symp. branches	Germany, Spain	Gramaje et al. 2012, Gierl & Fischer 2017
Collophorina rubra	*P. dulcis*, *P. persica*, *P. persica* var. *nucipersica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2010
Coniochaeta africana	*P. salicina*	symp. branches	South Africa	Damm et al. 2010
Coniochaeta prunicola	*P. armeniaca*, *P. laurocerasus*, *P. salicina*	symp. twigs and branches	Slovakia, South Africa	Damm et al. 2010, Ivanová & Bernadovičová 2013
Coniochaeta velutina	*P. armeniaca*, *P. salicina*	symp. branches	South Africa	Damm et al. 2010
Cryptovalsa ampelina	*P. armeniaca*, *P. avium*, *P. salicina*	symp. branches	South Africa, USA	Trouillas et al. 2010, 2012, Moyo et al. 2018
Cytospora amygdali	*P. dulcis*	symp. twigs and branches	USA	Lawrence et al. 2018
Cytospora californica	*P. dulcis*	symp. twigs and branches	USA	Lawrence et al. 2018
Cytospora chrysosperma	*P. persica*, *P. spinosa*	symp. twigs and branches	Germany, Iran	Bagherabadi et al. 2017, Gierl & Fischer 2017
Cytospora eucalypti	*P. dulcis*	symp. twigs and branches	USA	Lawrence et al. 2018
Cytospora leucostoma	*P. avium*, *P. domestica*, *P. persica*	symp. branches	Australia, Germany, USA	Trouillas et al. 2012, Gierl & Fischer 2017
Cytospora longispora	*P. domestica*	symp. twigs and branches	USA	Lawrence et al. 2018
Cytospora personata	*P. domestica*	twigs	Lithuania	Valiuškaitė 2002
Cytospora plurivora	*P. domestica*, *P. dulcis*, *P. persica*	symp. twigs and branches	USA	Lawrence et al. 2018
Cytospora populina	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Cytospora sorbicola	*P. armeniaca*, *P. avium*, *P. domestica*, *P. dulcis*, *P. persica*	symp. twigs and branches	USA	Lawrence et al. 2018
Cytosporina sp.	*Prunus* sp.	trunk and/ or twig	Europe	Brandenburger 1985
Dermea cerasi	*P. avium*, *P. cerasus*, *P. serotina*	trunk and/ or twig	Europe	Brandenburger 1985, Ellis & Ellis 1985
Dermea padi	*Prunus* sp.	trunk and/ or twig	Europe	Brandenburger 1985
Diaporthe amygdali	*P. dulcis*, *P. persica*	symp. twigs and branches	China, Portugal, Spain	Diogo et al. 2010, Gramaje et al. 2012, Tian et al. 2018
Diaporthe eres	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Diaporthe neotheicola	*P. armeniaca*, *P. dulcis*	symp. branches	Portugal	Diogo et al. 2010
Diaporthe perniciosa	*P. domestica*, *P. persica*, *P. spinosa*	branches	N/A	Ellis & Ellis 1985
Diaporthe spp.	*P. avium*, *P. domestica*, *P. dulcis*	healthy twigs and symp. branches	Hungary, Portugal	Diogo et al. 2010, Haddadderafshi et al. 2011

Diatrype oregonensis	*P. armeniaca*	symp. branches	USA	Trouillas et al. 2010
Didymella glomerata	*P. cerasus*, *P. domestica*	twigs	Lithuania	Valiuškaitė 2002
Diplodia africana	*P. persica*	symp. branches	South Africa	Damm et al. 2007a
Diplodia mutila	*P. armeniaca*, *P. salicina*	symp. branches	Germany, South Africa	Damm et al. 2007a, Gierl & Fischer 2017
Diplodia olivarum	*P. dulcis*	symp. branches	Spain	Gramaje et al. 2012
Diplodia pinea	*P. persica*	symp. branches	South Africa	Damm et al. 2007a
Diplodia pseudoseriata	*P. persica*	symp. twigs and branches	Uruguay	Sessa et al. 2016
Diplodia seriata	*P. armeniaca*, *P. laurocerasus*, *P. dulcis*, *P. persica*, *P. persica* var. *nucipersica*, *P. salicina*	symp. twigs and branches	Germany, Italy, South Africa, Spain, Uruguay, USA	Damm et al. 2007a, Inderbitzin et al. 2010, Gramaje et al. 2012, Quaglia et al. 2014, Sessa et al. 2016, Gierl & Fischer 2017
Dothiorella sarmentorum	*P. dulcis*	symp. branches	USA	Inderbitzin et al. 2010
Dothiorella viticola	*P. persica* var. *nucipersica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2007a
Embellisia sp.	*P. avium*	healthy twigs	Hungary	Haddadderafshi et al. 2011
Epicoccum nigrum	*P. avium*, *P. cerasus*	healthy twigs and branches	Czech Republic, Hungary	Haddadderafshi et al. 2011, Hortová & Novotný 2011
Eutypa cremea	*P. armeniaca*, *P. salicina*	symp. branches	South Africa	Moyo et al. 2018
Eutypa lata	*P. armeniaca*, *P. avium*, *P. dulcis*, *P. salicina*	symp. branches	Australia, Greece, South Africa, Spain, USA	Carter 1957, 1982, Moyo et al. 2018, Trouillas & Gubler 2010, Gramaje et al. 2012, Trouillas et al. 2012
Eutypa petrakii var. *petrakii*	*P. spinosa*	living or dead wood	Switzerland	Trouillas & Gubler 2010
Eutypella citricola	*P. armeniaca*, *P. salicina*	symp. branches	South Africa	Moyo et al. 2018
Eutypella microtheca	*P. armeniaca*, *P. salicina*	symp. branches	South Africa	Moyo et al. 2018
Fusarium spp.	*P. avium*	healthy twigs	Hungary	Haddadderafshi et al. 2011
Hypoxylon fuscum	*P. padus*	N/A	N/A	Ellis & Ellis 1985
Jattaea mookgoponga	*P. persica* var. *nucipersica*	symp. branches	South Africa	Damm et al. 2008a
Jattaea prunicola	*P. salicina*	symp. branches	South Africa	Damm et al. 2008a
Lasiodiplodia plurivora	*P. salicina*	symp. branches	South Africa	Damm et al. 2007a
Lylea tetracoila	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Macrophomina phaseolina	*P. avium*, *P. dulcis*	healthy twigs and symp. branches	Hungary, USA	Inderbitzin et al. 2010, Haddadderafshi et al. 2011
Microsphaeropsis olivacea	*P. avium*, *P. cerasus*	healthy twigs	Iran	Abdolli Aghdam & Fotouhifar 2016
Minutiella tardicola	*P. armeniaca*	symp. branches	South Africa	Damm et al. 2010
Monilinia laxa	*P. armeniaca*, *P. cerasus*, *P. domestica*, *P. persica*	N/A	N/A	Ellis & Ellis 1985
Nectria cinnabarina	*P. avium*, *P. cerasus*, *P. domestica*	twigs	Lithuania	Valiuškaitė 2002

Neocosmospora solani	*P. avium*	healthy twigs	Hungary	Haddadderafshi et al. 2011
Neocucurbitaria cava	*P. avium*, *P. cerasus*, *P. domestica*	twigs	Lithuania	Valiuškaitė 2002
Neofusicoccum australe	*P. armeniaca*, *P. domestica*, *P. dulcis*, *P. persica*, *P. salicina*	symp. branches	South Africa, SPain	Damm et al. 2007a, Slippers et al. 2007, Gramaje et al. 2012
Neofusicoccum mediterraneum	*P. dulcis*	symp. branches	USA	Inderbitzin et al. 2010
Neofusicoccum nonquaesitum	*P. dulcis*	symp. branches	USA	Inderbitzin et al. 2010
Neofusicoccum parvum	*P. dulcis*, *P. persica*	symp. twigs and branches	Spain, Uruguay, USA	Inderbitzin et al. 2010, Gramaje et al. 2012, Sessa et al. 2016
Neofusicoccum vitifusiforme	*P. persica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2007a
Neophaeomoniella zymoides	*P. salicina*	symp. branches	South Africa	Damm et al. 2010
Nitschkia collapsa	*P. spinosa*	N/A	N/A	Ellis & Ellis 1985
Pallidophorina paarla	*P. avium*, *P. cerasus*, *P. persica*, *P. salicina*	healthy twigs and symp. branches	Germany, Iran, South Africa	Damm et al. 2010, Abdolli Aghdam & Fotouhifar 2016, Gierl & Fischer 2017
Paraconiothyrium brasiliense	*P. persica*, *P. persica* var. *nucipersica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2008c
Paraconiothyrium variabile	*P. persica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2008c
Peyronellaea obtusa	*P. domestica*, *P. persica*, *Prunus* sp.	symp. branches	South Africa	Slippers et al. 2007
Pezicula cinnamomea	*Prunus × yedoensis*	twigs	Japan	Ooki et al. 2003
Pezicula sp.	*P. cerasus*	healthy branches	Czech Republic	Hortová & Novotný 2011
Phaeoacremonium africanum	*P. armeniaca*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium aleophilum	*P. armeniaca*	symp. branches	Iran	Arzanlou et al. 2014
Phaeoacremonium amygdalinum	*P. dulcis*	symp. branches	Spain	Gramaje et al. 2012
Phaeoacremonium australiense	*P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium fraxinopennsylvanica	*P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium fuscum	*P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium griseo-olivaceum	*P. armeniaca*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium griseorubrum	*P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium iranianum	*P. armeniaca*, *P. dulcis*	symp. branches	South Africa, Spain	Damm et al. 2008b, Gramaje et al. 2012
Phaeoacremonium minimum	*P. armeniaca*, *P. persica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium pallidum	*P. armeniaca*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium parasiticum	*P. armeniaca*, *P. avium*	symp. branches	Greece, South Africa	Rumbos 1986, Damm et al. 2008b
Phaeoacremonium prunicola	*P. salicina*	symp. branches	South Africa	Damm et al. 2008b

Phaeoacremonium scolyti	*P. armeniaca*, *P. persica*, *P. persica* var. *nucipersica*, *P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium spp.	*P. avium*	symp. branches	USA	Trouillas et al. 2012
Phaeoacremonium subulatum	*P. armeniaca*	symp. branches	South Africa	Damm et al. 2008b
Phaeoacremonium venezuelense	*P. armeniaca*	symp. branches	Spain	Olmo et al. 2014
Phaeoacremonium viticola	*P. salicina*	symp. branches	South Africa	Damm et al. 2008b
Phoma herbarum	*P. cerasus*, *P. domestica*	twigs	Lithuania	Valiuškaitė 2002
Phomopsis amygdaliana	*Prunus* sp.	trunk and/ or twig	Europe	Brandenburger 1985
Phomopsis prunorum	*P. avium*, *P. cerasus*, *P. domestica*	twigs	Lithuania	Valiuškaitė 2002
Podosphaera tridactyla	*P. domestica*, *P. laurocerasus*, *P. padus*, *P. spinosa*	twigs	N/A	Ellis & Ellis 1985
Pseudocamarosporium africanum	*P. persica*	symp. branches	South Africa	Damm et al. 2008c
Pseudofusicoccum sp.	*P. persica*	symp. twigs and branches	Uruguay	Sessa et al. 2016
Quambalaria cyanescens	*P. avium*, *P. cerasus*	healthy twigs	Iran	Abdolli Aghdam & Fotouhifar 2016
Sarcinomyces sp.	*P. cerasus*	healthy branches	Czech Republic	Hortová & Novotný 2011
Sarcopodium circinatum	*P. avium*, *P. cerasus*, *P. domestica*	wood	Lithuania	Valiuškaitė 2002
Sarocladium strictum	*P. avium*, *P. cerasus*	healthy twigs	Iran	Abdolli Aghdam & Fotouhifar 2016
Sporidesmium altum	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Stigmina carpophila	*P. amygdalus*, *P. avium*, *P. cerasus*, *P. domestica*, *P. laurocerasus*, *P. persica*	twigs and branches	Lithuania	Ellis & Ellis 1985, Valiuškaitė 2002
Truncatella angustata	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Tympanis prunastri	*Prunus* sp.	trunk and/ or twig	Europe	Brandenburger 1985
Valsa pustulata	*Prunus* sp.	N/A	N/A	Ellis & Ellis 1985
Valsaria insitiva	*P. avium*, *P. domestica*	symp. branches	Italy	Romanazzi et al. 2012
Basidiomycota				
Schizophyllum commune	*P. avium*	symp. branches	California US	Trouillas et al. 2012
Trametes versicolor	*P. avium*	symp. branches	Australia, California US	Trouillas et al. 2012
Chondrostereum sp.	*P. avium*	symp. branches	Australia	Trouillas et al. 2012
Stereum hirsutum	*P. dulcis*	symp. branches	Germany	Gierl & Fischer 2017

[1] symp.: symptomatic; N/A: information not available.

1.5 The German Barcoding of Life project

The German Barcoding of Life (GBOL; https://www.bolgermany.de) project aims at the acquisition of all German species diversity of animals, fungi and plants. Taxonomists concerned with the different organism groups collaborate to establish a DNA-barcode library of the German species. Furthermore, strategies of efficient acquisition of DNA-barcodes and their scientific and economic applications as well as applications relevant to nature conservation are developed. A DNA-barcode library provides a tool for species identification for both experts and non-experts. The use of all GBOL data is free of charge and allows various applications like monitoring of general biodiversity, endangered or invasive species, pests and pathogens or species identification based on life stages (e.g. larvae, eggs, spores) or organism fragments (e.g. single insect legs, roots, fungal hyphae) that are hard to determine morphologically. The DNA-barcodes could be used, for example, for biodiversity conservation, customs control of illegal trade or trace analyses in forensics and criminalistics.

In a second funding phase of the GBOL-project (GBOL-II), the DNA-barcode library was expanded and innovative and efficient instruments for specific problems in agriculture, horticulture, monitoring as well as health and consumer protection were developed.

Within the GBOL-II project several research groups from different universities and scientific institutes collaborated on fungi in the project part "Fungal pathogens and necrotic fungi in orchards". In the context of a sustainable and ecological production, a distinct and fast identification of plant pathogens is of increasing importance. Therefore, one aim of this project part was the development of a diagnostic tool for fast and easy identification of fungal plant pathogens in form of a microarray chip (Harjes & Rambold 2016). The basis of an efficient application of this chip was an extensive reference database containing DNA-sequences of primarily pathogenic and endophytic fungi isolated from different *Rosaceae* hosts.

This study directly contributed to the GBOL-II-project by provision of DNA-sequences of fungi isolated from *Prunus* wood for the establishment of the DNA-barcode library and for the development of the microarray chip.

1.6 Objectives of the study

Despite the economic significance of the genus *Prunus* in Germany, little is known about the fungal diversity inhabiting above-ground woody parts of *Prunus* species, especially necrotic tissue. Therefore, the aim of this study was to reveal the present potentially pathogenic fungal diversity by extensive isolations from symptomatic branches of the three most important *Prunus* species in Germany, *P. avium*, *P. cerasus* and *P. domestica*. Furthermore, a selection of primarily anamorphic fungal genera is treated in-depth by means of morphological and phylogenetic methods in order to clarify their taxonomic relationships and to provide tools for fast and reliable species identification. Sampling took place in three important fruit production areas in

Germany. The sampling sites comprise commercial orchards in the vicinity of Görlitz and the fruit production areas south of Dresden, Saxony, the 'Alte Land' in Lower Saxony and the 'Rhein Ebene' in Baden-Württemberg.

1.7 References

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2 Manuscript I

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Novel collophorina-like genera and species from *Prunus* trees and vineyards in Germany

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Key words

Collophora
morphology
multi-locus phylogeny
new taxa
species diversity
systematics

Abstract Strains with a yeast-like appearance were frequently collected in two surveys on the biodiversity of fungi in Germany, either associated with necroses in wood of *Prunus* trees in orchards in Saxony, Lower Saxony and Baden-Württemberg or captured in spore traps mounted on grapevine shoots in a vineyard in Rhineland-Palatinate. The morphology of the strains was reminiscent of the genus *Collophorina*: all strains produced aseptate conidia on integrated conidiogenous cells directly on hyphae, on discrete phialides, adelophialides and by microcyclic conidiation, while in some strains additionally endoconidia or conidia in conidiomata were observed. Blastn searches with the ITS region placed the strains in the *Leotiomycetes* close to *Collophorina* spp. Analyses based on morphological and multi-locus sequence data (LSU, ITS, *EF-1α*, *GAPDH*) revealed that the 152 isolates from wood of *Prunus* spp. belong to five species including *C. paarla*, *C. africana* and three new species. A further ten isolates from spore traps belonged to seven new species, of which one was isolated from *Prunus* wood as well. However, a comparison with both LSU and ITS sequence data of these collophorina-like species with reference sequences from further *Leotiomycetes* revealed the genus *Collophorina* to be polyphyletic and the strains to pertain to several genera within the *Phacidiales*. *Collophorina paarla* and *C. euphorbiae* are transferred to the newly erected genera *Pallidophorina* and *Ramoconidiophora*, respectively. The new genera *Capturomyces*, *Variabilispora* and *Vexillomyces* are erected to accommodate five new species isolated from spore traps. In total nine species were recognised as new to science and described as *Collophorina badensis*, *C. germanica*, *C. neorubra*, *Capturomyces funiculosus*, *Ca. luteus*, *Tympanis inflata*, *Variabilispora flava*, *Vexillomyces palatinus* and *V. verruculosus*.

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INTRODUCTION

The coelomycetous genus *Collophora* (*Tympanidaceae*, *Phacidiales*, *Leotiomycetes*) was described from necrotic wood of several *Prunus* species (*P. dulcis*, *P. persica*, *P. persica* var. *nucipersica*, *P. salicina*) in South Africa (Damm et al. 2010). After the previously described plant genus *Collophora* Mart. 1830 (*Apocynaceae*) was incorporated in the plant list, the fungal name was recognised as illegitimate being a later homonym (Art. 53.1, McNeill et al. 2015) and renamed as *Collophorina* (Wijayawardene et al. 2017). Five species were originally described by Damm et al. (2010) based on a combination of morphological and DNA sequence data, namely *C. africana*, *C. capensis*, *C. paarla*, *C. pallida* and *C. rubra*. However, based on multi-locus sequence data, *C. pallida* and *C. capensis* were later synonymised with *C. paarla* and *C. africana*, respectively (Gramaje et al. 2012). A further three species have subsequently been described, namely *C. hispanica* from *P. dulcis* in Spain (island of Mallorca), *C. aceris* from *Acer glabrum* var. *douglasii* in the North West of the USA and *C. euphorbiae* from *Euphorbia polycaulis* in Iran (Gramaje et al. 2012, Xie et al. 2013, Nasr et al. 2018).

Collophorina spp. (mostly as *Collophora*) have also been reported from necrotic and symptomless wood and leaves of *Prunus* spp. in Germany, Iran, Slovakia and Spain (Benavides et al. 2013, Ivanová & Bernadovičová 2013, Aghdam & Fotouhifar 2016, Arzanlou et al. 2016, Gierl & Fischer 2017), from necrotic wood of *Castanea sativa* (Yurkewich et al. 2017), from leaves of forest trees and grapevine in France (Fort et al. 2016) and from roots of *Caluna vulgaris* and *Holcus lanatus* in Germany (Kreyling et al. 2012). In addition to plant hosts, there is also one report of *Collophorina* from an animal, namely from the beak of a hummingbird (Belisle et al. 2012). *Collophorina* species were also repeatedly isolated from spore traps (Fischer et al. 2016, Fort et al. 2016, Gierl & Fischer 2017).

Species of *Collophorina* produce whitish, cream or red pigmented colonies and aseptate conidia originating from reduced conidiogenous cells resembling those of the genus *Coniochaeta* (syn. *Lecythophora*), from conidiomata or by microcyclic conidiation. A sexual morph has not been observed. Sanoamuang et al. (2013) discussed *Gelatinomyces* as possible sexual morph of *Collophorina*, but dismissed this assumption after a thorough molecular and morphological comparison.

In two surveys aiming to reveal the diversity of fungi either associated with wood necroses of *Prunus* trees in Germany or captured in spore traps in vineyards in Germany, fungi with a yeast-like appearance and reduced conidiogenous cells were frequently isolated that were tentatively placed in the genus *Collophorina* by ITS sequence comparison. The objective of this study was to investigate the phylogenetic relationships of these strains using molecular phylogenetic analyses of LSU, ITS, *EF-1α* and *GAPDH* sequences and to characterise the species based on molecular, morphological and physiological data.

MATERIALS AND METHODS

Sampling and fungal isolation

Branches with symptomatic wood (e.g., canker, necroses, wood streaking, damaged bark, gummosis) were sampled from plum

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Table 1 List of strains included in this study, with collection details and GenBank accession numbers.

Species	Accession no.[1]	Host / substrate	Country	GenBank no.[2]				References
				LSU	ITS	*EF-1α*	*GAPDH*	
Alatospora acuminata	CCM F-02383	stream foam	Great Britain	KC834018	AY204587	–	–	Baschien et al. (2013)
Alatospora pulchella	CCM F-502*	stream, *Athyrium filix-femina* frond	Czech Republic	KC834019	KC834039	–	–	Baschien et al. (2013)
Aotearoamyces nothofagi	ICMP 21868	unidentified wood	New Zealand	MG807386	KM677202	–	–	Quijada et al. (2018)
	PDD 106298	*Nothofagus fusca*, bark of dead wood	New Zealand	MG807388	MG807392	–	–	Quijada et al. (2018)
Cadophora luteo-olivacea	CBS 141.41*	waste water	Sweden	MH867586	AY249066	KM497089	JN808849	Harrington & McNew (2003)
Capturomyces funiculosus	GLMC 1846*	spore trap on *Vitis vinifera*	Germany	**MK314599**	**MK314552**	**MK314517**	**MK314493**	This study
	GLMC 1848	spore trap on *Vitis vinifera*	Germany	**MK314600**	**MK314553**	**MK314518**	**MK314494**	This study
Capturomyces luteus	GLMC 1842*	spore trap on *Vitis vinifera*	Germany	**MK314603**	**MK314554**	**MK314519**	**MK314495**	This study
Claussenomyces olivaceus	NB-479	*Picea rubens*, resin on branch stub	Canada	KY633629	KY633590	–	–	Tanney & Seifert (2018)
Claussenomyces prasinulus	KL218	rotten wood	Estonia	KX090815	–	–	–	Pärtel et al. (2017)
'*Collophorina*' *aceris*	PC 23	*Acer glabrum* var. *douglasii*	USA	–	KF057075	–	–	Xie et al. (2013)
Collophorina africana	CBS 120872*	*Prunus salicina*, necrotic wood	South Africa	**MK314588**	GQ154570	GQ154643	GQ154648	Damm et al. (2010), this study
	CBS 120879	*Prunus salicina*, necrotic wood	South Africa	GQ154610	GQ154571	GQ154644	GQ154649	Damm et al. (2010)
	GLMC 1736	*Prunus domestica*, necrotic wood	Germany	**MK314581**	**MK314542**	**MK314507**	**MK314474**	This study
	GLMC 1777	*Prunus domestica*, necrotic wood	Germany	**MK314582**	**MK314543**	**MK314508**	**MK314475**	This study
	GLMC 462	*Prunus domestica*, necrotic wood	Germany	**MK314583**	**MK314537**	**MK314504**	**MK314470**	This study
	GLMC 464	*Prunus domestica*, necrotic wood	Germany	**MK314584**	**MK314538**	**MK314509**	**MK314471**	This study
	GLMC 466	*Prunus domestica*, necrotic wood	Germany	**MK314586**	**MK314540**	**MK314505**	**MK314476**	This study
	GLMC 551	*Prunus domestica*, necrotic wood	Germany	**MK314585**	**MK314539**	**MK314510**	**MK314472**	This study
	GLMC 600	*Prunus domestica*, necrotic wood	Germany	**MK314587**	**MK314541**	**MK314506**	**MK314473**	This study
Collophorina badensis	GLMC 1684*	*Prunus domestica*, healthy wood	Germany	**MK314594**	**MK314546**	**MK314503**	**MK314482**	This study
	GLMC 1686	*Prunus domestica*, necrotic wood	Germany	**MK314591**	**MK314547**	**MK314502**	**MK314483**	This study
	GLMC 1546	*Prunus domestica*, necrotic wood	Germany	**MK314589**	**MK314544**	**MK314499**	**MK314479**	This study
	GLMC 1637	*Prunus domestica*, necrotic wood	Germany	**MK314590**	**MK314545**	**MK314500**	**MK314480**	This study
	GLMC 1639	*Prunus domestica*, necrotic wood	Germany	**MK314592**	**MK314549**	**MK314501**	**MK314481**	This study
	GLMC 1844	spore trap on *Vitis vinifera*	Germany	**MK314593**	**MK314548**	**MK314498**	**MK314484**	This study
Collophorina germanica	GLMC 1445*	*Prunus avium*, necrotic wood	Germany	**MK314595**	**MK314550**	**MK314515**	**MK314477**	This study
	GLMC 1769	*Prunus avium*, necrotic wood	Germany	**MK314596**	**MK314551**	**MK314516**	**MK314478**	This study
Collophorina hispanica	CBS 128568*	*Prunus dulcis*, branch	Spain	**MK314597**	MH864962	JN808852	JN808845	Gramaje et al. (2012), this study
	CBS 128566	*Prunus dulcis*, branch	Spain	–	JN808839	JN808850	JN808843	Gramaje et al. (2012)
	CBS 128569	*Prunus dulcis*, branch	Spain	MH876412	JN808842	JN808853	JN808846	Gramaje et al. (2012)
Collophorina neorubra	GLMC 929*	*Prunus avium*, necrotic wood	Germany	**MK314604**	**MK314533**	**MK314511**	**MK314485**	This study
	GLMC 1669	*Prunus avium*, necrotic wood	Germany	**MK314606**	**MK314536**	**MK314514**	**MK314488**	This study
	GLMC 1587	*Prunus avium*, necrotic wood	Germany	**MK314605**	**MK314534**	**MK314512**	**MK314486**	This study
	GLMC 1588	*Prunus avium*, necrotic wood	Germany	**MK314607**	**MK314535**	**MK314513**	**MK314487**	This study
Collophorina rubra	CBS 120873*	*Prunus persica*, necrotic wood	South Africa	**MK314598**	GQ154547	JN808855	JN808848	Damm et al. (2010), Gramaje et al. (2012), this study
	CBS 121441	*Prunus persica* var. *nucipersica*, necrotic wood	South Africa	GQ154607	GQ154551	GQ154642	GQ154647	Damm et al. (2010)
Crinula caliciiformis	AFTOL-ID 272	N/A	N/A	AY544680	KT225524	–	–	Lutzoni et al. (2004)
Epiglia gloeocapsae	M193	moss	Finland	EU940128	EU940204	–	–	Stenroos et al. (2010)
Epithamnolia xanthoriae	HA92	N/A	Iceland	KY814508	KY814526	–	–	Suija et al. (2017)
	HA90	N/A	Netherlands	KY814513	KY814532	–	–	Suija et al. (2017)
Flagellospora curvula	CB-M13	*Cladrastis kentukea*, submerged leaf	USA	KC834024	KC834045	–	–	Baschien et al. (2013)
Flagellospora leucohynchus	CCM F-14183	stream foam	Slovakia	KC834025	KC834049	–	–	Baschien et al. (2013)
Gelatinomyces siamensis	KKUK1*	*Bambusa nutans*	Thailand	JX219381	JX219379	–	–	Sanoamuang et al. (2013)
	KKUK2	*Bambusa nutans*	Thailand	JX219382	JX219380	–	–	Sanoamuang et al. (2013)
Gorgomyces honrubiae	CCM F-12003*	stream foam	Spain	KC834028	KC834057	–	–	Baschien et al. (2013)
Holwaya mucida	AFTOL-ID 272	N/A	N/A	AY544680	KT225524	–	–	Lutzoni et al. (2004)
	B 70 0009352	N/A	N/A	DQ257356	DQ257357	–	–	Wang et al. (2006)

Table 1 (cont.)

Species	Accession no.[1]	Host / substrate	Country	GenBank no.[2]				References
				LSU	ITS	*EF-1α*	*GAPDH*	
Mniaecia jungermanniae	M145	moss	Finland	EU940109	EU940185	–	–	Stenroos et al. (2010)
Mniaecia nivea	M167	moss	Finland	EU940115	EU940188	–	–	Stenroos et al. (2010)
Pallidophorina paarla	CBS 120877*	*Prunus salicina*, necrotic wood	South Africa	**MK314610**	GQ154586	GQ154646	GQ154651	Damm et al. (2010), this study
	CBS 120878	*Prunus salicina*, necrotic wood	South Africa	GQ154611	GQ154575	JN808854	JN808847	Damm et al. (2010), Gramaje et al. (2012)
	GLMC 452	*Prunus cerasus*, healthy wood	Germany	**MK314608**	**MK314555**	**MK314524**	**–**	This study
	GLMC 1282	*Prunus domestica*, necrotic wood	Germany	**MK314609**	**MK314561**	**MK314529**	**–**	This study
	GLMC 780	*Prunus domestica*, necrotic wood	Germany	**MK314611**	**MK314559**	**MK314525**	**–**	This study
	GLMC 791	*Prunus cerasus*, necrotic wood	Germany	**MK314612**	**MK314560**	**MK314527**	**–**	This study
	GLMC 892	*Prunus avium*, necrotic wood	Germany	**MK314614**	**MK314556**	**MK314528**	**–**	This study
	GLMC 1230	*Prunus avium*, necrotic wood	Germany	**MK314615**	**MK314557**	**MK314526**	**–**	This study
	GLMC 1497	*Prunus avium*, necrotic wood	Germany	**MK314613**	**MK314558**	**MK314530**	**–**	This study
Ramoconidiophora euphorbiae	CBS 141018*	*Euphorbia polycaulis*	Iran	**MK314602**	MG592739	MG592735	MG592733	Nasr et al. (2018), this study
	IBRC-M 30208	*Euphorbia polycaulis*	Iran	**MK314601**	MG592740	MG592736	MG592734	Nasr et al. (2018), this study
Tympanis abietina	CBS 350.55	*Abies balsamea*	Canada	**MK314617**	**MK314563**	**–**	–	This study
Tympanis acericola	CBS 351.55[aut]	*Acer spicatum*	Canada	**MK314631**	**MK314564**	**–**	–	This study
'*Tympanis*' *alnea*	CBS 352.55	*Alnus*	Canada	**MK314635**	**MK314580**	**–**	–	This study
Tympanis amelanchieris	CBS 353.55*	*Amelanchier*	Canada	MH869048	MH857508	–	–	Vu et al. (2019)
Tympanis confusa	CBS 354.55	*Pinus resinosa*	USA	**MK314619**	**MK314568**	**–**	–	This study
Tympanis conspersa	CBS 355.55	*Malus sylvestris*	USA	**MK314618**	**MK314573**	**–**	–	This study
Tympanis diospyri	CBS 356.55*	*Diospyros virginana*	USA	MH869049	MH857509	–	–	Vu et al. (2019)
Tympanis fasciculata	CBS 357.55	*Viburnum cassioides*	Canada	**MK314620**	**MK314565**	**–**	–	This study
Tympanis hansbroughiana	CBS 358.55*	*Pseudotsuga menziesii*	USA	MH869050	MH857510	–	–	Vu et al. (2019)
Tympanis inflata	GLMC 1856*	spore trap on *Vitis vinifera*	Germany	**MK314625**	**MK314566**	**MK314532**	**MK314496**	This study
Tympanis laricina	CBS 360.55	*Larix laricina*	Canada	**MK314621**	**MK314570**	**–**	–	This study
'*Tympanis*' *malicola*	CBS 221.69	*Malus sylvestris*	Netherlands	**MK314632**	**MK314579**	**–**	–	This study
Tympanis piceae	CBS 361.55*	*Picea glauca*	Canada	MH869051	MH857511	–	–	Vu et al. (2019)
Tympanis piceina	CBS 362.55[aut]	*Picea abies*	Sweden	MH869052	MH857512	–	–	Vu et al. (2019)
Tympanis pitya	CBS 363.55	*Pinus resinosa*	USA	**MK314623**	**MK314569**	**–**	–	This study
Tympanis prunicola	CBS 364.55[aut]	*Prunus*	Canada	MH869053	MH857513	–	–	Vu et al. (2019)
'*Tympanis*' *pseudotsugae*	CBS 365.55*	*Pseudotsuga menziesii*	USA	**MK314633**	MH857514	–	–	Vu et al. (2019), this study
	CBS 463.59	*Pseudotsuga menziesii*	Canada	**MK314634**	**MK314578**	**–**	–	This study
Tympanis saligna	CBS 366.55	*Salix discolor*	Canada	**MK314626**	**MK314567**	**–**	–	This study
Tympanis spermatiospora	CBS 367.55	*Populus*	Canada	**MK314624**	**MK314571**	**–**	–	This study
Tympanis truncatula	CBS 368.55	*Abies balsamea*	Canada	**MK314622**	**MK314572**	**–**	–	This study
Tympanis tsugae	CBS 369.55*	*Tsuga canadensis*	Canada	MH869054	MH857515	–	–	Vu et al. (2019)
'*Tympanis*' *xylophila*	CBS 133220	*Fraxinus excelsior*, decayed branch	Luxembourg	MH877529	MH866059	–	–	Vu et al. (2019)
Variabilispora flava	GLMC 1858*	spore trap on *Vitis vinifera*	Germany	**MK314616**	**MK314562**	**MK314531**	**MK314497**	This study
Vexillomyces palatinus	GLMC 1852*	spore trap on *Vitis vinifera*	Germany	**MK314627**	**MK314574**	**MK314520**	**MK314489**	This study
Vexillomyces verruculosus	GLMC 1854*	spore trap on *Vitis vinifera*	Germany	**MK314629**	**MK314576**	**MK314522**	**MK314492**	This study
	GLMC 1840	spore trap on *Vitis vinifera*	Germany	**MK314630**	**MK314577**	**MK314523**	**MK314491**	This study
	GLMC 1838	spore trap on *Vitis vinifera*	Germany	**MK314628**	**MK314575**	**MK314521**	**MK314490**	This study

[1] AFTOL: Assembling the Fungal Tree of Life project, USA; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; GLMC: Culture collection of Senckenberg Museum of Natural History Görlitz, Görlitz, Germany; IBRC: Iranian Biological Center, Tehran, Iran; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; KKUK: Culture collection of Khon Kaen University, Khon Kaen, Thailand; PDD: New Zealand Fungal Herbarium, Auckland, New Zealand

[2] LSU: nuclear large subunit ribosomal DNA; ITS: internal transcribed spacers and intervening 5.8S nrDNA; *EF-1α*: translation elongation factor 1-α gene; *GAPDH*: partial glyceraldehyde-3-phosphate dehydrogenase gene. Sequences generated in this study are emphasised in **bold**.

*ex-type cultures; [aut]authentic strains; N/A not available.

(*P. domestica*), sour cherry (*P. cerasus*) and sweet cherry (*P. avium*) orchards in Saxony, Lower Saxony and Baden-Württemberg, Germany, in 2015 and 2016. Wood pieces (5 × 5 × 5 mm) from the transition zone of symptomatic to non-symptomatic wood tissue as well as pieces of the same size from non-symptomatic wood of the same branch were surface sterilised (30 s in 70 % ethanol, 1 min in 3.5 % NaOCl, 30 s in 70 % ethanol) washed for 1 min in sterilised water and plated on synthetic nutrient-poor agar medium (SNA, Nirenberg 1976) as well as oatmeal agar medium (OA; Crous et al. 2009), both supplemented with 100 mg/L penicillin, 50 mg/L streptomycin sulphate and 1 mg/L chloramphenicol.

Additionally, glass slides covered with petroleum jelly (Balea Vaseline, DM, Karlsruhe, Germany) were attached to vines of *Vitis vinifera* in a research vineyard of Julius-Kühn-Institute Siebeldingen, Rhineland-Palatinate, Germany, in 2016 and 2017. The slides were exchanged on a weekly basis and washed for 10 s with 30 mL washing solution (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4, 0.01 % Tween® 80) followed by filtration first with a 5 µm filter, followed by a 0.45 µm filter. Further 500 µL washing solution were used for washing off the spores and particles from the 0.45 µm filter that were subsequently plated out on each two malt-yeast agar plates (MYA, 250 µL per plate; Crous et al. 2009).

After incubation for several days at 25 °C, hyphal tips of developing fungi were transferred to SNA medium with a sterilised needle. Single-conidial isolates were obtained from the strains for further study. Reference strains are maintained in the culture collections of the Senckenberg Museum of Natural History Görlitz, Germany (GLMC), the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (CBS) and the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (DSMZ). Specimens (dried cultures), including type specimens were deposited in the fungarium of Senckenberg Museum of Natural History Görlitz (GLM).

Morphological analysis

To enhance sporulation autoclaved filter paper and double-autoclaved pine needles were placed on the surface of the SNA medium. The cultures were incubated at 25 °C. Colony growth on SNA and OA were noted after 2 and 4 wk, colony characters on SNA and OA were noted after 4 wk. Colony colours were rated according to Rayner (1970). Microscopic preparations were made after 4 wk in clear lactic acid and observations and measurements (30 measurements per structure) were made with a Nikon SMZ18 stereomicroscope (SM) or with a Nikon Eclipse N*i*-U light microscope with differential interference contrast (LM). Photographic images were captured with Nikon Digital Sight DS-Fi2 cameras installed on the above-mentioned microscopes making use of the Nikon NIS-Elements software (v. 4.30).

Phylogenetic analysis

Genomic DNA of the isolates was extracted using the method of Damm et al. (2008). A partial sequence of the 28S nrDNA (LSU) and the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 were amplified and sequenced using the primer pairs LROR (Rehner & Samuels 1994) + LR5 (Vilgalys & Hester 1990) and ITS-1F (Gardes & Bruns 1993) + ITS-4 (White et al. 1990), respectively. Additionally, a partial sequence of the translation elongation factor 1α (*EF-1α*) and a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were amplified using the primer pairs EF1-728F + EF1-986R (Carbone & Kohn 1999) and GDF1 + GDR1 (Guerber et al. 2003), respectively.

The reaction mixture for PCR contained 1 µL of 1 : 10 DNA template, 2.5 µL 10× buffer (Peqlab, Erlangen, Germany), 1 µL of each primer (10 mM), 2.5 µL $MgCl_2$ (25 mM), 0.1 µL *Taq* polymerase (0.5U, Peqlab, Erlangen, Germany) and 2.5 µL of 2 mM dNTPs. Each reaction was made up to a final volume of 20 µL with sterile water. DNA amplifications of ITS were carried out in a Mastercycler® pro S (Eppendorf, Hamburg, Germany) programmed for an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94, 51 and 72 °C for 30, 30 and 60 s, respectively, with a 3 min extension at 72 °C on the final cycle. For DNA amplifications of LSU the PCR conditions were set according to Paulin & Harrington (2000). The PCR conditions for the amplification of the *EF-1α* and *GAPDH* were those as described in the respective references listed above. The PCR products were visualised on a 1 % agarose gel and sequenced by the Senckenberg Biodiversity and Climate Research Centre (BiK-F) laboratory (Frankfurt, Germany). The forward and reverse sequences were assembled by using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall 1999).

Sequences of all *Collophorina* species as well as other reference sequences of *Leotiomycetes*, especially those of the ex-type strains, were downloaded from GenBank and added to the sequences generated in this study and those of the outgroup *Cadophora luteo-olivacea* CBS 141.41. Two sequence datasets were compiled. In dataset 1 the sequences from this study as well as sequences of *Collophorina* species were combined with other sequences from *Leotiomycetes* for a two gene phylogeny (LSU, ITS) to resolve the generic placement of the strains. In dataset 2 the sequences generated in this study were combined with sequences from all formerly described species of *Collophorina* for a four gene phylogeny (LSU, ITS, *EF-1α*, *GAPDH*) to determine species identification. The datasets were aligned automatically using MAFFT v. 7.308 (Katoh et al. 2002, Katoh & Standley 2013) and manually adjusted where necessary.

The phylogenetical analyses were conducted using Bayesian Inference (BI), Maximum Likelihood (ML) and maximum parsimony (MP). For BI analyses, the best fit model of evolution was estimated by MEGA7 (Kumar et al. 2016) for each partition. Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) as implemented in Geneious v. 10.2.2 (Kearse et al. 2012), using the estimated models of evolution. Four simultaneous Markov chains were run for 1 Mio generations and trees were sampled every 100th generation. The first 2000 trees, which represent the burn-in phase of the analyses, were discarded and the remaining 8000 trees were used to calculate posterior probabilities in the majority rule consensus tree. The ML analyses were performed by RAxML v. 8.2.11 (Stamatakis 2006, 2014) as implemented in Geneious v. 10.2.2 (Kearse et al. 2012) using the GTRGAMMA model with the rapid bootstrapping and search for best scoring ML tree algorithm including 1000 bootstrap replicates. The MP analyses were performed with MEGA7 (Kumar et al. 2016) using tree-bisection-reconnection (TBR) as branch-swapping algorithm. The robustness of the trees was evaluated by 1000 bootstrap replicates and 10 random sequence additions. Tree length, consistency index, retention index and composite index were calculated for the resulting trees. The DNA sequences generated in this study were deposited in GenBank (Table 1), the alignments in TreeBASE (https://treebase.org/treebase-web/home.html) (23717) and taxonomic novelties in MycoBank (www.mycobank.org; Crous et al. 2004).

RESULTS

Phylogenetic analyses

In total 152 out of 1018 isolates from necrotic wood of *Prunus* spp. and 10 out of 810 isolates from spore traps mounted on grapevine vines were tentatively identified as *Collophorina* spe-

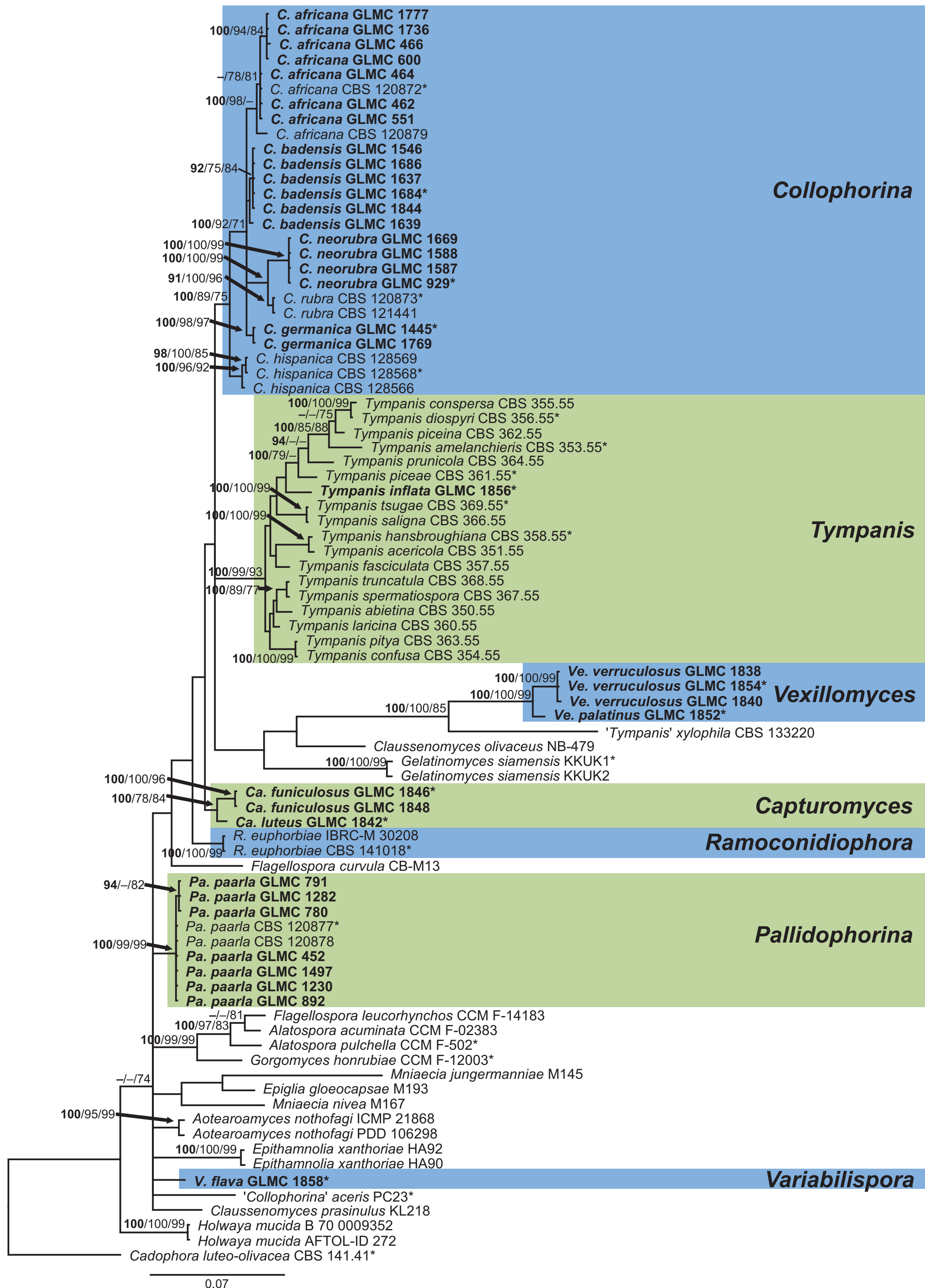

Fig. 1 Phylogeny obtained by bayesian inference analysis of the combined LSU and ITS sequence alignment of species from *Tympanidaceae*. BI posterior probability support values above 90 % (**bold**), ML and MP bootstrap support values above 70 % are shown at the nodes. *Cadophora luteo-olivacea* strain CBS 141.41 is used as outgroup. Numbers of ex-type strains are emphasised with an asterisk (*). Strains analysed in this study are emphasised in **bold**.

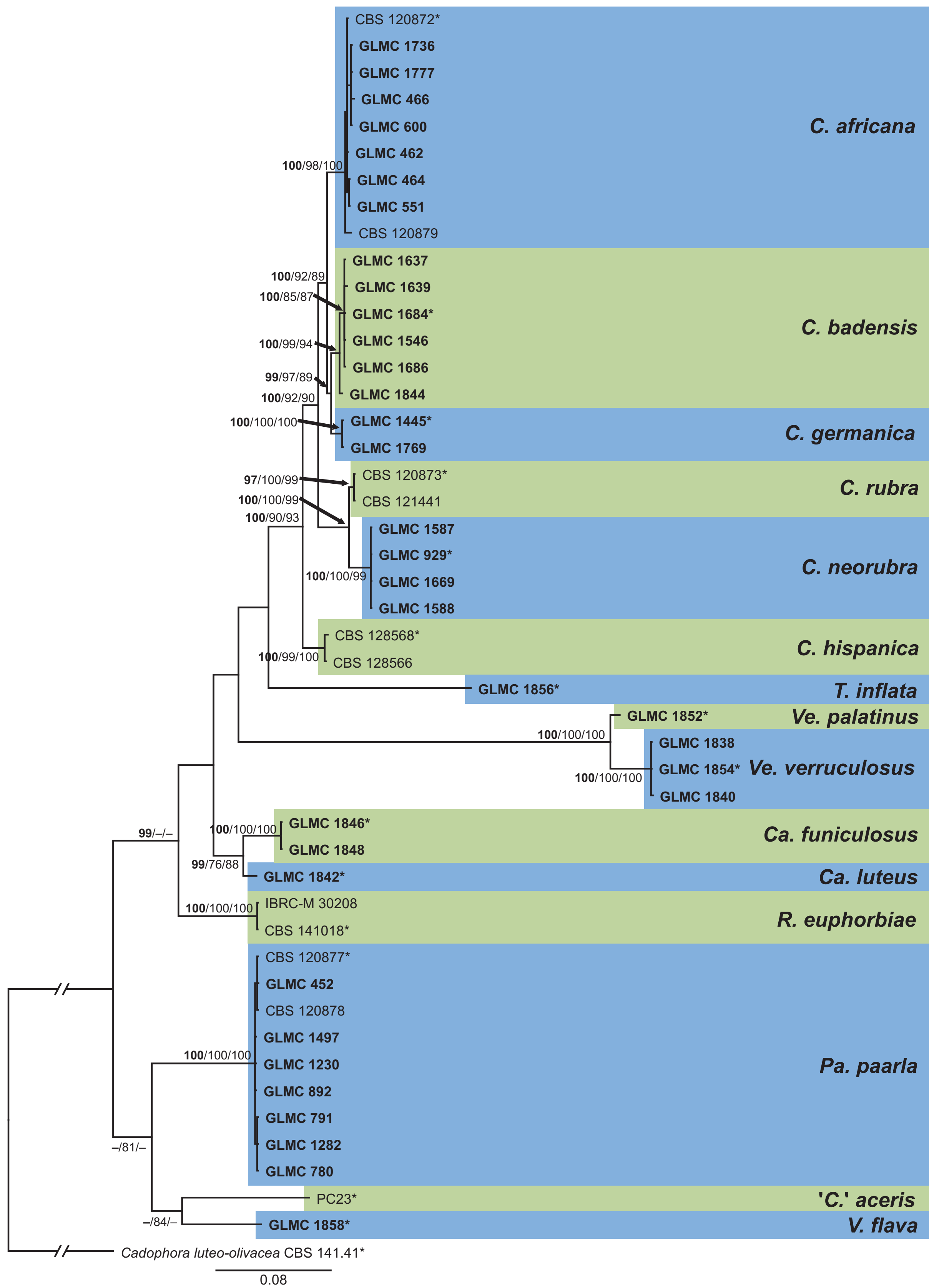

Fig. 2 Phylogeny obtained by bayesian inference analysis of the combined LSU, ITS, *EF-1α*, and *GAPDH* sequence alignment of collophorina-like species. BI posterior probability support values above 90 % (**bold**), ML and MP bootstrap support values above 70 % are shown at the nodes. *Cadophora luteo-olivacea* strain CBS 141.41 is used as outgroup. Numbers of ex-type strains are emphasised with an asterisk (*). Strains analysed in this study are emphasised in **bold**. Branches that are crossed by diagonal lines are shortened by 50 %.

cies based on morphological similarities and blastn searches with the ITS region. One hundred and twelve of the 152 isolates from *Prunus* wood showed a high morphological and sequence similarity with *C. paarla*. The 10 isolates from spore traps and 25 randomly chosen isolates from *Prunus* wood were selected for DNA sequence analyses.

The combined sequence dataset 1 consisted of 84 isolates including the outgroup and comprised 1 484 characters, of which 322 characters were parsimony-informative, 425 parsimony-uninformative and 896 constant. The gene boundaries in the multi-locus alignment were as follows: LSU: 1–903, ITS: 904–1484. The most parsimonious tree was generated by MP analysis with tree length: 854 steps, consistency index: 0.423423, retention index: 0.810250 and composite index: 0.385201 and 0.343079 for all sites and parsimony informative sites, respectively. The BI phylogeny obtained by bayesian inference including BI posterior probability values as well as ML (lnL = -8073.184749) and MP bootstrap support values is shown in Fig. 1.

