Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

New records of *Penicillium* and *Aspergillus* from withered grapes in Italy, and description of *Penicillium fructuariae-cellae* sp. nov.

Marilinda LORENZINI[1,*], Maria Stella CAPPELLO[2], Giancarlo PERRONE[3], Antonio LOGRIECO[3], Giacomo ZAPPAROLI[1]

[1] *Università degli Studi di Verona, Dipartimento di Biotecnologie, 37134 Verona, Italy*
[2] *Istituto di Scienze delle Produzioni Alimentari, CNR, 73100 Lecce, Italy*
[3] *Istituto di Scienze delle Produzioni Alimentari, CNR, 70126 Bari, Italy*
*Corresponding authors: lorenzini.marilinda@gmail.com

Summary. *Penicillium* and *Aspergillus* are common pathogenic fungi of grapes, that occur frequently on withered berries used in the Italian passito wine production. Members of these genera isolated from withered grapes were identified using molecular and morphological approaches. The isolates were examined by amplification of internal transcribed spacer region, β-tubulin, calmodulin and RNA polymerase II second largest subunit. *Penicillium bilaiae, Aspergillus pallidofulvus* and *A. puulaauensis* are reported for the first time from *Vitis vinifera.* Two *Penicillium* isolates showed a distinct phylogenetic position and different morphological characteristics from *P. bissettii* and *P. vasconiae*, the two most closely related species. These isolates are assigned to the new species *Penicillium fructuariae-cellae*, that is here described. An *in vitro* pathogenicity assay was carried out to evaluate the infectivity to grape berries by *Penicillium* and *Aspergillus* isolates recovered in this study. All examined isolates colonized the berries when artificially inoculated, but to a lesser extent than *Botrytis cinerea*. This suggests that these fungi may contribute, with other pathogenic species, to the onset of post-harvest diseases of grapes.

Keywords. Saprophytic/pathogenic fungi, grapes, phylogeny, taxonomy, post-harvest diseases.

OPEN ACCESS

Citation: Lorenzini M., Cappello M.S., Perrone G., Logrieco A., Zapparoli G. (2019) New records of *Penicillium* and *Aspergillus* from withered grapes in Italy, and description of *Penicillium fructuariae-cellae* sp. nov.. *Phytopathologia Mediterranea* 58(2): 323-340. doi: 10.14601/Phytopathol_Mediter-10619

Accepted: June 115, 2019

Published: September 14, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Dimitrios I. Tsitsigiannis, Agricultural University of Athens, Greece.

INTRODUCTION

Fungal contamination of grapes causes severe and economically important losses for world food and beverage industries. Table, wine and raisin grapes can be infected by several species of fungi, on grapevines and/or during post-harvest processes. Grapes for Italian passito wine production are particularly vulnerable to fungal infections, during withering carried out in fruit drying rooms (*fruttaio*) (off-vine withering) (Mencarelli and Tonutti, 2013).

Penicillium and *Aspergillus* spp. are among the most frequent saprophytic fungal pathogens on withered grapes (Torelli *et al*., 2006; Lorenzini *et al*., 2016; Stefanini *et al*., 2017). Their presence is very important since they are

causal agents of bunch rot and can be mycotoxin producers (Torelli *et al*., 2006; Somma *et al*., 2012). In addition, grape contamination by these fungi can lower the quality of the resulting wines. The detrimental effects of withered grapes infected by *P. expansum* and *P. crustosum* on the quality of Amarone wine, a dry red passito wine, has recently been documented (Zapparoli *et al*., 2018).

During previous surveys on fungi associated with withered grapes (Lorenzini *et al*., 2016, 2018), eight species of *Penicillium* (*P. adametzoides*, *P. expansum, P. crocicola*, *P. crustosum, P. glabrum*, *P. griseofulvum*, *P. oxalicum* and *P. ubiquetum*) and five species of *Aspergillus* (*A. flavus*, *A. sydowii, A. tubingensis*, *A. uvarum* and *A. welwitschiae*) were identified by phylogenetic analyses. The placements of two isolates (*Penicillium* sp. P3 and *Aspergillus* sp. AS100) were not clear enough for reliable species delimitations.

In the present study, isolates P3 and AS100 were phylogenetically and morphologically analyzed to clarify their taxonomic positions. Three isolates of *Penicillium* and *Aspergillus*, recovered from withered grapes during the current survey, were also identified. These fungi belonged to species that are reported for the first time from *Vitis vinifera*. Two isolates were assigned to a new species of *Penicillium*. The pathogenicity of fungi isolated from grape berries was also assayed.

MATERIALS AND METHODS

On the basis of our previous studies (Lorenzini *et al*., 2016; 2018) carried out on grape berries of the Garganega and Corvina varieties, collected from fruit-drying rooms located in two Northern Italian winemaking areas (Soave and Valpolicella), five representative strains of *Aspergillus* and *Penicillium* were isolated and identified. Three isolates recovered from withered grapes in this study (designated Pdb1, Pls8 and ASls13) were isolated according to Lorenzini *et al.* (2016). The other two isolates (P3 and AS100) were obtained during a previous sampling (Lorenzini *et al.,* 2016). These isolates are deposited at the Westerdijk Fungal Biodiversity Institute (CBS, Utrecht, the Netherlands) and ITEM Agro-Food Microbial Culture Collection of the Institute of Science and Food Production (CNR-ISPA, Bari, Italy) (Table 1).

DNA was extracted from pure culture of each isolate as previously described (Lorenzini and Zapparoli, 2014). Each DNA extract was used to amplify the internal transcribed spacer (ITS) region, using primers ITS1/ITS4 (White *et al.,* 1990), partial β-tubulin gene (*benA*), using primers Bt2a/Bt2b (Glass and Donaldson, 1995), partial calmodulin gene (*CaM*), using primers cmd5/cmd6 (Hong *et al*., 2006), and parts of the second largest subunit of RNA polymerase II (*rpb2*), using primers fRPB2-5F2/fRPB2-7C (Liu *et al.,* 1999). The amplified products were purified using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel), and were sequenced in both directions using the same primers applied for amplification (Eurofins Genomics, Edersberg, Germany). The generated sequences were deposited at GenBank (Table 2).

Combined and individual analyses were conducted using the partial DNA sequences of five isolates recovered from withered grapes, and other reference taxa belonging to the genus *Penicillium* (sections *Lanata–divaricata* and *Sclerotiora*) and *Aspergillus* (*A. versicolor* clade and section *Circumdati*), retrieved from GenBank (Table 2). Maximum Likelihood (ML) analysis of the combined data sets was performed using MEGA7 v. 7.0.25 software. The combined data sets were analysed as three or four distinct partitions. For each individual data set, the most optimal substitution model was calculated in MEGA7 (Kumar *et al*., 2016) using the Akaike Information Criterion (AIC). Maximum Likelihood analyses of the individual data sets were also conducted using MEGA7, and robustness of the trees was evaluated by 1,000 bootstrap (BS) replicates. A second measure for statistical support was performed using Bayesian Evolutionary Analysis Sampling Trees (BEAST) Version v1.10.1, 2002-2018 (Drummond and Rambaut, 2007), and the previously obtained most optimal substitution model was used in the analyses. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. Burn-in was set to 25%

Table 1. Fungus isolates recovered from withered grape berries in this study.

Specie	Isolate designation	CBS number	ISPA number	Grape variety
Penicillium fructuariae-cellae	P3	CBS 145110^{T}	ITEM 18276^{T}	Corvina
Penicillium fructuariae-cellae	Pdb1	CBS 145111	ITEM 18277	Garganega
Penicillium bilaiae	Pls8	CBS 145112	ITEM 18278	Garganega
Aspergillus pallidofulvus	ASls13	CBS 145108	ITEM 18279	Garganega
Aspergillus puulaauensis	AS100	CBS 143103	ITEM 18280	Corvina

Table 2. Isolates used in phylogenetic analyses.

Species	Strain identification	Source	Locality	GenBank numbers			
				ITS	β-tubulin	Calmodulin	*rpb2*
Aspergillus section *Circundati*							
A. affinis	ATCC MYA-4773^T	Leaf litter	Italy	GU721090	GU721092	GU721091	-
A. auricomus	NRRL 391^T	ni	ni	EF661411	EF661320	EF661379	-
A. bridgeri	NRRL 13000^T	ni	ni	EF661404	EF661335	EF661358	-
A. cretensis	NRRL 35672^T	ni	ni	FJ491572	AY819977	FJ491534	-
A. elegans	NRRL 4850^T	ni	ni	EF661414	EF661349	EF661390	-
A. fresenii	NRRL 4077^T	Soil	India	EF661409	EF661341	EF661382	-
A. insulicola	NRRL 6138^T	Soil	Venezuela	EF661430	EF661353	EF661396	-
A. melleus	NRRL 5103^T	Soil	India	EF661425	EF661326	EF661391	-
A. muricatus	NRRL 35674^T	Grassland soil	Philippines	EF661434	EF661356	EF661377	-
A. neobridgeri	NRRL 13078^T	Soil	USA	EF661410	EF661345	EF661359	-
A. occultus	CBS 137330^T	Air sample	Netherlands	KJ775443	KJ775061	KJ775239	-
A. ochraceopetaliformis	NRRL 4752^T	Scalp lesion	Brazil	EF661429	EF661350	EF661388	-
A. ochraceus	NRRL 398^T	ni	ni	EF661419	EF661322	EF661381	-
A. ostianus	NRRL 420^T	ni	ni	EF661421	EF661324	EF661385	-
A. pallidofulvus	NRRL 4789^T	ni	ni	EF661423	EF661328	EF661389	-
*A. pallidofulvus**	ITEM 18279 = CBS 145108	Vitis vinifera	Italy	MK039437	MK045335	MK045340	-
A. persii	NRRL 35669^T	Toenail	Italy	FJ491580	AY819988	FJ491559	-
A. pseudoelegans	CBS 112796^T	Soil	Costa Rica	FJ491590	AY819962	FJ491552	-
A. pseudosclerotiorum	UTHSCSA DI15-13^T	Lung biopsy	-	LT574713	LT574748	LT574783	-
A. pulvericola	CBS 137327^T	Indoor hause	Micronesia	KJ775440	KJ775055	KJ775236	-
A. robustus	NRRL 6362^T (outgroup)	Soil	Kenya	EF661176	EU014101	EF661357	-
A. roseoglobulosus	NRRL 4565^T	Decaing leaves	Bahamas	FJ491583	AY819984	FJ491555	-
A. salwaensis	DTO 297B3^T	Soil	Qatar	KJ775447	KJ775056	KJ775244	-
A. sclerotiorum	NRRL 415^T	Apple	USA	EF661400	EF661337	EF661384	-
A. sesamicola	CBS 137324^T	Sesame sead	Denmark	KJ775437	KJ775063	KJ775233	-
A. steynii	NRRL 35675^T	Grean bean coffee	India	EF661416	EF661347	EF661378	-
A. subramanianii	NRRL 6161^T	Shelled brazil nuts	Canada	EF661403	EF661339	EF661397	-
A. tanneri	NRRL 62425^T (outgroup)	Human lung	USA	JN853798	JN896582	JN896583	-
A. westerdijkiae	NRRL 3174^T = CBS 112803	*Andropogum sorghum*	South Africa	EF661427	EF661329	EF661360	-
A. westlandensis	CBS 137321^T	Air sample	Netherlands	KJ775434	KJ775066	KJ775230	-
A. versicolor clade							
A. amoenus	NRRL 4838^T	ni	ni	NR_137462	EF652304	EF652392	-
A. austroafricanus	NRRL 233^T	ni	South Africa	NR_135443	JN853963	JN854025	-
A. creber	NRRL 58592^T	Indoor air sample	USA	NR_135442	JN853980	JN854043	-

(Continued)

Table 2. (Continued).

