Greeneria uvicola, cause of bitter rot of grapes, belongs in the Diaporthales

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Bitter rot of grapes, a cosmopolitan disease, is caused by *Greeneria uvicola*, an asexually reproducing fungus with no known sexual state. Based on molecular sequence data, specifically the large subunit of the nuclear ribosomal DNA, it was determined that *G. uvicola* is a member of the Diaporthales. *Greeneria uvicola* is re-described and illustrated based on two recent collections and comparison with an authentic specimen. Data are also presented that suggest the family Magnaporthaceae, including *Magnaporthe grisea* (anamorph: *Pyricularia grisea*) and *Gaeumannomyces graminis* (anamorph: *Harpophora* sp.), plant pathogens of wheat, rice, and other grass crops, should be excluded from the Diaporthales.

Keywords: anamorph, *Diaporthe*, *Discula*, *Harpophora*, Magnaporthaceae, mitotic fungi, *Valsa*.

The majority of plant-associated microfungi reproduce asexually and lack any known sexual state as reported by Rossman (1993) based on data from Farr & al. (1989) for the United States. Although most of the mitotic fungi are derived from ascomycetes, their relationships within the sexually based taxonomic scheme are generally unknown. These ascomycetous microfungi include many devastating plant pathogens, thus knowledge of their taxonomic affinities is crucial for developing disease control measures. With increased use of molecular sequence data for reconstructing fungal evolutionary relationships at all levels (Kohn, 1992), the affinities of mitotic fungi with their sexually reproducing relatives can be determined.

The Diaporthales are a group of ascomycetes that includes about 98 genera of plant-associated fungi, particularly of woody plants as well as many herbaceous crops (Hawksworth & al., 1995). A number of serious plant diseases are caused by members of the Diaporthales including chestnut blight caused by *Cryphonectria parasitica* (Murrill) Barr and stem canker of soybeans caused by *Diaporthe*

phaseolorum (Cooke & Ellis) Sacc. The known asexual states of members of the Diaporthales are generally coelomycetous bearing their phialidic conidiogenous cells and conidia in acervuli or pycnidia with or without a well-developed stroma. At present, the extent to which the asexual states of diaporthalean fungi have become independent entities without known sexual states is unknown. However, studies of the large genus *Diaporthe* Nitschke and its asexual state *Phomopsis* (Sacc.) Bubák reveal that many asexual species of *Phomopsis* exist that are apparently no longer closely related to sexual states (Mostert & al., 2001; unpublished data).

Greeneria uvicola causes bitter rot of grapes, a disease that is common on muscadine and bunch grapes throughout the world. The fungus overwinters on stem lesions and mummified berries. Although *G. uvicola* can infect leaves, tendrils and stems, this disease primarily damages fruit especially if rainy weather persists into the harvest season. The fungus is known to attack several species of *Vitis* including *V. aestivalis* Michx., *V. labrusca* L., *V. rotundifolia* Michx. and *V. vinifera* L. *Greeneria uvicola* is an asexually reproducing fungus with no known sexual state. In order to determine its taxonomic affinities, an isolate of *G. uvicola* was included in a study of the molecular systematics of perithecial ascomycetes.

Material and methods

Isolation and maintenance of cultures

Newly sequenced isolates used in this study are listed in Tab. 1. GenBank sequence numbers from previously sequenced isolates are included with the species name in Fig. 1. Isolates obtained from specimens were grown from single ascospores or conidia plated on agar of 1.7% Difco Corn Meal (CM) plus 0.2% dextrose and antibiotics. Germinated spores were transferred to 3.9% Difco Potato Dextrose Agar (PDA) plates for observation. *Greeneria uvicola* was isolated from surface-sterilized diseased stem lesions of *Vitis labrusca* plated on PDA. The tissue was washed in sterile distilled water, surface sterilized in 10% chlorox, and washed two more times in sterile distilled water. All isolates were maintained on CM agar slants and as plugs in 10% glycerol-water cultures at 4 C (Burdsall & Dorworth, 1994). The colony description is based on cultures grown on PDA incubated at 25 C with 12 h periods of fluorescent cool white light. To stimulate fruiting body production, cultures were grown on autoclaved 20–40 × 1–2 mm stems of alfalfa (*Medicago sativa* L.) on water agar and incubated at 25 C with 12 h periods of fluorescent cool white light. For examination of micro-

scopic features, material was mounted in 3% KOH. Conidiomata were sectioned using a freezing microtome and the sections mounted in lactic acid with cotton blue. Colors were determined using Kornerup & Wanscher (1978). Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with bright-field and fluorescence illumination. Calcofluor was used as the fluorescent dye. Measurements were taken using a Spot digital camera (Diagnostic Instruments, Sterling Heights, Michigan) and ImagePro software (Media Cybernetics, Silver Spring, Maryland).

