



***Cytospora* species associated with canker disease of three anti-desertification plants in northwestern China**

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Abstract

Cytospora species are important phytopathogens causing severe canker disease with a worldwide distribution and broad host range. However, identification of taxa to species level is difficult due to poor phylogenetic understanding and lack of sequenced type species. Morphological and phylogenetic studies have been carried out on several important hosts such as *Eucalyptus* and *Malus* in China, Iran, and South Africa. In this study destructive canker diseases of the anti-desertification plants, *Elaeagnus angustifolia*, *Hippophae rhamnoides*, and *Salix psammophila*, were investigated in northwest China. Multilocus phylogenetic analyses of ITS, nrLSU, RPB2, and ACT gene regions, combined with detailed morphological analyses and comparison with ex-type strains revealed six *Cytospora* species, *C. chrysoesperma*, *C. elaeagni*, *C. hippophaes*, *C. nivea*, *C. populina* comb. nov. and *C. gigaspora* sp. nov. causing cankers on these hosts. The novel species *C. gigaspora* has flat multiple locules with a conceptacle and unusually long 12 µm conidia. Detailed descriptions and molecular data for the *Cytospora* species causing cankers on the three psammophilic host plants are provided. *Cytospora elaeagni* and *C. hippophaes* have previously been recorded from *Elaeagnus angustifolia* and *Hippophae rhamnoides*, whereas the other species causing *Cytospora* canker of *Elaeagnus angustifolia* and *Salix psammophila* are new records.

Key words: Ascomycota, Diaporthales, Morphology, New species, Phylogeny

Introduction

The genus *Cytospora* (Ascomycota: Diaporthales) was established by Ehrenberg (1818). It includes important phytopathogens that cause dieback and canker disease on a wide range of plants, causing severe commercial and ecological damage and significant losses worldwide (Adams *et al.* 2005, 2006). *Cytospora* has been categorized under several coelomycetous genera in the dual-classification system, including asexual states of *Leucostoma*, *Valsa*, *Valsella*, and *Valseutypella* (Fries 1823; Saccardo 1884; Deng 1963; Tai 1979; Wei 1979; Spielman 1985; Wang *et al.* 2011; Adams *et al.* 2002). All the genera were recently combined under *Valsa*, either as subgenera or species with no additional infrageneric rank (Adams *et al.* 2005). The current International Code of Nomenclature for Algae, Fungi, and Plants (ICN) requires a single-name for pleomorphic taxa, and the dual-nomenclature system has become redundant (Hawksworth 2011). A single name for complex genera such as *Diaporthe/Phomopsis*, *Glomerella/Colletotrichum*, *Pestalotia/Pestalotiopsis*, and *Phyllosticta/Guignardia* have followed the oldest or the most conserved name (Hyde *et al.* 2009; Wikee *et al.* 2011; Huang *et al.* 2013; Wei *et al.* 2013; Udayanga *et al.* 2014). *Cytospora* (1818) is an older name than *Valsa* (1849) and the asexual state more common in nature; therefore, we chose to adopt *Cytospora* and treat *Valsa* species as synonyms for *Cytospora*. More than 560 species epithets named *Cytospora* have been recorded in Index Fungorum (2014) with an estimated 110 species in Kirk *et al.* (2008). Ex-type sequence data, is however, available for only a very few species and many taxa need epitypifying, thus identification to species level is difficult. Therefore, research towards a backbone tree for *Cytospora* species is needed so that backbone trees to species can be updated as in previous studies in other genera (Hyde *et al.* 2014).

