

An overview of problems in the classification of plant-parasitic *Clavicipitaceae*

James F. White, Jr., Raymond Sullivan, Melinda Moy, Rachna Patel and Russell Duncan

Department of Plant Pathology, Cook College–Rutgers University, New Brunswick, New Jersey 08901

Correspondence: J. F. White <jwhite@aesop.rutgers.edu>

Abstract: The current status of the taxonomy of the plant-infecting *Clavicipitaceae* is evaluated. Particular emphasis is placed on current problems in classification, including the paraphyletic relationship of the *Clavicipitales* with the *Hypocreales* and the definition of the genera *Balansia*, *Claviceps*, *Epichloë*, *Neotyphodium*, *Parepichloë*, and *Ustilaginoidea*. The use of a phylogenetic approach is advocated to assist in the definition of genera. The morphological and ecological criteria employed to define species among the plant-infecting *Clavicipitaceae* are also discussed.

Key words: *Balansia*, *Claviceps*, *Epichloë*, *Neotyphodium*, *Parepichloë*, *Ustilaginoidea*, ITS rDNA, 18S rDNA, *Hypocreales*, endophytes

Introduction

Species of the family *Clavicipitaceae* (*Hypocreales*) have numerous impacts on humans. For example, *Cordyceps subsessilis* Petch (anamorph *Tolyposcladium inflatum* W. Gams) is a source of the powerful immunosuppressant drug cyclosporin, used to prevent rejection after organ transplantations (Drefuss & Chapela, 1994; Hodge *et al.*, 1996). Alkaloids produced by *C. purpurea* (Fr.) Tul. have been employed in western medicine for hundreds of years (Lewis & Elvin-Lewis, 1977). Ergonovine and other indole alkaloids, employed in the treatment of migraine headaches and to induce uterine contractions, are derived from this fungus (Lewis & Elvin-Lewis, 1977). This fungus was responsible for ergotism, which historically afflicted human populations consuming sclerotia mixed with grain (Alexopoulos *et al.*, 1996). The same fungus was the original source of lysergic acid diethylamide (LSD₂₅), a fungal product that gave rise to the psychedelic era of the 1960's (Schultes & Hoffmann, 1973). Anthropological evidence demonstrates that another member of the *Clavicipitaceae*, *Balansia cyperi* Edg., is employed among women of the amazonian Achuar Jivaro tribe to facilitate child birth (Lewis & Elvin-Lewis, 1990; Plowman *et al.*, 1990). In addition to pharmaceutical uses and potentials, recent investigations of

the graminicolous *Clavicipitaceae* have suggested ecological benefits to the host plants because of the increased resistance to herbivory by insects and mammals (Clay, 1988, 1989; West *et al.*, 1990), increased drought tolerance (West *et al.*, 1990), and increased fungus disease resistance (Clarke *et al.*, in press). Clay (1988) has characterized the relationship between mycosymbiont and host as a defensive mutualism.

Rogerson (1970) included 13 genera of the graminicolous *Clavicipitaceae* in his outline of the family. Diehl's (1950) monograph of the *Balansieae* is the most complete work available on the graminicolous *Clavicipitaceae* but treated only three genera, paying very little attention to important genera such as *Claviceps* Tul., *Epichloë* (Fr.) Tul., and *Ustilaginoidea* Bref. Diehl's monograph is now very dated.

Among the important systematic problems in the graminicolous *Clavicipitaceae* are: 1) resolution of suprageneric classification; 2) definition of genera; and 3) definition of species. We believe that these systematic problems will be resolved only by taking a holistic approach to systematics of the entire family. The following treatment expands on each of these problems and provides summary data on the state of the systematics to date. Entomogenous members of the *Clavicipitaceae*, which comprise the other large

ecological group of the family, are not discussed here.

Ordinal classification and centrum development

There is a historical and ongoing debate concerning classification of the *Clavicipitaceae*, with perithecial ontogeny being a crucial element. Historically, the *Clavicipitaceae* have been classified with the *Hypocreaceae* because of the production of colourful ascomata and well-defined perithecial walls. For example, in an 1865 treatise on fungi, *Selecta Fungorum Carpologia*, the Tulasne brothers placed members of both families in their section *Nectriei* of the order Pyrenomycetes (Grove *et al.*, 1931). Ellis & Everhart (1890) classified members of both families in the 'suborder' *Hypocreaceae* (order = Pyrenomycètes) following the system devised by Winter (1887). Saccardo (1891) placed both families in his family *Hypocreaceae*, but classified them in distinct sections, *Scolecosporae* for the *Clavicipitaceae* and *Hyalodidymae* for the *Hypocreaceae*.

Nannfeldt (1932) was the first to separate the *Clavicipitaceae* into a distinct order, the *Clavicipitales*, but he recognized its affinity to the *Hypocreaceae*. Luttrell (1951) examined centrum ontogeny and perithecial structure in pyrenomycetous ascomycetes and concluded that the *Clavicipitaceae* bear affinity to the *Xylariales* because of similarities in centrum ontogeny. He classified the centrum of the *Clavicipitaceae* as a *Xylaria*-type, and that of the *Hypocreales* as the *Nectria*-type. Chadeaud (1960) removed the *Clavicipitaceae* from the *Xylariales* into the *Clavicipitales*, based on the many unique features of the group. Rogerson (1970) classified them in distinct orders, *Clavicipitales* and *Hypocreales*, because of differences in perithecial ontogeny, but considered both orders to be part of a 'hypocrealean complex'.

Recent studies on small and large subunit rDNA (Spatafora & Blackwell, 1993; Rehner & Samuels, 1995) suggest that the *Clavicipitales* bear closer relationship to fungi of the *Hypocreales* than to those of the *Xylariales*, thus supporting the classification of *Clavicipitaceae* and *Hypocreaceae* in the same order. Spatafora & Blackwell (1993) concluded that centrum ontogeny and perithecial structures in the *Clavicipitaceae* were misinterpreted by earlier investigators (e.g. Luttrell, 1951; Mhaskar & Rao, 1976) who weighted perithecial ontogeny heavily in determining ordinal classification (Reynolds, 1981).

