Mycologia, 103(5), 2011, pp. 1133–1145. DOI: 10.3852/11-031

Periglandula, a new fungal genus within the Clavicipitaceae and its association with Convolvulaceae

Ulrike Steiner
Sarah Leibner

Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES)-Phytomedizin, Rheinische Friedrich Wilhelms-Universität Bonn, Nussallee 9, D-53115 Bonn, Germany

Christopher Lewis Schardl

Department of Plant Pathology, 201F Plant Sciences Building, 1405 Veterans Drive, University of Kentucky, Lexington, Kentucky 40546-0312

Adrian Leuchtmann

Plant Ecological Genetics, Institute of Integrative Biology, ETH Zürich, Universitätstrasse 16, CH-8092 Zürich, Switzerland

Eckhard Leistner[1]

Institut für Pharmazeutische Biologie, Rheinische Friedrich Wilhelms-Universität Bonn, Nussallee 6, D-53115 Bonn, Germany

Abstract: We describe two newly discovered fungi living on the adaxial leaf surface of plants belonging to the Convolvulaceae, *Ipomoea asarifolia* and *Turbina corymbosa*. The fungi apparently are epibionts because hyphae were never observed to penetrate epidermal cells or stomata of their respective host plants, and most remarkably are intimately associated with secretory glands on the leaf surface. Hyphae and structures resembling chlamydospores and synnemata (but lacking conidia), formed by both fungal species are phenotypically nearly indistinguishable after in vitro growth or when examined in vivo on the leaf surface. Phylogenetic trees based on aligned DNA sequences from nuclear genes for β-tubulin (*tubB*) and RNA Polymerase II subunit 1 (*rpbA*), and the mitochondrial gene for ATP synthase F0 subunit A (*atp6*), grouped the fungal species in a clade within the Clavicipitaceae. Clavicipitaceous fungi isolated from the two different plant species could be distinguished by their *atp6* and *rpbA* sequences, and nuclear genes for γ-actin (*actG*), translation elongation factor 1–α (*tefA*), and 4–(γ,γ-dimethylallyl)tryptophan synthase (*dmaW*), the determinant step in ergoline (i.e. ergot) alkaloid biosynthesis. Based on these findings we propose a new fungal genus, *Periglandula*, gen. nov., and describe two new species, *Periglandula ipomoeae* sp. nov., from host plant *I. asarifolia*, and *Periglandula turbinae* sp. nov., from *T. corymbosa*.

Key words: Clavicipitaceae, Ergoline alkaloids, ergot alkaloids, *Ipomoea asarifolia*, molecular systematics, *Periglandula ipomoeae*, *P. turbinae*, symbiosis, *Turbina corymbosa*

INTRODUCTION

Fungi belonging to the Clavicipitaceae (Hypocreales) occur as symbionts of invertebrates (Gams and Zare 2003, Suh et al. 2001, Sullivan et al. 2000), parasites of hart's truffle fungi (*Elaphomyces* spp.) (Bischoff and White 2005, Fukatsu and Nikoh 2003), or epibiotic or endophytic symbionts of monocotyledonous plants in the families Cyperaceae and Poaceae (Poales) (Schardl and Clay 1997, Schardl et al. 2004). A characteristic of many Clavicipitaceae that are symbionts of vascular plants is ergoline (syn. ergot) alkaloid production (Schardl et al. 2006). Although ergoline alkaloids are known from fungi in other orders, those produced by Clavicipitaceae play roles in the ecological interactions of plants with their environment (Bischoff and White 2003, Bush et al. 1997, Clay and Schardl 2002, Pazoutová 2003, Saikkonen et al. 2004, Schardl and Leuchtmann 2005, Tudzynski et al. 2001, Tudzynski and Scheffer 2004), and episodically cause poisoning of livestock or humans but also have medical applications (Gröger and Floss 1998, Hofmann 1961, Schardl et al. 2006, Schultes and Hofmann 1979). Hofmann (1961, 2006), having found that some Convolvulaceae also contain ergoline alkaloids, discussed the possibility that these dicotyledonous plants might host an alkaloid producing fungus. This finding met with great interest as a potential explanation for the hallucinogenic properties of *ololiuqui*, used in religious ceremonies by Native Americans (Hofmann 1961, Schultes and Hofmann 1979). However up to that time no symbiotic Clavicipitaceae had been reported in convolvulaceous plants.

The presence of ergoline alkaloids in taxonomically unrelated taxa, such as fungi and dicotyledonous plants, seemed to contradict the principle of chemotaxonomy that similar or even identically structured natural products are features of taxonomically related taxa. A horizontal gene transfer or the repeated invention of the biosynthetic pathway leading to ergoline alkaloids was suggested but remained un-

Submitted 23 Jan 2011; accepted 23 Mar 2011.
[1] Corresponding author. E-mail: eleistner@uni-bonn.de

TABLE I. Plants and seeds of different origin symbiotic with *Periglandula* species[a]

Fungal species	Host plant	Origin of plant
P. ipomoeae	*I. asarifolia (w)*	1. Plants raised at PB Bonn from seeds supplied by E. Eich, PB Berlin
		2. Plant and seeds collected by E. Eich, PB Berlin, at a site (''Los Cedros'', ''La Cumbre'') in Ecuador in 1991
		3. Plants grown from cuttings at PB Bonn
P. ipomoeae	*I. asarifolia (r)*	4. Plants grown at PB Bonn from seeds collected by E. Eich, PB Berlin, from a site (''Cherube'') in Panama in 1995
		5. Plants grown at PB Bonn from seeds collected by E. Eich, PB Berlin, from a site (''Cherube'') in Panama in 1997
		6. Plants raised at PB Bonn from cuttings
P. turbinae	*T. corymbosa*	7. Plant and seeds collected at a site (''Altos de Campana'') in Panama by E. Eich, PB Berlin, in 1995
		8. Plants raised at PB Bonn from seeds obtained from RKD
		9. Plants obtained from BGM (AKZ, 200208625)

[a] Plant materials were identified by colleagues shown in parenthesis. No. 2 (DF Austin, Texas, USA), Nos. 4, 5, 7 (E Eich, PB Berlin, Germany), No. 9 (R. Omlor, BGM, Mainz, Germany). Plant specimens infected by the respective fungi were deposited at the Atkinson Herbarium at Cornell University (CUP) (Nos. 1, 3, 5, 8, 9), PB Berlin (Nos. 2, 7) and PB Bonn (Nos. 3, 4, 6, 9). Microscopic slides also were prepared from fungi collected from all plants and deposited at CUP.

Abbreviations: *w* = white blooming, *r* = red blooming, Pharmazeutische Biologie Bonn, Germany (PB Bonn), Pharmazeutische Biologie Berlin, Germany (PB Berlin), Rühlemanns Kräuter und Duftpflanzen, Horstedt, Germany (RKD), Botanischer Garten Universität Mainz, Germany (BGM), accession number of the Botanischer Garten Universität Mainz, Germany (AKZ).

proven (Gröger and Floss 1998, Tudzynski et al. 2001, Clay and Schardl 2002). Experiments leading to the present study did not support such assumptions. We showed instead that clavicipitaceous fungi colonize Convolvulaceae, such as *Ipomoea asarifolia* (Desr.) Roem.& Schult., *Ipomoea* tricolor L.[2] and *Turbina corymbosa* (L.) Raf. (Ahimsa-Müller et al. 2007), and provided evidence that these symbionts are responsible for the presence of ergoline alkaloids within these plants (Kucht et al. 2004).

The purpose of the present study was to provide a valid description of ergoline-alkaloid-producing clavicipitaceous fungi symbiotic with Convolvulaceae. Based on host specificity, morphological characterization and molecular phylogenetic evidence, a new genus is proposed, named *Periglandula*, because these fungi are closely associated with secretory glands on the adaxial surfaces of host leaves (Kucht et al. 2004, Leistner and Steiner 2009). New species described within this genus are *Periglandula ipomoeae* from *I. asarifolia* and *Periglandula turbinae* from *T. corymbosa*. These are the first ergoline alkaloid-producing Clavicipitaceae described that are apparently mutualistic symbionts of dicotyledonous plants (Leistner and Steiner 2009).

[2] In a previous publication (Ahimsa-Mueller et al. 2007) we reported on experiments with *Ipomoea violacea* L. This plant however was misidentified and represents in fact *Ipomoea tricolor* Cav. We are grateful for the help of Dr Rick Elton Miller, Southeastern Louisiana University, Hammond, LA 70402, who brought this to our attention.