Species	Strain identification	Source	Locality	GenBank numbers			
				ITS	β-tubulin	Calmodulin	*rpb2*
A. cvjetkovicii	NRRL 227^{T}	Soil	USA	EF652440	EF652264	EF652352	-
A. fructus	NRRL 239^{T}	Date fruit	USA	EF652449	EF652273	EF652361	-
A. griseoaurantiacus	DTO_267D8^{T}	Indoor air dust	Micronesia	KJ775553	KJ775086	KJ775357	-
A. hongkongensis	HKU49^{T}	Human nail	Hong Kong	AB987907	LC000552	LC000565	-
A. jensenii	NRRL 58600^{T}	Indoor air sample	USA	NR_135444	JN854007	JN854046	-
A. multicolor	NRLL 4775^{T} (outgroup)	ni	ni	EF652477	EF652301	EF652389	-
A. protuberus	NRRL 3505^{T}	Brined meat	UK	NR_135353	EF652284	EF652372	-
A. puulaauensis	NRRL 35641^{T}	Dead hardwood branch	USA	NR_135445	JN853979	JN854034	-
A. puulaauensis	**ITEM 18280 = CBS 145103**	**Fruit of *Vitis vinifera***	Italy	MK039438	MK045336	KU554606	-
A. sydowii	NRRL 250^{T}	ni	n	EF652450	EF652274	EF652362	-
A. tabacinus	NRRL 4791^{T}	ni	ni	NR_135361	EF652302	EF652390	-
A. tennesseensis	NRRL 13150^{T}	Ex toxic dairy feed	USA	NR_135447	JN853976	JN854017	-
A. venenatus	NRRL 13147^{T}	Toxic dairy feed	USA	NR_135448	JN854003	JN854014	-
A. versicolor	NRRL 238^{T}	ni	USA	EF652442	EF652266	EF652354	-
A.subversicolor	NRRL 58999^{T}	Coffee berry	India	NR_135446	JN853970	JN854010	-
Penicillium section *Lanata–divaricata*							
P. abidjanum	CBS 246.67^{T}	Soil	Ivory Coast	NR_111502	GU981650	KF296383	JN121469
P. amphipolaria	DAOMC 250551^{T} = KAS 2555 = CBS 140997	Leaves	Panama	KT887872	KT887833	KT887794	na
P. annulatum	CV 37 = CBS 135126^{T}	Soil	South Africa	NR_138303	JX091514	JX141545	KF296410
P. araracuarense	CBS 113149^{T}	Leaf litter	Colombia	GU981597	GU981642	KF296373	KF296414
P. austrosinenese	CGMCC 3.18797^{T} = CBS 144505	Acidic soil	China	KY495003	KY495112	KY494943	KY495061
P. bissettii	DAOMC 167011^{T} = KAS1951 = CBS 140972	Soil	Canada	KT887845	KT887806	KT887767	KY495055
P. brasilianum	CBS 253.55^{T}	Herbarium specimen	Brazil	AF178512	GU981629	AB667857	KF296420
P. brefeldianum	CBS 235.81^{T}	Human alimentary tract	ni	GU981580	GU981623	AB667857	KF296421
P. camponotum	KAS2177 = DAOMC 250557^{T} = CBS 140982	Ants	Canada	KT887855	KT887816	KT887777	na
P. caperatum	CBS 443.75^{T}	Soil	Australia	KC411761	GU981660	KF296392	KF296422
P. cataractum	CBS 140974 = KAS2145 = DAOMC 250534^{T}	Nuts of *Carya cordiformis*	Canada	KT887847	KT887808	KT887769	na
P. fructuariae-cellae	**ITEM 18276^{T} = CBS 145110^{T}**	**Fruit of *Vitis vinifera***	**Italy**	**MK039434**	KU554679	MK045337	MK520927
P. fructuariae-cellae	**ITEM 18277 = CBS 145111**	**Fruit of *Vitis vinifera***	**Italy**	**MK039435**	**MK045333**	**MK045338**	**MK520928**
P. cluniae	CBS 326.89^{T}	Soil	Spain	KF296406	KF296471	KF296402	KF296424
P. coeruleum	CBS 141.45^{T}	ni	ni	NR_138293	GU981655	KF296393	KF296425
P. cremeogriseum	CBS 223.66^{T}	Soil	Ukraine	NR_111505	GU981624	KF296403	KF296426
P. curticaule	CV2842 = CBS 135127^{T}	Soil	South Africa	FJ231021	JX091526	JX141536	KF296417
P. daleae	CBS 211.28^{T}	Soil	Poland	NR_111503	GU981649	KF296385	KF296427
P. echinulonalgiovense	CBS 328.59^{T}	Soil	Japan	GU981587	GU98163	KX961269	KX961301

(Continued)

Table 2. (Continued).

Species	Strain identification	Source	Locality	GenBank numbers			
				ITS	β-tubulin	Calmodulin	*rpb2*
P. ehrlichii	CBS 324.48^T	ni	Poland	GU981578	KF296464	KF296395	KF296428
P. elleniae	CBS 118135^T	Leaf litter	Colombia	GU981612	GU981663	KF296389	KF296429
P. excelsum	HF-2015 = CCT 7772^T	Nut shell	Brazil	KR815341	KP691061	KR815342	na
P. flaviroseum	CGMCC 3.18805^T = CBS 144479	Acidic soil	China	KY495032	KY495141	KY494972	KY495083
P. glabrum	CBS 125543^T (outgroup)			GU981567	GU981619	KM089152	JF417447
P. glaucoroseum	NRRL 908^T = CBS 138908	Soil	USA	KF296407	KF296469	KF296400	KF296430
P. globosum	CGMCC 3.18800^T = CBS 144639	Acidic soil	China	KY495014	KY495123	KY494954	KY495067
P. griseoflavum	CGMCC 3.18799^T = CBS 144525	Acidic soil	China	KY495011	KY495120	KY494951	KY495064
P. griseopurpureum	CBS 406.65^T	Soil	England	KF296408	KF296467	KF296384	KF296431
P. guangxiense	CGMCC 3.18793^T = CBS 144526	Soil	China	KY494986	KY495095	KY494926	KY495045
P. hainanense	CGMCC 3.18798^T = CBS 144527	Acidic soil	China	KY495009	KY495118	KY494949	KY495062
P. infrabuccalum	KAS2181^T= DAOMC 250537	Ants	Canada	KT887817	KT887817	KT887778	na
P. janthinellum	CBS 340.48^T	Soil	Nicaragua	NR_111504	GU981625	KF296401	JN121497
P. javanicum	CBS 341.48^T	Rut of *Cammelia sinensis*	Indonesia	NR_111511	GU981657	KF296387	JN121498
P. jianfenglingense	CGMCC 3.18802^T = CBS 144640	Soil	China	KY495016	KY495125	KY494956	KY495069
P. koreense	KACC 47721^T	Soil	Korea	KJ801939	KM000846	na	na
P. laevigatum	CGMCC 3.18801^T = CBS 144481	Acidic soil	China	KY495015	KY495124	KY494955	KY495068
P. levitum	CBS 345.48^T	Modeling clay	USA	NR_111510	GU981654	KF296394	KF296432
P. limosum	CBS 339.97^T	Marine sediment	Japan	NR_111496	GU981621	KF296398	KF296433
P. lineolatum	CBS 188.77^T	Soil	Japan	NR_111500	GU981620	KF296397	KF296434
P. ludwigii	CBS 417.68^T	Polished rice	Japan	NR_138339	KF296468	KF296404	KF296435
P. malacosphaerulum	CV2855 = CBS 135120^T	Soil	South Africa	FJ231026	JX091524	JX141542	KF296438
P. mariae-crucis	CBS 271.83^T	*Secale cereale*	Spain	NR_111506	GU981630	KF296374	KF296439
P. meloforme	CBS 445.74^T	Soil	Papua New Guinea	NR_153203	GU981656	KF296396	KF296440
P. ochrochloron	CBS 357.48^T	Copper sulphate solution	USA	NR_111509	GU981672	KF296378	KF296445
P. onobense	CBS 174.81^T	Soil	Spain	NR_111497	GU981627	KF296371	KF296447
P. ortum	CV 102 = CBS 135669^T	Soil	South Africa	NR_138304	JX091520	JX141551	KF296443
P. oxalicum	CBS 219.30^T (outgroup)	Soil	USA	MH855125	KF296462	KF296367	JN121456
P. panissanguineum	DAOMC 250562^T = KAS 2209 = CBS 140989	Termite mounds	Tanzania	KT887862	KT887823	KT887784	na
P. paraherquei	ATCC 22354 = CBS 338.59^T	Soil	Japan	AF178511	KF296465	KF296372	KF296449
P. pedernalense	CBS 140770^T	Waste compost	Ecuador	KU255398	KU255396	KY494968	KY495079
P. penarojense	CBS 113178^T	Leaf litter	Colombia	NR_138289	GU981646	KF296381	KF296450
P. piscarium	CBS 362.48^T	Cod-liver oil emulsion	Norway	NR_111507	GU981668	KF296379	KF296451
P. pulvillorum	CBS 280.39^T	Acidic soil	UK	NR_138292	GU981670	KF296377	KF296452
P. raperi	NRRL 2674 = CBS 281.58^T	Soil	UK	AF033433	GU981622	KF296399	KF296453

(Continued)

Table 2. (Continued).