Nucleic acid extraction and PCR amplification

Mycelium for DNA extraction was grown in shaker flasks at 125 rpm for 5–10 d in 100 mL liquid CYM (Raper & Raper, 1972) at room temperature under ambient light conditions. Mycelium was harvested by vacuum filtration on Whatman No. 1 filter paper and freeze-dried prior to DNA extraction. Alternatively, DNA was extracted directly from actively growing surface mycelium scraped from PDA plates. DNA was extracted with the Plant DNeasy Mini kit (Qiagen Inc., Chatsworth, California) according to the manufacturer's instructions using approximately 15 mg dried tissue or 50 mg fresh mycelium.

A fragment consisting of approximately 1400 base pairs of the 5' end of the large subunit nuclear ribosomal DNA (nLSU) was amplified in 50 µL reactions on a GeneAmp 9700 thermal cycler (PEBio/ABI, Foster City, California) under the following reaction conditions: 10–15 ng of genomic DNA, 200 mM each dNTP, 2.5 units Amplitaq Gold (PEBiosystems, Foster City, California), 25 pmoles each of primers LR0R and LR7 (Vilgalys & Hester, 1990) and the supplied 10X PCR buffer with 15 mM $MgCl_2$. The thermal cycler program was as follows: 10 min at 95 C followed by 35 cycles of 30 sec at 94 C, 30 sec at 55 C, 1 min at 72 C, with a final extension period of 10 min at 72 C. Following amplification, the PCR products were purified with QIAquick columns (Qiagen Inc., Chatsworth, California) according to the manufacturer's instructions. Amplified products were sequenced with the Big Dye kit (PEBiosystems, Foster City, California) on an ABI 310 or ABI 377 automated DNA sequencer using the following primers: LR0R, LR3R, LR5R, LR7, LR5, LR3 (Vilgalys & Hester, 1990).

Sequence analysis

Raw sequences were edited using Sequencher version 4.05 for Windows (Gene Codes Corporation, Ann Arbor, Michigan). Alignments were manually adjusted using GeneDoc 2.6.001 (Nicholas & Nicholas, 1997). Trees were inferred by the neighbor-joining (NJ)

Tab. 1. – Isolates of newly sequenced diaporthalean fungi.

Scientific Name	Locality	Host	Strain and Herbarium Specimen Number	GenBank Accession Number
Coniella fragariae (Oudem.) B. Sutton*	Minnesota: St. Paul	*Lythrum salicaria*, isol. E. Katovich	ATCC PTA-275 (= AR 3382, BPI 747949	AF362553
Diaporthe arctii (Lasch) Nitschke	New Jersey: Union Co., Clark	*Ambrosia trifida*, coll. & isol. G. Bills	AR 3450 (= GB 6421, = CBS 109490), BPI 747273	AF362562
Diaporthe eres Nitschke	Austria: Niederoesterreich, Losenheim	*Corylus avellana*, coll. W. Jaklitsch, isol. A. Rossman	AR 3519 ex WJ 1605, = CBS 109497, BPI 747936	AF362565
Diaporthe medusaea Nitschke	Austria: Vienna, Risenbergbach-Weg	*Laburnum anapyroides*, coll. W. Jaklitsch, isol. A. Rossman	AR 3422 ex WJ 1443, = CBS 109492, BPI 748231	AF362560
Discula destructiva Redlin*	Maryland: Catoctin Mt. Park	*Cornus florida*, isol. S. Redlin, ex-type	ATCC 76230, BPI 1107735	AF362568
Endothia gyrosa (Schwein. : Fr.) Fr.*	Maryland: Anne Arundel Co.	*Quercus* sp., on roots, coll. & isol. A. Rossman	AR 3396, = CBS 109494, BPI 748238	AF362555
Gaeumannomyces graminis (Sacc.) Arx & D.L. Olivier var. *avenae* (E.M. Turner) Dennis*	Great Britain	*Avena* sp., from Ann Osbourn via Joan Henson	AR 3400	AF362556
Gaeumannomyces graminis var. *graminis**	Georgia	*Glycine* sp., from Craig Rothrock via Joan Henson	AR 3401	AF362557
Gnomonia setacea (Pers. : Fr.) Ces. & De Not.	New Jersey: Huntendon Co., Califon	*Quercus prinus*, coll. & isol. G. Bills	AR 3451 (= GB 6418, = CBS 109523), BPI 747274	AF362563
Gnomoniella fraxinii (Peck) Redlin & Stack*	Maryland: Crofton	*Fraxinus pennsylvanica*, isol. S. Redlin	AR 2789 (= SR 96003, = CBS 109498)	AF362552
Greeneria uvicola (Berk. & M. A. Curtis) Punith.*	Ohio: Perryville	*Vitis labrusca*, 'Concord', isol. from diseased stems by Omer Erincik	OH-35, = CBS 109667, BPI 748239	AF362570

