

## Epitypification of *Graphium penicillioides* Corda, with comments on the phylogeny and taxonomy of graphium-like synnematosus fungi<sup>1</sup>

Gen Okada<sup>a</sup>, Karin Jacobs<sup>b</sup>, Thomas Kirisits<sup>c</sup>, Gerry W. Louis-Seize<sup>d</sup>, Keith A. Seifert<sup>d</sup>, Takashi Sugita<sup>a2</sup>, Akiko Takematsu<sup>a2</sup>, and Michael J. Wingfield<sup>b</sup>

<sup>a</sup> Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan

<sup>b</sup> Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Biological and Agricultural Sciences, University of Pretoria, Pretoria 0002, Republic of South Africa

<sup>c</sup> Institute of Forest Entomology, Forest Pathology and Forest Protection, Universität für Bodenkultur, Hasenauerstrasse 38, A-1190 Vienna, Austria

<sup>d</sup> Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Research Branch, Ottawa, Ontario K1A 0C6, Canada

Correspondence: Gen Okada <okada@jcm.riken.go.jp> and Keith A. Seifert <seifertk@em.agr.ca>

**Abstract:** Graphium-like anamorphs have previously been known in three groups of ascomycetes, including the *Microascales* (*Graphium sensu stricto*), the *Ophiostomatales* (anamorphs now classified in *Pesotum*), and the *Chaetothyriales*. In this paper, the modern interpretation of the classical hyphomycete genus *Graphium* is fixed by epitypification of the type species, *G. penicillioides*, using a culture derived from the original host and near the original location where the holotype was collected more than 160 years ago. The epitype culture is described and illustrated, and a comparison is made with the remnants of the holotype specimen. Neighbor joining analyses of small subunit (SSU/18S) rDNA sequences confirm that the phylogenetic disposition of the epitype strain is near others identified as *G. penicillioides*, in the *Microascales* clade. Sequences of the internal transcribed spacer (ITS) region of the epitype and other strains identified as *G. penicillioides* confirm earlier results that this is a species aggregate, including at least four species. Comments on the phylogenetic relationships of some additional species sometimes referred to *Graphium* are included, and a fourth group of graphium-like anamorphs, phylogenetically related to the discomycetes, is briefly mentioned. The following new combinations are proposed: *Dendrostilbella smaragdina* (Alb. & Schw.) Seifert, *Exophiala calicioides* (Fr.) Okada & Seifert, *Graphium basitruncatum* (Mats.) Seifert & Okada, and *Pesotum erubescens* (Mathiesen) Okada (see Appendix).

**Key words:** *Microascales*, *Ophiostomatales*, *Chaetothyriales*, discomycetes, *Dendrostilbella*, *Exophiala*,

<sup>1</sup> Part 12 in a series on the taxonomy of synnematosus fungi by Gen Okada.

<sup>2</sup> Present addresses: Akiko Takematsu: Suwahara-danchi 2-3-204, Wako, Saitama 351-0103, Japan.  
Takashi Sugita: Department of Microbiology, Meiji Pharmaceutical University, Kiyose, Tokyo 204-8588, Japan.

*Pesotum*, ITS, SSU (18S) rDNA.

## Introduction

The anamorph genus *Graphium* Corda (1837), lectotypified by *G. penicillioides* Corda (Hughes, 1958), has traditionally included species with darkly pigmented, determinate synnemata, percurrently proliferating conidiogenous cells and slimy, aseptate, hyaline to pale brown conidia (Ellis, 1971; Crane & Schoknecht, 1973). Although the teleomorph of the lectotype species *G. penicillioides* is unknown, the genus was long believed to have ophiostomatoid affinities (Goidànich, 1935; Upadhyay, 1981; Seifert & Okada, 1993). An historical overview of *Graphium*, including a morphological survey of representative species, and a discussion of their known and supposed teleomorph connections was presented by Seifert & Okada (1993). Their broad morphological generic concept, accommodating plasticity in conidium ontogeny and synnema pigmentation, incorporated species formerly disposed into much more narrowly defined genera. Similar conclusions were presented by Wingfield *et al.* (1991) and Mouton *et al.* (1993). However, as noted by Seifert & Okada (1993), even the most restrictive morphological generic concept for *Graphium* (limiting the genus to species with percurrently proliferating conidiogenous cells) includes anamorphs of three orders of the *Ascomycota*.