Species	Strain identification	Source	Locality	GenBank numbers			
				ITS	β-tubulin	Calmodulin	*rpb2*
P. reticulisporum	NRRL 3447 = CBS 122.68^T	Soil	Japan	AF033437	GU981665	KF296391	KF296454
P. rolfsii	CBS 368.48^T	Pineapple	USA	JN617705	JU981667	KF296375	KF296455
P. rubriannulatum	CGMCC 3.18804^T = CBS 144641	Acidic soil	China	KY495029	KY495138	KY494969	KY495080
P. simplicissimum	CBS372.48^T	Flannel bag	South Africa	NR_138290	GU981632	KF296368	JN121507
P. singorense	DTO 133C6 = CBS 138214^T	House dust	Thailand	KJ775674	KJ775167	KJ775403	na
P. skrjabinii	CBS 439.75^T	Soil	Russia	NR_111498	GU981626	KF296370	EU427252
P. soliforme	CGMCC 3.18806^T = CBS 144482	Acidic soil	China	KY495038	KY495147	KY494978	KY495047
P. stolkiae	NRRL 5816 = CBS 315.67^T (outgroup)	ni	ni	NR_121233	JN617717	AF481135	JN121488
P. spiliferum	CGMCC 3.18807^T = CBS 14483	Acidic soil	China	KY495040	KY495149	KY494980	KY495090
P. subrubescens	DTO188-D6 = CBS 132785^T	Soil	Finland	KC346350	KC346327	KC346330	KC346306
P. svalbardense	CBS 122416^T	Glacial ice	Greenland	GU981603	KC346325	KC346338	KF296457
P. tanzanicum	DAOMC 250514^T = KAS 1946 = CBS 140968	ni	Tanzania	KT887841	KT887802	KT887763	KY495066
P. terrarumae	CBS 131811^T	Soil	China	KC346349	KC346326	KC346339	KC346316
P. vanderhammenii	CBS126216^T	Leaf litter	Colombia	NR_137577	GU981647	KF296382	KF296458
P. vasconiae	CBS 339.79^T	Soil	Spain	NR_138291	GU981653	KF296386	KF296459
P. viridissimum	CGMCC 3.18796^T = CBS 14484	Acidic soil	China	KY495004	KY495113	KY494944	KY495059
P. wotroi	CBS 118171^T	Leaf litter	Colombia	GU981591	GU981637	KF296369	KF296460
P. yunnanense	CGMCC 3.18794^T = CBS 14485	Acidic soil	China	KY494990	KY495099	KY494930	KY495048
P. zonatum	CBS 992.72^T	Coastal marsh soil	USA	NR_111501	GU981651	KF296380	KF296461
Penicillium section *Sclerotiora*							
P. adametzii	CBS 209.28^T	Soil	Canada	NR_103661	JN625957	KC773796	-
P. adametzioides	CBS 313.59^T	Soil	Japan	JN686433	JN799642	JN686387	-
P. alexiae	DTO 118H8^T	Soil	Tunisia	KC790400	KC773778	KC773803	-
P. amaliae	CV 1875 = CBS 134209^T	*Protea repens*	South Africa	JX091443	JX091563	JX141557	-
P. angulare	CBS 130293^T	Polypore on dead conifer	USA	KC773828	KC773779	KC773804	-
P. arianeae	DTO 20B8^T	Soil	Netherlands	KC773833	KC773784	KC773811	-
P. austrosinicum	HMAS 248734^T = CGMCC 3.18410	ni	ni	NR_153272	KX885041	KX885051	-
P. bilaiae	NRRL 3391^T	Soil	Ukraine	NR_111679	JN625966	JN626009	-
P. bilaiae	**ITEM 18278 = CBS 145112**	**Fruit of *Vitis vinifera***	**Italy**	**MK039436**	**MK045334**	**MK045339**	**-**
P. brocae	CBS 116113^T	Coffee berry	Mexico	NR_111868	KC773787	KC773814	-
P. cainii	DAOM 239914^T	Nuts of *Juglans nigra*	Canada	NR_120000	JN686366	JN686389	-
P. choerospondiatis	HMAS 248813^T = CGMCC 3.18411	ni	ni	NR_153274	KX885043	KX885053	-
P. daejeonium	CNU 100097 = KACC 46609^T	Grape fruit	South Korea	JX436489	JX436493	JX436491	-
P. exsudans	HMAS 248735^T = CGMCC 3.18412	ni	ni	NR_153273	KX885042	KX885052	-
P. guanacastense	DAOM 239912^T	Gut of caterpillar	Costa Rica	NR_111673	JN625967	JN626010	-

(Continued)

Table 2. (Continued).

Species	Strain identification	Source	Locality	GenBank numbers			
				ITS	β-tubulin	Calmodulin	*rpb2*
P. herquei	CBS 336.48^T	Leaf of *Agauria pirifolia*	France	JN626101	JN625970	JN626013	-
P. hirayamae	CBS 229.60^T	Milled rice	Thailand	JN626095	JN625955	JN626003	-
P. jacksonii	DAOM 239937^T	Forest soil	Australia	NR_111675	JN686368	JN686391	-
P. johnkrugii	DAOM 239943^T	Forest soil	Malaysia	NR_111676	JN686378	JN686401	-
P. jugoslavicum	CBS 192.87^T	Seed of *Helianthus annuus*	Ex Yugoslavia	NR_120269	KC773789	KC773815	-
P. lilacinoechinulatum	CBS 454.93^T	Soil	Japan	KC773837	KC773790	KC773816	-
P. malachiteum	CBS 647.95^T	Soil	Japan	NR_120271	KC773794	KC773820	-
P. mallochii	DAOM 239917^T	Caterpillar	Costa Rica	NR_111674	JN625973	JN626016	-
P. maximae	NRRL 2060^T	Cellophane	USA	NR_121343	KC773795	KC773821	-
P. restingae	MS-2014 = URM 7075^T	Soil	ni	KF803354	KF803349	KF803352	-
P. sanshaense	HMAS 248820^T = CGMCC 3.18413	ni	ni	NR_153276	KX885050	KX885060	-
P. sclerotiorum	NRRL 2074^T	Air	Indonesia	JN626132	JN626001	JN626044	-
P. vanoranjei	DTO 99H6^T	Soil	Tunisia	KC695696	KC695686	KC695691	-
P. verrucisporum	HMAS 248819^T = CGMCC 3.18415	ni	ni	KX885069	KX885049	KX885059	-
P. viticola	FKI-4410 = JCM 17636^T	Grape	Japan	AB606414	AB540174	AB540173	-
P. levitum	CBS 345.48^T (outgroup)	ni	ni	NR_111510	GU981654	KF296394	-

* Strains studied in this paper are in bold type;
- Sequences not used in this study;
^{T} = Ex-type strain; ni = no information about the source and locality; na = not available
ATCC, American Type Culture Collection (Manassas, VA); CBS, Westerdkjik Fungal Biodiversity Institute (Utrecht, the Netherlands); DTO, Applied and Industrial Mycology Department Collection, Westerdkjik Fungal Biodiversity Institute (Utrecht, Netherlands); NRRL, Agriculture Research Service Culture Collection (Peoria, NY); UTHSCSA, University of Texas Health Science Center (San Antonio, TX); HKU, University of Hong Kong, (Hong Kong, China). ITEM, Agro-Food Microbial Culture Collection of Institute of Science and Food Production (ISPA) (Bari, Italy); JCM, Japan Collection of Microorganism (Saitama, Japan); CGMCC. Chinese General Microbiological Culture Collection Center (Beijing, China); URM, University Recife Mycology Culture Collection (Recife, Brazil); KACC, Korean Agricultural Culture Collection (Suwon, South Korea); CCT, Coleção de Cultura Tropical (Campinas, Brazil); DAOMC, Canadian Collection of Fungal Cultures (Ottawa, Canada); DAOM, Canadian National Mycological Herbarium (Ottawa, Canada).

and Tracer v. 1.5.0 (Rambaut and Drummond, 2009) was used to confirm the convergence of chains. The phylograms obtained through the ML analyses were used for presenting the data. Phylograms were redrawn from the tree files using FigTree v1.4 2006-2012. Bootstrap values less than 70% and posterior probability (pp) values less than 0.95 were removed from the phylograms. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA7. All isolates of *Aspergillus* and *Penicillium* (Pdb1, P3, Pls8, ASls13 and AS100) were inoculated onto Czapek yeast extract agar (CYA), yeast extract sucrose agar (YES), creatine sucrose agar (CREA) (Visagie *et al.* 2014) and malt extract agar (MEA, 2% w v^{-1} malt extract, 0.1% w v^{-1} peptone, 2% w v^{-1} dextrose, 1.5% w v^{-1} agar) at 25°C in the darkness. The colony diameters (mm) were measured daily for 7 d (three replicate plates for each isolate) and the experiments were performed twice. The colony diameters were also measured on CYA at 15, 30 and 37°C. The cultural characteristics and micro-morphology of each strain was examined on CYA, YES and MEA after 7, 14 or 30 d at 25°C. Colony morphology was also examined on CREA after 7 d incubation.

Growth tests for the fungal isolates were carried out under acidic, neutral and alkaline conditions, as reported by Diao *et al.* (2018).

Preparations for microscopy were made from colonies grown on CYA, YES and MEA after 7, 14 or 30 d. Lactic acid (60% v v^{-1}) was used as mounting fluid and excess conidia were washed away with ethanol (70% v v^{-1}). ethanol. Characters were recorded and analyzed using stereomicroscopy (Leica EZ4D, Leica Microsystems). Measurements of fungal components were carried out using light microscopy (Leica DM750) equipped with camera module (Leica ICC50W). Lengths and widths were determined for 20 conidiophores, metulae and phialides, 50 conidia, 20 cleistothecia and 30 ascospores (when present) from each isolate.

