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New species of *Phaeomoniellales* from a German vineyard and their potential threat to grapevine (*Vitis vinifera*) health

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Abstract: Recently, the order *Phaeomoniellales* was established that includes fungi closely related to *Phaeomoniella chlamydospora*, a phytopathogen assumed to be the main causal agent of the two most destructive grapevine trunk diseases, Petri disease and esca. Other species of this order are reported as pathogens of other economically important crops, like olive, peach, apricot, cherry, plum, rambutan, lichee or langsat. However, they are rarely isolated and hence, little is known about their ecological traits and pathogenicity. During a 1-yr period of spore trapping in a German vineyard divided in minimally and intensively pruned grapevines, 23 fungal strains of the *Phaeomoniellales* were collected. Based on morphological and molecular (ITS, LSU and *tub2*) analyses the isolated strains were assigned to eight different species. Two species were identified as *P. chlamydospora* and *Neophaeomoniella zymoides,* respectively. The remaining six species displayed morphological and molecular differences to known species of the *Phaeomoniellales* and are newly described, namely *Aequabiliella palatina*, *Minutiella simplex*, *Moristroma germanicum, Mo. palatinum, Neophaeomoniella constricta* and *N. ossiformis*. A pathogenicity test conducted in the greenhouse revealed that except for *P. chlamydospora,* none of the species of the *Phaeomoniellales* isolated from spore traps is able to induce lesions in grapevine wood.

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INTRODUCTION

To separate *Phaeomoniella chlamydospora* (synonym *Phaeoacremonium chlamydosporum*; Crous *et al*. 1996) from the genus *Phaeoacremonium* based on significant morphological and molecular differences, Crous & Gams (2000) established the hyphomycete genus *Phaeomoniella*. *Phaeomoniella chlamydospora* is considered to be one of the main causal agents of Petri disease and esca, two grapevine trunk diseases (GTDs), which lead to high yield losses in grapevine industry all over the world (Bertsch *et al*. 2012, Fontaine *et al*. 2016, Gramaje *et al*. 2018). After the introduction of the genus *Phaeomoniella*, further species in this genus were described (Lee *et al*. 2006, Crous *et al*. 2008, 2011, Damm *et al*. 2010). Additionally, further unidentified *Phaeomoniella* species and related fungi appeared in many surveys focusing on fungal endophytes (Arnold *et al*. 2007, Botella & Diez 2010, Sánchez Márquez *et al.* 2011, Gueidan *et al*. 2014). Chen *et al.* (2015) established the order *Phaeomoniellales* to assemble all fungi with close affinity to the genus *Phaeomoniella*. Shortly thereafter, Crous *et al*. (2015) combined several *Phaeomoniella* species into new genera, and to date, the order *Phaeomoniellales* comprises 35 species in the following 12 genera: *Aequabiliella*, *Celerioriella*, *Celothelium*, *Dolabra*, *Minutiella*, *Moristroma*, *Neophaeomoniella*, *Paraphaeoisaria*, *Paraphaeomoniella*, *Phaeomoniella*, *Pseudophaeomoniella* and *Xenocylindrosporium* (Chen *et al*. 2015, Crous *et al.* 2015, 2016, 2018). Kirk (2015) described the family *Phaeomoniellaceae* with the type genus *Phaeomoniella* and an identical circumscription to the order. However, *Celothelium*, the type genus of the family *Celotheliaceae* (Aptroot *et al*. 2008) is also included in the circumscription of the family *Phaeomoniellaceae*. As the older name of the family has priority, *Phaeomoniellaceae* is an illegitimate name [ICN (Shenzhen) Art. 11.3 and Art. 52.1]. Moreover, in the phylogeny in Chen *et al*. (2015) there are no clades that would support the existence of two families.

Almost all known species of the *Phaeomoniellales* were isolated from plants. Several species, besides *P. chlamydospora*, are associated with wood diseases of economically important fruit crops. For example, *Pseudophaeomoniella oleae* and *Ps. oleicola* were isolated from discoloured xylem of wilting olive trees in Italy (Nigro *et al*. 2013, Crous *et al*. 2015). *Aequabiliella effusa*, *Minutiella tardicola*, *M. pruni-avium*, *Celerioriella dura*, *C. prunicola,* and *Neophaeomoniella zymoides* were isolated from necrotic wood of *Prunus* trees in South Africa and Germany (Damm *et al*. 2010, Bien & Damm 2020). *Dolabra nepheliae* is associated with stem canker disease of rambutan (*Nephelium lappaceum*; Booth &

Ting 1964) and lychee (*Litchi chinensis*; Rossman *et al*. 2010), as well as corky bark disease of langsat (*Lansium domesticum*; Keith *et al*. 2013). Other species, such as *Paraphaeomoniella capensis*, *Xenocylindrosporium kirstenboschense*, *N. niveniae* and *C. petrophiles* were isolated from symptomatic leaves of various host plants (Crous *et al*. 2008, 2009, 2011, 2016). Other species were reported from wood or other substrates without association to a symptom or disease. For example, ascostromata of *Moristroma quercinum* and *Mo. japonicum* were discovered by Nordén *et al.* (2005) on canes and old stumps of oak trees. Moreover, *N. zymoides* and *P. pinifoliorum* as well as *N. eucalypti* were isolated from pine needles (*Pinus densiflora*) and stems of *Eucalyptus globulus*, respectively (Lee *et al*. 2006, Crous *et al*. 2015). *Neophaeomoniella corymbiae* and *N. eucalyptigena* were both isolated from eucalypt leaves, *Corymbia citriodora* and *Eucalyptus pilularis*, respectively (Crous *et al.* 2018). *Paraphaeoisaria alabamensis* was reported from acial galls of *Cronartium quercuum* (de Hoog & Morgan-Jones 1978). Furthermore, unknown species with affinity to the *Phaeomoniellales* were detected in a screening for lichen-associated fungi and multiple times in leaves of pine trees in Arizona (Peršoh & Rambold 2012, Bowman & Arnold 2018).

Despite efforts in revealing the ecological role and phylogeny of the *Phaeomoniellales*, the number of isolates and thus the information currently available is insufficient for concrete conclusions (Chen *et al*. 2015). Consequently, the isolation and examination of more fungi belonging to the *Phaeomoniellales* is indispensable.

During a 1-yr period of spore trapping in a German vineyard planted with minimally and intensively pruned grapevines cv. Riesling, several fungi were isolated that were presumed to belong to the *Phaeomoniellales* based on preliminary blastn searches with ITS sequences. Therefore, one objective of this study was to clarify the relationship of these fungi based on molecular data, to characterise the species morphologically and by means of DNA sequence data and monitor their occurrence depending on season and pruning method. In addition, since many species of the *Phaeomoniellales* can induce necrosis in woody tissue of their hosts and since the fungi were collected from spore traps located in vineyards, their potential threat to grapevine (*Vitis vinifera*) was investigated.

MATERIALS AND METHODS

Isolates

Fungal spores were trapped using glass slides coated with petroleum jelly (Balea Vaseline, DM, Karlsruhe, Germany) attached close (2 cm; Fig. 1) to branches of grapevine (*Vitis vinifera*) cv. Riesling. The vineyard was located close to the Julius Kühn-Institute in Siebeldingen, Germany (49°13'11.5"N 8°02'32.3"E). Half of the grapevines were trained in semi minimal pruned hedge (SMPH; Fig. 1A) and the other half in vertical shoot positioning (VSP; Fig. 1B). For each training system, four spore traps were placed randomly in the field.

Spore trapping was carried out from February 2016 to February 2017. Each week, glass slides were replaced by new ones. A sterile washing solution (NaCl 136.9 mM; KCl 2.7 mM; Na_2HPO_4 7.9 mM; KH_2PO_4 1.5 mM; Tween® 80, 0.01 %; in distilled water) was used to release the spores from the coated glass slides. Under sterile conditions, 25 mL washing solution was added to a 50 mL reaction tube containing one glass slide each. The tube was shaken vigorously by hand for about 30 s. Subsequently, the washing solution was passed through a filter system consisting of a 5.0 µm and a 0.45 µm filter (mixed cellulose ester membrane filter, ADVANTEC MFS Inc., Japan). Since fungi of the order *Phaeomoniellales* produce small conidia (< 5.0 µm), the 0.45 µm filter was used for further analysis. The filter was placed into a 2 mL reaction tube and washed with 500 µL washing solution. Subsequently, the washing solution was spread equally on two plates of malt yeast agar (MYA; 20 g/L malt extract, 1 g/L yeast extract, 20 g/L agar, 2.5 µg/mL chloramphenicol; Carl Roth, Karlsruhe, Germany) and incubated for 2 wk at 20 °C. Growing colonies were transferred to new MYA plates and identified by morphological and molecular analyses.

Cultures of newly described species are maintained in the culture collections of the Julius Kühn-Institute (JKI; www.julius-kuehn.de), the CBS culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (WI; www.wi.knaw.nl) and the Senckenberg Museum of Natural History, Görlitz, Germany (GLMC; www.senckenberg.de). Type specimens of the species studied are deposited in the fungarium of the Senckenberg Museum of Natural History Görlitz, Germany (GLM). All descriptions are based on ex-holotype cultures, if not stated otherwise.

Local weather data (precipitation and temperature) were provided by the DLR Rhineland-Palatinate (www.dlr.rlp.de).

DNA extraction, PCR and sequencing

DNA extraction was performed according to the method of Tillett & Neilan (2000) from 2-wk-old cultures growing on MYA at 20 °C. Quality and quantity of the DNA were determined using a Spectrophotometer (Nanodrop 2000c, Thermo Fisher Scientific, Waltham, MA, USA). DNA was diluted to a final concentration of 100 µg/mL in distilled water.

For phylogenetic studies, three primer pairs were used to perform a polymerase chain reaction (PCR) amplifying the internal transcribed spacer regions 1 and 2 and intervening 5.8S rRNA (ITS: ITS5 and ITS4; White *et al.* 1990), the 28S rRNA (LSU: NL1 and NL4; O´Donnell 1993), and the beta-tubulin gene (*tub2*: BT2A and BT2B; Glass & Donaldson 1995). PCR reactions were set up as described in the KAPAHiFi™ hot start polymerase user manual (PEQLAB Biotechnologie GmbH, Erlangen, Germany). The reaction mixture consisted of 15.25 µL H_2O, 5.0 µL 5× KAPAHiFi™ buffer, 0.75 µL KAPAHiFi™ dNTP mix (10 mM), 0.75 µL forward primer (10 µM), 0.75 µL reverse primer (10 µM), 0.5 µL KAPAHiFi™ HotStart DNA polymerase (1 U/µL) and 2 µL DNA template (5 ng/µL). For DNA amplification the following reaction steps were implemented on a SimpliAmp™ thermal cycler (Applied Biosystems, Darmstadt, Germany): 95 °C initial denaturation (5 min), 98 °C denaturation (20 s), 58 °C annealing (15 s), 72 °C extension (20 s), 72 °C final extension (1 min). The main amplification steps (denaturation - annealing - extension) were repeated 35 times. Afterwards electrophoresis was carried out for 45 min at 110 V using a 1.5 % agarose gel to check the quality and quantity of the amplicons. Amplicons were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing reactions were set up using the ABI Prism Big Dye Terminator v. 3.1 cycle sequencing ready reaction kit (PE Biosystems, Foster City, CA, USA). Sequencing was performed on an ABI Prism 3130XL DNA sequencer. Sequence analyses and alignments were done with the program CLC Main Workbench v. 8.0 (Qiagen). ITS, *tub2* and LSU sequences of the newly described fungi were deposited at NCBI GenBank (www.ncbi.nlm.nih.gov/genbank/).

