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

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Key words
Ozonium
Pezizales
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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 corticioid basidiomycetes *Phanerochaete omnivora* (*Polyporales*) and *Sistotrema brinkmannii* (*Cantharellales*; both *Agaricomycetes*) have been suggested as telemorphs 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 *Pilopezia* and *Rhizina*.

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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 *Phymatrichum* 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 attributes). This species is capable of infesting 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).

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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 nonsporulating 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 *Hydnium omnivorum* Shear (1925), once again based on a different type specimen (C.L. Shear 5267, BPI 259732) from that of *Ozonium omnivorum* or *Phymatrichum 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 *Phymatrichum*, and Weresub & LeClair (1971) considered this report to be based on a homothallic culture contaminant.

The type species of *Phymatrichum, P. gemellum* Bonord., was shown to be a member of *Botrytis* by Hennebert (1973). Hennebert (1973) believed that the name *Phymatrichum 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.
| Species                        | Voucher | SSU Accession Numbers | ITS Accession Numbers | LSU Accession Numbers | RPB2 Accession Numbers | β-tub Accession Numbers |
|-------------------------------|---------|-----------------------|-----------------------|-----------------------|------------------------|-------------------------|
| Aleuria aurantia              | OSC 100018 | AY544698              | –                     | AY544654              | DQ247785               | –                       |
| Anthracobia sp.               | OSC 100026 | AY544704              | –                     | AY544660              | –                      | –                       |
| Ascomobolus carbonarius       | KH.00.008(C) (dubl. OSC 100079) | AY544720              | –                     | AY545036              | –                      | –                       |
| Ascomobolus crunulatus        | KH.02.005(C) (dubl. OSC 100082) | AY544721              | –                     | AY545037              | –                      | –                       |
| Ascodesmis nigricans          | CBS 389.68 | –                     | –                     | –                     | –                      | –                       |
| Ascodesmis sp.                | RK 95.55 (O) | U53372               | –                     | –                     | –                      | –                       |
| Balsamia magnata              | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Balsamia crenulata            | U42657 | –                     | –                     | U42684                | –                      | –                       |
| Ascobolus carbonarius         | KH 00.008 (C) (dubl. OSC 100079) | AY544720              | –                     | AY500526              | –                      | –                       |
| Ascobolus crenulatus          | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Ascodesmis nigricans          | CBS 389.68 | –                     | –                     | –                     | –                      | –                       |
| Ascodesmis sp.                | RK 95.55 (O) | U53372               | –                     | –                     | –                      | –                       |
| Balsamia magnata              | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Balsamia crenulatus           | U42657 | –                     | –                     | U42684                | –                      | –                       |
| Ascodesmis nigricans          | CBS 389.68 | –                     | –                     | –                     | –                      | –                       |
| Ascodesmis sp.                | RK 95.55 (O) | U53372               | –                     | –                     | –                      | –                       |
| Balsamia magnata              | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Balsamia crenulatus           | U42657 | –                     | –                     | U42684                | –                      | –                       |
| Ascodesmis nigricans          | CBS 389.68 | –                     | –                     | –                     | –                      | –                       |
| Ascodesmis sp.                | RK 95.55 (O) | U53372               | –                     | –                     | –                      | –                       |
| Balsamia magnata              | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Balsamia crenulatus           | U42657 | –                     | –                     | U42684                | –                      | –                       |
| Ascodesmis nigricans          | CBS 389.68 | –                     | –                     | –                     | –                      | –                       |
| Ascodesmis sp.                | RK 95.55 (O) | U53372               | –                     | –                     | –                      | –                       |
| Balsamia magnata              | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Balsamia crenulatus           | U42657 | –                     | –                     | U42684                | –                      | –                       |
| Ascodesmis nigricans          | CBS 389.68 | –                     | –                     | –                     | –                      | –                       |
| Ascodesmis sp.                | RK 95.55 (O) | U53372               | –                     | –                     | –                      | –                       |
| Balsamia magnata              | JMT 1320 (OSC) | U42664               | –                     | U42683                | –                      | –                       |
| Balsamia crenulatus           | U42657 | –                     | –                     | U42684                | –                      | –                       |

