Studies on some Indian Soil Fungi-II.

On some new or interesting Ascomycetes.

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With Plate I.

In a previous paper *) an account of some members of the Sphaeropsidales from Indian soils were described. During these studies, several ascomycetes were also isolated, and detailed cultural work on these have been carried out. Of particular interest are the species of the genera Emericellopsis and Microascus. Species of former like E. terricola var. glabra and E. salmosynnemata are known to produce the valuable antibiotic synnematin. Consequently search for new species of Emericellopsis and the conidial stage Cephalosporium is being made in screening programme for new antibiotics. Considerable confusion has resulted in working out the speciation of Emericellopsis. Since new mutant strains arise readily and these show considerable variation in spore sizes, each author develops his own concept of limits of variability for differentiating species.

Species of Microascus have been reported as saprophytes in soil, weak plant and animal parasites. The deadly human pathogen Hormodendrum (Phialophora) pedrosoi is now shown to be the conidial stage of Microascus (Fuentes & Wolf, 1956 and 1956a). In the present paper some species of Microascus isolated from soil are being reported for the first time in India. Type cultures of new species are deposited at Mycology Division, I.A.R.I., New Delhi, C.M.I. Kew, England, A.T.C.C. Washington D.C. and Centraalbureau Voor Schimmelkultur, Baarn, Holland.

1. Microascus trigonosporus Emmons and Dodge (1931).

This fungus was isolated from a soil sample from Pimpri and was cultured on glucose-yeast agar. The flat, more or less floccose, and slow growing colonies are white at first, but with the development of the conidia, they soon become ash-coloured, and finally as the perithecia develop, they become brownish-black in colour. After about a month or two, when the mature ascospores are discharged in the form of long brown cirrhi the colony surface becomes reddish brown.

The conidia representing Scopulariopsis are similar to those described by Emmons and Dodge (1931) for M. trigonosporus. The phialides appear to be developing directly as small branch of the hyphae, hyaline and constricted at the base, broadest near about the middle

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region and tapering towards the apex. They measure 4.5—9 \times 2—3 \mu (Fig. 1). The conidia are subglobose, to obpyriform, truncate at the base with a collar around a basal-pore smooth, measuring 3.5—4.5 \times \times 3—4 \mu (Fig. 2).

The perithecia are globose to pyriform with ostioles, which are often beaked (fig. 3). They are black, carbonous and are mostly on the surface of the medium (but may often be embedded in the upper layers of the media), lying with their ostioles directed in any direction, and measuring 87.5—245 \times 73.5 — 245 \mu. The wall is composed of many layers of pseudoparenchymatous cells, with outer 3—4 layers being thick-walled and dark, while the inner layers having thin and hyaline walls. The asci are broadly clavate to obpyriform 8-spored and measure 9.5—13.5 \times 6.5—7.5 \mu (fig. 4). At maturity the walls of the asci deliquisce leaving the ascospores, still associated in groups of eight, scattered within the perithecial cavity. The mature ascospores are triangular with rounded ends and slightly inwardly curved sides (fig. 5) and measure 4.5—5.5 \times 3.5—4.5 \mu. No thickening of the side walls, resulting into the formation of a fourth angle or projection, as described by Emmons and Dodge, could be observed in the present fungus. The mature ascospores are discharged from the perithecia in long contorted cirrhi, which can be prominently seen in old cultures.

The present fungus closely resembles *Microascus trigonosporus* Emmons & Dodge and is being recorded for the first time in India. Whitehead et al (1948) described *M. trigonosporus* from seed, but their illustration of the perithecia indicate the presence of a fairly long beak—rather a rostrum. It is manifest that the fungus described by Whitehead et al (1948) should not be placed under *M. trigonosporus*.


This fungus was isolated from a soil sample from Pimpri. The colonies are more or less floccose in the central region but submerged at margins and are white at first, but becomes ash coloured with the development of conidial structures. Small black perithecia, more or less close-set, begin to appear after 8—10 days and impart a slightly darker colour to the colony. After about a month, the discharge of the mature ascospores in long contorted cirrhi from the perithecia turns the colony surface yellowish to reddish brown. Reverse of the colony is dark in the centre but more or less white at the margin.

