

## **Heat-tolerant fungi and applied research: On the taxonomic position of some overlooked thermophilic fungi**

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**Abstract** – Despite recent progress achieved in the stabilisation of the systematic of thermophilic fungi, a few names remained unassessed. Among the three zygomycetous taxa considered *Mucor miehei* var. *minor* and *M. thermohyalospora* are regarded as possible synonyms of *Rhizomucor miehei*; *Mucor thermoaerospora* and its variant name *M. thermoaerosporus* represent a later synonym of the same. The ascomycete *Talaromyces thermocitrinus* is regarded as a possible synonym of *T. thermophilus*, the only obligate heat-tolerant fungus with a *Penicillium* anamorph. Similarly the synnematosous hyphomycete *Synnukerjiomyces thermophilus* proved to be a later name of *Remersonia thermophila*; the former was introduced as the type species of the new genus *Synnukerjiomyces*, a generic entity invalidly published due to violation of several articles of the latest International Code of Botanical Nomenclature.

**fungi / taxonomy / thermophilic / zygomycete / Ascomycete / *Mucor* / *Rhizomucor* / *Synnukerjiomyces* / *Talaromyces***

### **INTRODUCTION**

In 1964, Cooney & Emerson published the first comprehensive account on the taxonomy, biology and economic importance of thermophilic fungi. Both authors then proposed the following definition for these microorganisms: a thermophilic fungus develops maximum growth at a temperature of 50°C or above and minimum growth at 20°C or above; by comparison a thermotolerant fungus is able to grow well below 20°C with a maximum growth temperature value at about 50°C. Eleven thermophiles were then considered with few being new to science. The numbers of known thermophilic and of thermotolerant fungi have expanded rapidly in the last decades. However, no updated edition of the Cooney & Emerson' monograph was yet undertaken (Mouchacca, 2000b).

Recently, two contributions attempted to clarify the systematic of thermophilic fungi. Mouchacca (1997) introduced a new genus and reduced to synonyms of already existing taxa several species and varieties of thermophiles described in the last decades. He subsequently redefined the taxonomic concept of taxonomically valid thermophiles, proposed additional synonymies and underlined cases awaiting clarification (Mouchacca, 1999). These two papers were the foundation for a synopsis of name changes and synonymies of thermophiles

most commonly cited in the literature of fundamental and applied work and of those having complex taxonomic histories (Mouchacca, 2000b). The synopsis was addressed for applied research workers to foster the continuous use of taxonomically valid names of accepted thermophiles. A strict adherence to this rule aims to prevent redundancies in name citation by using binomials of established synonyms. The synopsis should also suppress reference to ghost binomials having no nomenclatural or taxonomic statuses. However, despite the availability of this synthetic document synonymous names are still reported in the recent overview on obligate heat-tolerant fungi prepared by Rawat & Johri (2003).

Mycologists and workers of applied research were also not consistent in the application of Cooney & Emerson's (1964) concepts distinguishing thermophiles from simple thermotolerants. A survey of the literature dealing with biotechnological studies had underlined simple heat-tolerant fungi were commonly referred to as thermophiles (Johri *et al.*, 1999; Maheshwari *et al.*, 2000; Mehrotra, 1985). Mouchacca (2000a) analysed in detail recent information on the taxonomy and physiology of thermotolerant fungi erroneously reported as possessing thermophilic attributes. He critically reviewed 130 fungal names of such pseudothermophiles and produced a list of about 30 taxonomically valid names of strictly thermotolerant fungi. Subsequent efforts in this line developed a critical review of another group of 20 pseudothermophilic taxa (Mouchacca, 2007a) and the establishment of a second list of fifty species possessing simple thermotolerant abilities (Mouchacca, 2007b).

Despite recent progress aiming to stabilise the taxonomic positions of heat-tolerant fungi a limited number of known thermophiles have remained overlooked. The present note considers the taxonomic position of three Zygomycetes, an Ascomycete and one anamorphic fungus.

