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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*

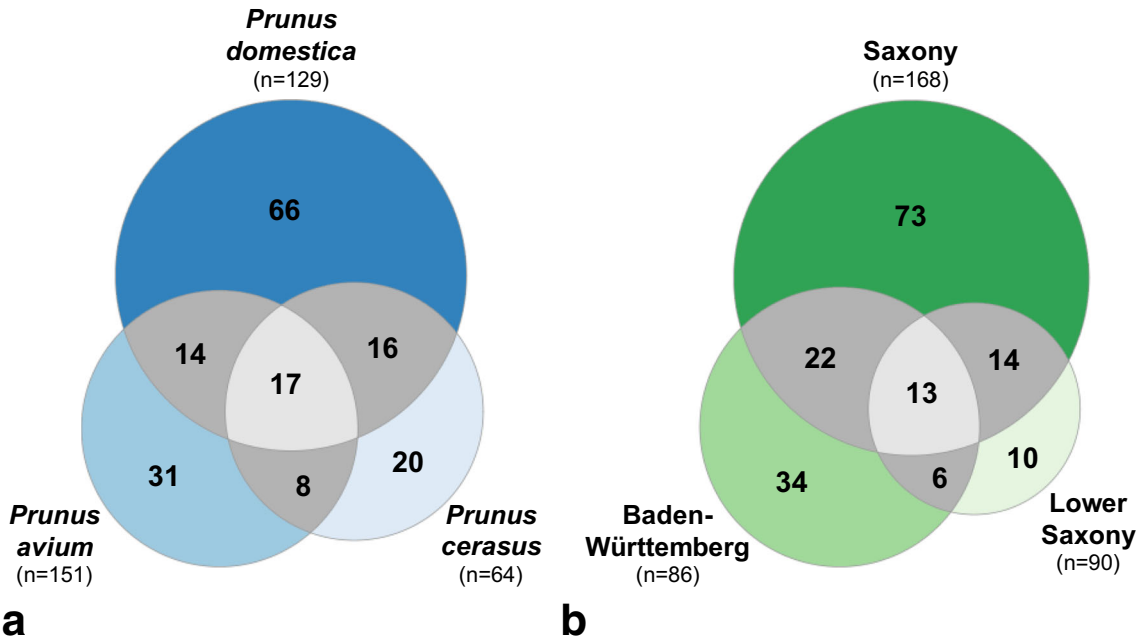


Fig. 1 Number of species isolated from *Prunus* wood in Germany **a** per host species and **b** per sampling region. *n*, number of sampled branches

(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,

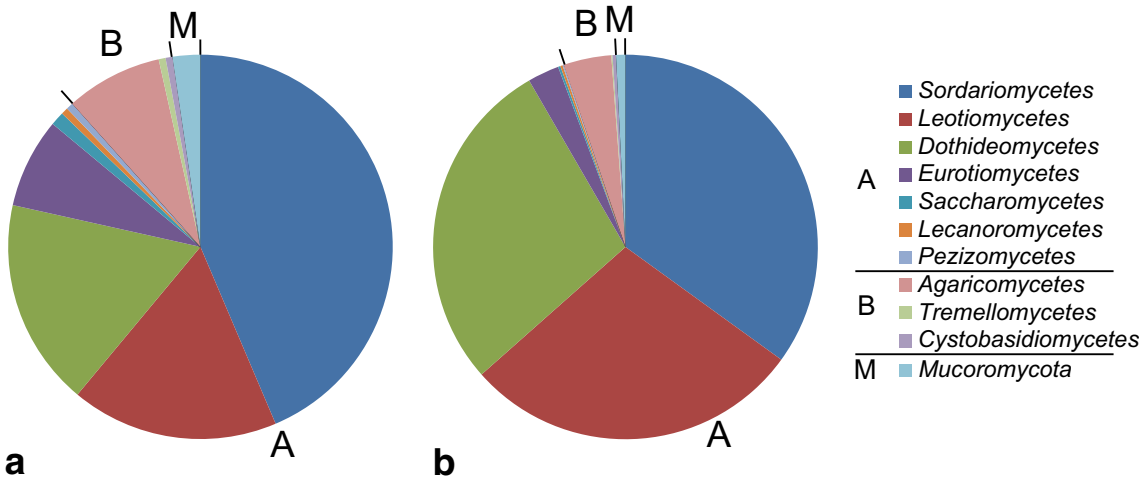


Fig. 2 Percentage **a** of taxa per class and phylum and **b** of strains per class and phylum isolated from *Prunus* wood in Germany. A, *Ascomycota*; B, *Basidiomycota*; M, *Mucoromycota*