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                                      | SSU+LSU                  | LSU+RPB2                  | RPB2                        |
|----------------------------------------------|--------------------------|---------------------------|------------------------------|
| Neoumula pouchetii                           | NSW 6435 (OSC)           |                           |                              |
| Octospora hygrohypnophila                    | KH.03.30 (FH)            | DQ064539                  | DQ20379                     |
| Orbilia parietina                            | C-F-2444 (C)             | DQ062998                  | DQ062988                     |
| Orbilia auricolor                            | CBS 54763 (dubl. OSC)    | DQ471001                  | DQ470983 / DQ470903         |
| Otidea oreadica                              | SSU: mh 665 (FH), LSU: KH-98-107 (C) | AF006308                  | AF015121                     |
| Pacychella cyphista                          | FH No. 387 (FH)          | DQ064540                  | AY050042                     |
| Pacychypholeus melanoanthus                  | SSU: 1255 (UP), LSU: Gardner & Healy 195 (FH) | AF054899                  | DQ191674                     |
| Parascutellinia cameranosanguinea             | KH.03.34 (FH)            | DQ064541                  | DQ20388                     |
| Paurocotylis pila                            | SSU: UME 32030, LSU: Trappe 1283 (OSC) | US3382                  | AY568337                     |
| Peziza badidasca                             | SSU+LSU: ALTA 9333, RP21: KH-98-12 (C) | AF133175                  | AF133162 / AY050049         |
| Peziza echniniopora                          | SSU: DHP #136 (C), LSU+RP21: Jukka Vauras 9110F (TURA) | AF006309                  | AF335138 / AY050046         |
| Peziza gerardii                              | KH-97-90 (C)             | DQ064543                  | AF335143                     |
| Peziza ibotulata                             | KH.03.157 (C)            | DQ064544                  | AY950548                     |
| Peziza michellii                             | TL-5692 (C)              | DQ064545                  | AY050551                     |
| Peziza polatipapulata                        | KH-98-11 (C)             | DQ064546                  | AY335164                     |
| Peziza quelepoidella                         | NRRRL 22/05              | U26069                    | U42693                      |
| Peziza sullatellina                          | SSU: ALTA 9029, LSU: Winterhoff 8544 (herb. Winterhoff) | AF133144                  | AY335164                     |
| Peziza succosa                               | SSU: UME 29677 (U), LSU: KH-98-07 (C) | US3383                  | AY335166                     |
| Peziza vesiculosa                            | SSU: OSC 100074 (OSC), SSU+LSU: OSC 126 (OSC), LSU+RP21: JV 95-652 (C) | AF133175                  | AY500552 / DQ470948 / AY500499 |
| Phillipia domingensis                        | SSU: mh 663 (FH)         | AF006313                  |                             |
| Phillipia crispata                           | LSU+RP21: T. Læssoe AAI-44695a (AAU, C) | -                      | AY945845 / DQ017599         |
| Phymatotrichopsis omnivora                   | ATCC 22316               | EF441991 / EF494042 / EF441991  |                             |
| Pseudombrophila guldeniae                    | Kongsv. 85.10B (C)       | EF441992                  | EF441993                     |
| Pseudombrophila theiodilcica                  | C-F-70057 (C)            | EF439405 / EF494043 / EF494040 / EF494070  |                             |
| Pseudophaeilla minuscula                     | SSU: mh 673 (FH), LSU: mh 675 (FH) | EF419494 / EF419494       | EF419494 / EF494056         |
| Pseudoplectania nigrella                     | SSU: Japan, LSU: KH-97-28 (FH) | AF104345                  | AY945852                     |
| Pulcopeza cf. nummularisis                   | TL-11785 (QCNE, dubl. C) | EU722510                  | EU722509                     |
| Pulcopeza deligata                           | KH-98-13 (FH)            | DQ494047                  | EF494005 / EF494063         |
| Pulcopeza jurusiskas                         | T. Læssoe AAU-44912 (QCA, dubl. C) | DQ494040 / DQ494040 / DQ494040 / DQ494065 |                             |
| Pulchronyceae fimbriatellata                 | ATCC 18595 (dubl. CBS 127.69, CUP 49531) | EF442001 / EF439405 / EF494059 / EF494069 / EF494066 |                             |
| Pulvilocamptus simplex                        | ATCC 36770 (dubl. IFAS-F 316) | EF442002 / EF494061 / EF494068 / EF494062 |     |
| Pseudonidularia rubrifera                    | SSU: DAOI 195928, LSU: BAP 458 (FH) | U42612                    | DQ20389 / DQ20397           |
| Pylluma confinis                             | TL-11685 (QCNE, dubl. C) | DQ494045                  | DQ20391 / EF494071         |
| Pyrematopsis domesticus                      | SSU: ARON 1766, LSU+RP22: CBS 666.88 (dubl. OSC 100503) | U42610                    | U42687                      |
| Reddeletomyceae donkii                       | JMT 13292 (OSC)          | DQ494044                  | DQ20410                     |
| Rhizina undulata                             | SSU: NRRL 22168, LSU: KH-02.44 (FH) | U42684                    | DQ20410                     |
| Rhizodiscarella Rossae                       | KH-03.107 (FH)           | DQ494050                  | DQ20413                     |
| Sarcocystpha austriaca                       | SSU: mh 667 (FH), LSU: mh 670 (FH) | AF006318                  | AY945856                     |
| Sarcocystpha coccinea                        | spiot 03-02 (dubl. OSC 100003) | AY54589                             | AY544755                     |
| Sarcosphaera coronaria                       | SSU+LSU: OSC 100049, SSU: ALTA 9605, LSU: KS-94-24A (C), RP21: KS-94-19 (C) | AF544712 / AF133157      | AY54468 / AY500555 / AY500523  |
| Scabrepetrea scabrosa                        | Pfister 13.8.83 (FH)     | AF133158                  | AY133173                     |
### Table 1