### LIST OF TREATED SPECIES

For the cited culture collections the following acronyms were used. ATCC: American Type Culture Collection, USA; CBS: Centraalbureau voor Schimmelcultures, The Netherlands; HACC: Hindustan Antibiotics Culture Collection; IMI: International Mycological Institute (now CAB International), United Kingdom. Authors of fungal names were abbreviated following Kirk & Ansell (2003, electronic version).

### ZYGOMYCETES

***Mucor miehei* var. *minor* Subrahm. & Gopalkr.** – Indian Botanical Reporter, Prof. K.B. Deshpande Commemoration Volume: 33. 1984. Typification. Only the following simple note is provided: HACC No. M5, ATCC No. 36018.

The original strain of the new variety was isolated from an aeroslide exposed at the city of Pimpri, Poona, India (Subrahmanyam & Gopalkrishnan, 1984). It was reported to have a growth temperature range of 28-55°C with an optimum at 45°C. Further the 'smaller sporangiospores clearly distinguished the isolate from *Mucor miehei* Cooney & Emerson'.

Few years before the description of this variety *minor*, Schipper (1978) had reintroduced the genus *Rhizomucor* (Lucet & Costantin) Wehmer:Vuill. to

accommodate the three known thermophilic species of *Mucor* Fresen. *Rhizomucor* was regarded to be distinct by the formation of stolons and rudimentary rhizoids and by its obligate temperature requirements. The heterothallic *Rhizomucor pusillus* (Lindt) Schipper was selected as type species. The two other accepted members were the homothallic *Rhizomucor miehei* (Cooney & Emerson) Schipper and *R. tauricus* (Milko & Schkurenko) Schipper.

The Indian variety of *Mucor miehei* was overlooked in recent taxonomic studies on thermophilic fungi (Mouchacca, 1997, 1999). It was not even cited in the review chapter 'Ecology and Distribution' authored by A. Subrahmanyam in the book 'Thermophilic Moulds in Biotechnology' (eds.: B.N. Johri, T. Satyanarayana & J. Olsen 1999). The protologue of the variety depicts a homothallic zygomycete with sporangiospores hyaline, globose to oval, delicately echinulate, 2.2-3.3  $\mu\text{m}$  in diam.; zygospores are abundant, globose to oval, dark brown to black, tuberculate, 30-50  $\mu\text{m}$ ; azygospores occasionally present.

In the same note introducing the variety *minor*, Subrahmanyam & Gopalkrishnan (1984) indicated having isolated from bat droppings at Vijayawada, India, an isolate referable to *Mucor miehei* Cooney & Emerson. This isolate was then regarded as the first record of the thermophile in the Indian subcontinent. The corresponding strain revealed a comparatively narrow growth temperature range of 37-55°C with an optimum at 45°C; the developing sporangiospores are golden yellow, spherical to slightly oval, delicately echinulate, and 4.4-6.6  $\mu\text{m}$  in diam. A comparison of the descriptions provided by the two Indian authors for this strain and for the variety *minor* discloses the existence of a continuum (and partial overlap) in their respective values of growth temperature ranges and, more particularly, in the range of their spore dimensions.

*Mucor meihei* was initially reported by Cooney & Emerson (1964) to produce sporangiospores colorless, subspherical to oval, 4-6  $\times$  3-5  $\mu\text{m}$ . Schipper (1978) after examining a large number of strains of this thermophile concluded that the sporangiospores are subglobose, a few ellipsoidal, 3-4  $\mu\text{m}$  in diam., a few being larger (3-5  $\mu\text{m}$  following Domsch *et al.*, 1980). The same strains also disclosed a growth temperature range of 24-55°C. A critical comparison of available information on *Rhizomucor meihei* and *Mucor miehei* var. *minor* favours the hypothesis the variety *minor* is a possible synonym of the type variety.

***Mucor thermohyalospora* Subrahm.** [as '*thermo-hyalospora*'] – *Bibliotheca Mycologica* 91: 421. 1983. Typification. The following simple note is provided: HACC No. AS MPV 5: The type culture is deposited at ATCC. No relevant serial number given.