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

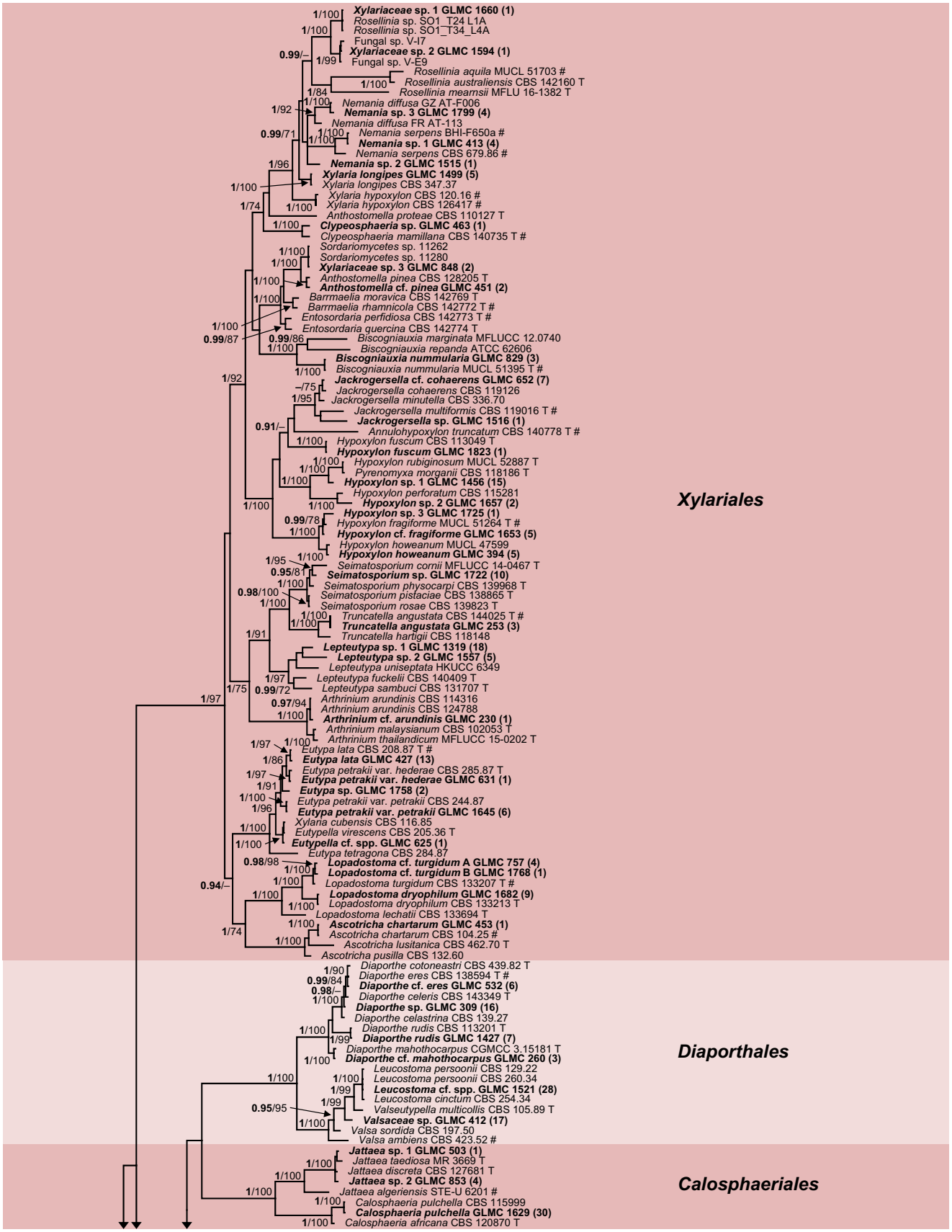
In total, 102 taxa were assigned to a particular species with high (82 taxa) or low (20 taxa) certainty. A further 57 species were determined to genus level. Thirteen species could not be assigned to any genus and were identified to family (six), order (five) or class (two), level, respectively. Almost 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


