

Short title: Taxonomy of *Epichloë*

Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*

Adrian Leuchtman¹

Institute of Integrative Biology, ETH Zürich, CH-8092 Zürich, Switzerland

Charles W. Bacon

Toxicology and Mycotoxin Research, USDA-ARS-SAA, Athens, Georgia 30605-2720

Christopher L. Schardl,

Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0312

James F. White, Jr.

Mariusz Tadych²

Department of Plant Biology and Pathology, Rutgers University, New Brunswick, New Jersey

08901-8520

Abstract: Nomenclatural rule changes in the International Code of Nomenclature for algae, fungi and plants, adopted at the 18th International Botanical Congress in Melbourne, Australia, in 2011, provide for a single name to be used for each fungal species. The anamorphs of *Epichloë* species have been classified in genus *Neotyphodium*, the form genus that also includes most asexual *Epichloë* descendants. A nomenclatural realignment of this monophyletic group into one genus would enhance a broader understanding of the relationships and common features of these grass endophytes. Based on the principle of priority of publication we propose to classify all members of this clade in the genus *Epichloë*. We have reexamined classification of several described *Epichloë* and *Neotyphodium* species and varieties and propose new combinations and states. In this treatment we have accepted 43 unique taxa in *Epichloë*,

including distinct species, subspecies, and varieties. We exclude from *Epichloë* the two taxa *Neotyphodium starrii*, as *nomen dubium*, and *Neotyphodium chilense*, as an unrelated taxon.

Key words: anamorph, Ascomycota, classification, Clavicipitaceae, endophyte, fungi, grass, nomenclature, pleomorph, Poaceae, systematic, taxonomy, teleomorph

INTRODUCTION

A major shift in the rules governing the nomenclature of pleomorphic fungi was adopted recently at the 18th International Botanical Congress in Melbourne, Australia, 2011 (McNeill et al. 2012). This included the modification and implementation of Article 59 by the International Code of Nomenclature for algae, fungi, and plants establishing among other changes, the principle of “one fungus = one name” (Hawksworth 2004, Hawksworth et al. 2011a, 2011b; Norvell 2011). This principle states that each species should have a single name, applicable to all growth and spore states of that organism. Here we propose to address the question for a fungal group that has an extensive molecular phylogenetic underpinning and for which evolutionary relationships between sexual and asexual forms have been very well studied. This group of fungi within the Clavicipitaceae includes the species of *Epichloë* and its anamorphic *Neotyphodium* species that are symbionts of grasses.

TAXONOMIC HISTORY OF *EPICHLÖE* AND ITS ANAMORPHS

Persoon (1798, 1801) described the type species of genus *Epichloë* as *Sphaeria typhina* Pers. Fries (1849) proposed the subgenus *Epichloë* Fr. to accommodate *Sphaeria typhina* and distinguished it from other species in *Sphaeria*. The brothers L.-R. and C. Tulasne elevated *Epichloë* to genus rank (Tulasne and Tulasne 1865). Historically, since its first description and according to Seaver (1910), *Epichloë typhina* has been subjected to several designations first as *S. typhina* Pers., followed by *Sphaeria spiculifera* Sow., *Dothidea typhina* Fries,

Stromatosphaeria typhina Greville, and *Cordyceps typhina* Fries. The genus *Epichloë* remained monotypic until symptomless infection in forage grasses were related to livestock toxicities (Bacon et al. 1977, Hoveland et al. 1980, Fletcher and Harvey 1981, Latch and Christensen 1982) and the responsible endophytes described as separate species (Morgan-Jones and Gams 1982, Latch et al. 1984).

Over the years numerous African, Asian and South American species of plant-infecting Clavicipitaceae with epibiotic mycelia were described in *Epichloë*. Asian species included: *E. bambusae* Pat., *E. cinerea* Berk. & Br., *E. kyllingiae* Rac., *E. sasae* Hara, *E. sclerotica* Pat.; African species included *E. cynodontis* Syd., *E. oplismani* P. Henn., *E. volkensisii* P. Henn., *E. warburgiana* Magn.; American species included *E. bertonii* Speg. White and Reddy (1998) segregated the majority of African and Asian species of *Epichloë* into a new genus, *Parepichloë* J.F. White & P. Reddy, based on phylogenetic placement and morphological features. The Asian species *E. warburgiana* and *E. kyllingiae* were excluded from both *Epichloë* and *Parepichloë* (White 1994a) on the basis of morphological features. Sullivan et al. (2000) transferred the species *E. bertonii* to a new genus *Hyperdermium* J.F. White, Sullivan, Bills & Hywel-Jones, based on phylogeny and morphological features. Meanwhile, numerous sexual *Epichloë* species were being described from within *E. typhina* based on morphology, reproductive incompatibility and phylogeny (White 1993, 1994b; Leuchtmann et al. 1994; Leuchtmann and Schardl 1998; Schardl and Leuchtmann 1999; Li et al. 2006; Tadych et al. 2012), and many more *Neotyphodium* species and varieties were being described based on conidial morphology or origin through interspecific hybridization (Schardl 2010).

