Epitypification of *Graphium penicillioides* Corda, with comments on the phylogeny and taxonomy of graphium-like synnematous fungi ¹

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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 *Pestalotia*), 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 calcioides* (Fr.) Okada & Seifert, *Graphium bastruncatum* (Mats.) Seifert & Okada, and *Pestalotia erubescens* (Mathiesen) Okada (see Appendix).

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

¹ Part 12 in a series on the taxonomy of synnematous fungi by Gen Okada.

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Introduction

The anamorph genus *Graphium* Corda (1837), lec-totypified by *G. penicilliioides* 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 lec-totype species *G. penicilliioides* is unknown, the genus was long believed to have ophiostomatoid affinities (Goidanich, 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 conidiigenous cells) includes anamorphs of three orders of the *Ascomycota*.

Okada et al. (1998) demonstrated that several cultures identified as *G. penicilliioides* 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 & Massue 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. penicilliioides* and *G. putredinis*, were considered species aggregates by Seifert & Okada (1993) and Okada et al. (1998).

The correct application of the name *G. penicilliioides* 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. penicilliioides* 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 basiirucatrum* Mats. from a culture isolated from soil from the Solomon Islands; Sutton (1973) later synonymized this species with *G. penicilliioides* (cf. Matsushima, 1975). Furthermore, the CBS culture collection catalogue (http://www.cbs.knaw.nl/databse.html) lists two isolates from *Prinus armeniacus* in Tunisia (CBS 318.72, 319.72). Do all of these populations actually represent the same species, and if not, how should *G. penicilliioides sensu stricto* be defined?

The holotype of *G. penicilliioides* 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. penicilliioides* 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.
**Materials and Methods**

**MORPHOLOGY AND CULTURAL CHARACTERISTICS**

Colours of morphological structures and colonies were determined using the charts of Rayner (1970, numeric-alphabetical codes in the form 19f) or Kornetup & Wanscher (1978, numeric-alphabetical-numeric codes in the form 26A2).

The optimal growth temperature of *Graphium penicillioides* was determined by inoculating ten plates of 2% malt extract agar (MEA; 20 g Bioblab malt extract, 20 g Bioblab agar and 1000 ml distilled water) with 6 mm diameter agar disks taken from the actively growing margins of two-week-old isolates. The plates were incubated at temperatures ranging from 10 to 30°C at 5°C intervals. Cultural descriptions were made using colonies grown on MEA and homemade oatmeal agar (OA; Gams et al., 1998).

Microscopic dimensions were based on 25 measurements and are given as arithmetic means ± standard error.

Cycloheximide tolerance was determined by inoculating 5 MEA plates amended with increasing concentrations of cycloheximide (0.0.01, 0.05, 0.1, 0.5 g/l) and incubated at 25°C. Colony diameters were measured after eight days and mean growth was calculated.

For scanning electron microscopy (SEM), sporulating material from agar media was fixed and dehydrated using the methods of Cole & Samson (1979) or simpler methods of Nakagiri (1999). After critical-point drying with Hitachi HCP-2, the materials were coated with Pt-Pd (ca 100–200 Å thick) in an Eiko ion coater (IB-3) and observed with Hitachi (S-430 or S-2400) or JEOI (JSM-T20) scanning electron microscopes at 10–20 kV.

**STRAINS USED FOR DNA SEQUENCING**

In addition to the 18S rDNA sequences already determined by Okada et al. (1995) for *G. penicillioides* and other Graphium species, the following strains were used for DNA sequencing in this study. JCM – Japan Collection of Microorganisms, RIKEN, Saitama, Japan (http://www.jcm.riken.go.jp/). DDBJ/EMBL/GenBank accession numbers are shown in square brackets.

**18S rDNA:** *Graphium album* Corda Sac. JCM 9744 (CBS 276.54) [AB007657]; *G. erubescens* Mathiesen JCM 9747 (CBS 278.54, ex-type) [AB007658]; *G. eumorphum* Saed JCM 9718 (CBS 987.73) [AB007684]; *G. fructicola* El. & Em. Marchal JCM 9750 (CBS 107.68) [AB007695]; *G. penicillioides* aggregate JCM 8083 (G. Okada OFC 3534, ex soil in Japan) [AB038421]; JCM 10499 (T. Kirisits No. 1, ex *Populas nigra* cv. *italica* in the Czech Republic) [AF179069, not used in Fig. 1]; JCM 10498 (T. Kirisits No. 3, ex *Populas nigra* cv. *italica* in the Czech Republic) [AB038423, AF178010 (not used in Fig. 1)]; JCM 10499 (T. Kirisits No. 4, ex *Populas nigra* cv. *italica* in the Czech Republic) [AF178011, not used in Fig. 1]; *G. rubrum* Rumbold JCM 9751 (CBS 210.34, ex-type according to CBS database) [AB007600]; *G. silvaticum* God. JCM 9752 (CBS 266.37) [AB007651]; *G. tectorum* C. Boeth JCM 9753 (CBS 127.84, ex-type) [AB007622]; *philographium-like* unidentified fungus JCM 8069 (G. Okada OFC 3528) [AB038422].

**ITS rDNA:** *G. penicillioides* aggregate JCM 7440 (= CBS 506.85, ex *Ulmus procera* in the UK, representative of the European *Ulmus* population) [AB038424]; JCM 8083 [AB038425]; JCM 9299 (= CBS 470.71, ex *Fagus Sylvatica* in Germany) [AB038426]; JCM 9300 (= CBS 320.72, ex-type of *Graphium basitricatum* (Mats.) Seifert & Okada (see Appendix), ex forest soil in the Solomon Islands) [AB038427]; JCM 9301 (= CBS 408.84, ex *Salix sp.* in the Netherlands) [AB038428]; JCM 9331 (= CBS 781.85, South Africa (substratum not identified), tentatively identified as *Graphium pseudosinercium* M. Moatton & M. J. Wingfield) [AB038429]; JCM 10494 [AB038430]; JCM 10497 (= T. Kirisits No. 2, ex *Populas nigra* cv. *italica* in the Czech Republic) [AB038431]; JCM 10498 [AB038432]; JCM 10499 [AB038433].

