More *Rhizomucor* causing human mucormycosis from China: *R. chlamydosporus* sp. nov.

Ru-Yong Zheng[1], Xiao-Yong Liu[2] & Ruo-Yu Li[3]

[1, 2] Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
[3] Dermatological Department, First Hospital of Beijing Medical University Beijing 100034, China
e-mails: zhengry@im.ac.cn, liuxiaoyong@im.ac.cn & lrymm@medmail.com.cn

Zheng R.Y., Liu X.Y. & Li R.Y. (2009) More *Rhizomucor* causing human mucormycosis from China: *R. chlamydosporus* sp. nov. – Sydowia 61 (1): 135–147.

In China, *Rhizomucor pusillus*, *R. variabilis* var. *variabilis* and *R. variabilis* var. *regularior* were known to be pathogenic to human beings. A new species, *R. chlamydosporus* sp. nov., was recently identified as the fourth member of the pathogenic *Rhizomucor* found in China. Distinguishing characteristics of the new species as well as its similarities and dissimilarities to the known taxa of the same genus are discussed. Reasons for raising *R. variabilis* var. *regularior* to specific level as *R. regularior* stat. & comb. nov. as well as for reducing *R. tauricus* to varietal status as *R. pusillus* var. *tauricus* stat. & comb. nov. in this study are also discussed.

Keywords: Taxonomy, morphology, molecular systematics, maximum growth temperature, mating experiments.

Rhizomucor is a small genus of the Mucoraceae established by Lucet and Costantin in 1900. In Index Fungorum (Anonymous 2004), ten names pertaining to *Rhizomucor* were listed: *R. endophyticus* R.Y. Zheng & H. Jiang (1995), *R. miehei* (Cooney & R. Emers.) Schipper (1978), *R. nainitalensis* M.C. Joshi (1982), *R. pakistanicus* M. Qureshi & J.H. Mirza (1983), *R. parasiticus* (Lucet & Costantin) Lucet & Costantin (1900), *R. pusillus* (Lindt) Schipper (1978), *R. septatus* (Bezold) Lucet & Costantin (1901), *R. tauricus* (Milko & Schkur.) Schipper (1978), *R. variabilis* R.Y. Zheng & G.Q. Chen var. *variabilis* (1991), and *R. variabilis* var. *regularior* R.Y. Zheng & G.Q. Chen (1993). Three of them, *R. pakistanicus*, *R. parasiticus* and *R. septatus* are synonyms of *R. pusillus* (Schipper 1978, Zheng & Chen 1991). The remaining seven taxa are tenable (Zheng & Jiang 1995). Besides, a name assigned to *Mucor*, *M. miehei* Cooney & R. Emers. var. *minor* Subrahmanyam & Gopalkrishnan (1984) should be treated as a synonym of *R. miehei*, since *M. miehei* is the basionym of *R. miehei*; also that *M. miehei* var. *minor* differs from *R. miehei* only in the slightly smaller size of sporangiospores which does not warrant recognition even at the varietal rank (Zheng & Chen 1991).

Recently, a culture (Rm-33) isolated from the wounded right backsides of a patient which has been identified as '*Mucor rouxianus*' (with no author citation) by someone was sent to us for re-identification from Dr. Cao Yu-Chun of the Department of Dermatology, Tongji Hospital, Wuhan, Hubei province. The re-identification resulted in a discovery of another *Rhizomucor* closely related to *R. variabilis* var. *variabilis* and *R. variabilis* var. *regularior*. After comparative studies on morphology, maximum growth temperature, and mating compatibility among all the *Rhizomucor* taxa we have at hand, the strain Rm-33 was proved to be a new species named *R. chlamydosporus* sp. nov.; *R. variabilis* var. *regularior* is raised to specific level as *R. regularior* stat. & comb. nov., and *R. tauricus* is reduced to varietal status as *R. pusillus* var. *tauricus* stat. & comb. nov. Molecular systematics (comprehensive studies on *cox1*, *cox2*, *cox3*, *pyrG*, SSU, and ITS rDNA) will be published in detail in a separate paper, whereas results of molecular studies concerning the validity of the taxa mentioned above will be discussed briefly in the present paper.