Xylariaceae sp. 1 GLMC 1660 (1)
Rosellinia sp. SO1_T24 L1A
Rosellinia sp. SO1_T34_L4A
Fungal sp. V-I7
Xylariaceae sp. 2 GLMC 1594 (1)
Fungal sp. V-E9
Rosellinia aquila MUCL 51703 #
Rosellinia australiensis CBS 142160 T
Rosellinia mearnsii MFLU 16-1382 T
Nemania diffusa GZ AT-F006
Nemania sp. 3 GLMC 1799 (4)
Nemania diffusa FR AT-113
Nemania serpens BHI-F650a #
Nemania sp. 1 GLMC 413 (4)
Nemania serpens CBS 679.86 #
Nemania sp. 2 GLMC 1515 (1)
Xylaria longipes GLMC 1499 (5)
Xylaria longipes CBS 347.37
Xylaria hypoxylon CBS 120.16 #
Xylaria hypoxylon CBS 126417 #
Anthostomella proteae CBS 110127 T
Clypeosphaeria sp. GLMC 463 (1)
Clypeosphaeria mamillana CBS 140735 T #
Sordariomycetes sp. 11262
Sordariomycetes sp. 11280
Xylariaceae sp. 3 GLMC 848 (2)
Anthostomella pinea CBS 128205 T
Anthostomella cf. pinea GLMC 451 (2)
Barrmaelia moravica CBS 142769 T
Barrmaelia rhamnicola CBS 142772 T #
Entosordaria perfidiosa CBS 142773 T #
Entosordaria quercina CBS 142774 T
Biscogniauxia marginata MFLUCC 12.0740
Biscogniauxia repanda ATCC 62606
Biscogniauxia nummularia GLMC 829 (3)
Biscogniauxia nummularia MUCL 51395 T #
Jackrogersella cf. cohaerens GLMC 652 (7)
Jackrogersella cohaerens CBS 119126
Jackrogersella minutella CBS 336.70
Jackrogersella multiformis CBS 119016 T #
Jackrogersella sp. GLMC 1516 (1)
Annulohypoxylon truncatum CBS 140778 T #
Hypoxylon fuscum CBS 113049 T
Hypoxylon fuscum GLMC 1823 (1)
Hypoxylon rubiginosum MUCL 52887 T
Pyrenomyxa morganii CBS 118186 T
Hypoxylon sp. 1 GLMC 1456 (15)
Hypoxylon perforatum CBS 115281
Hypoxylon sp. 2 GLMC 1657 (2)
Hypoxylon sp. 3 GLMC 1725 (1)
Hypoxylon fragiforme MUCL 51264 T #
Hypoxylon cf. fragiforme GLMC 1653 (5)
Hypoxylon howeanum MUCL 47599
Hypoxylon howeanum GLMC 394 (5)
Seimatosporium cornii MFLUCC 14-0467 T
Seimatosporium sp. GLMC 1722 (10)
Seimatosporium physocarpi CBS 139968 T
Seimatosporium pistaciae CBS 138865 T
Seimatosporium rosae CBS 139823 T
Truncatella angustata CBS 144025 T #
Truncatella angustata GLMC 253 (3)
Truncatella hartigii CBS 118148
Lepteutypa sp. 1 GLMC 1319 (18)
Lepteutypa sp. 2 GLMC 1557 (5)
Lepteutypa uniseptata HKUCC 6349
Lepteutypa fuckelii CBS 140409 T
Lepteutypa sambuci CBS 131707 T
Arthrinium arundinis CBS 114316
Arthrinium arundinis CBS 124788
Arthrinium cf. arundinis GLMC 230 (1)
Arthrinium malaysianum CBS 102053 T
Arthrinium thailandicum MFLUCC 15-0202 T
Eutypa lata CBS 208.87 T #
Eutypa lata GLMC 427 (13)
Eutypa petrakii var. hederae CBS 285.87 T
Eutypa petrakii var. hederae GLMC 631 (1)
Eutypa sp. GLMC 1758 (2)
Eutypa petrakii var. petrakii CBS 244.87
Eutypa petrakii var. petrakii GLMC 1645 (6)
Xylaria cubensis CBS 116.85
Eutypella virescens CBS 205.36 T
Eutypella cf. spp. GLMC 625 (1)
Eutypa tetragona CBS 284.87
Lopadostoma cf. turgidum A GLMC 757 (4)
Lopadostoma cf. turgidum B GLMC 1768 (1)
Lopadostoma turgidum CBS 133207 T #
Lopadostoma dryophilum GLMC 1682 (9)
Lopadostoma dryophilum CBS 133213 T
Lopadostoma lechatii CBS 133694 T
Ascotricha chartarum GLMC 453 (1)
Ascotricha chartarum CBS 104.25 #
Ascotricha lusitanica CBS 462.70 T
Ascotricha pusilla CBS 132.60
Diaporthe cotoneastri CBS 439.82 T
Diaporthe eres CBS 138594 T #
Diaporthe cf. eres GLMC 532 (6)
Diaporthe celeris CBS 143349 T
Diaporthe sp. GLMC 309 (16)
Diaporthe celastrina CBS 139.27
Diaporthe rudis CBS 113201 T
Diaporthe rudis GLMC 1427 (7)
Diaporthe mahothocarpus CGMCC 3.15181 T
Diaporthe cf. mahothocarpus GLMC 260 (3)
Leucostoma persoonii CBS 129.22
Leucostoma persoonii CBS 260.34
Leucostoma cf. spp. GLMC 1521 (28)
Leucostoma cinctum CBS 254.34
Valseutypella multicollis CBS 105.89 T
Valsaceae sp. GLMC 412 (17)
Valsa sordida CBS 197.50
Valsa ambiens CBS 423.52 #
Jattaea sp. 1 GLMC 503 (1)
Jattaea taediosa MR 3669 T
Jattaea discreta CBS 127681 T
Jattaea sp. 2 GLMC 853 (4)
Jattaea algeriensis STE-U 6201 #
Calosphaeria pulchella CBS 115999
Calosphaeria pulchella GLMC 1629 (30)
Calosphaeria africana CBS 120870 T
Xylariales
Diaporthales
Calosphaeriales