Fig. 1. Spore traps attached to grapevine plants cv. Riesling trained in SMPH (**A**) and VSP (**B**). Arrows indicate the spore traps (close-up views in the inserts).

Phylogenetic analyses

A concatenated ITS-LSU sequence alignment was constructed by separately aligning the sequences of the two loci and manually trimming the ends to achieve a uniform length of all sequences. Data of the *tub2* gene were excluded from the phylogenetic analyses, since sequences of only a few reference strains were available.

MEGA 7 (Molecular Evolutionary Genetics Analysis v. 7.0; Kumar *et al*. 2015) was used for Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses. A test was performed with MEGA 7 to find the most robust model for ML. Then a ML tree using tree bisection and reconnection (TBR) as branch-swapping algorithm was generated. Tree length, consistency index, retention index and composite index were calculated for the resulting tree. Finally, a ML tree containing bootstrap values (1 000 replications) calculated by both ML and MP analyses was chosen to visualise the phylogenetic relationship of the fungi investigated. Alignment and phylogenetic tree were lodged in TreeBASE (www.treebase.org, Study ID: 25809).

Morphological characterisation

To improve sporulation and pycnidial formation, fungal strains were cultivated on autoclaved grapevine wood pieces and autoclaved pine needles placed on SNA (synthetic nutrient-poor agar) medium (Nirenberg 1976) and incubated at 25 °C for up to 3 mo. Microscopic preparations were done in lactic acid with a Nikon Eclipse Ni-U light microscope with differential interference contrast (LM), or with a Nikon SMZ18 stereomicroscope (SM). Pictures were captured with Nikon Digital Sight DS-Fi2 cameras installed on the two microscopes mentioned above. In order to determine the size of fungal structures, 20 structures each were measured. Interpretation of colony characters was done after 2 wk of growth on potato dextrose agar (PDA; 40 g/L potato extract glucose agar, 5 µg/mL chloramphenicol; Carl Roth, Karlsruhe, Germany), oat meal agar (OA; 30 g/L oatmeal infusion, 20 g/L agar, 2.5 µg/mL chloramphenicol; Carl Roth, Karlsruhe, Germany) and MYA at 25 °C in the dark. The colour chart of Rayner (1970) was used to determine the colour of fungal colonies. To determine thermal tolerance and growth optima, the radial growth on MYA (three replicates) was measured at different temperatures, from 5 °C to 35 °C at 5 °C intervals, after 2 wk in the dark.

Pathogenicity

To investigate the potential threat of the trapped fungi to grapevine health, pathogenicity studies were performed on potted grapevines cv. Pinot noir and Müller-Thurgau in a greenhouse. Cuttings were hot-water treated at 50 °C for 30

min and then stored for 10 d at 10 °C for recovery. They were cut into smaller pieces with three buds each and planted into plastic boxes containing sterile soil. Incubation conditions in the greenhouse were 16 °C night time temperature, 24 °C day time temperature and 30 % relative humidity for 5 mo.

Young grapevine plants with shoot development were surface wounded with a sterilised scalpel by a diagonal cut of about 1 cm in length between the second and the third bud. After inoculating the wounds with a 20 µL spore suspension (~1 000 spores per 20 µL sterilised rainwater), they were sealed with plastic film to avoid evaporation and cross infection. One isolate of each species was tested, including *Phaeomoniella chlamydospora* as positive control. As negative control, plants were inoculated with sterile rainwater. For each species five plants were inoculated, and the experiment was repeated three times. Six months after inoculation, plants were cut longitudinally and lesions in the wood were measured up- and downwards from the inoculation point. Plants, which had dried out at the inoculation point, were excluded from the experiment. In a laminar flow cabinet, material from the lesion was removed with a sterile scalpel and placed on two MYA plates to verify the presence of the particular fungus. Fungi growing out from the lesion material were identified microscopically based on morphological characteristics.

For statistical evaluation of lesion length from the pathogenicity test, an analysis of variance (ANOVA) was conducted using the program RStudio v. 1.1.383 (RStudio Team 2016). Additionally, the re-isolation rate of the tested fungi, *i.e.* the percentage of the plants of which the fungus could be re-isolated, was determined.

RESULTS

Phylogenetic analyses

In total 23 fungal strains of the *Phaeomoniellales* were isolated in this study (Table 1). The phylogenetic analyses of the concatenated ITS-LSU sequence alignment comprised 45 strains, including reference strains and the outgroup *Capronia*

Table 1. Strains of the *Phaeomoniellales* studied with collection details and GenBank accession numbers.

	Accession no.[a]					GenBank no.[c]		
Species	**JKI**	**CBS**	**GLMC**	**Collection date**	**Training system[b]**	**ITS**	**LSU**	***tub*2**
Aequabiliella palatina	JKI-Mz48	CBS 145007	GLMC 1904	10 Mar. 2016	SMPH	MH999505	MH999528	MK070468
	JKI-Ap36*	CBS 145018	GLMC 1905	21 Apr. 2016	SMPH	MH999506	MH999529	MK070469
	JKI-May29	–	GLMC 1906	5 May 2016	SMPH	MH999507	MH999530	MK070470
Minutiella simplex	JKI-Jn27*	CBS 145008	GLMC 1907	9 Jun. 2016	SMPH	MH999508	MH999531	MK070471
	JKI-Jn38	CBS 145009	GLMC 1908	9 Jun. 2016	SMPH	MH999509	MH999532	MK070472
Moristroma germanicum	JKI-Feb06*	CBS 145012	GLMC 1911	3 Feb. 2017	SMPH	MH999512	MH999535	MK070475
Mo. palatinum	JKI-Feb17*	CBS 145010	GLMC 1909	23 Feb. 2017	SMPH	MH999510	MH999533	MK070473
	JKI-Au2	CBS 145011	GLMC 1910	9 Aug. 2016	VSP	MH999511	MH999534	MK070474
Neophaeomoniella constricta	JKI-Mz35*	CBS 145015	GLMC 1915	3 Mar. 2016	SMPH	MH999516	MH999539	MK070479
N. ossiformis	JKI-May02	CBS 145014	GLMC 1912	6 May 2016	SMPH	MH999513	MH999536	MK070476
	JKI-May03*	CBS 145013	GLMC 1913	6 May 2016	VSP	MH999514	MH999537	MK070477
	JKI-May30	–	GLMC 1914	6 May 2016	SMPH	MH999515	MH999538	MK070478
N. zymoides	JKI-Mz20	–	GLMC 1916	3 Mar. 2016	SMPH	MH999517	MH999540	MK070480
	JKI-Mz21	CBS 145156	GLMC 1917	3 Mar. 2016	SMPH	MH999518	MH999541	MK070481
	JKI-S09	–	GLMC 1918	11 Feb. 2016	VSP	MH999519	MH999542	MK070482
	JKI-Mz56	–	GLMC 1919	17 Mar. 2016	SMPH	MH999520	MH999543	MK070483
	JKI-Mz38	–	GLMC 1920	3 Mar. 2016	VSP	MH999521	MH999544	MK070484
	JKI-Mz40	–	GLMC 1921	3 Mar. 2016	VSP	MH999522	MH999545	MK070485
	JKI-Mz41	CBS 145155	GLMC 1922	3 Mar. 2016	VSP	MH999523	MH999546	MK070486
	JKI-Mz34	–	GLMC 1923	3 Mar. 2016	VSP	MH999524	MH999547	MK070487
Phaeomoniella chlamydospora	JKI-Ap04	CBS 145016	GLMC 1924	7 Apr. 2016	SMPH	MH999525	MH999548	MK070488
	JKI-Feb08	CBS 145017	GLMC 1925	9 Feb. 2017	SMPH	MH999526	MH999549	MK070489
	JKI-May05	–	GLMC 1926	6 May 2016	VSP	MH999527	MH999550	MK070490

[a]JKI: Culture collection of the Julius Kühn-Institute, Siebeldingen, Germany; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; GLMC: Culture collection of the Senckenberg Museum of Natural History Görlitz, Görlitz, Germany.

[b]Training system of the grapevine at which the trap was attached to. SMPH: semi minimal pruned hedge; VSP: vertical shoot positioning.

[c]ITS: internal transcribed spacers and intervening 5.8S DNA; LSU: 28S rDNA; *tub2*: partial beta-tubulin gene.

*Ex-type cultures.

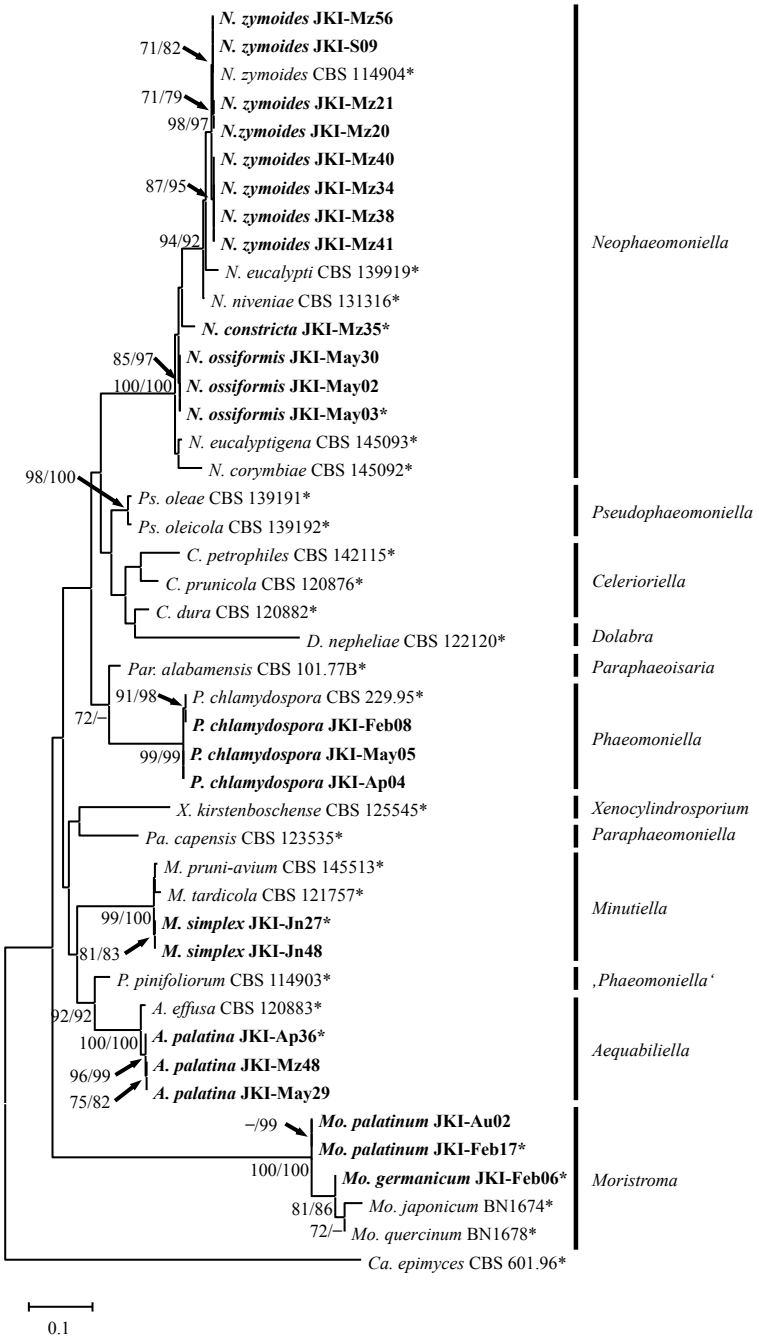

Fig. 2. Maximum likelihood tree based on the concatenated ITS-LSU sequence alignment of the *Phaeomoniellales*. Bootstrap support values above 70 % of ML/MP analyses are shown at the nodes. *Capronia epimyces* strain CBS 601.96 was used as outgroup. Isolates analysed in this study are emphasised in bold. Numbers of types and ex-type cultures are marked with an asterisk.