The conidial states of currently accepted *Epichloë* species and most of their asexual descendants are very similar but under previous nomenclature rules were classified in a form

genus distinct from the *Epichloë* teleomorph. Saccardo (1881) applied the name *Sphacelia typhina* Sacc. to a conidial state associated with stromata of *Epichloë*. Morgan-Jones and Gams (1982) examined herbarium material and descriptions of specimens examined by Saccardo and concluded that they did not represent the anamorph of *Epichloë typhina*. Morgan-Jones and Gams (1982) considered the name *Sphacelia typhina* to be misapplied to the anamorph of *E. typhina* and thus invalid. Diehl (1950) applied the informal name “typhodial” to the conidial state of *E. typhina* and a similar state in *Atkinsonella* Diehl. The genus *Typhodium* Link (Link 1826) is generally considered synonymous with *Epichloë* (Clements and Shear 1931), and its application as a name for the anamorph also would be invalid. To classify the anamorphs of *Epichloë*, Morgan-Jones and Gams (1982) created a new section, *Albo-lanosa* Morgan-Jones & W. Gams within the genus *Acremonium* Link. Glenn et al. (1996) conducted a phylogenetic examination of *Epichloë* anamorphs and other species classified in *Acremonium* and concluded that the anamorphic species of *Epichloë* were placed inappropriately in the polyphyletic genus *Acremonium* (Rykard et al. 1982, Vigalys et al. 1993, Gams 1995). Glenn et al. (1996) proposed the monophyletic genus *Neotyphodium* Glenn, C.W. Bacon & Hanlin to accommodate the anamorphs of *Epichloë*. Based on molecular phylogenetic relationships, reproductive compatibilities, host ranges and morphological characters, numerous additional teleomorph and anamorph species have been proposed (Leuchtman 2003; Schardl and Leuchtman 2005; Li et al. 2006; Moon et al. 2007; Chen et al. 2009; Iannone et al. 2009, 2011; Ji et al. 2009; Kang et al. 2009, 2011; Zhang et al. 2009; Ghimire et al. 2011; Charlton et al. 2012; Tadych et al. 2012).

THE BIOLOGY OF *EPICHLÖE* SPECIES

Modern taxonomic treatment includes as sexual *Epichloë* species those that produce a very similar yellow-orange stroma on the leaf sheath surrounding the immature and developmentally

arrested grass inflorescence (FIG. 1A, B). Once mature, filamentous ascospores are forcibly ejected. On substrate, these germinate directly to produce rows of conidiophores (FIG. 1C). The structures of the conidiophores and conidia (FIG. 1D) closely resemble those of most *Neotyphodium* species observed in cultures and in association with epiphyllous hyphal nets (Moy et al. 2000). Most teleomorphic *Epichloë* species in association with most of their hosts only sporulate on some tillers, leaving others to develop normal, albeit *Epichloë*-infected, seeds. The ability of *Neotyphodium* species and most *Epichloë* species to grow in leaves (FIG. 1E), inflorescences (FIG. 1F) and seeds (FIG. 1G) enables remarkably efficient vertical transmission, a key to the evolution of mutualism (Clay and Schardl 2002).

The biology of *Epichloë* species has long been a topic of investigation and is still being explored. Their potential as plant pathogens was recognized by Prillieux, who first described a disease caused by *E. typhina* on *Phleum pratense* (Prillieux 1897). Nonpathogenic, seed-transmissible endophytes previously had been identified in other cool-season grasses, and particularly well characterized in *Lolium temulentum* (Guérin 1898, Vogl 1898, Freeman 1904). Other studies of this fungus were made by de Bary (1863, 1887) who made the connection between the intercellular hyphae of *Epichloë* species and the external stromatic hyphae that clearly determined that they were endobiotic, rather than epibiotic, on leaves and stems as suggested by Bail (1861). Similar endophytic and intercellular growth of *Epichloë* species was documented by Sampson (Sampson 1933, 1935) who also suggested that in some hosts the infections were latent, giving perennially symptomless symbioses (Sampson 1933, 1937). In ensuing decades, similar symptomless infections were substantiated and extended to include several other host grasses where only intercellular infections were visible under microscopic examination (Bacon et al. 1977, White and Cole 1986, White 1987).

