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Microascus brevicaulis sp. nov., the teleomorph of *Scopulariopsis brevicaulis*, supports placement of *Scopulariopsis* with the Microascaceae

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Abstract: Five isolates of *Scopulariopsis brevicaulis* have been found to form perithecia in addition to typical conidia. The teleomorph is described as *Microascus brevicaulis* sp. nov., characterized by small (70–130 μm diam), black, ostiolate ascocarps that are papillate or very short-necked, and with a peridium of *textura angularis*. The ascospores are reniform, 5–6 × 3.5–4.5 μm, smooth, and pale orange in mass. Although other species of *Microascus* produce a *Scopulariopsis* state in culture, this discovery now definitively links *S. brevicaulis*, the type species of *Scopulariopsis*, to the Microascaceae.

Key Words: anamorph-teleomorph connections, Ascomycota, holomorph, hyphomycetes, Microascales, systematics

INTRODUCTION

Scopulariopsis brevicaulis (Sacc.) Bainier, described by Saccardo (1881) as *Penicillium brevicaule* Sacc., is an ubiquitous, saprobic mold found worldwide in soil, plant and animal matter, and air (Domsch et al., 1980). It has proteolytic and cellulolytic abilities, and is an occasional agent of superficial human infection. Although the connection between *Scopulariopsis* Bainier and *Microascus* Zukal (Microascaceae, Microascales) has been established for some species (e.g., Sopp, 1912 as *Acaulium*; Emmons and Dodge, 1931; Barron et al., 1961, Morton and Smith, 1963), a sexual state for the type species of *Scopulariopsis, S. brevicaulis,* has never been reported. Morton and Smith (1963) reported the presence of black 'sclerotia', which resembled perithecia but lacked ascospores, in two strains of *S. brevicaulis*. Many other *Scopulariopsis* species remain unconnected to sexual states.

An isolate of *Scopulariopsis brevicaulis* producing perithecia was recovered by air sampling in a honeybee (*Apis mellifera*) overwintering facility, along with many strictly anamorphic isolates (Sigler et al., 1996). Since the initial discovery in 1994, two other ascocarpic isolates have been recovered from indoor and outside air. A thorough reexamination of 65 strains of *S. brevicaulis* deposited in the University of Alberta Microfungus Collection and 25 unaccessioned isolates revealed two additional sexually reproducing isolates from Alberta.

The small, black, ostiolate ascocarps and reniform, pale orange ascospores place the teleomorph of *S. brevicaulis* in the genus *Microascus*, and it is here described as a new species. A discussion of its relationship to other *Microascus* species is provided.

MATERIALS AND METHODS

Ninety isolates of *Scopulariopsis brevicaulis* were examined for propensity to produce a sexual stage by growing them on oatmeal salts agar (OAT; Weitzman and Silva-Hutner, 1967) and monitoring them for 25 wk. Five teleomorphic and six anamorphic isolates selected from diverse substrata and geographic origins (see *Specimens examined*), were compared to determine if there were differences between ascocarpic and nonascocarpic isolates. Colony diam and morphologies of the selected isolates were examined on potato dextrose agar (PDA; Difco Laboratories, Detroit, Michigan) and pablum cereal agar (CER; Sigler, 1992), and thermotolerance was assessed on PDA at 37 C. Tolerance to fungal inhibitors was determined by measuring colony diam on mycosel agar containing cycloheximide at 400 μg/mL (MYC; Becton Dickinson Microbiology Systems, Cockeysville, Maryland) and on PDA supplemented with 2 μg/mL of benomyl at 25 C after 7 and 14 d. For these tests, a sterile needle was inserted into a suspension of conidia prepared for each strain in semisolid detergent agar

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(Pitt, 1973) and then stab inoculated into the center of 100 mm-diam petri dishes containing one of the described media. Conidial morphology was observed in CER slide-culture preparations, and all mounts were prepared in polyvinyl alcohol or lactofuchsin mounting medium (Sigler, 1992). Selected specimens were either air dried or fixed in 2% osmium tetroxide vapor and critical point dried for examination with a Hitachi S-2500 scanning electron microscope (SEM).

Holotype herbarium material and ex-type culture of the type strain, and living and dried material of the other strains are maintained in the University of Alberta Microfungus Collection and Herbarium (UAMH). Isotype herbarium material is deposited in the Herbarium, Royal Botanic Gardens, Kew (K).