The five isolates were tested for their ability to cause disease on grape berries (white fresh table and red withered wine grapes). The berries were surface sterilized by immersion for 5 min in 0.5% NaOCl solution, then rinsed twice with sterile distilled water and placed in compartmentalized square culture dishes. Suspensions of conidia were prepared, adjusted to 10^4 conidia mL^{-1} and then inoculated by berry piercing, as reported by Lorenzini and Zapparoli (2014). Mock inoculation (controls) consisted of berries wounded and inoculated with sterile water, and a positive control consisted of berries wounded and inoculated with *Botrytis cinerea* ITEM 17200. The experiment was performed twice, each with three replicates, which each consisted of 25 berries. After 7 and 14 d at 22°C, the disease index (DI) was assessed on a scale of 0 to 4, as previously described (Lorenzini and Zapparoli, 2014). Variance analysis (ANOVA) was used for the DI data to evaluate isolate differences in pathogenicity. Tukey's multiple comparison test (Tukey, HSD) was applied to determine statistically significant differences.

RESULTS

Phylogenetic analysis

Using the BLASTn tool in GenBank, the *benA*, *CaM*, ITS and *rpb2* gene sequences of Pdb1 (ITEM 18277) showed 99% similarity to *Penicillium* sp. P3 (ITEM 18276^T) (Lorenzini *et al.* 2016) for *benA*, greater than 89% similarity to *P. bissettii* and *P. annulatum* for *CaM*, greater than 98% similarity to different *Penicillium* species (e.g. *P. janthinellum, P. reticulisporum, P. ochrochloron, P. bissettii and P. javanicum*) for ITS, and 95% similarity to *Penicillium* sp. for *rpb2*. The alignment results of the *benA*, *CaM*, ITS and *rpb2* gene sequences of P3 were similar to those of Pdb1. Based on these data, the phylogenetic position of both isolates (Pdb1 and P3) was evaluated using members of *Penicillium* section *Lanata-divaricata*, according to Diao *et al.* (2018). The ML combined phylogenetic tree (*benA*+*CaM*+ITS+*rpb2*) with the greatest log likelihood (-21693.88) is shown in Figure 1a. The Pdb1 and P3 isolates were grouped together (BS/pp = 100/1), and were distantly related to *P. bissettii* DOAMC 167011 and *P. vasconiae* CBS 339.79. Data from phylogenetic analyses using *benA*, *CaM*, ITS and *rpb2* individually (data not shown) were in concordance with those based on the combine dataset. However, analyses of the combined dataset provided greater support than the individual datasets. The molecular identification of all isolates recovered in this study is based, therefore, on phylogeny from the combined dataset of gene sequences.

The comparative analysis by GenBank database of *benA*, *CaM* and ITS gene sequences of isolate Pls8 (ITEM 18278) showed 99% similarity to different strains of *Penicillium bilaiae* (section *Sclerotiora*). The phylogenetic position of Pls8 was therefore evaluated using members of *Penicillium* section *Sclerotiora*, reported by Wang *et al.* (2017). The combined phylogenetic tree (*benA*+*CaM*+ITS) with the greatest log likelihood (-11066.6111) confirmed the identification (Figure 1b), as the Pls8 (ITEM 18278) was grouped with *P. bilaiae* NRRL3391 (BS/pp = 100/1).

Comparative analysis using GenBank of *benA*, *CaM* and ITS gene sequences of ASls13 (ITEM 18279) showed 99% similarity to *A. melleus, A. pallidofulvus, A. sulphureus* and *A. petrakii* for *benA*, 99% similarity to *A.*

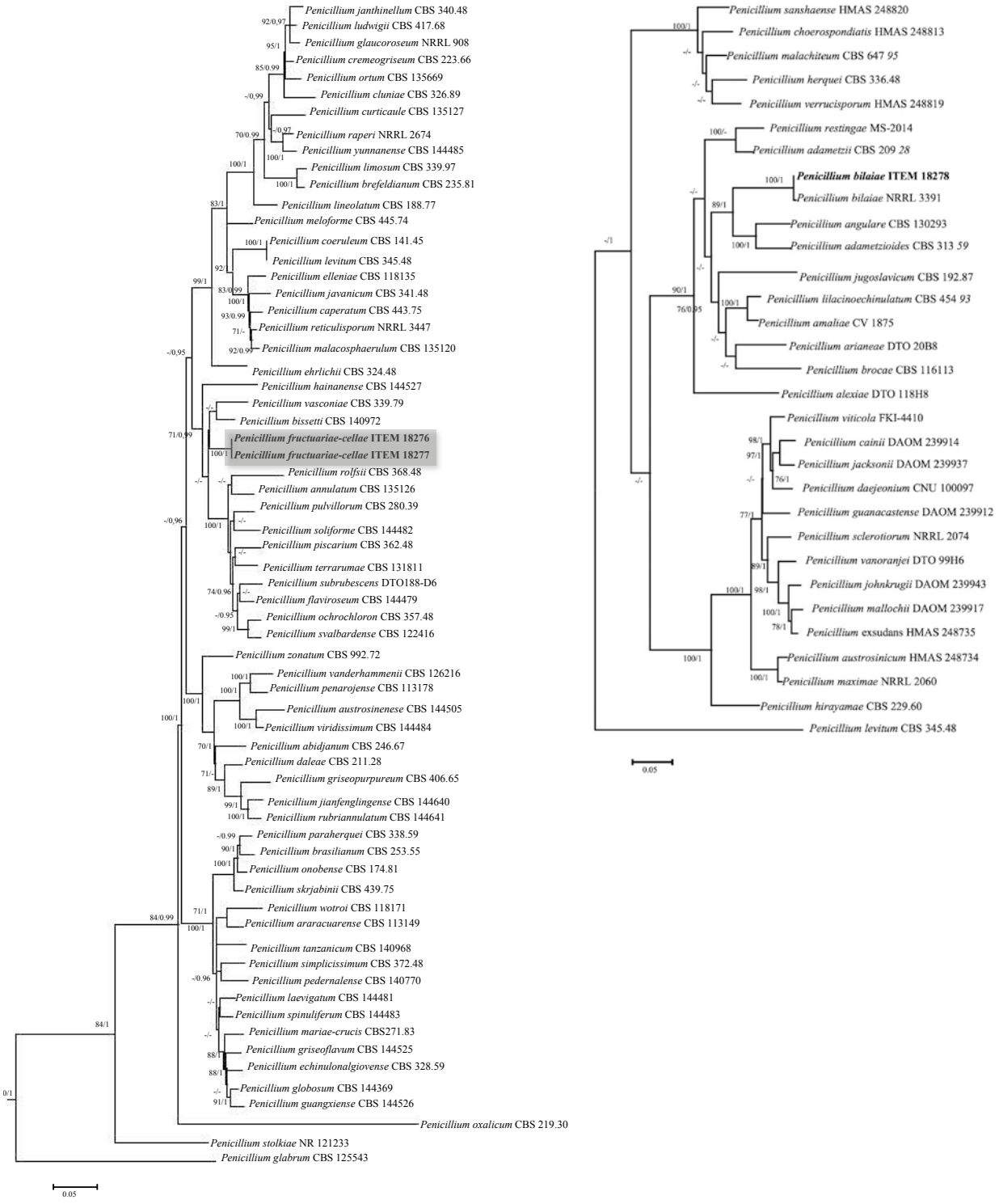
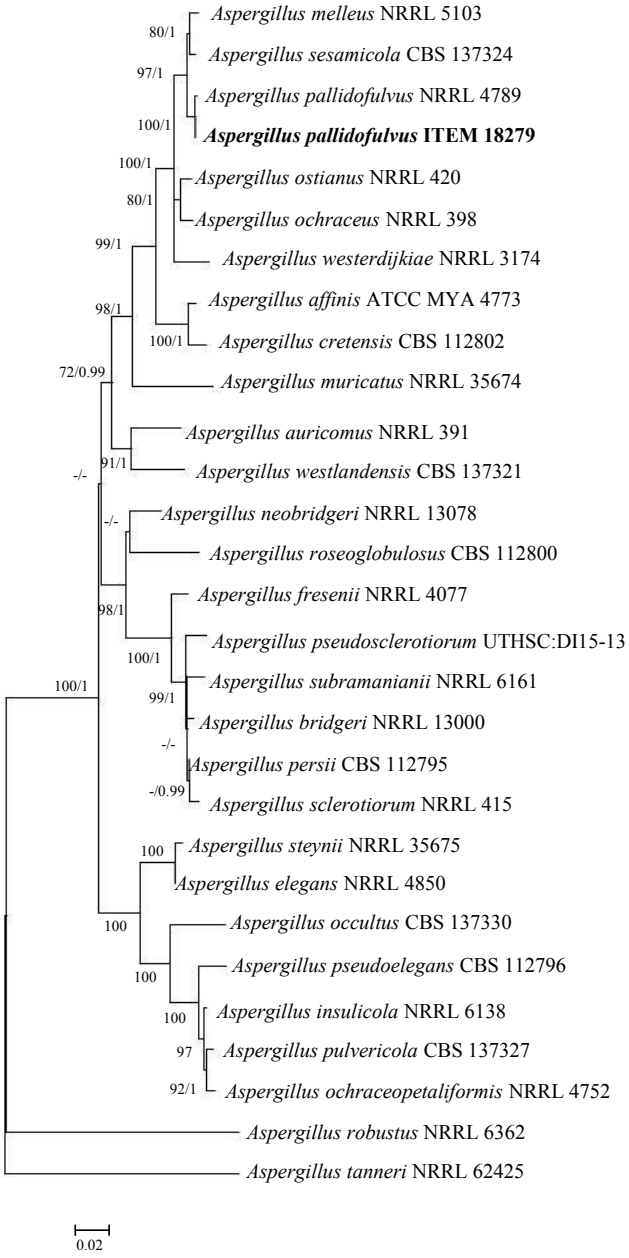


Figure 1. Phylogenetic tree of combined sequences of *Penicillium* spp. (a) Maximum Likelihood combined (*benA+CaM+ITS+rpb2*) tree of *P. fructuariae-cellae* ITEM 18277 (Pdb1) and ITEM 18276^{T} (P3) and representative taxa of *Penicillium* section *Lanata–divaricata*. *Penicillium stolkiae* CBS 315.67, *P. oxalicum* CBS 219.30 and *P. glabrum* CBS 125543 are outgroups. (b) Maximum Likelihood combined (*benA+CaM+ITS+rpb2*) tree of *P. bilaiae* ITEM 18278 (Pls8) and representative taxa of *Penicillium* section *Sclerotiora*. *Penicillium levitum* CBS 345.48 is outgroup. The BI posterior probabilities values and bootstrap percentages of the ML analysis are indicated above the nodes (BS/pp). Values less than 70% bootstrap support in the ML analysis and less than 0.95 posterior probability in the Bayesian analysis are indicated with a hyphen. Branches with high support are thickened.



