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Pycnopulvinus aurantiacus gen. et sp. nov., a new sporocarp-forming member of Pucciniomycotina

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Abstract

An unusual fungus producing minute orange stilboid sporocarps was found on a palm leaf mid-rib in a Neotropical forest. Morphological observations could not place this collection into any previously described species or genus and, due to an absence of sexual structures, even higher level placement was uncertain. Phylogenetic analysis of a portion of the large subunit and the internal transcribed spacer of the nuclear ribosomal DNA indicated that this fungus is related to *Heterogastridium pycnidioideum* and belongs to Heterogastridiales, Microbotryomycetes (Pucciniomycotina). A new genus and species, *Pycnopulvinus aurantiacus*, are proposed here to accommodate this fungus.

Key words

Ceratocystis, Fungal biodiversity, litter fungi, palm fungi, *Pycnobasidium*, tropical mycology

Introduction

The majority of Pucciniomycotina (Basidiomycota) species have life cycles that include the production of microscopic fruiting structures (e.g., spermogonia of rust fungi), but only a few species within the subphylum form macroscopic fruiting bodies (Swann et al. 2001; Aime et al. 2014). Although these sporocarps vary in form (e.g., see Aime et al. 2014), many are stilboid or pycnidioid with spore masses produced at the base of the sporocarp and exiting through the tip of the neck. Fungi with such fruiting bodies can be found mostly in Atractiellomycetes (Oberwinkler and Bandoni 1982) and, as is true for many other members of Pucciniomycotina, little is known about their biology or biodiversity.

Heterogastridium pycnidioideum Oberw. & R. Bauer (anamorph *Hyalopycnis blepharistoma* (Berk.) Seeler) is one such fungus that produces stilboid fruiting bodies. It is a strictly filamentous species with simple septal pores and specialized organelles–colacosomes–associated with mycoparasitism (Oberwinkler et al. 1990). *Heterogastridium* is placed in Heterogastridiaceae and Heterogastridiales (Oberwinkler et al. 1990), which are now placed in Microbotryomycetes (Weiss et al. 2004). Bauer et al. (2006) placed three genera in Heterogastridiaceae in addition to *Heterogastridum*–*Atractocolax* R. Kirschner, R. Bauer & Oberw., *Colacogloea* Oberw. & R. Bauer, and *Krieglsteinera* Pouzar. Of these, neither sequence data nor reference cultures are available for two of the genera, *Atractocolax* and *Krieglsteinera*, both of which are monotypic. *Colacogloea*, on the other hand, has been demonstrated several times to form a separate clade of Microbotryomycetes, distant from Heterogastridiales (e.g. Aime et al. 2006; Kurtzman et al. 2011). Thus, at present, only *Heterogastridium* and its sole species, *H. pycnidioideum*, can be confidently assigned to Heterogastridiales with molecular data.

An unusual stilboid fungus was discovered on the mid-rib of a palm leaf in litter that could not be confidently assigned to any previously described genus. DNA sequence data and phylogenetic analyses indicated that the collection represents a new member of Heterogastridiales. Herein we describe and illustrate *Pycnopulvinus aurantiacus* gen. et sp. nov. and provide a phylogenetic analysis of Heterogastridiales.

Methods

The specimen (PUL F2679) was collected near Bilsa Biological Station in Ecuador, in the vicinity of N0.350444, W79.732075, on 3 May 2004, where it was growing on the mid-rib of a dead palm leaf in the litter. The fungus was photographed and described in the field after which small pieces of substrate bearing the fruiting bodies were dried on an herbarium drier. Color designations refer to Kornerup and Wanscher (1978). Duplicates are deposited in the Kriebel Herbarium at Purdue University (PUL) and the herbarium of the Pontificia Universidad Catolica del Ecuador (QCA).

Morphological characters of the fruiting bodies were observed first with an Olympus SZ61 dissecting microscope. With the aid of the dissecting microscope, a few fruiting bodies were carefully removed from the substrate and placed in a sterile water droplet on a microscope slide, permitting five minutes of rehydration before further preparation. Thereafter microscopic characters were examined with a Nikon Eclipse 80i microscope with standard differential interference contrast (DIC) settings and with 10×, 20×, 40× and 100× objectives. The length and width of 20 spores was measured from three different fruiting bodies with an ocular micrometer using 100× oil-immersion objective. Images were taken with Nikon Digital Sight DS-Fi1 camera setup and measurements were calibrated with a stage micrometer.