RESULTS

Microascus brevicaulis S.P. Abbott, sp. nov. FIGS. 1–7

Ascomycota, Microascales, Microascaceae

Status anamorphosis: *Scopulariopsis brevicaulis* (Sacc.) Bainier. 1907. Bull. Soc. Mycol. Fr. 23: 99.

Peritheciis 80–150 × 70–130 μm, globosis vel subglobosis, ostiolatis, papillatis, nigris; peridiis textura angularis; ascis octosporis, globosis vel subglobosis, deliquescentibus; ascosporis 5–6 × 3.5–4.5 μm, reniformis, laevis, subhyalinis vel aurantiis en masse; conidiophora annellata; conidiis 6–9 × 5.5–9 μm, subglobosis, verrucosis, pallido-brunneis.

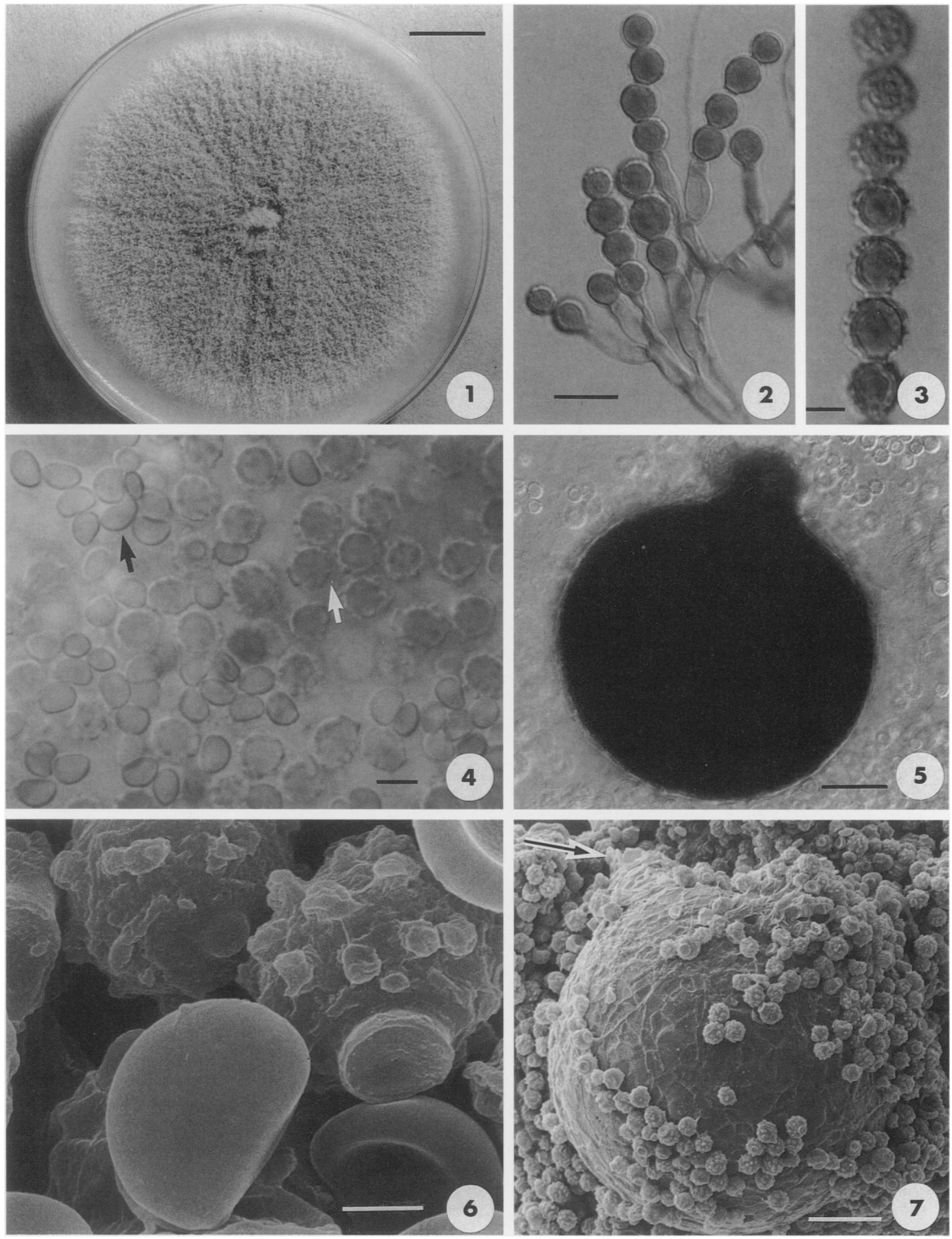
Perithecium 80–150 × 70–130 μm, globose to subglobose, with a papillate to short- necked (up to 20 μm) ostiolar region, black; peridium of *textura angularis,* cells 5–9 μm diam; appendages lacking. *Asci* 8–10 μm diam, subglobose to slightly irregular, octosporous, deliquescent at a very early stage and infrequently observed. *Ascospores* 5–6 × 3.5–4.5 μm, broadly reniform (plano-convex to concavo-convex) in face view and flattened, 2.5–3 μm in end view, orange in mass, appearing subhyaline in transmitted light, smooth, de Bary bubbles and guttules lacking, germ pore not evident by light or scanning microscopy. *Conidia* 6–9 × 5.5–9 μm, globose to subglobose, with a truncate base, base may be slightly protruding (lightbulb-shaped), pale brown, occasionally smooth or only finely ornamented but the majority of spores verrucose with coarse irregular warts at maturity, produced in dry chains from simple or branched annellidic conidiogenous apparatus; annellides 10–25 × 3–5 μm, elongate ampulliform, hyaline.

