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The first confirmed host record of *Colletotrichum gloeosporioides* on *Citrus reticulata* subsp. *unshiu* in the humid subtropics of Russia

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Abstract

A fresh collection of *Colletotrichum gloeosporioides* was recovered from *Citrus reticulata* Blanco in the humid subtropics of the Krasnodar region (Russia). Morphological examinations were performed and multi-loci phylogenies based on DNA sequences derived from actin (ACT), internal transcribed spacers (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase (CHS) and β-tubulin (TUB2) were generated to identify the species and investigate its evolutionary relationships to extant species. Our study provides the first confirmed host record of *Colletotrichum gloeosporioides* on *Citrus reticulata* Blanco subsp. *unshiu* in the humid subtropics of Russia. A brief introduction on *Citrus reticulata* and the impact of *Colletotrichum gloeosporioides* are discussed. Illustrations of the new host record are also provided.

Keywords – Asexual fungi – Coelomycetes – Garden Museum "Tree of Friendship" – Krasnodar Region – Phylogeny – Satsuma mandarin – Sochi – Taxonomy

Introduction

Mandarin orange or *Citrus reticulata* Blanco (*Rutaceae*) is a spontaneous hybrid that is highly diversified and is an economically important crop (Phetkul et al. 2013, Usman & Fatima 2018). It has a wide distribution, high production and high demand on the market (Sharma & Sharma. 2009, Phetkul et al. 2013, Usman & Fatima 2018). Mandarin is a common fruit in tropical and subtropical regions, especially the Mediterranean region, including its northern-eastern edge – the Black Sea Coast of Caucasus within Russia (Sanabam et al. 2015, Raldugina & Kulyan 2018, Usman & Fatima 2018).

Citrus fruits confer health benefits as they are rich with physiologically active compounds (Tennant et al. 2009, Ahmed et al. 2020). They consist of phytochemicals that have anti-cancer and

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antifungal properties against species such as *Alternaria*, *Rhizoctonia*, *Curvularia* and *Fusarium* (Lota et al. 2000, Chutia et al. 2009, Tennant et al. 2009).

Citrus reticulata Blanco subsp. *unshiu* (Marcov.) D. Rivera Nunez et al. (syn. *C. unshiu* (Swingle) Marcov.) is a semi-seedless and easy-peeling *Citrus* species, also known as Satsuma mandarin, unshu mikan, or cold hardy mandarin (Fujii et al. 2016). It was named after its original location Wenzhou (Unsyu in Japanese spelling) in China, but introduced to the West via Japan (Fujii et al. 2016). This host is considered as a separate mandarin species by the Tanaka classification system, but it is treated as a group of mandarin varieties based on the Swingle system (Froelicher et al. 2011). Modern phylogenetic studies have shown the Satsuma mandarin is a highly inbred mandarin-pomelo hybrid (Wu et al. 2018).

Citrus reticulata subsp. *unshiu* are the most widely grown *Citrus* in the Black Sea coastal area of the Western Caucasus in Russia and Abkhazia because of their market desirability and winter-hardiness (Ryndin & Kulyan 2013, Raldugina & Kulyan 2018, Volk et al. 2018). Large-scale plantings of Satsuma mandarins and other *Citrus* in the Black Sea Coast of Caucasus since the mid-1900s demonstrated that this *Citrus* could be a profitable crop in the humid subtropics of Abkhazia and Krasnodar region of Southern European Russia and adjacent Abkhazia and Georgia (Kulyan 2013, Ryndin et al. 2014). *Citrus* production has increased and yields have become more predictable in the Black Sea Coast of Russia since the middle of the 20th century. The breeding of new cold-hardy varieties of Satsuma mandarin has led to a more adaptable *Citrus* culture to the local conditions of the humid subtropics of the Russian Black Sea Coast for growers (Ryndin et al. 2014, Raldugina & Kulyan 2018, Volk et al. 2018).

Scientists of the Subtropical Scientific Center of the Russian Academy of Sciences in Sochi (former Russian Research Institute of Floriculture and Subtropical Crops) created new adapted local varieties of Satsuma mandarin and other *Citrus* species. The Center has the largest *Citrus* collections in Russia: 132 varieties of several *Citrus* species: citron (*C. medica* L.), grapefruit (*C. paradisi* Macfad.), Ichang papeda (*C. cavaleriei* H. Lév.), lemon (*C. limon* (L.) Burm. fil.), mandarin (*Citrus reticulata* Blanco, including parthenocarpic varieties of *Citrus reticulata* var. *unshiu*), pomelo (*Citrus grandis* (L.) Osbeck), kumquat (*Citrus japonica* Thunb.), sour orange (*Citrus* × *aurantium* L.), sweet orange (*Citrus* × *sinensis* (L.) Osbeck), trifoliate orange (*Citrus trifoliata* L.), and yuzu (*Citrus junos* Sieb. ex Tanaka), which were brought from Japan, USA, Italy, Spain, Nicaragua and Abkhazia (Ryndin & Kulyan 2016). The unique experimental garden-museum "Tree of Friendship" in Sochi is one of the main bases for the research and breeding of *Citrus* species and their varieties in Russia, including the unique object – Tree of Friendship. This tree was planted by breeder F.M. Zorin in 1934 as a scientific experiment to create a new mandarin hybrid, but soon the Tree became a unique collection of 45 different *Citrus* species and varieties grafted on this *Citrus* tree by many famous people from many countries. The tree was officially named "Tree of Friendship" in 1957, and in 1981 the former experimental breeding base around the Tree (2 ha) was transformed into a public Garden-Museum "Tree of Friendship" with the special Museum building (Kravtsov 2009).