Materials and Methods

Cultures

The strains studied are listed in Tab. 1. In all the *Rhizomucor* taxa we recognized, only one, *R. nainitalensis*, was not available for study. Ex-type cultures of *R. miehei* and *R. pusillus*, were obtained through exchange from Centraalbureau voor Schimmelcultures (CBS), and *R. pusillus* var. *tauricus* (originally published as *R. tauricus* by Schipper 1978) from the USDA Agricultural Research Service Collection (NRRL); those of *R. endophyticus*, *R. variabilis*, and *R. regularior* (originally published as *R. variabilis* var. *regularior* by Zheng & Chen 1993) are from our own research group; the ex-type culture of *R. chlamydosporus* (Rm-33) was supplied by the Tongji Hospital, Hubei Province. Ex-type cultures of the seven taxa are preserved in the Culture Collection of the Key Laboratory of Systematic Mycology & Lichenology (with a Rm- prefix) or the AS Culture Collection (with acronym AS), dried cultures are deposited in the Herbarium Mycologicum Instituti Microbiologici Academiae Sinicae (HMAS), both of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. Other non-ex-type cultures were mainly isolated by our research group.

Isolation

Most of the strains were supplied by culture collections (CBS 182.67, CBS 231.29, CBS 354.68, NRRL 3695), hospitals (Rm-6, Rm-7,

Table 1. Strains of *Rhizomucor* studied

Species or variety	Strain	Mating type	Substratum	Origin
R. chlamydosporus	Rm-33 (T)	+	backside lesion of a patient	Hubei Prov.
R. endophyticus	Rm-8 (T)	?	wheat leaf	Beijing
R. miehei	CBS 182.67 (T)	?	retting guayule shrub	USA
R. pusillus var. *pusillus*	CBS 231.29	+	——	Switzerland
R. pusillus var. *pusillus*	CBS 354.68 (NT)	?	cornmeal	Netherlands
R. pusillus var. *pusillus*	Rm-1	+	hen-house grasses	Beijing
R. pusillus var. *pusillus*	Rm-2	?	——	Japan
R. pusillus var. *pusillus*	Rm-4	+	cornmeal protein	Shaanxi Prov.
R. pusillus var. *pusillus*	Rm-5	–	hill soil	Xinjiang Prov
R. pusillus var. *tauricus**	NRRL 3695 (T)	?	forest soil	Ukraine
*R. regularior***	Rm-7 (T)	?	facial lesion of a patient	Hubei Prov.
R. regularior	Rm-20	?	fermented bean curd	Sichuan Prov.
R. variabilis	Rm-6 (T)	?	hand lesion of a patient	Jiangsu Prov.
R. variabilis	Rm-9	?	human deep mycosis lesion	Shandong – Prov.
R. variabilis	Rm-10	+	nose secretion of a patient	Sichuan Prov.
R. variabilis	Rm-11	–	human rhinocere-bral lesion	Sichuan Prov.

* Originally published as *R. tauricus* (Schipper 1978).
** Originally published as *R. variabilis* var. *regularior* (Zheng & Chen 1993).

Rm-9, Rm-10, Rm-11, Rm-33), or isolated from samples supplied by friends (Rm-1, Rm-2, Rm-4, Rm-5, Rm-8, Rm-20). For detailed isolation method of the wheat leaf endophyte (Rm-8) see Zheng & Jiang (1995). Plant materials or food stuffs (Rm-1, Rm-2, Rm-4, Rm-20) were isolated by placing directly on agar plates, while soil particles (Rm-5) were isolated by using the soil plate method of Warcup (1950).

Media and cultivation

Modified SMA (Hesseltine's modified synthetic *Mucor* agar which contains dextrose 20 g, asparagine 2 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, thiamine chloride 0.5 mg, agar 20 g in 1000 mL distilled water, pH 7) was used for morphological studies. PDA adjusted to

pH 7 was used for establishing temperature-growth relationships and L-PDA (PDA added with 3% lecitin) for mating experiments. Cultivation period and temperature were 4-10 days at 40–42 °C for the thermophilic strains of *R. miehei*, *R. pusillus*, and *R. pusillus* var. *tauricus* (= *R. tauricus*), and at 26–28 °C for the mesophilic strains of *R. chlamydosporus*, *R. endophyticus*, *R. variabilis* and *R. regularior* (= *R. variabilis* var. *regularior*) for morphological studies; 14–21 days at 35–36 °C for the thermophilic strains and at 30–31 °C for the mesophilic strains for mating experiments; 4–7 days at 48-60 °C for the thermophilic strains and at 30–42 °C for the mesophilic strains for determining the temperature maximum.

