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ASPERGILLUS CAELATUS, A NEW SPECIES IN SECTION *FLAVI*

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ABSTRACT

Aspergillus caelatus, a new species belonging to subgenus *Circumdati* section *Flavi*, is described from agricultural soils and peanut seeds in southwestern Georgia, USA. The species is similar to *A. tamarii*, but differs in colony color and smaller conidiophore structures as well as in its mycotoxin profile.

Species belonging to *Aspergillus* subgenus *Circumdati* section *Flavi* are among the most studied fungi owing to their importance in food fermentations (Cook and Campbell-Platt, 1994) and in the formation of mycotoxins (Kozakiewicz, 1994). The carcinogenic aflatoxins produced by *A. flavus* Link, *A. parasiticus* Speare, and *A. nomius* Kurtzman et al. have been subjects of intensive biochemical and medical research (Eaton and Groopman, 1994). In the peanut-growing regions of southwestern Georgia, an undescribed species of *Aspergillus* in section *Flavi* is frequently isolated from agricultural soils and also occurs in peanut seeds from insect-damaged pods (Horn and Greene, 1995; Horn et al., 1995). This distinctive species was originally designated as *A. tamarii* type B pending its formal description (Horn and Greene, 1995; Horn et al., 1995, 1996) and is herein described.

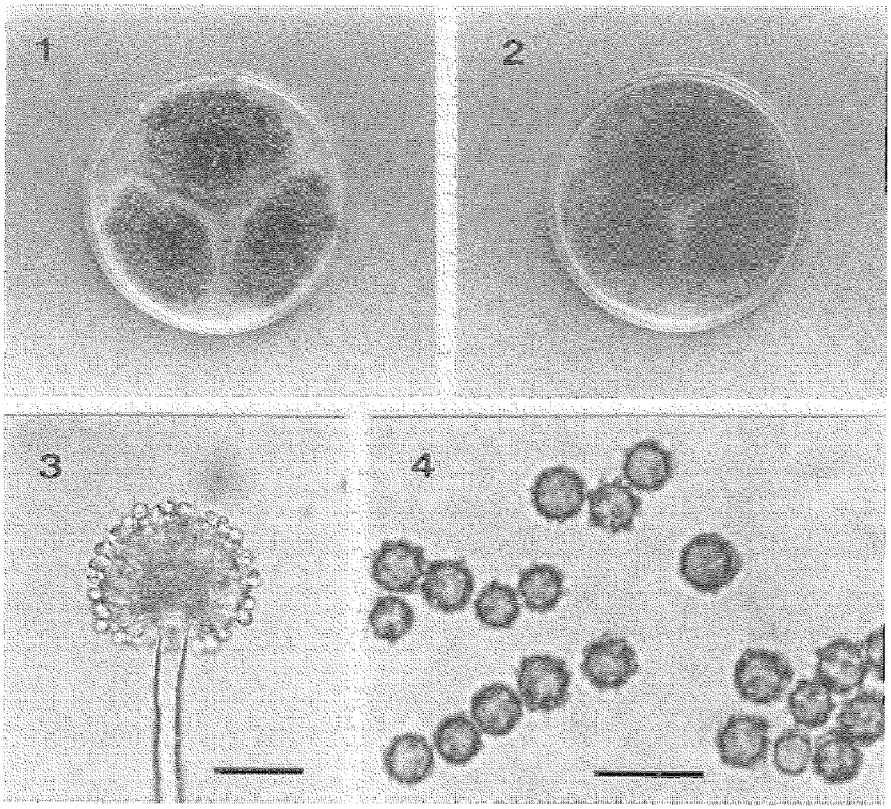
Aspergillus caelatus B. W. Horn, sp. nov. Figs. 1-4

Coloniae in agaro Czapekii ad 25 C post 7 dies usque ad diametrum 3.0-4.5 (-5.5) cm crescunt; ad 37 C post 7 dies ad diametrum 3.5-5.5 cm. Superficies coloniarum in aspectu velveti, ex capitulis conidialibus abundantibus composita (Fig. 1). Capitula conidialia in toto visa olivacea (1-2E5; Kornerup et Wanscher, 1978) post 7 dies (ad 25 C), post 14 dies olivacea brunnescentia et fuscantia (2E4-F4; Kornerup et Wanscher, 1978). Pars aversa flavido-brunnea; agar pigmentum diffluens concolor continens; isolationes sclerotiales exsudatum pellucidum vel subroseo-brunneum continentes. Sclerotia absentia vel abundantia, primo alba, deinde roseo-brunnea, prostremo purpureo-atra, subglobosa vel elongata vel saepe in ambitu irregularia, 400-1700 µm longa, 200-1100 µm lata. Capitula conidialia uni- vel biseriata, radiata, plerumque in columnas laxas divergentes findentia, diametro usque ad 600 µm. Stipes hyalini, subexasperati, longitudine variabili plerumque (100-) 200-800 (-1000) µm, diametro statim sub vesicula (6-) 8-12 (-14) µm. Vesiculae globosae vel subglobosae, diametro (12-) 15-38 µm, in parte distali 3/4 fertiles; metulae 4.5-11.5 x 2.5-6.0 µm; phialides 5.0-11.0 x 3.0-6.0 µm (Fig. 3). Conidia globosa, crassotunicata, grano sporae diametrum (4.5-) 5.0-6.0 (-7.0) µm attingenti; ornamentatio grossa, e tuberculis et transtris brevibus composita, <1.5 µm alta (Fig. 4).