**DNA ISOLATION, PCR AMPLIFICATION, AND SEQUENCING OF RIBOSOMAL DNA**

18S rDNA and ITS sequences for the four new isolates of *G. penicillioides* from the Czech Republic were determined independently in Japan and South Africa.

In Japan, the methods described by Okada et al. (1997) were used for isolation, amplification, cloning and sequencing of 18S rDNA of *G. album*, *G. erubescens*, *G. eumorphum*, *G. fructicola*, *G. penicillioides* aggregate (JCM 8083), *G. rubrum*, *G. silvaticum*, *G. tectorum* and the *philographium-like* unidentified fungus. The primers shown in Table 1 of Okada et al. (1997) were used for amplifying and sequencing 18S rDNA. For JCM 10498 in the *G. penicillioides* aggregate, DNA was obtained by heating mycelium scraped from a slant culture in a lysing solution containing detergent (Makimura et al., 1994) in a 1.5 ml microtube shaken on a vortex mixer with 0.34 mm diam sterile glass beads for 5–10 min. Following the methods of Sugita & Nakase (1999), the 18S rDNA and ITS-1 and ITS-2 regions were amplified by PCR using the universal primers P1 (cf. Suh & Nakase, 1995; Nishida & Sugiyama, 1993) and ITS4 (White et al., 1990). The PCR product was purified using an E.Z.N.A.™ Cycle-Pure kit (Omega Biotech, Doraville, GA, USA), and directly sequenced using a Takara Ex Taq™ sequencing kit (Takara, Shiga, Japan) with the following primers (cf. Suh et al., 1996; Sugita & Nakase, 1999) in addition to P1: 570 (5'–CGCGTAGAATTTCCAGCTCCA–3'); 934 (5'–GTCGAAAGGCAATTTGGCCAAGG–3'; Sugita & Nakase, 1999); 1315 (5'–CGATAAGCAGAACGTCTT–3'); U1 (5'–GGTGAATCCGCGCCGTGCTGCCAC–3'); U2 (5'–GGCCTACTTCCTTTAGTTTCCAGGC–3'); U3 (5'–GGCGCGAGGTTGTCACAAAGGC–3'); ITS2 (5'–GGCGGTTCCTTACGATGC–3').

DNA sequence reactions were analyzed with an ABI PRISM 377 DNA sequencer (Perkin Elmer Applied Biosystems, CA, USA). ITS regions including 5.8S rDNA were directly sequenced using a SequiTherm™ Long-Read™ Cycle sequencing kit (Epigenetics Technologies, Wisconsin, USA) or a Takara Ex Taq™ sequencing kit with the following primers: pITS-1 (Sugita et al., 1998); ITS1 (5'–GTGCTGATAACAGGTTCCCTTGGTAGG–3') and ITS4 (White et al., 1990). Other procedures or conditions
followed Sugita et al. (1998). DNA sequencing reactions were analyzed with an ALFexpress DNA sequencer (Phar- 
macia Biotech, Uppsala, Sweden) or an ABI PRISM 377 DNA sequencer.

In South Africa, DNA was extracted from two-week-
old cultures grown in malt extract broth (ME). A small 
amount of mycelium was ground to a fine powder in 
liquid nitrogen and 1.0 µl extraction buffer (1% CTAB) 
was added, followed by incubation in a 60°C waterbath 
for 1 h. Proteins were removed with phenol and chloro-
form (1:1), followed by a series of chloroform steps, until 
the interface was clean. DNA was precipitated with cold 
100% ethanol, left overnight at -20°C, pelleted at 15000 
rpm for 30 min, washed with cold 70% ethanol and dis-
dissolved in 100–200 µl sterile water. Part of the 18S rDNA 
were amplified using a Hybaid™ Touchdown Thermocycler 
system (Ashford, UK) using primers shown in Table 1 
of Okada et al. (1997). Reactions were done in 100 µl 
containing 10 µl 10X PCR buffer, 20 µl of 25 mM MgCl2, 
10 mM dNTPs, 20 pmol of each primer, 0.5 µl DNA and 
1.75U Expand Taq polymerase (Boehringer Mannheim, 
Germany). The PCR conditions were as follows: 2 min at 
94°C, annealing at 48°C for 1 min, 10 s at 62°C, 2 min at 
72°C with an increase of 5°C s^{-1}, 40 cycles with a final 
elongation step at 72°C for 8 min. The resulting products 
were purified with the High Pure™ PCR product purification 
kit (Boehringer Mannheim, Germany). Sequencing was 
performed on an ABI 377 automated sequencer using the Thermo Sequenase Dye Terminator Cycle Sequencing 
Pre-Mix kit (Perkin Elmer Applied Biosystems, CA, USA) 
with the primers listed in Table 1 of Okada et al. (1997).

Sequence data were edited in Sequence Navigator (Perkin 
Elmer Applied Biosystems, CA, USA). The ITS-2 region 
and part of the LSU rDNA were amplified with the 
primers ITS3 and LR3 (White et al., 1990) and sequenced 
with the primers ITS5, LR3 and 404X (5'-CCCTTTCAA 
CAATTTCAC-3'). Other procedures or conditions were 
identical to those for used for 18S rDNA.

MOLECULAR PHYLOGENETIC ANALYSIS
The newly determined 18S rDNA sequences, excluding 
iintrons, were aligned with sequences mentioned by Okada 
et al. (1998) using the multiple sequence alignment pro-
gram CLUSTAL W version 1.74 (Thompson et al., 1994) 
and adjusted manually. The following additional sequences 
from the nucleotide sequence databases (GenBank, EMBL 
and DDBJ) were included in the alignment: Ascochlorospora 
apis (Maassen ex Claussen) Olive & Spilbor M83264; 
Byssyochara nivae Westling M83256; Cryphonectria 
radicis (Schw.) Barr L42442; Ctenomyces serratus Eiden 
U29391; Gymnoascospora petalosporus Orr, Roy & 
Gosh U29392; Hypoecia putea (Tode) Petch D14407; 
Leucosporidium parasitum (Nitschke) Höhnel M83259; Reni-
spora flavissima Sigler, Gaur, Lichtwardt & Carmichael 
U29392; Saccharomyces fibuligerus (Lindner) Kloeck 
X69841; Sordaria fimicola (Roberge) Ces. & De Not. 
X69851; Talaromyces flavus (Kloeckler) Stolk & Samson 
M83262; Thermosascus crustaceus (Apinis & Chesters)

Stolk M83263; Unimacorpus raesi Sigler & Orr U29394; 
Xylorella carpophila (Pers.) Fr. Z49785. The alignment file 
has been deposited in TreeBASE (http://www.herbaria.
harvard.edu/treebase/index.html).