For molecular characterization, five dry fruiting bodies were carefully removed from the leaf surface to avoid the inclusion of leaf material and potential contaminants. These were used for DNA extraction with the E.Z.N.A. High Performance

DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA), following the manufacturers' instructions for samples with lower DNA content (protocol 3). PCR reactions were carried out in 25 µL reactions that contained 12.5 µL of Apex Taq RED Master Mix (Genesee Scientific, San Diego, CA, USA), 1.25 µL of each primer (10 µM), 5 µL of molecular grade water and 5 µL of template DNA. Amplification of the ITS region was conducted with primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), and the LSU region was amplified with LR0R and LR5 (Vilgalys and Hester 1990). Amplification conditions followed Toome et al. (2013). Sequencing of amplified fragments was performed by Beckman Coulter, Inc. (Danvers, MA), using the same primers that were used for amplification. Sequences were edited with Sequencher 5.2.3 (Gene Codes Corporation, Ann Arbor, MI, USA) and are deposited in GenBank.

A BLASTn analysis in GenBank (http://www.ncbi.nlm.nih.gov) was used to locate similar sequences for phylogenetic analyses. For the LSU dataset, sequences sharing >92% identity with PUL F2679 were selected and for the ITS dataset sequences sharing >85% identity with PUL F2679 were included. Sequences from *Rhodotorula hylophila* and *R. javanica* were added based on their relatedness according to the analyses of Kurtzman et al. (2011) and *R. yarrowii* was included for rooting purposes. Only the sequences from type strains were retained for previously described species. Accession numbers for sequences used are indicated on Figure 1.

Both datasets were aligned separately using the Muscle algorithm in MEGA 5.2 (Kumar et al. 2008), yielding 695 bp and 605 bp alignments for the ITS and the partial LSU region, respectively. These two alignments were subsequently concatenated for phylogenetic analyses. The final alignment is available in TreeBASE (http://treebase.org) under accession number 15679.

Phylogenetic analyses were performed via the CIPRES Science Gateway (Miller et al. 2010). The maximum likelihood (ML) analyses were conducted in RAxML-HPC2 7.6.3 using the -k option for bootstrap analysis. Bayesian posterior probability analyses were conducted with MrBayes 3.2.2 with parameters set to 10 000 000 generations, two runs and four chains. The resulting phylogenetic tree was edited in Incscape (http://www.inkscape.org).

Results

Sporocarps of PUL F2679 had a swollen cushion-like basal region, measuring 0.2 to 1 mm in diameter after drying. The basal region was pigmented, ranging from light to dark orange when fresh and appearing orange-brown after drying. This base supports a narrow synnemata-like structure with a 1 to 2 mm long neck. At the apex of the neck, a light yellow to orange mucous droplet of spores is formed (Figure 2 a–f). While the size of sporocarps was variable, all of them produced a spore-containing droplet. Although it could not be definitely determined, the spores appear to be asexually produced. Also, despite numerous attempts to isolate the fungus into pure culture from revived

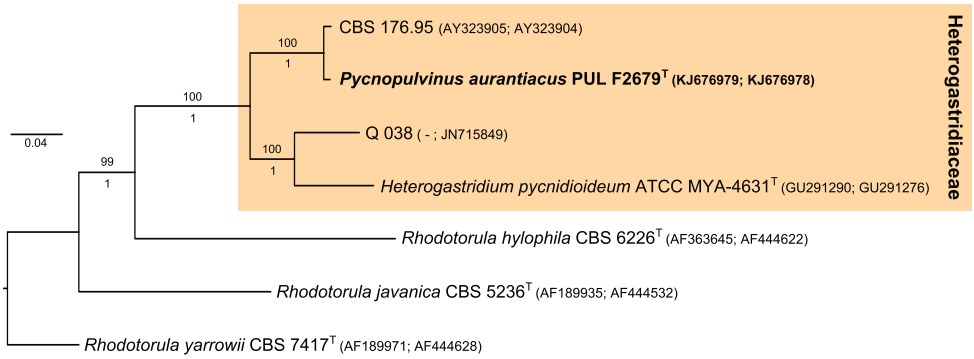

Figure 1. Maximum likelihood tree illustrating the placement of *Pycnopulvinus aurantiacus* in relation to other known members of Heterogastridiaceae. The analysis was performed with combined LSU and ITS sequence data and the topology was rooted with *Rhodotorula yarrowii*. The numbers above and below branches show the bootstrap and posterior probability values, respectively. LSU and ITS GenBank accession numbers of each used strain are given in the brackets. Superscript letter T indicates sequence data that originate from the type.

spores, no isolate was obtained. Only an isolate of *Ceratocystis paradoxa* (deposited in GenBank as AY821864; CBS 116770) was frequently recovered during these attempts.

