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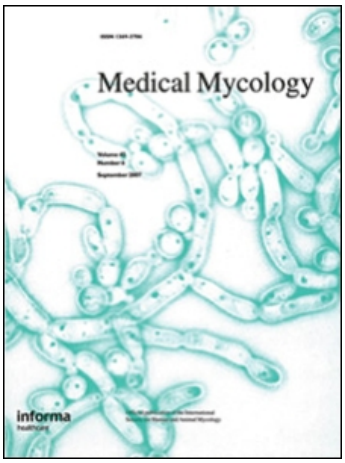

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Ajellomyces crescens sp. nov., taxonomy of Emmonsia spp., and relatedness with Blastomyces dermatitidis (teleomorph Ajellomyces dermatitidis)

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Ajellomyces crescens sp. nov., taxonomy of *Emmonsia* spp., and relatedness with *Blastomyces dermatitidis* (teleomorph *Ajellomyces dermatitidis*)

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Adiaspiromycosis is known primarily as a pulmonary infection of small burrowing mammals and rarely of humans, in which the tissue spore form consists of a large, globose, thick-walled, non-proliferating structure called an adiaspore. The causative agents have been placed in *Emmonsia* or *Chrysosporium* and treated as either two species or varieties. *Emmonsia parva* (= *Chrysosporium parvum* var. *parvum*) has been distinguished from *E. crescens* (= *C. parvum* var. *crescens*) by differences in maximum growth temperature, size of adiaspores, host range and geographical distribution. Phenotypic similarities between *Emmonsia* spp. and *Blastomyces dermatitidis* and chance observation of *Ajellomyces*-type ascomatal hyphae led to the hypothesis that the teleomorph of *Emmonsia* spp. could occur in *Ajellomyces*. Isolates preliminarily identified as *E. parva* or *E. crescens* were examined by morphology and physiology and tested for compatibility in mating experiments. *Ajellomyces crescens* Sigler sp. nov. is described for the teleomorph of *Emmonsia crescens* based on compatibility among 12 of 22 strains, stellate gymnothecial ascomata composed of obtuse diamond-shaped cells, helically coiled appendages and small, globose, muriculate ascospores. The agents of adiaspiromycosis are here treated as species with adiaspore size and morphology and temperature of induction as their major defining features. The species differ also in cycloheximide tolerance and in their abilities to form a teleomorph. With evidence of a connection between *Emmonsia crescens* and a teleomorph in *Ajellomyces*, *Emmonsia* is favoured over *Chrysosporium* as the correct name for the agents of adiaspiromycosis. This finding also corroborates earlier suggestions of a close phylogenetic relationship between *Emmonsia* spp. and the dimorphic pathogens *Blastomyces dermatitidis* and *Histoplasma capsulatum*.

Keywords adiaspiromycosis, *Ajellomyces* species, *Blastomyces dermatitidis*, *Emmonsia* species

Introduction

Adiaspiromycosis is known primarily as a pulmonary infection of rodents and small burrowing mammals in which the tissue spore form consists of a large globose, thick-walled, non-proliferating structure called an adiaspore. Human infections are rare, usually presenting as a diffuse pulmonary infection, and often diagnosed from histopathology [1]. A recent report documents extrapulmonary infection involving the bone in a patient with AIDS [2].

The classification of the causative agent has been controversial and either two species or two varieties are recognized [3–8]. *Emmonsia parva* (Emmons & Ashburn) Ciferri & Montemartini [= *Emmonsia parva* var. *parva* = *Chrysosporium parvum* (Emmons & Ashburn)

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Carmichael var. *parvum*] is known from relatively few species of animals in narrow geographical ranges within North America, Asia, Australia and Czechoslovakia, whereas *Emmonsia crescens* Emmons & Jellison [= *E. parva* var. *crescens* (Emmons & Jellison) van Oorschot = *Chrysosporium parvum* var. *crescens* (Emmons & Jellison) Carmichael] is known from over 96 species of animals and from the soil worldwide [9]. The species are distinguished by maximum growth temperature and the different sizes of adiaspores [3,4,7,8]. Most cases of human infection have been attributed to *E. crescens* with the maximum temperature for growth at 37 °C, and larger adiaspores, often 100 μm or more in diameter [4,7,8].

Morphological similarities between the species of *Emmonsia* Ciferri & Montemartini and *Blastomyces dermatitidis* Gilchrist & Stokes, the causative agent of blastomycosis, have been observed by various authors [3,4,7, 10], but these taxa have been maintained in separate genera largely as a result of the emphasis placed on their *in vivo* parasitic forms (i.e. non-replicative adiaspores in *Emmonsia* and broad-based budding yeast cells in *B. dermatitidis*). Using mating experiments, McDonough & Lewis [11,12] discovered the teleomorph of *B. dermatitidis* which they named *Ajellomyces dermatitidis* McDonough & Lewis. This heterothallic ascomycete is classified in the family Onygenaceae, order Onygenales [13]. Chance observation of *Ajellomyces*-type ascomatal hyphae in an unusual isolate of *Emmonsia* from the lung of an Australian wombat [14] suggested that the *Emmonsia* could be the anamorph of an *Ajellomyces* spp. [Sigler in ref. 15]. Moreover, molecular data obtained from sequencing 18S rDNA genes strongly supported grouping *E. crescens* (as *C. parvum*) with *B. dermatitidis* and *Histoplasma capsulatum* Darling, which also has a teleomorph in *Ajellomyces* [Bowman in refs 15,16].

This study examined 33 strains identified preliminarily as either *E. parva* or *E. crescens* to evaluate current species concepts. Strains were assessed for degree of distinction based on morphological features, for their responses in some physiological tests, and for their abilities to produce a teleomorph. Three additional strains were included in mating experiments.

Materials and methods

Source of strains

A total of 33 isolates, preliminarily identified as *E. parva*, *E. crescens* or of uncertain affinity, were on deposit at the University of Alberta Microfungus Collection and Herbarium (UAMH). Each strain was recevered from lyophilized material onto Pablum cereal agar (CER) [17]. Stock plates 14–28 days old were used as inoculum for all tests. The stock plates were maintained at 5 °C. Isolates received during the course of the study, one from New Zealand [18] and two from Israel [19], were included only in the mating experiments.

