

TWO NEW *ASPERGILLUS NIDULANS* GROUP MEMBERS FROM WYOMING SOILS

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SUMMARY

Two new species in the *Aspergillus nidulans* group are described and compared with known related taxa. *Aspergillus spectabilis*, with tall conidiophores, broad green conidial columns and early-ripening cleistothecia shallowly embedded in a vinaceous Hülle-cell crust, may be most closely related to *A. multicolor*, *A. crustosus* (both nonascosporic), *A. striatus* and *Emericella desertorum*. *Aspergillus bicolor* is distinctive among all other recognized species in the complex in its development of tall conidiophores and columnar green heads on dilute media and in its production on malt agar of a floccose yellow felt which forms vinaceous Hülle-cell-cleistothecium aggregations aerielly after growth for 4–6 wk. Ascospores in the latter species are produced sparingly, mature at 2–3 mo, are orange red, smooth or delicately roughened and have very low equatorial crests.

Two strikingly colorful aspergilli, recently isolated from *Artemisia* grassland and coal-spoil soils in Wyoming, appear to be previously undescribed species in the *Aspergillus nidulans* group. Both isolates have tall-growing brown-tinged conidiophores; are biseriate and produce cylindrical columns of leaf-green conidia; and both develop *A. nidulans*-type cleistothecia encased in vinaceous Hülle-cell masses. In RMF H429 the ascospores mature within 10–14 da and the eurotiaceous nature of this fungus was determined on first examination. RMF 2058, in contrast, was stored for several years as "*Aspergillus* sp. *nidulans* group." Its cleistothecia develop tardily in a pinkish-violet, granular overgrowth and maturation of the ascosporic state requires incubation for 60 to 90 da.

Aspergillus spectabilis Christensen & Raper, sp. nov. status perfectus
sit judicatus **Emericella spectabilis** Christensen, sp. nov.