Eighteen strains from *Prunus* wood and one strain from a spore trap (GLMC 1844) isolated in this study form a well-supported clade (100/81/74 % support) together with the ex-type and further strains of the type species of the genus *Collophorina*, *C. rubra*, as well as *C. africana* and *C. hispanica*. This clade consists of six subclades, of which most of them are well-supported. Strain GLMC 1856 isolated from a spore trap integrates in a well-supported clade (100/99/93 % support) of strains of different *Tympanis* species, including one of the type species *T. saligna*. Four strains, GLMC 1838, GLMC 1840, GLMC 1854 and GLMC 1852, isolated from spore traps, form a well-supported clade (100/100/99 % support) next to a strain identified as *Tympanis xylophila* (CBS 133220) which is, however, separated from the *Tympanis* main clade. Strains GLMC 1848, GLMC 1846 and GLMC 1842 isolated from spore traps form a well-supported clade (100/78/84 % support) next to the *C. euphorbiae* clade, including its ex-type strain. A further seven strains from *Prunus* wood form a well-supported clade (100/99/99 % support) with strains of *C. paarla*, including its ex-type strain. Strain GLMC 1858 isolated from spore traps does not integrate into any clade formed by reference strains. Within this phylogeny, previously described species of *Collophorina* do not form a monophyletic clade. A well-supported clade (100/89/75 %) including the type species, *C. rubra*, as well as *C. africana*, *C. hispanica* and three new species recognised in this study is formed excluding *C. aceris*, *C. euphorbiae* and *C. paarla*. Therefore, the genus *Collophorina* is recognised as polyphyletic. The clades, the strains studied in this paper belong to, are consistent in both single LSU and single ITS phylogenies calculated with all three algorithms (BI/ML/MP). Clades consisting only of collophorina-like species are separated by clades formed by strains of *Tympanis*, *Gelatinomyces*, *Aotearoamyces* and strains identified as *Alatospora*, *Epithamnolia*, *Flagellospora*, *Gorgomyces* and *Claussenomyces* spp. No further grouping of the seven main clades consisting of or including collophorina-like species was supported in the LSU-ITS tree.

The combined sequence dataset 2 consisted of 47 isolates including the outgroup and comprised 1 829 characters, of which 405 characters were parsimony-informative, 536 parsimony-uninformative and 1 202 constant. The gene boundaries in the multi-locus alignment were as follows: LSU: 1–858, ITS: 859–1409, *EF-1α*: 1410–1680, *GAPDH*: 1681–1829. Two most parsimonious trees were generated by MP analysis with tree length: 737 steps, consistency index: 0.639319, retention index: 0.894570, and composite index: 0.611755 and 0.571916 for all sites and parsimony informative sites, respectively. The BI phylogeny obtained by bayesian inference including BI posterior probability values as well as ML (lnL = -7839.866374) and MP bootstrap support values is shown in Fig. 2.

The phylogeny exhibits 15 clades, six of them representing previously defined species. Each seven isolates from necrotic wood of *Prunus* spp. formed well-supported clades with the ex-type strains of *C. africana* and *C. paarla*, respectively (100/98/100 % and 100/100/100 % support). Five isolates from necrotic wood of *P. domestica* sampled in Baden-Württemberg together with an isolate from a spore trap in Rhineland-Palatinate formed a distinct clade (100/99/94 % support), sister to a clade (100/100/100 % support) formed by two isolates from necrotic wood of *P. avium* sampled in Baden-Württemberg and Lower Saxony (GLMC 1445, GLMC 1769), respectively. Four isolates from necrotic wood of *P. avium* sampled in Saxony, Lower Saxony and Baden-Württemberg form a distinct clade (100/100/99 % support), sister to a clade formed by *C. rubra*. Further six distinct clades were formed by isolates from spore traps in Rhineland-Palatinate, two of them by two or three isolates, respectively, and four of them by a single strain each. All single- and multi-locus BI/ML/MP phylogenies showed similar tree typologies.

TAXONOMY

Based on the phylogenetic analyses and sequence comparisons, the strains studied here belong to 11 species in six genera. Nine species that were isolated from necrotic wood of *Prunus* spp. or from spore traps mounted on *Vitis vinifera* vines in Germany were revealed to be new to science and therefore described below. *Collophorina euphorbiae* and *C. paarla* are combined in two newly erected genera, respectively. Further three genera are newly described.

Capturomyces S. Bien, C. Kraus & Damm, *gen. nov.* — MycoBank MB829151

Etymology. Name reflects the way all strains of this genus were retrieved through capture (Lat.: *captura*) of spores with spore traps.

Type species. *Capturomyces funiculosus* S. Bien, C. Kraus & Damm.

Colonies slow-growing, moist, white, buff or yellow colours on oatmeal agar medium, with sparse or lacking aerial mycelium. *Sporulation* conidia formed in conidiomata, on hyphal cells and by microcyclic conidiation. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to discrete phialides, short adelophialides or more often with collarettes formed directly on hyphal cells. *Conidia* aggregated in masses around the hyphae and on the agar surface. *Conidiomata* solitary or aggregated, immersed to superficial, subglobose, uni- to multilocular, dehiscence irregular, appearing cup-shaped when mature. *Conidiophores* hyaline, branched or unbranched, septate. *Conidiogenous cells* enteroblastic, hyaline, conidiogenous loci formed laterally in each cell just below the septum as well as terminally (acropleurogenous). *Conidia* of conidiomata and intercalary hyphal cells small, hyaline, 1-celled, oblong or cylindrical to ellipsoidal, straight or slightly curved.

Capturomyces funiculosus S. Bien, C. Kraus & Damm, *sp. nov.* — MycoBank MB829153; Fig. 3a–b, 4

Etymology. Named after the funiculose mycelium on OA medium.

Typus. Germany, Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 17 Mar. 2016, *C. Kraus* (GLM-F112544 holotype; GLMC 1846 = CBS 144840 = DSM 107778 = JKI-Mz50 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, 1–2.5 µm wide, smooth-walled, lacking

chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple, mostly reduced to conidiogenous cells, directly formed on hyphae, conidiogenous loci formed terminally, 4–10 × 2–3 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, often reduced to mere openings with collarettes formed directly on hyphal cells, adelophialides and discrete phialides, navicular to subcylindrical, often constricted at the base, 3.5–9.5 × 1.5–2 µm; collarettes hardly visible, short tubular, 0.5–1 µm long, opening 1–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong to ellipsoidal, sometimes slightly curved, with both ends rounded, sometimes with a barely visible scar on one end, 3–5.5(–8.5) × (1–)1.5–2(–2.5) µm, mean ± SD = 4.4 ± 1.2 × 1.7 ± 0.3 µm, L/W ratio = 2.6. *Conidiomata* and *endoconidia* not observed. *Microcyclic conidiation* occurs, from minute collarettes at one or sometimes both ends of primary conidia that develop into swollen mother cells, often thick-walled, sometimes septate, > 5 µm long, 2–3.5 µm wide.

Colonies on OA flat to very low convex with entire to undulate margin, whitish to buff, mycelium on the surface appressed funiculose, aerial mycelium appearing after > 4 wk at the outer margin of the culture, sparse, villose, white to brown; reverse same colours, 8–20 mm diam in 2 wk, 26–36 mm diam in 4 wk; *on SNA* flat with entire to dentate margin, lacking aerial mycelium; whitish to buff; reverse same colours; 8–14 mm diam in 2 wk, 16–28 mm diam in 4 wk.

Additional material examined. Germany, Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 17 Mar. 2016, *C. Kraus*, GLM-F112545, culture GLMC 1848 = CBS 144841 = DSM 107779 = JKI-Mz53.

Notes — Isolates of *Capturomyces funiculosus* from spore traps in Rhineland-Palatinate did not produce any pigments on OA medium, similar to '*Collophorina*' *aceris*, *Pallidophorina*

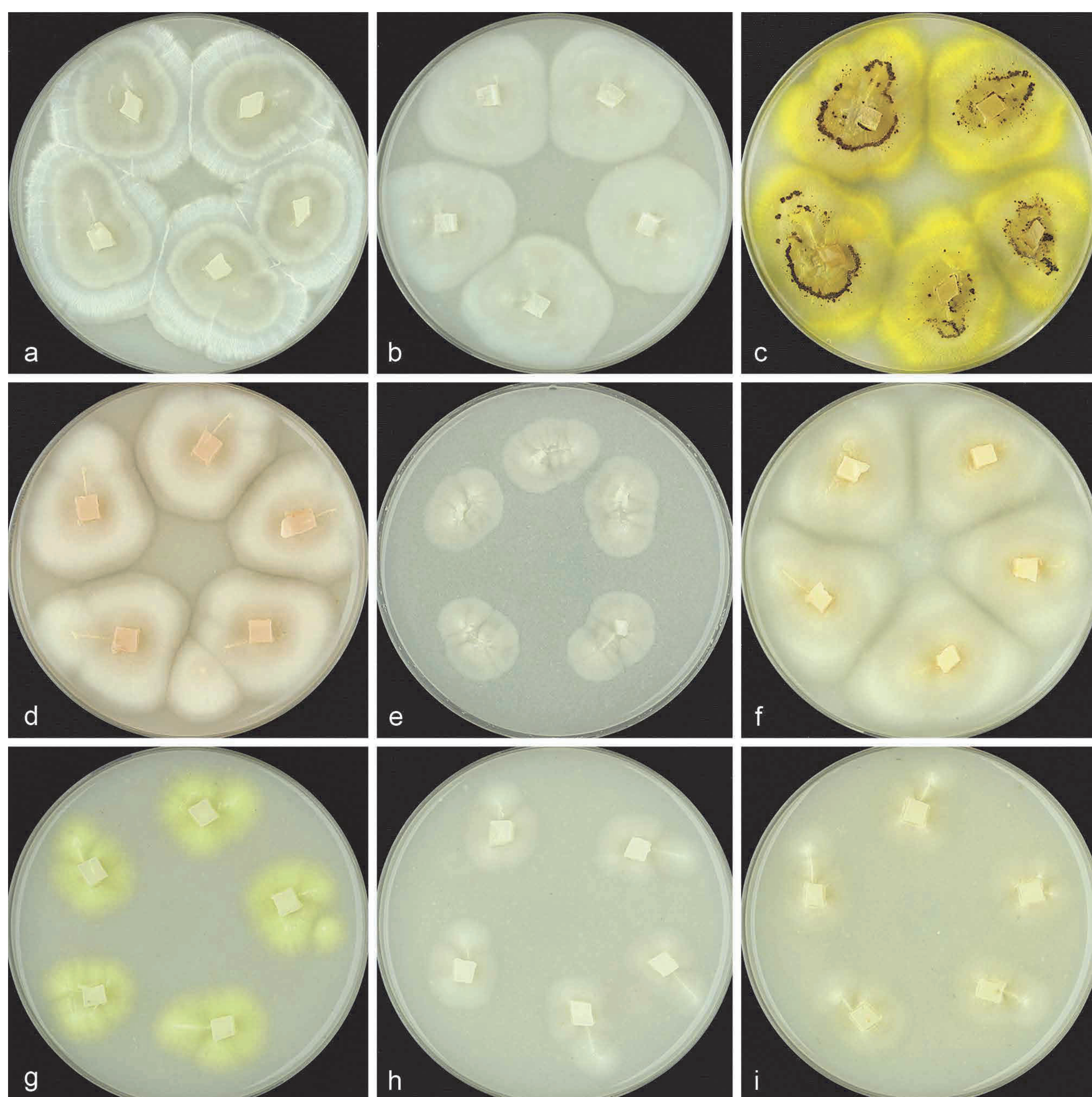

Fig. 3 Colony surface of collophorina-like species on OA medium after 4 wk. a. *Capturomyces funiculosus* GLMC 1848; b. *Ca. funiculosus* GLMC 1846*; c. *Ca. luteus* GLMC 1842*; d. *Pallidophorina paarla* CBS 120877*; e. *Ramoconidiophora euphorbiae* CBS 141018*; f. *Tympanis inflata* GLMC 1856*; g. *Variabilispora flava* GLMC 1858*; h. *Vexillomyces palatinus* GLMC 1852*; i. *Ve. verruculosus* GLMC 1854*. Strains with an asterisk are ex-type cultures.

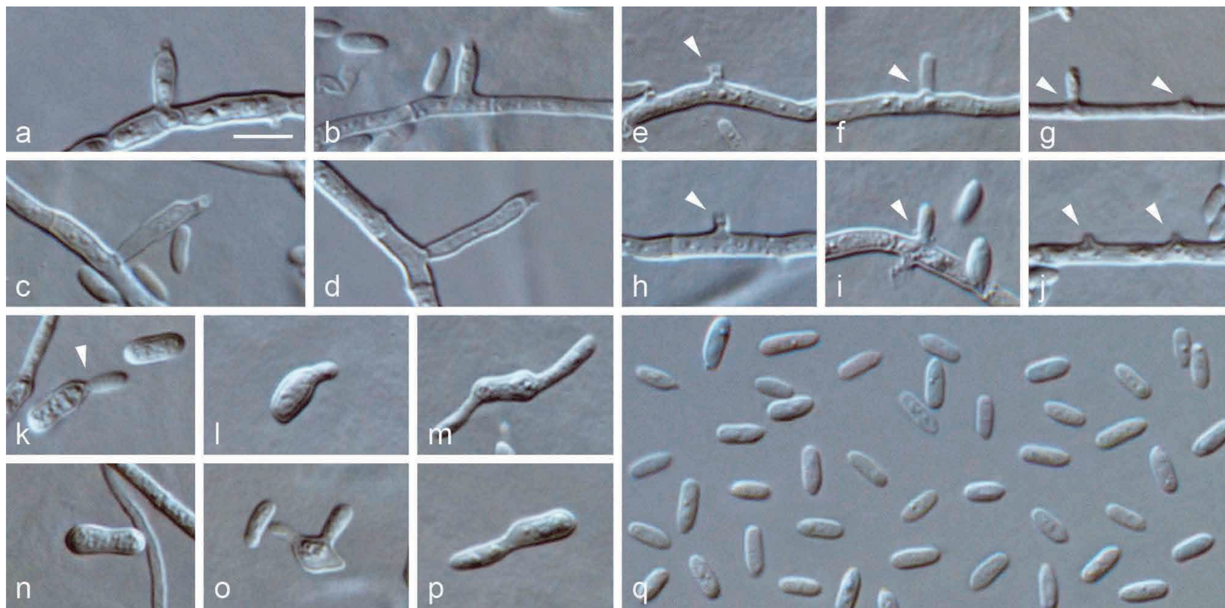

Fig. 4 *Capturomyces funiculosus*. a–j. Conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings or short necks); k–p. mother cells (arrow indicates conidiogenous opening); q. conidia formed on hyphal cells. a–q. From SNA. a–q. LM. — Scale bar: a = 5 µm; scale bar of a applies to b–q.

paarla, *Ramoconidiophora euphorbiae*, *Tympanis inflata*, *Vexillomyces palatinus* and *Ve. verruculosus*. The closest relative is *Capturomyces luteus* with one, 13, 22 and 11 nucleotide differences in the LSU, ITS, *EF-1α* and *GAPDH* sequences, respectively. In contrast to *Ca. luteus*, *Ca. funiculosus* lacks a yellow pigment in OA cultures and conidiomata were not observed. In a blastn search in GenBank, the ITS sequence of *Ca. funiculosus* showed 100 % identity with an unidentified ascomycete from a stump of *Picea abies* in Finland (MG190490, 92 % coverage, J Kaitera & HM Henttonen unpubl. data) and a *Leotiomycetes* sp. from bark tissue of *Tsuga canadensis* in Canada (KX589233, 90 % coverage, KM Complak et al. unpubl. data).

Capturomyces luteus S. Bien, C. Kraus & Damm, *sp. nov.* — MycoBank MB829154; Fig. 3c, 5

Etymology. Named after its luteous colonies on OA medium.

Typus. Germany, Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 6 May 2016, *C. Kraus* (GLM-F112542 holotype; GLMC 1842 = CBS 144839 = DSM 107780 = JKI-Mai12 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, lacking chlamydospores, 1–2.5 µm wide. *Sporulation* abundant, conidia formed directly on hyphal cells, in conidiomata and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple, constricted at the base, conidiogenous loci formed terminally, mostly reduced to conidiogenous cells, directly formed on hyphae. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, mostly reduced to mere openings with collarettes or short necks formed directly on hyphal cells, discrete phialides and adelophialides rarely observed, subcylindrical to navicular, constricted at the base, 4–6 × 2 µm; necks short, cylindrical, 1–1.5 × 1–1.5 µm; collarettes rarely visible, tubular, 0.5–1 µm long, opening 0.5–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong to ellipsoidal, mostly straight, sometimes slightly curved, with both ends rounded, sometimes with a prominent scar on one end, 3–5.5(–7) × 1.5–2(–2.5) µm, mean ± SD = 4.3 ± 1.1 × 1.7 ± 0.3 µm, L/W ratio = 2.5. *Conidiomata* produced on pine needles, on OA and on SNA in > 4 wk; solitary or aggregated, subglobose, uni- to multilocular, immersed to superficial, 60–400 µm wide, light brown to dark brown, sometimes nearly glabrous, but mostly densely covered with hairs, opening with an irregular rupture, often showing a light-coloured inner part with a darker, central dot or elongated stripe. *Conidiophores* hyaline, smooth-walled, septate, sometimes branched at the base and above, straight or slightly zigzag-shaped, often constricted at the septa, 10–35 µm long, conidiogenous loci formed terminally as well as intercalary, immediately below the septum. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, 5–7.5 × 1.5–2.5 µm; collarettes tubular, often inconspicuous, < 0.5–1 µm long, opening 0.5–1 µm, periclinal thickening sometimes visible. *Conidia* hyaline, smooth-walled, cylindrical to ellipsoidal, sometimes slightly curved, with both ends rounded, 3–4(–4.5) × 1.5–2 µm, mean ± SD = 3.6 ± 0.4 × 1.6 ± 0.1 µm, L/W ratio = 2.3. *Endoconidia* not observed. *Microcyclic conidiation* occurs from minute collarettes at one or both ends of primary conidia that develop into swollen mother cells, often thick-walled, sometimes septate, > 5 µm long, 3–4 µm wide.

Colonies on OA flat to very low convex with entire to undulate margin, lacking aerial mycelium; buff, with scattered sienna to umber spots due to conidiomata formation, spore mass oozing from conidiomata buff, after > 4 wk colony pale luteous, luteous to amber; reverse same colours, 16–20 mm diam in 2 wk, 26–34 mm diam in 4 wk; *on SNA* flat with fimbriate to rhizoid margin, lacking aerial mycelium; initially white, after > 4 wk with fulvous to sienna spots due to conidiomata formation; reverse same colours; 6–10 mm diam in 2 wk, 12–16 mm diam in 4 wk.

Notes — *Capturomyces luteus* differs from all other species of collophorina-like fungi by a luteous pigment formed in OA cultures. The closest relative is *Ca. funiculosus* with one, 13, 22 and 11 nucleotide differences in the LSU, ITS, *EF-1α* and the partly generated *GAPDH* sequence (only the second half of the *GAPDH* sequence is available), respectively. Although morphologically similar, *Ca. luteus* can be easily differentiated from *Ca. funiculosus* by the yellow pigment produced on OA as well as by the abundant development of conidiomata. In a blastn search in GenBank, the ITS sequence of *Ca. luteus* showed 100 % identity with strains (HM240822, 89 % cover-

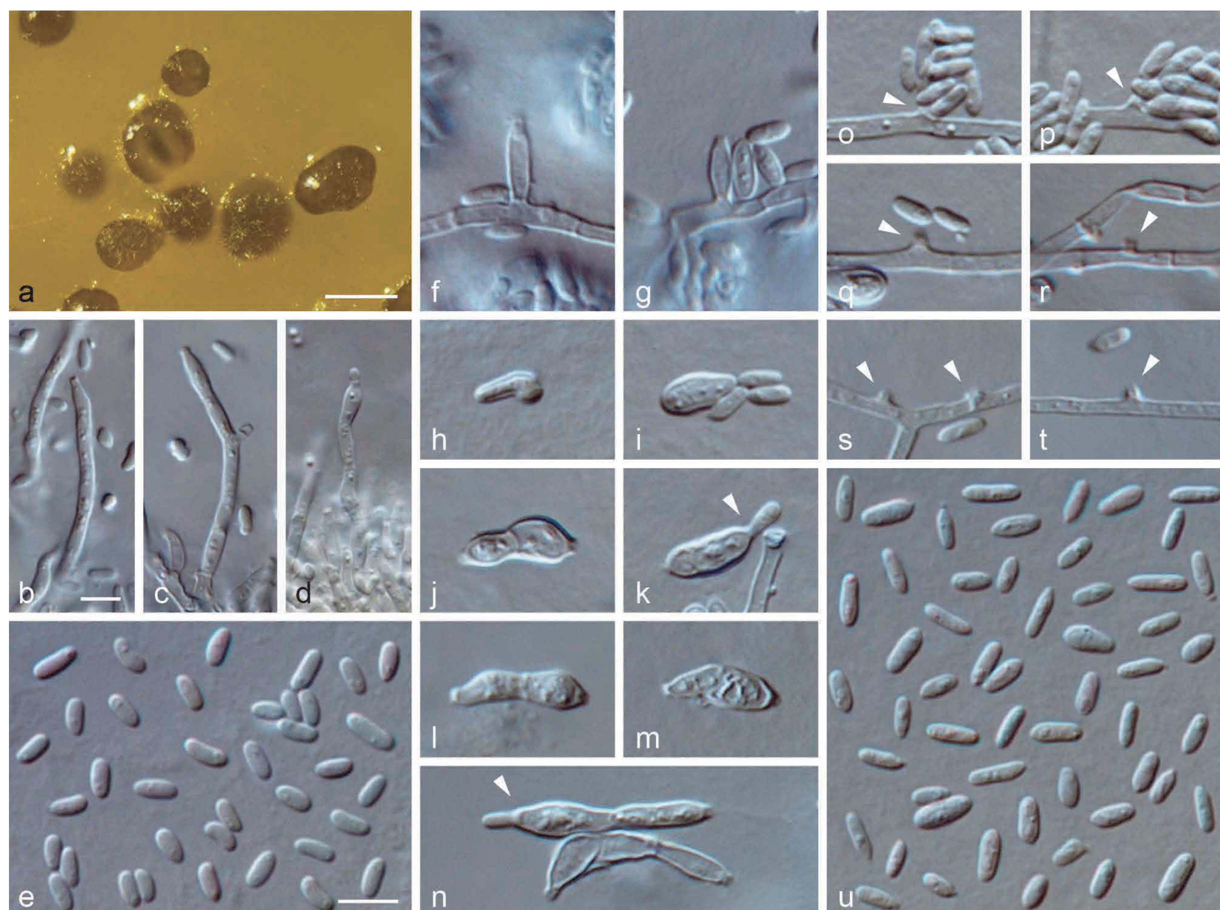

Fig. 5 *Capturomyces luteus*. a. Conidiomata; b–d. conidiogenous cells lining the inner wall of a conidioma; e. conidia formed in conidiomata; f–g, o–t. conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings or short necks); h–n. mother cells (arrows indicate microcyclic conidiation); u. conidia formed on hyphal cells. a–e. From OA; f–u. from SNA. a. SM; b–u. LM. — Scale bars: a = 300 µm, b, e = 5 µm; scale bar of b applies to c–d, scale bar of e applies to f–u.

age; MG190551, 92 % coverage,) isolated from a needle and a stump, respectively, of *Pinus sylvestris* in Finland (Terhonen et al. 2011; J Kaitera & HM Henttonen unpubl. data). An isolate from healthy twigs of *P. sylvestris* in Spain (JX421713) showed a 99 % identity (4 nucleotide differences, 95 % coverage, Sanz-Ros et al. 2015).

Collophorina africana (Damm & Crous) Damm & Crous, Fungal Diversity 86: 111. 2017; Fig. 6a

Basionym. Collophora africana Damm & Crous, Persoonia 24: 65. 2010.
Synonym. Collophora capensis Damm & Crous, Persoonia 24: 67. 2010.

Typus. South Africa, Western Cape Province, Paarl, from reddish brown necrosis in wood of *P. salicina*, 10 June 2004, *U. Damm* (CBS H-19993 holotype; CBS 120872 = STE-U 6113 = GLMC 1882 culture ex-type).

A description is provided in Damm et al. (2010).

Additional materials examined. Germany, Baden-Württemberg, in orchard south of Oppenau, N48°27'58.4" E8°09'26.7", from brown wedge-shaped necrosis in wood of *P. domestica*, 24 Aug. 2016, *S. Bien*, GLM-F110819, culture GLMC 1736 = CBS 144835 = DSM 107849; Baden-Württemberg, in orchard south of Oppenau, N48°27'58.4" E8°09'26.7", from brown wedge-shaped necrosis in wood of *P. domestica*, 24 Aug. 2016, *S. Bien*, GLM-F110860, culture GLMC 1777 = CBS 144837 = DSM 107850; Saxony, in orchard north of Wölkau, N50°58'42.3" E13°49'40.0", from brown wedge-shaped necrosis in wood of *P. domestica*, 2 Mar. 2016, *S. Bien*, GLM-F106312, culture GLMC 462; Saxony, in orchard north of Wölkau, N50°58'42.3" E13°49'40.0", from brown wedge-shaped necrosis in wood of *P. domestica*, 2 Mar. 2016, *S. Bien*, GLM-F106314, culture GLMC 464; Saxony, in orchard north of Wölkau, N50°58'42.3" E13°49'40.0", from brown wedge-shaped necrosis in wood of *P. domestica*, 2 Mar. 2016, *S. Bien*, GLM-F106316, culture GLMC 466; Saxony, in orchard north of Wölkau, N50°58'42.3" E13°49'40.0", from brown wedge-shaped necrosis in wood of *P. domestica*, 2 Mar. 2016, *S. Bien*, GLM-F106401, culture GLMC 551; Saxony, in orchard north of Wölkau, N50°58'42.3" E13°49'40.0", from brown wedge-shaped necrosis in wood of *P. domestica*, 2 Mar. 2016, *S. Bien*, GLM-F106450, culture GLMC 600.

Notes — *Collophorina africana* was isolated seven and 14 times from wood of *P. domestica* in Saxony and Baden-Württemberg, respectively. It was not found in spore traps.

Collophorina badensis S. Bien & Damm, *sp. nov.* — MycoBank MB829147; Fig. 6b, 7

Etymology. Named after the geographical region in southern Germany, in which most isolates including the ex-type strain were isolated.

Typus. Germany, Baden-Württemberg, orchard west of Nussbach, N48°31'55.8" E8°00'52.4", from non-symptomatic wood of *P. domestica*, 23 Aug. 2016, *S. Bien* (GLM-F110767 holotype; GLMC 1684 = CBS 144833 = DSM 107769 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, 1.5–3 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells, in conidiomata and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple or septate, 3–30 µm long, mostly reduced to conidiogenous cells, directly formed on hyphae, conidiogenous loci formed terminally and sometimes intercalary, immediately below the septum. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, 3–9 × 1.5–3 µm, often reduced to mere openings formed directly on hyphal cells, discrete phialides or adelophialides, ampulli-

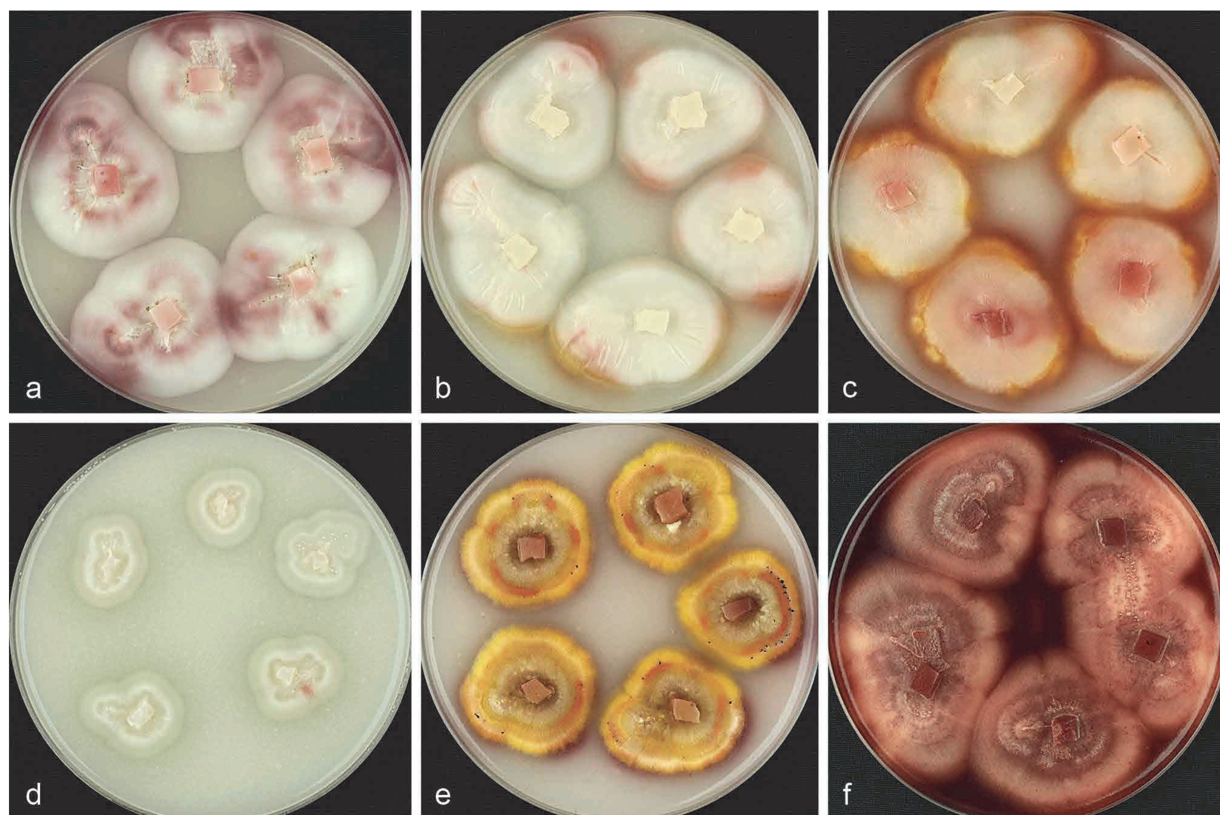

Fig. 6 Colony surface of *Collophorina* species on OA medium after 4 wk. a. *Collophorina africana* CBS 120872*; b. *C. badensis* GLMC 1684*; c. *C. germanica* GLMC 1445*; d. *C. hispanica* CBS 128568*; e. *C. neorubra* GLMC 929*; f. *C. rubra* CBS 120873*. Strains with an asterisk are ex-type cultures.

form to navicular, sometimes reduced to short necks, with short tubular to funnel-shaped collarettes, opening 0.5–1.5 μm diam, collarettes minute, < 0.5 μm long, opening 0.5–1.5 μm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, cylindrical to oblong, mostly straight, sometimes slightly curved, with obtuse ends, sometimes with a barely visible scar on one end, (3–)3.5–5.5(–6) × 1.5–2(–2.5) μm, mean ± SD = 4.4 ± 0.9 × 1.8 ± 0.3 μm, L/W ratio = 2.4. *Conidiomata* produced on OA in 2–4 wk, solitary or aggregated, subglobose, uni- to multilocular, immersed to erumpent, 50–300 μm wide, after > 4 wk up to 700 μm wide, light to dark brown, opening with an irregular rupture. *Conidiophores* hyaline, smooth-walled, straight, septate, often constricted at the septa, sometimes branched at the base and above, conidiogenous loci formed intercalary, immediately below the septum as well as terminally, 10–30 μm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, 4.5–8.5 × 2–2.5 μm; collarettes cylindrical, often inconspicuous, < 1 μm long, opening 0.5–1 μm, periclinal thickening sometimes visible. *Conidia* hyaline, after > 4 wk some of the conidia become reddish, smooth-walled, cylindrical to ellipsoidal, with both ends rounded, sometimes slightly curved, (2–)2.5–4(–5) × 1–2 μm, mean ± SD = 3.4 ± 0.7 × 1.5 ± 0.3 μm, L/W ratio = 2.3. *Endoconidia* not observed. *Microcyclic conidiation* occurs from minute collarettes at one or rarely both ends of primary conidia that develop into swollen mother cells, often thick-walled, sometimes septate, > 5 μm long, 2–4.5 μm wide. *Colonies on OA* flat to very low convex with entire margin; initially buff, sometimes with cinnamon to black spots due to conidiomata, after > 4 wk colony turning scarlet to bay, reddish pigment released into surrounding medium, spore mass from conidiomata pale luteous, after > 4 wk turning to blood colour; aerial mycelium sparse, white; reverse same colours; 6–12 mm diam in 2 wk, 20–32 mm diam in 4 wk; *on SNA* flat to very low convex with entire to undulate margin, whitish; lacking aerial mycelium; reverse same colours; 8–12 mm diam in 2 wk, 18–26 mm diam in 4 wk.

Additional materials examined. Germany, Baden-Württemberg, orchard west of Nussbach, N48°31'55.8" E8°00'52.4", from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug. 2016, *S. Bien*, GLM-F110769, culture GLMC 1686 = CBS 144834 = DSM 107770; Baden-Württemberg, orchard east of Nussbach, N48°31'57.3" E8°01'49.6", from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug. 2016, *S. Bien*, GLM-F110626, culture GLMC 1546; Baden-Württemberg, orchard west of Nussbach, N48°31'55.8" E8°00'52.4", from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug. 2016, *S. Bien*, GLM-F110717, culture GLMC 1637; Baden-Württemberg, orchard west of Nussbach, N48°31'55.8" E8°00'52.4", from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug. 2016, *S. Bien*, GLM-F110719, culture GLMC 1639; Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 25 May 2016, *C. Kraus*, GLM-F112543, culture GLMC 1844 = JKI-Mai59.

Notes — *Collophorina badensis* produces a red pigment like *C. africana*, *C. germanica*, *C. hispanica*, *C. neorubra* and *C. rubra*. The species is closely related to *C. germanica* with at least two, five, nine and four nucleotide differences in the LSU, ITS, *EF-1α* and *GAPDH* sequences, respectively. Conidia of *C. badensis* produced on hyphae and by microcyclic conidiation are less often curved than those of *C. germanica*. Strains of this species were almost exclusively isolated from *P. domestica* in Baden-Württemberg, while *C. germanica* is so far only known from wood of *P. avium*. One strain was isolated from a spore trap in Rhineland-Palatinate. The *EF-1α* sequence of this isolate differs in five nucleotides from that of the other isolates, while the LSU, ITS and the *GAPDH* sequences are identical.

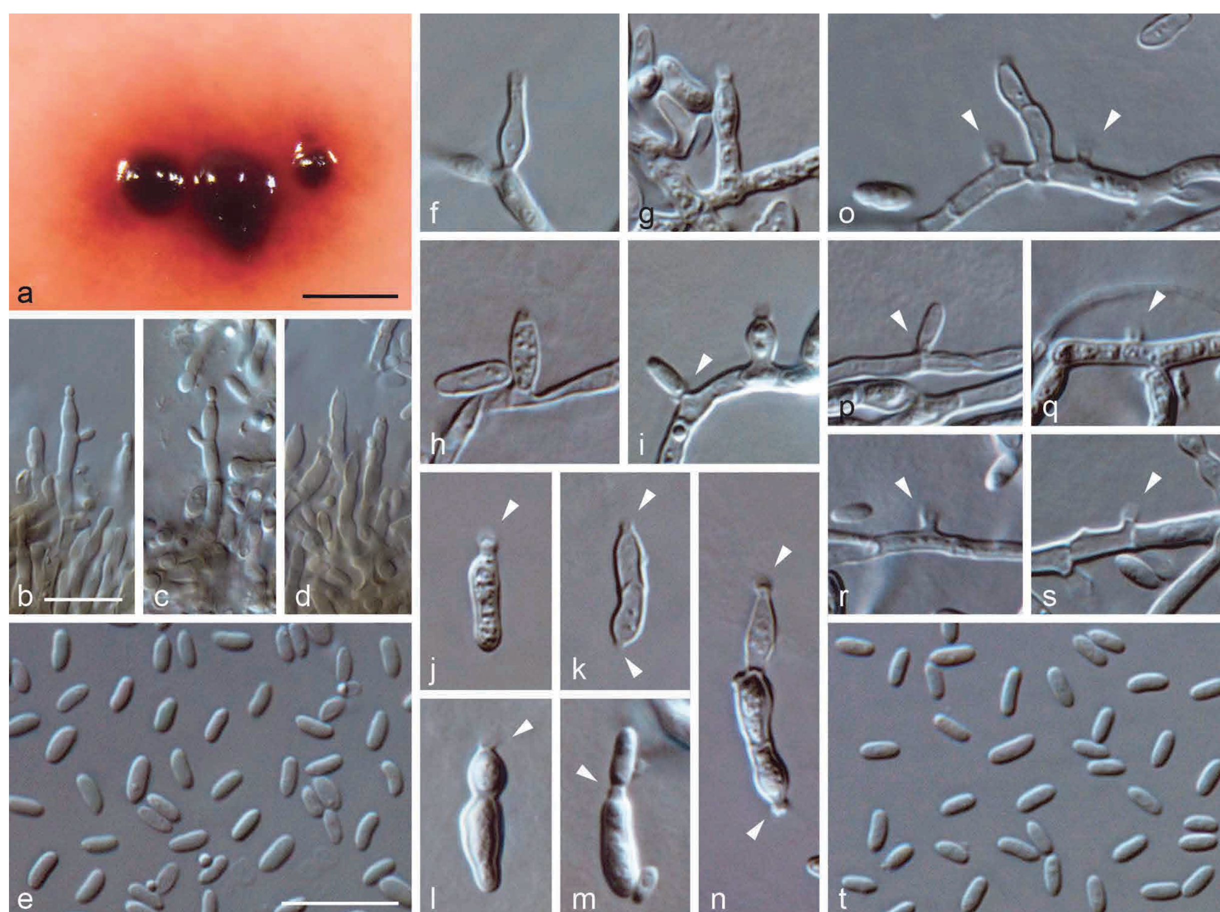

Fig. 7 *Collophorina badensis*. a. Conidiomata; b–d. conidiogenous cells lining the inner wall of a conidioma; e. conidia formed in conidiomata; f–i, o–s. conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings or short necks); j–n. mother cells (arrows indicate conidiogenous openings); t. conidia formed on hyphal cells. a–e. From OA; f–t. from SNA. a. SM; b–t. LM. — Scale bars: a = 400 µm, b, e = 10 µm; scale bar of b applies to c–d, scale bar of e applies to f–t.

Collophorina germanica S. Bien & Damm, *sp. nov.* — MycoBank MB829148; Fig. 6c, 8

Etymology. Named after the country of isolation.

Typus. Germany, Lower-Saxony, Hollern-Twielenfleth, orchard, N53°35'16.1" E9°34'23.7", from brown necrosis in wood of *P. avium*, 8 Oct. 2015, *S. Bien* (GLM-F110545 holotype; GLMC 1445 = CBS 144831 = DSM 107771 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, 1–3.5 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells, in conidiomata and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple or septate, mostly reduced to conidiogenous cells, directly formed on hyphae, conidiogenous loci formed terminally and sometimes intercalary, immediately below the septum, 3–30 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, often reduced to mere openings formed directly on hyphal cells, discrete phialides ampulliform to navicular, sometimes reduced to short necks, 2.5–10 × 2–2.5 µm, collarettes tubular to funnel-shaped, < 0.5–1 µm long, opening 1–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong to allantoid, rarely sigmoid, with obtuse ends, 4–8.5(–12) × 1.5–2(–2.5) µm, mean ± SD = 6.1 ± 2.3 × 1.8 ± 0.2 µm, L/W ratio = 3.4. *Conidiomata* produced on OA, rarely on SNA, in 4–8 wk; solitary or aggregated, subglobose, uni- to multilocular, immersed to superficial, 80–230 µm wide, dark brown to black, nearly glabrous to completely covered with hairs, opening with an irregular rupture. *Conidiophores* hyaline, smooth-walled, septate, constricted at the septa, straight, sometimes branched at the base and above, often not terminating in phialides, but with sterile, mostly pointed, sometimes inflated cells, 10–30 µm long, conidiogenous loci formed terminally or rarely intercalary, immediately below the septum. *Conidiogenous cells*, enteroblastic, hyaline, smooth-walled, 4–7 × 2–3 µm, collarettes cylindrical, often inconspicuous, < 1 µm long, opening 0.5–1.5 µm, periclinal thickening sometimes visible. *Conidia* hyaline to very pale brown, smooth-walled, cylindrical to ellipsoidal, with both ends rounded, 2.5–3.5 × (1–)1.5–2 µm, mean ± SD = 3 ± 0.3 × 1.6 ± 0.1 µm, L/W ratio = 1.9. *Endoconidia* not observed. *Microcyclic conidiation* occurs from minute collarettes at one or sometimes both ends of primary conidia that develop into swollen mother cells, often thick-walled, sometimes septate, > 6 µm long, 2.5–3.5 µm wide.

Colonies on OA flat to very low convex with entire to undulate margin; aerial mycelium not observed, buff to pale luteous in the centre, apricot to scarlet towards the margin, with black spots due to conidiomata formation, conidiomata oozing buff spore mass, reddish pigment released into surrounding medium, after > 4 wk whole colony becoming darker (up to bay); reverse same colours, 12–20 mm diam in 2 wk, 22–32 mm diam in 4 wk; *on SNA* flat to very low convex with entire, undulate, dentate or fimbriate margin, lacking aerial mycelium, white to luteous; reverse same colours; 10–12 mm diam in 2 wk, 12–22 mm diam in 4 wk.

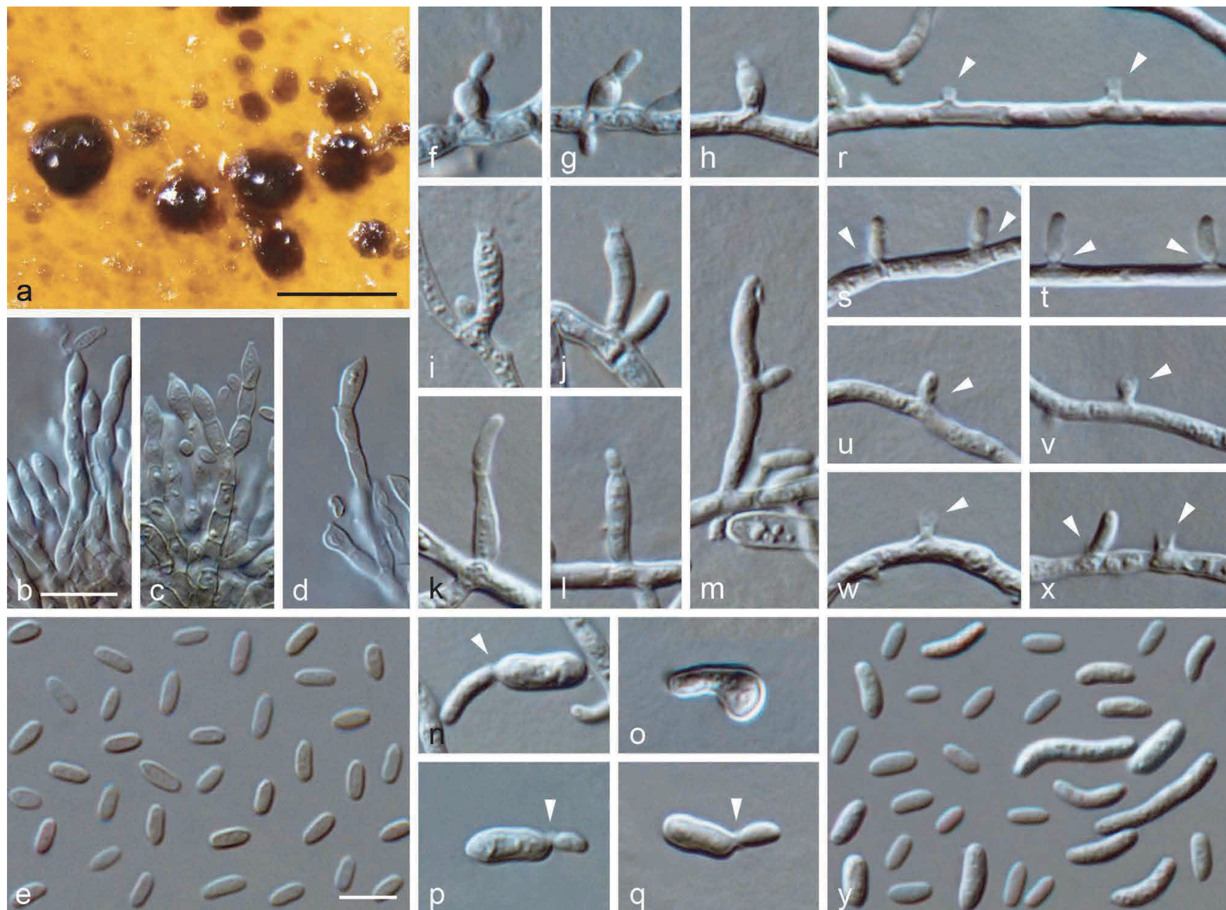

Fig. 8 *Collophorina germanica*. a. Conidiomata; b–d. conidiogenous cells lining the inner wall of a conidioma; e. conidia formed in conidiomata; f–m, r–x. conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings or short necks); n–q. mother cells (arrows indicate conidiogenous openings); y. conidia formed on hyphal cells. a–e. From OA; f–y. from SNA. a. SM; b–y. LM. — Scale bars: a = 300 µm, b = 10 µm, e = 5 µm; scale bar of b applies to c–d, scale bar of e applies to f–y.

Additional material examined. Germany, Baden-Württemberg, orchard south of Oppenau, on hill, N48°27'57.6" E8°09'11.0", from brown necrosis in wood of *P. avium*, 24 Aug. 2016, *S. Bien*, GLM-F110852, culture GLMC 1769 = CBS 144836 = DSM 107772.

Notes — *Collophorina germanica* produces a red pigment like *C. africana*, *C. badensis*, *C. hispanica*, *C. neorubra* and *C. rubra*. The closest relative is *C. badensis* with two, five, nine and four nucleotide differences in the LSU, ITS, *EF-1α* and *GAPDH* sequences, respectively. However, conidia produced on hyphae are more often allantoid to sigmoid than those of *C. badensis*. The two isolates originate from necrotic wood of *P. avium* in the most northern and most southern sampling areas in Germany.

Collophorina neorubra S. Bien & Damm, *sp. nov.* — MycoBank MB829149; Fig. 6e, 9

Etymology. Named based on the closest relative, *C. rubra*.

Typus. Germany, Saxony, orchard east of Gombson, N50°57'17.6" E13°47'19.3", from dark brown necrosis in wood of *P. avium*, 11 Aug. 2015, *S. Bien* (GLM-F106779 holotype; GLMC 929 = CBS 144829 = DSM 107773 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, 1.5–3.5 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed directly on hyphal cells, in conidiomata and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple or septate, conidiogenous loci formed terminally and sometimes intercalary, immediately below the septum, mostly reduced to conidiogenous cells, directly formed on hyphae, 3–30 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, often reduced to mere openings formed directly on hyphal cells, discrete phialides, ampulliform to navicular, 3–8 × 2–3 µm, collarettes tubular to funnel-shaped, 0.5–1.5 µm long, opening < 1–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong to ellipsoid, sometimes slightly curved, with obtuse ends, (3–)3.5–5(–7) × (1–)1.5–2.5 µm, mean ± SD = 4.3 ± 0.8 × 1.8 ± 0.5 µm, L/W ratio = 2.4. *Conidiomata* produced on OA in 2–4 wk; solitary or aggregated, uni- to multilocular, immersed to superficial, light to dark brown, subglobose, nearly glabrous to completely covered with hyaline to brown hairs, 100–600 µm wide, opening with an irregular rupture. *Conidiophores* hyaline, smooth-walled, septate, sometimes branched at the base and above, straight or slightly zigzag-shaped, often constricted at the septa, 10–30 µm long, conidiogenous loci formed intercalary, immediately below the septum as well as terminally. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, 5–7.5 × 1.5–2.5 µm, collarettes cylindrical, short, often inconspicuous, 0.5–1 µm long, opening 0.5–1 µm, periclinal thickening sometimes visible. *Conidia* hyaline, later turning to pale red, smooth-walled, cylindrical to ellipsoidal, sometimes slightly curved, with both ends rounded, 3–4(–4.5) × 1–1.5 µm, mean ± SD = 3.4 ± 0.4 × 1.5 ± 0.1 µm, L/W ratio = 2.3. *Endoconidia* not observed. *Microcyclic conidiation* occurs from minute collarettes at one or both ends of primary conidia that develop into swollen mother cells, often thick-walled, sometimes septate,

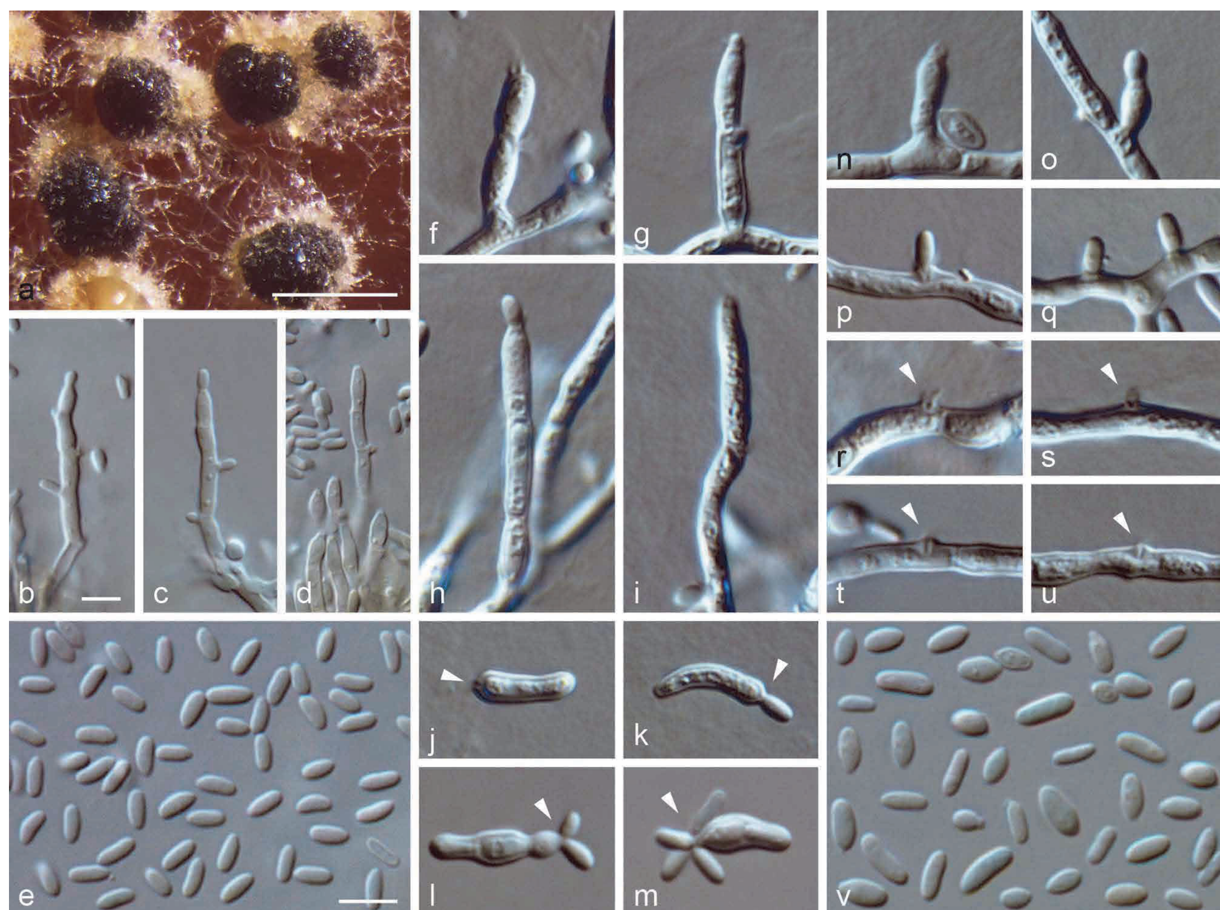

Fig. 9 *Collophorina neorubra*. a. Conidiomata; b–d. conidiogenous cells lining the inner wall of a conidioma; e. conidia formed in conidiomata; f–i, n–u. conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings); j–m. mother cells (arrows indicate conidiogenous openings); v. conidia formed on hyphal cells. a–e. From OA; f–v. from SNA. a. SM; b–v. LM. — Scale bars: a = 300 µm, b, e = 5 µm; scale bar of b applies to c–d, scale bar of e applies to f–v.