Okada *et al.* (1998) demonstrated that several cultures identified as *G. penicillioides* actually had phylogenetic affinities with *Graphium putredinis* (Corda) S. Hughes in the *Microascales*, based on phylogenetic analysis of 18S rDNA sequences. This necessitated the abandonment of the name *Graphium* for anamorphs of species of *Ophiostoma* Syd. & P. Syd. and the reassignment of the former morphological concept of this genus to the name *Pesotum* J. L. Crane & Schokn.. In addition, the phylogenetic affinities of *G. calicioides* (Fr.) Cooke & Masee were shown to be with the *Chaetothyriales*, not the *Chaetosphaeriaceae* as speculated earlier by Seifert & Okada (1993). Okada *et al.* (1998) provided provisional nomenclators for accepted species of *Graphium* and *Pesotum*.

Identification of *Graphium* and *Pesotum* species is difficult in the absence of teleomorphs, primarily because of the paucity of modern descriptions for most species. Morphologically similar sibling species (Brasier, 1993) exist for the teleomorphs of *Pesotum* species, but have rarely been critically compared using morphological techniques. The two best known species of *Graphium sensu stricto*, *G. penicillioides* and *G. putredinis*, were considered species aggregates

by Seifert & Okada (1993) and Okada *et al.* (1998).

The correct application of the name *Graphium penicillioides* has been complicated by the suspicion that the name has been used for more than one species. The original description by Corda (1837) was based on a specimen collected in Prague on *Populus nigra* cv. *italica*. Ellis (1971) listed the fungus as occurring on *Populus* wood in Europe and North America. Sutton & Laut (1970) and Sutton (1973) described specimens identified as *G. penicillioides* as a common secondary colonizer of bark beetle tunnels in *Ulmus* trees killed by Dutch elm disease in Manitoba and Saskatchewan, and this fungus is still common in this niche in Canada (Seifert, unpublished). Meanwhile, Matsushima (1971) described *Stilbum basitruncatum* Mats. from a culture isolated from soil from the Solomon Islands; Sutton (1973) later synonymized this species with *G. penicillioides* (cf. Matsushima, 1975). Furthermore, the CBS culture collection catalogue (<http://www.cbs.knaw.nl/database.html>) lists two isolates from *Prunus armeniaca* in Tunisia (CBS 318.72, 319.72). Do all of these populations actually represent the same species, and if not, how should *G. penicillioides sensu stricto* be defined?

The holotype of *G. penicillioides* is depauperate and has been examined by two of the authors of this paper (K.A.S., M.J.W.) as well as by Hughes (1958), who deposited a slide in herb. DAOM, and Crane & Schoknecht (1973), who deposited a slide in herb. ILLS. A few synnemata remain on the holotype, enough to briefly characterize the conidiomata, the conidiogenous cells, and the conidia. Subsequent to the Tokyo version of the International Code of Botanical Nomenclature (Greuter *et al.*, 1994), the concept of epitypification (Art. 9.7) allows the designation of a specimen and/or a culture. Such material can serve as a proxy for the holotype in the determination of morphological, physiological or molecular characteristics that cannot be determined from the holotype. In 1998, one of us (T.K.) visited the Czech Republic and took core samples from living trees of *Populus nigra* cv. *italica*. Four cultures conforming with the morphological characters of the holotype of *G. penicillioides* were isolated. In this paper, one of these cultures is designated as epitype for this species, formally fixing the application of the name and allowing confirmation of its phylogenetic relationships.

