

## A revision of the genus *Blakeslea* from India.

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With Plates IV—VII.

The genus *Blakeslea* was established by Thaxter in 1914 with the type species *B. trispora*. He distinguished this genus from *Choanephora* by the presence of few-spored sporangiola on sporangioli-ferous heads, and the absence of conidia. Both these genera have many characters in common, i. e., the presence of multi-spored and few-spored sporangia; the presence of striations and appendages in the sporangiospores; and the formation of zygospores between the tips of entwining branches. Later Sinha (1940), working on *B. trispora* noticed the production of one-spored sporangiola, besides few-spored sporangiola, under poor nutritional conditions. From this he concluded, that the monosporous sporangiola of *B. trispora* were identical with the conidia of *Choanephora*. He, therefore, suggested that *Blakeslea* must be reduced to *Choanephora*. Later Poitras (1955), also noticed one-spored sporangiola, besides the normal few-spored sporangiola in *B. trispora*. Further, he studied the conidia of the different species of *Choanephora* by subjecting them to various treatments. He found, that under certain conditions, conidia of *Choanephora* showed empty spaces. He demonstrated that there is an outer wall of the sporangium and a separate coloured inner wall of the endospore. Furthermore, he studied some of the germinating conidia of *Choanephora* and observed that during germination, the outer thin sporangiolic membrane was drawn out for a short distance with the germ tube. This further indicated that the inner spore wall was separate from the outer sporangiolic membrane. From these findings, he arrived at the conclusion, that the principal difference between *Blakeslea* and *Choanephora* was not of generic significance and, therefore, the correct epithet for the species having multi-spored (usually 3—5) sporangiola is *Choanephora trispora* (Thaxter) Sinha. Recently Naganishi and Kawakami (1955), described a new species of *Blakeslea*, viz., *B. circinans* from Japan. This species is characterized by the presence of only multi-spored and few-spored columellate sporangia with appendaged sporangiospores and the absence of few-spored sporangiola or conidia. Later Hesseltine and Benjamin (1957), studied the mating reaction of this species and found that

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the zygospores were similar to the other species of *Choanephora*. In view of the findings of Poitras (1955) and their own, they transferred this species to the genus *Choanephora*.

The author working on *Mucorales* in India isolated three different species of *Blakeslea*. These were *B. trispora* Thaxter, *B. circians* (Naganishi and Kawakami) Mehrotra and *B. tandonii* Mehrotra. The present isolate of *Blakeslea*, viz., *B. circinans* was found to develop few-spored sporangia on the sporangioliferous heads like *B. trispora* though rarely. Such structures were reported to be absent in this species by the previous workers. The present findings with this isolate now confirm its position in *Blakeslea*. It, however, differs from *B. trispora* in the presence of characteristic sporangia and colour, size and shape of the sporangiospores. Besides it differs from *B. trispora* in presence of generally more than 3—5 spored sporangia.

Another species of *Blakeslea*, viz., *B. tandonii*, which has been recently described by the author resembles *B. circinans* in the following characters.

1. Presence of few-spored sporangia on the sporangioliferous heads. — 2. Presence of generally more than 3—5 spored sporangia.

It, however, differs from that in the following characters:

1. Presence of septate sporangiophores. — 2. Size, shape and colour of the sporangiospores. — 3. Presence of appendages all over the entire surface of a number of sporangiospores.

The author personally feels, that considered from a practical viewpoint the retention of the genus *Blakeslea* now becomes indispensable. The presence of monosporous sporangia in different species of *Blakeslea* certainly shows, how evolution has taken place from multi-spored sporangial forms (viz., *Mucor*, *Absidia* and *Rhizopus*) to forms developing one-spored sporangia (different species of *Choanephora*). On the basis of this character, which is of theoretical importance, the reduction of *Blakeslea* to *Choanephora* seems to be impossible and would be rather erroneous. In view of the above facts, the author has retained the genus *Blakeslea* which stands out in one essential character, viz., the presence of few-spored sporangia, from the genus *Choanephora*. It is here represented by the following three species, viz., *Blakeslea trispora*, *B. circinans* and *B. tandonii*.