> 5 µm long, 2–3.5 µm wide, often more than one conidium attached to an opening.

Colonies on OA flat to very low convex with entire to undulate margin, pale luteous to luteous, apricot to orange, margin white; with saffron to black spots due to conidiomata formation, spore mass pale oozing from conidiomata luteous to bay, reddish pigment released into surrounding medium, aerial mycelium sparse, white, after > 4 wk colony turning to bay; reverse same colours; 12–16 mm diam in 2 wk, 20–24 mm diam in 4 wk; *on SNA* flat to very low convex with entire, undulate, dentate to fimbriate margin, lacking aerial mycelium; white to luteous; reverse same colours; < 1–2 mm diam in 2 wk, < 1–2 mm diam in 4 wk.

Additional materials examined. Germany, Baden-Württemberg, orchard west of Nussbach, N48°32'11.3" E8°01'01.3", from brown necrosis in wood of *P. avium*, 23 Aug. 2016, *S. Bien*, GLM-F110752, culture GLMC 1669 = CBS 144832 = DSM 107774; Lower-Saxony, Hollern-Twielenfleth, orchard, N53°35'16.1" E9°34'23.7", from brown necrosis in wood of *P. avium*, 8 Oct. 2015, *S. Bien*, GLM-F110667, culture GLMC 1587; Lower-Saxony, Hollern-Twielenfleth, orchard, N53°35'16.1" E9°34'23.7", from brown necrosis in wood of *P. avium*, 8 Oct. 2015, *S. Bien*, GLM-F110668, culture GLMC 1588.

Notes — *Collophorina neorubra* is closely related to *C. rubra* with nine, four, two and seven nucleotide differences in the LSU, ITS, *EF-1α* and *GAPDH* sequences, respectively. It produces a red pigment like *C. africana*, *C. badensis*, *C. germanica*, *C. hispanica* and *C. rubra*. Damm et al. (2010) described the phialides of the closely related *C. rubra* as particularly short with a maximum of 4 µm in length. However, up to 8 µm long phialides were observed in *C. neorubra*. A unique feature of this species is the frequent attachment of two to several conidia at the conidiogenous openings of the mother cells during microcyclic conidiation. *Collophorina neorubra* has only been isolated from wood of *P. avium*, but in all the production areas sampled, Baden-Württemberg, Saxony and Lower Saxony.

Pallidophorina S. Bien & Damm, *gen. nov.* — MycoBank MB829160

Etymology. Name refers to the pale (Lat.: *pallidus*) appearance of the culture on oatmeal agar medium and the resemblance to *Collophorina*.

Type species. *Pallidophorina paarla* (Damm & Crous) S. Bien & Damm.

Colonies slow-growing, moist, white or cream colours on oatmeal agar medium, with sparse or lacking aerial mycelium. *Sporulation* conidia formed in conidiomata, on hyphal cells and by microcyclic conidiation. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to short adelophialides, discrete phialides or more often to openings with collarettes formed directly on hyphal cells. *Conidia* aggregated in masses around the hyphae and on the agar surface. *Conidiomata* solitary or aggregated, subglobose, superficial or semi-immersed, uni- to multilocular, dehiscence irregular. *Conidiophores* hyaline, simple or branched, septate, filiform. *Conidiogenous cells* enteroblastic, hyaline, often short necks formed laterally in each cell just below the septum as well as terminally (acropleurogenous). *Conidia* of conidiomata and intercalary hyphal cells small, hyaline, 1-celled, cylindrical to ellipsoidal.

Pallidophorina paarla (Damm & Crous) S. Bien & Damm, *comb. nov*. — MycoBank MB829162; Fig. 3d

Basionym. Collophora paarla Damm & Crous, Persoonia 24: 67. 2010.
Synonyms. Collophora pallida Damm & Crous, Persoonia 24: 69. 2010.
Collophorina paarla (Damm & Crous) Damm & Crous, Fungal Diversity 86: 111. 2017.

Typus. South Africa, Western Cape Province, Paarl, from dark brown necrosis in wood of *P. persica*, 10 June 2004, *U. Damm* (CBS H-19996 holotype; CBS 120877 = STE-U 6114 = GLMC 1884 culture ex-type).

A description is provided in Damm et al. (2010).

Additional materials examined. Germany, Saxony, orchard north of Kunnerwitz, N51°07'27.5" E14°56'36.3", from dark brown necrosis in wood of *P. cerasus*, 15 Jan. 2015, *S. Bien*, GLM-F106302, culture GLMC 452 = CBS 144828 = DSM 107775; Lower-Saxony, orchard in Hollern-Twielenfleth, N53°36'13.6" E9°31'50.8", from brown necrosis in wood of *P. domestica*, 8 Oct. 2015, *S. Bien*, GLM-F107132, culture GLMC 1282 = CBS 144830 = DSM 107776; Saxony, orchard east of Borthen, N50°58'20.9" E13°48'48.1", from dark brown necrosis in wood of *P. domestica*, 11 Aug. 2015, *S. Bien*, GLM-F106630, culture GLMC 780; Saxony, orchard east of Lungkwitz, N50°56'12.4" E13°47'36.6", from dark brown necrosis in wood of *P. cerasus*, 11 Aug. 2015, *S. Bien*, GLM-F106641, culture GLMC 791; Saxony, orchard east of Gombson, N50°57'19.3" E13°47'22.0", from dark brown necrosis in wood of *P. avium*, 11 Aug. 2015, *S. Bien*, GLM-F106742, culture GLMC 892; Lower-Saxony, orchard in Hollern-Twielenfleth, N53°36'13.6" E9°31'50.8", from brown necrosis in wood of *P. avium*, 8 Oct. 2015, *S. Bien*, GLM-F107080, culture GLMC 1230; Baden-Württemberg, orchard east of Erlach, N48°34'17.3" E8°02'13.6", from brown necrosis in wood of *P. avium*, 23 Aug. 2016, *S. Bien*, GLM-F110577, culture GLMC 1497.

Notes — *Pallidophorina paarla* was the most frequently isolated species from wood of *Prunus* spp. in this study; 112 isolates belonged to this species, of which seven were included

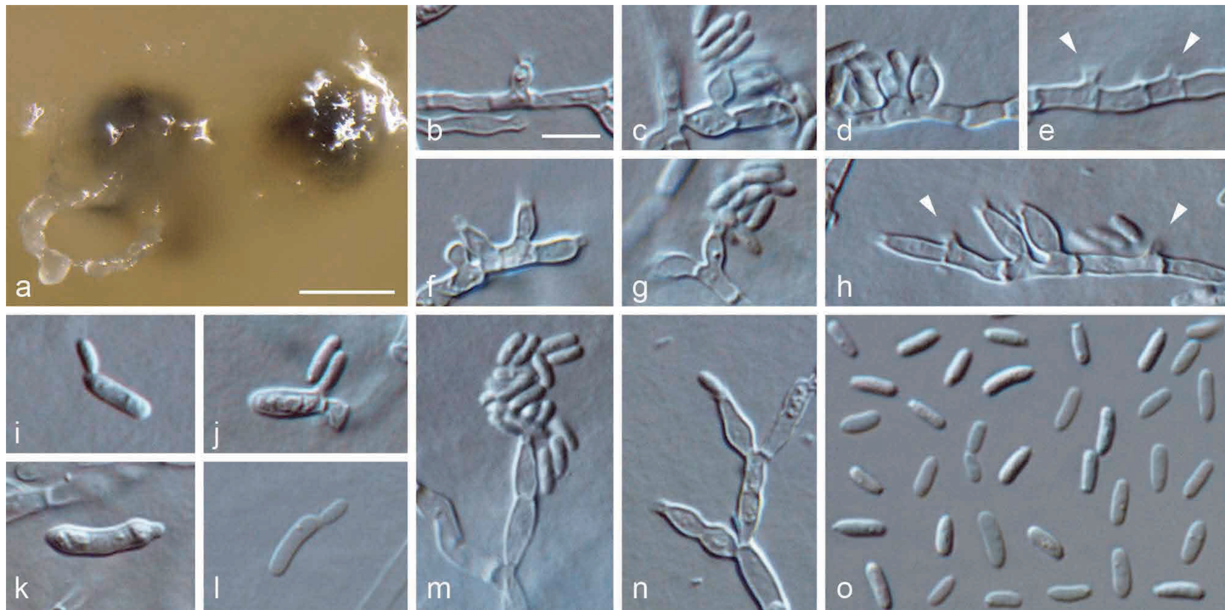

Fig. 10 *Tympanis inflata*. a. Conidiomata; b–h, m–n. conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings); i–l. mother cells (arrows indicate conidiogenous openings); o. conidia formed on hyphal cells. a. From OA; b–o. from SNA. a. SM; b–o. LM. — Scale bars: a = 200 µm, b = 5 µm; scale bar of b applies to c–o.

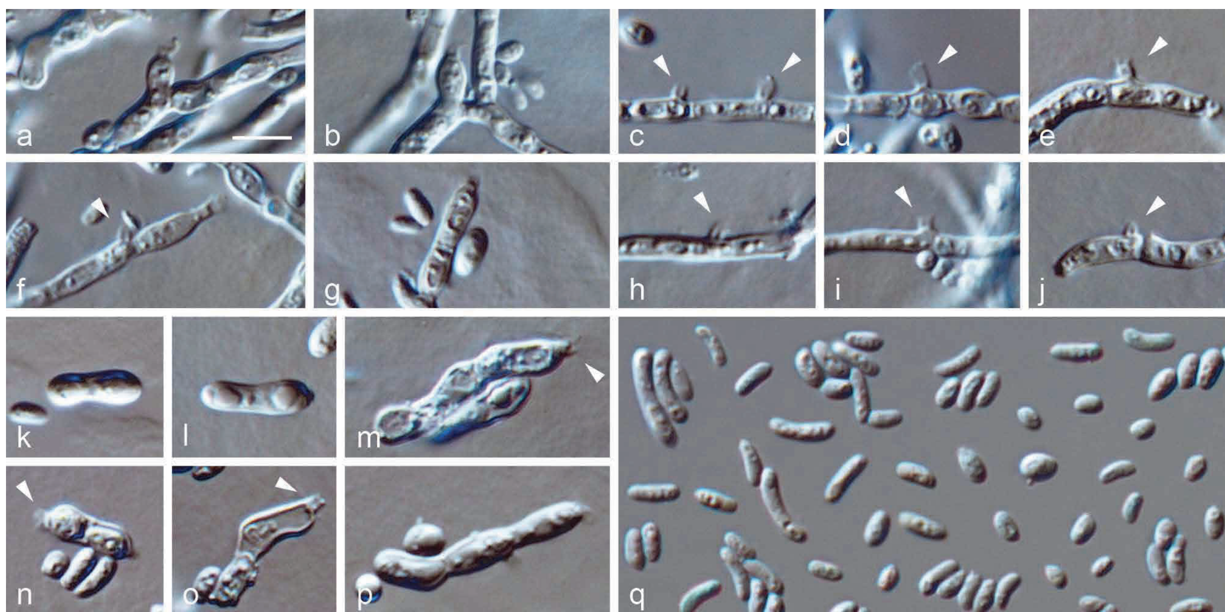

Fig. 11 *Variabilispora flava*. a–j. Conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings or short necks); k–p. mother cells (arrows indicate conidiogenous openings); q. conidia formed on hyphal cells. a–q. From SNA. a–q. LM. — Scale bar: a = 5 µm; scale bar of a applies to b–q.

in the molecular study (GAPDH sequences of strains isolated in this study are not available). It was isolated from *P. avium*, *P. cerasus* and *P. domestica* in Saxony, Lower Saxony and Baden-Württemberg. It was not found in any spore traps in this study, however in spore traps attached to *Prunus* trees in the study of Fischer et al. (2016).

Ramoconidiophora S. Bien & Damm, *gen. nov.* — MycoBank MB829161

Etymology. Name reflects the frequently branched conidiophores in conidiomata (*ramus* Lat. = branch).

Type species. *Ramoconidiophora euphorbiae* (S. Nasr et al.) S. Bien & Damm.

Colonies slow-growing, moist, white, buff or cream, lacking aerial mycelium. *Sporulation* conidia formed in conidiomata, on hyphal cells and by microcyclic conidiation. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to discrete phialides or more often with collarettes formed directly on hyphal cells. *Conidia* aggregated in masses around the hyphae and on the agar surface. *Conidiomata* solitary or aggregated, immersed to superficial, subglobose, unilocular, wall composed of angular to roundish cells, dehiscence irregular, appearing cup-shaped when mature. *Conidiophores* hyaline, branched, septate, constricted at the septa. *Conidiogenous cells* enteroblastic, hyaline, conidiogenous loci formed laterally in each cell just below the septum as well as terminally (acropleurogenous). *Conidia* of conidiomata and intercalary hyphal cells small, hyaline, 1-celled, cylindrical, straight or slightly curved.

Ramoconidiophora euphorbiae (S. Nasr et al.) S. Bien & Damm, *comb. nov*. — MycoBank MB829163; Fig. 3e

Basionym. *Collophorina euphorbiae* S. Nasr et al., Mycol. Progr. 17: 762. 2018.

A description is provided by Nasr et al. (2018).

Tympanis inflata S. Bien, C. Kraus & Damm, *sp. nov.* — MycoBank MB829150; Fig. 3f, 10

Etymology. Named after the inflated phialides.

Typus. Germany, Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 10 Nov. 2016, *C. Kraus* (GLM-F112546 holotype; GLMC 1856 = CBS 144844 = DSM 107852 = JKI-Nov7 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, 1–2.5 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple or septate, sometimes branched, often reduced to conidiogenous cells, directly formed on hyphae, constricted at the septa and at the base, conidiogenous loci formed terminally and rarely intercalary, immediately below the septum, 5–30 × 2–3 µm, rarely up to 60 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, often reduced to mere openings with collarettes formed directly on hyphal cells, adelophialides or discrete phialides, mostly ampulliform, sometimes navicular, often constricted at the base, 2–9 × 2–3 µm, with short tubular to funnel-shaped collarettes, opening 1–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong, often slightly curved, with obtuse ends, sometimes with a prominent scar on one end, (3–)3.5–4.5(–5) × (1–)1.5–2 µm, mean ± SD = 4 ± 0.6 × 1.5 ± 0.1 µm, L/W ratio = 2.7. *Conidiomata* on OA and SNA after > 8 wk rare, immersed to erumpent, 120–350 µm, brown to black, remaining sterile. *Endoconidia* not observed. *Microcyclic conidiation* occurs from flaring collarettes at one end of conidia that have developed into mother cells, often thick-walled, sometimes septate, > 5 µm long, 2–3 µm wide.

Colonies on OA flat to very low convex with entire margin, lacking aerial mycelium, whitish to buff, reverse same colours, 18–20 mm diam in 2 wk, 32–36 mm diam in 4 wk; *on SNA* flat with entire to dentate margin, lacking aerial mycelium, white, reverse same colour; 4–6 mm diam in 2 wk, 8–10 mm diam in 4 wk.

Notes — This species was isolated only once from a spore trap in Rhineland-Palatinate. Like other species of collophorina-like fungi described in this study, namely *Capturomyces funiculosus*, '*Collophorina*' *aceris*, *Pallidophorina paarla*, *Ramoconidiophora euphorbiae*, *Vexillomyces palatinus* and *Ve. verruculosus*, it does not produce any pigments on OA medium. Phylogenetic analyses places this species in the genus *Tympanis* with the closest relatives being *T. saligna* and *T. tsugae* with 13 and 19 nucleotide differences in the LSU and ITS, respectively. *Tympanis inflata* frequently produces small inflated phialides, which distinguishes it from any other species of collophorina-like fungi. Conidia are relatively small and narrow, similar to those of species of *Vexillomyces* described in this study, however less curved. Conidial stages of several *Tympanis* species are described as conidiomatal (Groves 1952); morphological comparison with them is hindered since observed conidiomata in *T. inflata* remained sterile. In a blastn search in GenBank, the ITS sequence of *T. flava* showed a 98 % identity (100 % coverage) with a fungus (KP990974) isolated from a healthy leave of *Juniperus deppeana* in the US (Huang et al. 2016).

Variabilispora S. Bien, C. Kraus & Damm, *gen. nov.* — MycoBank MB829155

Etymology. Named after the variable spore formes (*variabilis* Lat. = variable).

Type species. *Variabilispora flava* S. Bien, C. Kraus & Damm.

Colonies slow-growing, moist, sulphur to pure yellow colours on oatmeal agar medium, lacking aerial mycelium. *Sporulation* conidia formed on hyphal cells and by microcyclic conidiation. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to short adelophialides and discrete phialides or more often with collarettes formed directly on hyphal cells. *Conidia* aggregated in masses around the hyphae and on the agar surface. *Conidia* of intercalary hyphal cells small, hyaline, 1-celled, subglobose, ellipsoidal, oblong to allantoid, often slightly curved.

Variabilispora flava S. Bien, C. Kraus & Damm, *sp. nov.* — MycoBank MB829156; Fig. 3g, 11

Etymology. Named after its yellow (Lat.: *flavus*) colonies on OA.

Typus. Germany, Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 23 Nov. 2016, C. *Kraus* (GLM-F112547 holotype; GLMC 1858 = CBS 144845 = DSM 107777 = JKI-Nov103 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, 1–3.5 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, simple or septate, constricted at the septa and at the base, mostly reduced to conidiogenous cells, directly formed on hyphae, conidiogenous loci formed terminally and sometimes intercalary, immediately below the

septum, 5–10 × 1.5–2 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, mostly reduced to mere openings with collarettes or short necks formed directly on hyphal cells, discrete phialides and adelophialides rare, hyaline, smooth-walled, ampulliform, navicular to subulate, often constricted at the base, 2–9 × 1.5–2.5 µm; short cylindrical necks rare, 1–2 × 1–2.5 µm; collarettes tubular or funnel-shaped, < 1–1.5 µm long, opening < 1–2 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, subglobose, ellipsoidal, oblong to allantoid, often slightly curved, with both ends rounded, (2–)2.5–6.5(–9) × (1–)1.5–2(–2.5) µm, mean ± SD = 4.5 ± 1.8 × 1.8 ± 0.3 µm, L/W ratio = 2.5. *Conidiomata* and *endoconidia* not observed. *Microcyclic conidiation* occurs from flaring or hardly visible collarettes at one or sometimes both ends of conidia that have developed into mother cells, often thick-walled, sometimes septate, > 5 µm long, 2.5–3.5 µm wide.

Colonies on OA flat to very low convex with dentate to fimbriate margin, lacking aerial mycelium; sulphur yellow to pure yellow; reverse same colours, 6–10 mm diam in 2 wk, 12–16 mm diam in 4 wk; *on SNA* flat with fimbriate to rhizoid margin, lacking aerial mycelium, whitish; reverse same colour; 2–4 mm diam in 2 wk, 4–6 mm diam in 4 wk.

Notes — One isolate of *V. flava* has been isolated from a spore trap in Rhineland-Palatinate. It differs from any other species of collophorina-like fungi by its sulphur yellow to pure yellow colour on OA and conidia that are very variable in shape, from almost globose, elongated to allantoid. The closest relatives of *V. flava* are *Aotearoamyces nothofagi*, ‘*Collophorina*’ *aceris* and *Pallidophorina paarla*. In contrast to *A. nothofagi* that produces vermiform conidia on well-developed conidiophores arranged in small synnematous structures (Quijada et al. 2018), *V. flava* produces subglobose, ellipsoidal, oblong to allantoid conidia directly on hyphal cells, on reduced conidiophores or by microcyclic conidiation. Both species differ in 15, 21, 69 and 29 nucleotide differences in the LSU, ITS, *EF-1α* and *GAPDH* sequences, respectively. *Variabilispora flava* differs from ‘*Collophorina*’ *aceris*, by a lack of dark sclerotia on OA. Only the ITS sequence of ‘*C.*’ *aceris* is available, which differs in 36 nucleotides from *V. flava*. In contrast to *Pa. paarla* the conidia of *V. flava* are very variable in shape and neither endoconidia nor conidiomata were observed. *Variabilispora flava* differs from *Pa. paarla* in 11 and 25 nucleotides in the LSU and ITS sequences, respectively. In a blastn search in GenBank, the ITS sequence of *V. flava* showed a 100 % identity (92 % coverage) with an uncultured fungus (HE998707) found in a dead branch of *Fagus sylvatica* in Greifswald, Germany (Unterseher et al. 2013).

Vexillomyces S. Bien, C. Kraus & Damm, *gen. nov.* — MycoBank MB829157

Etymology. Name refers to the pronounced flag-like collarettes (Lat.: *vexillum* = flag).

Type species. *Vexillomyces verruculosus* S. Bien, C. Kraus & Damm.

Colonies slow-growing, moist, white or buff colours on oatmeal agar medium, lacking aerial mycelium. *Sporulation* conidia formed on hyphal cells, by microcyclic conidiation or endoconidiation. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to short adelophialides, discrete phialides or more often with collarettes formed directly on hyphal cells, collarettes mostly flaring or short tubular. *Conidia* aggregated in masses around the hyphae and on the agar surface, small, hyaline, 1-celled, cylindrical to ellipsoidal. *Vegetative hyphae* and *phialides* smooth-walled or verruculose.

Vexillomyces palatinus S. Bien, C. Kraus & Damm, *sp. nov.* — MycoBank MB829158; Fig. 3h, 12

Etymology. Named after the geographical region in Germany, in which the species was isolated.

Typus. Germany, Rhineland-Palatinate, west of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from spore a trap mounted on vine of *V. vinifera*, 31 Mar. 2016, *C. Kraus* (GLM-F112541 holotype; GLMC 1852 = CBS 144842 = DSM 107851 = JKI-Mz74 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled to verruculose, 1–3.5 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled to verruculose, simple or septate, rarely branched, constricted at the septa

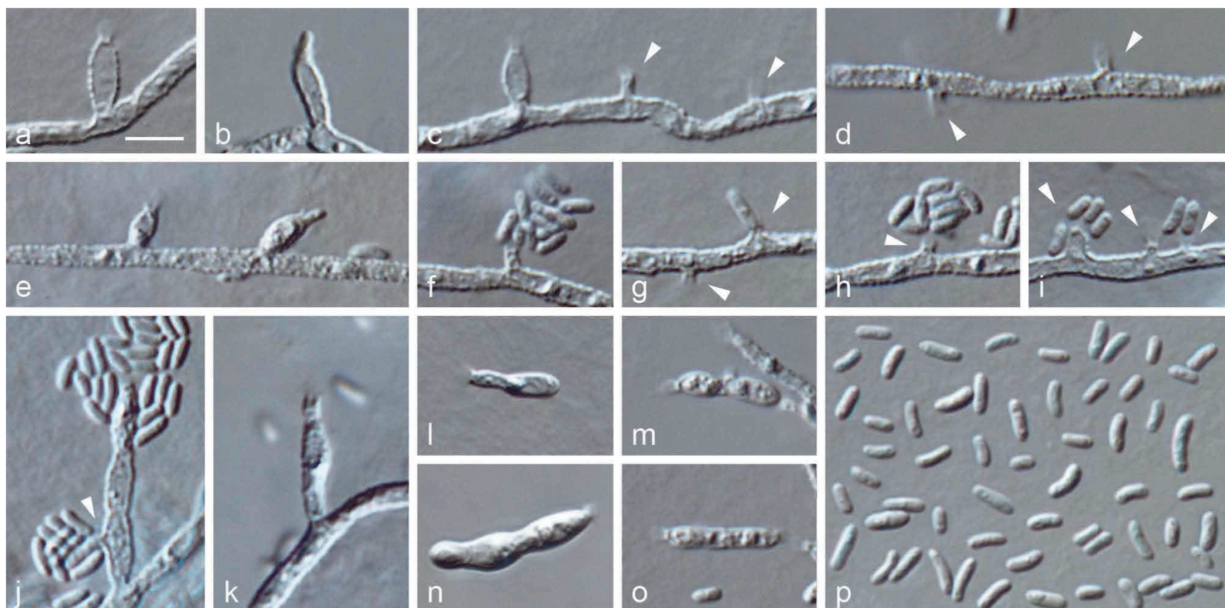

Fig. 12 *Vexillomyces palatinus*. a–k. Conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings or short necks); l–o. mother cells; p. conidia formed on hyphal cells. a–p. From SNA. a–p. LM. — Scale bar: a = 5 µm; scale bar of a applies to b–p.

and at the base, conidiogenous loci formed terminally and sometimes intercalary, immediately below the septum, mostly reduced to conidiogenous cells, directly formed on hyphae, 3–25 × 1.5–2 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled to verruculose, often reduced to mere openings with collarettes or short necks formed directly on hyphal cells, discrete phialides or adelophialides, navicular to subulate, often constricted at the base, 3–13 × 1.5–3 µm, short necks cylindrical, 0.5–2 × 1–1.5 µm, collarettes mostly flaring or short and tubular, 0.5–2.5 µm long, opening 1–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong, often curved, with both ends rounded, sometimes with barely visible papillate appendage on one end, (2.5–)3–4(–4.5) × 1–1.5(–2) µm, mean ± SD = 3.4 ± 0.6 × 1.3 ± 0.2 µm, L/W ratio = 2.6. *Conidiomata* and *endoconidia* not observed. *Microcyclic conidiation* occurs from flaring collarettes at one or sometimes both ends of conidia that have developed into mother cells, > 5 µm long, 2–3 µm wide, sometimes septate.
Colonies on OA flat to very low convex with entire margin, lacking aerial mycelium; whitish to buff, after > 4 wk sometimes fulvous to sepia; reverse same colours, 4–6 mm diam in 2 wk, 12–18 mm diam in 4 wk; *on SNA* flat with crenated to dentate, sometimes rhizoid margin, lacking aerial mycelium; whitish, reverse same colour; 1–3 mm diam in 2 wk, 2–3 mm diam in 4 wk.

Notes — *Vexillomyces palatinus* was only isolated once from a spore trap in Rhineland-Palatinate. The OA cultures of *Ve. palatinus* have a pigmentless, pale appearance, similar to those of *Ve. verruculosus* and the species *Capturomyces funiculosus*, '*Collophorina*' *aceris*, *Pallidophorina paarla*, *Ramoconidiophora euphorbiae* and *Tympanis inflata*. Hyphae and phialides are often verruculose; and the collarettes of phialides, intercalary hyphal openings and of conidia mother cells during microcyclic conidiation are often considerably pronounced. These features are mostly identical with its closest relative *Ve. verruculosus*, which was also isolated from spore traps. However, endoconidia have not been observed in *Ve. palatinus*; conidia of *Ve. palatinus* are on average smaller than those of *Ve. verruculosus*. Moreover, LSU, ITS, *EF-1α* and *GAPDH* sequences of the two species differ in three, 15, 16 and 12 nucleotides, respectively. In a blastn search on GenBank, the ITS sequence of *Ve. palatinus* showed 99 % identity (4 nucleotide differences, 92 % coverage, HQ611305) with an uncultured unidentified fungus from logs of *Picea abies* in Sweden (Lindner et al. 2011), as well as with an uncultured *Collophora* sp. (6 nucleotide differences, 82 % coverage, HE998707) found in a dead branch of *Fagus sylvatica* in Greifswald, Germany (Unterseher et al. 2013).

Vexillomyces verruculosus S. Bien, C. Kraus & Damm, *sp. nov.* — MycoBank MB829159; Fig. 3i, 13

Etymology. Named after the verruculose hyphae and phialides.

Typus. Germany, Rhineland-Palatinate, east of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 31 Mar. 2016, *C. Kraus* (GLM-F112540 holotype; GLMC 1854 = CBS 144843 = DSM 107853 = JKI-Mz75 culture ex-type).

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled to verruculose, 0.5–3 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on, rarely also inside, hyphae (endoconidia) and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled to verruculose, simple or septate, constricted at the septa and at the base, conidiogenous loci formed terminally and sometimes intercalary, immediately below the septum, mostly reduced to conidiogenous cells, directly formed on hyphae, 3–20 × 1.5–3 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled to verruculose, often reduced to mere openings with collarettes or short necks formed directly on hyphal cells, discrete phialides or adelophialides, navicular to subulate, often constricted at the base, 2–12 × 1.5–3 µm; necks cylindrical, 0.5–1.5 × 1–1.5 µm; collarettes mostly flaring or short tubular, 0.5–2.5 µm long, opening 1–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, often curved, oblong with both ends rounded, (2.5–)3.5–6.5(–9.5) × 1–1.5(–2) µm, mean ± SD = 5.1 ± 1.4 × 1.3 ± 0.2 µm, L/W ratio = 3.9. *Conidiomata* not observed. *Endoconidia* rarely observed, hyaline, smooth-walled, aseptate, oblong with both

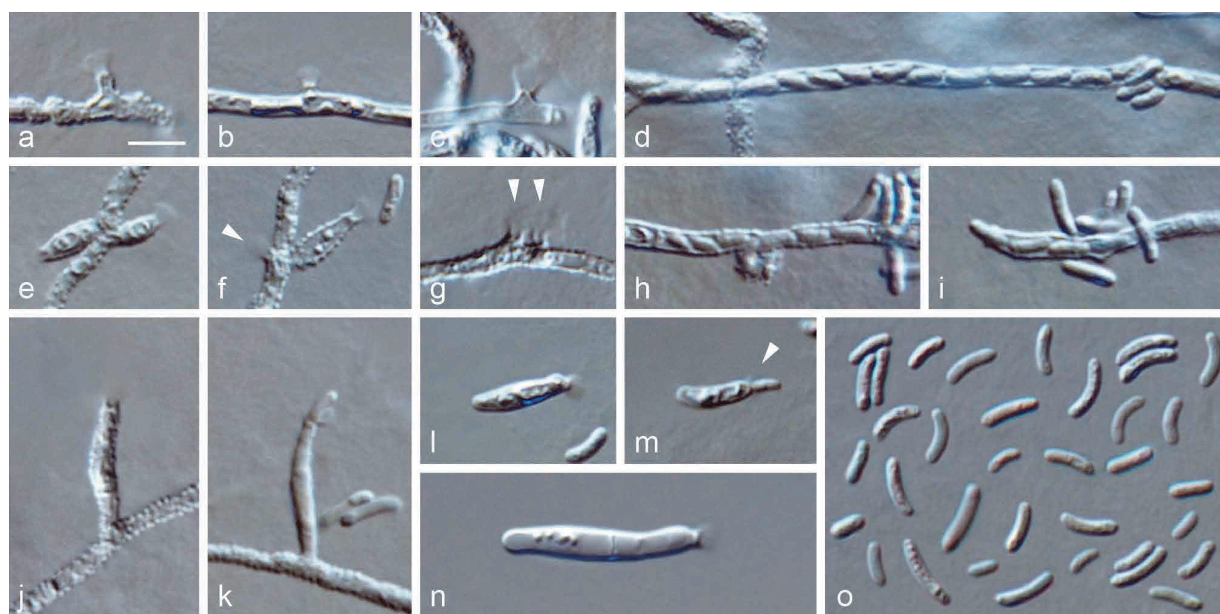

Fig. 13 *Vexillomyces verruculosus*. a–c, e–g, j–k. Conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings); d, h–i. endoconidia; l–n. mother cells (arrow indicates conidiogenous openings); o. conidia formed on hyphal cells. a–o. From SNA. a–o. LM. — Scale bar: a = 5 µm; scale bar of a applies to b–o.

ends rounded, 2.5–3.5 × 1–1.5 µm. *Microcyclic conidiation* occurs from flaring collarettes at one or sometimes both ends of conidia that have developed into mother cells, sometimes septate, > 5 µm long, 2–3 µm wide.

Colonies on OA flat to very low convex with entire to undulate margin, lacking aerial mycelium; whitish to buff, after > 4 wk sometimes fulvous to sepia; reverse same colours, 4–6 mm diam in 2 wk, 10–14 mm diam in 4 wk; *on SNA* flat with rhizoid margin, lacking aerial mycelium; whitish, reverse same colour; < 1–2 mm diam in 2 wk, 2–8 mm diam in 4 wk.

Additional materials examined. Germany, Rhineland-Palatinate, east of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 23 Feb. 2017, *C. Kraus*, GLM-F112539, culture GLMC 1840 = CBS 144838 = DSM 107854 = JKI-Feb24; Rhineland-Palatinate, east of Albersweiler, research vineyard of Julius-Kühn-Institute Siebeldingen, N49°13'11.5" E8°02'34.6", from a spore trap mounted on vine of *V. vinifera*, 23 Feb. 2017, *C. Kraus*, GLM-F112538, culture GLMC 1838 = JKI-Feb21.

Notes — Three isolates of *Ve. verruculosus* were isolated from spore traps in Rhineland-Palatinate. Cultures of *Ve. verruculosus* have a pale appearance on OA medium, similar to *Ve. palatinus* and the species *Capturomyces funiculosus*, '*Collophorina*' *aceris*, *Pallidophorina paarla, Ramoconidiophora euphorbiae* and *Tympanis inflata*. Hyphae and phialides of *Ve. verruculosus* are often verruculose; and the collarettes of phialides and integrated hyphal openings as well as on conidia mother cells during microcyclic conidiation are often considerably pronounced. These features are mostly identical to its closest relative *Ve. palatinus*, which was also isolated from a spore trap. However, conidia of *Ve. verruculosus* are on average bigger than those of *Ve. palatinus.* Moreover, LSU, ITS, *EF-1α* and *GAPDH* sequences of the two species differ in three, 15, 16 and 12 nucleotides, respectively.

DISCUSSION

In all phylogenies calculated from the LSU-ITS alignment of collophorina-like species and their closest relatives, well-supported clades of formerly described *Collophorina euphorbiae* and *C. paarla* are separated from a clade containing *C. africana*, *C. hispanica* and *C. rubra* by clades of *Tympanis*, *Gelatinomyces* and a strain identified as *Claussenomyces olivaceus*. Damm et al. (2010) already discussed the formation of two clades in the original description of the genus *Collophorina* (syn. *Collophora*). Inclusion of all species into one genus was based on similar morphological features and close relatedness, as well as a lack of sequence data of further related taxa. Quijada et al. (2018), who included *C. africana*, *C. rubra* and *C. paarla* in their analyses, discussed a necessary splitting of the genus, however erroneously described the current situation of *Collophorina* as paraphyletic. Our results are in agreement with those of Quijada et al. (2018) leading to the conclusion that *Collophorina* is polyphyletic, to the separation of *C. euphorbiae* and *C. paarla* from *Collophorina*, and to the description of the new genera *Pallidophorina* and *Ramoconidiophora*. All *Collophorina* species formed a monophyletic clade and can be distinguished from *Pa. paarla*, *R. euphorbiae* and all other collophorina-like species studied here by a red pigment produced on oatmeal agar medium. Although *C. aceris* also seems to represent a different genus, we refrain from erecting a new genus in this study as neither the strain nor LSU sequence data are available.

Phylogenetic analyses recognised a high diversity of collophorina-like species in both necrotic wood of *Prunus* trees and in spore traps mounted on grapevine shoots with nine previously unknown species. Three of them are described within *Collophorina*. *Capturomyces* and *Vexillomyces* are described with two new species each, which were isolated from spore traps. One species from a spore trap did not cluster with any of the other genera, for which the new genus *Variabilispora* is described.

One isolate from a spore trap proved to belong to the genus *Tympanis* based on DNA sequence data. The genus *Tympanis* was described by Tode (1790), sanctioned by Fries (1822) and today comprises around 60 species. *Tympanis* species are inoperculate discomycetes, forming dark, gelatinised apothecia and are saprophytes or weak parasites of twigs, branches or main trunks of woody plants (Yao & Spooner 1996). In culture, *Tympanis* species form slimy, yeast-like colonies (De Hoog & McGinnis 1987) resembling cultures of *Collophorina*. The asexual morph is described as flask-shaped, erumpent and aggregated pycnidia forming dark, branched, cylindrical and filiform conidiophores. Minute conidia are produced at the apices of the conidiophores and along the sides, immediately below the septa (Groves 1952, Sutton & Funk 1975), reminiscent of those formed by *Collophorina* species. However, the isolate from this study only produced few conidiomata that remained sterile. In the past, species of *Tympanis* have been described based on the morphology of their sexual morph only. Therefore, comparison with previously described species based on morphology was not possible. Groves (1952) assessed conidial states of *Tympanis* as of no value for species identification. There is no type specimen of the type species *T. saligna* available, but Groves (1952), who revised the genus, regarded one of the two *Tympanis* species known from *Salix* at that time as *T. saligna*, which is represented by strain CBS 366.55 in our study. Most of the *Tympanis* strains included in our study originated from the study of Groves (1952) and were sequenced by Vu et al. (2019) or in this study. This is the first phylogenetic study of the genus *Tympanis*. The majority of the strains including the type species form a monophyletic clade. However, the strain '*Tympanis*' *xylophila* CBS 133220 is separated from the main clade of *Tympanis*.

A further four strains that had been identified or described as *Tympanis alnea*, *T. malicola* and *T. pseudotsugae*, including the ex-type strain of the latter (listed in Table 1) were revealed to belong to *Sordariomycetes*, *Dothideomycetes* and *Lecanoromycetes*, respectively, based on blastn searches restricted to type sequences and preliminary phylogenetic analyses (data not shown). Therefore, we excluded the respective sequences from our phylogeny. According to these results, the genus *Tympanis* is also polyphyletic. Further studies are necessary to clarify the taxonomy of these strains.

Based on the recent study on *Phacidiales* by Quijada et al. (2018), *Collophorina* s.str., *Gelatinomyces* (as *Myriodiscus*), *Pallidophorina* (as *C. paarla*), *Aotearoamyces* and *Claussenomyces prasinulus* seem to belong to the core taxa of *Tympanidaceae* (clade H in that study). The inclusion of *Holwaya*, *Mniaecia* and *Epithamnolia* in the family is questionable as the respective backbone clades were not supported. This was apparently the reason for them not to draw any taxonomic consequences from their molecular study either to confirm or to correct the previous systematics of the order (Jaklitsch et al. 2016). The same problem was encountered in our study. All taxa studied here belong to a clade sister to *Holwaya* that corresponds to clades H and K in Quijada et al. (2018). However, as we included more possible *Tympanidaceae* taxa in our phylogeny, the backbone became even more unstable, and even well-supported clades corresponding to I, J and H in Quijada et al. (2018) became blurred. As we concentrated on the collophorina-like species collected and taxa intermingling with them, we cannot make a clear circumscription of *Tympanidaceae* either.

During our survey on necrotic wood of *Prunus* spp. in Germany, collophorina-like fungi were the most abundant, with the dominating species being *Pallidophorina paarla* (syn: *C. paarla*). *Col-*

lophorina africana and *Pa. paarla* were previously reported from Germany: *C. africana* from spore traps in *Prunus armeniaca* orchards and from wood of *P. dulcis*; and *Pa. paarla* from wood of *P. persica* and *P. cerasus* and from spore traps in *Prunus* sp. (Fischer et al. 2016, Gierl & Fischer 2017). In this study, *C. africana* occurred exclusively on *P. domestica*. This is the first report of *Pa. paarla*, *C. africana* and the genus *Collophorina* in general on *P. domestica*. In contrast, *C. hispanica* that was also detected in Germany by Gierl & Fischer (2017), was not found in any of the *Prunus* orchards sampled in this study and is so far only known from *P. armeniaca* and *P. dulcis* (Gramaje et al. 2012, Arzanlou et al. 2016, Gierl & Fischer 2017).

All collophorina-like species studied here can be identified by each of the three loci, ITS, *EF-1α* and *GAPDH*. With all species, sequences of the three loci showed differences in at least four, but often more than ten nucleotides, except for the *EF-1α* sequences of *C. rubra* and *C. neorubra*, which differed in only two nucleotides.

Compared to molecular data, morphological and cultural characters were found to be less suitable for species delimitation. Single features usually apply to several collophorina-like taxa, e.g., SNA and OA cultures of all species are slow growing. However, *Collophorina* can be distinguished from all other collophorina-like genera, by the red pigmentation of OA medium (Damm et al. 2010, Gramaje et al. 2012, Xie et al. 2013, Nasr et al. 2018, this study) and forms a well-supported clade in the phylogenies. In contrast, cultures of '*C.*' *aceris*, *R. euphorbiae* (syn: *C. euphorbiae*), *Pa. paarla* (syn: *C. paarla*) and the newly described *Capturomyces funiculosus*, *Tympanis inflata*, *Vexillomyces palatinus* and *Ve. verruculosus* remain white to cream, while OA cultures of *Variabilispora flava* and *Capturomyces luteus* are yellow pigmented. The latter two species are, however, not closely related to each other.

Microscopical features are often difficult to recognise. All species of collophorina-like fungi studied here, in Damm et al. (2010), Gramaje et al. (2012) and Nasr et al. (2018) produce conidia on intercalary conidiogenous cells, on discrete phialides or adelophialides as well as by microcyclic conidiation. In most species these structures are very similar; only some of the collophorina-like species form unique features. For example, both members of the new genus *Vexillomyces*, *Ve. palatinus* and *Ve. verruculosus*, form pronounced collarettes and verruculose hyphae and phialides. *Tympanis inflata* forms short, inflated phialides, and in the microcyclic conidiation of *C. neorubra* often two or more conidia remain attached to the conidiogenous opening of the mother cells. Endoconidia have previously been found in *Ramoconidiophora euphorbiae*, *C. hispanica* and *Pallidophorina paarla*, in this study only in *Vexillomyces verruculosus*. They are therefore not regarded as a genus-specific feature. Moreover, endoconidia were only rarely observed in these four species; it is possible that other species are also able to produce endoconidia, but they were just not observed in the cultures or not formed on the substrates studied. Among the newly described species, *C. badensis*, *C. germanica*, *C. neorubra* and *Capturomyces luteus* produced fertile conidiomata. Morphology of conidiomata, conidiophores and conidiogenous cells are not distinct from those of previously described species of collophorina-like fungi, except for *Ca. luteus* in which an elongated darker area was visible in the centre of ruptured conidiomata. *Ramoconidiophora euphorbiae* differs by its conidiomatal conidiophores that predominantly develop branches at almost each septum, instead of conidiogenous openings; conidiogenous openings are almost exclusively formed terminally. *Pallidophorina* differs by its very long, tuft-like/funnel-shaped collarettes, while those of *Collophorina* and *Ramoconidiophora* are short cylindrical or even inconspicuous. There is no information on collarettes in conidiomata of *Tympanis* (Groves 1952).

Collophorina-like species were most frequently isolated from *Prunus* wood. Furthermore, they were frequently found in association with wood necroses or other wood diseases, for example on *Prunus* wood in South Africa (Damm et al. 2010), Spain (Gramaje et al. 2012), Slovakia (Ivanová & Bernadovičová 2013), Iran (Arzanlou et al. 2016) and Germany (Gierl & Fischer 2017). Additionally, *C. hispanica* was isolated on *Castanea sativa* in Spain in association with the Chestnut Red Stain disease. However, the authors argued it would be more likely that *Fistulina hepatica*, which was co-isolated with *C. hispanica*, was the causal agent of the disease (Yurkewich et al. 2017). With the exception of '*C.*' *aceris* and *R. euphorbiae*, pathogenicity was confirmed for all previously described collophorina-like species (Damm et al. 2010, Olmo et al. 2015, Arzanlou et al. 2016). In contrast, some collophorina-like species have been found in symptomless plant tissue, namely *Pallidophorina paarla* from *Prunus avium* and *P. cerasus* (Aghdam & Fotouhifar 2016), '*C.*' *aceris* from *Acer glabrum* var. *douglasii* (Xie et al. 2013) and *R. euphorbiae* from *Euphorbia polycaulis* (Nasr et al. 2018), indicating an endophytic lifestyle in at least part of their life cycle. In our survey on *Prunus* wood, most of the isolates of collophorina-like fungi originated from the transition zone of symptomatic to non-symptomatic wood tissue, while sometimes the same species was isolated from non-symptomatic wood of the same branch, which supports the assumption of a life style transition.

All species of *Collophorina* isolated from wood in this study were isolated either only from *P. avium* or only from *P. domestica*. *Pallidophorina paarla* was isolated from all hosts sampled in this study. All species, except for *C. badensis*, were isolated either only from *Prunus* wood or only from spore traps mounted on grapevine shoots. Moreover, the species from spore traps in vineyards have not previously been reported from grapevine yet, neither in Germany (Fischer et al. 2016) nor in any other country (Farr & Rossmann 2018); and no sequences of these species from grapevine tissue could be found by blastn searches on GenBank. This raises the question where these species live. Only one of the species from spore traps in vineyards, *C. badensis*, was isolated from *Prunus* wood as well, but with five nucleotides difference in *EF-1α*. However, the ITS sequences of some of these species, namely *Variabilispora flava*, *Capturomyces funiculosus*, and *Ca. luteus*, are identical with those of fungi detected in *Fagus sylvatica*, *Picea abies*, *Tsuga canadensis*, and *Pinus sylvestris* in Germany, Finland and Canada, respectively (Terhonen et al. 2011, Unterseher et al. 2013, KM Complak et al. unpubl. data, J Kaitera & HM Henttonen unpubl. data). It is therefore more likely, that all or some of these species live in adjacent fruit orchards or other trees in the neighbourhood than in grapevine.

Species of collophorina-like fungi have often been found in woody tissue. Comparatively small spores and a space-saving conidiogenesis directly on or within hyphae could be an adaption to a life inside wood and a distribution within the plant body by means of the vascular tissue system. Findings in spore traps raise the question of the distribution strategy between host plants, which becomes even more obscure as there is no proof of these species from spore traps in grapevine tissue. Usually, object slides covered with Vaseline are used as spore traps (Fischer et al. 2016, Gierl & Fischer 2017, this study). Collophorina-like species can be considered as yeast-like because of its slimy spore masses. Although distribution of fungi via air currents is well-known (Brown & Hovmøller 2002), yeast cells and spores of fungi forming moist conidia masses are more likely to be distributed by water flow, rain splash, or insects as vectors (Kluth et al. 2002, Lachance 2011). If these species live in grapevine tissue, spores are more likely to be transported from plant parts to spore traps by raindrops than by air flow. However,

if they do not live in grapevine tissue, the distribution by rain splash is unlikely as it works only over small distances. Small flies trapped in the Vaseline of the spore traps were observed during collection of the object slides (Kraus unpubl. data). This observation and a finding of *Pallidophorina paarla* in galleries of the borer *Xylotrechus arvicola* (*Coleoptera*, *Cerambycidae*) in *Prunus pisardi* (Benavides et al. 2013) support the idea of a distribution strategy via insect vectors. A report of *Collophorina* from a spore trap analysing air-borne particles of air flow (Coriolis air sampler) should be considered as doubtful as the identification of the fungus is based on an identity of the ITS2 sequence with *C. hispanica* of only 85.6 % (Fort et al. 2016). Additionally, reports of *Collophorina* from roots of *Holcus lanatus* and *Caluna vulgaris* (Kreyling et al. 2012) as well as from sedimentary rock samples from a glacier in Antarctica (Barahona et al. 2016) should also be considered doubtful, because the ITS sequence identities were < 90 %.

The high species number detected in this study and the high incidence in necrotic wood of fruit trees observed in this study and in the study of Damm et al. (2010), along with reports of collophorina-like species from four continents, demonstrate that this group of fungi is widespread, abundant and diverse. Reports of the pathogenicity of some of the species underline their potential threat at least to economically important fruit trees. Damm et al. (2010) already discussed reasons why these fungi had not been discovered for such a long time; most notably they were overlooked due to their slow growth and yeast-like appearance. Xie et al. (2013) extracted the metabolic compound Collophorin from '*C.*' *aceris*, which inhibits the growth of plant pathogens belonging to *Ascomycota*, *Basidiomycota*, *Oomycota* as well as Gram-positive and -negative bacteria. This indicates the potential importance of the compounds of these poorly studied fungi and their possible applications.

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3 Manuscript II

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Arboricolonus simplex gen. et sp. nov. and novelties in *Cadophora*, *Minutiella* and *Proliferodiscus* from *Prunus* wood in Germany

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Abstract

During a survey on fungi associated with wood necroses of *Prunus* trees in Germany, strains belonging to the Leotiomycetes and Eurotiomycetes were detected by preliminary analyses of ITS sequences. Multi-locus phylogenetic analyses (LSU, ITS, *TUB*, *EF-1a*, depending on genus) of 31 of the 45 strains from *Prunus* and reference strains revealed several new taxa, including *Arboricolonus* **gen. nov.**, a new genus in the Helotiales (Leotiomycetes) with a collophorina-like asexual morph. Seven *Cadophora* species (Helotiales, Leotiomycetes) were treated. The 29 strains from *Prunus* belonged to five species, of which *C. luteo-olivacea* and *C. novi-eboraci* were dominating; *C. africana* **sp. nov.**, *C. prunicola* **sp. nov.** and *C. ramosa* **sp. nov.** were revealed as new species. The genus *Cadophora* was reported from *Prunus* for the first time. *Phialophora bubakii* was combined in *Cadophora* and differentiated from *C. obscura*, which was resurrected. Asexual morphs of two *Proliferodiscus* species (Helotiales, Leotiomycetes) were described, including one new species, *Pr. ingens* **sp. nov**. Two *Minutiella* species (Phaeomoniellales, Eurotiomycetes) were detected, including the new species *M. pruni-avium* **sp. nov**. *Prunus avium* and *P. domestica* are reported as host plants of *Minutiella*.

Keywords

Ascomycota, Eurotiomycetes, Leotiomycetes, new taxa, phylogeny, systematics

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Introduction

In order to study the mycobiome of wood necroses of economically important *Prunus* species in Germany, a survey has been conducted using isolation techniques. Based on preliminary analyses of generated ITS sequences, several strains belonging to the Leotiomycetes and Eurotiomycetes were detected. Some of them were recently identified as species of *Collophorina* and related genera (Bien et al. 2020). Further strains showed morphological or genetical affiliation to the genera *Cadophora*, *Proliferodiscus* (Leotiomycetes) and *Minutiella* (Eurotiomycetes).

Leotiomycetes and Eurotiomycetes are both ecologically and morphologically highly diverse classes (Geiser et al. 2006, 2015; Wang et al. 2006b; LoBuglio and Pfister 2010; Johnston et al. 2019). Members of the Leotiomycetes have been described as plant pathogens, especially wood rot fungi, endophytes, nematode-trapping and mycorrhiza-forming fungi, as well as terrestrial and aquatic saprobes (Wang et al. 2006a; Hustad and Miller 2011). Eurotiomycetes are commonly known as saprotrophs and parasites of plants and animals; however, the number of pathogens is relatively low, compared to Sordariomycetes or Dothideomycetes (Geiser et al. 2006, 2015).