The aligned data sets for the 18S rDNA were analyzed 
using CLUSTAL W with options set to exclude gaps and 
correct for multiple substitutions. Phylogenetic trees 
were constructed using the neighbor joining method (NJ; 
Saitou & Nei, 1987) based on the comparison of 1485 
sites in the aligned 18S rDNA data set. To evaluate the 
statistical significance of the resulting NJ trees, bootstrap 
tests of 1000 random resamplings were performed 
(Felsenstein, 1985), with identical sequences pruned from 
the data set.

Phylogenetic analyses of ITS rDNA of ten strains of the 
G. penicillioides aggregate were done using heuristic 
searches with PAUP version 3.1.1 (Swoford, 1993), using 
Pseudallescheria boydii (GenBank AF181558) as an out-
group, using default settings. Confidence intervals were 
determined by 1000 bootstrap replicates using heuristic 
searches.

Sequence similarities in the ITS-1 and ITS-2 regions 
in rDNA were determined visually in pairwise alignments 
using CLUSTAL W (Sugita et al., 1999). The alignment 
was also deposited in TreeBASE.

Results

MORPHOLOGY OF GRAPHIUM-LIKE SYNEMATOUS FUNGI

The new isolates from the type host and locality (JCM 
10496–10499) were morphologically typical (Figs 3–17, 21) of the Graphium penicillioides aggregate (Figs 3–25) and possessed similar characters to those that can be determined from the holotype (see Table 4). These isolates also produced mononematous conidiophores abundantly, in which conidial 
sizes were considerably broader than in the synema-
tous conidiomata. The new isolates from the Czech 
Republic were quite similar to JCM 9301 (= CBS 
408.84, isolated from Salix in the Netherlands, cf. 
Figs 18–20), and produced orange to brownish colon-
ies, especially on the reverse, on PDA (Difco or 
Nissui, Fig. 21) and straight (rather than curved) 
conidia with a truncate base (Figs 3, 4–7, 14–17; cf. 
Figs 18–20). Although it was not always clear using 
light microscopy (Figs 3, 8–10), both dense (Figs 15, 
17–19) and nodular (Figs 15, 16, 18, 19) annellations 
were observed in these cultures using SEM. The 
thick-walled pale brown conidia produced by these 
strains are more pigmented than the typically hyaline, 
thin-walled conidia of Pseudotum species, and rather 
similar in pigmentation and wall structure to the 
conidia of species of the G. putredinis aggregate. A complete description of G. penicillioides sensu stricto,
based on the epitype specimen and culture, is provided below. Supplemental morphological observations on other members of the *Graphium penicillioides* and *G. puredinis* aggregates, ophiostomatoid *Pestonum* species, and other graphium-like hyphomycetes are also provided below.

**CULTURAL CHARACTERS OF *GRAPHIUM PENICILLIOIDES SENSU STRICO**

The results of the temperature and cycloheximide studies using the Czech strains of *G. penicillioides sensu stricto* are shown in Tables 1 and 2, respectively. The fungus has an optimal growth temperature of 25–30°C. All strains tolerated cycloheximide and grew at 0.5 g/l, although some strains were more inhibited than others at this high concentration.

**PHYLOGENY OF GRAPHIUM-LIKE SYNNETMEN Fungi IN THE ASCOMYCETES BASED ON 18S rDNA SEQUENCES**

The NJ analysis, using representatives of the Hemi-ascomycetes as the outgroup (Fig. 1), leads to the following conclusions: (i) The newly sequenced strains of the *G. penicillioides* aggregate belong to the same clade as the other members of this species aggregate sequenced previously (Okada et al., 1998), supported with a 100% bootstrap value; (ii) In the *G. penicillioides* clade, some subclustering is evident (i.e., JCM 9301, 10498 and JCM 8083, 9300, supported with 89% and 71% bootstrap values, respectively; (iii) *Graphium eumorphum* (JCM 9748), *G. fructicola* (JCM 9750) and *G. tectonae* (JCM 9753, ex-type) belong to the *G. puredinis* aggregate subclade (Okada et al., 1998; see also Issakainen et al., 1997) of the *Microascales* clade, supported by a 99% bootstrap value; (iv) The *G. penicillioides* aggregate and *G. puredinis* aggregate clades are sister groups, with 100% bootstrap support, as shown previously by Okada et al. (1998); (v) *Graphium album* (JCM 9744) and *G. erubescens* (JCM 9747, ex-type) are included in the *Ophiostomatales* clade, supported with a 100% bootstrap value; (vi) *Graphium rubrum* (JCM 9751, ex-type), *G. silvum* (JCM 9752) and a phialoascomycete-like fungus (JCM 8069) belong to the discoymete clade supported with a 82% bootstrap value; (vii) *Graphium calicioides* clusters with some species of *Capronia* Sacc. and *Exophiala* J. W. Carmich., supported by a 100% bootstrap value, as noted previously (Okada et al., 1998).

Thus, at present, graphium-like synnematous fungi occur in four phylogenetically different groups, the *Microascales* (*Graphium*), *Ophiostomatales* (*Pestonum*), *Chaetothyriales* (*Exophiala*, see below) and probably the discomyces (undescribed genus/genera, or *Dendrostilbella*).

**SEQUENCES OF THE ITS rDNA OF THE *GRAPHIUM PENICILLIOIDES* AGGREGATE**

The gene tree for the ITS of ten sequenced strains of the *G. penicillioides* aggregate is shown in Fig. 2, with *Pseudallescheria boydii* as an outgroup. The data set included 519 characters, of which 35 were phylogenetically informative in the ingroup. The phylogenetic tree is one of six equally parsimonious trees 52 steps long (CI = 0.971, HI = 0.029, RI = 0.914, RC = 0.888).

