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SPECIES OF *PHOMA* ISOLATED FROM MARINE SOILS IN INDIA

BY V. H. PAWAR, P. N. MATHUR AND
M. J. THIRUMALACHAR

Antibiotics Research Centre, Hindustan Antibiotics Limited, Pimpri, Poona

(With 2 Text-figures)

Three new species, *Phoma multipora*, *P. capitulum*, *P. ostiolata*, and one new variety, *P. ostiolata* var. *brunnea* isolated from salt marsh sediments near Bombay coasts, are described and illustrated.

Marine fungi are gaining importance in recent years following the search for micro-organisms producing new and commercially valuable metabolic products. As pointed out by Wolf & Wolf (1947), the knowledge of marine fungi available to students of biology was, until recently, non-existent. While no reasons could be attributed to the lack of interest in the marine fungi, the cause may partially be due to the difficulty in isolating them from unusual habitats. Recently, however, Ritchie (1954), Vishniac (1955), Sparrow (1937), Barghoorn & Linder (1944), Pugh (1961, 1962), and others have made large-scale surveys of these fungi and have shown their importance not only from the viewpoint of phylogeny but also in biochemical studies such as nutrition, etc.

There have been a few records of fungi reported from marine soil in India (Pawar, Padhye & Thirumalachar, 1963; Pawar, Rahalkar & Thirumalachar, 1965). The present studies are confined to species of *Phoma* isolated from salt-marsh sediments from the back-water area near the Bombay coast where the soil is a heavy clay type and poorly aerated, and mangrove plants like *Rhizophora mucronata* Lamk., *Memphis acidula* Forst. and *Avicennia* sp. grow profusely, adding organic material which permits the establishment of other vascular plants. The type of study of such habitats is similar to that of Elliott (1930) who isolated marine fungi from salt marsh sediments which had an association of vascular plants such as *Salicornia*, *Glyceria*, *Armeria*, *Festuca* and *Juncus*.

Species of *Phoma* in marine habitats have been reported by previous workers on marine fungi. Cooke (1890) and D'Yakonov (1925) recorded *P. laminariae* Cooke & Massee and *P. navium* Woronichin respectively. Lloyd (1952), Ritchie (1954, 1957), Höhnk (1956), Nilsson (1957), Nicot (1958), Gold (1959), Goodman (1959), Meyers & Reynolds (1959), Kohlmeyer (1960), Siepmann & Johnson (1960), Pugh (1961, 1962), Pugh, Blakeman, Morgan-Jones & Eggins (1963) and Jones & Oliver (1964) reported *Phoma* sp. from marine habitats. Pady & Kapica (1955) found *Phoma* spores in air over the Atlantic ocean. Borut & Johnson (1962) reported *P. glomerata* (Corda) Wollenw. & Hochstetl and *P. hibernica*

Grimes, O'Connor & Cummins from estuarine sediments. In the present studies three new species and one new variety of *Phoma* isolated from marine soil are described.

