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Morphologic and Physiologic Studies of Three Dematiaceous Pathogens

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Uncertainty in identifying a dematiaceous mold from a human bronchial washing precipitated a study of *Dactylaria gallopava* (Cooke) Bhatt et Kendrick and its relationship to *Scolecobasidium constrictum* Abbott. Morphologic and physiologic studies were conducted with representative isolates and subcultures derived from the isolates used to prepare the type specimens of these two fungi, as well as a third dark mold, *Ochroconis constricta* (Abbott) de Hoog et von Arx. All test isolates were morphologically similar in that two-celled, cylindrical blastoconidia with a rhexolytic mode of dehiscence were the predominant and most consistent anamorphic structures. Sympodial development of the conidiophore was too variable to distinguish the isolates. Based on their morphologic similarity, we propose a new combination within the genus *Dactylaria* as amended by Bhatt and Kendrick, *D. constricta* (Abbott) Dixon et Salkin. However, *D. constricta* could be differentiated into two groups on the basis of physiologic tests — a subgroup in which isolates grow at 37 and 45°C, give a delayed positive gelatin reaction, and are inhibited on Mycosel agar; and a second subgroup in which isolates grow on Mycosel agar and give a rapid positive gelatin reaction, but do not grow at 37 or 45°C. We recommend that *D. constricta* be viewed as a potential pathogen when isolated from clinical specimens.

A dematiaceous hyphomycete was isolated in 1981 from a human bronchial washing and identified as a species of *Arthrobotrys* (Corda) Schenck, Kendrick et Pramer (M1864-81; Mycology Laboratory, Wadsworth Center for Laboratories and Research, New York State Department of Health). Reevaluation of the morphologic and physiologic characteristics of the isolate in three reference laboratories resulted in its reidentification as *Scolecobasidium constrictum* Abbott. However, its maximum temperature of growth and formation of a diffusible pigment were inconsistent with the published descriptions of *S. constrictum* (1, 3).

Two species of *Scolecobasidium* Abbott have been reported as animal pathogens. Both *S. humicola* Barron et Bush and *S. tshawytschae* (Doty et Slater) McGinnis et Ajello have been described as etiologic agents of systemic phaeohyphomycosis in fish (2, 10, 13, 17). While external evidence of infection was variable, lesions were evident internally on all major organs except those of the central nervous system. Histopathologic examination revealed granulomatous reactions with lymphocyte infiltration. Although branching, septate, dematiaceous hyphae were evident in many organs, especially massive invasion was noted in the kidneys. *S. humicola* has also been implicated as a systemic pathogen in frogs (11) and as a cause of cutaneous lesions in a tortoise (22).

These *Scolecobasidium* species are morphologically similar in vitro to another dematiaceous pathogen, *Dactylaria gallopava* (Cooke) Bhatt et Kendrick. All have two-celled, dematiaceous, blastic conidia with a similar mode of dehiscence. However, *D. gallopava*, as a pathogen, is more dangerous than the *Scolecobasidium* species, having been reported as the etiologic agent of fatal encephalitis in turkeys and chickens (6, 12). Because of the morphologic similarities, de Hoog and von Arx (9) and later de Hoog (7) suggested transferring *S. humicola*, *S. tshawytschae*, and *D. gallopava* to a new genus, *Ochroconis* de Hoog et von Arx.

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The dematiaceous fungi, a complex group of organisms, are frequently quite difficult to identify. A review of the literature revealed considerable confusion surrounding *Dactylaria* (Sacc.) Bhatt et Kendrick, *Ochroconis*, and *Scolecobasidium*. In view of the increasing recognition of these three genera as animal pathogens and their possible isolation in the clinical laboratory, we undertook the present study to identify isolate M1864-81 definitively and, in so doing, to clarify the confusion associated with these dematiaceous molds. We concluded that all isolates studied could be accommodated within the genus *Dactylaria* as amended by Bhatt and Kendrick (5).

MATERIALS AND METHODS

Test organisms. All test isolates (Table 1) were maintained on Sabouraud glucose agar (SGA) at 30°C and transferred at monthly intervals to fresh medium.