Low production of *Citrus* fruits may often be due to unfavorable weather conditions, pests and diseases caused by many microorganisms, especially fungi and viruses (Sharma & Sharma 2009, Tennant et al. 2009, Usman & Fatima 2018). The symptoms of the diseases can be observed on the aerial parts of the host such as the leaves, branches and fruits (Sharma & Sharma 2009). Diseases that are common among *Citrus* are anthracnose, cankers *Citrus* gummosis, the decay of fibrous root, crown rot, foot rot, brown rot, necrosis of tissue and canopy blight (Jiang et al. 2012, Naqvi 2004). Different fungal species are the most numerous and significant pathogens of *Citrus* species in the Black Sea Coast of Russia. Nowadays at least 35 plant pathogenic fungi were registered on *Citrus* species in the humid subtropics of Krasnodar Region and adjacent Abkhazia (Aiba et al. 2018). The most widespread fungal pathogens among them are *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (anthracnose of leaves and twigs). Others, but most devastating pathogens are *Plenodomus tracheiphilus* (Petri) Gruyter, Aveskamp & Verkley, *Phytophthora citricola* Sawada, and *Verticillium albo-atrum* Reinke & Berthold, which can infect

many parts of *Citrus* trees and ultimately cause the death of the trees (Aiba et al. 2018).

Sometimes, *Citrus* fruits may be affected by *Alternaria alternata* (Fr.) Keissl., *Aspergillus niger* Thiegh., *Botrytis cinerea* Pers., *Elsinoe fawcettii* Bitanc. & Jenkins, *Phyllosticta citricarpa* (McAlpine) Aa, *Trichothecium roseum* (Pers.) Link, and several *Aspergillus*, *Fusarium* and *Penicillium* species. Leaf spots could be caused by *Mycosphaerella gibelliana* (Pass.) Jacz. and *Phyllosticta citricola* Sacc. and sooty moulds in *Citrus* leaves by *Aithaloderma citri* (Briosi & Pass.) Woron. and *Meliola citricola* Syd. & P. Syd. are usual sooty moulds on *Citrus* leaves (Aiba et al. 2018). *Macrophoma mantegazziana* (Penz.) Berl. & Voglino, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Nectria cinnabarina* (Tode) Fr., *Sphaeropsis tumefaciens* Hedges, and several *Dothiorella* spp. cause blight and branch necrosis of *Citrus* trees, and *Citrus* roots rot may occur due to *Armillaria mellea* (Vahl) P. Kumm., *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr., *Irpex lacteus* (Fr.) Fr., *Ganoderma applanatum* (Pers.) Pat., *G. lucidum* (Curtis) P. Karst., *Sclerotinia sclerotiorum* (Lib.) de Bary, and several *Phytophthora* species cause *Citrus* root rots (Aiba et al. 2018).

Colletotrichum species and their importance

Corda (1831) introduced *Colletotrichum*, the only member of *Glomerellaceae* (Réblová et al. 2011, Maharachchikumbura et al. 2015, Jayawardena et al. 2016a, Hyde et al. 2020a). The genus was initially accommodated in *Phyllachoraceae*, and later it was placed in *Glomerellaceae* in 1984 by Locquin. The placement was validated when phylogenetic data became available and Réblová et al. (2011) clarified the placement (Hyde et al. 2020a). Species in the C*olletotrichum* are well-known pathogens on plants and humans (Jayawardena et al. 2017, Bhunjun et al. 2019, Jayawardena et al. 2021). The Species Fungorum database counts over 200 species names for *Colletotrichum* since August 2020 (http://www.speciesfungorum.org). *Colletotrichum* species are usually misidentified because of overlapping morphological characteristics and differences in taxonomists' perceptions. Previously, scientists had the misconception that *Colletotrichum* is host-specific which resulted in species nomenclatural problems (Farr et al. 2006, Jayawardena et al. 2016a, b). Also, several species lack type specimens of living strains to carry out DNA based identifications (Jayawardena et al. 2016b). DNA based multi-loci phylogenetic analyses have largely improved species identification and this has also led to species synonymy and provided much better taxonomic insights into the naming of species based on hosts (Farr et al. 2006, Lopes da Silva et al. 2019).

Colletotrichum life modes can be saprobic, pathogenic and endophytic. Some *Colletotrichum* species can change their lifestyle depending on nutritional requirements and environmental conditions (Farr et al. 2006, Nesher et al. 2008, Cannon et al. 2012, Jeewon et al. 2013, Jayawardena et al. 2016a, b, Jeewon et al. 2017, Samarakoon et al. 2018). Conidia and ascospores of *Colletotrichum* spread with the help of water splashes during rainfalls (Farr et al. 2006, Cannon et al. 2012). Several species of *Colletotrichum* can cause infection by forming appressorium that will penetrate the host and facilitate germination (Farr et al. 2006, Cannon et al. 2012, Jayawardena et al. 2016a, b).