The genus *Cadophora* (Ploettnerulaceae, Helotiales, Leotiomycetes) was established in 1927 based on *C. fastigiata* (Lagerberg et al. 1927). Melin and Nannfeldt (1934) added five new species to *Cadophora*, while Davidson (1935) described two additional species that were isolated from stained wood or pulpwood products. Subsequently, Conant (1937) determined that *Phialophora* and *Cadophora* were congeneric and transferred the eight species of *Cadophora* to *Phialophora*. In the monograph of *Phialophora*, Schol-Schwarz (1970) included *C. fastigiata* and *C. malorum*, as well as *Margarinomyces bubakii* in *Phialophora*. Gams (2000) reinstated the genus *Cadophora* for phialophora-like species with more or less pigmented hyphae and pale phialides (*C. fastigiata*, *C. melinii*, *C. malorum*, *C. repens*). For some of these, a relationship with sexual morphs in some discomycete genera, such as *Mollisia* and *Pyrenopeziza*, has been demonstrated (Le Gal and Mangenot 1960, 1961). This connection was confirmed by LSU sequence analysis by Harrington and McNew (2003). However, to date, the type species of none of the genera has been epitypified. Currently, the genus *Cadophora* comprises 17 species. Species of *Cadophora* have been isolated from plants (e.g. Di Marco et al. 2004; Gramaje et al. 2011; Crous et al. 2015; Travadon et al. 2015; Walsh et al. 2018), soil (Kerry 1990; Hujslová et al. 2010; Agustí-Brisach et al. 2013) and decaying wood (Nilsson 1973; Blanchette et al. 2004, 2010).

Haines and Dumont (1983) compared specimens of *Dasyscyphus inspersus* (syn. *Peziza inspersa*) with the type species of the genera *Dasyscyphus* and *Lachnum* and revealed this species not to be congeneric with either of them. Based on spore, hair, paraphysis and subiculum morphology, they erected the new genus *Proliferodiscus* within the family Hyaloscyphaceae (Helotiales, Leotiomycetes). Today, the genus *Proliferodiscus* comprises eight species and is known from woody hosts in tropical and temperate regions worldwide (Haines and Dumont 1983; Spooner 1987; Cantrell and Hanlin 1997; McKenzie et al. 2000; Hofton et al. 2009; Han et al. 2014a; Haelewaters et al. 2018; Ekanayaka et al. 2019).

Crous and Gams (2000) described the genus *Phaeomoniella* (Celotheliaceae, Phaeomoniellales, Eurotiomycetes) based on *Pa. chlamydospora*, the causal agent of Esca disease of grapevine wood (Bertsch et al. 2013; Fontaine et al. 2016; Gramaje et al. 2018). Damm et al. (2010) discovered several new *Phaeomoniella* species from *Prunus* wood in South Africa that were combined in new genera by Crous et al. (2015). One of them, *Minutiella tardicola* (syn. *Pa. tardicola*), was characterised by the very slow growth of the cultures and minute pycnidia (Damm et al. 2010). Most members of Celotheliaceae have been found on *Prunus* (Damm et al. 2010) or other woody hosts from angiosperms and gymnosperms (Crous and Gams 2000; Nordén et al. 2005; Lee et al. 2006; Crous et al. 2008, 2009, 2015, 2016; Zhang et al. 2012; Úrbez-Torres et al. 2013; Yurkewich et al. 2017).

In this study, we aim to (1) systematically place strains isolated from necrotic wood of *Prunus* trees in Germany, as well as some additional strains tentatively identified as *Cadophora* within Leotiomycetes and Eurotiomycetes and (2) formally describe new taxa.

Methods

Sampling and fungal isolation

Branches with wood symptoms (e.g. canker, necroses, wood streaking, gummosis) were sampled from plum (*Prunus domestica*), sour cherry (*P. cerasus*) and sweet cherry (*P. avium*) orchards in Saxony, Lower Saxony and Baden-Württemberg, Germany, in 2015 and 2016. Additionally, a wood sample from a sour cherry tree located in a garden in Bavaria, as well as three strains previously isolated from wood of *P. salicina* in South Africa and two *Phialophora bubakii* strains, all tentatively identified as *Cadophora* spp. in preliminary analyses, were included. Wood pieces (5 × 5 × 5 mm) from the transition zone of symptomatic to non-symptomatic wood tissue, as well as pieces of the same size from non-symptomatic wood of the same branch, were surface sterilised (30 s in 70% ethanol, 1 min in 3.5% NaOCl, 30 s in 70% ethanol), washed for 1 min in sterilised water and placed on synthetic nutrient-poor agar medium (SNA; Nirenberg 1976), as well as oatmeal agar medium (OA; Crous et al. 2019), both supplemented with 100 mg/l penicillin, 50 mg/l streptomycin sulphate and 1 mg/l chloramphenicol. After incubation for several days at 25 °C, hyphal tips of developing fungi were transferred to SNA medium with a sterilised needle. Single-conidial isolates were obtained from the strains for further study. Sampling and isolation of the strains from South Africa was similar (Damm et al. 2007).

The strains are maintained in the culture collections of the Senckenberg Museum of Natural History Görlitz, Germany (GLMC), the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (CBS) and the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (DSMZ). Specimens (dried cultures), including type specimens were deposited in the fungarium of the Senckenberg Museum of Natural History Görlitz (GLMC).

Morphological analysis

To enhance sporulation, autoclaved filter paper and double-autoclaved pine needles were placed on the surface of the SNA medium. The cultures were incubated in the dark at 25 °C. Colony growth and characters on SNA and OA, for some strains additionally on potato dextrose agar (PDA; Crous et al. 2019) and malt extract agar (MEA; Oxoid Ltd., England; 1.5% agar, Difco, USA), were noted after 2 and 4 wk. Colony colours were rated according to Rayner (1970). After 2 or 4 wk, microscopic preparations were made in clear lactic acid and observations and measurements (30 measurements per structure) were made with a Nikon SMZ18 stereomicroscope (SM) or with a Nikon Eclipse N*i*-U light microscope with differential interference contrast (LM). Photographic images were captured with Nikon Digital Sight DS-Fi2 cameras installed on the above-mentioned microscopes, making use of the Nikon NIS-Elements software (v.4.30).

DNA extraction, PCR amplification and sequencing

Of the forty-two strains isolated from *Prunus* wood in Germany, three strains from *Prunus* wood in South Africa, as well as two strains of *Phialophora bubakii* that were included in this study, 34, 4 and 8 strains had been identified as species of *Cadophora*, *Minutiella* and *Proliferodiscus*, respectively, in preliminary analyses based on ITS sequences. Twenty-two *Cadophora* strains, six *Proliferodiscus* strains, all *Minutiella* strains as well as an unidentified Leotiomycete strain were selected for phylogenetic analyses (Table 1).

Genomic DNA of the isolates was extracted using the method of Damm et al. (2008). A partial sequence of the 28S nrDNA (LSU) and the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS1 and ITS2 (ITS) were amplified and sequenced using the primer pairs LR0R (Rehner and Samuels 1994) + LR5 (Vilgalys and Hester 1990) and ITS1F (Gardens and Bruns 1993) + ITS-4 (White et al. 1990), respectively. Additionally, partial sequence of the β-tubulin gene (*TUB*) and the translation elongation factor 1α (*EF-1a*) of strains belonging to the genus *Cadophora* were generated using the primer pairs BTCadF + BTCadR (Travadon et al. 2015) and EF1-688F + EF1-1251R (Alves et al. 2008), respectively. The β-tubulin gene of the genus *Minutiella* was sequenced using the primer pair Bt2a + Bt2b (Glass and Donaldson 1995).

The PCR reaction mixture contained 1 µl of 1:10 DNA template, 2.5 µl 10X buffer (Peqlab, Erlangen, Germany), 1 µl of each primer (10mM), 2.5 µl $MgCl_2$ (25mM), 0.1 µl *Taq* polymerase (0.5 U, Peqlab, Erlangen, Germany) and 2.5 µl of 2mM dNTPs. Each reaction was made up to a final volume of 20 µl with sterile water. DNA amplifications were carried out in a Mastercycler pro S (Eppendorf, Hamburg, Germany). The amplification conditions for ITS and *EF-1a* were: initial denaturation at 95 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s and extension at 72 °C for 60 s; and a final extension step at 72 °C for 3 min. The amplification conditions for the primer pair Bt2a + Bt2b were: initial denaturation at

94 °C for 4 min; followed by 38 cycles of denaturation at 94 °C for 60 s, annealing at 61 °C for 60 s and extension at 72 °C for 45 s; and a final extension step of 5 min at 72 °C. For amplifications of LSU and *TUB* with the primer pair BTCadF + BTCadR, the PCR conditions were set according to Paulin and Harrington (2000) and Travadon et al. (2015), respectively.

The PCR products were visualised on a 1% agarose gel and sequenced using the same primers by the Senckenberg Biodiversity and Climate Research Centre (BiK-F) laboratory (Frankfurt, Germany). The forward and reverse sequences were assembled by using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall 1999).

Phylogenetic analysis

For the phylogenetic analyses, sequences, especially those of ex-type strains, were downloaded from GenBank and added to the sequences generated in this study and those of the appropriate outgroup sequences in four datasets. In order to determine the generic placement of strain GLMC 459, sequences of close matches from blastn searches with its LSU and ITS sequences were combined with sequences of the phylogenetic reassessment of Hyaloscyphaceae by Han et al. (2014b) (dataset 1). Three datasets were generated to determine the systematic position of strains of the genera *Cadophora* (ITS, *TUB*, *EF-1α*; dataset 2), *Minutiella* (LSU, ITS, *TUB*; dataset 3) and *Proliferodiscus* (LSU, ITS; dataset 4). The datasets were aligned automatically using MAFFT v. 7.308 (Katoh et al. 2002, Katoh and Standley 2013) and manually adjusted where necessary.

The phylogenetical analyses were conducted using Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). For BI analyses, the best fit model of evolution for each partition was estimated by MEGA7 (Kumar et al. 2016). Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) as implemented in Geneious v. 10.2.2 (Kearse et al. 2012), using the estimated models of evolution. For each dataset, four simultaneous Markov chains were run for 1 million generations and trees were sampled every 100th generation. The first 2000 trees, which represent the burn-in phase of the analyses, were discarded and the remaining 8000 trees were used to calculate posterior probabilities in the majority rule consensus trees. The ML analyses were performed by RAxML v. 8.2.11 (Stamatakis 2006, 2014) as implemented in Geneious v. 10.2.2 (Kearse et al. 2012), using the GTRGAMMA model with the rapid bootstrapping and search for best scoring ML tree algorithm, including 1000 bootstrap replicates. The MP analyses were performed with MEGA7 (Kumar et al. 2016) using tree-bisection-reconnection (TBR) as the branch-swapping algorithm. The robustness of the trees was evaluated by 1000 bootstrap replicates and 10 random sequence additions. Tree length, consistency index, retention index and composite index of the resulting trees were calculated. The DNA sequences generated in this study were deposited in GenBank (Table 1), the alignments in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S24703).

Table 1. List of strains analysed in this study, with collection details and GenBank accession numbers.

Species	Accession no.[1]	Host/ substrate	Country	GenBank no.[2]			
				LSU	ITS	*TUB*	*EF1-α*
Arboricolonus simplex	GLMC 459[T]	*Prunus domestica*	Germany	**MN232924**	**MN232935**	–	–
Cadophora africana	CBS 120890[T]	*Prunus salicina*	South Africa	–	**MN232936**	**MN232967**	**MN232988**
Cadophora bubakii (as *Phialophora bubakii*)	CBS 198.30[T]	margarine	Czech Republic	–	MH855111	–	**MN232989**
Cadophora luteo-olivacea	GLMC 517	*Prunus domestica*	Germany	–	**MN232937**	**MN232968**	**MN233003**
	GLMC 1264	*Prunus domestica*	Germany	–	**MN232938**	**MN232969**	**MN233004**
	GLMC 1310	*Prunus domestica*	Germany	–	**MN232939**	**MN232970**	**MN233005**
	GLMC 1517	*Prunus domestica*	Germany	–	**MN232940**	**MN232971**	**MN233006**
	GLMC 1545	*Prunus domestica*	Germany	–	**MN232941**	**MN232972**	**MN233007**
Cadophora novi-eboraci	GLMC 239	*Prunus cerasus*	Germany	–	**MN232942**	**MN232973**	**MN232990**
	GLMC 273	*Prunus cerasus*	Germany	–	**MN232943**	**MN232974**	**MN232991**
	GLMC 274	*Prunus cerasus*	Germany	–	**MN232944**	**MN232975**	**MN232992**
	GLMC 342	*Prunus cerasus*	Germany	–	**MN232945**	**MN232976**	**MN232993**
	GLMC 688	*Prunus cerasus*	Germany	–	**MN232946**	**MN232977**	**MN232994**
	GLMC 1472	*Prunus cerasus*	Germany	–	**MN232947**	**MN232978**	**MN232995**
Cadophora obscura (as *Phialophora bubakii*)	CBS 269.33	fresh water	Sweden	–	**MN232948**	–	**MN232996**
Cadophora prunicola	CBS 120891[T]	*Prunus salicina*	South Africa	–	**MN232949**	**MN232979**	**MN232997**
	STEU 6103	*Prunus salicina*	South Africa	–	**MN232950**	–	–
	GLMC 276	*Prunus cerasus*	Germany	–	**MN232951**	**MN232980**	**MN232998**
	GLMC 362	*Prunus domestica*	Germany	–	**MN232952**	–	–
	GLMC 735	*Prunus cerasus*	Germany	–	**MN232953**	**MN232981**	**MN232999**
	GLMC 1574	*Prunus domestica*	Germany	–	**MN232954**	**MN232982**	**MN233000**
	GLMC 1633	*Prunus domestica*	Germany	–	**MN232955**	**MN232983**	**MN233001**
Cadophora ramosa	GLMC 377[T]	*Prunus cerasus*	Germany	–	**MN232956**	**MN232984**	**MN233002**
Minutiella pruni-avium	GLMC 1624[T]	*Prunus avium*	Germany	**MN232925**	**MN232957**	**MN232985**	–
	GLMC 1667	*Prunus avium*	Germany	**MN232926**	**MN232958**	**MN232986**	–
Minutiella sp.	GLMC 1636	*Prunus domestica*	Germany	**MN232927**	**MN232959**	–	–
	GLMC 1687	*Prunus domestica*	Germany	**MN232928**	**MN232960**	**MN232987**	–
Proliferodiscus ingens	GLMC 1751[T]	*Prunus avium*	Germany	**MN232929**	**MN232961**	–	–
Proliferodiscus sp.	GLMC 460	*Prunus domestica*	Germany	**MN232930**	**MN232962**	–	–
	GLMC 470	*Prunus domestica*	Germany	**MN232931**	**MN232963**	–	–
	GLMC 502	*Prunus domestica*	Germany	**MN232932**	**MN232964**	–	–
	GLMC 1301	*Prunus domestica*	Germany	**MN232933**	**MN232965**	–	–
	GLMC 1761	*Prunus avium*	Germany	**MN232934**	**MN232966**	–	–

[1]CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; GLMC: Culture collection of Senckenberg Museum of Natural History Görlitz, Görlitz, Germany; STEU: University of Stellenbosch, Stellenbosch, South Africa.
[2]LSU: nuclear large subunit ribosomal DNA; ITS: internal transcribed spacers and intervening 5.8S nrDNA; *TUB*: β-tubulin gene; *EF1-α*: translation elongation factor 1-α gene.
Sequences generated in this study are emphasised in bold face. [T]ex-type cultures.

Results

Phylogenetic analyses

The combined sequence dataset 1 consisted of 59 isolates including the outgroup *Geoglossum nigritum* strain AFTOL-ID 56 and comprised 1540 characters, of which 436 characters were parsimony-informative, 578 variable and 885 constant. The gene boundaries on the LSU-ITS multi-locus alignment were as follows: LSU: 1–890 and ITS: 891–1540. The final ML optimisation likelihood of ML analysis was: lnL = -15669.074659. One most parsimonious tree was generated by MP analysis with tree length: 693 steps, consistency index: 0.298780, retention index: 0.555126 and composite index: 0.186644 and 0.165861 for all sites and parsimony informative sites, respectively. The BI phylogeny, including BI posterior probability values as well as ML and MP bootstrap support values, is shown in Fig. 1.

The clades in Fig. 1 represent all clades of the multi-locus phylogeny of the "Hyaloscyphaceae" by Han et al. (2014b) as well as clades formed by sequences of the closest matches from blastn searches with the ITS and LSU sequences of strain GLMC 459 in GenBank. Strain GLMC 459 from *P. domestica* in Germany forms a long single-strain clade that does not belong to any of the above-mentioned clades and is located close to *Polydesmia pruinosa* TNS-F-12764, strains belonging to Clade 9 in Han et al. (2014b) and a clade formed by three strains of *Polyphilus*. The clade, formed by GLMC 459 and these taxa, is not supported.

The combined sequence dataset 2 of *Cadophora* consisted of 70 isolates including the outgroup *Hyaloscypha finlandica* CBS 444.86 and comprised 1594 characters, of which 498 characters were parsimony-informative, 692 variable and 859 constant. The gene boundaries in the multi-locus alignment were as follows: ITS: 1–575, *TUB*: 576–1133 and *EF-1a*: 1134–1594. Five most parsimonious trees were generated by MP analysis with tree length: 205 steps, consistency index: 0.536145, retention index: 0.931189 and composite index: 0.581425 and 0.499252 for all sites and parsimony informative sites, respectively. The BI phylogeny, including BI posterior probability values as well as ML (lnL = -9335.686864) and MP bootstrap support values, is shown in Fig. 2.

The phylogeny consists of two main clades belonging to 21 clades representing different *Cadophora* species. The two main clades are formed by BI and ML analyses; both are supported by BI (100%); however, only the second clade is supported by ML and MP analyses (100% and 74%, respectively). In the first main clade, six strains isolated from *P. cerasus* in Saxony and Bavaria form a well-supported clade (100/100/83% BI posterior probability, ML and MP bootstrap support values, respectively) with strains of *C. novi-eboraci* including its ex-type strain. A further five strains from *P. cerasus* and *P. domestica* in Saxony and Baden-Württemberg and two strains from *P. salicina* in South Africa form a well-supported clade (100/99/78%) that does not include any previously described species. One strain isolated from *P. salicina* in South Africa (CBS 120890) and a strain referred to as *C.* "*novi-eboraci*" (CBS 101359) form single-strain clades and belong to a well-supported clade with *C. novi-eboraci* and *C. prunicola* (100/81/99%). One strain isolated from *P. cerasus* in Saxony (GLMC 377) forms a well-supported clade (100/100/94%) with four strains referred to as *C.* "*spadicis*". Within the second main clade, five strains isolated from *P. domestica* in all three sampling areas in Germany form a well-supported clade (100/94/–%) with 16 strains of *C. luteo-olivacea* including its ex-type strain. Two strains of *Phialophora bubakii* CBS 198.30 and CBS 837.69, both originating from margarine, form a well-supported clade (100/100/99%) sister to a third strain (CBS 269.33) from fresh water in Sweden that forms a single-strain clade. The clade formed by all three strains is well-supported (100/93/–%) as well.

The combined sequence dataset 3 consisted of 29 isolates of the Celotheliaceae and the outgroup *Capronia fungicola* CBS 614.96 and comprised 1904 characters, of which 486 characters were parsimony-informative, 685 variable and 1182 constant. The gene boundaries in the multi-locus alignment were as follows: LSU: 1–840 and ITS: 841–1482, *TUB*: 1483–1904. One most parsimonious tree was generated by MP analysis with tree length: 384 steps, consistency index: 0.558989, retention in-

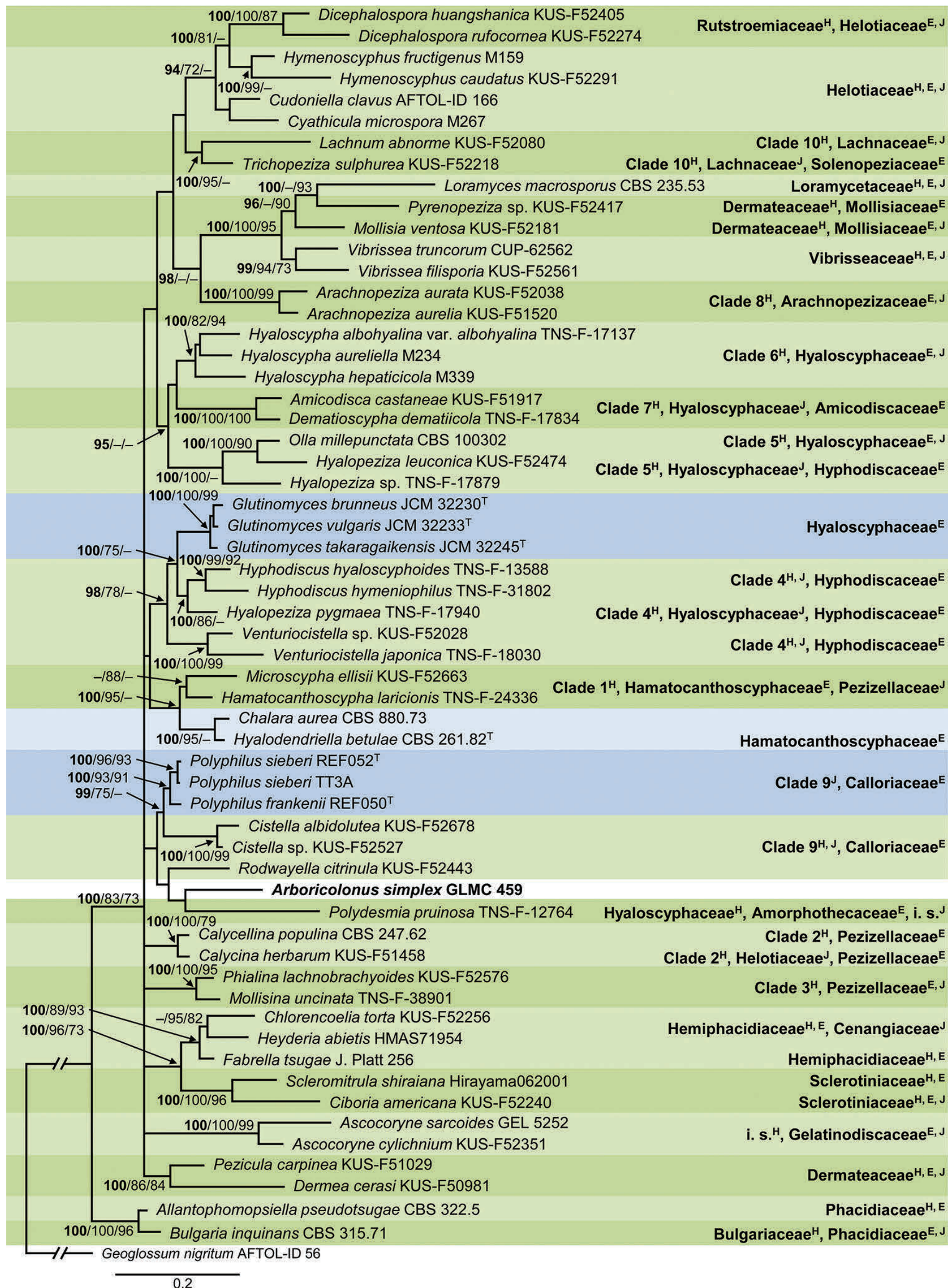

Figure 1. Phylogeny of dataset 1 obtained by Bayesian Inference analysis of the combined LSU and ITS sequence alignment for generic placement of strain GLMC 459. *Geoglossum nigritum* strain AFTOL-ID 56 is used as outgroup. BI posterior probability support values above 90% (bold) and ML and MP bootstrap support values above 70% are shown at the nodes. The strain, analysed in this study, is emphasised in bold. Green backgrounds indicate sequences included in the analyses of Han et al. (2014b). Blue backgrounds indicate close matches of GLMC 459 in blastn searches. Clades 1–10 of Hyaloscyphaceae, according to the analyses of Han et al. (2014b), are listed to the right. Family names are listed to the right, according to Han et al. (2014b, superscript H), Ekanayaka et al. (2019, superscript E) and Johnston et al. (2019, superscript J). Branches that are crossed by diagonal lines are shortened by 50%.

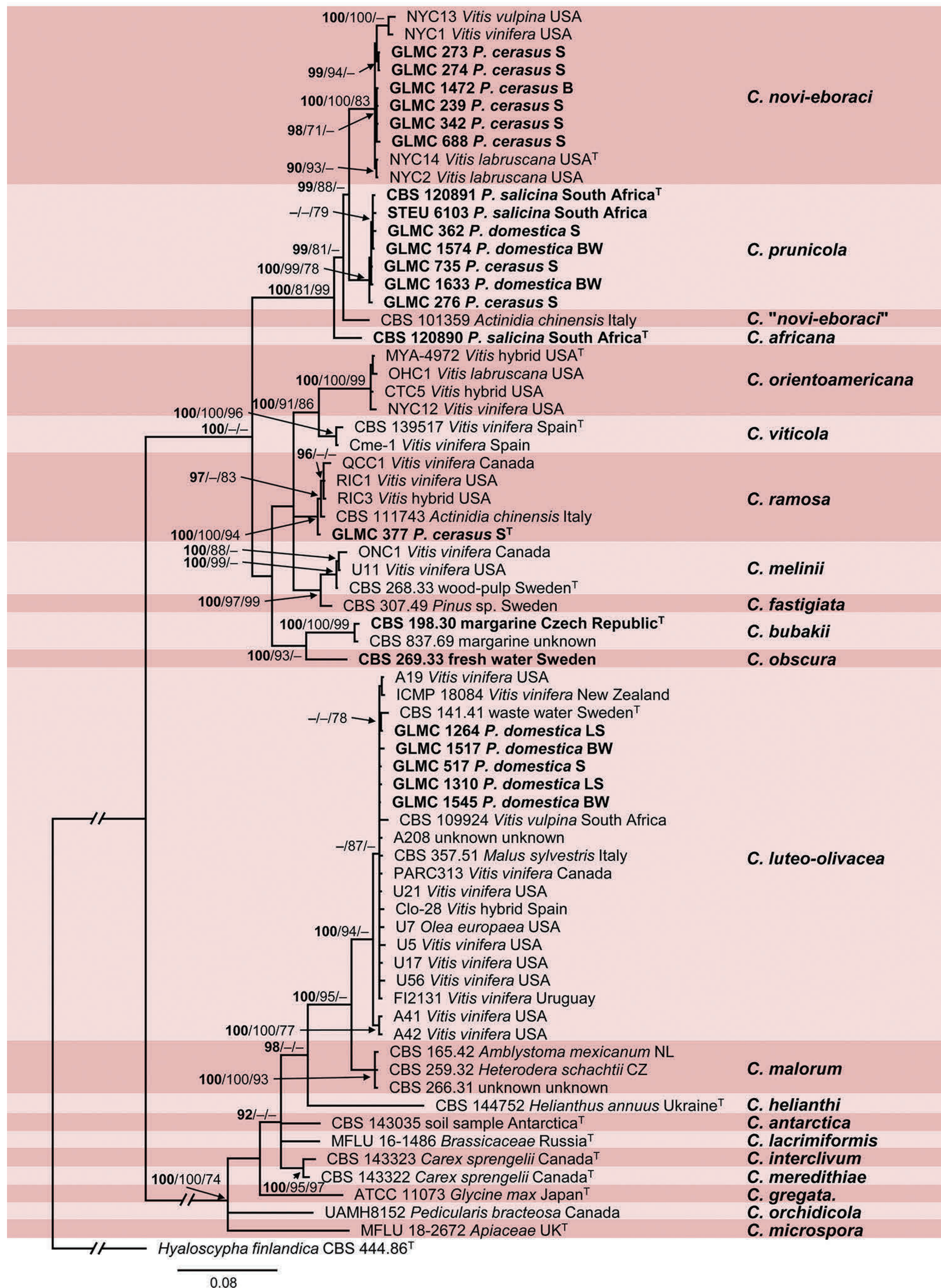

Figure 2. Phylogeny of dataset 2 obtained by Bayesian Inference analysis of the combined ITS, *TUB*, *EF-1a* sequence alignment of *Cadophora*. *Hyaloscypha finlandica* strain CBS 444.86 is used as outgroup. Host plant or substrate and country of isolation are given for every strain. For strains isolated from *Prunus* spp. in Germany, the German Federal State is given in abbreviation as location. Species names are listed to the right. BI posterior probability support values above 90% (bold), ML and MP bootstrap support values above 70% are shown at the nodes. The strains, analysed in this study, are emphasised in bold. Numbers of ex-type and ex-isotype strains are emphasised with a superscript T. Branches that are crossed by diagonal lines are shortened by 50%. B: Bavaria; BW: Baden-Württemberg; LS: Lower Saxony; S: Saxony.

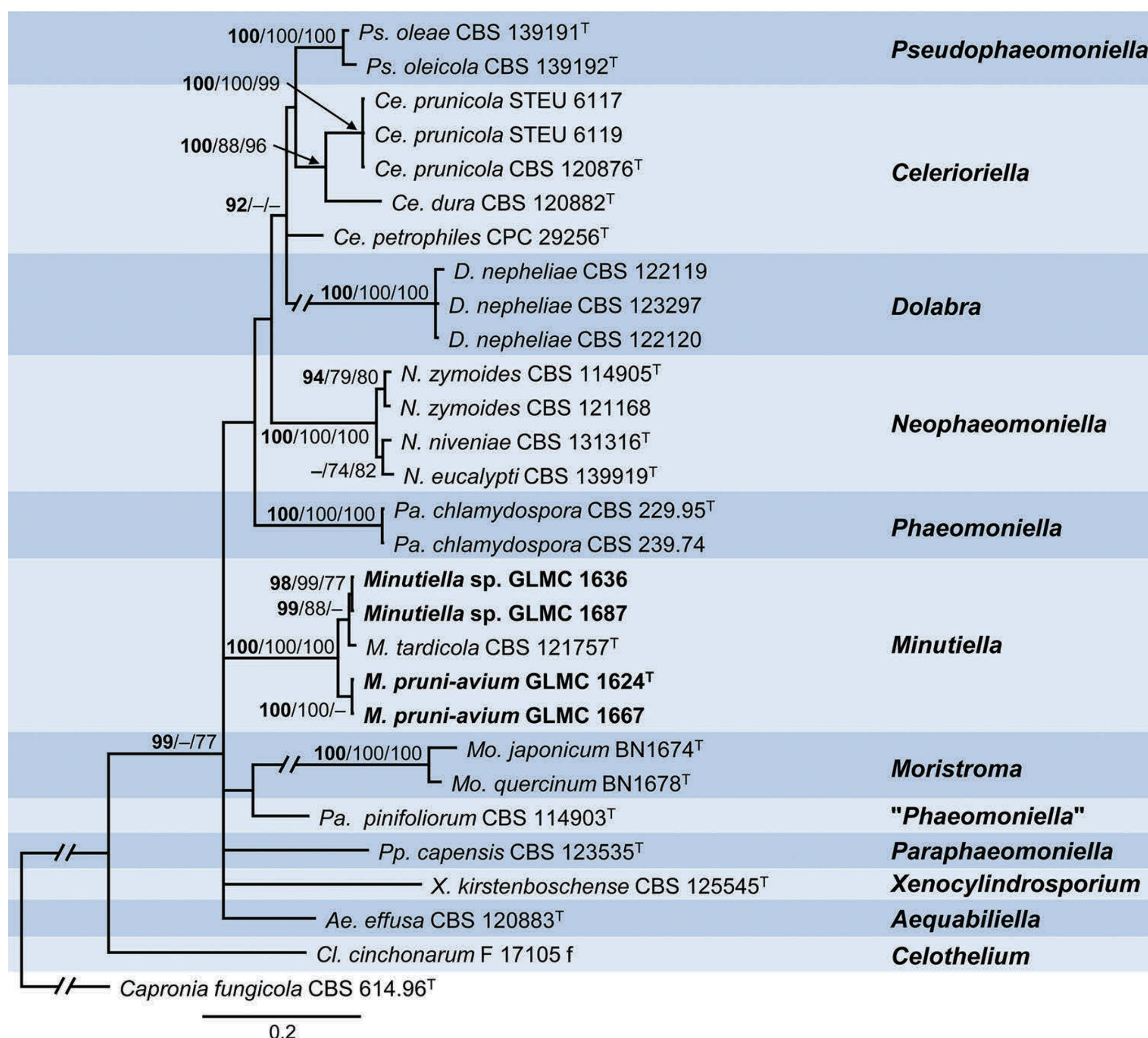

Figure 3. Phylogeny of dataset 3 obtained by Bayesian Inference analysis of the combined LSU, ITS, *TUB* sequence alignment of Phaeomoniellales, including *Minutiella*. *Capronia fungicola* strain CBS 614.96 is used as outgroup. BI posterior probability support values above 90% (bold), ML and MP bootstrap support values above 70% are shown at the nodes. The strains analysed in this study are emphasised in bold. Numbers of ex-type strains are emphasised with a superscript T. Branches that are crossed by diagonal lines are shortened by 50%.

dex: 0.779804 and composite index: 0.458467 and 0.435901 for all sites and parsimony informative sites, respectively. The BI phylogeny, including BI posterior probability values as well as ML (lnL = -9719.124620) and MP bootstrap support values, is shown in Fig. 3.

The 12 main clades of the phylogeny represent genera of the Celotheliaceae; all species for which sequences are available, are included. Four isolates from this study group in a well-supported clade (100/100/100%) with *Minutiella tardicola*. Two of the strains isolated from *P. domestica* form a well-supported sister clade (98/99/77 %) to the single-strain clade formed by the ex-type strain of *M. tardicola*. A further two strains isolated from *P. avium* form a well-supported clade (100/100/–%), sister to the clade consisting of *M. tardicola* and *Minutiella* sp.

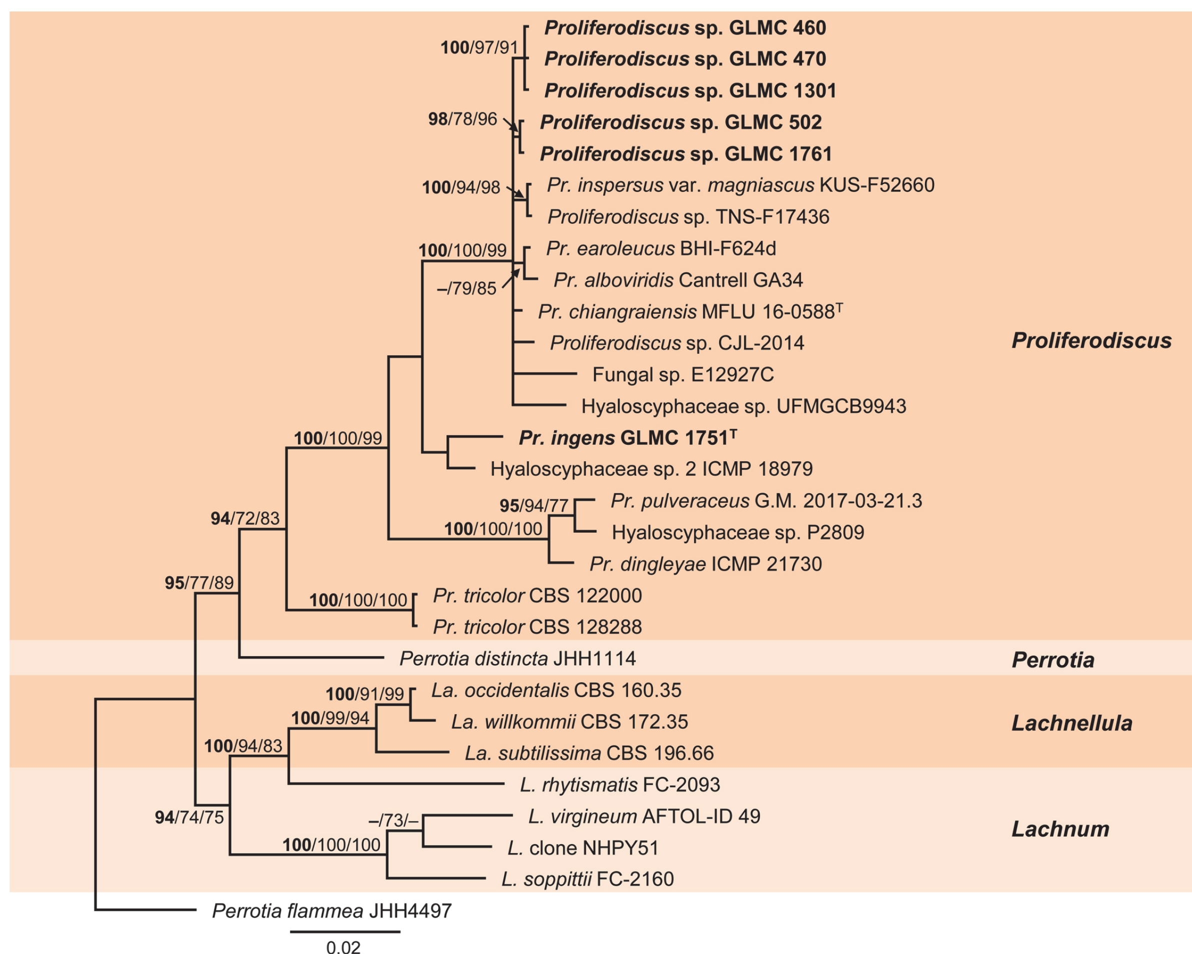

Figure 4. Phylogeny of dataset 4 obtained by Bayesian Inference analysis of the combined LSU, ITS sequence alignment of *Proliferodiscus* and close relatives. *Perrotia flammea* strain JHH4497 is used as outgroup. BI posterior probability support values above 90% (bold), ML and MP bootstrap support values above 70% are shown at the nodes. The strains, analysed in this study, are emphasised in bold. Numbers of ex-type strains are emphasised with a superscript T.

The combined sequence dataset 4 consisted of 29 isolates of *Proliferodiscus* and closely related genera including the outgroup *Perrotia flammea* JHH4497 and comprised 1385 characters, of which 152 characters were parsimony-informative, 204 variable and 1174 constant. The gene boundaries in the multi-locus alignment were as follows: LSU: 1–854 and ITS: 855–1385. Seven most parsimonious trees were generated by MP analysis with tree length: 263 steps, consistency index: 0.651341, retention index: 0.807611 and composite index: 0.526030 and 0.482422 for all sites and parsimony informative sites, respectively. The BI phylogeny obtained by Bayesian Inference, including BI posterior probability values as well as ML (lnL = -4019.800817) and MP bootstrap support values, is shown in Fig. 4.

The main clades represent closely related genera. Six strains from *Prunus* wood in Germany group in the *Proliferodiscus* clade. Five of them, from *P. avium* and *P. domestica*, cluster with seven ambiguously identified strains and the type strain of *Pr. chiangraiensis* in a well-supported clade (100/100/99%). Strain GLMC 1751 forms a single-strain clade sister to "Hyaloscyphaceae sp. 2" ICMP 18979.

Taxonomy

Based on DNA sequence data and morphology, the 33 strains studied (Table 1) are assigned to four genera, of which seven species belong to *Cadophora*, two species to *Minutiella* and two species to *Proliferodiscus*, including 5 species that proved to be new to science and are described. Two strains, referred to as *Phialophora bubakii*, proved to belong to two distinct species within *Cadophora*. Strain GLMC 459 could not be assigned to any known genus and is therefore described as new genus. All species studied in culture are characterised below.

Arboricolonus **S.Bien & Damm, gen. nov.**

MycoBank No: 832106

Type species. *Arboricolonus simplex* S.Bien & Damm.

Etymology. Referring to the life inside tree wood (*arbor* Lat. = tree + *colonus* = settler).

Description. *Colonies* slow-growing, moist, white or buff colours on oatmeal agar medium, lacking aerial mycelium. *Sporulation* conidia formed on hyphal cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to short discrete phialides or, more often, collarettes formed directly on hyphal cells, collarettes short tubular to funnel-shaped. *Conidia* aggregated around the hyphae, small, hyaline, 1-celled, cylindrical, ovoidal to allantoid. *Vegetative hyphae and phialides* hyaline, smooth-walled, septate, branched.

Arboricolonus simplex **S.Bien & Damm, sp. nov.**

MycoBank No: 832107

Figures 5A, 6

Type. Germany, Saxony, orchard north of Wölkau, 50°58'42.3"N, 13°49'40.0"E, from brown wedge-shaped necrosis in wood of *Prunus domestica*, 16 Jan 2015, S. Bien leg., GLM-F106309 – ***holotype***; GLMC 459 = CBS 145520 = DSM 109147 – culture ex-type.

Etymology. Named after the simple, reduced conidiophores.

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–3 µm wide, sometimes hyphal cells inflated and constricted at the septa, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, integrated or terminal, discrete phialides, ampulliform to navicular, 4–7 × 2–3 µm, often reduced to small necks or openings on hyphae, opening 0.5–1 µm wide, collarettes short tubular to funnel-shaped, 0.5–1 µm long, periclinal thickening sometimes visible. *Conidia* aggregated in heads or slimy masses around hyphae, hyaline, smooth-walled, aseptate, straight to ± curved, cylindrical,

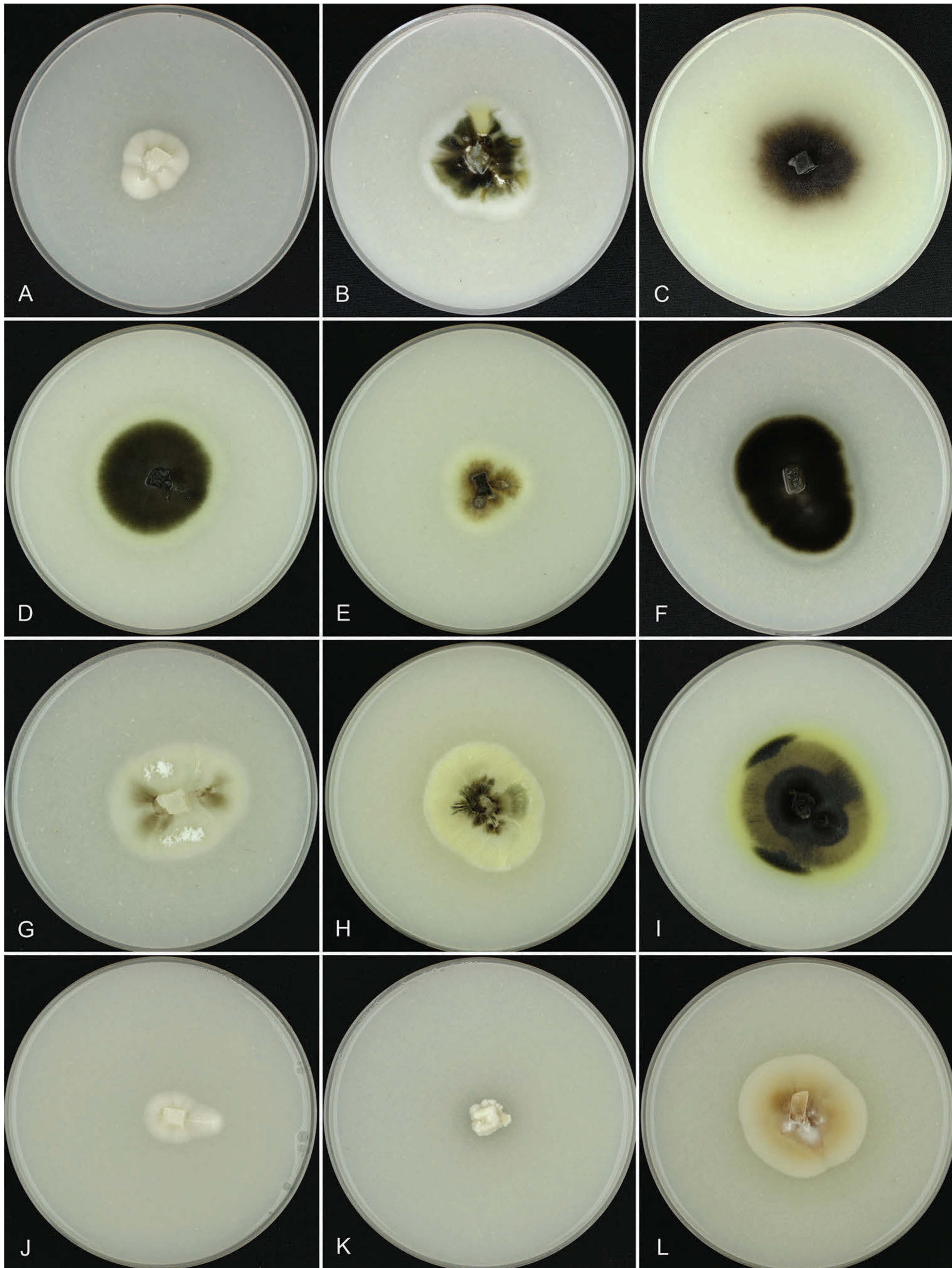

Figure 5. Colony surface of analysed strains on OA medium. **A** *Arboricolonus simplex* GLMC 459^{T} **B** *Cadophora africana* CBS 120890^{T} **C** *C. bubakii* CBS 198.30^{T} **D** *C. luteo-olivacea* GLMC 1264 **E** *C. novi-eboraci* GLMC 1472 **F** *C. obscura* CBS 269.33 **G** *C. prunicola* CBS 120891^{T} **H** *C. prunicola* GLMC 1633 **I** *C. ramosa* GLMC 377^{T} **J** *Minutiella pruni-avium* GLMC 1624^{T} **K** *Proliferodiscus ingens* GLMC 1751^{T} **L** *Proliferodiscus* sp. GLMC 460. Cultures **A, J–L** after 4 wk. Cultures **B–I** after 2 wk. Strains with a superscript T are ex-type cultures.

elongate ovoidal to allantoid, with one end rounded, the other end rounded to truncate, 3–4(–4.5) × 1–1.5(–2) µm, mean ± SD = 3.6 ± 0.6 × 1.3 ± 0.2 µm, L/W ratio = 2.8.

Culture characteristics. *Colonies on OA* flat to slightly raised with an entire to undulate margin, hyaline, whitish to buff, lacking aerial mycelium, reverse same colours, 2–4 mm diam. in 2 wk, 6–10 mm diam. in 4 wk; *on SNA* flat to slightly raised with an entire to undulate margin, hyaline to whitish, lacking aerial mycelium, reverse same colours, 1–2 mm diam. in 2 wk, 3–6 mm diam. in 4 wk.

Notes. The morphology of *Arboricolonus simplex* is reminiscent of collophorina-like species regarding the colonies that are slow growing, the lack of aerial mycelium and the conidiogenous cells that are mostly reduced to short necks or openings with collarettes on hyphae (Damm et al. 2010; Bien et al. 2020). In contrast to these genera, microcyclic conidiation has not been observed in *Arboricolonus.* This genus belongs to the Leotiomycetes as well; however, it is not closely related to *Collophorina* and collophorina-like genera (Phacidiales) treated by Bien et al. (2020). A class-wide phylogenetic analysis of LSU-ITS places it within the order Helotiales (data not shown).

A blastn search with the ITS sequence of *A. simplex* in GenBank resulted in uncultured and unidentified strains with ≤ 92% identity, for example, an uncultured Helotiales clone from soil in the USA (HQ021771, JH Vineis et al., unpubl. data), while the closest matches with strains, identified at least to the genus level, were strains of *Glutinomyces vulgaris* with 90% identity (e.g. LC218288; Nakamura et al. 2018). The closest matches in a blastn search with the LSU sequence were, with ≤ 97% identity, the ex-type strain of *Hyalodendriella betulae* (EU040232; Crous et al. 2007), a strain identified as *Chalara aurea* (MH872551; Vu et al. 2019) and strains belonging to *Polyphilus sieberi* (e.g. MG719708; Ashrafi et al. 2018).

Cadophora africana Damm & S.Bien, sp. nov.

MycoBank No: 832108
Figures 5B, 7

Type. South Africa, Western Cape Province, Franschhoek, from necrosis in wood of *Prunus salicina* close to old pruning wound, 10 June 2004, U. Damm leg., CBS H-19984 – ***holotype***; GLM-F117479 – ***isotype***; CBS 120890 = STE-U 6203 = GLMC 1892 – culture ex-type.

Etymology. Named after the continent of origin, Africa.

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–3 µm wide, hyphal cells sometimes inflated and constricted at the septa, sometimes becoming brown with age, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* hyaline, smooth-walled, mesotonously branched, occasionally with acropleurogenous branching, up to 35 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, discrete conidiogenous cells cylindrical to navicular, often constricted and sometimes widened at the base, 8–18 × 1.5–3 µm, necks cylindrical, 1–2 × 1–1.5 µm, collarettes

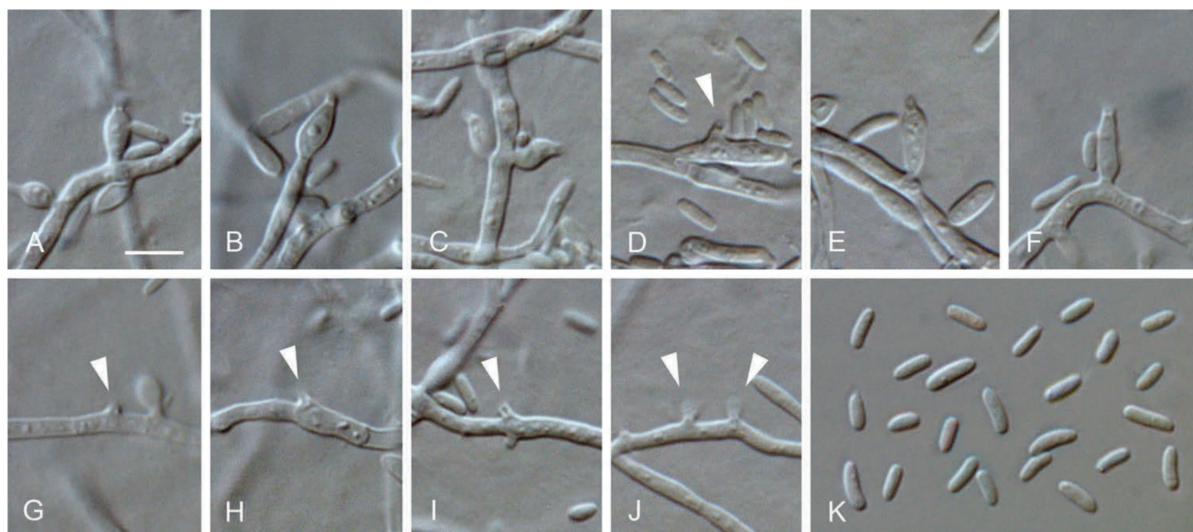

Figure 6. *Arboricolonus simplex* gen. et sp. nov. **A–J** conidiogenous cells (arrows indicate conidiogenous openings or short necks) **K** conidia **A–K** from SNA **A–K** LM. Scale bar: 5 µm (**A** applies to **B–K**).

distinct, cylindrical to narrowly funnel-shaped, 0.5–1.5 µm long, 1–1.5 µm wide at the upper edge, opening 1–1.5 µm wide, periclinal thickening observed. *Conidia* aggregated in heads, hyaline, smooth-walled, aseptate, mostly globose to subglobose or obovoid to tear-shaped, sometimes ellipsoidal, (2–)2.5–4(–4.5) × (1.5–)2–2.5(–3) µm, mean ± SD = 3 ± 0.5 × 2.1 ± 0.2 µm, L/W ratio = 1.4.