Growth studies

Growth rates of 33 isolates were tested at 28, 37 and 40 °C on phytone yeast extract agar (PYE; Becton Dickinson Microbiology Systems, Cockeysville, MD). Diameters and colonial features were recorded weekly for 21 days. Tolerance to cycloheximide at a concentration of 400 μg ml^{-1} was evaluated by measuring growth rates of each strain grown on mycosel agar (MYC; Becton Dickinson) compared with PYE at 28 °C. Colonial features and growth rates at 21 ± 2 °C were observed also on potato glucose agar (PDA; Difco Laboratories, Detroit, MI). Colony colours correspond to the colour charts of Kornerup & Wanscher [20]. Development of adiaspores on PYE was assessed by microscopic examination of residual inoculum at the highest temperature at which growth was strongly inhibited. To determine whether adiaspore production might be enhanced on enriched media, selected strains were grown also on brain heart infusion agar slants with and without blood (Difco) at 37 and 40 °C. Strains were also evaluated for their responses on several media used in the dermatophyte diagnostic including bromcresol purple-milk solids-glucose agar (BCP-MS-G), Christensen's urea and Trichophyton agars numbers 1–5 as these tests have been shown useful in discriminating among some members of the genus *Chrysosporium* Corda [Sigler in ref. 17].

Mating tests

Several attempts were made to mate available strains. The first experiment included 34 strains. For each species, strains were paired in all possible combinations, including self–self pairings, on Takashio agar [17] and incubated at room temperature in the dark. Each test strain was streaked in a straight line across the centre of a 100 mm Petri plate; the second test strain was streaked at right angles to the first. No fertile ascomata were observed, but pairings among nine isolates of *E. crescens* showed evidence of ascomatal hyphae after 10 weeks incubation. These nine strains (126, 127, 128, 129, 349, 4076, 4077, 7268, 7365) were included in the second experiment in which soil extract–yeast extract agar (SEA + YE) was used and the inoculum consisted of a suspension of conidia in sterile distilled water. Two or three drops of the conidial suspension were pipetted onto the surface of plates of SEA + YE; a few drops of a suspension from a second strain were added and the inocula mixed. Strains were mated in all possible combinations and plates were

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incubated at room temperature in alternating light and dark. The ingredients of SEA were modified from Kwon-Chung [21] and consisted of clear soil extract 1 l, glucose 2 g, yeast extract 5 g, $MgSO_4.7H_2O$ 1 g, KH_2PO_4 1·5 g, $NaNO_3$ 1 g, agar 15 g. Plates were examined weekly for 6 weeks and biweekly thereafter for the presence of ascomata. From this experiment, which yielded fertile ascomata, two strains were designated to be '−' mating type (129, 349) and the remaining seven strains were designated as '+' mating types. Plates were held for 5 months before being discarded as negative. Two subsequent mating experiments were conducted in an attempt to improve compatibility and fertility. They employed the same medium and inoculation procedures. For *E. crescens* matings, two strains designated + (126, 7365) and two designated − (129, 349) were mated with 13 additional strains of *E. crescens* and all strains of *E. parva*. In addition, *E. parva* strains were mated in all possible combinations.

For scanning electron microscopy, fertile ascomata on blocks of agar were soaked in phosphate buffer under vacuum, fixed in 4% glutaraldehyde, dehydrated through ethanol series into amyl acetate and then dried to the critical point. A coating of gold-paladium was applied with a sputter coater and specimens were examined and photographed with a Cambridge S-250 SEM.

Results

Features of the anamorph *spp.*

While the colonial morphologies of the isolates on PDA were within the range of reported variation [3,4,8], they did divide into several groups of typical morphologies. Two groups of 6 and 5 strains each are typical of the variation in *E. crescens*. Group 1 colonies (Fig. 1) (UAMH 126, 127, 128, 140, 4076, 4077) grew faster (75–80 mm diameter after 28 days) and were yellowish-white to orange-white (4A2–5B3), densely woolly in the centre with small exudate droplets, and with broad (10–12 mm) glabrous margin, reverse greyish brown (6D3). Group 2 colonies (Fig. 2) (UAMH 135, 137, 349, 7268, 7365) grew moderately fast (48–58 mm in 28 days) and were coarsely powdery with pale orange to greyish orange aerial mycelium (5A3/B5) over reddish-grey surface mycelium (8D4), margin irregular, reverse reddish brown (8E4). Other strains showed intermediate forms (Figs 3 and 4). Generally, colonies varied in topography from flat to umbonate or rugose, in texture from glabrous to woolly, velvety or coarsely powdery, in colour from white to yellowish-white or orange-white. Reverse pigmentation was pale grey to greyish-brown. Clear or yellowish exudate droplets were present or absent. Isolates of *E. parva* showed similar variation. One group of *E. parva* isolates (Fig. 5) (UAMH 125, 130, 134, 434, 2304, 7425, 7426) resembled group 1 of *E. crescens* in being woolly with a broad glabrous margin, but grew more slowly (35–61 mm in 21 days); a group of granular *E. parva* strains (Fig. 6) (UAMH 4489, 4770, 6312) resembled group 2 but were faster growing (80–85 mm). Sporulation of both species was enhanced on PDA or CER.

Twenty-one isolates were confirmed as *E. crescens* by their lack of hyphal growth at 37 °C and larger adiaspores (range 20–140 μm) (Fig. 21) formed on PYE (Table 1). Twelve isolates were confirmed as *E. parva* by their growth at 37 °C (colony diameter range 9–77 mm after 21 days) and by smaller adiaspores (range 8–20 μm) produced at 40 °C. Adiaspore production was not enhanced on BHIA or BHIA supplemented with blood. Strains of each species showed similar growth rates on PYE at 28 °C. Colony diameters ranged from 48 to 82 mm for *E. crescens* and from 36 to 85 mm for *E. parva* after 21 days. They differed in their tolerance to cycloheximide with *E. crescens* being less tolerant (Fig. 19). Sixteen of 21 strains of *E. crescens* were inhibited to $\leq$10 mm in 7 days, whereas only two of 12 strains of *E. parva* were inhibited to $\leq$10 mm in 7 days (Table 1). Strains of both species were similar in their production of urease and in growth on BCP-MS-G. Isolates grew slowly, lacked aerial mycelium and showed no proteolytic activity (Table 1). No strain demonstrated requirements for inositol, thiamine or nicotinic acid on *Trichophyton* agars. Of the three strains acquired during the study, the New Zealand isolate (7365) [18] was determined to be *E. crescens* and the Israeli isolates (7425, 7426) [19] were found to be atypical isolates of *E. parva*.

