New host record of *Nothophoma quercina* (Didymellaceae, Pleosporales) from *Ulmus minor × Ulmus pumila* in Russia

Chaiwan N¹, Manawasinghe IS¹, Doilom M², Bulgakov TS³, Karunarathna SC², Hyde KD¹,² and Jayawardena RS¹

¹Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand
²Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, People’s Republic of China
³Department of Plant Protection, Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences, Sochi 354002, Krasnodar region, Russia
⁴Honghe Innovation Center for Mountain Futures, Kunming Institute of Botany, Honghe County 654400, Yunnan, People’s Republic of China

Chaiwan N, Manawasinghe IS, Doilom M, Bulgakov TS, Karunarathna SC, Hyde KD, Jayawardena RS 2020 – New host record of *Nothophoma quercina*. (Didymellaceae, Pleosporales) from *Ulmus minor × Ulmus pumila* in Russia. Asian Journal of Mycology 3(1), 307–315, Doi 10.5943/ajom/3/1/5

Abstract

A fungus was collected from a dead twig of *Ulmus minor × Ulmus pumila* in Southern European Russia. The phylogenetic analysis based on combined gene regions of Internal transcribed spacer (ITS), the large subunit of nuclear ribosomal RNA (LSU), beta-tubulin (TUB2) and the second-largest subunit of nuclear RNA polymerase II (RPB2) sequence dataset shows that species clusters within the genus *Nothophoma*. Morphological characteristics and phylogenetic results confirmed that the fungus is *Nothophoma quercina*. Since this is the first report of *N. quercina* from *Ulmus*, we report this species as a new host record from *Ulmus minor × Ulmus pumila* in Russia. A detailed description, colour photographs and a phylogenetic tree to show the placement of *N. quercina* are given.

Key words – Ascomycota – phylogeny – saprobe – single spore isolation – taxonomy

Introduction

*Ulmus* (elms) are deciduous and semi-deciduous trees, widely distributed over most of the Northern Hemisphere as components of natural forests, inhabiting the temperate and tropical-montane regions of North America and Eurasia, presently ranging southward across the Equator into Indonesia (Richens 1983). The genus *Ulmus* comprises 20–40 species (Sherman-Broyles 1997) and many of them are traditional landscape trees with multiple uses, such as a source of good quality wood and the main tree species for artificial forest belts (Borlea 2004). During the 19th and early 20th centuries, many species and cultivars of *Ulmus* were planted as ornamental in street, garden, and park trees in Europe, North America, and some parts of the Southern Hemisphere (Richens 1983).

*Ulmus minor* Mill., or the field elm, is one of the most common and polymorphic European elm species. Its natural range is predominantly extending from southern Europe to Asia Minor and Iran. The tree’s typical habitat is low-lying forests along the rivers, growing in association with oak...
and ash, where it tolerates floods as well as droughts (Richens 1983). Ulmus pumila L., the Siberian elm, also known as the Asiatic elm and dwarf elm, is a tree native to Central Asia, Eastern Siberia, India (northern Kashmir), Korea, Mongolia, Northern China, the Russian Far East and Tibet (Fu et al. 2002). This elm is the last tree species encountered in the semi-desert regions of central Asia (Solla et al. 2005). Nowadays, U. pumila is widely cultivated throughout Asia, Argentina, North America and Southern Europe (including steppe zone of European Russia), becoming naturalized in many places (Lykholat et al. 2018) and naturally hybridized with aboriginal elm species, especially with U. minor in some South and East European countries, such as Balkan countries, Italy, Russia, Spain and Ukraine (Zalapa et al. 2010, Brunet et al. 2013, Hirsch et al. 2017). The resulting hybrid has not yet been given a formal botanical name (Brunet et al. 2013), and we, therefore, name it as “Ulmus minor × Ulmus pumila” in this paper.