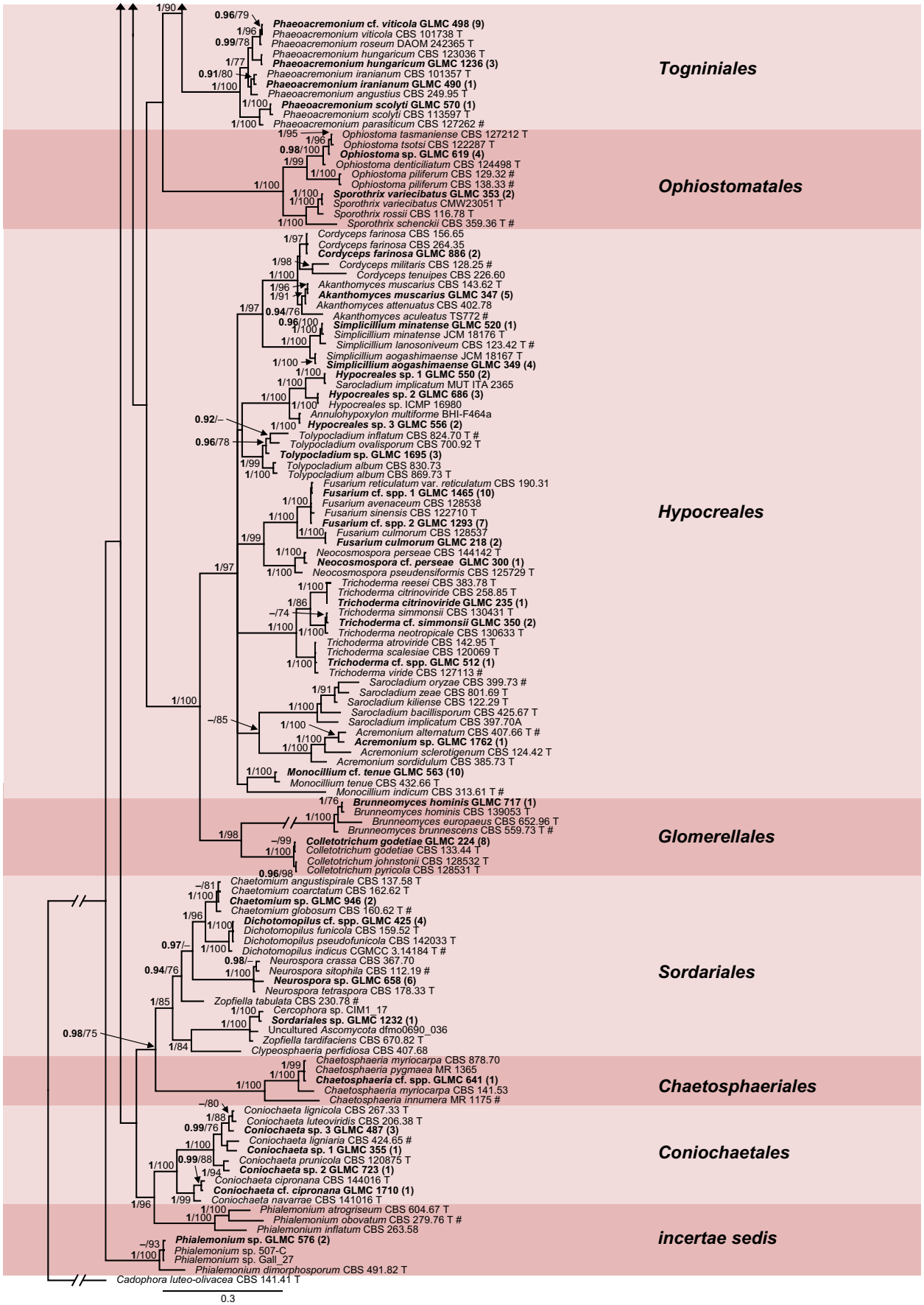


Fig. 3 (continued)

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).