epimyces (Table 2) and 919 characters. The ML tree with both ML and MP bootstrap values (1 000 replicates) is shown in Fig. 2 and consists of eleven main clades representing the ten genera of the *Phaeomoniellales* and *Phaeomoniella pinifoliorum*. *Celothelium cinchonarum* was excluded from the analysis due to missing sequence data. Strains JKI-Mz56, JKI-S09, JKI-Mz21, JKI-Mz20, JKI-Mz40, JKI-Mz34, JKI-Mz38, and JKI-Mz41 grouped with *N. zymoides* (ML/MP bootstrap support values: 98/97) in the *Neophaeomoniella* clade (100/100). Strains JKI-May02, JKI-May03 and JKI-May30 formed a sub-clade (85/97) within this clade, sister to the single strain sub-clade JKI-Mz35, and the sub-clade formed by *N. corymbiae*, *N. eucalyptigena*, *N. eucalypti*, *N. niveniae* and *N. zymoides*. The isolates JKI-Feb08, JKI-May05 and JKI-Ap04 grouped with *P. chlamydospora* in the *Phaeomoniella* clade (99/99). Strains JKI-Mz48, JKI-May29 and JKI-Ap36 grouped with *Aequabiliella effusa* in the *Aequabiliella* clade (100/100). However, the latter represented a single strain sub-clade. The same situation was found for strains JKI-Jn27 and JKI-Jn48 that formed a clade with *Minutiella pruni-avium* and *M. tardicola*, which formed single strain sub-clades within the *Minutiella* clade (99/100). Together with *Moristroma japonicum* and *Mo. quercinum*, strains JKI-Au02, JKI-Feb17 and JKI-Feb06 formed the *Moristroma* clade, in which isolate JKI-Feb06 grouped with *Mo. quercinum* and *Mo. japonicum* (81/86), while isolates JKI-Au02 and JKI-Feb17 formed a separate sub-clade within *Moristroma* (–/99). The *Moristroma* clade (100/100) was on a long branch, basal to all other genera in the *Phaeomoniellales*, except for *Dolabra nepheliae* strain CBS 122120.

Table 2. Reference strains of the *Phaeomoniellales* with collection details and GenBank accession numbers.

				GenBank no.[b]		
Species	**Accession no.[a]**	**Host**	**Country**	**ITS**	**LSU**	***tub2***
Aequabiliella effusa	CBS 120883*	*Prunus salicina*	South Africa	GQ154598	GQ154618	KR260451
Celerioriella dura	CBS 120882*	*Pr. salicina*	South Africa	GQ154597	GQ154617	–
C. petrophiles	CBS 142115*	*Petrophile teretifolia*	Australia	KY173394	KY173487	–
C. prunicola	CBS 120876*	*Pr. salicina*	South Africa	GQ154590	GQ154615	–
Dolabra nepheliae	CBS 122120*	*Nephelium lappaceum*	Malaysia	JQ004281	GU332516	–
Minutiella pruni-avium	CBS 145513*	*Pr. avium*	Germany	MN232957	MN232925	MN232985
M. tardicola	CBS 121757*	*Pr. armeniaca*	South Africa	GQ154599	GQ154619	KR260454
Moristroma japonicum	BN1674*	*Quercus mongolica*	Japan	AY254052	AY254052	–
Mo. quercinum	BN1678*	*Q. robur*	Sweden	AY254051	AY254051	–
Neophaeomoniella corymbiae	CBS 145092*	*Corymbia citriodora*	Australia	MK047457.1	MK047507.1	–
N. eucalypti	CBS 139919*	*Eucalyptus globulus*	USA	NR_138001	KR476782	–
N. eucalyptigena	CBS 145093*	*E. pilularis*	Australia	MK047458.1	MK047508.1	MK047584.1
N. niveniae	CBS 131316*	*Nivenia stokoei*	South Africa	JQ044435	JQ044454	–
N. zymoides	CBS 114904*	*Pinus densiflora*	Korea	GQ154600	GQ154620	KR260455
Paraphaeoisaria alabamensis	CBS 101.77B*	*Cronartium quercuum*	USA	MH861028	MH872801	–
Paraphaeomoniella capensis	CBS 123535*	*Encephalartos altensteinii*	South Africa	NR_137711	FJ372408	–
Phaeomoniella chlamydospora	CBS 229.95*	*Vitis vinifera*	Italy	FJ530942	AF353609	–
P. pinifoliorum	CBS 114903*	*Pi. densiflora*	Korea	DQ270240	DQ270250	–
Pseudophaeomoniella oleae	CBS 139191*	*Olea europaea*	Italy	NR_137966	KP635971	–
Ps. oleicola	CBS 139192*	*O. europaea*	Italy	KP411807	KP635970	–
Xenocylindrosporium kirstenboschense	CBS 125545*	*En. friderici-guilielmi*	South Africa	NR_132841	GU229891	–
Capronia epimyces[c]	CBS 601.96*	*Picea* sp.	Canada	AY156968	AF050245	–

[a] BN: Botanical Institute, Göteborg University, Göteborg, Sweden; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

[b] ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: 28S rDNA; tub2: partial beta-tubulin gene.

[c] Outgroup.

*Types and ex-type cultures.

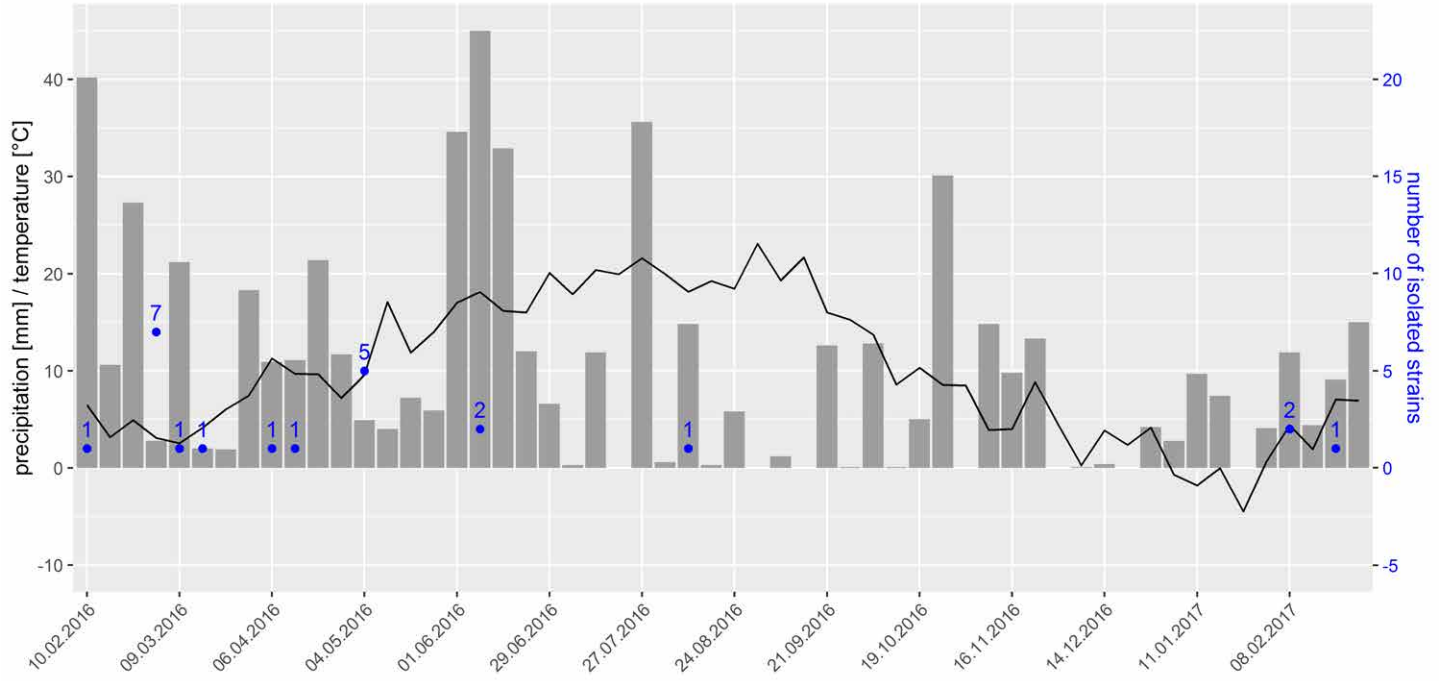

Fig. 3. Weekly precipitation [mm] (grey columns), average temperature [°C] (black line) and number of fungal strains collected (emphasised in blue) during the time of spore trapping.

Diversity and occurrence

The strains were assigned to eight species in five genera: *Aequabiliella*, *Minutiella*, *Moristroma,* *Neophaeomoniella* and *Phaeomoniella*. Fifteen strains belonging to eight species originated from spore traps attached to SMPH trained grapevines and eight strains belonging to four species were isolated from traps attached to VSP trained grapevines. Spores were trapped between February and June, with one exception in August (JKI-Au2; Fig. 3).

Taxonomy

Six of the eight *Phaeomoniellales* species found in this study exhibited significant morphological and molecular differences to known species and are therefore described as new species (Figs 4–10).

Aequabiliella palatina C. Kraus, Damm, S. Bien, Vögele & M. Fisch., ***sp. nov.*** MycoBank MB828284. Figs 4A, 5.

Etymology: Named after the federal state of Germany, Rhineland-Palatinate, in which the species was isolated (palatinus, adjective of Palatinatus = Pfalz).

Typus: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 21 Apr. 2016, *C. Kraus* (GLM-F117490 **holotype**, culture ex-type JKI-Ap36 = CBS 145018 = GLMC 1905).

Vegetative hyphae hyaline, smooth-walled, 1.5–2.5 µm wide, septate, chlamydospores not observed. *Sporulation* abundant; conidia formed on hyphae, in pycnidia and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, mainly reduced to conidiogenous cells, rarely 2- or 3-celled, cylindrical to lanceolate, 13–41.5 × 2–2.5 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, most frequently reduced to openings formed directly on hyphal cells, discrete phialides and adelophialides occasionally observed; collarettes inconspicuous; openings on hyphal cells 0.5–1.5 µm wide, periclinal thickening visible; discrete phialides cylindrical to cigar-shaped, 4.5–14 × 1.5–3 µm, adelophialides cylindrical, 1–8 × 1–3 µm. *Conidia* accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, cylindrical to oblong-elliptical, sometimes slightly curved, smooth-walled, (3–)4(–6) × (1–)1.5(–2) µm, L/W ratio = 2.7.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, elongated obovate to oblong-elliptical, (5–)6(–6.5) × (1–)2(–2.5) µm, L/W ratio = 2.7.