Morphology by light microscopy. To evaluate the microscopic morphology of M1864-81 and the comparative isolates under similar conditions, multiple fields in two slide culture preparations were observed in each of two laboratories. A 2-mm^3 portion of growth of each isolate was aseptically removed from a stock culture and transferred to the surface of corn meal agar (CMA), malt-extract agar, potato dextrose agar, or SGA in 100-mm plastic petri plates and culture tubes (18 by 150 mm). All media were obtained from BBL Microbiology Systems, Cockeysville, Md. Colony morphologies were examined after incubation for 14 days at 30°C on one or more of the above nutrient media. Conidial morphology and ontogeny were investigated with 5- to 7-day-old potato dextrose agar or CMA slide cultures which had been incubated in the dark at 30°C. Observations were made with a Zeiss Universal phase contrast light microscope.

Physiologic studies. A 2-mm^3 portion of growth of each isolate was aseptically removed from a stock culture, transferred to the surface on each of the experimental media, and incubated at 30°C (except in temperature tolerance studies).

TABLE 1. Morphologic characteristics of *Dactylaria constricta*

Strain no.[a]	Conidial dimensions (μm)		Morphologic character[b]							
	Mean	Range	1-Se	Cons	Sco	Blas	Rhex	Symp	Red	Brown
Unknown species										
DMD 381 (M1864-81)	9.0 × 3.2	6.5–13.0 × 2.5–4.0	+	+	+	+	+	+	+	−
Dactylaria gallopava										
DMD 405 (ATCC 26822)	9.9 × 3.1	5.8–14.9 × 2.1–4.2	+	+	+	+	+	+	+	−
DMD 411 (ATCC 16027, CDC B-579)[c]	9.7 × 4.0	7.5–14.1 × 3.3–5.8	+	+	+	+	+	+	+	−
DMD 412 (ATCC 28841)	8.0 × 3.6	4.2–10.8 × 2.5–4.5	+	+	+	+	+	+	+	−
DMD 413 (CDC B-1300)	10.4 × 3.2	8.3–13.3 × 2.5–4.2	+	+	−	+	+	+	+	−
DMD 414 (CDC B-1302)	11.9 × 3.3	10.0–16.6 × 2.5–4.2	+	−	+	+	+	−	+	−
DMD 415 (CDC B-1303)	8.7 × 2.9	3.3–13.2 × 2.4–3.2	+	+	+	+	+	+	+	−
Ochroconis gallopavum										
DMD 422 (CBS 437.64)[d]	9.3 × 2.9	5.8–12.4 × 2.5–4.2	+	+	+	+	+	−	+	−
Ochroconis constricta										
DMD 407 (CBS 202.27)[e]	7.7 × 3.0	5.0–10.8 × 1.7–4.2	+	+	−	+	−	−	−	+
DMD 410 (ATCC 11419)[f]	9.1 × 2.9	7.5–10.8 × 2.5–3.3	+	+	+	+	+	−	+	−
DMD 418 (CBS 124.65)	6.6 × 3.0	5.0–10.0 × 2.5–4.2	+	−	−	+	−	+	+	−
DMD 419 (CBS 211.53)	7.3 × 2.8	5.0–9.1 × 1.7–3.3	+	+	+	+	+	−	−	−
DMD 420 (CBS 269.61)	7.6 × 3.0	6.6–10.0 × 1.7–3.3	+	+	+	+	+	+	+	−
Scolecobasidium constrictum										
DMD 409 (ATCC 48579)	7.6 × 3.8	4.2–10.0 × 3.3–5.0	+	+	+	+	+	+	+	−

[a] DMD, Collection of Dennis M. Dixon, Loyola College; ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; CDC, Centers for Disease Control; M, Mycology Laboratory, Wadsworth Center. Genera and species are those listed by the source.
[b] Character: 1-Se, 1-septate; Cons, constricted; Sco, scolecospores; Blas, blastic; Rhex, rhexolytic; Symp, sympodial; Red and Brown, diffusible pigment.
[c] Subculture derived from the isolate used to prepare the type specimen (12).
[d] Subculture derived from the isolate used to prepare the type specimen (7).
[e] Subculture derived from the isolate used to prepare the type specimen (1, 9).
[f] Subculture derived from the isolate used to prepare the type specimen (3).

To evaluate cycloheximide sensitivity, the isolates were inoculated onto Mycosel slants (BBL; 0.5 mg of antifungal agent per ml of medium) in culture tubes (20 by 150 mm). Growth was assessed after 7 and 14 days.

To determine temperature tolerance, portions of each isolate were transferred to five SGA slants and incubated at 30, 37, 42, 45, or 50°C. Growth at each temperature was assessed at 7 and 14 days.

