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Cytospora tristicha (De Not.) Mlčoch *comb. nov.*, a lesser-known pathogen of wild roses

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Abstract: Stem canker of *Rosa pendulina*, collected in south-eastern Switzerland (canton of Graubünden) in August 2022, was studied in details. The causal agent of the disease was first identified as *Valseutypella tristicha* (*Valsaceae*, Diaporthales, Ascomycota) based on microscopic characteristics. The subsequent molecular analysis classified it into the genus *Cytospora* and indicated that it is identical with *C. rosicola*, described from China in 2020 based on the molecular data, however, *V. tristicha* is an older name than *C. rosicola*, so this has priority. Thus, a new combination of the name is proposed for this fungus.

Keywords: phylogeny; *Rosa* spp.; taxonomy; Valsaceae; rose stem canker

Rose dieback and canker have been reported from Europe since the early 1800s and from the United States of America (USA) since the 1910s. Numerous species of ascomycetes are known as the causal agents, often having an economic impact in horticulture, e.g., *Coniothyrium wernsdorffiae* and *Paraconiothyrium fuckelii* (Pleosporales, Dothideomycetes) or *Cryptosporella umbrina, Cytospora ceratosperma, C. leucostoma* (Diaporthales, Sordariomycetes) can be mentioned from the family Valsaceae (Pataky 1990; Fotouhifar et al. 2010; Pan et al. 2020; Caio et al. 2021). In this study, the taxonomical position of one of these species was re-evaluated.

Originally determined as *Diatrype tristicha* De Notaris (1867) from rose branches in Italy, the species was later combined under name *Valseutypella tristicha* (De Not.) Höhnel (1919) based on J.F. Brencler's collection from North Dakota in 1914, as a type of species of a new genus *Valseutypella* Höhnel (1919). Almost seven decades later, two more species of the genus were described, i.e., *Valseutypella khandalensis* Vaidya (1981) on dead wood of *Mimusops elengi* from Maharashtra (India) and *V. multicollis* Checa, G. Moreno & M.E. Barr (1986) from dead branches of *Quercus ilex* subsp. *rotundifolia* in Spain. Nevertheless, the initial phylogenetic revision of the genus *Cytospora* and related taxa from the family Valsaceae re-classified the species *V. multicollis* into an extensive clade of *Cytospora s.s.* (Norphanphoun et al. 2017).

Cytospora, in the modern concept, is a monophyletic, highly diversified genus of phytopathogenic fungi (Valsaceae, Diaporthales, Sordariomycetes, Ascomycota), causing the canker of branches and trunks of various woody plants, which often leads to large-scale dieback in host monocultures (Adams et al. 2005; Norphanphoun et al. 2017). The genus was originally described for anamorphs

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of *Valsa* spp. and has also been considered as the asexual morph of other genera such as *Leucocytospora*, *Leucostoma*, *Valsella* and *Valseutypella* (Bulgakov 2010; Rossman et al. 2015; Norphanphoun et al. 2017). However, extensive phylogenetic analyses have revealed that all the genera belong to a single clade within the genus *Cytospora* Ehrenberg (Norphanphoun et al. 2017; Fan et al. 2020; Pan et al. 2020). According to the modern concept, two basic morphotypes of stromata can be distinguished within the genus *Cytospora*: *(i)* stroma with a white, whitish or even yellow erumpent ectostromatic disc and barely protruding dark ostioles, which is usually delimited by a black stromatic zone at the basis (Spielmann 1984) that is characteristic for the species previously classified in the genus *Leucostoma* and some species of genera *Valsa* and *Valsella*, in the older concept; *(ii)* dark disc stroma with poorly developed entostroma and consisting of a higher number of conical to filiform ostioles piercing the bark, significantly protruding above the stroma surface and usually not delimited below by a black stromatic zone (Spielman 1984) found in species previously classified in the genera *Valsa* and *Valseutypella* (which may, thus, resemble the stroma of *Eutypella* spp., according to the author's observations). Both the conidia as well as the ascospores of *Cytospora* spp. according to the recent concept are allantoid, aseptate and often hyaline or yellowish (Bulgakov 2010; Rossman et al. 2015; Norphanphoun et al. 2017; Fan et al 2020; Pan et al 2020).

In recent study, we provide morphological and molecular data supporting a new taxonomic position for the causal agent of rose canker *Valseutypella tristicha.*

MATERIAL AND METHODS

The morphological, molecular, and taxonomic studies conducted within this work are based on the collection of Lucie Zíbarová from the Swiss Alps (see below), which was recently deposited in the mycological herbarium of the Moravian Museum in Brno (BRNM 844603). Five additional specimens from the Czech herbaria were compared (see the Material revised).

Material examined. Switzerland. BRNM 844603, Ratische Alpen, Bivio (Graubünden canton), Plaz, valley of the river Gelgia (GPS: 46°28'16.122"N, 9°39'7.129"E, 1 750 m a.s.l.), on the dead wood of *Rosa pendulina*, August 8, 2022, col. L. Zíbarová, det. P. Mlčoch as *Valseutypella tristicha* (Figure 1).