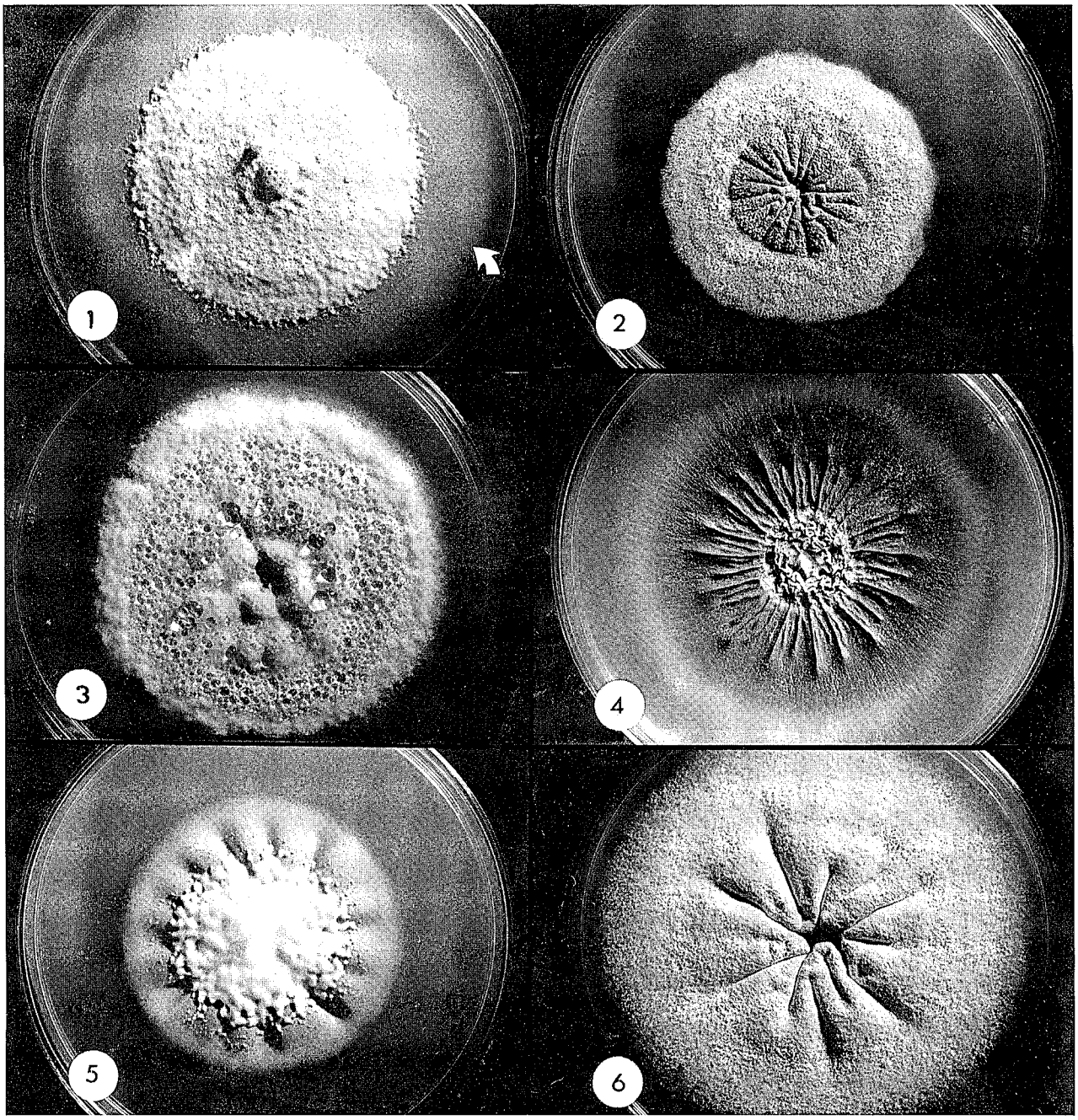
Microscopically, the two species appear indistinguishable. Conidia are sessile or borne at the ends of short, narrow stalks usually < 1 μm wide. The stalks occur at right angles to the hyphae and are either straight-sided or slightly swollen at the distal end (end nearest to the conidium). Each stalk bears a single terminal conidium, or the swollen end may bear one to three secondary spine-like pegs which in turn form a solitary conidium (Fig. 20). Conidia are subglobose or ovoid, appearing slightly flattened and broader than long, or are pyriform and have narrow basal scars. They measure 2·5–4 μm long by 3–5 μm wide, sometimes swelling in age. The wall is smooth or finely roughened in age.

Description of teleomorph

Ajellomyces crescens Sigler sp. nov. (Figs 7–16)

Ascomycotina, Onygenales, Onygenaceae

Fungus heterothallicus. Ascomata (gymnothecia) pallide brunnea, globosa vel irregulariter stallata,



Figs 1–6 Colonial variation in *Emmonsia* spp. grown on PDA after 28 days at 21 °C. Figs 1–4: *E. crescens*. **Fig. 1** Woolly colony with broad glabrous margin (arrow) (UAMH 126). **Figs 2–4** Powdery, woolly and glabrous colonies (2-349; 3-129; 4-1067). **Figs 5–6** *E. parva*. **Fig. 5** Woolly colony with broad glabrous margin (434). **Fig. 6** Powdery colony (4770). (× 0·83)

flavo-brunnea, parva; peridium compositum de hyphis et mensura et forma inaequalibus et apud septum constrictis; appendices ascomaton torsivae, spiris numerosis, parietibus crassis, flavo-brunneae, laeves, aseptatae. Asci octospori, irregulariter dispositi, globosi vel subglobosi, evanescentes. Ascosporae globosae, hyalinae, laevaes, per SEM visae ordinate punctatum-muricatae, 1–1·5 μm.

