



Multigene phylogeny and morphology reveal *Phaeobotryon rhois* sp. nov. (Botryosphaerales, Ascomycota)

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Abstract

The family *Botryosphaeriaceae* encompasses important plant-associated pathogens, endophytes and saprobes with a wide geographical and host distribution. Two dark-spored botryosphaeriaceous taxa associated with *Rhus typhina* dieback and canker disease were collected from Ningxia Province, in northwestern China. Morphology and multigene analysis (ITS, LSU and EF-1 α) clearly distinguished this clade as a distinct species in the genus. *Phaeobotryon rhois* is introduced and illustrated as a new species in this paper. The species is characterized by its globose, unilocular fruiting bodies and small, brown, 1-septate conidia. It can be distinguished from the similar species *P. cercidis*, *P. cupressi*, *P. mamane* and *P. quercicola* based on host association and conidial size and colour.

Key words: biodiversity, Botryosphaeriaceae, molecular phylogeny, new species, taxonomy

Introduction

Phaeobotryon (*Botryosphaeriaceae*) was established by Theissen & Sydow (1915) to accommodate *Dothidea cercidis* Cooke and subsequently entered a long period of confusion with a broad concept of the *Botryosphaeria* species. Phillips *et al.* (2008) redefined *Phaeobotryon* using the characteristic of 2-septate, brown ascospores, with bipolar conical apiculi. *Phaeobotryon* is a monophyletic genus with a single name for both sexual and asexual morphs (Phillips *et al.* 2008, Liu *et al.* 2012). Seven *Phaeobotryon* epithets are listed in Index Fungorum (2015) while Kirk *et al.* (2008) estimated there are four species. Sequence data or living cultures, are however, available for only a very few species and many taxa need verification. Recent studies suggest that this genus comprises four species (*P. cercidis* Cooke, *P. cupressi* Abdollahzadeh *et al.*, *P. mamane* Crous & Phillips and *P. quercicola* (Phillips) Crous & Phillips), of which only two species (*P. cupressi* and *P. mamane*) have been verified and studied based on available living cultures (Phillips *et al.* 2005, 2008, 2013; Abdollahzadeh *et al.* 2009). Until now, *Phaeobotryon* has been recorded from *Cercis canadensis* L., *Cupressus sempervirens* L., *Juniperus scopulorum* Sargent, *Quercus* sp. and *Sophora chrysophylla* (Salisb.) Seem. only in Germany, Iran and the USA (Abdollahzadeh *et al.* 2009, Phillips *et al.* 2013). Although *Phaeobotryon* species have been isolated from diseased plants, their pathogenicity has not been tested and their role as causal agents of disease is unresolved.

During an investigation of forest pathogens that cause canker or dieback disease in China, two *Phaeobotryon* specimens were collected from *Rhus typhina* L. in Yinchuan City, Ningxia Province, China. This species is characterized by globose, unilocular, fruiting bodies and brown, 22 × 11 μ m, ellipsoid to oblong, 1-septate conidia. Phylogenetic analysis inferred from combined ITS, LSU and EF-1 α sequence data provided strong support that this is a new species. We introduce *Phaeobotryon rhois* sp. nov. in this paper with a description and illustrations and compare it with other species in the genus.

it has not been determined if this species is pathogenic, as is true of other *Phaeobotryon* species. The taxonomy of species requires a robust sampling from a wide distribution range and consequent pathogenicity testing as in other well-studied botryosphaeriaceous fungi. Future studies should clarify the species diversity in this genus and improve the understanding of its disease importance.

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References

- Abdollahzadeh, J., Goltapeh, E.M., Javadi, A., Shams-Bakhsh, M., Zare, R. & Phillips, A.J.L. (2009) *Barriopsis iraniana* and *Phaeobotryon cupressi*: two new species of the *Botryosphaeriaceae* from trees in Iran. *Persoonia* 23: 1–8.
<http://dx.doi.org/10.3767/003158509X467552>
- Alves, A., Crous, P.W., Correia, A. & Phillips, A.J.L. (2008) Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* 28: 1–13.
- Chomnunti, P., Hongsanan, S., Aguirre-Hudson, B., Tian, Q., Peršoh, D., Dhamsi, M.K., Alias, A.S., Xu, J., Liu, X., Stadler, M. & Hyde, K.D. (2014) The sooty moulds. *Fungal Diversity* 66: 1–36.
<http://dx.doi.org/10.1007/s13225-014-0278-5>
- 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.
- Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F., Phillips, A.J.L., Alves, A., Burgess, T., Barber, P. & Groenewald, J.Z. (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* 55: 235–253.
<http://dx.doi.org/10.3114/sim.55.1.235>
- Desjardins, P., Hansen, J.B. & Allen, M. (2009) Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer. *Journal of Visualized Experiments* 33: 1–3.
<http://dx.doi.org/10.3791/1610>
- Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321.
- Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
<http://dx.doi.org/10.1093/sysbio/42.2.182>
- Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H.A., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., Hoog, S.D., Kang, J.C., Knudsen, K., Li, W.J., Li, X.H., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A., Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yan, J.Y., Yacharoen, S. & Zhang, M. (2013) Families of dothideomycetes. *Fungal Diversity* 63: 1–313.
<http://dx.doi.org/10.1007/s13225-013-0263-4>
- Katoh, K. & Toh, H. (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* 26: 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.