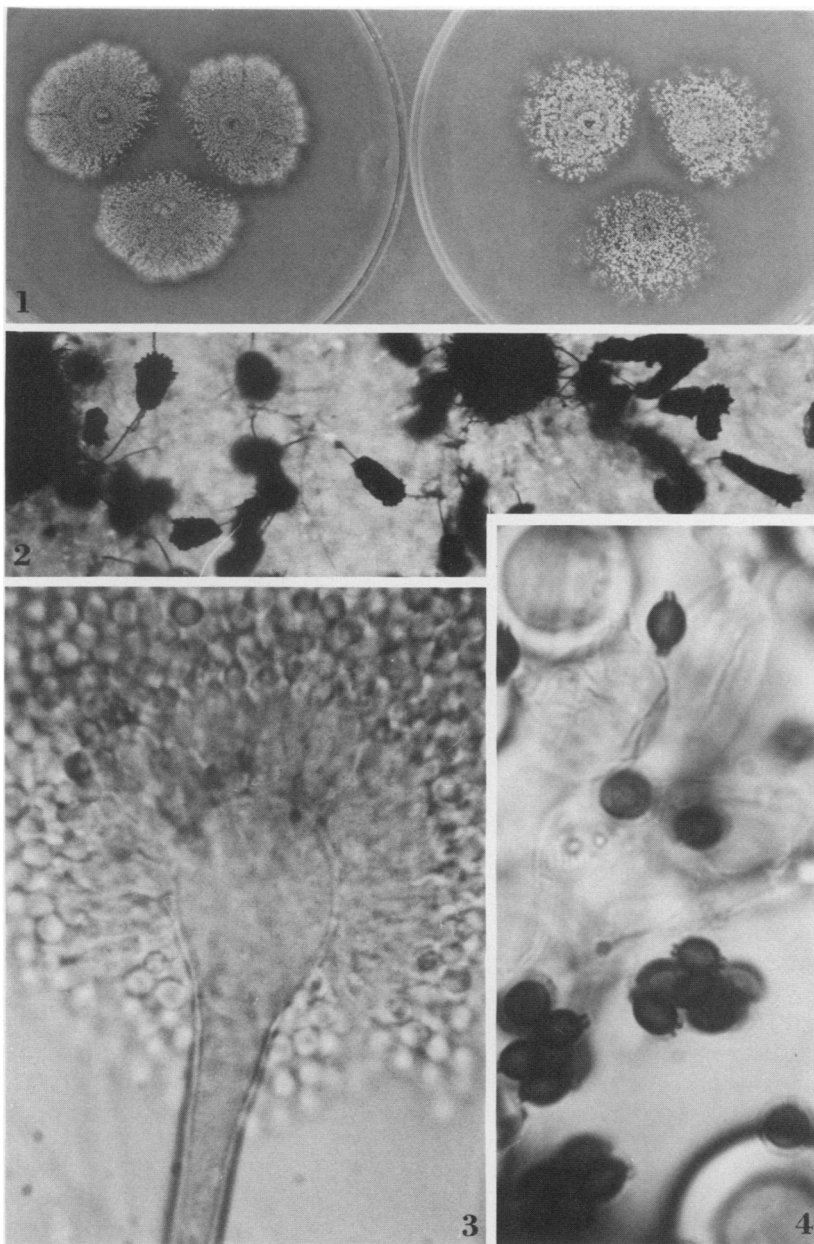
FIGS. 1-5, 7

Coloniae in agar Czapekii 3.2-3.6 cm 14 dies 24-26 C crescentes, tenues, marginibus lobosis, granulares, pallide vino-griseae; reverso rufo-vinaceo (2, XXXIX). Capitula conidica, primo globosa, deinde dense columnaria, cylindrata, usque ad $200 \times 100-125 \mu\text{m}$, biseriata; conidiophorae $190-364 \times 5.7-10.3 \mu\text{m}$, membranis usque ad $2 \mu\text{m}$ crassis, aequaliter flavo-brunneae, enodes; vesiculae admodum elongatae, $23.0-26.2 \times 16.0-19.4 \mu\text{m}$; metulae $5.7-7.0 \times 3.6-4.0 \mu\text{m}$; phialides $7.8-8.0 \times 2.3 \mu\text{m}$; conidia subglobosa, viridia, leviter rugosa, $2.6-3.4 \mu\text{m}$ diam. "Hülle" cellulae plurimae in agar maltoso, subviolaceae cum in massis productae sint, globosae vel subglobosae, plerumque $22-28 \mu\text{m}$ diam. Cleistothecia in agar maltoso post 2 hebdomades matura, violaceo-rubicunda, sphaeroidea, $170-310 \mu\text{m}$ diam, membranis $3.5-4.5 \mu\text{m}$ crassis, inclusa in parte superiore crustae paene continuae cellularum "Hülle" $250-530 \mu\text{m}$ crassis; asci octospori, $10-14 \times 10-12 \mu\text{m}$; ascosporae lenticulares, violaceae, cristis aequatorialibus 2, $1.0-1.2 \mu\text{m}$ latis ornatae, superficies convexae enodes, ascospororum corpora $3.6-4.6 \times 2.8-3.4 \mu\text{m}$.

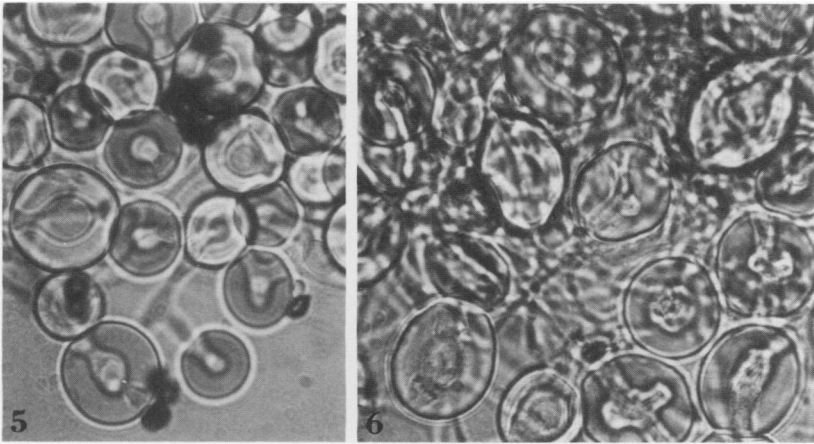
Typica cultura: RMF H 429, in anno 1976 collecta et isolata e solo superfodinam carbonariam effossam panso; in herbario NY deposita.