Material revised. Slovakia: PRC 9408, High Tatras, Holubyho Dolina Valley, tourist trail on Čarda, the side in front of the bench, on the dead wood of *Rosa pendulina,* July 22, 1959, col. Z. Urban, det. Z. Urban as *Valseutypella tristicha*. PRC 8435, High Tatras, Holubyho Dolina Valley, tourist trail on Čarda, under Lúčná skála rock, on the dead wood of *Rosa pendulina*, July 26, 1959, col. Z. Urban, det. Z. Urban as *V. tristicha*. PRM 519025, Belian Tatras, valley of the Seven Springs, Hlboký potok river (1 075 m a.s.l.), on the dead wood of *Rosa canina* s. l., July 31, 1959, col. Z. Urban, det. Z. Urban as *V. tristicha* USA. PRM 862024, North Dakota, Whitestone Gully, on the dead wood of *Rosa* sp., May 1, 1920, col. J. F. Brenckle, det. F. Petrak as *V. tristicha.* 5PRM 164652, North Dakota, Whitestone Gully, on the dead wood of *Rosa* sp., May 16, 1920, col. J. F. Brenckle, det. F. Petrak as *V. tristicha.*

Analysis of the morphological characteristics. Macrophotographs of the collections were taken with a Panasonic Lumix DMC-FZ300 camera (Panasonic, P.R. China) equipped with a Raynox DRC-250 conversion lens (Raynox, Japan). Subsequently, focus stacks were processed in CombineZP software (version 1.0) (Hadley 2012) and edited in Zoner Photo Studio software (version 17). Fungal structures (asci, spores) were examined in distilled water by light microscope (Bresser Trino, Germany), their microphotographs were taken with a USB 2.0 YW500 digital camera (ShenZhen YangWang Technology Co., Ltd., China) and their sizes were measured with PIXIMÉTRE software [(version 5.10 R 1541) – March 2020; Henriot 2020].

DNA extraction, PCR amplification and DNA sequencing. Recently collected material (BRNM 844603) was used for the molecular analysis. A sufficient quantity of material (2–3 stromata) was placed into a 2 mL Eppendorf tube, subsequently, this sample was cooled in liquid nitrogen and homogenised by a sterile plastic pestle and overlayed with a 0.5 M Tris-NaOH buffer (pH 8.0) to extract the DNA. Subsequently, the sample was moved to a new Eppendorf tube and 145 µL 100 mM of the Tris-HCl buffer (pH 8.0) was added. The DNA was purified using paramagnetic particles used to Beckam Coulter^{TM} SPRIselect (Berensmeier 2006). A standard polymerase chain reaction (PCR) analysis was performed. For the PCR amplification primers, ITS1/ITS4 (White et al. 1990) and LROR and LR5 (Vilgalys & Hester 1990) were used. The amplification

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Figure 1. *Cytospora tristicha* (De Not.) Mlčoch (BRNM 844603) on the dead branches of *Rosa penduline*
A, C – macroscopic view on the stroma; B – cross-section of the stroma and view on the perithecia; F, G – ascospores; D, F – asci in distilled water; E – asci in Lugol reagents; bar represents: A, B, C – 1 mm; D, E, F – 10 µm; G – 5 µm

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products were sequenced in Biocev (Czech Republic) by a 3 500 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA, USA).

Phylogenetic analyses. MEGA X software (version 10) (Kumar et al. 2018), library Ape in R Studio software (version 1.2.5033 (Paradis & Schliep 2019), IQ-Tree (version 2.2.0) (Trifinopoulos et al. 2016; Minh et al. 2020) and FigTree (version 1.4.4) [http://tree.bio.ed.ac.uk/software/figtree/ (accessed February 4, 2024)] were utilised for the bioinformatic processing of the analysed sequences. The obtained sequences of the Internal Transcribed Spacer (ITS) and Large Sub-unit (LSU) genes were first edited and then subjected to comparative analyses in the online algorithm of BLASTn to evaluate their similarity and percent identity with those sequences already present in the National Center for Biotechnology Information (NCBI) database. The newly derived sequences were subsequently incorporated into a dataset compiled mainly with the published sequences (Table 1). The sequences were loaded from the NCBI database in R Studio and then alignments were created for the individual genes using the multiple alignment using fast Fourier transform (MAFFT) algorithm in the R program. The final alignment matrix was put together in the TextPad software (version 9.3.1) (Helios Software Solutions). Phylogenetic analyses used the Maximum Likelihood Algorithm with the utilisation of the General Time reversible model and the XXX (ML) heuristic method of the Nearest Neighbour Interchange. The initial tree for the Maximum Likelihood was the Neighbour-Joining method. The phylogeny test was performed by the bootstrap method with 1 000 replications. The phylogram was prepared in IQ-Tree and final adjustments were performed in the FigTree software.

RESULTS

A detailed morphological examination of rose canker based on a collection from Switzerland from 2022 was performed, and the causal agent was determined as *Valseutypella tristicha*, which was in agreement with the revision of three collections from Slovakia (from 1959) and two collections from the USA (from 1920) preserved in the Prague herbaria (Herbarium of Charles University, Herbarium of National Museum). Based on the subsequent DNA sequence analysis, its taxonomical position was revised and the new combination *Cytospora tristicha* (De Not.) Mlčoch *comb. nov.* is proposed.