The mating system of the *Epichloë* species has been shown to be bipolar heterothallic, whereby conidia produced on stromata may be vectored by symbiotic flies (*Botanophila* species) between opposite mating types of the stromata in a process that is akin to pollination (Bultman and White 1987, White and Bultman 1987, Bultman et al. 1995). The process results in formation of perithecia embedded in stromata and culminates in forcible ejection of ascospores that may initiate infections of uninfected plants (Chung and Schardl 1997, Brem and Leuchtman 1999). Experimental mating can be conducted by transferring conidia from a stroma or culture plate to the stroma of an opposite mating type, and this capacity to evaluate reproductive compatibility of distinct collections of *Epichloë* has facilitated a biological species concept to circumscribe species of *Epichloë* (White 1993, 1994b; Leuchtman and Schardl 1998; Leuchtman 2003).

An important characteristic in the evolution of grass-*Epichloë* symbioses is efficient vertical transmission via host seeds. The process of symbiont vertical transmission in *L. temulentum* was well documented by Freeman (1904), with confirmatory evidence from several later studies on *L. perenne* (Philipson 1989) and several other grasses (White and Cole 1985, 1986; White et al. 1991). The degree of vertical transmission varies between host species-symbiont species combinations, and in natural associations a majority of seeds typically bear the fungus (Afkhani and Rudgers 2008, Gundel et al. 2012). In some, vertical transmission is at or near 100%. The fungus proliferates throughout most shoot and inflorescence primordia and persists as host cells elongate, apparently by intercalary hyphal growth (Christensen et al. 2008). As florets differentiate, giving rise to ovaries and ovules, the fungus grows within these maternal tissues as well (Freeman 1904). After ovary fertilization the fungus proliferates in the remnant nucellus as well as in association with the embryo. In early seed germination, the fungus

continues its growth through the embryonic scutellum into the shoot region of the embryo and continues endophytic growth in the aerial structures of the seedling to complete the vertical transmission cycle.

In contrast to the detailed descriptions of intercellular growth and vertical transmission (Freeman 1904, Christensen et al. 2008), the precise way that sexual or asexual states of *Epichloë* move between host lineages has long eluded biologists. It is now clear that many *Epichloë* species produce conidia, not only on stromata, but also on the surfaces of leaf blades and other aerial parts of plant hosts (White et al. 1996, Moy et al. 2000, Dugan et al. 2002, Tadych et al. 2012). Recent studies also have supported the hypothesis that some *Epichloë* species may spread contagiously via water-dispersed conidia or wind-dispersed ascospores that germinate to form conidia (Bacon and Hinton 1988, 1991; Brem and Leuchtman 1999; Tadych and White 2007; Tadych et al. 2007, 2012; Leyronas and Raynal 2008), with the most likely sites of infection being meristematic zones of seedlings and tillers (Brem and Leuchtman 1999, Leyronas and Raynal 2008, Tadych et al. 2012).

Of important ecological consequence for *Epichloë* species and their hosts is the almost universal occurrence of one or more different classes of fungal alkaloids with neurotropic activities in invertebrates or vertebrates (FIG. 2). Of interest, the symptomless symbiotes are apparently more adept at the production of these alkaloids (Bacon 1988; Leuchtman et al. 2000; Panaccione 2005; Schardl et al. 2007, 2012, 2013; Torres et al. 2008). The alkaloids of *Epichloë* species generally are believed to function defensively to protect hosts from herbivores (Clay 1988), and some are toxic to livestock (Bacon et al. 1977, Fletcher and Harvey 1981). Protective characteristics such as anti-herbivore alkaloids, other benefits such as protection from nematodes (Timper et al. 2005, Bacetty et al. 2009) and drought stress (Malinowski and Belesky 2000), and

the capability of efficient vertical transmission in host seeds (Saikkonen et al. 2002, Majewska-Sawka and Nakashima 2004, Gundel et al. 2012) form a strong evolutionary underpinning for the many mutualistic grass-*Epichloë* symbioses.

IMPORTANCE, MOTIVATION AND JUSTIFICATION

Once an obscure group of fungi, the species of *Epichloë* were brought to prominence due to their presence in valuable forage grasses and associated toxicity to grazing animals (Bacon et al. 1977). Subsequently it was discovered that these symbionts play a role in host plant defense and ecology (Omacini et al. 2001, Clay and Schardl 2002). International and highly multidisciplinary research and extension groups attend meetings, symposia and conferences held periodically with their primary focus on biological, agronomical and biotechnological exploitation of clavicipitaceous endophytes (Young et al. 2012). Microbial endophytes in general are increasingly viewed as important components of sustainable agriculture, and studies on clavicipitaceous endophytes have served as one model available that can demonstrate to the public that natural associations can be useful for biological plant protection. There have been numerous patents issued for clavicipitaceous grass endophytes, their genes and their metabolites, on the basis of their beneficial effects on plants.