Colonies on Czapek's solution agar (FIG. 1) attaining diam of 3.2-3.6 cm in 14 da at 24-26 C, thin, marginally lobed, becoming pale lavender and granular as Hülle-cell masses with cleistothecia arise intermixed with less conspicuous dark green conidial heads; reverse near russet vinaceous to light russet vinaceous (2, XXXIX) and with that pigment diffusing; no odor. Trailing hyphae often with tiny droplets of amber exudate which may collect on the agar surface. *Conidial heads* becoming compact columns near $200 \times 100-125 \mu\text{m}$, cylindrical to very slightly flared (FIG. 2), biseriate; *conidiophores* arising directly from the substrate, $190-364 \mu\text{m}$ in length and uniformly $5.7-10.3 \mu\text{m}$ in diam, nonseptate, with walls brown pigmented, smooth and $1-2 \mu\text{m}$ thick; *vesicles* somewhat elongate (FIG. 3), $23.0-26.2 \times 16.0-19.4 \mu\text{m}$, thin walled in upper portion or more often with wall equaling that of the conidiophore and $1-2 \mu\text{m}$ thick throughout, fertile over the upper three-fourths; *metulae* and *phialides* crowded, metulae $5.7-7.0 \times 3.6-4.0 \mu\text{m}$, phialides $7.0-8.0 \times 2.3 \mu\text{m}$; *conidia* subglobose, delicately roughened, green, $2.6-3.4 \mu\text{m}$ in diam. *Hülle cells* globose to subglobose (FIG. 5), mostly $22-28 \mu\text{m}$ but up to $35 \mu\text{m}$ in diam with very thick walls finely radially striate when viewed with phase illumination, hyaline by transmitted light, distinctly purplish in mass by reflected light, in aggregations $400-500 \mu\text{m}$ in diam usually enveloping single cleistothecia. *Cleistothecia* on this medium dimensionally and morphologically equal to those produced on malt agar but less consistently formed and with only scant development of a subtending Hülle-cell crust.

Colonies on malt agar (FIG. 1) 2.6-3.4 cm in diam after 14 da at 24-26 C, surface granular and slightly raised consisting of a centrally and subcentrally continuous cleistothecium-Hülle-cell crust vinaceous gray (2, L) in color with a submarginal admixture of dark green



FIGS. 1-4. *Aspergillus spectabilis* RMF H429. 1. Colonies on malt and Czapek agars, respectively, grown for 14 da at 24-26 C. 2. Conidial heads and Hülle-cell



FIGS. 5-6. Hülle cells of the two new species. 5. *Aspergillus spectabilis* RMF H429; cells mostly globose. $\times 800$. 6. *A. bicolor* RMF 2058; subglobose to elongate cells characteristically intermixed with vegetative hyphae. $\times 800$.

