



## Introducing *Chaetothyriothecium*, a new genus of *Microthyriales*

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### Abstract

The order *Microthyriales* comprises foliar biotrophs, epiphytes, pathogens or saprobes that occur on plant leaves and stems. The order is relatively poorly known due to limited sampling and few in-depth studies. There is also a lack of phylogenetic data for these fungi, which form small black spots on plant host surfaces, but rarely cause any damage to the host. A "Microthyriaceae"-like fungus collected in central Thailand is described as a new genus, *Chaetothyriothecium* (type species *Chaetothyriothecium elegans* sp. nov.). Phylogenetic analyses of LSU gene data showed this species to cluster with other members of *Microthyriales*, where it is related to *Microthyrium microscopicum* the type of the order. The description of the new species is supplemented by DNA sequence data, which resolves its placement in the order. Little molecular data is available for this order, stressing the need for further collections and molecular data.

**Key words:** foliar epiphytes, *Micropeltidaceae*, *Microthyriaceae*, phylogenetic analyses

### Introduction

Fungal foliar epiphytes are a polyphyletic group found on plants worldwide (Schoch *et al.* 2009, Li *et al.* 2011, Wu *et al.* 2011, Hyde *et al.* 2013). The group has been poorly studied, few cultures are available in culture collections, and DNA sequence data is lacking in public databases. One major contributing factor is the fact that many of these species are obligate parasites and cannot be cultured (Wu *et al.* 2011).

The order *Microthyriales* comprises foliar epiphytes, which mainly form small, inconspicuous, black spots on host leaves. The spots consist of flattened thyriothecia with various ostiole forms, while the basal wall is usually poorly developed. Ascii are bitunicate, fissitunicate, saccate to subglobose, obclavate to fusiform, or rarely cylindro-clavate, and ascospores are uni- to multi-septate, and hyaline or brown (Arnaud 1918, Luttrell 1973, von Arx & Müller 1975, Barr 1987, Kirk *et al.* 2008, Wu *et al.* 2011, Hyde *et al.* 2013). Little molecular data is presently available for the order and in an overview of Dothideomycetes (Schoch *et al.* 2009), *Microthyriales* was represented by a single strain, *Microthyrium microscopicum*, as a long branch sister to *Stomiopeltis betulae*. More recently, Wu *et al.* (2011) added sequence data for three "Microthyriaceae"-like species—*Paramicrothyrium* and *Neomicrothyrium* were introduced as new genera and *Micropeltis zingiberaccola* as a new species; molecular analysis indicated they can probably be placed in *Microthyriales*. Hyde *et al.* (2013) placed *Microthyrium microscopicum* (*Microthyriaceae* type species) and *Stomiopeltis betulae* (*Micropeltidaceae*) in *Microthyriales*, and the families *Microthyriaceae* and *Micropeltidaceae* were accepted based on morphological data. The family *Microthyriaceae* includes foliar biotrophs and saprotrophs, with easily removed thyriothecia comprising dark, mostly cuboid or angular cells, arranged in parallel rows from the prominent central ostiole to the periphery, and a poorly developed base. Ascii are fusiform to cylindrical or elongate and ascospores are uniseptate and hyaline (Dodge 1942, Müller & von Arx 1962, Luttrell 1973, Barr 1987, Hofmann & Piepenbring 2006, Hofmann 2010, Wu *et al.* 2011, Hyde *et al.* 2013). Wu *et al.* (2011) recognized seven genera of *Microthyriaceae*, while a further

smooth-walled. On PDA, mycelium growing very slowly, colonies reaching 1.5 cm diam after 30 days, raised, comprising raised dark grey mycelium, white to greyish at the margin and also partly covering colonies, surface of colonies velvety. Asexual state: not observed.

**Material examined:**—THAILAND. Nakhon Nayok Province: Khao Yai National Park, on dead leaves of *Castanopsis* sp., 16 June 2012, *Narumon Tangtheerasunun* (MFLU13-0091!—**holotype**, ex-type living culture = MFLUCC12-0399 (MFU) = CPC 21375 = CBS 136075).