Tab. 1 (cont.). – Isolates of newly sequenced diaporthalean fungi.

Scientific Name	Locality	Host	Strain and Herbarium Specimen Number	GenBank Accession Number
Leucostoma nivea (Hoffm. : Fr.) Höhn.	Russia: Primorsky Territory, Vladivostok vicinity	*Populus* sp., coll. L. Vasilyeva, isol. A. Rossman	AR 3413, = CBS 109489, BPI 748232	AF362558
Magnaporthe grisea (Cooke) Sacc.*	Pennsylvania	*Lolium perenne* L., isol. W. Uddin	AR 3390	AF362554
Melanconis alni Tul.	Russia: Sakhalin Island	*Duschekia maximowiczii*, coll. L. Vasilyeva, isol. A. Rossman	AR 3529, = CBS 109496, BPI 748233	AF362566
Melanconis stilbostoma (Fr.) Tul.	Russia: Sakhalin Island	*Betula* sp., coll. L. Vasilyeva, isol. A. Rossman	AR 3548, = CBS 109493, BPI 748234	AF362567
Schizoparme straminea Shear	Virginia: Arlington Farms	*Rosa rugosa*, coll. & isol. C. L. Shear, ex-type	CBS 149.22, BPI 797000	AF362569
Valsa ambiens (Pers. : Fr.) Fr.	Austria: Niederoesterrich, Losenheim	*Fagus sylvatica*, coll. W. Jaklitsch, isol. A. Rossman	AR 3516 ex WJ 1607, = CBS 109491, BPI 748237	AF362564
Valsa germanica Nitschke	Austria: St. Margareten	*Salix alba*, coll. W. Jaklitsch, isol. A. Rossman	AR 3427 ex WJ 1426, = CBS 109495, BPI 748236	AF362561
Valsa mali Miyabe & G. Yamada	Russia: Primorsky Territory, Vladivostok vicinity	*Malus* sp., coll. L. Vasilyeva, isol. A. Rossman	AR 3417, = CBS 109499, BPI 748235	AF362559

* Anamorph isolates.

method (Kimura 2-parameter distance calculation) and by maximum parsimony (MP) using the heuristic search option with the random addition sequence (10 replications) and the branch swapping (tree bisection-reconnection) option of PAUP 4.0b8 (Swofford, 1998). For both types of analyses, ambiguously aligned characters and extremely variable regions were excluded. Additionally for parsimony analysis, only the remaining parsimony informative characters were included in the analysis. All molecular characters were unordered and given equal weight during the analysis. Gaps were treated as missing data in the parsimony analysis and, for the neighbor joining analysis, missing or ambiguous sites were ignored for affected pairwise comparisons. Relative support for the branches was estimated with 100 bootstrap replications (Felsenstein, 1985). The sequence alignment was deposited as TreeBASE S613.

Results

The alignment consisted of 900 total characters of which 124 positions were excluded. Of the remaining 776 characters, 202 were parsimony informative. The NJ tree (Fig. 1) and the consensus MP tree (not shown) were not markedly different in branching order among the diaporthalean taxa, differing only in the placement of *Endothia gyrosa* as basal to the *Melanconis/Gnomonia* group in the MP analysis. The MP analysis grouped the Sordariales with the Xylariales, the Microascales with the *Plectosphaerella/Glomerella/Verticillium* group and did not group the Microascales, Halosphaeriales and Hypocreales. Parsimony analysis of the nLSU data set resulted in two most parsimonious trees (length = 758, CI = 0.420, RI = 0.705, and RC = 0.296) using 38 ingroup and two outgroup taxa, differing from one another only in the branching order of the *Valsa* taxa (trees not shown). Including all available characters in the alignment in the analyses did not affect overall topology in NJ or MP analysis (trees not shown), although, for MP analysis of parsimony informative characters only (277 characters), the number of MP trees increased to 4 and the tree scores decreased slightly (CI = 0.413, RI = 0.675, RC = 0.279). Bootstrap support for the major lineages did not markedly differ when including all available characters in the analyses.