To evaluate the decomposition of selected amino acids and starch, each isolate was transferred to 100-mm petri plates containing xanthine, tyrosine, casein, or starch (14) (BBL). Decomposition, indicated by the clearing of the medium, was assessed after incubation for 7, 14, and 21 days.

Gelatin liquefaction was investigated in culture tubes (20 by 150 mm) containing 5 ml of 14% nutrient gelatin (BBL). The ability of each isolate to liquefy the substrate was noted after 7, 14, and 21 days.

RESULTS

Based on our examination of the morphologic characteristics of the test isolates, we propose:

Dactylaria constricta (Abbott) Dixon et Salkin, comb. nov.
Basionym: *Scolecobasidium constrictum* Abbott, Mycologia **19**:30, 1927
Synonyms:

≡ *Ochroconis constricta* (Abbott) de Hoog and von Arx, Kavaka **1**:55–60, 1973.

= *Heterosporium terrestre* Atkinson, Mycologia **44**:813–822, 1952.

= *Diplorhinotrichum gallopavum* Cooke, in Georg, Bierer, and Cooke, Sabouraudia **3**:239–244, 1964.

≡ *Dactylaria gallopava* (Cooke) Bhatt and Kendrick, Can. J. Bot. **46**:1253–1257, 1968.

≡ *Ochroconis gallopavum* (Cooke) de Hoog, in Howard, *Fungi Pathogenic for Humans and Animals*, part A, p. 181–182, 1983.

We found that M1864-81 (DMD 381) grew readily on CMA, reaching colony diameters of 5.0 and 7.0 cm after 3 weeks of incubation at 23 to 25 and 30°C, respectively. Growth was more rapid at 37°C on all media tested, with colonies generally attaining diameters of 8.5 cm in 2 weeks. Colonies were olivaceous-gray except on CMA, where they were light brown. On SGA a striking diffusible pigment was produced at all incubation temperatures except 50°C. The pigment corresponded to a bordeaux color standard (16).

DMD 381 produced cylindrical, 1-septate conidia, which averaged 9.0 by 3.2 μm (range, 6.5 to 13.0 by 2.5 to 4.0 μm). The conidia had been formed holoblastically and were delimited by septa from the conidiogenous cells. Separation from the conidiogenous cell occurred typically at a point proximal to the terminal septum, leaving a characteristic threadlike remnant (Fig. 1). Dehiscence was by a rhexolytic mode, i.e., the release of the conidium through the rupture of the wall of the cell below the conidium (Fig. 2). One-celled, globose phialoconidia produced from phialides with fine collarettes were seen in the initial slide cultures but were seemingly lost with serial maintenance of the organism on SGA.

The morphologic characteristics of DMD 381 clearly resembled those of the other test isolates (Table 1). All had two-celled, dematiaceous, blastic conidia of similar dimensions and shape, with a rhexolytic mode of dehiscence. In all, threadlike denticles were invariably found between the

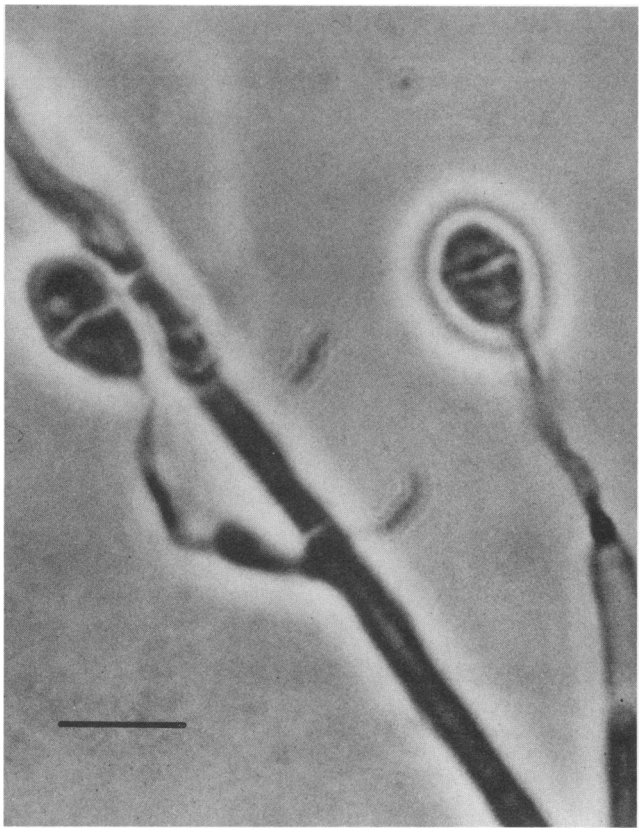

FIG. 1. Two-celled globose-to-cylindrical conidia borne on threadlike denticles. Bar, 5 μm.