*Mucor thermohyalospora* was isolated by A. Subrahmanyam from curd sampled in the local market also at Pimpri, Poona (Subrahmanyam, 1983). The zygomycete is clearly thermophilic with growth starting at 24°C, optimum at 45°C and maximum at 55°C. Following the protologue, 'careful study of monosporic cultures showed that it closely resembles *Mucor tauricus* Milko & Schkurenko, but differs from it in being homothallic. Therefore it is described as a new species'.

As underlined before, Schipper (1978) had already transferred *Mucor tauricus* Milko & Schkurenko to *Rhizomucor* (Lucet & Costantin) Wehmer:Vuill. in her attempt to accommodate the three known thermophilic species of *Mucor* Fresen. The reintroduction of *Rhizomucor* was thus overlooked by Subrahmanyam (1983).

The type culture of *Rhizomucor tauricus* develops very poor growth on beerwort agar (Schipper 1978). The original authors of this *Rhizomucor* described their species on prune-agar which, according to Schipper (1978), seems to have the same influence on the zygomycete as that of beerwort agar. The description

provided by Schipper (1978) for the original strain was based on colonies grown on beerwort agar but enriched with 20% saccharose. Milko & Schkurenko (1970) have distinguished their species from *Rhizomucor pusillus* and *R. miehei* mainly by its swollen sporangiophores and by its lower temperature maximum of 47-49°C. Schipper (1978) noted that on agar media enriched with saccharose, *Rhizomucor tauricus* and *R. pusillus* have the same maximum growth temperature: 55°C. Also on this medium a low percentage of narrow sporangiophores resembling those of *Rhizomucor pusillus* could be observed.

According to Schipper (1978) since only the type culture of *Rhizomucor tauricus* was available for examination, it is difficult to decide whether it represents a deviating strain of *R. pusillus* or a true species. The validity of *Rhizomucor tauricus* as a good species was also subsequently questioned by Vagvolgyi *et al.* (1999). These authors favour the hypothesis the still only available culture of this microorganism is a mutant heterothallic strain of the type species *Rhizomucor pusillus*. A comprehensive description of the latter was recently provided by Iwen *et al.* (2005) based on a strain inducing sinus-orbital zygomycosis in a patient with acute myelogenous leukemia.

*Mucor thermohyalospora* makes good growth on common media and develops characters matching those of the two other thermophilic Rhizomucors. A comparative analysis of the morphological features of these zygomycetes was not undertaken by Subrahmanyam (1983). Based on the provided description including the basic development of zygospores, *Mucor thermohyalospora* should be regarded as a possible synonym of *Rhizomucor miehei*.

***Mucor thermoaerospora* Subrahm. & B.S. Mehrotra**, in Subrahmanyam A. – Memoir of a D.Sc. Thesis, Kumanun University, Nainital, India, pp. 83-85, 1984; *nom. nud.*, Art. 32.1c ICBN.

= *Mucor thermoaerosporus* Subrahm., in S. Nagalakshmi & A. Subrahmanyam [as '*thermoaerospora*'] – Journal of Economic and Taxonomic Botany 26: 760. 2002. Typification. The following simple indication is provided: ATCC No. 36019; *nom. invalid.*, Art. 37.5 ICBN.

The binomial *Mucor thermoaerospora* first appeared in a memoir presented by A. Subrahmanyam for the award of a D.Sc. Degree at Kumanun University, India. It was then reported as *Mucor thermoaerosporus* with the corresponding authors' name being A. Subrahmanyam & B.S. Mehrotra (Subrahmanyam, 1984). The same binomial was again simply mentioned in the review chapter 'Ecology and Distribution' authored by A. Subrahmanyam in the book 'Thermophilic Moulds in Biotechnology' appearing in 1999 (eds.: B.N. Johri, T. Satyanarayana & J. Olsen). The original strain was then indicated as being isolated from air at Pune, India, hence the proposed specific epithet (Subrahmanyam, 1999: 23). Beside the absence of any published description no information depicting the proper thermotolerance levels of this zygomycete were then provided. In 2000, Mouchacca suggested the fungus should better be regarded as a thermotolerant organism.