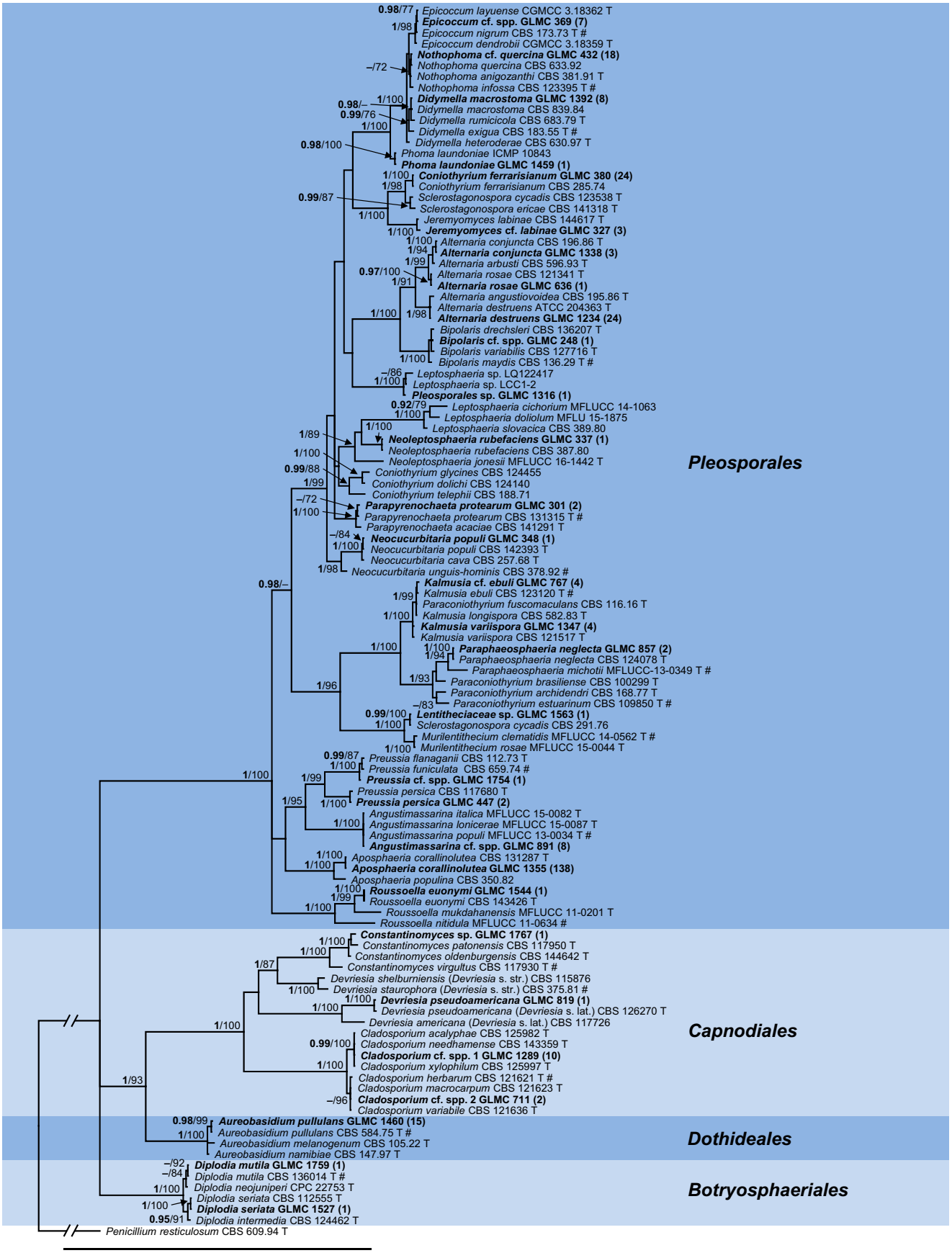
Part of the taxa isolated in this study probably represent first reports for the genus *Prunus*, for specific *Prunus* species or for Germany. We conducted a search of the 82 taxa identified to species level with high certainty on the USDA database (Farr and Rossman 2019). For 41 of these taxa, no previous 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 #
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
Aureobasidium pullulans GLMC 1460 (15)
Aureobasidium pullulans CBS 584.75 T #
Aureobasidium melanogenum CBS 105.22 T
Aureobasidium namibiae CBS 147.97 T
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
Penicillium resticulosum CBS 609.94 T
Pleosporales
Capnodiales
Dothideales
Botryosphaeriales
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