In all the examined strains of the *G. penicillioides* aggregate, the 5.8S rDNA sequences were identical and 158 bp long. In ITS-1 and ITS-2, the sequences were identical or very similar among JCM 9301, 10496–10499 (ITS-1: 148 bp in JCM 9301, 149 bp in JCM 10497, 150 bp in JCM 10496, 10496, 10499, ITS-2: 175 bp in JCM 10496–10499, 174 bp in JCM 9301). Sequences were identical in JCM 7440, 9299 (ITS-1: 167 bp, ITS-2: 163 bp), although the colonies of these strains on PDA were considerably different in colour and growth rate (Fig. 21). In JCM 8083 and 9300 the lengths of ITS-1 and ITS-2 were the same (132 bp ITS-1 and 172 bp ITS-2), but there was one bp difference in the ITS-2 sequences. ITS rDNA sequence similarities are shown in Table 3 and % similarity values in additional combinations are as follows. ITS-1: 99.3% (149/150 bp) between JCM 10496/10498/10499 and 10497, 98.7% (148/150 bp) between JCM 9301 and 10496/10498/10499, 99.3% (148/149 bp) between JCM 9301 and 10497. ITS-2: 98.9% (173/175 bp) between JCM 9301 and 10496/10497/10498/10499, 99.4% (171/172 bp) between JCM 8083 and 9300. Based on the sequences of the ITS regions mentioned above, the examined strains in *G. penicillioides* aggregate were divided into four groups.

The four groups that could be visually extracted from the alignment (cf. TreeBASE) correspond with the four clades marked in Fig. 2. These clades may represent phylogenetically distinct species, three of which can presently be named (see Fig. 2, Table 3 and Appendix). The clustering in the ITS gene tree is consistent with the less finely dissecting subclustering in the 18S neighbours joining tree (see Fig. 1). The low bootstrap support in Fig. 2 reflects the relatively few phylogenetically informative sites available for subsampling. However, the clades can be considered relatively robust because of the high consistency index, low homoplaspy index, and their occurrence in the strict consensus tree.
Fig. 1. 18S rDNA sequence-based phylogenetic tree derived using neighbour-joining, showing the disposition of graphula-like synnematous fungi in the Ascomycota. Non-italicized Latin names indicate DNA sequences newly determined in this study. The scale bar indicates one base change per 100 nucleotide positions. Bootstrap values were calculated from 1000 replicates. Bold lines indicate lineages with more than 95% bootstrap support. *A. capsulata*, *Ascosporea* = *A. apis*, *Axenochyta* = *A. zeffriniana*, *Blaumeria* = *B. graminis*, *Byssoclamys* = *B. nivea*, *Capronia* = *C. pilosella*, *Chaetotrichum* = *C. eutatus*, *Cryphonectria* = *C. radicalis*, *Ctenomyces* = *C. serratus*, *Debaryomyces* = *D. hansenii*, *Dipodascopsis* = *D. unimicella*, *Eremascus* = *E. albus*, *Eurotium* = *E. rubrum*, *Gymnoascuroides* = *G. petalo sporus*, *Hypocrea* = *H. lutea*, *Hypomycetes* = *H. chrysospermus*, *Leotia* = *L. lubrica*, *Leptosphaeria* = *L. bicolor*, *Leucosporidium* = *L. persoonii*, *Lophiostoma* = *L. cretae*, *Microascus* = *M. cirrospous*, *Monascus* = *M. purpureus*, *Neurospora* = *N. crassa*, *Pleospora* = *P. r. rudis*, *Podospora* = *P. anserina*, *Pseudallescheria* = *P. boydii*, *Rispora* = *R. flavissima*, *Saccharomyces* = *S. cerevisiae*, *Saccharomycoptis* = *S. fibuligera*, *Sclerotinia* = *S. sclerotiorum*, *Sordaria* = *S. fimicola*, *Spalniula* = *S. flavida*, *Sporormia* = *S. lignicola*, *Sporobolus* = *S. scheinii*, *Talaromyces* = *T. flavus*, *Thermoaerascus* = *T. crustaceus*, *Uncinocarpus* = *U. resedii*, *Xylaria* = *X. carpophila.*
**Taxonomy**

The four strains isolated from wood cores of different trees of *Populus nigra* cv. *italica* in České Budějovice, Czech Republic, are almost identical in morphology and in ITS rDNA sequences and are thus considered to represent one species. Because they agree well with the brief, original description of *G. penicilloides* (Corda, 1837), we arbitrarily designate one of them, JC M10498 (= T. Kiriisits No. 3), as epitype strain of *G. penicilloides*. A dried culture grown on *Populus* twigs, the epitype specimen, has been deposited as PR M 842988 and epitype strains have been deposited in Centraalbureau voor Schimmelcultures (CBS 102652) and Japan Collection of Microorganisms (JCM 10498). The species description provided below is based entirely on the epitype specimen and culture of *G. penicilloides*.

*Graphium penicilloides* Corda, l.c. Fung. 1: 18; 1837.  
Colonies on OA after 7 days at 25°C in the dark 1.4–1.7 cm diam, olive-brown (4DF6–7) to dark brown (5F5–7), planar, aerial mycelium sparse, appearing minutely glabrous because of synnemata, lacking soluble pigments, margin more or less invisible, reverse brownish grey to grayish brown (5DE2–3). Colonies on MEA after 8 days at 25°C up to 1.2 cm diam, buff (19Y) becoming darker with age. Hyphae immersed in medium with sparse aerial mycelium, hyaline to light to olivaceous, smooth, (0.5–)3–4 μm diam.

Synnemata on water agar with *Populus* twigs (50–)75–167 (95.4 ± 3.8) μm tall, scattered but abundantly produced, generally single, sometimes in pairs or triplets. Arising from the agar or twig surface, sometimes from aerial mycelium on twigs, with cylindrical, dark brown to black stipes 10–15 μm wide, and divergent light brown to grey capitula, surmounted by watery conidial masses, at first colourless, then white, but quickly becoming olive-brown to almost black, 25–100 (–250) μm, becoming confluent, especially near the inoculum. Synnemata sometimes originating from one or two single, clavate hyphae, giving rise to whorls of hyphae that comprise the synnema stipe; other synnemata arising from multiple hyphae and lacking basal clavate hyphae. Hyphae of stipe 2–3.5 μm wide, to 5 μm at the base, olivaceous to dark olivaceous, frequently constricted at the septa, the walls thin to slightly thickened; rhizoids absent *Conidiophore branching* generally biverticillate, with whorls of 2–4 metulae, 10–11.5 × 1.5–2.5 μm, with the apex swollen up to 5 μm diam; basal cells of the branching apparatus slightly brown. *Conidiogenous cells* in whorls of 2–4, 7–18 (–26.5) (13.7 ± 0.9) μm long, 1–2 μm wide, cylindrical to subulate, straight or sometimes gently curved, the conidiogenous zone up to about 5 μm long, annellations inconspicuous, sometimes with one or two geraniums. Conidia 3–4 (–6) × 1–1.5 (–2.5) (3.7 ± 0.1 × 1.5 ± 0.1) μm, L/W ratio 2–3 (–4), hyaline, aseptate, cylindrical to obovoid, with rounded apices and subtruncate to truncate bases.