#### Experimental.

Isolations from one inch of the soil surface of different types of soils were done by direct inoculation method of Waksman (1931), employed for isolating the fungi from soils. The following culture media were tried for the isolation and identification of the present organisms.

1. Potato Dextrose Agar Medium: Potato, 200 gm; dextrose, 20 gm; dist. water, 1000 ml.

2. Synthetic Mucor Agar Medium: Dextrose, 40 gm; asparagine, 2 gm;  $\text{KH}_2\text{PO}_4$ , 0,5 gm;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0,25 gm; thiamine hydrochloride, 0,5 mg; agar agar, 20 gm; dist. water, 1000 ml.

3. Blakeslee's Medium: Dry malt extract, 20 gm; dextrose, 20 gm; peptone, 1 gm; agar agar, 20 gm; dist. water, 1000 ml.

4. Barnett and Lilly's Medium: Dextrose, 3 gm; asparagine, 1 gm;  $\text{KH}_2\text{PO}_4$ , 1 gm;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0,5 gm; microelements as sulphates (Zn, 0,2 mg; Fe, 0,2 mg; Mn, 0,1 mg); thiamine hydrochloride, 100  $\mu\text{g}$ ; agar agar, 20 gm; dist. water, 1000 ml.

*Blakeslea trispora* Thaxter.

Colony cottony, white when young becoming yellowish on aging, growing rapidly; hyphae non-septate, granular upto 13  $\mu$  in width, showing tight twisted knots of mycelium; sporangiophores of sporangia arising from surface hyphae, unbranched, often bent or circinate below the sporangium, quite variable in length; sporangia at first white and later becoming brownish black, spherical, or slightly flattened 20—160  $\mu$ ; wall hyaline, persistent, tuberculate breaking open to give two equal halves; reduced sporangia borne on smaller sporangiophores, generally few-spored, rarely one-spored sporangia also seen; columellae elongate, oval to pyriform, with a collar, 45—120  $\times$  14—59  $\mu$ ; sporangioliferous sporangiophores may end into a primary vesicle which directly bears typically 3 spored sporangiola, rarely 1 or five spored sporangiola also produced, primary vesicle may be absent and the sporangiophore may bifurcate at its tip giving rise to secondary vesicles, at times the stalks may branch dichotomously, the ultimate branches terminate into sporangiola bearing vesicles; sporangiola oval 12—15  $\times$  11—13  $\mu$ , mostly 3 spored, rarely 1 or 4 spored borne on rounded sterigmata on the vesicles; sporangiola-spores like those of sporangia 8—15  $\times$  4—9,5  $\mu$ ; chlamydospores oval or fusiform, 11—20  $\times$  13—30  $\mu$ , usually present in old cultures, solitary or in chains; zygospores not seen.

Isolated from the soils collected from various parts of India. Culture No. 1., on which the above description is based, is with the author. It will also be deposited at NURD, Peoria, Illinois, U. S. A.

*Blakeslea circinans* (Naganishi an Kawakami) Mehrotra.

Colony white, growing slowly, with irregular margin, aerial mycelia, made up of lax and tangled hyphae sporulating poorly; sporangiophores bearing few-spored sporangiola on the sporangioliferous heads, arising from surface hyphae, unbranched, non-septate, hyaline; sporangiola spherical to oval, at first colourless later light brown in colour, reaching a diameter of 28  $\mu$ ; sprangiospores spherical to ellipsoid, 3,75—22,5  $\times$  3,75—15  $\mu$ , striate, appendaged at either end; sporangiophores bearing sporangia mostly unbranched, non-

septate; sporangia at first white then yellowish and finally brownish black, globose or slightly flattened, 30—180  $\mu$  in diam., sporangial wall purplish in colour, with surface smooth; wall breaking into pieces leaving a distinct broad collar at the base; columellae smooth-walled, faintly brown in colour, oval to pyriform, always longer than broad and the larger ones always pyriform, 20—100  $\times$  17,5—60  $\mu$  in size; reduced sporangia borne on smaller sporangiophores, generally few-spored, rarely one-spored sporangia also developed; sporangiospores irregular in shape, mostly oval, striate, appendaged, 3,5—14,75  $\times$  3,5—8,0  $\mu$ , chlamyospores 9,5—18  $\times$  16—29,75  $\mu$ ; zygo-spores not seen.