In order to reexamine structural and centrum ontogenetic features of the *Clavicipitaceae*, a study

was conducted on perithecia of five collections of *Epichloë* from Europe and North America (White, 1997). *Epichloë* (*Clavicipitaceae*) has a centrum development that is distinct from both the *Xylaria*-type centrum and the *Nectria*-type centrum development. It is distinguishable from the *Nectria* centrum in several features. The perithecial primordium in *Epichloë* is oblong to ovate rather than spherical, as seen in the *Nectria*-type centrum (Hanlin, 1961; Samuels, 1973). This is reflected in subsequent differences in the pattern of perithecial wall elongation. In the *Nectria*-type centrum, the perithecial wall expands evenly, so that a spherical primordium develops. In *Epichloë*, apical expansion occurs more rapidly than lateral expansion, so that an ovate primordium gradually gives rise to an ovate perithecium. The ascogenous mycelium in the clavicipitalean centrum remains as a central mound at the base of the perithecial cavity, rather than spreading out over the base and sides of the inner wall of the perithecium, as seen in the *Nectria*-type centrum (Luttrell, 1951; Hanlin, 1961). In the *Nectria*-type centrum, a subhymenial parenchyma layer is often present just beneath the asci (Samuels, 1973), while in *Epichloë*, the ascogenous mycelium is appressed directly to the wall of the perithecium. In the *Nectria*-type centrum, an apical meristem gives rise to the paraphyses (Luttrell, 1951). In *Epichloë*, the paraphyses bear no relation to apical or lateral meristems; instead, they form early in the process of cavity development. In the *Nectria*-type centrum, paraphyses intermix with asci (Samuels, 1973), while in *Epichloë* the paraphyses do not occur within the cluster of asci at any stage of development. A final developmental difference between the *Nectria*-type centrum and that seen in *Epichloë* relates to the origin of periphyses bordering the ostiole. In both, the ostioles form in a similar way. At the apex of the perithecial primordium, a pore develops through initiation of a meristem in the perithecial wall. In many species of *Nectria* and related genera, the determinate hyphae of the meristem that gave rise to the ostiole become the periphyses. In *Epichloë*, the hyphae of the meristem that formed the ostiole become the collar that borders the ostiole at the apex of the perithecial neck; the periphyses appear to be derived from short paraphyses that developed on the wall along the apex of the perithecial primordium.

Spatafora & Blackwell (1993) proposed that the clavicipitalean centrum was derived from the *Nectria*-type centrum through modification. If this hypothesis is correct, it may be possible to find stages of this evolutionary modification in members of the *Clavicipitaceae* that show evidence of being basally-

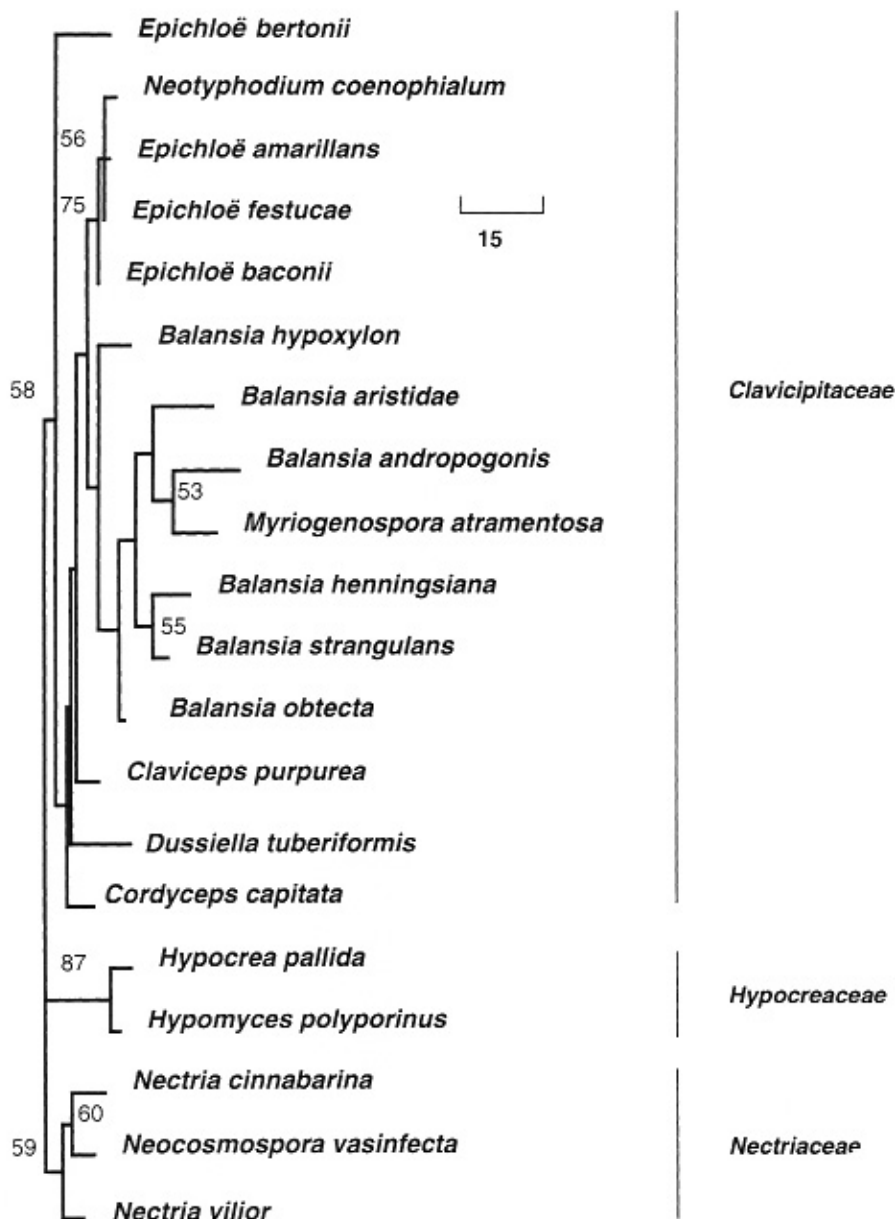


Fig. 1. Phylogenetic analysis of 18S rDNA from selected *Hypocreales* showing placement of *Epichloë bertonii* (arrow) and *Myriogenospora atramentosa*. The most parsimonious tree resulting from a heuristic analysis (135 steps; CI = 0.75; RI = 0.71). Numbers near branches are bootstrap values (250 replicates).

derived in phylogenetic analysis. Less derived species may exhibit a greater number of plesiomorphic (ancestral) features. *Epichloë bertonii* Speg. appears to be the most basally-rooted species in our 18S rDNA analysis (Fig. 1). This species shows the typical clavicipitalean features of thickened ascus tip (Fig. 2A) and filamentous ascospores. A detailed study of centrum ontogeny in *E. bertonii* may help to resolve the apparent conflict between centrum ontogeny and molecular phylogenetic data.

