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Heterothallism in the Microascaceae demonstrated by three species in the *Scopulariopsis brevicaulis* series

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Abstract: *Scopulariopsis* anamorphs are known for many species of the genus *Microascus* (Ascomycota, Microascaceae), but teleomorph connections for anamorphic species within the '*Scopulariopsis brevicaulis* series' are tenuous or lacking. *Microascus brevicaulis* was recently described as the teleomorph of the type and commonest species, *S. brevicaulis,* but only a few isolates yielded fertile perithecia. To investigate whether paucity of sexual reproduction was the result of heterothallism, mating experiments were conducted among isolates representing the species *S. brevicaulis, S. candida, S. asperula, S. fusca* and *S. koningii.* Results demonstrated heterothallism within three species and confirmed that two taxa could be reduced to synonymy. Three holomorph species, *M. brevicaulis, M. manginii,* and *M. niger,* are recognized to include anamorphs *S. brevicaulis* (synonym *S. koningii*), *S. candida,* and *S. asperula* (synonyms *S. arnoldii, S. bestae, S. fusca,* and *S. roseola*), respectively. *Microascus niger* is redescribed and a neotype proposed. The three species are most readily recognized by colony color (sandy tan to avellaneous in *M. brevicaulis,* white to cream in *M. manginii,* and medium to dark fuscous brown in *M. niger*). These colonial distinctions correlate generally with conidia that are coarsely roughened, smooth, or finely roughened, respectively. However, conidium ornamentation, previously considered a reliable taxonomic character, is shown to be variable.

Key Words: anamorph-teleomorph connections, Ascomycota, holomorph, hyphomycetes, *Microascus*

INTRODUCTION

Members of the ascomycete family Microascaceae are found primarily on cellulosic and protein-rich substrata (soil, plant litter, wood, dung, animal remains), and have a worldwide distribution in tropical, temperate and polar regions (Barron et al 1961, Morton and Smith 1963, Domsch et al 1993, Abbott 2000). The genus *Microascus* was established by Zukal in 1885, but the Microascaceae were not united at the family level until 1951 (Luttrell 1951, Malloch 1970). Axenic culture studies first provided evidence of connections between the anamorphic genus *Scopulariopsis* and teleomorphs within *Microascus* (Emmons and Dodge 1931, Barron et al 1961, Morton and Smith 1963), but many *Scopulariopsis* species remain unconnected or tenuously connected to teleomorphs.

Most species of the *Microascus* have been thought to be homothallic (Barron et al 1961), but the mating behavior has rarely been investigated. Emmons and Dodge (1931) showed that 20 single ascospore isolates of *M. trigonosporus* Emmons & Dodge developed fertile perithecia. In 1998, we demonstrated sexual reproduction in five isolates of *Scopulariopsis brevicaulis* (Sacc.) Bainier and described the teleomorph *M. brevicaulis* S.P. Abbott. This discovery established a definitive link between the type species of the anamorph genus *Scopulariopsis* and the Microascaceae (Abbott et al 1998), but raised the question as to why the sexual stage of such a well known species had hitherto been unreported. *Scopulariopsis brevicaulis* has been known since 1881 and is the commonest *Scopulariopsis* species worldwide. To address this question, we investigated the possibility of heterothallism in this and other taxa within the '*Scopulariopsis brevicaulis* series' in which sterile, perithecium-like structures devoid of ascospores had been occasionally reported (Morton and Smith 1963).

The '*Scopulariopsis brevicaulis* series' includes species having large, subglobose, smooth or ornamented, white to brown conidia, produced from highly branched conidiophores bearing annellides that are broad at the apex (3 μm) and not abruptly swollen at the base (Morton and Smith 1963). Species included are *S. brevicaulis, S. asperula* (Sacc.) S. Hughes, *S. flava* (Sopp) Morton & G. Smith, *S. candida* Vuillemin, *S. koningii* (Oudemans) Vuillemin, and *S. fusca* Castellani. Connections to teleomorphs, where known, are tenuous. Many isolates of *S. candida* produce sterile perithecia-like structures (Abbott et al 1998), and this species has been connected

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to *M. manginii* (Loub.) Curzi based on conidial similarity (Morton and Smith 1963). Morton and Smith tentatively accepted a connection between *S. asperula* and *M. niger* (Sopp) Curzi, but recommended that the teleomorph described as *Acaulium nigrum* Sopp (Sopp 1912) be disregarded since there was no extant type material and the species had not been seen since the original description.

To test the hypothesis of heterothallism and to clarify holomorph concepts, mating trials among strains of 5 of 6 species in the '*S. brevicaulis* series' were performed. *Scopulariopsis flava* was not included because of the lack of available authentic material (see Morton and Smith 1963). Disposition of *S. flava* is the topic of a separate paper. The status of *S. koningii* and *S. fusca* as distinct species was questioned because of morphological similarities to *S. brevicaulis* and *S. asperula,* respectively. Authentic material of these species was included in mating trials to aid in the evaluation of their taxonomic status. Neither has previously been connected to a sexual state. The mating tests provided experimental evidence for a reappraisal of accepted species within the '*S. brevicaulis* series' and for their connections to species of *Microascus.*

MATERIALS AND METHODS

Morphology.—Living cultures and herbarium material of wild-type strains, single ascospore isolates, and mated pairs are maintained in the University of Alberta Microfungus Collection and Herbarium (UAMH). Colonial features were recorded and colony diameters were measured on oatmeal salts agar (OAT; Kane et al 1997) and potato dextrose agar (PDA; Difco Laboratories, Detroit, Michigan) at 25 C after 7, 14, 21, and 28 d. Colors were determined using the color standards of Kornerup and Wanscher (1978). Microscopic mounts were prepared in polyvinyl alcohol or lactofuchsin mounting medium using the slide culture method for observation of conidiogenesis and squash mounts for observation of ascomata (Kane et al 1997). Material for scanning electron microscopy (SEM) was fixed in 2% OsO_4 vapor, and critical-point dried for examination with a Hitachi S-2500.

