The genus *Uncinocarpus* (Onygenaceae) and its synonym *Brunneospora*: new concepts, combinations and connections to anamorphs in *Chrysosporium*, and further evidence of relationship with *Coccidioides immitis*[1]

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Abstract: The genus *Uncinocarpus* (Onygenales, Onygenaceae) is emended to include keratinophilic fungi with discrete, globose gymnothecial ascomata without differentiated ascomatal hyphae and bearing uncinate, helical, or no appendages; oblate, punctate ascospores sometimes with irregular reticulations; bulbous initials, and *Malbranchea* or *Chrysosporium* anamorphs. The new combination *Uncinocarpus orissi* is proposed for *Pseudoarachniotus orissi*; *Gymnoascus arxii* is shown to be a synonym. New records show that the fungus has a wide distribution from North America, Europe, Asia, and the Middle East. The teleomorph is formed under laboratory conditions by mating representative isolates. The anamorph has been described under the names *Chrysosporium zonatum* and *Chrysosporium gourii*. *Chrysosporium queenslandicum* is morphologically similar. Its teleomorph *Apinisia queenslandica* is transferred also to the genus *Uncinocarpus* as *Uncinocarpus queenslandicus*. *Brunneospora reticulata*, the type species of the genus *Brunneospora*, is a synonym. *Orromyces spiralis* appears to be another name applied to this fungus. Development of helical coils in an isolate of *Coccidioides immitis* provides further evidence of a possible relationship between this dimorphic human pathogen and this group of ascomycetes.

Key words: *Uncinocarpus*, Onygenales, systematics, keratinophiles, human pathogen.

Résumé : Les auteurs amendent le genre *Uncinocarpus* (Onygénales, Onygénacées) pour y inclure des champignons kératinophiles comportant de petits ascomata gymnothèques globulaires sans hyphes ascomatales différenciées, et portant ou non des appendices uncinés ou hélice, des ascospores ponctuées, aplaties aux pôles, quelquefois avec des réticulations, des (cellules) initiales bulbeuses, et des anamorphes de types *Malbranchea* ou *Chrysosporium*. On propose la nouvelle combinaison *Uncinocarpus orissi* pour remplacer le *Pseudoarachniotus orissi*; on montre que le *Gymnoascus arxii* est un synonyme. De nouvelles mentions montrent que ce champignon est largement répandu en Amérique du Nord, en Europe, en Asie et au Moyen-Orient. On obtient la formation du téléomorphe au laboratoire, en croisant des isolats représentatifs. L'anamorphe a été décrit sous les noms de *Chrysosporium zonatum* et de *Chrysoporium gourii*. Le *Chrysoporium queenslandicum* est morphologiquement similaire. On transfère son téléomorphe *Apinisia queenslandica* également au genre *Uncinocarpus* devenant l'*Uncinocarpus queenslandicus*. Le *Brunneospora reticulata*, espèce type du genre *Brunneospora*, est un synonyme. Il semble que l'*Orromyces spiralis* soit un autre nom appliqué à ce champignon. Le développement d'un boudin en hélice chez un isolat du *Coccidioides immitis* constitue un autre élément de preuve de la relation possible entre ce pathogène humain dimorphe et ce groupe d'ascomycètes.

Mots clés : *Uncinocarpus*, Onygénales, systématique, kératinophyles, pathogène humain.

[Traduit par la Rédaction]

Received December 18, 1997.

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[1] This paper is dedicated to Dr. Stanley J. Hughes, a collegial and prodigious worker internationally acclaimed as a brilliant systematist whose theories on classification of conidial fungi have influenced scores of other workers. A model to us all, Stan has followed an exemplary career of 31 years of service and research in Canada and the United Kingdom with several years of dedicated research in retirement. We honor and congratulate him on his 80th birthday.

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Introduction

A recent review of the Onygenales (Scott et al. 1993) presented a key to 11 genera in which the ascomata possess uncinate, curved, or helical appendages. The genera *Uncinocarpus, Brunneospora*, and *Apinisia* were distinguished in part by ascospore shape: oblate for *Uncinocarpus*, ellipsoidal for *Brunneospora*, and globose for *Apinisia*. Sigler and Carmichael (1976) described the genus *Uncinocarpus* for *Uncinocarpus reesii* Sigler & G.F. Orr, a soil fungus having reddish brown, oblate, smooth ascospores that were later shown by scanning electron microscopy (SEM) to be ornamented with small puncta (pits or depressions) (Currah 1985). Other distinguishing features of *U. reesii* included heterothallic compatibility, bulbous initials, and discrete globose gymnothecial ascomata composed of a loose network of uncinate appendages. The species also produced a distinctive *Malbranchea* anamorph, which had first attracted the attention of mycologists because of morphological and habitat similarities with the dimorphic human pathogenic fungus *Coccidioides immitis* Stiles (Sigler and Carmichael 1976). Recent molecular studies have confirmed a close relationship between these species (Pan et al. 1994; Bowman et al. 1996), but no meiotic stage is known for the latter.

Guarro et al. (1987) introduced the monotypic genus *Brunneospora* Guarro & Punsola for a keratinophilic fungus isolated by hair bait of soil samples in Spain. The genus was distinguished by globose gymnothecial ascomata with helical appendages, reddish brown, reticulate, ellipsoidal ascospores, and a *Chrysosporium* anamorph. *Brunneospora reticulata*, the type species, was represented by two collections, of which only the type was obtained in axenic culture and it consisted mainly of the anamorph. *Orromyces spiralis* B. Sur & G.R. Ghosh (Ghosh and Sur 1985), known only from the original description, appears to be similar.

Brunneospora was distinguished from the genus *Apinisia* La Touche (1968) by ascospore shape. However, a second species, *Apinisia queenslandica* Apinis & R.G. Rees (1976) differs from the type species *Apinisia graminicola* La Touche in ascospore shape and substrate preferences. Previous workers (Currah 1985; Guarro et al. 1991) have suggested that *A. queenslandica* be redisposed. There appear to be no records of *B. reticulata* or *A. queenslandica* other than from the original descriptions, but the anamorph *Chrysosporium queenslandicum* Apinis & R.G. Rees is occasionally listed in surveys of keratinophilic fungi.

Our interest in the preceding taxa arose from difficulties in identifying several isolates of a keratinophilic *Chrysosporium* species obtained mainly from soils in India. These isolates produced yellowish white colonies that darkened to buff, clavate or broadly obovoid, broadly truncate aleurioconidia that were borne on short and often curved stalks, and infrequent arthroconidia. *Chrysosporium zonatum* Al-Musallam & C.S. Tan (1989) resembled our isolates in colonial features but was reported to produce abundant arthroconidia and to degrade cellulose as well as keratin. Also, the distinction between *C. zonatum* and the *Chrysosporium* anamorphs of *B. reticulata* and *A. queenslandica* was unclear. Two additional teleomorphic fungi, *Gymnoascus arxii* Cano & Guarro (1989) and *Pseudoarachniotus orissi* B. Sur & G.R. Ghosh (Ghosh and Sur 1985), also expressed similar anamorphs.

The purpose of this study was to evaluate the relationship among and between these taxa, to define species concepts, and to redescribe cultural features We used mating tests to confirm relationships among our *Chrysosporium* isolates and the ex-type strain of *C. zonatum* and between them and ex-type strains of *G. arxii* and *P. orissi*. Neither *Gymnoascus* nor *Pseudoarachniotus* is suitable for disposition of the teleomorph of *C. zonatum*. Therefore, it is redisposed here in *Uncinocarpus* and the genus is emended.

