

Taxa of *Pilaira* (Mucorales, Zygomycota) from China

by

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With 2 figures and 2 tables

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Abstract: Four taxa of the genus *Pilaira*, *P. anomala*, *P. moreaui* var. *caucasica* comb. nov., *P. praeampla* sp. nov., and *P. subangularis* sp. nov., were newly recorded from four different provinces of China. Descriptions, Latin diagnoses, and line drawings are provided for the four Chinese taxa. A tentative key to all recognizable taxa of the genus is also provided.

Key words: taxonomy, morphology, new record, new combination, new species.

Introduction

Pilaira Tiegh. (1875) is one of the three closely related small genera comprising the family Pilobolaceae of the order Mucorales. Like *Pilobolus* Tode (1784): Fr. and *Utharomyces* Boedijn (1959), all members of *Pilaira* are coprophilous and possess hemispherical and cutinized sporangia. However, *Pilobolus* has both subsporangial swellings and trophocysts, and the sporangia are forcibly shot away when mature; *Utharomyces* has also subsporangial swellings and trophocysts, but the sporangia are not violently discharged; whilst in *Pilaira*, neither subsporangial swellings nor trophocysts are formed, and the sporangia are not actively discharged. More distinctions among the three genera were summarized in detail by Alexopoulos et al. (1996). Since *Pilaira* and *Pilobolus* are so similar in their growth habitat and morphology, Fitzpatrick (1930) doubted that *Pilaira* may only represent abnormal material of *Pilobolus*. In order to support the validity of *Pilaira*, Anderson (1933) isolated *Pilaira anomala* (Ces.) J.Schröt. and *Pilobolus crystallinus* Tode from rabbit dung and proved them to be distinct through carefully designed experiments. Curiously,

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multigene phylogenetic analyses of the Mucorales by O'Donnell et al. (2001) placed *Pilaira anomala* distantly from *Pilobolus umbonatus*, close to *Mucor mucedo*.

All species of *Pilaira* seem to be very little studied especially taxonomically. *P. anomala* has been reported from many European and American countries (Anderson 1933, Bisby 1938, Grove 1934, Ling 1930, Mil'ko 1970, Namyslowski 1910, Oudemans & Koning 1902, Saccardo 1888, Sumstine 1910, etc.), but recent studies mainly concern its biology and not taxonomy (Harper & Webster 1964, Fletcher 1969, 1970, 1971, 1973, Wood & Cooke 1986, 1987). Other taxa, i.e. *P. caucasica* Milko (1970), *P. dimidiata* Grove (1884), *P. fimetaria* (Link) Pound (1894), *P. inosculans* Grove (1884), *P. moreaui* Y.Ling (1926), *P. nigrescens* Tiegh. (1875), and *P. saccardiana* Morini (1905), have not been rediscovered since they were described.

In Asia, the only species found is *P. anomala* from India (Nand & Mehrotra 1969), while in China, none has been reported until the present study.

Materials and methods

Cultures

The isolates studied are listed in Table 1. The Chinese strains were isolated and identified by us. Two of them, Pi-1 and Pi-2, died shortly after their morphology had been studied but before their maximum growth temperature and mating ability could be tested. Cultures from NRRL were kindly supplied by Dr. K.O'Donnell. Two of the lyophilized preparations of NRRL A-21850 and NRRL A-23502 did not survive when arrived and were not available for study. All living cultures are maintained in the Culture Collection of the Key Laboratory of Systematic Mycology and Lichenology (with a Pi-prefix) or the AS Culture Collection (with acronym AS), both of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. Dried cultures are deposited in the Herbarium Mycologicum Instituti Microbiologici Academiae Sinicae (HMAS).

Isolation

Almost all of the strains found in China were isolated from mouse or rabbit dung except one strain that was isolated from soil under cow dung. For dung samples, a small part of each was soaked in sterilized distilled water for 5-6 hours before using different dilutions to pour plates. For isolation from soil, Warcup's soil plates (Warcup 1950) were used.

Media and cultivation

Potato dextrose agar (PDA) adjusted to pH 7 was used for morphological studies, isolating, establishing temperature-growth relationships, and mating experiments. Cultivation period and temperature were 4-7 days at 18°C for isolating, 4-14 days at 18°C for morphological studies, 4 days at temperatures of 25-35°C for determining the maximum growth temperature, and 7-20 days at 18°C for mating experiments. To assess length of sporangiophores, cultures were prepared in 4 cm high glass Petri dishes. Microscopic measurements given with extreme values in brackets (without statistical treatment) are based on fresh material from culture mounted in water.

Results and discussion

Maximum growth temperature

The 21 living cultures, i.e. all strains listed in Table 1 except Pi-1 and Pi-2, were tested twice for their maximum growth temperature. The 5 taxa pertaining to the 4

Table 1. Species, varieties and strains of *Pilaira* studied.