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References

- 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.
<http://dx.doi.org/10.1071/AP06058>
- Adams, G.C., Surve-Iyer, R.S. & Iezzoni, A.F. (2002) Ribosomal DNA sequence divergence and group I introns within the *Leucostoma* species *L. cinctum*, *L. personii*, and *L. parapersoonii* sp. nov., ascomycetes that cause *Cytospora* canker of fruit trees. *Mycologia* 94: 947–967.
<http://dx.doi.org/10.2307/3761863>
- 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.
- Carbone, I. & Kohn, L. (1999) A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia* 91: 553–556.
<http://dx.doi.org/10.2307/3761358>
- Chen, M.M. (2002) *Forest fungi phytogeography, forest fungi phytogeography of China, North America, and Siberia and international quarantine of tree pathogens*. Sacramento, California, USA.
- Crous, P.W., Gams, W., Stalpers, J.A., Robert, V. & Stegehuis, G. (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* 50: 19–22.
- Deng, S.Q. (1963) *Fungi of China*. Beijing, China. [in Chinese]
- Desjardins, P., Hansen, J.B. & Allen, M. (2009) Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer. *Journal of Visualized Experiments* 33: 1–3.
- Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.
- Ehrenberg, C.G. (1818) *Sylvae Mycologicae Berolinenses*. Formis Theophili Brusckke, Berlin, Germany. [In Latin]
- Fan, X.L., Liang, Y.M., Ma, R. & Tian, C.M. (2014) Morphological and phylogenetic studies of *Cytospora* (Valsaceae, Diaporthales) isolates from Chinese scholar tree, with description of a new species. *Mycoscience* 55: 252–259.
<http://dx.doi.org/10.1016/j.myc.2013.10.001>
- Fotouhifar, K.B., Hedjaroude, G.A. & Leuchtmann, A. (2010) ITS rDNA phylogeny of Iranian strains of *Cytospora* and associated teleomorphs. *Mycologia* 102: 1369–1382.
<http://dx.doi.org/10.3852/10-034>
- Fries, E.M. (1823) *Systema mycologicum* vol 2. Greifswald, Germany. [in Latin]
- Gonzalez, F.R. (1916) Bosquejo de una Florula Hispalense de Micromicetos. *Trabajos del Museo Nacional de Ciencias Naturales. Serie Botanica* 10: 1–221.
- Hawksworth, D.L. (2011) Naming *Aspergillus* species: progress towards one name for each species. *Medical Mycology* 49: S70–S76.
<http://dx.doi.org/10.3109/13693786.2010.504753>
- Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol* 42: 182–192.
<http://dx.doi.org/10.2307/2992540>
- Huang, F., Chen, G.Q., Hou, X., Fu, Y.S., Cai, L., Hyde, K.D. & Li, H.Y. (2013) *Colletotrichum* species associated with cultivated citrus in China. *Fungal Diversity* 61: 61–74.
<http://dx.doi.org/10.1007/s13225-013-0232-y>
- Hyde, K.D., Cai, L., Cannon, P.F., Crouch, J.A., Crous, P.W., Damm, U., Goodwin, P.H., Chen, H., Johnston, P.R., Jones, E.B.G., Liu, Z.Y., McKenzie, E.H.C., Moriwaki, J., Noireung, P., Pennycook, S.R., Pfenning, L.H., Prihastuti, H., Sato, T., Shivas, R.G., Tan, Y.P., Taylor, P.W.J., Weir, B.S., Yang, Y.L. & Zhang, J.Z. (2009) *Colletotrichum*-names in current use. *Fungal Diversity* 39: 147–182.