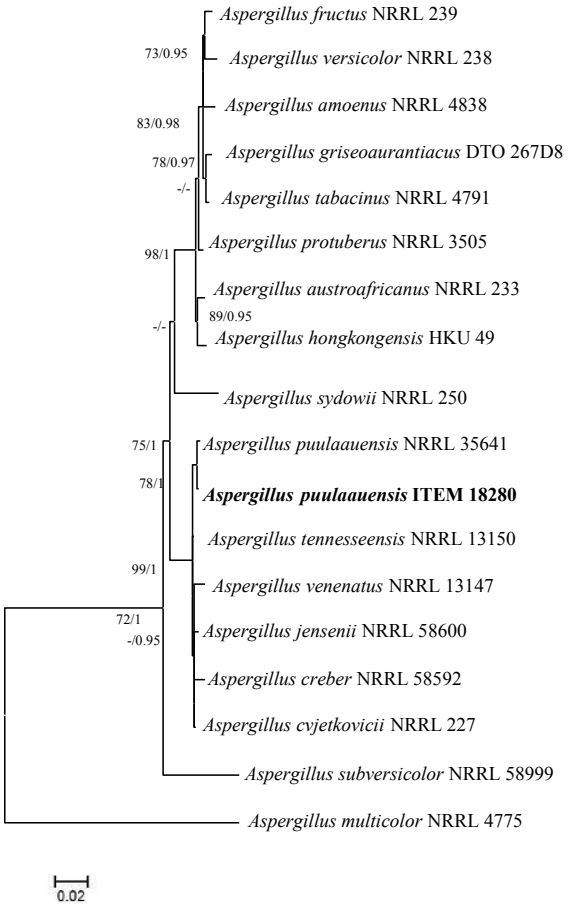


Figure 2. Phylogenetic tree of combined *benA*, *CaM* and ITS sequences of *Aspergillus* spp. (a) Maximum Likelihood tree of *Aspergillus pallidofulvus* ITEM 18279 (ASls13) and representative taxa of *Aspergillus* section *Circumdati*. *Aspergillus robustus* NRRL 6362 and *A. tanneri* NRRL 62425 are outgroups. (b) Maximum Likelihood tree of *A. puulaauensis* ITEM 18280 (AS100) and representative taxa of *Aspergillus* species belonging to *A. versicolor* clade. *Aspergillus subversicolores* NRRL 58999 and *A. multicolor* NRRL 4775 are outgroups. The BI posterior probabilities values and bootstrap percentages of the ML analysis are indicated above the nodes (BS/pp). Values less than 70% bootstrap support in the ML analysis and less than 0.95 posterior probability in the Bayesian analysis are indicated with a hyphen. Branches with high support are thickened.

pallidofulvus and different strains of *Aspergillus* sp. for *CaM*, and 99% similarity to different *Aspergillus* species (e.g. *A. ochraceus, A. melleus* and *A. pallidofulvus*) for ITS. Based on these results, the phylogenetic analysis was performed using the *Aspergillus* taxa of section *Circumdati*, according to Siqueira *et al.* (2017). The ML combined phylogenetic tree (*benA*+*CaM*+ITS) with the greatest log likelihood (-3612.040) showed that ASls13 strongly belongs to *A. pallidofulvus*, as it grouped with the relevant strain, NRRL4789 (BS/pp = 100/1) (Figure 2a).

The GenBank comparative analysis of *benA*, *CaM* and ITS sequences of AS100 (ITEM 18280) showed 99% similarity to *A. puulaauensis, A. versicolor* and *A. jensenii* for *benA*, and 99% similarity to different species of *Aspergillus* (e.g. *A. puulaauensis, A. cvjetkovicii*, *A. tennesseensis*, *A. versicolor*, *A. jensenii* and *A. creber*) for *CaM* and ITS. In the ML combined tree (*benA*+*CaM*+ITS) with the greatest log likelihood (-3938.2941), the clustering of AS100 with *A. puulauensis* NRRL35641 was highly supported (BS/pp = 78/1), as

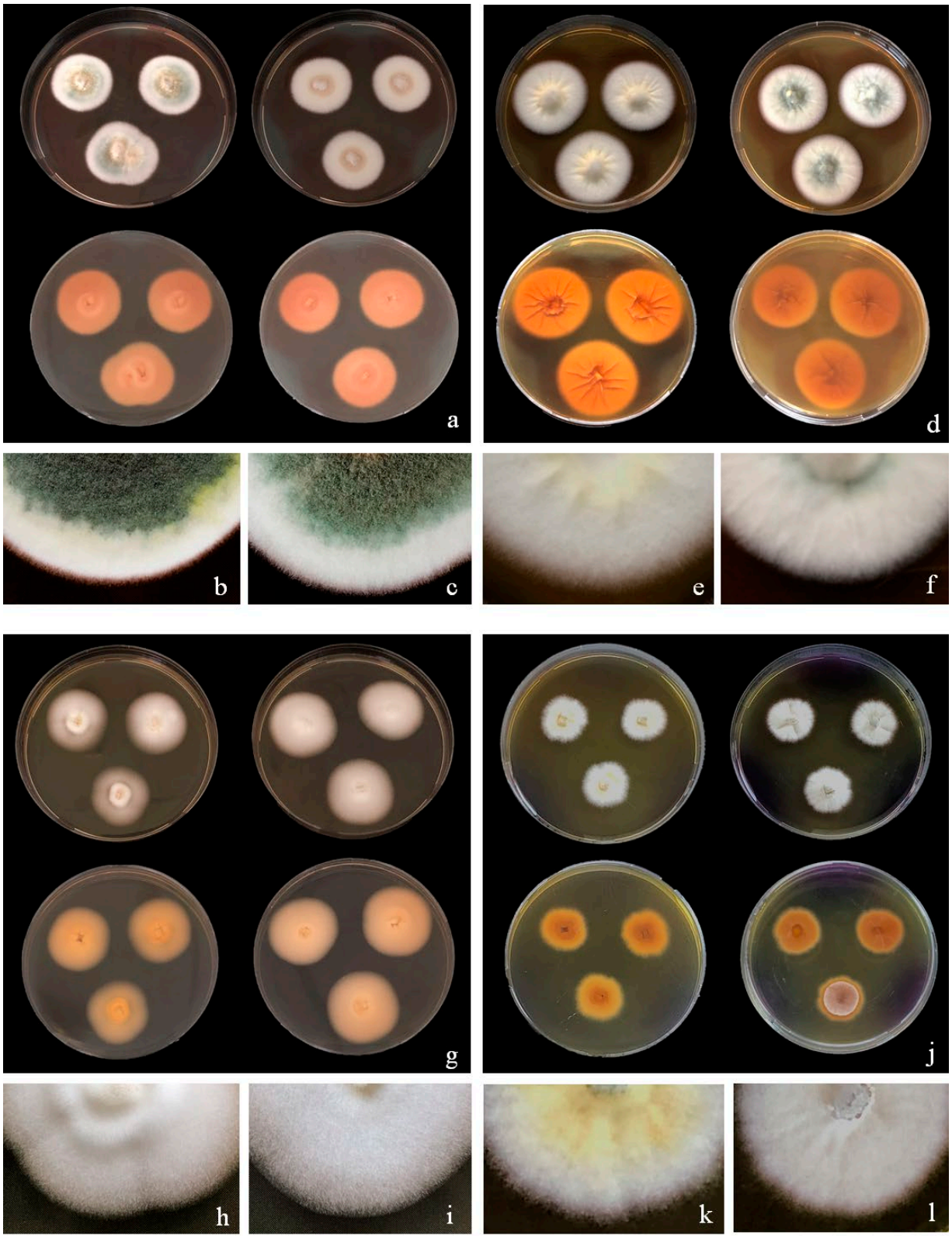


Figure 3. Macro-morphology of *Penicillium fructuariae-cellae* on culture media. Colony upper surfaces (top row) of ITEM 18277 (isolate Pdb1, left) and ITEM 18276^{T} (isolate P3, right), and reverse sides (bottom row) on CYA (a), YES (d), MEA (g) and CREA (j). Colony texture of ITEM 18277 (Pdb1) (b, e, h, k) and ITEM 18276^{T} (P3) (c, f, i, l) on, respectively, CYA, YES, MEA or CREA.

Table 3. Micromorphological characteristics of *Penicillium* and *Aspergillus* isolates after 7 d growth on different agar media. All dimensions are in µm. Numbers of metulae or phialides are indicated in parentheses..