Materials and methods

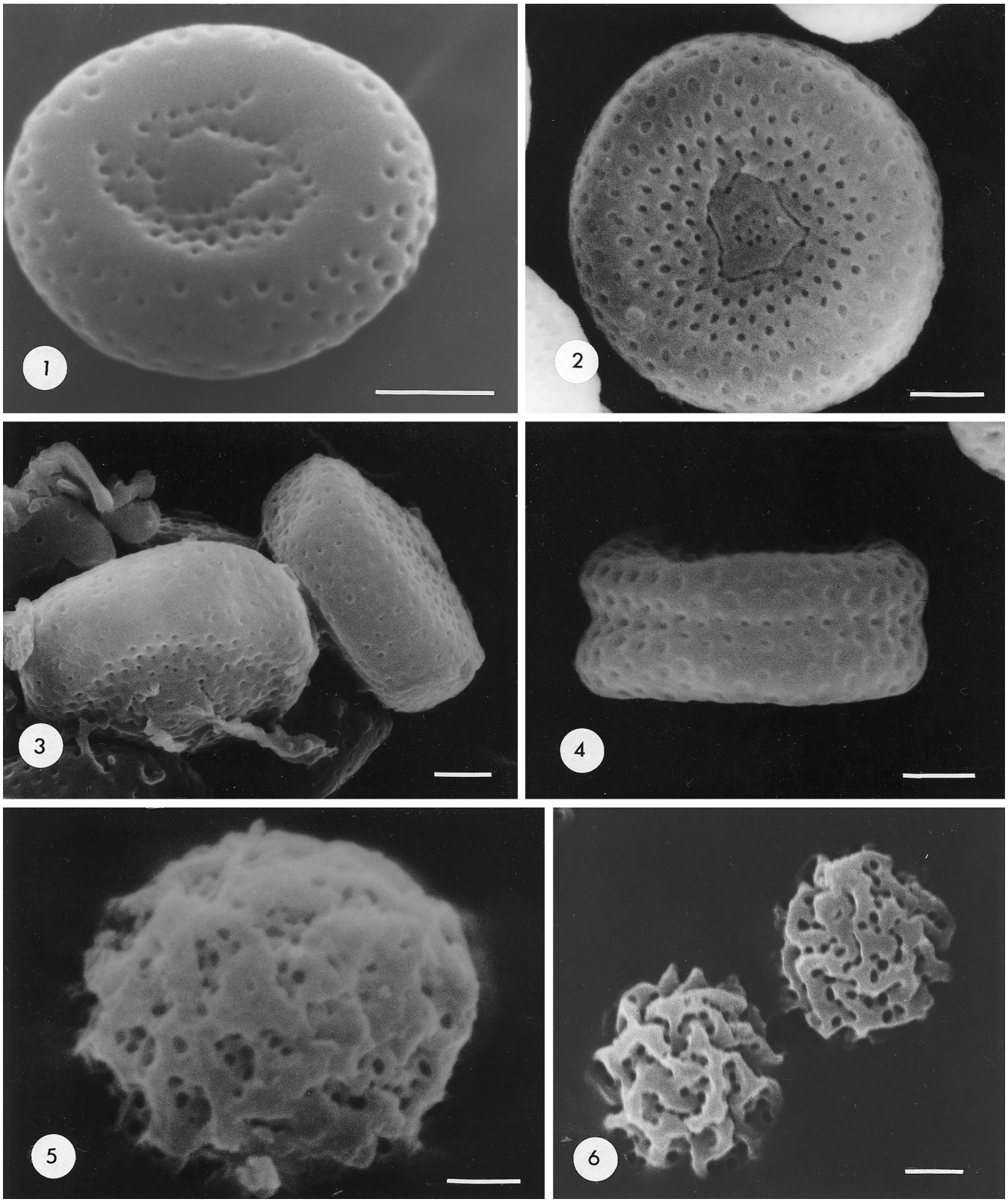
Studied strains were on deposit at the University of Alberta Microfungus Collection and Herbarium (UAMH) and included 15 strains preliminarily identified as *Chrysosporium* sp. IX and ex-type strains of *C. zonatum* (UAMH 6617), *Chrysosporium gourii* (UAMH 4436), *P. orissi* (UAMH 6950), *G. arxii* (UAMH 6611), *A. queenslandica* (UAMH 4319), and *B. reticulata* (UAMH 5704). Type material of the latter three species was obtained on loan.

Repeated attempts were necessary to mate available strains because of low fertility among tested strains. Changes were made to growth conditions including medium and temperature and to the methods of inoculation, using methods described by Sigler (1996). Initial experiments used autoclaved garden soil plus autoclaved human hair or horsehair and incubation at room temperature (22–25°C). Strains were paired in all possible combinations, including self–self pairings. Subsequent experiments used soil extract agar, both without and with glucose (recipe in Sigler 1996 but without yeast extract), oatmeal salts agar, or Takashio agar (recipes in Kane et al. 1997). All agar media were supplemented with sterile human hair or horsehair. The latter two media and incubation at 28–30°C were found to be optimal. Plates were held for 3–6 months before being discarded as negative. The first experiment, which included nine strains (2041, 2538, , 3728, 4044, 4426, 4427, 6499, and 6500), yielded fertile crosses among three pairings (6499 × 6500, 4426 × 4427, and 4427 × 6500). Seven single ascospore isolates were obtained from the cross of 4427 × 6500 and checked for fertility on their own and in backcrossings with the parent strains. Two single ascospore isolates (6635 and 9097) were designated as plus mating strains and two (6636 and 9098) as minus mating strains and were used in subsequent mating experiments. Each was nonfertile in self–self pairings but fertile when backcrossed with one of the parent strains.

For SEM, ascospores were obtained from dried type material and fresh cultural material. Ascospores were affixed directly onto Avery label glue coated onto stubs or onto glue that had been dissolved in chloroform. A coating of gold or gold–palladium was applied with an Edwards sputter coater (S150B) and specimens were examined and photographed with either a Cambridge S-250 or an Hitachi S-2500 SEM.

Growth studies were conducted on selected strains. Colony diameters and characteristics at 25 and 37°C were recorded after 7, 14, and 21 days on potato dextrose agar (PDA, Difco Laboratories, Detroit, Mich.). Tolerance for cycloheximide at a concentration of 400 μgmL^{-1} was evaluated by measuring growth rates of the same strains on mycosel agar (MYC, Becton Dickinson Microbiology Systems, Cockeysville, Md.) at 25°C. Terms for colony colors are from the charts of Kornerup and Wanscher (1978). Strains were also evaluated for their responses on several media used to aid identification of dermatophytes including bromcresol purple – milk solids – glucose agar (BCP-MS-G), Christensen's urea broth, trichophyton agars, and dermatophyte test medium (Sigler 1996, 1997). Capacity to degrade human hair was assessed using described methods (Carmichael 1962; Sigler 1997). Microscopic features of the anamorph were examined using slide culture preparations grown on Pablum cereal agar (Sigler 1997).

Figs. 1–6. Ascospores of *Uncinocarpus* species viewed by SEM. Fig. 1. *Uncinocarpus reesii* (UAMH 3882). Oblate ascospore with small puncta (shallow pits). Figs. 2–4. *Uncinocarpus orissi* (Figs. 2 and 4 from cross of 6499 × 6500; Fig. 3 from FMR 2105, isotype of *Gymnoascus arxii*). Oblate, punctate ascospores, sometimes with shallow equatorial furrow and bipolar wall thickenings. Figs. 5 and 6. *Uncinocarpus queenslandicus* (Fig. 5 from FMR 784, holotype of *Brunneospora reticulata*; Fig. 6 from holotype of *Apinisa queenslandica*). Broadly oblate, irregularly punctate–reticulate ascospores. Bars = 1 μm.



Figs. 7–11. *Uncinocarpus reesii*. Fig. 7. Bulbous initials (UAMH 3484). Fig. 8. Initial and asci (UAMH 3881). Fig. 9. Oblate ascospores (UAMH 2002 × 2847). Fig. 10. Terminal and intercalary conidia of *Malbranchea* anamorph (UAMH 3257). Fig. 11. *Malbranchea* anamorph and uncinate appendage (UAMH 160). **Figs. 12–14.** *Uncinocarpus orissi*. Fig. 12. Bulbous initial (UAMH 6635 × 9067). Figs. 13 and 14. Asci (UAMH 6635 × 9067). Bars = 10 μm.

Figs. 15–21. *Uncinocarpus orissi*. Fig. 15. Ascoma surrounded by undifferentiated hyaline hyphae (UAMH 6617 × 6635). Fig. 16. Oblate ascospores (UAMH 6635 × 6636). Fig. 17. Oblate ascospores and aleurioconidia from isotype of *Gymnoascus arxii* (FMR 2105). Fig. 18. Colony on PDA after 14 days at 37°C (UAMH 6635). Figs. 19 and 20. *Chrysosporium zonatum* anamorph in slide culture preparations. Aleurioconidia borne sessile or at the ends of short, often curved branches (arrow) (UAMH 6499). Fig. 21. In vitro hair digestion showing perforating bodies. Bars = 10 μm in Figs. 15–17 and 19–21; bar = 1 cm in Fig. 18.

Taxonomy

Uncinocarpus Sigler & G.F. Orr, in Sigler and Carmichael, Mycotaxon, 4: 461. 1976. Emend. Sigler.

Gymnothecial ascomata are solitary, discrete, globose, rarely aggregated, 200–1100 μm in diameter, forming within a thin covering of hyaline undifferentiated hyphae, and initially white, becoming reddish brown with maturation of ascospores. Appendages are uncinate, helically coiled or undulate, smooth, subhyaline to pale brown, or lacking. Ascomatal initials arise as bulbous lateral branches. Asci are 8-spored, obovoid to subglobose, 8–14 μm, evanescent. Ascospores are oblate, with truncate or rounded (obtuse) apices, sometimes with shallow furrow, minutely punctate to irregularly punctate–reticulate, yellowish or reddish brown.

ANAMORPH: *Malbranchea* or *Chrysosporium*.

TYPE SPECIES: *Uncinocarpus reesii* Sigler & G.F. Orr.