MATERIALS AND METHODS

Biological materials.—Seeds and plant material (TABLE I) were obtained from E. Eich at the Institut für Pharmazeutische Biologie, Freie Universität Berlin, Germany (PB Berlin), R. Omlor of the Botanical Garden, Universität Mainz, Germany (BGM) and Rühlemanns Kräuter und Duftpflanzen (RKD), Horstedt, Germany. *I. asarifolia* and *T. corymbosa* plants, from different origins (TABLE I), were grown in the greenhouse at the Institut für Pharmazeutische Biologie, Universität Bonn, Germany. (PB Bonn) through multiple generations since 2002. These plants were always associated with epibiotic clavicipitaceous fungi and contained ergoline alkaloids except after fungicide treatments (Kucht et al. 2004).

Plant specimens (TABLE I) infected by the respective fungi were deposited in the Atkinson Herbarium at Cornell University (CUP), PB Berlin and PB Bonn. Microscopic slides of leaves with the epiphyllous mycelium on adaxial leaf surfaces were prepared from all plants examined (TABLE I) and deposited at CUP. As the fungi, *P. ipomoeae* (accession number CUP-067884) and *P. turbinae* (accession number CUP-067885), are hitherto unculturable for long term, no living cultures are maintained.

Fungal strains were designated according to their plant hosts: *P. ipomoeae* strains IasaF13 on white blooming *I. asarifolia* and IasaredF01 on red blooming *I. asarifolia* and *P. turbinae* TcorF01 on *T. corymbosa*. The fungi were isolated from plants grown at PB Bonn (TABLE I) from young, unexpanded leaves. Closed leaves were surface disinfected with 1.3% sodium hypochlorite for 1 min, rinsed in sterile water and opened under sterile conditions. Mycelium samples from the adaxial surfaces of the leaves were transferred onto potato dextrose agar (PDA, Becton,

TABLE II. Primers employed in PCR reactions and sequencing, and accession numbers of PCR-products from *P. ipomoeae* on white (IasaF13) and red (IasaredF01) blooming *I. asarifolia* and *P. turbinae* (TcorF01) on *T. corymbosa*

Gene, product	Primers	Reference	Approximate size (bp) of PCR product (electrophoresis)	Fungus	Accession no. of PCR product
actG, γ-actin	Act1-exon1d-1 Act1-exon6u-1	Moon et al. 2002	—		
	Act-nes1 Act-nes2	this study[a]	1250	TcorF01	HQ702607
				IasaF13	HQ702608
				IasaredF01	HQ702609
tubB, ß-tubulin	T12 T22	Sung et al. 2007	780	TcorF01	HQ702604
				IasaF13	HQ702605
				IasaredF01	HQ702606
tefA, translation elongation factor- 1α	Tefl-exon5u-1 Tefl-exon1d-1	Moon et al. 2002	800	TcorF01	HQ702601
				IasaF13	HQ702602
				IasaredF01	HQ702603
Atp6, mitochondrial ATP synthase subunit 6	mtATP6-1A mtATP6-2A	Sung et al. 2007	700	TcorF01	HQ702613
				IasaF13	HQ702614
				IasaredF01	HQ702615
rpbA, RNA polymerase II large subunit	CRPB1 RPB1G	Sung et al. 2007	800	TcorF01	HQ702610
				IasaF13	HQ702611
				IasaredF01	HQ702612

[a] Nested primers Act-nes1 (CGT TCT GAC GGT TTC TA) and Act-nes2 (TAC ATG GGC ACG TGA GT) were newly developed for this study.

Dickinson & Co, Sparks, Maryland) containing antibiotics (50 mg L^{-1} penicillin, 50 mg L^{-1} chlortetracycline, 50 mg L^{-1} streptomycin). Cultures were incubated at 23 C in the dark. Fungal morphological characters were observed and radial growth rates were measured after 4 wk incubation.

Histology.—Microscopic investigations employed a stereomicroscope MZ 16F and a photomicroscope DMRB (Leica, Bensheim, Germany) equipped with Nomarski interference contrast. Fresh samples of mycelium from agar plates were mounted directly on slides with a cover slip. Leaf samples were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.3 for 24 h (Karnovsky 1965). The tissue was dehydrated and embedded in L.R. White acrylic embedding medium (Polysciences Inc., Warrington, Pennsylvania), and 1 µm sections were cut with glass knives. Sections were stained with toluidine blue (0.1% aqueous).

For scanning electron microscopy the specimens were rinsed with distilled water and dehydrated through a graded series of ethanol, then critical-point dried from CO_2 in eight cycles according to Svitkina et al. (1984) with a Balzers CPD 030 (BAL-TEC, Schalksmühlen, Germany). Dried specimens were mounted on aluminum sample holders and sputter coated with 2 nm platinum/palladium with a HR 208 coating device (Cressington, Watford, UK) SEM was performed with a XL 30 SFEG (Philips, Eindhoven, the Netherlands) equipped with a through lens secondary electron detector.

DNA Sequencing.—Plant DNA was extracted with the Wizard Magnetic DNA Purification Kit for Food (Promega Molecular Diagnostics, Mannheim, Germany) from plants grown at PB Bonn. The template DNA used for PCR (Steiner et al. 2006) was dissolved in 100 µL sterile water and kept at −20 C. The rDNA internal transcribed spacers (ITS) of the red blooming and the white blooming *I. asarifolia* plants were amplified by PCR with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990).

Fungal mycelia were collected from the adaxial leaf surfaces of young, folded leaves from 10 individual plants each of *T. corymbosa* and white blooming and red blooming varieties of *I. asarifolia* grown at PB Bonn. Mycelium from the plants was pooled and DNA was extracted with the Wizard Magnetic DNA Purification Kit for Food (Promega Molecular Diagnostics, Mannheim, Germany). This procedure was repeated to obtain replicate DNA samples for each species and variety. The replicate DNA samples were subjected to PCR. Control PCR reactions contained DNA extracted from a plant devoid of fungi (Kucht et al. 2004) and a blank with water instead of template DNA. The PCR primers used are provided (TABLE II). PCR reactions for partial internal transcribed spacers (ITS), translation elongation factor-1α (*tefA*), β-tubulin (*tubB*), β-actin (*actG*), mitochondrial ATP synthase F0 subunit A (*atp*6), and RNA polymerase II subunit 1 (*rpbA*) were carried out in a 25 µL volume containing 3 µL template DNA (5–20 ng/µL), forward and reverse primer (10 µM each), 1 unit GoTaq DNA polymerase (Promega Molecular Diagnostics, Mannheim, Germany), dNTPs (150 µM each), 5× Green GoTaq reaction buffer (1.5 mM Mg^{2+}) (Promega Molecular Diagnostics, Mannheim, Germany). The temperature regime was 9 min at 95 C, 35 cycles of 95 C for 60 s, 55 C for 60 s, 72 C for 60 s. Products were electrophoresed in 1% agarose gel and

isolated from the gel with the QIAGEN (Hilden, Germany) gel extraction kit. The approximate lengths of the PCR products are provided (TABLE II). The *actG* PCR product was obtained by using nested primer (Act-nes 1 and Act-nes 2) following PCR with primers Act 1–exon1d-1 and Act 1–exon6u-1 (TABLE II).

PCR products were subjected to sequencing reactions with the DTCS (Dye Terminator Cycle Sequencing) Quick Start Kit (Beckman Coulter) and sequencing primers employed in the PCR reaction (TABLE II). The reaction products were cleaned by magnetic bead purification with the CleanSEQ Kit (Agencourt Clean Seq, Beckman Coulter, Krefeld, Germany) and analyzed by capillary electrophoresis on a CEQ 8800 (Beckman-Coulter, Krefeld, Germany). Both strands of each PCR product were sequenced. Evaluation of the data, alignments and analysis of sequences were performed with Beckman-Coulter software.