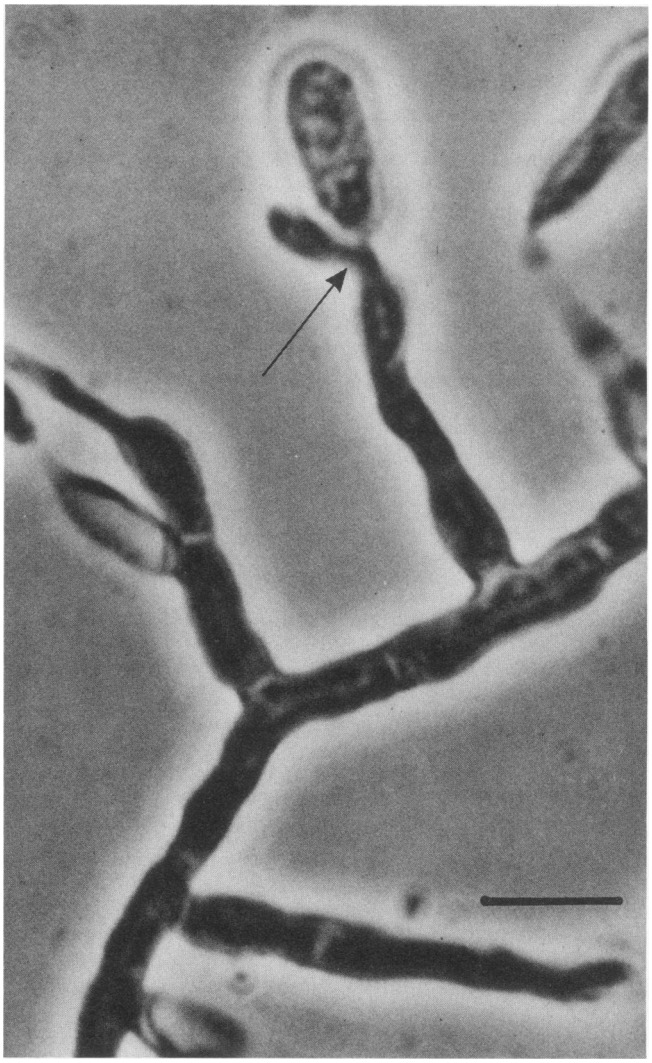

FIG. 2. Holoblastic, cylindrical, two-celled conidia. Sympodial growth is evident in the central conidiophore (arrow), while rhexolytic dehiscence is seen in the two adjacent conidia. Bar, 5 μm.

conidia and conidiogenous cells. Sympodial proliferation of the conidiophore was quite variable. Pigment production occurred on SGA at 30°C with all isolates but was especially pronounced with DMD 381, 405, 409, 411 through 415, and 422.

Conversely, the physiologic features of all isolates were more diverse. Only DMD 407, 410, and 418 through 420 grew on Mycosel after 14 days of incubation. DMD 381, 405, 409, 411 through 415, and 422 grew at temperatures up to 45°C, but DMD 407, 410 and 418 through 420 only at 30°C. No isolate decomposed xanthine; DMD 410 and 419 decomposed tyrosine and casein; and all other isolates decomposed only tyrosine. All isolates liquefied gelatin, but a pronounced delay (up to 21 days) was found with DMD 381, 405, 409, 411 through 415, and 422.

DISCUSSION

Dematiaceous fungi are gradually being recognized as important agents of human and animal diseases. Clinical laboratorians are confronted with the task of identifying an increasing number of such dark molds and assessing their clinical significance.

We have attempted to clarify the relationship of selected members of three dematiaceous genera whose history is one of taxonomic confusion. Abbott established *Scolecobasidium* in 1917 for *S. constrictum*, a dematiaceous mold with cylindrical, two-celled conidia constricted at the septum, and *S. terreum* (as the type of the genus) with T- or Y-shaped two-celled conidia (1). Since *Heterosporium terrestre* Atkinson was described (3) with essentially the same characteristics as *S. constrictum*, Barron and Busch (4) placed it in synonymy with the latter mold. de Hoog and von Arx proposed in 1973 the new genus *Ochroconis* to accommodate species of *Scolecobasidium*, including *S. constrictum* but not *S. terreum* (9).

