CZECH MYCOL. 54 (3–4), 2003

# Relationship of Cerebella to Epicoccum and their closest relatives among Dothideales

SYLVIE PAŽOUTOVÁ and RENÁTA KOLÍNSKÁ

Laboratory of Genetics, Physiology and Bioengineering of Fungi,
Institute of Microbiology, Czech Academy of Sciences,
142 20 Prague, Czech Republic

Pažoutová S. and Kolínská R. (2003): Relationship of Cerebella to Epicoccum and their closest relatives among Dothideales. – Czech Mycol. 54: 155–160

The Czech isolate of *Cerebella* sp. was confirmed as *C. andropogonis*, as its RAPD patterns were identical to those of Australian and African isolate of this species. Also, rDNA (ITS1–5.8S-ITS2) sequences of African *C. andropogonis* and the Czech isolate (AJ306620 and AJ400905) were identical except for a single transition A-G at position 47 of ITS1. Comparison of the sequence with databases yielded 24 closely related sequences with 96.5–98.9 % identity to *Cerebella*. The highest similarity was found between *Cerebella* and *Epicoccum nigrum/ Phoma epicoccina* isolates, two other related groups were: *Phoma herbarum*, *P. medicaginis*, *Phomopsis* sp., and *P. glomerata/Ampelomyces* sp.

**Key words**: *Cerebella andropogonis*, *Epicoccum*, phylogeny, rDNA sequence

Pažoutová S. a Kolínská R. (2003): Vztah rodů Cerebella a Epicoccum a jejich nejbližší příbuzní mezi Dothideales. – Czech Mycol. 54: 155–160

RAPD prokázalo, že český izolát *Cerebella* sp. náleží k druhu *C. andropogonis*, zastoupenému australským a africkým izolátem. Sekvence rDNA (ITS1–5.8S-ITS2) afrického a českého izolátu (AJ400905 a AJ306620) byly totožné s výjimkou transice A-G v pozici 47 spaceru ITS1. V databázích bylo nalezeno 24 příbuzných sekvencí rDNA které byly se sekvencí *C. andropogonis* z 96.5–98.9% totožné. Nejpříbuznější byly sekvence *Epicoccum nigrum/Phoma epicoccina*, další příbuzné skupiny tvořily *Phoma herbarum*, *P. medicaginis*, *Phomopsis* sp. a *P. glomerata/Ampelomyces* sp.

## INTRODUCTION

*Cerebella andropogonis*, a hyperparasite colonising sphacelial stages of various *Claviceps* species was once considered a plant pathogen and almost any new collection was named after the grass species where the sporodochium occurred. However, Langdon (1955) after thorough revision of herbarium specimens from the entire world reduced these names to synonyms of *Cerebella andropogonis* Cesati. Schol-Schwarz (1959) suggested transfer of *C. andropogonis* into the genus *Epicoccum*, as *E. andropogonis*, but this was not widely accepted by later authors. One reason for it may be distinct fungal hyperparasitism of *Cerebella* and the fact that the name reflects very vividly the morphology of convoluted sporodochia resembling a brain surface.

Recently, several studies elucidated relationship between *Epicoccum*, *Phoma* and another fungal hyperparasite, *Ampelomyces* using rDNA sequence analyses. Kiss and Nakasone (1998) found that slow-growing isolates of *Ampelomyces* are related to *Leptosphaeria microscopica* and *L. nodorum*, whereas fast-growing isolates were closer to *Epicoccum*. The pycnidia of *Phoma glomerata* and related *Ampelomyces* isolates were sessile, whereas the slow-growing *Leptosphaeria*-related isolates were characterized by stipitate pycnidia. Sullivan and White (2000) identified the rapidly growing isolates as *Phoma glomerata*. These isolates are hyperparasites of powdery mildew fungi and were formerly classified as *Ampelomyces heraclei, A. humuli* and *A. quercinus*. The closest teleomorphic species were *Didymella bryoniae* and *D. lycopersici*. Arenal et al. (2000) confirmed *Epicoccum nigrum* and *Phoma epicoccina* as the same biological species, where the *E. nigrum* isolates probably lost the ability of pycnidium formation. Other rDNA sequences related to *Phoma epicoccina/Epicoccum* were those of *Phoma americana*, *P. macrostoma* and also *Didymella* which places this group among mitosporic Dothideales.