Phylogenetic analysis.—The sequences from the symbionts were submitted as queries for sensitive (low complexity filter off, minimal word size) BLAST queries (Altschul et al. 1990) at the NCBI server http://www.ncbi.nlm.nih.gov/blast/Blast.cgi, and sequences aligning over at least 90% of the query sequence were retrieved for representatives of Clavicipitaceae s.s., other Hypocreales, and other Sordariomycetes. Sequence alignments and phylogenetic analyses were conducted on the Website http://www.phylogeny.fr/. Alignments were conducted with MUSCLE (Edgar 2004) and inspected by eye. The alignments were trimmed of regions of missing information for some taxa, as well as regions (generally intronic) of ambiguous alignment. Then the edited alignment was submitted to PhyML with HKY85 substitution model and branch support estimated by the SH-like approximate likelihood ratio test (aLRT) (Anisimova and Gascuel 2006). Sequences and trees were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11183).

RESULTS

Morphological characteristics.—No sexual fruiting structures were detected in these mycelia (FIGS. 1–3). Visual inspection of manually opened young leaves showed that the fungi were well established as dense white mycelial layers on the adaxial leaf surfaces of both *I. asarifolia* and *T. corymbosa*. The mycelium consisted of tightly packed hyphae in the cavity between the leaf halves (FIGS. 1, 2). Colonies on the adaxial side of fully developed young leaves of *T. corymbosa* were visible to the naked eye, and on older leaves colony distribution mainly followed the veins of the leaves, in contrast to the seemingly more random distribution of the mycelium on *I. asarifolia* leaves. No such mycelial masses were observed on the abaxial side of the leaves. The hyphae on both hosts had similar characteristics. They were approx. 1–1.5 μm across and were hyaline, thin-walled, frequently septate and variable in width. Hyphae occasionally are swollen to ca. 3.5 μm wide, chlamydospore-like structures (FIG. 1). Synnema-like structures also were observed but lacked conidia (FIG. 2C, D). The mycelia were closely associated with the leaf secretory glands, which in the Convolvulaceae consist of one basal cell, one stalk cell, up to eight glandular secretory cells and a sub-cuticular oil storage cavity that is derived from the cuticle of the secretory cells. As leaves expanded and matured, the hyphae were evident as only microscopically visible clumps, often near or around these peltate glandular cells (FIG. 2E, F). Sections through colonized tissue revealed that fungal mycelium was entirely superficial (FIG. 2G, H). No fungal hyphae penetrated the plant cell wall, and no necrosis or degradation of plant cells was detectable.

Mycelium taken from the adaxial side of young, folded leaves of both *I. asarifolia* and *T. corymbosa* grew slowly on PDA at 23 C to produce minute white colonies (FIG. 3). After 4 wk colonies of *P. ipomoeae* were 80–310 μm diam and colonies of *P. turbinae* were 290–760 μm diam. Hyphae were narrow (ca. 1–1.5 μm), hyaline, thin-walled, frequently septate, sparsely branched, and variable in width. No conidiophores were observed in culture. Hyphae that formed at the colony border ran almost parallel and seldom crossed each other (FIG. 3C, D). After more than 4 wk incubation both fungi formed synnema-like structures up to 50 μm long for *I. asarifolia* and up to 85 μm for *T. corymbosa*, but lacking conidiogenous cells and conidia (FIG. 3E, F). No sexual structures were observed.

Molecular phylogenetic relationships.—Sequences from one mitochondrial gene (*atp*6) and five nuclear gene loci (*actG*, *dmaW*, *rpbA*, *tefA* and *tubB*) of the three fungi, IasaF13 and IasaredF01 from *I. asarifolia* and TcorF01 from *T. corymbosa*, were compared (TABLE III). Only a single nucleotide substitution difference in *rpbA* was observed between the two isolates from *I. asarifolia*, but substantial differences both in nucleotide sequences and alignment gaps were observed among these and isolate TcorF01 for all except *tubB*.

Sequences of *atp6* and three nuclear genes (*dmaW*, *rpbA*, *tubB*) were alignable over sufficient length with sequences from Clavicipitaceae and related families for robust phylogenetic analysis. The phylogenetic relationships of *dmaW* gene products from three fungal families, including the fungal isolates herein designated as *Periglandula* spp. (as ''Clavicipitaceae''), have been published (Liu et al. 2009). The inferred phylogenies of *tubB*, *rpbA*, and *atp6* are illustrated (FIGS. 4, 5, 6 respectively). All these phylogenies placed the fungal isolates in a single subclade, hereafter designated as the *Periglandula*

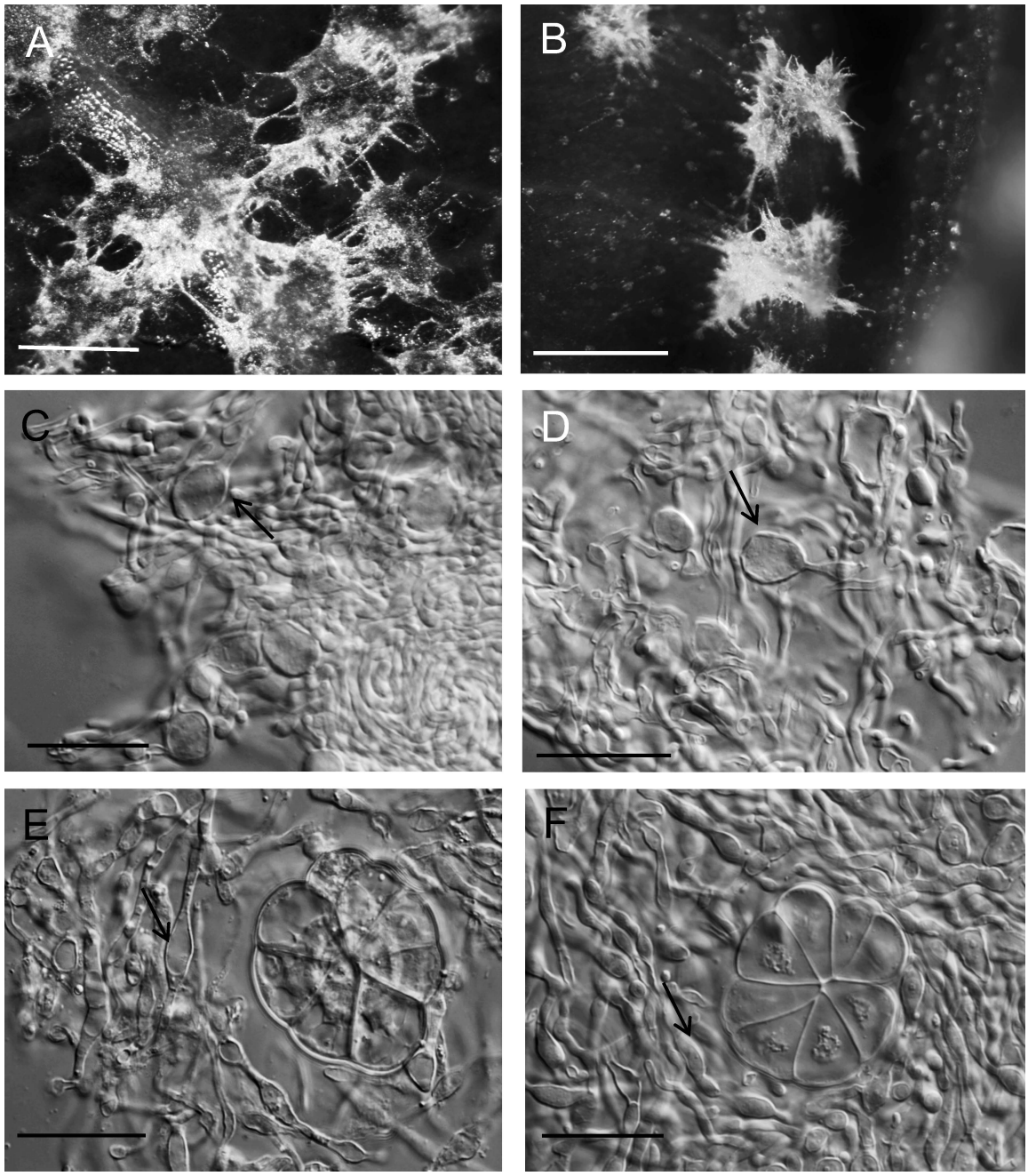

FIG. 1. Mycelial structures of *P. ipomoeae* (A, C, E) and *P. turbinae* (B, D, F) on plants: typical white colonies on the adaxial leaf surface of unfolded young leaves (A, B); septate hyphae with chlamydospore-like structures (C, D; arrows); variable in width hyphae typically encircle peltate glandular cell of the plant (E, F; arrows); Bars: A = 250 μm, B = 500 μm, C, D = 20 μm, E, F = 20 μm.

subclade, within a well supported clade that included all other plant-associated Clavicipitaceae analyzed. Support for this subclade was stronger for *tubB* (0.96; FIG. 4) than for *rpbA* (0.84; FIG. 5), probably because there was greater representation of plant-associated Clavicipitaceae among the *rpbA* sequences. The *atp6* gene tree (FIG. 6) showed less support for the *Periglandula* subclade (0.65), probably because of the similarity of sequences in plant-associated Clavicipitaceae, along with the *Metacordyceps taii*.