Highly pathogenic *Colletotrichum* species are reported to affect several important berries and fruit crops such as avocado (*Persea americana*), *Citrus*, banana (*Musa* spp.), mango (*Mangifera indica*), strawberry (*Fragaria ananassa*) and tomato (*Solanum lycopersicum*) (Cannon et al. 2012, Huang et al. 2013, Guarnaccia et al. 2017) that can cause diseases such as root rot, crown rot, brown blotch and anthracnose diseases (Farr et al. 2006, Cannon et al. 2012, Ahmed et al. 2020). Some examples of pathogenic *Colletotrichum* species are *C. acutatum sensu lato, C. fructicola, C. gloeosporioides, C. nymphaeae* and *C. viniferum* (Cannon et al. 2012, Jayawardena et al. 2017, Hyde et al. 2020b).

Colletotrichum gloeosporioides is also known as a preharvest pathogen, which later can transit to necrotrophs during harvest or post-harvest. Furthermore, pathogenic members of *Colletotrichum* exist as endophytes and often may be introduced to other countries by plants trading (Farr et al. 2006). This can result in yield loss or destruction of the crops if the infection propagates

(Jiang et al. 2012, Lopes da Silva et al. 2019). *Colletotrichum* species can attack the host plant leaves regardless of the stage of plant growth (Jiang et al. 2012).

Colletotrichum gloeosporioides can form lesions on some hosts and are seen as small, circular spots that can be water-soaked and sunken and constantly increasing in size (Jiang et al. 2012). The examples of some high-susceptible crops to pathogenic strains of *Colletotrichum* are *Citrus sinensis*, *Juglans* regia, *Litchi chinensis* and *Manihot esculenta* amongst others (Jiang et al. 2012). As studies reported, *Colletotrichum gloeosporioides* can be the usual plant anthracnose pathogen on apple (*Malus domestica*), olive (*Olea europea*), ivy (*Hedera taurica*), privet (*Ligustrum vulgare*) and prickly Russian thistle (*Kali tragus*) in the Republic of Crimea (Dudka et al. 2004) and Krasnodar Region of Russia (Kolomiets et al. 2008). This species is also well-known plant pathogen of many *Citrus* species in Mediterranian countries: Italy (Aiello et al. 2015), Greece and Spain (Guarnaccia et al. 2017), Portugal (Ramos et al. 2016), Morocco (Benyahia et al. 2003), Algeria (Mahiout et al. 2018), Tunisia (Rhaiem & Taylor 2016), Turkey (Huseyinov & Selcuk et al. 2001). This species was recorded on citruses in the western part of the South Caucasus region: Republic of Georgia (Zambettakis & Dzagania 1986), Abkhazia and Black Sea Coast of Russia (Aiba et al. 2018), however, these records were not confirmed by molecular phylogenetic methods.

Materials & Methods

Sample collection, isolation and identification

Leaves of *Citrus reticulata* Blanco with spots associated with a microfungus was collected in Khostinsky City District, the Garden Museum "Friendship Tree" (Russia, Krasnodar Region, Sochi, Khostinsky City District). Specimens were observed using a Motic SMZ 168 series dissecting stereo-microscope and morphological structures were examined using a stereomicroscope (Zeiss Discovery v8) fitted with Axio Cam ERc5S and photo-captured with a Leica DM2500 compound microscope attached with a Leica MC190 HD camera. Single spore isolations were carried out and pure cultures were obtained following the method described in Chomnunti et al. (2014) and Senanayake et al. (2020). The cultures were incubated for 10 to 15 days at 25°C with frequent observation. Sporulation was observed after 2 weeks, after which fungal morphology characters were examined as described by Cai et al. (2009). Morphological measurements were made using Tarosoft® Image Frame Work program and the images were processed and photo plates were made with Adobe Photoshop CC 2019.

DNA extraction, PCR amplification and sequencing

Fresh mycelium was collected from the margin of colonies on MEA plates and transferred into 1.5 ml microcentrifuge tubes for genomic DNA extraction. Modified CTAB method was used during Genomic DNA extraction from fresh mycelia described by Guo et al. (2000).

DNA amplification was accomplished using known primer pairs, ITS1/ITS4 to obtain sequence for internal transcribed spacer (ITS) of the rRNA gene (White et al. 1990). GDF/GDR was used for the gene coding for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Templeton et al. 1992). And ACT512F/ACT783R was used for actin (ACT) (Carbone & Kohn 1999). During, polymerase chain reactions (PCR) a total volume of 25 µl using PCR mixtures containing 16.2 µl of ddH2O, 1 µl of each primer, 3.0 µl of dNTPs (TaKaRa, China), 2.5 µl of 10x Ex-Taq buffer (TaKaRa, China), 1 µl of genomic DNA, and 0.3 µl of TaKaRa Ex-Taq DNA polymerase (TaKaRa, China) were used. For PCR amplification, A BIORAD C1000 TouchTM Thermal Cycler was used (Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: initial denaturation 95°C for 3 min, 35 cycles of denaturation at 95°C for 30 s, annealing for 48 s, elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. The temperature for the annealing step was 59°C for ITS, 54°C for GAPDH and 56°C for the ACT. To visualize the PCR products, ethidium bromide (EtBr) stain was used on 1% agarose electrophoresis gels. DNA sequencing of the required genes was done using the same PCR primers by Beijing Biomed Gene Technology Co., Ltd, China.

Phylogenetic analyses

Phylogenetic analyses were carried out using a combined dataset of ITS, GAPDH, ACT, CHS-1 and TUB2. Separate ITS, GAPDH, and ACT DNA sequences were subjected to the BLAST search engine of NCBI to verify and select taxa for phylogenetic analyses. Taxa used in the analyses were found from the latest publications (Bhunjun et al. 2019).