Using a member of the Eurotiales and the loculoascomycetous Pleosporales as outgroups, the perithecial ascomycetes, or class Sordariomycetes (Eriksson & al., 2001), grouped together with 100% bootstrap for both NJ and MP analyses. This tree includes representatives of the major orders of pyrenomycetous ascomycetes each of which are relatively well supported. The remaining members of the group are three somewhat disparate elements, *Glomerella cingulata* and *Plectosphaerella cucumerina*, each considered a member of the

Sordariomycetes of uncertain disposition (Eriksson & al., 2001), and the closely allied asexual species, *Verticillium dahliae*. The Hypocreales, represented by members of the three families, Hypocreaceae, Clavicipitaceae, and Nectriaceae, group together with 86% bootstrap for NJ analysis and 79% for MP analysis. As an order the Xylariales with three included taxa are well-supported, grouping together with 98% and 81% bootstrap values for NJ and MP analyses, respectively.

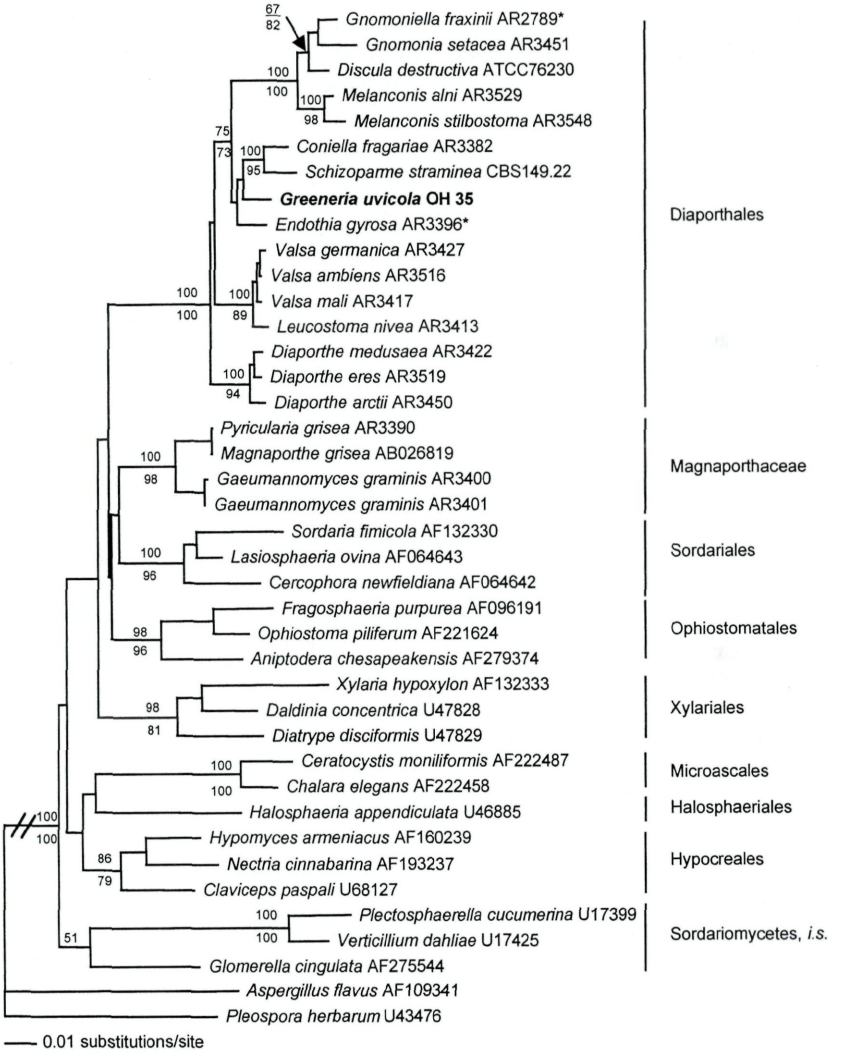


Fig. 1. – Phylogenetic analysis of large subunit nuclear rDNA sequence data. – Bootstrap values are indicated above and below branches leading to major lineages. NJ = above; MP = below. Asterisks indicate that anamorph isolates were sequenced.