TAXONOMY

Periglandula U. Steiner, E. Leistner et Leuchtm., gen. nov.

MycoBank MB561039

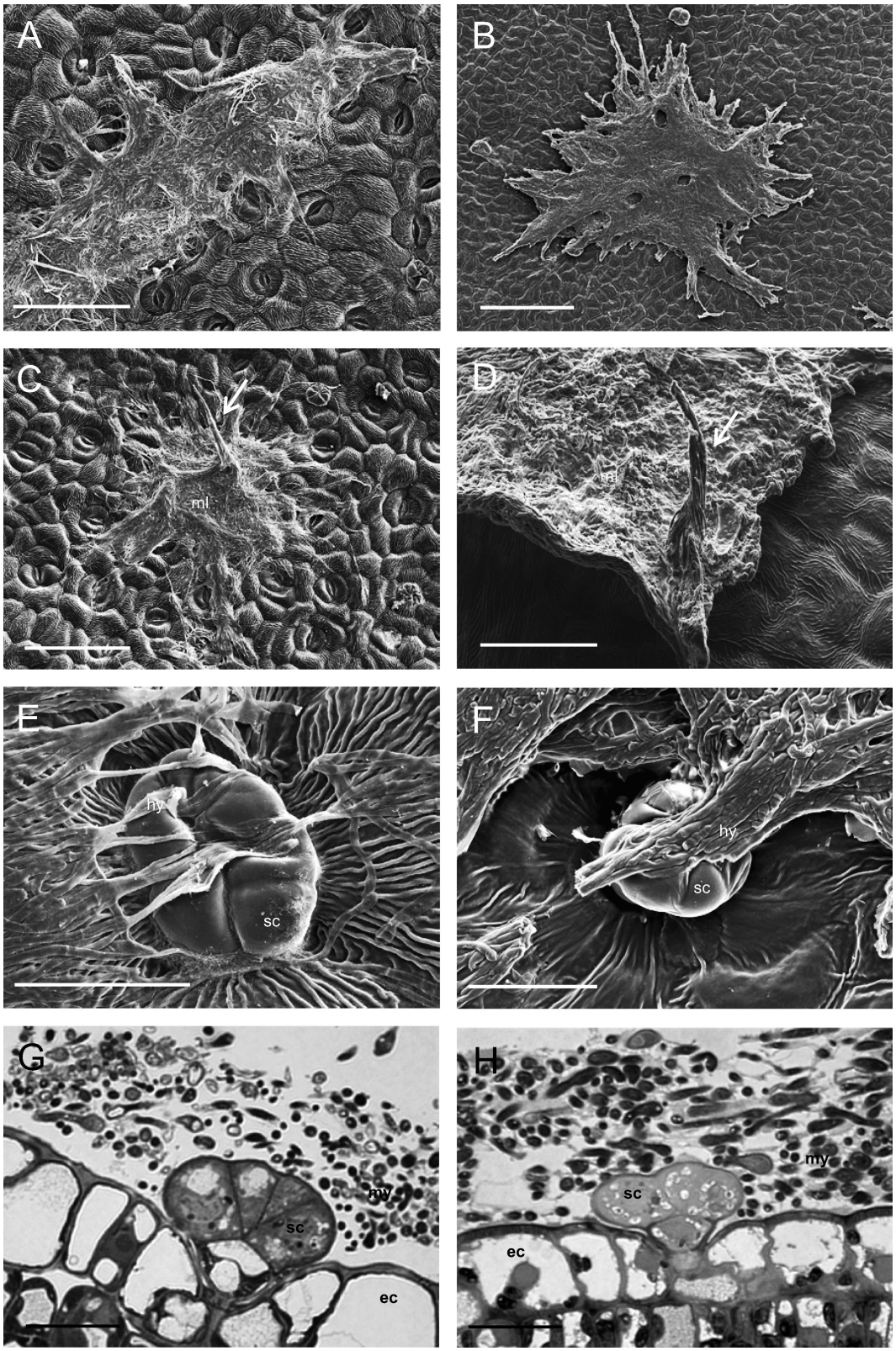

FIG. 2. Mycelial structures of *P. ipomoeae* on the adaxial leaf surface of *I. asarifolia* (A, C, E, G) and *P. turbinae* on *T. corymbosa* (B, D, F, H): epiphytic fungal colony adhered to the cuticle (A, B); synnema-like structure arising from the mycelium layer (ml) (C,D; arrows); hyphae (hy) typically encircling and overgrowing the peltate glandular cell (sc) of the leaf (E,F); cross-section of peltate glandular cell composed of basal cell, stalk cell and secretory cells (sc) showing the embedding by

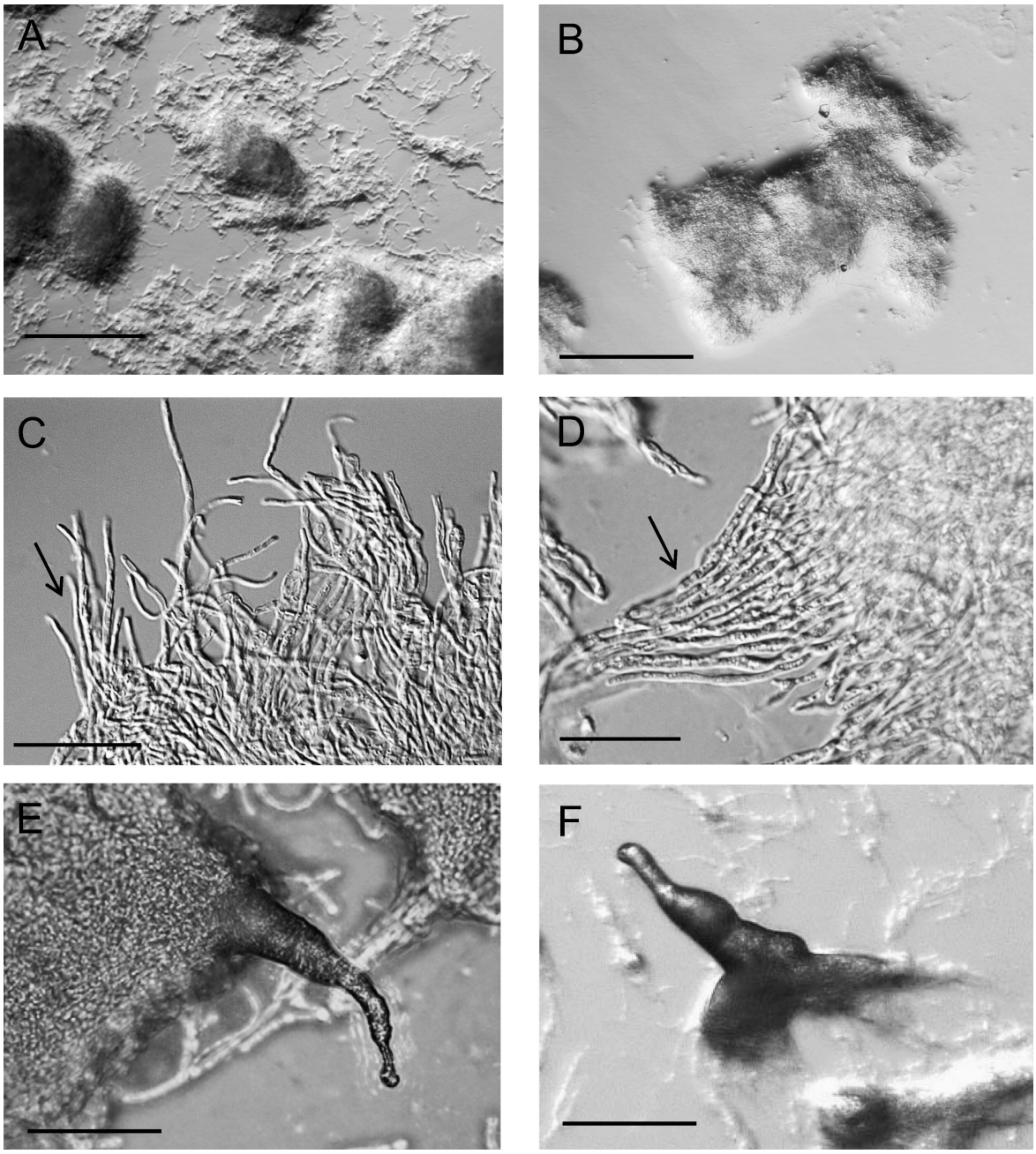

FIG. 3. Mycelial structures of *P. ipomoeae* (A, C, E) and *P. turbinae* (B, D, F) on PDA culture after 5 d at 23 C (A,B); parallel hyphae growth at the colony border in 7 d old cultures (C, D); synnema-like structures in 4 wk old cultures (E, F). Bars: A, B = 200 µm, C, D = 20 µm, E, F = 50 µm.