BioEdit v. 7.0.5.2 (Hall 1999) was used to merge the single sequence datasets (ITS, GAPDH, ACT, CHS-1 and TUB2) into a concatenated dataset. The phylogenetic analyses were based on maximum likelihood (ML), Bayesian posterior probability (BYPP) and Maximum parsimony analysis (MP). MrModeltest 2.2 (Nylander 2004) was used to select the best-fit nucleotide substitution models under the Akaike information criterion (AIC) for Bayesian analysis. Maximum likelihood analyses were generated using RaxMl-HPC2 on XSEDE (8.2.12) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010), using the GTR+I+G model of evolution.

Maximum parsimony analysis was performed using the PAUP1.0b10 software with the heuristic search option 1,000 random replicates (Swofford 2004). Maxtrees were set up to 5000 and branches of zero length were collapsed and all multiple parsimonious trees were saved. Length tree (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for trees generated under different optimality criteria. The validness of the most parsimonious trees was calculated by 1000 bootstrap replications resulting from maximum parsimony analysis (Hillis & Bull 1993). Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed to determine whether the trees were significantly different.

Bayesian analysis was carried out using MrBayes v 3.1.2 (Huelsenbeck & Ronquist 2001) to generate a posterior probability. For 10 x 10^6 generations, six simultaneous Markov chains were used and trees sampling were done at every 100^{th} generation. The stationary phase was examined by checking the distribution of log-likelihood scores and thus, it was decided, if extra runs were needed to achieve convergence, using the program Tracer 1.4 (Drummond & Rambaut 2007). To generate a reliable tree, 20% of generated trees were discarded and the remaining 80% were used to calculate posterior probabilities for the final tree.

Maximum likelihood bootstrap value (BS) equal to or greater than 50%, Bayesian Posterior Probabilities (BYPP) equal to or greater than 0.90 is given below or above each node. Figtree V.14 was used to visualize the tree (Rambaut 2012) and was edited using Microsoft Office PowerPoint 2016. Facesoffungi numbers are acquired as in Jayasiri et al. (2015). The new sequences generated from this study were submitted to GenBank (Table 1).

Results

Phylogenetic analyses

The combined alignment dataset comprised 55 taxa. *Colletotrichum boninense*)CBS 123755(and *Colletotrichum catinaense* (CBS 142417) were used as outgroup taxa. The MP dataset had 2111 characters of which 1375 were constant, 288 variable characters were parsimony-uninformative, and 448 characters were counted as a parsimony-informative character. The most parsimonious tree had scores as follows: TL = 1411, CI = 0.65, RI = 0.76, RC = 0.50, HI = 0.35.

RAxML analysis yielded a best scoring tree)Fig. 1) with a final ML optimization likelihood value of -10818.124024. The matrix had 874 distinct alignment patterns, with 18.92% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.225580 C = 0.302893, G = 0.242537, T = 0.228990; substitution rates: AC = 1.097064, AG = 2.844604, AT = 1.056356, CG = 0.921213, CT = 4.560703 and GT = 1.000000; gamma distribution shape parameter α = 1.103448. After 2x10^6 generations, the Bayesian analysis resulted in 10 000 trees. The first 1000 trees were discarded as it represented the burn-in phase of the analysis. The remaining 9 000 trees were used to calculate posterior probabilities in the majority rule consensus tree.

The MP tree generated indicates that our strain groups with other *Colletotrichum gloeosporioides*)with strong bootstrap support 99% ML and 0.99 BYPP.

Table 1 Taxa used in the phylogenetic analyses and their GenBank accession numbers. A newly generated sequence for *Colletotrichum gloeosporioides* is indicated in **bold red**. Ex-type strains are indicated in **black bold** and type species are indicated by *.

Taxa	Host	Location	Culture accession No.	GenBank Accession No.				
				ITS	GAPDH	ACT	CHS-1	TUB2
C. aenigma	*Persea Americana*	Israel	**ICMP 18608***	JX010244	JX010044	JX009443	JX009774	JX010389
C. aeschynomenes	*Aeschynomene virginica*	Arkansas	**ICMP 17673***	JX010176	JX009930	JX009483	JX009799	JX010392
C. alatae	*Dioscorea alata*	Rajasthan	**ICMP 17919***	JX010190	JX009990	JX009471	JX009837	JX010383
C. alienum	*Malus domestica*	New Zealand North	**ICMP 12071***	JX010251	JX010028	JX009572	JX009882	JX010411
C. aotearoa	*Coprosma*	New Zealand North	**ICMP 18537***	JX010205	JX010005	JX009564	JX009853	JX010420
C.artocarpicola	*Artocarpus heterophyllus*	Thailand	**MFLUCC 18-1167***	MN415991	MN435568	MN435570	MN435569	MN435567
C. asianum	*Coffea arabica*	Thailand	**ICMP 18580***	FJ972612	JX010053	JX009584	JX009867	JX010406
C.boninense	*Crinum asiaticum*	Ogasawara-shoto	**CBS 123755***	JQ005153	JQ005240	JQ005501	JQ005327	JQ005588
C. camelliae	*Camellia sinensis*	China	**CGMCC 3.14925***	KJ955081	KJ954782	KJ954363	-	KJ955230
C. catinaense	*Citrus reticulata*	Italy, Catania	**CBS 142417***	KY856400	KY856224	KY855971	KY856136	KY856482
C. chengpingense	*Fragaria × ananassa*	China	**MFLUCC 15-0022***	KP683152	KP852469	KP683093	KP852449	KP852490
C. citri-maximae	*Citrus maximum*	China	**AGMy0254***	KX943582	KX943578	KX943566	KX943571	KX943586
C. conoides	*Capsicum annuum*	China	**CAUG17***	KP890168	KP890162	KP890144	KP890156	KP890174
C. cordylinicola	*Cordyline fruticose*	Thailand	**ICMP 18579***	JX010226	JX009975	HM470235	JX009864	JX010440
C.endophytica	*Pennisetum purpureum*	Thailand	**MFLUCC 13-0418***	KC633854	KC832854	KF306258	-	-
C. fructicola	*Coffea arabica*	Thailand	**ICMP 18581***	JX010165	JX010033	FJ907426	JX009866	JX010405
C. fructivorum	*Vaccinium macrocarpon*	New Jersey	**Coll1414***	JX145145	-	-	-	JX145196
C. gloeosporioides	*Citrus bergamia*	Greece	CPC 27129	KY856425	KY856249	KY855998	KY856165	KY856507
C. gloeosporioides	*Citrus sinensis*	South Africa	AGMy0026b	KX578790	KX578774	-	-	KX578806
C. gloeosporioides	*Citrus limon*	Vietnam	AGMy0250	KX578801	KX578785	-	-	KX578817
C. gloeosporioides	*Citrus sinensis*	Italy	**CBS 112999***	JX010152	JX010056	JX009531	JX009818	JX010445