The species described below are united in *Uncinocarpus* on the basis of (*i*) globose gymnothecial ascomata without differentiated ascomatal hyphae, (*ii*) oblate yellowish brown or reddish brown ascospores ornamented with puncta (pits or depressions), (*iii*) bulbous ascomatal initials, (*iv*) anamorphs characterized by clavate terminal aleurioconidia and (or) intercalary arthroconidia, (*v*) capacity to digest hairs in vitro and produce perforating bodies, and (*vi*) thermotolerance. Although we consider the similarities among them to be sufficient to combine these species within a single genus, we acknowledge that their differences have led other workers to place them in separate genera. Ascomata of *U. reesii* and *Uncinocarpus queenslandicus* bear uncinate, helically coiled, or loosely curved appendages whereas those of *Uncinocarpus orissi* are devoid of appendages. Unlike *U. reesii* and *U. orissi*, which have minutely punctate, flattened ascospores with truncate apices (Figs. 1–4), *U. queenslandicus* has punctate–reticulate, broadly oblate ascospores with rounded apices (Figs. 5 and 6). The anamorphs of *U. orissi* and *U. queenslandicus* are very similar but differ from *U. reesii* in forming mainly terminal (Figs. 19, 20, 22, 28–30, and 32) rather than intercalary conidia (Fig. 10). The broader generic concept presented here appears to be a suitable approach for disposition of these species, which appear to be closely related. Close relationship between the type species *U. reesii* and the human pathogenic species *C. immitis* has been suggested by molecular evidence (Pan et al. 1994; Bowman et al. 1996). As illustrated here, some isolates of *C. immitis* produce helically coiled hyphae (Figs. 39–41). This observation provides additional support for possible relationship with members of the genus *Uncinocarpus*, but the meiotic stage of *C. immitis* remains unknown. One other species is currently accommodated in the genus. *Uncinocarpus uncinatus* (Eidam) Currah, which differs in forming a reticuloperidium, needs reevaluation, but the only available culture has not been reexamined in this study.

Uncinocarpus reesii. Figs. 1 and 7–11.

A brief description is provided here for ease of comparison (see also Sigler and Carmichael 1976; Sigler 1997). Ascomata are spherical, reddish brown, composed of intertwined uncinate or occasionally loosely helical appendages that extend beyond the perimeter. Ascomatal initials are bulbous (Figs. 7 and 8). Asci (Fig. 8) are obovoid or subglobose, evanescent. Ascospores (Figs. 1 and 9) are flattened and oblate with flattened or slightly rounded apices and ornamented on the surface with small pits that are hardly discernible by light microscopy. Arthroconidia (Figs. 10 and 11) are borne in straight, short or long, lateral branches and in the broader primary hyphae. Arthroconidia are usually separated by one or more segments of irregular length; separating cells are often somewhat collapsed. Arthroconidia are cylindrical or barrel shaped, 3–6 (8) × 2–4 μm, mostly 3.5–6 × 2.5–3.5 μm, but often round up in age. Anamorphic isolates often produce uncinate appendages in association with the arthroconidia (Fig. 11) but form ascomata only when mated.

Uncinocarpus orissi (B. Sur & G.R. Ghosh) Sigler & Flis, comb.nov. Figs. 2–4, 12–14, and 15–22.

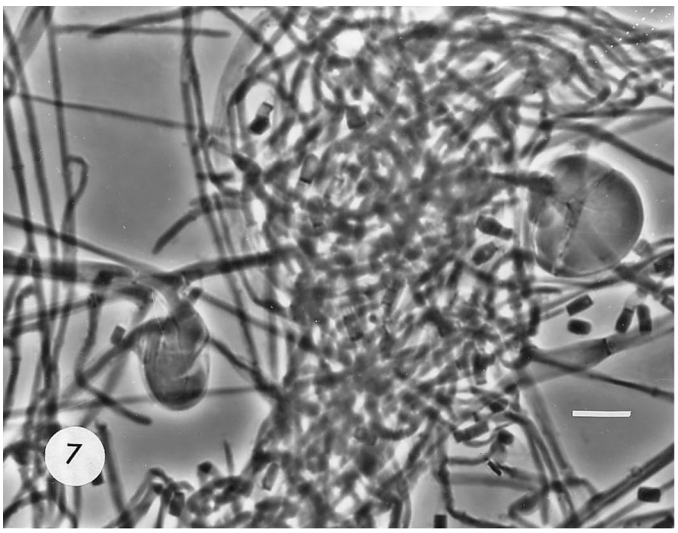
≡*Pseudoarachniotus orissi* B. Sur & G.R. Ghosh, in Ghosh and Sur, Kavaka, 12: 67. 1985. (Basionym)

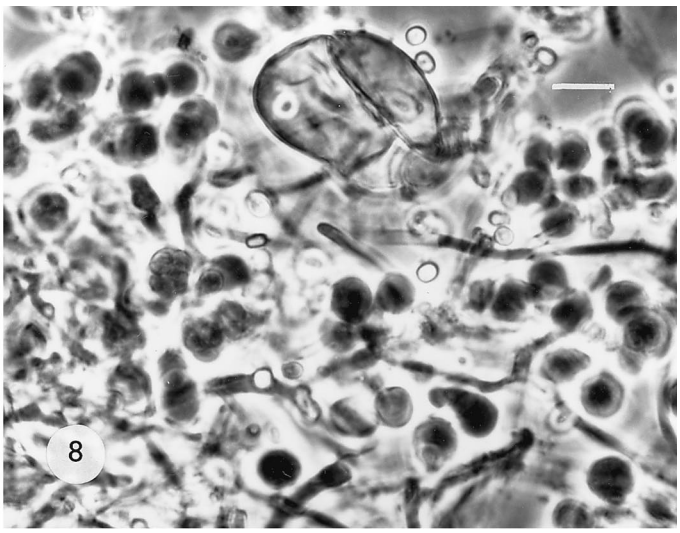
=*Gymnoascus arxii* Cano & Guarro, Stud. Mycol. 31: 61. 1989.

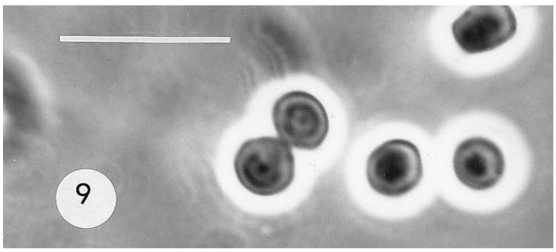
ANAMORPH: *Chrysosporium zonatum* Al-Musallam & C.S. Tan, Persoonia, 14: 69. 1989.

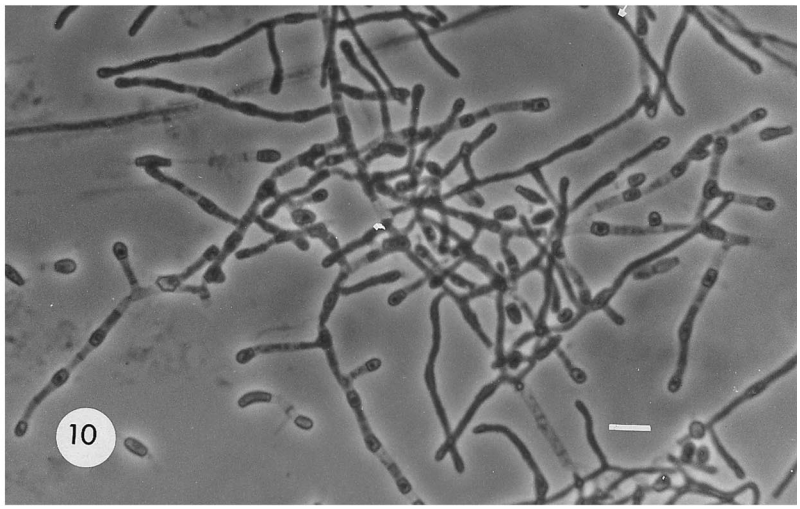
=*Chrysosporium gourii* P.C. Jain, Deshmukh & S.C. Agarwal, Mycoses, 36: 77. 1993.

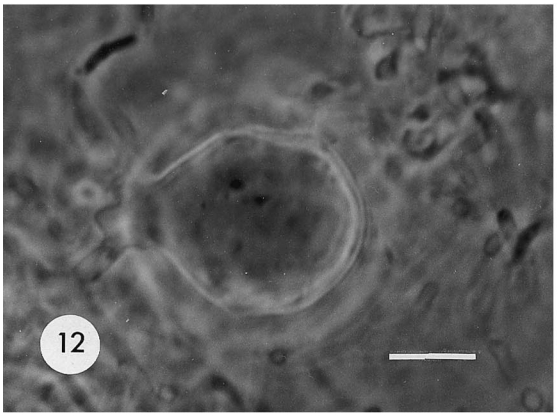
Heterothallic. Gymnothecial ascomata (Fig. 15) are more or less globose, discrete, solitary, rarely aggregated, 200–1100 μm, initially white and covered with clear exudate droplets, becoming dense, reddish brown, and surrounded by a loose weft of undifferentiated hyaline racquet hyphae often bearing conidia on lateral branches. Appendages are lacking. Ascomatal initials are bulbous (Fig. 12), arising as swollen lateral cells, 20–36 × 13–23 μm. Asci (Figs. 13 and 14) are 8-spored, subglobose, 8–14 × 7–10 μm, quickly evanescent. Ascospores (Figs. 2–4, 16, and 17) are oblate with truncate apices, sometimes with a shallow equatorial furrow (Fig. 4) and bipolar wall thickenings (Fig. 3). The surface appears smooth to minutely roughened under light microscopy but pitted under SEM, reddish brown, 4.5–7 × 3.0–4.5 μm.