Genera of graminicolous *Clavicipitaceae*

USTILAGINOIDEA

The anamorph genus *Ustilagoidea* Bref. includes

species that infect tropical grass species of variable economic importance. *Ustilagoidea virens* (Cooke) Takah. is a significant and common parasite of rice, causing a disease known as 'false smut'. Infections by *U. virens* result in slight to significant reductions in yield. Yield losses are especially high in newer high-yielding rice cultivars (Verma & Singh, 1988). *Ustilagoidea virens* infects rice wherever it is grown, with reports from Africa, Asia, Central America, South America, and North America (BPI Collections). It also infects corn, and sporadic outbreaks of false smut on corn have been reported from Central America and North America (Haskell & Diehl, 1929). *Ustilagoidea* species may also be a source of anticancer drugs. A family of cyclic peptides, called ustilo-

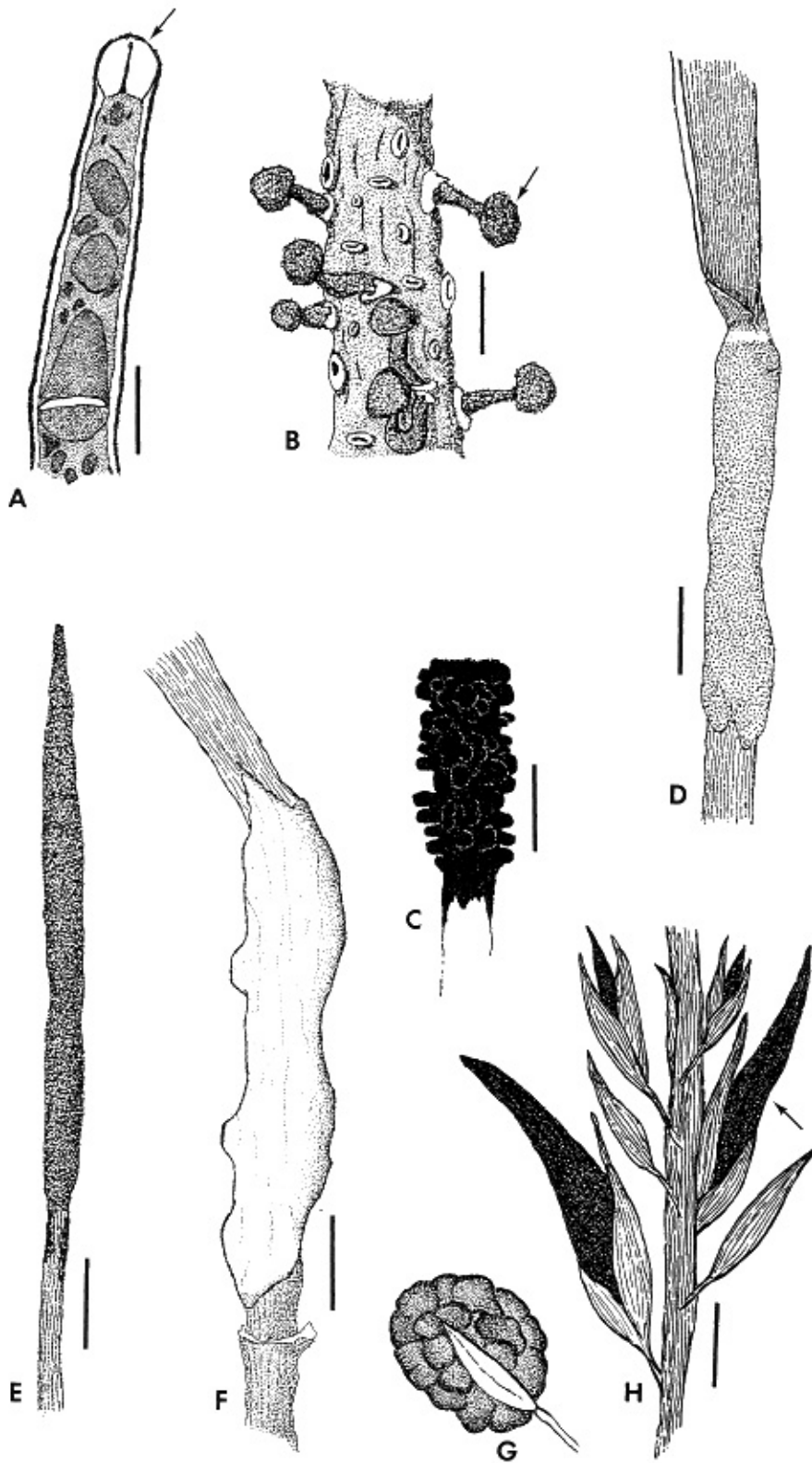


Fig. 2. Morphological features of *Clavicipitaceae*: A) typical refractive ascus tip (arrow) on immature ascus, bar = 7 μm ; B) stroma of *Balansia claviceps* showing stipitate ascomatal stromata (arrow), bar = 3 mm; C) stroma of *Balansia aristidae* showing a flattened layer of perithecia on the stroma, bar = 2 mm; D) stroma of *Epichloë baconii*, bar = 3 mm; E) stroma of *Parepichloë cinerea*, bar = 6 mm; F) stroma of *Epichloë bertonii*, bar = 5 mm; G) sclerotium of *Ustilaginoidea virens*, bar in H; H) sclerotia (arrow) of *Claviceps purpurea* on *Spartina* sp., bar = 4 mm.

xins, produced by *U. virens*, are potent microtubule inhibitors (Koiso *et al.*, 1992) and are under investigation as potential anticancer agents (Ludueña *et al.*, 1994). Examination of additional species in this genus may yield new potentially useful secondary products.

The genus *Ustilaginoidea* was established by Brefeld (1896) to accommodate *U. oryzae* (Pat.) Bref. and *U. setariae* Bref. Since its inception, the genus has come to include more than a dozen species names, although it is unknown how many distinct species are actually represented. Previous investigators (Takahashi, 1896) have shown that Brefeld's type of genus *Ustilaginoidea*, *U. oryzae* (Patouillard, 1887), is a nomenclatural synonym of *U. virens* (Cooke, 1879). Marchionatto (1940) examined *Ustilaginoidea strumosa* (Cooke) Ciferri, *Munkia martyris* Speg., and *Shropshira chusqueae* Stevens, concluding that they were conspecific. This needs to be confirmed because, if appropriate, the correct name of the species would employ the basionym of *Munkia martyris* Speg., the first described species (unless the name *U. virens* is proposed for conservation).

