Persoonia 22, 2009: 63–74
www.persoonia.org

RESEARCH ARTICLE

doi:10.3767/003158509X430930

# Molecular systematics of the cotton root rot pathogen, *Phymatotrichopsis omnivora*

S.M. Marek[1], K. Hansen[2], M. Romanish[3, 4], R.G. Thorn[3]

**Key words**

*Ozonium*
*Pezizales*
Phylogeny
Phymatotrichum root rot
*Pulchromyces fimicola*
rDNA
RPB2
Texas

**Abstract** Cotton root rot is an important soilborne disease of cotton and numerous dicot plants in the south-western United States and Mexico. The causal organism, *Phymatotrichopsis omnivora* (= *Phymatotrichum omnivorum*), is known only as an asexual, holoanamorphic (mitosporic) fungus, and produces conidia resembling those of *Botrytis*. Although the corticoid basidiomycetes *Phanerochaete omnivora* (*Polyporales*) and *Sistotrema brinkmannii* (*Cantharellales*; both *Agaricomycetes*) have been suggested as teleomorphs of *Phymatotrichopsis omnivora*, phylogenetic analyses of nuclear small- and large-subunit ribosomal DNA and subunit 2 of RNA polymerase II from multiple isolates indicate that it is neither a basidiomycete nor closely related to other species of *Botrytis* (*Sclerotiniaceae*, *Leotiomycetes*). *Phymatotrichopsis omnivora* is a member of the family *Rhizinaceae*, *Pezizales* (*Ascomycota*: *Pezizomycetes*) allied to *Psilopezia* and *Rhizina*.

**Article info** Received: 29 May 2008; Accepted: 23 February 2009; Published: 11 March 2009.

## INTRODUCTION

A devastating disease of cotton in Texas, which caused large numbers of plants in affected areas to suddenly wilt and die, was first reported in the 1880s (Pammel 1888, 1889). The disease has been variably called cotton root rot (after the major crop host), Texas root rot (for the centre of distribution), or Ozonium or Phymatotrichum root rot (for the former names of the causal organism). It has since remained a considerable economic concern, causing up to $ 100 million in annual losses to the US cotton crop alone (based on disease loss estimates and price data for 1980–2008; provided by the National Cotton Council of America, www.cotton.org). The average loss of raw cotton fibre yield has been estimated to be 3.5 % in Texas and 2.2 % in Arizona, with losses ranging from 8–13 % in severely infested areas (Kenerley & Jeger 1992). The causal agent is a soilborne fungus known as *Phymatotrichopsis omnivora* or, more commonly, *Phymatotrichum omnivorum* (Streets & Bloss 1973, Kenerley & Jeger 1992, Kirkpatrick & Rothrock 2001; see below for taxonomic authorities). This species is capable of infecting more than 2 000 species of dicots (Streets & Bloss 1973), arguably the largest host range of any plant pathogen. It also causes severe losses in alfalfa, vegetable crops, grapes, and fruit and nut orchards throughout its range, which stretches from eastern Texas and southern Oklahoma west through Arizona and south into Mexico (Streets & Bloss 1973). Generally, infected plants quickly wilt in the summer, and almost inevitably die, usually in large circular patches in the field (Fig. 1a, b). Below ground, the taproots of wilted plants are rotted and usually covered with mycelial strands of the causal fungus (Fig. 1c).

[1] Department of Entomology and Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078-3033, USA.
[2] Harvard University Herbaria, 22 Divinity Ave., Cambridge, MA 02138, USA; Present address: Swedish Museum of Natural History, Department of Cryptogamic Botany, P.O. Box 50007, 104 05 Stockholm, Sweden.
[3] Department of Biology, University of Western Ontario, London, Ontario, N6A 5B7, Canada; corresponding author e-mail: rgthorn@uwo.ca.
[4] Present address: Department of Medical Genetics, University of British Columbia, Vancouver, BC, V5Z 1L3, Canada.

### *Taxonomy*

The confused taxonomic history of the cotton root rot fungus goes back more than a century. The causal agent was first identified by W.G. Farlow as *Ozonium auricomum* Link, based on nonsporulating mycelium associated with diseased roots (Pammel 1888). However, this name now applies to the asexual state of *Coprinellus* (*Coprinus*) *domesticus* and related species (Shear 1907, Orton & Watling 1979, Redhead et al. 2001). The cotton root rot fungus was described as a new species of *Ozonium, O. omnivorum* Shear (1907), again based on non-sporulating mycelium associated with diseased roots. Later, a conidial stage was found forming sporemats on soil surrounding diseased plants and was named *Phymatotrichum omnivorum* (Shear) Duggar (1916).

A hydnoid homobasidiomycete fruiting body was found associated with diseased plants and named *Hydnum omnivorum* Shear (1925), once again based on a different type specimen (C.L. Shear 5267, BPI 259732) from that of *Ozonium omnivorum* or *Phymatotrichum omnivorum*. Later, a corticioid homobasidiomycete fruiting body was discovered in a culture of *Phymatotrichum omnivorum* and identified as *Sistotrema brinkmannii* (Baniecki & Bloss 1969). Basidiospores of the *Sistotrema* failed to form the mycelium of *Phymatotrichum*, and Weresub & LeClair (1971) considered this report to be based on a homothallic culture contaminant.

The type species of *Phymatotrichum*, *P. gemellum* Bonord., was shown to be a member of *Botrytis* by Hennebert (1973). Hennebert (1973) believed that the name *Phymatotrichum omnivorum* should be attributed to Duggar alone since it was based on different specimens than examined by Shear (1907) when he described *Ozonium omnivorum*, and because the distinguishing features described by Duggar (the conidia) were not present in the type of *Ozonium omnivorum* (C.L. Shear 1447, BPI 455660). *Phymatotrichum omnivorum* was transferred to *Phymatotrichopsis omnivora* (Duggar) Hennebert and *Phymatotrichum fimicola* Dring to *Pulchromyces fimicola* (Dring) Hennebert.

**Table 1** Species used in molecular phylogenetic analyses, specimen information and GenBank accession numbers. New sequences generated for this study are indicated with GenBank numbers in **bold**.