| Species Vouchers, Isolates, Strains (Herbarium) | GenBank Accession Numbers |
|----------------------------------------------|---------------------------|
| **SSU** | **ITS** | **LSU** | **RPB2** |
| Scutellinia scutellata | SSU: ARON 2188, SSU+RPB2: KH03212003-1 (dubl. OSC 100015), LSU: KS-94-035H (C) U53387/DQ247814 – DQ220421 DQ247796 – | |
| Sowerbyella imperialis | | | |
| Sphaerosporella brunnea | LSU: KH.03.04 (FH) SSU: UME 31147 U53388 – DQ220433 – – | |
| Strobiloscypha keliae | SSU: NSW 7333 (OSC), LSU: NSW 6387 (OSC) AF006310 – DQ220437 – – | |
| Tarzetta catinus | SSU: UME 29731, LSU: KS.94.10A (C) U53390 – DQ062984 – – | |
| Terfezia arenaria | SSU: 1217-1 (UP) AF054898 – – – – | |
| Terfezia claveryi | LSU: Trappe 3195 (FH, dubl. OSC)  – – AY500558 – – | |
| Tricharina praecox | SSU: UME 29738, LSU: KH.04.39 (FH, dubl. DBG) U53390 – DQ220454 – – | |
| Trichophaea hybrida | SSU: –, LSU: DHP 04-599 (FH) AF104664 – AY945859 – – | |
| Trichophaea woolhopeia | KH.01.33 (C)  DQ646553 – DQ220460 – – | |
| Trichophaeopsis bicuspis | SSU: ARON 2222 (O), LSU: NSW 8316 (OSC)  U53391 – DQ220461 – – | |
| Tuber gibbosum | | | |
| Underwoodia columnaris | | | |
| Urnula craterium | SSU: mh 671 (FH, dubl. DEB #278082), LSU+RPB2: DHP 04-511 (FH) AF104347 – AY945851 DQ017595 – | |
| Verpa bohemica | | | |
| Verpa conica | | | |
| Wolfina aurantiopsis | | | |
| Wynnella silvicola | | | |