Results and Discussion

Maximum growth temperature

All of the 16 strains representing the seven taxa of *Rhizomucor* used in this study were tested twice for their maximum growth temperature. The three earlier published taxa, *R. miehei*, *R. pusillus*, and *R. pusillus* var. *tauricus* (= *R. tauricus*) were already known as thermophiles. The maximum growth temperature for them as indicated by Schipper (1978) was respectively 57 °C, 56 °C, and 56 °C. In our study, *R. miehei* also has a maximum growth temperature of 57 °C, *R. pusillus* has a wider range of (53–) 56 (–57) °C, while that of *R. pusillus* var. *tauricus* was much lower (46 °C), although the strain we used (NRRL 3695) is the same as Schipper (1978) has used (CBS 179.69). These two cultures are the same fungus preserved in two different culture collections with two different numbers. The other four species found in China are all mesophiles with the following temperature maximum: *R. chlamydosporus* 39 °C, *R. endophyticus* 32–34 °C, *R. regularior* 36–38 °C, and *R. variabilis* 34–36 °C. This result is concordant with our molecular studies: the phylogenetic analyses of ITS rDNA of this genus supports the thermophilic group as being a monophyletic lineage on the phylograms with 0.99–1.00 BP and 100% PP supports; while the mesophilic taxa form a monophyletic group analyzing members of *Rhizomucor* only (1.00 BP and 100% PP).

Mating experiments

Among the seven taxa used in this study, four of them, *R. chlamydosporus*, *R. endophyticus*, *R. miehei*, and *R. pusillus* var. *tauricus* are represented by one strain only and do not have a mating partner to test with. The other three, i.e. *R. pusillus*, *R. regularior*, and *R. variabilis*, have respectively 6, 2, and 4 strains and are capable of

crossing with each other intraspecifically. Results obtained are as follows: zygospores were not formed between the two strains of *R. regularior*; but were formed by two pairs of *R. pusillus* strains [CBS 231.29 (+) × Rm-5 (–) and Rm-4 (+) × Rm-5 (–)] and one pair of *R. variabilis* strains [Rm-10 (+) × Rm-11 (–)]; likewise, azygospores were not formed in *R. regularior*, but were formed by one pair of *R. pusillus* strains [Rm-1 (+) × Rm-5 (–)], as well as by one pair of *R. variabilis* strains [Rm-9 (mating type unknown) × Rm-6 (mating type unknown)]. In interspecific matings, all strains of the seven taxa were crossed with strains from other taxa with the following results: azygospores were only formed in *R. chlamydosporus* [Rm-33 (+)] × *R. pusillus* [Rm-5 (–)]; while gametangia were only formed in *R. variabilis* [Rm-10 (+)] × *R. pusillus* [Rm-5 (–)]. As seen from the results stated above, in either intraspecific or interspecific crosses, Rm-5 seems to be the strain which has the strongest mating ability. Before mating experiments were conducted among the *Rhizomucor* strains we have at hand, only *R. pusillus* CBS 231.29 is known to be of (+) mating type, and the only strain that reacted with this strain is Rm-5, thus revealing it to be of (-) mating type. Rm-5 reacted with Rm-4 in intraspecific matings, and with Rm-10 and Rm-33 in interspecific matings, revealing Rm-4, Rm-10, and Rm-33 are of (+) mating type. Furthermore, since Rm-10 is known to be (+) and to form zygospores with Rm-11, Rm-11 is therefore of (–) mating type. On the other hand, although Rm-6 and Rm-9 formed azygospores, both of them did not react with any other strain, hence the mating type of neither of them could be determined.

Studying *Cunninghamella*, we have found that when two strains of opposite mating types are brought together, their ability to react with each other depended on their mating ability and their affinity (compatibility). If their mating ability is strong enough and their affinity is close enough, zygospores can be formed in both intraspecific and interspecific matings; usually the ability to form true zygospores is inheritable in intraspecific matings, but non-inheritable (azygospores) in interspecific matings. When their mating ability is not so strong or their affinity not so close, then, for instance, gametangia or zygosporangia will form instead of zygospores (Zheng & Chen 2001). This is also the case with *Rhizomucor*.