Species and variety identified	Strain	Substratum	Origin	Remarks
<i>P. anomala</i>	Pi-2	mouse dung	Mengyuan, Qinghai	dead
<i>P. anomala</i>	NRRL 2526	-	-	
<i>P. anomala</i>	NRRL 2527	-	-	
<i>P. anomala</i>	NRRL A-5067	-	-	received as <i>P. cesatii</i>
<i>P. anomala</i>	NRRL A-5068	-	-	received as <i>P. cesatii</i>
<i>P. anomala</i>	NRRL A-7646	-	-	
<i>P. anomala</i>	NRRL A-9310	-	-	received as <i>P. sp.</i>
<i>P. anomala</i>	NRRL A-9789	-	-	
<i>P. anomala</i>	NRRL A-16347	-	-	
<i>P. anomala</i>	NRRL A-16348	-	-	
<i>P. anomala</i>	NRRL A-16356	-	-	
<i>P. anomala</i>	NRRL A-23912	-	-	
<i>P. moreaui</i>	Pi-1	mouse dung	Aletai, Xinjiang	dead
var. <i>caucasica</i>				
<i>P. moreaui</i>	Pi-4	dung of plateau pika	Hongyagou, Qinghai	
var. <i>caucasica</i>	= AS 3.6636			
<i>P. moreaui</i>	Pi-6	mouse dung	Xilinguolemeng, Inner Mongolia	
var. <i>caucasica</i>	= AS 3.6654			
<i>P. moreaui</i>	Pi-7	mouse dung	Xilinguolemeng, Inner Mongolia	
var. <i>caucasica</i>	= AS 3.6655			
<i>P. moreaui</i>	Pi-8	mouse dung	Xilinguolemeng, Inner Mongolia	
var. <i>caucasica</i>	= AS 3.6656			
<i>P. moreaui</i>	NRRL 6282(T)	mouse dung	Armenia, USSR	received as <i>P. caucasica</i>
var. <i>caucasica</i>				
<i>P. moreaui</i>	NRRL 6283	-	-	
var. <i>moreaui</i>				
<i>P. moreaui</i>	NRRL 6284	-	-	
var. <i>moreaui</i>				
<i>P. moreaui</i>	NRRL A-7399	-	-	received as <i>P. anomala</i>
var. <i>moreaui</i>				
<i>P. praeampla</i>	Pi-5	soil under	Poyanghu, Jiangxi	
= AS 3.6638(T)		cow dung		
<i>P. subangularis</i>	Pi-3	dung of plateau pika	Hongyagou, Qinghai	
= AS 3.6637(T)				

species had a very similar range of maximum growth temperature [(29-)30-33°C] (Table 2). This may reflect the close affinity of these taxa to each other. On the other hand, the number of strains is too small to assess the exact range for each taxon. Since the results obtained are not distinctive enough to differentiate taxa, the maximum growth temperature was not used in this study.

Mating experiments

The same 21 living cultures mentioned above were crossed in all combinations for zygospore formation, all with negative results except NRRL 2526(+) × NRRL 2527(-). Gametangia were found abundantly in crossing between NRRL A-5067(+) × NRRL

Table 2. Maximum growth temperature of the *Pilaira* species.

Species and strain	°C	Species and strain	°C
<i>P. anomala</i>	(29-)30-32	<i>P. moreaui</i> var. <i>moreaui</i>	(30-)31-32
NRRL 2526	32, 31	NRRL 6283	30, 31
NRRL 2527	32, 32	NRRL 6284	32, 32
NRRL A-5067	29, 31	NRRL A-7399	32, 31
NRRL A-5068	30, 32	<i>P. moreaui</i> var. <i>caucasica</i>	(29-)32-33
NRRL A-7646	30, 31	Pi-4	29, 30
NRRL A-9310	32, 31	Pi-6	32, 33
NRRL A-9789	31, 31	Pi-7	32, 33
NRRL A-16347	30, 31	Pi-8	32, 33
NRRL A-16348	32, 33	NRRL 6282 (T)	32, 31
NRRL A-16356	31, 32	<i>P. praeampla</i>	30-32
NRRL A-23912	31, 31	Pi-5 (T)	30, 32
		<i>P. subangularis</i>	31

2527(-), but scarcely between NRRL 2526(+) × NRRL A-16356(-), NRRL 2526(+) × NRRL A-23912(-), and NRRL A-16347(+) × NRRL 2527(-). All these strains belong to *P. anomala*, for which only the mating types of NRRL 2526(+) and NRRL 2527(-) were known when received. According to gametangial formation, the mating types of the other strains could be inferred.

Morphological studies

Ling (1926) was the first author who provided a key for dividing the species in *Pilaira*. Morphological features used in his key included only the shape and length of the sporangiospores, and whether the sporangiophores are ramified or not. Grove (1934) Zycha (1935), and Zycha et al. (1969) accepted this key. Naumov (1939) added the diameter of sporangia and the color of sporangiospores to his key. In our study, besides the characteristics just mentioned, we found the width, septation, and shape (straight, curved, undulate, or geniculate) of sporangiophores, with or without swellings, the height of the sporangia, size, shape and color of the columellae, with or without a papilla, presence or absence and distinctness of the collar, angulation of the sporangiospores to be useful morphological features for distinguishing *Pilaira* taxa. Although the height of the sporangiophores may be affected by environmental conditions, the range of height in each taxon is rather stable, when measured for cultures grown on the same medium and under the same temperature and light conditions.

Taxonomy

Eight strains isolated from samples collected in China were studied and identified as *P. anomala* (Pi-2), *P. moreaui* var. *caucasica* (Milko) R.Y.Zheng & X.Y.Liu (Pi-1, Pi-4, Pi-6, Pi-7, and Pi-8), *P. praeampla* R.Y.Zheng & X.Y.Liu (Pi-5), and

P. subangularis R.Y.Zheng & X.Y.Liu (Pi-3). Fifteen strains from NRRL with names already provided were restudied: *P. anomala* [NRRL 2526, NRRL 2527, NRRL A-7646, NRRL A-9789, NRRL A-16347, NRRL A-16348, NRRL A-16356, and NRRL A-23912; NRRL A-5067 and NRRL A-5068 (received as *P. cesatii* (Coem.) Tiegh.), and NRRL A-9310 (received as *P. sp.*)], *P. moreaui* var. *moreaui* [NRRL 6283, NRRL 6284, and NRRL A-7399 (the latter received as *P. anomala*)], and *P. moreaui* var. *caucasica* [NRRL 6282 (received as *P. caucasica*)].

The four taxa isolated from China are described in detail and reasons for their taxonomic treatments are discussed under each description as follows:

Piaira anomala (Ces.) J.Schröt., in: Cohn's Krypt.-Fl. Schlesien 2: 211 (1886).