| Species | Vouchers, Isolates, Strains (Herbarium[1])[2] | GenBank Accession Numbers | | | | |
|---|---|---|---|---|---|---|
| | | SSU | ITS | LSU | RPB2 | β-tub[3] |
| *Aleuria aurantia* | OSC 100018 | AY544698 | – | AY544654 | DQ247785 | – |
| *Anthracobia* sp. | OSC 100026 | AY544704 | – | AY544660 | – | – |
| *Ascobolus carbonarius* | KH 00.008 (C) (dubl. OSC 100079) | AY544720 | – | AY500526 | – | – |
| *Ascobolus crenulatus* | KH.02.005 (C) (dubl. OSC 100082) | AY544721 | – | AY500527 | – | – |
| *Ascodesmis nigricans* | CBS 389.68 | – | – | DQ168335 | – | – |
| *Ascodesmis sphaerospora* | RK 95.55 (O) | U53372 | – | – | – | – |
| *Balsamia magnata* | JMT 13020 (OSC) | U42656 | – | U42683 | – | – |
| *Barssia oregonensis* | RF 533 (OSC) | U42657 | – | U42684 | – | – |
| *Boudiera acanthospora* | ARON 2167 (O) | U53373 | – | – | – | – |
| *Boudiera tracheia* | Rana 79.049 (C) | – | – | AY500530 | – | – |
| *Byssonectria terrestris* | SSU: UME 29218, LSU: KS-94-4 (C) | Z30241 | – | AY500531 | AY500504 | – |
| *Caloscypha fulgens* | DJ053103-2 | DQ247807 | – | DQ247799 | DQ247787 | – |
| *Cazia flexiascus* | JMT 12993 (OSC) | U42666 | – | U42694 | – | – |
| *Cheilymenia stercorea* | KH04282003-4 (dubl. OSC 100034) | AY544705 | – | AY544661 | DQ471123 | – |
| *Chorioactis geaster* | SSU: mh 694 (FH), LSU: H.W. Keller & K.C. Rudy s.n. (FH) | AF104340 | – | AY307944 | – | – |
| *Choiromyces venosus* | JMT 7014 (OSC) | U42661 | – | U42688 | – | – |
| *Cookeina tricholoma* | SSU: mh 686 (FH), LSU: 1D-D5 (FH) | AF006311 | – | AY945860 | – | – |
| *Desmazierella acicola* | SSU: 'Norway' (FH), LSU: RK 95.12 (Herb. Roy Kristiansen) | AF104341 | – | AY945854 | – | – |
| *Dingleya verrucosa* | JMT 12617 (OSC) | U42659 | – | U42686 | – | – |
| *Discina macrospora* | NSW 4498 (MICH) | U42651 | – | U42678 | – | – |
| *Disciotis venosa* | OSC 100045 (dubl. NRRL 22213) | U42643/AY544711 | – | U42670/AY544667 | DQ470892 | – |
| *Donadinia* sp. | mh 669 (FH) | AF104342 | – | DQ220329 | – | – |
| *Eleutherascus lectardii* | CBS 626.71 | DQ062997 | – | DQ168334 | DQ470918 | – |
| *Fischerula subcaulis* | JMT 1889 (OSC) | U42646 | – | U42673 | – | – |
| *Galiella rufa* | mh 101 (FH) | AF004948 | – | AY945850 | – | – |
| *Genea harknessii* | Trappe 11775 (FH, dubl. OSC) | DQ646526 | –,DQ220335 | – | – | |
| *Geopora* cf. *cervina* | KH.03.61 (FH) | DQ646527 | – | DQ220344 | – | – |
| *Geopora cooperi* f. *gilkeyae* | Trappe 18034 (FH, dubl. OSC) | DQ646528 | – | DQ220342 | – | – |
| *Geopyxis carbonaria* | SSU: _ (FH), LSU: C F-49793 (C) | AF104665 | – | DQ168336 | – | – |
| *Glaziella aurantiaca* | PR-5954 (FH) | DQ062996 | – | DQ220351 | – | – |
| *Gyromitra californica* | OSC 100068 | AY544717 | – | AY544673 | DQ470891 | – |
| *Gyromitra esculenta* | NRRL 20925 (dubl. CBS 335.73) | U42648 | – | U42675 | AY641045 | – |
| *Gyromitra melaleucoides* | NSW 7196 (OSC) | U42653 | – | U42680 | – | – |
| *Helvella* cf. *compressa* | OSC 100019 (OSC) | AY544699 | – | AY544655 | DQ497613 | – |
| *Humaria hemisphaerica* | KH.03.100 (FH) | DQ646529 | – | DQ220353 | – | – |
| *Hydnotrya cerebriformis* | NSW 6494 (OSC) | U42649 | – | U42676 | – | – |
| *Iodophanus carneus* | SSU: ARON 2102, LSU+RPB2: JHP 00.027 (C) | U53380 | – | AY500534 | AY500506 | – |
| *Iodowynnea auriformis* | 18510 PAN (FH) | DQ646530 | – | AF335118 | – | – |
| *Labyrinthomyces varius* | JMT 14825 (OSC) | U42662 | – | U42689 | – | – |
| *Lamprospora ascoboloides* | KH.03.54 (FH) | DQ646531 | – | DQ220358 | – | – |
| *Lasiobolidium orbiculoides* | CBS 344.73 | DQ063000 | – | DQ062995 | – | – |
| *Lasiobolidium spirale* | CBS 782.70 | DQ646533 | – | DQ220363 | – | – |
| *Lasiobolus ciliatus* | KS-94-005 (C) | DQ646532 | – | DQ167411 | – | – |
| *Leucangium carthusianum* | JMT 7205 (OSC) | U42647 | – | U42674 | – | – |
| *Marcelleina persoonii* | KH.00.07 (C) | DQ646534 | – | AY500536 | – | – |
| *Marcelleina tuberculispora* | All-94-8 (C) | DQ646535 | – | AF335120 | – | – |
| *Melastiza contorta* | KH.01.06 (C) | DQ646536 | – | AY500539 | – | – |
| *Melastiza cornubiensis* | KH.03.43 (FH) | DQ646537 | – | DQ646524 | – | – |
| *Miladina lecithina* | KH.03.156 (FH) | DQ646538 | – | DQ220371 | – | – |
| *Morchella elata* | SSU+LSU+RPB2: NRRL 25405, SSU+LSU: NRRL 22447 (dubl. OSC 100042) | U42641/AY544709 | – | U42667/AY544665 | AF107810 | – |
| *Morchella esculenta* | SSU: NRRL 22335, SSU+LSU: MV3 (dubl. OSC 100041), LSU+RPB2: ATCC 10968 | U42642/AY544708 | – | AY544664/AF279398 | AY641054 | – |
| *Nanoscypha tetraspora* | mh PR61 (FH) | AF006314 | – | DQ220374 | – | – |
| *Neolecta vitellina* | SSU: UME 29192 (U), LSU: JP 176 (F) | Z27393 | – | AF279401 | – | – |
| *Neottiella rutilans* | SSU: ARON 2690 (U), LSU: KH.03.55 (FH) | AF061720 | – | DQ220377 | – | – |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Neournula pouchetii* | NSW 6435 (OSC) | AF104666 | – | AY307940 | – | – |
| *Octospora hygrohypnophila* | KH.03.30 (FH) | DQ646539 | – | DQ220379 | – | – |
| *Orbicula parietina* | C F-24441 (C) | DQ062998 | – | DQ062988 | – | – |
| *Orbilia auricolor* | CBS 547.63 (dubl. OSC) | DQ471001 | – | DQ470953 | DQ470903 | – |
| *Otidea onotica* | SSU: mh 685 (FH), LSU: KH-98-107 (C) | AF006308 | – | AF335121 | – | – |
| *Pachyella clypeata* | FH No. 387 (FH) | DQ646540 | – | AY500542 | – | – |
| *Pachyphloeus melanoxanthus* | SSU: 1255 (UP), LSU: Gardner & Healy 195 (FH) | AF054899 | – | DQ191674 | – | – |
| *Parascutellinia carneosanguinea* | KH.03.34 (FH) | DQ646541 | – | DQ220388 | – | – |
| *Paurocotylis pila* | SSU: UME 30230, LSU: Trappe 12583 (OSC) | U53382 | – | DQ168337 | – | – |
| *Peziza arvernensis* | SSU+LSU: ALTA 9353, RPB2: KH-98-12 (C) | AF133175 | AF133175 | AF133162 | AY500497 | – |
| *Peziza badiofusca* | KH-98-113 (C) | DQ646542 | – | AF335132 | – | – |
| *Peziza echinispora* | SSU: DHP #136 (C), LSU+RPB2: Jukka Vauras 9110F (TURA) | AF006309 | – | AF335138 | AY500496 | – |
| *Peziza gerardii* | KH-97-90 (C) | DQ646543 | – | AF335143 | – | – |
| *Peziza lobulata* | KH.03.157 (FH) | DQ646544 | – | AY500548 | – | – |
| *Peziza michelii* | TL-5692 (C) | DQ646545 | – | AY500549 | – | – |
| *Peziza polaripapulata* | KH-96-11 (C) | DQ646546 | – | AY500551 | – | – |
| *Peziza quelepidotia* | NRRL 22205 | U42665 | – | U42693 | – | – |
| *Peziza subisabellina* | SSU: ALTA 9029, LSU: Winterhoff 8844 (herb. Winterhoff) | AF133144 | – | AF335164 | – | – |
| *Peziza succosa* | SSU: UME 29567 (U), LSU: KH-98-07 (C) | U53383 | – | AF335166 | – | – |
| *Peziza vesiculosa* | SSU: OSC 100074 (OSC), SSU+LSU: OSC 126 (OSC), LSU+RPB2: JV 95-652 (C) | AFTOL-202/DQ470995 | – | AY500552/DQ470948 | AY500489 | – |
| *Phillipsia domingensis* | SSU: mh 688 (FH) | AF006315 | – | – | – | – |
| *Phillipsia crispata* | LSU+RPB2: T. Læssoe AAU-44895a (AAU, C) | – | – | AY945845 | DQ017599 | – |
| *Phymatotrichopsis omnivora* | ATCC 22316 | **EF441991** | **EF441991 / EF494042** | **EF441991** | – | – |
| | ATCC 28960 | **EF441992** | **EF441993** | – | – | – |
| | ATCC 32445 | **EF494052** | **EF494043** | **EF494060** | **EF494070** | – |
| | ATCC 32446 | **EF441994** | **EF441994** | **EF441994** | – | – |
| | ATCC 32448 | **EF494048** | **EF494038** | **EF494056** | – | – |
| | ATCC 48084 | **EF441995** | **EF441995** | – | – | – |
| | M Olsen #1 | – | **EF441996** | – | – | – |
| | M Olsen #2 | **EF441997** | **EF441997** | **EF441997** | – | – |
| | M Olsen #3 | – | **EF441998** | – | – | – |
| | M Olsen #4 | **EF441999** | **EF441999** | – | – | – |
| | M Olsen #5 | **EF442000** | **EF442000** | – | – | – |
| | PC04 | **EF494049** | **EF494039** | **EF494057** | – | **EF494064** |
| | PP04 | **EF494047** | **EF494037** | **EF494055** | – | **EF494063** |
| | TAMDC04 | **EF494050** | **EF494040** | **EF494058** | – | **EF494065** |
| | NFAlf | **EF494051** | **EF494041** | **EF494059** | **EF494069** | **EF494066** |
| | OKAlf8 | **EF494045** | **AY549456** | **EF494053** | **EF494067** | **EF494061** |
| | TXCO3-9 | **EF494046** | **AY549455** | **EF494054** | **EF494068** | **EF494062** |
| | BMD Type sporemat (GLH 2868) (FH) | – | **FJ013259** | – | – | – |
| *Pseudombrophila guldeniae* | Kongsv. 85.10B (C) | DQ063001 | – | DQ062993 | – | – |
| *Pseudombrophila theioleuca* | C F-70057 (C) | DQ062999 | – | DQ062989 | – | – |
| *Pseudopithyella minuscula* | SSU: mh 673 (FH), LSU: mh 675 (FH) | AF006317 | – | AY945849 | – | – |
| *Pseudoplectania nigrella* | SSU: 'Japan', LSU: KH-97-28 (FH) | AF104345 | – | AY945852 | – | – |
| *Psilopezia* cf. *nummularialis* | TL-11785 (QCNE, dubl. C) | **EU722510** | – | **EU722509** | – | – |
| *Psilopezia deligata* | KH-99-13 (FH) | DQ646547 | **EF494044** | DQ220390 | **EF494071** | – |
| *Psilopezia juruensis* | T. Læssøe AAU 44912 (QCA, dubl. C) | DQ646548 | – | DQ220391 | – | – |
| *Pulchromyces fimicola* | ATCC 18595 (dubl. CBS 127.69, CUP 49531) | **EF442001** | **EF442001** | **EF442001** | – | – |
| | ATCC 36770 (dubl. IFAS-F 316) | **EF442002** | **EF442002** | **EF442002** | – | – |
| *Pulvinula archeri* | SSU: DAOM 195928, LSU: BAP 458 (FH) | U62012 | – | DQ220392 | – | – |
| *Pyronema confluens* | TL-11685 (QCNE, dubl. C) | DQ646549 | – | DQ220397 | – | – |
| *Pyronema domesticum* | SSU: ARON 1766, LSU+RPB2: CBS 666.88 (dubl. OSC 100503) | U53385 | – | DQ247805 | DQ247795 | – |
| *Reddellomyces donkii* | JMT 13292 (OSC) | U42660 | – | U42687 | – | – |
| *Rhizina undulata* | SSU: NRRL 22168, LSU: KH.02.44 (FH) | U42664 | – | DQ220410 | – | – |
| *Rhodotarzetta rosea* | KH.03.107 (FH) | DQ646550 | – | DQ220413 | – | – |
| *Sarcoscypha austriaca* | SSU: mh 667 (FH), LSU: mh 670 (FH) | AF006318 | – | AY945856 | – | – |
| *Sarcoscypha coccinea* | spat 03-02 (dubl. OSC 100003) | AY544691 | – | AY544647 | AY544755 | – |
| *Sarcosphaera coronaria* | SSU+LSU: OSC 100049, SSU: ALTA 9605, LSU: KS-94-24A (C), RPB2: KS-94-19 (C) | AY544712/AF133157 | – | AY544668/AY500555 | AY500523 | – |
| *Scabropezia scabrosa* | Pfister 13.8.83 (FH) | AF133158 | – | AF133173 | – | – |