Ustilaginoidea is an anamorph genus with confused phylogenetic affinities. Studies of *U. virens* (Hashioka *et al.* 1951) suggest affinities with the teleomorph genus *Claviceps*, because the fungus produces sclerotia that replace ovaries (Fig. 2 G, H) and stipitate ascumata, as do species of *Claviceps*. The teleomorphic binomial *Claviceps oryzae-sativae* Hashioka was established on the basis of sclerotial and ascumatal features of *U. virens*. An examination of the features of *U. strumosa* (Cooke) Ciferri (Marchionatto, 1940) suggests affinities with the genus *Balansia* Speg.. Because *U. strumosa* forms sclerotized stromata on culms of *Chusquea* species and produces flattened ascumata as seen in species of *Balansia* Speg. (see Brefeld, 1891), Hennings (1904) classified it in that genus, proposing the teleomorphic binomial *Balansia chusqueicola* P. Henn. Diehl (1950) recognized that the possession of an *Ustilaginoidea* anamorph was problematic for classification in *Balansia*, because *Balansia* species possess *Ephelis* Fr. anamorphs. Similarly, *Claviceps* species are known to produce *Sphacelia* Lévl. anamorphs. The anamorph is considered an important taxonomic criterion in most cases. The *Ustilaginoidea* anamorph is characterized by elongate, cylindrical conidiogenous cells that bud sphaeroid conidia pleurogenously along its length (see Fig. 3B). The *Ephelis* anamorph is characterized by narrow, cylindrical conidiogenous cells that bud holoblastic filamentous conidia at an apical locus (Fig. 3A). The *Sphacelia* anamorph (Fig. 3C) is characterized by isodiametric conidiogenous cells that

may elongate by a tube-like extension to form phialidic, ovate to cylindrical, conidia at an apical locus. Recent work in the *Balansieae* (Kuldau *et al.*, 1997; White, unpublished) has suggested that possession of the *Ephelis* anamorph defines a monophyletic group, underscoring the value of the anamorph for identifying phylogenetically-related groups of species. The possession of an *Ustilaginoidea* anamorph may be a strong indication that *U. virens*, *U. strumosa*, and similar species should be classified in a genus other than *Claviceps* or *Balansia*. Preliminary analysis of rDNA sequences of ITS1, 5.8S, and ITS2 regions place *Ustilaginoidea* intermediate between several *Balansia* species and *Claviceps purpurea*, with some bootstrap support (67%) suggesting a stronger linkage to genus *Balansia* than to *Claviceps* (Fig. 3). A more extensive study of taxonomic features and sequence analysis of additional representatives of the genera *Balansia*, *Claviceps*, and *Ustilaginoidea* are necessary to clarify whether teleomorphs of *Ustilaginoidea* belong in *Balansia*, *Claviceps*, or instead represent a distinct teleomorphic genus.

CLAVICEPS

Claviceps species affect many of the world's cereal and forage crops. *Claviceps purpurea* and *C. paspali* Stevens & Hall commonly infect forage species in the southern United States and Central and South America, frequently resulting in toxic syndromes in grazing animals. *Claviceps africana* Frederickson, Mantle & De Milliano has recently become epidemic wherever sorghum is grown. This species is considered a global threat, with seed losses up to 80% in some places.

Claviceps was erected in 1853 for *C. purpurea* (Saccardo, 1883). Since that time, the genus has come to contain almost a hundred species worldwide. The criteria for classification of a clavicipitaceous fungus in *Claviceps* is the formation of a sclerotium or sclerotium-like stroma in florets of grasses or sedges. The paucity of additional criteria has resulted in the lumping of species that are likely not closely related in *Claviceps*. For example, some species, such as *C. diadema* (Moell.) Diehl and *C. flavella* Berk. & M.A. Curtis, envelop part of the host inflorescence in a mycelial stroma and produce ascumata while still attached to the plant. These are features exhibited in species of the genus *Balansia* (e.g. *B. claviceps* Speg.). In *Claviceps* species, the sclerotium is contained within the floret (Fig. 2H) and is dispersed with the seeds. The possibility that species like *C. diadema* and *C. flavella* belong in other genera needs to be evaluated. *Claviceps*

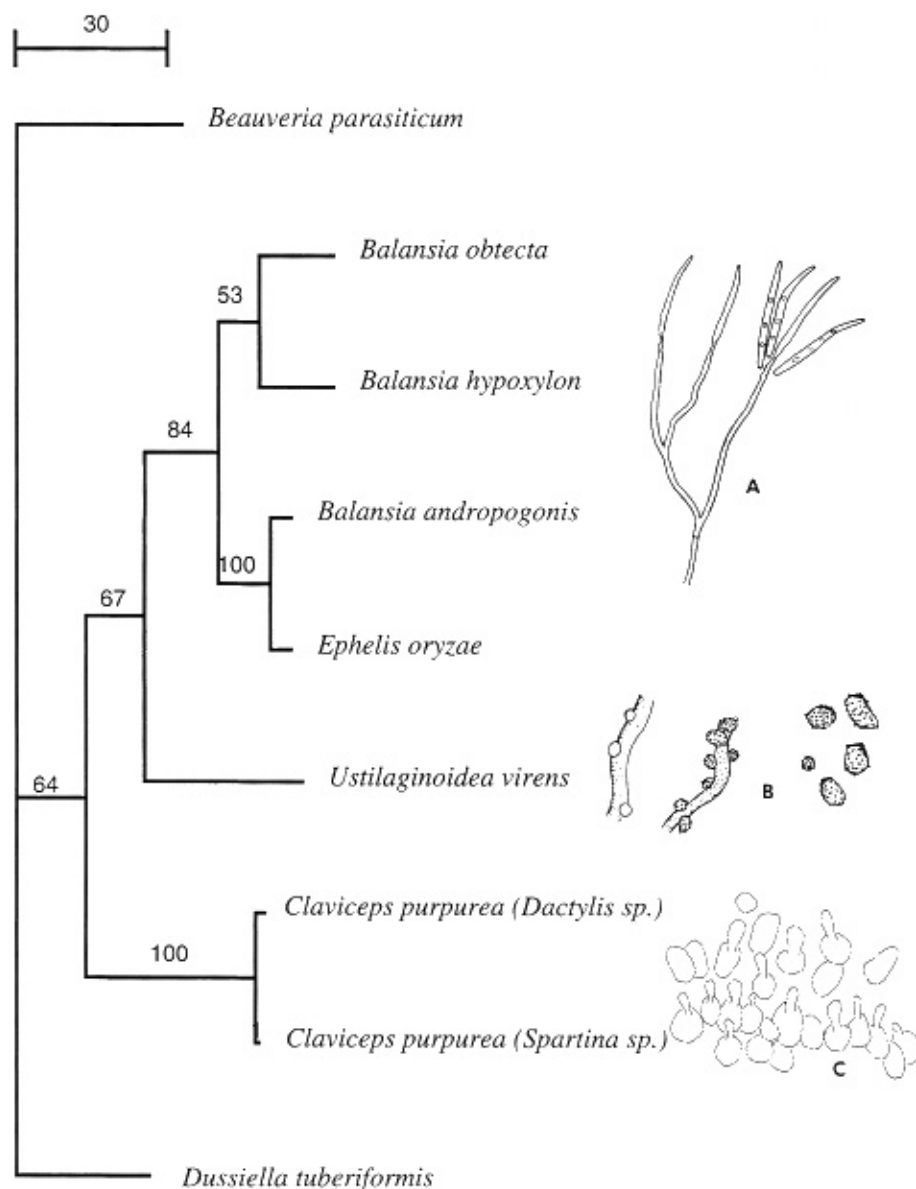


Fig. 3. Phylogram based on analysis of ITS1–5.8S–ITS2 rDNA showing placement of *Ustilaginoidea*. The phylogram was the most parsimonious tree resulting from a heuristic analysis (572 steps; CI = 0.72; RI = 0.64). Numbers near branches are bootstrap values (250 replicates). A–C anamorphs: A) *Ephelis* sp.; B) *Ustilaginoidea* sp.; C) *Sphacelia* sp.

purpurea has been reported on 50 genera distributed among 17 tribes of grasses. Collections of *C. purpurea* need to be examined using morphological and molecular approaches to determine if the species as it is now understood might contain multiple species or subspecies.