The dual naming system has proven to be more of an impediment than a benefit for most scientists working on the *Epichloë* species, as evidenced by the pervasive bad habit of distinguishing the sexual and apparently asexual species among them as “*Epichloë* endophytes” and “*Neotyphodium* endophytes” respectively. These terms not only reflect a misuse of taxonomic nomenclature but more importantly they belie the true and interesting diversity of evolutionary histories, life histories and host interactions in the *Epichloë* clade. For example, although stroma formation is required for sexual reproduction, at least one described

Neotyphodium species, *N. stromatolongum* (Ji et al. 2009), forms stromata on its host, although its stromata have not been observed to complete a sexual stage. The same situation is evident for *E. poae* strains identified as symbionts of *Poa secunda* subsp. *juncifolia* (Tadych et al. 2009, 2012). Many *Neotyphodium* species are interspecific hybrids lacking capacity for sexual reproduction (Moon et al. 2007), yet a hybrid *Epichloë* endophyte of *Poa pratensis* was described as *E. liyangensis* (Kang et al. 2011) based on its production of sparse perithecia with ascospores. We support a single generic classification in genus *Epichloë* following priority rules and emphasize the need for thorough characterization of the life history traits of each species and its various strains and host interactions.

We propose here that all previously described *Neotyphodium* species be synonymized under genus *Epichloë*, with the exceptions of *Acremonium chilense* (= *Neotyphodium chilense*) and *Neotyphodium starrii*. Our treatment includes 10 teleomorph-typified species and 24 anamorph-typified species, recognizes three subspecies and six varieties and involves 25 new combinations. We believe that this realignment of the described *Neotyphodium* species with *Epichloë*, and comprehensive reassessment of the taxonomy of this group, will provide a logical framework for future descriptions of taxa in the genus, as well as significantly enhancing communication of new research findings and a broader understanding of these fungi by the wider community.

TAXONOMY AND ACCEPTED *EPICHOË* TAXA

Taxonomic details (SUPPLEMENTARY TABLE I).

Teleomorph-typified species

Epichloë amarillans J.F. White, Mycologia 86:573. 1994.

Mycobank MB362599

Epichloë baconii J.F. White, Mycologia 85:449. 1993.

MycoBank MB360372

≡ *Acremonium typhinum* var. *bulliforme* J.F. White, Mycologia 84:433. 1992.

Epichloë brachyelytri Schardl & Leuchtm., Mycologia 91:104. 1999.

MycoBank MB450151

Epichloë bromicola Leuchtm. & Schardl, Mycol Res 102:1179. 1998.

MycoBank MB446562

= *Epichloë yangzii* W. Li & Z.W. Wang, Mycologia 98:562. 2006.

Epichloë elymi Schardl & Leuchtm., Mycologia 91:101. 1999.

MycoBank MB450254

Epichloë festucae Leuchtm., Schardl & M.R. Siegel, Mycologia 86:809. 1994.

MycoBank MB363267

var. *lolii* (Latch, M.J. Chr. & Samuels) C.W. Bacon & Schardl, stat. nov. et comb. nov.

MycoBank MBxxxxxx

≡ *Acremonium lolii* (as *Acremonium loliae*) Latch, M.J. Chr. & Samuels, Mycotaxon 20:537. 1984 (basionym).

≡ *Neotyphodium lolii* (Latch, M.J. Chr. & Samuels) Glenn, C.W. Bacon & Hanlin, Mycologia 88:377. 1996.

Epichloë glyceriae Schardl & Leuchtm., Mycologia 91:103. 1999.

MycoBank MB450322

Epichloë liyangensis Z.W. Wang, Y. Kang & H. Miao, Mycologia 103:1344. 2011.

MycoBank MB518666

Epichloë sylvatica Leuchtm. & Schardl, Mycol Res 102:1178. 1998.

MycoBank MB446561

Epichloë sylvatica subsp. *pollinensis* Leuchtm. & M. Oberhofer, Mycologia 105. 2013 (in press).

MycoBank MB802905

Epichloë typhina (Pers.) Tul. & C. Tul., Sel Fung Carpol 3:24. 1865.

MycoBank MB165057

≡ *Sphaeria typhina* Pers., Icones et Descriptiones Fungorum Minus Cognitorum 1:21. 1798 (basionym).

= *Acremonium typhinum* var. *fasciculatum* J.F. White, Mycologia 84:435. 1992.

var. *ammophilae* (J.F. White & Morgan-Jones) J.F. White, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium typhinum* var. *ammophilae* J.F. White & Morgan-Jones, Am J Bot 79:473. 1992 (basionym).

Epichloë typhina subsp. **clarkii** (J.F. White) Leuchtm. & Schardl, stat. nov.

MycoBank MBxxxxxxx

≡ *Epichloë clarkii* J.F. White, Mycologia 85:449. 1993 (basionym).

Epichloë typhina subsp. **poae** (Tadych, K.V. Ambrose, F.C. Belanger & J.F. White) Tadych, stat. nov.