Figs 1.1-1.3

≡ *Pilobolus anomalus* Ces., in: Klotzsch, Herb. viv. mycol. no. 1542 (1851) (basionym).

= *Ascophora cesatii* Coem., Monographie du genre *Pilobolus* Tode, p. 63 (1861).

≡ *Pilaira cesatii* (Coem.) Tiegh., Ann. Sci. Nat. Bot. Sér. 6, 1: 52 (1875).

= ?*Pilobolus mucedo* Bref., Bot. Unters. Schimmelpilze. Part 1: 27 (1872).

COLONIES on PDA floccose, filling the Petri dish in 6-7 days at 18°C, grayish white, reverse grayish yellow, 15 mm high. ODOR none. HYPHAE branching, nonseptate when young, septate in age, aerial hyphae (3.5-)5.5-8(-10) µm, submerged hyphae (6-)9-23(-32) µm wide. STOLONS absent. RHIZOIDS absent. SPORANGIOPHORES arising from submerged mycelia, simple, 10-60 mm high, (14-)23.5-47 µm wide, usually equal in width throughout, straight to substraight when young, soon shriveling and collapsed, nonseptate, subhyaline to dirty white, sometimes pale brownish, terminating in a sporangium. SUBSPORANGIAL SWELLINGS absent. TROPHOCYSTS absent. SPORANGIA hemispherical, upper part dark, thick, and cutinized, below hyaline, thin and gelatinous, when mature greatly distended and disappearing, (30-)45-90 µm high, (60-)82-175 (-210) µm diam., not forcibly discharged when mature. COLUMELLAE mostly applanate, (35-)44-95 × (53-)70-140 µm, pale to light orange, rarely conical and reaching a height of 120 µm, mostly without collar, rarely with a small collar. APOPHYSES present, orange to brownish. SPORANGIOSPORES ovoid, quite uniform in shape, (5.5-)7-12.5 × (4-)5.5-8 µm, not becoming irregular in shape at 28-30°C, subhyaline to yellowish. ZYGOSPORANGIA globose and 105-180 µm diam., or ovoid and 125-160 × 105-150 µm, subhyaline to pale brownish yellow, smooth. ZYGOSPORES globose and 85-150 µm diam., or ovoid and 108-130 × 85-120 µm, containing a large oil globule, hyaline. SUSPENSORS tong-shaped, 14-37 µm wide, the basal part of the two suspensors supporting a zygosporangium twisted tightly with each other, subhyaline. Heterothallic.

STRAINS STUDIED: P-2, isolated from mouse dung collected in Menyuan county, Qinghai Prov. by Wang Xue-wei (8952b), 27 June 2002 (not surviving after morphological studies). NRRL 2526, 2527, A-5067, A-5068, A-7646, A-9310, A-9789, A-16347, A-16348, A-16356, A-23912, all with unknown localities, unknown collectors, and unknown date of collections (see Table 1).

REMARKS: *P. anomala* is a common species in the genus. However, in China only one isolate has been found. This isolate agrees well with the foreign strains provided by NRRL in morphology, but did not react with any of them in mating experiments.

Inclusion of *P. saccardiana* Morini (1905) as one of the synonyms of this species by Zycha (1935) and Anonymous (2004) is not followed here. The diagnosis of *P. saccardiana* clearly indicated that the sporangiophores are simple or branched, rarely emerging from a rudimentary trophocyst, the columellae are short-conical and deep violet in color, and that the sporangiospores are hyaline but with a pallid violaceous membrane. Branching sporangiophores or trophocysts have never been reported in *P. anomala* or any other taxon, columellae are mostly applanate and never deep violet in color, and a pallid violaceous membrane of the sporangiospores is always absent in *P. anomala*. The two names could not apply to the same species.

In Index Fungorum (Anonymous 2004), the names *Pilaira mucedo* Bref. and *Pilobolus mucedo* Bref. were cited respectively in the species lists of the genera of *Pilaira* and *Pilobolus* without indicating the years of publication. A search in the literature reveals that *Pilaira mucedo* Bref. has never been reported, but *Pilobolus mucedo* Bref. was reported in *Botanische Untersuchungen über Schimmelpilze*, Vol. 1: 27, 1872. Saccardo (1888), Grove (1934), and Mil'ko (1970) considered *Pilobolus mucedo* synonymous with *Pilaira anomala*. The diagnosis of *Pilobolus mucedo* Bref. (Brefeld, 1872) is extremely short and meaningless, and no type material is available for study; it is hard to affirm whether it is the same fungus as *P. anomala*.

In *Pilaira*, a zygosporic state has been reported from *P. anomala* only. Van Tieghem (1875) was perhaps the first author who discovered, described, and drew zygosporangia of *P. anomala*. The diameter of the zygosporangia (as 'zygosporangia') was described as 100-120 µm, but the size of the suspensors was not mentioned. In this study, a zygosporic state was also found in *P. anomala* only, the range of zygosporangial diameter was found to be much larger than given by van Tieghem. True zygosporangia and suspensors are described here for the first time.

Pilaira moreaui* var. *caucasica (Milko) R.Y.Zheng & X.Y.Liu, comb. nov.

Figs 1.4, 1.5

≡ *Pilaira caucasica* Milko, Mikol. Fitopatol. 4 (3): 262 (1970) (basionym).