The family Didymellaceae (Pleosporales) was established by De Gruyter et al. (2009) and is characterized by the dark pseudothecial ascomata, filamentous pseudoparaphyses, 8-spored, fissitunicate, clavate to saccate asci, and hyaline, fusiform to biconical ascospores. Currently, there are 27 genera assigned to this family based on morphological and phylogenetic evidence (Chen et al. 2015, 2017, Abdel-Wahab et al. 2017, Thambugala et al. 2017, Valenzuela-Lopez et al. 2018, Wijayawardene et al. 2018, Wanasinghe et al. 2018). Species in this family are distributed in a broad range of geographical and climatic areas worldwide (Aveskamp et al. 2010, Jianyu et al. 2016) and limited studies have been carried out regarding the sexual morph of this family (Chen et al. 2015, Thambugala et al. 2017). Species in this family are endophytic, fungicolous, lichenicolous, saprobic and plant pathogenic (Aveskamp et al. 2008, Schoch et al. 2009, Jianyu et al. 2016, Doilom et al. 2018, Liu et al. 2018, Hyde et al. 2019).

Nothophoma was introduced by Chen et al. (2015) with N. infossa (Ellis & Everh.) Qian Chen & L. Cai as the type species. As described by Chen et al. (2015), the main morphological features of this genus are aseptate spores which are ovoid, oblong to ellipsoidal. In addition to Nothophoma several other genera in the family Didymellaceae including Didymella, Paraboeremia, Phoma and Phomatodes also develop aseptate conidia. However, these genera have unique characters rather than the spore shape to distinguish them from other genera in the family (Chen et al 2015). Nothophoma now comprises eleven species viz., N. anigozanthi (Tassi) Qian Chen & L. Cai, N. arachidis-hypogaeae (V.G. Rao) Qian Chen & L. Cai, N. gossypiicola (Gruyter) Qian Chen & L. Cai, N. infossa (Ellis & Everh.) Qian Chen & L. Cai, N. macrospora Valenz.-Lopez, Stchigel, Cano & Deanna A. Sutton, N. multilocularis Abdel-Wahab, N. pruni Chethana, J.Y. Yan, X.H. Li & K.D. Hyde, N. quercina (Syd. & P. Syd.) Qian Chen & L. Cai, N. raiii Roh. Sharma N. variabilis Valen.-Lopez, Cano, Guarro & Stchigel and N. spiraeae L.X. Zhang & X.L. Fan (Index Fungorum 2020).

In the present study, we report a new host record of Nothophoma quercina collected from Ulmus minor × Ulmus pumila in Russia based on both morphological characteristics and molecular phylogenetic data. This is the first report of N. quercina from Ulmus.

Materials & Methods

Fungal isolation and morphology

A dead twig of Ulmus minor × Ulmus pumila was collected from the European part of Russia (Rostov region) and observed using a stereomicroscope (Motic SMZ-171). Micro-morphological structures were photographed with a Canon EOS 600D digital camera fitted on to the Nikon Eclipse compound microscope. Measurements were made using Tarosoft (R) Image Frame Work program. Figures were processed with an Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA).

Single spore isolation was done following the method described in Chomnunthi et al. (2014). Germinating spores were aseptically transferred to fresh potato dextrose agar (PDA) plates and incubated at 28 °C. Cultures were grown for one week and morphological characteristics, such as colony colour, and texture were recorded. The herbarium material was deposited in the Herbarium
of Mae Fah Luang University (Herb. MFLU) and living culture was deposited in the Culture Collection of Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand. Faces of fungi and Index Fungorum numbers were obtained as mentioned in Jayasiri et al. (2015) and Index Fungorum (2020) respectively.