Degenerate synnemata or mononematous conidio- phores present, especially on MEA, with conidio- phores and conidia basically identical in shape to those found in synnemata, but much more variable in conidial dimensions.

Optimal growth on MEA at 25 or 30°C (Table 1). Resistant to high concentrations of cycloheximide with a 50% reduction in growth on 0.5 g/l cycloheximide in MEA at 25°C (Table 2).

Teleomorph unknown, but the species has affini- ties to the *Microascales* based on 18S rDNA sequences (Fig. 1).
ITS-1 (uppercase) – 5.8S rDNA (lowercase) – ITS-2 (uppercase) sequences of the epitype culture: CCGA GTTTCACCTC AAAAAACTG TGGACCT TACCAGTCTGGTCTCGGCGGCGC CAACCGCCGCGCGCGCGCGAC CCAAACCTTTATATTCTACCATCGTCTCTTCT GATAGCAAAAAGACAAACAAATCAaaacactcaacacg gatactctgttgggacatgatgacgagcaagcagtaactaataaa tggattacgtaattcagctgatgacgacattcgcce gctgtattcggcggaggtgtgtctcagagcgtattgcGTCCTACA AGCCCGCCCGCTTGTTGGGCACCCCGCGCA ACGCCCGGGGGTCGGCAGGCGCAGCCCAAT GCATCGGCGTCGCCGGCTCCCTGCGT

AGTAGAACCTTTCTGCATCGGGGTCCCGGCC GGCAGGCAGGCAGGCTAAGGCCCCCAATTCTGACCAAAC

**HOLOTYPE:** Graphium penicillioides, Prague, on "Populus it." (= P. nigra cv. italica fide Holubová-Jechová in litt. to K.A.S., 14 Dec. 1988) (PR 155518). Isotype, a slide from the holotype (DAOM 51800).

**EPITYPE:** PRM 842988 [JCM 10498 = CBS 102632 = T. Kirisits No. 3; epitype designated here-with], Czech Republic. České Budějovice, isolated from wood core of Populus nigra cv. italica, 3 Sep. 1998, T. Kirisits.

**Table 1.** Colony diameters of the Czech strains of Graphium penicillioides on MEA after eight days at temperatures ranging from 10 to 30°C*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>JCM 10496</td>
<td>2.1 (± 0.1)</td>
</tr>
<tr>
<td>JCM 10497</td>
<td>2.6 (± 0.1)</td>
</tr>
<tr>
<td>JCM 10498</td>
<td>2.1 (± 0.1)</td>
</tr>
</tbody>
</table>

* Colony diameters shown in mm ± standard error represent the means of eight measurements in ten plates, excluding the minimum and the maximum measurements.

**Table 2.** Colony diameters of the Czech strains of Graphium penicillioides after eight days at 25°C on MEA amended with increasing concentrations of cycloheximide*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentrations of cycloheximide (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>JCM 10496</td>
<td>12.3 (± 0.2)</td>
</tr>
<tr>
<td>JCM 10497</td>
<td>11.8 (± 0.3)</td>
</tr>
<tr>
<td>JCM 10498</td>
<td>11.8 (± 0.4)</td>
</tr>
<tr>
<td>JCM 10499</td>
<td>2.7 (± 0.1)</td>
</tr>
</tbody>
</table>

* Colony diameters represent the means of ten measurements in five plates, shown in mm ± standard error.


**EXCLUDED STRAINS TENTATIVELY TREATED AS OTHER GRAPHIUM SPECIES IN THE G. PENICILLIOIDES AGGREGATE.**—JCM 7440 (= CBS 506.86), JCM 9299 (= CBS 470.71); JCM 8083, JCM 9300 (= CBS 320.72); JCM 9331 (= CBS 781.85). Other strains are discussed in the Appendix.

**Discussion**

The overall phylogenetic relationships of graphium-like fungi demonstrated here conform with those shown by Okada et al. (1998), namely:

a) Graphium penicillioides and *G. putreadis* form sister clades allied with the *Microascales*. Because this
Table 3. Number of nucleotide differences in ITS-1 and ITS-2 in the *Graphium penicillioides* aggregate.\(^1\)

<table>
<thead>
<tr>
<th>Strain groups (species)</th>
<th>No. of nucleotide differences (bp length)</th>
<th>% similarity of ITS-1 + 2 in each strain group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ITS-1</td>
<td>ITS-2</td>
</tr>
<tr>
<td><em>G. penicillioides</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCM 10498 (^2)</td>
<td>(150)</td>
<td>(175)</td>
</tr>
<tr>
<td>JCM 10496</td>
<td>(150)</td>
<td>(175)</td>
</tr>
<tr>
<td>JCM 10499</td>
<td>(150)</td>
<td>(175)</td>
</tr>
<tr>
<td>JCM 10497</td>
<td>(149)</td>
<td>(175)</td>
</tr>
<tr>
<td>JCM 9301</td>
<td>(148)</td>
<td>(174)</td>
</tr>
<tr>
<td>Unidentified <em>Graphium</em> species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCM 7440 (^3)</td>
<td>(167)</td>
<td>(163)</td>
</tr>
<tr>
<td>JCM 9290 (^3)</td>
<td>(167)</td>
<td>(163)</td>
</tr>
<tr>
<td><em>G. hastruncatum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCM 9300 (^4)</td>
<td>(132)</td>
<td>(172)</td>
</tr>
<tr>
<td>JCM 8383</td>
<td>(132)</td>
<td>(172)</td>
</tr>
<tr>
<td>&quot;G. pseudomiticum&quot; (^5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCM 9331</td>
<td>(175)</td>
<td>(165)</td>
</tr>
</tbody>
</table>

\(^1\) The nucleotide sequences in ITS rDNA are identical and 138 bp long in all the strains used.
\(^2\) Ex-epitype strain.
\(^3\) Colonies on PDA were considerably different each other in colour and growth rate.
\(^4\) Ex-type strain.
\(^5\) Tentatively identified as *G. pseudomiticum*.