COLONIES on PDA floccose, filling the Petri dish in 7-8 days at 18°C, grayish white, reverse grayish yellow, 10-15 mm high. ODOR none. HYPHAE branching, nonseptate when young, septate in age, aerial hyphae (3-)5-7(-14) µm, submerged hyphae (5.5-)14-18.5(-32) µm wide. STOLONS absent. RHIZOIDS absent. SPORANGIOPHORES arising from submerged mycelia, simple, (10-)20-30 mm high, (14-)30-60(-85) µm wide, subequal in width throughout, sometimes swollen at places, slightly, or not attenuated in the uppermost part, nonseptate, hyaline to pale brownish, terminating in a sporangium. SUBSPORANGIAL SWELLINGS absent. TROPHOCYSTS absent. SPORANGIA hemispherical, upper part dark, thick, and cutinized, below hyaline, thin and gelatinous, when mature greatly distended and disappearing, (23-)47-120 µm high, (47-)95-235 (-295) µm diam., not forcibly discharged when mature. COLUMELLAE mostly applanate, rarely roundish conical, (17-)35-60(-100) × (35-)70-117(-260) µm, upper portion orange to brownish orange, lower portion deep orange or brown, usually without collar, sometimes with a small collar. APOPHYSES present. SPORANGIOSPORES ellipsoid to oblong-ovoid, (7-)9-14(-16) × (4.5-)5.5-7(-8) µm, not becoming irregular in shape at 28-30°C, subhyaline to yellowish. ZYGOSPORES unknown, probably heterothallic.

STRAINS STUDIED: P-1, isolated from mouse dung collected in the suburb of Aletai, Xinjiang Uygur Autonomous Region, by Wang Xue-wei (8683a), 19 June 2002 (not surviving after morphological studies); Pi-4 (= AS 3.6636), isolated from dung of plateau pika (*Ochotona curzoniae*) collected in Yuanzhecun, Hongyagou, Qinghai Prov. by Lei Yan-ming (8944f), 5 August 2002 ; Pi-6 (= AS 3.6654), Pi-7 (= AS 3.6655), and Pi-8 (= AS 3.6656), isolated from different samples of mouse dung collected in Xilinguolemeng, Inner Mongolia Autonomous Region by Liu Xiao-yong (10657b, 19658, 10659, respectively), on the same day of 13 July 2003. NRRL 6282 (T), isolated from mouse dung collected in Armenia by A.A.Mil'ko.

REMARKS: According to our comparative studies among the 5 Chinese strains (Pi-1, 4, 6, 7, 8) and the ex-type strain (NRRL 6282) of *Pilaira caucasica* Milko and the 3 foreign strains including the type of *P. moreaui* Y.Ling [NRRL 6283 (T), 6284, A-7399], we found that *P. caucasica* resembles *P. moreaui* in many aspects. For example, both of them possess cylindrical-ellipsoid sporangiospores which are not found in any other taxon of the genus, similar range of sporangiophore width [(14-)30-60(-85) μm in the former and (11.5-)23.5-70(-95) μm in the latter]; similar range of sporangial diameter [(47-)95-235(-295) μm in the former and (53-)118-200(-305) μm in the latter], and similar shape of the columellae (mostly appanate). Hence, *P. caucasica* is reduced to varietal rank under *P. moreaui*. Var. *caucasica* and var. *moreaui* can be distinguished mainly by sporangiospore length, which is (8-)10-14(-16) μm in the former and 18-20(-24) μm in the latter; and the height of the sporangiophores which is 10-20(-30) mm in the former and 100-120 mm in the latter.

***Pilaira praeampla* R.Y.Zheng & X.Y.Liu, sp. nov.**

Fig. 2

COLONIAE in PDA in 18°C floccosae, 15-18 mm altae, primum albae deinde sordide albae. HYPHAE aeriae (5-)8-11.5(-15.5) μm , submersae (5.5-)11-16(-23) μm latae. STOLONES absentes. RHIZOIDEA absentia. SPORANGIOPHORA ex hyphis submersis enata, simplicia, 6-12(-18) mm alta, (12-)23-95 (-220) μm lata, pluries subito inflata, vetusta 1-3(-5)-septata, hyalina ad dilute aurantiaca vel brunneolo-aurantiaca. INTUMESCENTIAE SUBSPORANGIALES absentes. TROPHOCYSTES absentes. SPORANGIA hemisphaerica, parte superiore nigra et cutinisata, parte inferiore hyalina et gelatinosa, (24-)95-245 μm alta, (35-)130-325 μm diam., matura non ejicientia. COLUMELLAE rotundo-conicae ad breviter ellipsoideae, 64-165 \times 123-200 μm , interdum globosae ad subglobosae, 65-180 μm diam., raro appanatae in minoribus et 17-72 \times 23-130 μm , dilute vel intense aurantiacae ad brunneolo-aurantiacae, plerumque collari distincto praeditae. APOPHYSES conspicuae. SPORANGIOSPORAE ovoideae ad ellipsoideo-ovoideae, 7-10.5(-15) \times 5.5-7 μm 18°C, 28-30°C irregulares, ad 60 \times 25 μm . ZYGOSPORAE ignotae, verosimile heterothallicae.

ETYMOLOGY: praeampla; referring to the very wide sporangiophores.

COLONIES on PDA floccose, filling the Petri dish in 7-8 days at 18°C, dirty white in the upper portion, Apricot yellow in the lower portion and reverse, 15-18 mm high. ODOR none. HYPHAE branching, nonseptate when young, septate in age, aerial hyphae (5-)8-11.5(-15.5) μm , submerged hyphae (5.5-)11-16(-23) μm wide. STOLONS absent. RHIZOIDS absent. SPORANGIOPHORES arising from submerged mycelia, simple, 6-12(-18) mm high, soon collapsing, (12-)23-95(-220) μm wide, equal in width throughout but tapering at the uppermost portion and then constricted just beneath the sporangium, often suddenly swollen, straight, or often curved to undulate, rarely geniculate, nonseptate when young, 1-3(-5)-septate in age, hyaline to light orange or brownish orange, terminating in a sporangium. SUBSPORANGIAL SWELLINGS absent. TROPHOCYSTS absent. SPORANGIA hemispherical, upper part dark, thick, cutinized,

and not deliquescing, below hyaline, thin, gelatinous, at maturity greatly distended and disappearing, (24-)95-245 μm high, (35-)130-325 μm diam., not projecting when mature. COLUMELLAE roundish conical to short-ellipsoid and 64-165 \times 123-200 μm , sometimes globose to subglobose and 64-182 μm diam., rarely applanate in the smaller ones and 17-72 \times 23-130 μm , light or deep orange to brownish orange, mostly with a disitinct collar. APOPHYSES distinct. SPORANGIOSPORES ovoid to ellipsoid-ovoid, 7-10.5(-15) \times 5.5-7 μm at 18°C, irregular, reaching 60 μm in length and 25 μm in width at 28-30°C, yellowish when single, yellow to orange in mass. ZYGOSPORES unknown, probably heterothallic.