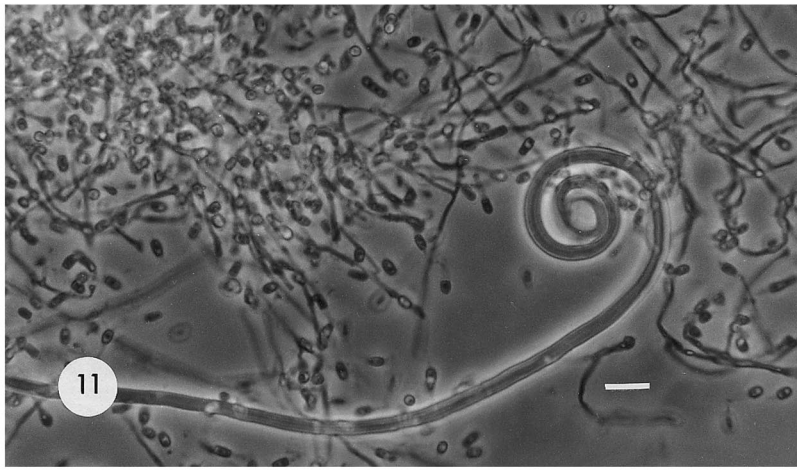


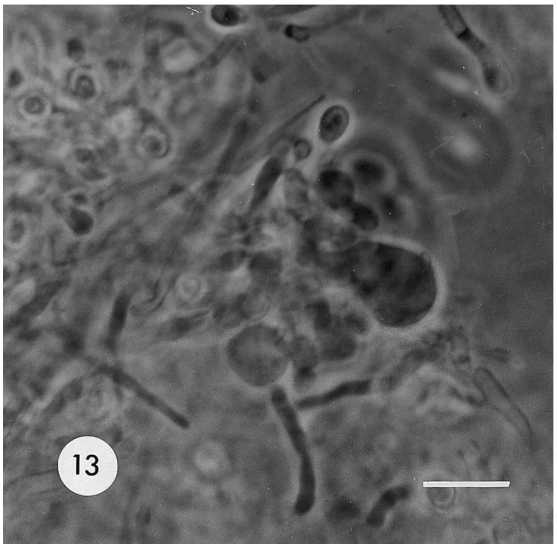


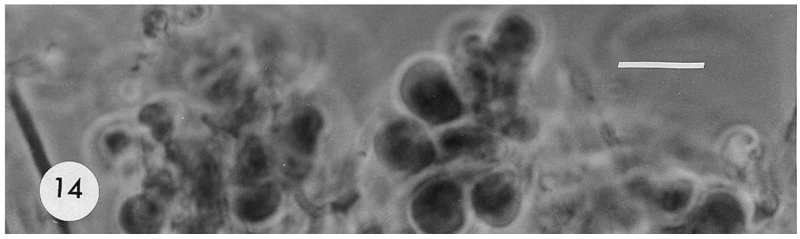


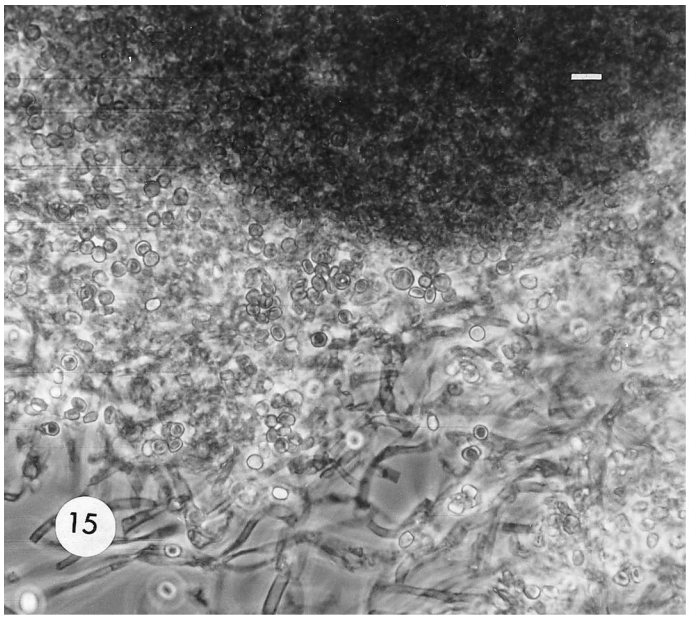


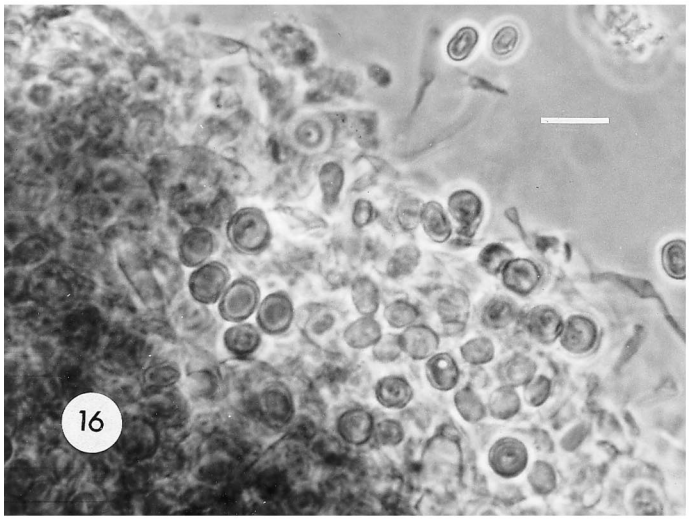


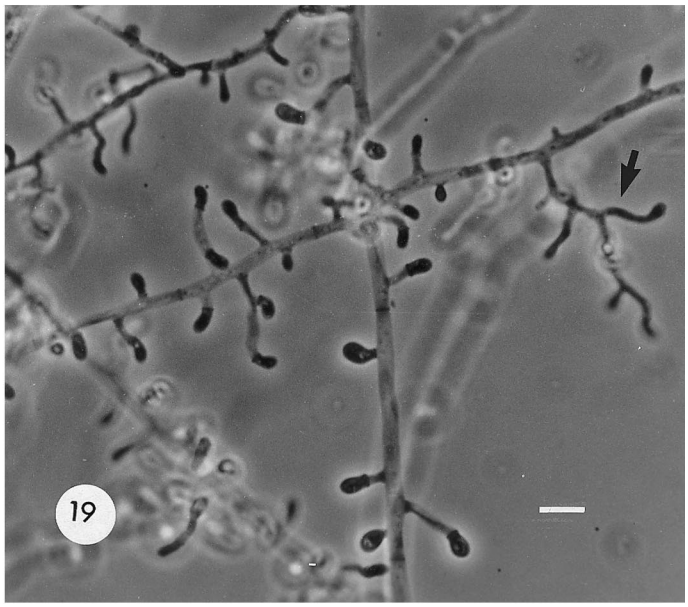


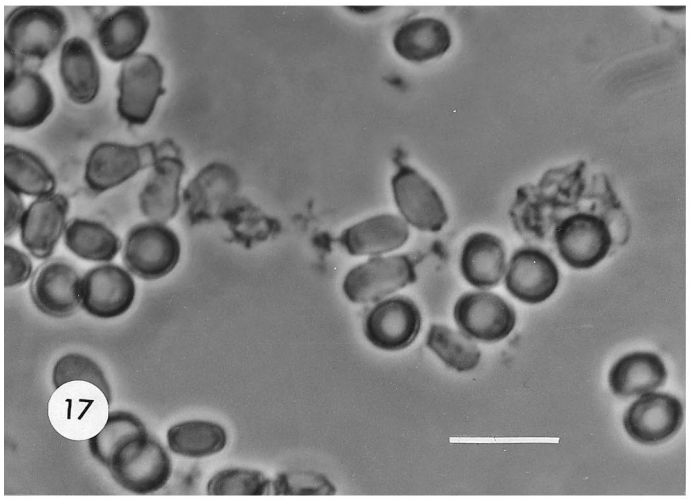


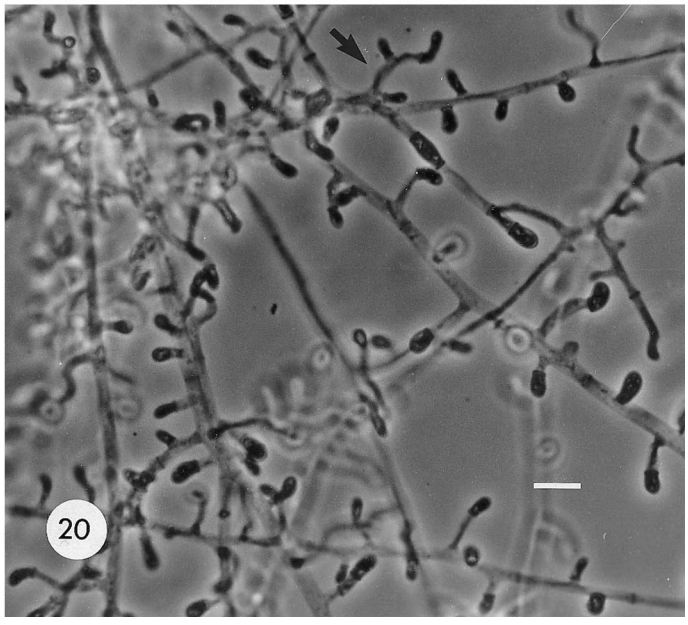












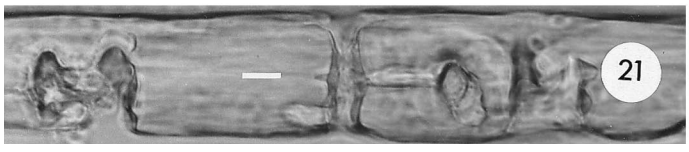
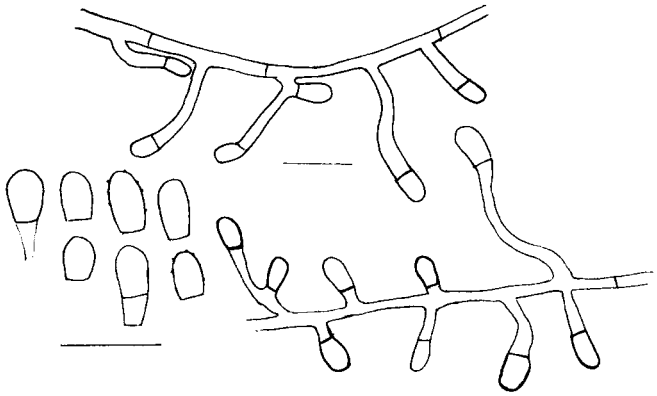


Fig. 22. *Chrysosporium zonatum* anamorph. Aleurioconidia borne sessile or at the ends of short, often curved branches. Note that conidia are clavate or broadly obovoid, rounded at the tip, and have a broad truncate base. Bars = 10 μm.