Phylogeny. The phylogenetic analysis performed in the IQ Tree software combined 52 concatenated sequences of ITS and LSU regions with 853 nucleotide sites. The alignment contained 715 constant sites, 715 invariant sites, 92 parsimony informative sites and 151 distinct site patterns. For the phylogram visualisation, a TIM3e+I+G4 model was used. The root of the tree was manually set with *Diaporthe vaccinii* as an outgroup (Figure 2). The molecular analysis of the ITS gene from the Swiss collection revealed 99.5 % identity with the recently described *Cytospora rosicola* M. Pan & X.L. Fan (Pan et al. 2020). The reconstruction of the phylogeny based on the ITS and LSU genes proved its position in the genus *C. sensu stricto* by the modern concept (Figure 2).

Taxonomy. Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Diaporthales, Valsaceae

Cytospora tristicha (De Not.) Mlčoch *comb. nov.*
MycoBank No. 854605
Basionym: Diatrype tristicha De Not. (1867)
= *Valseutypella tristicha* (De Not.) Höhn. (1919)
≡ *Valsa tristicha* (De Not.) Lar. N. Vassilyeva (1994)
= *Cytospora rosicola* M. Pan & X.L. Fan (2020)

Sexual morph. Stroma pulvinate, semi-globose, erumpent to bark, dark brown to black, 2–3 (3.5) mm in diameter (Figure 1). Ascoma perithecial, narrowly cylindrical, standing parallel, numerous in stroma (about 20 to 30), 1 mm long and 0.3–0.5 mm in diameter. The ascomal wall is composed of several layers of pseudoparenchymatous cells. Hamathecium composed of numerous, narrow, cellular colourless pseudoparaphyses, in mature absent (unobserved in the collection sequenced). Asci unitunicate inoperculate, 8-spored, clavate, short stalked, IKI (Lugol's iodine) –, 43–51 × 8–11.5 µm; *n* = 15. Ascospores biseriate in asci, colourless to yellowish, allantoid, aseptate, without gelatinous sheath and ornamentation, (14–)15–21(–22) × 3.3–4.5(–5.5) µm; *n* = 20.

Asexual morph. Not observed in the studied material. Pan et al (2020) described the anamorph of this species as *Cytospora rosicola*: Conidiomata pycnidial, immersed in bark, erumpent through the surface of bark when mature, solitary, scattered, breaking through the outer branch. Locules multiple, circular to ovoid, arranged vesicularly with common walls, 620–710 mm in diameter. Ectostromatic disc brown to black, circular, disc dark brown, 380–660 mm in diameter. Ostiole conspicuous,

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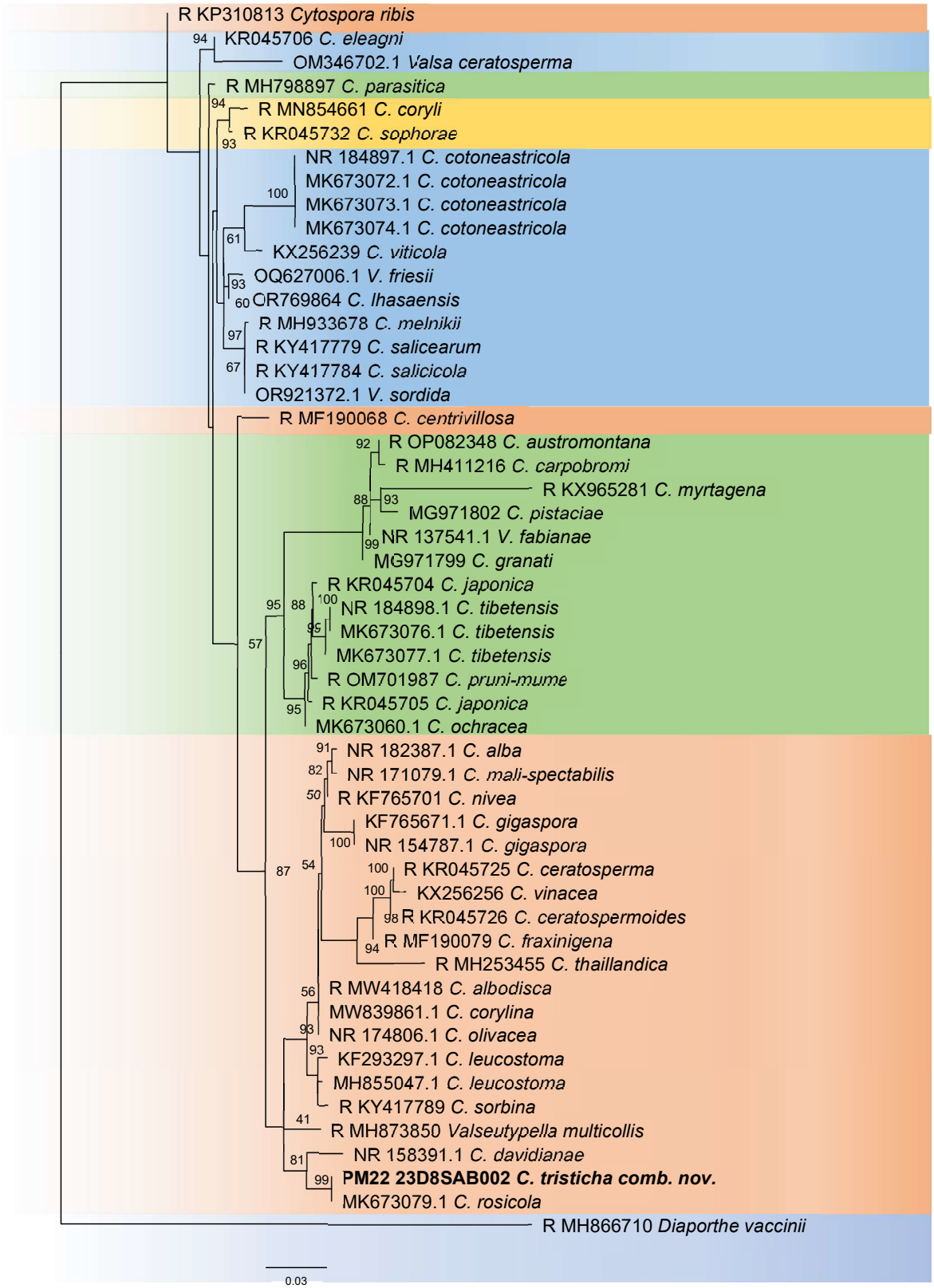

