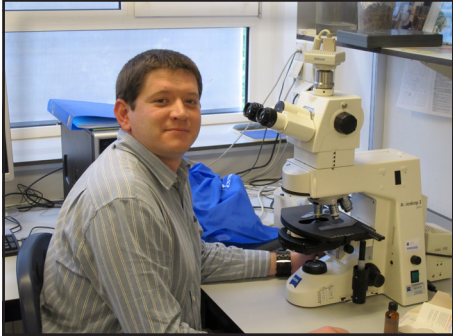


GENERIC CONCEPTS IN *Nectriaceae*



Lorenzo Lombard¹, Nicolaas A. van der Merwe²,
 Johannes Z. Groenewald¹, Pedro W. Crous¹
¹CBS-KNAW Fungal Biodiversity Centre, P.O. Box
 85167, 3508 AD Utrecht, The Netherlands
²Department of Genetics and Forestry and Agricultural
 Biotechnology Institute (FABI), University of Pretoria,
 Pretoria 0002, South Africa.
 l.lombard@cbs.knaw.nl

The ascomycete family *Nectriaceae* (*Hypocreales*, *Hypocreomycetidae*, *Sordariomycetes*, *Pezizomycotina*, *Ascomycota*) includes numerous important plant and human pathogens, as well as several species used extensively in industrial and commercial applications as biodegraders and biocontrol agents. Members of this family are unified by phenotypic characters such as uniloculate ascomata that are yellow, orange-red to purple which change colour in KOH and not immersed in a well-developed stroma. They are associated with phialidic asexual morphs producing ameroporous to phragmosporous conidia (Rossman *et al.* 1999, Rossman 2000). The *Nectriaceae* consists of around 55 asexual- and sexual morph genera, which include approximately 900 species (www.mycobank.org; www.indexfungorum.org). The majority of these species are weak to virulent soil-borne plant pathogens and/or saprobes while some are fungicolous and insecticolous (Rossman *et al.* 1999, Rossman 2000, Chaverri *et al.* 2011, Gräfenhan *et al.* 2011, Schroers *et al.* 2011). Several species have also been reported as important human pathogens (de Hoog *et al.* 2000, Chang *et al.* 2006, Guarro 2013) while others produce mycotoxins of medical concern (Rossman 1996).

Prior to the advent of DNA sequencing studies, most sexual morph genera recognised in the *Nectriaceae* were placed in *Nectria s. lat.* (Rehner & Samuels 1995, Rossman *et al.* 1999). The genus *Nectria s. str.*, however, is restricted to the type species *N. cinnabarina* with tubercularia-like asexual morphs (Rossman 2000, Hirooka *et al.* 2012). Recently, several studies have treated taxonomic concepts within *Nectriaceae* based on multi-gene phylogenetic inference (Lombard *et al.* 2010, 2012a,b, 2014a,b, Chaverri *et al.* 2011, Gräfenhan *et al.* 2011, Schroers *et al.* 2011, Hirooka *et al.* 2012). In these studies, well-known and important plant and human pathogenic genera have been segregated into several new genera, with some older generic names resurrected to genus level (Chaverri *et al.* 2011, Gräfenhan *et al.* 2011, Schroers *et al.* 2011, Hirooka *et al.* 2011, 2012). This has resulted in extensive debates (Geiser *et al.* 2013, O'Donnell *et al.* 2013, Aoki *et al.* 2014) pertaining to the use of some generic names for species of agricultural and medical importance. Furthermore, several genera have been excluded from these studies, although they are traditionally classified in the *Nectriaceae*.

The generic concepts in *Nectriaceae* are poorly defined, since DNA sequence data have not been available for many of these genera. To address this issue, a multi-gene phylogenetic analysis was done using partial sequences for the 28S large subunit nrDNA, the internal transcribed spacer region and intervening 5.8S nrRNA gene, the large subunit of the ATP citrate lyase, the RNA polymerase II largest subunit, RNA polymerase II second largest subunit, α -actin, β -tubulin, calmodulin, histone H3, and translation elongation factor 1-alpha gene regions for available type and authentic strains representing known genera in *Nectriaceae*, including several genera for which no sequence data were previously available. Nomenclatural changes due to the implementation of the new *International Code of Nomenclature for algae, fungi and plants* (ICN; McNeill *et al.* 2012), are also considered and the taxonomy of some genera is re-evaluated.