A formal description but of *Mucor thermoaerosporus* Subrahm. was subsequently provided by Nagalakshmi & Subrahmanyam (2002). The zygomycete was relocated in the genus *Mucor* despite its high temperature requirements and the presence of simple rhizoids at the base of the sporangiophores. As previously underlined these two characters were used by Schipper (1978) to substantiate the re-introduction of the genus *Rhizomucor* for the three thermophilic rhizoidal species of *Mucor*. Nagalakshmi & Subrahmanyam (2002) then based their decision on the statement forwarded by B.S. Mehrotra in his 1985 Jeersanndhi award lecture at the Indian Phytopathological Society (Mehrotra, 1985). This

statement was reported as a simple undocumented infra note pagination: Recognition of the genus *Rhizomucor* for thermophilic and rhizoidal species of *Mucor* is debatable (Indian Phytopathology 38: 217. 1985).

*Mucor thermoaerospora* was invalidly published following Art. 37.5 of the ICBN: For the name of a new taxon of the rank of genus or below published on or after 1 January 1990, indication of the type must include one of the words 'typus' or 'holotypus', or its abbreviation, or its equivalent in a modern language. Following Nagalakshmi & Subrahmanyam (2002), the new *Mucor* resembles the equally homothallic *M. miehei* in 'several respects but smaller size of the sporangiospores clearly distinguishes from it'. Indeed sporangiospores of the former are reported to measure 2.2-3.3  $\mu\text{m}$  vs 3-4 (5)  $\mu\text{m}$  for spores of *Rhizomucor miehei*. This assumed slight difference in spore size had already been evoked by A. Subrahmanyam to substantiate for the introduction of the variety *minor* for the same species. As stressed earlier in the case of the variety, this assumed argument do not warrant the proposal of a valid new species distinguishable from *Rhizomucor miehei*.

Recently O'Donnell *et al.* (2001) constructed comprehensive molecular and morphological data sets for 63 species representing 54 of the 56 genera and all 13 families of *Mucorales sensu* Hawksworth *et al.* (1995). *Rhizomucor pusillus* comes out in a clade quite separate from the one with the *Mucor* species tested, a clear indication the two entities are not congeneric.

*Rhizomucor meihei* is well known for the production of a protease currently used to replace calf-rennet in cheese industry (Garg & Johri, 1999). The search for more productive strains is a reasonable goal definitely not to be achieved *via* the proposal of speculative synonymous names of the same thermophile.

## ASCOMYCETE

***Talaromyces thermocitrinus* Subrahm. & Gopalkr.** [as '*thermocitrinum*'] – Indian Botanical Reporter, Deshpande Commemoration Volume: 35. 1984. Typification. The simple following note is provided: HACC No. Y 12, ATCC 36868. anamorph: no name designated.

This new species of *Talaromyces* C.R. Benj. was not considered in recent publications dealing with the genus (Frisvad *et al.*, 1990; Pitt *et al.*, 2000). The name was even omitted by A. Subrahmanyam (1999) in his review chapter 'Ecology and Distribution' of the book 'Thermophilic Moulds in Biotechnology' (eds.: B.N. Johri, T. Satyanarayana & J. Olsen 1999). It was also overlooked in recent notes dealing with the ecology of thermophilic and thermotolerant fungi (Mouchacca, 1999, 2000 a, b).