Langdon (1954) proposed that physiological and ecological adaptation are the key factors driving diversification in the genus *Claviceps*. In preliminary studies on populations of *C. purpurea* on the hosts *Spartina alterniflora*, *Festuca rubra*, *Dactylis glomerata*, and *Lolium perenne*, this principle seems well-established. Sclerotia on the semiaquatic plant *S. alterniflora* are large and contain numerous intercellular air spaces, allowing them to float. Sclerotia

on terrestrial hosts are smaller, lack the abundance of internal intercellular spaces, and also lack the capacity for flotation. They thus appear adapted for dispersal with the seeds in the terrestrial environment. A comparison of ITS sequences of an individual from the *Spartina* population and that of an individual from *Dactylis glomerata* demonstrated only three nucleotide substitutions in the rDNA ITS1, 5.8S, and ITS2 regions (White, unpublished). The paucity of base substitutions between the two populations may be an indication of the recency of divergence of the two populations.

It is generally considered that *C. purpurea* is homothallic, with populations being isolated genetically. However, similar rDNA sequences might be

explainable if there were gene flow between the populations with adaptive features being maintained through stabilizing selection on particular traits. *Claviceps purpurea* would have ample opportunity for genetic exchange because it has a 'honeydew stage', in which conidia of the sphaelial anamorph are exposed to insects that visit florets. There is no evidence that isolating mechanisms exist that would exclude the mixing of sphaelial conidia from different populations and subsequent parasexual recombination. If such a mechanism is operational in *Claviceps*, the evidence of it might be detectable in the form of polymorphic genes, as has been observed in numerous anamorphic endophyte populations in the genus *Neotyphodium* Glenn, Bacon & Hanlin. One way to screen for the presence of polymorphisms is to conduct isozyme analysis. Isozyme studies could be followed by cloning and sequencing of certain of the polymorphic genes, such as that for β -tubulin, to determine their origins. The presence of such a mechanism for genetic exchange might explain the diversity and adaptability of *Claviceps purpurea*.

EPICHLÖE

Epichloë is a genus of parasites of grasses adapted to predominantly temperate regions. Species of *Epichloë* perennate as endophytic mycelium in leaf sheaths and culm tissues of their hosts. They are often also seed-transmitted, first by growth of the mycelium from the maternal plant into developing ovules, followed by penetration of the embryo within the seed. The teleomorphic *Epichloë* morph is characterized by production of a stromatal mycelium on the culm, which embeds the living inflorescence primordium and a leaf sheath in a stroma (Fig. 2D). Spermatia, followed by perithecia, are produced on the surface of the stroma. Several species of *Epichloë* have been well characterized (see Table 1).

Examination of herbarium material suggests that additional morphologically distinct species of *Epichloë* are yet to be described. One such species has been collected exclusively in the states of Morelos and Hidalgo in Mexico on an unknown grass. This species is distinguished from all known species of *Epichloë* by its perithecia, which are deeply embedded within the stroma, while those of other species are not or only partially embedded within the stroma tissues. The Mexican *Epichloë* species appears to be adapted for growth in an arid environment; perhaps deeply embedded perithecia aid in conservation of water during development. In previous studies (White & Camp, 1995), we demonstrated that high water losses occur from the surface of stromata of *E. ama-*

rillans. In an arid environment, considerable selective pressure may stimulate adaptive changes in stromata to conserve water. It is interesting that even the behavior of the symbiotic fly *Botanophila* sp. (= *Phorbia*) appears adapted to the arid conditions of growth of the Mexican *Epichloë* species. Adult females of *Botanophila* species vector spermatia between mating types of *Epichloë*, depositing them along with eggs on the stroma. In most species of *Epichloë*, the *Botanophila* eggs hatch and the larvae emerge to crawl around on the surface of the stroma to consume developing perithecia (Bultman *et al.*, 1995). However, in the Mexican *Epichloë* species, eggs hatch and larvae immediately burrow into the stroma to feed exclusively on the internal mycelium of the stroma. The larvae are thus protected from the dry arid air by the developing ascumatal stroma that covers them. Because sequence data has played a key role in defining the species of *Epichloë* documented to date, it is logical to employ sequence data to test the hypothesis that the Mexican *Epichloë* species is distinct from all previously established species.

Three life cycle patterns of *Epichloë/Neotyphodium* endophytes have been described (White, 1988; Table 1). Type I endophytes are those species of *Epichloë* that are not seed-transmitted; a high percentage of the host culms bear stromata. Type II endophytes have stroma development on some culms and seed transmission on other culms. The percentage of culms that bear stromata may be a function of environmental conditions such as soil fertility (Funk & White, 1997). Seasonal variation is also evident. In some years, grass crops of fine fescues containing *E. festucae* produce many stromata, while in other years fewer stromata are formed (White, unpublished). As yet unknown environmental conditions may alter the balance between endophyte and plant and affect the degree of stroma development. Type III endophytes very rarely or never form stromata. These endophytes appear to rely on seed transmission as the primary means of dispersal. These *Epichloë* species are typically classified by their anamorphs in the genus *Neotyphodium* and are frequently referred to as *Neotyphodium* endophytes.

Biogeographic patterns in the distribution of endophyte types are evident. For example, all three types of life cycles are found on native grasses in the Northern Hemisphere wherever cool-season grasses are grown. However, in South America only Type III endophytes have been identified on native grasses. Perhaps, *Epichloë* originated in the Northern Hemisphere as a parasite of cool-season grasses. Founder populations that colonized South America may have been exclusively of the Type III variant. The tropical

Table 1. *Epichloë/Neotyphodium* species, hosts, and life cycle type.