Morphological studies

Being a genus segregated from *Mucor*, *Rhizomucor* has many morphological similarities with this genus. The main difference is that *Rhizomucor* forms well-developed rhizoids which may arise from mycelia, sporangiophores, sporangia, columellae, while *Mucor* usually does not form rhizoids. Furthermore, *Rhizomucor* forms sto-

lons, and *Mucor* does not. The two genera can be distinguished very well not only by morphology but by molecular data as well (O'Donnell *et al*. 2001, and unpublished data from our research group).

Morphological characteristics for dividing species or varieties in *Rhizomucor* include: color (light or deep) of the colonies; development (poor or abundant) and place of origin (mycelia, sporangiophores, sporangia or columellae) of rhizoids; swollen or not swollen, diameter, length, fertility, branching type, length of branches, of sporangiophores; diameter of sporangia; presence or absence of collar; shape and size of columellae; shape and size of sporangiospores; type of sexual reproduction; type, shape, size of suspensors; type, shape, size, color, ornamentation of zygosporangia; and shape, size, ornamentation, edge of zygospores.

Taxonomy

When *R. variabilis* var. *regularior* was reported in 1993, molecular studies on the genus *Rhizomucor* have not been carried out in this laboratory, also that the morphological similarities of this fungus to *R. variablilis* var. *variabilis* were overemphasized; hence it was treated as a variety of the latter, although many dissimilarities have been noticed between them (Zheng & Chen 1993).

The newly found strain Rm-33, *R. chlamydosporus* is likewise closely related to both the two above-mentioned taxa. All the three of them cause human mucormycosis, the maximum growth temperatures of them do not exceed 39 °C (Tab. 2), their colonies are all bright yellow, and at least a portion of their columellae are irregularly shaped. A re-consideration on the morphological, phylogenetical, and physiological characteristics among these three fungi, we now believe that it is more natural to treat all the three of them as distinct species. According to the data obtained by our research group, in molecular analyses the Bayesian 50% majority-rule consensus tree illustrating molecular phylogeny of *Rhizomucor* inferred from ITS rDNA sequences has shown that *R. chlamydosporus*, *R. endophyticus*, *R. miehei*, *R. regularior*, *R. variabilis* belong to different clades, with *R. pusillus* and *R. tauricus* clustered in the same clade. Therefore, *R. chlamydosporus* is doubtless a new species, and *R. variablilis* var. *regularior* should be raised to species level as *R. regularior*. (Lengths of ITS regions are listed in Tab. 3)

Regarding the relationship between *R. pusillus* and *R. tauricus*, Vágvölgyi *et al*. (1999) and Vastag *et al*. (2000) believe *R. tauricus* to be a mutant of *R. pusillus* and not a true species based on the similarities of their isoenzyme, ITS-RFLP and RAPD patterns. In our opinion, *R. tauricus* is morphologically distinct from *R. pusillus*, does not sexually react with it, and its maximum growth tempera-

ture is much lower. But phylogenetically it clustered with *R. pusillus* in the same clade; therefore, it is better to treat it as *R. pusillus* var. *tauricus*.

As can be seen from the following key, the members of *Rhizomucor* can easily be distinguished by distinct sets of characters.