Scopulariopsis brevicaulis.—Nine single ascospore isolates were obtained from UAMH 8627 ex-paratype culture of *M. brevicaulis.* One perithecium was removed and washed repeatedly with sterile water to remove adhering conidia, then transferred into 10 mL of sterile water and crushed to release the ascospores. Approximately 1 mL of the ascospore suspension was spread onto each of 10 PDA plates that were incubated at 22 C for 3–7 d to allow germination. From each of 9 colonies resulting from germination of a single ascospore, a hyphal tip was excised and transferred to individual OAT plates. These plates were used as the source of inoculum (conidia) for mating tests.

Conidial suspensions in 1.5 mL of sterile water were prepared for each of the 9 single ascospore isolates. Self-self crosses and crosses in all combinations were made by pipeting one drop of suspension onto the center of an OAT plate and mixing with a drop from a second strain. Plates were incubated at 22 C, examined after 7 wk and periodically for 14 mo. Plates were examined macroscopically for the presence of submerged ascomata around the edge of the plate. Plates were examined also with a dissecting microscope but because heavy conidial growth often obscured ascomata forming at the agar surface, some of the surface growth in the central areas was removed prior to examination. Microscopic mounts of developing perithecia were made periodically to monitor for ascospore production.

Two single ascospore isolates that yielded a fertile cross were designated as plus (UAMH 9090) and minus (UAMH 9092) mating types. These were mated with six nonascocarpic wild-type strains of *S. brevicaulis* examined in Abbott et al (1998) and with two strains originally received as *S. koningii* and one as *S. flava.* Conidia of each strain were suspended in semisolid detergent agar (SSD; Pitt 1973). Each test strain was streaked onto one half of an OAT plate opposite to the mating type streak, allowing for a central zone of contact as the isolates grew. Plates were incubated at 22 C and monitored after 6 wk and periodically thereafter for 12 mo for development of ascomata within the central zone.

Scopulariopsis candida.—Nine wild-type strains of *S. candida* were crossed in all possible combinations (including self-self pairings). Conidia were suspended in SSD and streaked onto OAT as described above. Plates were examined over 19 mo. Strains were chosen to represent the broad range of variation observed among the 35 strains held at UAMH, and included a strain that routinely exhibited sterile perithecia (UAMH 3568) as well as strictly conidial isolates. Two wild-type isolates that yielded a fertile cross were designated as plus (UAMH 3568) and minus (UAMH 9004) mating types. These were back crossed to the ex-type culture of *M. manginii,* received from the Centraalbureau voor Schimmelcultures (CBS 170.27 =UAMH 9135), that had produced only the anamorph in culture. The perithecia and ascospores produced in positive crosses were compared with those produced by eight isolates of *M. manginii* held at UAMH.

Scopulariopsis asperula.—Nine strains that represented type, authentic, and recent isolates of *S. asperula, S. fusca, S. roseola* Inagaki, *S. arnoldii* (Mangin & Patouillard) Vuillemin and *S. bestae* (Pollacci) Nannizzi, were crossed in all combinations as described for *S. candida* above. These were selected to represent the extremes of morphological variation found among 25 strains with similar gross morphologies. Subcultures of three fertile crosses were accessioned into the UAMH (9489, 9490, 9491).

Crosses between species.—To test for fertility between species, plus and minus mating type strains determined in each experiment above were crossed. Crosses were done as described above between *S. brevicaulis* (UAMH 9090, 9092), *S. candida* (UAMH 3568, 9004), and *S. asperula* (UAMH 8362, 9037) in all combinations, and observed periodically for 13 mo.

RESULTS

Microascus brevicaulis S.P. Abbott. 1998. Mycologia 90:298. (HOLOTYPE UAMH 7770)

FIGS. 1, 4, 7, 10

Anamorph. *Scopulariopsis brevicaulis* (Saccardo) Bainier. 1907. Bulletin Societé Mycologique de France 23:99.

≡ *Penicillium brevicaule* Saccardo. 1881. Fungi Italici No. 893.

= *Scopulariopsis koningii* (Oudemans) Vuillemin. 1911. Bulletin Societé Mycologique de France 27:143.

≡ *Monilia koningii* Oudemans. 1902. in Oudemans and Koning, Archives Néerlandaises des Sciences, Sér 2, 7: 287.