The ash-coloured colonies produce abundant conidia of the *Scopulariopsis*-type. The phialides may develop directly upon the hyphae or upon branched conidiophore in a verticil at its apex along its sides. The phialides are typical of *Scopulariopsis*, constricted at the base, more or less inequilaterally thickened in the middle and tapering towards the apex where they produce long conidial chains (fig. 6). They measure 16.5—39.5 \times 1.5—3 \mu. The smooth walled hyaline
Conidia are lemon shaped to obpyriform in shape, with a truncate base having a distinct collar surrounding the basal pore (fig. 7). The successive conidia in the chain are separated by a ring which is clearly seen in early stages. Mature conidia measure $3 - 5 \times 2 - 4 \mu$.

The carbonaceous smooth walled perithecia are flask-shaped with very long hairy beak (fig. 8). Owing to the presence of the stiff hairs, which are pointing upwards or downwards the surface of the beak appear to be rough. In some perithecia a tuft of hairs is present at the tip also, and give the appearance of *Melanospora* sp. The perithecia measure from $181.5 - 303.5 \times 221 - 303.5 \mu$ (excluding the long beaks which measure from $52 - 264 \times 33 - 66 \mu$). A large number of asci are produced in the perithecium. They are more or less clavate when young but as the ascospores mature, they gradually become oval in shape $8 - 13.5 \times 6 - 8 \mu$ (fig. 9). The ascus wall is hyaline, membranous and evanescent, disappearing even before the spores mature, so that, the ascospores held in groups without the ascus wall can be seen. Mature ascospores are extruded out of the ostiole in a long contorted brown cirrhus, which is sometimes as long as $1.5 - 2.0 \ mm$ (in length) and as much as $33 - 42 \mu$ broad. (fig. 8) The ascospores are pale-brown, smooth reinform with rounded ends (fig. 10), measuring $4.5 - 5.5 \times 2.5 - 3 \mu$.

In having long beaked perithecia, the present fungus resembles *Microascus longirostris* Zukal (1885), *M. variabilis* Massee and Salmon (1901), *M. intermedius* Emmons and Dodge (1931), *M. lunasporus* Jones (1936) *M. cirrosus* Curzi (1930) and *M. doqueti* Moreau (1953) in having beaked perithecia. Excepting *M. lunasporus* and *M. doqueti* none of the above mentioned species have been reported to produce the conidial structures and hence the present fungus cannot be compared regarding conidial stages. The ascospores in *M. lunasporus*, though lunate in shape, are very much large in size ($8 - 14 \times 4 - 7 \mu$) as compared to those of the present fungus ($4.5 - 5.5 \times 2.5 - 3 \mu$). Besides this, Jones original sketches of the perithecium of *M. lunasporus* does not indicate the occurrence of any long beak in that fungus, where as in the present fungus the beak is very long, sometimes as long as the venter-portion of the perithecium and is distinctly setose. Thus the present fungus is quite distinct from *M. lunasporus*. The hairy long beak and the lunate ascospores brings the present fungus very close to *M. doqueti* Moreau, but in the structure of the conidia the two appear to be clearly distinct. Moreau (1953) has described the presence of characteristic longitudinal ridges (or markings) on the conidia which are completely different from the smooth walled conidia of the present fungus. Hence the present one under study is distinct from other *Microascus* species so far described and the name *Microascus griseus* is proposed.
Microascus griseus Mathur & Thirum. sp. nov.

Colonies on glucose-yeast agar flat, floccose, white at first turning greyish after production of conidia. Perithecia developing in 8—10 days imparting a darker colour to the colonies, and yellowish-brown after the extrusion of ascospores in cirri. Reverse white at first later on becoming black in centre. Phialides developing directly on mycelia or upon simple or branched conidiophores singly or in verticels, 16.5—39.5 × 1.5—3 μ constricted at the base, and tapering upwards from the inequilaterally broad middle region. Conidia hyaline, smooth, lemon shaped to obpyriform, truncated at the base, with a distinct collar surrounding a basal pore, 3—5 × 2—4 μ produced in long chain. Perithecia abundantly produced flask-shaped 181.5—303.5 × 221—303.5 μ carbonous, with very long hairy beak, hairs stiff directed upwards or downwards. Asei abundant 8-spored, ovate to globose, 8—13.5 × 6—8 μ. Ascus wall hyaline, membranous, and evanescent; ascospores reniform, pale yellowish-brown 4.5—5.5 × 2.5—3 μ, discharged in long contorted yellowish to reddish brown, cirri, 34—42 μ thick and 1.5—2 mm long.