Table 2 Continued.

Taxa	Host	Location	Culture accession No.	GenBank Accession No.				
				ITS	GAPDH	ACT	CHS-1	TUB2
C. gloeosporioides	*Citrus sinensis* '*Lanelate*'	Spain	CBS 142408	KY856402	KY856226		KY856142	KY856484
C. gloeosporioides	***Citrus reticulata***	**Russia**	**MFLUCC 20-0148**	**MT947088**	**MW192774**	**MW192773**	**-**	**-**
C. gloeosporioides	*Citrus limon*	Italy	CPC 26373	KY856406	KY856230	KY855979	KY856146	KY856488
C. gloeosporioides	*Citrus paradise*	Italy	CPC 26376	KY856407	KY856231	KY855980	KY856147	KY856489
C. gloeosporioides	*Citrus limon*	Italy	CPC 26381	KY856408	KY856232	KY855981	KY856148	KY856490
C.gloeosporioides	*Citrus sinensis*	Zimbabwe	AGMy0246	KX578797	KX578781	-	-	KX578813
C.gloeosporioides	*Citrus sinensis*	Brazil	AGMy0229a	KX578796	KX578780	-	-	KX578812
C. gloeosporioides	*Citrus floridana*	Italy	CPC 28155	KY856442	KY856266	KY856015	KY856182	KY856524
C. gloeosporioides	*Citrus medica*	Italy	CPC 26515	KY856412	KY856236	KY855985	KY856152	KY856494
C. grevilleae	*Grevillea* spp	Italy	**CBS 132879***	KC297078	KC297010	KC296941	KC296987	KC297102
C. hebeiense	*Vitis vinifera*	China	**MFLUCC 13-0726***	KF156863	KF377495	KF377532	KF289008	KF288975
C. henanense	*Camellia sinensis*	China	**CGMCC 3.17354***	KJ955109	KJ954810	KM023257	-	KJ955257
C. horii	*Diospyros kaki*	Japan	**ICMP 10492***	GQ329690	GQ329681	JX009438	JX009752	JX010450
C. hystricis	*Citrus hystrix*	Italy	**CPC 28153 ***	KY856450	KY856274	KY856023	KY856190	KY856532
C. jiangxiense	*Camellia sinensis*	China	**CGMCC 3.17363***	KJ955201	KJ954902	KJ954471	-	KJ955348
C. kahawae	*Coffea arabica*	Kenya	**ICMP 17816***	JX010231	JX010012	JX009452	JX009813	JX010444
C. ledongense	*Hevea brasiliensis*	China	**LD1680***	MG242008	MG242016	MG242014	-	KX893580
C. musae	*Musa* spp.	USA	**ICMP 19119***	JX010146	JX010050	JX009433	JX009896	HQ596280
C. nupharicola	*Nuphar lutea*	Washington	**ICMP 18187 ***	JX010187	JX009972	JX009437	JX009835	JX010398
C.pandanicola	*Pandanus* spp.	Thailand	**MFLUCC 17-0571***	MG646967	MG646934	MG646938	MG646931	MG646926
C. proteae	*Protea* spp.	South Africa	**CBS 132882***	KC297079	KC297009	KC296940	KC296986	KC297101
C. pseudotheobromicola	*Vitis* spp.	China	**JZB330119***	MG763975	MG812553	MG812544	-	MG812559
C. psidii	*Psidium* spp.	Italy	**ICMP 19120***	JX010219	JX009967	JX009515	JX009901	JX010443
C. queenslandicum	*Carica papaya*	Australia	**ICMP 1778***	JX010276	JX009934	JX009447	JX009899	JX010414
C. rhexiae	*Rhexia virginica*	Delaware	**Coll1026***	JX145128	-	-	-	JX145179
C. salsolae	*Salsola tragus*	Hungary	**ICMP 19051***	JX010242	JX009916	JX009562	JX009863	JX010403
C. siamense	*Coffea arabica*	Thailand	**ICMP 18578***	JX010171	JX009924	FJ907423	JX009865	JX010404
C. syzygicola	*Syzygium samarangense*	Thailand	**MFLUCC 10-0624***	KF242094	KF242156	KF157801	-	KF254880
C. temperatum	*Vaccinium macrocarpon*	New York	**Coll883***	JX145159	-	-	-	JX145211

Table 3 Continued.