The original strain was isolated from dust accumulating on unused books at Pimpri, Poona. According to the two Indian authors 'the new species resembles *Talaromyces thermophilus* in several respects including the small conidiophores and nature of penicilli. Both of them produce cleistothecia. The Indian isolate, however, can be distinguished by the presence of delicately echinulate, larger conidia, and smaller cleistothecia. Irregular ridges ornamenting the ascospores of *Talaromyces thermophilus* are absent in the present form having ascospores provided with a central furrow. The most relevant important feature is the development of cleistothecia on all agar media incubated up to 45°C; asci are globose, 8-spored; ascospores are globose, smooth, pale yellow with a central furrow, 4.4-4.4  $\mu\text{m}$  in diam. Growth rapid at 37°C: 9 cm in 5 d, slower and restricted at 60°C, minimum at 28°C'. In the published protologue no indication was however reported as to whether the asci are produced in chains or not. Further, no binomial was provided for the developing associated anamorph.

The ascomycete *Talaromyces thermophilus* Stolk is related to the sole thermophilic *Penicillium* state producing greenish conidia: *P. dupontii* Griffon & Maublanc (Stolk, 1965; Mouchacca, 1997). The anamorph was originally described exclusive of the teleomorph since the ascocarps are seldom produced on agar media but on sterile oat grains at 45°C and only occasionally (Raper & Thom, 1949; Cooney & Emerson, 1964; Stolk, 1965; Stolk & Samson, 1972); it follows simply the *Penicillium* state is usually encountered in studies focusing on thermophilic fungi (Mouchacca, 1999).

Cooney & Emerson (1964) had also noted 'the perfect stage does not ordinarily occur on agar cultures and apparently requires some rather special conditions for its initiation. Observed cleistothecia are formed regularly and abundantly on moist, chopped guayule shrub in small pure-culture rets. Ascospores tend to mature in cultures 10-14 days old. Ascospores are distinctively pale orange or tan in mass, and very pale yellow when viewed singly. They are 3.5-5.0 × 2.5-3.5 µm and thick-walled. Ascospores are generally lenticular and with a fairly well defined equatorial furrow flanked by low, smooth or somewhat jagged ridges. The convex surfaces are smooth or show occasional ridges and irregularities' (see Cooney & Emerson, 1964 – Thermophilic Fungi: 32, figs. 10-14).

The former description elaborated by Cooney & Emerson (1964) was based on Emerson strain No. 26 (CBS 236.58, NRRL 2155) ex type of *Talaromyces thermophilus*, isolated by R. Emerson from retting guayule shrub (*Parthenium argentatum*) in California, USA, 1945. Emerson was the first to observe the development of the teleomorph in culture; he then prepared an emended description of both states of *Penicillium dupontii* to be incorporated by Raper & Thom in their first 'Manual of the Penicillia' (1949: 573). Emerson did not, however, provide a binomial for the teleomorphic state.

Stolk (1965) using the same CBS 236.58 obtained the teleomorph on sterile oat grains. Asci were found to develop in chains, a character also omitted by Cooney & Emerson (1964). Ascospores are rather ellipsoidal, 3.5-4.5 × 2.2-3.5 µm, ornamented by 2 to 6 somewhat jagged, irregular, usually longitudinal ridges, about 0.2 µm wide. A clear central furrow is definitely absent (Stolk & Samson, 1972). However, the disposition of some ridges may give the impression of such a furrow (see Pitt – The Genus *Penicillium*: 515, fig. 132 E). No *Talaromyces* species is presently known to develop ascospores with a central furrow. This character is disclosed by some members of the genus *Eupenicillium* F. Ludwig which, however, includes no thermophilic member. It follows *Talaromyces thermocitrinus* should better be considered as a possible synonym of *T. thermophilus*.

In connection with the above debated zygomycetous and ascomycetous taxa introduced from the Indian subcontinent the following indication is worth to be underlined: the practice of depositing holotype cultures only at institutions selling them at a cost beyond the budget of many researchers is counter-productive. Even when the describing author does not maintain a culture collection he or she may be able to deposit the material in a less costly place as well as at ATCC.