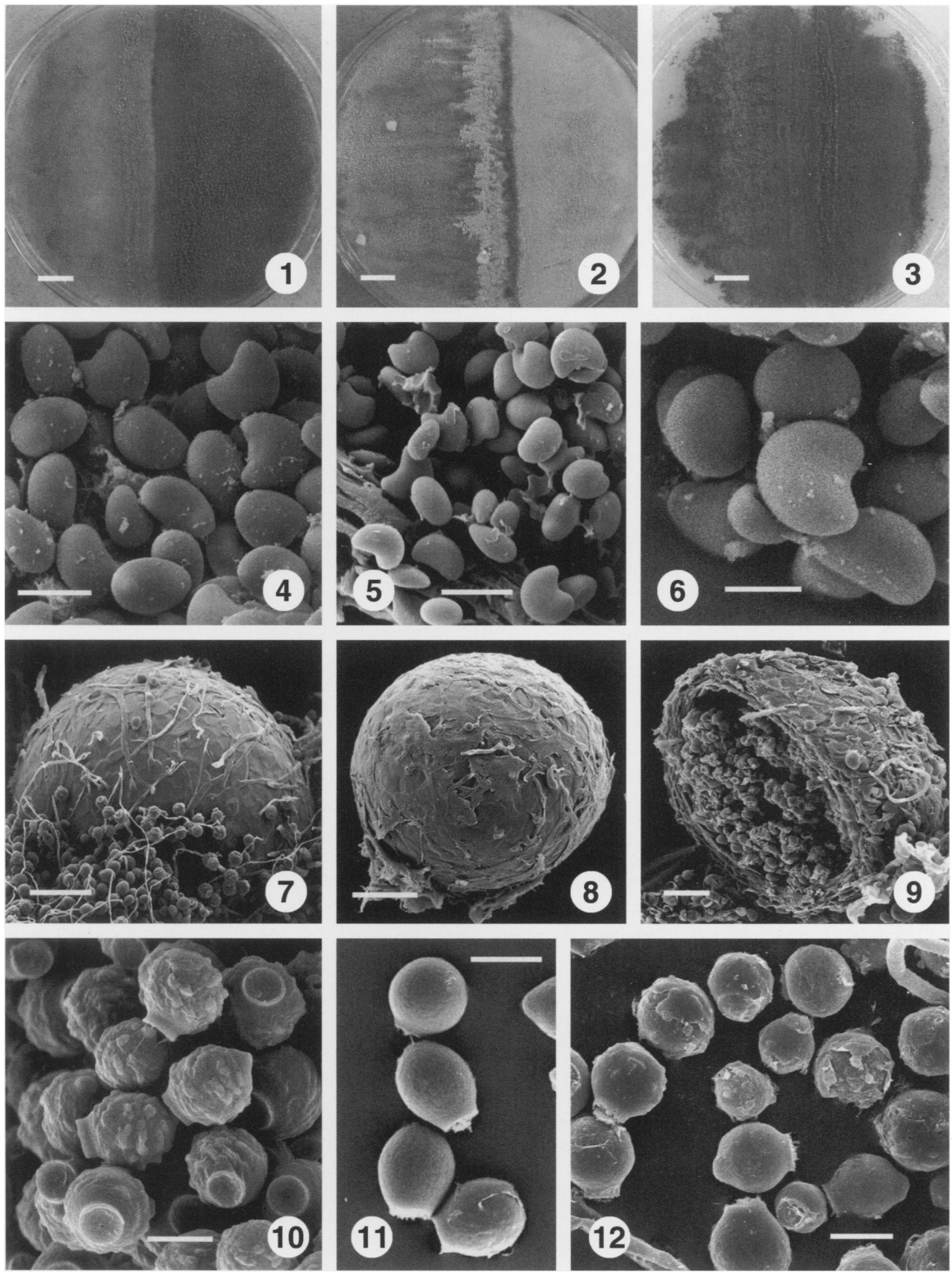
Other synonyms listed in Morton and Smith (1963).

The results of the mating tests demonstrated that *M. brevicaulis* is heterothallic (TABLES I AND II). The nine single ascospore isolates were sterile in self crosses and produced fertile ascomata when crossed (TABLE I). All strains produced fertile ascomata in some crosses (12 fertile crosses total), but six pairings produced only infertile ascomata along the contact zone. Three isolates were designated as plus mating type strains and six as minus strains. The nine anamorphic wild-type *S. brevicaulis* isolates which were back crossed with plus (UAMH 9090) and minus (UAMH 9092) mating type stains yielded fertile ascomata with only one mating type (TABLE II).

This is the first account of heterothallism in the Microascaceae and is confirmed in *M. brevicaulis* by: i) absence of a sexual stage in single ascospore isolates, ii) fertility between single ascospore isolate pairs, and iii) fertility between single ascospore isolates and wild-type strains. *Microascus brevicaulis* behaves as expected for a single gene (unifactorial) heterothallic mating system, with strains being either plus or minus, and mating only with the opposite mating types in all cases. Infertile ascomata were found in one-third of the single ascospore pairings (TABLE I) for which the production of fertile ascomata was expected. We observed that perithecia may form within one to two months, but that some required a lengthy period for ascospore development (results given at 14 mo). It is possible that the infertile ascomata would have eventually proceeded to maturity.