conidial heads; reverse brownish gold near antique brown (2, III) to antimony yellow (2, XV) marginally; odor faint; amber exudate drops sparse. Conidial structures duplicating in form, color and dimensions those described on Czapek's agar but chains of conidia generally shorter and heads becoming short flared masses expanded in diam from near $50\text{ }\mu\text{m}$ to about $120\text{ }\mu\text{m}$; conidiophores often arising from stolon-like substrate hyphae. *Cleistothecia* proper evident as dark violet spheres loosely covered by Hülle cells and a thin weft of pale lavender hyphae, from 170 to $310\text{ }\mu\text{m}$ but mostly 240 – $280\text{ }\mu\text{m}$ in diam, easily freed of Hülle cells by manipulation on an agar surface, with wall 3.5 – $4.5\text{ }\mu\text{m}$ thick consisting of two to three layers of loosely meshed hyphae and an outer single layer of irregularly lobed interlocking flattened hyaline cells about 7 – 14×5 – $9\text{ }\mu\text{m}$; *asci* 8-spored, 10 – 14×10 – $12\text{ }\mu\text{m}$; *ascospores* maturing rapidly (10 – 14 da), vinaceous by transmitted light when developed on malt agar but orange red on Czapek's agar, lenticular with smooth convex walls and two equatorial crests which are pleated, entire and about 1.0 – $1.2\text{ }\mu\text{m}$ wide, spore body 3.6 – 4.6×2.8 – $3.4\text{ }\mu\text{m}$ commonly $4.5 \times 3.3\text{ }\mu\text{m}$ (FIG. 4). *Cleistothecia* on malt agar characteristically embedded in the upper surface of a purplish-lavender Hülle-cell layer about $250\text{ }\mu\text{m}$ to $530\text{ }\mu\text{m}$ thick (FIG. 7); number of

masses on Czapek agar at 16 da showing characteristic columnar to obconical head shape. $\times 30$. 3. Typical vesicle and biserial appendages bearing subglobose conidia. $\times 1,400$. 4. Hülle cells, asci, and ascospores in face and edge view. $\times 1,500$.

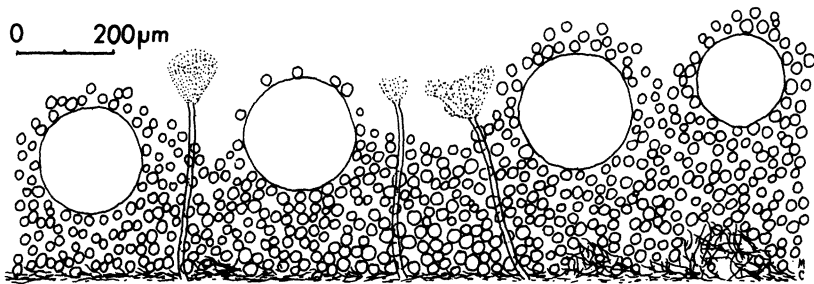


FIG. 7. Cross section of *Aspergillus spectabilis* colony on malt agar showing subtending crust of Hülle cells, cleistothecia in a single layer, and tall-growing conidiophores. Semidiagrammatic; scale approximate.

Hülle cells in lower crust increasing with age thereby elevating the single layer of cleistothecia while surrounding and burying conidial structures.

Colonies on both malt and Czapek's agar at 30 C growing somewhat more rapidly than at room temperature, lighter pigmented and with less exudate, and on malt agar with an odor suggestive of a temperate seashore.

The perfect state may be regarded as *Emericella spectabilis* Christensen.

The outstanding characteristics of this species are its 1) tall-growing (to 360 μm) heavy-walled brownish conidiophores with dark green columns of conidia, 2) large (to 310 μm) cleistothecia in a single layer in the upper portion of a purplish Hülle-cell crust, and 3) quickly maturing ascospores that are vinaceous with smooth convex walls and entire pleated crests up to 1.2 μm wide.

The type culture, RMF H429, was isolated from coal-mine-spoil material shaped and seeded in 1976 at the Seminole No. 1 mine near Hanna, Wyoming. The HOLOTYPE, a dried plate culture, has been placed in the Cryptogamic Herbarium of The New York Botanical Garden. Living cultures of *A. spectabilis* RMF H429 are on deposit in the American Type Culture Collection, Rockville, Maryland (ATCC 36105), at the Commonwealth Mycological Institute, Kew, Surrey, England (IMI 216611), and with the Centraalbureau voor Schimmelfcultures, Baarn, Netherlands (CBS 429.77).