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

**MATERIALS AND METHODS**

**Cultures**

Phymatotrichopsis omnivora, Pulichromyces fimicola and Sistotrema brinkmannii were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultures of Phanerochaete omnivora and Phanerochaete chrysorhiza 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 & Kennerly 1992) and maintained on modified ATCC medium 1078 (M1078), containing per 1 000 mL distilled water: 1 g NH₄NO₃; 0.75 g MgSO₄; 0.4 g KH₂PO₄; 0.9 g K₂HPO₄; 0.1 g CaCl₂; 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.
primers (PoITSA 5′-CCTGCGGAAGATCATTAAA-3′ and PoITSB 5′-GGGGGTTCCTTTGTAGGGG-3′; developed in this study). Hand-sectioned sporematas 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 (sporemata on soil pedils 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)°;  
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. ined., 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 bead homogenization. Genomic DNA was isolated following Zolan & Pukkila (1986). Molecular methods #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 electro-phoresis in 0.7% agarose gels in Tris acetate EDTA (TAE) buffer followed by electrophoresis (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 mm³ 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), LR0 and LR7 (for LSU) or SGG and LR5 (for LSU to SSU) (Vilgalys & Hester 1990, White et al. 1990, Hauser et al. 1993). Two successive PCR reactions were used to amplify the ITS region from the *P. omnivora* herbarium specimens. 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 was used as template in 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 RP2B from *P. omnivora* isolates were amplified in two overlapping segments using the primer pairs RP2B-Ds3F (5′-WSYGRAGAAGHTBYTATGCRCAAGCGC-3′) and RP2B-7cR, and RP2B-Ds6F (5′-TGGGGWYSGTHTGTYCCCGWC-3′) and RP2B-11aR. A region of the β-tubulin gene spanning three introns was amplified and sequenced with primers Bt2a and Btsp (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, Hauser et al. 1993); and RP2B-Ds3F, RP2B-5F, RP2B-5R, RP2B-Ds6F, RP2B-7cF, RP2B-7cR, RP2B-980F, RP2B-1014R, RP2B-1554R, RP2B-1599F, RP2B-2488F, RP2B-2568R and RP2B-11aR for RP2B (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, McCinnis & 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*, *Pulchoromycetes fimicola* and an additional species of *Psilotepoo*, *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 (Rambout 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 *Pulchoromycetes fimicola* within *Pezizales*, a data matrix including an additional gene, β-tubulin, was compiled representing a subset of the taxa from the combined LSU and SSU dataset. Amino acid sequences of RP2B 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 2005) 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 DNA 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, OAIlf8, formed typical sporemats on the surface of black clay (Fig. 1g), in which OAIlf8-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 conidiophores 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 pods 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 deliquata (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–Tubercaceae) and C (Pyronemataceae–Ascodesmidaceae–Glaziellaceae–Sarc-
69S.M. Marek et al.: Systematics of Phymatotrichopsis 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. 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 omnivora; 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.
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.
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 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 Rhizinaeae with Psilopezia and Rhizina. Rhizinaeae 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 Rhizinaeae 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 Rhizinaeae to group with lineage B (with or without Caloscyphaeae) or lineage C. Based on SSU and LSU sequences, Pulchrolyces fimbicola (formerly Phymatrichotum fimbicola) is also a member of the class Pezizomycetes, but is clearly not congeneric with Phymatrichotipsis. Instead, it is closely related to members of the C-lineage, possibly in Pyronemataceae or Ascodesmidae. Pulchrolyces 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 Pulchrolyces, namely Ascodesmis, Lasiodiplodiium, Lasiodolus and Pseudomorphalia (Fig. 2), are similarly fimbicolic, although the fimbicolic 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 Pulchrolyces at the family level.