	Media	*P. fructuariae-cellae* ITEM 18276^T (P3)	*P. fructuariae-cellae* ITEM 18277 (Pdb1)	*P. bilaiae* ITEM 18278 (Pls8)	*A. pallidofulvus* ITEM 18279 (ASls13)	*A. puulaauensis* ITEM 18280 (AS100)
Cleistothecia*	CYA	121-221 × 80-194	74-120 × 67-100	-	-	-
	YES	88-192 × 88-178	126-277 × 120-231	-	-	-
	MEA	124-191 × 77-133	35-87 × 25-56	-	-	-
Ascospore	CYA	2.5–4.5	2.5–4	-	-	-
Conidiophores/Stipes	CYA	56-200 × 2-3	55-313 × 2-3.5	34-110 × 2-3.5	155-760 × 4.5-10	115-360 × 4.5-7.5
	YES	137-413 × 2.5-4	68-227 × 2.5-5	26-97 × 2-3.5	288-630 × 4.5-10.5	168-325 × 3.5-9.5
	MEA	92-129 × 2.5-3.5§	86-120 × 2-3§	31-123 × 2-3.5	200-890 × 5-9	190-810 × 4-7
Vescicles	CYA	-	-	3.5-5	12-30	11-19.5
	YES	-	-	2.5-4.5	20-51	15-22
	MEA	-	-	3-4	13-31	9-19
Metulae/Branches	CYA	9-16 × 2-3 (2-4)	10-47 × 2-3.5 (2-4)	-	5.5-8 × 2.5-4.5	4.5-6.5 × 2-3
	YES	13-24 × 2 -4 (2-3)	8.5-18 × 2-4.5 (2-4)	-	6-12 × 3-6	3.5-7 × 2.5-4
	MEA	12-19 × 2.5-4 (2-4) **	10-26 × 2-4 (2-3) **	-	7.5-10 × 3-6	4-8 × 2-3.5
Phialides	CYA	3.5-8 × 2-3 (2-10)	4.5-10 × 2-3 (3-10)	6-9 × 2.5-3.5 (3-8)	5-8 × 2-3.5	4.5-7.5 × 2-3.5
	YES	7.5-15 × 2-4 (2-7)	5.5-11 × 2-3.5 (3-9)	5-10 × 2-3 (2-6)	6-9 × 2.5-4	4-7 × 2-3.5
	MEA	6-9.5 × 2-3 (3-5) **	5-9.5 × 2-3.5 (3-5) **	4.5-8.5 × 2-3.5 (2-4)	6.5-8 × 2.5-4	4.5-7 × 2-3.5
Conidia	CYA	2-3.5	2-3.5	2-3.5	2.5-4	2-3.5
	YES	2-4	2-3	2-3	2-3.5	2-3.5
	MEA	2-3.5 **	2-3.5 **	2-3.5	2.5-4.5	2-3.5

- = structures absent; § n < 4; * measurements based on 14 d old cultures; ** measurements based on 30 d old cultures.

shown in Figure 2b. Phylogenetic analysis based on the ITS dataset placed AS100 in a group containing most of the *Aspergillus* taxa of the *A. versicolor* clade (data not shown).

Culture and morphological characteristics

Penicillium isolates Pdb1 (ITEM 18277) and P3 (ITEM 18276^T) had similar colony morphology on the different media (Figure 3). On CYA, colonies were compact, velvety, with entire margins, and were white; initially yellowish (for Pdb1) or cream (for P3) then becoming gray-green due to abundant sporulation. Spherical or suboval cleistothecia were observed, often covered with a network of hyphae. The cleistothecia matured after three or more weeks, containing evanescent asci and hyaline ascospores, which were smooth–walled and globose to subglobose (Table 3). The colonies were surrounded by diffused soluble pigment in the agar developing as a red-brown colony halos. Hyaline exudates were also observed. Reverse sides of the colonies were light brown and pale cream shades (Figures 3a, b and c).

The growth test revealed little variability among the isolates. The colony diameters at 25°C were 36 to 41 mm for isolate Pdb1 and 43 to 48 mm for P3, at 15°C were 16 to 20 mm for Pdb1 and 17 to 20 mm for P3 mm, and at 30°C were 46 to 49 mm for Pdb1 and 47 to 49 mm for P3. The colony diameters at 37°C were 8 to 10 mm for Pdb1 and 10 to 13 mm for P3. On YES, the colonies were moderately deep and radially sulcate, with regular margins. The mycelium was white and pale yellow in the centre for Pdb1, or white and greenish to grayish in the centre for P3. Sporulation was moderate and pale yellow for Pdb1 and pale gray-green for P3. The cleistothecia were spherical or suboval. The colonies were surrounded by diffused soluble pigment in the agar, as a faint yellow zones surrounded by faint purpuric-brown halos. The colony textures were pubescent and exudates were not observed. The reverse sides of the colonies were orange to brown in colour (Figures 3b, e and f). The colony diameters were 43 to 50 mm for isolate Pdb1 and 42 to 46 mm for P3. On MEA, the colonies were compact, velvety, sometimes radially wrinkled, with entire and plane margins. The mycelia were whitish and non-sporulating mycelium. The cleistothecia were spherical or suboval, and hyaline exudates sometimes were observed. The reverse sides of colonies were yellow and white for Pdb1 and white to pale cream for P3 (Figures 3g, h and i). The colony diameters were 38 to 40 mm for isolate Pdb1 and

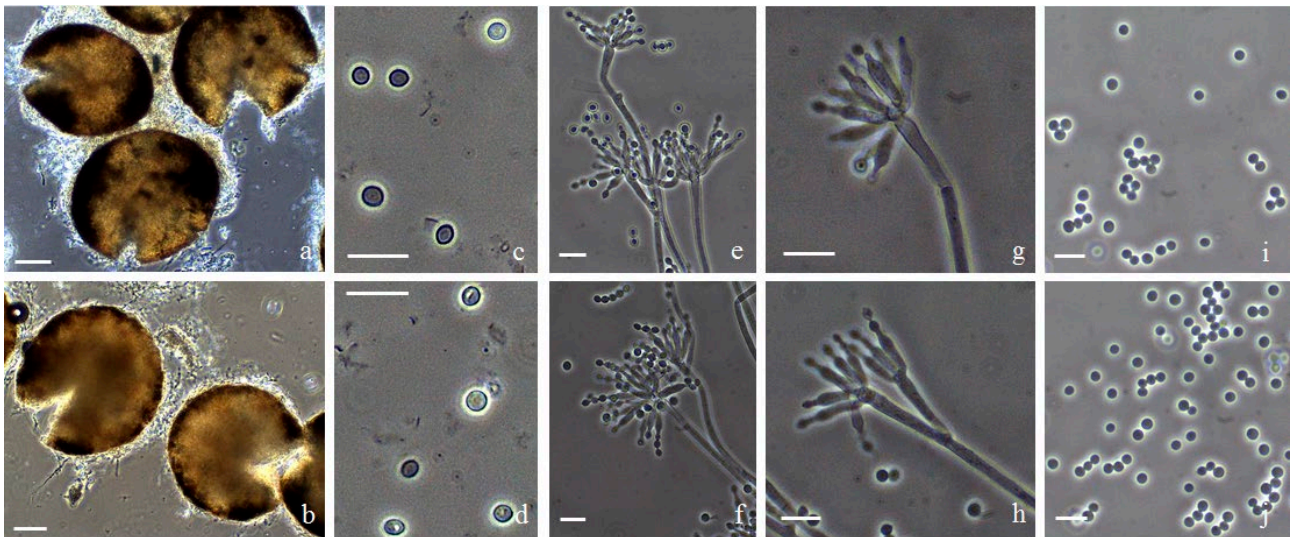


Figure 4. Micro-morphology of *Penicillium fructuariae-cellae* on CYA. Cleistothecia (a and b), ascospores (c and d), conidiophores (e to h), and conidia (i and j) of, respectively, ITEM 18277 (isolate Pdb1, top row) and ITEM 18276^{T} (isolate P3, bottom row). Bars = 50 µm (a and b), or 10 µm (c to j).

43 to 45 mm for P3. The colony diameters at 25°C after 7 d on PDA pH 4 were 37 to 40 mm for Pdb1 and 39 to 41 for P3, on ¼ strength PDA pH 7 were 35 to 38 mm for Pdb1 and 38 to 42 mm for P3, and on Horikoshi agar pH 10 were 29 to 30 mm for Pdb1 and 32 to 33 mm for P3. On CREA, the isolates grew well and produced acid (Figures 3j, k, l). On CYA and YES, conidiophores were monoverticillate or biverticillate, and a minor proportion were divaricate. Metulae were oblong and divergent, phialides were ampuliform, and conidia were smooth walled and globose to subglobose (Figure 4; Table 3). On MEA, conidiophores were rarely observed.

On CYA, colonies of *P. bilaiae* Pls8 were convex, with concentric folds, conidia were abundantly produced, and aerial mycelium was lanose and floccose and grey-green to white. The colony margins were margin entire and white and sporulation was heavy. Exudates were dark, superficial or embedded, and the colonies were surrounded by diffused soluble pigment into agar as faint purpuric-brown halos. The colony reverse sides were orange-yellow. On YES, colonies were heavily wrinkled, white and pubescent at the margins, green-gray in colour, and felty in the centres. The colonies sporulated heavily. The margins entire and exudates were not observed. The colonies were surrounded by soluble pigment diffused into the agar as faint yellow zones surrounded by faint purpuric-brown halos. The colony reverse sides were orange to brown. On MEA, the colonies were velutinous, floccose and raised in the centre, with villose white aerial mycelium and entire margins which were entire, low, plane and white. The colonies were; sporulating heavily, without exudates, and were surrounded by diffused soluble pigment into the agar as faint yellow zones. The reverse sides of the colonies were yellow to pale orange. Colony diameters were 22 to 26 mm on CYA, 20 to 22 mm on YES and 16 to 20 mm on MEA. On CREA, the fungal growth was weak with good acid production. On CYA, YES, and MEA the conidiophores were monoverticillate, the stipes were smooth walled and mostly globose vesiculate or subglobose to ellipsoidal. The phialides were ampulliforms and wide at the bases. Conidia were globose to subglobose (Table 3).

On CYA, colonies of *A. pallidofulvus* ASls13 was velutinous, the mycelium was white, and sporulation was pale yellow to cream, without exudates. Reverse sides of colonies were light brown to brown. On YES, the colonies were moderately powdery to velutinous, the mycelium was white without exudates, and sporulation was light yellow Colony reverse sides were pale brown to yellow. On MEA, the colonies were velutinous, felty and floccose and the mycelium was white with light yellow sporulation, without exudates. Colony reverse sides were cream to pale brown. Colony diameters were 56 to 60 mm on CYA, 70 to 75 mm on YES and 55 to 61 mm on MEA. On CREA, growth was weak with no acid production. On CYA, YES and MEA, the conidiophores were biseriate, stipes were hyaline to pale brown, vesicles were globose, metulae were oblong covering entire vesicles, and phialides were ampulliform. Conidia were globose, subglobose to ovoid and smooth (Table 3).

Table 4. Disease index (%) on grape berries after 14 d, for three isolates of *Penicillium* (P3, Pb1b and Pls8) and two of *Aspergillus* (ASls13 and AS100), all recovered from withered grapes, and for *B. cinerea* ITEM 17200 as positive control.