MycoBank MBxxxxxxx

≡ *Epichloë poae* Tadych, K.V. Ambrose, F.C. Belanger & J.F. White, Fungal Divers 54:122. 2012 (basionym).

var. *aonikenkana* Iannone & Schardl, Mycologia (in review)

MycoBank MBxxxxxxx

var. *canariensis* (C.D. Moon, B. Scott & M.J. Chr.) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

= *Neotyphodium typhinum* var. *canariense* C.D. Moon, B. Scott & M.J. Chr., Mycologia 92:1113. 2000.

var. *huerfana* (J.F. White, G.T. Cole & Morgan-Jones) Tadych & Leuchtm., stat. nov. et comb. nov.

MycoBank MBxxxxxxx

= *Acremonium huerfanum* J.F. White, G.T. Cole & Morgan-Jones, Mycologia 79:150. 1987.

= *Neotyphodium huerfanum* (J.F. White, G.T. Cole & Morgan-Jones) Glenn, C.W. Bacon & Hanlin, Mycologia 88:378. 1996.

Anamorph-typified species

Epichloë aotearoae (C.D. Moon, C.O. Miles & Schardl) Leuchtm. & Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium aotearoae* C.D. Moon, C.O. Miles & Schardl, Mycologia 94:703. 2002 (basionym).

Epichloë australiensis (C.D. Moon & Schardl) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium australiense* C.D. Moon & Schardl, Mycologia 94:705. 2002 (basionym).

Epichloë cabralii Iannone, M.S. Rossi & Schardl, Mycologia (in review)

MycoBank MBxxxxxxx

Epichloë canadensis N.D. Charlton & C.A. Young, Mycologia 104:1195. 2012.

MycoBank MB563748

Epichloë chisosa (J.F. White & Morgan-Jones) Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Acremonium chisosum* J.F. White & Morgan-Jones, Mycotaxon 28:183. 1987 (basionym).

≡ *Neotyphodium chisosum* (J.F. White & Morgan-Jones) Glenn, C.W. Bacon & Hanlin, Mycologia 88:377. 1996.

Epichloë coenophiala (Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Acremonium coenophialum* Morgan-Jones & W. Gams, Mycotaxon 15:313. 1982 (basionym).

≡ *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin, Mycologia 88:377.
1996.

Epichloë danica Leuchtm. & M. Oberhofer, Mycologia 105. 2013 (in press).

MycoBank MB802904

Epichloë disjuncta Leuchtm. & M. Oberhofer, Mycologia 105. 2013 (in press).

MycoBank MB802903

Epichloë funkii (K.D. Craven & Schardl) J.F. White, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium funkii* K.D. Craven & Schardl, Mycologia 99:899. 2007 (basionym).

Epichloë gansuensis (C.J. Li & Nan) Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium gansuense* C.J. Li & Nan, Mycotaxon 90:142. 2004 (basionym).

var. **inebrians** (C.D. Moon & Schardl) Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium gansuense* var. *inebrians* C.D. Moon & Schardl, Mycologia 99:899. 2007 (basionym).

Epichloë guerinii (Guillaumin, Ravel & C.D. Moon) Leuchtm. & Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium guerinii* Guillaumin, Ravel & C.D. Moon, Mycologia 99:897. 2007 (basionym).

Epichloë hordelymi Leuchtm. & M. Oberhofer, Mycologia 105. 2013 (in press).

MycoBank MB802902

Epichloë melicicola (C.D. Moon & Schardl) Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium melicicola* C.D. Moon & Schardl, Mycologia 94:706. 2002 (basionym).

Epichloë mollis (Morgan-Jones & W. Gams) Leuchtm. & Schardl, comb. nov. et nom. nov.

MycoBank MBxxxxxxx

≡ *Acremonium typhinum* Morgan-Jones & W. Gams, Mycotaxon 15:315. 1982 (basionym).

≡ *Neotyphodium typhinum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin, Mycologia 88:377. 1996.

Epichloë occultans (C.D. Moon, B. Scott & M.J. Chr.) Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium occultans* C.D. Moon, B. Scott & M.J. Chr., Mycologia 92:1113. 2000 (basionym).

Epichloë pampeana (Iannone & Cabral) Iannone & Schardl, Mycologia (in review)

MycoBank MBxxxxxxx

≡ *Neotyphodium pampeanum* Iannone & Cabral, Mycologia 101:347. 2009 (basionym).

Epichloë schardlii (Ghimire, Rudgers & K.D. Craven) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium* × *schardlii* Ghimire, Rudgers & K.D. Craven, *Mycologia* 103:77. 2011 (basionym).

Epichloë sibirica (X. Zhang & Y.B. Gao) Tadych, comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium sibiricum* X. Zhang & Y.B. Gao, *FEMS Microbiol Lett* 301:14. 2009 (basionym).