Morton and Smith (1963) accepted *S. koningii* based on the smooth conidia and pale avellaneous colonies and listed UAMH 363 as a representative strain. Raper and Thom (1968) considered *S. koningii* a synonym of *S. brevicaulis* but provided no rationale for their decision. Our mating results indicate that the species are conspecific. Two strains (UAMH 363 and 9040) that fit Morton and Smith's concept of *S. koningii* produced fertile ascomata in crosses with the mating type isolates of *M. brevicaulis* (TABLE II). Our observations suggest a correlation between smooth conidia and paler colonies, since the greatest concentration of pigment is revealed by light microscopy to be in the protuberances of ornamented conidia, thus accounting for associated colonial coloration. Isolates of *S. koningii* have occasionally been reported from human sources (e.g., Sartory 1916), but Morton and Smith suspected that such isolates were merely smooth-spored representatives of *S. brevicaulis* that produced rough conidia tardily. Since *S. koningii* is here reduced to synonymy, reports in the medical mycology literature concerning this species may now be attributed to *S. brevicaulis* which is the most common human pathogenic species (Kane et al 1997, Sigler and Kennedy 1998).

Specimens examined. Microascus brevicaulis. CANADA. ALBERTA: Scandia, indoor air of honeybee (*Apis mellifera*) overwintering facility, 11 Mar 1994, *S.P. Abbott OHS 428,* (HOLOTYPE and ex-type culture UAMH 7770, ISOTYPE K); Calgary, indoor air from basement of home, 10 Jan 1995, *S.P. Abbott SA-M26,* (PARATYPE UAMH 7880); Barrhead, outside air, 20 Mar 1996, *S.P. Abbott SA-M76,* (PARATYPE UAMH 8627); Alberta Game Farm east of Edmonton, straw of birdhouse roosts, 8 Nov 1961, *J.W. Carmichael 16–12-a,* (PARATYPE UAMH 1197); Lethbridge, dead housefly larvae, 1974, *R.G. Bell.* (PARATYPE UAMH 3753); Elk Island National Park, wood, well rotted log of white spruce (*Picea glauca*), 28 Sep 1998, *T. Lumley* and *S.P. Abbott EI-02-S1D,* (UAMH 9367); 30 km east of Nordegg, dung of moose (*Alces alces*), 30 Sep 1996, *S.P. Abbott SA-M136,* (UAMH 9458); UAMH, single ascospore isolates ex UAMH 8627, *S.P. Abbott* (+) isolates *Mb4* (UAMH 9090), *Mb8* (UAMH 9407), *Mb9* (UAMH 9091); (−) isolates *Mb1* UAMH 9406), *Mb3* (UAMH 9092), *Mb7* (UAMH 9093). *Scopulariopsis brevicaulis.* AUSTRALIA. QUEENSLAND: Innisfail, atmosphere, cleared site, 1985, *J. Upsher,* obtained from Australian National Collection of Biodeterioration Microfungi as AMRL 1675, (UAMH 8702). CANADA. ALBERTA: north of Mariana Lake, burnt wood of black spruce (*Picea mariana*), 16 Aug 1996, *S.P. Abbott SA-M137,* (UAMH 8628); Edmonton, hairs from neck, *J.W. Carmichael* 1955, (UAMH 363; =LSHB Sc.114, =IMI 86929). MANITOBA: Winnipeg, outside air, 21 Dec 1994, *S.P. Abbott SA-M31,* (UAMH 9040). KOREA. CHUNCHEON: Meju, Korean fermented soybeans, *J.D. Lee A-1–2,* obtained from Japan Collection of Microorganisms as JCM 2619, (UAMH 8497). NETHERLANDS. Pupa of *Pteronus pini,* 1935, *J. Rozsypal,* obtained from Centraalbureau voor Schimmelcultures as *S. flava,* CBS 335.35, (UAMH 9139). UNITED KINGDOM. Manchester, 1930, obtained from International Mycological Institute as IMI 61424, (UAMH 8785). VENEZUELA. Caracas, Sep 1955, *C.B. Pinto 43–3,* obtained from United States Department of Agriculture as NRRL A-6185, (UAMH 943). ZAIRE. Mount Hawa, silk worm chrysalis, 1952, *R.L. Steyaert,* obtained from International Mycological Institute as IMI 49528, (UAMH 644).



FIGS. 1–12. *Microascus brevicaulis, M. manginii* and *M. niger.* 1. Mated strains of *M. brevicaulis* on OAT after 12 mo at 25 C showing central contact zone and confluent conidial growth. Note paler colony at left is typical for *S. koningii* and darker at right is typical for *S. brevicaulis* (UAMH 363 left × 9092 right). 2. Mated strains of *M. manginii* on OAT after 19 mo at 25 C showing central contact zone and confluent growth of *S. candida* anamorph (UAMH 3568 left × 934 right). 3. Mated strains of *M. niger* on OAT after 19 mo at 25 C showing central contact zone and confluent growth of *S. asperula* anamorph (UAMH 8362 left × 9037 right). 4. *M. brevicaulis* ascospores (UAMH 9139 × 9092). 5. *M. manginii* ascospores (UAMH 3568 × 9135). 6. *M. niger* ascospores (UAMH 9489; NEOTYPE). 7. *M. brevicaulis* ascoma, with conidia on surface

Microascus manginii (Loubière) Curzi. 1931. Bollettino Stazione di Patologia Vegetale di Roma (NS) 11:60. FIGS. 2, 5, 8, 11

≡ *Nephrospora manginii* Loubière. 1923. Comptes Rendus Academie des Sciences (Paris) 177:209. (ex-type culture UAMH 9135)

= *Scopulariopsis alboflavescens* Zach. 1934. Österreichische Botanische Zeitschrift 83:177. (ex-type culture UAMH 934)