Figure 2. Phylogram of *Cytospora* spp. based on the combined sequences of the Internal Transcribed Spacer (ITS) and Large Sub-unit (LSU) genes

Maximum Likelihood (ML) bootstrap support values (n = 1 000) equal or above 75% are shown; the tree was rooted to *Diaporthe vaccinii* (CBS 160.32)

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Table 1. Taxa used in the phylogenetic analysis with their corresponding GenBank numbers

Species	Internal transcribed spacer	Large Sub-unit genes	Citation	Strain	Host
Cytospora alba	NR_182387.1	na	Lin et al. (2022)	CFCC 55462^{T}	na
C. albodisca	NR_172745.1	MW418418.1	Pan et al. (2021)	CFCC 53161^{T}	na
C. austromontana	ON989593.1	OP082348.1	Mertin et al. (2022)	RBG 7239	*Eucalyptus* sp.
C. carpobromi	MH382812	MH411216	Fan et al. (2020)	CMW 48981^{T}	*Carpobrotus edulis*
C. centrivillosa	MF190122	MF190068	Fan et al. (2020)	MFLUCC 16-1206^{T}	*Sorbus domestica*
C. ceratosperma	KR045646	KR045725	Fan et al. (2020)	CFCC 89624	*Juglans regia*
C. ceratospermoides	KR045647	KR045726	Fan et al. (2020)	CFCC 89626^{T}	*Juglans regia*
C. coryli	MN854450.1	MN854661.1	Zhu et al. (2020)	CFCC 53162^{T}	na
C. corylina	MW839861.1	na	Gao et al. (2021)	CFCC54684	na
C. cotoneastricola	NR_184897.1	na	Fan et al. (2020)	CF 20197031^{T}	na
C. cotoneastricola	MK673074.1	na	Fan et al. (2020)	CF 20197030	na
C. cotoneastricola	MK673073.1	na	Fan et al. (2020)	CF 20197028	na
C. cotoneastricola	MK673072.1	na	Fan et al. (2020)	CF 20197027	na
C. davidianae	NR_158391.1	na	Whang et al. (2015)	MUCL 55127^{T}	na
C. eleagni	KR045626	KR045706	Fan et al. (2020)	CFCC 89632	*Eleagnus angustifolia*
C. fraxinigena	MF190134	MF190079	Fan et al. (2020)	MFLU 17-0880	*Fraxinus ornus*
C. gigaspora	KF765671.1	na	Fan et al. (2020)	CFCC 89634^{T}	*Salix psammophila*
C. gigaspora	NR_154787.1	na	Fan et al. (2015)	CFCC 89635^{T}	na
C. granati	MG971799	na	Fan et al. (2020)	CBS 144237^{T}	*Punica granatum*
C. japonica	KR045624	KR045704	Fan et al. (2020)	CFCC 89956	*Prunus cerasifera*
C. japonica	KR045625	KR045705	Fan et al. (2020)	CFCC 89960	*Prunus cerasifera*
C. leucostoma	KF293297.1	na	Fotouhifar et al. (2010)	na	*Persica* sp.
C. leucostoma	MH855047.1	*	Adams et al. (2002)	na	*Prunus* sp.
C. lhasaensis	OR769864	na	Li et al. (2024)	na	*Rosa omeiensis* f. *pteracantha*
C. mali-spectabilis	NR_171079.1	na	Pan et al. (2020)	CFCC 53181^{T}	*Malus spectabilis*
C. melnikii	MH933644	MH933678	Fan et al. (2020)	CFCC 89984	*Rhus typhina*
C. myrtagena	AY347363	KX965281.1	Fan et al. (2020)	CBS 116843^{T}	*Tibouchiina urvilleana*
C. nivea	KF765685	KF765701	Fan et al. (2020)	CFCC 89643	*Salix psammophila*
C. ochracea	MK673060.1	na	Pan et al. (2020)	CFCC 53164.	*Cotoneaster* sp.
C. olivacea	NR_174806.1	na	Pan et al. (2020)	CFCC 53176^{T}	*Sorbus tianschanica*
C. parasitica	MH798884	MH798897	Fan et al. (2020)	XJAU 2542-1	*Malus* sp.
C. pistaciae	MG971802	na	Fan et al. (2020)	CBS 144238^{T}	*Pistacia vera*
C. pruni-mume	NR_171080.1	OM701987.1	Pan et al. (2020)	CFCC 53180^{T}	*Prunus mume*
C. ribis	KP281267	KP310813	Fan et al. (2020)	CFCC 50026	*Ulmus pumila*
C. rosicola	MK673079.1	na	Pan et al. (2020)	BJFC CF 20197024^{T}	*Rosa* sp.
C. salicearum	KY417745	KY417779	Fan et al. (2020)	MFLUCC 15-0861	*Salix* × *fragilis*
C. salicicola	KY417750	KY417784	Fan et al. (2020)	MFLUCC 15-0862^{T}	*Salix alba*
C. sophorae	KR045653	KR045732	Fan et al. (2020)	CFCC 50047	*Styphnolobium japonicum*
C. sorbicola	KY417755	KY417789	Fan et al. (2020)	MFLUCC 16-0584^{T}	*Acer pseudoplatanus*
C. thaillandica	MG975776	MH253455	Fan et al. (2020)	MFLUCC 17-0262^{T}	*Xylocarpus moluccensis*
C. tibetensis	NR_184898.1	na	Pan et al. (2020)	BJFC CF 20197032^{T}	na
C. tibetensis	MK673076.1	na	Pan et al. (2020)	BJFC CF 20197026	na