HOLOTYPE: HMAS 89523-1~11, dried culture prepared from Pi-5 (= AS 3.6638), isolated from soil under cow dung collected in Poyanghu district, Jiangxi Prov. by Wang Xue-wei (10210), 11 April 2002, are preserved in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS). Living cultures (Pi-5, = AS 3.6638) are deposited in the AS Culture Collection, also of the Institute of Microbiology, Chinese Academy of Sciences.

REMARKS: *Pilaira praeampla* differs strikingly from all the other species or varieties in the genus by having sporangiophores which are swollen in parts and reaching 200 μm wide, 1-3(-5)-septate when aged; and sporangia which are 95-245 μm high; the columellae are much higher (reaching 165 μm) and variously shaped, globose, ovoid, roundish conical, or campanulate, applanate only in the smaller ones, and mostly with a distinct collar; the sporangiospores are ovoid to ellipsoid-ovoid and regular in shape at 18-20°C, but irregular and reaching 60 μm in length at 28-30°C. In other taxa of *Pilaira*, the sporangiophores generally lack swellings, are less wide, and remain nonseptate; sporangia are much less high, not exceeding 150 μm diam.; the columellae usually do not exceed 100 μm , and are consistently applanate to roundish conical, mostly without, or only seldom with a small collar; the sporangiospores are not irregular in shape at 28-30°C.

***Pilaira subangularis* R.Y.Zheng & X.Y.Liu, sp. nov.**

Figs 1.6, 1.7

COLONIAE in PDA 18°C floccosae, 15 mm altae, dilute griseolo-albae. HYPHAE aerae (3-)5.5-7 (-8.5) μm , submersae (4.5-)9-13.5(-15) μm latae. STOLONES absentes. RHIZOIDEA absentia. SPORANGIOPHORA ex hyphis submersis enata, simplicia, 20-30 mm alta, cito tremulo collapsa, 7-15.5 μm lata, nonseptata, hyalina ad pallide brunneola. INTUMESCENTIAE SUBSPORANGIALES absentes. TROPHOCYSTES absentes. SPORANGIA hemisphaerica, parte superiore nigra et cutinisata, parte inferiore hyalina et gelatinosa, 23-95 μm alta, 47-150(-210) μm diam., matura non ejicientia. COLUMELLAE applanatae, 17-26(-35) \times 35-60(-80) μm , subhyalinae vel pallide brunneae ad pallide aurantiacae, plerumque collari absente. APOPHYSES conspicuae, intense luridae vel brunneae, granulosae. SPORANGIOSPORAE globosae ad subglobosae, 5-6(-8) μm diam., breviter ovoideae et 5.5-8 \times 5-7 μm , plerumque subangulares, 28-30°C non irregulares. ZYGOSPORAE ignotae, verosimile heterothallicae.

ETYMOLOGY: subangularis; referring to the angulation of the sporangiospores.

COLONIES on PDA floccose, filling the Petri dish in 6-7 days at 18°C, light grayish white, reverse light grayish yellow, 15 mm high. ODOR slightly aromatic. HYPHAE branching, nonseptate when young, septate in age, aerial hyphae (3-)5.5-7(-8.5) μm , submerged hyphae (4.5-)9-13(-15) μm wide. STOLONS absent. RHIZOIDS absent. SPORANGIOPHORES arising from submerged mycelia, simple, 20-30 mm high, soon shivering and collapsed, 7-15.5 μm wide, equal in width throughout, rarely swollen at places, straight to substraight, nonseptate, hyaline to pale or light brown, terminating in a sporangium. SUBSPORANGIAL SWELLINGS absent. TROPHOCYSTS absent. SPORANGIA