**DNA extraction, PCR amplification and sequencing**

The DNA was extracted from the mycelium grown on PDA for 14–21 days old culture using the E.Z.N.A. Forensic DNA Kit (OMEGA® biotek, China). The internal transcribed spacer region of ribosomal DNA (ITS: ITS5/ITS4) (White et al. 1990), 28s large subunit nuclear ribosomal DNA (LSU: LROR/LR5) (Vilgalys & Hester 1990), the partial beta-tubulin gene (TUB2: Bt-2a/Bt-2b) (Glass & Donaldson 1995) and the second-largest subunit of nuclear RNA polymerase II (RPB2: fRPB2-5f/fRPB2-7cR) gene (Liu et al. 1999) were amplified. The amplification reactions were performed in 25 μl final volumes containing 8.5 μl of sterilized ddH2O, 12.5 μl of Easy Taq PCR Super Mix (Tsingke company, China), 1 μl of each forward and reverse primer, and 2 μl of the DNA template. The PCR thermal cycle program for ITS, LSU and RPB2 amplification was provided as initially 94 °C for 3 mins, followed by 35 cycles of denaturation at 94 °C for 30 secs, annealing at 55 °C for 50 secs, elongation at 72 °C for 90 secs, and a final extension at 72 °C for 10 mins. The RPB2 was not successfully amplified in this study even after several attempts. The PCR thermal cycle program for TUB2 gene amplification was provided as initially 94 °C for 3 mins, followed by 35 cycles of denaturation at 95 °C for 30 secs, annealing at 53 °C for 30 secs, elongation at 72 °C for 45 secs, and a final extension at 72 °C for 90 secs. Purification and sequencing of PCR products were carried out by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China). The chromatograms of the sequences were checked to ensure the quality of the sequence using BioEdit v5 (Hall 2004). Then the BLAST search was performed and the closest taxa were identified. The sequences generated in this study are deposited in NCBI GenBank (Table 1). The nucleotide sequence data acquired were deposited in GenBank (Table 1). The finalized alignment and tree were deposited in TreeBASE, submission ID (25018).

**Phylogenetic analyses**

The sequence data of Nothophoma and closely related taxa in the Didymellaceae were retrieved from NCBI GenBank (Table 1) following blast results and recent publications (Chen et al. 2015, Valenzuela-Lopez et al. 2018, Chethana et al. 2019, Zhang et al. 2020). Downloaded sequences were aligned with the sequences obtained in the current study using MAFFT (Katoh & Standley 2013). Sequences were manually improved with BioEdit 7.0.1. when necessary (Hall 2004).

The phylogenetic analyses were performed using maximum likelihood (ML) generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. The maximum parsimony analysis (MP) was carried out with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 with the following parameter settings: characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, and branches collapsing if the maximum branch length was zero. The gaps in the alignment were treated as missing characters in the analysis of the combined data set, where they occurred in relatively conserved regions. Trees were inferred using the heuristic search option with 1000 random sequence additions, with maxtrees set at 1000. Descriptive tree statistics for parsimony including tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC) and homoplasy index (HI) were calculated for trees generated. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether trees were significantly different. Maximum likelihood (ML) and maximum parsimony (MP) bootstrap values equal to or greater than 60% are given at each node. Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate Bayesian posterior probability.
(BYPP) (Rannala & Yang 1996) by Markov chain Monte Carlo sampling GTR+I+G was used in the command. Six simultaneous Markov chains were run for 2000000 generations and trees were sampled at every 200th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.4 (Rambaut & Drummond 2007). First, 10% of generated trees were discarded and the remaining 90% of trees were used to calculate posterior probabilities in the majority rule consensus tree. The BYPP values greater than or equal to 0.95 are given at each node. The phylogenetic trees were visualised in the FigTree v. 1.4 (Rambaut 2012) and edited using Microsoft Office PowerPoint 2007 and Adobe Illustrator CS3 (Adobe Systems Inc., USA).

Results

Phylogenetic analyses

The combined sequence alignment comprised of 21 taxa, including Phoma herbarum (CBS 615.75) and Vacuiphoma bulgarica (CBS 357.84) as the outgroup taxa. The dataset comprised of 2365 characters (ITS, LSU, TUB2 and RPB2 sequence data).