Culture characteristics. *Colonies on SNA* flat with an entire to undulate margin, white to buff, sometimes grey olivaceous to olivaceous, lacking aerial mycelium, reverse same colours, 6–14 mm diam. in 2 wk (25 °C in the dark); *Colonies on OA* flat with an entire to undulate margin, primrose to amber, grey olivaceous to olivaceous black, often with a white margin, partly covered by floccose white aerial mycelium, reverse buff to grey olivaceous, 22–30 mm diam. in 2 wk (25 °C in the dark); *Colonies on PDA* flat to raised, entire edge, short aerial mycelium, pale buff, after > 2 wk with pale olivaceous to pale olivaceous grey patches or sectors, reverse same colours, 30 mm diam. in 2 wk (20 °C). *Colonies on MEA* flat to low umbonate, with entire edge, abundant velvety aerial mycelium, mycelium and surface white to very pale smoke-grey; reverse very pale luteous, ochreous to buff, in diffuse daylight with concentric oliveceous-grey rings, 30 mm diam. in 2 wk (20 °C).

Notes. *Cadophora africana* was isolated once from *P. salicina* in South Africa. *Cadophora africana*, as well as *C. bubakii* and *C. ramosa*, form subglobose conidia. However, conidia of *C. africana* are mostly globose to subglobose, sometimes even tear-shaped, while those of *C. ramosa* are often ellipsoidal, elongate-ellipsoidal to cylindrical and the portion of subglobose conidia in *C. bubakii* is comparatively low. Therefore, conidia of both species are on average longer (4.9 µm and 3.6 µm, respectively) than those of *C. africana* (3 µm) and with a larger L/W ratio (2.2 and 2.1, respectively; *C. africana*: 1.4).

The ITS sequence of *C. africana* strain CBS 120890 differs in eleven nucleotides from the ex-type strain of *C. prunicola* and in nine nucleotides, both from the ex-type strain of *C. novi-eboraci* NYC14 and from strain CBS 101359. The differences to these

Figure 7. *Cadophora africana* sp. nov. **A–G** conidiophores and conidiogenous cells (arrow indicates a short neck) **H** conidia **A–H** from SNA **A–H** LM. Scale bar: 5 µm (**A** applies to **B–H**).

strains exceed 30 and 18 nucleotides in the *TUB* and *EF-1a* sequences, respectively. The closest match in a blastn search with the ITS sequence of *C. africana* is strain NYC13 of *C. novi-eboraci* (identity 98.48%), which is included in our phylogeny.

Cadophora bubakii (Laxa) Damm & S.Bien, comb. nov.

Figures 5C, 8

Margarinomyces bubakii Laxa, Zentbl. Bakt. ParasitKde, Abt. II 81: 392. 1930. (Basionym)
≡ *Phialophora bubakii* (Laxa) Schol-Schwarz, Persoonia 6 (1): 66. 1970.

Type. Czech Republic, Prague, from a margarine factory, margarine, O. Laxa leg., collection date unknown (isolated by O. Laxa, deposited in CBS collection by O. Laxa probably 1930), CBS H-491, CBS H-7316, GLM-F117482 – ***isotypes***; CBS 198.30 = IMI 24000 = NCTC 3273 = VKM F-162 = LM 288 = LM 793 = GLMC 1895 – culture ex-isotype.

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–3 µm wide, sometimes becoming brown with age, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* hyaline, smooth-walled, occasionally with acropleurogenous branching, up to 26 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, discrete conidiogenous cells cylindrical to navicular, often slightly inflated having a flask-shaped appearance, often constricted at the base, 3–12 × 1.5–3.5 µm, necks cylindrical, 1–2.5 × 1–2 µm, collarettes distinct, cylindrical to funnel-shaped, 0.5–1 µm long, 1–1.5 µm wide at the upper edge, opening 1–1.5 µm wide, periclinal thickening observed. *Conidia* aggregated in heads, hyaline, smooth-walled, aseptate, subglobose to ellipsoidal or cylindrical with both ends rounded, straight or slightly curved, (2–)2.5–4.5(–6) × 1.5–2 µm, mean ± SD = 3.6 ± 0.9 × 1.7 ± 0.2 µm, L/W ratio = 2.1.

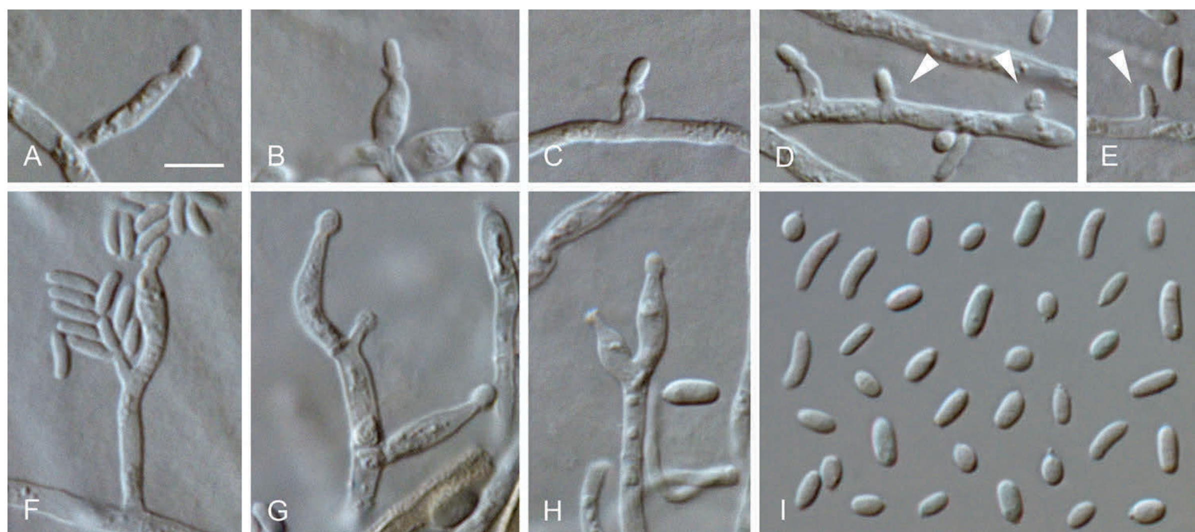

Figure 8. *Cadophora bubakii* comb. nov. **A–H** conidiophores and conidiogenous cells (arrows indicate short necks) **I** conidia **A–I** from SNA **A–I** LM. Scale bar: 5 µm (**A** applies to **H–I**).

Culture characteristics. *Colonies on SNA* flat with an entire to undulate margin, white, lacking aerial mycelium, reverse same colour, 36–56 mm diam. in 2 wk (25 °C in the dark); *Colonies on OA* flat with an entire to undulate margin, olivaceous to olivaceous black, sometimes covered by floccose aerial mycelium, olivaceous grey, reverse same colours, 24–27 mm diam. in 2 wk (25 °C in the dark).

Notes. The genus *Margarinomyces* was described 1930 with *Ma. bubakii* as type species after causing problems in a margarine factory in Czech Republic by forming greenish-black spots on and in margarine cubes that smelled like bitter-almond (benzaldehyde) (Laxa 1930). The fungus was shown to survive 20 min at 60 °C and to be resistant to organic preservatives such as sodium benzoate that was, however, only tolerated in margarine up to a concentration of 0.2% (Laxa 1930). According to the CBS website, strain CBS 198.30 is ex-isotype of *Ma. bubakii*. Schol-Schwarz (1970) included *Ma. bubakii* in *Phialophora* and considered *C. obscura* as a synonym. The genus *Margarinomyces* had been included in *Phialophora* by Gams and McGinnis (1983), though excluded by Cole and Kendrick (1973), but *Ma. bubakii* has never been considered as a species of *Cadophora* before. All nine *Margarinomyces* species had been combined in other genera, most of them in *Coniochaeta* (http://www.indexfungorum.org).

Cadophora bubakii (strain CBS 198.30) differs from *C. obscura* (strain CBS 269.33) by forming conidiogenous cells that are often slightly inflated and therefore flask-shaped, while those of CBS 269.33 are mostly narrow cylindrical. Conidia of strain CBS 198.30 are sometimes subglobose and, on average, distinctly shorter than the ellipsoidal to cylindrical conidia of CBS 269.33. Moreover, colonies of CBS 198.30 grow faster. Van Beyma (1943) compared *Ma. bubakii* and *Ph. obscura* and mentioned flask-shaped conidiogenous cells and a faster colony growth rate of *Ma. bubakii* and narrow phialides of *Ph. obscura* as well. However, the conidia shape of both species was described and illustrated as rod-shaped.

The ITS sequences of the two *C. bubakii* strains included in the phylogeny of this study, CBS 198.30 and CBS 837.69, are identical but differ both in 19 nucleotides from that of the *C. obscura* strain CBS 269.33. The *EF-1a* sequence of the two species differs in 31 nucleotides. The *TUB* sequences of CBS 198.30 and CBS 269.33 were not able to be aligned with each other and the rest of the dataset and therefore excluded from the phylogeny.

A blastn search with the ITS sequence of CBS 198.30 resulted in high similarities (99.82% and 99.64%) with "*Ph. bubakii*" strains CBS 837.69 (included in our analysis) and CBS 836.69, both isolated from margarine, as well as CBS 834.69, isolated from wood pulp of *Populus tremula* (Vu et al. 2019).

Cadophora luteo-olivacea (J.F.H.Beyma) T.C.Harr. & McNew

Figures 5D, 9

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–10 µm wide, hyphal cells often, sometimes very strongly inflated and constricted at the septa, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* hyaline, smooth-walled, simple or septate and branched, up to 40 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, cylindrical to ± inflated, 3–14 × 1.5–4 µm, sometimes integrated, necks cylindrical, 0.5–3 µm long, collarettes funnel-shaped, 1–1.5 µm long, 1–2 µm wide at the upper edge, opening 1–1.5 µm wide, periclinal thickening not observed. *Conidia* aggregated in heads, hyaline, smooth-walled, aseptate, cylindrical, rarely ellipsoidal, straight, sometimes slightly curved, both ends rounded, conidia of strain GLMC 1310 measure (2–)4–7(–8) × 1.5–2.5 µm, mean ± SD = 5.3 ± 1.4 × 2.0 ± 0.3 µm, L/W ratio = 2.7, while those of GLMC 1264 are longer, measuring (3–)5–8(–10) × 1.5–2 µm, mean ± SD = 6.4 ± 1.6 × 1.8 ± 0.2 µm, L/W ratio = 3.5.

Culture characteristics. *Colonies on SNA* flat with an entire margin, hyaline, sometimes filter paper partly pale olivaceous to olivaceous, lacking aerial mycelium, reverse same colours, strains GLMC 1264 and GLMC 1310 5–15 mm diam., strains GLMC 517 and GLMC 1501 32–43 mm diam. in 2 wk (25 °C in the dark); *Colonies on OA* flat with an entire margin, buff, olivaceous buff, olivaceous to olivaceous black, lacking aerial mycelium or partly covered by pale grey aerial mycelium, reverse same colours, 28–44 mm diam. in 2 wk (25 °C in the dark).

Notes. In total, 12 strains of *C. luteo-olivacea* were isolated from *Prunus domestica* in Baden-Württemberg (3), Lower Saxony (8) and Saxony (1). Two strains from Baden-Württemberg, two strains from Lower Saxony and the strain from Saxony had been selected for the phylogenetic analyses. The complete sequence dataset of *C. luteo-olivacea*, including reference strains, exhibits a variation of up to five nucleotides within ITS, up to nine nucleotides within *TUB* and up to 16 nucleotides within *EF-1a* sequences. The ITS sequences of the strains from this study are identical with those of the ex-type strain, except for GLMC 1517, which differs in five nucleotides, while

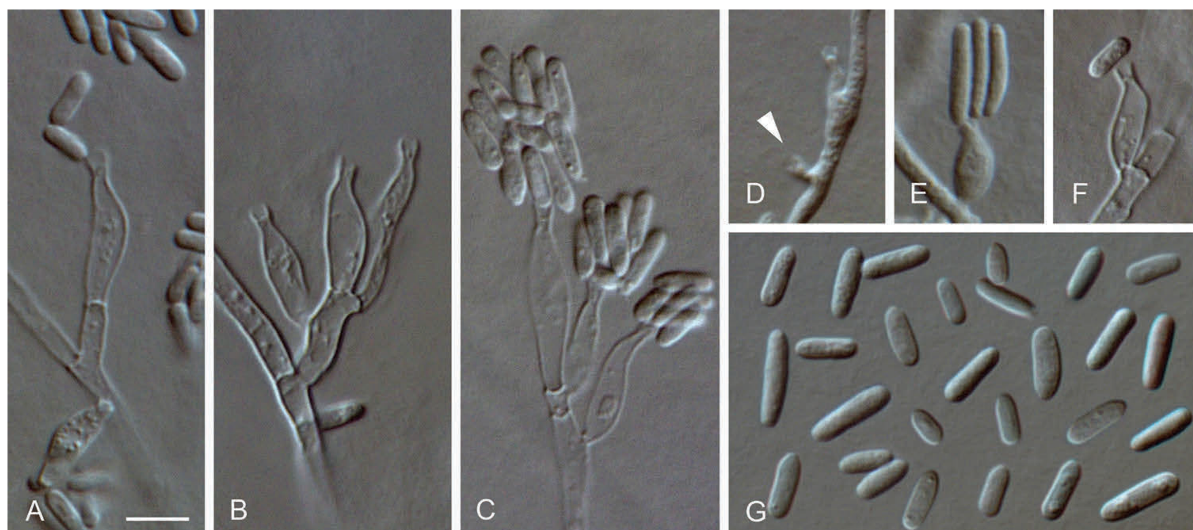

Figure 9. *Cadophora luteo-olivacea.* **A–F** conidiophores and conidiogenous cells (arrow indicates a short neck) **G** conidia **A–G** from SNA **A–G** LM. Scale bar: 5 μm (**A** applies to **B–G**).

all *TUB* sequences of our isolates differ in eight to nine nucleotides from the ex-type strain. The *EF-1a* sequence of all strains from this study differs in five nucleotides from the ex-type strain, except for GLMC 1264 with no differences.

Material examined. Germany, Lower Saxony, Hollern-Twielenfleth, orchard, 53°36'13.6"N, 9°31'50.8"E, from brown wedge-shaped necrosis in wood of *Prunus domestica*, 8 Oct. 2015, S. Bien leg., GLM-F107114, culture GLMC 1264 = CBS 145524 = DSM 109143; Lower Saxony, Hollern-Twielenfleth, orchard, 53°36'13.6"N, 9°31'50.8"E, from brown wedge-shaped necrosis in wood of *P. domestica*, 8 Oct 2015, S. Bien leg., GLM-F107160, culture GLMC 1310 = CBS 145525 = DSM 109142; Saxony, in orchard north of Wölkau, 50°58'42.3"N, 13°49'40.0"E, from brown wedge-shaped necrosis in wood of *P. domestica*, 16 Jan 2015, S. Bien leg., GLM-F106367, culture GLMC 517; Baden-Württemberg, orchard west of Nussbach, 48°31'55.8"N, 8°00'52.4"E, from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug 2016, S. Bien leg., GLM-F110581, culture GLMC 1501; Baden-Württemberg, orchard east of Nussbach, 48°31'57.3"N, 8°01'49.6"E, from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug 2016, S. Bien leg., GLM-F110597, culture GLMC 1517 = CBS 145526 = DSM 109141.

Cadophora novi-eboraci **Travadon, D.P.Lawr., Roon.-Lath., Gubler, W.F.Wilcox, Rolsh. & K.Baumgartner**
Figures 5E, 10

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–4 μm wide, sometimes hyphae inflated and constricted at the septa, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* hyaline, smooth-walled, mostly simple, rarely

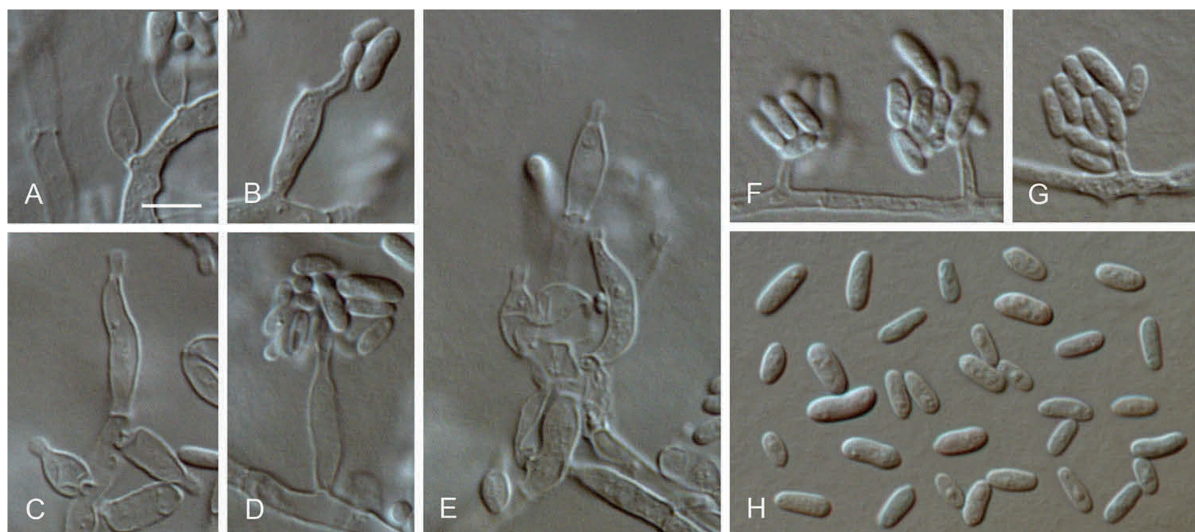

Figure 10. *Cadophora novi-eboraci* **A–G** conidiophores and conidiogenous **H** conidia **A–H** from SNA **A–H** LM. Scale bar: 5 µm (**A** applies to **B–H**).

septate and branched, up to 20 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, often integrated, discrete conidiogenous cells ampulliform, ellongate-ampulliform to navicular, 7–17 × 1.5–3 µm, necks cylindrical, 1–1.5 × 1.5–5.5 µm, collarettes cylindrical to narrowly funnel-shaped, 1.5–2 µm long, 0.5–1.5 µm wide at the upper edge, opening 0.5–1 µm, periclinal thickening sometimes observed. *Conidia* aggregated in heads, hyaline, smooth-walled, aseptate, cylindrical, elongate-ellipsoidal to ellipsoidal, straight, rarely slightly curved, with both ends rounded, (3–)4.5–6.5(–8.5) × 1.5–2(–2.5) µm, mean ± SD = 5.4 ± 1.1 × 1.8 ± 0.4 µm, L/W ratio = 2.9.

Culture characteristics. *Colonies on SNA* flat with an entire to undulate margin, hyaline to pale smoke grey, filter paper partly pale luteous to very pale smoke grey, lacking aerial mycelium, reverse same colours, 5–7 mm diam. in 2 wk (25 °C in the dark); *Colonies on OA* flat with an entire to undulate margin, fawn to umber with a pale luteous to luteous margin, partly covered by floccose white aerial mycelium, reverse fawn, pale olivaceous to pale luteous, 18 mm diam. in 2 wk (25 °C in the dark).

Notes. In total, eight strains of *C. novi-eboraci* were isolated from *Prunus cerasus* in Saxony (7) and Bavaria (1). Five of the strains from Saxony and the strain from Bavaria had been selected for the phylogenetic analyses. The complete sequence dataset of *C. novi-eboraci* exhibits a certain amount of variation in the loci analysed. The ITS and *EF-1a* sequences exhibited a maximum of one and two nucleotide differences to those of the ex-type strain NYC14, respectively. The *TUB* sequences were more variable; the *TUB* sequence of strain NYC13 differs in 15 nucleotides from that of NYC14. The *TUB* sequences of the strains from this study only differ with a maximum of two nucleotides from the ex-type strain.

Material examined. Germany, Bavaria, in garden east of Wolferszell, 48°57'38.8"N, 12°38'24.9"E, from non-symptomatic wood of *Prunus cerasus*, 2 Oct 2016, J. Simmel leg., GLM-F110552, culture GLMC 1472 = CBS 145758 = DSM 109145.

Cadophora obscura **Nannf., Svenska Skogsvårdsföreningens Tidskrift 50: 418 (1934)**
Figures 5F, 11

≡ *Phialophora obscura* (Nannf.) Conant, Mycologia 29(5): 598 (1937)

Type. Sweden, Umeå, Sofiehem, Sofiehems trämassefabrik, from fresh water, E Melin leg., collection date unknown, UPS F-153532 – ***holotype*** (not seen); unknown source, E Melin, collection date unknown (isolated by E Melin and JA Nannfeldt No. 389:11, deposited in CBS collection by E Melin probably 1933), CBS H-7589, CBS H-7590, GLM-F117483 – ***isotypes***; CBS 269.33 = GLMC 1896 – culture ex-isotype.

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–3.5 µm wide, sometimes becoming brown with age, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, discrete conidiogenous cells cylindrical to navicular, often bent, sometimes constricted at the base, 3–19 × 2–3 µm, necks cylindrical, 1–3.5 × 1.5–2 µm, collarettes distinct, cylindrical to funnel-shaped, 0.5–1.5 µm long, 1–1.5 µm wide at the upper edge, opening 1–1.5 µm wide, periclinal thickening observed. *Conidia* aggregated in heads, hyaline, smooth-walled, aseptate, ellipsoidal to cylindrical, mostly slightly curved, with both ends rounded, (3–)3.5–6(–7) × 1.5–2(–2.5) µm, mean ± SD = 4.8 ± 1.2 × 1.7 ± 0.3 µm, L/W ratio = 2.8.

Culture characteristics. *Colonies on SNA* flat with an entire to fimbriate margin, white to cinnamon, filter paper buff to olivaceous, lacking aerial mycelium, reverse same colours, 14–16 mm diam. in 2 wk (25 °C in the dark); *Colonies on OA* flat with an entire margin, olivaceous black to greenish-black, with honey to white margin, sometimes covered by floccose, olivaceous grey aerial mycelium, reverse same colours, 14–20 mm diam. in 2 wk (25 °C in the dark).

Notes. *Cadophora obscura* was originally described by Melin and Nannfeldt (1934) from freshwater in Sweden. According to the CBS website, strain CBS 269.33 is an "ex-isotype" culture. However, as Melin and Nannfeldt (1934) only isolated this species once and stated that they handed the strains from their study over to the Centraalbureau voor Schimmelcultures in Baarn now Westerdijk Fungal Biodiversity Institute, this can only be the ex-holotype strain. However, we were not able to allocate this strain to the holotype without doubt.

This species had previously been regarded as belonging to the genus *Phialophora* (Medlar 1915) and as a synonym of *Phialophora bubakii* (Schol-Schwarz 1970). However, based on the phylogeny of this study, both species are distinct species of the genus *Cadophora*. *Cadophora obscura* (CBS 269.33) differs from *C. bubakii* (CBS 198.30) by forming conidiogenous cells that are mostly narrow cylindrical, while those of CBS 198.30 are often flask-shaped. Conidia of *C. obscura* are distinctly longer than those of *C. bubakii*; subglobose-shaped conidia were not observed. Colony growth is slower compared to *C. bubakii*.

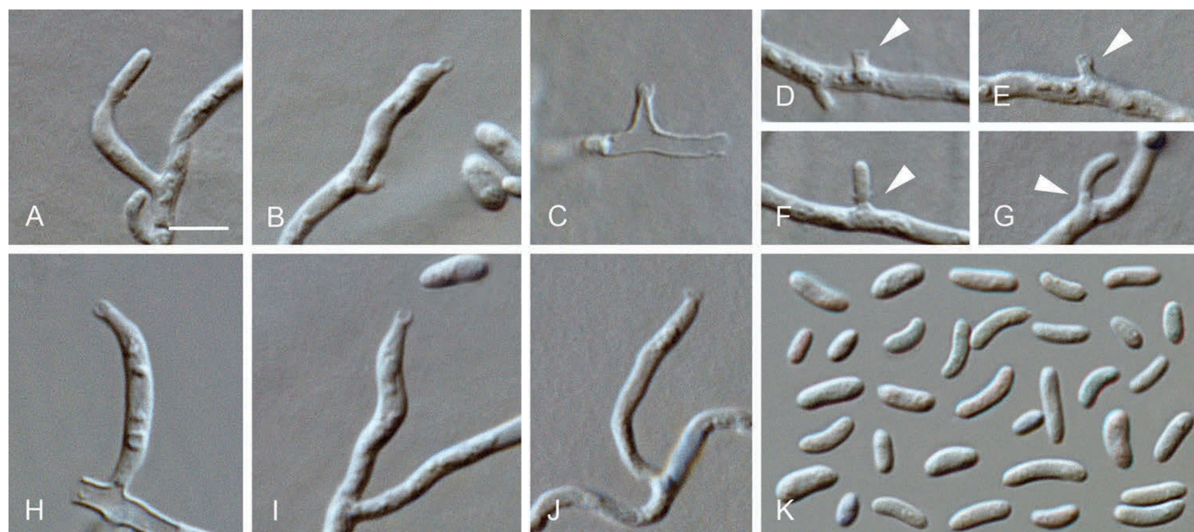

Figure 11. *Cadophora obscura* **A–J** conidiophores and conidiogenous cells (arrows indicate short necks) **K** conidia **A–K** from SNA **A–K** LM. Scale bar: 5 µm (**A** applies to **B–K**).

The ITS and *EF-1a* sequences of the ex-type strains of *C. bubakii* and *C. obscura* differ in 19 and 31 nucleotides, respectively. The *TUB* sequences of the two species were excluded from the analyses (see Notes of *C. bubakii*).

The ITS sequence of CBS 269.33 is 100% identical with three strains isolated from archaeological wood in Greenland (586-C, 592-B, 588-A, NB Pedersen et al., unpubl. data).

Cadophora prunicola Damm & S.Bien, sp. nov.

MycoBank No: 832109
Figures 5G, H, 12

Type. South Africa, Western Cape province, Franschhoek, from reddish-brown necrosis in wood of *Prunus salicina* close to an old pruning wound, 10 June 2004, U. Damm leg., CBS H-19985 – ***holotype***; GLM-F117487 – ***isotype***; CBS 120891 = STE-U 6202 = GLMC 1902 – culture ex-type.

Etymology. Named after its host genus, *Prunus* + suffix -cola (dweller).

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, septation hardly visible, branched, 1–3 µm wide, sometimes becoming brown with age, chlamydospores absent, hyphae of strain GLMC 735 in some parts inflated and restricted at the septae and up to 5 µm wide. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* hyaline, simple or septate and branched, up to 50 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, cylindrical, often inflated and bent in the upper part or attenuated at the base, delicate (disintegrating quickly), 4–15 × 1.5–2 µm, in strains GLMC 735 and GLMC 1574 sometimes integrated, necks cylindrical, 3–3.5 × 1 µm, collarettes distinct, funnel-shaped, cylindrical, 1–3 µm long, 1–2 µm wide at the upper edge, opening 1–1.5 µm wide, periclinal thickening sometimes observed. *Conidia* aggregated in heads, hyaline, smooth-

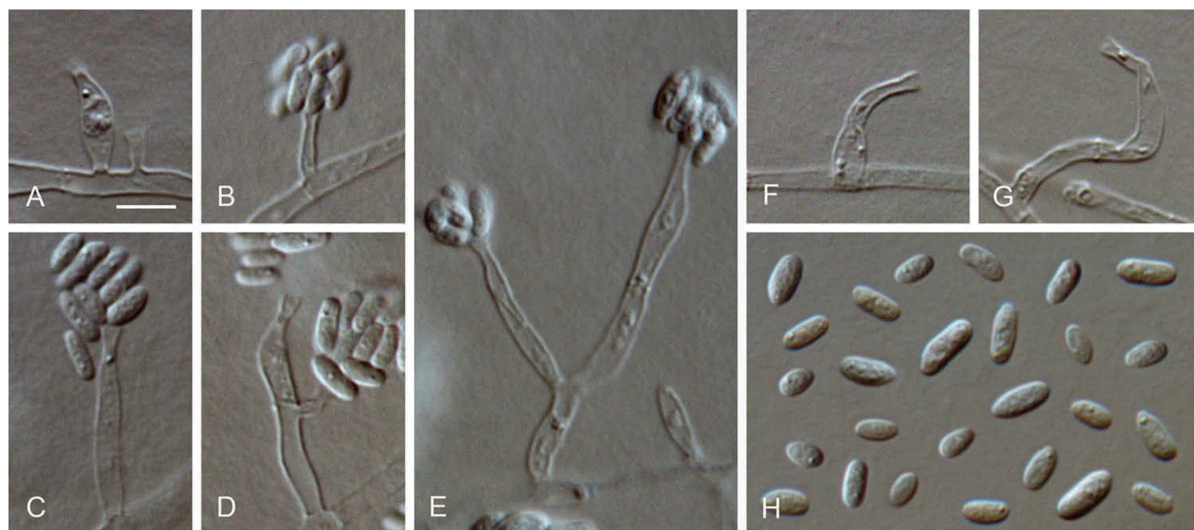

Figure 12. *Cadophora prunicola* sp. nov. **A–G** conidiophores and conidiogenous cells **H** conidia **A–H** from SNA **A–H** LM. Scale bar: 5 µm (**A** applies to **B–H**).

walled, aseptate, ellipsoidal, cylindrical to ovoidal, straight, rarely slightly curved, both ends rounded, (2.5–)3–6.5(–9) × 1.5–2 µm, mean ± SD = 4.9 ± 1.8 × 1.7 ± 0.3 µm, L/W ratio = 2.8, conidia of strain GLMC 1574 smaller, measuring (2.5–)3.5–5(–6.5) × 1.5–2.5(–3) µm, mean ± SD = 4.2 ± 0.7 × 1.4 ± 0.4 µm, L/W ratio = 2.1.

Culture characteristics. *Colonies on SNA* (strains GLMC 735, GLMC 1574 and GLMC 1633) flat with an entire to undulate margin, whitish, lacking aerial mycelium, reverse same colours, 18–27 mm diam. in 2 wk (25 °C in the dark). *Colonies on OA* (strains GLMC 735, GLMC 1574 and GLMC 1633) flat with an entire margin to undulate margin, buff, very pale luteus to cinnamon, lacking aerial mycelium, except for strain GLMC 1574 that was partly covered by white woolly aerial mycelium, reverse buff to fawn, 20–27 mm diam. in 2 wk (25 °C in the dark). *Colonies on PDA* (CBS 120891) flat to raised, entire margin, mycelium and surface white to very pale smoke-grey, with age turning isabelline to olivaceous in the centre, abundant velvety aerial mycelium, reverse straw to pure yellow, 18 mm diam. in 2 wk (25 °C). *Colonies on MEA* (CBS 120891) raised with entire margin, mycelium and surface white to very pale luteous, with age turning isabelline, abundant velvety aerial mycelium, reverse buff, honey to salmon, in diffuse daylight with a concentric apricot ring between centre and margin, 24 mm diam. in 2 wk (25 °C).

Notes. *Cadophora prunicola* was isolated from *Prunus salicina* (2) in the Western Cape Province of South Africa, from *P. cerasus* (3) and *P. domestica* (2) in Saxony and *P. domestica* (3) in Baden-Württemberg, Germany. The strains from South Africa, as well as three strains from both hosts from Saxony and two strains from Baden-Württemberg, were selected for the phylogenetic analyses. This species is similar to *C. novi-eboraci* and *C. africana*, but differs by forming conidiophores of up to 50 µm length and conidiogenous cells that are often inflated. Subglobose or tear-shaped conidia as in *C. africana* have not been observed. The ITS, *TUB* and *EF-1a* sequences of *C. prunicola* differ in 8, 29 and 9 nucleotides, respectively, from *C. novi-eboraci* and in 11, 30 and 20 nucleotides, respectively, from *C. africana*.

A blastn search with the ITS sequence of *C. prunicola* in GenBank showed a 100% identity with an uncultured *Cadophora* from dead wood of *Fagus sylvatica* in Germany (LC015696, Floren et al. 2015).

Additional material examined. Germany, Saxony, orchard east of Lungkwitz, 50°56'12.4"N, 13°47'36.6"E, from brown wedge-shaped necrosis in wood of *Prunus cerasus*, 11 Aug 2015, S. Bien leg., GLM-F106569, culture GLMC 735 = CBS 145521 = DSM 109135; Baden-Württemberg, orchard west of Nussbach, 48°31'55.8"N, 8°00'52.4"E, from brown necrosis in wood of *P. domestica*, 23 Aug 2016, S. Bien leg., GLM-F110714, culture GLMC 1633 = CBS 145522 = DSM 109146; Baden-Württemberg, orchard east of Nussbach, 48°31'57.3"N, 8°01'49.6"E, from brown wedge-shaped necrosis in wood of *P. domestica*, 23 Aug 2016, S. Bien leg., GLM-F110654, culture GLMC 1574; South Africa, Western Cape province, Franschhoek, from necrosis in wood of *P. salicina* close to old pruning wound, 10 June 2004, U. Damm leg., STE-U 6103.

Cadophora ramosa S.Bien & Damm, sp. nov.

MycoBank No: 832110
Figures 5I, 13

Cadophora spadicis Travadon, D.P.Lawr., Roon.-Lath., Gubler, W.F.Wilcox, Rolsh. & K.Baumgartner, *Fungal Biology* 119(1): 62 (2015). nom. inval., Art. 40.6 (Shenzhen)(Synonym).

Type. Germany, Saxony, orchard north of Kunnerwitz, 51°07'27.5"N, 14°56'36.3"E, from dark brown necrosis in wood of *Prunus cerasus*, 15 Jan 2015, S. Bien leg., GLM-F106227 – ***holotype***; GLMC 377 = CBS 145523 = DSM 109144 – culture ex-type.

Etymology. Named after the often densely branched conidiophores (*ramosus* Lat. = branching).

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative mycelium* hyaline, smooth-walled, septate, branched, 1–5 µm wide, chlamydospores absent. *Sporulation* abundant, conidia formed on hyphal cells. *Conidiophores* hyaline, smooth-walled, septate, often densely branched, up to 50 µm long. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, flask-shaped, 4.5–11.5 × 2.5–3.5 µm µm, collarettes narrowly funnel-shaped, 1.5–2 µm long, 1–1.5 µm wide at the upper edge, opening 0.5–1 µm, periclinal thickening sometimes observed. *Conidia* aggregated in heads, hyaline, smooth-walled, aseptate, subglobose, ovoidal, ellipsoidal to elongate-ellipsoidal, straight, with both ends rounded, different spore-shapes formed from the same conidiogenous cells, sporulation often inside the medium, (3.5–)4–6(–9) × 2–2.5(–3) µm, mean ± SD = 4.9 ± 1.2 × 2.2 ± 0.3 µm, L/W ratio = 2.2, rarely up to 15 × 2.5 µm.

Culture characteristics. *Colonies on SNA* flat with an entire margin, hyaline, filter paper partly pale olivaceous to olivaceous, lacking aerial mycelium, reverse same col-

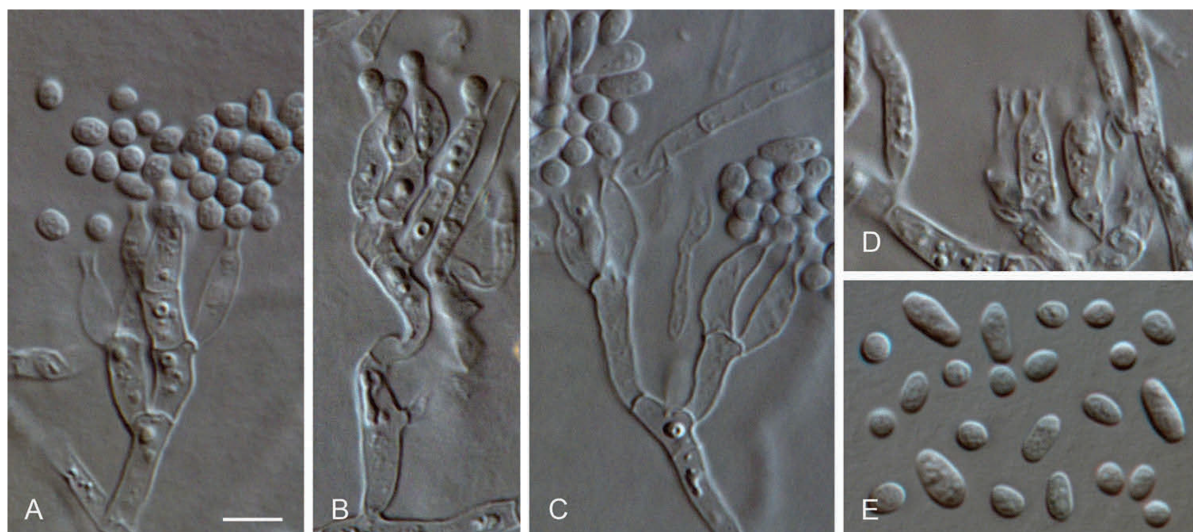

Figure 13. *Cadophora ramosa* sp. nov. **A–D** conidiophores and conidiogenous cells **E** conidia **A–E** from SNA **A–E** LM. Scale bar: 5 µm (**A** applies to **B–E**).

ours, 32–40 mm diam. in 2 wk (25 °C in the dark). *Colonies on OA* flat with an entire margin, pale cinnamon, with an umber inner and pale luteous outer margin, partly covered by woolly white to grey aerial mycelium, reverse pale cinnamon, with a citrine inner and pale luteous outer margin, 24–28 mm diam. in 2 wk (25 °C in the dark).

Notes. *Cadophora ramosa* was previously described from grapevine in North America as *C. spadicis* (Travadon et al. 2015). Although Travadon et al. (2015) indicated *C. spadicis* as a new species, they listed a basionym and added the authorities of that basionym in brackets with the new name, as if they would combine an already existing species in a new genus, which was not the case. As Travadon et al. (2015) described *C. spadicis* as a new species, they should have listed a holotype (Art. 40.6, Art. 9.1, Turland et al. 2018); however, they listed a neotype, although original material was available (Art. 9.8). Therefore, the name *C. spadicis* is invalid. Moreover, the "neotype" listed is a living strain and not a (metabolically inactive) specimen. The species listed as "basionym" of *C. spadicis* by Travadon et al. (2015), *C. melinii*, was based on a wrong identification of strain CBS 111743 by Prodi et al. (2008), the strain that was listed as "neotype" of *C. spadicis*. However, the ex-type strain of *C. melinii*, CBS 268.33, was included in the study of Travadon et al. (2015) and belonged to a different clade in the phylogeny of that publication. Moreover, the authors listed as authorities of the "basionym" are the authors of the publication in which strain CBS 111743 was wrongly identified (Prodi et al. 2008) and not the authorities of *C. melinii*. Finally, although probably not intended as the whole name, prior to the authorities and "sp. nov.", Travadon et al. (2015) listed "*Cadophora spadicis* CBS 111743", which could be interpreted as not being a binary combination consisting of the name of the genus followed by a single specific epithet (Art. 23.1).

As the name *C. spadicis* is invalid, we described the species newly as *C. ramosa* on the basis of a specimen from *Prunus cerasus* in Saxony, Germany, collected in this study. The morphology of the ex-type strain of *C. ramosa* shows a high morphological

concordance with the strains described as *C. spadicis* by Travadon et al. (2015). Conidiophores, conidiogenous cells, conidia and collarettes have similar shapes and sizes. The ITS, *TUB* and *EF-1a* sequences of *C. ramosa* differ at most in two, four and two nucleotides, respectively, which is a lower genetic variation than in *C. luteo-olivacea* and *C. novi-eboraci*.

Minutiella pruni-avium **S.Bien & Damm, sp. nov.**

MycoBank No: 832111
Figures 5J, 14

Type. Germany, Baden-Württemberg, orchard west of Nussbach, 48°31'55.8"N, 8°00'52.4"E, from brown necrosis in wood of *Prunus avium*, 23 Aug 2016, S. Bien leg., GLM-F110704 – ***holotype***; GLMC 1624 = CBS 145513 = DSM 109150 – culture ex-type.

Etymology. Name refers to the host species, *Prunus avium*.

Description. *Sexual morph* not observed. *Asexual morph on SNA. Vegetative hyphae* hyaline, smooth-walled, septate, branched, 1–3 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed directly on hyphal cells, in conidiomata and by microcyclic conidiation. *Conidiophores on hyphae* reduced to conidiogenous cells, conidiogenous loci formed terminally. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, mostly reduced to mere openings with collarettes formed directly on hyphal cells, discrete phialides rare, navicular, constricted at the base, 5.5–14.5 × 1.5–2.5 µm; collarettes rarely visible or flaring, < 0.5–3 µm long, opening 0.5–1.5 µm, periclinal thickening sometimes visible. *Conidia* aggregated in masses around the hyphae, hyaline, smooth-walled, aseptate, oblong to ellipsoidal, mostly straight, sometimes slightly curved, with both ends rounded, sometimes with a prominent scar on one end, (2.5–)3–5(–6) × 1–1.5(–2) µm, mean ± SD = 3.9 ± 0.9 × 1.4 ± 0.2 µm, L/W ratio= 2.8. *Conidiomata* produced on OA in 2–4 wk; solitary or aggregated, globose to subglobose, unilocular, immersed to superficial, 50–340 µm wide, olivaceous to black, mostly glabrous, sometimes with a few hairs, opening with an irregular rupture. *Conidiophores* reduced to conidiogenous cells. *Conidiogenus cells* enteroblastic, hyaline, smooth-walled, conidiogenous loci formed terminally, discrete phialides, globose to ampulliform or navicular, 3.5–7.5 × 2–3.5 µm, opening 0.5–1 µm, periclinal thickening sometimes visible. *Conidia* hyaline, smooth-walled, cylindrical to ellipsoidal, sometimes slightly curved, with both ends rounded, (2.5–)3–4.5(–6) × (1–)1.5–2(–3) µm, mean ± SD = 3.8 ± 0.8 × 1.7 ± 0.4 µm, L/W ratio = 2.2. *Microcyclic conidiation* occurs from minute collarettes at one or both ends of primary conidia that develop into swollen mother cells, often thick-walled, sometimes septate, > 5 µm long, 2–3.5 µm wide.

Culture characteristics. *Colonies on OA* flat with entire margin, white to saffron, with scattered umber spots due to conidiomata formation, aerial mycelium lacking, spore masses oozing from conidiomata buff, reverse white to buff, 4–8 mm diam. in 2

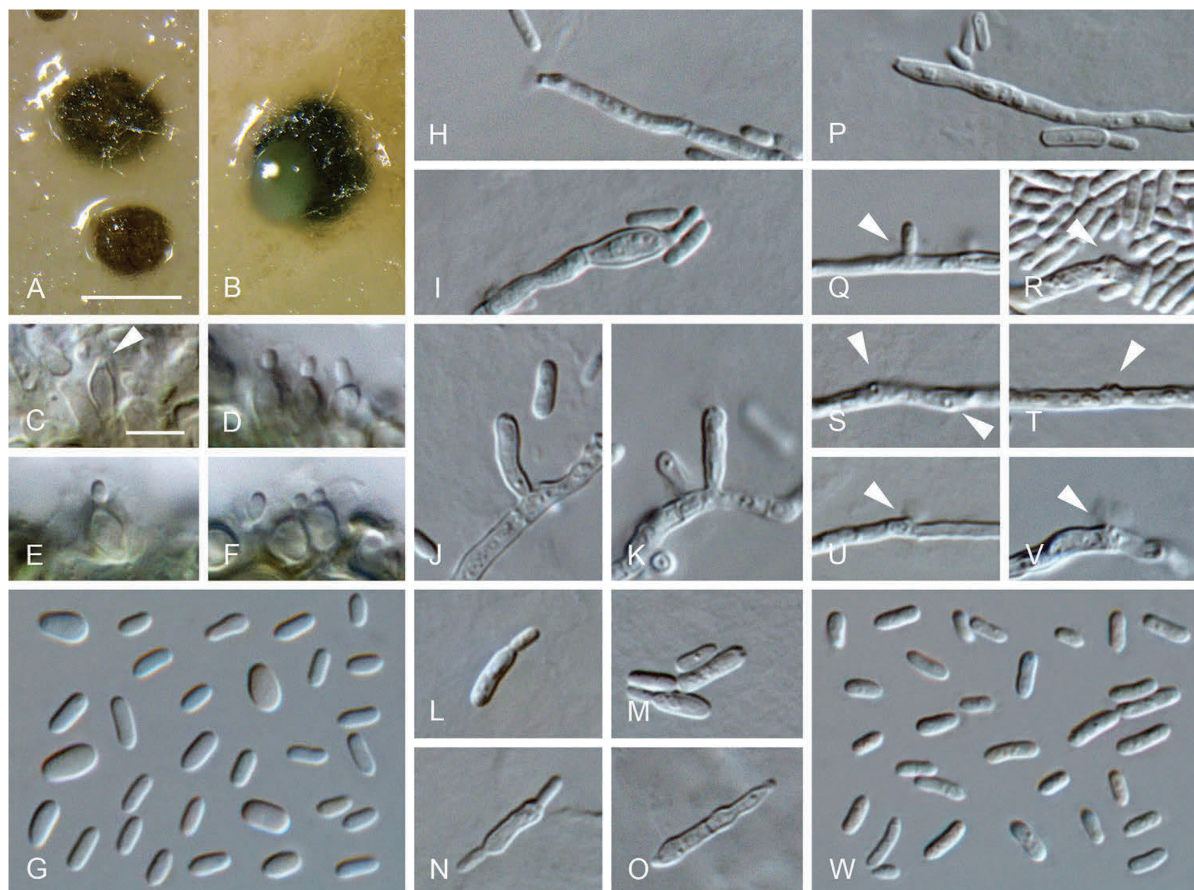

Figure 14. *Minutiella pruni-avium* sp. nov. **A, B** conidiomata **C–F** conidiogenous cells lining the inner wall of a conidioma **G** conidia formed in conidiomata **H–K, P–V** conidiogenous cells formed on hyphal cells (arrows indicate conidiogenous openings) **L–O** mother cells **W** conidia formed on hyphal cells **A–G** from OA **H–W** from SNA **A, B** SM, **C–W** LM. Scale bars: 200 µm (**A** applies to **B**), 5 µm (**C** applies to **D–W**).

wk, 6–10 mm diam. in 4 wk. *Colonies on SNA* flat with entire margin, white, lacking aerial mycelium, reverse same colour; < 1–2 mm diam. in 2 wk, 6–8 mm diam. in 4 wk.

Notes. Two strains of *Minutiella pruni-avium* were isolated from wood of *Prunus avium*. The LSU sequences of these strains differ in three and one nucleotides from those of *M. tardicola* and *Minutiella* sp., respectively. The ITS region shows 11 differences to *M. tardicola* and 9 differences to *Minutiella* sp. The *TUB* sequence of *M. tardicola* and *Minutiella* sp. differ in one nucleotide, however, in 35 and 33 nucleotides compared to *M. pruni-avium*. *Minutiella pruni-avium* differs from *Minutiella tardicola* and the strains of *Minutiella* sp. by forming larger conidiomata, longer discrete phialides and flaring collarettes of up to 3 µm.

The closest match in a blastn search with the ITS sequence of strain GLMC 1624 is the type strain of *Minutiella tardicola* CBS 121757 with 97.9% identity (NR132006, Damm et al. 2010).

Additional material examined. Germany, Baden-Württemberg, orchard west of Nussbach, 48°32'11.3"N, 8°01'01.3"E, from brown necrosis in wood of *Prunus avium*, 23 Aug 2016, S. Bien leg., GLM-F110750, culture GLMC 1667 = CBS 145514 = DSM 109149.

Proliferodiscus ingens **S.Bien & Damm, sp. nov.**
MycoBank No: 832112
Figures 5K, 15

Type. Germany, Baden-Württemberg, orchard south of Oppenau, on a hill, 48°27'57.6"N, 8°09'11.0"E, from necrotic wood of *Prunus avium*, 24 Aug 2016, S. Bien leg., GLM-F110834 – ***holotype***; GLMC 1751 = CBS 145519 = DSM 109148 – culture ex-type.

Etymology. Named after the comparatively huge conidiomata (*ingens* Lat. = huge).

Description. *Sexual morph* not observed. *Asexual morph on OA. Vegetative hyphae* hyaline, smooth-walled, septate, branched, 1.5–3 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed in conidiomata. *Conidiomata* produced on OA in 2–4 wk, solitary or aggregated, subglobose, unilocular, superficial, 250–1000 µm wide, dull green to grey olivaceous, almost glabrous to completely covered with hairs, opening with an irregular rupture. *Conidiophores* hyaline, smooth-walled, septate, sometimes branched at the base and above, conidiogenous loci formed terminally. *Conidiogenus cells* enteroblastic, hyaline, smooth-walled, navicular to subulate, tapering towards apices, 8–15 × 1–2 µm; collarettes hardly visible, cylindrical, < 1 µm long, opening 0.5–1 µm, periclinal thickening sometimes visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to ellipsoidal, straight, with both ends rounded, 2.5–3(–3.5) × 1–1.5 µm, mean ± SD = 2.9 ± 0.2 × 1.4 ± 0.1 µm, L/W ratio = 2.1.

Culture characteristics. *Colonies on OA* raised with entire to crenated margin, buff to pale olivaceous grey, white at the margin, with umber to black spots due to conidiomata, aerial mycelium sparse, white, reverse buff to cinnamon, 1–2 mm diam. in 2 wk, 2–3 mm diam. in 4 wk. *Colonies on SNA* flat to very low convex with entire to fimbriate margin, white, lacking aerial mycelium, reverse same colour; 1–2 mm diam. in 2 wk, 2–3 mm diam. in 4 wk.

Notes. Strain GLMC 1751, described here as *Proliferodiscus ingens*, was isolated from *Prunus avium* in Baden-Württemberg. Only the asexual morph of this fungus was observed. Asexual morphs have previously rarely been observed in the genus *Proliferodiscus* and no complete description is available. However, Baral and Kriegelsteiner (1985) and Dennis (1949) mention an asexual morph of *Pr. pulveraceus*. Dennis (1949) observed multilocular pycnidia with slender conidiophores (10–12 µm long) and spherical conidia (1 µm diam.), whereas Baral and Krieglsteiner (1985) described oval conidia, measuring 1.5–1.7 × 1.2–1.4 µm, produced on verticillately branched conidiophores. In contrast to the description of Dennis (1949), the strains observed here produce unilocular pycnidia. Conidia of *Pr. ingens* are larger than conidia of *Pr. pulveraceus* in both descriptions. The asexual morph of *Pr. ingens* differs from that of the other *Proliferodiscus* strains observed in this study by producing larger, darker conidiomata, a different conidial shape and a lower growth rate.

The closest match in a blastn search with the ITS sequence of strain GLMC 1751 with 97.7% identity is "Hyaloscyphaceae sp. 2" strain ICMP 18979 from symptomless leaves of *Nothofagus fusca* in New Zealand (JN225935, Johnston et al. 2012).