Specimens examined. Microascus brevicaulis. HOLOTYPE. CANADA, ALBERTA: Scandia. Dried colony on OAT at 25 wk ex indoor air of honeybee (*Apis mellifera*) overwintering facility, 11 Mar. 1994, *S.P. Abbott OHS 428,* (UAMH 7770). ISOTYPE. (K). PARATYPES. CANADA: ALBERTA, Calgary. indoor air from basement of home, 10 Jan. 1995, *S.P. Abbott SA-M26* (UAMH 7880); Barrhead. Outside air, 20 Mar. 1996, *S.P. Abbott SA-M76,* (UAMH 8627); Alberta Game Farm east of Edmonton. Straw of birdhouse roosts, 8 Nov. 1961, *J.W. Carmichael 16-12-a,* (UAMH 1197); Lethbridge. Dead housefly larvae, 1974, *R.G. Bell.* (UAMH 3753).

Scopulariopsis brevicaulis. ZAIRE: Mount Hawa. Silk worm chrysalis, 1952, *R.L. Steyaert,* obtained from International Mycological Institute as IMI 49528, (UAMH 644). VENEZUELA: Caracas. Sep. 1955, *C.B. Pinto 43-3,* obtained from United States Department of Agriculture as NRRL A-6185, (UAMH 943). KOREA: Chuncheon. Meju, Korean fermented soybeans, *J.D. Lee A-1-2,* obtained from Japan Collection of Microorganisms as JCM 2619, (UAMH 8497). CANADA: ALBERTA, north of Mariana Lake. burnt wood of black spruce (*Picea mariana*), 16 Aug. 1996, *S.P. Abbott SA-M137,* (UAMH 8628). AUSTRALIA: QUEENSLAND, Innisfail. atmosphere, cleared site, 1985, *J. Upsher,* obtained from Australian National Collection of Biodeterioration Microfungi as AMRL 1675, (UAMH 8702). UNITED KINGDOM: Manchester. 1930, obtained from International Mycological Institute as IMI 61424, (UAMH 8785).

Cultural properties.—Macroscopic and microscopic morphological characters, as well as colony diameters under various conditions (medium, temperature, antifungal compounds) were comparable among the eleven strains and no distinction between ascocarpic and nonascocarpic strains was discerned (TABLE I). Colonies were light sandy tan or avellaneous brown, typically with a white, entire margin, shallowly convex, fasciculate to velutinous, occasionally with a floccose mycelial overlay. Colonies on CER (FIG. 1) were more distinctly fasciculate, and the color was more pronounced than on PDA. Colony diam were greater, and there was less between-isolate variation among strains on CER than on PDA in 14 d at 25 C. All isolates grew more slowly at 37 C, with colony diam 33–86% of those at 25 C. All isolates were tolerant to benomyl at 2 μg/mL, with growth rates comparable to those on unamended media. Strains showed no inhibition when grown on PDA amended with 10 μg/mL benomyl (data not shown). On medium containing 400 μg/mL cycloheximide, isolates grew slowly but demonstrated good sporulation. Conidial size varied over a narrow range (extremes 5.5–7.5 vs. 6.5–9 μm diam) among strains.