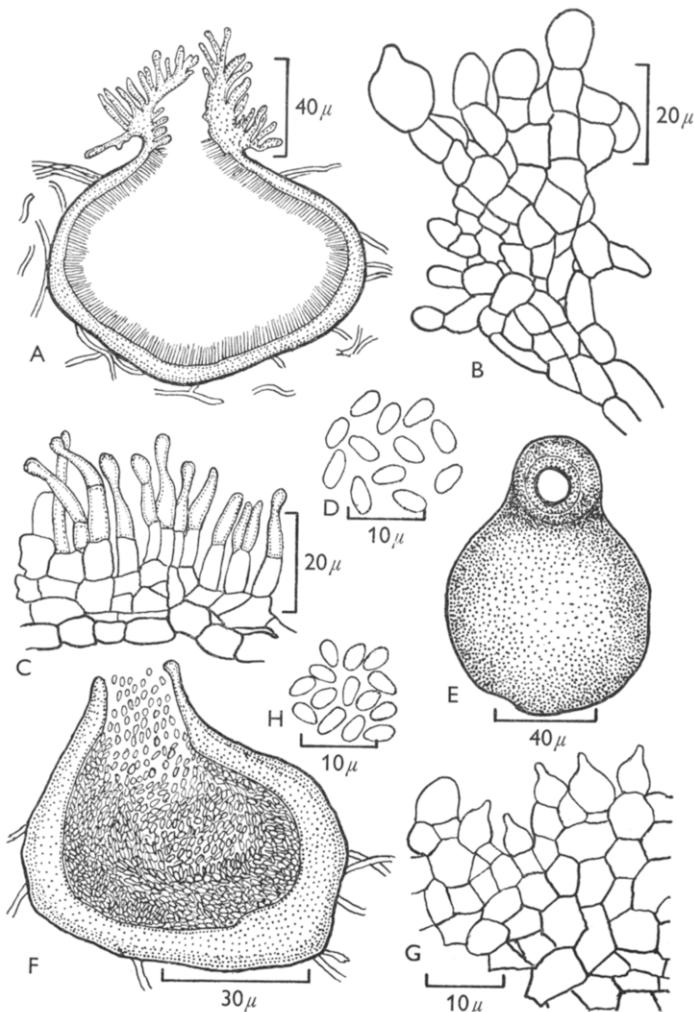


Fig. 1. A-D, *Phoma multipora*. A, Pycnidium; B, wall cells in the ostiolar region; C, pycnidial wall showing conidiophores; D, conidia. E-H, *Phoma capitulum*. E, F, Pycnidia; G, section of pycnidial wall; H, conidia.

1. *Phoma multipora* sp.nov. (Fig. 1 A-D)

Coloniae albae vel purpureo-albae vel violaceae. Lente crescentes in culturis plurimis, sed producentes pycnidia copiosa. Pycnidia globosa, subglobosa vel irregularia, violacea, non carbonacea, $72.5-330 \times 69.5-264\mu$, ostiolata; ostiolis uno, duobus vel pluribus, amplis, $26.5-56\mu$ diam., circumdatis ordinibus brevibus cellularum (filamentis brevibus) desinentibus in cellulas relative maiores, parietibus gracilibus ornatas. Cavitas pycnidii

unica; conidiophora brevia, simplicia, hyalina, terminaliter supportantia conidia. Conidia minuta, ovalia vel oblonga, hyalina, in massa purpurascens, $3\text{--}5\cdot4 \times 1\cdot5\text{--}3\mu$. Typus HACC-164 lectus in solo salino, Bandra, Bombay, India, 15. i. 1958.

Colony white to pinkish white or violet. Slow growing on most media, but producing abundant pycnidia. *Pycnidia* globose, subglobose to irregular, violet, not carbonous, $72\cdot5\text{--}330 \times 69\cdot5\text{--}264\mu$, ostiolate; ostioles one, two or more, large, $26\cdot5\text{--}56\mu$ diam, surrounded by short rows of cells (short filaments) terminated by relatively large, thin-walled cells. Pycnidial cavity single; conidiophores short, simple, hyaline, bearing conidia terminally. *Conidia* small, oval to oblong, hyaline, pinkish in mass, $3\text{--}5\cdot4 \times 1\cdot5\text{--}3\mu$.

Habitat: Type HACC-164 isolated from saline soils, Bandra, Bombay, India, 15 January 1958.

Colonies on Czapek's agar slow-growing, $4\cdot2$ cm diam in 10 days at 28°C , flat, submerged, with little subaerial mycelium and abundant pycnidial production, pinkish white and with pinkish reverse; growth slow on glucose-yeast extract agar and potato-dextrose agar, with colony diam. $3\cdot8$ and $3\cdot5$ cm respectively, in 10 days at 28° . Growth was poor at 32° and the fungus failed to grow at 37° . The mycelium is composed of slender, hyaline to slightly pinkish, profusely branched hyphae up to 6μ thick. The pycnidial wall is composed of three to four layers of thin-walled hyaline cells. The cells round the ostiole are slightly coloured. The spores are formed from the innermost layer of the pycnidial wall, and are embedded in a gelatinous matrix.

This fungus cannot be matched with any of the known species of *Phoma*. It differs from *P. hibernica* Grimes, O'Connor & Cummins, *P. humicola* Gilman & Abbott and *P. glomerata* (Corda) Wollenw. & Hochapfel in having smaller spores, peculiar short hyphae swollen at the tips surrounding the ostioles, and more than one ostiole in the majority of the pycnidia.

2. *Phoma capitulum* sp.nov. (Fig. 1 E-H)

Coloniae lente crescentes in Czapek agaro. Mycelium hyalinum, gracile, septatum. Pycnidia copiosa, hyalina vel sub-brunnea, erumpentia, sphaerica, subsphaerica vel paulum irregulare, $56\text{--}145 \times 46\cdot5\text{--}82\cdot5\mu$, ostiolata, rostris papillae similibus vel brevibus et curvis, uno vel pluribus, ad $16\cdot5\mu$ diam. Conidia terminaliter insidentia papillis, minutis, parva, hyalina, ovales, $3\text{--}4\cdot5 \times 1\cdot5\text{--}2\cdot5\mu$, liberata in massa mucosa purpurascenscentia.