Taxa	Host	Location	Culture accession No.	GenBank Accession No.				
				ITS	GAPDH	ACT	CHS-1	TUB2
C. theobromicola	*Theobroma cacao*	Panama	**ICMP 18649***	JX010294	JX010006	JX009444	JX009869	JX010447
C. ti	*Cordyline* spp.	New Zealand	**ICMP 4832***	JX010269	JX009952	JX009520	JX009898	JX010442
C. tropicale	*Theobroma cacao*	Panama	**ICMP 18653***	JX010264	JX010007	JX009489	JX009870	JX010407
C. viniferum	*Vitis* p.	China	**GZAAS5.08601***	JN412804	JN412798	JN412795	-	JN412813
C. wuxiense	*Camellia sinensis*	China	**CGMCC 3.17894***	KU251591	KU252045	KU251672	KU251939	KU252200
C. xanthorrhoeae	*Xanthorrhoea preissii*	Australia	**ICMP 17903***	JX010261	JX009927	-	JX009823	JX010448

Abbreviation: CBS = Centraalbureau voor Schimmelcultures, MFLUCC = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, ICMP = International Collection of Microorganisms from Plants, CGMCC = China General Microbiological Culture Collection Center

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., Atti Inst. Veneto Sci. lett., ed Arti, Sér. 6 2(5): 670 (1884)
≡ *Vermicularia gloeosporioides* Penz., in Saccardo, Michelia 2 (no. 8): 450 (1882)

Index Fungorum number: IF158410; Facesoffungi number: FoF 09424

Saprobic on *Citrus reticulata* (*Rutaceae*) leaves. Sexual morph: undetermined. Asexual morph: Black conidiomata observed in the culture from the centre of the edge. *Conidiomata* 155–182 × 232–378 μm ($\bar{x}$ = 166× 285 μm, n = 6) abundant, pycnidial, wide ostiole. *Conidiophores* 2–3 × 12–30 μm ($\bar{x}$ = 2 × 21 μm, n = 2) reduced to conidiogenous cell, hyaline and smooth**.** *Conidiogenous cells* 10–39 μm ($\bar{x}$ = 21, n = 6) hyaline, smooth wall, aseptate, hyaline, slightly rounded at the centre or cylindrical, rounded at the apex and base and guttulated or granular, at times annelids. Appressoria and chlamydospores not observed.

Culture Characteristics – Colonies growing from single conidia on MEA plates white mycelium. Grey in the centre and mycelium changes to white in periphery. The culture reached its maximum diameter (80 mm) in 14 days at 22°C. White mycelium, grey to white cottony mycelium, conidial masses developed from orange to black, being black in the centre and white in the periphery. After 14 days, conidia masses could be observed and conidiomata formation was seen after 20 days.

Material examined – RUSSIA, Krasnodar Region, Sochi, Khostinsky City District, Garden Museum "Friendship Tree", on leaves of *Citrus reticulata* Blanco subsp. *unshiu* (Marcow.) D. Rivera Nunez et al., 04 August 2018, Timur S. Bulgakov, T-7269, MFLUCC 20-0148 living culture**.**

Notes – The collected specimen morphologically resembles *Colletotrichum gloeosporioides* with hyaline spores measuring 10–39 μm. Based on, phylogenetic analyses, our strain grouped with all *Colletotrichum gloeosporioides* species with strong bootstrap support (99% and 0.99 BYPP). Upon pairwise alignment of nucleotides, no base pair differences were observed in ITS and ACT regions and hence, we identify it as *C. gloeosporioides* based on the recommendations of Jeewon and Hyde (2016). Our species was also recognized as the first record on *Citrus reticulata* in Russia.