Mycelium epiphyllum album, stratum densum formans; hyphae 1–1.5 µm latae, hyalinae, temue tunicatae, plerumque septatae, latitudine variantes, aliquando structuris chlamydosporis similes ad ca. 3.5 µm latis inflatae; structurae synnematoideae adsunt, sine conidiis; structurae sexuales non observatae.

Mycelium epibiotic, forming a dense layer on the adaxial leaf surfaces, white; hyphae 1–1.5 µm across, hyaline, thin-walled, frequently septate and variable in width, occasionally swollen to ca. 3.5 µm wide chlamydospore-like structures; synnema-like struc-

←
epiphytic mycelium (my) without penetration of epidermal cells (ec) Bars: A = 100 µm, B = 100 µm, C = 100 µm, D = 20 µm, E = 20 µm, F = 20 µm, G = 20 µm, H = 20 µm.

TABLE III. Numbers of nucleotide substitution differences and alignment gaps compared to sequences of *P. ipomoeae* IasaF13[a]

	atp6	*actG*	*dmaW*	*rpbA*	*tefA*	*tubB*
Length compared	648	1617	1608	700	772	770
Length of noncoding sequence	0	617	115	67	458	64
Isolate IasaredF01[b]	0;0	0;0	0;0	1;0	0;0	0;0
Isolate TcorF01[b]	11;0	110;28	135;3	46;2	94;39	0;0

[a] GenBank accession numbers DQ121453, DQ121454, DQ647955 (*dmaW*) and HQ702601–HQ702615 (see TABLE II).
[b] Data indicate number of substitutions followed by number of alignment gaps.

tures present, but lacking conidia; no sexual structures observed.

Type. **Periglandula ipomoeae** U. Steiner, E. Leistner et Schardl

Etymology. Referring to the close association with the secretory glands of host leaves.

Periglandula ipomoeae U. Steiner, E. Leistner et Schardl, sp. nov. FIGS. 1A, C, E; 2A, C, E, G; 3A, C, E
MycoBank MB561040

Mycelium epiphyllum album, in venis foliorum veterum restrictum; hyphae chlamydosporis similes ca. 3.5 µm latis; structurae synnematoideae adsunt, sine conidiis; structurae sexuales non observatae. Coloniae in PDA albae, tarde crescentes, 80–310 µm diam aetate 4 hebdomadum ad 23 C; conidia vel structurae sexuales non observatae.

Mycelium epibiotic, on adaxial surfaces of young leaves, restricted to veins of older leaves, white; hyphae with chlamydospore-like structures ca. 3.5 µm wide; synnema-like structures present that lack conidia; no sexual structures observed. Colonies on PDA white, slow-growing, 80–310 µm diam after 4 wk at 23 C; no conidia or sexual reproduction observed.

HOLOTYPE. ECUADOR: ''Los Cedros'', ''La Cumbre'', on *Ipomoea asarifolia* (Desr.) Roem. et Schult., leg. E. Eich, 1991 (CUP-067884).

Etymology. Referring to the host genus *Ipomoea*

Periglandula turbinae U. Steiner, E. Leistner et Schardl, sp. nov.

FIGS. 1B, D, F, 2B, D, F, H and 3B, D, F
MycoBank MB561041

Mycelium epiphyllum album, in foliis veteribus fortuite distributum; hyphae chlamydosporis similes ca. 3.5 µm latis; structurae synnematoideae adsunt, sine conidiis; structurae sexuales non observatae. Coloniae in PDA albae, tarde crescentes, 290–760 µm diam aetate 4 hebdomadum ad 23 C; conidia vel structurae sexuales non observatae. Differt genetice ab *P. ipomoeae* sequentiis acidi nucleici *atp6*, *actG*, *dmaW*, *rpbA* et *tefA*.

Mycelium epibiotic, on adaxial surfaces of young leaves, more randomly distributed on older leaves, white; hyphae with chlamydospore-like structures ca. 3.5 µm wide; synnema-like structures present that lack conidia; no sexual structures observed. Colonies on PDA white, slow-growing, 290–760 µm diam after 4 wk at 23 C; no conidia or sexual reproduction observed. Genetically different from *P. ipomoeae* in sequences of genes *atp6*, *actG*, *dmaW*, *rpbA* and *tefA*.

HOLOTYPE. PANAMA: ''Altos de Campaña'', on *Turbina corymbosa* (L.) Raf., leg. E. Eich, 1995 (CUP-067885).

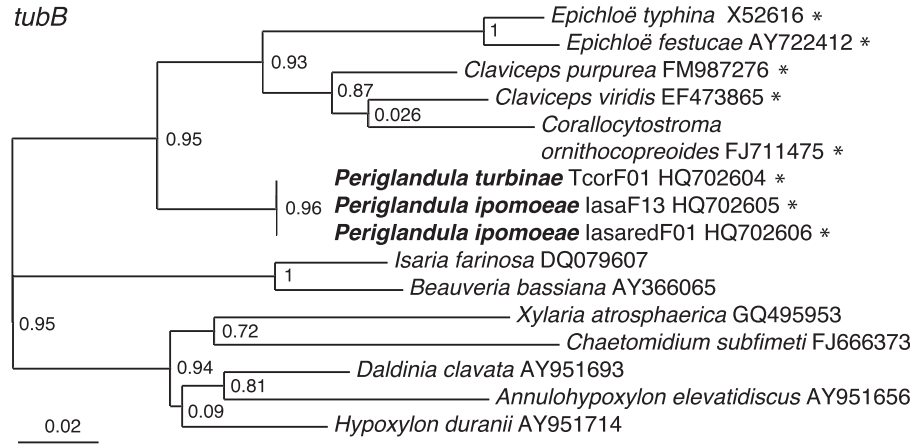

FIG. 4. Maximum likelihood phylogeny inferred from alignment of *tubB* sequences for *Periglandula* species, representatives of Hypocreales and other filamentous ascomycetes. Midpoint root is at the left edge. Values indicated at nodes are aLTR support values. GenBank accessions follow species names. Asterisks indicate plant-associated Clavicipitaceae.