Selected Species	Selected Hosts	Assoc. Type	Reference
<i>E. amarillans</i>	<i>Agrostis hiemalis</i>	Type II	White, 1994
<i>E. baconii</i>	<i>Agrostis stolonifera</i>	Type I	White, 1993
<i>E. brachyelytri</i>	<i>Brachyletrum erectum</i>	Type II	Schardl & Leuchtman, 1999
<i>E. bromicola</i>	<i>Bromus ramosus</i>	Type II	Leuchtman & Schardl, 1998
<i>E. clarkii</i>	<i>Holcus lanatus</i>	Type I	White, 1983
<i>E. elymi</i>	<i>Elymus canadensis</i>	Type II	Schardl & Leuchtman, 1999
<i>E. festucae</i>	<i>Festuca rubra</i>	Type II	Leuchtman <i>et al.</i> , 1994
<i>E. glyceriae</i>	<i>Glyceria striatica</i>	Type I	Schardl & Leuchtman, 1999
<i>E. sylvatica</i>	<i>Brachypodium sylvaticum</i>	Type II	Leuchtman & Schardl, 1998
<i>E. typhina</i>	<i>Dactylis glomerata</i> <i>Lolium perenne</i> <i>Poa pratensis</i>	Type I	White, 1983
<i>N. coenophialum</i>	<i>Festuca arundinacea</i>	Type III	Morgan-Jones & Gams, 1982
<i>N. lolii</i>	<i>Lolium perenne</i>	Type III	Latch <i>et al.</i> , 1984
<i>N. starrii</i>	<i>Festuca arizonica</i>	Type III	White <i>et al.</i> , 1987
<i>N. uncinatum</i>	<i>Festuca pratensis</i>	Type III	Gams <i>et al.</i> , 1990
<i>N. tembladerae</i>	<i>Poa huecu</i> <i>Festuca argentina</i>	Type III	Cabral <i>et al.</i> , 1999

zone along the equator may serve as a barrier to the movement of endophytes between Northern and Southern Hemispheres because of the absence of the cool-season grass hosts (Cabral *et al.*, 1999).

Sexually-reproducing species of *Epichloë* (Types I and II endophytes) have given rise to the asexual *Neotyphodium* endophytes (Type-III endophytes) (White, 1988). Asexual *Neotyphodium* species perennate in the leaves, rhizomes, and culms of infected grasses and grow into seeds when they are produced, infecting the embryos within. It has long been assumed that *Neotyphodium* endophytes lack the capacity for external spread or genetic interaction and that they remain endophytic at all times. We have observed that many grasses infected by endophytes commonly have an epiphyllous network of mycelium on which conidia are produced (White *et al.*, 1996; unpublished). This suggests that *Neotyphodium* endophytes may possess an external, potentially contagious stage.

Many Type-III endophytes show polymorphisms in several genes, while Types I and II endophytes tend not to show such polymorphisms (Tsai *et al.*, 1994; Schardl *et al.*, 1997; Leuchtman & Schardl, 1998; Cabral *et al.*, 1999). This has been interpreted by Tsai *et al.* (1994) as evidence of past hybridization events, involving Types I and II endophytes, that gave rise to Type-III endophytes. Thus, gene polymorphisms are suggested to be remnants of species origin events. It seems equally plausible to hypothes-

ize that gene polymorphisms may be the result of a parasexual process that is a function of the epiphyllous stages of endophytes. Anastomosis of the mycelium of endophytes on leaf surfaces is a frequently observed phenomenon (White *et al.*, 1996). Chung & Schardl (1997) demonstrated that *Epichloë/Neotyphodium* endophytes show interspecies vegetative compatibility. It may be that anastomoses on leaf surfaces are part of a parasexual process that endophytes employ to compensate for the loss of stromata and sexual reproduction. This may be an important means by which asexual endophytes evolve and change genetically.

Epichloë bertonii Speg. was described by Spegazzini (1922) to accommodate a fungus that formed its stroma on living twigs of *Mikania scandens* (Asteraceae) in Paraguay. We have studied collections of what appears to be *E. bertonii* on species of *Asteraceae* from Costa Rica, Indonesia, and Thailand. This species is readily excluded from *Epichloë* for morphological and ecological reasons. Stromata of *E. bertonii* often form bright orange to red collars of stromatic tissues that surround the stem of its host and superficially resemble stromata of *Epichloë* (Fig. 2F). However, *E. bertonii* appears to be exclusively epibiotic on nonmeristematic tissues of dicots, whereas *E. typhina* and other graminicolous species of *Epichloë* are endophytic, forming the stroma on the developing inflorescence primordium and a leaf sheath that surrounds the primordium (Fig. 2E).

Differences in the anamorphs provide another indication that *E. bertonii* should not be classified in the genus *Epichloë*. An examination of the anamorph of *E. bertonii* shows that conidia progressively develop several septa as they mature, while in *E. typhina* and other graminicolous species conidia are aseptate. Preliminary phylogenetic analysis of 18S rDNA sequences demonstrates that *E. bertonii* does not group with *Epichloë* (Fig. 1). Instead, it is more deeply rooted in the *Clavicipitaceae*. The correct generic placement of *E. bertonii* remains to be evaluated. Genera to which *E. bertonii* may show affinity include *Podocrella* Seaver, *Dussiella* Pat., *Fleischeria* Penzig & Sacc., *Konradia* Racib., *Oomyces* Berk. & Broome, *Mycomalus* A. Möller, *Ascopolyporus* A. Möller, and *Globulina* Velen.

BALANSIA

Balansia includes both endophytic and epibiotic species. The majority of the endophytes are classified in the subgenus *Dothichloë*, and the epibionts in subgenus *Eubalansia*. Subgenus *Dothichloë* is characterized by the possession of flattened ascromatal stromata that develop on leaves or culms of grasses (Fig. 2C), while subgenus *Eubalansia* was defined to include species with pulvinate or stipitate ascromatal stromata (Fig. 2B). Future research will need to address whether the subgenera in *Balansia* represent natural (i.e., phylogenetic) groupings.

Several genera have been allied with the genus *Balansia*, including *Atkinsonella* Diehl, *Balansiopsis* Hohn., and *Myriogenospora* Atk. Diehl (1950) defined genera largely on the basis of their anamorphs. Species were classified in *Balansia* if they possessed only an *Ephelis* anamorph, and in *Atkinsonella* if they possessed an *Ephelis* anamorph and an acremonium-like synanamorph. *Balansiopsis* included species that did not appear to produce any anamorph. *Myriogenospora* was distinguished from these genera on the basis of differences in perithecial, ascus, and ascospore morphology (White & Glenn, 1994). A recent phylogenetic analysis (Kuldau *et al.*, 1997) indicated that species of genera bearing the *Ephelis* anamorph form a monophyletic clade (Fig. 1). Additional work is needed on more complete data sets to determine whether lumping these genera into a single genus is warranted.

Conclusions

The graminicolous *Clavicipitaceae* comprise several genera that are relatively well-defined. Systematic problems that need to be addressed include rec-

tification of the apparent conflict between perithecial ontogeny and DNA sequence-based phylogenetic data. This problem may extend beyond the *Clavicipitaceae*, because centrum ontogeny is weighted heavily in classification of ascomycetes. Among the problem genera in the *Clavicipitaceae* are *Myriogenospora* and *Ustilaginoidea*.