Of previously sequenced species, PUL F2679 shared the most sequence identity with *H. pycnidioideum* (= *H. blepharistoma*) at 92% (414 bp of 452 bp) in the LSU region and 86% (515 bp of 596 bp) in the ITS region. The only other close match was to a previously sequenced but undescribed isolate, CBS 196.95 (GenBank no. LSU–AY323905; ITS–AY323904), which shared 98% identity (446 bp of 453 bp) in the LSU region and 99% identity (609 bp of 616 bp) in the ITS region. Results of phylogenetic analyses are presented in Figure 1.

Taxonomy

Pycnopulvinus **Toome & Aime, gen. nov.**
MycoBank MB808523

Diagnosis. Member of Heterogastridiaceae, Heterogastridiales, Microbotryomycetes, Pucciniomycotina. *Pycnopulvinus* is similar to *Heterogastridium*, but differs in possessing a distinct basal cushion, segmented spores, and pigmented sporocarps. *Pycnopulvinus* can also be distinguished with rDNA sequence data.

Type. *Pycnopulvinus aurantiacus* Toome & Aime

Description. Minute, pigmented stilboid sporocarps with a swollen basal region and long tubular neck bearing a mucoid droplet of spores at the tip.

Ecology and distribution. On palm litter in South America (Ecuador); known from sequence data in Central America (Costa Rica).

Etymology. *pycno-* = dense, compact, and *pulvinus* = cushion, for the distinctive cushion-like base of the sporocarp.

Discussion. The genus is closely related to *Heterogastridium*, but has orange-colored fruiting bodies that are larger and form a distinct basal cushion. Of the other three genera currently recognized in Heterogastridiales (Kirk et al. 2008, Bauer et al. 2006), *Pycnopulvinus* can be readily separated from *Colacogloea* on the basis of DNA sequence data (Aime et al. 2006), and the absence of stilboid fructifications or septate spores in the latter, from *Krieglsteinera* which does not form fruiting bodies or multicelled spores and parasitizes ascocarps, and from *Atractocolax* which forms smaller (140 µm diam.), gelatinous, hyaline sporocarps, produces unicellular spores and is associated with bark beetles. Currently, *Pycnopulvinus* is a monotypic genus. A previously undescribed isolate, CBS 176.95 (isolated from the tropical forest of Costa Rica and accessioned in the GenBank as "*Pycnobasidium* sp." – a generic name that has not been validly published – appears to represent a member of this genus. In the absence of morphological or other additional data, it cannot be determined whether CBS 176.95 is conspecific with *P. aurantiacus* or represents a second species of *Pycnopulvinus*. Despite the rapid accumulation of environmental sequencing data, no studies thus far have published sequences referable to *Pycnopulvinus*, indicating that members of the genus are probably not widely dispersed in commonly sampled habitats.

***Pycnopulvinus aurantiacus* Toome & Aime, sp. nov.**
MycoBank MB808524
Figure 2

Diagnosis. Sporocarps minute, orange, with a swollen basal cushion (up to 3 mm wide) and long narrow neck (up to 3 mm long) subtended by a light yellow to orange mucous droplet (up to 1.5 mm diam.) of hyaline, 2–4 celled spores, averaging 3.25 × 11.8 µm. Found on palm leaf litter.

Type. ECUADOR. Manabi Division, near Bilsa Biological Station, in the vicinity of N0.350444, W79.732075, on palm leaf mid-rib in the litter, 3 May 2004, M.C. Aime, MCA 2548 (holotype PUL F2679; isotype QCA). GenBank no. KJ676978 (ITS), KJ676979 (LSU).

Description. Gregarious, light to dark orange (ca. 5A6–7), superficial, stilboid sporocarps with swollen globose base (0.5–3 mm wide in fresh specimen, drying to 0.2–1 mm), surrounded by hyphae with globular apical cells, 20–30 µm wide and 45–55 µm long. Sporocarp necks erect, long (0.5–2 mm), narrow (up to 110 µm at base, 50–70 µm at middle and widening up to 130 µm at tip), tubular, light yellow to orange, smooth. Hyphae on the outer layer of the neck 5 µm wide, septate; hyphae at the base and inside the neck 2–2.5 µm wide, septate. Ostiolar hyphae extend from the outer layer of the neck cells, hyaline, non-septate, 10–12 µm wide, narrowing at the tip. Clamp connections not observed. Spores accumulate in pale to orange mucous droplets at tips