Epichloë siegelii (K.D. Craven, Leuchtm. & Schardl) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium* × *siegelii* K.D. Craven, Leuchtm. & Schardl, *Sydowia* 53:64. 2001 (basionym).

Epichloë sinica (Z.W. Wang, Y.L. Ji & Y. Kang) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium sinicum* Z.W. Wang, Y.L. Ji & Y. Kang, *Mycologia* 101:213. 2009 (basionym).

Epichloë sinofestucaae (Y.G. Chen, Y.L. Ji & Z.W. Wang) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium sinofestucaae* Y.G. Chen, Y.L. Ji & Z.W. Wang, *Mycologia* 101:682. 2009 (basionym).

Epichloë stromatolonga (Y.L. Ji, L.H. Zhan & Z.W. Wang) Leuchtm., comb. nov.

MycoBank MBxxxxxxx

≡ *Neotyphodium stromatolongum* Y.L. Ji, L.H. Zhan & Z.W. Wang, *Mycologia* 101:202. 2009 (basionym).

Epichloë tembladerae (Cabral & J.F. White) Iannone & Schardl, *Mycologia* (in review)

MycoBank MBxxxxxxx

≡ *Neotyphodium tembladerae* Cabral & J.F. White, *Mycologia* 91:321. 1999 (basionym).

Epichloë uncinata (W. Gams, Petrini & D. Schmidt) Leuchtm. & Schardl, comb. nov.

MycoBank MBxxxxxxx

≡ *Acremonium uncinatum* W. Gams, Petrini & D. Schmidt, *Mycotaxon* 37:68. 1990 (basionym).

≡ *Neotyphodium uncinatum* (W. Gams, Petrini & D. Schmidt) Glenn, C.W. Bacon & Hanlin, *Mycologia* 88:378.

1996.

Excluded taxa

Neotyphodium starrii (J.F. White & Morgan-Jones) Glenn, C.W. Bacon & Hanlin, nomen dubium, *Mycologia* 88:377. 1996.

MycoBank MB415544

≡ *Acremonium starrii* J.F. White & Morgan-Jones, *Mycotaxon* 30:88. 1987 (basionym).

Acremonium chilense Morgan-Jones, J.F. White & Piont., *Mycotaxon* 39:444. 1990.

MycoBank MB127783

≡ *Neotyphodium chilense* (Morgan-Jones, J.F. White & Piont.) Glenn, C.W. Bacon & Hanlin, *Mycologia* 88:378. 1996.

DISCUSSION

Moving to a single genus classification of *Epichloë* and *Neotyphodium* species amounts to formal recognition of their membership in one monophyletic clade (Schardl et al. 2013), similar morphologies and common behaviors such as intercellular, systemic colonization of aerial plant tissues, seed transmissibility (in most strains) and the ability of many to produce various protective alkaloids including some that are rare (lolines) or unknown (peramine) outside this group of fungi (Schardl et al. 2013). Our taxonomic treatment recognizes 34 described *Epichloë* species, as well as several subspecies and varieties. We have adopted a species concept that is based on phylogeny and mating compatibility. In making new combinations in *Epichloë* we have sought to preserve original taxon names whenever possible. The exception is *E. mollis* for which a new name is proposed. In some cases based on phylogenetic placement (FIG. 3), current species have been assigned to subspecies or variety rank or are treated as synonyms. These include *N. lolii* as *E. festucae* var. *lolii* and three species of the *E. typhina* complex, *E. clarkii* as subsp. *clarkii*, *E. poae* as subsp. *poae* and *N. huerfanum* as var. *huerfana*. Furthermore, *E. yangzii* is considered a synonym of *E. bromicola*. In organizing the taxon list, we separated teleomorph-

typified taxa with known sexual states and anamorph-typified taxa with unknown or absent sexual states, although taxonomically this distinction is arbitrary.

Efficient vertical transmission in host seeds is a hallmark of this genus, and typifies the vast majority of *Epichloë* species, subspecies and varieties. Taxa that propagate only clonally (asexually) include *E. coenophiala*, *E. uncinata* and numerous other interspecific hybrids (Tsai et al. 1994, Moon et al. 2004). In contrast, *E. baconii* and *E. glyceriae* are known to propagate only horizontally (contagiously), whereas *Epichloë bromicola* and *E. typhina* are capable of vertical transmission in some of their hosts but are contagious in other host species (Leuchtman and Schardl 1998, Schardl and Leuchtman 1999). Although all asexual *Epichloë* species including interspecific hybrids are vertically transmitted, horizontal transmission is also a possibility for some. Epiphytic hyphal nets provide for sparse sporulation, and some asexual strains, such as *E. typhina* subsp. *poae* on *Poa secunda*, produce stromata with abundant conidia, thus providing a possible means of horizontal transmission in addition to efficient vertical transmission (Tadych et al. 2012). Dual horizontal and vertical transmission is well established for the majority of sexually reproducing *Epichloë* species, and in most hosts these fungi produce the sexual stromatal stage (“choke”) only on some host tillers while transmitting vertically in seeds produced on asymptomatic tillers (Leuchtman et al. 1994, Leuchtman and Schardl 1998, Schardl and Leuchtman 1999). Modes of transmission have been the subject of research for many decades (Sampson 1933), so it is reasonable to include some taxonomic distinction, for example at the variety level, where there are clear life-history differences. Thus, *E. typhina* subsp. *poae* includes varieties that are transmitted only vertically (as far as is known) in *Bromus setifolius* (var. *aonikenkana*), *Lolium edwardii* (var. *canariensis*) and *Festuca arizonica* (var. *huerfana*). Likewise, we have reclassified *N. lolii* as a variety of *E. festucae* in recognition of its