Conidiomata pycnidial produced superficially on pine needles, grapevine wood and immersed in SNA medium after four wk, solitary or in groups, globose to subglobose, 110–330 µm diam, unilocular, opening by irregular rupture, pycnidial wall composed of *textura angularis*, 6–13 µm thick, 2–4 cell layers. *Conidiophores* reduced to conidiogenous cells lining the inner wall of pycnidia. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, discrete phialides, obpyriform 3.5–6.5 × 2.5–4 µm, collarettes inconspicuous; opening 0.5–2 µm. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, elliptical to oblong-elliptical, (2.5–)3(–3.5) × (1–)1.5(–2) µm, L/W ratio = 1.9.

Culture characteristics: Colonies on PDA flat to raised, with entire to crenated margin, moist, buff to primrose, sparse, whitish, funiculose aerial mycelium in centre; reverse same colours. Colonies on OA flat, with entire to crenated margin, moist, olivaceous buff to olivaceous grey, olivaceous buff to olivaceous black in the centre, buff at the margin, aerial mycelium sparse; reverse same colours. Colonies on MYA flat, with crenated margin, moist, honey in centre, with a grey olivaceous to olivaceous black ring and a buff margin, aerial mycelium sparse, reverse same colours; 27–27.5 mm diam after 14 d on MYA (25 °C, in the dark), min 10 °C, max 30 °C, opt 25 °C.

Additional materials examined: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 10 Mar. 2016, *C. Kraus*, JKI-Mz48 = CBS 145007 = GLMC 1904; Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6 May 2016, *C. Kraus*, JKI-May29 = GLMC 1906.

Notes: *Aequabiliella palatina* is closely related to *A. effusa*, however, *tub2*, ITS and LSU sequences of the ex-type strain are only 94 % (21 nucleotides different), 98 % (9 nucleotides different) and 99 % (1 nucleotide different), respectively, identical to that of *A. effusa* (Damm *et al*. 2010, Úrbez-Torres *et al*. 2015). A blastn search of the ITS sequence resulted in 99 % (2–3 nucleotides different) accordance with unidentified fungal isolates from wood samples of Norway spruce and Scots pine taken in Finland (MG190556, Müller *et al*. 2018) and Western white pine taken in Montana, USA (JF705946, Larkin *et al*. 2012). Colonies of *A. effusa* are herbage-green, dark herbage-green to olivaceous on PDA, while those of *A. palatina* are buff to primrose. Microcyclic conidiation was only observed in *A. palatina*. Additionally, the temperature depending growth range differs between the two species: *A. effusa* can grow at temperatures between 5 °C and 35 °C, with an optimum at 30 °C, while the growth range of *A. palatina* is narrower, ranging from 10 °C to 30 °C, with an optimum at 25 °C.

Minutiella simplex C. Kraus, Damm, S. Bien, Vögele & M. Fisch., ***sp. nov.*** MycoBank MB828285. Figs 4B, 6.

Etymology: Named after the simple conidiogenous cells on hyphae.

Typus: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 9 Jun. 2016, *C. Kraus* (GLM-F117492 **holotype**, culture ex-type JKI-Jn27 = CBS 145008 = GLMC 1907).

Vegetative hyphae hyaline, smooth-walled, 2–3.5 µm wide, septate, chlamydospores not observed. *Sporulation* abundant, conidia formed on hyphae, in pycnidia and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, almost exclusively simple openings on hyphal cells, rarely discrete phialides, 10–18 × 2–3 µm; collarettes inconspicuous; openings on hyphal cells 0.5–1 µm wide. *Conidia* accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, cylindrical, sometimes slightly curved, smooth-walled, (3–)4(–5) × (1–)1.5(–2) µm, L/W ratio = 2.4.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, cylindrical, obovate to

oblong-elliptical, often curved, (5–)6(–7) × (1.5–)2(–2.5) µm, L/W ratio = 3.1.

Conidiomata pycnidial produced superficially on pine needles, grapevine wood and immersed in SNA medium after four to eight wk, mainly solitary, globose, subglobose to ellipsoidal, 120–300 µm diam, unilocular, opening by irregular rupture, pycnidial wall composed of *textura angularis*, 8–18 µm thick, 3–6 cell layers. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, hyaline, discrete phialides; collarettes visible; discrete phialides obpyriform to ampulliform, 4–7 × 2–5 µm, openings 0.5–1.5 µm. *Conidia* hyaline, smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, (2.5–)3(–4) × (1–)1.5(–2) µm, L/W ratio = 2.2.

Culture characteristics: Colonies on PDA raised, with undulate to lobate margin, moist, buff to straw, lacking aerial mycelium, reverse same colours; on OA flat, with entire to crenated margin, moist, whitish to buff, funiculose to felty aerial mycelium in centre; reverse same colours; on MYA flat to raised, with crenated margin, moist, buff, hyaline, sparse aerial mycelium; reverse buff to luteous; 3–6.5 mm diam after 14 d on MYA (25 °C, in the dark), min 10 °C, max 25 °C, opt 20 °C.

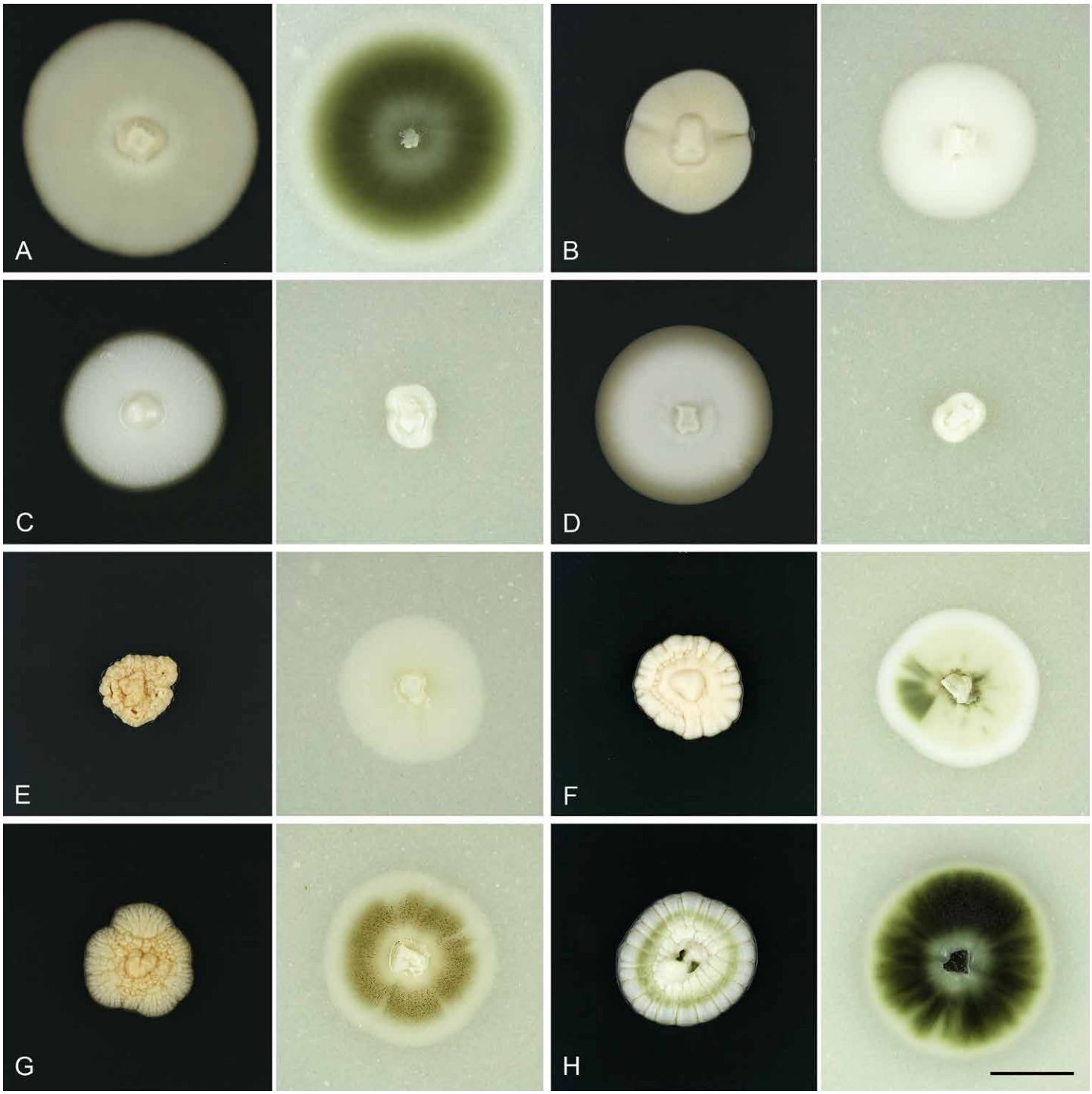

Fig. 4. Cultures of *Phaeomoniellales* grown on PDA (left) and OA (right) for 4 wk at 20 °C in the dark. **A.** *Aequabiliella palatina* strain JKI-Ap36*. **B.** *Minutiella simplex* strain JKI-Jn27*. **C.** *Moristroma germanicum* strain JKI-Feb06*. **D.** *Mo. palatinum* strain JKI-Feb17*. **E.** *Neophaeomoniella constricta* strain JKI-Mz35*. **F.** *N. ossiformis* strain JKI-May03*. **G.** *N. zymoides* strain JKI-Mz41. **H.** *Phaeomoniella chlamydospora* strain JKI-Ap04. Scale bar: H = 15 mm; H applies to A–H. * Ex-type cultures.