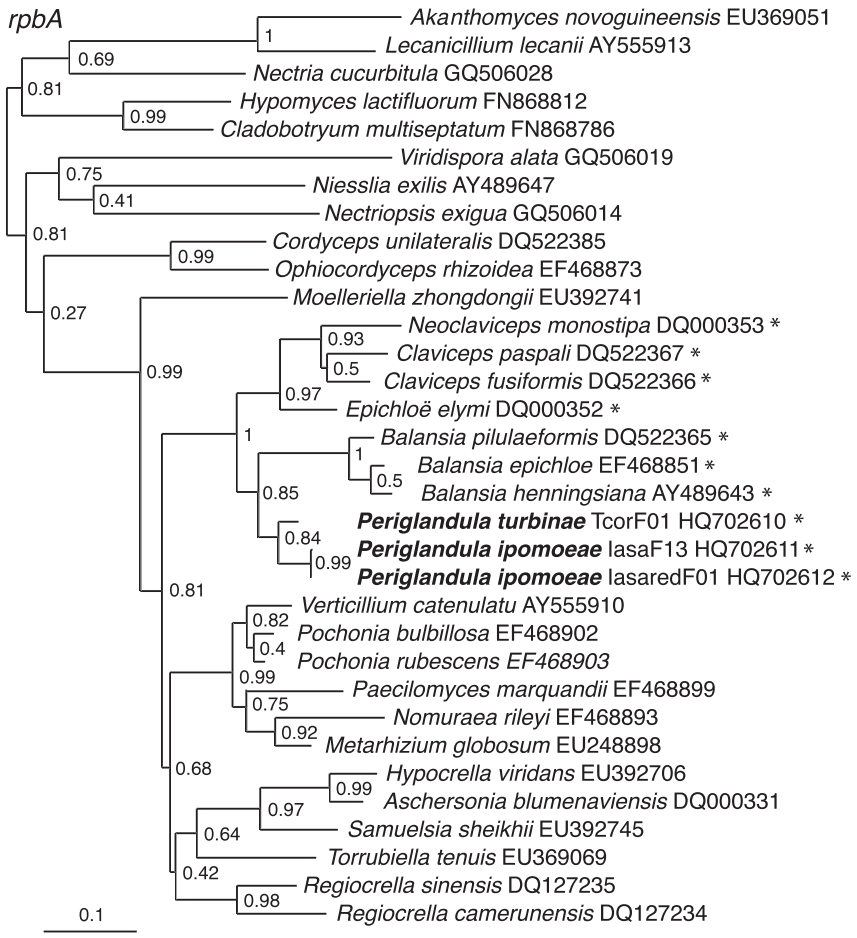

FIG. 5. Maximum likelihood phylogeny inferred from alignment of *rpbA* sequences for *Periglandula* species, representatives of Hypocreales. Midpoint root is at the left edge. Values indicated at nodes are aLTR support values. GenBank accessions follow species names. Asterisks indicate plant-associated Clavicipitaceae.

Etymology. Referring to the host genus, *Turbina*

DISCUSSION

Host plants.—Species of the newly described genus *Periglandula* are epibiotic fungi symbiotic with plants belonging to the Convolvulaceae, and the two newly described species are associated with different host species, *P. ipomoeae* on white (IasaF13) or red (IasaredF01) blooming *I. asarifolia* and *P. turbinae* (TcorF01) on *T. corymbosa* (Kucht et al. 2004, Steiner et al. 2006, Ahimsa-Müller et al. 2007, Steiner et al. 2008, Markert et al. 2008, Leistner and Steiner 2009). The host plants apparently include three taxa, two from *I. asarifolia* (white or red blooming) and one of *T. corymbosa*. Although the two *I. asarifolia* taxa may be different subspecies or varieties, they have not been described as such, yet are different with respect to chemotaxonomic criteria. The white-blooming plants apparently lack the ability to synthesize anthocyanins. The ITS sequences (accessions: *I.asarifolia* white HQ832593, *I. asarifolia* red HQ832594; 622 bp) obtained from both *I. asarifolia* taxa were identical and might indicate that the white- and the red-blooming *I. asarifolia* plants represent different chemovarieties of the same species.

Sequencing of the small subunit ribosomal RNA (18SrDNA) and the internal transcribed spacers (ITS) showed that a clavicipitaceous fungus also is present in seeds of *I. tricolor*[2]. The seeds contain ergoline alkaloids (Ahimsa-Mueller et al. 2007). In fact the occurrence of ergoline alkaloids in Convolvulaceae is a common observation. Eich (2008) lists 23 ergoline alkaloid containing *Ipomoea* species. It is to be expected that these plants also harbor clavicipitaceous fungi. Among the 12 endophytic and one epibiotic fungal isolates obtained from the white-blooming *I. asarifolia* plants, the epibiont (*P. ipomoeae*) was most abundant (FIG. 4 in Steiner et al. 2006). This parallels the situation of *P. turbinae* present on *T. corymbosa* (Ahimsa-Mueller 2007). A phylogenetic analysis of the 18S rDNA from all 13

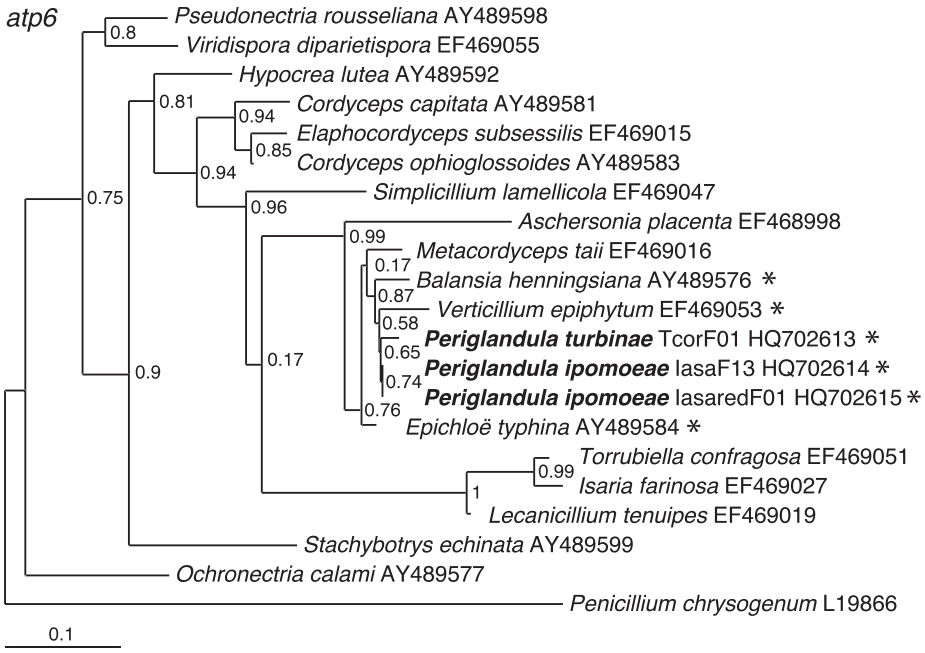

FIG. 6. Maximum likelihood phylogeny inferred from alignment of *atp6* sequences for *Periglandula* species, representatives of Hypocreales, and the outgroup taxon *Penicillium chrysogenum*. Values indicated at nodes are aLTR support values. GenBank accessions follow species names. Asterisks indicate plant-associated Clavicipitaceae.

fungal isolates from the white blooming *I. asarifolia* plants showed that the epibiont was the only member of the Hypocreales.

Plant-Periglandula *symbiosis*.—Results of single strand conformational polymorphism (SSCP) analysis have indicated that the newly described fungi also are present within the seeds (Steiner et al. 2006, Ahimsa-Mueller et al. 2007), which also contain ergoline alkaloids. When a *T. corymbosa* or *I. asarifolia* plant was grown from a surface-sterilized seed on an artificial medium under axenic conditions, only these fungi were associated with their respective host plants and the plants contained ergoline alkaloids (Steiner et al. 2006). These results further support the conclusion that *Periglandula* species are the sources of the ergoline alkaloids found in these Convolvulaceae. Plants devoid of such symbiotic fungi lack ergoline alkaloids (Kucht et al. 2004).

In a further test of the role of both *P. ipomoeae* and *P. turbinae* we attempted unsuccessfully to re-inoculate the fungicide-treated, alkaloid-free host plants. The fungi showed no growth on the plants and consequently ergoline alkaloids were undetectable (Steiner et al. 2008). An asymptomatic association between *Penicillium roquefortii* and *I. asarifolia* however has been reestablished experimentally (Steiner et al. 2006, Steiner et al. 2008). The association between *I. asarifolia* and *P. roquefortii* however did not lead to any detectable accumulation of ergoline alkaloids including isofumigaclavine A in the plant (Steiner et al. 2006, Steiner et al. 2008). Isofumigaclavine A is known to be produced by this fungus in vitro (Scott et al. 1976).

The *I. asarifolia*-*P. ipomoeae* symbiosis also could be maintained in plant callus and cell-suspension cultures (Steiner et al. 2006). The fungus apparently survived the sterilization procedure during establishment of the plant cell culture. Microscopic investigation, SSCP analysis and sequencing of the ribosomal internal transcribed fungal ITS DNA clearly indicated the presence of the fungus in the callus and the cell suspension culture. The fungus was not visible to the naked eye, and the plant cells showed a normal and asymptomatic growth. Of interest, ergoline alkaloids were undetectable in the callus or in the cell-suspension culture even when grown in various media and under different conditions (Hussein 2004). When the hormonal regime of a callus culture was changed by lowering the concentration of benzylaminopurine, the culture developed an intact shoot that formed roots after placement in half-strength Murashige and Skoog medium (Steiner et al. 2006). The new *I. asarifolia* plantlet was covered with the fungus and its identity was confirmed by SSCP. Chemical analysis of the plantlet by thin-layer chromatography and by a combined gas chromatograph-mass spectrometry revealed ergoline alkaloids (Steiner et al. 2006, Steiner et al. 2008). The investigators have concluded that the plant integrates *P. ipomoeae* into

its own developmental program and that the production of ergoline alkaloids by the fungus depends on an intact differentiated *I. asarifolia* plant.