From soil Pimpri, India, Culture N. 1252 (type).

3. Sporormia indica Mathur & Thirum, sp. nov.

This fungus was isolated from a soil sample from Chinchawad, near Poona. On glucose-yeast agar the colonies grow fairly rapidly producing a white floccose growth which gradually turns pinkish yellow. Under the hyphal mat small black perithecial bodies begin to develop within a week. The perithecia are not visible superficially. The reverse of the colony is colourless at first but gradually becomes dull reddish brown.

The mycelium is composed of white, septate profusely branched hyphae, which especially towards the surface, become yellowish pink. No conidial structures have been observed in the present fungus. The perithecia are small black, spherical to subspherical in shape and measure 277—495 μ in diameter. The perithecial wall is thin, membranous, composed of brownish polygonal cells. A large number of asci
are produced within a perithecium. They are ovate to obpyriform in shape (fig. 11) and have a hyaline, membranous, and evanescent wall, which disappears releasing the ascospores within the perithecial cavity. They measure 16.5—22.5 × 12—19.5 μ. The ascospores are dark-brown coloured, 4-celled, cylindrical with rounded ends and are constricted at the septa and smooth (fig. 12). They measure 13.5—15 × 3 μ. The cells of a spore separate from each other while the spores are still enclosed within the ascus, and the separating flat ends become rounded. The individual cells as found in the perithecium after the dissolution of the ascus wall range from 4—18 × 3 μ. It seems that after separation from their sister cells the individual spore cells elongate and divide (fig. 12) resulting in a large number of smaller cells.

_Sporomia indica_ Mathur & Thirum, sp. nov.

Colonies on glucose-yeast extract agar rapid growing, floccose to fluffy, orbicular, white at first, later turning pinkish-yellow, reverse white at first, turning dirty reddish brown. Perithecia globose, black, smooth, 277—495 μ in diameter, covered over by the aerial mycelium, wall thin, composed of brownish polygonal cells. Ascii abundant, ovate to obpyriform, 16.5—22.5 × 12—19.5 μ; ascus wall membranous, hyaline, evanescent; ascospores dark brown, 4-celled, cylindrical with rounded ends, constricted at the septa, 13.5—15 × 3 μ, the cells separating early, individual cells oblong with rounded ends, 4—18 × × 3μ, proliferating by elongating and dividing.

From soil, Chinchwad, Poona, India, Culture No. 1253 (Type).

_Caespitulis mycelii floccosis vel puberulis, orbicularibus, primum albidis, postea roseolo flavidis, subtus primum albidis, postea rubro-brunneis; perithecia globosa, nigra, levia, 277—495 μ diam., mycelio obvoluta et plus minusve obtecta; pariete tenui, e cellulis angulosis, brunneis composito; asci numerosi, ovoidei vel obpyriformes, 16.5—22.5 × 12—19.5 μ tenuiter tuniciati, mox diffusantes; sporae obscure brunneae, 3-septatae, cylindraceae, utrinque obtusae, ad septa plus minusve constrictae, 13.5—15 × 3 μ, mox in cellulas oblongas utrinque rotundatas secedentes.

4. _Emericellopsis humicola_ (Cain) Cain.

This fungus was isolated from a soil sample from Pimpri (Poona). On glucose-yeast agar the fungus grows rather slowly producing a floccose white colony exhibiting shallow radial depressions or striations and a slightly raised central region. The colony is not wet and does not exhibit the development of funiculose mycelial strands. There is no zonation in the colony though sectoring, especially in the cleistothecia producing region, is sometimes seen. No diffusible pigments in the media have been observed. At a temperature of 24° C, the colony is raised and cottony white and exhibit deep striations. In 10 to 15 days
cleistothecia begin to develop in the central region giving it a dark appearance. Optimum temperature for the production of cleistothecia has been found to be 28° C.