In our previous work (Pažoutová and Kolínská 1999), we described the Czech isolate of dematiaceous hyphomycete *Cerebella* sp. differing slightly in the spore morphology from typical *C. andropogonis* found in Brazil. Obviously, the morphological observations cannot add more to the correct species identification of the Czech *Cerebella* isolate or to the elucidation of *Cerebella-Epicoccum* relationship. Therefore, RAPD fingerprinting which is commonly used for differentiation between isolates of the same species, as well as rDNA sequence comparison, were applied to DNA from the Czech isolate of *Cerebella* sp. and *C. andropogonis* isolates from Africa and Australia. To elucidate the *Cerebella* taxonomical relatedness, its rDNA sequence was compared to fungal sequences from EMBL and GenBank databases.

## MATERIAL AND METHODS

### Isolates:

*Cerebella andropogonis* CZ was isolated from the sphacelial stage of *Claviceps purpurea* on *Festuca arundinacea*, in 1998 (Trutnov, Czech Republic) (Pažoutová and Kolínská 1999). *C. andropogonis* AU was isolated from *Sorghum bicolor* colonised by *Claviceps africana* (1999, Warwick, Queensland, Australia, coll. and det. M. Ryley, isol. S. Pažoutová), *Cerebella andropogonis* AF was isolated from *Heteropogon contortus* colonised by *C. pusilla* (2000, Matopos, Zimbabwe, coll. D. Frederickson, isol. and det. S. Pažoutová).

### DNA analysis:

Mycelium for DNA preparation was grown for 4–5 days on RK agar plate overlaid with cellophane. Mycelium was then scraped and pulverised in liquid nitrogen

**Table 1.** Sequences related to *C. andropogonis*

| Organism | Accession No. | Reference |
|---|---|---|
| *Cerebella andropogonis* CZ | AJ400905 | this paper |
| *Cerebella andropogonis* AF | AJ306620 | this paper |
| *Epicoccum nigrum* strain EP5 | AF149926 | Arenal et al. (2000) |
| *Epicoccum nigrum* strain EP22 | AF149927 | " |
| *Epicoccum nigrum* strain EP27 | AF149928 | " |
| *Epicoccum nigrum* strain EP33 | AF149929 | " |
| *Epicoccum nigrum* strain EP34 | AF149930 | " |
| *Phoma epicoccina* strain PE20002 | AF149931 | " |
| *Phoma epicoccina* strain PE20003 | AF149932 | " |
| *Phoma epicoccina* strain PE20028 | AF149933 | " |
| *Phoma epicoccina* strain PE20044 | AF149934 | " |
| *Epicoccum nigrum* strain CBS 318.8 | AJ279448 | Wirsel et al. (2001) |
| *Epicoccum* sp. isolate A9 | AJ279452 | " |
| *Epicoccum* sp. 5.8S isolate 6/97–74 | AJ279463 | " |
| *Epicoccum* sp. 4/97–60 | AJ279486 | " |
| *Phomopsis* sp. IW-75 | AF079770 | Rosskopf et al. (2000) |
| *Phomopsis* sp. BG-96 | AF079771 | " |
| *Phomopsis* sp. FP1–96 | AF079772 | " |
| *Phomopsis* sp. FP3–96 | AF079773 | " |
| *Phoma medicaginis* | AF079775 | " |
| *Phoma herbarum* | AF218792 | Bradner et al., unpublished |
| *Ampelomyces quercinus* | AF035778 | Kiss and Nakasone (1998) |
| *Ampelomyces humuli* | AF035779 | " |
| *Phoma glomerata* | AF126816 | Sullivan and White (2000) |
| *Phoma glomerata* | AF126819 | " |
| *Microsphaeropsis amaranthi* | AF079774 | Rosskopf et al. (2000) |

by mortar and pestle. DNA extraction, RAPD analysis, and rDNA amplification were carried out as in Pažoutová et al. (2000). RAPD analysis was performed using primers 8F (GCTCTGAGATTGTTCCGGCT), 5R (TTTGTCCGGCT-CAGAAAC), and 30F (GAGGACGATTCATCAACC). The rDNA of *Cerebella andropogonis* CZ and *C. andropogonis* AF containing the ITS1–5.8S-ITS2 region was sequenced at Microsynth (Balgach, Switzerland) and the sequences deposited in EMBL Nucleotide Sequence Database under the Accession No. AJ400905 and AJ306620, respectively.