Status anamorphosis: *Emmonsia crescens* Emmons & Jellison 1960

Holotypus: Coloniae exsiccatae UAMH 8089, ex cruce UAMH 349 (−) × 7365 (+)

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Table 1 Comparison of *Emmonsia crescens* with *E. parva* by growth rates, physiology and adiaspore size

	E. crescens ($n = 21$)	*E. parva* ($n = 12$)
Mean colony diameter (mm)		
PYE (28 °C) after 21 days	65	69
PYE (37 °C)	0 to trace	36
PYE (40 °C)	ND	0 to trace
MYC (28 °C)		
Day 7	8	17
Day 21	36	58
Adiaspore size (PYE) (μm)		
Mean	59 (37 °C)	12 (40 °C)
Range	20–140	8–20
Urease	+	+
Bromcresol purple-milk solids-glucose agar		
pH change at 14 days	None	None*
Growth rate	Slow	Slow

ND, not determined; PYE, phytone yeast extract agar; MYC, mycosel agar.
*Two strains (4770, 6312) showed trace alkalinity; one strain (2304) showed trace acidity.

Isotypus: DAOM 221108

Heterothallic. Ascocarps are discrete, pale brown, globose or irregular in shape, small, 80–250 μm in diameter, composed of branched anastomosing pale brown peridial hyphae in which individual cells are swollen near the centre and constricted at the septa resulting in an obtuse diamond shape; appendages pale brown, helically coiled, thick-walled, yellowish brown. Asci are subglobose or club-shaped, evanescent and contain eight ascospores. Ascospores are small, globose, hyaline and measure 1–1·5 μm diameter. They appear muriculate (having short, hard outgrowths) by scanning electron microscopy but smooth under light microscopy. Conidial state: *Emmonsia crescens*.

Fertile ascomata occurred only in pairings among 12 isolates of *E. crescens* grown on SEA + YE after 6 weeks or longer incubation (Table 2). Cultures were held for up to 5 months before being discarded as negative. No teleomorph was produced when strains were grown alone. One strain (7365) showed signs of stimulation, i.e. producing coils and ascomatal hyphae, in pairings with both + and − strains, but ascospores were produced only in crosses with − mating strains. The ex-type culture of *E. crescens* (UAMH 3008 = ATCC 13704) initially was degenerate and failed to sporulate. Sporulation was recovered on PDA, but it failed to mate. On SEA + YE, many strains produced crystalline deposits of reddish brown pigment either on the surface or embedded in the agar.

Discussion

Taxonomy and nomenclature of the anamorph

The unique nature of the *in vitro* parasitic form has led mycologists to consider the agents of adiaspiromycosis distinct from the dimorphic pathogens despite morphological convergence. Adiaspores are globose, thick-walled, uni- or multinucleate cells commonly found in enlarged cyst-like formations in the lungs of rodents and other animals. The term adiaspore was derived from the Greek verb '-speirein-' to scatter, with 'adia-' being the negative (M. Hertwig, personal communication) and proposed for the spherule which enlarges from the inhaled conidium [7]. Adiaspiromycosis describes the infection in which there is no multiplication or dissemination of the fungus from the original site [7]. Jellison [22] suggested that Kirschenblatt in 1939 may have been the first to publish a report of cyst-like bodies in a rodent and to recognize the structures as being of fungal origin. Kirschenblatt named the organism *Rhinosporidium pulmonale*, but he neither confirmed the fungal cause by culture nor provided a Latin diagnosis. Although Jellison [22] observed adiaspores in preserved lung tissue from a rodent trapped in Sweden in 1845, Emmons & Ashburn [6] first described the fungus from rodents in Arizona trapped during a study to delineate the natural reservoir of *Coccidioides immitis*. A survey of 303 animals recovered *C. immitis* from 8%, 'an apparently related' new fungus from 33%, and both fungi from 2% of specimens. In sections of the lung, the spores appeared as spherical non-budding cells reaching a diameter up to 14 μm. Emmons & Ashburn [6] described their fungus within the 'phycomycete' genus *Haplosporangium* under the name *H. parvum* for its small size compared with other species. At that time, the internal replication of the spherule in *C. immitis* had been interpreted also as phycomycetous by Emmons [23] and others. Emmons & Ashburn speculated on a genetic relationship between *C. immitis* and *H. parvum* on the basis of resemblance of tissue forms, serological cross-reactions and the occasional finding of mixed infections. Although they questioned whether *H. parvum* might be a mutant of *C. immitis* 'despite great morphological differences', they noted a remarkable resemblance to *B. dermatitidis* and *Histoplasma capsulatum*.

Later, Dowding [10] observed large pearl-like cysts containing fungal cells up to 300 μm diameter in lungs of 14 of 275 rodents surveyed in Alberta. Although the Alberta fungus resembled *H. parvum* culturally and microscopically, it differed in forming larger cells *in vivo* and in producing large chlamydospores when grown at 37 °C. She suspected that *H. parvum* was closely related to *B. dermatitidis*, *Blastomyces (Paracoccidioides) brasiliensis* and *H. capsulatum*, but was less closely related to