Isolated from the soils collected from various parts of India. Culture No. 2., on which the above description is based, is with the author. It will also be deposited at NURD, Peoria, Illinois, U. S. A.

*Blakeslea tandonii* Mehrotra.

Colony colourless when young, becoming yellowish on aging, growing rapidly; aerial mycelium profusely developed; hyphae septate, branching irregularly; sporangiophores bearing few-spored sporangiola on the sporangioliferous heads, arising from surface hyphae, unbranched, septate, hyaline; sporangiola spherical to oval, normally with 5—10 spores, borne on long stalks, at first colourless later brownish black, reaching a diameter of 40  $\mu$ , wall colourless, smooth, breaking into pieces; sporangiospores irregular 5,0—30  $\times$  5,0—18,5  $\mu$ , finally striate, light coloured, few with a hyaline structure at one end, appendaged mostly at either end and several provided with innumerable appendages at several points on the surface; sporangiophores of the reduced sporangia arising from surface hyphae, hyaline, unbranched, septate; reduced sporangia reaching a diameter of 50  $\mu$ , brownish black, columellate or non-columellate; columellae globose to pyriform; zygospores not seen.

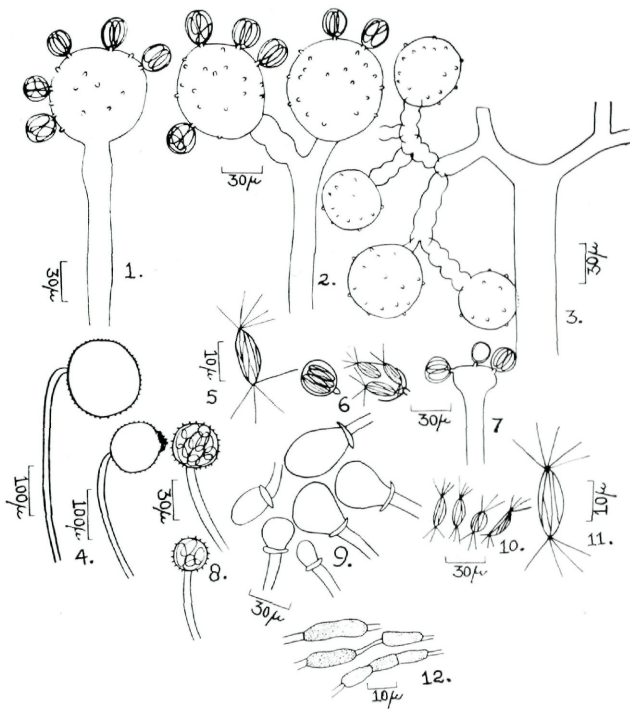
Isolated from a soil sample obtained from Dr. S. S. Khanna who collected it from a garden, January, 1963, at Nainital. Culture No. 3., on which the above description is based is with the author. It will also be deposited at NURD, Peoria, Illinois, U. S. A.

#### Acknowledgements.

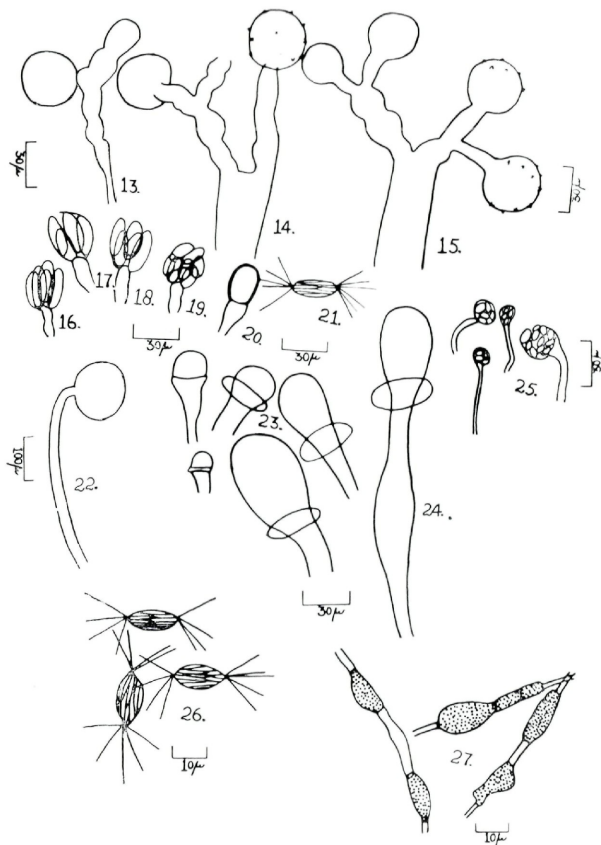
The author is indebted to Dr. S. S. Khanna, assistant professor in Government Degree College, Nainital for obtaining the soil sample. He is also grateful to Shri Govind Prasad for his help and to Prof. R. N. Tandon for the laboratory facilities.