- Liu, J.K., Phookamsak, R., Doilom, M., Wikee, S., Li, Y.M., Ariyawansa, H., Boonmee, S., Chomnunti, P., Dai, D.Q., Bhat, J.D., Romero, A.I., Zhuang, W.Y., Monkai, J., Jones, E.B.G., Chukeatirote, E., Ko-Ko, T.W., Zhao, Y.C., Wang, Y. & Hyde, K.D. (2012) Towards a natural classification of *Botryosphaeriales*. *Fungal Diversity* 57: 149–210.
<http://dx.doi.org/10.1007/s13225-012-0207-4>.
- O'Donnell, K. (1993) *Fusarium* and its near relatives. Wallingford, UK.
- Phillips, A.J.L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M.J., Groenewald, J.Z. & Crous, P.W. (2013) The *Botryosphaeriaceae*: genera and species known from culture. *Studies in Mycology* 76: 51–167.
<http://dx.doi.org/10.3114/sim0021>.
- Phillips, A.J.L., Alves, A., Correia, A. & Luque, J. (2005) Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. *Mycologia* 97: 513–529.
<http://dx.doi.org/10.3852/mycologia.97.2.513>.
- Phillips, A.J.L., Alves, A., Pennycook, S.R., Johnston, P.R., Ramaley, A., Akulov, A. & Crous, P.W. (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. *Persoonia*: 21: 29–55.
<http://dx.doi.org/10.3767/003158508X340742>.
- 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.
- 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>.
- Slippers, B., Boissin, E., Phillips, A.J.L., Groenewald, J.Z., Lombard, L., Wingfield, M.J., Postma, A., Burgess, T. & Crous, P.W. (2013) Phylogenetic lineages in the *Botryosphaeriales*: a systematic and evolutionary framework. *Studies in Mycology* 76: 31–49.
<http://dx.doi.org/10.3114/sim0020>.
- Swofford, D.L. (2003) PAUP*: *Phylogenetic analysis using parsimony, * and other methods*. Version 4.0b10. Sinauer Associates, Sunderland.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
- 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.
- Wijayawardene, N.N., Crous, P.W., Kirk, P.M., Hawksworth, D.L., Boonmee, S., Braun, U., Dai, D.-Q., D'souza, M.J., Diederich, P., Dissanayake, A., Doilom, M., Hongsanan, S., Jones, E.B.G., Groenewald, J.Z., Jayawardena, R., Lawrey, J.D., Liu, J.-K., Luecking, R., Madrid, H., Manamgoda, D.S., Muggia, L., Nelsen, M.P., Phookamsak, R., Suetrong, S., Tanaka, K., Thambugala, K.M., Wanasinghe, D.N., Wikee, S., Zhang, Y., Aptroot, A., Ariyawansa, H.A., Bahkali, A.H., Bhat, D.J., Gueidan, C., Chomnunti, P., De Hoog, G.S., Knudsen, K., Li, W.-J., McKenzie, E.H.C., Miller, A.N., Phillips, A.J.L., Piatek, M., Raja, H.A., Shivas, R.S., Slippers, B., Taylor, J.E., Tian, Q., Wang, Y., Woudenberg, J.H.C., Cai, L., Jaklitsch, W.M. & Hyde, K.D. (2014) Naming and outline of Dothideomycetes-2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69: 1–55.
<http://dx.doi.org/10.1007/s13225-014-0309-2>
- Young, N.D. & Healy, J. (2003) GapCoder automates the use of indel characters in phylogenetic analysis. *Bmc Bioinformatics* 4: 6.