Supported by morphological observations, the data resolved 47 genera in the *Nectriaceae*, of which three genera, namely *Calostilbe*, *Corallonectria* and *Dematiocladium*, are represented by single lineages due to the paucity of cultures. For 11 of these genera no to very limited DNA sequence data have been available prior to this study. These include *Curviocladium*, *Cylindrocarpostylus*, *Cylindrodendrum*, *Flagellospora*, *Ophionectria*, *Paracremonium*, *Penicillifer*, *Sarcopodium*, *Xenoacremonium*, *Xenocylindrocladium*, and *Xenogliocladopsis*. All 11 genera were shown to form monophyletic clades and will form the basis for new studies, as some of these (*e.g.* *Paracremonium* and *Xenoacremonium*) represent important human pathogens (Gams 1971). The remaining nine genera are for the most part regarded as endophytes and saprobes of mostly woody plant hosts (Ranzoni

1956, Crous & Kendrick 1994, Kirschner & Oberwinkler 1999, Rossman *et al.* 1999), which might play an important role in future industrial applications. We re-evaluated the generic status of several genera, which resulted in the introduction of six new genera (*Aquanectria*, *Bisifusarium*, *Coccinonectria*, *Paracremonium*, *Rectifusarium* and *Xenoacromonium*) to accommodate species that were initially classified as members of the genera *Acromonium*, *Fusarium* and *Pseudonectria*, based solely on morphological characters. Several generic names are also proposed for synonymy based on the abolishment of dual nomenclature. Additionally, a new family (*Tilachlidiaceae*) is introduced for two genera that were previously accommodated in the *Nectriaceae*.

This study provides a broad phylogenetic backbone and framework for future studies of the *Nectriaceae*. Members of this family are commonly found in various environments, where they play an important socio-economic role in human endeavours in the fields of agriculture, industry and medicine. Therefore, the phylogenetic foundation set here will form the basis for further investigation of several genera and identification of novel taxa in existing and new fungal groups in this family. Although several taxonomic issues are clarified for some genera, this study also highlights some taxonomic problems relating to the *Nectriaceae*. To our knowledge, this study represents the largest sampling of nectriaceous fungi subjected to multi-locus sequence analyses to date. This also highlights the importance of maintaining living cultures in public culture collections, as many of the genera included were subjected to molecular analysis for the first time, and several recently described taxa, were also unavailable for inclusion.