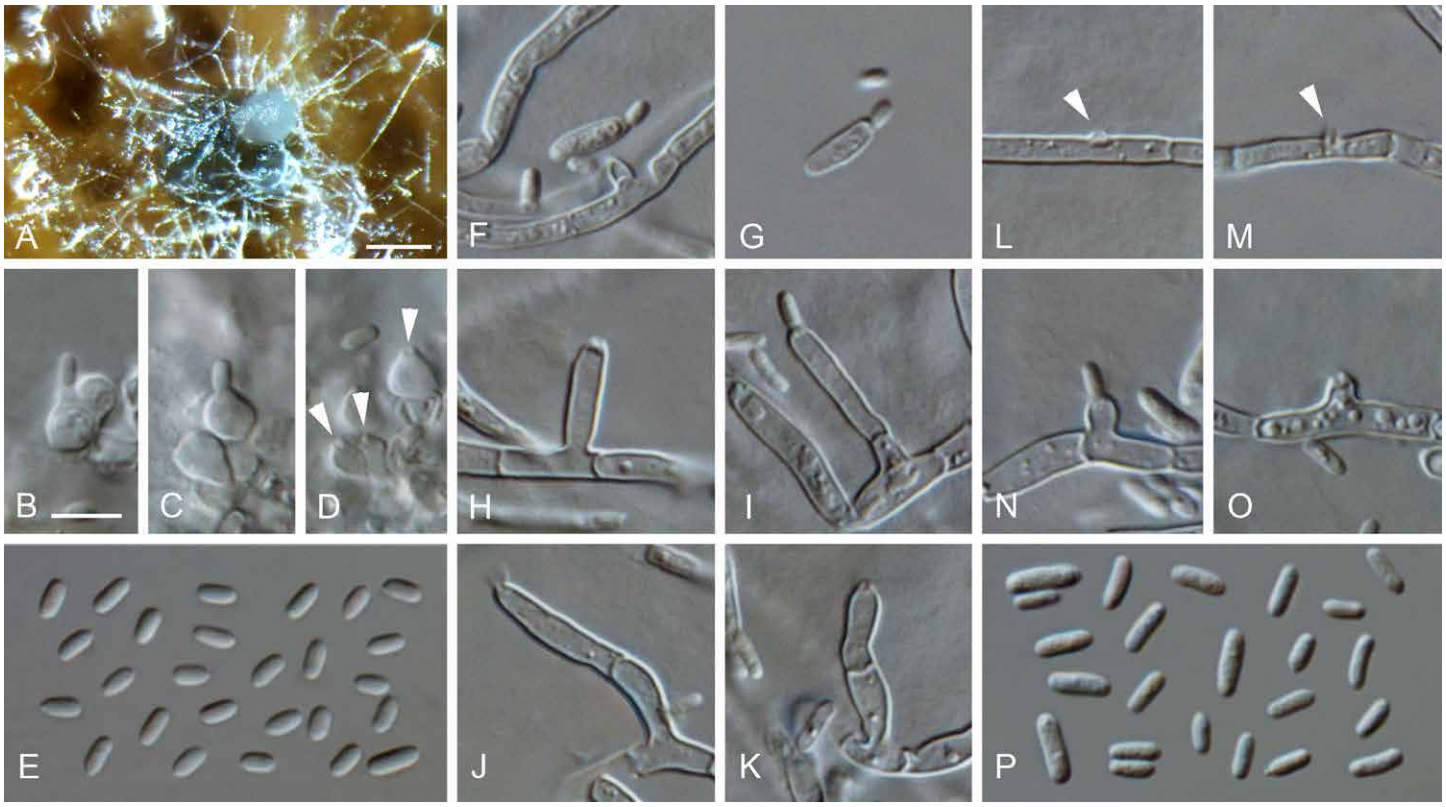

Fig. 5. *Aequabiliella palatina* (ex-type culture JKI-Ap36). **A.** Conidia oozing from a pycnidium on grapevine wood. **B–D.** Conidiogenous cells lining the inner cell wall of pycnidia (arrows indicate conidiogenous openings). **E.** Conidia formed in pycnidia. **F–G.** Microcyclic conidiation. **H–O.** Conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings on hyphal cells). **P.** Conidia generated on hyphal cells. A: SM; B–P: LM. Scale bars: A = 100 µm; B = 5 µm; B applies to B–P.

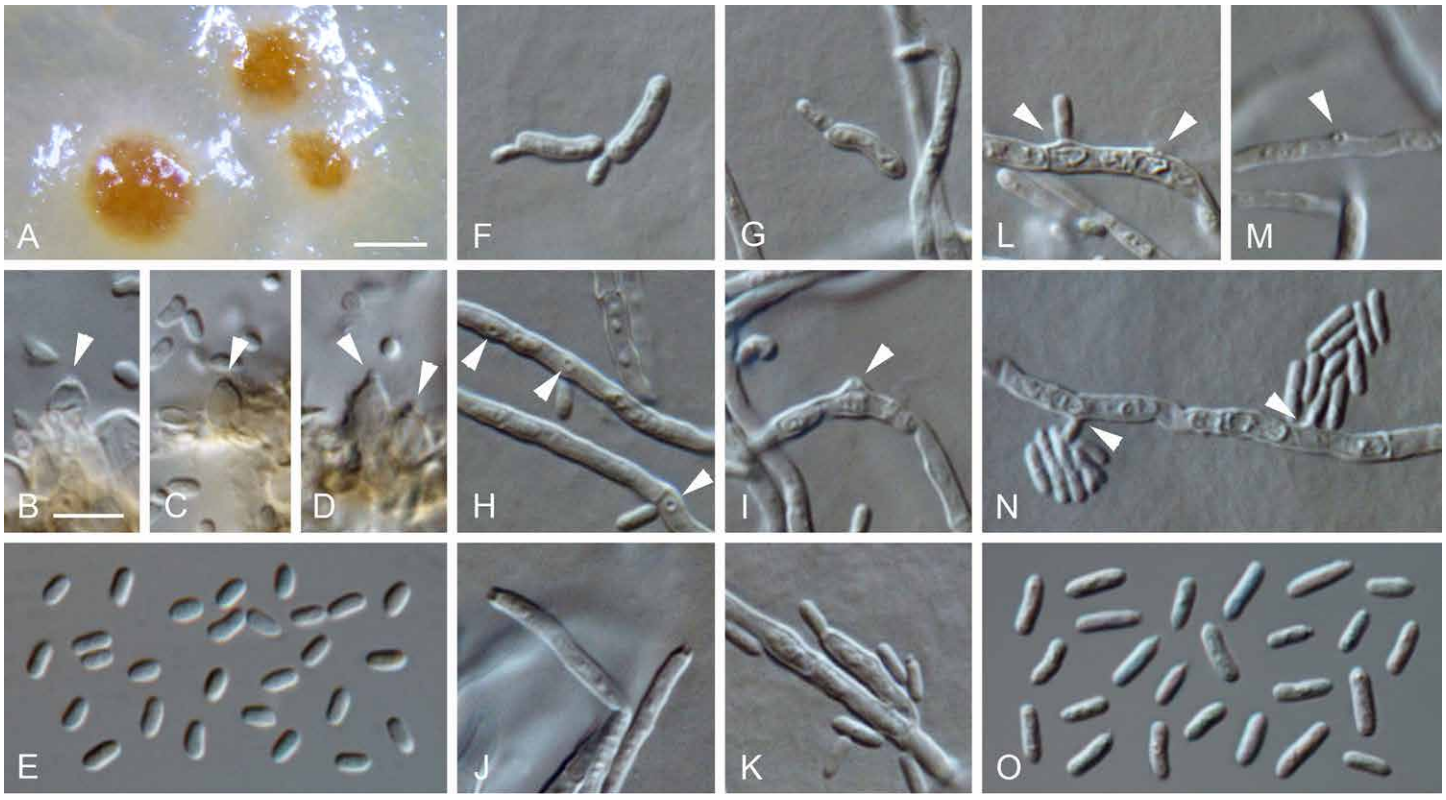

Fig. 6. *Minutiella simplex* (ex-type culture JKI-Jn27). **A.** Pycnidia immersed in SNA. **B–D.** Conidiogenous cells lining the inner cell wall of pycnidia (arrows indicate conidiogenous openings). **E.** Conidia formed in pycnidia. **F–G.** Microcyclic conidiation. **H–N.** Conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings). **O.** Conidia generated on hyphal cells. A: SM; B–O: LM. Scale bars: A = 200 µm; B = 5 µm; B applies to B–O.

Additional material examined: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 9 Jun. 2016, *C. Kraus*, culture JKI-Jn38 = CBS 145009 = GLMC 1908.

Notes: *Minutiella simplex* is morphologically similar to *M. tardicola* and *M. pruni-avium*, however, differs in all three loci sequenced. Large subunit sequences of *M. simplex* differ in four and one nucleotides, ITS sequences in six and nine nucleotides and *tub2* sequences in one and 33 nucleotides from those of *M. tardicola* and *M. pruni-avium*, respectively (Damm *et al*. 2010; Úrbez-Torres *et al*. 2015; Bien & Damm 2020). Furthermore, colonies of *M. simplex* have a higher growth rate than *M. tardicola* and form larger pycnidia. Additionally, *M. simplex* can grow between 10 °C and 25 °C with an optimum at 20 °C, while *M. tardicola* grows between 15 °C and 30 °C, preferring 25 °C (Damm *et al*. 2010).

Moristroma germanicum C. Kraus, Damm, S. Bien, Vögele & M. Fisch., ***sp. nov.*** MycoBank MB828287. Figs 4C, 7.

Etymology: Named after the country the species was found in, Germany.

Typus: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 3 Feb. 2017, *C. Kraus* (GLM-F117494 **holotype**, culture ex-type JKI-Feb06 = CBS 145012 = GLMC 1911).

Vegetative hyphae hyaline, smooth-walled, septate, 1–2.5 µm wide, partly inflated up to 5.5 µm, chlamydospores not observed. *Sporulation* abundant, conidia formed on hyphae and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, mainly reduced to conidiogenous cells, rarely 2–7-celled, cylindrical, 17–71 × 1–2 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, often reduced to openings directly formed on hyphal cells, adelophialides and discrete phialides frequently observed; adelophialides cylindrical to lanceolate, sometimes conical, 2–21 × 1–2 µm; discrete phialides cylindrical to naviculate, 2.5–15.5 × 1.5–4 µm; collarettes conspicuous, cylindrical, 0.5–1.5 µm long, opening 0.5–1 µm, periclinal thickening observed. *Conidia* accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, cylindrical, oblong-elliptical to obovate, (2.5–) 3(–5) × (1–)1.5(–2) µm, L/W ratio = 2.1.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, sometimes 2-celled, irregularly shaped or obovate (3–)4(–5) × (2–)2.5(–3) µm, L/W ratio = 1.6.

Conidiomata not observed.

Culture characteristics: Colonies on PDA flat to raised, with entire margin, moist, whitish to buff, aerial mycelium sparse; reverse same colours; on OA flat, with undulate margin, moist, whitish to buff, aerial mycelium sparse; reverse same colours; on MYA flat, with entire margin, moist, buff to primrose, aerial mycelium in the centre dense, whitish, funiculose; reverse same colours; 12.5–14 mm diam after 14 d on MYA (25 °C, in the dark), min 15 °C, max 30 °C, opt 25 °C.

Notes: Frequently occurring discrete phialides and the presence of inflated hyphal cells distinguishes *Mo. germanicum* from *Mo. palatinum*. The *tub2* sequence of *Mo. germanicum* shows 96 % identity with *Mo. palatinum* (15 nucleotides different). The alignment displays 97 % identity (14 nucleotides different) of the ITS sequence of *Mo. germanicum* with that of *Mo. quercinum*, 93 % (31 nucleotides different) with that of *Mo. palatinum* and 93 % (33 nucleotides different) with that of *Mo. japonicum*. The LSU sequence of *Mo. germanicum* is two nucleotides different from *Mo. quercinum* (99 % identical), 9 nucleotides different (98 % identical) from *Mo. japonicum* and 11 nucleotides different (98 % identical) from *Mo. palatinum*. Due to missing sequence data and morphological data of the asexual morph, *Mo. multisporum* and *Mo. polysporum*, that were described from dead wood of *Terminalia arjuna* in India and from decorticated wood of *Eucalyptus viminalis* in Argentina, respectively, cannot be compared with the two *Moristroma* species described in this study (Sivanesan *et al*. 1988, Romero & Samuels 1991, Boonmee *et al*. 2011, Zhang *et al*. 2012).

Moristroma palatinum C. Kraus, Damm, S. Bien, Vögele & M. Fisch., ***sp. nov.*** MycoBank MB828286. Figs 4D, 8.

Etymology: Named after the federal state of Germany, Rhineland-Palatinate, in which the species was isolated.