Ascocarps were produced on several media but were most abundant on OAT in 6–25 wk. They occurred on the agar surface and submerged in the upper 5 mm but their presence was obscured by the confluent conidial stage. Ascospores typically remained within perithecia for prolonged periods, but were eventually exuded in a droplet from the ostiole after 6–11 mo as the medium dried. Unlike many



FIGS. 1–7. *Microascus brevicaulis*. 1. Colony on CER 14 d at 25 C showing confluent growth of anamorph (UAMH 7880), bar = 15 mm. 2. Conidiophore and young, smooth to slightly roughened conidia (UAMH 1197), bar = 10 μm. 3. Mature, coarsely ornamented conidia (UAMH 943), bar = 5 μm. 4–7. UAMH 7770, TYPE. 4. Ascospores (black arrow) and conidia (white arrow), bar = 5 μm. 5. Perithecium, bar = 25 μm. 6. Smooth ascospores and ornamented conidia showing slightly protruding and truncate base, SEM, bar = 1.5 μm. 7. Perithecium, note ostiole (arrow) and peridium of *textura angularis*, SEM, bar = 20 μm.

TABLE I. Comparison of conidium size and colony diam in 5 ascocarpic and 6 nonascocarpic strains

		Colony diam at 14 d (mm)				
Ascocarps	Conidium diam (μm)	PDA at 25 C	PDA + benomyl at 25 C	PDA at 37 C	CER at 25 C	MYC at 25 C
+	5.5–9	46–57	43–54	17–32	75–78	30–41
−	5.5–9	38–75	37–75	15–42	72–80	17–38

other species of *Microascus,* no prominent cirrus was produced. Perithecia were produced less abundantly on MYC, CER, phytone yeast extract agar, Takashio agar (Takashio, 1972), and corn meal agar. Cultures of UAMH 7770 grown for one year on 2% malt extract agar, soil extract agar, potato dextrose agar, Sabouraud dextrose agar or on pieces of sterile wood (aspen and pine) placed on tap water agar failed to produce fertile ascocarps.

DISCUSSION

Considering the long history, widespread distribution, and frequent isolation of *S. brevicaulis,* the discovery of five sexually reproducing isolates is remarkable. There are several possible explanations for the teleomorph being hitherto unknown. (i) Ascocarps are produced only after considerable time (6–25 wk). Because the colonies grow rapidly and conidia are produced abundantly within a week, isolates frequently are not retained long enough for ascocarp production. (ii) Ascocarps are obscured by heavy mycelial and conidial growth (FIG. 1), and their inconspicuousness is complicated by small size (70–130 μm diam) (FIGS. 5, 7), lack of prominent cirrus, and production at the agar surface or submerged in the medium. Even when ascocarps were nearly confluent they were difficult to see, and were often first noticed submerged in the agar against the side of the petri dish. (iii) Sporulating strains produced ascocarps on a number of media, but ascocarps were reduced, delayed or lacking on malt extract agar, potato dextrose agar and Sabouraud dextrose agar, routinely used for fungal isolation.

None of the other 85 strains of *S. brevicaulis* produced ascocarps under any condition. These were from diverse sources from across North America, South America, Europe, Asia, Africa and Australia. All teleomorphic strains have been isolated from Alberta. It is possible that there is a geographic restriction of a potentially sexually reproducing population, as is known for *Hypomyces cervinigenus* Rogerson & Simms which has a widespread anamorph, *Mycogone cervina* Ditmar (Rogerson and Simms, 1971). However, many other strains from Alberta exhibit typical anamorphic states only, including isolates recovered at the same sites as ascocarpic ones. Also, the two isolates reported to produce 'sclerotia' or abortive perithecia (Morton and Smith, 1963) were from the U.K. These two isolates (UAMH 8785, 8786) were studied by us but have not produced any ascomatal structures; however, each has been maintained in culture collections for about 70 yr.

Examination of herbarium material consisting of dried colonies and permanent mounts of UAMH 1197 revealed the presence of primarily immature perithecia of which a small proportion produced a limited number of ascopores. When the isolate was regrown from a lyophilized ampoule (prepared 1962) and from an agar slant frozen at −20 C (prepared 1971), cultures were very slow (6 mo) to produce a few ascocarps. Subsequent subculture from ascocarp-producing areas of the colonies enhanced perithecium production and fertility. In contrast, UAMH 3753 recovered from a lyophilized ampoule (prepared 1975) produced abundant perithecia within 6 wk, although maturity required additional time. Particular attention was paid to preserve the three recent isolates of *M. brevicaulis* (UAMH 7770, 7880, 8627) from colonies with mature perithecia, and all have demonstrated the ability to produce fertile ascocarps upon recovery from storage.