Aspergillus spectabilis resembles *Aspergillus multicolor* Sappa, *A. crustosus* Raper & Fennell and *Emericella desertorum* Samson & Mouchacca in general aspect, and *A. multicolor*, *A. eburneo-cremeus* Sappa and *A. heterothallicus* Kwon, Raper & Fennell in its production

of robust conidiophores with relatively broad conidial columns (1, 3). It differs from three of the above-named species in being ascosporic; it differs from *A. heterothallicus* in being homothallic; and it differs from *E. desertorum* in producing abundant, well developed conidiophores and in ascospore size and morphology. The species herein described is very similar to Eidam's original *Aspergillus nidulans* var. *nidulans* in ascospore size and morphology but differs radically in conidiophore size and morphology and in Hülle-cell prominence and color in mass. *Aspergillus spectabilis* resembles *Aspergillus striatus* Rai, Tewari & Mukerji in cleistothecium distribution in relation to Hülle cells, and these two species, with cleistothecia elevated and embedded as a single layer in the upper portion of a Hülle-cell crust, are unique in this respect among members of the *Emericella-Aspergillus nidulans* group. *Aspergillus spectabilis* differs from *A. striatus*, *E. desertorum* and *A. violaceus*, all ascosporic and perhaps its closest relatives, in conidial state morphology and in ascospore size and ornamentation. Additionally, Hülle-cell color in mass in *A. spectabilis* and *E. desertorum* (as in the nonascosporic *A. multicolor* and *A. crustosus*) is vinaceous; in *A. striatus*, *A. violaceus*, *A. purpureus* Samson & Mouchacca (4) and *A. nidulans*, by contrast, Hülle cells in mass are pale yellow to straw colored.

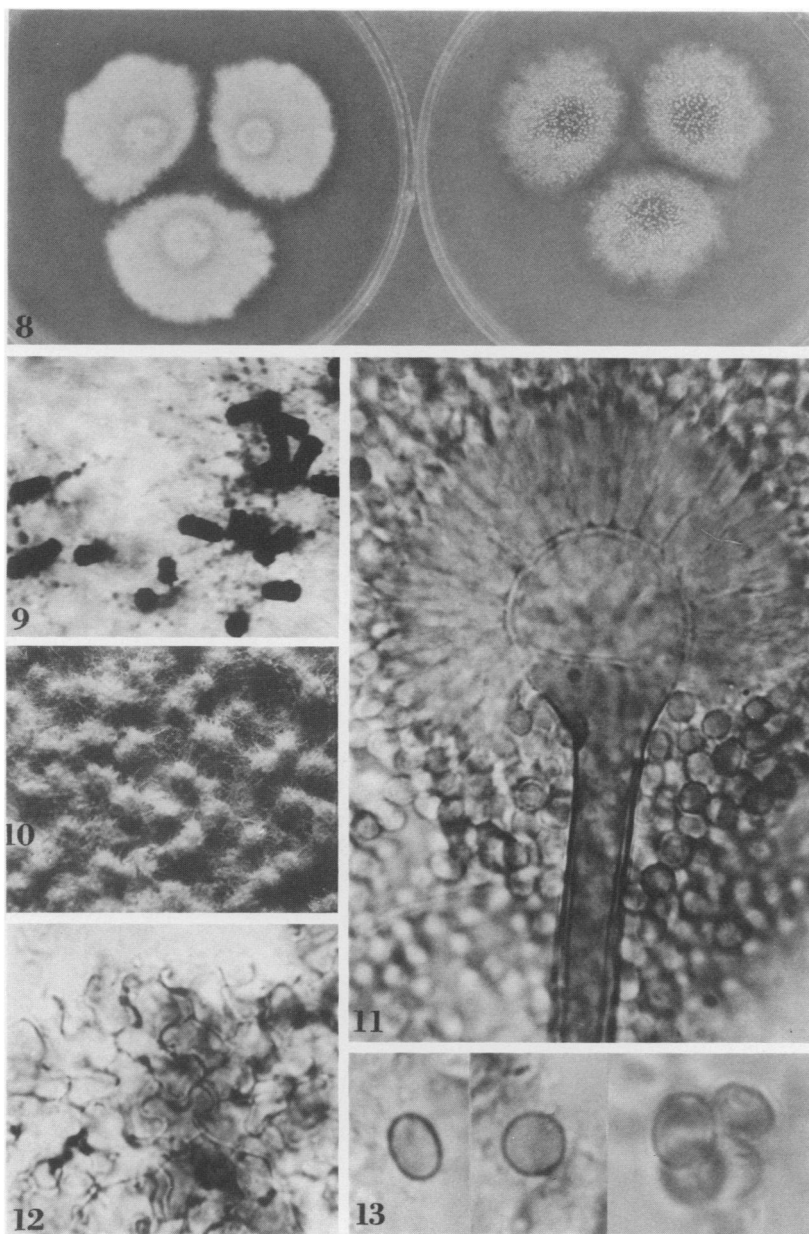
Aspergillus bicolor Christensen & States, sp. nov. status perfectus sit
judicatus ***Emericella bicolor*** Christensen & States, sp. nov.