## Discussion

*Chaetothyriothecium* differs from other genera in the *Microthyriales* that have thyrothelial ascomata (Wu *et al.* 2011), because of the darkened rim surrounding the central ostiole, which is made up of long radiating setae. In other aspects, *Chaetothyriothecium elegans* is typical of *Microthyriales* in having thyrothecia comprising radiating cells but with a poorly developed base, ovoid bitunicate asci with short pedicels and bi-celled, hyaline ascospores. The phylogenetic analyses of LSU sequence data indicate that *Chaetothyriothecium* is a well resolved genus in *Microthyriales*.

There is a lack of sequence data for *Microthyriales* available in GenBank and this group is also morphologically relatively poorly studied. A putative strain of *Microthyrium microscopicum*, which represents the type of the order indicates that the *Microthyriales* is well resolved (Schoch *et al.* 2009, Wu *et al.* 2011, Hyde *et al.* 2013). *Chaetothyriothecium elegans* is related to *Microthyrium microscopicum* and *Stomiopeltis betulae* and can be included in *Microthyriaceae* as it is morphologically similar and this is supported in the phylogenetic analysis. It is important that more gene sequences are obtained for taxa in this order so that a natural classification can be obtained.

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## References

- Arnaud, G. (1918) Lés Asterinées. *Annals d'École National d'Agriculture de Montpellier Série 2* 16: 1–288.
- Arx, J.A. von & Müller, E. (1975) A re-evaluation of the bitunicate ascomycetes with key to families and genera. *Studies in Mycology* 9: 1–159.
- Barr, M.E. (1987) New taxa and combinations in the Louculoascomycetes. *Mycotaxon* 29: 501–505.
- Batista, A.C. (1959) Monografia dos fungos Micropeltaceae. *Publicações Instituto de Micologia da Universidade do Recife* 56: 1–519.
- Cai, L., Guo, X.Y. & Hyde, K.D. (2008) Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* 20: 53–58.  
<http://dx.doi.org/10.3767/003158508x314732>
- Cai, L., Jeewon, R. & Hyde, K.D. (2006) Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. *Mycological Research* 110: 137–150.  
<http://dx.doi.org/10.1016/j.mycres.2005.09.014>
- Cai, L., Wu, W.P. & Hyde, K.D. (2009) Phylogenetic relationships of *Chalara* and allied species inferred from ribosomal DNA sequences. *Mycological Progress* 8(2): 133–143.  
<http://dx.doi.org/10.1007/s11557-009-0585-5>
- Chomnunti, P., Schoch, C.L., Aguirre-Hudson, B., Ko-Ko, T.W., Hongsanan, S., Jones, E.B.G., Kodsueb, R., Phookamsak, R., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) *Capnodiales*. *Fungal Diversity* 51: 103–134.  
<http://dx.doi.org/10.1007/s13225-011-0145-6>
- Clements, F.E. & Shear, C.L. (1931) *Genera of fungi* 2 Vol. i–vii. H.W. Wilson, USA, New York, 496 pp.
- Doidge, E.M. (1942) South African *Microthyriaceae*. *Bothalia* 4: 273–344.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hofmann, T.A. (2010) *Plant parasitic Asterinaceae and Microthyriaceae from the Neotropics (Panama)*. PhD thesis, The