A variety of species of Microascaceae, including *S. brevicaulis,* have demonstrated tolerance to benomyl (Summerbell, 1993; Valmaseda et al., 1987). Our results confirm the uniformity of tolerance among isolates. Tolerance to cycloheximide is also common in the family, but there is much greater variation between species and among individual strains (Abbott, unpublished data), as was shown for this species (TABLE I).

Close relationship of *M. brevicaulis* to *M. manginii* (Loub.) Curzi, the teleomorph of *S. candida* (Guéguen) Vuillemin, is suggested by similarities in ascocarps, ascospores and conidial states. In both species, ascocarps are small (up to 150 μm diam) and papillate. Ascospores are broadly reniform, 4–5 × 2.5–4 μm in *M. manginii* and 5–6 × 3.5–4.5 μm in *M. brevicaulis.* Conidia are similar in shape, but easily distinguished by surface texture (smooth in *S. candida* and ornamented in *S. brevicaulis*) and coloration (white to cream colonies with hyaline conidia in *S. candida* and sandy/tan brown colonies with distinctly pigmented conidia in *S. brevicaulis*). Similarities in condia and conidiophores were noted by Morton and Smith (1963) who included both species within the 'brevicaulis series' of *Scopulariopsis.* Morton and Smith (1963) rarely observed peritheciu-

like bodies in both *S. candida* and *S. brevicaulis*. The propensity of *S. candida* to produce non-fertile perithecia was frequently observed in our isolates (e.g., UAMH 3568, 4065, 4367, 7882, 8683), but not in the 90 strains of *S. brevicaulis*.

Two other species of *Microascus* with conidial states similar to *M. brevicaulis* were described by Sopp (1912), as *Acaulium nigrum* Sopp and *A. flavum* Sopp. Although *Acaulium* Sopp is considered a synonym of *Scopulariopsis* by most authors (e.g., Barron et al., 1961; Morton and Smith, 1963), Sopp described perithecia in three species. No type material or cultures exist of Sopp's specimens (fide Morton and Smith, 1963), but the original descriptions and illustrations are sufficient to distinguish them from our new species. *Acaulium nigrum* was treated in *Microascus* by Curzi (1931), as *M. niger* (Sopp) Curzi. Although no perithecia have been observed since the original description, *M. niger* was tentatively accepted by Barron et al. (1961) and regarded as the teleomorph of *S. asperula* (Sacc.) S. Hughes by Morton and Smith (1963). The anamorph is distinguished from *M. brevicaulis* in its darker, fuscous-brown colonies and conidia, and the ascospores, in the original description, are larger (7 × 5 μm). Morton and Smith treated *A. flavum* as a species of *Scopulariopsis* since their material failed to produce a sexual stage. This species differs from *M. brevicaulis* by paler, buff colonies and conidia (Morton and Smith, 1963), and larger (6–7 μm) ascospores (Sopp, 1912). Both of Sopp's species were isolated from insect larvae, as was one strain of *M. brevicaulis* (UAMH 3753).

Recent molecular phylogenetic studies (Hausner et al., 1993; Spatafora and Blackwell, 1994; Cassar and Blackwell, 1996; Berbee and Taylor, 1992) have supported the Microascaceae as monophyletic, but the analyses have included few representatives of only teleomorphic taxa. Although the connection between *Microascus* and *Scopulariopsis* has been recognized by many authors, the discovery of a teleomorph for the type species of *Scopulariopsis* allows this anamorph genus to be placed among the Microascaceae. Anamorphic form-genera are primarily artificial taxonomic entities based on structural similarity serving as a practical means of identifying and naming asexual fungi (Gams, 1995), but many authors (e.g., Seifert, 1993) have favored a phylogenetic approach for the classification of anamorphic taxa. Form-genera are not strictly monophyletic units, especially when combined in a phylogenetic framework with teleomorphic taxa, but we support the view that unrelated and morphologically divergent taxa should be excluded where practicable (Gams, 1995; Seifert, 1993). In this case, the form-genus *Scopulariopsis* can be restricted to anamorphs of the Microascaceae. Many morphologically distinct species with different affinities have already been transferred to other form-genera including *Sagenomella* W. Gams, *Basipetospora* G.T. Cole & W.B. Kendr., *Polypaecilum* G. Sm., and *Gliomastix* Guég. Although some authors (e.g., Taylor, 1995) have taken an extreme position and advocated the abandonment of anamorphic names when phylogenetic position can be clearly established, form genera continue to serve a useful purpose in routine identification, especially in a case such as this where the *Microascus* teleomorph is rarely seen.

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