Colonies on PDA (Fig. 18) at 14 days reach diameters of 6.7–8.5 cm at 37°C (average daily growth rate 5.2 mm) and 5.5–7.8 cm at 25°C (average daily growth rate 4.6 mm). At 37°C, colonies at 7 days are yellowish white (4A2) but darken within 14–21 days to buff or hazel (greyish or brownish orange, 6A3, 6B/C5) with light to dark brown reverse (greyish orange to brown, 5A2–B5, 6E8). Colonies are flat, dense, somewhat zonate with peripheral zones darker than central ones, coarsely powdery, margin thin. Colonies on PDA and MYC at 25°C after 14 days appear similar in growth rate (average daily growth rate 4.2 mm) and appearance, appearing woolly to coarsely powdery, yellowish white (4A2) with uncolored reverse. Darkening is slower, occurring within 21 days, buff (greyish orange, 5B3–6B/C3–4) on the obverse and light brown (5B3–6D5) on the reverse. The urease test is positive (fuchsia color) within 4 days. At 11 days on BCP-MS-G, the isolates grow profusely and show no pH change or slight alkalinity (trace purple). They show no requirements for vitamins inositol or thiamine. On dermatophyte test medium, they produce a color change in the indicator from yellow to red in 7 days. Isolates are strongly keratinolytic, digesting hairs with the aid of perforating bodies within 7 days (Fig. 21).

Aleurioconidia (Figs. 19, 20, and 22) are borne at the ends of short or long stalks that are characteristically curved, or are sessile. They are hyaline, buff or tan in mass, smooth or warty, single celled, rarely 1-septate, clavate or broadly obovoid, rounded at the tip and with a broad, truncate base. They measure (3.5) 4–8 (13) × (2.5) 3–5 μm, with a basal scar, 2.5–3.5 μm wide. Intercalary arthroconidia are uncommon in most isolates. When present, they measure 7–11 × 2–3 μm. Racquet hyphae are common.

Results of matings (Table 1) demonstrate the relationship between *P. orissi*, *G. arxii*, and the anamorphs *C. zonatum* and *C. gourii*. No isolate, including the ex-type cultures of *P. orissi* and *G. arxii*, fruited under any condition in self–self pairings. The ex-type of *G. arxii* (6611) mated with the ex-types of *P. orissi* (6950) and *C. zonatum* (6617) and with several wild-type isolates but not with any single ascospore isolate whereas the ex-type of *P. orissi* mated with only one wild type (4426) and one single-ascospore (6635) isolate. The ex-type cultures of *C. zonatum* and *C. gourii* (4436) demonstrated greater fertility, mating with both wild-type and single-ascospore isolates.

Appropriate generic disposition of the teleomorph has been problematic. Ghosh and Sur (1985) favored *Pseudoarachniotus* because of the undifferentiated ascocarp and shape of the ascospores. *Pseudoarachniotus* is now considered a synonym of *Gymnascella*, which is not keratinophilic and lacks distinct anamorphs (Currah 1985). Some *Gymnascella* species have ascospores of similar shape, but they are smooth or irregularly furrowed rather than punctate. Cano and Guarro (1989) placed their species in *Gymnoascus* (Gymnoascaceae) following von Arx's (1986, 1987) broad circumscription of the genus, which included many species ascribed to *Gymnascella* as well as several other genera. However, the punctate or pitted ascospores, well-defined *Chrysosporium* anamorph, and keratinolytic activity are incompatible with the genus *Gymnoascus* and the family Gymnoascaceae as circumscribed by Currah (1985).

Cano and Guarro (1989) considered the ascospores of *G. arxii* to be most similar to those of *Gymnoascus desertorum* (Moustafa) Arx, but they recognized differences in peridial hyphae and anamorphs. The peridial hyphae of *G. desertorum* were loosely interwoven, swollen, and lightly pigmented whereas *G. arxii* had a membranous peridium composed of a layer of hyaline flattened cells. However, we did not observe the membranous peridium. The arthroconidia reported by Moustafa (1973) have not been confirmed by others (Orr et al. 1977; Cano and Guarro 1989).

The ascospores of *U. reesii* (Figs. 1 and 9) are similar in color, shape, and surface ornamentation to those of *U. orissi* but lack the shallow furrow. Other similarities between the species include heterothallism, buff to tan colonies, initials (compare Figs. 7, 8, and 12), and thermotolerance. However, *U. reesii* shows optimal growth at 25°C (Sigler and Carmichael 1976) whereas *U. orissi* grows optimally at 37°C. *Uncinocarpus reesii* differs in having ascomata with uncinate or loosely spiraled appendages and in forming an anamorph with a preponderance of intercalary arthroconidia (*Malbranchea*). Arthroconidia are infrequent in the anamorph of *U. orissi*, which has been described independently as *C. zonatum* and *C. gourii*. A similar situation has occurred in *Aphanoascus*, which now encompasses anamorphs represented by solitary aleurioconidia (*Chrysosporium*) or alternate arthroconidia (*Malbranchea*) (Cano and Guarro 1990). As explained by Sigler (1997), there are subtle distinctions between some members of the genera *Chrysosporium* and *Malbranchea*.

Chrysosporium zonatum is distinguished from *C. queenslandicum* (described below) by its colonies that darken to buff and clavate or broadly obovoid, broadly truncate aleurioconidia borne on short characteristically curved stalks. Arthroconidia are infrequent but may be common in some strains. Isolates are vigorous in degrading human hair or horsehair with the aid of perforating bodies (Fig. 21). Al-Musallam and Tan (1989) reported that their isolate degraded cellulose, but none of the isolates that we examined decomposed cellophane membranes, assessed by the method described previously (Sigler and Carmichael 1976). *Chrysosporium zonatum* has a broad distribution. UAMH records indicate that it has been recovered, usually by hair bait, from soil and sand, sewage sludge, horsehair in horse dung, rabbit and cow dung, and

Figs. 23–31. *Uncinocarpus queenslandicus*. Fig. 23. Ascoma with coiled appendages from holotype of *Apinisia queenslandica* (Herb. IMI 121675). Fig. 24. Bulbous initial in ex-type culture (UAMH 4319). Fig. 25. Broadly oblate ascospores (Herb. IMI 121675). Fig. 26. Ascospores and undulate hyphae from *Brunneospora reticulata* (FMR 811). Fig. 27. Broadly oblate ascospores from holotype of *Brunneospora reticulata* (FMR 784). Figs. 28–30. *Chrysosporium queenslandicum* anamorph in slide culture preparations. Aleurioconidia borne sessile or at the ends of short, straight or slightly curved stalks. Intercalary arthroconidia also present (Figs. 28 and 29 = UAMH 5704; Fig. 30 = UAMH 4319). Fig. 31. Colony on PDA after 14 days at 37°C (UAMH 4319). Bars = 10 μm in Figs. 23–30; bar = 1 cm in Fig. 31.