The Sordariales is also distinctly supported by 100% and 96% bootstrap values for NJ and MP analyses, respectively.

The family Magnaporthaceae P. F. Cannon is represented by two varieties of *Gaeumannomyces graminis* and one asexual isolate of *Pyricularia grisea* (teleomorph: *Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udagawa) as well as a GenBank sequence for *M. grisea*. All representative isolates of this family group together in a strongly supported taxon with bootstrap values of 100% for NJ and 98% for MP analyses. Although previously considered a member of the Diaporthales, the Magnaporthaceae falls outside of the Diaporthales grouping with the Sordariales in the NJ analysis, although without any bootstrap support.

The Diaporthales represented here by 16 teleomorphic and anamorphic taxa is a strongly supported group with 100% bootstrap values for both NJ and MP analyses. Within the order several distinct groups are evident that correlate with established lineages. Three species of *Diaporthe* are represented including *D. eres*, the type of the genus, which grouped together with bootstrap values are 100% and 94% for NJ and MP analyses, respectively. Likewise, the genus *Valsa* Fr. including the type, *V. ambiens*, and two other species, and the closely related *Leucostoma* (Nitschke) Höhn. represented by *L. nivea* form a strongly supported group with bootstrap values of 100% and 89% for NJ and MP analyses, respectively. The anamorphic species, *Greeneria uvicola*, falls clearly within the Diaporthales and groups with the lineage that also includes *Coniella fragariae, Schizoparme straminea*, and *Endothia gyrosa* although the bootstrap value for this group as a whole is less than 50%. The genus *Schizoparme* represented by the type species, *S. straminea,* groups closely with the anamorphic species *Coniella fragariae* having 100% and 95% bootstrap values for NJ and MP analyses, respectively. Two species of *Melanconis*, the type, *M. stilbostoma*, and *M. alni*, group together with 100% and 98% bootstrap values for NJ and MP analyses, respectively. Three taxa, including the type species of *Gnomonia* Ces. & De Not., *G. setacea*, a conidial isolate of *Gnomoniella fraxini* and an isolate of the asexual fungus *Discula destructiva*, are representative of the Gnomoniaceae and form a group with 67% and 82% bootstrap values for NJ and MP analyses, respectively. This family is allied with the two species of *Melanconis* at a 100% bootstrap value for both kinds of analyses.

Taxonomy

Greeneria uvicola (Berk. & M. A. Curtis) Punith., Mycol. Pap. 136: 6. 1974. – Figs. 1–12.

≡ *Phoma uvicola* Berk. & M.A. Curtis, Grevillea 2: 82. 1873.

= *Greeneria fuligineum* Scribn. & Viala, Compt. Rend. 105: 473. 1887.

≡ *Melanconium fuligineum* (Scribn. & Viala) Cavara, Atti Ist. Bot. Univ. Pavia, ser. 2, 1: 359. 1888.