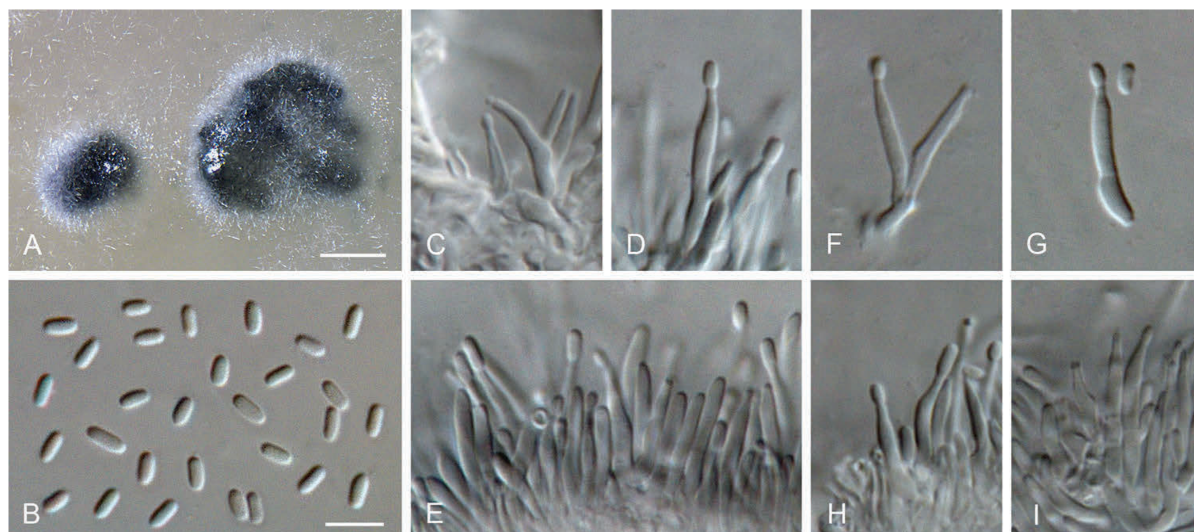

Figure 15. *Proliferodiscus ingens* sp. nov. **A** conidiomata **B** conidia formed in conidiomata **C–E, H, I** conidiogenous cells lining the inner wall of a conidioma **F, G** detached conidiogenous cells **A–I** from OA **A** SM **B–I** LM. Scale bars: 300 µm (**A**), 5 µm (**B** applies to **C–I**).

Proliferodiscus sp.

Figures 5L, 16

Description. *Sexual morph* not observed. *Asexual morph on OA. Vegetative hyphae* hyaline, smooth-walled, septate, branched, 1.5–3 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed in conidiomata. *Conidiomata* produced on OA, SNA and pine needles in 2–4 wk, solitary or aggregated, subglobose, unilocular, superficial, 125–500 µm wide, luteous, almost glabrous to completely covered with hairs, opening with an irregular rupture. *Conidiophores* hyaline, smooth-walled, septate, simple or branched, conidiogenous loci formed terminally. *Conidiogenus cells* enteroblastic, hyaline, smooth-walled, navicular to subulate, tapering towards apices, 9–14 × 1–2 µm, collarettes cylindrical, < 1 µm long, opening 0.5–1 µm, periclinal thickening sometimes visible, conidiogenous cells often extend to form new conidiogenous openings, extensions flask-shaped to navicular. *Conidia* hyaline, smooth-walled, aseptate, mostly globose to obovoid, sometimes cylindrical to ellipsoidal, straight, with both ends rounded, 1.5–2(–3) × 1.5(–2) µm, mean ± SD = 1.9 ± 0.3 × 1.5 ± 0.1 µm, L/W ratio = 1.2.

Culture characteristics. *Colonies on OA* flat to effuse with entire to fimbriate margin, white to buff, cinnamon to sienna at the margin, aerial mycelium sparse, white, reverse buff, cinnamon to sienna; 6–14 mm diam. in 2 wk, 16–32 mm diam. in 4 wk. *Colonies on SNA* flat to effuse with entire to fimbriate margin, white, lacking aerial mycelium, reverse same colour; 6–18 mm diam. in 2 wk, 20–34 mm diam. in 4 wk.

Notes. In total, five strains of *Proliferodiscus* sp. have been isolated from wood of *Prunus domestica* in Saxony (3) and Lower Saxony (1) as well as *P. avium* in Baden-Württemberg (1). Two subclades are formed by these strains, which differ in one and four nucleotides in the LSU and ITS sequences, respectively. No morphological differences were noticed between strains of the two subclades. They form a well-supported clade (100/100/99%) with eight strains retrieved from GenBank, including the ex-type

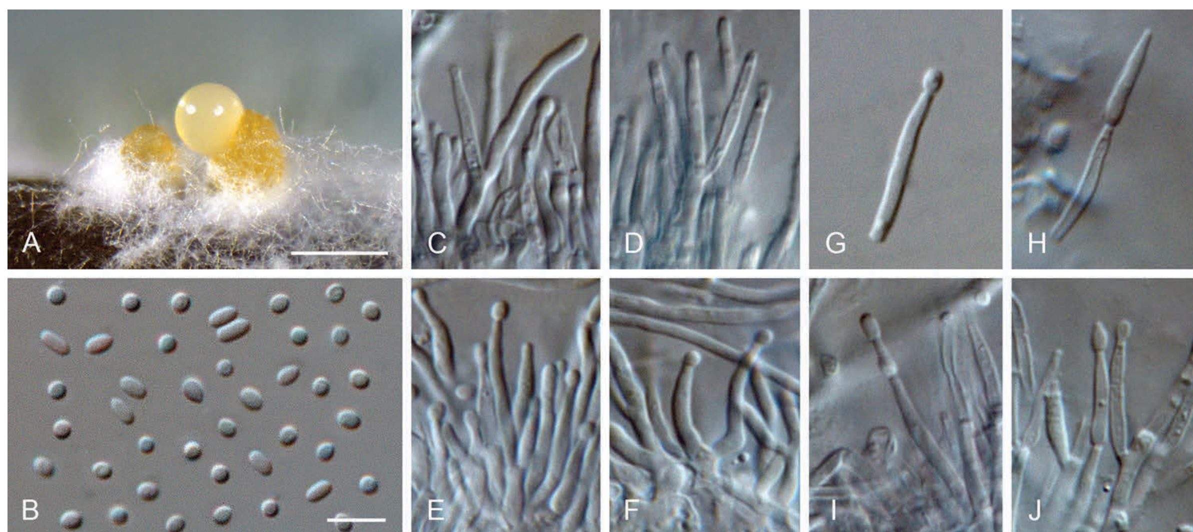

Figure 16. *Proliferodiscus* sp. **A** conidiomata **B** conidia formed in conidiomata **C–F, I–J** conidiogenous cells lining the inner wall of a conidioma **G, H** detached conidiogenous cells **H–J** extensions on conidiogenous cells **A–J** from OA **A** SM **B–J** LM. Scale bars: 300 µm (**A**), 5 µm (**B** applies to **C–J**).

strain of the recently described *Pr. chiangraiensis*. The conidial shape of these strains is similar to that of the asexual morph of *Pr. pulveraceus* observed by Baral and Krieglsteiner (1985); the conidia are slightly larger, but the measurements overlap. However, we cannot link these strains to *Pr. pulveraceus* on this basis, because the species was described based on its sexual morph and no sequences of type material are available. Moreover, a recently published sequence, designated as *Pr. pulveraceus* (MN066320, G Marson unpubl.), belongs to a different clade in our phylogeny.

One striking feature was observed in our collections: new conidiogenous cells grow out of conidiogenous openings (Fig. 16H–J). This feature has previously been observed in species of other genera, for example, *Fusarium graminearum* (Domsch et al. 2007) and several *Colletotrichum* species (Damm et al. 2012, 2019).

The anamorphic states of the observed strains of *Proliferodiscus* sp. differ from *Pr. ingens* (strain GLMC 1751, this study) by the colour and the smaller size of conidiomata, faster culture growth rate on OA and SNA and the shape of the conidia.

Material examined. Germany, Saxony, in orchard north of Wölkau, 50°58'42.3"N, 13°49'40.0"E, from brown necrosis in wood of *Prunus domestica*, 16 Jan 2015, S. Bien leg., GLM-F106310, culture GLMC 460 = CBS 145517 = DSM 109138; Baden-Württemberg, orchard west of Nussbach, 48°31'55.8"N 8°00'52.4"E, from necrotic wood of *P. avium*, 23 Aug 2016, S. Bien leg., GLM-F110844, culture GLMC 1761 = CBS 145518 = DSM 109137; Saxony, in orchard north of Wölkau, 50°58'42.3"N, 13°49'40.0"E, from brown necrosis in wood of *P. domestica*, 16 Jan 2015, S. Bien leg., GLM-F106320, culture GLMC 470; Lower Saxony, Hollern-Twielenfleth, orchard, 53°36'13.6"N, 9°31'50.8"E, from brown wedge-shaped necrosis in wood of *P. domestica*, 8 Oct 2015, S. Bien leg., GLM-F107151, culture GLMC 1301; Saxony, in orchard north of Wölkau, 50°58'42.3"N, 13°49'40.0"E, from brown necrosis in wood of *P. domestica*, 16 Jan 2015, S. Bien leg., GLM-F106352, culture GLMC 502.

Discussion

The new genus *Arboricolonus* is described, based on one strain, GLMC 459, that could not be assigned to any known genus. Closest matches of ITS (90% identity) and LSU (97% identity) sequences of this fungus with strains identified at least to the genus level were strains of *Glutinomyces vulgaris*, *Chalara aurea*, *Hyalodendriella betulae* and *Polyphilus sieberi*; (Ashrafi et al. 2018; Crous et al. 2007; Nakamura et al. 2018; Vu et al. 2019). The asexual morph of *Arboricolonus simplex* clearly differs from these genera. The monotypic genus *Hyalodendriella* forms pigmented micro- and macro-conidiophores directly on hyphae as well as pigmented limoniform to ellipsoid and prominently apiculate conidia (Crous et al. 2007), while *Chalara* is characterised by forming sessile or stalked phialides with basal ventres, long collarettes and deep-seated conidiogenous loci; conidia are cylindrical and often produced in basipetal chains (Holubová-Jechová 1984; Kowalski 2006). However, *Chalara*, is highly polyphyletic; species have been placed in different classes of Ascomycota (Paulin and Harrington 2000; Koukol 2011). As the type species belongs to the Sordariomycetes (*Chalara fusidioides*), strain CBS 880.73 probably does not even belong in *Chalara* s. str. The genera *Polyphilus* and *Glutinomyces* were described, based on sequence data and colony morphology only (Ashrafi et al. 2018; Crous et al. 2017; Nakamura et al. 2018); no asexual morphs are available that could be compared to *Arboricolonus*.

For systematic placement of the genus on order level, we conducted a class-wide phylogenetic analysis of LSU-ITS with reference sequences of Leotiomycetes which clearly places it in the order Helotiales (data not shown). The closest matches from LSU and ITS blastn searches indicate the relationship of this new genus to the Hyaloscyphaceae, the largest family of the Helotiales that is mainly circumscribed by features of sexual morphs (Jaklitsch et al. 2016). Han et al. (2014b) and Johnston et al. (2019) demonstrated the polyphyly of the family. For the placement of the new genus on family level, we included LSU and ITS sequences of selected sequences from all clades of Hyaloscyphaceae and closely related families recognised by Han et al. (2014b) in their multi-locus ML analyses, as well as the closest matches from the blastn searches. The clades in our phylogeny are mostly well-supported and in agreement with the clades in Han et al. (2014b). However, most of these clades are placed on a polytomous branch. Due to the lack of a stable backbone, the exact placement of clades in relation to each other shown by the ML analyses of Han et al. (2014b) could not be confirmed and remains inconclusive. Furthermore, as shown in Fig. 1, family designation of the included strains according to Han et al. (2014b), Johnston et al. (2019) and Ekanayaka et al. (2019) is highly problematic.

The new genus *Arboricolonus* clusters in our phylogeny with sequences of *Polyphilus*, *Cistella*, *Rodwayella* and *Polydesmia*, however, on short branches, they lack posterior probability or bootstrap support. Therefore, we consider the placement of the genus as of uncertain taxonomic position on family level (Helotiales, incertae sedis). We did not find any record of asexual morphs of *Cistella* and *Rodwayella* for morphological comparison with the new genus. In contrast to *Arboricolonus simplex*, *Polydesmia pruinosa*

(asexual morph: *Brefeldochium pruinosum*) produces septate falcate conidia in sporodochia (Verkley 2005).

Except for the lack of microcyclic conidiation, the genus *Arboricolonus* morphologically resembles *Collophorina* and related genera by forming slow growing cultures, conidiophores that are reduced to short phialides or openings on hyphae with minute to flaring collarettes and cylindrical to ellipsoid conidia with obtuse ends (Bien et al. 2020). A similar morphological appearance could be explained with a similar lifestyle within plant wood and is therefore regarded as a result of convergent evolution. The possibility of morphological adaptation of collophorina-like species to the habitat within the woody plant body has previously been discussed by Bien et al. (2020).

In total, 29 strains of *Cadophora* have been isolated from wood in Germany, all from *Prunus cerasus* and *P. domestica*, of which 17 were included our phylogeny. A further three strains included in the analyses originated from wood of *P. salicina* in South Africa.

The strains of *C. novi-eboraci* from this study were all isolated from wood of *P. cerasus* in Saxony and Bavaria, Germany. *Cadophora novi-eboraci* was described from decaying wood of *Vitis* spp. in the USA (Travadon et al. 2015) and recently reported from necrotic wood of *Malus domestica* in Germany (Gierl and Fischer 2017). To our knowledge, this is the first report of *C. novi-eboraci* from *Prunus* wood worldwide.

Strain CBS 101359 from stained wood of *Actinidia chinensis* in Italy had been referred to as *C. malorum* by Di Marco et al. (2004) and Prodi et al. (2008). Travadon et al. (2015) identified it as *C. novi-eboraci*. In our phylogeny, it is placed distantly from both species. We therefore regard this strain as a different taxon.

Cadophora luteo-olivacea was originally isolated from wastewater in Sweden (Van Beyma 1940). This species has been reported mostly from *Vitis vinifera* and several other woody hosts worldwide (Farr and Rossman 2019), but not from *Prunus*. Fischer et al. (2016) isolated this species from grapevine nurseries and vineyards in Germany. In this study, *C. luteo-olivacea* was isolated from *Prunus domestica* in all three sampling areas. Therefore, this is the first report of *C. luteo-olivacea* from *Prunus* wood worldwide. *Cadophora luteo-olivacea* seems to be not only widely distributed, but also very variable. Gramaje et al. (2011) observed a high variability of colony pigmentation within *C. luteo-olivacea*, which was also observed by Harrington and McNew (2003), not only in this, but also in other species of the genus. We noticed that the *TUB* and *EF-1a* sequences of the ex-type strain CBS 141.41 and strain A42, identified as *C. luteo-olivacea* by Travadon et al. (2015), differed in 8 and 16 nucleotides, respectively. Strains, isolated from *Prunus* wood in this study, show up to nine and five nucleotide differences in the *TUB* and *EF-1a* sequences, respectively. In the resulting single-locus trees (not shown), subgroups are formed that are, however, not concordant and therefore do not represent independent evolutionary lineages. This phenomenon was previously studied in the highly variable species *Colletotrichum siamense* (Liu et al. 2016), that had been regarded as species complex, based on single-locus analyses. Except for a small cluster formed by two strains from the study of Travadon et al. (2015), no subgroups are formed in the multi-locus phylogeny.

Three of the *Cadophora* species we isolated from *Prunus* wood, namely *C. luteo-olivacea*, *C. novi-eboraci* and the newly described *C. ramosa* (syn. *C. spadicis*), were previously associated with wood diseases like cankers or Petri disease of *Vitis* spp., (e.g. Casieri et al. 2009; Halleen et al. 2007; Fischer et al. 2016; Travadon et al. 2015; Pintos et al. 2018). Several other fungal species are reported both from *Vitis* and fruit trees as well, for example, several species belonging to the Botryosphaeriaceae, Diatrypaceae and the genus *Phaeoacremonium* (Damm et al. 2007, 2008; Moyo et al. 2018); fruit trees were referred to as alternative hosts of grapevine trunk disease pathogens. Similar to these fungi, *Cadophora* species could be transferred to grapevine plants from *Prunus* trees grown in close vicinity to vineyards. To our knowledge, the genus *Cadophora* has never been reported from *Prunus* before (Farr and Rossman 2019 as well as all references listed in this study). Moreover, we found new species on this host genus. One of them, *C. prunicola*, was isolated from three different *Prunus* species, *P. cerasus*, *P. domestica* and *P. salicina*, both in Germany and South Africa. A second new species, *C. africana*, is so far only known from *P. salicina* in South Africa. Based on a blastn search with its ITS sequence, *C. prunicola* was detected as an uncultured *Cadophora* in dead wood of *Fagus sylvatica* in Germany (Floren et al. 2015), but so far, there is no report of any of these two species in *Vitis* wood.

In addition to the strains isolated from *Prunus* trees in Germany and South Africa, we included strains of *Phialophora bubakii*, because we noticed a close affinity to the genus *Cadophora* by preliminary sequence comparisons (not shown). *Phialophora bubakii* that was originally described from margarine as *Margarinomyces bubakii* (Laxa 1930) and combined in *Cadophora* in this study, had previously been reported from wood of *Pinus strobus* and *Populus* sp. (Ellis 1976), where it caused blue stain on timber, from *Betula pendula* in Poland (Mulenko et al. 2008), further from subcutaneous infections (Porto 1979) and from corneal ulcers (Eiferman et al. 1983), both in humans and dogs in several countries (Hoog et al. 2000). An ITS sequence of a strain from wood pulp of *Populus tremula*, identical to that of the ex-isotype strain, is available in GenBank (Vu et al. 2019), confirming the occurrence of *Ph. bubakii* on *Populus* wood. The remaining reports lack sequence data and are therefore doubtful. Some of the reports could actually refer to other species that could have been confused due to similar morphology. None of the *C. bubakii* and *C. obscura* strains, included in this study, originated directly from wood or infections of mammals. However, the ex-isotype strain of *C. obscura* apparently originates from water in a "trämassefabrik" (trämassa = wood pulp) and therefore possibly from the processed wood itself. An identical ITS sequence from archaeological wood in Greenland (NB Pedersen et al., unpubl. data) suggests the occurrence of *C. obscura* in wood as well.

The *TUB* sequences of *C. bubakii*, *C. obscura* and *C. viticola* were excluded from the phylogenetic analyses, because all of them differed tremendously from each other and from the rest of the dataset. Furthermore, sequencing *TUB* of *C. obscura* (CBS 269.33), using either the forward or the reverse primer, generated two vastly differing sequences (data not shown). Sequencing *TUB* of *Aspergillus* spp. by Peterson (2008) and Hubka and Kolarik (2012) also resulted in different sequences from the same species, which were regarded as possible paralogous gene fragments. Based on the data in this study, we assume a similar situation in *Cadophora*.

All *Cadophora* species treated in this study can be distinguished by all single loci analysed (data not shown). Due to the high genetic variation within some of them, the use of more than one locus is recommended for further studies on this genus.

The genus *Minutiella* was isolated for the first time from wood of *Prunus armeniaca* in South Africa and described as *Phaeomoniella tardicola* (= *M. tardicola*) (Damm et al. 2010). This is the first report of the genus *Minutiella* and the Phaeomoniellales, in general, from *P. avium* and *P. domestica* worldwide. The genus *Minutiella* is, so far, only known from wood of *Prunus* trees. More specifically, *M. tardicola* is known from *P. armeniaca* in South Africa, the new species *M. pruni-avium* from *P. avium* in Germany and *Minutiella* sp. from *P. domestica* in Germany (Damm et al. 2010; this study). This genus also forms reduced conidiogenous cells; probably an adaption to the living conditions inside wood like *Collophorina* and related species and *Arboricolonus* (Bien et al. 2020; this study).

The two *Minutiella* strains GLMC 1636 and GLMC 1687 are morphologically indistinguishable from *M. tardicola*, however, differ in LSU, ITS and *TUB* sequences from this species. The description of this further new species is in preparation (C. Kraus, pers. comm.).

The LSU-ITS-*TUB* phylogeny of the Celotheliaceae shows a high similarity to the previously compiled LSU phylogeny in Chen et al. (2015). In this study, we provide the first multi-locus phylogeny of the family. For a conclusive placement of genera within this family, more data is needed, since all phylogenies lack a deep node support (Chen et al. 2015; this study). "*Phaeomoniella*" *pinifoliorum* apparently represents a separate genus. A new genus was, however, not described, as no strain and only ITS sequence data were available for characterisation of this genus.

Proliferodiscus has been reported from wood and bark of several woody hosts worldwide (Albertini and Schweinitz 1805; Dennis 1949; Baral and Krieglsteiner 1985; Spooner 1987; Weber and Bresinsky 1992; Haelewaters et al. 2018; Farr and Rossman 2019). Dennis (1949) lists *Prunus insititia* as one of the hosts of *Proliferodiscus pulveraceus.* In this study, two *Proliferodiscus* species have been collected from wood of *P. avium* and *P. domestica*.

The species delimitation in the genus *Proliferodiscus* was previously based on the morphology of the sexual morphs only (e.g. Haines and Dumont 1983; Baral and Krieglsteiner 1985; Spooner 1987; Hofton et al. 2009). Six of the species had been described even before 1900 in other genera and were transferred to this genus later. Only few morphological treatments of *Proliferodiscus* species contained information on asexual morphs (Dennis 1949; Baral and Krieglsteiner 1985). The genus has not been treated in modern terms yet.

There are sequences of ten strains/specimens identified as *Proliferodiscus* in GenBank, none of them is ex-type, except for the recently described *Pr. chiangraiensis*. The available sequences of *Proliferodiscus* belong to three main clades in our phylogeny. One well-supported clade in our phylogeny contains several apparently closely related strains/specimens, for which different names have been applied.

Most of our strains from *Prunus*, belonging to two subclades of the same main clade, did not show any morphological differences of the asexual morph and only differed in few nucleotides from each other and from the remaining specimens/sequences

within this clade. Therefore, we refrained from describing two new species in this clade and refer to the strains as *Proliferodiscus* sp. In order to allow comparison with asexual morphs in this genus in the future, we provided a description of this species, as well as of the newly described species *Pr. ingens*. In order to provide a solid basis for identifications and detections of new species, *Proliferodiscus* species need to be epitypified and data of both sexual and asexual morphs, as well as sequence data, need to be provided.

Conclusion

The isolation of fungal strains from necrotic wood of *Prunus* species in Germany and South Africa revealed several unknown taxa within Leotiomycetes and Eurotiomycetes. Based on morphology and multi-locus molecular analyses, we described one new genus and six new species in four genera. Although previously unknown from wood of *Prunus* trees, the genus *Cadophora* was revealed to be a common wood inhabitant of *P. cerasus* and *P. domestica* in Germany, but apparently not of *P. avium*. The genus *Minutiella*, originally described from *P. armeniaca* in South Africa, also occurs in *Prunus* wood in Germany and, thus, belongs to the common genera in *Prunus* wood as well. Our analyses of the genus *Proliferodiscus* also contributes to the knowledge of this genus by the first detailed descriptions of asexual morphs of this genus. The results underline the sparse knowledge of several fungal genera from wood and of the wood mycobiome of the economically important host genus *Prunus*. The morphological data presented here and the up-to date molecular frameworks will provide a basis for further studies on these genera and on wood diseases of *Prunus* trees.

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Supplementary material 1

Complete list of strains included in this study, with collection details, GenBank accession numbers and references

Authors: Steffen Bien, Ulrike Damm

Data type: molecular data

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4 Manuscript III

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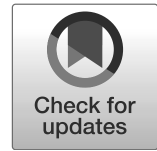

ORIGINAL ARTICLE

Prunus trees in Germany—a hideout of unknown fungi?

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Abstract

Prunus belongs to the economically most important genera of fruit crops in Germany. Although wood pathogens possess the capability to damage the host substantially, the knowledge of the fungal pathogenic community and the mycobiome of *Prunus* wood in general is low. During a survey in important fruit production areas in Germany, branches with symptoms of fungal infection were sampled in *Prunus avium*, *P. cerasus* and *P. domestica* orchards, and 1018 fungal isolates were obtained primarily from the transition zone of symptomatic to non-symptomatic wood. By a combination of blastn searches and phylogenetic analyses based on ITS and LSU sequences with a strong focus on reliable reference data, a diversity of 172 fungal taxa belonging to *Ascomycota*, *Basidiomycota* and *Mucoromycota* were differentiated. The majority of the strains belonged to three classes of *Ascomycota*, namely *Sordariomycetes*, *Leotiomycetes* and *Dothideomycetes*. The dominant species were *Aposphaeria corallinolutea* (*Dothideomycetes*) and *Pallidophorina paarla* (*Leotiomycetes*) that were isolated more than a hundred times each, while all other taxa were isolated ≤ 30 times. Only part of them could be identified to species level. Because of the high plasticity of species boundaries, the identification certainty was divided into categories based on nucleotide differences to reference sequences. In total, 82 species were identified with high and 20 species with low (cf.) certainty. Moreover, about 70 species could not be assigned to a known species, which reveals *Prunus* wood to represent a habitat harbouring high numbers of potentially new species, even in a well-explored region like Germany.

Keywords Cultivation · Fungal community · Stone fruit trees · Systematics · Wood inhabitants

Introduction

Fungal pathogens inhabiting the woody plant body can plug vessels and necrotise tissue, which causes wilting, inhibition of blossoming and dieback of branches and whole trees. The resulting decrease in fruit or timber yield can ruin the productivity of orchards, vineyards and forests and can even require replanting. Additionally, some of the pathogens can reduce the quality of fruits, which causes further yield losses. Moreover, trees in forests and orchards are usually grown in monocultures and are therefore especially threatened by fungal plant pathogens, both due to the increasing global plant trade (Roy et al. 2014, Ghelardini et al. 2017) and effects of climate change (Anderson et al. 2004, Gange et al. 2011, Luck et al. 2011, Fisher et al. 2012, Altizer et al. 2013). An example for the threat an exotic pathogen can pose to native trees is *Hymenoscyphus fraxineus*, the causal agent of ash dieback that moved from eastern Asia to Europe, encountering ash tree species being more susceptible (McMullan et al. 2018). Due to extreme conditions like drought, trees become also more susceptible to fungi that are already living as endophytes inside their wood, so-called weak parasites. They include species of *Botryosphaeriales* that have frequently been isolated from *Prunus* trees in South Africa (Damm et al. 2007a, b). In Germany, one of these species, *Diplodia pinea*, has been reported to cause serious damage to pine trees that suffered from drought stress and had been attacked by bark beetles (Heydeck and Dahms 2012, Petercord 2017). Furthermore, trees can become more susceptible to pathogens or encounter new potential pathogens if they are planted outside their typical growing region, for example by the northward expansion

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of European crop production due to global warming (Maracchi et al. 2005, Santos et al. 2017). In order to allow an early detection and control of known and new threats for the fruit industry, knowledge of the wood mycobiome of fruit trees is crucial.

Fungal communities inside wood have frequently been studied using culture-independent high-throughput sequencing (HTS) (e.g. Kubartová et al. 2012, Hoppe et al. 2016, Purahong et al. 2018) and isolation techniques (e.g. Kowalski 1983, Butin and Kowalski 1986, Lygis et al. 2005, Santamaría and Diez 2005, Simeto et al. 2005, Cloete et al. 2011, Markakis et al. 2017, Fischer et al. 2016). However, many studies focused on endophytic fungi (e.g. Barengo et al. 2000, Fröhlich et al. 2000, Gonthier et al. 2006) or were restricted to grapevine wood (e.g. Hofstetter et al. 2012, Pancher et al. 2012, Bruez et al. 2014, 2016). Sweet cherry (*Prunus avium*), sour cherry (*P. cerasus*) and plum (*P. domestica*) are the most important stone fruit crops in German fruit industry (Garming et al. 2018). In 2018, more than 350,000 t of sweet cherry, sour cherry and plum fruit were produced on an area of around 12,000 ha (FAO 2020). In spite of this economic importance, there are only a few studies on the fungal diversity of aboveground woody parts of *Prunus* trees (e.g. Bernadovičová and Ivanová 2011, Haddadderafshi et al. 2011, Hortová and Novotný 2011, Gramaje et al. 2012, Abdollahi Aghdam and Fotouhifar 2016, 2017). Most of these studies are limited by a small sample size, by a narrow sampling area or by relying solely on morphological features for species identification.

The most extensive work so far has been conducted in a survey on the fungal diversity of *Prunus* species in South Africa (Damm et al. 2007a, b, 2008a, b, c, 2010, Moyo et al. 2018, Bien and Damm 2020). More than 40 taxa were reported, predominantly within *Botryosphaeriales* (nine species) and *Phaeoacremonium* (14 species). During this survey, 24 species of *Botryosphaeria-ceae*, *Calosphaeriaceae*, *Togniniaceae*, *Montagnulaceae*, *Coniochaetaceae*, *Celotheliaceae*, *Tympanidaceae* and *Ploettnerulaceae* were recognised as new to science. However, these publications aimed only on selected, very abundant or specifically interesting taxa of wood-inhabiting fungi from *Prunus* wood in South Africa; the complete diversity collected was not evaluated. Moreover, no comprehensive study has been done on the mycobiome of *Prunus* trees in Germany. In a study on several tree species in the vicinity of a vineyard in Germany, only a selection of eight fungal species (belonging to *Botryosphaeriaceae*, *Stereaceae*, *Tympanidaceae* and *Valsaceae*) isolated from wood of six *Prunus* species (including *P. cerasus* and *P. domestica*) was reported (Gierl and Fischer 2017).

With an extensive study such as the evaluation of a mycobiome, time is the most limiting factor. For the selection of an appropriate approach for identification, quantity and quality have to be balanced against each other. Uncertainties in identifications of fungi can arise due to deficiencies of both morphological and molecular approaches. Morphological identification of fungal cultures is hindered or impossible, if strains do not develop identification-relevant features (fruiting structures) or show phenotypic plasticity (Slepecky and Starmer 2009), belong to a complex of cryptic species that cannot be differentiated by morphological features (e.g. Damm et al. 2012) or species had been described based on one morph only, usually the sexual morph, that does not develop in culture (e.g. Bien and Damm 2020). Even if morphological identification is possible, each genus requires a certain amount of expertise (Hofstetter et al. 2019), as well as time to obtain necessary literature and reference/type material. If many taxa extending over the entire fungal kingdom need to be identified in a reasonable time frame, an overall morphology-based approach is not appropriate; identification based on sequence data is the method of choice.

Fungal identification solely based on blastn searches with ITS sequences is common practice (Hughes et al. 2009, Hofstetter et al. 2019); however, it has a lot of shortcomings as well. Although the ITS region is considered as the universal barcode region for fungi and the most commonly sequenced locus in mycology, it is not suitable for species delimitation in each genus (Schoch et al. 2012). Species identification in surveys using HTS is even less certain, because the sequences generated are very short, and the high number of sequences generated puts even more time pressure on identification, allowing only unquestioned/unvalued blastn searches. Moreover, identification results cannot be verified by morphology as no cultures are available. Therefore, species can often only be identified up to genus level (LoBuglio and Pfister 2010, Johnston et al. 2014, Ekanayaka et al. 2017, Pärtel et al. 2017, Purahong et al. 2018) or result in doubtful identifications like those of *Collophorina* species that are discussed in Bien et al. (2020).

The purpose of this study was to reveal the mycobiome of *Prunus* trees in a temperate climate focusing on potential pathogens associated with wood necroses of *P. avium*, *P. cerasus* and *P. domestica* in three important fruit production areas in Germany. Some of the genera isolated within this study, belonging to the *Leotiomycetes* and *Eurotiomycetes*, have previously been analysed in depth and several new taxa were revealed (Bien et al. 2020, Bien and Damm 2020). The aim of this study was to give an overview of the complete fungal diversity based on LSU and ITS sequences, to highlight the possible depth of identification based on these loci as part of a mycobiome study and to detect potential new taxa. A culture-dependent approach allowed verifying results by morphology, if necessary, and facilitates further taxonomic studies.

Materials and methods

Sampling and fungal isolation

Branches with wood symptoms (e.g. canker, necroses, wood streaking, gummosis) were collected from *Prunus domestica* (61 branches), *P. cerasus* (64) and *P. avium* (43) orchards in Saxony; from *P. domestica* (30) and *P. avium* (60) orchards in Lower Saxony; and from *P. domestica* (38) and *P. avium* (48) orchards in Baden-Württemberg, Germany, in 2015 and 2016. Additionally, a symptomatic wood sample from a *P. cerasus* tree located in a private garden in Bavaria was included. From each of these 345 branches, ten wood pieces (5 × 5 × 5 mm) from the transition zone of symptomatic to non-symptomatic wood tissue as well as each three pieces of the same size from non-symptomatic wood of the same branch were surface sterilised 30 s in 70% ethanol, 1 min in 3.5% NaOCl and 30 s in 70% ethanol and washed for 1 min in sterilised water. Five pieces from symptomatic tissue were placed on synthetic nutrient-poor agar (SNA, Nirenberg 1976) medium, and the remaining five pieces from symptomatic tissue as well as the three pieces from non-symptomatic tissue on oatmeal agar (OA; Crous et al. 2019) medium both supplemented with 100 mg/L penicillin, 50 mg/L streptomycin sulphate and 1 mg/L chloramphenicol. After incubation for several days at 25 °C, hyphal tips of developing fungi were transferred to SNA medium with a sterilised pine needle. Single-spore or single-hyphae isolates were obtained from the fungi for further study.

The resulting strains are preserved in cryotubes containing sterile distilled water with 10% glycerol at − 80 °C and in sterile distilled water at + 4 °C in the culture collection of the Senckenberg Museum of Natural History Görlitz, Germany (GLMC). Specimens (dried cultures) were deposited in the fungarium of the Senckenberg Museum of Natural History Görlitz (GLM).

Phylogenetic analysis

Genomic DNA of the isolates was extracted using the method of Damm et al. (2008b). A partial sequence of the 28S nrDNA (LSU) and the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 (ITS) were amplified and sequenced using the primer pairs LROR (Rehner and Samuels 1994) + LR5 (Vilgalys and Hester 1990) and ITS-1F (Gardes and Bruns 1993) + ITS-4 (White et al. 1990), respectively.

The PCR mixture contained 1 μL of 1:10 DNA template, 2.5 μL 10X buffer (Peqlab, Erlangen, Germany), 1 μL of each primer (10 mM), 2.5 μL $MgCl_2$ (25 mM), 0.1 μL *Taq* polymerase (0.5 U, Peqlab, Erlangen, Germany) and 2.5 μL of 2 mM dNTPs. Each reaction was made up to a final volume of 20 μL with sterile water. DNA amplifications were carried out in a Mastercycler® pro S (Eppendorf, Hamburg, Germany). Conditions for the amplification of LSU and ITS were set according to Paulin and Harrington (2000) and Bien et al. (2020), respectively. The PCR products were visualised on a 1% agarose gel and sequenced by the Senckenberg Biodiversity and Climate Research Centre (BiK-F) laboratory (Frankfurt, Germany). The forward and reverse sequences were assembled by using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall 1999).

All strains were grouped based on comparison of their ITS sequences. One strain of each group with an identical ITS sequence was selected for blastn searches and phylogenetic analysis. For generic determination of the isolates and selection of reference strains, blastn searches were performed on the NCBI GenBank (www.ncbi.nlm.nih.gov) and EPPO-Q-Bank (qbank.eppo.int) databases. For each genus, sequences of strains identified to species level, preferably of ex-type strains and strains of the type species, with at least 97% identity were included as reference strains in the phylogenetic analyses. If no type strains were available, strains with a CBS (culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands) number were favoured. Strains without species determination were only used, if blastn searches did not result in any close match with a strain identified to species level.

For the phylogenetic analyses, the sequences downloaded were added to the sequences generated in this study and those of the appropriate outgroup sequences in five LSU-ITS datasets depending on phylum and class. Four datasets were assembled for species of the *Ascomycota* classes *Sordario-mycetes*, *Dothideomycetes*, *Leotiomycetes* and *Eurotiomycetes*, respectively. A fifth dataset encompasses species of the classes *Agaricomycetes*, *Tremellomycetes* and *Cystobasidiomycetes* (*Basidiomycota*); *Lecanoromycetes*, *Pezizomycetes* and *Saccharomycetes* (*Ascomycota*); as well as the subdivision *Mucoromycotina* of the *Mucoromycota*. The datasets of each locus were aligned automatically using MAFFT v. 7.308 (Katoh et al. 2002, Katoh and Standley 2013), manually adjusted where necessary and subsequently concatenated using Geneious v. 10.2.2 (Kearse et al. 2012).

The phylogenetic analyses were conducted using Bayesian inference (BI) and maximum likelihood (ML) as described in Bien et al. (2020). The DNA sequences generated in this study were deposited in GenBank (Table 1) and the alignments in TreeBASE (treebase.org/treebase-web/home.html; TB2:S25316). The complete list of strains included in the phylogenetic analyses is provided in the supplementary material table (suppl. material tab.).

Table 1 List of taxa isolated from *Prunus* wood in Germany with novelties and potential new reports, numbers of strains per wood tissue, host species and sampling region, representative strains and GenBank numbers

Taxon	Nov.	Strains	sy.	n-sy.	P.d.	P.c.	P.a.	Sa	LSa	BW	Ba	Rep. strain	GenBank no.[1]	
													LSU	ITS
Ascomycota														
Dothideomycetes														
Alternaria conjuncta	G, a, c, d	3	3		1	1	1	1	2			GLMC 1338	MT156154	MT153704
Alternaria destruens	G, P	24	24		8	7	9	13	7	4		GLMC 1234	MT156155	MT153705
Alternaria rosae	G, P	1	1		1			1				GLMC 636	MT156156	MT153706
Angustimassarina cf. spp.		8	8			3	5	4		1	3	GLMC 891	MT156157	MT153707
Aposphaeria corallinolutea	G, P	138	125	13	99	18	21	72	41	23	2	GLMC 1355	MT156159	MT153708
Aureobasidium pullulans	d	15	15		11	2	2	10	1	4		GLMC 1460	MT156164	MT153709
Bipolaris cf. spp.		1	1			1		1				GLMC 248	MT156165	MT153710
Cladosporium cf. spp. 1		10	8	2	2	1	7	2	3	5		GLMC 1289	MT156192	MT153711
Cladosporium cf. spp. 2		2	1	1	2			2				GLMC 711	MT156193	MT153712
Coniothyrium ferrarisianum	G, P	24	13	11	6	14	4	24				GLMC 380	MT156201	MT153713
Constantinomyces sp.		1	1		1					1		GLMC 1767	MT156202	MT153714
Devriesia pseudoamericana	P	1		1	1			1				GLMC 819	MT156209	MT153715
Didymella macrostoma	a, c, d	8	8		2	5	1	7	1			GLMC 1392	MT156215	MT153716
Diplodia mutila	d	1	1		1					1		GLMC 1759	MT156216	MT153717
Diplodia seriata		1	1		1					1		GLMC 1527	MT156217	MT153718
Epicoccum cf. spp.		7	7		2	5		5		2		GLMC 369	MT156218	MT153719
Jeremyomyces cf. *labinae*		3	2	1	1	2		2	1			GLMC 327	MT156244	MT153720
Kalmusia cf. *ebuli*		4	4			3	1	4				GLMC 767	MT156245	MT153721
Kalmusia variispora	G, P	4	4		4			1	3			GLMC 1347	MT156246	MT153722
Neocucurbitaria populi	G, P	1	1		1			1				GLMC 348	MT156266	MT153723
Neoleptosphaeria rubefaciens	G, P	1	1			1		1				GLMC 337	MT156269	MT153724
Nothophoma cf. *quercina*		18	17	1	7	11		11	1	6		GLMC 432	MT156271	MT153725
Paraphaeosphaeria neglecta	P	2	2				1	1				GLMC 857	MT156275	MT153726
Parapyrenochaeta protearum	G, P	2	1	1		1	1	1		1		GLMC 301	MT156276	MT153727
Phoma laundoniae	G, a	1	1				1			1		GLMC 1459	MT156298	MT153728
Preussia persica	G, P	2	2		2			1	1			GLMC 447	MT156301	MT153731
Preussia cf. spp.		1	1				1			1		GLMC 1754	MT156302	MT153732
Roussoella euonymi	G, P	1	1				1			1		GLMC 1544	MT156304	MT153733
Lentitheciaceae sp.		1	1		1					1		GLMC 1563	MT156299	MT153729
Pleosporales sp.		1	1		1				1			GLMC 1316	MT156300	MT153730
Eurotiomycetes														
Aspergillus chevalieri	G, P	2	2				2	2				GLMC 899	MT156162	MT156109
Aspergillus glaucus	G, d	1	1		1			1				GLMC 771	MT156163	MT156110
Capronia sp.		1	1				1		1			GLMC 1254	MT156189	MT156111
Exophiala sp.		3	1	2	3					3		GLMC 1670	MT156225	MT156112
Minutiella pruni-avium	N	2	2				2			2		GLMC 1624	MN232925	MN232957
Minutiella sp.	P	2	2		2					2		GLMC 1636	MN232927	MN232959
Penicillium angulare	G, P	1	1		1					1		GLMC 1646	MT156277	MT156114
Penicillium brevicompactum	G, P	6	6				6		3	3		GLMC 1661	MT156278	MT156115
Penicillium glabrum	G, P	2	2				2		2			GLMC 1400	MT156279	MT156116
Penicillium cf. spp.		1	1		1				1			GLMC 1288	MT156280	MT156117
Rhinocladiella cf. *quercus*		3	3			2	1	2		1		GLMC 1752	MT156303	MT156118
Talaromyces sp.		2	2		2					2		GLMC 1678	MT156312	MT156119
Herpotrichiellaceae sp.		1	1			1		1				GLMC 914	MT156229	MT156113

Table 1 (continued)

Taxon	Nov.	Strains	sy.	n-sy.	P.d.	P.c.	P.a.	Sa	LSa	BW	Ba	Rep. strain	GenBank no.[1]	
													LSU	ITS
Lecanoromycetes														
Lecanoromycetes sp.		2	2			1	1	1		1		GLMC 1733	MT156247	MT156137
Leotiomycetes														
Arboricolonus simplex	N	1	1		1			1				GLMC 459	MN232924	MN232935
Botrytis cinerea		4	1	3	4			3	1			GLMC 635	MT156168	MT156090
Cadophora luteo-olivacea	P	12	12		12			1	8	3		GLMC 1264	MT156172	MN232938
Cadophora novi-eboraci	P	8	8			8		7			1	GLMC 1472	MT156181	MN232947
Cadophora prunicola	N	8	8		5	3		5		3		GLMC 1633	MT156183	MN232955
Cadophora ramosa	N	1	1			1		1				GLMC 377	MT156187	MN232956
Collophorina africana	d	21	20	1	21			7		14		GLMC 1736	MK314581	MK314542
Collophorina badensis	N	10	10		10					10		GLMC 1684	MK314594	MK314546
Collophorina germanica	N	2	2				2		1	1		GLMC 1445	MK314595	MK314550
Collophorina neorubra	N	7	7				7	3	2	2		GLMC 929	MK314604	MK314533
Dermea cerasi A	G	4	4				4			4		GLMC 1760	MT156207	MT156093
Dermea cerasi B	G	1	1				1	1				GLMC 862	MT156206	MT156092
Dermea sp.		2	2				2	2				GLMC 867	MT156208	MT156094
Monilinia laxa	G	4	4		4			2	2			GLMC 1290	MT156255	MT156091
Neofabraea vagabunda	G, P	1	1		1			1				GLMC 718	MT156268	MT156100
Neofabraea sp.		8	8		5	3		7	1			GLMC 1284	MT156267	MT156099
Oidiodendron cf. *griseum*		8	7	1	6	2		8				GLMC 602	MT156272	MT156101
Oidiodendron sp. 1		1		1	1			1				GLMC 469	–	MT156102
Oidiodendron sp. 2		7	7		7			7				GLMC 485	MT156273	MT156103
Pallidophorina paarla	a, c, d	112	110	2	17	12	83	33	64	15		GLMC 452	MK314608	MK314555
Pezicula cf. *carpinea*		4	4		3	1		4				GLMC 416	MT156283	MT156095
Pezicula eucrita	P	2	2		2			2				GLMC 643	MT156284	MT156096
Pezicula sporulosa	G, P	4	4				4	4				GLMC 1224	MT156286	MT156097
Pezicula sp.		14	14		14				5	9		GLMC 1726	MT156285	MT156098
Phialocephala piceae	G, P	26	26		12	2	13	25		1		GLMC 331	MT156294	MT156105
Phialocephala sp. 1		1	1				1	1				GLMC 803	MT156295	MT156106
Phialocephala sp. 2		2	2		2			2				GLMC 385	MT156296	MT156107
Phialocephala sp. 3		6	6		5		1	6				GLMC 833	MT156297	MT156108
Proliferodiscus ingens	N	1	1				1			1		GLMC 1751	MN232929	MN232961
Proliferodiscus sp.		7	7		6		1	3	3	1		GLMC 460	MN232930	MN232962
Leotiomycetes sp.		1	1			1		1				GLMC 792	MT156248	MT156104
Pezizomycetes														
Trichophaeopsis bicuspis	G, P	1	1				1			1		GLMC 1596	MT156319	MT156139
Saccharomycetes														
Nakazawaea cf. *holstii*		1	1		1				1			GLMC 1309	MT156261	MT156138
Wickerhamomyces silvicola	G, P	1	1				1			1		GLMC 1708	MT156324	MT156140
Sordariomycetes														
Acremonium sp.		1	1				1			1		GLMC 1762	MT156152	MT153618
Akanthomyces muscarius	G, P	5	5		5			5				GLMC 347	MT156153	MT153619
Anthostomella cf. *pinea*		2	1	1		1	1	2				GLMC 451	MT156158	MT153620
Arthrinium cf. *arundinis*		1	1			1		1				GLMC 230	MT156160	MT153621
Ascotricha chartarum	G, P			1		1		1				GLMC 453	MT156161	MT153622
Biscogniauxia nummularia	a, d	3	1	2	2		1	3				GLMC 829	MT156166	MT153623
Brunneomyces hominis	G, P	1	1		1			1				GLMC 717	MT156169	MT153624

Table 1 (continued)

Taxon	Nov.	Strains	sy.	n-sy.	P.d.	P.c.	P.a.	Sa	LSa	BW	Ba	Rep. strain	GenBank no.[1]	
													LSU	ITS
Calosphaeria pulchella	G, d	30	30			3	27	29		1		GLMC 1629	MT156188	MT153625
Chaetomium sp.		2	1	1			2	1	1			GLMC 946	MT156190	MT153626
Chaetosphaeria cf. spp.		1	1		1			1				GLMC 641	MT156191	MT153627
Clypeosphaeria sp.		1	1		1			1				GLMC 463	MT156194	MT153628
Colletotrichum godetiae	c	8	8			8		8				GLMC 224	MT156195	MT153629
Coniochaeta cf. *cipronana*		1	1				1			1		GLMC 1710	MT156196	MT153633
Coniochaeta sp. 1		1	1		1			1				GLMC 355	MT156197	MT153630
Coniochaeta sp. 2		1	1			1		1				GLMC 723	MT156198	MT153632
Coniochaeta sp. 3		3	3		3			3				GLMC 487	MT156199	MT153631
Cordyceps farinosa	P	2	2				2	2				GLMC 886	MT156151	MT153634
Diaporthe cf. *eres*		6	6			6		6				GLMC 532	MT156210	MT153637
Diaporthe cf. *mahothocarpus*		3	3			3		3				GLMC 260	MT156211	MT153635
Diaporthe rudis	P	7	7		4		3		3	4		GLMC 1427	MT156212	MT153638
Diaporthe sp.		16	16		13	3		11	3	2		GLMC 309	MT156213	MT153636
Dichotomopilus cf. spp.		4	4			4		4				GLMC 425	MT156214	MT153639
Eutypa lata	c	13	13		6	7		11		2		GLMC 427	MT156219	MT153640
Eutypa petraki var. *hederae*		1	1		1			1				GLMC 631	MT156220	MT153641
Eutypa petraki var. *petrakii*		6	6		6			2		4		GLMC 1645	MT156221	MT153642
Eutypa sp.		2	2		2					2		GLMC 1758	MT156222	MT153643
Eutypella cf. spp.		1	1		1			1				GLMC 625	MT156223	MT153644
Fusarium culmorum	c	2	2			2		2				GLMC 218	MT156226	MT153645
Fusarium cf. spp. 1		10	10		4	6		6	1	3		GLMC 1465	MT156227	MT153647
Fusarium cf. spp. 2		7	7		3	4		4	3			GLMC 1293	MT156228	MT153646
Hypoxylon cf. *fragiforme*		5	4	1	3		2	2	1	2		GLMC 1653	MT156234	MT153653
Hypoxylon fuscum	d	1	1		1					1		GLMC 1823	MT156235	MT153656
Hypoxylon howeanum	a, d	5	3	2	3		2	4		1		GLMC 394	MT156236	MT153651
Hypoxylon sp. 1		15	12	3	7		8	1	2	12		GLMC 1456	MT156237	MT153652
Hypoxylon sp. 2		2	2				2			2		GLMC 1657	MT156238	MT153654
Hypoxylon sp. 3		1	1		1					1		GLMC 1725	MT156239	MT153655
Jackrogersella cf. *cohaerens*		7	6	1	3	1	3	3		4		GLMC 652	MT156240	MT153657
Jackrogersella sp.		1	1		1					1		GLMC 1516	MT156241	MT153658
Jattaea sp. 1		1	1		1			1				GLMC 503	MT156242	MT153659
Jattaea sp. 2		4	4				4	3		1		GLMC 853	MT156243	MT153660
Lepteutypa sp. 1		18	18		18				12	6		GLMC 1319	MT156249	MT153661
Lepteutypa sp. 2		5	5			4	1			1	4	GLMC 1557	MT156250	MT153662
Leucostoma cf. spp.		28	25	3	17	2	9	7		21		GLMC 1521	MT156251	MT153663
Lopadostoma dryophilum	G, P	9	9		3	5	1	7		2		GLMC 1682	MT156254	MT153665
Lopadostoma cf. *turgidum* A		4	4		3		1	4				GLMC 757	MT156252	MT153664
Lopadostoma cf. *turgidum* B		1	1				1			1		GLMC 1768	MT156253	MT153666
Monocillium cf. *tenue*		10	10		8	2		10				GLMC 563	MT156256	MT153667
Nemania sp. 1		4	4		2	1	1	4				GLMC 413	MT156262	MT153668
Nemania sp. 2		1	1		1					1		GLMC 1515	MT156263	MT153669
Nemania sp. 3		4	3	1	3		1			4		GLMC 1799	MT156264	MT153670
Neocosmospora cf. *perseae*		1	1			1		1				GLMC 300	MT156265	MT153671
Neurospora sp.		6	5	1	1	4	1	5	1			GLMC 658	MT156270	MT153672
Ophiostoma sp.		4	4		3	1		4				GLMC 619	MT156274	MT153673
Phaeoacremonium hungaricum	G, P	3	3		1		2	1	2			GLMC 1236	MT156288	MT153677

Table 1 (continued)

Taxon	Nov.	Strains	sy.	n-sy.	P.d.	P.c.	P.a.	Sa	LSa	BW	Ba	Rep. strain	GenBank no.[1]	
													LSU	ITS
Phaeoacremonium iranianum	G, d	1	1		1			1				GLMC 490	MT156289	MT153674
Phaeoacremonium scolyti	G	1	1		1			1				GLMC 570	MT156290	MT153676
Phaeoacremonium cf. *viticola*		9	9		2	7		9				GLMC 498	MT156287	MT153675
Phialemonium sp.		2	2			2		2				GLMC 576	MT156293	MT153678
Seimatosporium sp.		10	10		7	3		1		7	2	GLMC 1722	MT156305	MT153679
Simplicillium aogashimaense	G, P	4	4		4			4				GLMC 349	MT156306	MT153681
Simplicillium minatense	G, P	1		1	1			1				GLMC 520	MT156307	MT153680
Sporothrix variecibatus	G, P	2	2		2			2				GLMC 353	MT156310	MT153683
Tolypocladium sp.		3	3				3			3		GLMC 1695	MT156313	MT153684
Trichoderma citrinoviride	c	1	1			1		1				GLMC 235	MT156317	MT153685
Trichoderma cf. *simmonisii*		2	2		2			2				GLMC 350	MT156316	MT153686
Trichoderma cf. spp.		1	1		1			1				GLMC 512	MT156318	MT153687
Truncatella angustata	P	3	3		1	2		2		1		GLMC 253	MT156320	MT153688
Xylaria longipes	P	5	2	3			5			5		GLMC 1499	MT156328	MT153690
Valsaceae sp.		17	15	2	15	1	1	15	2			GLMC 412	MT156323	MT153689
Xylariaceae sp. 1		1		1			1			1		GLMC 1660	MT156325	MT153693
Xylariaceae sp. 2		1	1				1			1		GLMC 1594	MT156326	MT153692
Xylariaceae sp. 3		2	1	1	1		1	2				GLMC 848	MT156327	MT153691
Hypocreales sp. 1		2	1	1	2			2				GLMC 550	MT156231	MT153648
Hypocreales sp. 2		3	3		3			3				GLMC 686	MT156232	MT153650
Hypocreales sp. 3		2	1	1	2			2				GLMC 556	MT156233	MT153649
Sordariales sp.		1		1			1		1			GLMC 1232	MT156309	MT153682
Basidiomycota														
Agaricomycetes														
Bjerkandera cf. *adusta*		5	5		3	1	1	3		2		GLMC 431	MT156167	MT156120
Coniophora puteana	G, d	1	1		1			1				GLMC 420	MT156200	MT156121
Coprinellus cf. spp.		1	1			1		1				GLMC 737	MT156203	MT156122
Coriolopsis gallica	G, d	1	1		1				1			GLMC 1308	MT156204	MT156123
Exidia glandulosa	c	1	1			1		1				GLMC 374	MT156224	MT156125
Heterobasidion annosum	d	1	1		1				1			GLMC 1320	MT156230	MT156126
Mycoacia fuscoatra	G, P	1	1		1			1				GLMC 1268	MT156260	MT156127
Peniophora cinerea	a, c, d	13	13		1	11	1	12	1			GLMC 947	MT156281	MT156128
Peniophora quercina		2	2		2					2		GLMC 1640	MT156282	MT156129
Phellinus tuberculosus A	d	4	4		4			3		1		GLMC 396	MT156291	MT156130
Phellinus tuberculosus B	a, d	3	3		2		1			3		GLMC 1755	MT156292	MT156131
Sistotrema sp.		2	2		2					2		GLMC 1593	MT156308	MT156132
Stereum cf. spp.		3	3		3			3				GLMC 475	MT156311	MT156133
Trametes hirsuta		1	1		1			1				GLMC 467	MT156314	MT156134
Trametes versicolor		2	2		1		1		1	1		GLMC 1717	MT156315	MT156135
Cystobasidiomycetes														
Cystobasidium pinicola	G, P	3	3		2		1			3		GLMC 1603	MT156205	MT156124
Tremellomycetes														
Udeniomyces sp.		1	1		1				1			GLMC 1365	MT156321	MT156136
Mucoromycota														
Mucor circinelloides	a	1	1				1			1		GLMC 1405	MT156257	MT156141
Mucor hiemalis	P	1	1				1		1			GLMC 1395	MT156258	MT156142
Mucor sp.		1	1			1		1				GLMC 656	MT156259	MT156143

Table 1 (continued)

Taxon	Nov.	Strains	sy.	n-sy.	P.d.	P.c.	P.a.	Sa	LSa	BW	Ba	Rep. strain	GenBank no.[1]	
													LSU	ITS
Umbelopsis isabellina	G, P	5	3	2	5			5				GLMC 521	MT156322	MT156144
# branches sampled					129	64	151	168	90	86				

Nov., novelties and potential first reports during this survey; *sy.*, from symptomatic wood tissue; *n-sy.*, from non-symptomatic wood tissue; *P.d.*, from *Prunus domestica*; *P.c.*, from *P. cerasus*; *P.a.*, from *P. avium*; *Sa*, from Saxony; *LSa*, from Lower Saxony; *BW*, from Baden-Württemberg; *Ba*, from Bavaria; *N*, newly described in Bien et al. 2020 or in Bien and Damm 2020; *G*, *P*, *a*, *c*, *d*, potential first report from Germany, *Prunus*, *P. avium*, *P. cerasus* or *P. domestica*, respectively; *rep. strain*, representative strain for the taxon

[1] *LSU*, 28S nrDNA; *ITS*, internal transcribed spacers and intervening 5.8S nrDNA

Identification

The strains were identified to species, genus or higher level, depending on the affinity to the available reference sequences. These identifications were assigned to a level of identification certainty based on an evaluation of the respective clades in the phylogenetic trees and nucleotide differences in the respective ITS alignments. A species was assigned to “identified with high certainty”, if the strain showed ≤ 4 nucleotide differences in the ITS sequence to a named reference sequence. Letters at the species name indicate a sequence variation within strains that were identified as the same species. A low certainty was indicated with “cf.”, if the ITS sequence of a strain differed in 5–10 nucleotides from the closest named reference sequence. The strain was assigned to a genus, but not to a species, if the ITS sequence differed in > 10 nucleotides from the closest named reference sequence or matched with more than one named reference sequence and marked with “sp.” or “cf. spp.”, respectively. If the strain belonged to a clade, for which no named reference sequence was available or with reference sequences belonging to more than one genus, the name of family, order or class was applied. Identifications of part of the taxa to genus level were verified based on microscopic examination of morphological features formed on the used standard media.