**Table 1** (cont.)

| Species | Vouchers, Isolates, Strains (Herbarium[1])[2] | GenBank Accession Numbers | | | | |
|---|---|---|---|---|---|---|
| | | SSU | ITS | LSU | RPB2 | β-tub[3] |
| *Scutellinia scutellata* | SSU: ARON 2188, SSU+RPB2: KH03212003-1 (dubl. OSC 100015), LSU: KS-94-035H (C) | U53387/DQ247814 | – | DQ220421 | DQ247796 | – |
| *Sowerbyella imperialis* | CL2004-105 (C) | DQ646551 | – | DQ220427 | – | – |
| *Sphaerosporella brunnea* | LSU: KH.03.04 (FH) SSU: UME 31147 | U53388 | – | DQ220433 | – | – |
| *Strobilioscypha kellae* | SSU: NSW 7333 (OSC), LSU: NSW 6387 (OSC) | AF006310 | – | DQ220437 | – | – |
| *Tarzetta catinus* | SSU: UME 29731, LSU: KS.94.10A (C) | U53389 | – | DQ062984 | – | – |
| *Terfezia arenaria* | SSU: 1217-1 (UP) | AF054898 | – | – | – | – |
| *Terfezia claveryi* | LSU: Trappe 3195 (FH, dubl. OSC) | – | – | AY500558 | – | – |
| *Tricharina praecox* | KH.03.101 (FH) | DQ646552 | – | DQ646525 | – | – |
| *Trichophaea hybrida* | SSU: UME 29738, LSU: KH.04.39 (FH, dubl. DBG) | U53390 | – | DQ220454 | – | – |
| *Trichophaea woolhopeia* | KH.01.33 (C) | DQ646553 | – | DQ220460 | – | – |
| *Trichophaeopsis bicuspis* | SSU: ARON 2222 (O), LSU: NSW 8316 (OSC) | U53391 | – | DQ220461 | – | – |
| *Tuber gibbosum* | NSW 7049 (OSC) | U42663 | – | U42690 | – | – |
| *Underwoodia columnaris* | Kanouse 1951 (MICH) | U42658 | – | U42685 | – | – |
| *Urnula craterium* | SSU: mh 671 (FH, dubl. DEB #278082), LSU+RPB2: DHP 04-511 (FH) | AF104347 | – | AY945851 | DQ017595 | – |
| *Verpa bohemica* | NRRL 20858 (dubl. CBS 551.72) | U42645 | – | U42672 | – | – |
| *Verpa conica* | NRRL 20856 (dubl. CBS 407.81) | U42644 | – | U42671 | – | – |
| *Wilcoxina mikolae* | SSU: ATCC 52684, LSU: WS 36 (SFSU) | U62014 | – | DQ220468 | – | – |
| *Wolfina aurantiopsis* | SSU: –, LSU: DHP 04-599 (FH) | AF104664 | – | AY945859 | – | – |
| *Wynnella silvicola* | NSW 6219 (OSC) | U42655 | – | U42682 | – | – |

[1] For herbaria abbreviations see Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

[2] When different Isolates were used as sources for different genes, the respective gene is indicated prior to the isolate designation, i.e. 'Gene: Isolate'.

[3] When different sequences were used for rDNA or rDNA+RPB2 trees, two sets of sequences for the same species will be listed.

The type specimen and cultures of *Hydnum omnivorum* were studied by Burdsall and Nakasone (1978) who transferred this species to *Phanerochaete* and distinguished it from *Phymatotrichopsis omnivora* and from *Phanerochaete chrysorhiza* on the basis of culture morphology. *Phanerochaete omnivora* has been found on dead stems and roots of angiosperm trees and shrubs in Arizona and Texas but has not been reported from cotton or most of the other hosts of *Phymatotrichopsis omnivora* (Burdsall & Nakasone 1978, Burdsall 1985). As of today, the name of this economically important plant pathogen is *Phymatotrichopsis omnivora* and, as far as is known, it is a holoanamorphic (solely asexual) fungus of unknown phylum (e.g., *Ascomycota*, *Basidiomycota* or *Zygomycota*).

More recent work has provided some clues to the phylogenetic identity of *Phymatotrichopsis omnivora*. It is sensitive to the fungicide benomyl at rates of 5 mg/L (Hine et al. 1969, Lyda & Burnett 1970), a concentration to which most members of the *Basidiomycota* are tolerant, whereas members of the *Ascomycota*, excepting *Pleosporales*, are sensitive (Edgington et al. 1971). Gunasekaran et al. (1974) examined the hyphal walls of *P. omnivora* using transmission electron microscopy (TEM). Unfortunately, they did not study septa, which could have conclusively indicated whether *P. omnivora* is an ascomycete (simple septal pore with Woronin bodies) or basidiomycete (simple or dolipore septa lacking Woronin bodies) (Bracker 1967, Bartnicki-Garcia 1987). However, the hyphal walls of *P. omnivora* clearly possessed the bilayered structure typical of *Ascomycota*, with a thick, translucent inner layer and a thin, electron-dense outer layer (Gunasekaran et al. 1974). In contrast, hyphal walls of most *Basidiomycota* show multiple thin translucent and electron-dense layers (Bartnicki-Garcia 1987). Woronin bodies, diagnostic of filamentous *Ascomycota*, were discovered by Dong et al. (1981) in the hyphae of *Phymatotrichopsis omnivora*. Despite this strong evidence to indicate that *P. omnivora* is actually a member of the *Ascomycota*, the Dictionary of the Fungi (Kirk et al. 2001) lists *Phymatotrichopsis* as "? anamorphic *Basidiomycota*". A preliminary phylogenetic analysis of the relationships among *P. omnivora* and other botryoblastosporic fungi using the nuclear ribosomal internal transcribed spacer (ITS) region was inconclusive (Riggs 1993). The purpose of the current study is to provide a more conclusive and precise systematic placement of the cotton root rot pathogen, *Phymatotrichopsis omnivora*, based on phylogenetic analyses of DNA sequence data from nuclear ribosomal DNA and protein-coding genes.

## MATERIALS AND METHODS

### *Cultures*

*Phymatotrichopsis omnivora, Pulchromyces fimicola* and *Sistotrema brinkmannii* were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultures of *Phanerochaete omnivora* and *Phanerochaete chrysosporium* from USDA-FPL (Madison, WI). Additional isolates of *P. omnivora* were obtained from Dr Mary Olsen, University of Arizona, Tucson (Table 1) or isolated from the roots of diseased cotton and alfalfa plants as previously described (Lyda & Kenerley 1992) and maintained on modified ATCC medium 1078 (M1078), containing per 1 000 mL distilled water: 1 g $NH_4NO_3$; 0.75 g $MgSO_4$; 0.4 g $KH_2PO_4$; 0.9 g $K_2HPO_4$; 0.1 g $CaCl_2$; 40 g glucose; 1 g yeast extract; 1 g peptone; 100 µL Vogel's trace elements (Vogel 1964) and 18 g agar. Cultures collected for this study will be deposited at ATCC.

Sporemats were recovered from pots of *Phymatotrichopsis omnivora*-inoculated plum trees grown in Houston black clay and were identified based on morphology and ITS-rDNA sequences amplified using *Phymatotrichopsis omnivora*-specific

primers (PoITSA 5'-CCTGCGGAAGGATCATTAAA-3' and PoITSB 5'-GGGGGTTTTCTTTGTTAGGG-3'; developed in this study). Hand-sectioned sporemats were mounted in lactoglycerol and examined using a Nikon Eclipse E800 microscope with PlanFluor objectives and a CCD camera (Qimaging, Burnaby, Canada). Digital micrographs were contrast-adjusted, cropped and scale bars inserted in Photoshop (Adobe Systems Inc., San Jose, USA).

Specimens of *P. omnivora* at the Farlow Herbarium (Harvard University, Cambridge, MA) studied and described by Duggar (1916) were examined microscopically and small fragments excised for DNA isolations. Specimens examined were labelled as follows:

1 "*Phymatotrichum omnivorum* (Shear) on soil in cotton field, Paris, Texas, Sept. 18, 1915, BMD, Received from Missouri Bot. Garden June 1916 (sporemat on soil peds mounted in slide box; insert: *Ostracoderma omnivorum, comb. nov. ined.*, TYPE SPECIMEN for the conidial state, Examinavit G.L. Hennebert 2868, Nov. 1961)";
2 "*Phymatotrichum omnivorum* (Shear) on Cultv. Cotton, Petty, Texas, Sept. 12, 1902, BMD, "Ozonium" stage, Recv. from Missouri Bot. Garden, June 1916 (insert 1: Shear Bull Torr. Bot Club 34: 305 1907, on root of cotton; insert 2: *Ozonium* state of *Ostracoderma omnivorum, comb. nov. ined.*, Examinavit G.L. Hennebert 2869, Nov. 1961)"; and
3 "*Phymatotrichum omnivorum* (Shear) Paris, Texas, Sept. 18, 1915, BMD, "*Ozonium*" stage on Cotton, Recd from Missouri Bot. Garden, June, 1916, See also Box (insert: *Ozonium* state of *Ostracoderma omnivorum*, *comb. nov. inedit.*, Examinavit G.L. Hennebert 2870, Nov. 1961)".