Saccardo established *Dactylaria* in 1880 for *D. purpurella*, which he had mistakenly described in 1877 as *Acrothecium purpurellum* (18, 19). *Diplorhinotrichum gallopavum* Cooke was described in 1964 as the etiologic agent of fungal meningitis in turkey poults (12). In 1968, however, Bhatt and Kendrick proposed the new combination *Dactylaria gallopava* after uniting *Diplorhinotrichum* under their amended definition of the genus *Dactylaria* (5). de Hoog has recently proposed to tranfer *D. gallopava* to *Ochroconis* to establish the combination *O. gallopavum* (7).

These taxonomic problems have resulted in part from the polymorphic nature of many dematiaceous fungi and the use of morphologic features to distinguish taxa. Conidial ontogeny and morphology are the primary characters now used to identify dematiaceous molds. These characters are

subject to environmentally induced variations (15, 20), as well as to variations in interpretation by different observers.

In the present investigation isolates were grown on several media and observed for differences in colony morphology. Some variation was found in colony color and the formation of a diffusible pigment, but the essential morphologic features remained constant. Two-celled blastoconidia with rhexolytic dehiscence were the predominant and most consistent anamorphic structures in all isolates.

Due to their morphologic similarity we proposed a new combination, *D. constricta*, within *Dactylaria* as amended by Bhatt and Kendrick. This amended definition broadly describes the genus as including dematiaceous molds which, among other characteristics, form nondarkly pigmented (hyaline) or pigmented conidia; these conidia may be cylindrical, spindlelike (fusiform), flasklike (ampulliform), narrowly clublike (clavate), or ellipsoidal. However, de Hoog and von Arx (9) and most recently de Hoog (8) seemingly further restrict the definition of *Dactylaria* to those that form usually hyaline conidia which are very narrowly fusiform or threadlike (filiform). Those dematiaceous molds that formed pigmented, cylindrical, or fusiform conidia were included in the genus *Ochroconis*. Thus, while we agree with de Hoog and von Arx and de Hoog (7) that *D. gallopava* and *S. constrictum* are members of the same genus, we believe that the appropriate genus is not *Ochroconis* but *Dactylaria*. Moving species now in *Scolecobasidium* and *Ochroconis* to *Dactylaria* would conform with the International Code of Botanical Nomenclature (Section 3, Article 11.2 [21]), since *Dactylaria*, the first of the three genera to be validly published, has priority. Similarly, the specific epithet *D. constricta* has priority as it was the first of the three used with this organism to be validly reported.

While morphologically similar, isolates of *D. constricta* could be differentiated into two groups on the basis of physiologic results. In the first subgroup (DMD 381, 405, 409, 411 through 415, and 422), isolates grow at 37 and 45°C, give a delayed positive gelatin reaction, and are inhibited on Mycosel agar. In addition, isolates in this subgroup have been reported as pathogens of turkeys and chickens and are neurotropic in experimentally infected mice (D. M. Dixon, T. J. Walsh, I. F. Salkin, P. Kelly, and A. Polak, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, F69, p. 408). Members of the second subgroup (DMD 407, 410, and 418 through 420) do not grow at 37 or 45°C but give a rapid positive gelatin reaction and readily grow on Mycosel agar. Isolates in this subgroup have never been implicated as the etiologic agents of disease in animals. Since, as noted, morphologic characters are presently the primary means of distinguishing taxa of dematiaceous molds and since all isolates studied were morphologically quite similar, we felt they should be accommodated within a single species despite their physiologic differences. However, such physiologic characteristics may be used to separate taxa below the species level, and consequently we plan to formally propose two varieties of *D. constricta* in a subsequent publication.

Dactylaria constricta is a known avian pathogen, a neurotropic agent in mice, and the cause (as *D. gallopava*) of a disseminated infection in an immunocompromised patient (A. A. Terreni, W. B. Crymes, P. R. Morris, S. Milligan, and H. Dowda, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, F40, p. 404). Therefore, accurate identification of *D. constricta*, through the morphologic characters outlined in this report, is of increasing importance. In addition, when isolated from a clinical specimen it should be viewed, in association with the patient history, as a potential pathogen.

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