

Pseudogymnoascus palmeri



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Pseudogymnoascus palmeri Rea-Ireland, Smyth, Lindner & Overton, *sp. nov.*

Etymology. Named after Jonathan M. Palmer, formerly of the United States Forest Service, for his many contributions to the study of *Pseudogymnoascus* and his contributions to establishing mating-type genes for the genus.

Classification — *Pseudeurotiaceae*, *Thelebolales*, *Leotiomycetes*.

On Rose Bengal Agar (RBA): *Ascomata* gymnothecial, solitary, globose, measuring 85.3–172.5 (av. = 125.3, n = 10) × 60–161.8 (av. = 112.9, n = 10) μm in size; grey orange (5B3-6; Kornerup & Wanscher 1978); developing rapidly and ripening within 10 d at 25 °C (12 h white fluorescent light / 12 h dark). *Ascomatal* initials coiled to irregular; peridium is a gymnothecium composed of *textura intricata*, the peridial hyphae darkly pigmented brownish yellow (5C7), smooth to minutely roughened with distinct appendages. *Asci* ovoid, 8-spored, 6.7–9.5 (av. = 7.6, n = 35) × 4.9–7.3 (av. = 5.9, n = 35) μm. *Ascospores* aseptate, fusoid to ellipsoid, smooth, grey orange (5B36-6); 2.9–4.1 (av. = 3.4, n = 80) × 1.8–3 (av. = 2.3, n = 80) μm.

Culture characteristics — (12 h white fluorescent light / 12 h dark at 25 °C): Colony colour analysed on Sabouraud dextrose agar (SAB) acidified with 120 μL 85 % lactic acid for optimal pigment production rather than RBA because the pink colour of the agar compromises interpretation of fungal pigmentation. Colony reverse at first yellow white (4A2), maturing to grey orange (5B3-6) with age after 10 d. On oatmeal salt sediment agar, colony reverse colour is diffuse light orange to orange (6A5-7).

Typus. USA, Pennsylvania, Centre County, Woodward Cave, from sediment, 2019, *B. Overton* LHU 407 (dried, non-metabolically active holotype CUP-70724, in Cornell University Plant Pathology Fungarium, metabolically active culture CBS 147159 in the CBS Collection of the Westerdijk Fungal Biodiversity Institute ITS, *rpb2*, and *tef1* sequences GenBank MT988150, MW054468, MW054467; MycoBank MB837413).

Notes — *Pseudogymnoascus palmeri* produces sexual structures on SNA and RBA in the presence of a bacterium co-isolated from the original sediment sample. A BLAST search of the bacterial co-isolate's 16S rDNA provided a 100 % match with *Pseudomonas moorei*. Culture became sterile after removal of the co-isolated bacterium using SAB acidified with 120 μL 85 % lactic acid. Sterility was maintained, even when the fungal isolate was re-plated onto RBA or SNA. Morphological analyses suggest that *P. palmeri* and *P. roseus* could be sister taxa. They are similar in the morphological characteristics of gymnothecial ascomata production and ascospore size. Samson (1972) described *P. roseus* as being characterised by pinkish to reddish ascomata, roughened appendages with spines or warts, and the presence of aleurioconidia. *Pseudogymnoascus palmeri* can be distinguished from *P. roseus* based on conidiogenesis (*P. palmeri* does not produce conidia) and colour (*P. palmeri* ascomata are grey orange). Samson (1972) did not describe

Colour illustrations. Background photo of Woodward Cave, Pennsylvania, USA. Confocal laser-scanning image of gymnothecium; DIC image of ascospores on synthetic nutrient-poor agar (SNA); colony back colour on SAB at 10 d; ascomatal initials on SNA at 10 d; asci and peridial hyphae on SNA. Scale bars = 20 μm (gymnothecium), 2 μm (ascomatal initial), 5 μm (all others).

the reverse colour of colony plates, but as a morphological character in *Pseudogymnoascus*, this should not be ignored (Crous et al. 2019c). Minnis & Lindner (2013) were the first to examine many *Pseudogymnoascus* taxa using modern phylogenetic methods. This work builds off their multi-gene approach, utilising three of the five phylogenetically informative loci useful for phylogenetic species resolution within the genus *Pseudogymnoascus* (Minnis & Linder 2013).

The three-locus phylogenetic analysis conducted in this study indicates strong support for the placement of *P. palmeri* (LHU 407) in a clade with isolate WSF 3629 (Minnis & Linder 2013). Phylogenetically, WSF 3629 is closely related to clade G in the *P. roseus* complex (Palmer et al. 2014). This isolate is also of significant interest due to its phylogenetic proximity to the white-nose syndrome pathogen, *P. destructans*.

The relationship of this complex to *P. destructans* has not been fully resolved, even with a five-gene analysis (Minnis & Linder 2013). WSF 3629 was suggested as a new species by Palmer et al. (2014) but has remained an undescribed species since the publication of their work. This study honours the work of Palmer, and formally describes the new species as *Pseudogymnoascus palmeri* sp. nov. and identifies a new strain of this species (LHU 407) from Pennsylvania.

In addition to morphology, phylogenetic analysis of a three-gene multi-locus alignment (ITS nrDNA, *rpb2* and *tef1*) support the description of isolates LHU 407 and WSF 3629 as the phylogenetic species *P. palmeri*, distinct from clade G in the *P. roseus* complex. This study generated the three-locus dataset for isolate LHU 407. The challenge in resolving the clade G in the *P. roseus* complex, as well as its association with *P. destructans*, highlights the need for greater biodiversity sampling of the genus *Pseudogymnoascus*. The multi-gene data for isolate WSF 3629, and the remainder of the *Pseudogymnoascus* species, were derived from Minnis & Linder (2013), as well as previous species descriptions for *P. lindneri* and *P. turneri* (Crous et al. 2019c). The outgroup taxon was *Gymnascella minnisii*, GenBank *rpb2* and *tef1* sequences MW054470, MW054469, also described in this issue of Fungal Planet.

Supplementary material

FP1156 Phylogenetic placement of *Pseudogymnoascus palmeri* sp. nov. on a strict consensus maximum parsimony tree with maximum likelihood/maximum parsimony bootstrap support values (based on 1000 bootstrap pseudo-replicates), was determined from analysis of a multi-gene alignment of rDNA (primers ITS1, ITS4; White et al. 1990), *rpb2* (primers RPB2-7cF, RPB2-11aR; Liu et al. 1999), and *tef1* (primers EF1-983F, EF1-2218R; Rehner & Buckley 2005). PAUP v. 4.0a build 167 (Swofford 2003) was used to conduct the maximum parsimony analysis. The parsimony analysis generated a single most parsimonious tree which was also the strict consensus. A maximum likelihood analysis was completed using GARLI v. 2.01 (Zwickl 2006) on the CiPRES Science Gateway (Miller et al. 2010). A consensus tree was generated from a single replicate ML analysis with 1000 bootstrap pseudo-replications. There were no significant topological differences between the parsimony and likelihood consensus trees. For maximum likelihood, the General Time Reversible (GTR) evolutionary model was utilised, the proportion of invariant sites was set to estimate, and the model of rate heterogeneity was set to gamma distribution. Bootstrap support values located at nodes are: Maximum Likelihood/Maximum Parsimony. Alignment and tree(s) are deposited in TreeBASE (study 27014).

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