Herbarium specimens will be referred to by the examination numbers given by G.L. Hennebert (e.g. GLH #2868, GLH #2869, and GLH #2870).

### *Molecular methods*

Genomic DNA was isolated following Zolan & Pukkila (1986). Some DNA preparations required further cleaning using glass milk (Gene Clean II, Bio101, La Jolla, California) or electrophoresis in 0.7 % agarose gels in Tris acetate EDTA (TAE) buffer followed by electroelution (GeBA flex-tube micro-dialysis kit, Gene Bio-Application Ltd, Kfar-Hanagid, Israel). Genomic DNA was also isolated from homogenized mycelia using a glass filter-based kit (UltraClean Microbial DNA, MoBio Laboratories, Inc., Carlsbad, CA). DNA was isolated from Farlow Herbarium specimens using a E.Z.N.A. Forensic DNA Extraction Kit (Omega Bio-tek, Doraville, GA) with the manufacturer's dried blood protocol with the following modifications: intact dried herbarium tissue (3–30 $mm^3$ piece) was incubated in 200 µL Buffer STL and 25 µL OB protease solution 45 min using a Thermomixer (Eppendorf, Westbury, NY), frozen over liquid nitrogen and thawed at 60 °C, twice, and incubated at 60 °C shaking at 500 rpm for 20 h. An additional 100 µL Buffer STL and 10 µL OB protease solution were added to each extraction tube, freeze-thawed as before and incubated at 60 °C shaking at 500 rpm for 20 h more. Softened herbarium tissue was then crushed with a sterile pestle in the lysis buffer and DNA isolated according to manufacturer's instructions with solution volumes adjusted for the additional 110 µL lysis buffer (STL + OB protease).

Nuclear rDNA (SSU, ITS and 5' LSU regions) was PCR amplified using the following primer pairs SSJ and NS8, NS1 and NS8 (for SSU), ITS4 and ITS5 (for ITS), PoITSA and ITS2 (for herbarium material), LROR and LR7 (for LSU) or SSG and LR5 (for SSU to LSU) (Vilgalys & Hester 1990, White et al. 1990, Hausner et al. 1993). Two successive PCR reactions were used to amplify the ITS region from the *P. omnivora* herbarium specimen. For the first PCR, 50 µL reactions were denatured at 95 °C for 3 min, followed by 41 cycles of 94 °C for 30 s, 50 °C for 45 s and 72 °C for 45 s and a final extension of 72 °C for 7 min. After observing a faint band by gel electrophoresis, 1 µL from each of the first PCRs were used as templates for a second 50 µL PCR with an initial denaturation of 95 °C for 3 min, 20 cycles of 94 °C for 30 s, 50 °C for 45 s, and 72 °C for 45 s, and a final extension of 72 °C for 7 min. Using the thermocycler program and reverse primers of Liu et al. (1999), sequences spanning conserved regions 3–11 in RPB2 from *P. omnivora* isolates were amplified in two overlapping segments using the primer pairs RPB2-Ds3F (5'-WSYGARAAGGTHYTBATYGCRCAAGAGCG-3') and fRPB2-7cR, and RPB2-Ds6F (5'-TGGGGWYTSGTHTGYCCWGC-3') and fRPB2-11aR. A region of the β-tubulin gene spanning three introns was amplified and sequenced with primers Bt2a and Btspect (Glass & Donaldson 1995, Paolocci et al. 2004). Sequences were obtained in an automated sequencer (ABI 377) using dye-terminator technology and the following primers: SSJ, NS1, NS2, NS3, NS4, NS5, SSG, NS8, ITS1, ITS4, ITS5, LS1R, LS1, LR3R, LR7, LR16, NL1, NL4 and LR3 for rDNA (Vilgalys & Hester 1990, White et al. 1990, Hausner et al. 1993); and RPB2-Ds3F, fRPB2-5F, fRPB2-5R, RPB2-Ds6F, fRPB2-7cF, fRPB2-7cR, RPB2-980F, RPB2-1014R, RPB2-1554R, RPB2-1599F, RPB2-2488F, RPB2-2568R and fRPB2-11aR for RPB2 (Liu et al. 1999, Reeb et al. 2004). Complementary strand sequences were aligned and corrected in SeqEd (ABI Software) or ChromasPro (Technelysium Pty Ltd) and combined with most similar sequences from GenBank determined using BLASTn (Altschul et al. 1990, McGinnis & Madden 2004). All newly derived sequences have been deposited in GenBank as accession numbers EF441991–EF442000, EF494037–EF494070 and FJ013259 (Table 1).

### *Phylogenetic analyses*

Large subunit and SSU rDNA sequences from *Phymatotrichopsis omnivora*, *Pulchromyces fimicola* and an additional species of *Psilopezia, Ps.* cf. *nummularialis*, were added to a data matrix containing 99 species of *Pezizales* (Hansen & Pfister 2006) by hand using the software Se-Al v. 2.0a11 (Rambaut 2002). The sequences represent all known sublineages within *Pezizales*, 82 genera and 14 families (out of c. 164 genera and 16 families; Table 1). *Neolecta vitellina* was used as outgroup. To substantiate the placement of *Phymatotrichopsis omnivora* and *Pulchromyces fimicola* within *Pezizales*, a data matrix including an additional gene, RPB2, was compiled representing a subset of the taxa from the combined LSU and SSU dataset. Amino acid sequences of RPB2 were deduced using a combination of BLASTx (Altschul et al. 1997) and the ExPASy translate tool (http://us.expasy.org/tools/dna.html). Multiple sequence alignments were generated using ClustalX (Thompson et al. 1997) or Muscle (Edgar 2004). The final alignments are available from TreeBASE (S2105).

Individual and combined analyses of the data matrices were performed using PAUP v. 4.0b10 (Swofford 2002) and MrBayes v. 3.1.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) on Macintosh computers. Maximum parsimony (MP) analyses with heuristic searches consisted of 1 000 or 5 000 (for the subset LSU-SSU-RPB2 datasets) random sequence addition replicates with tree bisection-reconnection (TBR) branch swapping, MULPARS in effect and saving all equally most parsimonious trees (MPTs). All characters were equally weighted and unordered. In MP analyses of the individual, larger SSU rDNA data matrix a two-step search was performed (due to an exceedingly large number of trees generated), as follows: First, 1 000 heuristic searches were performed with random sequence addition and TBR branch swapping, with MAXTREES unrestricted, and keeping only up to 15 trees per replicate. Second, exhaustive swapping was performed on all the MPTs

discovered with MAXTREES set to 15 000. Robustness of individual branches was estimated by parsimony bootstrap proportions (BP), using 500 (LSU-SSU dataset) or 1000 (LSU-SSU-RPB2 dataset) bootstrap replicates, each consisting of a heuristic search with 100 random addition sequence replicates, TBR branch swapping, and MAXTREES set at 100 (LSU-SSU) or unrestricted (LSU-SSU-RPB2).

The GTR+I+G model of nucleotide substitution was found to fit each of the rDNA datasets best using a hierarchical likelihood ratio test as implemented in the program MrModeltest v. 2.2 (Nylander 2004). In Bayesian analyses of the LSU-SSU-RPB2 combined dataset, rDNA nucleotide data and RPB2 amino acid data were specified as distinct partitions to allow the use of the GTR+I+G model of evolution for SSU and LSU sequences and an empirical amino acid model (Whelan & Goldman 2001) for RPB2 sequences. Bayesian analyses for the larger LSU-SSU dataset consisted of two parallel searches each run for 5 000 000 generations, whereas analyses of the LSU-SSU-RPB2 dataset consisted of two searches run for 2 000 000 generations. An incremental heating scheme for analyses used the default settings in MrBayes (i.e. three heated chains and one cold chain). For the LSU-SSU dataset, trees sampled prior to the chains reaching a split deviation frequency of 0.05 were discarded as the 'burn-in', while the remaining trees were used to calculate the Bayesian posterior probabilities (PP) of the clades. For the LSU-SSU-RPB2 dataset, trees prior to stabilizing at < 0.01 average standard deviation between chains were discarded as 'burn-in' and the remaining trees were used to calculate the Bayesian PPs of the clades.

Based upon the phylogenetic analyses, constraint parsimony analyses of the combined LSU-SSU-RPB2 dataset were constructed in which *Phymatotrichopsis* or *Rhizinaceae* were forced into monophyly with alternative distinct lineages or outside the *Pezizomycetes* (Table 2). Constraint topologies were manually specified in PAUP v. 4.0b10 and heuristic searches of 1 000 replicates, saving only those trees in agreement with the forced constraint, were conducted using the same settings as the parsimony searches described above. The resulting trees were compared using the nonparametric comparison test of Templeton (Templeton 1987).