clear phylogenetic relationship with that species, as well as its strict vertical transmission in *L. perenne* (Latch et al. 1984, Latch and Christensen 1985).

In recent times, teleomorph-typified *Epichloë* species have been circumscribed mainly on the basis of interfertility relationships, usually with supporting phylogenetic evidence based on intron sequences from single-copy genes (White 1993, 1994b; Leuchtman et al. 1994, Leuchtman and Schardl 1998, Schardl and Leuchtman 1999). The phylogenetic information is especially important when opportunities and biological materials for mating tests are limited, as is often the case for species of restricted range and sparse or haphazard expression of stromata. Increased sampling has helped address some ambiguous species circumscriptions, for example by indicating interfertility and co-phyletic relationships of *E. clarkii* with *E. typhina*, and of *E. poae* with *E. typhina* (see FIG. 3). In some cases certain genotypes are associated with certain host species, even though strains on different hosts may be interfertile and would be considered the same species under the phylogenetic species recognition concept (Taylor et al. 2000). For such situations, we have erected the subspecies, *E. typhina* subsp. *clarkii* and *E. typhina* subsp. *poae*, to accommodate the previously described *E. clarkii* and *E. poae* respectively (White 1993, Tadych et al. 2012). Furthermore, we synonymize *E. yangzii*, described from *Roegneria* species (Li et al. 2006), with the morphologically similar *E. bromicola*, described from *Bromus erectus* (Leuchtman and Schardl 1998), based on phylogenetic analysis of closely related strains from *Elymus repens* (syn. *Agropyron repens*), *Hordelymus europaeus*, *Hordeum brevisubulatum*, *Leymus chinensis* and additional *Bromus* species (Brem and Leuchtman 2003, Yanagida et al. 2005, Lembicz et al. 2010, Oberhofer and Leuchtman 2012, Zhu et al. 2013) and supported by mating tests involving most host strains (Leuchtman unpubl). Notwithstanding the importance of phylogenetic and biological species concepts in *Epichloë* taxonomy, phylogenies and

interfertility relationships are not always concordant. In particular, *E. baconii* does not constitute a single monophyletic clade, and *E. typhina* appears to be paraphyletic to *E. sylvatica*, with which it is not interfertile (Leuchtman and Schardl 1998).

Descriptions of new asexual species and varieties have been based on their clear phylogenetic relationships to the sexual *Epichloë* species. In particular, descriptions of hybrid species have been based in large part on genotypes of sexual *Epichloë* species in their pedigrees. It is legitimate to refer to such hybrids by formulas, whereby, for example, the Lp1 strain of *L. perenne* symbiont (Schardl et al. 1994) can be designated as *E. festucae* var. *lolii* x *E. typhina*. However, there are clear indications that hybridizations have arisen on multiple occasions in several hosts worldwide, and independent hybrid origins sometimes can be discerned only by specific genotypes (DNA sequences) of the ancestral species. Thus, for example, several *E. festucae* x *typhina* hybrids have been separately described and are recognized here as *E. australiensis*, *E. pampeana* and *E. tembladerae* (Moon et al. 2007, Iannone et al. 2012). It remains unclear, however, whether the broad host distribution of *E. tembladerae* reflects multiple hybridizations involving closely related ancestors or whether this species has an exceptional ability to move between disparate hosts (Iannone et al. 2012).

Our treatment excludes only two species from genus *Epichloë*. These are *Acremonium chilense*, also recombined as *N. chilense* on the basis of an assumed but untested relationship to *Epichloë* species (Glenn et al. 1996), and *N. starrii*, described from *Festuca subulata* as *Acremonium starrii*. For *N. starrii* no ex-type material was available to establish its status and relationship within *Epichloë*. The taxon could be a distinct species or a synonym of another *Festuca*-infecting species and is excluded from the list of *Epichloë* species as nomen dubium. The identification of *A. chilense* (Morgan-Jones et al. 1990), other *Acremonium* species (Naffaa

et al. 1998, Huang and Kelemu 2004), the penicillate p-endophytes (An et al. 1993) and other seed-transmissible systemic symbionts of grasses (White et al. 1990) illustrates a variety of similar host interactions and raises the question of what characteristics are unique to *Epichloë* species and what are pertinent to a wider variety of symbionts.