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Table 1. to be continued...

Species	Internal transcribed spacer	Large Sub-unit genes	Citation	Strain	Host
C. tibetensis	MK673077.1	na	Pan et al. (2020)	BJFC CF 20197029	na
C. tristicha	PP982419	PP982420	This study	na	*Rosa pendulina*
C. vinacea	KX256256	na	Fan et al. (2020)	CBS 141585^{T}	*Vitis* sp. *'Vidal'*
C. viticola	KX256239	na	Fan et al. (2020)	CBS 141586^{T}	*Vitis vinifera 'Cabernet Franc'*
Diaporthe vaccinii	NR_103701.1	MH866710.1	Fan et al. (2020)	CBS 160.32^{T}	na
Valsa ceratosperma	OM346702.1	na	Adams et al. (2006)	246	*Jacaranda* sp.
V. fabianae	NR_137541.1	na	Adams et al. (2005)	ATCC96150^{T}	*Eucalyptus* sp.
V. friesii	OQ627006.1	na	Frascella & Danti (2023)	CBS19442	*Abies* sp.
V. sordida	OR921372.1	na	Fotouhifar et al. (2010)	HMBF159	*Salix* sp.
Valseutypella multicollis	NR_145275.1	MH873850.1	Adams et al. (2006)	CBS105.89^{T}	*Quercus* sp.

The sequences newly generated in this study are indicated in bold, the type of materials are marked with ^{T}; na – information not available (Adams et al. 2002, 2005 and 2006; Fotouhifar et al. 2010; Wang et al. 2015; Fan et al. 2020; Pan et al. 2020; Gao et al. 2021; Lin et al. 2022, Mertin et al. 2022; Li et al. 2024; Frascella & Danti 2023)

circular to ovoid, dark brown to black at the same level as the disc surface, 280–350 mm in diameter. Conidiophores colourless, branched at the base or occasionally not branched. Conidiogenous cells enteroblastic, phialidic, sub-cylindrical to cylindrical. Conidia hyaline, allantoid, eguttulate, aseptate, thin-walled, (4.0–)4.5–5.0(–5.5) × 1–2 mm.

Culture characteristics. Prášil et al. (1974) cultivated anamorph from Urban's collection from *Rosa pendulina* (August 11, 1971, locality same as PRM 519025 in the Material revised). Following 3–4 weeks on KHG agar (Kern 1957) at 22 °C, a light grey-brown mycelium with asexual pycnidia of spherical shape formed, containing hyaline oval conidia (3–)4–5 ×

Table 2. *Cytospora* spp. causing canker on *Rosa* spp. with the corresponding citations