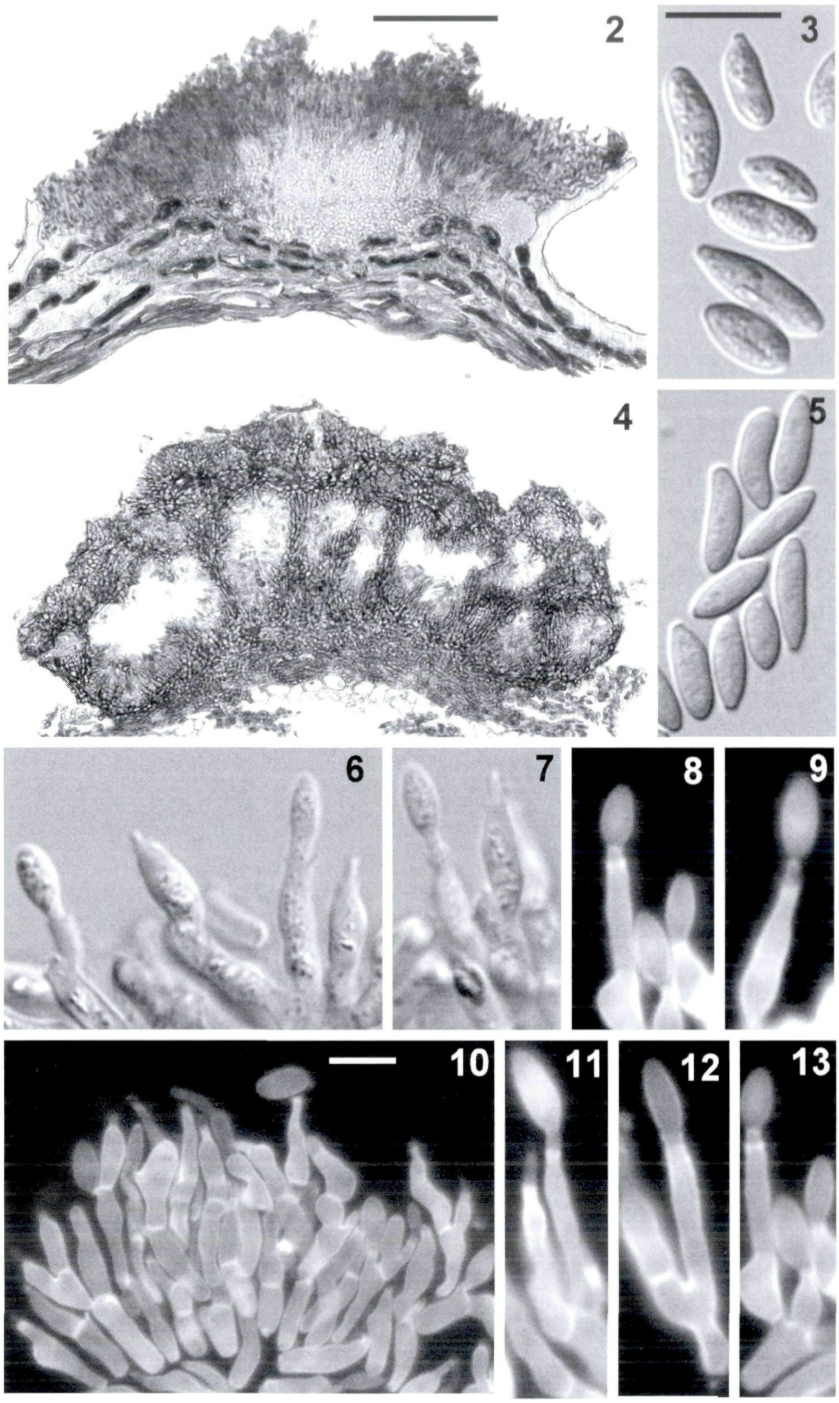
= *Myrothecium convexum* Berk. & M.A. Curtis, Grevillea 3: 99. 1875.

Conidiomata on berries 200–550 µm diam, subcuticular, acervular with a central, well-developed, pale brown, pseudoparenchymatous layer that becomes thinner or absent at the margin of the conidiomata. Branched conidiophores forming a compact layer above the pseudoparenchymatous tissue and extending beyond at the margins. Conidiomata in culture on alfalfa stems 300–500 µm diam, with a basal pseudoparenchymatous tissue becoming convoluted and multiloculate, dehiscence irregular. Conidiomata in culture pycnidial, scattered, pale brown, 140–200 µm, spherical, wall of *textura intricata*, 15–25 µm broad, hyphae of wall slightly thickened, dehiscence irregular. – Conidiogenesis phialidic. – Conidiogenous cells frequently proliferating percurrently, usually with thin periclinal thickenings, evident as flared collarettes. – Conidia pale brown, smooth, variously shaped ranging from fusiform, oval, to ellipsoidal, each with a truncate base and obtuse to bluntly pointed apex; conidia on berries 6.0–13.0 × 3.3–5.2 µm, on alfalfa stems in culture 6.6–9.4 × 2.8–3.7 µm.

Colony characteristics on PDA: surface mycelium dense with a felty to cottony surface, gray to orange gray (6B1–6B2), thick mat with four evenly spaced zones, each zone demarcated by a thin line of aerial hyphae, with numerous black dots from the masses of conidia scattered over the surface, reaching the edge of a 100 mm plate in 7 days at 25 C.