The phylogenetic tree obtained in the present study showed similar topology to the previous study by Zhang et al. (2020). The RAxML analysis of the combined dataset yielded the best-scoring tree (Fig. 1) with a final ML optimization likelihood value of -6638.164497. The matrix had 357 distinct alignment patterns, with 10.37% of undetermined characters or gaps. Parameters for the GTR+I+G model of the combined ITS, LSU, TUB2 and RPB2 were as follows: estimated base frequencies: A = 0.237920, C = 0.242917, G = 0.277199, T = 0.241963; substitution rates: AC = 0.998108, AG = 2.908111, AT = 1.251990, CG = 0.677457, CT = 7.169579, GT = 1.000000; gamma distribution shape parameter α = 0.832287. The Bayesian analysis resulted in 10000 trees after 2000000 generations. The first 1000 trees, representing the burn-in phase of the analysis, were discarded, while the remaining 9000 trees were used for calculating posterior probabilities in the majority rule consensus tree.

The maximum parsimonious dataset consisted of 2365 characters, of which 1959 were constant, 198 parsimony-informative and 208 parsimony-uninformative. The parsimony analysis of the data matrix resulted in one most parsimonious trees with a length of 595 steps (CI = 0.775, RI = 0.781, RC = 0.605, HI = 0.225) in the first tree. Tree topologies (generated under MP and Bayesian criteria) from single-gene datasets were also compared and the overall tree topologies were congruent to those obtained from the ML tree of the combined dataset (Fig. 1). The MP tree generated based on sequence analysis of the combined dataset indicated that our strain grouped with Nothophoma quercina (UTHSC: DI16–270) and N. quercina (CBS 633.92) (Fig. 1). The isolate obtained in the present study formed with a moderate supported clade (68% ML and 0.90 BYPP) with N. quercina (UTHSC: DI16–270).

Nothophoma quercina (Syd. & P. Syd.) Qian Chen & L. Cai, Stud. Mycol. 82: 213 (2015)

Index Fungorum number: IF551664; Faces of fungi number: FoF01302

Saprobic or weakly pathogenic on Ulmus minor × Ulmus pumila (Ulmaceae). Sexual morph: Undetermined. Asexual morph: Conidiomata 94.5–95.5 μm high, 93–95 μm diam. (x = 95 × 94 μm, n = 5), pycnidial, solitary or aggregate, dark brown, globose to subglobose, immersed, unilocular, thick-walled papillate. Peridium 20–25 μm wide, composed of 4–6 layers, with outer 2–3 layers comprising brown and inner 3–4 layers of pale brown to hyaline cells of textura angularis. Conidiophores reduced to conidigenous cells. Conidiogenous cells 13–15 μm long, 7–9 μm wide, broadly compressed, phialidic, lining the inner cavity, hyaline, smooth, ampulliform, apex with prominent periclinal thickening. Conidia 4–6 × 2–3 (x = 5.7 × 3 μm, n = 30), hyaline, ellipsoidal to obovoid, oblong, asetate, minute guttules, smooth-walled.

Culture characteristics – Colonies on PDA reach 80 mm diam. after 7 days at 28°C, with regular margin, Dull white brown aerial mycelium surface floccose to woolly, with brownish
olivaceous to olivaceous near the centre and reverse dark ochreous in the centre and white in the margin.

Material examined – RUSSIA, Rostov region, Shakhty, street trees, on a dead attached twig of Ulmus minor Mill. × Ulmus pumila L. (Ulmaceae), 6 May 2017, Timur S. Bulgakov, T-1824 (MFLU 17-2126, new host record); living culture MFLUCC 18-0124.