The asexual stage of the fungus belongs to the genus Cephalosporium. The conidiophores develop as short lateral branches of the subaerial hyphae (fig. 13). They are erect, simple and slender being broadest near the base and gradually tapering towards the apex, and measuring 25—66 × 1.5—2.5 μ. The conidia produced remain adhering together in a glistening mucous drop, sometimes as large as 36 μ in diameter. The conidia (fig. 14) are hyaline oblong with rounded end and measure 4—7.5 × 1.5—3 μ.

The perithecia are abundantly produced in culture and are covered over by the subaerial white mycelium (fig. 15). They are very much close set and appear as small rounded black bodies. The perithecia are astomous and measure 99—218 × 86—175 μ. A large number of asci (irregularly disposed) are produced in the perithecium, which are 8-spored, ovate in shape (fig. 16), and have thin hyaline and evanescent walls. They measure 9—11.5 × 7.5—9 μ. The ascospores are dark-brown to black ovate to oblong (fig. 17) and measure 4—5.5 × 2.5—3 μ (excluding the wings). They have three longitudinal (sometimes obliquely placed) wing-like appendages, extending from one pole to the other. They are clearly visible in the polar view of the spore. In one polar view, the wings appear to be diverging from a common centre. The wings are about 1—1.5 μ. wide, membranous and pale in colour. The wall of the asci disappears at an early stage releasing the ascospores, still adhering in groups of eight within the cleistothecia. The presence of a large number of black ascospores imparts the perithecium a dark colour, though its three to four cell-layer wall is almost hyaline or light brown in colour and transparent. There is no special mechanism for the discharge of ascospores.

The present fungus resembles the one described by Cain (1956) under the name *Saturnomyces humicola* in all respects except in the size of the perithecia. But since the size of the fruiting bodies may (as has correctly been remarked by Grosklags & Swift, 1947) vary markedly with the media used, a difference in this character alone should not to be taken into account in distinguishing the species. And hence the present fungus is identified as *Emericellopsis humicola*.

Further, since Cain’s fungus has not so far been formally assigned to the genus *Emericellopsis* Van Beyma (1939) with which it resembles in having black ascospores having three longitudinal ridges, it is proposed that the fungus *Saturnomyces humicola* as described by Cain be designated as *Emericellopsis humicola* (Cain) Cain.

On the basis of his cultural studies and extensive remeasurements of the spores of the known and freshly isolated members of the genus *Emericellopsis* Durrell (1959) suggests that *E. humicola* be regarded as

The present fungus was isolated from soil sample, from a poultry farm in Talegaon, near Poona, and was cultured on glucose-yeast agar.

The colonies are fairly rapid growing, wet submerged, bacterioid at first, later producing pinkish subaerial hyphae bearing conidiophores and conidia, and exhibiting deep radial furrows running from about half the radius of the colony to its margin, and with a floccose central region. The cleistothecia begin to develop after 12—15 days: They are produced most abundantly in colonies incubated at 28°C, none being produced in those kept at 32°C. Reverse of the colony is light pinkish in colour at 24°C but is slightly darker at 28°C, the central region becoming black with the production of cleistothecia.

The mycelium is composed of very fine, septate, slender, profusely branched, submerged hyphae closely developing to produce a thick crust or pellicle on the agar surface. Sub-aerial pinkish hyphae develop in patches over the colony surface and produce conidiophores and conidia. The conidial stages are typical of the species of *Cephalosporium* (fig. 18). The conidiophores developing as lateral branches of the subaerial hyphae, are long, erect, slender, spirally produced 33—79×1.5—2.5 µ. They are broadest near the base, gradually tapering upward towards the apex, producing a glistening mucilage drop forming the conidial head measuring upto 16.5 µ in diameter. The conidia (fig. 19) are hyaline, oblong, ovate to obpyriform, and measure 4.5—10.5×2.0 to 4.5 µ.