Species	Isolate designation	Disease index (%) on grape berries	
		White fresh table	Red withered wine
Penicillium fructuariae-cellae	P3	24 ± 0 a	44 ± 1 a
Penicillium fructuariae-cellae	Pdb1	23 ± 1 a[1]	44 ± 1 a
Penicillium bilaiae	Pls8	24 ± 2 a	44 ± 2 a
Aspergillus pallidofulvus	ASls13	22 ± 1 a	47 ± 2 a
Aspergillus puulaauensis	AS100	23 ± 1 a	43 ± 1 a
Botrytis cinerea	ITEM 17200	100 ± 0 b	98 ± 1 b

[1]Values (mean of three independent measurements ± standard deviation) with different letters are significantly different (ANOVA, Tukey HSD, $P < 0.05$)

On CYA, colonies of *A. puulaauensis* AS100 was sulcate and raised in the centre. Sporulation green to gray. Colony margins were regular, plane and white without exudates. Colony reverse sides were yellow to light orange. On YES, colonies were moderately powdery to felty, sulcate, raised in the centre, and white with green to grey and light pink shadows. Sporulation was green to gray. Colony margins were regular and no exudates were produced. Colony reverse sides were yellow to light brown. On MEA, colonies were sulcate, and raised in the centre. Mycelium was white and sporulation was light yellow. Colony margins were regular, plane and white, without exudates. Colony reverse sides were yellow to light orange. The colony diameters were 18 to 22 mm on CYA, 24 to 25 mm on YES and 18 to 20 mm on MEA. On CREA, growth was moderate growth without acid production. On CYA, YES and MEA, the conidiophores were biseriate, and stipes were smooth, and hyaline to light yellow. Vesicles were spatulate or subspherical, and metulae were oblong covering entire vesicles. Phialides were oblong from which globose or subglobose conidia developed (Table 3).

Pathogenicity assay

All *Penicillium* and *Aspergillus* isolates obtained in this study displayed ability to infect grape berries, although infection was much less than for *B. cinerea* (Table 4). Wounded inoculated berries initially showed mycelium around the inoculation sites and subsequently necrotic areas became visible, particularly in white fresh table berries. Red withered berries inoculated by *Penicillium* isolates Pdb1, P3 and Pls8 caused typical symptoms of *Penicillium* infection characterized by tufts of white and green mycelium erupting from the berry skins (Figure 5). Abundant sporulation was observed on berries infected by *A. pallidofulvus* isolate ASls13. The pathogens were re-isolated from inoculated berries, fulfilling Koch's postulates.

Taxonomy

The phylogenetic analysis based on four different loci and the morphological analysis showed that two isolates of *Penicillium* (Pdb1 and P3) recovered from withered grapes were distinct from any known species within the *Penicillium* section *Lanata–divaricata*. Therefore, these isolates are here described as members of a new *Penicillium* species.

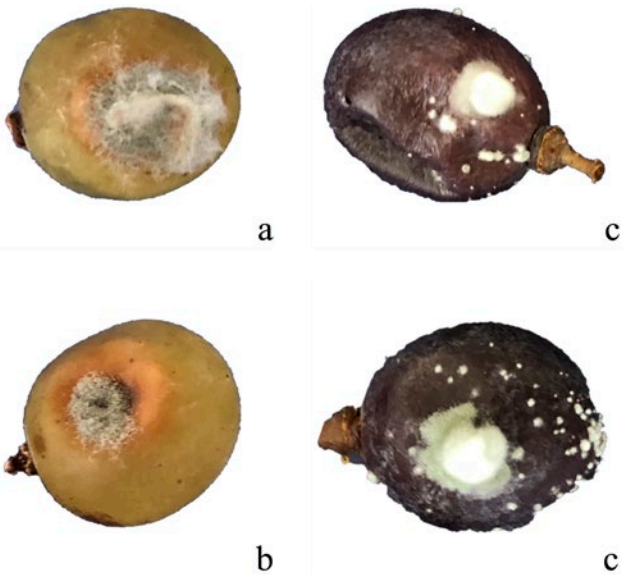


Figure 5. Details of the pathogenicity assay of ITEM 18277 (isolate Pdb1) (a and c) and ITEM 18276^T (isolate P3) (b and d) on white fresh table grapes (left) and red withered wine grapes (right).

Penicillium fructuariae-cellae Lorenzini, Zapparoli & Perrone **sp. nov.**

MycoBank: MB 831228 – Figures 3 and 4

In: subgenus *Aspergilloides*, section *Lanata–divaricata*.

ITS barcode: MK039434. Alternative markers: *benA* =KU554679; *CaM* = MK045337; *rpb2* = MK520927.

Etymology. Latin, *fructuariae-cellae*, meaning fruit-drying room for grape withering, the place where two representative strains were isolated.

Type specimen. ITALY, Verona, Marano di Valpolicella, on Corvina withered grapes stored in fruit-drying room, Dec. 2013, *coll*. M. Lorenzini and G. Zapparoli, *isol*. M. Lorenzini and G. Zapparoli, P3 (holotype CBS 145110^{T}; ex-type strain ITEM 18276^{T}).

Colony morphology. Colony diameters (mm), 7 d: CYA 43–48; YES 42–46; MEA 43–45; CREA 35–37; CYA 15°C 17–20; CYA 30°C 47–49; CYA 37°C 10–13.

Colonies on CYA after 7 d at 25°C were compact, velvety; margins entire and white; sporulation abundant, conidia *en masse* pale gray-green; cleistothecia spherical or suboval covered with networks of hyphae; asci evanescent, ascospores hyaline, smooth–walled, globose to subglobose; soluble red-brown pigments and hyaline exudates produced; colony reverse sides pale brown and pale cream. Colonies on YES after 7 d at 25°C were moderately deep, radially sulcate, with regular margins; mycelia white and green to gray; sporulation moderate, conidia *en masse* pale gray-green; cleistothecia observed; soluble yellow and faint purpuric-brown pigments produced from colonies; exudates absent; colony reverse sides orange to brown. Colonies on MEA after 7 d at 25°C were compact, sometimes radially wrinkled, with entire and plain margins with velvety texture; mycelia white; sporulation poor; cleistothecia observed; hyaline exudates sometimes observed; colony reverse sides white yellow-pale cream. Colonies grew well on CREA after 7 d at 25°C, with good acid production.

Conidiophores (on CYA) monoverticillate, biverticillate, with a minor proportion divaricate; stipes smooth, 55–313 × 2–3.5 µm, *metulae* divergent, 2–4 per stipe or branch, 9–47 × 2–3.5 µm; *phialides* ampulliform, 2–10 per metula, 3.5–10 × 2–3 µm; *conidia* smooth walled, globose to subglobose, 2–3.5 µm (mean = 2.5 µm ± 0.4 µm n = 50); *cleistothecia* covered with a network of hyphae, 74–221 × 67–194 µm (n = 20); asci evanescent; *ascospores* hyaline, smooth–walled, globose to subglobose 2.5–4.5 µm (mean = 3.0 µm ± 0.4 µm, n = 30).

Other strains examined. ITALY, Verona, Montecchia di Crosara, Garganega withered grapes stored in a fruit-drying room, Nov. 2017, *coll*. M. Lorenzini and G. Zapparoli, *isol*. M. Lorenzini and G. Zapparoli, Pdb1, ITEM 18277 = CBS 145111, ITS barcode: MK039435. Alternative markers: *benA* = MK045333; *CaM* = MK045338; *rpb2* = MK520928.

Notes. *Penicillium fructuariae-cellae* is classified in section *Lanata–divaricata*, and is distantly related to other *Penicillium* species. The multi-locus phylogeny placed it closed to *P. bissettii* KAS1951 and *P. vasconiae* CBS 339.79 (Figure 1a). *Penicillium fructuariae-cellae* produces red-brown pigments in CYA compared with *P. bissettii* and *P. vasconiae* that do not produce pigments. *Penicillium fructuariae-cellae* mainly differs from *P. vasconiae* and *P. bissettii* in conidiophore structure and size as it has longer conidiophores than *P. vasconiae*, and shorter conidiophores than *P. bissetti*. *Penicillium fructuariae-cellae* also differs from *P. bissettii* by having smooth stipes. *Penicillium fructuariae-cellae* differs from *P. vasconiae* in phialide shape (*P. vasconiae* has long tapped neck phialides) and having smooth and small conidia.

DISCUSSION

Phylogenetic analysis and morphological observations of the isolates recovered in this study from withered grapes compile the first report of *P. bilaiae*, *A. pallidofulvus* and *A. puulaauensis* from *Vitis vinifera*.

The species identification of Pls8 (ITEM 18278) as *P. bilaiae* was taxonomically clear due to its congruence with phylogenetic and morphological data from the *P. bilaiae* holotype NRRL 3391. However, isolate Pls8 showed slower growth in agar media and some micro-morphological differences (i.e. longer stipes, wider and more numerous phialides) than holotype PB-50 described by Pitt (1979) and Savard *et al.* (1994). Prior to the present study, *P. bilaiae* was detected in Portuguese grapes through morphological observations (Serra *et al*., 2005), a method that does not provide sufficient data for reliable identification at the species level. *Penicillium bilaiae* is morphologically similar to both *P. alexiae* and *P. adametzioides* (Visagie *et al*., 2013). Hence, the present study provides the first taxonomic evidence of the occurrence of *P. bilaiae* on *Vitis vinifera*.

The assignment of isolate ASls13 (ITEM 18279) to *A. pallidofulvus* was confirmed by its genealogy and macromorphology, according to the description of the holotype *A. pallidofulvus* NRRL 4749 (Visagie *et al*., 2014). However, micro-morphological observations of ASls13 showed differences in the size of its stipes and phialides (respectively smaller and shorter for ASls13 than the holotype), and with no production of sclerotia by ASls13, in contrast with the *A. pallidofulvus* holotype. This spe-

cies, recently introduced into section *Circumdati*, has also been isolated from green coffee beans in India and clinical samples (Visagie *et al*. 2014; Masih *et al*., 2016). The recovery of this species from grapes and the results of pathogenicity assays show that *A. pallidofulvus* may exhibit pathogenic behaviour in grapevine. Nevertheless, further investigation is required to determine occurrence for this fungus on withered grapes and its infectivity under post-harvest environmental conditions.