Species	Distribution	Host range	Citation
Cytospora ceratosperma (Tode) G.C. Adams & Rossman	Europe, Asia, North America	Dicotyledonous trees and shrubs, incl. *Rosa canina, R. alberti*	Fotouhifar et al. 2010; Sypabekkyzy 2024
C. donetzica Norph., Bulgakov, T.C. Wen & K.D. Hyde	Russia (Rostov Region)	Rosaceae, incl. *Rosa* sp.	Norphanphoun et al. 2017
C. leucostoma (Pers.) Sacc.	Europe, Asia, North America	Rosaceae, incl. *Rosa canina*	Fotouhifar et al. 2010
C. lhasaensis Ning Jiang	China (Tibet)	*Rosa omeiensis* f. *pteracantha*	Li et al. 2024
C. populina (Pers.) Rabenh.	worldwide	Polyphagous, incl. *Rosa* spp.	Teng 1963; Zhuang 2005; Sypabekkyzy 2024
C. sacculus (Schwein.) Gvrit.	China	*Olea europaea* and *Rosa* spp.	Zambettakis & Dzagania 1986
C. tamaricicola X.L. Fan & C.M. Tian	China	Associated with canker disease of *Rosa multiflora* and *Tamarix chinensis*	Fan et al. 2020
C. xinjiangensis M. Pan & X.L. Fan	China	*Rosa* spp.	Fan et al. 2020
Valsa salicina (Pers.) Fr. (syn. *Cytospora salicina* (Pers.) Sacc.)	worldwide	Mainly *Salix* spp.; Urban (1957) and Hayova and Minter (1998) listed *R. canina* and other hosts	Urban 1957; Hayova & Minter 1998
Valsaria insitiva (Tode) Ces. & De Not. (syn. *Cytospora cincta* Sacc.)	Europe, Asia, North America	Rosaceae, incl. *Rosa* spp., *Juglans regia* and *Vitis vinifera*	Gvritishvili 1982; Hayova & Minter 1998; Fotouhifar et al. 2010

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1.5–2 µm. After 6–8 weeks, stromata began to form, and, after 8–12 weeks, the authors reported also asci sized 50–55(–60) × 8–9 µm with four allantoid ascospores sized 11–16 × 3–4 µm.

Habitat. Dead branches of various *Rosa* spp. in the natural habitats, mainly *R. pendulina* and rarely *R. canina*. This species was not found in cultural habitats (gardens, parks, etc.). **Distribution.** Known from the temperate zone of the Northern hemisphere, mainly from mountain areas. The taxon was collected in North America (North Dakota, USA; this study), in Europe (Switzerland and Slovakia, this study) and in Asia (China – Pan et al 2020; Russia – Vassilyeva 1994). Vassilyeva and Scheuer (1996) also present one collection from Graz (Austria).

Additional *Cytospora* spp. infecting roses. In the literature, we found an additional ten species reported on cultivated and wild *Rosa* spp., some of these also infecting other host plants. The most widespread species are *Cytospora ceratosperma* (syn. *C. rosarum*), and *C. leucostoma* (Table 2).

DISCUSSION

The specimen collected by L. Zíbarová in Switzerland (BRNM 844603) was identified as *Valseutypella tristicha* based on the morphology. A comparison with other herbarium materials including two specimens from the PRM dessicate collection, which were collected in 1920 at the locality of type *V. tristicha* by the original collector J.F. Brenckle, proved the correctness of the recent material determination. The subsequent sequencing of the ITS and LSU genes revealed the phylogenetic position of this species within the genus *Cytospora* Ehrenb. A new name combination, *Cytospora tristicha* (De Not.) Mlčoch, is therefore proposed. Moreover, the ITS gene sequence showed 99.5% identity with *Cytospora rosicola* M. Pan & X.L. Fan, a species recently described from China (The Tibet Autonomous Region) based on the collection of anamorphs from branches of *Rosa* sp. (Pan et al. 2020). We found that the taxon was morphologically identical with the material examined in this work, matching the appearance and structure of the stroma, as well as the host genus. Therefore *C. rosicola* represents a synonym of the name *C. tristicha comb. nov.* proposed herein.

A review of herbarium collections and available data demonstrated that this species occurs throughout the temperate zone of the Northern Hemisphere. The pathogen was collected from wild roses so far, i.e., *Rosa pendulina, R. canina* and *Rosa* sp. (De Notaris 1867; Höhnel 1919; Prášil et al. 1974; Vassilyeva 1994; Vassilyeva & Scheuer 1996; Pan et al. 2020; this study). On the other hand, collections from cultivated roses have not been published. The data indicate that wild roses might be more susceptible to canker and dieback caused by *C. tristicha,* esp. when growing in mountains, where the fungus adapted to less favourable environmental conditions. Given the frequency of *C. tristicha* collections in Czech herbaria (PRC, PRM, BRNM) containing desiccate collections from around the world, it is probably a less common species in host populations. This species has not yet been mentioned in an overview of large-scale study focused on fungal taxa associated with Rosaceae (Wanasinghe et al. 2018).

Eleven *Cytospora* species are currently recognised on *Rosa* ssp. Microscopic ascomycetes may be overlooked until they cause symptoms, esp. in wild plant pathosystems and remote areas. Recently, a new species, *Cytospora lhasaensis* Ning Jiang, was described in Tibet from *Rosa omeiensis* f. *pteracantha* Rehd. & Wils, a common wild shrub in highland ecosystems (Li et al. 2024). It is highly probable that the real diversity of *Cytospora* spp. associated with *Rosa* spp. is much higher and our knowledge will increase with availability of NGS (new generation sequencing) which can reveal the presence of endophytic fungi in asymptomatic tissues. Nevertheless, a polyphasic approach to species determination and awareness of historic mycological studies and herbarium material is inevitable.