FIGS. 6, 8–14

Coloniae in agar Czapekii 3.2–3.5 cm 14 dies 24–26 C crescentes, tenues, pallide flavae, marginibus lobosis; reverso vinaceo, agarum colorante eodem colore. Capitula conidica, viridia (2, XLI), primo globosa, deinde columnaria, usque ad 150–300 × 80–120 µm, biseriata; conidiophorae 130–245 × 6.5–8.5 µm, membranis 1.0 µm crassis, enodibus, aequaliter brunneae; vesiculae rotundae, brunneae, 10.5–24.0 µm in diam; metulae 6.5–10 × 3.5–4.5 µm; phialides 6.5–8.0 × 2.8–3.2 µm; conidia subglobosa vel subelliptica, aspera, plerumque 3.5–4.0 × 2.5–3.2 µm raro vel 4.5 × 3.5 µm. Cleistothecia inclusa in cellulis "Hülle," tota massa appetens ad 500–600 µm in diam, violacea (2, XXXVI), crescentia in mycelio aereo subflavo in agar maltoso ad dies 30–50; cleistothecia globosa, 130–330 µm in diam, membranis flavis 3.5–5.4 µm crassis; asci postremo octospori, 9–14 µm in diam; ascosporae post dies 60–80 maturantes, rufo-aurantiis, lenticulares, bivalvatae, 4.2–5.8 × 3.0–3.5 µm, prope sine cristis orbicularibus; superficies convexae, leviter rugosa.

Typica cultura: RMF 2058, e solo campi Artemisio-graminei prope Jackson, Wyoming, USA, isolata; in herbario NY deposita.

Colonies on Czapek's solution agar (FIG. 8) 3.2–3.5 cm in diam after 14 da at 24–26 C, thin, lightly floccose to granular with development of pale yellow hyphae-Hülle-cell masses, producing scattered conidial heads at first radiate and light yellow green, later becoming



FIGS. 8-13. *Aspergillus bicolor* RMF 2058. 8. Colonies on malt and Czapek agars, respectively, grown for 14 da at 24-26 C. 9. Conidia in compact cylindrical columns on dilute hay infusion agar. $\times 25$. 10. Surface view of a portion of a