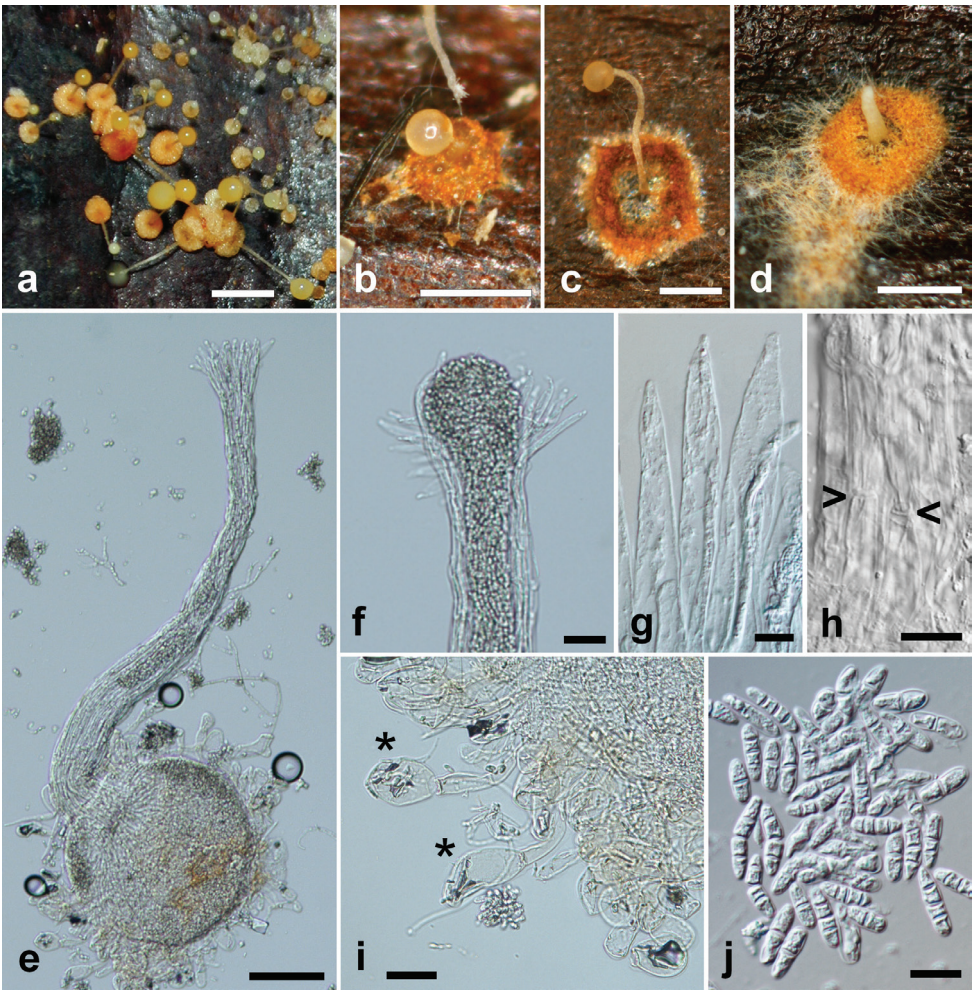

Figure 2. *Pycnopulvinus aurantiacus* (holotype PUL F2679). **a** Field photo of fresh sporocarps on palm leaf. Note the variable size and color of the sporocarps. Bar = 2 mm **b–d** Sporocarps of various stages and sizes after drying. Bars = 0.5 mm **e** Sporocarp as seen under the light microscope. Note the swollen pigmented base with surrounding large globose cells and the long tubular neck with a widening tip. Bar = 200 µm **f** Tip of the neck with spore mass exiting the sporocarp. Bar = 25 µm **g** Ostiolar hyphae at the tip of the neck. Bar = 10 µm **h** Outer surface of the neck, wide hyphae are visible, septa are marked with arrows. Bar = 10 µm **i** Globose cells (marked with asterisk) surrounding the base of the sporocarp. Bar = 25 µm **j** Multicellular spores produced inside the sporocarps. Note the four-celled spores on the left breaking into smaller compartments. Bar = 10 µm.

of sporocarp necks, approximately half of the size of the basal cushion in diam. (up to 1.5 mm diam. in fresh material). Spores hyaline, mostly 2–4 celled and cylindrical or somewhat fusiform, measuring 3–4 × 7–18 µm (average 3.25 × 11.8 µm), breaking into smaller compartments. No sporogenous cells were detected at the interior of the sporocarp, but the spores are likely asexually produced. Basidia not observed.