Typus HACC-167 lectus in solo salino, Bandra, Bombay, India, 20. iv. 1958.

Colonies slow-growing on Czapek's agar. Mycelium hyaline, slender, septate. *Pycnidia* abundant, hyaline to slightly brownish, erumpent, spherical, subspherical to slightly irregular, $56\text{--}145 \times 46\cdot5\text{--}82\cdot5\mu$ ostiolate, beaks as papillae or short and curved, one or more, up to $16\cdot5\mu$ diam. *Conidia* borne terminally on small papillae, small, hyaline, oval, $3\text{--}4\cdot5 \times 1\cdot5\text{--}2\cdot5\mu$, discharged in pinkish yellow gelatinous mass.

Habitat: Type HACC-167 isolated from saline soils, Bandra, Bombay, India, 20 April 1958.

On Czapek's agar the fungus grows slowly, producing a colourless flat colony $3\cdot5$ cm diam in 10 days at 28°C ; on potato-dextrose agar the fungus grows rapidly reaching $6\cdot5$ cm diam in 10 days at 28° ; on glucose-yeast extract agar growth is slow compared with that on potato-dextrose

agar. Pycnidia are pale coloured at first becoming slightly brownish later, especially near the ostiole. The pycnidial wall is composed of 4–6 layers of thin-walled, hyaline cells. The cells of the outer two to three layers become slightly thick-walled. The cells of the innermost wall layer produce the spores on small papillate conidiophores.

This fungus differs markedly from the known species of *Phoma*. It is distinguished from *P. humicola* by the size of the pycnidia and the spores, and from *P. hibernica* and *P. glomerata* in having smaller spores. The spore mass in *P. hibernica* is pinkish and in *P. glomerata* it is white, while in the present fungus it is orange. It differs from *P. pimprina* Mathur, Menon & Thirum. and *P. purpurea* Mathur & Thirum. in the colour of the spore mass and also in the colony characters. The possession of orange spore masses brings this fungus closer to *P. ostiolata* and its variety *brunnea*. It differs, however, from *P. ostiolata* in having smaller pycnidia and papillate ostioles. Besides this, the distinct, slender, peg-like conidiophores found in *P. ostiolata* are wholly unrepresented in the present fungus. *P. ostiolata* var. *brunnea* also differs from this fungus in having larger pycnidia and in the absence of beaked ostioles.

3. *Phoma ostiolata* sp.nov. (Fig. 2A–D)

Lente crescens in agar Czapek, thermophilicum, crescens sat faciliter ad 37 °C. Pigmenta solubilia nulla producta. Mycelium constans hyphis hyalinis, gracilibus, septatis, profuse ramosis. Pycnidia sphaerica vel subsphaerica, parva, 105–205 × 75–165 μ, ostiolata. Ostiolis 10–16 μ diam. Cellulis circum ostiola nigris, crassis. Conidio-phora parva, paxillata, hyalina, terminaliter supportantia sporas. Conidia parva, hyalina, ovales vel sphaerica vel ovato-oblonga, 3–5 × 1.8–3 μ, liberata in massa mucosa purpureo-lutea vel aurantiaca.

Typus HACC-165 lectus in solo salino, Bandra, Bombay, India, 15. iii. 1958.

Fungus slow-growing on Czapek's agar, thermophilic, growing and reproducing readily at 37°. No soluble pigments produced. Mycelium consists of hyaline, slender, septate and profusely branched hyphae. *Pycnidia* spherical, subspherical, small, 105–205 × 75–165 μ, ostiolate. Ostioles 10–16 μ diam. Cells bordering ostioles thick-walled, dark black. Conidiophores small, peg-like, hyaline, bearing spores terminally. *Conidia* small, hyaline, oval to spherical to ovate-oblong, 3–5 × 1.8–3 μ, discharged in a pinkish yellow or orange gelatinous mass.

Habitat: Type HACC-165 isolated from saline soil, Bandra, Bombay, India, 15 March 1958.

Colonies on Czapek's agar slow growing, 4.6 cm diam in 10 days at 28°. submerged to subvelvety, with little subaerial to aerial mycelium. The fungus grows rapidly on glucose–yeast extract agar and potato-dextrose agar producing white floccose colonies, 7.0 and 6.3 cm diam respectively, in 10 days at 32°. The optimum temperature for the growth of the fungus appears to be near 32°.