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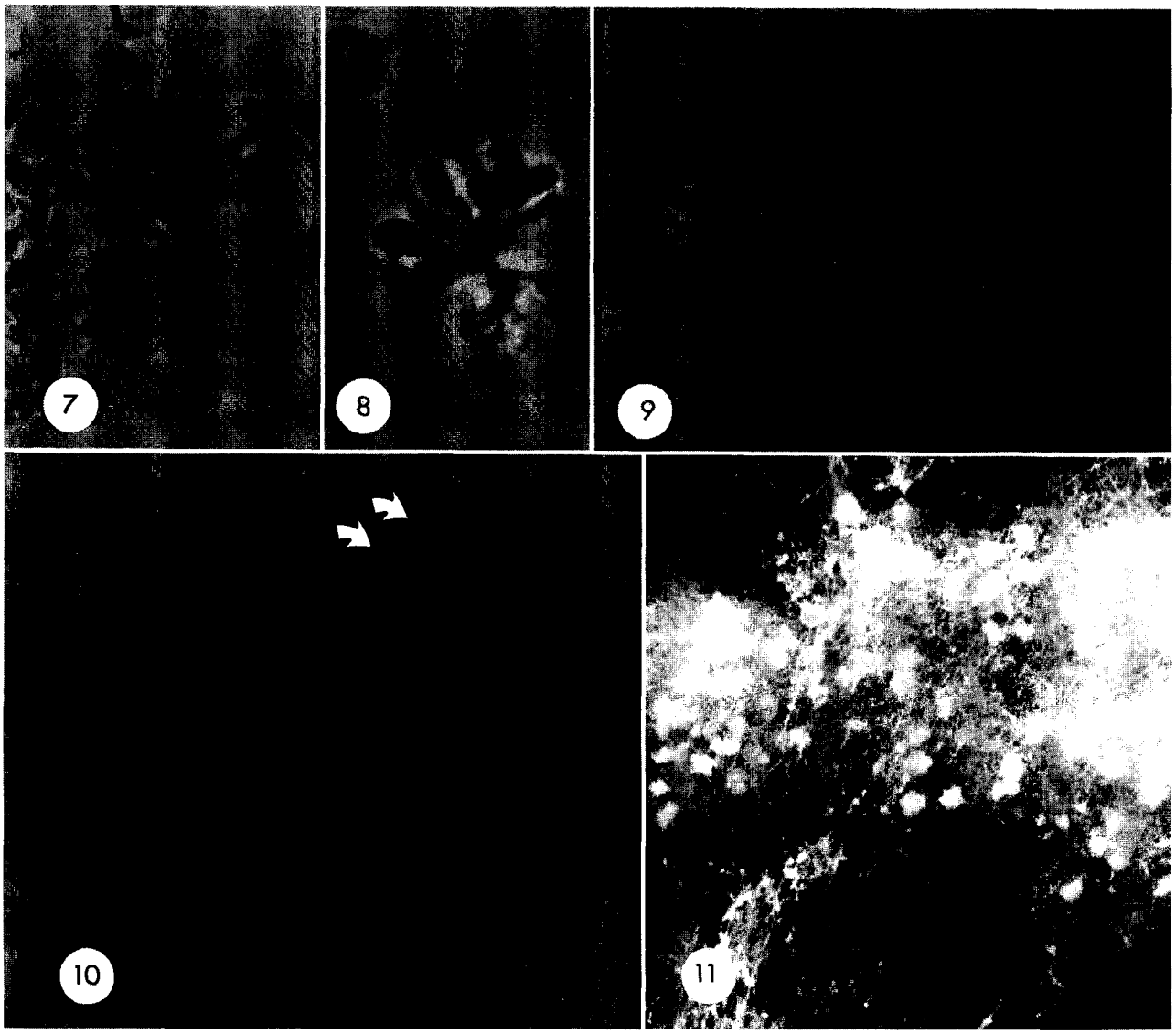

Fig 7–11 *Ajellomyces crescens* examined by light microscopy. **Fig. 7** Ascocarp initial (UAMH 128 × 129), × 1120. **Fig. 8** Cluster of club-shaped asci (349 × 7365), × 1400. **Fig. 9** Globose ascospores (128 × 349), × 1280. **Fig. 10** Immature ascocarp showing developing asci (arrows) and with helically coiled appendage (129 × 7268), × 1120. **Fig. 11** Ascomata developing on SEA + YE and examined under a dissecting microscope (349 × 7365), × 33.

C. immitis because the latter formed arthroconidia and in tissue developed cells (spherules) containing internal spores. She noted that *H. parvum* and *B. dermatitidis* appeared identical in colonial and microscopic features, but distinct in their tissue forms.

In 1951, Carmichael [3] suggested that *H. parvum* should be transferred from *Haplosporangium* because its conidia are released by fracture and often have remnants of the conidiophore attached and because its closest affinity was with *B. dermatitidis*. Although differences

Figs 12–18 *Ajellomyces crescens* and *A. capsulatus* viewed by scanning electron microscopy. **Figs 12–16** *A. crescens*. **Fig. 12** Irregularly stellate ascocarp showing helically coiled appendages (UAMH 127 × 349). Bar = 100 μm. **Fig. 13** Helically coiled appendage (127 × 349). Bar = 10 μm. **Fig. 14** Hyphae composing the ascocarp. Individual cells are swollen near the centre and constricted at the septa (128 × 129). Bar = 10 μm. **Fig. 15** Swollen cells of ascomatal hyphae (128 × 349). Bar = 4 μm. **Fig. 16** Muriculate ascospore (127 × 349). Bar = 1 μm. **Figs 17 and 18** *A. capsulatus* ex holotype specimen BPI 71811. **Fig. 17** Muriculate ascospore from dried specimen. Note surrounding ascospores show signs of collapse. Bar = 2 μm. **Fig. 18** Ascocarp with helically coiled appendages. Bar = 100 μm.