We propose to classify in genus *Epichloë* all sexual *Epichloë* species and all asexual *Neotyphodium* taxa accepted in this study, as well those described and published subsequent to this proposal. We base our proposal on the principle of priority of publication in which *Epichloë* has priority over *Typhodium* and *Neotyphodium*. It is hoped that our proposed system for classification in *Epichloë* will enable future exploration and discovery in the group. We anticipate that many new species and varieties are yet to be identified. Frontiers of *Epichloë* research include evaluation of population ecology and aspects of endophyte dissemination biology. There is also much more to discover concerning the hypha-plant cell interactions in planta, as well as the mechanisms of hybrid formation and subsequent processes of selection that may explain the abundance of interspecific hybrids. Further, we anticipate many new applications to enhance plant performance that employ *Epichloë* species. Among these future applications is the likely use of endophytes as vectors for plant growth or defensive enhancement genes. Finally, clarifying the systematics of fungi now classified in *Epichloë* will provide a model for systematic reorganization of other groups of fungi.

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LEGENDS

FIG. 1. Structures of *Epichloë* species. A. Stromata of *Epichloë amarillans* before fertilization (black arrow), and after fertilization and maturation (white arrow). B. Fertilized and partially mature stroma of *E. typhina* (on *Poa trivialis*) with a larval case (arrow) of the symbiotic *Botanophila* sp. fly, surrounded by feeding tracks. C. Germinating ascospores of *Epichloë elymi* giving rise to conidiophores bearing single or multiple conidia (arrow). D. Scanning electron micrograph of conidiophores from a mass of germinating *E. amarillans* ascospores. E. Intercellular growth of *Epichloë festucae* (arrow) in leaf sheath of *Lolium perenne*. F. Inflorescence primordium of *L. perenne* with endophytic growth of *E. festucae* (arrow). G. Endophytic growth of *E. festucae* in *L. perenne* seed, beneath seed coat (arrow), and around and within the embryo. C. DIC image taken by Sladana Bec. D. Scanning electron micrograph taken by Dorothy M. Hinton. E, F, G. Confocal optical slices of transformed *E. festucae* expressing green (E) or cyan (F, G) fluorescent protein, overlain with DIC images, taken by JingGe Liu.

FIG. 2. Alkaloids produced by *Epichloë* species. Ergovaline is associated with tall fescue toxicosis in grazing livestock. Loline alkaloids such as *N*-formyllooline, as well as peramine, have anti-insect activities. The indole-diterpene, lolitrem B, is associated with ryegrass staggers in livestock.

FIG. 3. Phylogram based on alignments of a portion of the beta-tubulin (*tubB*) gene from representatives of *Epichloë* and *Neotyphodium* species. Sequences including the first three introns of *tubB* for all taxa considered in this study were obtained from GenBank and aligned with MUSCLE (Edgar 2004) without G-blocks curation; tree was inferred by maximum likelihood with PhyML (Guindon and Gascuel 2003) implemented in the phylogeny.fr website (Dereeper et al. 2008). Branch support values were estimated by the approximate likelihood-ratio test (Anisimova and Gascuel 2006) with the SH-like option, at phylogeny.fr. Leaves are labeled with proposed names (*E.* = *Epichloë*), strain designations (or GenBank gi numbers, when strains were unknown) and host names. Hybrids are indicated with circles next to the leaf labels, where different gene copies are indicated by black, white and (where three copies are present) gray fill. Included are representatives of all described species and most varieties of *Epichloë* and *Neotyphodium*. Also included are some additional taxa that have not yet had formal taxonomic description, and these are designated by abbreviated host names followed by TG- (taxonomic group) and a number (for details see SUPPLEMENTARY TABLE II). Dashed lines denote clades, which are designated by Roman numerals where they correspond to previously determined mating populations (MP) (Leuchtman and Schardl 1998; Schardl and Leuchtman 1999), in some cases suffixed to distinguish clades within an MP. The clade designated Id / VII includes representatives that are interfertile with either *E. sylvatica* (MP VII) or *E. typhina* (MP I). The tree is

unrooted, but the midpoint is placed at the left edge of the left column, and for space consideration the tree is divided into two columns with the point of division indicated by arrowheads at the bottom of the left column and top of the right column. The sequence alignment was deposited at TreeBASE (<http://purl.org/phylo/treebase>; submission ID XXXXX).

FOOTNOTES

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¹Authors contributed equally to the planning, discussion and analysis of this research and to the writing of the manuscript.

²Corresponding author. E-mail: tadych@aesop.rutgers.edu