Large number of perithecia are produced in the culture within 12—15 days of incubation. They are so close set and crowded, as to impart a blackish appearance to the colony. The cleistothecia are covered over by the hyphal pellicle (fig. 20). They are globose to sub-
globose or variously shaped owing to mutual adpression, astomous, 66—125 µ in diameter. The cleistothecial wall is about 6—7 µ thick and is composed of 3-layers of cells, pseudoparenchymatous, hyaline. In older cleistothecia one or two pores are sometimes developed in the cleistothecial wall for releasing ascospores. Large number of asci are produced, irregularly scattered within the cleistothecium. They are spherical to ovate in shape (fig. 21) and measure 10—13.5 µ in diameter. Their walls are thin membranous hyaline, and evanescent. The mature ascospores come to lie freely in the cleistothecial cavity after the ascus wall disappears. The ascospores are dark-brown in colour, and oblong to elliptical in shape, with three rarely four longitudinally or slightly oblique running wings 1 to 2.25 µ wide (fig. 22). They are broad towards one pole of the ascospore and narrow towards the other pole. The ascospores measure 6—7.5 µ × 3—3.5 µ (excluding the wings).

The present fungus resembles *Emericellopsis minima* Stolk and *E. salmosynnemata* Grosklags & Swift in having a pinkish colony colour. It, however, differs from *Emericellopsis minima* in the following characters: 1. in having well marked deep striations in the colony and pinkish subaerial mycelium, 2. larger conidiophores and broader conidia, which are oval to obpyriform, 3. relatively thin cleistothecial wall which is 3-cell layered about 6—7 µ thick where as in *E. minima* it is upto 15 µ, thick, 4. in having slightly longer but narrower ascospores 6—7.5 × 3—3.5 µ, which markedly differ in appearance from those of *E. minima* (5—6 × 3.5—4 µ).

Absence of well developed synnemata, presence of smaller conidiophores, conidia and ascospores and the submerged growth of the colony with well marked deep striations distinguishes the present fungus from *E. salmosynnemata*.

The other species of *Emericellopsis* which have been described so far differ from the present fungus in having a white colony colour. Marked differences also occur in ascospore sizes and many other characters. Thus the present fungus represents an undescribed species of *Emericellopsis* and the name *E. pusillus* is assigned for its accommodation.

**Emericellopsis pusilla**, Mathur, Sukapure & Thirumalachar, sp. nov.

Colonies on glucose-yeast agar rapidly growing, submerged, wet, with deep striations all over the colony and pink in colour, developing pinkish subaerial hyphae, bearing conidia and conidiophores: cleistothecia develop abundantly giving a blackish appearance to the colony. Reverse pinkish black central region. Colonies floccose at 28° C with profuse cleistothecial development. Mycelium delicate, septate, profusely branched, forming a thick crust on the agar surface, pinkish subaerial hyphae bearing conidiophores and conidia. Conidiophores,
lateral, erect, slender, broadest at base, tapering upward, 33—79 × 1.5—2.5 μ; conidial head upto 16.5 μ in diameter. Conidia hyaline, oblong, ovate to obpyriform 4.5—10.5 × 2.25—4.5 μ. Cleistothecia abundant, covered by mycelium, globose, subglobose or variously shaped due to adpression, astomous 66—125 μ in diameter. Wall 3-cell-layered pseudoparenchymatous, 6—7 μ thick; one or two pores developing in wall of older cleistothecia. Asci abundant, irregularly arranged in the cleistothecium, spherical to ovate, 10—13 μ, in diameter and with hyaline, membranous and evanescent wall. Ascospores brown, oblong to elliptical in shape, 6—7.5 × 3.0—3.5 μ, with three, rarely four, longitudinally or slightly obliquely running wing-like ridges, upto 2.25 μ wide.

From soil, Talegeon, Poona, Culture No. 1254 (Type).

Mycelii hyphae septatae, irregulariter ramulosae, in superficie matricis crustam crassiusculam formantes; conidiophora lateralia recta, gracilia, at basim latissima, sursum attenuata, 33—79 × 1.5—2.5 μ; conidiq hyalina, oblonga, ovoidea vel obpiriformia, 4.5—10.5 × 2.25—4.5 μ; cleistothecia numerosa, in mycelio nidulantia, globosa, subgloboa vel e mutua pressione plus minusve irregularia, astoma, 66—125 μ diam., in maturitate poro singulo vel poris duobus aperta; pariete psedoparenchymatico, 6—7 μ crasso; asci numerosi, irregulariter dispositi, globosi vel ovoidei, 10—13 μ diam., tenuiter tunicati, mox mucosi; sporae, brunneae, oblongae vel ellipsoideae, 6—7.5 × 3—3.5 μ, longitudinaliter vel suboblique vittis aliformibus usque ad 2.25 μ latis praeditae.