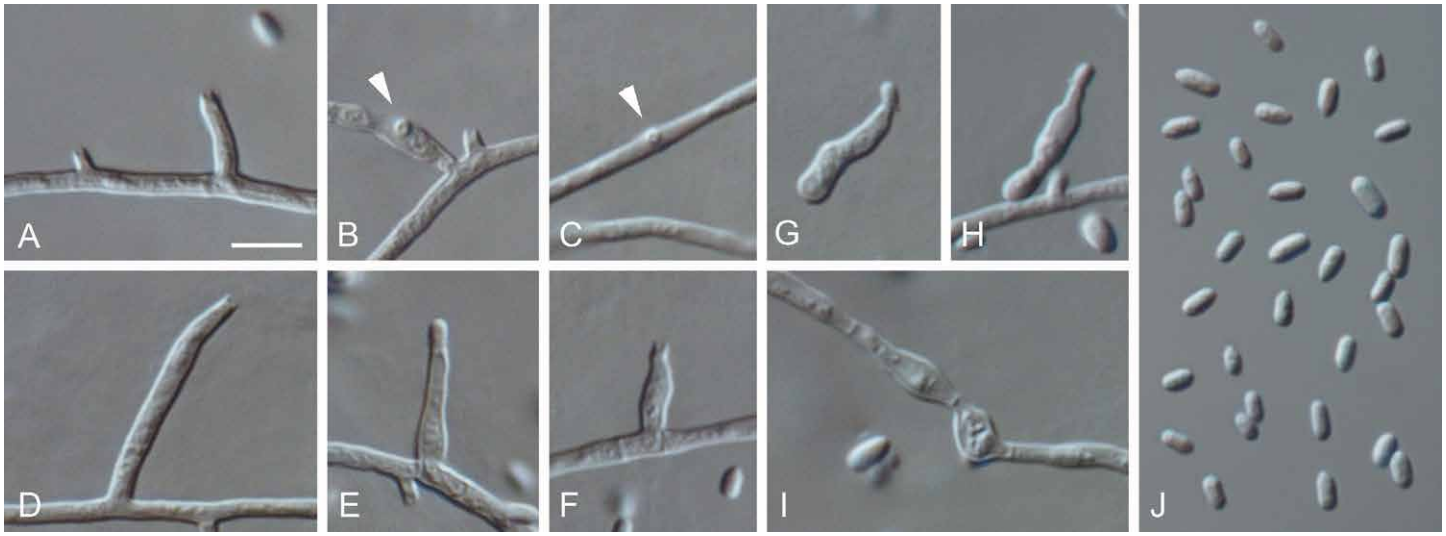

Fig. 7. *Moristroma germanicum* (ex-type culture JKI-Feb06). **A–F.** Conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings). **G–H.** Microcyclic conidiation. **I.** Inflated hyphal cells. **J.** Conidia formed on hyphal cells. A–J: LM. Scale bars: A = 5 µm; A applies to A–J.

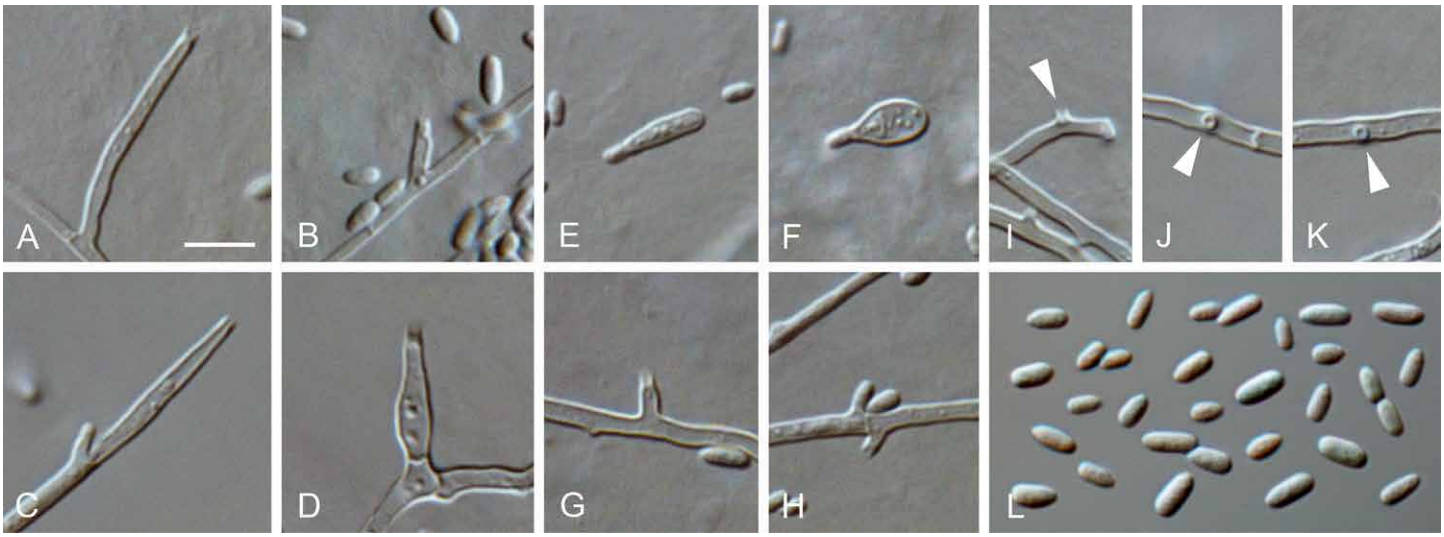

Fig. 8. *Moristroma palatinum* (ex-type culture JKI-Feb17). **A–D, G–K.** Conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings). **E–F.** Microcyclic conidiation. **L.** Conidia formed on hyphal cells. A–L: LM. Scale bars: A = 5 µm; A applies to A–L.

Typus: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 23 Feb. 2017, *C. Kraus* (GLM-F117495 **holotype**, culture ex-type JKI-Feb17 = CBS 145010 = GLMC 1909).

Vegetative hyphae hyaline, smooth-walled, 1–2.5 µm wide, septate, chlamydospores not observed. *Sporulation* abundant, conidia formed on hyphae, in pycnidia and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, mostly reduced to openings directly formed on hyphal cells and adelophialides, discrete phialides rare; collarettes conspicuous, cylindrical, 0.5–1.5 µm long, opening 0.5–1.5 µm diam; adelophialides cylindrical to lanceolate, sometimes conical, discrete phialides cylindrical to lanceolate, 1.5–17 × 1–2.5 µm. *Conidia* accumulated in heads around conidiogenous opening, hyaline, smooth-walled, aseptate, cylindrical, elliptical, oblong-elliptical to obovate, (2–)3(–3.5) × (1–)1.5(–2) µm, L/W ratio = 1.9.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, ellipsoidal to ovoidal, sometimes cylindrical, (5–)5.5(–6.5) × (2.5–)3(–4) µm, L/W ratio = 1.9.

Conidiomata pycnidial (not shown), rarely observed on pine needles after four wk, mainly solitary, superficial, ovoid, 40–90 µm diam, unilocular, with a central ostiole, pycnidial wall composed of *textura angularis,* 7–15 µm thick, 2–4 cell layers. *Conidiophores* reduced to conidiogenous cells lining the inner wall of the pycnidia. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, discrete phialides; collarettes conspicuous, cylindrical, 0.5–1 µm long; discrete phialides ampulliform, 3.5–6.5 × 1.5–3.5 µm, opening 0.5–1.5 µm. *Conidia* hyaline, smooth-walled, aseptate, oblong-elliptical to obovate, (2–)2.5(–3) × (1–)1.5(–2.0) µm, L/W ratio = 1.7.

Culture characteristics: Colonies on PDA flat, with entire to undulate margin, moist to slimy, buff, aerial mycelium sparse; reverse same colours; on OA flat, with entire margin, moist, whitish to buff, dense, funiculose aerial mycelium in centre; reverse same colours; on MYA flat to raised, with undulate margin, moist, buff to primrose, sparse aerial mycelium; reverse same colours; 15–18 mm diam after 14 d on MYA (25 °C, in the dark), min 10 °C, max 30 °C, opt 25 °C.

Additional material examined: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 9 Aug. 2016, *C. Kraus*, JKI-Au02 = CBS 145011 = GLMC 1910.

Notes: The ITS and LSU sequences of *Moristroma palatinum* are 93 % and 98 % identical with *Mo. germanicum* (31 and 11 nucleotides different), 91 % and 98 % with *Mo. quercinum* (42 and 13 nucleotides different) and 92 % and 97 % with *Mo. japonicum* (35 and 16 nucleotides different; Nordén et al., 2005), respectively. Additionally, the *tub2* sequence of *Mo. palatinum* differs from *Mo. germanicum* by 15 nucleotides (95 % identical). Moreover, the absence of inflated hyphal cells and the lesser occurrence of discrete phialides distinguish *Mo. palatinum* from *Mo. germanicum*. The pycnidia of *Mo. palatinum* are smaller and conidiogenous cells in pycnidia are longer than those of *Mo. quercinum* and *Mo. japonicum*.

Neophaeomoniella constricta C. Kraus, Damm, S. Bien, Vögele & M. Fisch., ***sp. nov.*** MycoBank MB828288. Figs 4E, 9.

Etymology: Named after the conidiogenous cells that are often constricted at the septa.

Typus: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 3 Mar. 2016, *C. Kraus* (GLM-F117500 **holotype**, culture ex-type JKI-Mz35 = CBS 145015 = GLMC 1915).

Vegetative hyphae hyaline, smooth-walled, 1–3 µm wide, septate, partly inflated up to 5.5 µm. *Sporulation* abundant; conidia formed on hyphae, in pycnidia and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, mainly reduced to conidiogenous cells, occasionally 2–6-celled conidiophores observed, often constricted at the septa, 4.5–32.5 × 2.5–3.5 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, mostly openings formed directly on hyphal cells;