Coloniae in extracto malti post 7 dies ad 25 C diametrum 6.0-8.0 cm attingentes. Superficies coloniae in aspectu velveti (Fig. 2). Capitula conidialia post 14 dies in toto visa olivacea (1-2E5; Kornerup et Wanscher, 1978). Sclerotia absentia vel sparsa.

HOLOTYPUS: cultura exsiccata NRRL 25528, in isolatione e solo agarvi *Arachidis hypogaeae*, Terrell Co., Georgia, USA, 8 die mensis Junii 1992 lecta, in National Fungus Collections, US Department of Agriculture, Beltsville, Maryland (BPI 737601) deposita. Culturae vivae NRRL 25528 in ARS Culture Collection, Peoria, Illinois depositae.

Colonies on Czapek agar attaining a diameter of 3.0-4.5 (-5.5) cm in 7 days at 25 C; growth at 37 C in 7 days reaching a



Figs. 1-4. *Aspergillus caelatus* (NRRL 25528). 1. Colonies on Czapek agar (14 days; 25 C). 2. Colonies on malt extract agar (14 days; 25 C). 3. Biseriate head. Bright-field microscopy; bar = 30 μm. 4. Conidia. Bright-field microscopy; bar = 10 μm.

diameter of 3.5-5.5 cm. Colony surface mostly velvety, consisting of abundant conidial heads (Fig. 1). Conidial heads en masse olive (1-2E5; Kornerup and Wanscher, 1978) at 7 days (25 C), shifting to a darker, browner olive (2E4-F4; Kornerup and Wanscher, 1978) by 14 days. Reverse pale yellow brown; agar medium with diffusable pigment of the same color; clear to light pink-brown exudate present in sclerotial isolates. Sclerotia absent to abundant, white becoming pink brown and finally purple black, subglobose to elongate and often irregular in shape, 400-1700 μm long and 200-1100 μm wide.

Conidial heads uniseriate or biseriate, radiate, usually splitting into loose divergent columns, up to 600 µm in diameter. Stipes hyaline, finely roughened; length variable, mostly (100-) 200-800 (-1000) µm; diameter immediately below vesicle (6-) 8-12 (-14) µm. Vesicles globose or subglobose, (12-) 15-38 µm in diameter, fertile over upper three-fourths; metulae 4.5-11.5 x 2.5-6.0 µm; phialides 5.0-11.0 x 3.0-6.0 µm (Fig. 3). Conidia globose and thick walled, with spore body (4.5-) 5.0-6.0 (-7.0) µm in diameter; ornamentation coarse, consisting of tubercles and short bars <1.5 µm high (Fig. 4).

Colonies on malt extract agar attaining a diameter of 6.0-8.0 cm in 7 days at 25 C. Colony surface velvety (Fig. 2). Conidial heads en masse olive (1-2E5; Kornerup and Wanscher, 1978) at 14 days. Sclerotia absent or sparse.

HOLOTYPE: dried plate culture of NRRL 25528, isolated from soil collected 8 June 1992 in a peanut field, Terrell Co., Georgia, USA, has been deposited with the National Fungus Collections, US Department of Agriculture, Beltsville, Maryland (BPI 737601). Living cultures of NRRL 25528 have been deposited in the ARS Culture Collection, Peoria, Illinois.

The specific epithet, which is from Latin meaning chiseled or carved, refers to the roughened ornamentation of the conidia. The description of *A. caelatus* is based on three isolates each from vegetative compatibility groups 1-3 which were obtained from soil in a peanut field in southwestern Georgia (Horn and Greene, 1995). NRRL 25528, ex holotype of *A. caelatus*, belongs to vegetative compatibility group 1 and produces numerous sclerotia. For this study, *A. caelatus* was compared to isolates of *A. tamarii* Kita from Horn and Greene (1995) (three isolates each from vegetative compatibility groups 1-3) as well as NRRL 20818 (= CBS 104.13), ex lectotype of *A. tamarii*, and NRRL 429 (= WB 429), used for the description of *A. tamarii* by Raper and Fennell (1965).