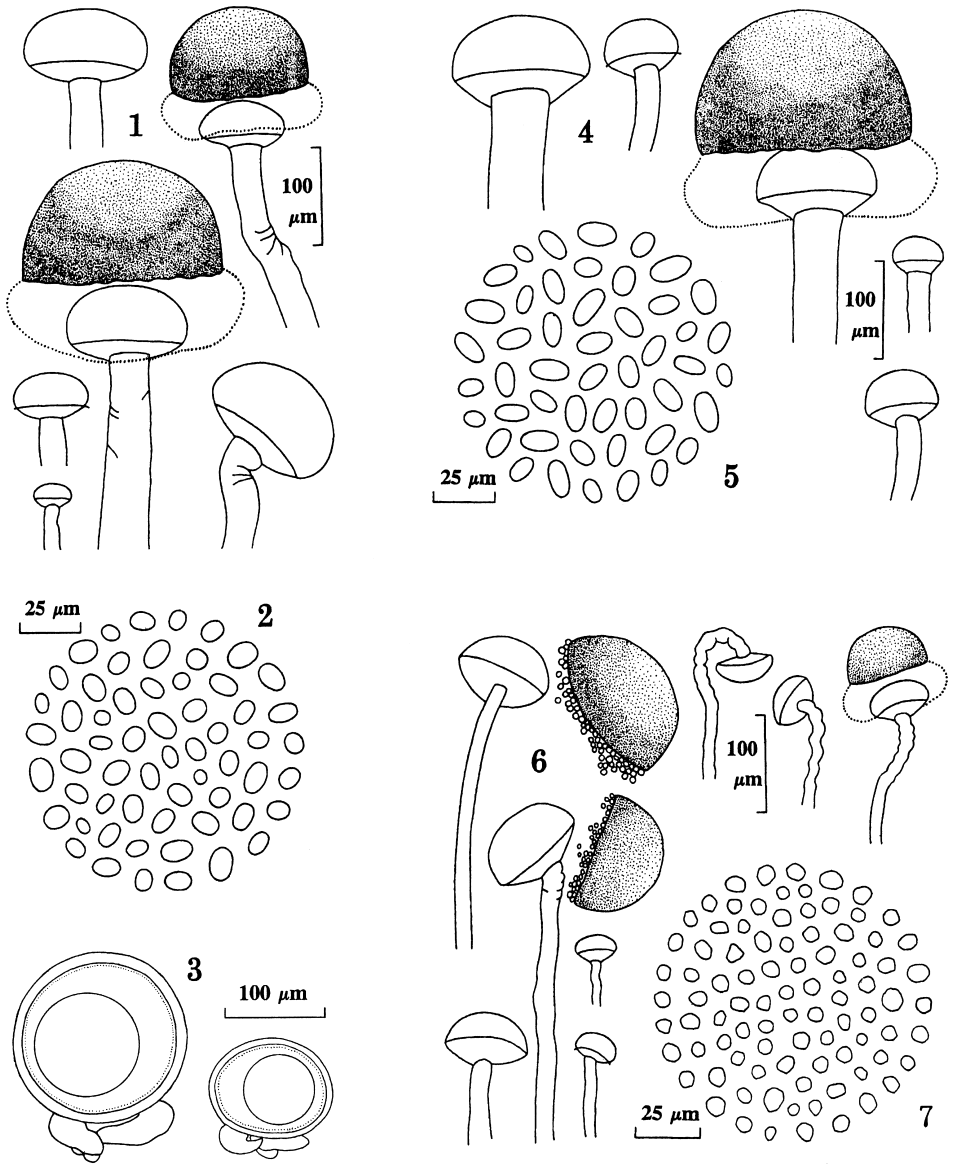


Fig. 1. *Pilaira* taxa. 1, 2, 3. *P. anomala* [Pi-2, NRRL 2526(+) × NRRL 2527(-)]; 4, 5. *P. moreauii* var. *caucasica* (Pi-4); 6, 7. *P. subangularis* (Pi-3, type). [1, 4, 6. Sporangioophores, sporangia, columellae, apophyses, and collars. 2, 5, 7. Sporangiospores. 3. Zygosporegia with suspensors].

hemispherical, upper part dark, thick and cutinized, lower part hyaline, thin, and gelatinous, when mature greatly distended and disappearing, 23-95 μm high, 45-150 (-210) μm diam., not projecting when mature. COLUMELLAE applanate, 17-26(-35) ×