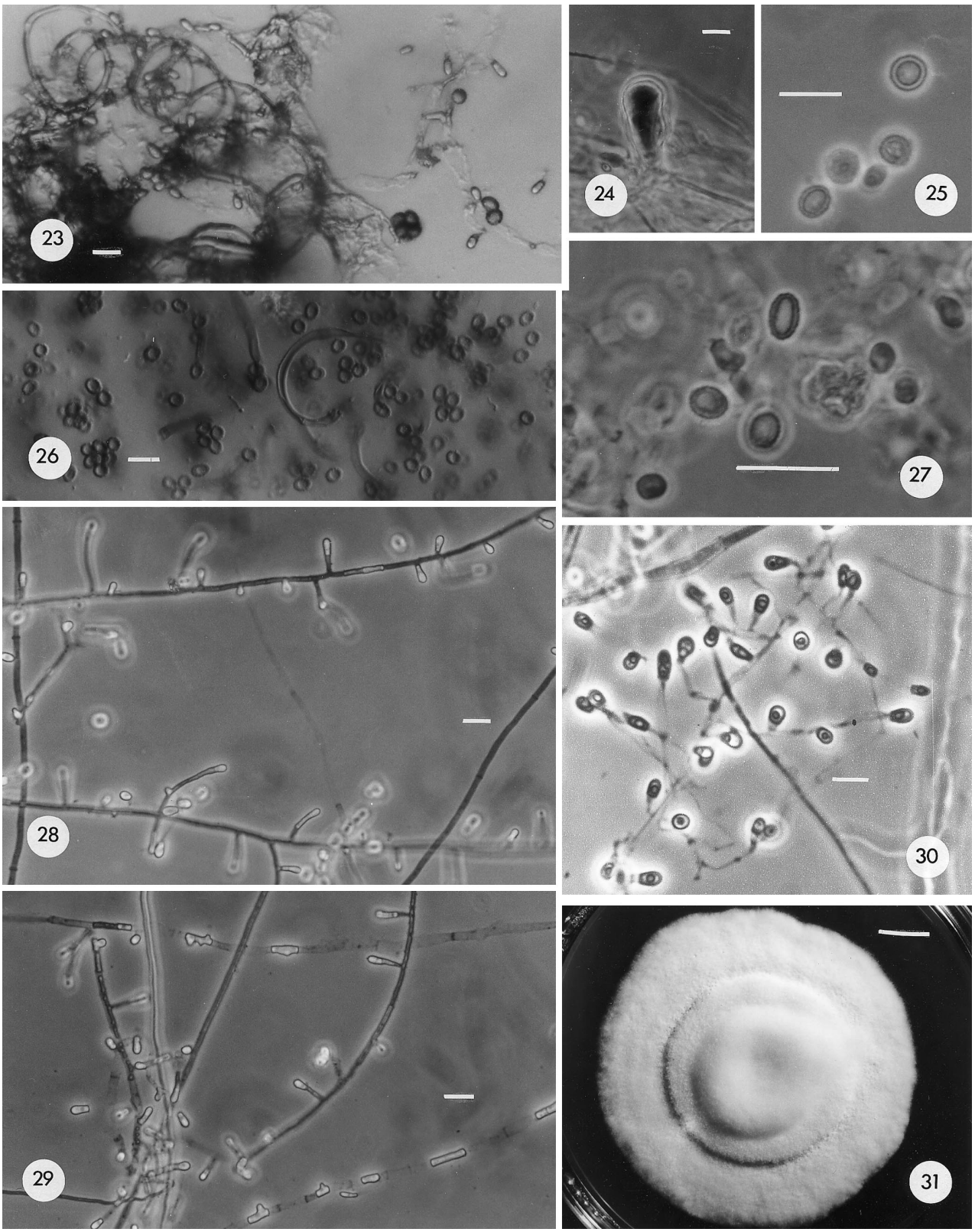
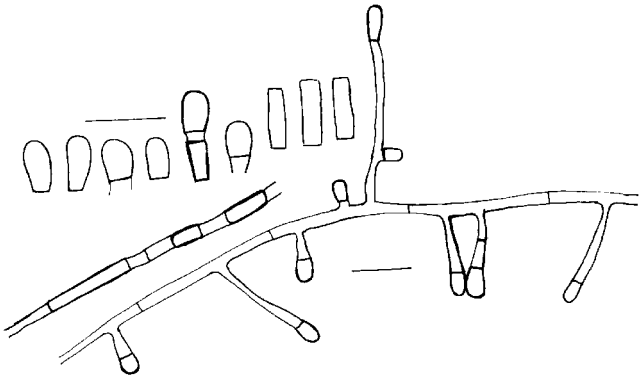


Fig. 32. *Chrysosporium queenslandicum* anamorph (ex-type UAMH 4319). Aleurioconidia are sessile or formed at the ends of straight or slightly curved stalks. Intercalary arthroconidia are rare to common.



human respiratory specimens from India, North America (Florida, Utah), Europe (Spain, Italy, Greece), the Middle East (Kuwait), and Asia (Japan). Cano et al. (1996) provided additional records from dung and river and beach sediments. In contrast with most Chrysosporia, which are not pathogenic, this thermotolerant species appears to be capable of colonizing cavitary lesions in the lung and may be capable of causing deep infection in immunosuppressed individuals (Sigler et al. 1998; Roilides et al. 1999).

MATERIAL EXAMINED: SPAIN: as *Gymnoascus arxii*, Castellon, Peniscola, soil, April 1987, J. Cano (isotype FMR 2105 and ex-type culture UAMH 6611). Living cultures: INDIA: as *Pseudoarachniotus orissi*, Cuttack, Victoria Goshala, soil, cattle resting place, B. Sur and G.R. Ghosh (ex-type culture ITCC 3177 = UAMH 6950; holotype in Indian Agricultural Research Institute, New Delhi, India, not seen); soil, S.C. Agarwal 23 (UAMH 4044); soil, P.C. Jain CP-1 (UAMH 4426); soil, P.C. Jain CP-3 (UAMH 4427); soil, P.C. Jain CP-15 (UAMH 4428); soil, P.C. Jain CP-7, (UAMH 4435); as *Chrysosporium gourii*, soil, P.C. Jain CP-21 (ex-type UAMH 4436); soil, R. Kushwaha CCD 1 (UAMH 4894); soil, S.K. Agnihotri S.K. 3 (UAMH 5080); poultry comb lesion, G.R. Ghosh S-11 (UAMH 6499); sand, Orissa, G.R. Ghosh, CLK 212 (UAMH 6500). ITALY: soil, E. Varsavsky EV I-22 (UAMH 2041); unknown: sewage sludge, K. Ulfig 9 (UAMH 4872). GREECE: sputum and bone, male 15 years, E. Bibashi (UAMH 8936). KUWAIT: as *Chrysosporium zonatum*: horsehair in dung, February 1986, A. Al-Musallam and C.S. Tan (CBS 437.88 = UAMH 6617). UNITED STATES: Florida, soil, 1965, G.F. Orr 118 (UAMH 2538); Utah, rabbit dung, G.F. Orr O-1287 (UAMH 3728); single-ascospore isolates ex-cross of UAMH 4427 × 6500 (UAMH 6635 (B), 6636 (G), 9097 (F), 9098 (E)). JAPAN: Chiba, bronchial lavage from female with pulmonary cavity (UAMH 9067); Kyushu, bronchial lavage from male with pulmonary cavity (9068).

Uncinocarpus queenslandicus (Apinis & R.G. Rees) Sigler comb.nov. Figs. 5, 6, and 23–32.

≡*Apinisia queenslandica* Apinis & R.G. Rees, Trans. Br. Mycol. Soc. 67: 524. 1976. (Basionym)

=*Brunneospora reticulata* Guarro & Punsola, in Guarro et al., Persoonia, 13: 387. 1987.

=*Orromyces spiralis* B. Sur & G.R. Ghosh, in Ghosh and Sur, Kavaka, 12: 63. 1985.

ANAMORPH: *Chrysosporium queenslandicum* Apinis & R.G. Rees, ibid.

≠*C. articulatum* Scharapov, Nov. Syst. Niz. Rast, 15: 146. 1978. Fide Oorschot (see Sigler et al. 1986).

Gymnothecial ascomata (Fig. 23) are solitary, discrete, globose, rarely aggregated, 350–1000 μm in diameter, within a thin covering of hyaline undifferentiated hyphae, initially white becoming yellowish brown. Peridial appendages are loosely or irregularly spiraled (Fig. 23), septate, yellowish brown, smooth, 1.5–4 μm in diameter. Ascomatal initials are bulbous (Fig. 24). Asci are 8-spored, short stalked, ovoid to subglobose, 8–14 μm, evanescent. Ascospores (Figs. 5, 6, and 25–27) are broadly oblate with rounded apices, irregularly punctate–reticulate, yellow to reddish orange or reddish brown in mass, 5–7 × 4–5 μm.

Colonies (Fig. 31) on PDA at 14 days reach diameters of 5.5–6.2 cm at 37°C (average daily growth rate 4.2 mm) and 4–4.7 cm at 25°C (average daily growth rate 3.1 mm). Growth on MYC at 25°C is slightly reduced (average daily growth rate 2.7 mm). Colonies on PDA at 37 and 25°C are similar and appear yellowish white (4A2), slightly raised with small central umbo, dense, velvety to cottony, sometimes with clear droplets of exudate near the centre. At 37°C, the reverse becomes tan to light brown (6D4) but remains uncolored at 25°C within 14 days. The urease test is positive (fuchsia color) within 7 days. At 11 days on BCP-MS-G, the isolates grow moderately, develop thin aerial mycelium, and show no pH change or slight acidity (trace yellow). They show no requirements for vitamins inositol and thiamine. On dermatophyte test medium, they produce a color change in the indicator from yellow to pale red in 7 days. Isolates digest hairs with the aid of perforating bodies within 14 days.

Aleurioconidia (Figs. 28–30 and 32) are sessile or borne at the ends of short straight or curved stalks. They are single celled, rarely 1-septate, clavate or broadly pyriform, rounded at the tip. They measure 4–8.5 × 2.5–4 μm, commonly 4–6 μm long, and have a broad basal scar, 2–3 μm wide. Arthroconidia are rare to common and are usually irregularly spaced. The arthroconidia measure 5–12 × 2.5–3 μm.