KEY TO THE SPECIES OF *RHIZOMUCOR*

1. Thermophilic, maximum growth temperature 46–57 °C; rhizoids relatively poor; collar absent; zygosporangia when formed not exceeding 70 µm in diam. 2
1. Mesophilic, maximum growth temperature 32–39 °C; rhizoids well-developed; collar evident; zygosporangia when formed reaching 100 µm in diam. 5
2. Maximum growth temperature 48 °C; colonies light gray to grayish yellow; sporangiospores irregular in shape ..... ***Rhizomucor nainitalensis***[1]
2. Maximum growth temperature 56–57 °C; colonies dark gray; sporangiospores regular in shape 3
3. Sporangiophores swollen, reaching a diameter of 35 µm; sporangia reaching 125 µm diam.; DNA length of entire ITS region (including 5.8S) 539–542 bp ... ***Rhizomucor pusillus* var. *tauricus***
3. Sporangiophores not swollen, not exceeding 15 µm diam.; sporangia usually not exceeding 100 µm diam. 4
4. Sporangia reaching 100 µm diam.; zygosporangia up to 70 µm diam.; usually heterothallic; DNA length of entire ITS region (including 5.8S) 540–542 bp ... *Rhizomucor pusillus* var. *pusillus*
4. Sporangia reaching 60 µm diam.; zygosporangia not exceeding 50 µm diam.; homothallic; DNA length of entire ITS region (including 5.8S) 615–616 bp ..... ***Rhizomucor miehei***
5. Homothallic; colonies dark gray to blackish owing to the formation of numerous zygosporangia; optimum, maximum, and minimum temperatures for growth 18–28 °C, 36 °C, and 5 °C respectively; branches of the sporangiophores usually very long, 300–1500 (–8000) µm in length, occasionally non-fertile and substituted by a sterile spine; columellae regular in shape; endophytic in higher plants; DNA length of entire ITS region (including 5.8S) 612 bp ..... ***Rhizomucor endophyticus***
5. Heterothallic; colonies bright yellow; optimum, maximum, and minimum temperatures for growth 24–30 °C, 38 °C, and 9 °C respectively; branches of the sporangiophores usually shorter, not exceeding 2500 µm in length, usually fertile and terminated by a

[1] No culture is available for testing the DNA length of this taxon.

sporangium; columellae very irregular in shape; causing human primary cutaneous mucormycosis 6

6. Rhizoids arising from stolons, mycelia, sporangiophores, sporangia, and columellae; main stems of the sporangiophores reaching 2000 μm in length and 23.5 μm in diam.; sporangia reaching 106 μm diam., non-apophysate to indistinctly or distinctly apophysate; sporangiospores very irregular in shape and size; DNA length of entire ITS region (including 5.8S) 548–549 bp ..... ***Rhizomucor variabilis***
6. Rhizoids arising from stolons, mycelia, and sporangiophores, but not from sporangia and columellae; main stems of the sporangiophores at most reaching 1350 μm in length and 16 μm in diam.; sporangia usually not exceeding 55 μm and reaching 70 μm diam., non-apophysate; sporangiospores quite regular in shape and size 7
7. Main stems of the sporangiophores not exceeding 1000 μm in length, often sympodially and successively branching 1–2 (–7) times, branches often subterminal; chlamydospores present on mycelia only, absent on sporangiophores; DNA length of entire ITS region (including 5.8S) 552 bp ..... ***Rhizomucor regularior***
7. Main stems of the sporangiophores reaching 1350 μm in length, with 1–3 lateral branches, branches alternate or sometimes in pairs, a few of the branches may repeatedly branching once, usually not subterminal; chlamydospores abundant on both mycelia and the main stems and branches of sporangiophores; DNA length of entire ITS region (including 5.8S) 540 bp ..... ***Rhizomucor chlamydosporus***

For descriptions of *R. miehei, R. pusillus*, and *R. pusillus* var. *tauricus* (= *R. tauricus*), see Schipper (1978), for *R. nainitalensis* see Joshi (1982), for *R. endophyticus* see Zheng & Jiang (1995), for *R. variabilis* see Zheng & Chen (1991), for *R. regularior* (= *R. variabilis* var. *regularior*) see Zheng & Chen (1993), and for *R. chlamydosporus* see below:

Rhizomucor chlamydosporus R.Y. Zheng, X.Y. Liu & R.Y. Li **sp. nov.** – Figs. 1, 2.
MycoBank no.: MB 512892

Coloniae lanatae, in SMA ad temp. 24–30 °C bene crescents, 1–1.5 mm altae, Petri-patellas in 8 vel 9 diebus implentes, incremento ad vel subter temp. 10 °C et ad vel super temp. 40 °C cessatae, primum albae, cito 'Light Buff. *Stolones* interdum praesentes. *Rhizoidea* copiosa, e hyphis, stolonibus, vel sporangiophoris enata. *Sporangiophora* cum stipitibus primis 306–1350 μm longis, (5.0–) 8.5–14 μm diam., eramosis vel sympodice 1 (–3) ramosis, ramis (47–) 106–482 μm longis et (3–) 5.5–9 μm diam., stipitibus primis et ramis cum sporangio terminalibus. *Sporangia*