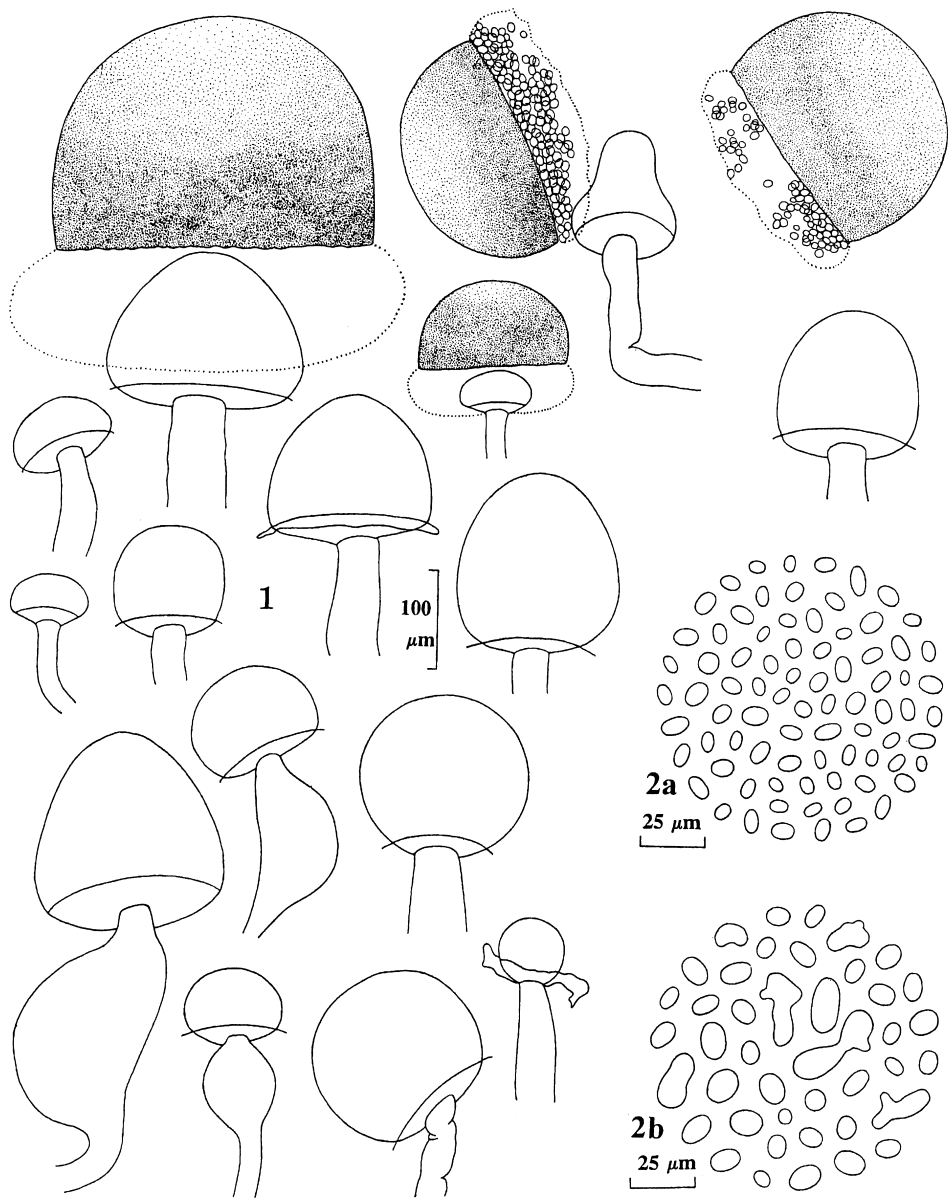


Fig. 2. *Pilaira praeampla*. 1. Sporangiophores, sporangia, columellae, and collars. 2a. Sporangiospores cultured at 18°C. 2b. At 28-30°C. [Pi-5, type].

35-60(-80) μm, subhyaline or pale brown to pale orange, usually without collar. APOPHYSES distinct, deep violet-brown or deep brown, granulate. SPORANGIOSPORES globose to subglobose and 5-6(-8) μm diam., short-ovoid and 5.5-8 × 5-7 μm, mostly

subangular, not becoming irregular in shape at 28-30°C, hyaline when single, light yellow in mass. ZYGOSPORES unknown, probably heterothallic.

HOLOTYPE: HMAS 89522-1~8, dried culture prepared from Pi-3 (= AS 3.6637), isolated from dung of *Ochotona curzonia* collected in Yuanzhecun, Hongyagou, Qinghai Prov. by Lei Yan-ming (8944a), 5 Aug. 2002, are preserved in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS). Living cultures (Pi-3, = AS 3.6637) are deposited in the AS Culture Collection, also of the Institute of Microbiology, Chinese Academy of Sciences.

REMARKS: Pi-3 and *P. nigrescens* are the only species of *Pilaira* that have globose sporangiospores. The two species differ in several respects: the spores of the former are slightly but distinctly angular, the sporangiophores are 20-30 mm high, and the columellae are mostly applanate and not papillate, subhyaline to pale brown or pale orange in color; the spores of the latter are not angular, the sporangiophores are 15-20 mm high, the columellae are roundish conical, papillate, and blackish-violaceous or bluish. For these reasons, Pi-3 is treated as a new species.

A tentative key to the species and varieties of *Pilaira**

1. Sporangiophores 12-95 µm wide, often swollen to 220 µm diam., 1-3(-5)-septate when aged, straight, curved, or undulate, sometimes geniculate; sporangia (24-)95-245 µm high and (35-)130-325 µm diam.; columellae 65-165 µm high and 123-200 µm diam., variously shaped, mostly with a distinct collar; sporangiospores ovoid to ellipsoid-ovoid, regular in shape at 18-20°C, at 28-30°C irregular and up to 60 µm long..... *P. praeampla*
1. Sporangiophores less wide, generally without swellings, usually remaining nonseptate, not undulate, never geniculate; sporangia and columellae smaller, the latter of regular shape, mostly without a recognizable collar; sporangiospores of regular shape even at 28-30°C..... 2
2. Spores globose to subglobose, angular in one species..... 3
2. Spores ovoid, oblong-ovoid or ellipsoid-fusoid..... 4
3. Sporangiophores 15-20 mm high; sporangia reaching 100 µm diam.; columellae roundish conical and papillate; sporangiospores globose, not angular..... *P. nigrescens*
3. Sporangiophores 20-30 mm high; sporangia 47-150(-210) µm diam.; columellae mostly applanate and not papillate; sporangiospores globose to subglobose, slightly angular..... *P. subangularis*
4. Sporangiophores short, at first 0.5-1 mm long, then bending towards the substratum and becoming 3-4 mm long, tapering upwards; the lower and thin-walled part and the upper and cutinized part of the sporangia nearly equal in height *P. dimidiata*
4. Sporangiophores usually longer than 10 mm; the lower and thin-walled part of the sporangia shorter than the upper cutinized part 5
5. Sporangiospores ovoid to ellipsoid, usually not exceeding 12.5 µm in length..... 6
5. Sporangiospores cylindrical-ellipsoid to fusoid, usually reaching 16 µm or more in length..... 7
6. Sporangiophores simple or branched, rarely emerging from a rudimentary trophocyst; columellae short-conical, deep violet; sporangiospores 7-10 µm long, hyaline but with a smooth pallid violaceous membrane..... *Pilaira saccardiana*
6. Sporangiophores always simple, never emerging from a trophocyst; columellae mostly applanate, pale to light orange; sporangiospores (5.5-)7-12.5 µm long, hyaline, yellowish in mass, membrane not pallid violaceous..... *P. anomala*
7. Sporangiophores 10-12 cm high; sporangia (80-)300-400 µm diam.; columellae 150-180 µm diam.; sporangiospores 18-20(-24) µm long..... *P. moreaui* var. *moreaui*
7. Sporangiophores not exceeding 3.0 cm; sporangia (47-)95-235(-295) µm diam.; columellae (35-)70-117(-260) µm diam.; sporangiospores (7-)9-14(-16) µm long..... *P. moreaui* var. *caucasica*

*The key includes 8 taxa, only five of them (*P. anomala*, *P. moreaui* var. *moreaui*, *P. moreaui* var. *caucasica*, *P. praeampla*, and *P. subangularis*) have been studied, the other three (*P. dimidiata*, *P. nigrescens* and *P. saccardiana*) were not available for study, characteristics for their differentiation were adopted from the original, usually very short diagnoses. This key is therefore only tentative.