The genus *Apinisia* is characterized by globose ascospores (La Touche 1968). The type and only collection of *A. graminicola* occurred on decaying grass cuttings and consists of large, white stromatic ascomata (up to 7000 μm) that are soft and contain globose ascospores (Figs. 33 and 34) among fragments of hyaline hyphae. Conidia belonging to two different *Scopulariopsis* species were also present. The ex-type culture (UAMH 4315) matches the original description in producing a few sessile aleurioconidia and intercalary arthroconidia (Fig. 36) as well as coiled hyphae (Fig. 35), but it failed to fruit under any condition. It differs from the other species described here in being mesophilic and unable to grow above 30°C and in failing to degrade human hair. The affinities of *A. graminicola* are not clear, but differences in substrate preferences and ascospore shape support a transfer of the keratinophilic *A. queenslandica* to another genus.

We consider the strong similarities between the anamorphs of *A. queenslandica* and *U. orisii* as one of the grounds for placing these two taxa within the genus *Uncinocarpus*. However, *B. reticulata* is a later name for *A. queens-*

Figs. 33–36. *Apinisia graminicola* (holotype Herb. IMI 126422). Figs. 33 and 34. Globose ascospores. Fig. 35. Coiled hyphae in ex-type culture (UAMH 4315). Fig. 36. Sessile conidia and arthroconidia in slide culture preparation (UAMH 4315). Bars = 10 μm in Figs. 33, 35, and 36; bar = 1 μm in Fig. 34.

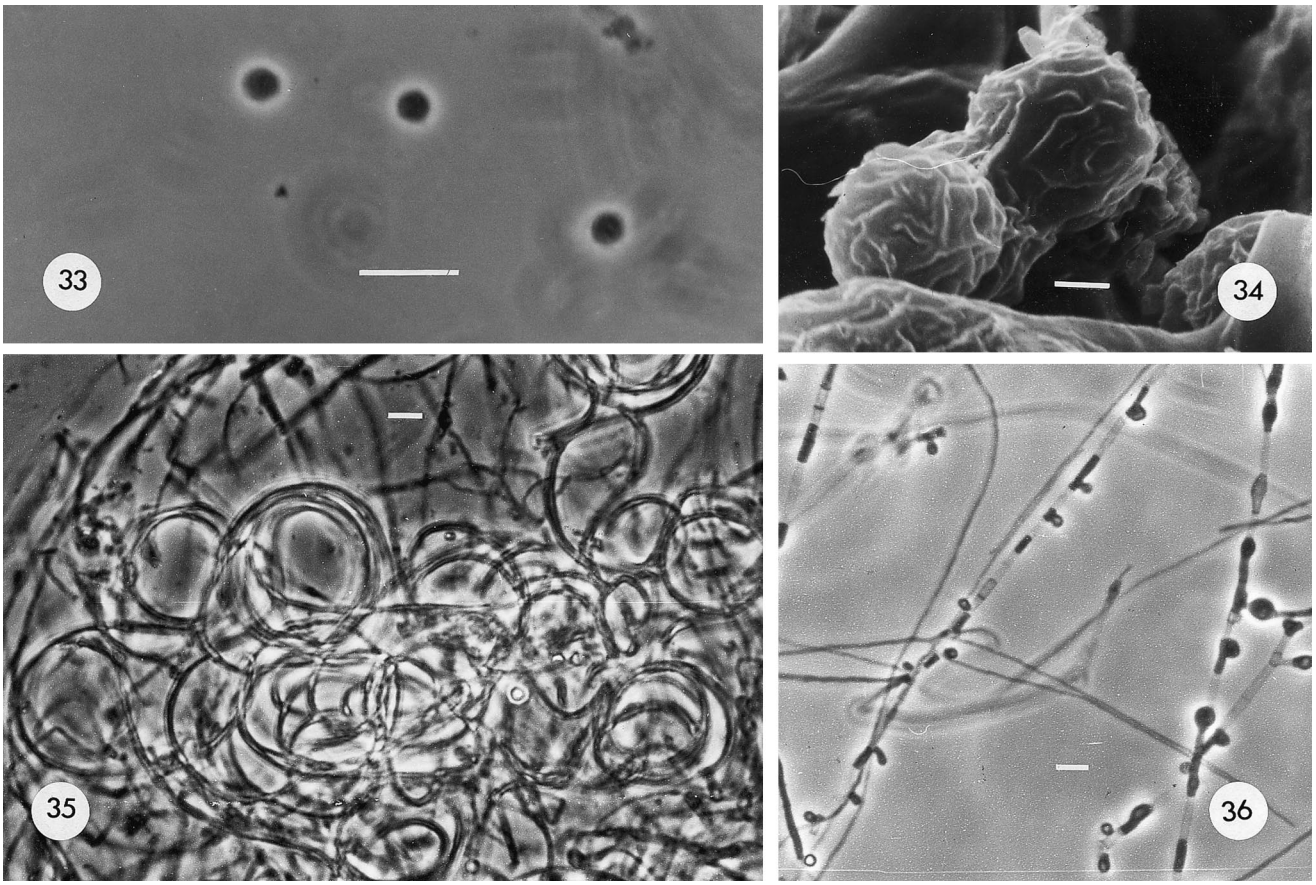
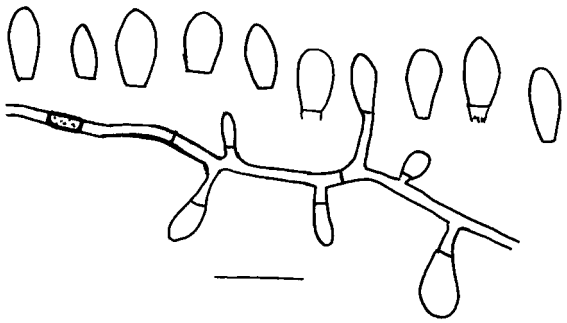


Fig. 37. *Chrysosporium tropicum* (ex-type UAMH 691). Pyriform or clavate aleurioconidia borne sessile or at the ends of short stalks. Note that conidia are tapered at the tip. Bar = 10 μm.



landica and the genus *Brunneospora* could be resurrected if a separate genus is warranted. The teleomorph is known so far only from the original collections and appears not to be readily formed in culture. The original authors reported that the ex-type cultures of *A. queenslandica* (4319) and *B. reticulata* (5704) fruited on some media, but in the case of the latter failed to produce the coiled appendages. Coiled appendages were not observed in isotype material of *B. reticulata*, but some were present in a second collection (Fig. 26, FMR 811). Similarly, coiled hyphae were observed only once in *A. queenslandica*. They were present on a single ascoma in a prepared slide from chicken feathers (Fig. 23) but not on ascomata in dried cultures. Unfortunately the condition of the mount precluded good observation. Compatibility is unknown, but presumed to be homothallic. The ex-type cultures failed to fruit on any medium at 25, 30, or 37°C. Matings between the ex-type cultures at 30 and 37°C were infertile, as were matings between *B. reticulata* (5709) and all of the isolates of *U. orissi* (Table 1) and between 4319 and single-ascospore isolates of *U. orissi*. Whether infertility in *U. queenslandicus* is a result of genetic factors, such as loss of one mating type, or to environmental conditions is unknown. Both ex-type cultures appeared somewhat degenerate initially, but incubation at higher temperatures promoted reversion from cottony to powdery colonies and increased sporulation. No type material of *O. spiralis* could be obtained for study. Several loan requests sent to the fungal herbarium, Botany Laboratory, Regional College of Education, Bhubaneswar, Orissa, India, went unanswered, so we are uncertain if the type is lost. However, the description provides convincing evidence that the fungus described is the same as *U. queenslandicus*.

Previous workers have struggled with the species concept

Table 1. Results of mating tests among isolates of *Chrysosporium zonatum*, *Gymnoascus arxii*, and *Pseudoarachniotus orissi* and with *Brunneospora reticulata*.

	Minus mating strain										
Plus mating strain	3728	4428	4435	6500	6617 (T) *C. zonatum*	6636 sai	6950 (T) *P. orissi*	8936	9067	9098 sai	5704 (T) *B. reticulata*
2041	+	–	–	–	+	+	–	NT	NT	+	–
2538	–	NT	NT	+	–	–	–	NT	NT	–	–
3676	–	NT	NT	–	+	+	–	NT	NT	+	–
4044	+	+	+	+	+	+	–	NT	NT	+	–
4426	+	+	–	+	+	–	+	NT	NT	+	–
4427	+	+	+	+	+	+	–	NT	NT	+	–
4436 (T) *C. gourii*	+	NT	NT	+	+	+	–	NT	NT	+	–
4446	+	NT	NT	+	+	+	–	NT	NT	+	–
5080	+	NT	NT	+	+	+	–	NT	NT	+	–
6499	+	–	+	+	–	–	–	NT	NT	–	–
6611 (T) *G. arxii*	+	–	+	+	+	–	+	NT	NT	–	–
6635 sai	+	+	+	+	+	+	+	+	+	+	–
9068	NT	NT	NT	NT	NT	–	NT	+	–	–	NT
9097 sai	+	+	–	+	+	+	–	NT	NT	+	–
5704 (T) *B. reticulata*	–	–	–	–	–	–	–	NT	NT	–	–

Note: T, ex-type culture; sai, single ascospores isolate derived from cross of 4427 × 6500; NT, not tested.

of *C. queenslandicum*. van Oorschot's (1980) synonymy with *Chrysosporium articulatum* Scharapov was refuted by Sigler et al. (1986) and by Al-Musallam and Tan (1989), who considered *C. queenslandicum* to be more closely related to *C. zonatum*. Our findings support their views. *Chrysosporium queenslandicum* is distinguished from *C. zonatum* by subtle differences. Arthroconidia are more abundant in *C. queenslandicum*, stalks supporting the aleurioconidia are less regularly curved, and colonies remain yellowish white rather than becoming buff or tan as in *C. zonatum*. Our findings are dissimilar to those of Al-Musallum and Tan (1989) in that both species are thermotolerant, showing enhanced growth at 37°C compared with 25°C, and form conidia that are smooth to slightly warty.