Anamorph. *Scopulariopsis candida* Vuillemin. 1911. Bulletin Societé Mycologique de France 27:143. (epitype culture selected UAMH 9004)

≡ *Monilia candida* auct., sensu Guéguen. 1899. Bulletin Societé Mycologique de France 15:271. (non Persoon; non Bonorden)

≠ *Monilia candida* Persoon. 1801. Synopsis methodica fungorum. (=*Aspergillus* fide Vuillemin 1911)

≠ *Monilia candida* Bonorden. 1851. Handbuch der allgemeinen mykologie. (=*Monilia bonordenii* Vuillemin. 1911)

= *Chrysosporium keratinophilum* var. *denticolum* Moreau. 1969. Mycopathologia et Mycologia Applicata 37:37. (nom invalid, ICBN Art. 36). (ex-type culture UAMH 8798)

Other synonyms listed in Morton and Smith (1963).

Although the mating trials demonstrated lower levels of fertility compared with *M. brevicaulis,* heterothallism was demonstrated also for this species. Nine of 18 crosses among nine anamorphic strains yielded fertile ascomata and four yielded infertile ascomata (TABLE III). Three isolates were designated as plus and six as minus mating type strains. One strain (UAMH 3568) that had previously produced infertile perithecia-like structures when grown alone, produced ascomata with ascospores when paired with all minus mating type isolates but no ascospores in self crosses. This isolate also crossed with the ex-type culture of *M. manginii* (UAMH 9135) that had failed to produce ascomata when received (TABLE III). Perithecia and ascospores produced in crosses among *S. candida* isolates were identical to those of self-fertile strains of *M. manginii.*

Strains included in the mating tests were chosen to represent a range of variation. UAMH 3568 was included because of its propensity to produce sclerotia-like structures randomly throughout the colony. This 'sclerotial' strain demonstrated a high level of fertility in matings (TABLE III), and in all crosses, the fertile ascomata formed within the central line of contact, thus enabling a positive result to be readily determined. Two strains (UAMH 931, 7924) were somewhat degenerate with glabrous colonies and sparse conidial sporulation, and their identity was questioned. UAMH 931 produced infertile ascomata in two pairings while 7924 produced fertile ascomata only with 3568 (TABLE III). Another strain, UAMH 8404, differed in having pale-yellow rather than typical white to cream colonies, but it produced fertile ascomata with 3568.

The connection between *S. candida* and *M. manginii* was demonstrated by Loubière (1923, as *Monilia candida* and *Nephrospora manginii*) and has been accepted by others (Thom 1930, Curzi 1931, Morton and Smith 1963). Zach (1934) described *S. alboflavescens* as including a sexual stage, and Barron et al (1961) treated it as a synonym of *M. manginii.* The ex-type culture of *S. alboflavescens* (UAMH 934) was strictly conidial when recovered from preserved stocks and in self crosses, but produced fertile ascomata when paired with two plus mating type strains (TABLE III). This behavior is similar to that observed in the ex-type culture of *M. manginii* as discussed above.

The epithet *candida* is problematic. *Monilia candida* described by Persoon (1801), is appropriately referred to *Aspergillus* (Vuillemin 1911). *Monilia candida,* described for a different species by Bonorden (1851), was recognized as a later homonym and renamed as *Monilia bonordenii* (Vuillemin 1911). Based on the branched conidial chains, the species illustrated in Bonorden (1851) is clearly not a *Scopulariopsis.* Guéguen (1899) described a variant of *Monilia candida* Bonorden, and this is the first description that corresponds to the fungus we recognize today as *S. candida.* By referring Guéguen's isolate to *Scopulariopsis,* Vuillemin (1911) created the unintentional nomen novum *S. candida* Vuillemin (ICBN Art. 33, Greuter et al 1994). Because of the nomenclatural confusion and since no type material is in existence, UAMH 9004 is chosen as epitype culture to stabilize the modern concept of *S. candida,* as first defined by Vuillemin.

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(UAMH 9040 × 9090). 8. *M. manginii* ascoma (UAMH 3568 × 9135). 9. *M. niger* mature ascoma in cross-section showing ascospores (UAMH 9489; NEOTYPE). 10. *M. brevicaulis* showing globose to subglobose verrucose conidia with slightly protruding and truncate base (UAMH 9139 × 9092). 11. *M. manginii* showing subglobose to ovoid, smooth conidia with slightly protruding and truncate base (UAMH 9004 × 938). 12. *M. niger* showing globose to subglobose, smooth to finely roughened conidia with slightly protruding and truncate base (UAMH 9489; NEOTYPE). Bars: 1–3 = 10 mm; 4, 10, 11 = 4 μm; 5, 12 = 5 μm; 6 = 3 μm; 7 = 25 μm; 8 = 40 μm; 9 = 15 μm.

TABLE I. Mating reactions between nine single ascospore isolates[a] of *Microascus brevicaulis* on OAT after 14 mo

	Plus mating type		
Minus mating type	UAMH 9090	UAMH 9091	UAMH 9407
UAMH 9092	I[b]	I	+
UAMH 9093	+[c]	+	+
UAMH 9406	+	+	+
Mb[d] 2	+	+	+
Mb5	I	I	+
Mb6	I	I	+

[a] Single ascospore isolates derived from UAMH 8627, ex-paratype culture.
[b] I, infertile ascomata lacking ascospores.
[c] +, ascomata with ascospores produced.
[d] Mb, single ascospore isolate not accessioned into UAMH.