Results

In total, 1018 fungal strains were isolated from *Prunus* wood, which belonged to 172 species. The numbers of species isolated per host species were as follows: 113 species from *Prunus domestica*, 70 from *P. avium* and 61 from *P. cerasus*

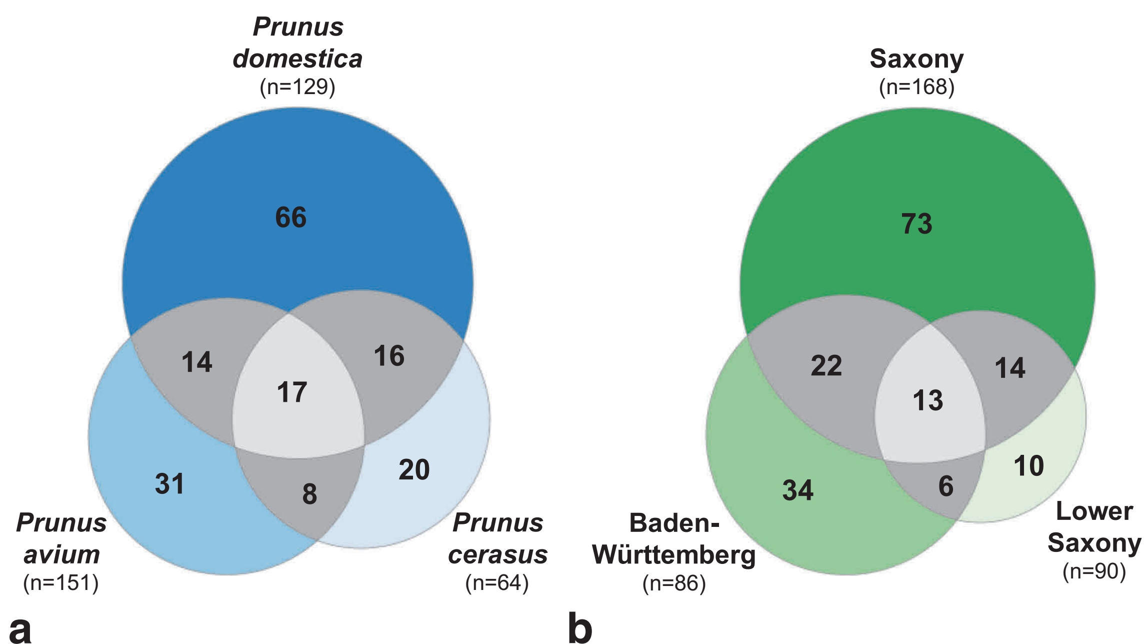


Fig. 1 Number of species isolated from *Prunus* wood in Germany **a** per host species and **b** per sampling region. *n*, number of sampled branches

(Fig. 1a). While 66, 31 and 20 species, respectively, were exclusively isolated from one of these hosts, 17 species occurred in all of them. Regarding the main sampling regions, 122, 75 and 43 species were isolated from *Prunus* wood collected in Saxony, Baden-Württemberg and Lower Saxony, respectively. While 73, 34 and 10 species, respectively, were exclusively isolated from wood collected from one of these regions, 13 species occurred in all of them (Fig. 1b). Five species were isolated from all three *Prunus* species and in all collection areas, namely *Alternaria destruens*, *Aposphaeria corallinolutea*, *Aureobasidium pullulans*, *Pallidophorina paarla* and *Cladosporium* cf. spp. 1 (Table 1). *Aposphaeria corallinolutea* and *Pa.* were isolated 138 and 112 times, respectively, all other taxa ≤ 30 times. Most of the taxa with 15–30 strains were isolated from at least two host species and in at least two collection regions, except for *Collophorina africana* and *Lepteutypa* sp. 1 that were collected only from *P. domestica*, and *Coniothyrium ferrarisianum* that was collected only from Saxony.

The majority of the species (166 species) was isolated from the transition zone between symptomatic and non-symptomatic tissue, 138 species exclusively from this tissue, while 34 species were isolated from asymptomatic tissue, six species (each one isolate) exclusively from asymptomatic tissue.

Of the 172 species, 152 species belonged to the *Ascomycota* (965 strains), 16 to the *Basidiomycota* (45 strains) and four to the *Mucoromycota* (eight strains). Within the *Ascomycota*, 75 species belonged to the *Sordariomycetes* (356 strains), 30 to the *Leotiomycetes* (290 strains), 30 to the *Dothideomycetes* (287 strains) and 13 to the *Eurotiomycetes* (27 strains), representing 43.6%, 17.4%, 17.4% and 7.6%, respectively, of the total diversity and 35%, 28.5%, 28.2% and 2.7%, respectively, of the abundance of the complete mycobiome of *Prunus* wood isolated in this study (Fig. 2a, b). The sequences of the four most abundant classes of *Ascomycota* were analysed in separate alignments, while the remaining classes of the *Ascomycota* were analysed together with *Basidiomycota* and *Mucoromycota.*

Phylogenetic analyses

The combined sequence dataset 1 of the *Sordariomycetes* consisted of 246 strains including the reference strains and the outgroup *Cadophora luteo-olivacea* strain CBS 141.41 (*Leotiomycetes*) and comprised 1884 characters (gene boundaries: LSU: 1–902, ITS: 903–1884, including gaps). The final ML optimisation likelihood of ML analysis was lnL = -39,461.875859. In total, 356 isolates from *Prunus* wood belonged to 75 taxa (Fig. 3). Thirty-one species (136 isolates) were placed in the order *Xylariales*, of which 15 taxa were determined to species, 13 to genus and three to family level. Six species (77 isolates) were placed in the *Diaporthales*; the generic determination of one of them was unclear. Three taxa (35 isolates) were placed in the *Calosphaeriales*, four (14 isolates) in the genus *Phaeoacremonium*, *Togniniales*, and two (six isolates) in the *Ophiostomatales*. Seventeen species (57 isolates) were placed in the order *Hypocreales*; the generic placement of three of them was unclear. Two taxa (nine isolates) were placed in the order *Glomerellales* and determined to species level. Four species (13 isolates) were placed in the order *Sordariales*, one of which not determined to genus level. One isolate was placed in the genus *Chaetosphaeria*, *Chaetosphaeriales*. Four species (six isolates) were placed in the genus *Coniochaeta*, *Coniochaetales*; one of them was identified to species level. One species (two isolates) was placed in a clade formed by strains of *Phialemonium* sp., sister to the single-strain clade of the ex-type strain of *Ph. dimorphosporum* (*incertae sedis*). With 30 strains,

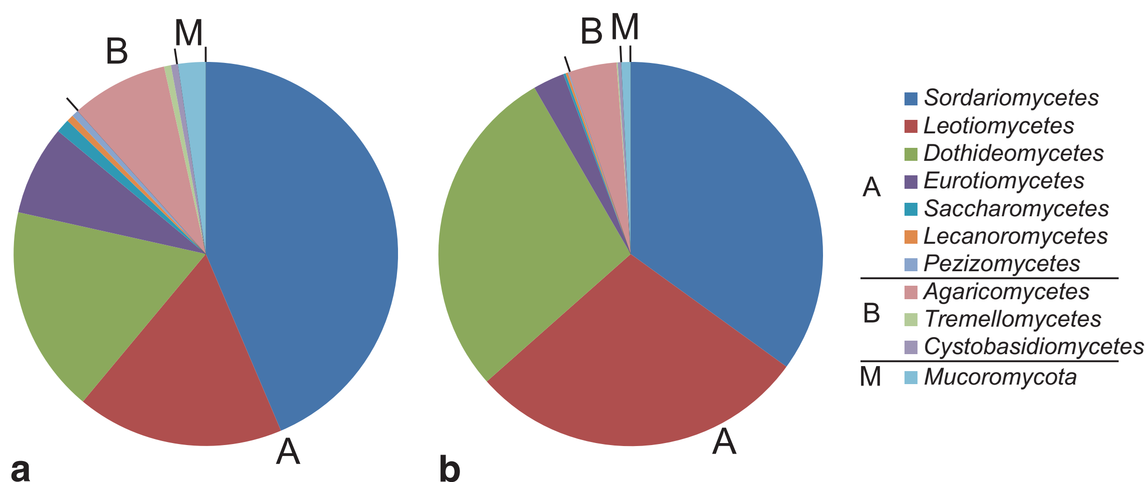


Fig. 2 Percentage **a** of taxa per class and phylum and **b** of strains per class and phylum isolated from *Prunus* wood in Germany. A, *Ascomycota*; B, *Basidiomycota*; M, *Mucoromycota*

Calosphaeria pulchella (*Calosphaeriales*) was the most frequently isolated species in the *Sordariomycetes.*

The combined sequence dataset 2 of the *Dothideomycetes* consisted of 113 strains including the outgroup *Penicillium resticulosum* strain CBS 609.94 (*Eurotiomycetes*) and comprised 1585 characters (gene boundaries: LSU: 1–870, ITS: 871–1585, including gaps). The final ML optimisation likelihood of ML analysis was lnL = - 17,660.376634. In total, 287 isolates belonged to 30 taxa (Fig. 4). Twenty-three taxa (258 isolates) were placed in the *Pleosporales*, of which 17 were determined to species, four to genus and each one to family and order level. Four taxa (14 isolates) were placed in the *Capnodiales* and determined to species or genus level. One taxon (15 isolates) of the *Dothideales* was identified as *Aureobasidium pullulans*. Each one isolate was identified as *Diplodia mutila* and *D. seriata* (*Botryosphaeriales*). With 138 strains, *Aposphaeria corallinolutea* (*Pleosporales*) was the most frequently isolated species in the *Dothideomycetes*.

The combined sequence dataset 3 of the *Leotiomycetes* consisted of 84 strains including the outgroup *Colletotrichum godetiae* strain CBS 133.44 (*Sordariomycetes*) and comprised 1557 characters (gene boundaries: LSU: 1–912, ITS: 913–1557, including gaps). The final ML optimisation likelihood of ML analysis was lnL = - 11,950.782384. In total, 290 isolates belonged to 30 taxa (Fig. 5). Twenty-four taxa (137 isolates) were placed in the *Helotiales*, of which 15 were determined to species and nine to genus level. Five taxa (152 isolates) were placed in *Phacidiales* and determined to species level. One strain remained in an uncertain taxonomic position on order level. With 112 strains, *Pallidophorina paarla* (*Phacidiales*) was the most frequently isolated species in the *Leotiomycetes*.

The combined sequence dataset 4 of the *Eurotiomycetes* consisted of 38 strains including the outgroup *Diplodia intermedia* strain CBS 124462 (*Dothideomycetes*) and comprised 1573 characters (gene boundaries: LSU: 1–908, ITS: 909–1573, including gaps). The final ML optimisation likelihood of ML analysis was lnL = - 9837.561743. In total, 27 isolates belonged to 13 taxa (Fig. 6). Seven taxa (15 isolates) were placed within *Eurotiales*, of which five were determined to species and two to genus level. Four taxa (eight isolates) were placed in *Chaetothyriales*; one was determined to species, two to genus and one to family level. Two taxa (four isolates) were placed in *Phaeomoniellales*, of which one was determined to species and one to genus level. All species of the *Eurotiomycetes* were isolated with low frequencies (≤ 6 strains).

The combined sequence dataset 5 of the remaining classes of the *Ascomycota*, as well as all *Basidiomycota* and *Mucoromycota* consisted of 105 strains including the outgroup *Entomophthora sphaerosperma* strain CBS 530.75 (*Entomophthoromycotina*, *Zoopagomycota*) and comprised 2058 characters (gene boundaries: LSU: 1–1115, ITS: 1116–2058, including gaps). The final ML optimisation likelihood of ML analysis was lnL = - 33,834.310764. Within the 16 taxa (45 strains) of *Basidiomycota*, 14 taxa (41 strains) belonged to the in *Agaricomycetes*, of which 11 taxa were identified to species and three to genus level (Fig. 7). One isolate of the *Tremellomycetes* and one taxon (three isolates) of the *Cystobasidiomycetes* were determined to genus and species level, respectively. With 13 strains, *Peniophora cinerea* was the most frequently isolated species in the *Basidiomycota*. Species of the other phyla were isolated with low frequencies (< 10 strains). One strain of the class *Pezizomycetes* (*Ascomycota*) was determined as *Trichophaeopsis bicuspis*. One taxon of the *Lecanoromycetes* (two strains) could not be further determined. Two strains of the *Saccharomycetes* were determined as *Nakazawaea* cf. *holstii* and *Wickerhamomyces silvicola*, respectively. Within the 8 strains of *Mucoromycota*, five strains were identified as *Umbelopsis isabellina*, two strains as *Mucor circinelloides* and *M. hiemalis*, respectively, while one further *Mucor* strain could not be assigned to a species.

Identification certainty

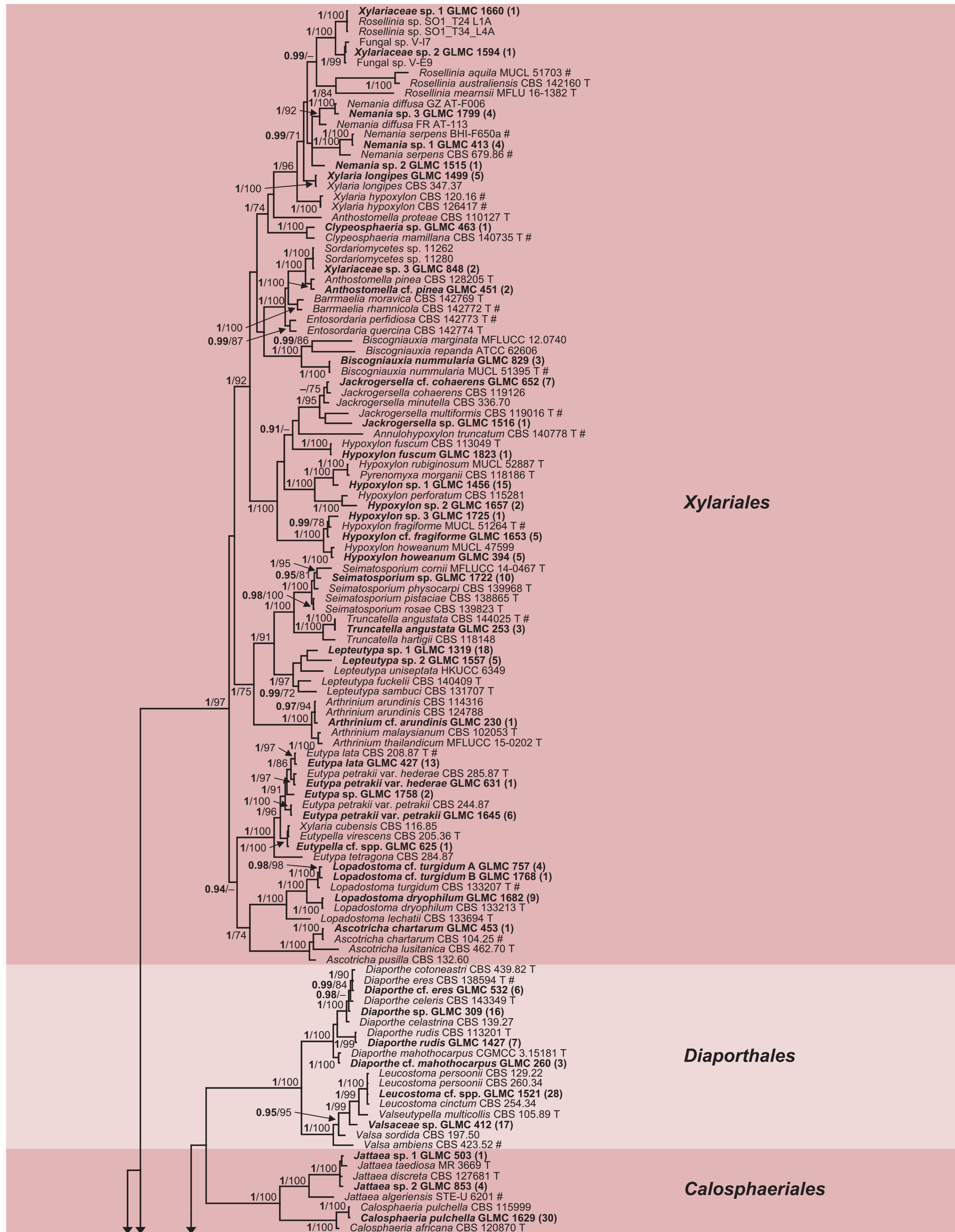
In total, 102 taxa were assigned to a particular species with high (82 taxa) or low (20 taxa) certainty. A further 57 species were determined to genus level. Thirteen species could not be assigned to any genus and were identified to family (six), order (five) or class (two), level, respectively. Almost all of the 70 taxa that were not identified to species level belonged to the *Ascomycota*, with the largest number of taxa belonging to the *Sordariomycete*s (39), followed by *Leotiomycetes* (ten), *Dothideomycetes* (nine) and *Eurotiomycetes* (five) (Fig. 8). Only few undetermined species belonged to *Basidiomycota*, *Mucoromycota* and to the remaining classes of *Ascomycota*.

Discussion

Fungal diversity of necrotic *Prunus* wood in Germany

In total, 172 fungal species were detected in the wood samples of *Prunus* trees studied. The diversity detected in this study far exceeds the number of taxa usually reported from isolation studies of woody plants. In many cases, not more than 30 taxa were reported (e.g. Barengo et al. 2000, Gonthier et al. 2006, Hortová and Novotný 2011, Markakis et al. 2017). Only in few studies up to or more than a hundred taxa were isolated

Fig. 3 Phylogeny of dataset 1 obtained by Bayesian inference analysis of ▶ the combined LSU and ITS sequence alignment of *Sordariomycetes*. *Cadophora luteo-olivacea* strain CBS 141.41 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% are shown at the nodes. The strains isolated in this study are emphasised in bold. Numbers in parentheses indicate the number of isolated strains per taxon. Branches that are crossed by diagonal lines are shortened by 50%. T, ex-type strain; #, type species


Xylariales
Diaporthales
Calosphaeriales

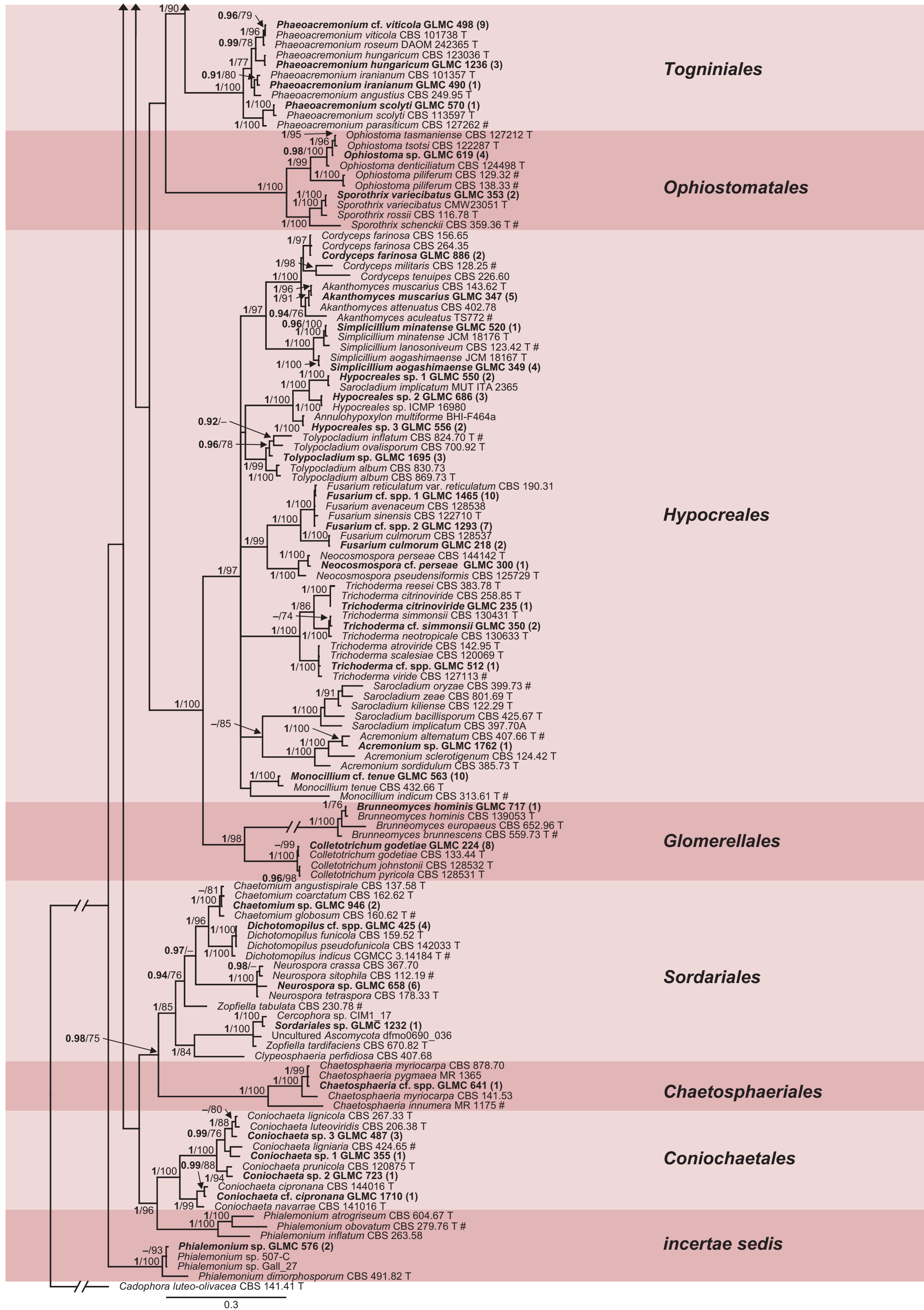


Fig. 3 (continued)

from wood (Lygis et al. 2005, Simeto et al. 2005, Hofstetter et al. 2012). The high number of detected taxa in our study presumably results from the high sample number of three different target host species over a wider geographical area, in contrast to most of the studies that display less diversity. However, the isolated taxa only encompass those fungi present at the time of sampling and accessible by isolation; a multitude of fungi cannot be cultured in general or on standard media (Allen et al. 2003, O'Brien et al. 2005, Tsui et al. 2011, Muggia et al. 2017). Therefore, studies using culture-independent high-throughput sequencing (HTS) techniques usually report much higher species numbers from the fungal diversity inside living or dead plant parts (up to 2000 operational taxonomic units, OTU) than studies using isolation techniques (e.g. Kubartová et al. 2012, Hoppe et al. 2016, Dissanayake et al. 2018, Jayawardena et al. 2018, Purahong et al. 2018). As most of the taxa were isolated in this study only once or few times, we would expect the number of taxa to increase tremendously, if the number of wood samples would be increased. The mycobiome of the wood of the three *Prunus* species in Germany is far from being complete.

The two most abundant species, *Aposphaeria corallinolutea* (*Dothideomycetes*, *Pleosporales*) and *Pallidophorina paarla* (*Leotiomycetes*, *Phacidiales*), were isolated > 100 times each and from all three host species and in all three collection areas. *Aposphaeria corallinolutea* was revealed as the most dominant inhabitant of *Prunus* wood in Germany in our study, while there are only five reports from previous studies: from *Fraxinus excelsior* and *Kerria japonica* in the Netherlands (de Gruyter et al. 2013), from decaying wood in Thailand (Li et al. 2016), from dead branches of *Prunus padus* in Russia (Tibpromma et al. 2017) and from needles of *Pseudotsuga menziesii* in the USA (Daniels 2017). The only ITS sequence of this species in GenBank originates from the study in Thailand. Thus, *A. corallinolutea* is known from several hosts, including *Prunus*, and from different countries, however, has not previously been reported from Germany or from any of the *Prunus* species studied here. The low number of reports could be explained by the lack of studies on its main host plants/substrates that, based on this study, includes necrotic wood of *Prunus* in Germany, but also by the facts that *A. corallinolutea* was described only 2013 (de Gruyter et al. 2013) and that the first and so far only ITS sequence of a strain identified as this species was submitted to GenBank only 2017 (Li et al. 2016). A blastn search with the ITS sequence of strain GLMC 1355 revealed a 100% match with an unidentified *Ascomycota* strain from leaves of *Fagus sylvatica* in Germany (Unterseher and Schnittler 2009) indicating the occurrence of this fungus on a further host as well as in Germany. In contrast, the second most abundant species, *Pa. paarla*, has previously frequently been reported from a number of *Prunus* species in several countries including Germany (Gierl and Fischer 2017, Bien et al. 2020).

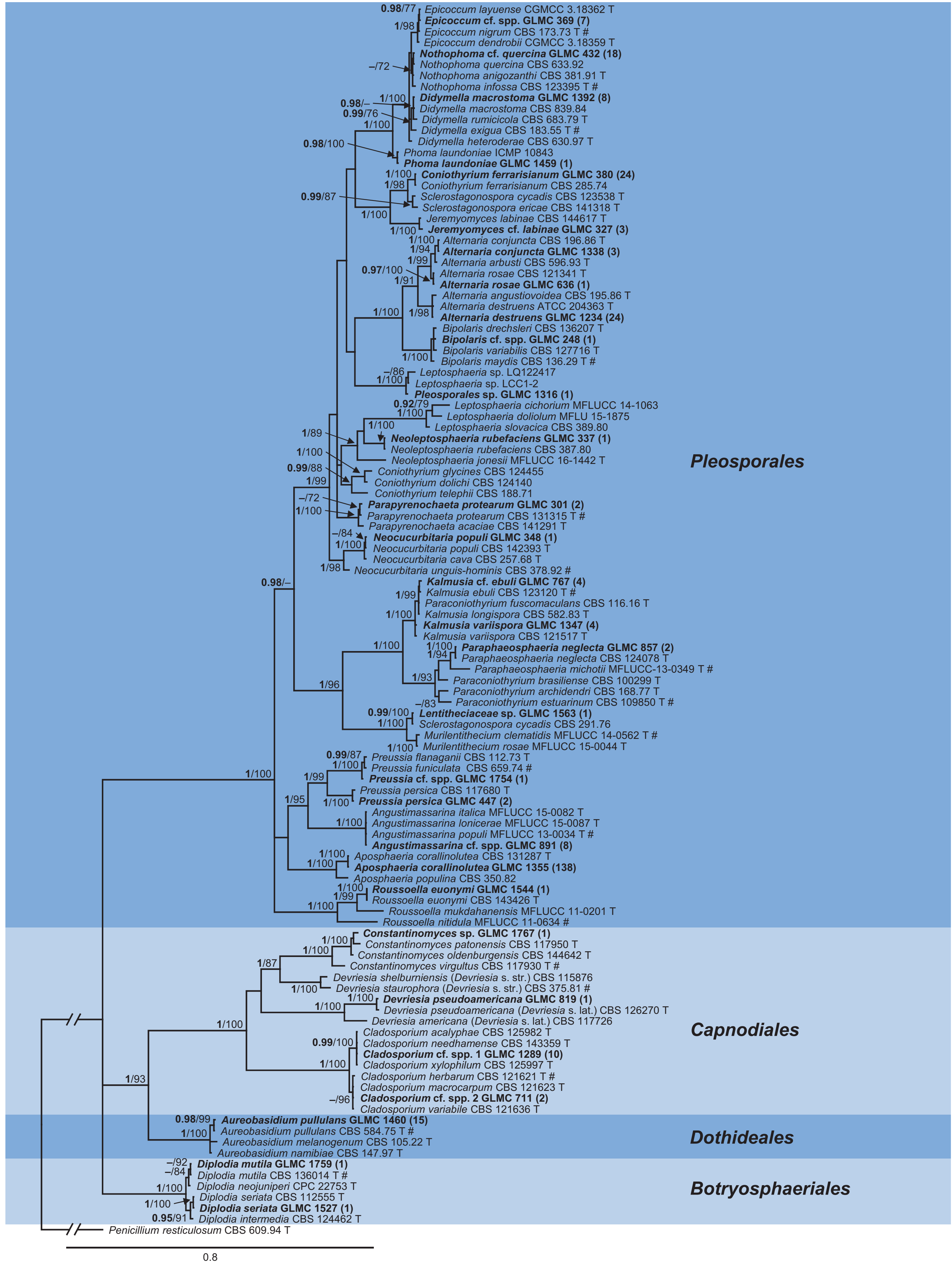
Part of the taxa isolated in this study probably represent first reports for the genus *Prunus*, for specific *Prunus* species or for Germany. We conducted a search of the 82 taxa identified to species level with high certainty on the USDA database (Farr and Rossman 2019). For 41 of these taxa, no previous report from Germany and for 40 taxa, no previous report from the host genus *Prunus* was listed (Table 1). Of further 25 taxa, there was no previous report from one or more of the *Prunus* hosts, on which they were collected from in our study. However, as some of the latest publications are missing, this database is apparently not complete. Therefore, and due to the unreliable identification results of many species, we consider these reports as potential first reports. They need to be confirmed by in-depth studies of the respective species, which was beyond the scope of this study.

The aim of this study was to reveal the mycobiome associated with necroses of *Prunus* wood in Germany as complete as possible in a reasonable time frame using a cultivation approach. As the study was based on commercial orchards, it was not possible to collect the exact amount of samples from each host species with the same age, same cultivars etc. at the same collection area. For some of the orchards, data like tree age and cultivar were not even available. Therefore, a direct comparison of the three collection areas and host species regarding strain or species numbers cannot be made as it is most probably biased by other factors.

Comparison with other studies from *Prunus*

The results obtained in this study could only be compared to a few other studies that used similar methods (culturing, sequence-based identification). However, most of them were conducted on different *Prunus* species and in different climates. The extensive survey of fungi in *Prunus* wood (*P. armeniaca*, *P. dulcis*, *P. persica*, *P. persica* var. *nucipersica*, *P. salicina*) in South Africa resulted in reports of 47 species in several publications by Damm et al. (2007a,b, 2008a,b,c, 2010), Moyo et al. (2018) and Bien and Damm (2020) focusing on specific genera. Gramaje et al. (2012) isolated nine fungal species from *Prunus dulcis* in Spain (Island of Mallorca) including five species belonging to the *Botryosphaeriales* as well as *Collophorina hispanica*, *Diaporthe amygdali*, *Eutypa lata* and *Phaeoacremonium amygdalinum*. The study of Inderbitzin et al. (2010) was restricted to *Botryosphaeriaceae* from *Prunus dulcis* in CA, USA, and that of Tian et al. (2018) to *Diaporthe amygdali* and *Botryosphaeria dothidea* of *P. persica* in Yangshan, China. The only study from Germany was that by Gierl and Fischer (2017), who reported only eight fungal species from symptomatic wood of six *Prunus* species, two of which were also sampled by us, namely *P. cerasus* and *P. domestica*.

Botryosphaeriales are known as pathogens and endophytes of various woody hosts (Slippers et al. 2007, Cloete et al.


Epicoccum layuense CGMCC 3.18362 T
Epicoccum cf. spp. GLMC 369 (7)
Epicoccum nigrum CBS 173.73 T #
Epicoccum dendrobii CGMCC 3.18359 T
Nothophoma cf. quercina GLMC 432 (18)
Nothophoma quercina CBS 633.92
Nothophoma anigozanthi CBS 381.91 T
Nothophoma infossa CBS 123395 T #
Didymella macrostoma GLMC 1392 (8)
Didymella macrostoma CBS 839.84
Didymella rumicicola CBS 683.79 T
Didymella exigua CBS 183.55 T #
Didymella heteroderae CBS 630.97 T
Phoma laundoniae ICMP 10843
Phoma laundoniae GLMC 1459 (1)
Coniothyrium ferrarisianum GLMC 380 (24)
Coniothyrium ferrarisianum CBS 285.74
Sclerostagonospora cycadis CBS 123538 T
Sclerostagonospora ericae CBS 141318 T
Jeremyomyces labinae CBS 144617 T
Jeremyomyces cf. labinae GLMC 327 (3)
Alternaria conjuncta CBS 196.86 T
Alternaria conjuncta GLMC 1338 (3)
Alternaria arbusti CBS 596.93 T
Alternaria rosae CBS 121341 T
Alternaria rosae GLMC 636 (1)
Alternaria angustiovoidea CBS 195.86 T
Alternaria destruens ATCC 204363 T
Alternaria destruens GLMC 1234 (24)
Bipolaris drechsleri CBS 136207 T
Bipolaris cf. spp. GLMC 248 (1)
Bipolaris variabilis CBS 127716 T
Bipolaris maydis CBS 136.29 T #
Leptosphaeria sp. LQ122417
Leptosphaeria sp. LCC1-2
Pleosporales sp. GLMC 1316 (1)
Leptosphaeria cichorium MFLUCC 14-1063
Leptosphaeria doliolum MFLU 15-1875
Leptosphaeria slovacica CBS 389.80
Neoleptosphaeria rubefaciens GLMC 337 (1)
Neoleptosphaeria rubefaciens CBS 387.80
Neoleptosphaeria jonesii MFLUCC 16-1442 T
Coniothyrium glycines CBS 124455
Coniothyrium dolichi CBS 124140
Coniothyrium telephii CBS 188.71
Parapyrenochaeta protearum GLMC 301 (2)
Parapyrenochaeta protearum CBS 131315 T #
Parapyrenochaeta acaciae CBS 141291 T
Neocucurbitaria populi GLMC 348 (1)
Neocucurbitaria populi CBS 142393 T
Neocucurbitaria cava CBS 257.68 T
Neocucurbitaria unguis-hominis CBS 378.92 #
Kalmusia cf. ebuli GLMC 767 (4)
Kalmusia ebuli CBS 123120 T #
Paraconiothyrium fuscomaculans CBS 116.16 T
Kalmusia longispora CBS 582.83 T
Kalmusia variispora GLMC 1347 (4)
Kalmusia variispora CBS 121517 T
Paraphaeosphaeria neglecta GLMC 857 (2)
Paraphaeosphaeria neglecta CBS 124078 T
Paraphaeosphaeria michotii MFLUCC-13-0349 T #
Paraconiothyrium brasiliense CBS 100299 T
Paraconiothyrium archidendri CBS 168.77 T
Paraconiothyrium estuarinum CBS 109850 T #
Lentitheciaceae sp. GLMC 1563 (1)
Sclerostagonospora cycadis CBS 291.76
Murilentithecium clematidis MFLUCC 14-0562 T #
Murilentithecium rosae MFLUCC 15-0044 T
Preussia flanaganii CBS 112.73 T
Preussia funiculata CBS 659.74 #
Preussia cf. spp. GLMC 1754 (1)
Preussia persica CBS 117680 T
Preussia persica GLMC 447 (2)
Angustimassarina italica MFLUCC 15-0082 T
Angustimassarina lonicerae MFLUCC 15-0087 T
Angustimassarina populi MFLUCC 13-0034 T #
Angustimassarina cf. spp. GLMC 891 (8)
Aposphaeria corallinolutea CBS 131287 T
Aposphaeria corallinolutea GLMC 1355 (138)
Aposphaeria populina CBS 350.82
Roussoella euonymi GLMC 1544 (1)
Roussoella euonymi CBS 143426 T
Roussoella mukdahanensis MFLUCC 11-0201 T
Roussoella nitidula MFLUCC 11-0634 #
Pleosporales
Constantinomyces sp. GLMC 1767 (1)
Constantinomyces patonensis CBS 117950 T
Constantinomyces oldenburgensis CBS 144642 T
Constantinomyces virgultus CBS 117930 T #
Devriesia shelburniensis (Devriesia s. str.) CBS 115876
Devriesia staurophora (Devriesia s. str.) CBS 375.81 #
Devriesia pseudoamericana GLMC 819 (1)
Devriesia pseudoamericana (Devriesia s. lat.) CBS 126270 T
Devriesia americana (Devriesia s. lat.) CBS 117726
Cladosporium acalyphae CBS 125982 T
Cladosporium needhamense CBS 143359 T
Cladosporium cf. spp. 1 GLMC 1289 (10)
Cladosporium xylophilum CBS 125997 T
Cladosporium herbarum CBS 121621 T #
Cladosporium macrocarpum CBS 121623 T
Cladosporium cf. spp. 2 GLMC 711 (2)
Cladosporium variabile CBS 121636 T
Capnodiales
Aureobasidium pullulans GLMC 1460 (15)
Aureobasidium pullulans CBS 584.75 T #
Aureobasidium melanogenum CBS 105.22 T
Aureobasidium namibiae CBS 147.97 T
Dothideales
Diplodia mutila GLMC 1759 (1)
Diplodia mutila CBS 136014 T #
Diplodia neojuniperi CPC 22753 T
Diplodia seriata CBS 112555 T
Diplodia seriata GLMC 1527 (1)
Diplodia intermedia CBS 124462 T
Botryosphaeriales
Penicillium resticulosum CBS 609.94 T
0.8

◀ **Fig. 4** Phylogeny of dataset 2 obtained by Bayesian inference analysis of the combined LSU and ITS sequence alignment of *Dothideomycetes*. *Penicillium resticulosum* strain CBS 609.94 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% are shown at the nodes. The strains isolated in this study are emphasised in bold. Numbers in parentheses indicate the number of isolated strains per taxon. Branches that are crossed by diagonal lines are shortened by 50%. T, ex-type strain; #, type species

2011). In previous studies, species of this order were reported to be very abundant in wood of *Prunus* trees in South Africa, the USA, Spain and China (Damm et al. 2007a, b, Inderbitzin et al. 2010, Gramaje et al. 2012, Tian et al. 2018). The dominating species in the studies from South Africa and Spain were *D. seriata* and *Neofusicoccum parvum*, respectively, while only *Botryosphaeria dothidea* was reported in that from China. Moreover, *D. pinea*, a pathogen of several *Pinus* species in many countries (Farr and Rossman 2019), that also cause serious damage to pine trees suffering from drought stress and bark beetle attacks in Germany (Heydeck and Dahms 2012, Petercord 2017), had frequently been isolated from *P. persica* in South Africa and tested positive for its pathogenicity on this host (Damm et al. 2007a). Therefore, host jumps from infected *Pinus* plantations to *Prunus* orchards in close vicinity are possible. However, *Botryosphaeriales* were surprisingly rare in this study. Only one strain each of *D. seriata* and *D. mutila* was detected in wood of *P. domestica* in the most southern sampling region in Germany; *D. pinea* was not isolated at all. Brodde et al. (2019) documented an outbreak of *Diplodia* tip blight on *Pinus sylvestris* stands in Sweden in 2016 caused by *D. pinea* and attributed it to the increased summer temperatures. An influence of different climatic conditions on distribution patterns of *Botryosphaeriales* species has also been observed in the USA and Australia (Taylor et al. 2005, Úrbez-Torres et al. 2006, Pitt et al. 2010). However, a climatical or geographical explanation in general can be ruled out, since species of this order have been detected from fruit trees and grapevine in Central Europe before, even in different parts of Germany, including a report of the same two species from *P. armeniaca* (Trapman et al. 2008, Quaglia et al. 2014, Fischer et al. 2016, Gierl and Fischer 2017). Based on the results in this study, species of *Botryosphaeriaceae* are currently not regarded as a threat for German *Prunus* orchards.

With 14 species, *Phaeoacremonium* was the genus with the highest diversity in the study on *Prunus* wood in South Africa (Damm et al. 2008b), while only four *Phaeoacremonium* species were isolated in Germany (this study). Three of them were isolated in both studies, namely *Pm. iranianum*, *Pm. scolyti* and *Pm. viticola*, provided the identification of the latter, which was with low certainty (cf.), is correct. Although the genera were found in *Prunus* wood in both countries, completely different species of *Coniochaeta* (*Coniochaetales*, *Sordariomycetes*), *Calosphaeria*, *Jattaea* (*Calosphaeriales*, *Sordariomycetes*), *Paraconiothyrium*/*Paraphaeosphaeria* (*Pleosporales*, *Dothideomycetes*) and *Phaeomoniellales* (*Eurotiomycetes*) were collected in Germany and in South Africa (Damm et al. 2008a, c, 2010, Bien and Damm 2020, this study). The latter order was much more diverse and frequent in *Prunus* wood in South Africa; in Germany, only two *Minutiella* species were collected. In contrast, *Cadophora* species were more frequently detected in wood of different *Prunus* species in Germany, but only rarely detected in South Africa; only *Ca. prunicola* was collected in *Prunus* wood in both countries (Bien and Damm 2020).

Collophorina (syn. *Collophora*) and *Pallidophorina* species were isolated frequently in *Prunus* wood both in South Africa and in Germany (Damm et al. 2010, Bien et al. 2020, this study). The dominating *Collophorina* species isolated from several *Prunus* species in South Africa was *C. rubra*, a species not reported from Germany, while the dominating one in Germany was *C. africana* (syn. *Collophora capensis*). The latter was originally found exclusively on wood of *P. salicina* in South Africa, while in our study, it was exclusively present on *P. domestica*. In the study by Damm et al. (2010), *Pa. paarla* (syn. *C. paarla*, *Collophora pallida*) was mostly isolated from *P. salicina* in South Africa, while this species was one of the two dominating species in this survey occurring in all *Prunus* species studied (Bien et al. 2020, this study). The *Collophorina* species isolated from *P. dulcis* wood in Spain (Gramaje et al. 2012), *C. hispanica*, was not found in our study. Gierl and Fischer (2017) isolated *Pa. paarla* from symptomatic wood of *P. cerasus* and *P. persica*, as well as *C. hispanica* and *C. africana* from *P. armeniaca* and *P. dulcis*, respectively.

Although five species of *Diatrypaceae* were collected in the surveys in Germany and South Africa, *Eutypa lata* was the only species found in both of them, in wood of *P. cerasus* and *P. domestica* in Germany, as well as in *P. armeniaca*, *P. avium*, *P. dulcis* and *P. salicina* in South Africa (Moyo et al. 2018, this study). It was also found in wood of *P. dulcis* in Mallorca (Gramaje et al. 2012). Furthermore, *Diaporthe* species have been isolated in all three studies as well. Based on preliminary studies, none of the species is overlapping with those found in this study (Gramaje et al. 2012, U. Damm, unpubl. data). The remaining taxa cannot be compared as no data were published from the survey in South Africa.

Function of the fungal species inside wood

Only for part of the species/genera isolated in this study information on lifestyle, like pathogenicity on *Prunus* species, is available. In the survey on *Prunus* wood in South Africa, preliminary pathogenicity tests on detached shoots revealed the majority of tested species belonging to *Botryosphaeriaceae*, *Celotheliaceae*, *Coniochaetaceae*, *Togniniaceae* and *Tympanidaceae* to be potentially

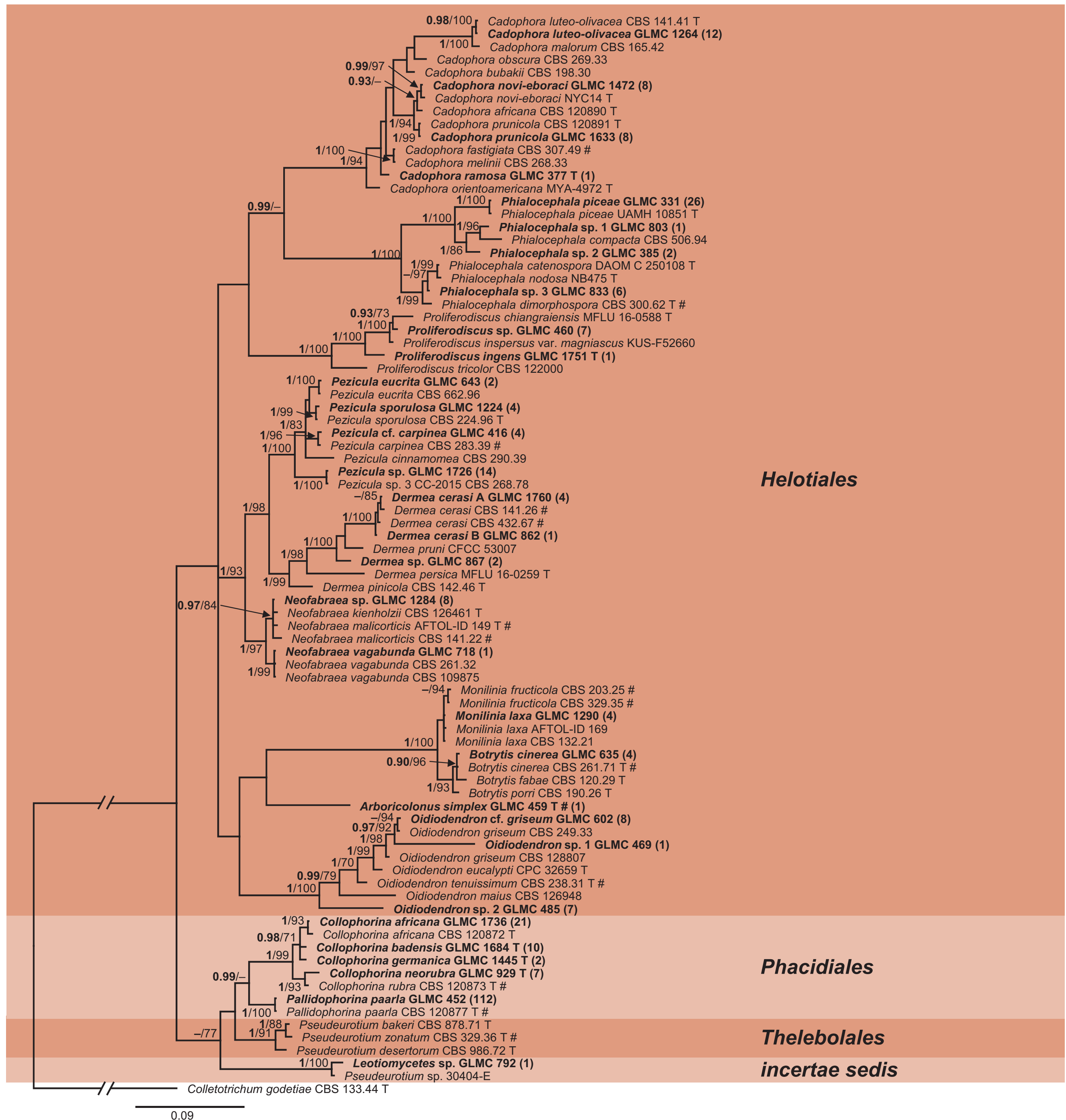


Fig. 5 Phylogeny of dataset 3 obtained by Bayesian inference analysis of the combined LSU and ITS sequence alignment of *Leotiomycetes*. *Colletotrichum godetiae* strain CBS 133.44 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% are shown at the nodes. The strains isolated in this study are emphasised in bold. Numbers in parentheses indicate the number of isolated strains per taxon. Branches that are crossed by diagonal lines are shortened by 50%. T, ex-type strain; #, type species

pathogenic to *P. persica* var. *nucipersica* and/or *P. salicina* (Damm et al. 2007a, 2008b, 2010). Species of all these families have been isolated in this study as well. However, apart from the fact that these pathogenicity tests were preliminary and not followed up by field tests, these results cannot be transferred to this study, because most of the fungal species isolated were different, and even the few species isolated in both studies, for example *Pa. paarla* and *C. africana*, were not isolated from the same *Prunus* species. Therefore, the pathogenicity of each fungal species isolated in this study would need to be tested on its host species in Germany.