The fungus is distinct from other known species of *Phoma*.

4. Var. **brunnea** var.nov. (Fig. 2 E-G)

Affinis *Phomae ostiolatae* characteribus generalibus coloniae et evolutionis. Pycnidia paulo maiora, 230–537 × 160–325 μ , ostiolata. Ostiolis 13–21.5 μ diam. Cellulis circumdantibus ostiolum crassis fusce brunneis. Conidia sessiles, ovales, hyalina, 2.5–4.8 × 2–3 μ , liberata in massa purpureo-lutea vel aurantiaca.

Typus HACC-166 lectus in solo salino, Bandra, Bombay, India, 15. xi. 1958.

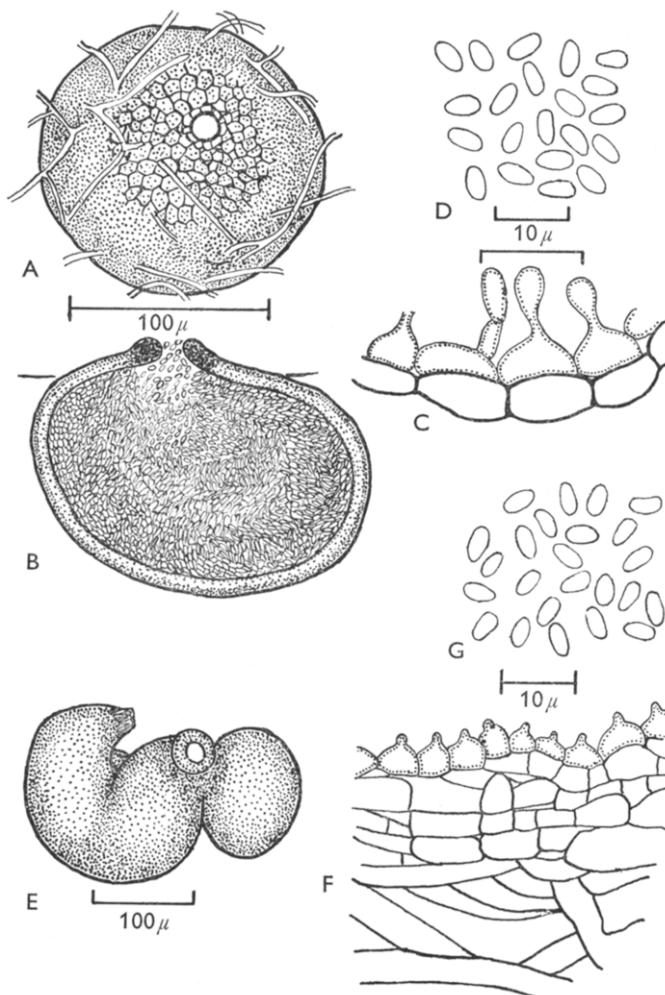


Fig. 2. A-D, *Phoma ostiolata*. A, B, Pycnidia; C, pycnidial wall showing spore development; D, conidia. E-G, *Phoma ostiolata* var. *brunnea*. E, Pycnidium; F, section of pycnidial wall; G, conidia.

The fungus resembles *P. ostiolata* in general colony characters and growth habits. Pycnidia similar to those of *P. ostiolata* but slightly larger in size, 230–537 × 160–325 μ diam. Cells bounding ostioles thick-walled, dark

brown. Conidia sessile, oval, hyaline, $2.5-4.8 \times 2-3 \mu$, discharged as a pinkish yellow or orange mass.

Habitat: Type HACC-166 isolated from saline soil, Bandra, Bombay, India, 15 November 1958.

The optimum temperature for the growth of the fungus seems to be near 32° . The fungus is capable of growing and reproducing at 37° . With the development of the lobes in the pycnidia the number of ostioles in each pycnidium also seem to increase to two, three or more, according to the number of the lobes.

The fungus resembles *P. ostiolata* in the spherical to subspherical to lobed appearance of the pycnidia, in the colour of the spore mass, in size of the spores and in ability to grow at high temperatures. It differs, however, from *P. ostiolata* in having large pycnidia, dark brown cells surrounding the ostioles, relatively large ostioles and by the absence of distinct conidiophores. Because of the general resemblance to *P. ostiolata* it has been considered best to refer it to this species. The points of difference shown above, however, seem to justify regarding it as a new variety.

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