Chrysosporium queenslandicum must be distinguished also from *Chrysosporium tropicum* J.W. Carmich., which is thermotolerant, forms yellowish white colonies, and has aleurioconidia of similar size and wall ornamentation (4–9 × 3–5 μm, mostly 6–7 × 3.5–4 μm) (Carmichael 1962; Sigler 1997) (Fig. 37). Again, the distinctions are subtle. The colonies of the former are slightly raised with small central umbo, velvety to cottony, rather than flat and coarsely powdery as in *C. tropicum*. Arthroconidia occur only sporadically in *C. tropicum*, the stalks bearing conidia are not characteristically curved, and the aleurioconidia vary slightly in shape, often being tapered at the tip (Fig. 37) rather than broadly rounded as in *C. queenslandicum* (Fig. 32) and *C. zonatum* (Fig. 22). These differences appear to be supported by a putative connection to *Aphanoascus verrucosus* Cano and Guarro (1990), which has a cleistothecial ascoma.

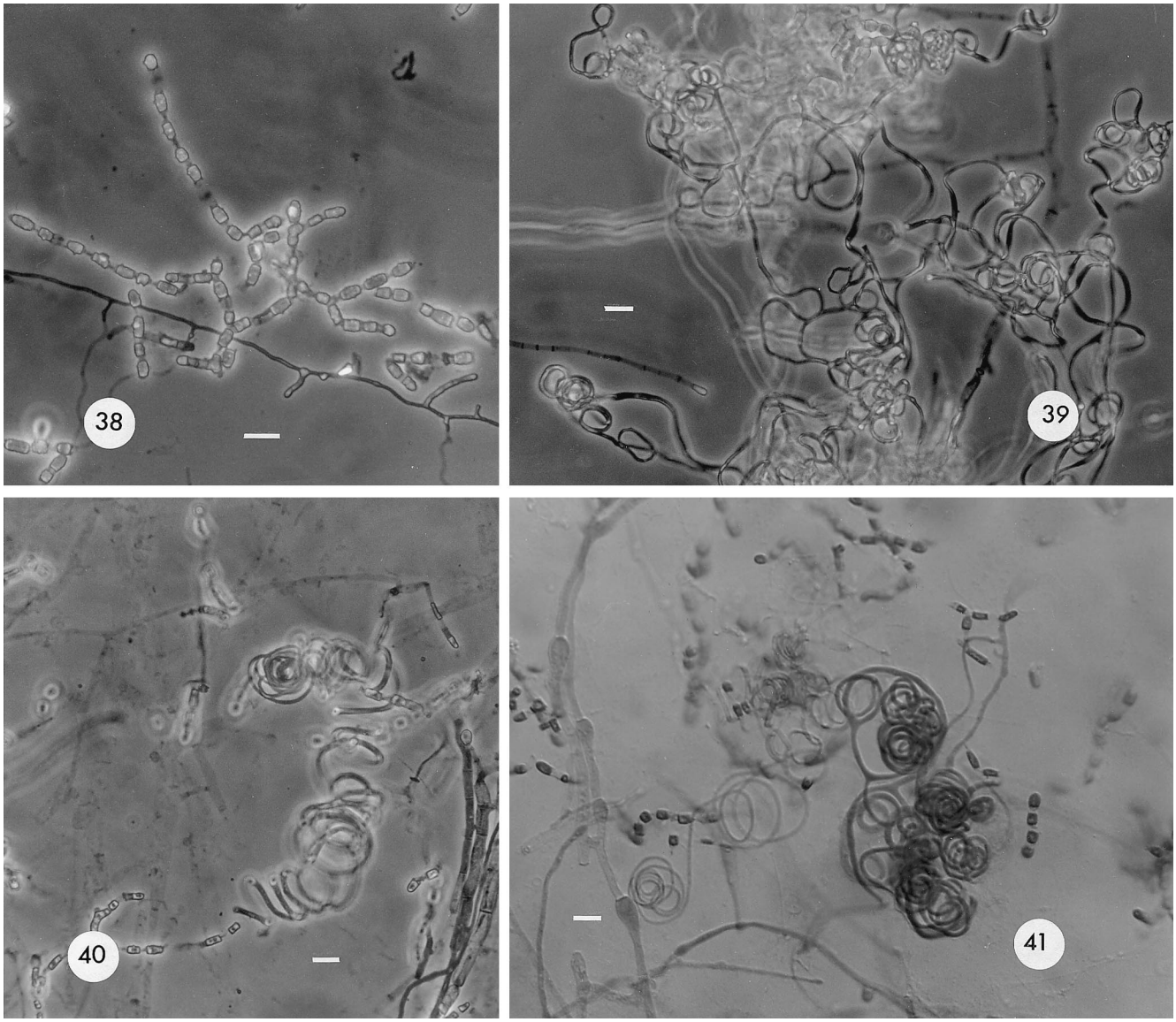
Although the concept for the anamorph *C. queenslandicum* has been unclear (Sigler et al. 1986; Al-Musallam and Tan 1989), the species is occasionally listed in surveys of keratinophilic fungi (e.g., Nigam and Kushwaha 1990). Given the uncertainty about the species, it is difficult to evaluate its distribution. Cano et al. (1996) used restriction analysis of mitochondrial DNA to examine a number of unidentified environmental strains. None yielded profiles consistent with the reference strain of *C. queenslandicum*, but several grouped with the reference strain of *C. zonatum*. This is in agreement with our finding of the broad distribution of the latter species. These authors disclaimed the value of the technique for evaluating relationships, but failed to point out that three of the four species evaluated had known or putative teleomorphs (Cano and Guarro 1990).

MATERIAL EXAMINED: AUSTRALIA: Queensland: as *Apinisia queenslandica*, Cunnamulla, domestic chicken feathers on cellulose agar, May 1965, A.E. Apinis and R.G. Rees (holotype Herb. IMI 121675 and ex-type culture UAMH 4319 = CBS as 280.77). SPAIN: Catalonia: as *Brunneospora reticulata*, Segria, arable soil, September 1980, L. Punsola (holotype FMR 784 and ex-type culture UAMH 5704); Baix Ebre, arable soil, August 1980, L. Punsola (FMR 811). UNITED KINGDOM: as *Apinisia graminicola*. Yorkshire, Leeds, grass (indet.), October 1964, C.J. La Touche (holotype Herb. IMI 126422 and ex-type culture UAMH 4315 = IMI 126422).

Coccidioides immitis. Figs. 38–41.

The taxonomic affinity of *C. immitis*, a respiratory pathogen with a unique parasitic cycle and no known meiotic phase, has been the subject of much speculation. The alternate arthroconidia of its saprobic phase, racquet hyphae, and colonial morphology are typical of some *Malbranchea* species (Sigler and Carmichael 1976) and it has been hypothesized that its closest relatives would be found among some keratinophilic species of *Malbranchea* having meiotic stages in the Onygenaceae (Sigler in McGinnis et al. 1992; Sigler 1993). These observations have been supported by molecular

Figs. 38–41. *Coccidioides immitis*. Alternate arthroconidia and helically coiled hyphae observed in slide culture preparations (Figs. 38 and 39 = herb. UAMH 9149; Figs. 40 and 41 = herb. UAMH 9150). Bars = 10 μm.



phylogenetic analyses (Pan et al. 1994; Bowman et al. 1996), which indicate that *U. reesii* is a close relative. That sexual reproduction may occur in *C. immitis* has been suggested by molecular evidence of recombination (Burt et al. 1996) and by the finding of helically coiled hyphae in two isolates we examined (Figs. 38–41). Development of helical coils in *C. immitis* as well as evidence that *U. orisii* may be weakly pathogenic provide further support for the close relationship between this dimorphic human pathogen and ascomycetes within the genus *Uncinocarpus*. Helical, spiral, or uncinate hyphae are usually found as appendages on ascomata, but often occur independently in cultures of anamorphs of heterothallic onygenalean fungi, where they are recognized as markers of possible sexual reproduction. Orr (1968) also reported the presence of helically coiled hyphae in cultures of *C. immitis* obtained by baiting soil samples with human hair, but his report has often been overlooked. Unfortunately, mating studies in *C. immitis* have been impeded by the laboratory hazards of working with the fungus.

Acknowledgements

Thanks are expressed to Dr. J. Guarro, the Centraalbureau voor Schimmelcultures, and the International Mycological Institute for providing cultures and specimens; to Linda and Sean Abbott for photographic and technical assistance; and to the Natural Sciences and Engineering Research Council of Canada for financial support (L.S.).

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