Chemotypic and molecular characterization of Periglandula.—Ergoline alkaloids from plants of the Convolvulaceae (*I. violacea* and *T. corymbosa*) first were isolated by Hofmann (1961). Chemical analysis showed the presence of ergoline alkaloids also for *I. asarifolia*, which contains chanoclavine, lysergic acid amide, lysergic acid α-hydroxyethylamide, ergobalansine and ergobalansinine (Jenett-Siems et al. 1994, Jenett-Siems et al. 2004) and in addition lysergic acid, isolysergic acid amide and ergometrine (Ahimsa-Müller et al. 2007). In *T. corymbosa* chanoclavine, lysergic acid α-hydroxyethylamide and its isoform, lysergic acid amide and its isoform, ergonovine, elymoclavine and agroclavine have been detected (Steiner et al. 2006, Ahimsa-Müller et al. 2007). Thus, in contrast to *I. asarifolia*, agroclavine and elymoclavine are constituents of *T. corymbosa*, and both plant species can be distinguished by their alkaloid spectra. Epibiotic clavicipitaceous fungi described herein are responsible for the occurrence of ergoline alkaloids in these plants (Kucht et al. 2004, Steiner et al. 2006, Markert et al. 2008, Leistner and Steiner 2009).

Genes responsible for ergoline alkaloid biosynthesis typical of Clavicipitaceae (Schardl et al. 2006) were detectable in *P. ipomoeae* (Markert et al. 2008). The *dmaW* gene, encoding 4–(γ,γ-dimethylallyl) tryptophan synthase, also was detected in *P. turbinae* (Markert et al. 2008). This gene is responsible for the first step in ergoline alkaloid biosynthesis and consists of three exons and two introns with exon two being strictly conserved to 122 base pairs in each case of the seven *dmaW* genes known to be present in different ergoline-alkaloid producing fungal species (Markert et al. 2008). The *P. ipomoeae dmaW* gene product has been expressed as a His-tagged fusion protein, purified to homogeneity and characterized. The substrate specificity and kinetic data were in full agreement with its function as a 4–(γ,γ-dimethylallyl)-tryptophan synthase enzyme (DMATS). A reverse genetic experiment conducted after mRNA extraction revealed that the *dmaW* gene is transcribed within the epibiotic fungus (Markert et al. 2008).

Part of the ergoline alkaloid biosynthetic gene cluster in *P. ipomoeae* has been sequenced (Markert et al. 2008) and found to be homologous to that of *Claviceps purpurea* and *C. fusiformis* (Lorenz et al. 2007). The order and orientation of the genes *ccsA* (encoding a component of chanoclavine cyclase; Lorenz et al. 2010), *easF* (encoding an *N*-methyltransferase; Rigbers and Li 2008), *easG* (encoding a reductase/dehydrogenase) and *dmaW* within the cluster parallels the arrangement of genes in *C. purpurea* and *C. fusiformis*, and differs from their arrangement in *Aspergillus fumigatus* (Schardl et al. 2006, Wallwey et al. 2010), a member of the only distantly related Trichocomaceae (Eurotiales).

Similarly to the other genes analyzed herein, the inferred *dmaW* gene products indicated identical amino acid sequences for the two *P. ipomoeae* isolates, and a closely related sequence from *P. turbinae* (Ahimsa-Mueller et al. 2007). Parsimony analysis of an alignment of the entire protein sequences or conserved blocks within them grouped these sequences in a DMATS clade with other genera of Clavicipitaceae (Liu et al. 2009). Here we have provided additional phylogenetic analysis, based on sequences of nuclear genes, *tubB* and *rpbA*, and the mitochondrial gene, *atp6*, as well as morphological data, which support the description of the new genus, *Periglandula*, encompassing the two new species, *P. ipomoeae* and *P. turbinae*.

Conclusions.—We have described a new genus with two new species of fungi that are symbiotic with certain dicotyledonous host plants, for which we propose the genus name *Periglandula* in reference to their associations with secretory glands on leaf adaxial surfaces and the species names *P. ipomoeae* and *P. turbinae* in reference to their respective hosts, *Ipomoea asarifolia* and *Turbina corymbosa* (Convolvulaceae). While a morphological distinction between both fungal species is hardly possible, the *P.turbinae* as opposed to the *P.ipomoeae* mycelium is embedded in a mucilage matrix (Leistner and Steiner 2009). Phylogenetic analyses of a mitochondrial gene, *atp6*, and the nuclear gene loci *tubB*, *rpbA* (this study) and *dmaW* (Markert et al. 2008, Ahimsa-Müller et al. 2007) support the monophyly of *Periglandula* within the Clavicipitaceae s.s., Hypocreales. These fungi possess genes for synthesis of ergoline alkaloids, and several studies have provided conclusive evidence that they are the source of these bioprotective alkaloids, long known to be present in their host plants. In contrast to other plant symbionts in the Clavicipitaceae, the *Periglandula* species are associated with dicots instead of with grasses or sedges that host *Balansia*, *Epichloë*, *Ephelis*, *Neotyphodium* and other plant-associated genera in the family (Bischoff and White 2003). Sequence comparisons of *atp6*, *actG*, *dmaW*, *rpbA* and *tefA* distinguish the two *P. ipomoeae* isolates from the *P. turbinae* isolate. Based on their related hosts and similar or identical gene sequences, we consider isolates IasaF13 and IasaredF01 to be members of one species, *P. ipomoeae*, and isolate TcorF01 represents a distinct congeneric species, *P. turbinae*.

ACKNOWLEDGMENTS

We wish to thank Drs. E. Eich and R. Omlor for the supply of plant material and herbarium specimens and the late Dr D. Groeger for a generous gift of ergoline alkaloids. Financial support was provided by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie (to EL) and by the United States National Institute of General Medical Science (grant 1R01GM086888-01 to CLS).

LITERATURE CITED

Ahimsa-Müller MA. 2007. The colonization of ergoline alkaloid containing Convolvulaceae plants by clavicipitaceous fungi [doctoral dissertation]. Bonn, Germany: Rheinische Friedrich-Wilhelms-Univ. Bonn.

———, Markert A, Hellwig S, Knoop V, Steiner U, Drewke C, Leistner E. 2007. Clavicipitaceous fungi associated with ergoline alkaloid-containing Convolvulaceae. J Nat Prod 70:1955–1960, doi:10.1021/np070315t

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Anisimova M, Gascuel O. 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Syst Biol 55:539–552, doi:10.1080/10635150600755453

Bischoff JF, White JF Jr. 2003. The plant infecting clavicipitaleans. In: White JF Jr, Bacon CW, Hywel-Jones NL, Spatafora JW, eds. Clavicipitalean fungi, evolutionary biology, chemistry, biocontrol, and cultural impacts. New York: Marcel Dekker. p 125–149.

———, ———. 2005. Evolutionary development of the Clavicipitaceae. In: Dighton J, White JF Jr, Oudemans P, eds. The fungal community, its organization and role in the ecosystems. 3rd ed. Boca Raton, Florida: CRC Taylor & Francis. p 505–518.

Bush LP, Wilkinson HH, Schardl CL. 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114:1–7.

Clay K, Schardl C. 2002. Evolutionary origin and ecological consequences of endophyte symbioses with grasses. Am Nat 160:S99–S127, doi:10.1086/342161

Edgar R. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113, doi:10.1186/1471-2105-5-113

Eich E. 2008. Solanaceae and Convolvulaceae: secondary metabolites. Biosynthesis, chemotaxonomy, biological and economic significance. Berlin and Heidelberg, Germany: Springer. 637 p.

Fukatsu T, Nikoh N. 2003. Interkingdom host shift in the *Cordyceps* fungi. In: White JF Jr, Bacon CW, Hywel-Jones NL, Spatafora JW, eds. Clavicipitalean fungi: evolutionary biology, chemistry, biocontrol and cultural impacts. Vol. 19. New and Basel: Marcel-Dekker Inc. p 311–327.