Specimens examined. – USA: North Carolina, Greenville, on fruit of *Vitis labrusca*, 16. 9. 1961, John McGrew (BPI 402079 as *Melanconium fuligineum*); Fayetteville, on *Vitis* sp., 7.1887, Scribner & Viala (BPI 1104521 as *Greeneria fuliginea* in Ellis & Everhart North American Fungi Second Series – 2071). Ohio, near Perryville, isolated from diseased stem of *Vitis labrusca* "Concord", 23. 6. 2000, Omer Erincik, OH-35, = CBS 109667 (BPI 748239).

Our morphological observations supplement the description provided by Sutton & Gibson (1977) in several ways. First, we observed that the conidiomata of *Greeneria uvicola* produce a well-defined, basal pseudoparenchymatous layer on both the fruit and alfalfa stems (Figs. 2, 4) that has not been illustrated previously. Secondly, although Sutton & Gibson (1977) described the conidiogenous cells as phialidic, we found that, in addition, they often proliferate percurrently as shown in Figs. 6–13. Finally, there is some variability in conidial size. While Sutton & Gibson (1977) stated that the conidia were 7.5–10 × 3–4 µm, the conidia of *G. uvicola* on fruit (BPI 402079) measured 6.0–13.0 × 3.3–5.2 µm while the culture from



Figs. 2–13. *Greeneria uvicola*. – 2. Conidiomata on fruit of *Vitis*. – 3. Conidia from fruit of *Vitis*. – 4. Conidiomata in culture on alfalfa stems. – 5. Conidia in culture on alfalfa stems. – Figs. 6–13. Conidiogenesis. – Figs. 2–3, 6–13. BPI 402079. Figs. 4–5. BPI 748239. – Scale bar: Figs. 2, 4 = 50 µm; 3, 5–9, 11–13 = 10 µm; 10 = 10 µm.

Ohio on alfalfa stems (BPI 748239) produced conidia that were 6.6–9.4 × 2.8–3.7 µm.

The monotypic genus *Greeneria* based on *G. fuligeneum* was described by Scribner & Viala (1887). Later, this species was transferred to *Melanconium* Link : Fr. (Cavara, 1888); the fungus was known as *Melanconium fuligineum* for many years and is often reported under that name. Punithalingam (1974) determined that *Phoma uvicola* provided an earlier epithet for this species, recognized that it was unlike many of species of *Melanconium,* and transferred the earlier epithet to *Greeneria* as *G. uvicola.*

Greeneria uvicola has been confused with another diaporthalean asexual fungus on grapes, namely *Phomopsis viticola* (Sacc.) Sacc., but *G. uvicola* is distinguished by the pale brown conidia and acervular fruiting bodies. The isolate used in this study was originally isolated as a *Phomopsis*. Similar to *G. uvicola*, isolates of *Phomopsis vaccinii* Shear are known to infect the fruits of blueberries causing a disease known as blueberry twig blight and fruit rot (Caruso & Ramsdell, 1995). In a study of the isolates of *Phomopsis vaccinii* Shear from *Vaccinium corymbosum* L. and *V. macrocarpon* Aiton, we determined that a strain isolated from fruit was identical to strains isolated from other parts of the plant (unpublished data). *Greeneria uvicola* is somewhat unusual for anamorphs in the Diaporthales in having pale brown conidia. Dark brown conidia are produced in a number of diaporthalean anamorphs including the anamorphs of *Melanconis* Tul & C. Tul., often described as species of *Melanconium*, as well as species of *Coniella* Höhn. including the anamorph of *Schizoparme straminea*. The placement of *G. uvicola* in the Diaporthales agrees with the general biology of members of the Diaporthales. Many species are facultative, occasionally virulent, pathogens, of woody plants while other species such as *S. straminea* occur on herbaceous plants as hemibiotrophs rarely as obligate biotrophs.