Table 4. Comparison of the epitype (growing on *Populus* twigs) and holotype specimens of *Graphium penicillioides*. All measurements in μm, based on 25 measurements for the epitype and variable numbers of measurements for the holotype.

<table>
<thead>
<tr>
<th></th>
<th>Epitype (PRM 842988)</th>
<th>Holotype (PR 155518)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synnema</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>height</td>
<td>(50–75–167 (95.4 ± 3.8)</td>
<td>up to 250</td>
</tr>
<tr>
<td>width</td>
<td>10–15</td>
<td>10–25 (–75)</td>
</tr>
<tr>
<td><strong>Hyphae of stipe</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>width</td>
<td>2–3.5</td>
<td>1.5–2</td>
</tr>
<tr>
<td><strong>Conidigenous cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>length</td>
<td>7–18 (–26.5) (13.7 ± 0.9)</td>
<td>15–26 (19.4 ± 2.7, n = 4)</td>
</tr>
<tr>
<td>width</td>
<td>1–2</td>
<td>1.5–2</td>
</tr>
<tr>
<td><strong>Conidia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>shape</td>
<td>cylindrical to obovoid</td>
<td>cylindrical to obovoid</td>
</tr>
<tr>
<td>apex</td>
<td>rounded</td>
<td>rounded</td>
</tr>
<tr>
<td>base</td>
<td>truncate</td>
<td>truncate</td>
</tr>
<tr>
<td>length</td>
<td>3–4 (–6.0) (3.7 ± 0.1)</td>
<td>4–5.5 (4.4 ± 0.1, n = 18)</td>
</tr>
<tr>
<td>width</td>
<td>1–1.5 (–2.5) (1.5 ± 0.1)</td>
<td>1.5–2 (1.6 ± 0.03, n = 18)</td>
</tr>
<tr>
<td>L/W</td>
<td>2–4</td>
<td>2.25–3.7</td>
</tr>
</tbody>
</table>
includes the type species of Graphium, the generic name should be restricted to fungi related to this ascomycete order.

b) The synnematus anamorphs of Ophiostoma species are phylogenetically unrelated to Graphium sensu stricto, and should be referred to the anamorph genus Pseudothecia.

c) The phylogenetic affinities of Graphium calicoides are with the Chaetothryciaceae.

To this overall pattern, we can add a fourth clade of graphium-like synnematus anamorphs that is probably related to the discomycetes. These includes Graphium rubrum (JCM 9751 = CBS 210.34, ex-type), G. sikamum (JCM 9752 = CBS 206.37, authentic strain isolated and identified by G. Goidanich) and a phialogriphium-like unidentified fungus (JCM 8069, common on rotten wood in Japan), which are related to the discomycetes based on their 18S rDNA sequences. In these strains, typical phialidic conidiogenesis was observed using SEM (Figs 33, 34) or light microscopy. This cannot be distinguished morphologically from the conidiogenesis of Pseudothecia sanguinoterpae (Upadhyay & Kendrick) Okada & Seifert (originally described in Phialogriphium), the anamorph of Ophiostoma sanguinoterpae (Wright & Cain) Solheim. Synnematus anamorphs are well-known, but rather sparsely dispersed in the discomycetes. Graphium-like synnematus anamorphs are classified in Crinula Fries (teleomorphs in Holwaya Sacc.; Seifert & Okada, 1993), Coryne Nees (teleomorphs in Ascochyrae J. W. Groves & D. E. Wilson; Seifert, 1989) and Dendrothrielia Höhnel (teleomorphs in Ciausenomyces Kirschst.; Seifert, 1985). In fact, one of these species, Dendrothrielia sanguinoterpae (Alb. & Schw.) Seifert (see Appendix), with dark green synnemata and phialidic conidiogenous cells, has often been referred to as Graphium sanguinoterpae (Alb. & Schw.) Sacc.

The convergent evolution demonstrated by these four groups of synnematus anamorphs is remarkable. As we have observed in the past, the anamorph of Ophiostoma columnare is very similar in micromorphological characters to G. penicilliodes, differing primarily in the pigmentation of the synnemata (Seifert & Okada, 1993). However, if fasciation of conidiophores is a banal evolutionary event, then the occurrence of such similar evolutionary events in different
clades of the Ascomycetes is unsurprising. As for conidium ontogeny, similar convergent evolution also exists in graphium-like hyphomycetes. Conidiogenous cells in Graphium sensu stricto exhibit percurrent proliferation, in common with other members in the Microascales (e.g. species of Scoepulariopsis Bainier, Cephalotrichum Link). In Posotum species, intermediate modes between percurrent and sympodial proliferations and phialidic conidiogenesis are frequently observed. Graphium calicoides, when grown in culture, is quite reminiscent of the so-called ‘black yeasts’, producing sessile pustules of slime on the agar surface, and the conidiogenous cells have a characteristic narrowing before percurrent proliferations begin that is quite similar to the conidiogenous cells of Exophiala species. Many anamorphs of discomycetes have percurrently proliferating or phialidic conidiogenous cells, which are similar to those illustrated here.

Okada et al (1998) provided preliminary generic diagnoses to distinguish the synnematous anamorphs of two of the three phylogenetic groups recognized. They emphasized differences in conidium ontogeny between the opphiostomalane and microascalean anamorphs. Pale brown conidia in G. penicillioides and the G. putredinis aggregate possibly reflect their phylogenetic relationships to the Microascales. The colours of conidia, conidiogenous cells, conidiophores and agar colonies sometimes reflect phylogenetic affinities in anamorphic fungi (Okada et al., 1997, 1998). In all the examined strains of the G. penicillioides aggregate, we have observed nodular annulations (Figs 15, 16, 18, 19, 22–25), as well as dense annulations (Figs 15, 17, 18, 19, 25). Nodular annulations were frequently observed especially at the base of the percurrently elongating part of the conidiogenous cells (Figs 15, 18, 19). In some strains, dense annulations were more frequently observed than nodular ones. Nodular annulations do not always suggest affinities to the Microascales (cf. Remersonia thermophila (Fergus) Seifert & Samson related to the Sordariaceae; Seifert et al., 1997).