Gams W, Zare R. 2003. A taxonomic review of the clavicipitaceous anamorphs parasitizing nematodes and other microinvertebrates. In: White JF Jr, Bacon CW, Hywel-Jones NL, Spatafora JW, eds. Clavicipitalean fungi, evolutionary biology, chemistry, biocontrol and cultural impacts. New York: Marcel Dekker. p 17–73.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118, doi:10.1111/j.1365-294X.1993.tb00005.x

Groeger D, Floss HG. 1998. Biochemistry of ergot alkaloids-achievements and challenges. In: Cordell GA, ed. The alkaloids-chemistry and biology. Vol. 50. New York: Academic Press. p 171–218.

Hofmann A. 1961. Die Wirkstoffe der mexikanischen Zauberdroge ''Ololiuqui''. Planta medica 9:354–367, doi:10.1055/s-0028-1100366

———. 2006. LSD-mein Sorgenkind, die Entdeckung einer Wunderdroge. 12th ed. München: Deutscher Taschenbuchverlag. 215 p.

Hussein YHA. 2004. Biochemical analysis of Convolvulaceae plant tissue cultures for the presence of ergoline alkaloids [doctoral dissertation]. Zagazid, Egypt: Faculty of Science, Zagazig Univ.

Jennett-Siems K, Kaloga M, Eich E. 1994. Ergobalansine/ergobalansinine, a prolin-free peptide-type alkaloid of the fungal genus *Balansia* is a constituent of *Ipomoea piurensis*. J Nat Prod 57:1304–1306, doi:10.1021/np50111a022

———, ———, ———. 2004. Ergobalansine/ergobalansinine, a prolin-free peptide-type alkaloid of the fungal genus *Balansia* is a constituent of *Ipomoea piurensis*. J Nat Prod 67:2160, doi:10.1021/np040195e

Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electronmicroscopy. J Cell Biol 27:137–138.

Kucht S, Groß J, Hussein Y, Grothe T, Keller U, Basar S, König WA, Steiner U, Leistner E. 2004. Elimination of ergoline alkaloids following treatment of *Ipomoea asarifolia* (Convolvulaceae) with fungicides. Planta 219:619–625, doi:10.1007/s00425-004-1261-2

Leistner E, Steiner U. 2009. Fungal origin of ergoline alkaloids present in dicotyledonous plants (Convolvulaceae). In: Anke T, Weber D, eds. The Mycota. Physiology and genetics: selected basic and applied aspects Vol. XV. Heidelberg, Germany: Springer. p 197–208.

Liu M, Panaccione DG, Schardl CL. 2009. Phylogenetic analyses reveal monophyletic origin of the ergot alkaloid gene *dmaW* in fungi. Evol Bioinform 5:15–30.

Lorenz N, Olsovska J, Sulc M, Tudzynski P. 2010. Alkaloid cluster gene *ccsA* of the ergot fungus *Claviceps purpurea* encodes chanoclavine I synthase, a flavin adenine dinucleotide-containing oxidoreductase mediating the transformation of *N*-methyl-dimethylallyltryptophan to chanoclavine I. Appl Environ Microbiol 76:1822–1830, doi:10.1128/AEM.00737-09

———, Wilson EV, Machado C, Schardl CL, Tudzynski P. 2007. Comparison of ergot alkaloid biosynthesis gene clusters in *Claviceps* species indicates loss of late pathway steps in evolution of *C. fusiformis*. Appl Environm Microbiol 73:7185–7191, doi:10.1128/AEM.01040-07

Markert A, Steffan N, Ploss K, Hellwig S, Steiner U, Drewke

C, Li S-M, Boland W, Leistner E. 2008. Biosynthesis and accumulation of ergoline alkaloids in a mutualistic association between *Ipomoea asarifolia* (Convolvulaceae) and a clavicipitalean fungus. Plant Physiol 147: 296–305, doi:10.1104/pp.108.116699

Moon CD, Craven KD, Leuchtmann A, Clement SL, Schardl CL. 2002. The evolutionary origins of three *Neotyphodium* endophyte species from grasses indigenous to the southern hemisphere. Mycologia 94:694–711, doi:10.2307/3761720

Pazoutová S. 2003. Evolutionary strategy of *Claviceps*. In: White JF Jr, Bacon CW, Hywel-Jones NL, Spatafora JW, eds. Clavicipitalean fungi, evolutionary biology, chemistry, biocontrol, and cultural impacts. New York: Marcel Dekker. p 329–354.

Rigbers O, Li S-M. 2008. Ergot alkaloid biosynthesis in *Aspergillus fumigatus*: overproduction and biochemical characterization of a 4–dimethylallyltryptophan *N*-methyltransferase. J Biol Chem 283:26859–26868, doi:10.1074/jbc.M804979200

Saikkonen K, Wäli P, Helander M, Faeth SH. 2004. Evolution of endophyte-plant symbioses. Trends Plant Sci 9:275–280, doi:10.1016/j.tplants.2004.04.005

Schardl CL, Clay K. 1997. Evolution of mutualistic endophytes from plant pathogens. In: Carroll GC, Tudzynski P, eds. The Mycota V: plant relationships B. Berlin: Springer-Verlag. p 221–238.

———, Leuchtmann A. 2005. The epichloë endophytes of grasses and the symbiotic continuum. In: Dighton J, White JF, Oudemans P, eds. The fungal community: its organization and role in the ecosystem. Vol. 23. Boca Raton, Florida: CRC Press. p 475–503.

———, ———, Spiering MJ. 2004. Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340, doi:10.1146/annurev.arplant.55.031903.141735

———, Pannacione DG, Tudzynski P. 2006. Ergot alkaloids—biology and molecular biology. In: Cordell GA, ed. The alkaloids: chemistry and biology. Vol. 63. New York: Academic Press. p 45–86.

Schultes RE, Hofmann A. 1979. Plants of the gods: their sacred, healing and hallucinogenic power. New York: McGraw-Hill. 208 p.

Scott PM, Merrien MA, Polonsky J. 1976. Roquefortin and isofumigaclavine A, metabolites from Penicillium roquefortii. Experientia 32:140–142, doi:10.1007/BF01937728

Steiner U, Ahimsa-Müller MA, Markert A, Kucht S, Groß J, Kauf N, Kuzma M, Zych M, Lamshöft M, Furmanowa M, Knoop V, Drewke C, Leistner E. 2006. Molecular characterization of a seed transmitted clavicipitaceous fungus occurring on dicotyledonous plants (Convolvulaceae). Planta 224:533–544, doi:10.1007/s00425-006-0241-0

———, Hellwig S, Leistner E. 2008. Specificity in the interaction between an epibiotic clavicipitalean fungus and its convolvulaceous host in a fungus/plant symbiotum. Plant Signal Behav 3:704–706, doi:10.4161/psb.3.9.6432

Sullivan RF, Bills GF, Hywel-Jones NL, White JF Jr. 2000. *Hyperdermium*: a new clavicipitalean genus for some tropical epibionts of dicotyledonous plants. Mycologia 92:908–918, doi:10.2307/3761587

Suh SO, Noda H, Blackwell M. 2001. Insect symbiosis: derivation of yeast-like endosymbionts within an entomopathogenic filamentous lineage. Mol Biol Evol 18:995–1000.

Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW. 2007. A multigene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Mol Phylogenet Evol 44:1204–1223, doi:10.1016/j.ympev.2007.03.011

Svitkina TM, Shevelev AA, Berschadsky AD, Gelfand VI. 1984. Cytoskeleton of mouse embryo fibroblasts. Electron microscopy of platinum replicas. Eur J Cell Biol 34:64–74.

Tudzynski P, Correia T, Keller U. 2001. Biotechnology and genetics of ergot alkaloids. Appl Microbiol Biotechnol 52:593–605, doi:10.1007/s002530100801

———, Scheffer J. 2004. *Claviceps purpurea*: molecular aspects of a unique pathogenic lifestyle. Mol Plant Pathol 5:377–388, doi:10.1111/j.1364-3703.2004.00237.x

Wallwey C, Matuschek M, Xie, Xiu-Lan, Li, Shu-Ming. 2010. Ergot alkaloid biosynthesis in *Aspergillus fumigatus*: conversion of chanoclavine-l aldehyde to festuclavine by the festuclavine synthase FgaFS in the presence of the old yellow enzyme FgaOx3. Org Biomol Chem 8: 3500–3508, doi:10.1039/c003823g

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.