## RESULTS

### *Phymatotrichopsis omnivora isolates*

Besides isolates from ATCC, several isolates were cultured from alfalfa and cotton fields displaying characteristic symptoms (Fig. 1a, b) and signs of Phymatotrichum root rot. Mycelial strands were often observed on infected cotton roots (Fig. 1c), but were less conspicuous on alfalfa roots (not shown). Under magnification, mycelial strands were hirsute with acicular hyphae (Fig. 1d), some of which displayed cruciform branching (Fig. 1e). Though strands were rhizomorphic in appearance, with a melanised rind consisting of polygonal plectenchymatous cells (Fig. 1f), no obvious apical meristems were observed, and so would be better termed 'mycelial cords' (Kirk et al. 2001). One isolate, OKAlf8, formed typical sporemats on the surface of black clay (Fig. 1g), in which OKAlf8-inoculated plum trees had been potted. These sporemats developed the characteristic globose conidiophores with botryose blastoconidia borne singly on denticles (Fig. 1h–k). In a few cases, clavate or moniliform conidophores with apically borne conidia formed (Fig. 1l, m), similar in appearance to the 'basidia' observed previously (Baniecki & Bloss 1969). Examined herbarium specimens from FH of *P. omnivorum* possessed either characteristic hirsute mycelial cords ('*Ozonium*' stage) on cotton roots (GLH #2869 and GLH #2870) or crustose sporemats adhering to peds of black clay (GLH # 2868). Upon microscopic examination, excised pieces from the sporemat were not found to possess any readily apparent conidiophores; however, characteristic hirsute mycelial cords were observed ramified throughout the soil underlying the sporemats (data not shown).

**Table 2** Impact of phylogenetic constraints on the position of *Phymatotrichopsis omnivora* (Po) within a 31-taxon dataset (Fig. 3) on the resulting tree scores (#MPTs = number of equally most parsimonious trees; CI = consistency index; *p* = probability from a non-parametric two-tailed test (Templeton 1987), where trees with $p < 0.05$ are rejected as significantly worse.

| Constraint | #MPTs | Length (steps) | CI | *p* |
|---|---|---|---|---|
| None | 18 | 3123 | 0.601 | best |
| *Rhizinaceae* with lineage B | 6 | 3123 | 0.601 | 0.995 |
| *Rhizinaceae* with lineage C | 6 | 3125 | 0.600 | 0.637–0.732 |
| *Rhizinaceae* and *Caloscypha* within lineage B | 18 | 3128 | 0.600 | 0.535–0.603 |
| *Rhizinaceae* with lineage A | 3 | 3163 | 0.593 | 0.0003 |
| *Rhizinaceae* with Pezizaceae | 15 | 3176 | 0.591 | < 0.0001 |
| Po only with lineage A | 6 | 3342 | 0.561 | < 0.0001 |
| Po only with lineage B | 3 | 3331 | 0.563 | < 0.0001 |
| Po only with lineage C | 3 | 3409 | 0.550 | < 0.0001 |
| Po only with *Caloscypha* | 6 | 3320 | 0.565 | < 0.0001 |
| Po only outside *Pezizomycetes* | 9 | 3341 | 0.562 | < 0.0001 |

### *Molecular data*

Fifty six new sequences were determined in this study from *Phymatotrichopsis omnivora*, *Pulchromyces fimicola*, *Psilopezia* cf. *nummularialis* and *Psilopezia deligata* (Table 1). Efforts to amplify RPB2 from *Ps. nummularialis* were unsuccessful. The six β-tubulin sequences from *P. omnivora* were determined to not be phylogenetically informative (data not shown) and thus not included in phylogenetic analyses. From the three herbarium specimens of *P. omnivorum*, a partial ITS sequence was amplified only from the sporemat specimen (GLH #2868) using one of four primer pairs attempted (data not shown). Based on the alignment of this sequence with ITS sequences from over one hundred other *P. omnivora* isolates, the herbarium specimen sequence was most similar to *P. omnivora* isolates from El Campo, TX (100 % identity, 302/302) and the ATCC 48084 isolate (99 % identity, 302/303), which belong to an ITS haplotype common in southern Oklahoma and throughout eastern and central Texas (data not shown).

### *LSU and SSU gene tree*

No supported conflict (BP ≥ 75 %, PP ≥ 95 %) was detected between the individual LSU and SSU gene trees. The combined dataset consisted of 2 743 characters of which 774 were parsimony informative. Parsimony analyses resulted in 6 equally most parsimonious trees (MPTs). The strict consensus tree of all MPTs was nearly completely resolved, except for a trichotomy of the three species of *Psilopezia* (indicated with an asterisk in Fig. 2). Nevertheless, many of the deeper branches have only low BP support. Bayesian analyses reached an average standard deviation of split frequencies below 0.05 after approximately 377 000 generations and the first 3 770 trees were excluded as the 'burn-in'. Bayesian PPs supported many of the terminal relationships in the phylogeny with confidence but, as with BPs, failed to support some of the deeper nodes.

*Phymatotrichopsis omnivora* and *Pulchromyces fimicola* were nested within the *Pezizales* (Fig. 2). *Phymatotrichopsis omnivora* formed a monophyletic group with *Rhizina undulata* and three species of *Psilopezia* (*Rhizinaceae*), although with only low support (BP 56 %, PP 72 %). The lineages B (*Morchellaceae–Discinaceae–Helvellaceae–Tuberaceae*) and C (*Pyronemataceae–Ascodesmidaceae–Glaziellaceae–Sarco-*


**Fig. 1** Phymatotrichum root rot and morphological characteristics of the causal fungus, *Phymatotrichopsis omnivora*. a. Disease foci in an alfalfa field (near Devol, OK); b. disease foci in a cotton field (near Austwell, TX); c. mycelial strands (arrows) on infected cotton root; d–f. mycelial strand showing acicular hyphae, cruciform hypha (arrow, inset e) and rectangular and polygonal cells (inset f); g. sporemat on soil surface; h–m. conidiophores and conidia borne on sporemat of *Phymatotrichopsis omnivore*; j. immature conidiophores produced from mycelial strand hyphae; k. botryoblastoconidia forming on conidiophores; l, m. 'basidium-like' conidiophores (arrows). — Scale bars: d = 100 µm; e = 50 µm; f, h = 25 µm; g = 5 mm; i = 20 µm; j–m = 10 µm.

*scyphaceae–Sarcosomataceae–Chorioactidaceae*), *Rhizinaceae* and *Caloscyphaceae* formed a strongly supported monophyletic group (BP 93 %, PP 100 %). Parsimony analyses suggested that *Caloscyphaceae* was a sister group to a clade of the lineages B and C and *Rhizinaceae* (BP 78 %). Lineage C was strongly supported (BP 96 %, PP 100 %), whereas the relationships between *Rhizinaceae* and the lineages B and C were without support. *Pulchromyces fimicola* was nested within lineage C, but its placement among members of *Pyronemataceae* and *Ascodesmidaceae* was uncertain (Fig. 2). LSU and SSU rDNA sequences from *Phymatotrichopsis omnivora* showed several substitutions or deletions (17/1404 bp in the LSU region (1.21 %), 18/1741 bp in the SSU region (1.03 %)). The two available isolates of *Pulchromyces fimicola* had identical sequences through 2 989 bases of the SSU, ITS, and 5'-LSU regions.

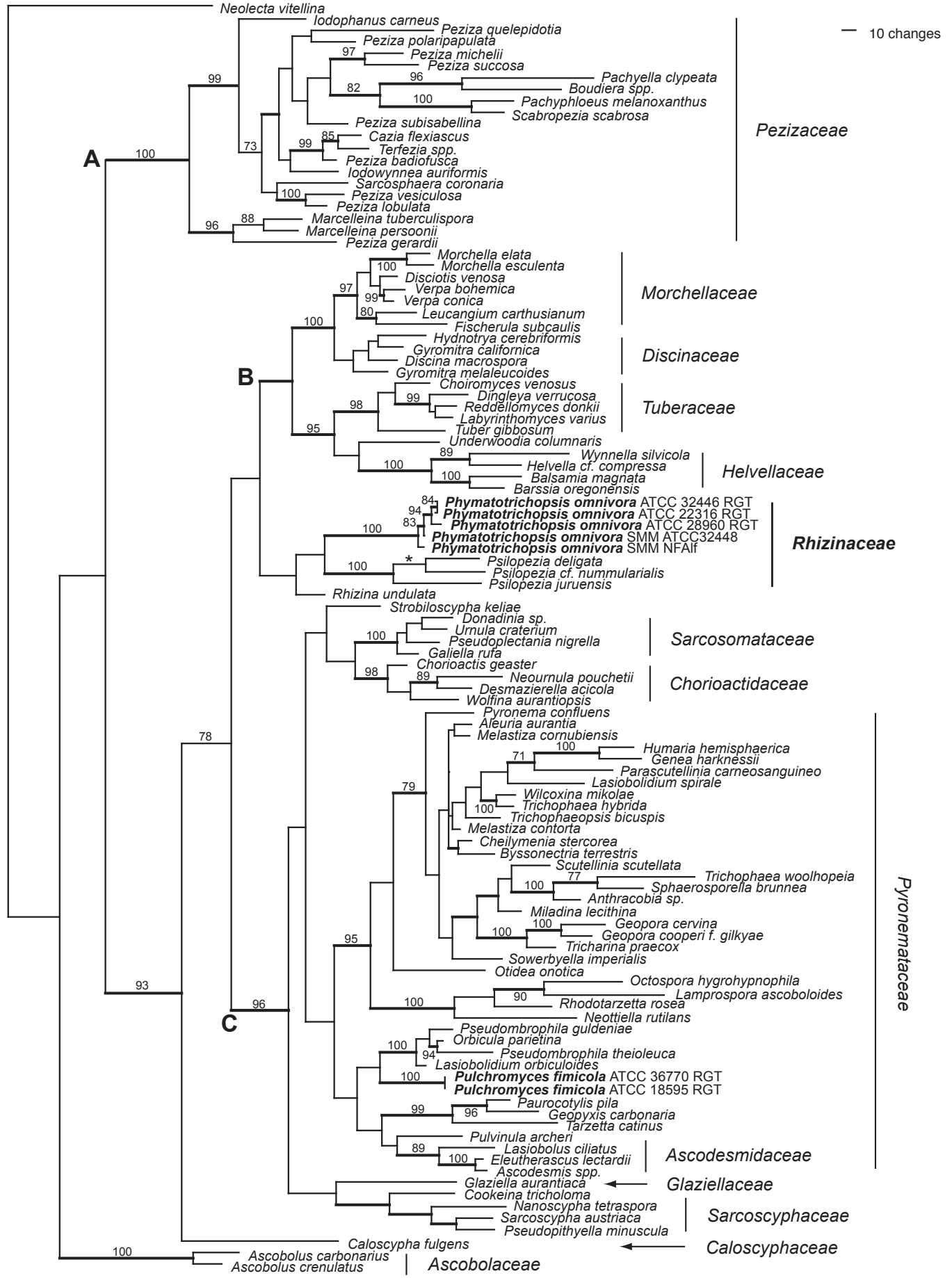


**Fig. 2** Phylogenetic relationships of *Phymatotrichopsis omnivora* and *Pulchromyces fimicola* among a broad sampling of *Pezizomycetes* inferred from combined analyses of LSU and SSU rDNA. One of 6 most parsimonious trees is shown here. Terminal taxa represent individual specimens (see Table 1). Only one branch, indicated with an asterisk, collapses in the strict consensus tree of all MP trees. Numbers by branches are MP bootstrap proportions ≥ 70 %. Thickened branches indicate Bayesian posterior probabilities ≥ 95 %, obtained from a 50 % majority rule consensus tree of the 46 230 trees sampled from a Bayesian MCMC analysis. The three primary lineages are labelled A, B and C for discussion.