Sequence-based phylogenetic analysis has the potential to provide better-defined taxa at all levels of classification. However, its optimal application demands that systematists have a broad knowledge of the organisms, their genetics, morphology and biology. Systematics that relies solely on molecular approaches is incomplete and runs the risk of producing erroneous results. With the advent of molecular phylogeny, mycologists now have the means to fuse anamorph taxa into teleomorph groupings. The application of anamorph names such as *Neotyphodium* for anamorphs of *Epichloë* is a practice that is rapidly becoming obsolete. The classification of these species in the genus *Epichloë*, even though some may lack a teleomorph (perithecia), is logical and inevitable.

Literature cited

- ALEXOPOULOS, C.J., MIMS, C.W. & BLACKWELL, M., 1996 — Introductory mycology. — John Wiley & Sons, New York, NY.
- BREFELD, O., 1891 — Ascomyceten II. Untersuchungen aus dem Gesamtgebiete der Mykologie. **10**: 155–378.
- BREFELD, O., 1896 — *Ustilaginoidea oryzae* nov. genus auf *Oryza sativa*. Untersuchungen aus dem Gesamtgebiete der Mykologie **12**: 194–202.
- BULTMAN, T.L., WHITE, J.F., JR., BOWDISH, T.I., WELCH, A.M. & JOHNSTON, J., 1995 — Mutualistic transfer of *Epichloë* spermatia by *Phorbia* flies. — *Mycologia* **87**: 182–189.
- CABRAL, D., CAFARO, M., SAIDMAN, B., LUGO, M., REDDY, P. V. & WHITE, J.F., JR., 1999 — Evidence supporting the occurrence of a new species of endophyte in some South American grasses. — *Mycologia* **91**: 315–325.
- CHADEFAUD, M., 1960 — Les végétaux non-vasculaires (Cryptogamie). — *Traité de Botanique systématique* **1**: 1–1018.
- CHUNG, K.R. & SCHARDL, C.L., 1997 — Vegetative compatibility between and within *Epichloë* species. — *Mycologia* **89**: 558–565.
- CLARKE, B.B., WHITE, J.F., JR., FUNK, C.R., JR., SUN, S., HUFF, D.R. & HURLEY, R.H., 2000 — Enhanced resistance to dollar spot in endophyte-infected fine fescues. — *Plant Disease*, in press.
- CLAY, K., 1988 — Fungal endophytes of grasses: a defensive mutualism between plants and fungi. — *Ecology* **69**: 10–16.

- CLAY, K., 1989 — Clavicipitaceous endophytes of grasses: Their potential as biocontrol agents. — *Mycol. Res.* **92**: 1–12.
- COOKE, M.C., 1879 — Some extra-European fungi. — *Grevillea* **7**: 13–15.
- DIEHL, W.W., 1950 — *Balansia* and the Balansiae in America. — U. S. Dep. Agric., Washington, DC.
- DREYFUSS, M.M. & CHAPELA, I.H., 1994 — Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. — In: Gullo, V. P. (Ed.): The discovery of natural products with therapeutic potential. Pp. 49–80. Butterworth-Heinemann Press, Stoneham Mass.
- ELLIS, J.B., & EVERHART, B.M., 1890 — North American Pyrenomycetes. — William C. Cloyd Press, Vineland, NJ.
- FUNK, C.R., & WHITE, J.F. JR., 1997 — Use of natural and transformed endophytes for turf improvement. — In: Bacon, C.W. & Hill, N. (Eds.): *Neotyphodium*/grass interactions. Pp. 229–239. Plenum Press, New York.
- GAMS, W., PETRINI, O. & SCHMIDT, D., 1990 — *Acremonium uncinatum*, a new endophyte in *Festuca pratensis*. — *Mycotaxon* **37**: 67–71.
- GROVE, W.B., BULLER, A.H.R. & SHEAR, C.L., 1931 — Tulasne's Selecta Fungorum Carpologia (English translation). — Oxford University Press, Oxford.
- HANLIN, R.T., 1961 — Studies in the genus *Nectria*. II. Morphology of *N. gliocladioides*. — *Amer. J. Bot.* **48**: 900–908.
- HASHIOKA, Y., YOSHINO, M., & YAMAMOTO, T., 1951 — Physiology of the rice false smut, *Ustilagoidea vires* (Cke.) Tak. — *Res. Bull. Saitama Agric. Exp. Sta.* **2**: 20.
- HASKELL, R. J. & DIEHL, W. W., 1929 — False smut of maize, *Ustilagoidea*. — *Phytopathology* **19**: 589–591.
- HENNINGS, P., 1904 — Einige neue Pilze aus Costarica und Paraguay. — *Hedwigia* **43**: 147–149.
- HODGE, K. T., KRASNOFT, S. B. & HUMBER, R. A., 1996 — *Tolypocladium inflatum* is the anamorph of *Cordyceps subsessilis*. — *Mycologia* **88**: 715–719.
- KOISO, Y., NATORI, M., IWASAKI, S., SATO, S., SONODA, Y., FUJITA, R., YAEGASHI, H. & SATO, Z., 1992 — Ustiloxin, a phytotoxin and mycotoxin from false smut balls on rice panicles. — *Tetrahedron Lett.* **33**: 4157–4160.
- KULDAU, G.A., LIU, J-S., WHITE, J.F. , JR., SIEGEL, M.R. & SCHARDL, C.L., 1997 — Molecular systematics of *Clavicipitaceae* supporting monophyly of genus *Epichloë* and form genus *Ephelis*. — *Mycologia* **89**: 431–441.
- LANGDON, R.F. N., 1954 — The origin and differentiation of *Claviceps* species. — *Trans. Brit. mycol. Soc.* **47**: 205–213.
- LATCH, G.C.M., CHRISTENSEN, M.J. & SAMUELS, G.J., 1984. — Five endophytes of *Lolium* and *Festuca* in New Zealand. — *Mycotaxon* **20**: 535–550.
- LEUCHTMANN, A., & SCHARDL, C.L., 1998 — Mating compatibility and phylogenetic relationships among two new species of *Epichloë* and other congeneric European species. — *Mycol. Res.* **102**: 1169–1182.
- LEUCHTMANN, A., SCHARDL, C.L. & SIEGEL, M.R., 1994 — Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. — *Mycologia* **86**: 802–812.
- LEWIS, W.H. & ELVIN-LEWIS, M.P.F., 1977 — *Medical Botany*. — John Wiley & Sons, New York.
- LEWIS, W.H. & ELVIN-LEWIS, M.P. F., 1990 — Obstetrical use of the parasitic fungus *Balansia cyperi* by Amazonian Jivaro women. — *Econ. Bot.* **44**: 131–133.
- LUDUEÑA, R.F., ROACH, M.C., PRASAD, V., BANERJEE, M., KOISO, Y., LI, Y. & IWASAKI, S., 1994 — Interaction of Ustiloxin A with bovine brain tubulin. — *Biochem. Pharmacol.* **47**: 1593–1599.
- LUTTRELL, E.S., 1951 — Taxonomy of the Pyrenomycetes. — University of Missouri, Columbia, Missouri.
- MARCHIONATTO, J.B., 1940 — Nota toxonómica sobre *Munkia martyris*, *Ustilagoidea strumosa* y *Shropshira chusqueae*. — *Rev. Argent. Agron.* **7**: 172–175.
- MHASKER, D.N. & RAO, V.G., 1976 — Development of the ascocarp in *Epichloë cinerea* (*Clavicipitaceae*). — *Mycologia* **68**: 994–1001.
- MORGAN-JONES, G. & GAMS, W., 1982 — Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloë typhina*, new taxa in one of two new sections of *Acremonium*. — *Mycotaxon* **15**: 311–318.
- NANNFELDT, J.A., 1932 — Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomyceten. — *Nova Acta Reg. Soc. Sci. Upsal. Ser. 4*, **8**: 1–368.
- PATOUILLARD, N., 1887 — Contributions à l'étude des champignons extra-européens. — *Bull. Soc. Mycol. Fr.* **2**: 119–131.
- PLOWMAN, T.C., LEUCHTMANN, A., BLANEY, C. & CLAY, K., 1990 — Significance of the fungus *Balansia cyperi* infecting medicinal species of *Cyperus* (*Cyperaceae*) from Amazonia. — *Econ. Bot.* **44**: 452–462.
- REHNER, S.A. & SAMUELS, G.J., 1995 — Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. — *Canad. J. Bot.* **73** (Suppl. 1): S816–S823.
- REYNOLDS, D.R., 1981 — Ascomycete systematics: the Luttrellian concept. — Springer-Verlag, New York.
- ROGERSON, C.T., 1970 — The hypocrealean fungi (Ascomycetes, Hypocreales). — *Mycologia* **62**: 865–910.
- SACCARDO, P.A., 1883 — *Sylloge fungorum omnium hucusque cognitorum*. Vol. **2**, p. 564. — Padua.
- SACCARDO, P.A., 1891 — *Sylloge Fungorum omnium hucusque cognitorum*. Vol. **9** — Padua.
- SAMUELS, G. J., 1973 — Perithecial development in *Hypomyces aurantius*. — *Amer. J. Bot.* **60**: 268–276.
- SCHARDL, C.L. & LEUCHTMANN, A., 1999 — Three new species of *Epichloë* symbiotic with North American grasses. — *Mycologia* **91**: 95–107.
- SCHARDL, C.L., LEUCHTMANN, A., CHUNG, K., PENNY, D.