Specimens examined. Microascus manginii. AUSTRIA. Diseased skin of man, *F. Zach,* obtained from Centraalbureau voor Schimmelcultures as CBS 399.34, ex-type culture of *Scopulariopsis alboflavescens,* (UAMH 934). BURMA. Milled rice, 1954, *S. Udagawa NHL 2278,* (UAMH 1923). CANADA. ALBERTA: Red Deer, outside air, 9 Mar 1995, *S.P. Abbott SA-M73* (UAMH 7921); Edmonton, head lesions from chicken, 1967, *J.W. Carmichael,* (UAMH 2710); Elk Island National Park, wood, log of white spruce (*Picea glauca*), 11 Feb 1997, *T. Lumley EI-09-S3E,* (UAMH 9147). ONTARIO: Guelph, chicken litter, 10 Jan 1966, *G. Barron 10490,* (UAMH 2642). FRANCE. *L. Mangin,* ex-type culture of *Nephrospora manginii,* obtained from Centraalbureau voor Schimmelcultures as CBS 170.27, (UAMH 9135). INDIA. Lucknow, 1967, *J.N. Rai,* obtained from International Mycological Institute as IMI 128461, (UAMH 8796). UNITED KINGDOM. Buckwheat chaff, 1974, *A. Donnelly,* obtained from International Mycological Institute as IMI 182498, (UAMH 8797). UNITED STATES. ARIZONA: Dung, 29 Aug 1958, *G.F. Orr O-425,* obtained from United States Department of Agriculture as NRRL A-8022, (UAMH 8977). *Scopulariopsis candida.* CANADA. ALBERTA: Edmonton, skin from chin of man, 1954, *J.W. Carmichael,* (UAMH 238); toe nail of man, 20 Feb 1995 *C. Sand,* (UAMH 7924). BRITISH COLUMBIA: Chilliwak, indoor air of office building, 27 Mar 1997, *S.P. Abbott SA-M175,* (EPITYPE UAMH 9004). ONTARIO: Wallaceburg, carpet dust from home, 14 Apr 1994, *D. Malloch 138–110.1,* (UAMH 8404). CHILE. Soil, (UAMH 3568). FRANCE. *C. Moreau* 1969, obtained from International Mycological Institute as IMI 139629 *Scopulariopsis candida,* ex-type of *Chrysosporium keratinophilum* var. *denticola,* (UAMH 8798). NETHERLANDS. Obtained from Centraalbureau voor Schimmelcultures as *S. rufulus,* (UAMH 931). UNITED KINGDOM. Soil, 1952, *BB 299,* (UAMH 961). UNITED STATES. USDA Bureau of Dairy Industry, abnormal cheese, Jun 1938, *L.A. Rogers,* (UAMH 938).

TABLE II. Mating reactions between mating types of *Microascus brevicaulis* and nine wild-type isolates of *Scopulariopsis brevicaulis* on OAT after 12 months

	Single ascospore isolates of *Microascus brevicaulis*	
Wild-type strains of *S. brevicaulis*	UAMH 9090 plus mating type	UAMH 9092 minus mating type
UAMH 363[b]	−	+[a]
UAMH 644	+	−
UAMH 943	+	−
UAMH 8497	−	+
UAMH 8628	+	−
UAMH 8702	−	+
UAMH 8785	−	+
UAMH 9040	+	−
UAMH 9139[c]	+	−

[a] +, ascomata with ascospores produced.
[b] Strain originally identified as *S. koningii* by Morton and Smith (1963).
[c] Strain originally received as *S. flava.*

Microascus niger (Sopp) Curzi. 1931. Bollettino Stazione di Patologia Vegetale di Roma (NS) 11:60. (NEOTYPE and ex-type culture selected UAMH 9489) FIGS. 3, 6, 9, 12

≡ *Acaulium nigrum* Sopp. 1912. Videnskaps Selskapets Skrifter. 1. Mat-Naturv Klasse 11:47.

Anamorph. *Scopulariopsis asperula* (Sacc.) S. Hughes. 1958. Canadian Journal of Botany 36:803. (EPITYPE culture selected UAMH 9037)

≡ *Torula asperula* Sacc. 1882. Michelia 2:560. (HOLOTYPE L)

= *Scopulariopsis fusca* Zach. 1934. Österreichische Botanische Zeitschrift 83:174. (ex-type culture UAMH 930).

= *Scopulariopsis bestae* (Pollacci) Nannizzi. 1934. Trattato di micopatologia umana 4:254.

≡ *Torula bestae* Pollacci. 1922. Riv Biol 4:317. (ex-type culture UAMH 924)

= *Scopulariopsis arnoldii* (Mangin & Patouillard) Vuillemin. 1911. Bulletin Societé Mycologique de France 27: 148.

≡ *Monilia arnoldii* Mangin & Patouillard. 1908. Bulletin Societé Mycologique de France 24:164. (?ex-type culture UAMH 923)

TABLE III. Crosses among wild-type isolates of *Scopulariopsis candida* and the ex-type culture of *Microascus manginii* on OAT after 19 months

	Plus mating type		
Minus mating type	UAMH 931	UAMH 938	UAMH 3568
UAMH 238	I[a]	+[b]	+
UAMH 934 ([T]*S. alboflavescens*)[c]	−	+	+
UAMH 961	−	I	+
UAMH 7924	−	−	+
UAMH 8404	I	I	+
UAMH 9004	−	+	+
UAMH 9135 ([T]*M. manginii*)	NT[d]	NT	+

[a] I, infertile ascomata formed.
[b] +, ascomata with ascospores produced.
[c] Species described as having a *Microascus* teleomorph.
[d] NT, not tested.
[T] Ex-type culture.