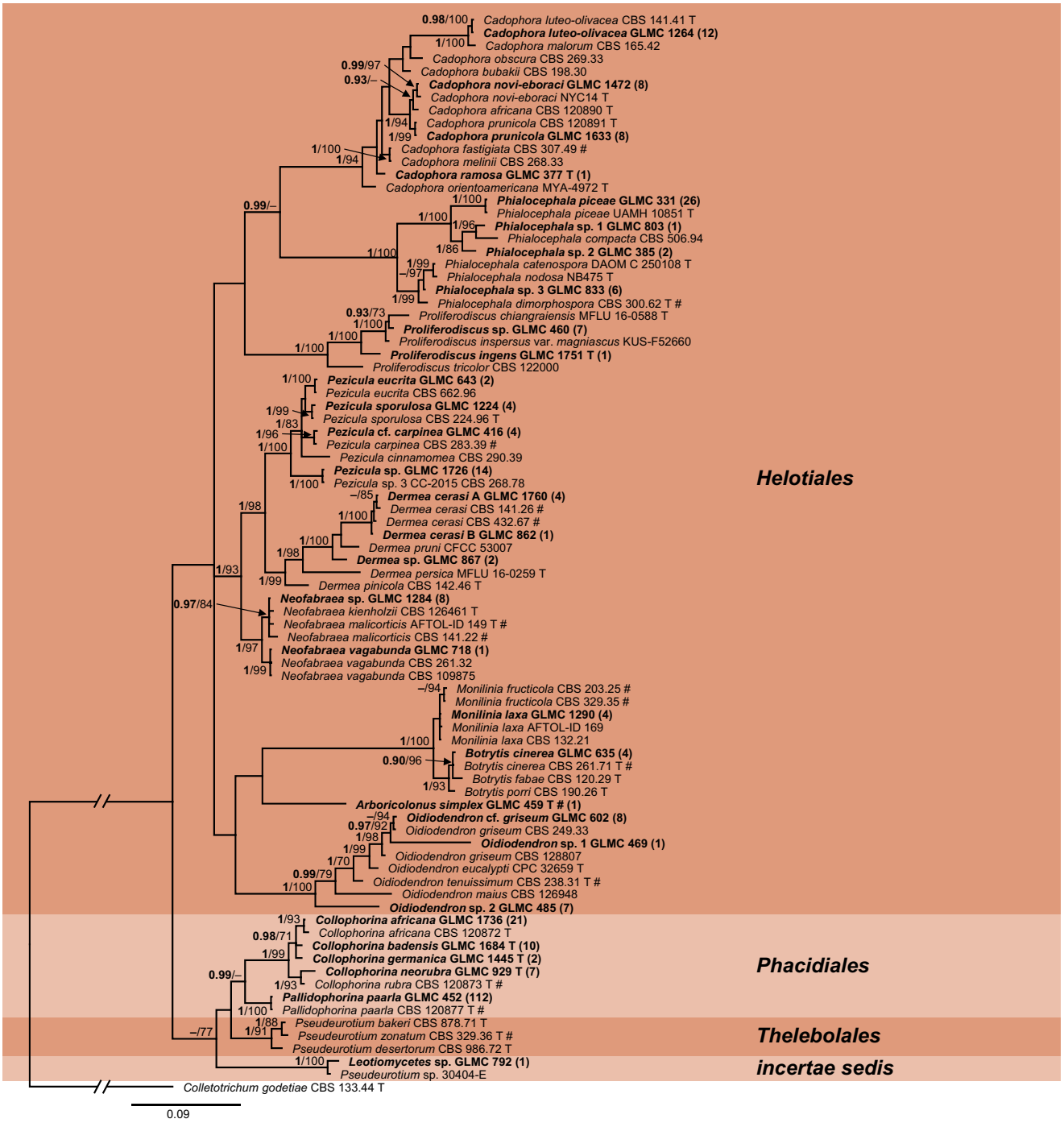


Fig. 5 Phylogeny of dataset 3 obtained by Bayesian inference analysis of the combined LSU and ITS sequence alignment of *Leotiomycetes*. *Colletotrichum godetiae* strain CBS 133.44 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% 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-