REFERENCES

- Aoki, T., O'Donnell, K. and Geiser, D.M. 2014. Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. *J Gen Plant Pathol* 80:189–201.
- Chang, D.C., Grant, G.B., O'Donnell, K., Wannemuehler, K.A., Noble-Wang, J., Rao, C.Y., Jacobson, L.M., Crowell, C.S., Sneed, R.S., Lewis, F.M., Schaffzin, J.K., Kainer, M.A., Genese, C.A., Alfonso, E.C., Jones, D.B., Srinivasan, A., Fridkin, S.K. and Park, B.J. 2006. Multistate outbreak of *Fusarium keratitis* associated with use of a contact lens solution. *JAMA* 296: 953–963.
- Chaverri, P., Salgado, C., Hirooka, Y., Rossman, A.Y. and Samuels, G.J. 2011. Delimitation of *Neonectria* and *Cylindrocarpon* (*Nectriaceae*, *Hypocreales*, *Ascomycota*) and related genera with *Cylindrocarpon*-like anamorphs. *Stud Mycol* 68: 57–78.
- Crous, P.W. and Kendrick, W.B. 1994. *Arnaudiella eucalyptorum* sp. nov. (Dothideales, Ascomycetes), and its hyphomycetous anamorph *Xenogliocladiopsis* gen. nov., from *Eucalyptus* leaf litter in South Africa. *Can J Bot* 72: 59–64.
- Gams, W. 1971. *Cephalosporium-artige Schimmelpilze (Hyphomycetes)*. Gustav Fischer Verlag, Stuttgart, Germany.
- Geiser, D.M., Aoki, T., Bacon, C.W., Baker, S.E., Bhattacharyya M.K., Brandt, M.E., Brown, D.W., Burgess, L.W., Chulze, S., Coleman, J.J., Correll, J.C., Covert, S.F., Crous, P.W., Cuomo, C.A., et al. 2013. One fungus, One Name: Defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology* 103: 400–408.
- Gräfenhan, T., Schroers, H.-J., Nirenberg, H.I. and Seifert, K.A. 2011. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Stud Mycol* 68: 79–113.
- Guarro, J. 2013. Fusariosis, a complex infection caused by a high diversity of fungal species refractory to treatment. *EJCMID* 32: 1491–1500.
- Hirooka, Y., Rossman, A.Y. and Chaverri, P. 2011. A morphological and phylogenetic revision of the *Nectria cinnabarina* complex. *Stud Mycol* 68: 35–56.
- Hirooka, Y., Rossman, A.Y., Samuels, G.J., Lechat, C. and Chaverri, P. 2012. A monograph of *Allantonectria*, *Nectria*, and *Pleonectria* (*Nectriaceae*, *Hypocreales*, *Ascomycota*) and their pycnidial, sporodochial, and synnematus anamorphs. *Stud Mycol* 71: 1–210.
- Hoog, G.S. de, Guarro, J., Gené, J., Figueras, M.J. 2011. *Atlas of clinical fungi* (3rd Ed., CD-ROM). CBS-KNAW fungal Biodiversity Centre, Utrecht, The Netherlands.
- Kirschner, R. and Oberwinkler, F. 1999. *Cylindrocarpostylus*, a new genus based on a hyphomycete rediscovered from bark beetle galleries. *Mycol. Res.* 103: 1152–1156.
- Lombard, L. and Crous, P.W. 2012a. Phylogeny and taxonomy of the genus *Gliocladiopsis*. *Persoonia* 28: 25–33.
- Lombard, L., Crous, P.W., Wingfield, B.D. and Wingfield, M.J. 2010a. Phylogeny and systematics of the genus *Calonectria*. *Stud Mycol* 66: 31–69.
- Lombard, L., Shivas, R.G., To-Anun, C., and Crous, P.W. 2012b. Phylogeny and taxonomy of the genus *Cylindrocladiella*. *Mycol Prog* 11: 835–868.
- Lombard, L., Serrato-Diaz, L.M., Cheewangkoon, R., French-Monar, R.D., Decock, C. and Crous, P.W. 2014a. Phylogeny and taxonomy of the genus *Gliocephalotrichum*. *Persoonia* 32: 127–140.
- Lombard, L., Merwe, N.A. van der, Groenewald, J.Z., and Crous, P.W. 2014b. Lineages in *Nectriaceae*: Re-evaluating the generic status of *Ilyonectria*. *Phyto Medit* 53: 515–532.
- McNiell, J., Barrie, F.F., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhol, K., Prado J., Prud'homme Van Reine, W.F., Smith, G.F., Wiersema, J.H. and Turland, N.J. (eds.). 2012. *International Code of Nomenclature for algae, fungi and plants (Melbourne Code)*. [Regnum Vegetabile no. 154] A.R.G. Gantner Verlag KG.
- O'Donnell, K., Rooney, A.P., Proctor, R.H., Brown, D.W., McCormick, S.P., Ward, T.J., Frandsen, R.J.N., Lysøe, E., Rehner, S.A., Aoki, T., Robert, V.A.R.G., Crous, P.W., Groenewald, J.Z., Kang, S. and Geiser D.M. 2013. Phylogenetic analyses of *RPB1* and *RPB2* supports a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fung Genet Biol* 52: 20–31.
- Ranzoni, F.V. 1956. The perfect stage of *Flagellospora penicillioides*. *Am J Bot* 43: 13–17.
- Rehner, S.A. and Samuels, G.J. (1995). Molecular systematics of the *Hypocreales*: a teleomorph gene phylogeny and the status of their anamorphs. *Can J Bot* 73: S816–S823.
- Rossman, A.Y. 1996. Morphological and molecular perspectives on systematics of the *Hypocreales*. *Mycologia* 88: 1–19.
- Rossman, A.Y. 2000. Towards monophyletic genera in the holomorphic *Hypocreales*. *Stud Mycol* 45: 27–34.
- Rossman, A.Y., Samuels, G.J., Rogerson, C.T. and Lowen, R. 1999. Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (*Hypocreales*, *Ascomycetes*). *Stud Mycol* 42: 1–248.
- Schroers, H.-J., Gräfenhan, T., Nirenberg, H.I. and Seifert, K.A. 2011. A revision of *Cyanonectria* and *Geejayessia* gen. nov., and related species with *Fusarium*-like anamorphs. *Stud Mycol* 68: 115–138.