The morphological data presented here support our previous contention (Seifert & Okada, 1993; Okada et al., 1998) that G. penicillioides should be regarded as a species aggregate. Sugita et al. (1999), working with species of Trichosporon Behrend, observed that conspecific strains have less than a 1% overall nucleotide difference in both the ITS-1 and ITS-2 regions. As indicated clearly in Fig. 2 and Table 3, the examined
Figs 14–20. Scanning electron micrographs of *Graphium pencillitodes* on OA (14–17, JCM 10498 (ex-epitype; 18–20, JCM 9361 (= CBS 408.84)). 14. Synnema. 15. Nodular (arrows) and dense (arrow heads) anellations on the conidiogenous cells. 16. Nodular anellations on a synnema. 17. Dense anellations on a synnema. 18,19. Nodular (arrows) and dense (arrow heads) anellations respectively at the base and apical part of the conidiogenous cells. 20. Conidia with a truncate base. Scale bars in Figs 15–20 = 5 μm, in Fig. 14 = 10 μm.
Figs 21–25. Graphium penicilliioides sensu lato. 21. Colonies on Difco PDA in 9 cm diam Petri dishes, 20°C, 2 wks; JCM 10498, 10496, 10499, 10497, 9301 from left to right in upper row; JCM 7440, 9299, 8083, 9300, 9331 from left to right in lower row; JCM 9300, 8083 = Graphium basitruncatum, see Fig. 2, Table 3 and Appendix. 22, 23. Nodular annellations in JCM 7440 (= CBS 506.86) and JCM 9299 (= CBS 470.71), respectively; on OA. 24. Nodular annellations of Graphium (big arrow) and scedosporium-like (small arrow) synanamorphs in G. basitruncatum JCM 9306 (= CBS 320.72); on OA. 25. Catenate conidia produced from dense/nodular annellides at the head of a synnema in JCM 9331 (= CBS 781.85), on OA; tentatively identified as G. pseudomiticum. Scale bars in Figs 22–25 = 5 μm.
Figs 26-30. Scanning electron micrographs of the *Graphium putredinis* aggregate (26-28, *G. tectona* ICMB 9753 (= CBS 127.84, ex-type), 29, *G. eumorphum* ICMB 9748 (= CBS 987.73), 30, *G. fructicola* ICMB 9750 (= CBS 107.68). 26. *Graphium* synanamorph, on Miura's agar. 27. Conidia from *Graphium* (arrow heads) and *Scedosporium* (arrow) synanamorphs, on Miura's agar. 28. Globose chlamydospores, on Miura's agar. 29, 30. Conidia produced from nodular anellides at the head of a synnemata, on PDA and OA, respectively. Scale bars in Figs 27-30 = 5 μm, in Fig. 26 = 20 μm.

Figs 31, 32. Scanning electron micrographs of ophiostomatoid graphium-like hyphomycete, *Pestumerubescens* ICMB 9747 (= CBS 278.54, ex-type of *Graphium rubescens*), on OA. 31. Anellated (arrow heads) and sympodula-like (arrows) conidiogenous cells. 32. Anellides (arrow heads) and phialide-like conidiogenous cells (arrows). Scale bars in Figs 31 and 32 = 5 μm.
strains in the *G. penicillioides* aggregate can be divided into four groups based on ITS rDNA sequences that could be treated as different phylogenetic anamorph species. In the LSU rDNA partial sequences, single-base substitutions or deletions were found among the Czech strains of *G. penicillioides* (ICM 10497–10499) (data not shown). However, a true test of phylogenetic species requires concordance between different gene trees (Taylor et al., 1999).

The precise application of the anamorph name *Graphium penicillioides sensu stricto* is now well-established. The epitype for *G. penicillioides* can now be used in place of the holotype for the intensive morphological and molecular studies needed to effectively differentiate between the four or more species in this clade. There are some discrepancies between the descriptions of specimens and cultures identified as *G. penicillioides* by Matsushima (1975), Sutton & Laut (1970), Ellis (1971), Matsushima (1971), Crane & Schoknecht (1973) and Sutton (1973), particularly in the length of the conidiogenous cells, as noted by Seifert & Okada (1993).

*Graphium putredinis* is another species aggregate (Seifert & Okada, 1993; Okada et al., 1998). Before Okada et al. (1998) analyzed this group using 18S rDNA sequences, Issakainen et al. (1997) reported, using the same molecule, that the ex-type strain of *G. tectum* (CBS 127.84) belongs to the microascelaean clade with *Scedosporium prolificans* (Hennebert & Desai) Guého & de Hoog and *Petriella setifera* (A. Schmidt) Curzi. They also mentioned that microascelaean fungi having *Graphium* anamorphs (= *G. putredinis* aggregate sensu Seifert & Okada, 1993) can be divided into at least three groups. Species concepts for both the *G. penicillioides* and *G. putredinis* aggregates should be investigated further, along with related microascelaean fungi, by the use of molecular techniques and studies of micromorphology. Characters that can be tested as possible indicators of speciation include conidium size and shape, morphology of detachment scars, colour of conidial slime, length of conidiogenous cells, height of syne-mata, growth rates and pigmentation in pure culture, occurrence of synanamorphs, and host/substratum relationships.

Little is known of the biology of *G. penicillioides sensu stricto*. Although the four cultures from the Czech Republic were isolated from different living trees with fully functional, wet sapwood, no inoculation trials have been done, nor were the trees examined for possible insect vectors.

The tolerance to cycloheximide by *G. penicillioides* is surprising, because this character is often considered indicative of a relationship with *Ophiostoma*. However, de Hoog et al. (1994) showed that some strains of *Pseudallescheria boydii* (Shear) McGinnis, Padhye & Ajello and the related species *Scedosporium prolificans* (both fungi in the Microascaceae) were tolerant to 0.05–0.1% cycloheximide. They mentioned that both species, based on DNA/DNA homology, included both sensitive and tolerant strains. Therefore, sensitivity to cycloheximide should be carefully applied as an indicator of phylogenetic affinities in the Ascomycota.