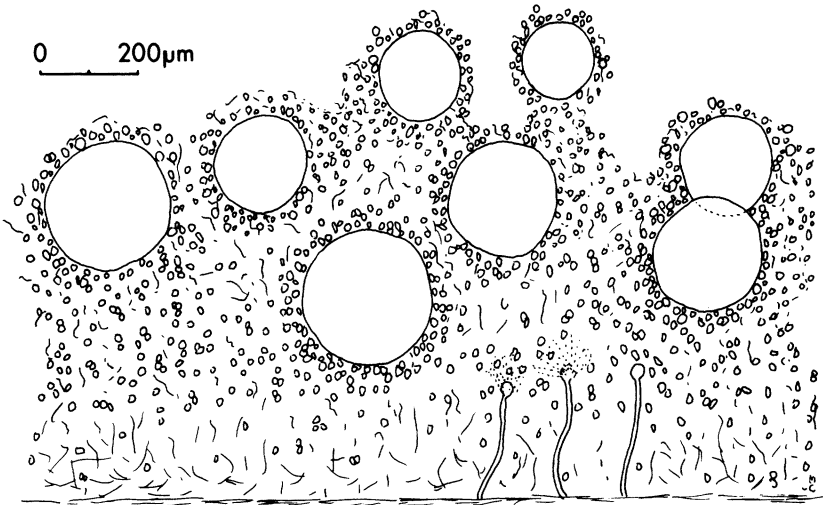


FIG. 14. Cross section of *Aspergillus bicolor* colony on malt agar showing cleistothecia developing at several levels in the aerial felt and with only loose envelopes of Hülle cells and associated hyphae; conidiophores submerged, from substrate hyphae. Semidiagrammatic; scale approximate.

Lincoln green (2, XLI), forming columns $150\text{--}180 \times 65\text{--}90\text{ }\mu\text{m}$ but these not affecting colony color macroscopically; colony margin irregular; reverse jasper to eugenia red (2, XIII) or honey yellow (2, XXX) with striking production and diffusion of vinaceous pigment into the agar. *Conidial heads* abundant on dilute hay infusion agar (1) at 10–14 da (FIG. 9), dark green, columnar, up to $200\text{--}300 \times 80\text{--}120\text{ }\mu\text{m}$, biserial; *conidiophores* on both media uniformly brown pigmented, straight, with wall up to $1.0\text{ }\mu\text{m}$ thick, smooth, $130\text{--}245 \times 6.5\text{--}8.5\text{ }\mu\text{m}$, uniform in diam; *vesicles* globose or nearly so (FIG. 11), brown, $10.5\text{--}24.0\text{ }\mu\text{m}$ in diam, fertile over the upper two-thirds; *metulae* conspicuously broad, $6.5\text{--}10.0 \times 3.5\text{--}4.5\text{ }\mu\text{m}$; *phialides* $6.5\text{--}8.0 \times 2.8\text{--}3.2\text{ }\mu\text{m}$; *conidia* subglobose to ellipsoidal, mostly $3.5\text{--}4.0 \times 2.5\text{--}3.2\text{ }\mu\text{m}$ rarely to $4.5 \times 3.5\text{ }\mu\text{m}$, bright green by transmitted light, roughened, separated by prominent connectives. Hülle-cell development scant on Czapek's agar and cleistothecia not formed.

Colonies on malt agar (FIG. 8) 3.4–3.8 cm in diam after growth

malt colony at 3 mo with developing cleistothecia and surrounding envelopes of Hülle cells; the subglobose masses are vinaceous in color thus contrasting with the yellowish aerial mycelium. $\times 15$. 11. Brown-walled conidiophore ending in a typical, subglobose vesicle with conspicuously broad metulae. $\times 1,400$. 12. Cleistothecium wall showing a portion of the discrete layer of lobed interlocking cells. $\times 1,000$. 13. Immature ascospores and ascus. $\times 2,000$.

for 14 da at 24–26 C, soft yellow near cream buff (2, XXX), floccose, conidial structures not present; reverse near antimony yellow (2, XV); odor not pronounced. Growth about one-third faster on malt at 30 C and central portion of colony becoming pale vinaceous earlier than at room temperature. Hülle cells and associated hyphae vinaceous in mass near varley's gray (2, XLIX), characteristically developing throughout colony centers at 14–21 da forming both beneath and loosely within the pale yellow, floccose aerial mycelium. *Hülle cells* subglobose up to 25 μm in diam or irregularly elongate to $32 \times 9 \mu\text{m}$ (FIG. 6), terminal and intercalary, with thick walls prominently but finely radially striate when viewed with phase illumination.