Notes – Our isolate (MFLUCC 18-0124) formed a clade with N. quercina isolates UTHSC: DI16–270 and CBS 633.92 with moderate statistical supports (68% ML and 0.90 BYPP). Our strain shows the characteristics of N. quercina by having brown, subglobose to oval or obtuse conidia (Chen et al. 2015). Our strain was found on Ulmus minor × Ulmus pumila whereas N. quercina (UTHSC:DI16–270) was found on Human superficial tissue (USA) and N. quercina (CBS 633.92) was found on Quercus sp. (Ukraine) (Chen et al. 2015, Valenzuela-Lopez et al. 2018). No base pair differences observed in the ITS and LSU sequences among the N. quercina isolates UTHSC: DI16–270, CBS 633.92 and our strain MFLUCC 18-0124.

Table 1 Taxa used in the phylogenetic analyses and their GenBank accession numbers. The new host record of Nothophoma quercina, its culture number and GenBank accession numbers are indicated in purple bold. Ex-type strains are indicated in black bold.

| Taxa                        | Culture accession No. | GenBank Accession No. |
|-----------------------------|------------------------|-----------------------|
| N. arachidis-hyposesae      | CBS 125.93             | GU237771              |
| N. gossypiciola             | CBS 377.67             | GU237845              |
| N. infossa                  | CBS 123395             | FJ427025              |
| N. macrospora               | CBS 1008-093           | LN880536              |
| N. multiocularis            | AUMC-12003             | -                     |
| N. pruni                   | MFLUCC 18-1600         | MH827007              |
| N. pruni                   | JZB380015              | MH827004              |
| N. quercina                | UTHSCDI16-270          | LT592929              |
| N. quercina                | CBS 633.92             | GU237900              |
| N. quercina                | MFLUCC 18-0124         | MN493947              |
| N. raii                    | MCC 1082               | MF664467              |
| N. spireae                 | CFCC 53928             | MN737833              |
| N. variabilis               | UTHSCDI16-285          | LT592939              |
| Phoma herbarum             | CBS 615.75             | FJ427022              |
| Vacuiphoma bulgarica       | CBS 305.45             | GU237837              |

Discussion

The genus Nothophoma was established by Chen et al. (2015) to accommodate five species of Phoma section Macrospora with Nothophoma infossa as the type species. Nothophoma is a close relative of Phoma characterized by aseptate, ovoid, oblong to ellipsoidal conidia (Chen et al. 2015). Recent publications have reported that Nothophoma comprises 11 species (Chen et al. 2015, Crous et al. 2017, Valenzuela-Lopez et al. 2018, Chethana et al. 2019).

Our strain groups within Nothophoma quercina clade with 68% ML and 0.90 BYPP statistical supports, and forms a sister group with N. spireae which has been reported as a canker pathogen of Spiraea salicifolia from Beijing, China (Zhang et al. 2020). Nothophoma quercina
causes trunk cankers on Crabapple (*Malus micromalus*) (Aveskamp et al. 2010, Liu et al. 2018) and brown spot disease of Jujube (*Ziziphus jujube*) in China (Jianyu et al. 2016).

**Fig. 1** – Phylogram generated from RAxML based on combined ITS, LSU, TUB2 and RPB2 sequence data. Bootstrap support values for maximum parsimony (MP, on left) and maximum likelihood (ML, middle) equal to or greater than 60% and Bayesian posterior probabilities (PP, right) equal to or greater than 0.90 are indicated above the nodes. The taxon belongs to the present study is in purple bold. Ex-type strains are indicated in black bold.

**Fig. 2** – *Nothophoma quercina* (MFLU 17-2126, new host record). a, b, c Appearance of pycnidia on host surface. d Pycnidium. e Neck of pycnidium. f Peridium. g, h Conidiogenous cells.
Conidia. i Colonies on PDA. m, n, o, p Conidia from culture. Scale bars: a = 2000 μm, b, c = 200 μm, d = 100 μm, f, e, g, h = 50 μm, i-k, m-p = 5 μm.