Durrell (1959) on the basis of series of ascospore measurement studies on species of Emericellopsis and their variants, concluded, that there are only two distinct species; one of them, the large-spored E. mirabilis (Malan) Stolk, and the small spored E. terricola van Beyma. For this purpose he took into consideration all the variants and mutants, which showed intergradation in the size of ascospores and tried to interpret that E. minima Stolk, E. salmosynnemata Grosklags & Swift, and E. humicola (Cain) Cain, are synonymous with E. terricola. He himself states that the sizes of ascospores in a particular species is fairly constant, irrespective of the media on which they are cultured. By taking into consideration the mutants with intergrading ascospore sizes, he builds up an artificial broad-based species in which several other species can be shown to merge, This is a very incorrect method, since the species described themselves are very distinct in cultural characters and spore measurements. The conidial stages are not taken into consideration at all. For instance in E. synnematicola Mathur & Thirum. (1961) showed a Stilbella type of conidial stage. The conidiophores of E. salmosynnemata are 45—100 μ long, while those of E. terricola are between 20—50 μ. Some of the species like E. salmosynnemata possess well developed mycelial strands grouped into synnemata which is a distinct character. Without considering all morphological and cultural characters together, the sizes of ascospores alone, that
too from abnormal variants, are overemphasised and misinterpreted to lump distinctly separate species under *E. terricola*.

Maag, Durrell and Payne (1959) have gone further to study the aminoacid and amide content of the different species to justify their conclusion that there can be only two *Emericellopsis* species. They showed that *E. mirabilis* has more lysine than other species, and hence there is a biochemical basis for taxonomic differentiation. It is needless to point out to those who are working with amino acid production from molds, that by enzyme block as a result of genetic variability amino acids may be made to accumulate in the medium, and this has no relation to the taxonomic status of the organism. While these biochemical studies may sometime help in elucidating classification problems, they should not be relied upon in case of important taxonomic considerations. It is therefore necessary to recognise *E. terricola*, *E. pusilla*, *E. synnematicola*, *E. humicola*, *E. mirabilis*, *E. minima* and *E. salmosynnemata* as distinct species of the genus *Emericellopsis*. The variants and mutants studied by Durrell have the status of hybrids and sports among higher plants.

In conclusion the authors wish to express their deep sense of gratitude to Prof. Dr, Franz Petrak for giving the latin diagnoses of the new species.

**Literature cited.**


— and —. *Microascus pedrosio* is *M. cinereus* — a correction. Mycologia 48: 466—448, 1956a.


Explanation Plate I.

1: Phialides of *Microascus trigonosporus* bearing conidia in chain. \(\times 1000\). — 2: Conidia \(\times 2000\). — 3: Perithecium \(\times 150\). — 4: Ascus \(\times 1250\). — 5: Ascospores \(\times 1500\). — 6: Phialides and conidiophores of *Microascus griseus* \(\times 1000\). — 7: Conidia \(\times 2000\). — 8: Perithecia showing long setose beak and the spores discharged in cirrhi \(\times 150\). — 9: Ascus \(\times 1250\). — 10: Ascospores \(\times 1500\). — 11: Asci of *Sporormia indica* \(\times 1000\). — 12: Ascospores of various ages. *S. indica* \(\times 1250\). — 13: Conidiophores of *Emericellopsis humicola* \(\times 500\). — 14: Conidia \(\times 750\). — 15: Cleistothecium \(\times 150\). — 16: Ascus \(\times 1500\). — 17: Ascospores \(\times 1750\). — 18: Conidiophores of *Emericellopsis pusilla* \(\times 500\). — 19: Conidia \(\times 750\). — 20: Cleistothecium embedded in hyphal pellicle \(\times 150\). — 21: Ascus \(\times 1500\). — 22: Ascospores showing wings \(\times 1750\).
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