globosa ad leniter depresso-globosa, fertilia et cum normibus sporis, raro cum 1-pluribus externis processibus, interdum irregulariter conformatis et cum abnormibus sporis, (19–) 23.5–47 μm diam., flavide vel griseole brunnea, parietibus subpersistentibus, lente tabidis vel interdum disruptis. *Apophyses* absenes. *Columellae* satis ordinatae in forma et magnitudine, depresso globosae, 9–23 × 13.5–28.5 μm, quando vetustae aliquae cum 1- pluribus valde irregularibus externis processibus e apice vel latere, hyalinae, interdum griseolae. *Collaria* absenia. *Sporangiosporae* praecipue globosae ad subglobosas et late ovoideas, (2.5–) 4–7 (–9.5) μm diam. vel (3–) 4.5–7 (–9) × (2.5–) 4–6 (–8) μm, raro oblongo-ovoideae ad elliptico-ovoideas et 6–9 (–14.5) × 3–4 (–5.5) μm, granulosae et hyalinae. *Chlamydosporae* valde copriosae, in myceliis aeries vel substrati et sporangiophoris. *Zygosporae* ignotae.

Holotypus: HMAS 175268-1–26 (cultura exsiccate e Rm33) isolatus e homin(is) de Provincia Hubei a Cao Yu-chun, 2005.

Colonies lanose, growing well at 24–30 °C on SMA, 1-1.5 mm high, filling the Petri dish in 8 or 9 days, development ceased at 10 °C or below and at 40 °C or above, at first white, soon becoming 'Light Buff' (Ridgway XV), reverse side 'Cream Buff' (Ridgway XXX). Hyphae branching, nonseptate when young, septate at age with simple septa, 2–12.5 μm diam. Stolons sometimes present. Rhizoids present but not very abundant, arising from the hyphae, stolons, and the middle or basal part of the sporangiophores, mostly root-like and branched, septate or nonseptate, straight, curved or tangled into balls, often embedded with chlamydospores. Sporangiophores erect, ascending or recumbent, arising from stolons, with opposite or non-opposite rhizoids, or more often arising directly from aerial hyphae and stolon-like; main stem of the sporangiophores (306–) 423–1350 μm long, (5–) 8.5–14 μm diam., with several to numerous septa and forming chlamydospores, often slightly enlarging upwards, then equal in width for some distances, slightly constricted just beneath the sporangia, rarely unequal throughout, colorless or with grayish contents, simple or with 1–3 side branches, some of which may rebranching once, primary branches mostly alternate, rarely in pairs, also septate and forming chlamydospores, (47–) 106–482 μm long and usually shorter than the main stems, (3–) 5.5–9 μm diam., equal in width and slightly, or not constricted at the uppermost part, mostly substraight and seldom curved, all terminated with a sporangium. Sporangia globose to slightly depressed-globose, some oblong-ovoid to peach-shaped, fertile or non-fertile, (19–) 23.5–47 μm diam., slowly dissolving, sometimes broken, yellowish brown to brownish gray, non-apophysate. Columellae depressed-globose, 9–23 × 13.5–28.5 μm, without or with 1-several irregular outgrowths arising from the apical or lateral portion similar to those of *R. variabilis*, hyaline, sometimes with grayish contents. Collar absent. Sporangiospores principally globose to subglobose and broadly ovoid, (2.5–) 4–7 (–9.5) μm diam.

or (3–) 4.5–7 (–9) × (2.5–) 4–6 (–8) μm, rarely oblong-ovoid to elliptic-ovoid and 6–9 (–14.5) × 3–4 (–5.5) μm, granulose and hyaline. Chlamydospores very abundant, solitary or in short chains on aerial or substrate mycelia, also on rhizoids, sporangiophores, sporangia, or columellae, 11.5–18.5 μm diam. or (7–) 9–18.5 (–25) × (5.5–) 7–14.5 (–18.5) μm. Zygospores unknown.

Maximum growth temperature: 39 °C.

DNA length of entire ITS region (including 5.8S): 540 bp.

Holotype: HMAS 175268-1–26 (dried cultures from Rm33) isolated from a man of Hubei Prov. by Cao Yu-chun, 2005.