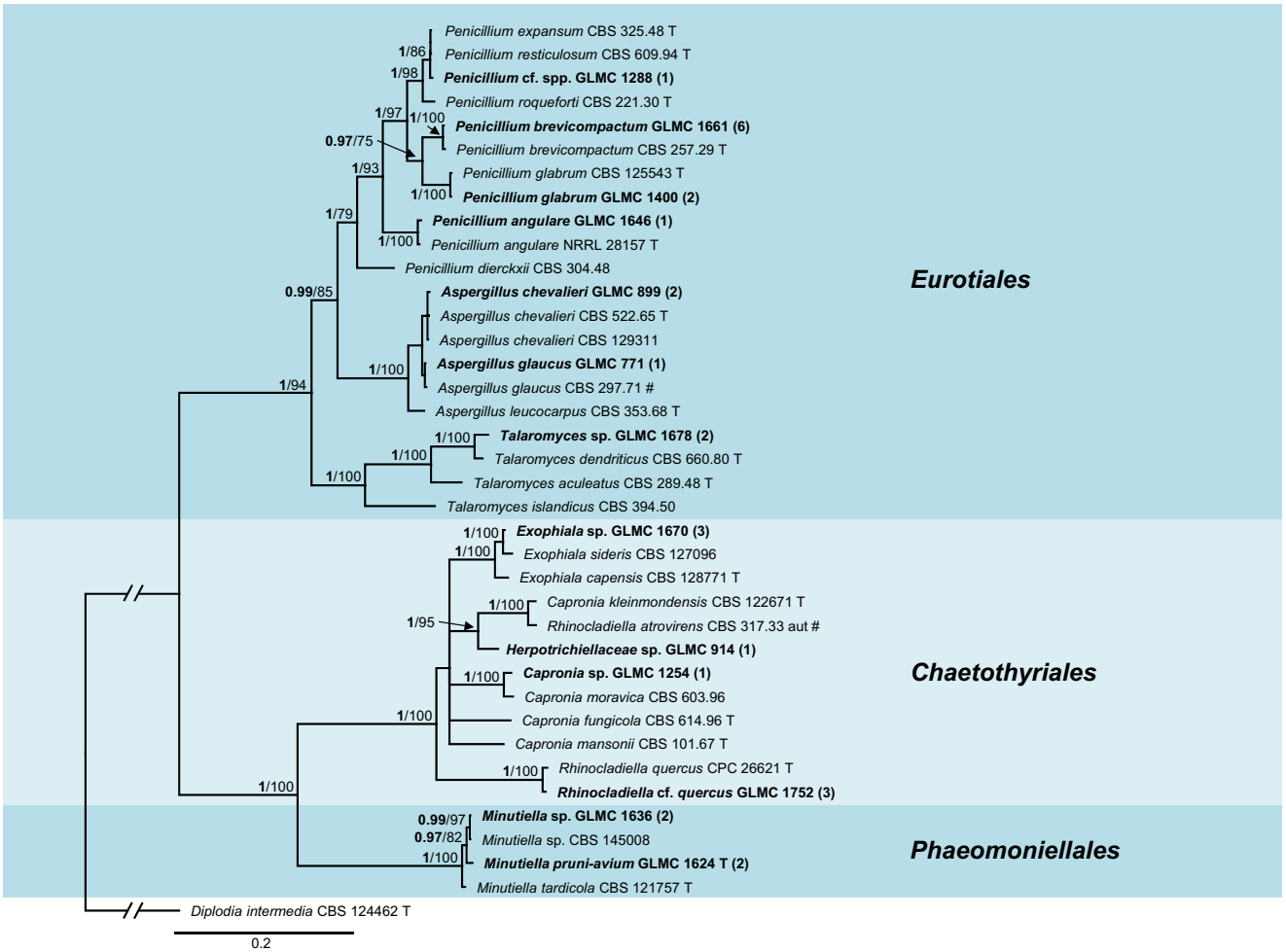
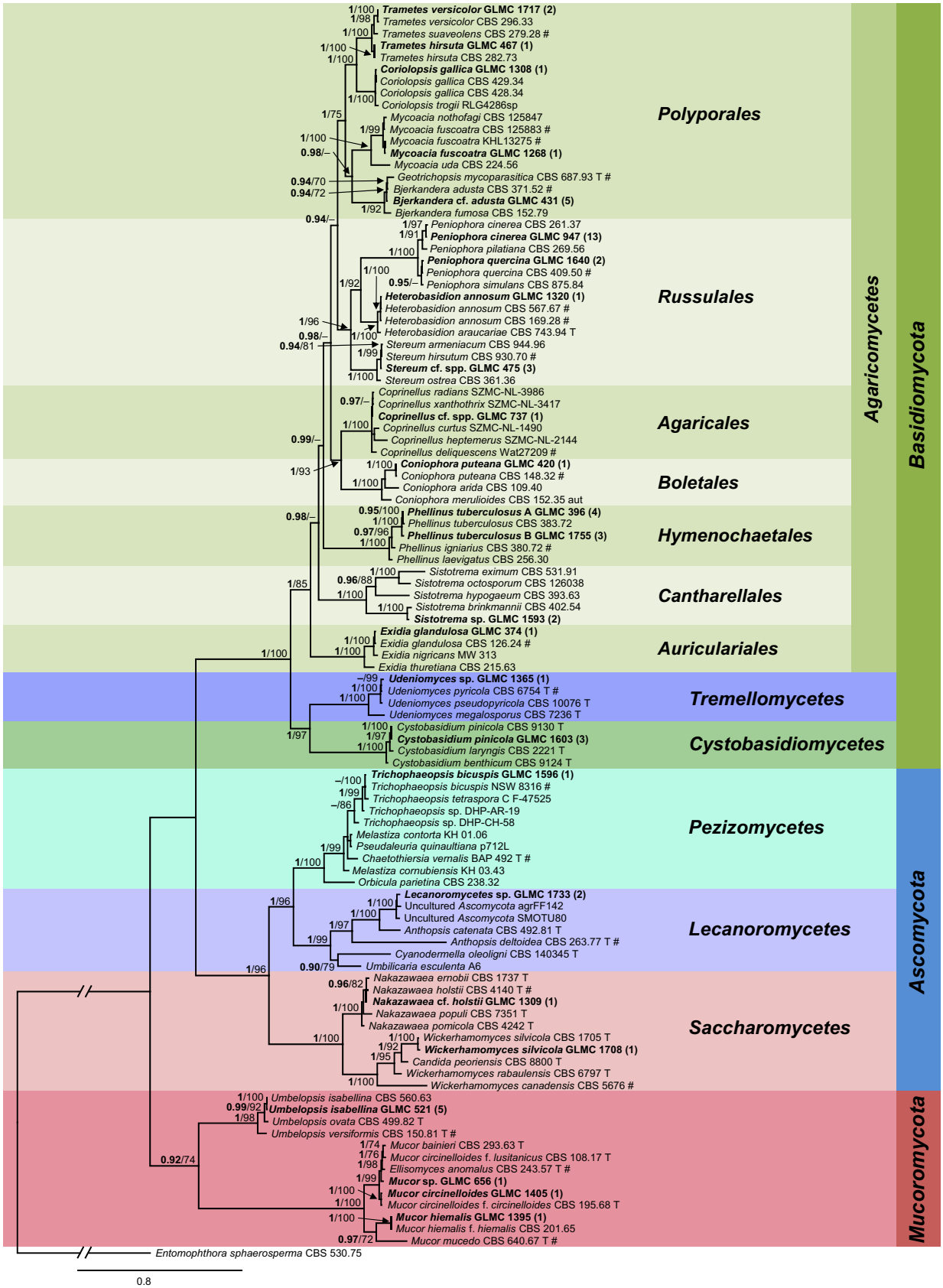