The host specificity of *Cytospora* spp. needs to be studied in detail. Data from available collections and records suggest that some species of the genus *Cytospora* may be relatively specific to their plant hosts. However, recent phylogenetic analyses indicated that many *Cytospora* species have broader host ranges (Norphanphoun et al 2017). Some species were originally found on one or several host plants and were thus considered of narrow host range. However, subsequent records of a species usually reveal a wider host range than originally assumed, e.g., *Cytospora leucostoma* (Pers.) Sacc was described from branches of *Prunus padus* by Saccardo (1881), while it was later also confirmed molecularly from the host plants of the genera *Betula, Cornus, Juglans, Prunus, Rosa, Salix,* and *Sorbus* (Zhu et al. 2020).

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The data published so far led us to the assumption that geographical and environmental factors are those which influence the *Cytospora* spp. occurrence strongly. The specificity to individual host plant taxa must be taken into account, but should be revised. More collections of different *Cytospora* species and availability of DNA data are required to expand our understanding of the host range and distribution of these sapro-parasitic ascomycetes.

CONCLUSION

Based on the DNA sequence data, the new name combination *Cytospora tristicha* (de Not.) Mlčoch was proposed. The revision of the available *C. tristicha* material, recent and historical specimens, together with its molecular match to *C. rosicola* from China, pointed to a pathogen occurrence in natural populations of wild *Rosa* spp. (*R. pendulina, R. canina* and *Rosa* sp.). All the studied collections are from natural habitats in mountains, while cultivated *Rosa* plants in man-made habitats have not been confirmed as hosts of this species yet. Thus, it seems that wild rose populations (especially those at higher altitudes) might be more susceptible to canker and dieback caused by *C. tristicha*.

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REFERENCES

Adams G.C., Surve-Iyer R.S., Iezzoni A. (2002): Ribosomal DNA sequence divergence and group I introns within *Leucostoma* species, *L. cinctum, L. persoonii*, and *L. parapersoonii* sp. nov., ascomycetes that cause *Cytospora* canker of fruit trees. Mycologia, 94: 947–967.

Adams G.C., Wingfield M.J., Common R., Roux J. (2005): Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (Ascomycota, Diaporthales, Valsaceae) from *Eucalyptus*. Studies in Mycology, 52: 1–144.

Adams G.C., Roux J., Wingfield M.J. (2006): *Cytospora* species (Ascomycota, Diaporthales, Valsaceae): introduced and native pathogens of trees in South Africa. Australasian Plant Pathology, 35: 521–548.

Berensmeier S. (2006): Magnetic particles for the separation and purification of nucleic acids. Applied Microbiology and Biotechnology, 73: 495–504.

Bulgakov T.S. (2010): Microfungi of *Leucostoma* and *Valsa* genera and their *Cytospora* anamorphs on arboreal plants in the steppe zone of Southern Russia // Actual problems of ecology: Mater. IV All-Russian Science Conference in Vladikavkaz, North Ossetian State University: 40–45. (in Russian)

Caio P., Ferreira B., Aucique-Perez C., Barreto R. (2021): *Diaporthe rosiphthora* sp. nov.: Yet another rose dieback fungus. Crop Protection, 139: 105365.

De Notaris G. (1867): *Diatrype tristicha*. Commentario della Societa Crittogamologica Italiana 2(fasc. 3): 481. (in Italian)

Fan X.L., Bezerra J.D.P., Tian C.M., Crous P.W. (2020): *Cytospora* (Diaporthales) in China. Persoonia, 45: 1–45.

Frascella A., Danti R. (2023): First Report on Health Conditions of the Endemic/Criticallyendangered species *Abies nebrodensis* in Sicily. IPSP, CNR: Firenze.

Fotouhifar K.B., Hedjaroude G.A., Leuchtmann A. (2010): ITS rDNA phylogeny of Iranian strains of *Cytospora* and associated teleomorphs. Mycologia, 102: 1369–1382.

Gao H., Pan M., Tian C., Fan X. (2021): *Cytospora* and *Diaporthe* species associated with hazelnut canker and dieback in Beijing, China. Frontiers in Cellular and Infection Microbiology, 11: 664366.

Gvritishvili M.N. (1982): The Fungal Genus *Cytospora* in the USSR. Izdatelstve Sabchota Sakarstvelo, Tbilisi.

Hadley A. (2012): CombineZP. Available at: https://combinezp.software.informer.com/ (accesssed December 16, 2024)

Hayova V.P., Minter D.W. (1998): *Leucostoma cinctum*. IMI Descriptions of Fungi and Bacteria. Set 137, No. 1361. CAB International, Wallingford, UK.

Henriot A. (2020): Piximétre: La mesure de dimensions sur images. Available at: http://www.piximetre.fr/ (in French, accessed December 16, 2024)

von Höhnel, F.X.R. (1919): Rehm: Ascomycetes exs. Fasc. 56 und 57. Annales Mycologici, 16: 209–224. (in German)

Kern H. (1957): Centersuchungen uber die Umgrenzung der Arten in der Ascomycetengattung *Leucostoma*. Phytopathologische Zeitschrift, 30: 149–180. (in German)

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018): MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549.