The use of multi-locus phylogenetic analysis (*CaM*, *benA* and ITS) resolved the taxonomic position of isolate AS100 (ITEM 18280), identifying it as *A. puulaauensis*. Assignment of the isolate to this species has previously proved impossible using only the *CaM* gene sequence (Lorenzini *et al*., 2016). Moreover, AS100 and the holotype *A. puulaauensis* NRRL 35641 (Jurjevic *et al*., 2012) both showed identical colony macro- and micro-morphological characters. The recovery of *A. puulaauensis* from grape berries further supports its worldwide and cosmopolitan distribution, since this species has previously been reported from disparate environments including Hawaiian plants, Atlantic sponges, Italian cheese, air samples in North America and clinical samples (Jurjevic *et al*., 2012; Siqueira *et al*., 2017; Bovio *et al*., 2018; Decontardi *et al*., 2018).

Based on multi-locus phylogenetic analyses, isolates Pdb1 (ITEM 18277) and P3 (ITEM 18276^{T}) represented a distinct species, herein named *P. fructuariae-cellae*. These two isolates form a phylogenetic cluster based on *benA*+*CaM*+ITS+*rpb2* combined gene genealogies, distinct from any currently described species in section *Lanata–divaricata,* which was recently updated with 13 new *Penicillium* species collected from Chinese acidic soils (Diao *et al*., 2018). *Penicillium fructuariae-cellae* is acid-preferential, like most of the new species described by Diao *et al.* (2018). This is a physiological characteristic congruent with the acidic habitat (grapes) from which it was isolated. Isolates of P3 and Pdb1 are distantly related to *P. vasconiae* CBS 339.79 (Ramírez and Martínez, 1980) and the recently described species *P. bissettii* (Visagie *et al*., 2016). Moreover, *P. fructuariae-cellae* displays some differences in macro-morphological characteristics (e.g. colony colour, texture and pigment production) compared with related species. The pigment production by these two isolates distinguishes them from *P. vasconiae* and *P. bissettii* that produce no pigments. *Penicillium fructuariae-cellae* produces monoverticillate, sometimes biverticillate conidiophores, whereas *P. vasconiae* is strictly monoverticillate and *P. bissettii* is biverticillate/terverticillate. The conidiophores of *P. fructuariae-cellae* are longer than those of *P. vasconiae* (< 50 μm; Ramírez and Martínez, 1980) and shorter than conidiophores of *P. bissettii* (190–670 μm; Visagie *et al.,* 2016). Its stipes are smooth, while in *P. bissettii* they are rough. Its phialides are also shorter than those of *P. vasconiae* (9–13 μm; Ramírez and Martínez, 1980). As well, of *P. fructuariae-cellae* produced smaller conidia than *P. vasconiae* (4–4.5 μm; Ramírez and Martínez, 1980). The conidial surfaces of *P. fructuariae-cellae* were smooth, while those of *P. vasconiae* are conspicuously echinulate (Ramírez and Martínez, 1980). These morphological differences together with phylogenetic information, support the uniqueness of *P. fructuariae-cellae*.

Based on the results of pathogenicity assays, the detrimental effects of these fungi on withered grapes was quite significant. Although these *Penicillium* and *Aspergillus* isolates were less pathogenic than *B. cinerea*, they could make major contributions to berry rotting. Decay of berry surfaces, like that observed on infected berries caused by each isolate due to mycelial growth and necrosis, is important for susceptibility to subsequent fungal infections by the same and other pathogens (Padgett and Morrison, 1990). Incidence and symptoms of grape diseases caused by these fungi under fruit-drying room conditions require further investigation.

In conclusion, this study describes isolates belonging to species of *Penicillium* and *Aspergillus* from withered grape berries that have not been previously reported from *V. vinifera*. A new species of *Penicillium* from this host, *P. fructuariae-cellae,* is herein described. The recovery of these species highlights the complexity of fungal species affecting withered grapes (Lorenzini *et al*., 2016; 2018). According to pathogenicity assays, *P. fructuariae-cellae*, *P. bilaiae*, *A. pallidofulvus* and *A. puulaauensis* are able to infect grapes but with much lower infectivity than *B. cinerea*, which is the most important pathogen occurring on withered grapes. It is likely that the species identified in this study are less pathogenic than other *Penicillium* and *Aspergillus* species frequently reported on withered grapes (e.g. *P. expansum, P. crustosum, A. tubingensis* and *A. uvarum*) (Lorenzini *et al*., 2016). Further investigations are necessary to ascertain the pathogenic role of *P. fructuariae-cellae, P. bilaiae, A. pallidofulvus* and *A. puulaauensis* under withering conditions, as well as their interaction with other causal fungal agents causing rots of grape berries.

LITERATURE CITED

Bovio E., Garzoli L., Poli A., Prigione V., Firsova D., … Varese G.C., 2018. The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balausformis* and *T. spongiae*. *Fungal Systematics and Evolution* 1: 141–167.

Decontardi S., Soares C., Lima N., Battilani P., 2018. Polyphasic identification of Penicillia and Aspergilli isolated from Italian grana cheese. *Food Microbiology* 73: 137–149.

Diao Y-Z., Chen Q., Jiang X.-Z., Houbraken J., Barbosa R.N., … Wu W.-P., 2018. *Penicillium* section *Lanata-divaricat*a from acidic soil. *Cladistics*, 0: 1–36.

Drummond A.J., Rambaut A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7: 214.

Glass N.L., Donaldson G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology,* 61: 1323–1330.

Hong S.B., Cho H.S., Shin H.D., Frisvad J.C., Samson R.A., 2006. Novel *Neosartorya* species isolated from soil in Korea. *International Journal of Systematics and Evolutionary Microbiology* 56: 477–486.

Jurjevic Z., Peterson S.W., Horn B.W., 2012. *Aspergillus* section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny. *IMA Fungus* 3: 59–79.

Kumar S., Stecher G., Tamura K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.

Liu Y., Whelen S., Hall B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.

Lorenzini M., Zapparoli G., 2014. Characterization and pathogenicity of *Alternaria* spp. strains associated with grape bunch rot during post-harvest withering. *International Journal of Food Microbiology* 186: 1–5.

Lorenzini M., Cappello M.S., Logrieco A., Zapparoli G., 2016. Polymorphism and phylogenetic species delimitation in filamentous fungi from predominant mycobiota in withered grapes. *International Journal of Food Microbiology* 238: 56–62.

Lorenzini M., Simonato B., Favati F., Bernardi P., Sbarbati A., Zapparoli G., 2018. Filamentous fungi associated with natural infection of noble rot on withered grapes. *International Journal of Food Microbiology* 272: 83–86.

Masih A, Singh P.K., Kathuria S., Agarwal K., Meis J.F., Chowdhary A., 2016. Identification by molecular methods and matrix-assisted laser desorption ionization–time of flight mass spectrometry and antifungal susceptibility profiles of clinically significant rare *Aspergillus* species in a referral chest hospital in Delhi, India. *Journal of Clinical Microbiology* 54: 2354–2364.

Mencarelli F., Tonutti P., 2013. *Sweet, Reinforced and Fortified Wines: Grape Biochemistry, Technology and Vinification.* John Wiley & Sons, Ltd, Chichester, UK.

Padgett M., Morrison J.C., 1990. Changes in grape berry exudates during fruit development and their effect on mycelial growth of *Botrytis cinerea. Journal of the American Society for Horticultural Science* 115: 269–273.

Pitt J.I., 1979. *The genus* Penicillium *and its teleomorphic states* Eupenicillium *and* Talalvmyces. London, Academic Press, UK.

Rambaut A., Drummond A.J., 2009. Tracer v. 1.5. Available from: http://tree.bio.ed.ac.uk/software/tracer/.

Ramìrez C., Martìnez A.T., 1980. Some new species of *Penicillium* recovered from the atmosphere in Madrid and from other substrata. *Mycopathologia* 72: 181–191.

Savard M.E., Miller J.D., Blais L.A., Seifert K.A., Samson R.A., 1994. Secondary metabolites of *Penicillium bilaii* strain PB-50. *Mycopathologia* 127: 19–27.

Serra R., Braga A., Venâncio A., 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Research in Microbiology* 156: 515–521.

Siqueira J.P.Z., Sutton D.A., Gené J., García D., Wiederhold, N., … Guarro J., 2017. Multilocus phylogeny and antifungal susceptibility of *Aspergillus* section *Circumdati* from clinical samples and description of *A. pseudosclerotiorum* sp. nov. *Journal of Clinical Microbiology* 55: 947–958.

Somma S., Perrone G., Logrieco A., 2012. Diversity of black Aspergilli and mycotoxin risks in grape, wine and dried vine fruits. *Phytopathologia Mediterranea* 51: 131–147.

Stefanini I., Carlin S., Tocci N., Albanese D., Donati C., … Cavalieri D., 2017. Core microbiota and metabolome of *Vitis vinifera* L. cv. Corvina grapes and musts. *Frontiers in Microbiology* 8: 457.

Torelli E., Firrao G., Locci R., Gobbi E., 2006. Ochratoxin A-producing strains of *Penicillium* spp. isolated from grapes used for the production of "passito" wines. *International Journal of Food Microbiology* 106: 307–312.

Visagie C.M., Houbraken J., Rodriques C., Silva Pereira C., Dijksterhuis J., … Samson R.A., 2013. Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal family. *Persoonia* 31, 42–62.

Visagie C.M., Varga J., Houbraken J., Meijer M., Kocsube S., … Samson S.A., 2014. Ochratoxin production and taxonomy of the yellow aspergilli (*Aspergillus* section *Circumdati*). *Studies in Mycology* 78: 1–61.

Visagie C.M., Renaud J.B., Burgess K.M.N., Malloch D.W., Clark D., … Seifert K.A., 2016. Fifteen new species of *Penicillium. Persoonia* 36: 247–280.

Wang X.C., Chen K., Zeng Z.Q., Zhuang W.Y., 2017. Phylogeny and morphological analyses of *Penicillium* section *Sclerotiora* (Fungi) lead to the discovery of five new species. *Scientific Reports* 7: 8233.

White T.J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a Guide to Methods and Applications* (M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White eds.), Academic Press San Diego, CA, USA, 315–322.

Zapparoli G., Lorenzini M., Tosi E., Azzolini M., Slaghenaufi D., … Simonato B., 2018. Changes in chemical and sensory properties of Amarone wine produced by *Penicillium* infected grapes. *Food Chemistry* 263: 42–50.