= *Scopulariopsis roseola* Inagaki. 1962. Transactions of the Mycological Society of Japan 4:1. (ex-type culture UAMH 8847)

Perithecium 130–190 × 110–170 μm, globose to subglobose or pyriform, with a papillate to short-necked (up to 20 μm) ostiolar region, black; peridium of *textura angularis*; appendages lacking. *Asci* 14 × 8 μm, irregularly ovoidal, 8-spored, deliquescent at a very early stage and infrequently observed. *Ascospores* 4.5–6.5 × 3.5–4 μm, broadly reniform (plano-convex or concavo-convex) in face view, flattened, orange in mass, appearing subhyaline in transmitted light, smooth, de Bary bubbles and guttules lacking, germ pore not evident by light microscopy or SEM. *Conidia* 6–8 μm diam, globose to subglobose, with a truncate base that may slightly protrude (lightbulb-shaped), brown, walls smooth to finely ornamented but often verrucose with prominent, irregular warts at maturity (degree of ornamentation may vary among strains), produced in dry chains from simple or branched annellidic conidiogenous apparatus; annellides 13–18 μm long, 3–3.5 μm diam at apex, 3–5 μm diam at base, elongate cylindrical to ampulliform, hyaline. *Colonies* dominated by conidia, typically medium to dark fuscous brown (brownish orange 6C4–5 to light brown or brown 6D5–6 to 6E5–7, Kornerup and Wanscher 1978), velutinous to fasciculate, plane, margin white, entire, moderately slow growing, 21–36 mm diam on OAT after 14 d.

Heterothallism was demonstrated in *S. asperula* by formation of fertile perithecia among crosses of nine isolates chosen to include material representative of *S. asperula, S. fusca, S. roseola, S. arnoldii* and *S. bestae* (TABLE IV). Four strains were designated as plus and five as minus mating type.

The holomorph obtained by mating agrees well in both conidial and sexual stages with Sopp's description of *Acaulium nigrum* (Sopp 1912). Curzi (1931)

TABLE IV. Mating reactions among nine isolates of *Scopulariopsis asperula* on OAT after 19 mo

	Plus mating type			
Minus mating type	UAMH 923 ([T]*S. arnoldii*)	UAMH 7879	UAMH 8362	UAMH 8847 ([T]*S. roseola*)
UAMH 924 ([T]*S. bestae*)	I[a]	+[b]	+	I
UAMH 930 ([T]*S. fusca*)	I	+	+	I
UAMH 8984	I	I	+	I
UAMH 9029	I	+	+	+
UAMH 9037	+	+	+	+

[a] I, infertile ascomata formed.
[b] +, ascomata with ascospores produced.
[T] Ex-type culture.

treated the species in *Microascus* as *M. niger,* and Thom (1930) and Barron et al (1961) accepted the species based on previous descriptions. Morton and Smith (1963) connected *M. niger* to *S. asperula* in the synonym list, yet argued that the name *M. niger* should be disregarded due to lack of supporting evidence. Although it is difficult to be certain that the teleomorph obtained by us in mating experiments is conspecific with Sopp's species, the name *M. niger* is in use and the connection with *S. asperula* has been accepted. Nomenclatural stability is best served by typifying the species based on its modern usage (Hawksworth 1993). Since there is no extant type specimen nor any living teleomorphic isolate (see Morton and Smith 1963), UAMH 9489 derived from a mated cross is selected as neotype of *M. niger* and the above redescription is provided.

Hughes (1958) recognized that Saccardo's (1882) species *Torula asperula* was a species of *Scopulariopsis* similar to, but distinct from, *S. brevicaulis.* Morton and Smith (1963) separated *S. asperula* from *S. fusca* by its coarsely ornamented, rather than smooth conidia, and both names are widely used at present (e.g., Domsch et al 1993). Based on morphological similarities between type and representative strains, we suspected that *S. asperula, S. fusca, S. arnoldii, S. bestae* and *S. roseola* were conspecific, and this is confirmed here by mating tests. More than 25 isolates representing these taxa were studied, and conidial wall ornamentaion ranged from smooth to coarsely ornamented. However, the majority of conidia exhibited an intermediate roughening somewhat less pronounced than that typically seen in *S. brevicaulis* (compare FIGS. 10 AND 12). Morton and Smith (1963) listed *S. arnoldii* as a synonym of *S. asperula* based on the verrucose conidia described in the protologue, but they disposed of an authentic culture of Mangin under *S. fusca* since it produced only smooth conidia. UAMH 923 is an authentic, probable ex-type culture of *S. arnoldii* and it exhibits both smooth and ornamented conidia, further demonstrating variation found within this species. Morton and Smith (1963) treated *S. bestae* as a synonym of *S. koningii* but the ex-type culture exhibits the typical fuscous brown colony coloration of *S. asperula,* rather than the sandy tan or avellaneous colors typical of *S. brevicaulis* isolates (including *S. koningii,* see discussion under *Microascus brevicaulis*).