Li J.T., Li J.R., Jiang N. (2024): Identification of *Cytospora* species isolated from branch canker diseases of woody plants in Tibet, China. Forests, 15: 121.

Lin L., Pan M., Tian C., Fan X. (2022): Fungal richness of *Cytospora* species associated with willow canker disease in China. Journal of Fungi (Basel), 8: 377.

https://doi.org/10.17221/122/2024-PPS

Mertin A.A., Laurence M.H., van der Merwe M., French K., Liew E.C.Y. (2022): The culturable seed mycobiome of two *Banksia* species is dominated by latent saprotrophic and multi-trophic fungi. Fungal Biology, 126: 738–745.

Minh B.Q., Schmidt H.A., Chernomor O., Schrempf D., Woodhams M.D., von Haeseler A., Lanfear R. (2020): IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution, 37: 1530–1534.

Norphanphoun C., Doilom M., Daranagama D.A., Phookamsak R., Wen T.C., Bulgakov T.S., Hyde K.D. (2017): Revisiting the genus *Cytospora* and allied species. Mycosphere, 8: 51–97.

Pan M., Zhu H., Bonthond G., Tian C., Fan X. (2020): High diversity of *Cytospora* associated with canker and dieback of Rosaceae in China, with 10 new species described. Frontiers in Plant Science, 11: 00690.

Pan M., Zhu H., Tian C., Huang M., Fan X. (2021): Assessment of *Cytospora* isolates from conifer cankers in China, with the descriptions of four new *Cytospora* species. Frontiers in Plant Science, 12: 636460.

Paradis E., Schliep K. (2019): Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics, 35: 526–528.

Pataky N.R. (1990): Rose cane cankers. University of Illinois, Report on Plant Disease, 626: 5.

Prášil K., Šašek V., Urban Z. (1974): *Valseutypella tristicha.* In: Izolace a kultivace některých lignikolních stromatických pyrenomycetů. II. Diaporthales (Isolation and cultivation of some stromatic lignicolous Pyrenomycetes. II. Diaporthales). Česká Mykologie (Czech Mycology), 28: 13. (in Czech)

Rossman A.Y., Adams G.C., Cannon P.F., Castlebury L.A., Crous P.W., Gryzenhout M., Jaklitsch W.M., Mejia L.C., et al. (2015): Recommendations of generic names in Diaporthales competing for protection or use. IMA Fungus, 6: 145–154.

Saccardo P.A. (1881): Fungi Veneti novi vel critici v. mycologiae Veneti addendi. Series XII. Michelia, 2: 241–301.

Spielman L.J. (1984): A monograph of *Valsa* on hardwoods in North America. Canadian Journal of Botany, 63: 1355–1378.

Sypabekkyzy G. (2024): Ecological and trophic differentiation of fungal diversity in Zailiysky and Kungei Alatau [Ph.D. thesis]. Al-Farabi Kazakh National University, Almaty, Republic of Kazakhstan. (in Russian)

Teng S.C. (1963): Fungi of China. Science Press, Beijing, China. (in Chinese)

Trifinopoulos J., Nguyen L.T., von Haeseler A., Minh B.Q. (2016): W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research, 44: W232–W235.

Urban Z. (1957): Vorläufige Mitteilung der Ergebnisse einer Revision der Gattungen *Valsa* and *Valsella*. Preslia, 29: 394–395. (in German)

Vassilyeva L.N. (1994): Pyrenomycetes of the Russian Far East. 2. Valsaceae. Institute of Biology and Pedology, Vladivostok.

Vassilyeva L.N., Scheuer C. (1996): Neuere Aufsammlungen stromatischer Pyrenomyceten aus Österreich, insbesondere der Steiermark. Naturwissenschaftlicher Verein für Steiermark, 126: 61–82. (in German)

Vilgalys R., Hester M. (1990): Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology, 172: 4238–4246.

Wanasinghe D.N., Phukhamsakda C., Hyde K.D., Jeewon R., Lee H.B., Jones E.B.G., Tibpromma S., Tennakoon D.S., et al. (2018): Fungal diversity notes 709–839: Taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Diversity, 89: 1–236.

Wang Y.L., Lu Q., Decock C., Li Y.X., Zhang X.Y. (2015): *Cytospora* species from *Populus* and *Salix* in China with *C. davidiana* sp. nov. Fungal Biology, 119: 420–432.

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

Zambettakis C., Dzagania A. (1986): Comparaison des caracteres morphologiques entre micromycetes phytopathogenes des regions subtropicales (Georgie) et Mediterraneennes. Bulletin trimestriel de la Société Mycologique de France, 102: 141–154. (in French)

Zhu H., Pan M., Bezerra J.D.P., Tian C., Fan X. (2020): Discovery of *Cytospora* species associated with canker disease of tree hosts from Mount Dongling of China. MycoKeys, 62: 97–121.

Zhuang W.Y. (2005): Fungi of Northwestern China. Mycotaxon, Ltd., New York.

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