- Faculty of Biological Sciences at the J.W. Goethe-University Frankfurt am in, Germany, 408 pp.
- Hofmann, T.A. & Piepenbring, M. (2006) New records and host plants of fly-speck fungi from Panama. *Fungal Diversity* 22: 55–70.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754–755.  
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansha, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P., 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., Nelsen, M.P., Phookamsak, R., Muggia, L., Pang, K.L., Senanayake, I., Shearer, C.A., Wijayawardene, N., Wu, H.X., Thambugala, M., Suetrong, S., Tanaka, K., Wikee, S., Zhang, Y., Hudson, B.A., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, L.J., Bhat, J.D., Camporesi, E., Chukeatirote, E., Hoog, S.D., Gueidan, C., Hawksworth, D.L., Hirayama, K., Kang, J.C., Knudsen, K., Li, W.J., Liu, Z.Y., McKenzie, E.H.C., Miller, A.N., Nadeeshan, D., Phillip, A.J.L., Mapook, A., Raja, H.A., Tian, Q., Zhang, M., Scheuer, C., Schumm, F., Taylor, J., Yacharoen, S., Tibpromma, S., Wang, Y., Yan, J. & Li, X. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313.  
<http://dx.doi.org/10.1007/s13225-013-0263-4>
- Katoh, K., Asimenos, G. & Toh, H. (2009) Multiple alignment of DNA sequences with MAFFT. *Methods in Molecular Biology* 537: 39–64.  
[http://dx.doi.org/10.1007/978-1-59745-251-9\\_3](http://dx.doi.org/10.1007/978-1-59745-251-9_3)
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's dictionary of the fungi*, 10<sup>th</sup> edition. CAB International, Wallingford, UK, 428 p.
- Li, Y.M., Haixia, Wu, Cheng, H. & Hyde, K.D. (2011) Morphological studies in Dothideomycetes: *Elsinoë* (*Elsinoaceae*), *Butleria* and three excluded genera. *Mycotaxon* 115: 507–520.  
<http://dx.doi.org/10.5248/115.507>
- Lumbsch, H.T. & Huhndorf, S.M. (2010) Outline of Ascomycota—2009. *Fieldiana Life Earth Science* 1: 1–60.
- Luttrell, E.S. (1973) Loculoascomycetes. In: Ainsworth, G.C., Sparrow, F.K. & Sussman, A.S. (eds) *The fungi. An advanced treatise*. Academic Press, New York and London, pp. 135–219.
- Müller, E. & Arx, J.A. von (1962) Die Gattungen der didymosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* 11(2): 1–922.
- Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581–583.  
<http://dx.doi.org/10.1093/bioinformatics/btm388>
- Page, R.D.M. (2001) TreeView: Tree drawing software for Apple Macintosh and Windows. Available at <http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>
- Pattengale, N.D., Alipour, M., Bininda-Emonds, O.R.P., Moret, B.M.E. & Stamatakis, A. (2009) How many bootstrap replicates are necessary? *LNCS* 5541: 184–200.
- 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>
- 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>
- Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W., Burgess, T.I., de Gruyter, J., de Hoog, G.S., Dixon, L.J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, S., Hirayama, K., Hosoya, T., Huhndorf, S.M., Hyde, K.D., Jones, E.B., Kohlmeyer, J., Kruys, A., Li, YM., Lucking, R., Lumbsch, H.T., Marvanova, L., Mbatchou, J.S., McVay, A.H., Miller, A.N., Mugambi, G.K., Muggia, L., Nelsen, M.P., Nelson, P., Owensby, C.A., Phillips, A.J., Phongpaichit, S., Pointing, S.B., Pujade-Renaud, V., Raja, H.A., Plata, E.R., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C.A., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wingfield, M.J., Wood, A.R., Woudenberg, J.H., Yonezawa, H., Zhang, Y. & Spatafora, J.W. (2009) A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1–15.  
<http://dx.doi.org/10.3114/sim.2009.64.01>
- Silvestro, D. & Michalak, I. (2012) RAxMLGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335–337.  
<http://dx.doi.org/10.1007/s13127-011-0056-0>
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.  
<http://dx.doi.org/10.1093/nar/25.24.4876>
- Wu, X., Schoch, C.L., Boonmee, S., Bahkali, A.H., Chomnunti, P., Hyde, K.D. (2011) A reappraisal of Microthyriaceae. *Fungal Diversity* 51(1): 189–248.  
<http://dx.doi.org/10.1007/s13225-011-0143-8>
- Zhaxybayeva, O. & Gogarten, J.P. (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3: 4.