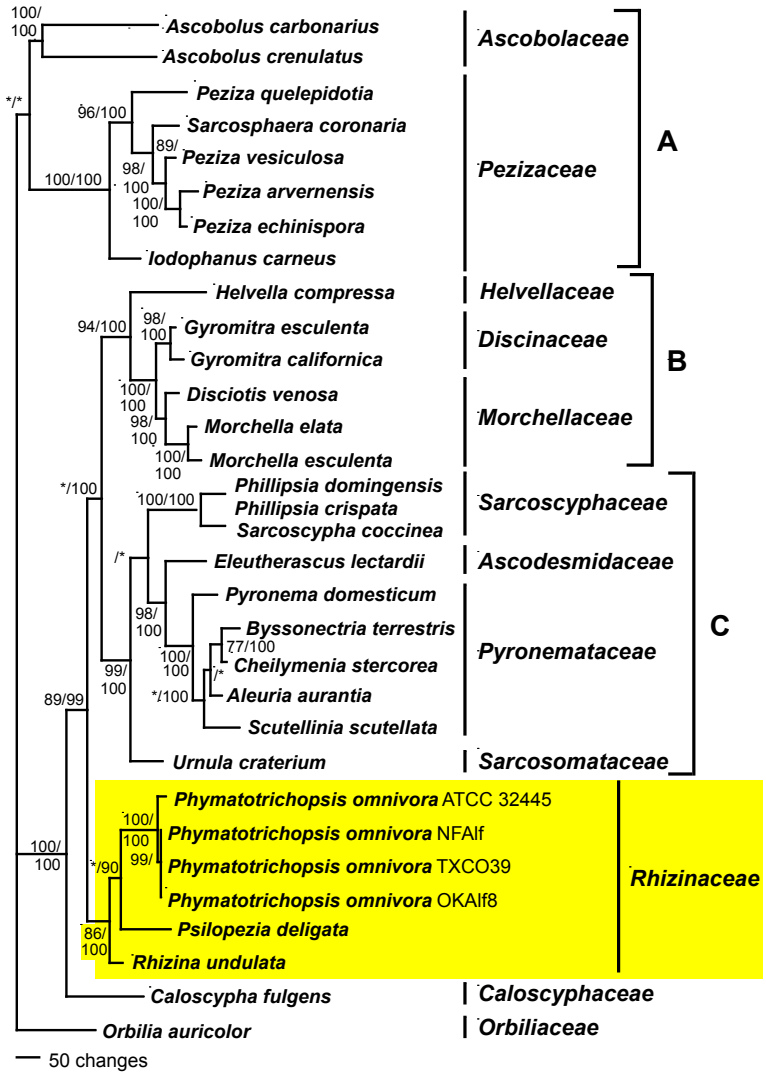


**Fig. 3** Phylogenetic relationships of *Phymatotrichopsis omnivora* with selected *Pezizomycetes* based on DNA sequences of SSU and LSU rDNA and deduced amino acid sequences of RPB2. One of 18 most parsimonious trees is shown here. Branch support at nodes are MP bootstrap proportions ≥ 70 % (number before '/') and Bayesian posterior probabilities ≥ 95 % (number after '/'). Branches that collapsed in a strict consensus of the MP trees or the trees retained in the Bayesian analysis are indicated by '*'. *Orbilia auricolor* (*Orbiliomycetes*) was used as the outgroup to root the tree (James et al. 2006). The three primary lineages are labelled A, B and C and the *Rhizinaceae* is shaded yellow for discussion.

### *Combined LSU, SSU genes and RPB2 protein tree*

Overall no supported conflict (BP ≥ 70 %, PP ≥ 90 %) was detected between the individual trees constructed from LSU and SSU rDNA and RPB2 amino acid sequences. The combined dataset consisted of 6 194 characters of which 757 were parsimony informative. Parsimony analyses resulted in 18 MPTs (Fig. 3). The strict consensus tree of all MPTs was highly resolved and the majority of nodes were well supported by BP. Bayesian analyses reached an average standard deviation of split frequencies below 0.01 after approximately 180 000 generations and the first 2 000 trees were excluded as the 'burn-in'. Bayesian PPs supported many of the terminal, as well as, deep nodes in the phylogeny with confidence.

Parsimony analyses of the combined LSU-SSU-RPB2 dataset recovered the same major lineages, with high BP support, as those found with support in analyses of the LSU-SSU alignment. *Phymatotrichopsis omnivora* was strongly supported within the family *Rhizinaceae* (BP 86 %, PP 100 %). Bayesian analyses suggested that *Rhizinaceae* was a sister group to the lineages B and C (PP 100 %), whereas the relationship between *Rhizinaceae* and lineages B and C was unresolved in MP analyses (Fig. 3). As in analyses of the LSU-SSU alignment, the *Ascobolaceae* and *Pezizaceae* were not supported as a distinct lineage (A). Nevertheless, the two families were resolved as sister taxa or successive sister taxa to the rest of the *Pezizales* (Fig. 2, 3).

Parsimony trees resulting from constraint analyses that forced *Phymatotrichopsis omnivora* to group outside of *Rhizinaceae*, with either lineage A, B or C, *Caloscyphaceae*, or outside *Pezizomycetes*, or with *Rhizinaceae* and lineage A were strongly rejected using the Templeton test ($P < 0.0001$; Table 2). However, those trees recovered from analyses forcing *Rhizinaceae* to form a monophyletic group with *Morchellaceae–Discinaceae–Helvellaceae* (lineage B), as seen in MP analyses of the LSU-SSU dataset (Fig. 2), could not be rejected ($p = 0.995$). Forcing *Rhizinaceae* with lineage C or with *Caloscyphaceae* and lineage B also could not be rejected ($p = 0.637–0.732$ or $p = 0.535–0.603$, respectively).

## DISCUSSION

Neither *Sistotrema brinkmannii* nor *Phanerochaete omnivora* represent the teleomorph of the cotton root rot pathogen. *Phymatotrichopsis omnivora* is not a member of the phylum *Basidiomycota*. Instead, *Phymatotrichopsis omnivora* is an anamorphic (mitosporic) member of the phylum *Ascomycota*, class *Pezizomycetes* (order *Pezizales*, operculate discomycetes). Our phylogenetic analyses place *Phymatotrichopsis omnivora*

in *Rhizinaceae* with *Psilopezia* and *Rhizina. Rhizinaceae* was resurrected as a monotypic family based on molecular data (O'Donnell et al. 1997), and recently, species of *Psilopezia* were suggested to belong to the family (Hansen & Pfister 2006). Whether *Rhizinaceae* represents an independent lineage within *Pezizomycetes*, as suggested by Hansen & Pfister (2006) and our Bayesian analyses (Fig. 3), is still uncertain, as we are unable to reject constraint topologies that force *Rhizinaceae* to group with lineage B (with or without *Caloscyphaceae*) or lineage C. Based on SSU and LSU sequences, *Pulchromyces fimicola* (formerly *Phymatotrichum fimicola*) is also a member of the class *Pezizomycetes*, but is clearly not congeneric with *Phymatotrichopsis*. Instead, it is closely related to members of the C-lineage, possibly in *Pyronemataceae* or *Ascodesmidaceae*. *Pulchromyces* has been found on the dung of mice, otters, bats and shrews, in temperate and tropical regions, in Ghana, Panama and the United States (Pfister et al. 1974). A number of genera shown to be closely related to *Pulchromyces*, namely *Ascodesmis*, *Lasiobolidium*, *Lasiobolus* and *Pseudombrophila* (Fig. 2), are similarly fimicolous, although the fimicolous habit has been multiply derived throughout the *Pezizomycetes* and many other groups of fungi. A better taxon sample of these minute *Pezizomycetes* and related anamorphs will be required to settle the taxonomic position of *Pulchromyces* at the family level.

The anamorphic morphology of *Phymatotrichopsis omnivora* partially supports its placement in the *Pezizomycetes*. The botryoblastoconidia produced by *Phymatotrichopsis omnivora* are also observed in many of the pleomorphic *Pezizomycetes* in which anamorph–teleomorph associations have been determined. For example, the anamorphic genera *Chromelosporium*, *Oedocephalum*, *Ostracoderma*, *Glischroderma* and *Dichobotrys*, are associated with the *Pezizomycetes* meiosporic genera, *Peziza* (first four) and *Trichophaea* (Paden 1972, Hennebert 1973, Hansen et al. 2001). However, botryoblastosporic reproduction occurs in several classes of both the *Ascomycota* and *Basidiomycota*. Such anamorphic genera are found in the *Leotiomycetes* (inoperculate discomycetes), in *Botrytis*, *Streptobotrys*, *Amphobotrys*, and *Veruccobotrys*, and in the *Agaricomycetes* (*Homobasidiomycetes*), in *Spiniger* (Hennebert 1973, Stalpers 1974, Kiffer & Morelet 2000). Thus, botryoblastosporic patterns of conidiogenesis arose several times during fungal evolution and may have limited value for taxonomic classifications above genus.