Figs 33, 34. Scanning electron micrographs of phialidic conidiogenesis (arrows) in non-synnematous conidiophores of discomyceteous anamorphs to be excluded from *Graphium*, on PDA. 33. *Graphium rubrum* ICM 9751 (= CBS 210.34, ex-type). 34. *Graphium silvum* ICM 9752 (= CBS 206.37). Scale bars in Figs 33 and 34 = 5 μm.
Based on phylogenetic relationships, we transfer *G. calicioides* to the genus *Exophiala* below. This species produces capitulate slender synnemata on decaying wood, but the conidiomata are poorly developed or absent in agar culture. Although the distinction between mononematous or synnematous conidiophores has often been used to separate anamorph genera in hyphomycetes, there are now several precedents for including anamorphs with mononematous and conidiomatal conidiophores in a single genus (e.g. *Aspergillus* Link, *Penicillium* Link, *Trichophyton* Shearer & Crane). Therefore, in contrast to our previous ideas (Okada et al., 1998), we elect not to create a new hyphomycete genus for *G. calicioides*. The teleomorph of *Exophiala calicioides* (Fr.) Okada & Seifert is a species of *Capronia* (see Uнтерее, this volume), but its description awaits collection of adequate material from temperate rain forests, such as a beech forest in autumn, and critical studies of existing herbarium specimens.

**Appendix. Some nomenclatural changes and taxonomic notes**

Following the emended concepts for the *Graphium* complex proposed by Okada et al. (1998), some of the new findings mentioned above based on 18S rDNA suggest additional formal taxonomic proposals and changes to the nomenclature. Where we have examined type or authentic material, this is indicated by an exclamation mark (!).

NEWLY ACCEPTED SPECIES OF *GRAPHIUM* Corda emend. Okada & Seifert:

*Graphium penicilliioides* aggregate:

*Graphium basitrunctatum* (Mats.) Seifert & Okada, *comb. nov.*


For the secedosporium-like synanamorph, see Fig. 3 of Okada et al. (1998) and Fig. 24 of this paper. Nodular annellations were observed in both the *Graphium* and secedosporium-like synanamorphs (Fig 24; Fig. 3 of Okada et al. 1998)). The strains JCM 8083 and 9300 are considered conspecific based on morphology and ITS rDNA sequences (cf. Fig. 2, Table 3, and the alignment of ITS rDNA deposited in TreeBASE).

In the *G. penicilliioides* aggregate, there are at least two other species (i.e., JCM 7440, 9299 and 9331) in addition to *G. penicilliioides sensu stricto* and *G. basitrunctatum* (cf. Fig. 2, Table 3, and the alignment of ITS rDNA deposited in TreeBASE). The strain JCM 9311, tentatively identified as *G. pseudomorphus* (cf. Mouton *et al.*, 1994, and Fig. 25 of this paper), produced conidiogenous cells with nodular annellations, as does an unidentified *Graphium* species (JCM 7440, 9299) (Figs 22, 23, 25; cf. Figs 1, 2 and Table 3). Dense annellations were also observed in JCM 7440 and 9331.

*Graphium puwedonis* aggregate:

*Graphium tectone* C. Booth, Mycol. Pap. 94: 5. 1964. (ex-type strain! JCM 9753 = CBS 12784 = IMI 0956734).

In addition to the *Graphium* anamorph (Fig. 26), *Secosporium* (Fig. 27) and sporothrix-like synanamorphs and chlamydospores (Fig. 28) were observed.

We can now add that *Graphium eumorphum* Sacc. (JCM 9748 = CBS 98773; Fig. 29) and *G. fruticola* El. Marchal & Em. Marchal (JCM 9750 = CBS 10768; Fig. 30) have affinities with the *G. puwedonis* aggregate, but it remains to be seen whether they are distinct species. In *G. eumorphum*, a *Secosporium* synanamorph has been observed. In *G. fruticola*, *Secosporium* and *Sporothrix* synanamorphs and an intermediate morph occur.

NEWLY ACCEPTED SPECIES OF *PESTOTUM* J.L. Crane & Schoknecht *sensu* Okada & Seifert (1998):

*Pestotum erubescens* (Mathiesen) Okada, *comb. nov.*


Using light microscopy, the verruculately arranged conidiogenous cells on the synnemata look like phialides with a collarette. Using SEM, however, clear percurrent proliferations were observed, in addition to an intermediate mode between sympodial proliferation and phialidic ontogeny (Figs 31, 32).

The culture identified as *Graphium album* (Corda) Sacc. (JCM 9744 = CBS 27654) is apparently a species of *Pestotum*, but must be reidentified at species level. There is no type of this species, based on *Ceratopodium album* Corda, in Corda's herbarium (Seifert, unpublished).

**CHAETOTHYRIALEAN ANAMORPHS:**

*Exophiala calicioides* (Fr.) Okada & Seifert, *comb. nov.*

= *Sporocysta calicioides* Fr., Syst. mycol. 3: 343. 1832 (basionym) (based on *Calciun hussellare* Achat., *cortypes* UPS, not seen, slides DAOI 500491, 500501).


For a description of the species see Ellis (1971), and
Seifert & Okada (1993) for another illustration. Our transfer of this species to Exophiala emends the concept of that genus to include species with synnematous conidiomata.

Discomycetous Anamorphs:

Dendrostilbella smaragdina (Alb. & Schw.) Seifert, comb. nov.
= Stilbium smaragdunum Alb. & Schw., Conspicue Fungorum, p. 355. 1805 (basionym).
For other synonyms, a description and illustration, see Seifert (1985), who neotypified this species, treating it under Tubercularia. This species has also been widely known as Graphium smaragdum (Alb. & Schw.) Sacc. The recombinere here is based on recent discovery of an as-yet undescribed teleomorph referable to Clavissomyces (Seifert, unpublished).

Species incertae sedis:

Graphium rubrum Rumbold, Phytopathology 24: 300. 1934 (ex-type strain! ICMP 9752 = CBS 210.34).


In both species, probably related to the discomycetes based on 18S rDNA sequences (see above), phialides were observed using SEM (Figs 33, 34) although no synnema were produced in culture. Periclinal thickening and collarettes were also observed at the conidigenous apertures using light microscopy.

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