Ecology and distribution. On palm leaf litter in tropical forest of Ecuador. Possibly occurring in association with other fungi. Known only from the type locale.

Etymology. *aurantiacus* = orange, for the color of fresh sporocarps.

Specimens examined. PUL F2679.

Discussion. The sporocarps most likely represent an asexual stage of *P. aurantiacus*. Anamorphic stilboid conidiomata have been described for other members of Pucciniomycotina, especially in Atractiellomycetes (e.g. Oberwinkler et al. 2006). However, multicellular spores have not previously been described for any sporocarp-forming member of Atractiellomycetes or Microbotryomycetes. The organic matter of palm trees is not a common substrate among Pucciniomycotina and only one other basidiocarp-forming genus, *Agaricostilbum* Wright, is known to specifically inhabit palm litter (Wright et al. 1981). The recovery of *C. paradoxa* from the material might hint on a possible mycoparasitic strategy for *P. aurantiacus*.

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References

Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, Piepenbring M, McLaughlin DJ, Szabo LJ, Begerow D, Sampaio JP, Bauer R, Weiss M, Oberwinkler F, Hibbett DS (2006) An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. Mycologia 98: 895–905. doi: 10.3852/mycologia.98.6.896

Aime MC, Toome M, McLaughlin DJ (2014) Pucciniomycotina. In: McLaughlin DJ, Spatafora JW (Eds) The Mycota: Systematics and Evolution, 2nd edition, vol 7A. Springer-Verlag, Berlin, 271–294.

Bandoni RJ, Oberwinkler F (1981) *Hyalopycnis blepharistoma*: a pycnidial basidiomycete. Canadian Journal of Botany 59: 1631–1620. doi: 10.1139/b81-219

Bauer R, Begerow D, Sampaio JP, Weiss M, Oberwinkler F (2006) The simple-septate basidiomycetes: a synopsis. Mycological Progress 5: 41–66. doi: 10.1007/s11557-006-0502-0

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhiza and rusts. Molecular Ecology 2: 113–118. doi: 10.1111/j.1365-294X.1993.tb00005.x

Kornerup A, Wanscher JH (1978) Methuen handbook of colour, 3rd ed. Richard Clay Ltd., Chichester.

Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics 9: 299–306. doi: 10.1093/bib/bbn017

Kurtzman CP, Fell JW, Boekhout T (2011) The Yeasts, a Taxonomic Study, 5th edition, vol 3. Elsevier, London, UK.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop. New Orleans, Louisiana, 1–8.

Oberwinkler F, Bandoni RJ (1982) A taxonomic survey of the gasteroid, auricularioid Heterobasidiomycetes. Canadian Journal of Botany 56: 1726–1750.

Oberwinkler F, Bauer R, Bandoni RJ (1990) Heterogastridiales: a new order of basidiomycetes. Mycologia 82: 48–58. doi: 10.2307/3759962

Oberwinkler F, Kirschner R, Arenal F, Villarreal M, Rubio V, Begerow D, Bauer R (2006) Two new pycnidial members of the Atractiellales: *Basidiopycnis hyalina* and *Proceropycnis pinicola*. Mycologia 98: 637–649. doi:10.3852/mycologia.98.4.637

Swann EC, Frieders EM, McLaughlin DJ (2001) Urediniomycetes. In: McLaughlin DJ, McLaughlin EJ, Lemke P (Eds) The Mycota: Systematics and Evolution, vol 7B. Springer-Verlag, Berlin, 37–56.

Toome M, Roberson RW, Aime MC (2013) *Meredithblackwellia eburnea* gen. et sp. nov., Kriegeriaceae fam. nov. and Kriegeriales ord. nov. – toward resolving higher-level classification in Microbotryomycetes. Mycologia 105: 486–495. doi: 10.3852/12-251

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. http://jb.asm.org/content/172/8/4238.full.pdf+html

Weiss M, Bauer R, Begerow D (2004) Spotlights on heterobasidiomycetes. In: Agerer R, Piepenbring M, Blanz P (Eds) Frontier in Basidiomycote Mycology. IHW-Verlag, Eching, 7–48.

White TJ, Bruns TD, Lee S, Taylor JW (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. Academic Press, San Diego, California, 315–322. doi: 10.1016/B978-0-12-372180-8.50042-1

Wright JE, Bandoni R, Oberwinkler F (1981) *Agaricostilbum*: an auricularioid basidiomycete. Mycologia 73: 880–886. doi: 10.2307/3759799