Rhizomorph-like, mycelial strands are formed by both *Phymatotrichopsis omnivora* (Lyda & Kenerley 1992) and, proposed confamilial, *Rhizina undulata* (Booth & Gibson 1998). Conspicuous mycelial strands are often found on the infected roots of host plants and are often used by plant pathologists to diagnose the root rots caused by either fungus. Besides soilborne dissemination, the mycelial strands connect the reproductive structures, sporemats of *Phymatotrichopsis omnivora* or apothecia of *Rhizina undulata*, to nutritional sources. The root-like nature of the apothecial mycelial strands of *Rhizina* was the namesake character of the genus (Fries 1822). The mycelial strands of *Phymatotrichopsis* eventually form long-lived, hypogeous sclerotia (King & Loomis 1929, Neal 1929, King et al. 1931), while sclerotia have not been reported for *Rhizina,* which survives as thick-walled ascospores that are stimulated to germinate by fire (Jalaluddin 1967b).

The majority of the *Pezizomycetes* traditionally have been considered saprobic, but the trophic strategies of most species are not well-studied and remain undocumented. The inclusion of the *Tuberales*, which are assumed to be mainly mycorrhizal, in the *Pezizales* (Trappe 1979, Læssøe & Hansen 2007) and molecular studies identifying numerous other *Pezizomycetes* as ectomycorrhizal associates (Dahlstrom et al. 1999, Fujimura et al. 2005, Tedersoo et al. 2006) has revealed mycorrhizae as a major ecological niche of many pezizalean fungi. On the other hand, the ecology of *Phymatotrichopsis omnivora*, a mostly hypogeous plant pathogen with an extensive dicotyledonous host range (Lyda 1978), is relatively rare among the *Pezizomycetes*. *Rhizina undulata* is also a plant pathogen that infects a wide range of conifers (Gremmen 1971). Other plant pathogenic *Pezizomycetes* include the conifer seed pathogen *Caloscypha fulgens* (Paden et al. 1978) and the Strumella canker fungus, *Conoplea globosa* (= *Strumella coryneoidea*; mitosporic *Urnula*) (Kopcke et al. 2002, Wang et al. 2005). Also, species of *Octospora*, *Lamprospora* and *Neottiella* form obligate associations with numerous bryophytes, which have been interpreted as parasitic (Döbbeler 1979, Benkert 1993, Davey & Currah 2006). Both *Phymatotrichopsis* and *Rhizina* also colonise dead plant debris in field situations, acting as facultative saprobes, and utilise these substrates for reproduction (Jalaluddin 1967a; Rush & Gerik 1989).

Very few similarities in apothecia morphology support a close relationship of *Psilopezia* with *Rhizina* (Hansen & Pfister 2006), and no obvious mitosporic or somatic similarities support a confamilial relationship with *Phymatotrichopsis*. The little that is known about the natural history of *Psilopezia* suggests a saprobic life style on wet, rotted wood (Pfister 1973), while *Rhizina* and *Phymatotrichopsis* are plant pathogens with a facultative saprobic phase. Nevertheless, based on our phylogenies of combined rDNA and RPB2 sequences, the monophyly of the *Rhizinaceae*, including *Rhizina undulata*, *Phymatotrichopsis omnivora* and *Psilopezia deligata*, was highly supported (BP 86 %, PP 100 %) and constraint topologies that forced *Phymatotrichopsis* to group outside *Rhizinaceae* were rejected. The relationships among *Psilopezia*, *Rhizina* and *Phymatotrichopsis* were, however, not resolved with confidence (the branch collapses in the strict consensus tree of all MPTs, and PP 90 %). *Psilopezia* may possess an as yet unrecognised pathogenic phase, or represents a saprotrophic sister group to a derived parasitic clade of *Rhizina* and *Phymatotrichopsis*. More members of the *Rhizinaceae* must be identified and characterized before further inferences on the evolution of their nutritional strategies can be clarified.

Knowledge of the correct phylogenetic placement of the cotton root rot pathogen as a member of the *Pezizomycetes* (*Ascomycota*), and not *Agaricomycetes* (*Basidiomycota*), will have significance in detecting the pathogen in the field and in developing methods of chemical or biological control. Also, it will facilitate current efforts to assemble and annotate the genome sequence of *Phymatotrichopsis omnivora* strain OKAlf8 (http://www.genome.ou.edu/fungi.html) through comparative genomics with related ascomycetes. In addition to *Phymatotrichopsis*, genomic projects of two other *Pezizomycetes*, *Tuber melanosporum* and *T. borchii*, are ongoing (Poma et al. 2006, Lazzari et al. 2007; http://mycor.nancy.inra.fr/IMGC/TuberGenome/index.html). The insights into the genetic underpinnings of this fascinating, but understudied, class of fungi should prove fruitful.

### *Nomenclature and typification*

Given the economic importance of *Phymatotrichopsis omnivora* and the presence of ITS sequence variation among strains of this species (data not shown), it is important that a consensus is reached as to the correct author citation and (therefore) typification of this species. Duggar (1916) explicitly transferred the species *Ozonium omnivorum* Shear to the genus *Phymatotrichum* because of the presence and nature of conidia in specimens of what he believed to be the same species as described by Shear (1907) and thus did not designate a type specimen among the various collections he referred to. The decision of Hennebert (1973) to attribute the name solely to Duggar

therefore left the species without a type specimen. The relevant sections of the International Code of Botanical Nomenclature (ICBN; McNeill et al. 2006) are Art. 7.4, 48.1 and 59.6. Article 7.4 states that "a new name formed from a previously published legitimate name (stat. nov., comb. nov.) is, in all circumstances, typified by the type of the basionym", unless the author(s) explicitly excluded the type of the basionym (Art. 48.1) or explicitly described a new morph, simultaneously meeting all the requirements for description of a new species (Art. 59.6) (McNeill et al. 2006). The decision by Hennebert (1973) rests on a narrow definition of Art. 59.6, that a conidial form should represent a new 'morph' separate from the 'sterile' mycelium that produced it, and goes against the growing consensus among mycologists of the principle of 'one fungus – one name' (Hennebert 1993). We therefore choose to treat the decision by Hennebert (1973) to attribute the basionym of *Phymatotrichopsis omnivora* to Duggar as an error to be corrected under Art. 33.6, resulting in the authorities for the combination of *Phymatotrichopsis omnivora* (Shear) Hennebert and the restitution of Shear's type specimen (C.L. Shear 1447, BPI 455660) as holotype. The living culture, strain OKAlf8 (ATCC MYA-4551; isolated from infected alfalfa roots growing near Belleville, OK by S. Marek, August 2003), which is currently the basis of genome sequencing (http://www.genome.ou.edu/fungi.html), provides a sound anchor for future molecular studies.

**Acknowledgements** We would like to thank the American Type Culture Collection (Manassas, VA), Mary Olsen (University of Arizona, Tucson) and the USDA Forest Products Laboratory (Madison, WI) for provision of cultures. SMM is grateful to James N. Enis, Madhavi Dhulipala and Tim Samuels for technical assistance and to Donald H. Pfister, Genevieve Lewis-Gentry, Lisa DeCesare and Matthew E. Smith of the Farlow Herbarium (Harvard University, Cambridge, MA) for ably assisting with type specimens and accompanying historical documents. During our final revisions, Walter Gams, David Hawksworth, Grégoire Hennebert and Scott Redhead made extensive and thoughtful contributions via email to the debate over the nomenclature and typification of synanamorphs, for which we are very grateful. This study was supported in part by USDA-NRI grant 98-35303-6776 and NSERC grant DG 238464-01 to RGT, funding from Oklahoma Agricultural Experiment Station Project 2536, Oklahoma Department of Agriculture, Food and Forestry and NSF-EPSCoR to SMM, and in part by NSF grant DEB-0315940 to KH.

## REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389–3402.

Baniecki JF, Bloss HE. 1969. The basidial stage of Phymatotrichum omnivorum. Mycologia 61: 1054–1059.

Bartnicki-Garcia S. 1987. The cell wall: a crucial structure in fungal evolution. In: Rayner ADM, Brasier CM, Moore D (eds), Evolutionary Biology of the Fungi: 389–403. Cambridge University Press, Cambridge, United Kingdom.

Benkert D. 1993. Bryoparasitic Pezizales: Ecology and systematics. In: Pegler DN (ed), Fungi in Europe: Investigation, recording and conservation: 147–156. Royal Botanical Gardens, Kew, United Kingdom.

Booth C, Gibson IAS. 1998. Rhizina undulata. IMI Descriptions of Fungi and Bacteria 33: 1–2.

Bracker CE. 1967. Ultrastructure of fungi. Annual Review of Phytopathology 5: 343–372.

Burdsall HH. 1985. A contribution to the taxonomy of the genus Phanerochaete (Corticiaceae, Aphyllophorales). Mycologia Memoir 10: 1–165.

Burdsall HH, Nakasone KK. 1978. Taxonomy of Phanerochaete chrysorhizon and Hydnum omnivorum. Mycotaxon 7: 10–22.

Dahlstrom JL, Smith JE, Weber NS. 1999. Mycorrhiza-like interaction by Morchella with species of the Pinaceae in pure culture synthesis. Mycorrhiza 9: 279–285.

Davey ML, Currah RS. 2006. Interactions between mosses (Bryophyta) and fungi. Canadian Journal of Botany 84: 1509–1519.

Döbbeler P. 1979. Studies on the moss parasites Pezizales related to Octospora. Nova Hedwigia 31: 817–864.