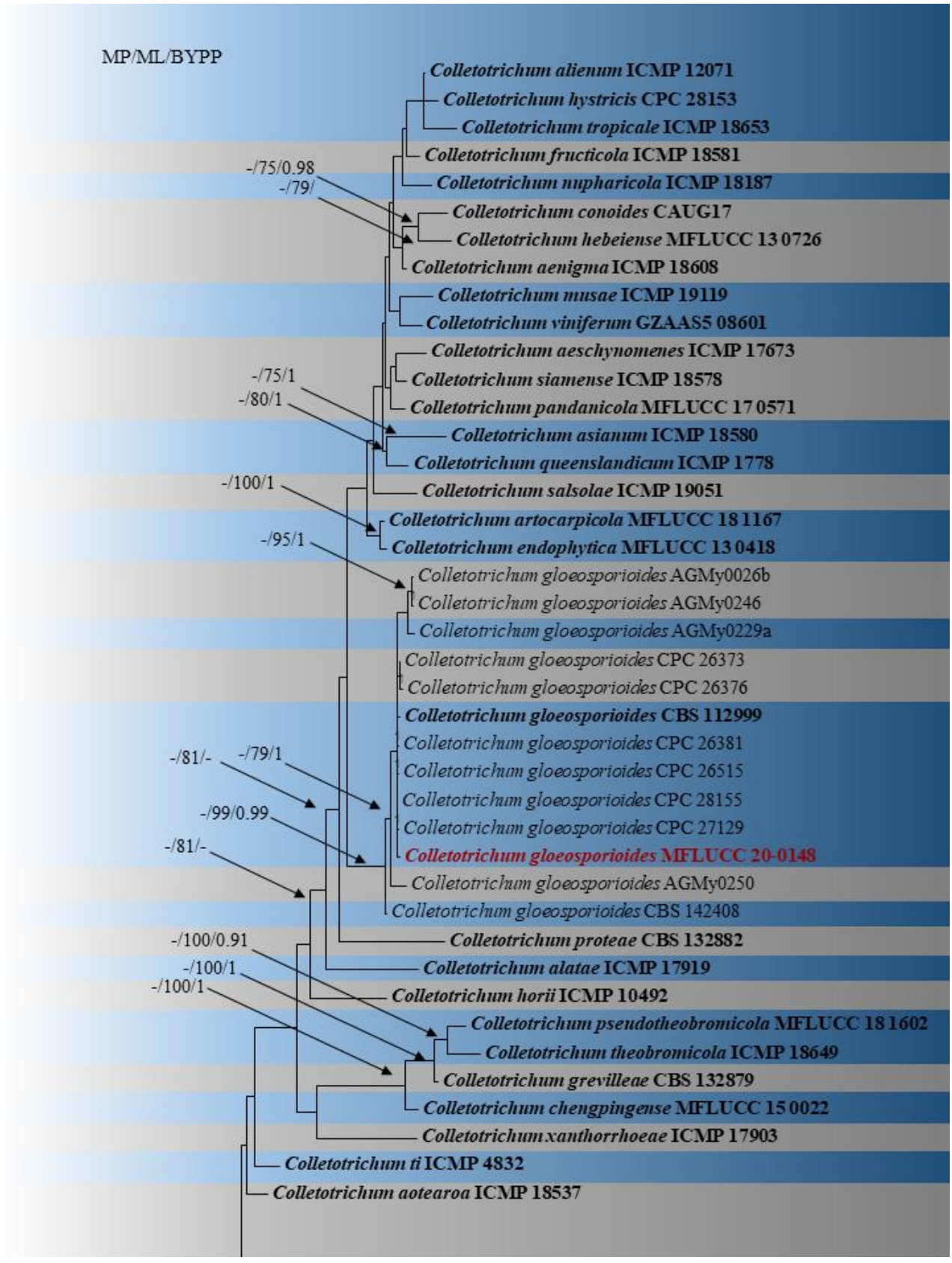

Fig. **1** – Phylogram generated from maximum parsimony (MP) analysis of combined ITS, GAPDH, ACT, CHS and TUB2 sequence data of *Colletotrichum*. Bootstrap (MP/ML) support values greater than or equal to 70% are given above the nodes and for BYPP greater than 0.90%. The culture accession number is given along with the species name, and the tree is rooted with *C. boninense* (CBS 123755) and *C. catinaense* (CBS 142417). The strain obtained in this study is in **red bold** and ex-types strains are in **black bold**.

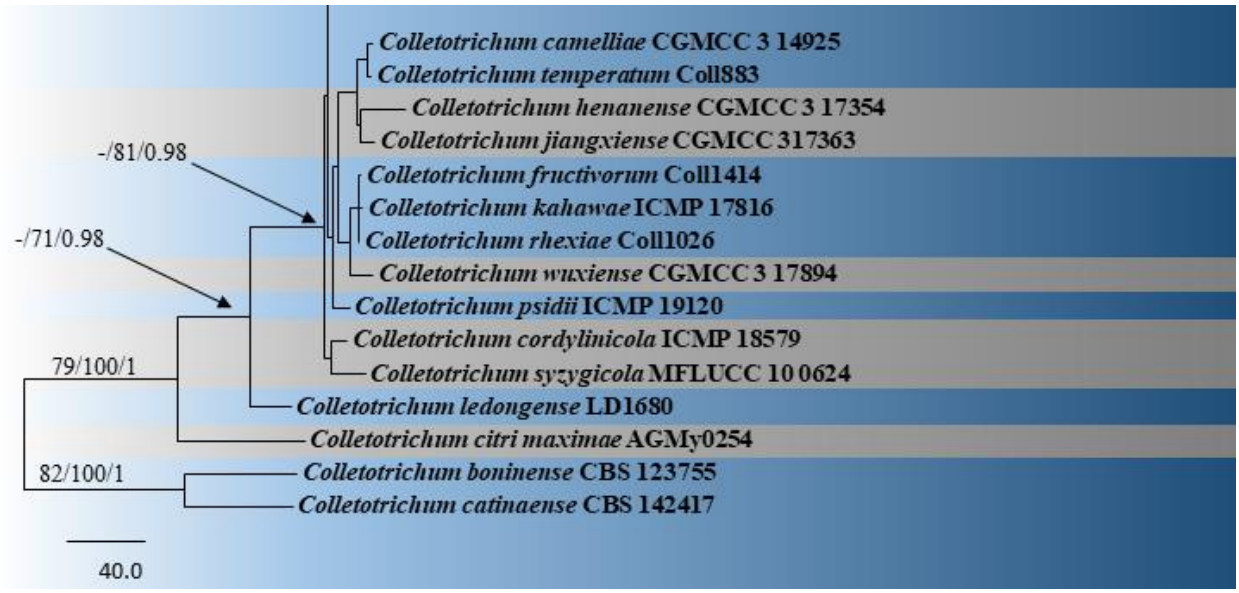

Fig. 1 – Continued.