Fig. 6 Phylogeny of dataset 4 obtained by Bayesian inference analysis of the combined LSU and ITS sequence alignment of *Eurotiomycetes*. *Diplodia intermedia* strain CBS 124462 is used as outgroup. BI posterior probability support values above 0.9 (bold) and ML bootstrap support values above 70% are 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
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
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 #
Coniophora puteana GLMC 420 (1)
Coniophora puteana CBS 148.32 #
Coniophora arida CBS 109.40
Coniophora merulioides CBS 152.35 aut
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
Sistotrema eximum CBS 531.91
Sistotrema octosporum CBS 126038
Sistotrema hypogaeum CBS 393.63
Sistotrema brinkmannii CBS 402.54
Sistotrema sp. GLMC 1593 (2)
Exidia glandulosa GLMC 374 (1)
Exidia glandulosa CBS 126.24 #
Exidia nigricans MW 313
Exidia thuretiana CBS 215.63
Udeniomyces sp. GLMC 1365 (1)
Udeniomyces pyricola CBS 6754 T #
Udeniomyces pseudopyricola CBS 10076 T
Udeniomyces megalosporus CBS 7236 T
Cystobasidium pinicola CBS 9130 T
Cystobasidium pinicola GLMC 1603 (3)
Cystobasidium laryngis CBS 2221 T
Cystobasidium benthicum CBS 9124 T
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
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
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 #
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 #
Entomophthora sphaerosperma CBS 530.75
0.8
Polyporales
Russulales
Agaricales
Boletales
Hymenochaetales
Cantharellales
Auriculariales
Tremellomycetes
Cystobasidiomycetes
Pezizomycetes
Lecanoromycetes
Saccharomycetes
Agaricomycetes
Basidiomycota
Ascomycota
Mucoromycota

◀ **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.

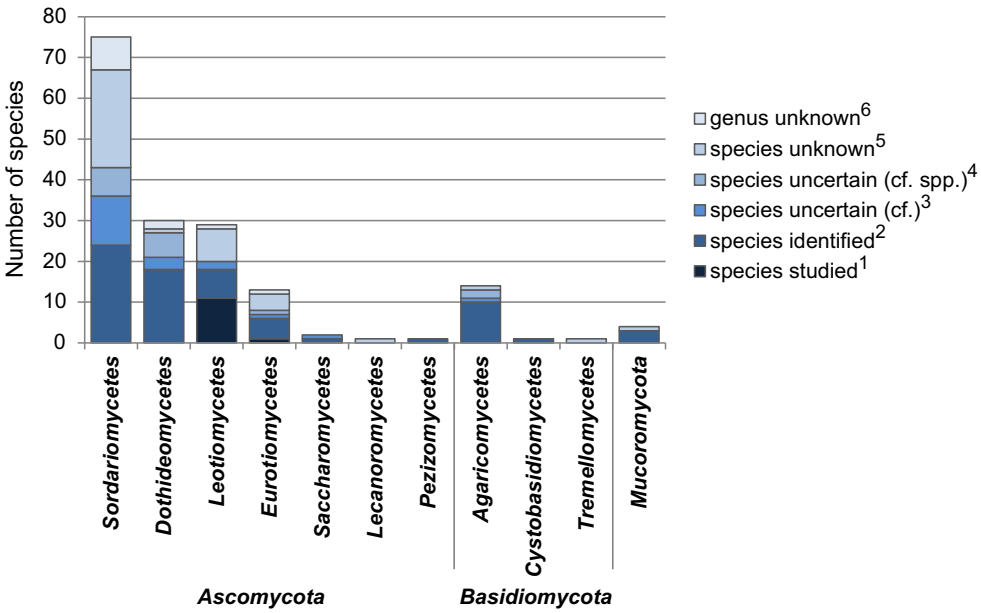


Fig. 8 Number of species in different classes of *Ascomycota*, *Basidiomycota* and *Mucoromycota* detected inside *Prunus* wood in Germany and level of certainty of identification based on ITS/LSU sequence comparisons. [1]Species treated in Bien et al. 2020 or Bien and Damm 2020, [2]ITS 0–4 nucleotide 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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