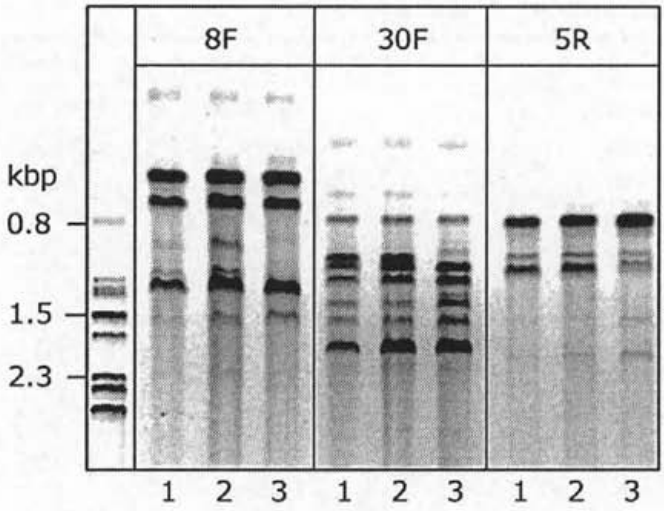


**Fig. 1** RAPD patterns of *C. andropogonis* isolates 1 – Australian, 2 – African, 3 – Czech

Phylogenetic methods:

rDNA sequences of *C. andropogonis* were compared with EMBL and GenBank sequence databases. The closest 24 sequences (Tab. 1) were used for further analysis. Sequences were aligned using BioEdit version 4.7.1 (T. Hall, Department of Microbiology, North Carolina State University, Raleigh, NC 27695). The sequence of *Microsphaeropsis amaranthi* (AF079774) was used as an outgroup. Phylogenetic analysis was performed using TREE-PUZZLE 5.0 (©1999–2000, H. A. Schmidt, K. Strimmer, M. Vingron, and A. von Haeseler), which reconstructs phylogenetic trees from molecular sequence data by maximum likelihood.

## RESULTS AND DISCUSSION

RAPD analysis of African and Australian *C. andropogonis* and Czech *Cerebella* sp. isolates with three primers (Fig. 1) revealed identical patterns for all three isolates. Therefore, we conclude that, despite small differences in conidial size, all three isolates belong to the same species, *C. andropogonis*. The species identity of the Czech isolate was also confirmed by comparison of its rDNA sequence to that of African *C. andropogonis*. Sequences were identical except for a single transition at position 47 of ITS1 (TAA –TGA).

Alignment of *C. andropogonis* with 24 related database sequences contained 469 sites, 51 of them variable. Quartet trees were based on approximate maximum likelihood values using the HKY model of substitution (Hasegawa et al. 1985) with

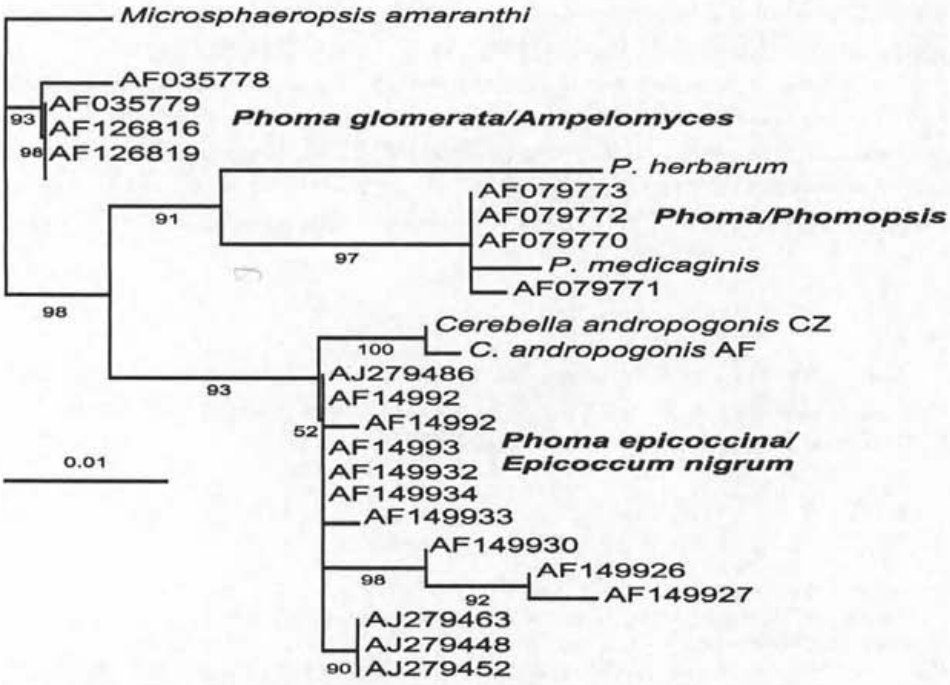


**Fig. 2** Phylogenetic relationships of *Cerebella*.
Quartet puzzling tree with maximum likelihood branch lengths and branch support values. Number of puzzling steps: 10000, analysed quartets: 14950, unresolved quartets: 3228 (= 21.6%), log likelihood = –995.40.