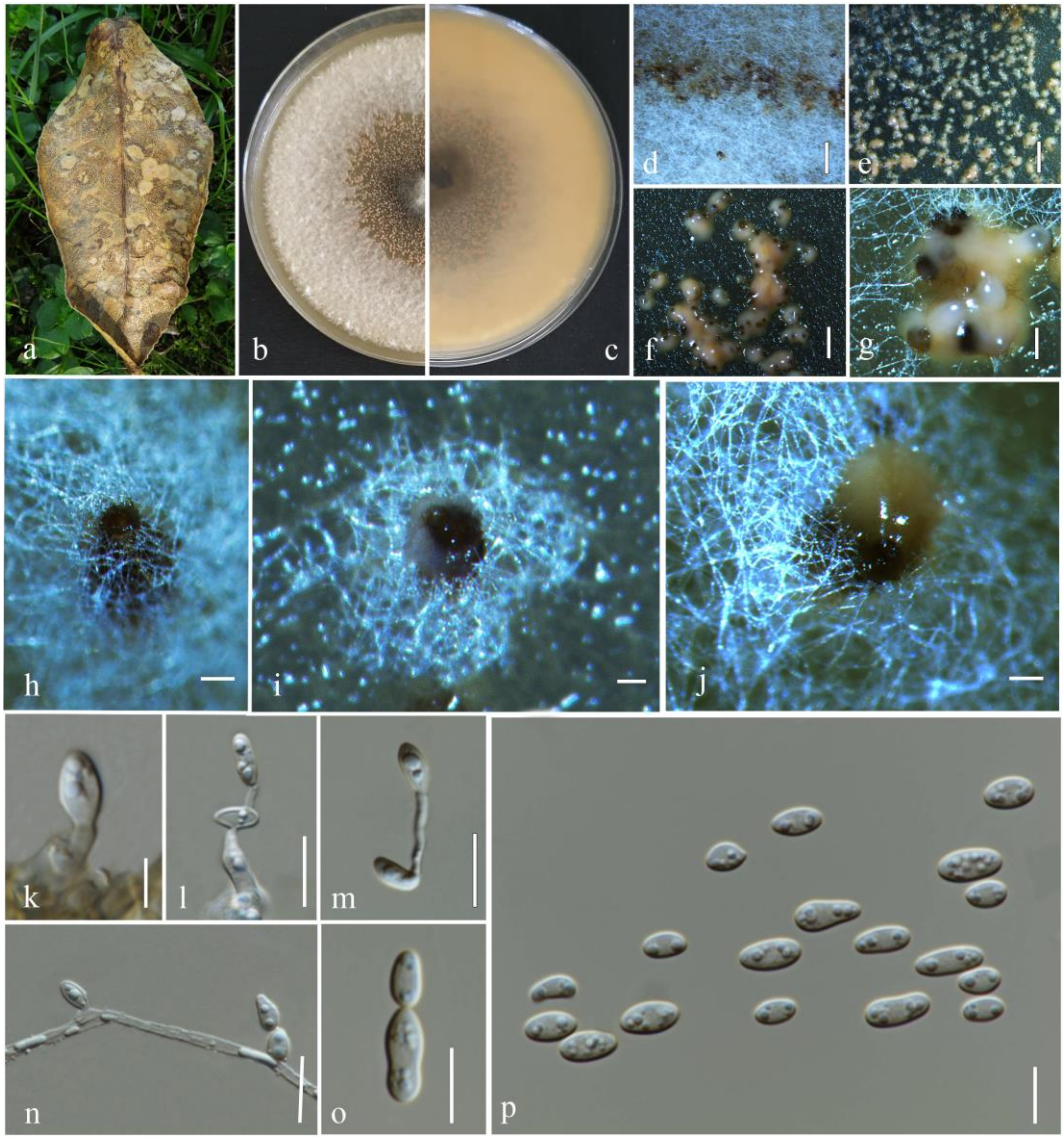

Fig. 2 – *Colletotrichum gloeosporioides* (MFLUCC 20-0148). a Specimen. b–c Culture on MEA.)a = above view, b = reverse view(. d–g Spore mass. h–j mature conidiomata. k conidiophores.

l–n conidiogenous cells and conidiogenesis. o–p conidia. Scale bars: d–h = 80 µm, i–j = 100 µm, k = 5 µm, l = 10 µm, m = 15 µm, n = 100 µm, o = 20 µm, p = 100 µm

Discussion

Colletotrichum gloeosporioides is known to inhabit several hosts in tropical and sub-tropical countries (Sanders & Korsten 2003, Rampersad et al. 2013, Douanla-Meli & Unger 2017, Guarnaccia et al. 2017, Fuentes-Aragón et al. 2018, Tovar-Pedraza et al. 2019, Sakthivel et al. 2020, Satapathy & Beura 2020). This indicates that *Colletotrichum gloeosporioides* is resistant to climatic variations (Douanla-Meli & Unger 2017, Guarnaccia et al. 2017). Since the identification of *Colletotrichum* is complex, it is important to carry out more sampling from *Citrus* hosts and on a wider geographical scale. This will help future research studies to identify locations that are at risk.

This is the first confirmed record of *Colletotrichum gloeosporioides* on *Citrus reticulata* Blanco in the humid subtropics of Russia, and it is worth noting that this fungal pathogen is already known in the Black Sea Coast of the Caucasus, where it causes anthracnose of many plants, including cultivated *Citrus* spp. (Aiba et al. 2018).

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