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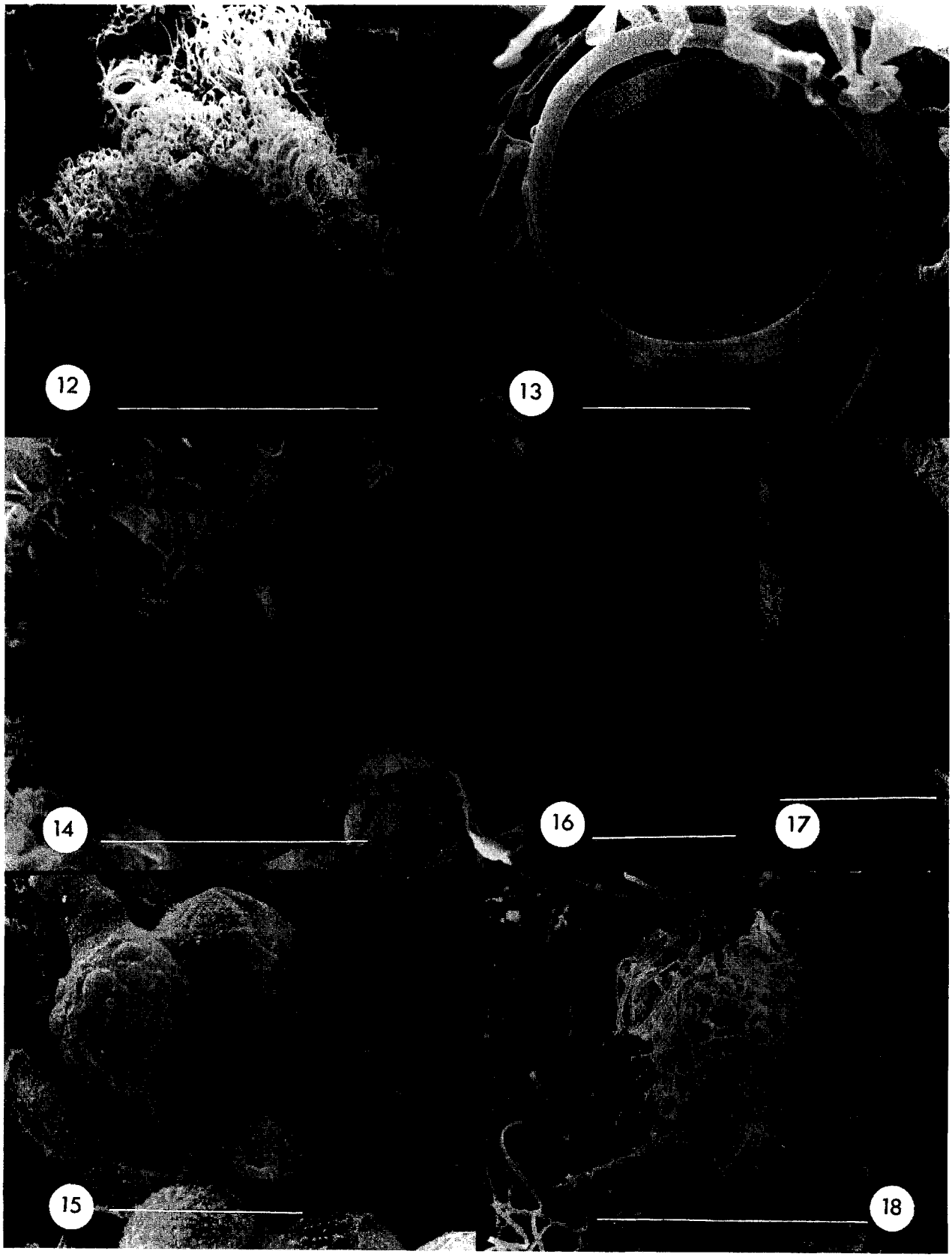

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Table 2 Results of mating tests among 12 strains of *Emmonsia crescens* on soil extract agar + yeast extract

	Plus mating strains								
	126	127	128	140	1067	4076	4077	7268	7365
Minus mating strains									
129	+	+	+	+	+	+	+	+	+
349	+	+	+	+	+	+	+	+	+
1140	+	NT	NT	NT	NT	NT	NT	NT	+

NT, not tested.

among strains from northern and southern rodents could allow separation into two or three species according to their differing responses to temperature, Carmichael recommended against such action [3]. The position of *H. parvum* within the phycomycetes was further challenged by the finding of chitin in its cell wall rather than cellulose [7].

In 1959, Ciferri and Montemartini [5] erected the genus *Emmonsia* for *H. parvum*, and in 1960, Emmons & Jellison [7] added *E. crescens* which they stated was indistinguishable by morphology. They placed emphasis on the size of the adiaspores (200–480 μm with walls 10–70 μm thick for *E. crescens* compared with 14–60 μm with walls 2 μm thick for *E. parva*) (Fig. 21), the lower temperature at which they formed (37 °C for *E. crescens* versus 40 °C for *E. parva*), and the nuclear condition of the adiaspores. Those of *E. crescens* were multinucleate and germinated by multiple germ tubes. *Emmonsia parva* adiaspores were uninucleate and germinated with a single germ tube. Later, Emmons [24] showed that a form of 'budding' could be induced in the adiaspores of *E. crescens* by manipulating growth conditions. If adiaspores with diameters of < 200 μm (8–12 days) were returned to room temperature for 4–8 h they began to form multiple germ tubes; on reincubation at 37 °C, the germ tubes become multiple buds [24].

In 1962, Carmichael [4] revised the classification of fungi which produce solitary single celled conidia that are released by disintegration of the supporting structure (aleurioconidia) and broadened the genus *Chrysosporium* to accept many of them. Teleomorphs of few species were known then, but he suggested that they would occur in the ascomycete family Gymnoascaceae (now Onygenales [13, 25]). He transferred the two species of *Emmonsia* and reduced them to varietal level as *Chrysosporium parvum* var. *parvum* and *C. parvum* var. *crescens*. He argued that both *B. dermatitidis* and *H. capsulatum* could be transferred to *Chrysosporium* on the basis of their formation of hyaline aleurioconidia, but formally proposed a transfer only for *B. dermatitidis*. The transfer was made on nomenclatural grounds as the name *B. dermatitidis* is invalid under the International Code of Botanical Nomenclature [4,8,25,26]. Neither this transfer nor an earlier proposal to take up the name *Zymonema* [4,8,25,26] has been widely adopted because the name *B. dermatitidis* is firmly entrenched in the literature.

Following von Arx [27], van Oorschot [8] retained *Emmonsia* with a single species having two varieties based on blastic conidial development rather than thallic developed as supposed for *Chrysosporium*. However, it has been suggested that there is a high level of variation in this character within species of *Chrysosporium* [28]. Van Oorschot also placed emphasis on the nature of the tissue form and on the ability to cause a mycosis as grounds for separating *Emmonsia* and the dimorphic pathogens from *Chrysosporium*. Both generic names continue to be used.