- Hyde, K.D., Henrik Nilsson, R., Aisyah Alias, S., Ariyawansa, H.A., Blair, J.E., Cai, L., de Cock, W.A.M.A., Dissanayake, A.J., Glockling, S.L., Goonasekara, I.D., Gorczak, M., Hahn, M., Jayawardena, R.S., van Kan, J.A.L., Laurence, M.H., André Lévesque, C., Li, X., Liu, J.-K., Maharachchikumbura, S.S.N., Manamgoda, D.S., Martin, F.N., McKenzie, E.H.C., McTaggart, A.R., Mortimer, P.E., Nair, P.V.R., Pawłowska, J., Rintoul, T.L., Shivas, R.G., Spies, C.F.J., Summerell, B.A., Taylor, P.W.J., Terhem, R.B., Udayanga, D., Vaghefi, N., Walther, G., Wilk, M., Wrzosek, M., Xu, J.-C., Yan, J.Y. & Zhou, N. (2014) One stop shop: backbone trees for important phytopathogenic genera: I (2014). *Fungal Diversity* 67: 21–125.
<http://dx.doi.org/10.1007/s13225-014-0298-1>
- Katoh, K. & Toh, H. (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* 26(15): 1899–1900.
<http://dx.doi.org/10.1093/bioinformatics/btq224>
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth and Bisby's dictionary of the fungi, 10th edn*. CAB International, Wallingford.
- Kobayashi, T. (1969) *Taxonomic studies of Japanese Diaporthaceae with special reference to their life-histories*. Tokyo, Japan.
- Matheny, P.B. (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Molecular Phylogenetics and Evolution* 35: 1–20.
<http://dx.doi.org/10.1016/j.ympev.2004.11.014>
- Mehrabi, M.E., Mohammadi, G.E. & Fotouhifar, K.B. (2011) Studies on *Cytospora* canker disease of apple trees in Semrom region of Iran. *Journal of Agricultural Technology* 7: 967–982.
- O'Donnell, K. (1993) *Fusarium and its near relatives*. Wallingford, UK.
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
<http://dx.doi.org/10.1093/bioinformatics/14.9.817>
- Rambaut, A. & Drummond, A. (2010) *FigTree v.1.3.1*. Institute of evolutionary biology, University of Edinburgh, Edinburgh, UK.
- Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311.
<http://dx.doi.org/10.1007/BF02338839>
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Saccardo, P.A. (1881) *Michelia vol 2*. Typis Seminarii, Italy. [in Latin]
- Saccardo, P.A. (1882) *Sylloge Fungorum vol 1*. Typis Seminarii, Italy. [in Latin]
<http://dx.doi.org/10.5962/bhl.title.80010>
- Saccardo, P.A. (1884) *Sylloge Fungorum vol 3*. Typis Seminarii, Italy. [in Latin]
- Saccardo, P.A. (1889) *Sylloge Fungorum vol 14*. Typis Seminarii, Italy. [in Latin]
- Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejia, L.C. & White, J.F. (2008) Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. *Studies in Mycology* 62: 1–79.
<http://dx.doi.org/10.3114/sim.2008.62.01>
- Spielman L.J. (1983). *Taxonomy and biology of Valsa species on hardwoods in North America, with special reference to species on maples*. Cornell University, New York, USA.
- Spielman, L.J. (1985) A monograph of *Valsa* on hardwoods in North America. *Canadian Journal of Botany* 63: 1355–1378.
<http://dx.doi.org/10.1139/b85-190>
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
<http://dx.doi.org/10.1093/bioinformatics/btl446>
- Swofford, D.L. (2003) *PAUP*: Phylogenetic analysis using parsimony, * and other methods. Version 4.0b10*. Sinauer Associates, Sunderland.
- Tai, F.L. (1979) *Sylloge Fungorum Sinicorum*. Beijing, China. [in Chinese]
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
<http://dx.doi.org/10.1093/molbev/msr121>
- Udayanga, D., Castlebury, L.A., Rossman, A.Y., Chukeatirote, E. & Hyde, K.D. (2014) Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. *Fungal Diversity* 67: 203–229.
<http://dx.doi.org/10.1007/s13225-014-0297-2>
- Wang, X.L., Kang, Z.S., Huang, L.L. & Yang, P. (2007) Pathogen identification of *Valsa* canker on pear tree: evidences from rDNA-ITS sequences and cultural characteristics. *Mycosystema* 26: 517–527. [in Chinese]
- Wang, X.L., Wei, J., Huang, L.L. & Kang, Z. (2011) Re-evaluation of pathogens causing *Valsa* canker on apple in China. *Mycologia* 103,

317–324.

<http://dx.doi.org/10.3852/09-165>

Wei, J.C. (1979) *Identification of Fungus Handbook*. Shanghai, China. [in Chinese]

Wei, J.G., Phan, C.K., Wang, L., Xu, T., Luo, J.T., Sun, X. & Guo, L.D. (2013) *Pestalotiopsis yunnanensis* sp. nov., an endophyte from *Podocarpus macrophyllus* (Podocarpaceae) based on morphology and ITS sequence data. *Mycological Progress* 12: 563–568.

<http://dx.doi.org/10.1007/s11557-012-0863-5>

White, T.J., Bruns, T., 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, San Diego, USA, pp. 315–322.

<http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>

Wikee, S., Udayanga, D., Crous, P.W., Chukeatirote, E., McKenzie, E.H.C., Bahkali, A.H., Dai, D.Q. & Hyde, K.D. (2011) *Phyllosticta*-an overview of current status of species recognition. *Fungal Diversity* 51: 43–61.

<http://dx.doi.org/10.1007/s13225-011-0146-5>

Yang, X., Zhang, K., Jia, B. & Ci, L. (2005) Desertification assessment in China: An overview. *Journal of Arid Environments* 63: 517–531.

<http://dx.doi.org/10.1016/j.jaridenv.2005.03.032>

Zhenda, Z. (1998) Concept, cause and control of desertification in china. *Quaternary Sciences* 2: 5.

Zhou, D. & Hyde, K.D. (2001) Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. *Mycological Research* 105: 1449–1457.

<http://dx.doi.org/10.1017/S0953756201004713>

Zhuang, W.Y. (2005) *Fungi of northwestern China*. Ithaca, New York, USA.