As we aimed at isolating pathogens causing necroses inside *Prunus* wood, the majority of wood pieces we isolated from were from the transition zone of symptomatic to non-

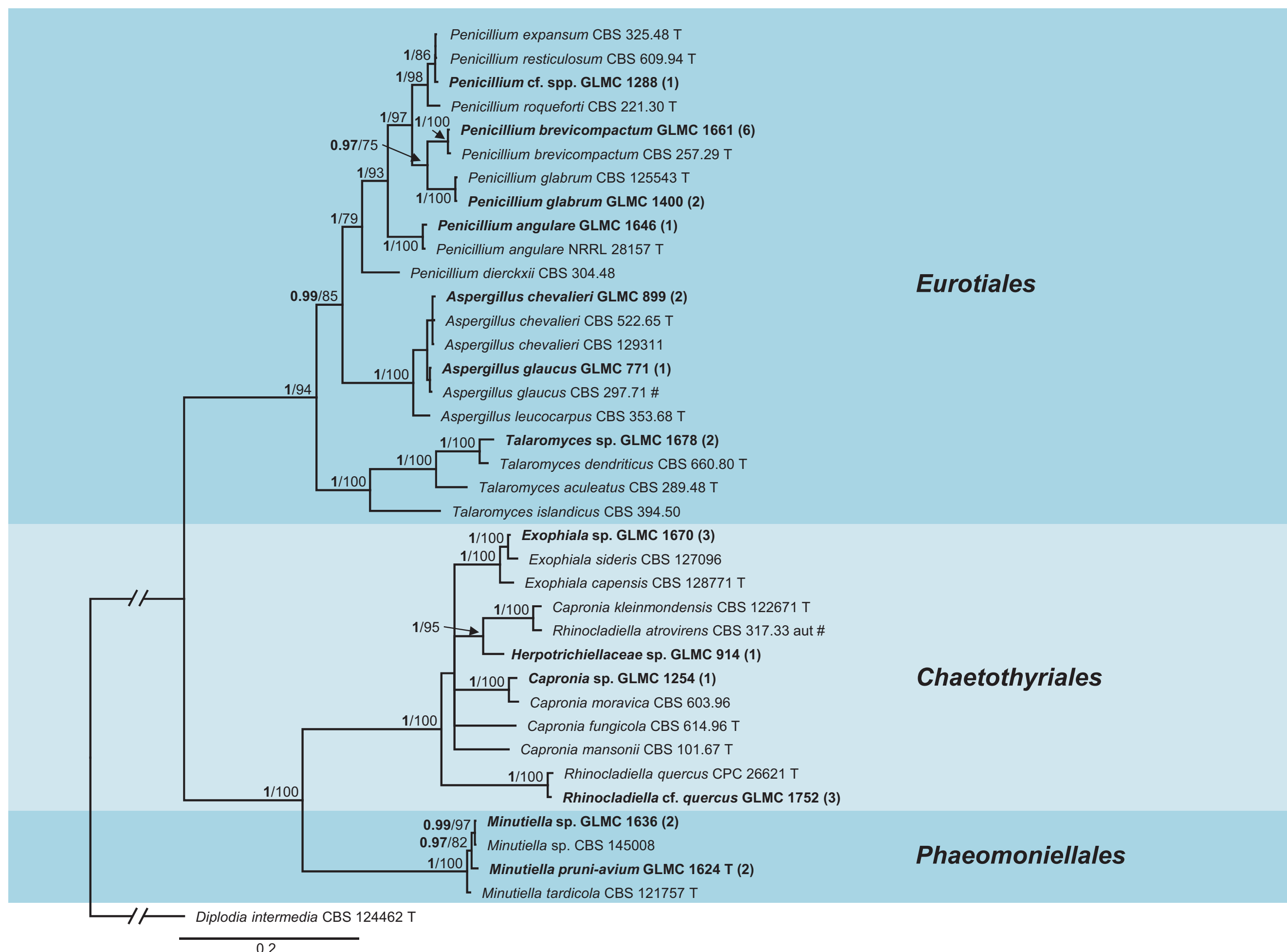
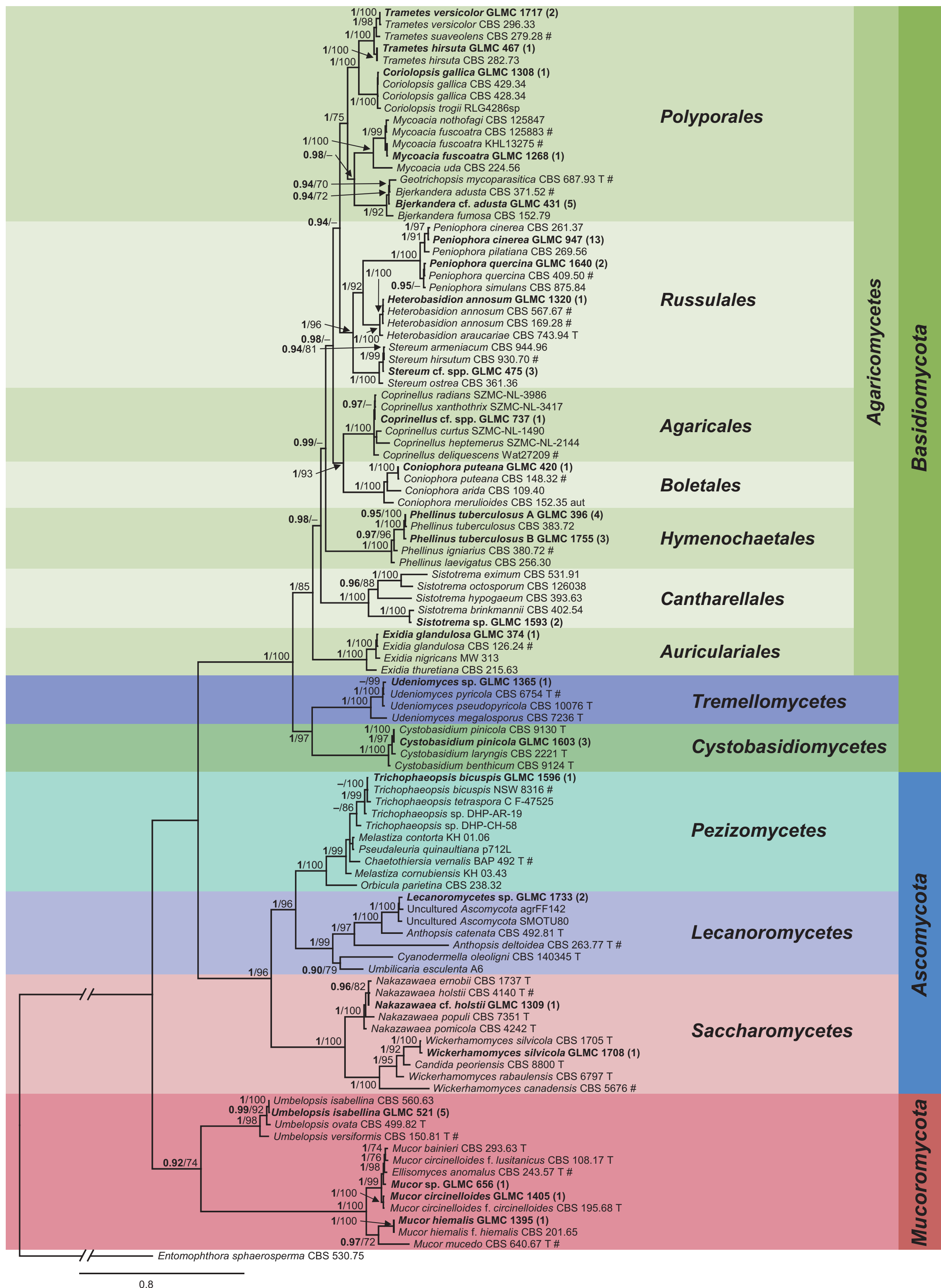


Fig. 6 Phylogeny of dataset 4 obtained by Bayesian inference analysis of the combined LSU and ITS sequence alignment of *Eurotiomycetes*. *Diplodia intermedia* strain CBS 124462 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% are shown at the nodes. The strains isolated in this study are emphasised in bold. Numbers in parentheses indicate the number of isolated strains per taxon. Branches that are crossed by diagonal lines are shortened by 50%. T, ex-type strain; #, type species

symptomatic wood tissue. From most of the wood samples, we isolated several fungi. Wood diseases are caused by a complex of fungal pathogens, which is known from grapevine trunk diseases like esca and Botryosphaeria dieback (Larignon and Dubos 1997, Bertsch et al. 2013). Therefore, more than one of the isolated fungi could be responsible for the symptoms on the respective branch. Moreover, transitions between different lifestyles have been shown in a high number of fungi (Promputtha et al. 2010, Álvarez-Loayza et al. 2011, Eaton et al. 2011, O'Connell et al. 2012, Kuo et al. 2014). As an example, many of the wood-inhabiting fungi, including *Botryosphaeriaceae*, are known as weak pathogens: they do not cause symptoms and live inside their host endophytically and become pathogenic, if the host plant is exposed to stress, e.g. drought (Desprez-Loustau et al. 2006, Slippers and Wingfield 2007). However, not only the presence of one or more pathogens decides, if a disease develops, but also the absence of other fungi or other organisms that prevent the disease and keep the tree healthy. Thus, in a study of Gennaro et al. (2003), the endophytic communities on declining oaks were less diverse than those on healthy trees, and endophyte communities of needles of Norway spruce have been proposed as indicators of tree health (Rajala et al. 2014). It is therefore hardly possible to draw conclusions concerning the particular role of the individual species within the temporal-spatial succession of fungal communities associated with wood necroses of *Prunus* trees in Germany.

We isolated fungi both from the transition zone of symptomatic to non-symptomatic tissue and from non-symptomatic tissue of the same branch providing that the sole isolation of a certain species from one of the two zones would indicate a certain life style, e.g. the sole isolation from non-symptomatic tissue would indicate an endophytic life style. However, the resulting data are not directly comparable, because the number of wood pieces of the non-symptomatic tissue of a branch with wood symptoms studied was lower than the number of wood pieces from the transition zone of symptomatic to non-symptomatic wood tissue. Moreover, in some branches, little



Trametes versicolor GLMC 1717 (2)
Trametes versicolor CBS 296.33
Trametes suaveolens CBS 279.28 #
Trametes hirsuta GLMC 467 (1)
Trametes hirsuta CBS 282.73
Coriolopsis gallica GLMC 1308 (1)
Coriolopsis gallica CBS 429.34
Coriolopsis gallica CBS 428.34
Coriolopsis trogii RLG4286sp
Mycoacia nothofagi CBS 125847
Mycoacia fuscoatra CBS 125883 #
Mycoacia fuscoatra KHL13275 #
Mycoacia fuscoatra GLMC 1268 (1)
Mycoacia uda CBS 224.56
Geotrichopsis mycoparasitica CBS 687.93 T #
Bjerkandera adusta CBS 371.52 #
Bjerkandera cf. adusta GLMC 431 (5)
Bjerkandera fumosa CBS 152.79
Polyporales
Peniophora cinerea CBS 261.37
Peniophora cinerea GLMC 947 (13)
Peniophora pilatiana CBS 269.56
Peniophora quercina GLMC 1640 (2)
Peniophora quercina CBS 409.50 #
Peniophora simulans CBS 875.84
Heterobasidion annosum GLMC 1320 (1)
Heterobasidion annosum CBS 567.67 #
Heterobasidion annosum CBS 169.28 #
Heterobasidion araucariae CBS 743.94 T
Stereum armeniacum CBS 944.96
Stereum hirsutum CBS 930.70 #
Stereum cf. spp. GLMC 475 (3)
Stereum ostrea CBS 361.36
Russulales
Coprinellus radians SZMC-NL-3986
Coprinellus xanthothrix SZMC-NL-3417
Coprinellus cf. spp. GLMC 737 (1)
Coprinellus curtus SZMC-NL-1490
Coprinellus heptemerus SZMC-NL-2144
Coprinellus deliquescens Wat27209 #
Agaricales
Coniophora puteana GLMC 420 (1)
Coniophora puteana CBS 148.32 #
Coniophora arida CBS 109.40
Coniophora merulioides CBS 152.35 aut
Boletales
Phellinus tuberculosus A GLMC 396 (4)
Phellinus tuberculosus CBS 383.72
Phellinus tuberculosus B GLMC 1755 (3)
Phellinus igniarius CBS 380.72 #
Phellinus laevigatus CBS 256.30
Hymenochaetales
Sistotrema eximum CBS 531.91
Sistotrema octosporum CBS 126038
Sistotrema hypogaeum CBS 393.63
Sistotrema brinkmannii CBS 402.54
Sistotrema sp. GLMC 1593 (2)
Cantharellales
Exidia glandulosa GLMC 374 (1)
Exidia glandulosa CBS 126.24 #
Exidia nigricans MW 313
Exidia thuretiana CBS 215.63
Auriculariales
Agaricomycetes
Udeniomyces sp. GLMC 1365 (1)
Udeniomyces pyricola CBS 6754 T #
Udeniomyces pseudopyricola CBS 10076 T
Udeniomyces megalosporus CBS 7236 T
Tremellomycetes
Cystobasidium pinicola CBS 9130 T
Cystobasidium pinicola GLMC 1603 (3)
Cystobasidium laryngis CBS 2221 T
Cystobasidium benthicum CBS 9124 T
Cystobasidiomycetes
Basidiomycota
Trichophaeopsis bicuspis GLMC 1596 (1)
Trichophaeopsis bicuspis NSW 8316 #
Trichophaeopsis tetraspora C F-47525
Trichophaeopsis sp. DHP-AR-19
Trichophaeopsis sp. DHP-CH-58
Melastiza contorta KH 01.06
Pseudaleuria quinaultiana p712L
Chaetothiersia vernalis BAP 492 T #
Melastiza cornubiensis KH 03.43
Orbicula parietina CBS 238.32
Pezizomycetes
Lecanoromycetes sp. GLMC 1733 (2)
Uncultured Ascomycota agrFF142
Uncultured Ascomycota SMOTU80
Anthopsis catenata CBS 492.81 T
Anthopsis deltoidea CBS 263.77 T #
Cyanodermella oleoligni CBS 140345 T
Umbilicaria esculenta A6
Lecanoromycetes
Nakazawaea ernobii CBS 1737 T
Nakazawaea holstii CBS 4140 T #
Nakazawaea cf. holstii GLMC 1309 (1)
Nakazawaea populi CBS 7351 T
Nakazawaea pomicola CBS 4242 T
Wickerhamomyces silvicola CBS 1705 T
Wickerhamomyces silvicola GLMC 1708 (1)
Candida peoriensis CBS 8800 T
Wickerhamomyces rabaulensis CBS 6797 T
Wickerhamomyces canadensis CBS 5676 #
Saccharomycetes
Ascomycota
Umbelopsis isabellina CBS 560.63
Umbelopsis isabellina GLMC 521 (5)
Umbelopsis ovata CBS 499.82 T
Umbelopsis versiformis CBS 150.81 T #
Mucor bainieri CBS 293.63 T
Mucor circinelloides f. lusitanicus CBS 108.17 T
Ellisomyces anomalus CBS 243.57 T #
Mucor sp. GLMC 656 (1)
Mucor circinelloides GLMC 1405 (1)
Mucor circinelloides f. circinelloides CBS 195.68 T
Mucor hiemalis GLMC 1395 (1)
Mucor hiemalis f. hiemalis CBS 201.65
Mucor mucedo CBS 640.67 T #
Mucoromycota
Entomophthora sphaerosperma CBS 530.75
0.8

◀ **Fig. 7** Phylogeny of dataset 5 obtained by Bayesian inference analysis of the combined LSU and ITS sequence alignment of miscellaneous *Ascomycota* (*Lecanoromycetes*, *Pezizomycetes*, *Saccharomycetes*), *Basidiomycota* and *Mucoromycota*. *Entomophthora sphaerosperma* strain CBS 530.75 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% are shown at the nodes. The strains isolated in this study are emphasised in bold. Numbers in parentheses indicate the number of isolated strains per taxon. Branches that are crossed by diagonal lines are shortened by 50%. T, ex-type strain; #, type species

non-symptomatic tissue was available due to the large expansion of the necroses and the "symptomless tissue" placed on OA for isolation was very closely located to the necrotic tissue. Biggs et al. (1983) detected hyphae of *Cytospora chrysosperma* up to 2 cm away from xylem tissue of *Populus* with visible necroses caused by this fungus. Therefore, isolation of a pathogenic fungus from nearby symptomless tissue cannot be excluded and a similar fungal diversity and abundance was expected. However, the number of fungi isolated from non-symptomatic tissue was exceptionally low compared to that from the transition zone of symptomatic to non-symptomatic tissue. The fungi isolated solely from symptomless tissue were isolated only once. And none of the few species isolated more often from symptomless than from symptomatic tissue was found more than five times in total. This cannot be explained by the lower subsample number of non-symptomatic wood pieces. We attribute this to the larger number of ecological niches of the wood pieces from the transition zone resulting in a temporal-spatial succession of fungal communities including endophytes, pathogens and saprobionts.

Uncertainties in identifications

Of the 172 species isolated from *Prunus* wood in this study, 102 could be assigned to a particular species with different levels of certainty. The ITS region of many species is highly variable, which decreases the similarity values and results in unjustified uncertainty (Nilsson et al. 2008, Simon and Weiß 2008, Hughes et al. 2009). In contrast, ITS sequences of closely related species can be identical or nearly so, which results in similarity values of up to 100% and therefore unjustified certainty (e.g. Houbraken et al. 2011, Damm et al. 2019). This means, on the one hand, some of the 70 taxa not assigned to a species could possibly be identified to species level by including the whole variability of the ITS sequences of the respective genus. On the other hand, some of the 102 species that were assigned to a particular species with high or low (cf.) certainty, even of those with identical ITS sequences, could represent new species or species with no sequence data in GenBank. This demonstrates how imprecise an identification based on solely ITS data is, even by availability of full-length sequences, careful selecting the reference data and inclusion of the nucleotide differences/identities.

The different inter- and intraspecific variability of ITS sequences is a dilemma of species identification in fungal diversity studies dealing with big and diverse sampling datasets. It is simply not possible to study the variability of each taxon and consider DNA variability of each species while defining a uniform threshold for species differentiation. On the one hand, a rigorous application of strict criteria for species delimitation ignores the variability of different fungal taxa. On the other hand, if no clear criteria are applied, species delimitation is to a certain degree subjective and the different reasons for a specific decision hard to compare.

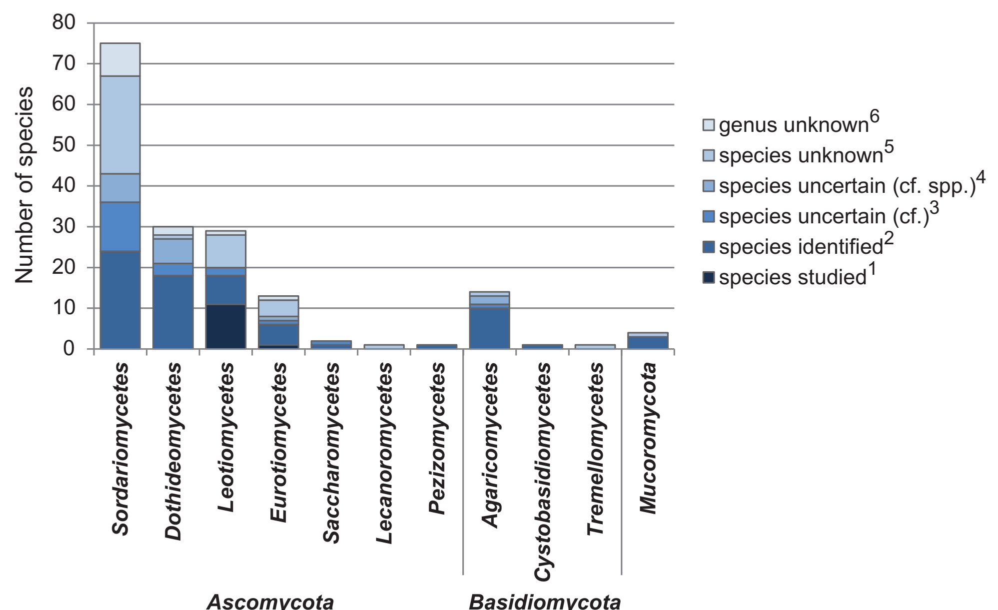


Fig. 8 Number of species in different classes of *Ascomycota*, *Basidiomycota* and *Mucoromycota* detected inside *Prunus* wood in Germany and level of certainty of identification based on ITS/LSU sequence comparisons. [1]Species treated in Bien et al. 2020 or Bien and Damm 2020, [2]ITS 0–4 nucleotide differences to a named reference sequence, [3]ITS 5–10 nucleotide differences to a named reference sequence, [4]ITS 0–4 nucleotide difference to reference sequences of ≥ 2 different species, [5]ITS > 10 nucleotide differences to a named reference sequence, [6]no reference sequence in the same clade

Moreover, blastn searches often not only result in uncertain but more importantly in wrong identifications due to the sequence data in NCBI GenBank, of which many are incomplete, include artefacts, are mixed up or derived from wrongly identified samples and therefore not suitable as reference data (Vilgalys 2003, Nilsson et al. 2006, Bidartondo 2008, Hyde et al. 2010, Ko et al. 2011). Therefore, only sequences of ex-type strains can be reliable references. However, even sequences of ex-type strains can be unreliable, if they are based on sequences with low quality or mixed up with other species, as revealed for example in *Colletotrichum hymenocallidicola* (Damm et al. 2019). Nonetheless, the main drawback of identification based on sequence data (in GenBank) is the limited part of the overall known fungal diversity with available sequence data, especially those from type material. It is possible that the number of species that were not identified to species level and regarded as new species is lower. That means, some of these 70 potentially new species as well as some of those with uncertainty identified species could represent species that had previously been described based on morphology, however, lacking sequence data in GenBank.

Species identified with high certainty

In total, 82 taxa (630 strains) were identified to species level with certainty. All species belonging to the genera *Arboricolonus*, *Cadophora*, *Collophorina*, *Pallidophorina* and *Proliferodiscus* in the *Leotiomycetes* and *Minutiella* in the *Eurotiomycetes* have previously been studied in detail morphologically as well as by multi-locus phylogenetic analyses (Bien et al. 2020, Bien and Damm 2020). Therefore, their identifications are reliable. Three and two species of *Cadophora* and *Collophorina*, respectively, and one species each of *Proliferodiscus* and *Minutiella*, as well as the genus *Arboricolonus* have been described in these two previous studies based on strains isolated from *Prunus* wood in Germany collected within our survey.

Strain GLMC 380 belonging to the *Dothideomycetes* shows that also strains assigned to a species with certainty can actually be of uncertain systematic position. The sequence of this strain (representing further 23 strains) was identical with that of a strain referred to as *Coniothyrium ferrarisianum* (CBS 285.74). Both strains form a clade sister to a clade formed by two ex-type strains of *Sclerostagonospora*. Other strains of *Coniothyrium*, *Co. dolichi*, *Co. glycines* and *Co. telephi*, formed a distant clade also within the *Pleosporales*. There is no DNA sequence of the type species *Co. palmarum* available; the genus *Coniothyrium* is currently regarded as polyphyletic (Verkley et al. 2004, 2014). Therefore, the systematic placement of the genus *Coniothyrium* as well as of the individual species, including *Co. ferrarisianum*, still needs to be clarified.

Species identified with low certainty

In total, 20 taxa (98 strains) were assigned to a species with low certainty, because the ITS sequences differed in 5–10 nucleotides from the closest named reference sequences. These are taxa that need to be studied in depth; species boundaries need to be evaluated, etc. It is possible that part of these taxa represent new species. Even the affiliation of some of the taxa to genus level still needs to be clarified, for example *Anthostomella* cf. *pinea* strain GLMC 451 (see below).

Taxa identified to genus level

In total, 57 taxa (255 strains) were assigned to a genus, but not to a species. Of these, 16 taxa (86 strains) matched with more than one named reference sequence (cf. spp.); these fungi are unlikely to represent new species and can probably be identified to species level based on secondary barcodes. It is also possible that one of the species in the respective clade represents a synonym that has previously not been revealed yet.

The ITS sequences of the other 41 taxa (169 strains) differed in > 10 nucleotides from the closest named reference sequence. Most of these taxa represent new species, unless the species was described only based on morphology and no ITS sequence is available.

Species not identified to genus level

Thirteen species could not be identified to genus level (35 strains), because they did not match with named reference sequences in blastn searches and were placed isolated within the phylogenies (e.g. *Leotiomycetes* sp. GLMC 792, *Lecanoromycetes* sp. GLMC 1733) or because the respective genus is polyphyletic and sequences of the type species are either not available or belong to a different clade within the phylogeny. The 13 taxa were therefore identified to family (six taxa), order (five taxa) or class (two taxa) level only; most of them belong to the *Sordariomycetes*.

Although some of the closest matches in blastn searches with the ITS sequences of strains GLMC 1660 (*Xylariaceae* sp. 1) and GLMC 1594 (*Xylariaceae* sp. 2) were strains previously identified as *Rosellinia* sp., we doubt these taxa belong to this genus, because sequences of ex-type strains of two species and of a strain of the type species, *R. aquila* (Wendt et al. 2018), belong to different clades. Affiliation of the strains isolated in this study to the genus *Rosellinia* cannot be clarified with the data at hand.

Strain GLMC 848 (*Xylariaceae* sp. 3) is placed together with two strains from *Juniperus deppeana* in the USA referred to as *Sordariomycetes* sp. (Hoffman and Arnold 2010). The clade formed by these strains is sister to a clade formed by strain GLMC 451 (*Anthostomella* cf. *pinea*, this study) and the ex-type strain of *Anthostomella pinea* (CBS 128205). However, the genus *Anthostomella* is polyphyletic

(Daranagama et al. 2015), which is confirmed here as the ex-type strain of another species, *An. proteae* (CBS 110127), belongs to a different clade. None of these clades was confirmed to represent the genus *Anthostomella*, because there is no sequence of the type species of the genus, *An. limitata*, available. Therefore, the affinity of both strains, GLMC 848 and GLMC 451, to *Anthostomella* is unclear.

Strain GLMC 1232 (*Sordariales* sp.) groups with a strain referred to as *Cercophora* sp. (CIM1_17, Mapperson and Dearnaley, unpubl. data), an uncultured *Ascomycota* (dfmo0690_036) from soil in the USA (O'Brien et al. 2005) and the ex-type strain of *Zopfiella tardifaciens* (CBS 670.82). A strain of the type species of *Zopfiella*, *Z. tabulata* (CBS 230.78), is placed in a single-strain clade sister to this group. The intergeneric relationships of *Lasiosphaeriaceae* genera including *Zopfiella* and *Cercophora* were described as inconclusive due to the uncertainty about the phylogenetic value of different morphological characters (Cai et al. 2005).

Strain GLMC 1316 (*Pleosporales* sp.) clustered with two strains referred to as *Leptosphaeria* sp. (LCC1-2, Li et al. unpubl.; LQ122417, Qiong et al., unpubl. data) that are distant from a clade formed by strains of three further *Leptosphaeria* species, none of which are ex-type strains. The affiliation of the isolated strain to this genus is therefore doubtful.

The ITS sequence of strain GLMC 1563 (*Lentitheciaceae* sp.) is identical with that of a strain previously identified as *Sclerostagonospora cycadis* (CBS 291.76). Both strains form a clade sister to a clade formed by two ex-type strains of *Murilentithecium* species, including the type species of the genus. As the ex-type strain of *S. cycadis* (CBS 123538) belongs to a different clade within the *Pleosporales*, sister to the ex-type strain of *S. ericae*, strain CBS 291.76 must have been wrongly identified. Both strains are likely to be a *Murilentithecium* species, which needs to be confirmed.

Strain GLMC 792 (*Leotiomycetes* sp.), belonging to the *Leotiomycetes*, grouped with strain 30404-E that had been isolated from wood in Greenland and identified as *Pseudeurotium* sp. (Pedersen et al., unpubl. data). However, the placement in this genus is doubtful, because this clade is distant from the *Pseudeurotium* clade formed by three ex-type strains including the type species of the genus.

This study highlights that a common substrate like wood of fruit trees in Germany actually represents an underexplored habitat and houses a widely unknown mycobiome with widely unknown host spectrum/specificity, distribution, conservation status, life cycle and function and probably large potentials for applications. We expect most of the taxa not assigned to a species and part of the species identified with more or less certainty to represent new species or even new genera. In order to clarify their identity, these species should be treated in depth in further follow-up studies by a polyphasic approach consisting of multilocus sequence analyses and sound morphological examinations.

Availability of data and material The DNA sequences generated in this study were deposited in GenBank (Table 1, suppl. material tab.). The datasets generated and analysed during the current study are available from the TreeBASE website, http://purl.org/phylo/treebase/phylows/study/TB2: S25316.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

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5 General discussion

5.1 Species delimitation and descriptions

During this survey, several taxa have been recognised as new to science by means of thorough morphological and molecular investigations. Eight species were described based on strains isolated from *Prunus* in Germany. A further seven species were described based on additional strains included in the analyses. Additionally, four new genera were discovered (*Arboricolonus*, *Capturomyces*, *Variabilispora*, *Vexillomyces*), while two previously described *Collophorina* species were revealed to belong to new genera (*Pallidophorina*, *Ramoconidiophora*) after recognising this genus to be polyphyletic.

Accurate species delimitation and identification is dependent on a presumed species concept and the underlying species recognition criteria (Taylor et al. 2000, de Queiroz 2007). In the past, species boundaries of fungi have primarily been based on morphological divergence (Morphological Species Recognition, MSR), intersterility (Biological Species Recognition, BSR) or the adaptation to a particular ecological niche (Ecological Species Recognition, ESR; Cai et al. 2011). However, features such as morphology, mating compatibility and host specificity have frequently proven to be inadequate for delineating fungal species (Harrington & Rizzo 1999, Crous et al. 2015). For example, morphological species recognition of microfungi is difficult as the morphology of many species is simple; they do not develop taxonomically informative structures (Wang & Guo 2007, Thomas et al. 2008, Rintoul et al. 2012). Taylor et al. (2000) stated that the MSR tends to lump two or more species and cannot be counted on to distinguish evolutionarily meaningful species of fungi, as it was demonstrated for example with collophorina-like fungi in chapter 2. With few exceptions the species of this group, even if belonging to different genera, are indistinguishable by their morphology. The application of the MSR furthermore fails, if no data on a particular morph are available. For example, *Tympanis* and *Proliferodiscus* species have in the past only been described based on their sexual morphs. Strains of these species isolated in this study (chapters 2 and 3) only produced the asexual morphs, which therefore did not allow morphological comparison. The BSR has been used to identify groups of compatible individuals in mating tests, which cannot be applied to fungi, for which a sexual morph is not known or that are homothallic and produce meiospores in absence of a partner (Taylor et al. 2000). For example, no sexual morph is known for the genera treated in chapter 2, except for the genus *Tympanis*. Another limitation of this concept is that sexual morphs of heterothallic fungi sometimes cannot be induced in culture or retain interspecific interbreeding as ancestral character (Zervakis et al. 2004, Dettman et al. 2008, Damm et al. 2012). In plant pathogenic fungi the ESR has been widely used in the past (Rossman & Palm-Hernández 2008, Cai et al. 2011, Vialle et al. 2013). However, with only single or few strains isolated from a limited selection of plant

hosts, the data set obtained from the genera treated in detail is not sufficient to include the ESR into consideration for species delimitation. Additionally, with the advances in molecular systematics, the approach to base taxonomic conclusions solely on ESR is now considered to be obsolete (Crous et al. 2015).

In recent years, molecular systematics, based for example on the Phylogenetic Species Concept (PSC), has been employed commonly by mycologists for species delimitation (Millanes et al. 2014, Crous et al. 2015). According to the PSC, species are the smallest monophyletic unit, generally defined as a group of organisms that includes the common ancestor and all of its descendants (Baum & Donoghue 1995, Rintoul et al. 2012). The concept of Genealogical Concordance Phylogenetic Species Recognition (GCPSR), an adaptation of the PSC, uses the phylogenetic concordance of unlinked genes to indicate a lack of genetic exchange and thus, evolutionary independence of lineages (Taylor et al. 2000). Phylogenetic gene topographies of multiple genes are compared, and the presence of separate species is supported by the concordance of branches, whereas the intersection of conflicting topographies indicates gene flow among individuals below the level of species (Taylor et al. 2000). This tool has proven valuable for species delimitation in fungi (de Jong et al. 2001, Wedin et al. 2004, Jargeat et al. 2010, Brown et al. 2013, Vialle et al. 2013, Damm et al. 2019). The concept has been considered as more accurate and convenient than MSR or BSR, especially for asexual fungi (Giraud et al. 2008, Brown et al. 2013, Vialle et al. 2013, Dai et al. 2015), like those analysed in chapter 2 and 3 of this thesis. The decisions for recognition of novel species in this study were primarily based on DNA sequence data and phylogenetic analyses applying the GCPSR. All further available data was included in the decision process, e.g. morphological features.

For all taxa treated in detail, the attempt was to include DNA sequences of as many loci as possible. The number of different loci, however, is dependent on the availability of the same loci from reference strains provided in public sequence databases. For instance, within the species level analyses of collophorina-like fungi four (LSU, ITS, EF-1α, GAPDH) and of *Cadophora* (ITS, TUB, EF-1α) and *Minutiella* (LSU, ITS, TUB) three different loci, respectively, could be included for a wide range of reference strains. In contrast, the family-wide analyses of collophorina-like fungi as well as the analyses of species of *Proliferodiscus* were restricted to ITS and LSU simply because no data of further loci were available from reference strains. In all these analyses, every new species recognised showed considerable nucleotide differences in the treated loci compared to closely related species, resulting in well-supported monophyletic clades in all single-gene trees as well as concordant topographies. The GCPSR provided clear evidence for delimitation on species as well as on genus level.

In fungal taxonomy, it is widely accepted good practice to include morphological, molecular and host-related/collection data and, if applicable, also data of secondary metabolites and pathogenicity into the decision process (Frisvad et al. 2008, Cai et al. 2009, Wittstein et al. 2020). This approach is referred to as polyphasic or

polythetic or integrative taxonomy. However, integration of large data sets into the taxonomic decision process is limited by the amount of data available in many fungal groups. For many fungal species only single strains or specimens are available (Begerow et al. 2010), which applies to several species newly described in this study (*Arboricolonus simplex*, *Cadophora africana*, *Capturomyces luteus*, *Proliferodiscus ingens*, *Tympanis inflata*, *Variabilispora flava*, *Vexillomyces palatinus*).

Rossman & Palm-Hernández (2008) advocated documentation of every research involving fungal pathogens with voucher specimens and living strains. Provision of all data and material gained within this survey allows later verification of every taxonomic decision made with more information, especially on hosts, substrates, geographic distribution, pathogenicity and secondary metabolites, available in the future.

5.2 Comparison of the fungal diversity in *Prunus* wood

When comparing the fungal diversity detected in this study with the fungal species previously reported from above ground woody tissue of *Prunus* species listed in chapter 1 (table 1), only a small overlap can be found. In this study, 102 species were identified to species level, while 118 species from living and/or symptomatic wood of *Prunus* and identified to species level were previously reported. Only 18 of them were overlapping (table 2). Looking at host species level, the overlap is even smaller. Most of the overlapping fungal species are known for their wide host range and geographical distribution (e.g. *Aureobasidium pullulans*, *Botrytis cinerea*, *Eutypa lata*; Rolshausen et al. 2004, Fournier et al. 2005, Zalar et al. 2008), which explains their presence in wood of *Prunus* spp. hosts in different surveys.

Of the 71 genera previously reported from *Prunus* wood, 32 genera were also detected in this study. Since many of the taxa isolated in this study could not be identified to species level with high certainty or only to genus, family, order or class level, additional concordances on species level could be revealed with further detailed analyses of the isolated species.

Apart from the incomplete list of species in table 1, the comparison between different studies has several limitations especially regarding the identification. The taxa in some of the previous studies were only identified based on morphology, including that of sexual morphs, leading to the possibility of wrong identifications in previous studies. Furthermore, the systematics used for identifications in the cited studies could have been revised in the meantime. Most of the taxa in this study were identified based on ITS-LSU sequences, only part of them in-depth, and sexual morphs that are usually formed only in nature, e.g. on the bark of the trees, were not available for comparison. Hence, species that have in the past only been treated by means of morphology and for which no DNA sequence data of the two loci is available could not be identified using the applied methodology.

Nevertheless, differences on species level are exceptionally conspicuous in certain taxonomic groups. Since high abundances of the *Botryosphaeriales* species have

Table 2. List of fungal species previously reported from wood of *Prunus* species and detected in this study

Fungal species	*Prunus* hosts in previous studies[1]	*Prunus* hosts in this study[1]
Aureobasidium pullulans	ar av ce du pe	av ce **do**
Botrytis cinerea	ce	**do**
Calosphaeria pulchella	av du ma pe	av **ce**
Collophorina africana	du sa	**do**
Dermea cerasi	av ce se	av
Diaporthe eres	N/A	**ce**
Diplodia mutila	ar sa	**do**
Diplodia seriata	ar du la pe pn sa	**do**
Eutypa lata	ar av du sa	**ce do**
Eutypa petrakii var. *petrakii*	sp	**do**
Hypoxylon fuscum	pa	**do**
Monilinia laxa	ar ce do pe	do
Pallidophorina paarla	av ce pe sa	av ce **do**
Phaeoacremonium iranianum	ar du	**do**
Phaeoacremonium scolyti	ar pe pn sa	**do**
Phaeoacremonium viticola	sa	**ce do**
Trametes versicolor	av	av **do**
Truncatella angustata	N/A	**ce do**

[1] ar: *Prunus armeniacae*; av: *P. avium*; ce: *P. cerasus*; do: *P. domestica*; du: *P. dulcis*; la: *P. laurocerasus*; ma: *P. mahaleb*; pa: *P. padus*; pe: *P. persica*; pn: *P. persica* var. *nucipersica*; sa: *P. salicina*; se: *P. serotina*; sp: *P. spinosa*; N/A: no information available; new host species are indicated in bold.

been detected in different studies on *Prunus* in China, North America, South Africa and Spain (Damm et al. 2007a, b, Inderbitzin et al. 2010, Gramaje et al. 2012, Tian et al. 2018), similar results were expected in Germany as well. However, the diversity and abundance of species of this order was very low, which has been discussed in chapter 4. Many species of *Phaeoacremonium* have been reported from different species of *Prunus*, especially in the study from South Africa by Damm et al. (2008), who reported 14 different species. In contrast, only four *Phaeoacremonium* species have been found in this study, three of them were overlapping with the study from South Africa, namely *Ph. iranianum*, *Ph. scolyti* and *Ph. viticola* (the latter identified with low certainty).

When comparing the different studies, several reasons for the observed incongruences become apparent. Probably one of the main reasons, already

mentioned in chapter 1, is the focus on specific taxonomic groups in different studies, while further detected species were not reported. Other possible reasons are differences between studies regarding the culturing technique, like culture media or culturing conditions (e.g. light, temperature), which decreases the detection of fungi with specific nutrient or environmental requirements. As discussed in chapter 2, collophorina-like fungi might have been overlooked or even ignored in studies prior to the study of Damm et al. (2010) due to their inconspicuous or yeast-like appearance. Additionally, slow-growing fungi might have been overlooked simply because they were overgrown by fast-growing fungi.

The species assembly of a particular habitat can be influenced by functional similarity and competition between the fungal species present, and therefore change over time (Halley et al. 1994, Maherali & Klironomos 2007). Certain fungal species might not have been recognised in particular studies including this one, because sampling took place at a certain point within community succession before or after the respective fungi are present (Peay et al. 2008).

Furthermore, the incongruences of identified species between this and the cited studies could be explained by a sampling bias. The three target host species of this study *P. avium*, *P. cerasus* and *P. domestica*, as well as the geographical region, Central Europe, were underrepresented in previous studies (see table 1). Additionally, some of the studies cited in table 1 focused on healthy rather than symptomatic wood or even remain unspecific about the sampled tissue. These differences between studies indicate influences of geography, climate, host specificity and lifestyle on the species composition inside the wood of *Prunus* species. Tedersoo et al. (2014) concluded that climate might be a main driver of fungal diversity, especially for soil inhabiting fungi. Wollan et al. (2008) found that temperature is a main predictor for the distribution of macrofungi. Jansa et al. (2014) showed the influence of geographic properties such as altitude and geographic distance on mycorrhizal fungi in agricultural soils. Even in species compositions of indoor fungi geographic patterns have been recognised (Amend et al. 2010). Ranelli et al. (2015) showed that host specificity has a great influence on patterns of fungal colonisation of perennial grasses.

The observed incongruences illustrate the high variability and lack of knowledge about fungal communities in *Prunus* wood worldwide, especially as many of the species in table 1 were newly described within the respective studies (e.g. Damm et al. 2008, 2010, Gramaje et al. 2012). The detection of fungal species from other *Prunus* species or from other countries that were missing in this study might indicate that these species are not (or not yet) present on the three examined host species and/or in Germany. The differences observed between geographic regions emphasise the importance of quarantine measures.

The orchards sampled in this study are located in different regions of Germany. However, comparisons of results from the different sampling regions should be handled with care as they can lead to wrong conclusions. In an experimental approach, the sites would need to be uniform in respect of e.g. host species and

cultivars, plant age, planting pattern, soil type etc., in order to allow comparisons (Bruelheide et al. 2014). However, there is no long-term field experiment with these parameters available for *Prunus*. Therefore, the specimens were collected from commercial orchards, which were not uniform regarding *Prunus* species, cultivar, etc., but reflect the variability of these parameters in Germany.

In order to answer ecological questions regarding the fungal species composition, methodologies generating big data sets in a comparatively short time frame, such as high-throughput sequencing (HTS) methods, are better suited than the time-consuming isolation procedure applied in this study. Using such methods, further factors can be measured and integrated in the analyses, as it has been done for example for the evaluation of different factors influencing the diversity of soil inhabiting organisms (Tedersoo et al. 2016). Sampling could be expanded over larger geographic area such as the continent or the global scale. HTS methods allow the comparison of large data sets, but neither the unambiguous identification of the individual species, nor their extended study or even application as discussed in chapter 4.

As the general purpose of this study was to reveal the current fungal diversity of symptomatic *Prunus* wood in Germany and to identify the species as accurate as possible, culturing of the fungi was a precondition.

5.3 General remarks on the diversity assessment and taxonomic studies

Considering the insufficient knowledge about the biodiversity of fungi, the research of fungal communities and the systematic study of fungal species with molecular methods are of uttermost importance. Estimates of fungal species living on earth range between half a million and 13.2 million species (Hawksworth 1991, 2001, O'Brien et al. 2005, Blackwell 2011, Mora et al. 2011, Hawksworth & Lücking 2017, Wu et al. 2019). However, the number of described species only encompasses around 120,000 species including synonyms (Hawksworth & Lücking 2017), and therefore represents only a fraction of the total fungal species diversity on earth. Part of the unknown fungal diversity is expected to be found in underexplored habitats, which does not necessarily mean exotic and understudied regions of the world, such as the tropics. Hawksworth & Rossman (1997) concluded that enormous numbers of unrecognised fungi could be found almost everywhere, including "one's own backyard". The results of this study, including seven newly described species, one newly described genus and a large number of further potentially new species detected in wood of common *Prunus* species in Germany confirm this assumption in an impressive manner. Furthermore, the fact that many species were isolated only rarely and probably by chance indicates that there are likely to be even more known and unknown species hidden in this habitat.

The taxonomic studies of the selected genera that were analysed in detail using molecular methods revealed new insights in all of them. Moreover, still existing knowledge gaps were uncovered. For example, the available data set of DNA

sequence data needs to be extended. For most of the *Proliferodiscus* and some of the *Tympanis* species, there is no sequence data available at all; for some species of the two genera sequences of only one or two loci are available, most of them are not from type material. In *Proliferodiscus*, sequences of only one ex-type strain were available prior this study. The example *Tympanis* illustrates that without molecular examination, polyphyly might remain undiscovered. The family-wide analyses of *Tympanidaceae* revealed most *Tympanis* strains to form a monophyletic clade, while some strains, including one ex-type strain (*T. pseudotsugae*), not to belong to this clade, some not even to the same class.

In the past many fungal genera were taxonomically treated only based on their sexual morph formed on the natural substrate rather than in culture, including many *Helotiales* like *Proliferodiscus* and *Tympanis*. For many species no living strains are available. This hinders comparisons with states formed in culture, especially asexual morphs, and the sequencing of different loci, which is necessary to clarify the phylogenetic placement of the species. For this reason the species need to be epitypified, which requires sampling of new material from the original locations and hosts. Furthermore, type specimens of many species are not available because they are lost or destroyed. In such cases, neotypification is needed to facilitate taxonomic evaluations of the respective species as well as taxa on higher rank (Hyde & Zhang 2008, Ariyawansa et al. 2014). Moreover, for many genera, including *Proliferodiscus* and *Tympanis*, the application of the generic name need to be fixed by clarifying the systematic position of their type species by epi- or neotypification as promoted in the project Genera of Fungi (Crous et al. 2014).

In contrast, type specimens with living cultures are available for most of the species in the other genera treated in detail in this study. For collophorina-like fungi, the genus *Minutiella* and many further species of the *Phaeomoniellales*, as well as many *Cadophora* species, data of several taxonomically informative loci are available. However, many questions remain unanswered in these genera as well. The results of chapter 2 and 3 indicate that such morphologically inconspicuous fungi are far more diverse and abundant than previously assumed. This is underlined by the fact that since the more or less recent discovery of these genera the number of species and reports increased quickly. After the data presented in chapter 2 and 3 have been published, further new species of the examined genera/families have been discovered, for example, six new species within the *Phaeomoniellales* isolated from spore traps, including an additional new species of *Minutiella* (Kraus et al. 2020), and at least seven new *Cadophora* species from roots of *Microthlaspi* species (Macia-Vicente et al. under review).

However, many aspects concerning the biology of the fungi from *Prunus* wood, especially those inconspicuous fungi, remain unclear, for example, their worldwide distribution, host-specificity, exact function within plant-fungus interactions and their ability to inhabit further substrates than woody tissue.

5.4 Outlook

The list of fungal species detected in this study gives a status report of the fungal diversity of *Prunus* trees in German fruit production areas. The data set obtained can serve as a basis for subsequent monitoring. Provision of morphological and DNA sequence data as well as living strains, especially of those genera analysed in-depth, allows quick recognition in subsequent surveys and facilitates further research on the studied species and systematic studies of the respective taxonomic ranks above (Hawksworth 2004).

Only a selection of taxa has been examined in detail (chapters 2 and 3) regarding morphology and phylogeny. Many more in-depth studies as well as further experiments, for example, pathogenicity tests and the study of secondary metabolites, are planned. Some of these experiments have already started: the first strains of collophorina-like fungi, isolated in this study, were already sent to the Julius Kühn Institute for Breeding Research on Fruit Crops in Dresden-Pillnitz, where they are tested for their pathogenicity to *Prunus* species grown in Germany.

5.5 References

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Statement on the personal contribution to the publications

Manuscript I

Bien, S., Kraus, C., Damm, U. (2020). Novel collophorina-like genera and species from *Prunus* trees and vineyards in Germany. Persoonia, 45, 46–67.

Material-collection	Steffen Bien (95%), Christian Kraus (5%)
Lab-work	Steffen Bien (55%), Michaela Schwager, Samantha Kühnel, Kristin Baber (40%), Christian Kraus (5%)
Analyses	Steffen Bien (100%)
Writing	Steffen Bien (80%), Ulrike Damm (20%)

Manuscript II

Bien, S., Damm, U. (2020). *Arboricolonus simplex* gen. et sp. nov. and novelties in *Cadophora*, *Minutiella* and *Proliferodiscus* from *Prunus* wood in Germany. MycoKeys, 63, 119–161.

Material-collection	Steffen Bien (95%), Ulrike Damm (5%)
Lab-work	Steffen Bien (55%), Michaela Schwager, Samantha Kühnel, Kristin Baber (40%), Ulrike Damm (5%)
Analyses	Steffen Bien (100%)
Writing	Steffen Bien (80%), Ulrike Damm (20%)

Manuscript III

Bien, S., Damm, U. (2020). *Prunus* trees in Germany – a hideout of unknown fungi? Mycological Progress, 19, 667–690.

Material-collection	Steffen Bien (100%)
Lab-work	Steffen Bien (60%), Michaela Schwager, Samantha Kühnel, Kristin Baber (40%)
Analyses	Steffen Bien (100%)
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06/10/ – 11/10/2018	DGfM Internationale Tagung 2018 Möhnesee, in Möhnesee, Germany
28/08/ – 01/09/2017	CBS Symposium 2017 at the Westerdijk Fungal Biodiversity Institute in Utrecht, The Netherlands
09/07/ – 10/07/2017	GBOL working group meeting in Braunschweig, Germany
03/07/ – 07/07/2017	10th International Workshop on Grapevine Trunk Diseases in Reims, France
09/09/ – 16/09/2016	DGfM Wolfgang-Beyer-Gedächtnis-Tagung and GBOL working group meeting in Bernried, Germany
02/06/ – 03/06/2016	GBOL working group meeting in Bochum, Germany

Publications

- **Bien, S.**, Damm, U. (2020). *Arboricolonus simplex* gen. et sp. nov. and novelties in *Cadophora*, *Minutiella* and *Proliferodiscus* from *Prunus* wood in Germany. MycoKeys, 63, 119–161.

- **Bien, S.**, Damm, U. (2020). *Prunus* trees in Germany – a hideout of unknown fungi? Mycological Progress, 19, 667–690.

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- Nasr, S., **Bien, S.**, Soudi, M. R., Alimadadi, N., Fazeli, S. A. S., Damm, U. (2018). Novel *Collophorina* and *Coniochaeta* species from *Euphorbia polycaulis*, an endemic plant in Iran. Mycological Progress, 17(6), 755–771.

Eidesstattliche Erklärung

Hiermit erkläre ich, dass die Arbeit mit dem Titel **"The mycobiome of symptomatic wood of *Prunus* trees in Germany"** bisher weder der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.
Ferner erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht. Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Ort, Datum, Unterschrift

Verteidigung der Dissertation am:

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