Dong S, Bloss HE, Alcorn SM. 1981. Ultrastructure and comparison of Phymatotrichum omnivorum and Sistotrema brinkmannii. Mycologia 73: 321–333.

Duggar BM. 1916. The Texas root rot fungus and its conidial stage. Annals of the Missouri Botanical Garden 3: 11–23.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797.

Edgington LV, Khew KL, Barron GL. 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology 61: 42–44.

Fries EM. 1822. Systema Mycologicum, vol. 2. Berling, Lund, Sweden.

Fujimura KF, Smith JE, Horton TR, Weber NS, Spatafora JW. 2005. Pezizalean mycorrhizas and sporocarps in ponderosa pine (Pinus ponderosa) after prescribed fires in eastern Oregon, USA. Mycorrhiza 15: 79–86.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Gremmen J. 1971. Rhizina undulata. A review of research in the Netherlands. European Journal of Forest Pathology 1: 1–6.

Gunasekaran M, Hess WM, Weber DJ. 1974. Lipids and ultrastructure of Phymatotrichum omnivorum. Transactions of the British Mycological Society 63: 519–525.

Hansen K, Læssøe T, Pfister DH. 2001. Phylogenetics of the Pezizaceae, with an emphasis on Peziza. Mycologia 93: 958–990.

Hansen K, Pfister DH. 2006. Systematics of the Pezizomycetes – the operculate discomycetes. Mycologia 98: 1029–1040.

Hausner G, Reid J, Klassen GR. 1993. On the subdivision of Ceratocystis s.l. based on partial ribosomal DNA sequences. Canadian Journal of Botany 71: 52–63.

Hennebert GL. 1973. Botrytis and Botrytis-like genera. Persoonia 7: 183–204.

Hennebert GL. 1993. Towards a natural classification of the fungi. In: Reynolds DR, Taylor JW (eds), The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics: 283–294. CAB International, Wallingford, United Kingdom.

Hine RB, Johnson DL, Wenger CJ. 1969. Persistency of 2 benzimidazole fungicides in soil and their fungistatic activity against Phymatotrichum omnivorum. Phytopathology 59: 798–801.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Jalaluddin M. 1967a. Studies on Rhizina undulata. II. Observations and experiments in East Anglian plantations. Transactions of the British Mycological Society 50: 461–472.

Jalaluddin M. 1967b. Studies on Rhizina undulata. I. Mycelial growth and ascospore germination. Transactions of the British Mycological Society 50: 449–459.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, et al. 2006. Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443: 818–822.

Kenerley CM, Jeger MJ. 1992. Fungal diseases of the root and stem. In: Hillocks RJ (ed), Cotton diseases: 161–190. CAB International, Wallingford, United Kingdom.

Kiffer E, Morelet M. 2000. The Deuteromycetes: Mitosporic fungi, classification and generic keys. English ed. Translated by Anantharaman L, Dube DC. Scientific Publishers Inc., Enfield, NH.

King CJ, Loomis HF. 1929. Further studies of cotton root rot in Arizona, with a description of a sclerotium stage of the fungus. Journal of Agricultural Research 39: 641–676.

King CJ, Loomis HF, Hope C. 1931. Studies on sclerotia and mycelial strands of the cotton root-rot fungus. Journal of Agricultural Research 42: 827–840.

Kirk PM, Cannon PF, David JC, Stalpers JA (eds). 2001. Dictionary of the Fungi. 9th ed. CABI Publishing, Wallingford, United Kingdom.

Kirkpatrick TL, Rothrock CS. 2001. Compendium of cotton diseases. 2nd ed. APS Press, St. Paul, MN.

Kopcke B, Weber RWS, Anke H. 2002. Galiellalactone and its biogenetic precursors as chemotaxonomic markers of the Sarcosomataceae (Ascomycota). Phytochemistry 60: 709–714.

Læssøe T, Hansen K. 2007. Truffle trouble: what happened to the Tuberales? Mycological Research 111: 1075–1099.

Lazzari B, Caprera A, Cosentino C, Stella A, Milanesi L, Viotti A. 2007. ESTuber db: an online database for Tuber borchii EST sequences. BMC Bioinformatics 8: S13.

Liu YJJ, Whelen S, Benjamin DH. 1999. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808.

Lyda SD. 1978. Ecology of Phymatotrichum omnivorum. Annual Review of Phytopathology 16: 193–209.

Lyda SD, Burnett E. 1970. Influence of benzimidazole fungicides on Phymatotrichum omnivorum and Phymatotrichum root rot of cotton. Phytopathology 60: 726–728.

Lyda SD, Kenerley CM. 1992. Phymatotrichum. In: Singleton LL, Mihail JD, Rush CM (eds), Methods for Research on Soilborne Phytopathogenic Fungi: 145–148. American Phytopathological Society, St. Paul, MN.

McGinnis S, Madden TL. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Research 32: W20–W25.

McNeill J, Barrie F, Burdet H, Demoulin V, Hawksworth D, Marhold K, Nicolson D, Prado J, Silva P, Skog J, Wiersema J, Turland N (eds). 2006. International code of botanical nomenclature (Vienna Code). Regnum Vegetabile 146. ARG Ganter Verlag, Ruggell, Liechtenstein.

Neal DC. 1929. The occurrence of viable cotton root rot sclerotia in nature. Science 70: 409–410.

Nylander JAA. 2004. MrModeltest 2.2 (distributed by the author). Evolutionary Biology Centre, Uppsala University, Sweden.

O'Donnell K, Cigelnik E, Weber NS, Trappe JM. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89: 48–65.

Orton PD, Watling R. 1979. British fungus flora. Agarics and Boleti. Coprinaceae Part 1, Coprinus. Royal Botanical Garden, Edinburgh, United Kingdom.

Paden JW. 1972. Imperfect states and the taxonomy of the Pezizales. Persoonia 6: 405–414.

Paden JW, Sutherland JR, Woods TAD. 1978. Caloscypha fulgens (Ascomycetidae, Pezizales): the perfect state of the conifer seed pathogen Geniculodendron pyriforme (Deuteromycotina, Hyphomycetes). Canadian Journal of Botany 56: 2375–2379.

Pammel LH. 1888. Root rot of cotton, or "cotton blight". Texas Agricultural Experiment Station Bulletin 4: 3–18.

Pammel LH. 1889. Cotton root-rot. Texas Agricultural Experiment Station Bulletin 7: 5–30.

Paolocci F, Rubini A, Riccioni C, Topini F, Arcioni S. 2004. Tuber aestivum and Tuber uncinatum: two morphotypes or two species? FEMS Microbiology Letters 235: 109–115.

Pfister DH. 1973. Psilopezioid fungi. III. The genus Psilopezia (Pezizales). American Journal of Botany 60: 355–365.

Pfister DH, Winn GJ, Cacavio GJ. 1974. Some comments on Pulchromyces fimicola from the Americas as noted by W. H. Weston. Mycotaxon 1: 137–141.

Poma A, Limongi T, Pacioni G. 2006. Current state and perspectives of truffle genetics and sustainable biotechnology. Applied Microbiology and Biotechnology 72: 437–441.

Rambaut A. 2002. Se-Al, Sequence Alignment Editor 2.0 (distributed by the author). Department of Zoology, University of Oxford, United Kingdom.

Redhead SA, Vilgalys R, Moncalvo JM, Johnson J, Hopple JS. 2001. Coprinus Pers. and the disposition of Coprinus species sensu lato. Taxon 50: 203–241.

Reeb V, Lutzoni F, Roux C. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Molecular Phylogenetics and Evolution 32: 1036–1060.

Riggs JL. 1993. Genomic variation of Phymatotrichum omnivorum (Shear) Duggar revealed by restriction fragment length polymorphism and random amplified polymorphic DNA. PhD thesis, Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Rush CM, Gerik TJ. 1989. Relationship between postharvest management of grain sorghum and Phymatotrichum root rot in the subsequent cotton crop. Plant Disease 73: 304–305.

Shear CL. 1907. New species of fungi. Bulletin of the Torrey Botanical Club 34: 305–317.

Shear CL. 1925. The life history of the Texas root rot fungus, Ozonium omnivorum Shear. Journal of Agricultural Research 30: 475–477.

Stalpers JA. 1974. Spiniger, a new genus for imperfect states of basidiomycetes. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, C 77: 402–407.

Streets RB, Bloss HE. 1973. Phymatotrichum root rot. American Phytopathological Society, St. Paul, MN.

Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods) 4.0b10. Sinauer Associates, Sunderland, MA, USA.

Tedersoo L, Hansen K, Perry BA, Kjoller R. 2006. Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytologist 170: 581–596.

Templeton AR. 1987. Nonparametric phylogenetic inference from restriction cleavage sites. Molecular Biology and Evolution 4: 315–319.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Trappe JM. 1979. Orders, families, and genera of hypogeous Ascomycotina (truffles and their relatives). Mycotaxon 9: 297–340.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.

Vogel HJ. 1964. Distribution of lysine pathways among fungi – Evolutionary implications. American Naturalist 98: 435–446.

Wang Y, Guo LD, Hyde KD. 2005. Taxonomic placement of sterile morphotypes of endophytic fungi from Pinus tabulaeformis (Pinaceae) in northeast China based on rDNA sequences. Fungal Diversity 20: 235–260.

Weresub LK, LeClair PM. 1971. On Papulaspora and bulbilliferous basidiomycetes Burgoa and Minimedusa. Canadian Journal of Botany 49: 2203–2213.

Whelan S, Goldman N. 2001. A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Molecular Biology and Evolution 18: 691–699.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR Protocols: A Guide to Methods and Applications: 315–322. Academic Press, San Diego, CA.

Zolan ME, Pukkila PJ. 1986. Inheritance of DNA methylation in Coprinus cinereus. Molecular and Cellular Biology 6: 195–200.