- & SIEGEL, M.R., 1997 — Coevolution by common descent of fungal symbionts (*Epichloë* spp.) and grass hosts. — *Mol. Biol. Evol.* **14**: 133–143.
- SCHULTES, R.E. & HOFFMANN, A., 1973 — The botany and chemistry of hallucinogens. — C. C. Thomas, Springfield, Illinois.
- SPATAFORA, J. W. & BLACKWELL, M., 1993 — Molecular systematics of unitunicate perithecial ascomycetes: The *Clavicipitales*–*Hypocreales* connection. — *Mycologia* **85**: 912–922.
- SPEGAZZINI, C., 1922 — *Epichloë bertonii*. *Anales Mus. Nac. Hist. Nat. Buenos Aires*. **31**: 416–417.
- TAKAHASHI, Y., 1896 — On *Ustilago virens* Cooke and a new species of *Tilletia* parasitic on rice plants. — *Bot. Mag., Tokyo* **10**: 16–20.
- TSAI, H.-F., LIU, J., STABEN, C., CHRISTENSEN, M. J., LATCH, G. C. M., SIEGEL, M. R. & SCHARDL, C.L., 1994 — Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloë* species. — *Proc. Nat. Acad. Sci.* **91**: 2542–2546.
- VERMA, R. K. & SINGH, R. A., 1988 — Variations in *Claviceps oryzae-sativae*: the incitant of false smut of rice. — *Indian Phytopathol.* **41**: 48–50.
- WEST, C. P., OOSTERHUIS, D. M. & WULLSCHLEGER, S. D., 1990 — Osmotic adjustment in tissues of tall fescue in response to water deficit. — *Environ. Exp. Bot.* **30**: 149–156.
- WHITE, J.F., JR., 1988 — Endophyte–host associations in forage grasses. XI. A proposal concerning origin and evolution. — *Mycologia* **80**: 442–446.
- WHITE, J.F., JR., 1993 — Endophyte–host associations in grasses. XIX. A systematic study of some sympatric species of *Epichloë* in England. — *Mycologia* **85**: 444–455.
- WHITE, J.F., JR., 1994 — Endophyte–host associations in grasses. XX. Structural and reproductive studies of *Epichloë amarillans* sp. nov. and comparisons to *E. typhina*. — *Mycologia* **86**: 571–580.
- WHITE, J.F., JR., 1997 — Perithecial structure in the fungal genus *Epichloë*: an examination of the clavicipitalean centrum. — *Amer. J. Bot.* **84**: 170–178.
- WHITE, J.F., JR. & CAMP, C. R., 1995 — A study of water relations of *Epichloë amarillans* White, an endophyte of the grass *Agrostis hiemalis* (Walt.) B.S.P. — *Symbiosis* **18**: 15–25.
- WHITE, J.F., JR., COLE, G.T. & MORGAN-JONES, G., 1987 — Endophyte–host associations in forage grasses. VI. A new species of *Acremonium* isolated from *Festuca arizonica*. — *Mycologia* **79**: 148–152.
- WHITE, J.F., JR. & GLENN, A.E., 1994 — A study of two fungal epibionts of grasses: structural features, host relationships, and classification in genus *Myriogenospora* Atk. (*Clavicipitales*). — *Amer. J. Bot.* **81**: 216–223.
- WHITE, J.F., JR., REDDY, P.V., GLENN, A.E. & BACON, C.W., 1997 — An examination of structural features and relationships in *Balansia* subgenus *Dothichloë*. — *Mycologia* **89**: 408–419.
- WHITE, J.F., JR., MARTIN, T. I. & CABRAL, D., 1996 — Endophyte–host associations in grasses. XXIII. Conidia formation by *Acremonium* endophytes in the phylloplanes of *Agrostis hiemalis* and *Poa rigidifolia*. — *Mycologia* **88**: 174–178.
- WINTER, G., 1887 — Die Pilze: Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. — E. Kummer, Leipzig.