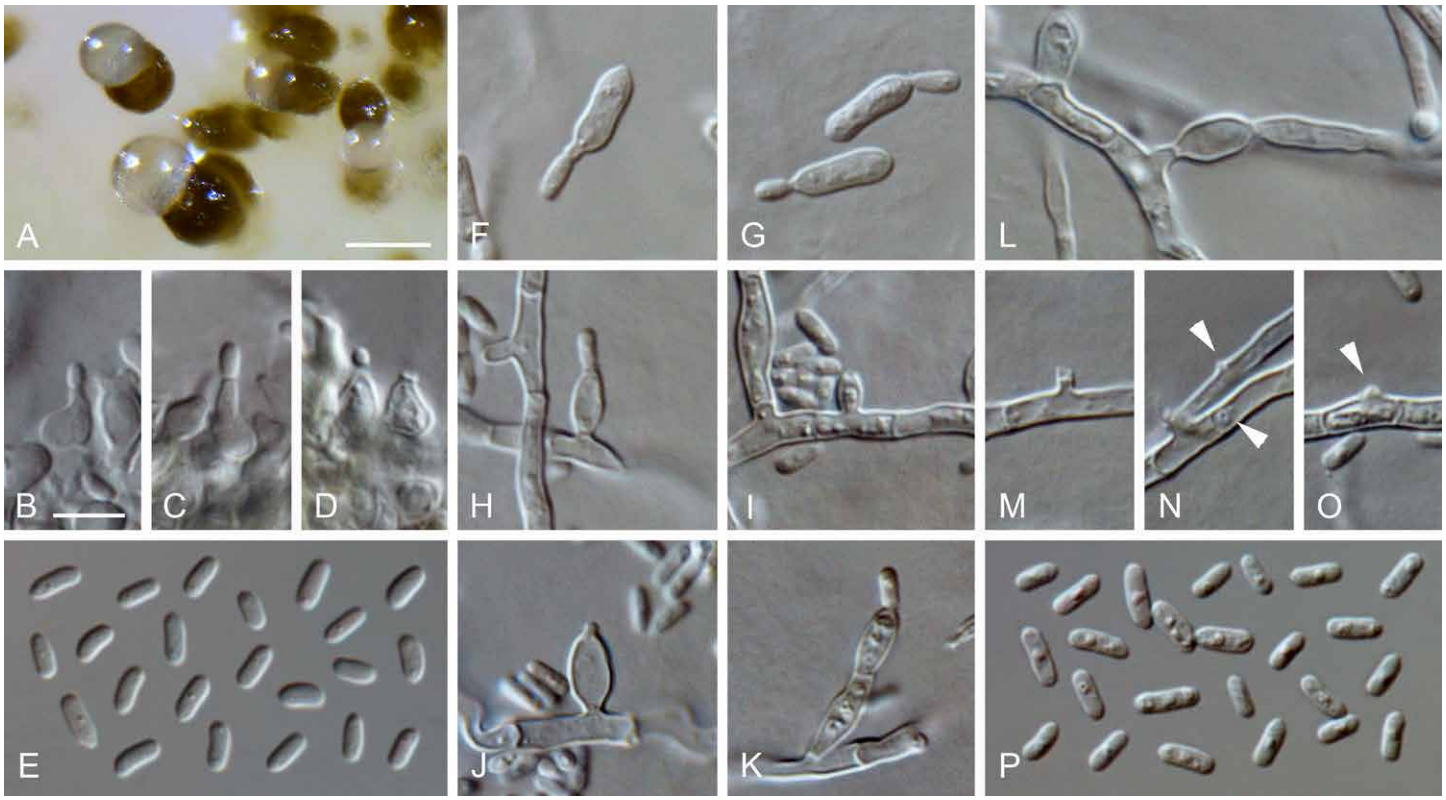

Fig. 9. *Neophaeomoniella constricta* (ex-type culture JKI-Mz35). **A.** Pycnidia formed on OA. **B–D.** Conidiogenous cells from the inner cell wall of pycnidia. **E.** Conidia formed in pycnidia. **F–G.** Microcyclic conidiation. **H–K, M–O.** Conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings). **L.** Inflated hyphal cell. **P.** Conidia formed on hyphal cells. A: SM; B–P: LM. Scale bars: A = 100 µm; B = 5 µm; B applies to B–P.

sometimes discrete phialides, ellipsoidal, ovoidal to navicular, rarely cylindrical, 3.5–7.5 × 2.5–4 µm; collarettes sometimes visible, periclinal thickening inconspicuous. *Conidia* accumulated in groups around conidiogenous openings, hyaline, smooth-walled, aseptate, cylindrical to oblong-elliptical, sometimes slightly curved, (3–)4(–5) × (1–)1.5(–2) µm, L/W ratio = 2.7.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, rarely 2-celled, cylindrical to oblong-elliptical, (5–)6.5(–8) × (1.5–)2(–3) µm, L/W ratio = 3.0.

Conidiomata pycnidial, observed on pine needles, grapevine wood, on SNA and OA after four wk, solitary or in groups, superficial or immersed, globose, subglobose to ellipsoidal, 20–150 µm diam, unilocular, opening by irregular rupture, pycnidial wall composed of *textura angularis*, 8–19 µm thick, 2–6 cell layers. *Conidiophores* reduced to conidiogenous cells lining the inner cavity of the conidiomata. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, phialides discrete, ampulliform, 3.5–8 × 2.5–4 µm, collarettes inconspicuous, opening 0.5–1.5 µm wide. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to oblong-elliptical, sometimes slightly curved, (3–)4(–5) × (1–)1.5(–2) µm, L/W ratio = 2.3.

Culture characteristics: Colonies on PDA raised, radially striate with lobate to crenated margin, moist, saffron to luteous, aerial mycelium sparse; reverse saffron; on OA flat, with entire margin, moist, in the centre greenish olivaceous to dark herbage green due to pycnidia formation, with a buff margin, aerial mycelium abundant, sometimes funiculose; reverse same colours; on MYA flat, with lobate margin, moist, saffron, aerial mycelium sparse; reverse same colours; 2–4.5 mm diam after 14 d on MYA (25 °C, in the dark), min 10 °C, max 25 °C, opt 20 °C.

Notes: The formation of up to 6-celled conidiophores, typically constricted at the septa, distinguishes *N. constricta* from its close relatives *N. ossiformis*, *N. corymbiae*, *N. eucalyptigena*, *N. eucalypti*, *N. niveniae* and *N. zymoides*. Within this group of slow-growing fungi studied here, *N. constricta* was the one with the slowest growth on PDA, which is an additional feature of this fungus (Fig. 4). Comparison of the *tub2*, ITS and LSU sequences showed that *N. constricta* is 93 % (24 nucleotides different), 98 % (10 nucleotides different) and 99 % (6 nucleotides different), respectively, identical with its closest relative *N. ossiformis*, while *N. zymoides* is 82 %, 95 % and 97 % identical. The ITS and LSU sequences of *N. constricta* are 93 % and 98 % identical with *N. corymbiae* (36 and 8 nucleotides different) and 97 % and 98 % with *N. eucalyptigena* (18 and 9 nucleotides different; Crous *et al*. 2018), respectively. A blastn search with the ITS sequence of *N. constricta* resulted in 97–99 % identity (8–16 nucleotides different) with sequences of eight fungal strains (identified as *Eurotiomycetes*, *Phaeomoniella* sp. or referred to as uncultured fungus), most of them described as endophytes of trees in Arizona (USA), New Zealand, New Mexico (USA) and Canada (KP202999, GQ999270, JN225892, KT264520, KT264593, KF742578, GQ153143, GQ153196; Hoffman & Arnold 2010, Johnston *et al*. 2012, Bérubé & Nicolas 2015, Chen *et al*. 2015). Additionally, 38 endophytic fungi isolated from leaves of pine trees in Arizona (USA) display a high accordance (98–99 %; 7–11 nucleotides different) with *N. constricta* (Bowman & Arnold 2018).

Neophaeomoniella ossiformis C. Kraus, Damm, S. Bien, Vögele & M. Fisch., ***sp. nov.*** MycoBank MB828289. Figs 4F, 10.

Etymology: Named after the shape of the conidia mother cells of the microcyclic conidiation that are sometimes ossiform.

Typus: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6 May 2016, *C. Kraus* (GLM-F117498 holotype, culture ex-type JKI-May03 = CBS 145013 = GLMC 1913).

Vegetative hyphae hyaline, smooth-walled, 1.5–3 µm wide, septate, partly inflated up to 8 µm wide. *Sporulation* abundant; conidia formed on hyphae, in pycnidia and by microcyclic conidiation. *Conidiophores on hyphae* hyaline, smooth-walled, mainly reduced to conidiogenous cells, occasionally 2–3-celled conidiophores observed, 6.5–22.5 × 2–3.5 µm. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, mostly reduced to openings directly formed on hyphal cells, discrete phialides sometimes observed, subglobose to elongate navicular, 4.5–11.5 × 2–3.5 µm, collarettes rarely observed, periclinal thickening inconspicuous. *Conidia* accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, cylindrical, sometimes slightly curved, (2.5–)3.5(–4.5) × (1–)1.5(–2) µm, L/W ratio = 2.7.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, obovate to oblong-elliptical, sometimes ossiform, (6–)8(–10) × (1.5–)2.5(–3) µm, L/W ratio = 3.3.

Conidiomata pycnidial, produced superficially on pine needles, grapevine wood and immersed in SNA medium after four wk, mainly solitary, globose, subglobose to ellipsoidal, 40–360 µm diam, unilocular, opening by irregular rupture, pycnidial wall composed of *textura angularis*, 10–16 µm thick, 3–6 cell layers. *Conidiophores* reduced to conidiogenous cells lining the inner cavity of the pycnidium. *Conidiogenous cells* enteroblastic, hyaline, smooth-walled, phialides discrete, ampulliform to flask-shaped, 3.5–7 × 2.5–5 µm, opening 0.5–1.5 µm wide, collarettes inconspicuous, periclinal thickening not observed. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to oblong-elliptical, sometimes slightly curved, (3–)3.5(–4) × (1–)1.5(–2) µm, L/W ratio = 2.3.

Culture characteristics: Colonies on PDA raised, radially striate with lobate to crenated margin, moist, buff, grey olivaceous in centre due to pycnidia formation, aerial mycelium in centre sparse, funiculose; reverse buff; on OA flat with entire edge, moist, buff to grey olivaceous with whitish margin, aerial mycelium sometimes formed in centre, sparse, funiculose; reverse same colours; on MYA raised, radially striate with lobate margin, moist, buff, grey olivaceous in centre due to pycnidia formation, aerial mycelium abundant, whitish, funiculose; reverse buff; 7–9 mm diam after 14 d on MYA (25 °C, in the dark), min 10 °C, max 25 °C, opt 20 °C.

Additional materials examined: **Germany**, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6 May 2016, *C. Kraus*, JKI-May02 = CBS 145014 = GLMC 1912; Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6 May 2016, *C. Kraus*, JKI-May30 = GLMC 1914.

Notes: *Neophaeomoniella ossiformis* can be distinguished from the closely related *N. constricta* by its faster growth and shorter

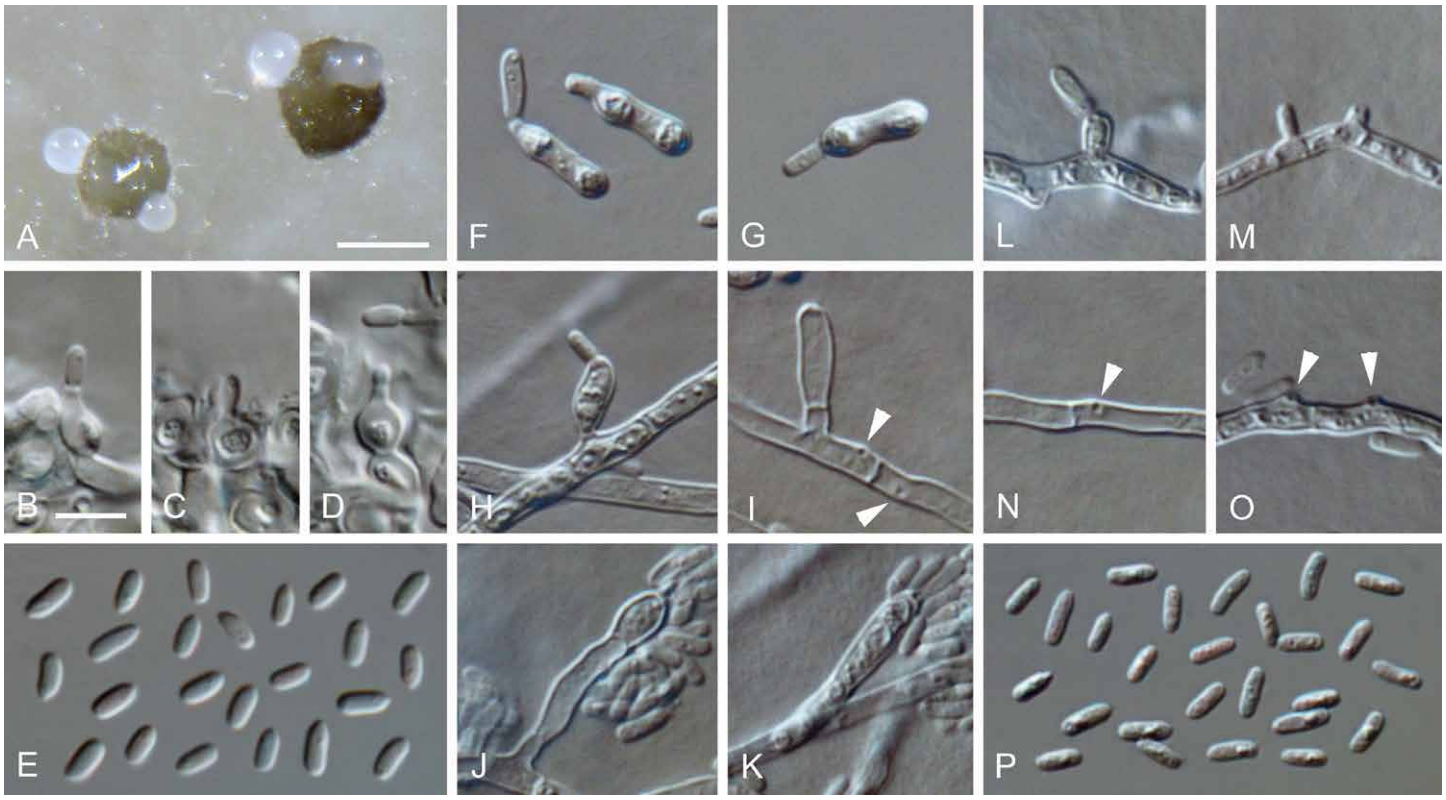

Fig. 10. *Neophaeomoniella ossiformis* (ex-type culture JKI-May03). **A.** Pycnidia formed on OA. **B–D.** Conidiogenous cells from the inner cell wall of pycnidia. **E.** Conidia formed in pycnidia. **F–G.** Microcyclic conidiation. **H–O.** Conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings). **P.** Conidia formed on hyphal cells. A: SM; B–P: LM. Scale bars: A = 100 µm; B = 5 µm; B applies to B–P.