uniform rate heterogeneity. Quartet puzzling was used to choose from the possible tree topologies and to simultaneously infer support values for internal branches (Fig. 2). For parameter estimation (substitution process and rate variation), the neighbour-joining tree was used. The transition/transversion parameter estimated from the data set was 2.88 (S. E. 0.87), expected transition/transversion ratio: 2.92, expected pyrimidine transition/purine transition ratio: 1.45.

High sequence similarity (96.5–98.9 % identity) caused that some clades were unresolved. The 5.8S rDNA gene was completely conserved among all taxa. The closest match was found between mycoparasitic *Cerebella* and various *Epicoccum nigrum* or *Phoma epicoccina* isolates which were on a highly supported clade (93 %). However, separation of *Cerebella* and *Phoma epicoccina*/*Epicoccum nigrum* clades was only weakly supported (52 %). Sequence similarity thus supports the placement of *Cerebella* into the genus *Epicoccum*. The second group of related fungi includes phytopathogens *Phoma herbarum* (Bradner et al. unpublished), *Phoma medicaginis* and *Phomopsis* sp. isolates (Rosskopf et al.

2000). The third group consisted of mycoparasitic *Phoma glomerata* and related *Ampelomyces* (Kiss and Nakasone 1998; Sullivan and White 2000).

The similarity of rDNA sequences between *Epicoccum*, *Cerebella*, *Phomopsis* and *Phoma glomerata/Ampelomyces* species is striking when compared to morphological differences and differences in life style of related species. It may reflect recent divergence of these fungi. Among the species related to *C. andropogonis*, saprophytes, necrotrophs, mycoparasites as well as plant pathogens were found.

## ACKNOWLEDGEMENTS

This work was partially supported by the British Mycological Society and the Czech Ministry of Education (Grant Project COST 835.30) and also by the Institutional Research Concept No. AV0Z5020903.

## REFERENCES

ARENAL F., PLATAS G., MONTE E. and PELÁEZ F. (2000): ITS sequencing supports that Epicoccum nigrum and Phoma epicoccina are the same biological species. – Mycological Research 104: 301–303.

BRADNER J. R., GILLINGS M. and NEVALAINEN K. M. H. (2000): A molecular identification of some Antarctic microfungi using nrDNA sequence data. Unpublished.

HASEGAWA M., KISHINO H. and YANO K. (1985): Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. – Journal of Molecular Evolution 22: 160–174.

KISS L. and NAKASONE K. K. (1998): Ribosomal DNA internal transcribed spacer sequences do not support the species status of Ampelomyces quisqualis, a hyperparasite of powdery mildew fungi. – Current Genetics 33: 362–367.

LANGDON R. F. N. (1955): The Genus Cerebella. – Mycological Papers 61: 1–18.

PAŽOUTOVÁ S. and KOLÍNSKÁ R. (1999): Record of Cerebella sp. in Czech Republic and of Cerebella andropogonis in Brazil. – Czech Mycology 52: 81–88.

PAŽOUTOVÁ S., OLŠOVSKÁ J., LINKA M., KOLÍNSKÁ R. and FLIEGER M. (2000): Claviceps purpurea populations, chemoraces and habitat specialization. – Applied and Environmental Microbiology 66: 5419–5425.

ROSSKOPF E. N., CHARUDATTAN R., SHABANA Y. M. and BENNY G. L. (2000): Phomopsis amaranthicola, a new species from Amaranthus sp. – Mycologia 92: 114–122.

SCHOL-SCHWARZ B. (1959): The genus Epicoccum Link. – Transactions of the British Mycological Society 42: 149–173.

SULLIVAN R. F. and WHITE J. F. (2000): Phoma glomerata as a mycoparasite of powdery mildew. – Applied and Environmental Microbiology 66: 425–427.

WIRSEL S. G. R., LEIBINGER W., ERNST M. and MENDGEN K. (2001): Genetic diversity of fungi closely associated with common reed (Phragmites australis). – New Phytologist 149: 589–598.