The olive, radiate conidial heads and the predominantly globose vesicles that are fertile over most of their surface, combined with the presence of metulae and the roughened hyaline stipes, place *A.*

caelatus in section *Flavi* (formerly the *A. flavus* group) of the subgenus *Circumdati* (Christensen, 1981; Gams et al., 1985; Raper and Fennell, 1965). Within the section, *A. caelatus* most closely resembles *A. tamarii*. Both species have thick-walled, coarsely roughened conidia that are similar in size (Christensen, 1981; Horn and Greene, 1995; Raper and Fennell, 1965). However, vesicle diameter (27 ± 4.9 μm) (± SD), stipe width (10 ± 1.7 μm), and stipe length (520 ± 206.3 μm) of biseriate heads of *A. caelatus* are significantly smaller ($P < 0.001$; $n = 36$) than those of *A. tamarii* (42 ± 6.7, 15 ± 1.4, and 1220 ± 251.7 μm, respectively) (Horn and Greene, 1995).

Colony color on Czapek agar is the easiest means of distinguishing *A. caelatus* from *A. tamarii*. In *A. caelatus*, conidial heads en masse at 14 days were olive (2E4-F4) (Kornerup and Wanscher, 1978) and in *A. tamarii*, heads ranged from olive brown (4E6-F6) in isolates from Horn and Greene (1995) to between olive brown (4E6-F6) and yellowish brown (5E6-F6) in NRRL 20818 and 429. Digital color image processing of colonies and filtered conidia of *A. caelatus* and *A. tamarii*, as well as *A. flavus* and *A. parasiticus*, also shows significant differences in the intensity of the primary colors red, green, and blue (Horn et al., 1996). In addition, *A. caelatus* typically produces a yellow-brown diffusable pigment in the medium; isolates of *A. tamarii* recovered by Horn and Greene (1995) lack this pigmentation. The small conidiophores of *A. caelatus* give colonies a close-textured appearance suggestive of *A. parasiticus* whereas colonies of *A. tamarii*, characteristically with larger conidiophores, are much coarser (Christensen, 1981; Horn and Greene, 1995; Raper and Fennell, 1965). Horn et al. (1996) also reported that 22 of the 32 *A. caelatus* isolates examined produced sclerotia; none of the 34 *A. tamarii* isolates obtained in the same study was sclerotial. *Aspergillus tamarii* has been reported to produce sclerotia (Raper and Fennell, 1965), though it is possible that some of those isolates represented *A. caelatus*.

Aspergillus caelatus and *A. tamarii* also differ in nonmorphological characters. In the study of soil populations of *Aspergillus* species from section *Flavi*, all isolates of *A. caelatus* were

vegetatively incompatible with those of *A. tamarii* (Horn and Greene, 1995). Furthermore, Horn et al. (1996) showed that isolates of *A. caelatus* (n = 25) produced kojic acid (116.3-226.8 μg/ml medium) but no detectable cyclopiazonic acid whereas *A. tamarii* isolates (n = 32) formed kojic acid at lower levels (5.9-70.3 μg/ml) and consistently produced cyclopiazonic acid (5.5-41.9 μg/ml). None of the *A. caelatus* and *A. tamarii* isolates examined produced aflatoxins.

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LITERATURE CITED

Christensen, M. 1981. A synoptic key and evaluation of the species in the *Aspergillus flavus* group. *Mycologia* 73: 1056-1084.

Cook, P. E., and G. Campbell-Platt. 1994. *Aspergillus* and fermented foods. Pp. 171-188. *In: The genus* Aspergillus: *from taxonomy and genetics to industrial application*. Eds., K. A. Powell, A. Renwick, and J. F. Peberdy. Plenum Press, New York.

Eaton, D. L., and J. D. Groopman, eds. 1994. *The toxicology of aflatoxins: human health, veterinary, and agricultural significance*. Academic Press, San Diego, California. 544 pp.

Gams, W., M. Christensen, A. H. Onions, J. I. Pitt, and R. A. Samson. 1985. Infrageneric taxa of *Aspergillus*. Pp. 55-62. *In: Advances in* Penicillium *and* Aspergillus *systematics*. Eds., R. A. Samson and J. I. Pitt. Plenum Press, New York.

Horn, B. W., and R. L. Greene. 1995. Vegetative compatibility within populations of *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii* from a peanut field. *Mycologia* 87: 324-332.

Horn, B. W., R. L. Greene, and J. W. Dorner. 1995. Effect of corn and peanut cultivation on soil populations of *Aspergillus flavus* and *A. parasiticus* in southwestern Georgia. *Appl. Environm. Microbiol.* 61: 2472-2475.

Horn, B. W., R. L. Greene, V. S. Sobolev, J. W. Dorner, J. H. Powell, and R. C. Layton. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii*. *Mycologia* 88: 574-587.

Kornerup, A., and J. H. Wanscher. 1978. *Methuen handbook of colour*. 3rd ed. Eyre Methuen, London, United Kingdom. 252 pp.

Kozakiewicz, Z. 1994. *Aspergillus*. Pp. 575-616. *In: Foodborne disease handbook. Diseases caused by viruses, parasites, and fungi.* Vol. 2. Eds., Y. H. Hui, J. R. Gorham, K. D. Murrell, and D. O. Cliver. Marcel Dekker, Inc., New York.

Raper, K. B., and D. I. Fennell. 1965. *The genus* Aspergillus. Williams and Wilkins Co., Baltimore, Maryland. 686 pp.