As new information convincingly establishes a close phylogenetic relationship between the agents of adiaspiromycosis and blastomycosis and with the agent of histoplasmosis a close relative (see Relationships), a strong case could be made for placing these fungi in the same anamorphic genus. However, such a decision is contraindicated by the need to maintain stability in nomenclature. With evidence of a connection between *Emmonsia crescens* and a teleomorph in *Ajellomyces*, *Emmonsia* is favoured over *Chrysosporium* as the correct generic name

Figs 19–26 *Emmonsia crescens* compared with *Blastomyces dermatitidis*. **Figs 19–21** *E. crescens*. **Fig. 19** Growth on medium without (top) and with cycloheximide at a concentration of 400 μg ml^{-1} (bottom) showing some sensitivity (UAMH 128), × 0·83. **Fig. 20** Slide culture preparation showing small, ovoid conidia borne sessile or at the ends of narrow stalks commonly swollen at the distal end. The swollen end may form one to three secondary conidia (arrow) (7365), × 460. **Fig. 21** Adiaspores formed on PYE at 37 °C (7365), × 460. **Figs 22–26** *Blastomyces dermatitidis*. **Fig. 22** Growth on medium without (top) and with cycloheximide showing tolerance (3539), × 0·83. **Figs 23 and 24** Slide culture preparation showing small pyriform conidia formed sessile or at the ends of narrow stalks which have straight sides or are swollen at the tip (Fig. 24, large arrow), × 610. Note enlarged conidium (small arrow) in Fig. 24. **Fig. 25** Enlarged conidium showing irregular wall protrusions (5438), × 770. **Fig. 26** Conversion to budding yeast *in vitro* (5438), × 610.

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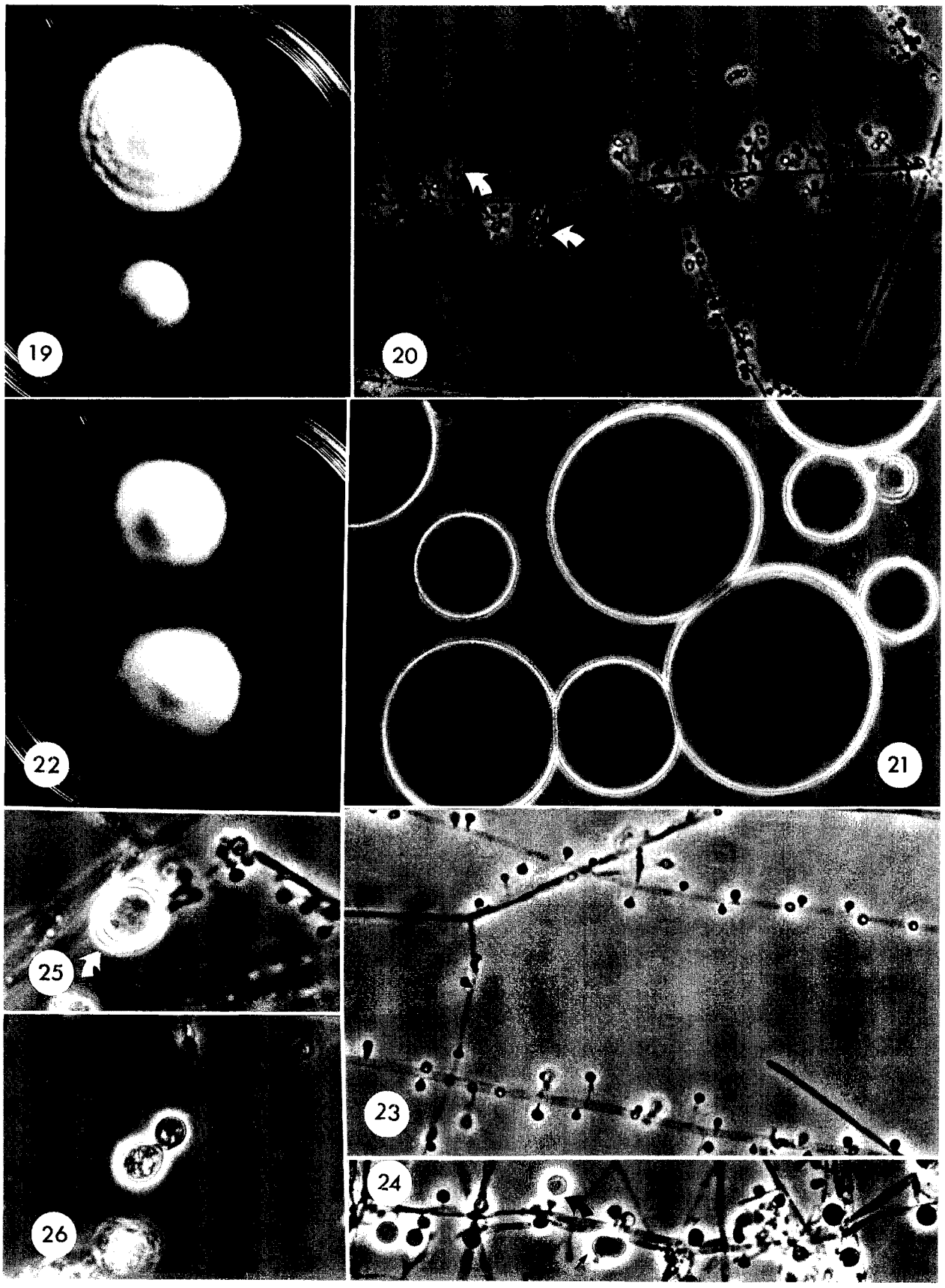

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for the agents of adiaspiromycosis, which are here treated as species rather than as varieties. Adiaspore size and morphology and temperature of induction are their major defining features. This study has shown also that the species differ in cycloheximide tolerance and in their abilities to form a teleomorph. Variations in colonial morphology occurred in both species, as has been observed previously [29], but there was no apparent link between colonial morphology and mating type among isolates of *E. crescens*.