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References

- ALEXOPOULOS, C.J., C.W. MIMS & M. BLACKWELL (1996): Introductory Mycology 4th ed. - John Wiley & Sons, Inc., New York.
- ANDERSON, R.S. (1933): The validity of the genus *Pilaira*. - University of Iowa Studies **15**: 3-5.
- ANONYMOUS (2004): The CABI Bioscience Database of Fungal Names (Index Fungorum, <http://www.indexfungorum.org/Names/Names.asp>).
- BERLESE, A.N. & J.B. DE TONI (1888): Phycomycetae. - In: SACCARDO, P.A. Sylloge Fungorum **7**: 181-233. R. Freidländer & Sohn, Leipzig.
- BISBY, G.R. (1938): The fungi of Manitoba and Saskatchewan. - Ottawa, National Research Council of Canada.
- BOEDIJN, K.B. (1958 [1959]): Notes on the Mucorales of Indonesia. - Sydowia **12**: 321-362.
- BREFELD, O. (1872): Botanische Untersuchungen über Schimmelpilze. Vol. 1. Zygomyceten. - Arthur Felix, Leipzig.
- FITZPATRICK, H.M. (1930): The Lower Fungi. Phycomycetes. - McGraw-Hill Book Co., New York.
- FLETCHER, H.J. (1969): The development and tropisms of the sporangiophores of *Pilaira anomala*. - Trans. Brit. Mycol. Soc. **53**: 130-132.
- FLETCHER, H.J. (1970): Problems in the nutrition of *Pilaira anomala*. - Trans. Brit. Mycol. Soc. **54**: 485-488.
- FLETCHER, H.J. (1971): Some aspects of the biology of *Pilaira anomala* - an extremely versatile fungus. - J. Biol. Educ. **5**: 229-237.
- FLETCHER, H.J. (1973): The sporangiophore of *Pilaira* species. - Trans. Brit. Mycol. Soc. **61**: 553-568.
- GROVE, W.B. (1884): New or noteworthy fungi. - J. Bot. **22**: 132.
- GROVE, W.B. (1934): A systematic account and arrangement of the Pilobolidae. - In: BULLER, A.H.R. (1934): Researches on Fungi, Vol. VI: 190-224. Longmans, Green and Co., London, United Kingdom.
- HARPER, J.E. & J. WEBSTER (1964): An experimental analysis of the coprophilous fungus succession. - Trans. Brit. Mycol. Soc. **47**: 511-530.

- LING, Y. (1926): Étude morphologique, cytologique, & microchimique d'une nouvelle Mucorinée, *Pilaira moreau* sp. nov. - Mem. Ac. Sc. Clermont-Ferrand: 1-15.
- LING, Y. (1930): Étude des phénomènes de la sexualité chez les Mucorinées. - Rev. Gén. Bot. **42**: 72-743. Faculté de Sciences de l'Université de Clermont, France, Clermont-Ferrand, G. de Bussac, Cours Sablou, France.
- MIL'KO, A.A. (1970): *Pilaira caucasica* Milko and related species - Mikol. Fitopatol. **4**: 262-264. (in Russian).
- MORINI, F. (1905): Materiali per una monografia delle Pilobolee. - Mem. Reale Accad. Sci. Ist. Bologna, Ser. 6, **3**: 111-129.
- NAMYSŁOWSKI, B. (1910): Studien über Mucorineen. - Bull. Int. Acad. Sci. Cracovie. Cl. Sci. Math., Sér. B, Sci. Nat. **1910**: 477-520.
- NAND, K. & B.S. MEHROTRA (1969): Species of *Pilobolus* and *Pilaira* from India. - Sydowia **22**: 299-306.
- NAUMOV, N.A. (1939): Clés des Mucorinées (Mucorales). - Encyl. Mycol. **9**: 1-137.
- O'DONNELL, K., F.M. LUTZONI, T.J. WARD & G.L. BENNY (2001): Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. - Mycologia **93**: 286-296.
- OUDEMANS, C.A.J.A. & C.J. KONING (1902): Prodrome d'une Flore mycologique obtenue par la culture sur gélatine préparée de la terre humeuse du Spanderswoud près de Bussum. - Arch. Néerl. Sci. Exact. Nat., Sér. 2, **7**: 266-298.
- POUND, R.A. (1894): A revision of the Mucoraceae with special reference to species reported from North America. - Minnesota Bot. Stud. **9**: 87-104.
- SACCARDO, P.A. (1888): Phycomyceteae. Sylloge Fungorum **7**: 181-233. Padova.
- SUMSTINE, D.R. (1910): The North American Mucorales-I. - Mycologia **2**: 125-154.
- TIEGHEM, P. VAN (1875): Nouvelles recherches sur les Mucorinées. - Ann. Sci. Nat., Bot., Sér. 6, **1**: 5-175.
- TODE, H.J. (1784): Beschreibung des Hutwerfers (*Pilobolus*). - Schriften Berlin. Ges. Naturf. Freunde **5**: 46-52.
- WARCUP, J.H. (1950): The soil-plate method for isolation of fungi from soil. - Nature **166**: 117-118.
- WOOD, S.N. & R.C. COOKE (1986): Effect of *Piptocephalis* species on growth and sporulation of *Pilaira anomala*. - Trans. Brit. Mycol. Soc. **86**: 672-674.
- WOOD, S.N. & R.C. COOKE (1987): Nutritional competence of *Pilaira anomala* in relation to exploitation of faecal resource units. - Trans. Brit. Mycol. Soc. **88**: 247-255
- ZYCHA, H. (1935): Mucorineae. - In: Kryptogamenflora der Mark Brandenburg, VIa: 1-264. Gebrüder Bornträger, Leipzig.
- ZYCHA, H., R. SIEPMANN & G. LINNEMANN (1969): Mucorales eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe. - Lehre, J. Cramer.

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