Discussion

Greeneria uvicola in the Diaporthales

The analyses of nLSU data as presented in Fig. 1 place *G. uvicola* in the Diaporthales. *Greeneria uvicola* is similar to most diaporthalean anamorphs in producing phialidic conidiogenous cells in acervuli or pycnidia that may or may not be associated with stromatic tissue. Although occasional reference is made to anamorphic states of Diaporthales being holoblastic (Barr, 1978; Kobayashi, 1970), the anamorphic states of diaporthalean fungi examined in detail have proven to be phialidic, with or without percurrent pro-

liferation (Farr & Rossman, 2001; Kobayashi, 1970; Mostert & al., 2001; Redlin, 1991; Redlin & Stack, 1988). The genus *Greeneria* is placed in the Phialostromatineae by Sutton (1980) for coelomycetous genera having acervuli and phialidic conidiogenous cells. The diaporthalean taxa allied with *Greeneria* in this study are *Endothia–Endothiella* and *Schizoparme–Coniella* and, more distantly, with *Gnomonia–Discula* and *Melanconis–Melanconium*. *Endothia gyrosa*, represented by its anamorph of *Endothiella gyrosa* Sacc., has phialidic conidiogeny produced in multiloculate pycnidial fruiting bodies (Sutton, 1980). The anamorphic genus *Coniella* produces phialides (Sutton, 1980) and is represented here by *Coniella fragariae* and *Schizoparme straminea* [anamorph *Coniella castaneicola* (Ellis & Everh.) Sutton; Samuels & al., 1993]. While the anamorph of *G. setacea* is unknown, *Gnomoniella fraxini,* represented by its anamorph *Discula fraxinea*, is similar to *Discula destructiva*, both having phialides with elongated, narrow, non-annellated necks (Redlin, 1993; Redlin & Stack, 1988). *Discula destructiva* is similar to *Greeneria uvicola* in lacking a known teleomorph (Zhang & Blackwell, 2001). The anamorph of *Melanconis stilbostoma, Melanconium bicolor* Nees ex Kuntze, and anamorph of the congeneric species, *M. alni*, both produce brown to olive-brown conidia borne on phialides (Kobayashi, 1970). More distantly related to *Greeneria* are the two large diaporthalean genera, *Valsa* and *Diaporthe,* each represented by several taxa including their respective type species. The anamorph of *Valsa* is *Cytospora* Ehrenb. while that of *Diaporthe* is *Phomopsis*. Both anamorph genera have phialidic conidiogenous cells developing in uni- to multiloculate pycnidia (Sutton, 1980).

In examining relationships among genera of the Diaporthales, the results presented here do not agree with placement of these genera in the currently accepted families. Based on the available literature, Eriksson & al. (2001) include *Melanconis* and *Schizoparme* in the Melanconidaceae while *Diaporthe*, *Endothia, Gnomonia, Leucostoma* and *Valsa* are placed in the Valsaceae. In agreement with Zhang & Blackwell (2001), our results suggest that *Gnomonia* and *Melanconis* form a lineage distinct from *Schizoparme, Endothia*, and the anamorph genus *Greeneria*. The large genera *Diaporthe* and *Valsa* also represent major lineages but are not especially closely related. Although one can speculate on the as-yet unfound sexual state of *G. uvicola*, the lack of bootstrap support grouping it with any of the taxa in our tree suggests that this anamorphic species is not closely related to any of the major lineages in this study.

Magnaporthaceae outside the Diaporthales

Although for many years the genera *Magnaporthe* R. A. Krause & R. K. Webster and *Gaeumannomyces* von Arx & D. A. Olivier were

included in the Diaporthales (Barr, 1978; Cannon, 1988; Monod, 1983; Yaegashi & Udagawa, 1978) or the Amphisphaeriales for *Magnaporthe* (Eriksson & Hawksworth, 1993), these genera are now placed in their own family, the Magnaporthaceae established by Cannon (1994). The asexual states of species of *Magnaporthe* are recognized as *Pyricularia* and "*Phialophora*" sensu lato while those of *Gaeumannomyces graminis*, previously in the heterogeneous *Phialophora* Medlar, have recently been placed in the genus *Harpophora* W. Gams (Gams, 2000). In describing this family with six genera, Cannon (1994) reviewed its ordinal placement and concluded only that it might be excluded from the Diaporthales based primarily on the biology of these fungi. Genera of the Magnaporthaceae are primarily virulent pathogens of grasses and other monocotyledonous plants, often attacking the plant roots. Members of the Diaporthales are primarily facultative pathogens sporulating on recently killed tissues, although a few serious pathogens are systemic causing cankers and dieback symptoms. In agreement with the findings of Zhang & Blackwell (2001), our molecular sequence data confirm the placement of *Gaeumannomyces* and *Magnaporthe* in a distinct family outside the Diaporthales. Despite attempts to determine their taxonomic affinities, members of the Magnoporthaceae were not found to be closely related to any major lineage within the Sordariomycetes.

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