Colonies on malt agar at 24–26 C for 1 mo 6.0 cm in diam, lightly floccose, warm buff (2, XV), deep lavender (2, XXXVI) to light purple drab (2, XLV) tinged and granular centrally to subcentrally as aerial Hülle-cell masses increase in size and number; reverse yellow-orange near yellow ocher (2, XV) to mars yellow (2, III); exudate clear and in small droplets; odor not pronounced. *Hülle-cell aggregations* (FIG. 10) mostly 275–330 μm in diam expanding with development of cleistothecia to 500–600 μm at 50 da, subglobose, with one to three cleistothecia. Freehand sections through mo-old colonies revealing the presence of a thin surface weft of yellow hyphae underlain by a deep layer of massed Hülle cells and loose interstitial hyphae (FIG. 14). The basal mycelium, chrome yellow in color, occasionally seen to have formed brown-stalked conidiophores and grass-green conidial columns equal in all respects to those already described; conidiophores conspicuous over the surface of the colony at about 6–8 wk, apparently forming as the culture dries. *Cleistothecia* encased in Hülle cells developing at several levels in the colony profile (FIG. 14) and at various stages of maturation after 4–6 wk; cleistothecia proper globose, 130–330 μm in diam mostly 260–290 μm , with a reddish wall 3.5–5.4 μm thick constructed of one or two layers of irregularly lobed interlocking cells (FIG. 12) with one or two layers of crisscrossing hyphae beneath as in *Aspergillus nidulans* and *A. spectabilis*; *asci* produced sparsely after about 40 da, subglobose, 9–14 μm in diam, eventually 8-spored; *ascospores* maturing slowly, infrequent at 60 da, orange red, lenticular with very low equatorial crests, convex walls smooth to delicately echinulate, $4.2\text{--}5.8 \times 3.0\text{--}3.5 \mu\text{m}$ (FIG. 13).

The perfect state may be regarded as *Emericella bicolor* Christensen & States.

The outstanding characteristics of this species are its 1) coloration on malt agar—pale yellow aerial mycelium with lavender-gray to vinaceous Hülle-cell-cleistothecial masses, 2) tall-growing (to 245

μm) brown conidiophores with globose brown vesicles, broad metulae and compact conidial columns sparse and inconspicuous on malt agar but abundant on hay infusion agar, 3) tardily forming cleistothecia, often underdeveloped, encased in elevated Hülle-cell masses, and 4) ascospores that are infrequent, orange red, smooth or delicately echinulate, have very low equatorial crests, and mature unevenly at 2–3 mo.

The type culture, RMF 2058, was isolated from *Artemisia*-grassland soil collected in the Teton Basin near Jackson, Wyoming. The HOLOTYPE, a dried plate culture, is deposited in the Cryptogamic Herbarium of The New York Botanical Garden. Cultures of *A. bicolor* RMF 2058 are in the American Type Culture Collection, Rockville, Maryland (ATCC 36104), at the Commonwealth Mycological Institute, Kew, Surrey, England (IMI 216612), and at the Centraalbureau voor Schimmelcultures, Baarn, Netherlands (CBS 425.77).

Aspergillus bicolor resembles *A. nidulans* var. *acristatus* Fennell & Raper in producing ascospores with very low equatorial crests; it differs greatly from that taxon, however, in many features, e.g., conidiophore size and morphology, ascospore surface ornamentation and Hülle-cell size, shape and color. *Aspergillus bicolor* is similar to *A. rugulosus* Thom & Raper in having cleistothecia of all ages loosely arranged and at several levels within the colony profile. It differs from *A. rugulosus* in conidiophore dimensions, color of Hülle-cell layer, Hülle-cell abundance, and ascospore size and morphology. Comparing the eight known members of the *Aspergillus nidulans* group with tall conidiophores, *A. bicolor* differs from *A. crustosus*, *A. aeneus* Sappa, *A. eburneo-cremeus* and *A. multicolor* primarily in being ascosporic but secondarily in colony color, growth rate and conidial state morphology (notably size of conidial columns, breadth of the metulae, and conidium size shape and surface). It differs from *A. varicolor* (Berk. & Br.) Thom & Raper, *A. heterothallicus*, *A. aurantiobrunneus* (Atkins, Hindson & Russell) Raper & Fennell, *A. spectabilis* and indeed all other ascosporic members of the *A. nidulans* group in ascospore morphology, colony color and particularly in the position and mode of development of its distinctive Hülle-cell-cleistothecium masses.

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