Relationships

Although some mycologists have suspected a possible relationship between the agent of adiaspiromycosis and the dimorphic pathogens, confirmatory evidence has been lacking. Sigler [15] put forward a hypothesis that the teleomorph of *Emmonsia* spp. would occur in *Ajellomyces*. Analysis of 18S rDNA sequences showed strong support for the grouping of *E. crescens* (as *C. parvum*) (UAMH 1067) with *B. dermatitidis* and *H. capsulatum* which differed from each other at only 12 positions within 1713 bases sequenced [Bowman in refs 15,16]. Both species are known to have teleomorphs in *Ajellomyces* (family Onygenaceae, order Onygenales), *A. dermatitidis* described for *B. dermatitidis* [11,12] and *A. capsulatus* [30] (Figs 17 and 18) described for *H. capsulatum* (as *Emmonsiella capsulata*) [31,32]. Analysis of large subunit ribosomal RNA from a broader representation of onygenalean fungi [33] confirmed that *E. parva* showed seven base differences from *B. dermatitidis* and grouped together with *H. capsulatum* and its varieties and with *Paracoccidioides brasiliensis*, thus confirming observations made by Dowding in 1947 [10]. In addition, recent studies have shown that *C. immitis* and its near relative *Uncinocarpus reesii* (*Malbranchea* anamorph) (Onygenaceae) occur as a monophyletic group apart from, but closely related to, *B. dermatitidis* and *H. capsulatum* [15,16,34]. Antigenic similarity has been demonstrated in exoantigen tests in which non-specific precipitin lines are produced between *E. parva* antisera and the A or H and M antigens of *B. dermatitidis* and *H. capsulatum*, respectively [35]. Studies of ubiquinones have shown that *H. capsulatum* and *Emmonsia* spp. have Q-10 (H_2) as the major ubiquinones [36,37]; whereas *B. dermatitidis* had ubiquinone-10 (Q-10) as the major component [36]. Although the significance of these differences in ubiquinone distribution is difficult to evaluate [38], all other data predict a close relationship, now confirmed by the discovery of a teleomorph for one of the species of *Emmonsia*.

Emmonsia spp. and *Blastomyces dermatitidis* share a number of features, including white or tan, downy, velvety, powdery or occasionally glabrous colonies, similar growth rates, formation of solitary, single-celled aleurioconidia which may be smooth or verrucose and dimorphism (Figs 19–26). *Blastomyces dermatitidis* differs in the following features. (1) It is not inhibited by cycloheximide (Fig. 22). (2) Conidia are usually solitary and formed sessile or at the ends of unswollen or slightly swollen stalks (Figs 23 and 24). Proliferation to form another conidium is rare in *B. dermatitidis* but common in *Emmonsia* spp. (3) Growth at 37 °C occurs in the form of thick-walled budding yeast cells (Fig. 26). While 'typical' conidia of *Emmonsia* spp., *B. dermatitidis* and *H. capsulatum* can be readily distinguished, intermediate forms are common. Conidia of *B. dermatitidis* may also be echinulate [39] and often inflate (Figs 24 and 25) sometimes approaching the size of the macroconidia of *H. capsulatum* and may show wall protrusions (Fig. 25) similar to those on the macroconidia of *H. capsulatum* as described by Berliner [40]. The macroconidia of *H. capsulatum* can be smooth in young primary isolates. The microconidia of *H. capsulatum* are smooth or verrucose as are the conidia of *B. dermatitidis* and *Emmonsia* spp. The exoantigen and DNA probe tests have been shown to be reliable in differentiating among the species [9,35,41]. In the one study in which isolates of *E. parva* (as *C. parvum*) have been included, the commercially available DNA probe was found to show cross-reactivity with *Paracoccidioides brasiliensis* but not with *E. parva* [41].

In a revised concept of the Onygenales, *Ajellomyces* has been placed in the family Onygenaceae [13]. Members of the Onygenaceae have punctate ascospores, abilities to degrade keratin and anamorphs in which the conidia dehisce by lytic degradation of the supporting cell (aleurioconidia or alternate arthroconidia). The genus *Ajellomyces* is unusual in the family in forming helically coiled appendages (Fig. 18) and minute ascospores ($< 2\,\mu$m diameter) which are globose and muriculate (having short hard outgrowths). The ascospores of *A. dermatitidis* and *A. capsulatus* were described originally as smooth [11,12, 31,32] but SEM examination of the holotype of *A. capsulatus* shows the ascospore wall ornamentation to be very similar to that of *A. crescens* (Fig. 17). Garrison *et al.* [42] examined the ascospores of *A. dermatitidis* by TEM and showed the surface to be covered with short, sharply pointed or blunt spines which measured approximately 0·19 μm in length. Unfortunately, the holotype of *A. dermatitidis* (NCDC B767d; original designation FAL × GRA) was not available for comparison. It appears to have been lost as there is no record of it at Centers for Disease Control, Atlanta (A. A. Padhye, personal communication). Attempts to obtain a neotype by crossing the mating type strains (UAMH 3538 = CDC B-784 = ATCC 18187; UAMH 3539 = CDC B-788 = ATCC 18188) have been unsuccessful.

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A fungus with possible affinity to the *Ajellomyces* spp. is *Polytolypa hystricis*, described from a single isolate from porcupine dung [43]. It has ascospores with a similar wall ornamentation described as punctate-muricate, and helically coiled appendages; however, its ascospores are ellipsoidal rather than globose, the ascomatal hyphae are unswollen rather than diamond-shaped (Fig. 14), and the anamorph consists of irregular alternate arthroconidia rather than solitary aleurioconidia.

Despite repeated attempts using different media and conditions of incubation, few strains of the *Emmonsia* spp. could be induced to mate. The factors responsible for this low level of fertility are unknown but it seems likely that nutritional rather than physical factors are involved. Response was improved when yeast extract was added to Takashio medium. Infertile ascomata were observed in the first experiment using this medium, but fertile ascomata were obtained in a later limited study among a few compatible strains grown on yeast extract-amended medium (data not shown). While this work was in progress, compatibility among several isolates of *B. dermatitidis* also was tested, but none of the isolates demonstrated compatibility. None appeared degenerate. Studies of sexuality are often impeded by infertility among isolates and by the hazards of working with living cultures over the long periods required to induce production of the sexual stages. Molecular approaches are safer as the DNA can be extracted from killed cultural material. A collaborative study is ongoing to sequence approximately 600 nucleotides at the 5′ end of the 28S rDNA covering the D1 and D2 domains (variable regions) of large subunit rRNA. The aim of this study is to further resolve interspecific relationships within the genus *Emmonsia* in the absence of a teleomorph for *E. parva*. Preliminary data appear to confirm that the agents of adiaspiromycosis should be treated as distinct species.

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