Our finding together with previous research findings show that Nothophoma may have a wide host range. More samplings from potential plant hosts of Nothophoma and a wider geographical range will be recommended for future research studies. This is the first record of Nothophoma from Ulmus.

Acknowledgements
N. Chaiwan thanks the Thailand Research Fund (PHD60K0147) for the financial support, Molecular Phylogeny Laboratory of Kunming Institute of Botany for providing the facilities for the phylogenetic work and Deiping Wei for her help with the molecular phylogenetic work. K.D. Hyde thanks the grants entitled: the future of specialist fungi in a changing climate: baseline data for generalist and specialist fungi associated with ants, Rhododendron species and Dracaena species (Grant number: DBG6080013) and the climate change grant: Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion (Grant number: RDG613001). M. Doilom thanks the 5th batch of Postdoctoral Orientation Training Personnel in Yunnan Province and the 64th batch of the China Postdoctoral Science Foundation. S.C. Karunarathna thanks the CAS President’s International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2018PC0006) and the National Science Foundation of China (NSFC) for funding this work under the project code 3185110759.

References
Abdel-Wahab MA, Bahkali AHA, El-Gorban AM, Hodhod MS. 2017 – Natural products of Nothophoma multilocularis sp. nov. an endophyte of the medicinal plant Rhazya stricta. Mycosphere 8, 1185–1200.
Aveskamp MM, De Gruyter J, Crous PW. 2008 – Biology and recent developments in the systematics of Phoma, a complex genus of major quarantine significance. Fungal Diversity 31, 1–18.
Aveskamp MM, De Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW. 2010 – Highlights of the Didymellaceae: A polyphasic approach to characterise Phoma and related pleosporalean genera. Studies in Mycology 65, 1–60.
Borlea GF. 2004 – Investigación Agraria: Sistemas y Recursos Forestales 13, 29.
Brunet J, Zalapa JE, Pecori F, Santini A. 2013 – Hybridization and introgression between the exotic Siberian elm, Ulmus pumila, and the native Field elm, U. minor, in Italy. Biological invasions 15, 2717–2730.
Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. 2015 – Resolving the Phoma enigma. Studies in Mycology 82, 137–217.
Chen Q, Hou LW, Duan WJ, Crous PW, Cai L. 2017 – Didymellaceae revisited. Studies in Mycology 82, 105–159.
Chethana KW, Jayawardene RS, Zhang W, Zhou YY et al. 2019 – Molecular characterization and pathogenicity of fungal taxa associated with cherry leaf spot disease. Mycosphere 10, 490–530.
Chomnunti P, Hongsanan S, Hudson BA, Tian Q. 2014 – The sooty moulds. Fungal Diversity 66, 1–36.
Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ et al. 2017 – Fungal Planet description sheets: 625–715. Persoonia 39, 270–467.
De Gruyter J, Aveskamp MM, Woudenberg JHC. 2009 – Molecular phylogeny of Phoma and allied anamorph genera: Towards a reclassification of the Phoma complex. Mycological Research 113, 508–519.
Doilom M, Hyde KD, Phookamsak R, Dai DQ et al. 2018 – Mycosphere Notes 225–274: types and other specimens of some genera of Ascomycota. Mycosphere 9, 647–754.

Fu L, Xin Y, Whittemore A. 2002 – Ulmaceae, in Wu, Z. & Raven, P. (eds) Flora of China, Vol. 5 (Ulmaceae through Basellaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, US.

Glass NL, Donaldson GC. 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.

Hall T. 2004 – BioEdit v. 7.0.1. Department of Microbiology, North Carolina State University. Available at www.mbio.ncsu.edu/BioEdit/bioedit.html

Hirsch H, Brunet J, Zalapa J, Von Wehrden H et al. 2017 – Intra- and interspecific hybridization in invasive Siberian elm. Biological Invasions, 19, 1889–1904.