The new species *R. chlamydosporus* differs from all the known taxa of the same genus by possessing numerous chlamydospores on all parts of the fungus. *Mucor racemosus* Fres. forms abundant chlamydospores too on its sporangiophores, sporangia, and columellae. However, *R. chlamydosporus* can easily be distinguished from *M. racemosus* by the following characters: (1) Colonies light buff and 1–1.5 mm high, instead of pale smoke gray and up to 20 mm high; (2) rhizoids and stolons present; (3) branching of sporangiophores simple or with 1-3 side branches, some of which may rebranching once, primary branches mostly alternate, rarely in pairs, instead of branching in a mixed sympodial and monopodial fashion; (4) sporangia globose, some oblong-ovoid to peach-shaped, up to 47 μm diam., instead of entirely globose and up to 90 μm diam.; (5) columellae depressed-globose, with or without 1-several irregular outgrowths, instead of obovoid, ellipsoidal, cylindrical-ellipsoid, slightly pyriform, never with irregular outgrowths.

Rhizomucor pusillus (Lindt) Schipper var. ***tauricus*** (Milko & Schkur.) R.Y. Zheng, X.Y. Liu & R.Y. Li **stat. & comb. nov.**

≡ *Mucor tauricus* Milko & Schkur., Novosti Sistematiki Nizshikh Rastenii 7: 139. 1970. (Basionym)

= *Rhizomucor tauricus* (Milko & Schkur.) Schipper, Studies in Mycology 17: 62. 1978.

MycoBank no.: MB 512911

Rhizomucor regularior (R.Y. Zheng & G.Q. Chen) R.Y. Zheng, X.Y. Liu & R.Y. Li **stat. & comb. nov.**

≡ *Rhizomucor variabilis* R.Y. Zheng & G.Q. Chen var. *regularior* R.Y. Zheng & G.Q. Chen, Mycosystema 6: 2, 9. 1993. (Basionym)