The anamorphic morphology of Phymatrichotipsis omnivora partially supports its placement in the Pezizomycetes. The botryoblastocnidia produced by Phymatrichotipsis omnivora are also observed in many of the pleomorphic Pezizomycetes in which anamorph–teleomorph associations have been determined. For example, for the anamorphic genera Chromelosporium, Oedoccephalum, Ostracoderma, Gischroderma and Dichobrys, are associated with the Pezizomycetes meiosporic genera, Peziza (first four) and Trichophyta (Paden 1972, Hennebert 1973, Hansen et al. 2001). However, botryoblastocnidal reproduction occurs in several classes of both the Ascomycota and Basidio-

mycota. Such anamorphic genera are found in the Leotiomycetes (inoperculate discomyceses), in Botryis, Streptothrix, Ambrophotrycs, and Verucobotrycs, and in the Agaricomycetes (Hombasidionymycetes), in Spingera (Hennebert 1973, Stalpers 1974, Kiffer & Morelet 2000). Thus, botryoblastocnidal patterns of conidio-
genesis arose several times during fungal evolution and may have limited value for taxonomic classifications above genus. Rhizomorph-like, mycelial strands are formed by both Phymatrichotipsis omnivora (Lyda & Kenerley 1992) and, proposed con-

familial, Rhizina undulata (Booth & Gibson 1998). Consipicuous 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 disemina-
tion, the mycelial strands connect the reproductive structures, sporemites of Phymatrichotipsis 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 Phymatrichotipsis eventually form long-lived, hypogeous scle-

rotia (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 mycorhizal, in the Pezizales (Trappe 1979, Læsøe & 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 Phymatrichotipsis 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 coryneoides; mitosporid Ursula) (Kopcke et al. 2002, Wang et al. 2005). Also, species of Octospora, Lamprospora and Neotilliota form obligate associations with numerous bryophytes, which have been interpreted as parasitic (Döbbeler 1979, Benkert 1993, Davey & Currah 2006). Both Phymatrichotipsis and Rhizina also colonise dead plant debris in field situations, as facultative saprobates, 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 Phymatrichotipsis. The little that is known about the natural history of Psilopezia suggests a sapro-
bic life style on wet, rotted wood (Pfister 1973), while Rhizina and Phymatrichotipsis are plant pathogens with a facultative saprobic phase. Nevertheless, based on our phylogenies of combined rDNA and RP2B sequences, the monophyly of the Rhizinaeae, including Rhizina undulata, Phymatrichotipsis omnivora and Psilopezia deligata, was highly supported (BP 86 %, PP 100 %) and constraint topologies that forced Phymatrichotipsis to group outside Rhizinaeae were rejected. The relationships among Psilopezia, Rhizina and Phymatrichotipsis 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 Phymatrichotipsis. More members of the Rhizinaeae must be identified and charac-
terized before further inferences on the evolution of their nutri-
tional strategies can be clarified.

Knowledge of the correct phylogenetic placement of the cot-
ton 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 gen-

genome sequence of Phymatrichotipsis omnivora strain OKAfl8 (http://www.genome.ou.edu/fungi.html) through comparative genomics with related ascomycetes. In addition to Phymatrichotipsis, genomic projects of two other Pezizomycetes, Tuber melanosporor and T. borchii, are ongoing (Poma et al. 2006, Lazzeri et al. 2007; http://mycor.nancy.inra.fr/IMGC/Tuber-

Genome/index.html). The insights into the genetic underpin-
ings of this fascinating, but understudied, class of fungi should prove fruitful.

Nomenclature and typification

Given the economic importance of Phymatrichotipsis 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 Oszonium omnivorum Shear to the genus Phymatotri-

chum because of the presence and nature of conidia in speci-

mens 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 (nom. cons.) is treated as new 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 ‘morp’ 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 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 OKAf8 (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.

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