Huelsenbeck JP, Ronquist F. 2001 – MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Hyde KD, Tennakoon DS, Jeewon R, Bhat DJ. 2019 – Fungal diversity notes 1036–1150: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 97, 1878–9129.

Index Fungorum. 2020 – Index Fungorum. Available from: http://www.indexfungorum.org/Names/Names.asp (Accessed on 19 April 2020)

Jayasiri SC, Hyde KD, Abd-Elsalam KA, Abdel-Wahab MA et al. 2016 – Occurrence and identification of Notophoma quercina causing brown spot of jujube in China. Canadian Journal of plant pathology 38, 527–532.

Jianyu B, Xiaoming W, Yanjiang S, Canxing D. 2016 – Occurrence and identification of Notophoma quercina causing brown spot of jujube in China. Canadian Journal of Plant Pathology 4, 527–532.

Katoh K, Standley DM. 2013 – MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772–780.

Kishino H, Hasegawa M. 1989 – Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoida. Journal of Molecular Evolution 29, 170–179.

Lykholat Y, Khromykh N, Didur O, Alexeyeva A et al. 2018 – Modeling the invasiveness of Ulmus pumila in urban ecosystems in conditions of climate change. Regulatory Mechanisms in Biosystems, 9, 161–166.

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

Liu M, Zhang W, Manawasinghe IS, Zhou Y et al. 2018 – First Report of Notophoma quercina Causing Trunk Canker on Crabapple (Malus micromalus) in China. Plant Disease 1462–1462.

Miller MA, Pfeiffer W, Schwartz T. 2010 – Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), November 14, 2010, New Orleans, Louisiana. 1–8.

Rambaut A, Drummond AJ. 2007 – Tracer v1.4, Available at: http://beast.bio.ed.ac.uk/Tracer

Rambaut A. 2012 – FigTree v1.4: Tree figure drawing tool. http://tree.bio.ed.ac.uk/software/figtree/

Rannala B, Yang Z. 1996 – Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43, 304–311.

Richens RH. 1983 – Elm. Cambridge University Press, Cambridge. 347 p.

Schoch CL, Crous PW, Groenewald JZS, Boehm EWA, Burgess TI. 2009 – A class-wide phylogenetic assessment of Dothideomycetes. Studies in Mycology 64, 1–15.

Sherman-Broyles SL. 1997 – Flora of North America North of Mexico, Flora of North America Editorial Committee, ed. New York and Oxford, vol. 3.
Solla A, Martín JA, Corral P, Gil L. 2005 – Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. New Phytologist, 166, 1025–1034.

Stamatakis A, Hoover P, Rougemont J. 2008 – A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57, 758–71.

Stamatakis A. 2014 – RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

Thambugala KM, Daranagama DA, Phillips AJL. 2017 – Microfungi on *Tamarix*. Fungal Diversity 82, 239–306.

Valenzuela-Lopez N, Cano-Lira JF, Guarro J, Sutton DA et al. 2018 – Coelomycetous Dothideomycetes with emphasis on the families Cucurbitariaceae and Didymellaceae. Studies in Mycology 90, 1–69.

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 DN, Jeewon R, Peršoh D, Jones EBG et al. 2018 – Taxonomic circumscription and phylogenetics of novel didymellaceous taxa with brown muriform spores. Studies in Fungi 3, 152–175.

White TJ, Burns T, Lee S, Taylor JW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, California, USA, 315–322.

Wijayawardene NN, Hyde KD, McKenzie EHC, Wang Y. 2018 – Notes for genera update – Ascomycota: 6822-6917. Mycosphere 9, 1222–1234.

Zalapa JE, Brunet J, Guries RP. 2010 – The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae). Evolutionary Applications 3, 157–168.

Zhang LX, Yin T, Pan M, Tian CM, Fan XL. 2020 – Occurrence and identification of *Nothophoma spiraeae* sp. nov. in China. Phytotaxa 430, 147–156.