MycoBank no.: MB 512893

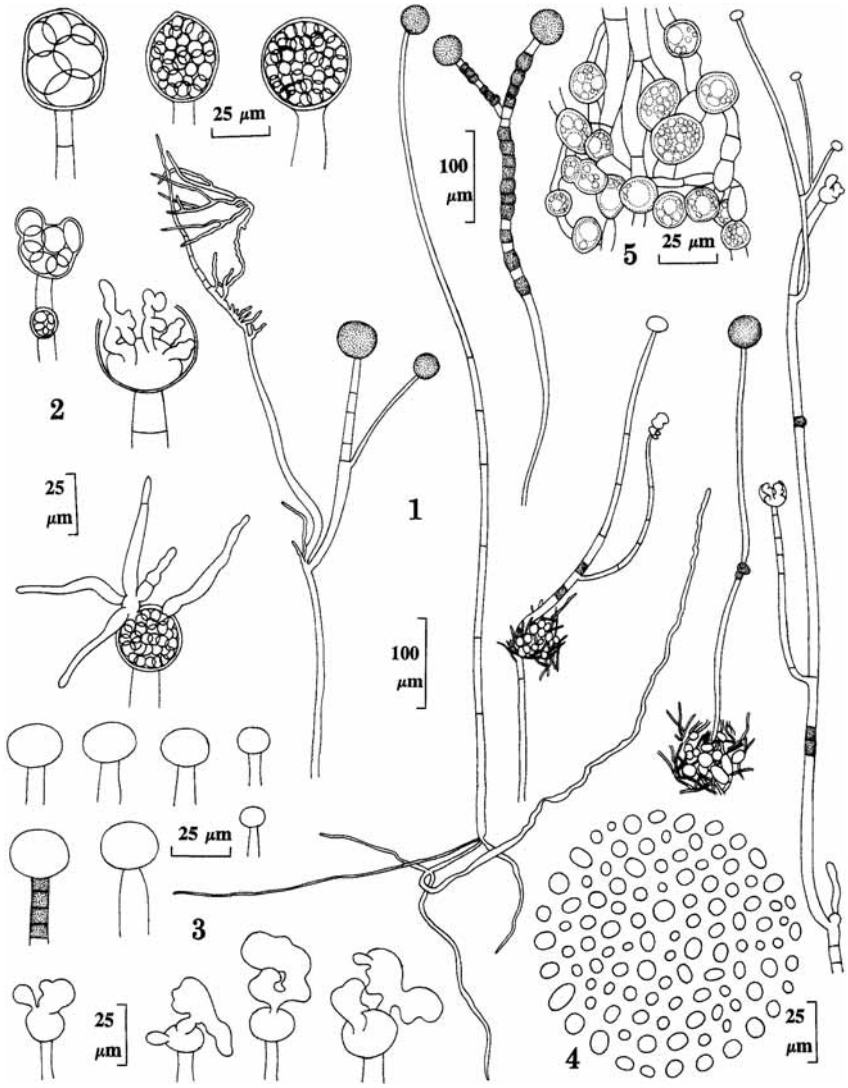

Fig. 1. – *Rhizomucor chlamydosporus* sp. nov. **1.** General characteristics of the sporangiophores. Note that rhizoids are formed on the basal or the intercalary parts of the sporangiophores; septa are more or less abundantly on the sporangiophores. Chlamydospores are formed solitary, in pairs, or in short to long chains and may be intermixed with rhizoids. **2.** Sporangia of various shapes. Note that the sporangiospores inside are uniform or differing markedly in size, shape, or number; some of the columellae have abnormal protrusions within the sporangia before or after they deliquesce. **3.** Columellae. Normal ones are usually depressed-globose, others with all kinds of protrusions. **4.** Sporangiospores. **5.** Chlamydospores in mass.

Table 2. Maximum growth temperature [°**C**] of *Rhizomucor* taxa and strains.

R. chlamydosporus **[39]**	Rm-33 (T) – [**39**, **39**]
R. endophyticus **[32-34]**	Rm-8 (T) – [**32**, **34**]
R. miehei **[57]**	CBS 182.67 (T) – [**57**, **57**]
R. pusillus var. *pusillus* **[(53-) 56 (-57)]**	CBS 231.29 – [**53**, **53**], CBS 354.68 (NT) – [**55**, **55**], Rm-1 – [**57**, **57**], Rm-2 – [**56**, **56**], Rm-4 – [**56**, **56**], Rm-5 – [**56**, **56**]
R. pusillus var. *tauricus* **[46]**	NRRL 3695 (T) – [**46**, **46**]
R. regularior **[36-38]**	Rm-7 (T) – [**36**, **37**], Rm-20 – [**36**, **38**]
R. variabilis **[34-36]**	Rm-6 (T) – [**35**, **36**], Rm-9 – [**34**, **34**], Rm-10 – [**35**, **36**], Rm-11 – [**36**, **36**]

Table 3. DNA length [bp] of entire ITS region (including 5.8 S) of *Rhizomucor* taxa and strains.

R. chlamydosporus **[540]**	Rm-33 (T) – [**540**]
R. endophyticus **[612]**	Rm-8 (T) – [**6**12]
R. miehei **[615-616]**	ATCC 26282 – [**615**], CBS 182.67 (T) – [**616**], NRRL 5901 – [**615**]
R. pusillus var. *pusillus* **[(540-) 542]**	ATCC 36606 – [**542**], CBS 354.68 (NT) – [**542**], CNRMA 03.1205 – [**542**], CNRMA 04.210 – [**542**], IBP M.p./1 – [**541**], MTCC 6597 – [**540**]
R. pusillus var. *tauricus* **[539-542]**	NRRL 3695 (T) – [**539**] (GenBank AJ278365), NRRL 3695 (T) – [**542**] (GenBank EU293849)
R. regularior **[552]**	Rm-7 (T) – [**552**], Rm-20 – [**552**]
R. variabilis **[548-549]**	CBS 103.93 (T) – [**548**], Rm-6 (T) – [**548**], Rm-9 – [**549**], Rm-10 – [**549**], Rm-11 – [**549**]

Acknowledgement

The project was partially supported by the National Science Foundation of China and the Foundation of the Knowledge Innovation Program of the Chinese Academy of Sciences. Cultures supplied by CBS and NRRL used in this study are gratefully acknowledged. Many friends and colleagues who supplied us with their samples and cultures are greatly appreciated. We also thank Ms Xiang-fei Zhu and Ms Hong-mei Liu, both of this laboratory, for respectively inking the line drawings and testing the maximum growth temperatures. Ms Wen-hua He is thanked for her devotion to the taking care of our *Rhizomucor* strains kept in the AS Culture Collection all through these years.

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(Manuscript accepted 2 April 2009; Corresponding Editor: R. Pöder)