## ANAMORPHIC FUNGUS

***Remersonia thermophila* (Fergus) Seifert & Samson**, in Seifert, Samson, Boekhout & Louis-Seize – Canadian Journal of Botany 75: 1160. 1997. Type species of *Remersonia* Seifert & Samson, in Seifert, Samson, Boekhout & Louis-Seize – Canadian Journal of Botany 75: 1160. 1997.

basionym: *Stilbella thermophila* Fergus - Mycologia 56: 277. 1964.  
 = *Synnmuikerjiomyces thermophilus* (Lindau) Aneja & Kumar [as 'thermophile'],  
 in Tewari, Lakhanpal, Singh, Gupta & Chamola (eds.) – Advances in Microbial  
 Biotechnology (New Delhi, India: APH Publishing): 1. 1999. Type species of  
*Synnmuikerjiomyces* Aneja & Kumar, in Tewari, Lakhanpal, Singh, Gupta &  
 Chamola (eds.) – Advances in Microbial Biotechnology (New Delhi, India: APH  
 Publishing): 1. 1999; *nom. invalid.*, Arts. 37.1 & 37.3 ICBN.  
 basionym: no basionym indicated; *nom. invalid.* Art. 33.3. No holotype could thus  
 be designated; *nom. invalid.*, Art. 36.1.

*Stilbella thermophila* was described by Fergus (1964) from straw and horse manure compost in Switzerland and placed in *Stilbella* Lindau on account of the synnematos habit of the conidiophores. Optimum growth was reported to lie between 35-50°C; at 55°C, slight development still occurs but such is not the case below 25°C. In culture, the fungus produces white synnemata, up to 300 µm high, bearing whitish glistening mucoid conidial heads; conidia are hyaline, continuous, oblong ellipsoid, 15-17 × 6-10 µm.

In his monographic treatment of *Stilbella*, Seifert (1985) re-examined authentic material. The slimy conidia were observed to develop from percurrently proliferating conidiogenous cells, i.e. annellophores, a feature enhancing its exclusion from the genus. The situation remained unchanged until Mouchacca (1997) reported additional work was being undertaken by Seifert (pers. comm.) to establish the correct taxonomic position of this holoanamorphic thermophile.

Seifert *et al.* (1997) introduced *Remersonia* Seifert & Samson to accommodate this obligate heat-tolerant *Stilbella*. The new genus was characterized by determinate synnemata with scarcely branched conidiophores, percurrently proliferating conidiogenous cells and relatively large ameroconidia accumulating in slime. The somewhat marked size of *Stilbella thermophila* spores, its occurrence in self-heating substrates, and thermophilic nature are further evidences the fungus is unrelated to the accepted species of *Stilbella*; these tend to have smaller conidia and mesophilic, saprobic habitats. *Remersonia thermophila* had since been isolated from similar composts used for cultivating mushrooms in Europe and also in Indonesia, India and New Zealand (Mouchacca, 1999).

Aneja & Kumar's (1999) isolation of a synnematos thermophile from north-Indian soils revealed to them the unsatisfactory generic placement of *Stilbella thermophila*; they proposed the new genus *Synnmuikerjiomyces* Aneja & Kumar (dedicated to the Indian mycologist Prof. K.G. Mukerji) to resolve the problem. The binomial of the type species had to be formulated then as *Synnmuikerjiomyces thermophilus* (Fergus) Aneja & Kumar. The relevant draft was most probably presented for review to publication a short time after the appearance of the note introducing the new genus *Remersonia* (Seifert *et al.*, 1997). Most probably also the content of this draft was subsequently re-adapted for inclusion in the book "Advances in Microbial Biotechnology". Such involved the replacement of the name Fergus by Lindau in the new coined combination *Synnmuikerjiomyces thermophile* (Lindau) Aneja & Kumar and the concomitant omission of a basionym binomial.

The new genus *Synnmuikerjiomyces* Aneja & Kumar should be regarded as invalidly published due to violation of several articles of the Code. First no Latin diagnosis was provided for the genus and no type species specified (Arts. 36.1 & 37.1 ICBN). Second the proposed new combination of the "presumed type species" is invalidly published in the absence of any indication about the basionym and its place of publication (Art. 33.3 ICBN).

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