Specimens examined. Microascus niger. CANADA. ALBERTA: UAMH, mated cross of UAMH 8362 × 9037, 17 Apr 1998, *S.P. Abbott* (NEOTYPE and ex-neotype culture UAMH 9489); mated cross of UAMH 7879 × 9037, 17 Apr 1998, *S.P. Abbott* (UAMH 9490); mated cross of UAMH 8847 × 9037, 17 Apr 1998, *S.P. Abbott* (UAMH 9491). *Scopulariopsis asperula.* AUSTRIA. Carcass of rabbit, *F. Zach,* ex-type culture of *Scopulariopsis fusca,* (UAMH 930). CANADA. ALBERTA: Girouxville, indoor air of honeybee (*Apis mellifera*) overwintering facility, 30 Jan 1994, *S.P. Abbott OHS 207,* (UAMH 7879); Leduc, dung of striped skunk (*Mephitis mephitis*), 10 Jun 1997, *S.P. Abbott SA-M183,* (UAMH 9029). SASKATCHEWAN: Saskatoon, outside air, 28 Nov 1994, *S.P. Abbott SA-M24,* (UAMH 9037). ONTARIO: Ottawa, surface swab from home, Dec 1995, *D. Malloch M22–2C,* (UAMH 8362). FRANCE. *L. Mangin,* authentic, probable ex-type culture of *Scopulariopsis arnoldii,* obtained from Centraalbureau voor Schimmelcultures, (UAMH 923). ITALY. *Pollacci,* ex-type culture of *Torula bestae,* (UAMH 924). JAPAN. Wheat flour, 1962, *N. Inagaki I-391,* obtained from Institute for Fermentation, Osaka as IFO 7564, ex-type culture of *Scopulariopsis roseola,* (UAMH 8847). UNITED STATES. MISSOURI: Hay, 12 Feb 1992, *D.T. Wicklow DTW-001,* obtained from United States Department of Agriculture as NRRL A-28654, (UAMH 8984).

DISCUSSION

Mating trials provided evidence of heterothallism and confirmed that two of the six taxa included within the '*S. brevicaulis* series' could be reduced to synonymy. Holomorph taxa *M. brevicaulis, M. manginii,* and *M. niger* include anamorphs *S. brevicaulis* (synonym *S. koningii*), *S. candida,* and *S. asperula* (synonyms *S. fusca, S. arnoldii, S. bestae, S. fusca,* and *S. roseola*), respectively. No responses were observed between mating strains representing each holomorph species. The status of *S. flava* was unresolved in this study due to a lack of authentic strains.

The three taxa are easily separable by their colonial coloration. Colonies of *M. brevicaulis* are pale to medium sandy tan or avellaneous. Those of *M. niger* are typically much darker brown and always exhibit a pronounced fuscous or violaceous tint. *M. manginii* colonies are white to cream or very pale yellow if heavily conidial but are occasionally dominated by black perithecia. In contrast, perithecia are obscured by dominant conidial growth in isolates of *M. brevicaulis* and *M. niger.* Conidial wall ornamentation, previously a primary character for defining anamorph taxa, was found to be not very reliable and probably accounts for much of the previous confusion resulting in application of different names for isolates with slight variation.

Positive crosses were easiest to detect when strains were streaked onto opposite halves of the petri plate, rather than mixed together. Strains typically grew robustly on their respective sides but formed a central contact zone with sparse conidial growth. Fertile ascomata formed within this central zone, sometimes submerged in the agar, and were best observed using a dissecting microscope. Frequently, perithecia of *M. brevicaulis* and *M. niger* were detected at the edge of

the petri dish submerged in the agar in the central zone. Ascomata were more difficult to detect when conidial suspensions were mixed, since ascomata occurred in a random fashion, in clusters and radiating lines throughout the colony, and were frequently obscured by heavy conidial overgrowth.

Mating systems in the ascomycetes vary. Homothallism is most common, but both heterothallism and homothallism occur among different orders, families and genera of ascomycetes. Yun et al (1999) presented a good argument that homothallism is a derived state and that heterothallism is ancestral in the ascomycetes. Most heterothallic ascomycetes demonstrate a simple (single-locus, two-idiomorph) mating system, although a more complex heterothallic system (unbalanced heterothallism) controlled by multiple, multiallelic loci was demonstrated in *Glomerella* (Vaillancourt et al 2000). Compatibility has rarely been investigated within the Microascaceae. Homothallism was reported in *M. trigonosporus* (Emmons and Dodge 1931) but this is the first report of heterothallism within the genus *Microascus*. The results of mating trials are consistent with a single-locus, two-idiomorph mating system (see TABLE II). The mating systems of species within other microascaceous genera (*Kernia, Lophotrichus, Petriella, Pseudallescheria*) are poorly known. No fertility has been found in crosses among isolates of *Scedosporium apiospermum* (Sacc.) Castell. & Chalmers, the anamorph of *Pseudallescheria boydii* (Shear) McGinnis, Padhye & Ajello (Mann 1981). The results of the present study prompted investigation of one species from the genus *Kernia*. Nine single ascospore isolates obtained from *K. pachypleura* Malloch & Cain (UAMH 8857) were strictly anamorphic, but failed to produce ascomata in back crossings after 11 mo on OAT (Abbott unpubl). Further investigation is needed to elucidate the genetics of sexual reproduction in other members of the family.

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