conidiophores that are usually less strongly constricted at the septa. The *tub2*, ITS and LSU sequences of *N. ossiformis* are 93 % (24 nucleotides different), 98 % (10 nucleotides different) and 99 % (6 nucleotides different) identical with the sequences of *N. constricta*. The ITS and LSU sequences of *N. ossiformis* are 94 % (32 nucleotides different) and 99 % (2 nucleotides different) identical with those of *N. corymbiae*, respectively, and 96 % (20 nucleotides different) and 99 % (4 nucleotides different) with those of *N. eucalyptigena*, respectively (Crous *et al*. 2018). A blastn search of the ITS sequence of *N. ossiformis* produced similar results as for *N. constricta* (see above).

Pathogenicity

Until the day of evaluation, *i.e.* after six months, infected plants showed no external symptoms of esca or other GTDs. However, longitudinal sections of the trunks unveiled discolorations at the inoculation site in all plants. The measured mean lesion length revealed significant differences between *P. chlamydospora* (22.7 ± 13.8 mm and 20.4 ± 11.2 mm, respectively) and the water control (6.1 ± 0.9 mm and 6.5 ± 1.7 mm, respectively) for both cultivars, Pinot noir and Müller-Thurgau (Table 3). However, the lesion length of the seven other examined species of the *Phaeomoniellales* showed no differences to the water control. For Pinot noir the produced mean lesions of the tested fungi ranged from 6.0 ± 1.2 mm (*Mo. palatinum*) to 7.5 ± 2.4 mm (*N. zymoides*). Similarly, for Müller-Thurgau the mean lesion varied between 6.2 ± 1.5 mm (*Mo. palatinum*) and 10.5 ± 4.1 mm (*N. zymoides*).

Wood pieces from the induced lesions were placed on MYA plates to verify the presence of the inoculated fungi in order to fulfil Koch's postulates. *Phaeomoniella chlamydospora* was re-isolated most frequently (46.7 %) from infected Pinot noir plants. The seven other studied fungi reached a re-isolation rate between 14.3 % (*A. palatina*) and 30.0 % (*Mo. germanicum*). *Moristroma palatinum* could not be re-isolated from Pinot noir plants. Concerning the Müller-Thurgau vines, *P. chlamydospora* again showed the highest re-isolation rate with 66.7 %. The remaining fungi were recovered from 6.7 % (*Mo. palatinum*) to 61.5 % (*M. simplex*).

DISCUSSION

During this study, spore traps made of sticky glass slides were positive for fungi of the *Phaeomoniellales* in eleven out of 56 monitored wk (20 %). A similar trapping method was applied in Californian vineyards that were monitored from February to July; spores of *Phaeomoniella chlamydospora* were detected in nine out of 24 wk (38 %) (Eskalen & Gubler 2001), while Larignon & Dubos (2000) detected *P. chlamydospora* spores in 15 wk (15 %) during a 2-yr study with a sampling size of thirty collected glass slides each week. Both studies focused on *P. chlamydospora* spores and do not report the detection of other *Phaeomoniellales*. Since the detection rate was low in all three studies, it is assumed that in general the spore concentration of *Phaeomoniellales* in the air is relatively low or that the applied spore trapping method does not enable a higher trapping rate. Especially the trapping technique seems to be a critical point for successful monitoring of *Phaeomoniellales*. Although *P. chlamydospora* is common in South African vineyards, it was not detected by spore trapping, which could be due to the use of air sampling traps specifically made for the collection of wind distributed spores, *e.g.* Hirst or Burkard spore traps (Hirst 1952, Mostert *et al*. 2006, van Niekerk *et al*. 2010). However, slimy spores like those of *P. chlamydospora* and its relatives are more likely to be distributed by rain droplets, pruning scissors or even insects than by air (Aroca *et al*. 2010, Moyo *et al*. 2014). That may be the reason why glass slides with a sticky surface, closely attached to grapevines, are more effective in trapping spores of *Phaeomoniellales* than strict air sampling devices.

Only two of the eight identified species of the family *Phaeomoniellales*, namely *P. chlamydospora* and *N. zymoides*, were known species. *Phaeomoniella chlamydospora* is a known pathogen almost exclusively associated with grapevine; only once it was isolated from symptomatic wood of olive trees and from *Convolvulus arvensis* (Agustí-Brisach *et al*. 2010, Úrbez-Torres *et al*. 2013, Farr & Rossman 2018). *Neophaeomoniella zymoides* was first described from healthy pine needles and later isolated from necrotic wood of *Prunus salicina* (Lee *et*

Table 3. Results of the pathogenicity test performed with eight species of the *Phaeomoniellales*. The test was conducted with grapevine varieties Pinot noir and Müller-Thurgau in a greenhouse. Shown is the mean total lesion length measured up- and downwards from the inoculation point in mm and the re-isolation rate. Significant differences ($p < 0.001$) between mean lesion lengths according to an ANOVA are indicated by different letters.

		Pinot noir		Müller-Thurgau	
Accession no.[a]	Fungal species	Mean lesion length [mm]	Re-isolation rate [%]	Mean lesion length [mm]	Re-isolation rate [%]
JKI-Ap04	*Phaeomoniella chlamydospora*	22.7 ± 13.8^{A}	46.7	20.4 ± 11.2^{A}	66.7
JKI-Ap36*	*Aequabiliella palatina*	6.1 ± 2.4^{B}	14.3	7.2 ± 3.8^{B}	7.7
JKI-Jn27*	*Minutiella simplex*	6.6 ± 2.6^{B}	21.4	8.2 ± 2.9^{B}	61.5
JKI-Feb06*	*Moristroma germanicum*	6.0 ± 1.4^{B}	30	6.8 ± 1.1^{B}	38.5
JKI-Feb17*	*Mo. palatinum*	6.0 ± 1.2^{B}	0	6.2 ± 1.5^{B}	6.7
JKI-Mz35*	*Neophaeomoniella constricta*	7.2 ± 4.0^{B}	15.4	8.1 ± 2.2^{B}	14.3
JKI-May03*	*N. ossiformis*	7.1 ± 1.9^{B}	27.3	8.6 ± 3.1^{B}	50
JKI-Mz41	*N. zymoides*	7.5 ± 2.4^{B}	15.4	10.5 ± 4.1^{B}	50
–	Control (water)	6.1 ± 0.9^{B}	0	6.5 ± 1.7^{B}	0

[a] JKI: Culture collection of the Julius Kühn-Institute, Siebeldingen, Germany.

*Ex-type cultures.

al. 2006, Damm *et al*. 2010). Two of the newly described species, *N. constricta* and *N. ossiformis*, are closely related to *N. corymbiae*, *N. eucalypti*, *N. eucalyptigena*, *N. niveniae* and *N. zymoides*, while *Aequabiliella palatina* and *Minutiella simplex* are closely related to *A. effusa*, *M. pruni-avium* and *M. tardicola*, respectively. The latter three were also associated with symptomatic wood of *Prunus* trees (Damm *et al*. 2010, Bien & Damm 2020). *Moristroma germanicum* and *Mo. palatinum* were assigned to the genus *Moristroma*; the other four species of this genus, *Mo. japonicum*, *Mo. quercinum*, *Mo. multisporum* and *Mo. polysporum*, had been described from branches of *Quercus mongolica* var. *grossoserrata*, *Q. robur*, dead wood of *Terminalia arjuna* and decorticated wood of *Eucalyptus viminalis*, respectively (Sivanesan *et al*. 1988, Romero & Samuels 1991, Nordén *et al.* 2005).

This is the first report of *Phaeomoniellales* other than *P. chlamydospora* in vineyards. Due to the harm, which this esca associated pathogen can cause, the question emerged, if the species detected are also pathogenic to grapevine. However, the pathogenicity test showed that only *P. chlamydospora* is able to induce necrosis in the wood of potted grapevine plants. This led to the conclusion that these species are not pathogenic to grapevine. Furthermore, it is likely that grapevine wood is not the natural habitat of these species, since they have to date never been found in or on any parts of grapevine plants (Casieri *et al*. 2009, González & Tello 2011, Hofstetter *et al*. 2012, Pancher *et al*. 2012, Bruez *et al*. 2014, Pinto *et al*. 2014, Bruez *et al*. 2016, Travadon *et al*. 2016, Farr & Rossman, 2018, Kraus *et al*. 2018). Based on the habitats of previously described *Phaeomoniellales* species, the new species might originate from woody plants. Five species belonging to four genera of the *Phaeomoniellales* have previously been collected from wood of *Prunus* trees in South Africa (Damm *et al*. 2010). One of these species, *N. zymoides,* was originally described from pine needles in Korea (Lee *et al*. 2006) and was the most abundant species in our study. Moreover, Bien & Damm (2020) recently isolated a fungus with high similarity to the ITS (100 % identical) and *tub2* (99.8 % identical, 1 nucleotide different) sequence of *M. simplex* from necrotic wood of *P. domestica* in the region of Baden-Württemberg, Germany. Therefore, the *Prunus* trees planted close (in about 100 m distance) to the vineyard sampled could be one possible origin of the collected spores in this study. Two independent investigations on endophytic fungi in wood of pine trees in Montana, USA and Finland revealed unidentified fungal strains with high similarity (99 %, 2–3 nucleotides different) to the ITS sequence of *A. palatina* (Larkin *et al*. 2012, Müller *et al*. 2018). These findings may indicate that *A. palatina* also appears endophytically and that pine trees represent a possible host genus.

With the data generated in this study, the comparatively new order *Phaeomoniellales* will be extended by six new species. However, with the information at hand, the origin of the trapped fungal spores and their ecological traits remain basically unknown. The pathogenicity test conducted in this study does not support them to be pathogens of grapevine. However, this is no proof that these fungi do not live in or on grapevine wood. Further studies are necessary to reveal the origin and life style of the newly described species and their possible impact for example on economically important fruit crops.

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