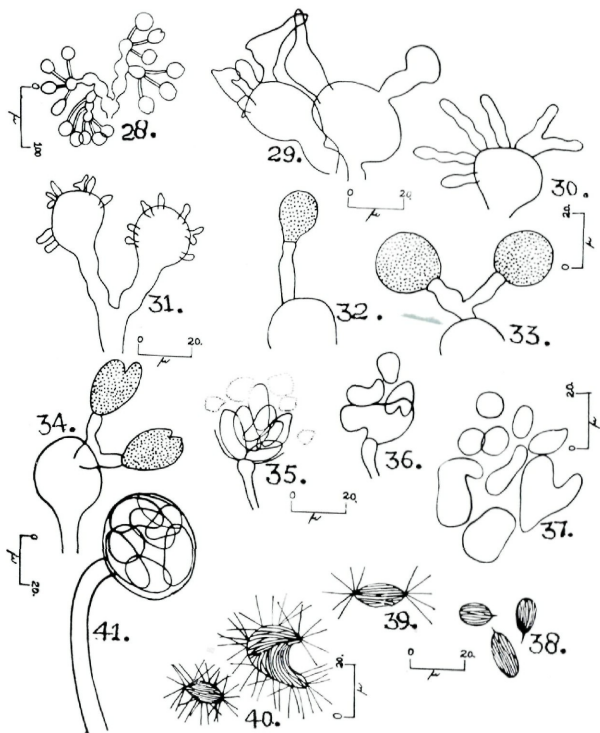
#### Literature cited.

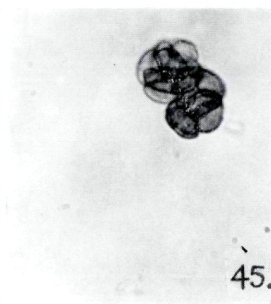
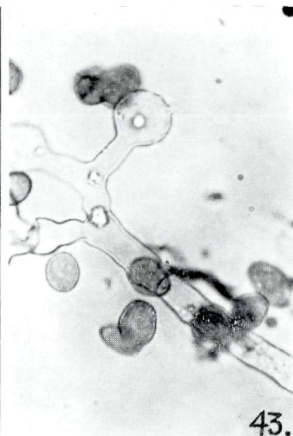
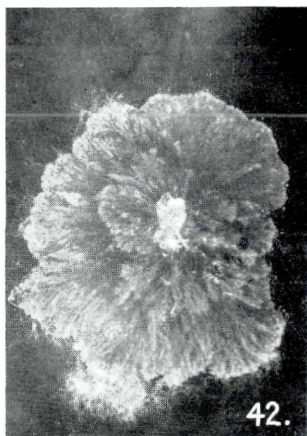
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#### Explanation of Plate IV—VII.

Plate IV. 1—12. *Blakeslea trispora*. 1. A sporangiophore with a primary vesicle bearing few-spored sporangiola. 2. A sporangiophore with bifurcated primary vesicle, the secondary vesicles bearing few-spored sporangiola. 3. A sporangiophore showing secondary vesicles borne on dichotomously branched stalks. 4. Two multispored sporangia borne on the circinate sporangiophores. 5. One sporangiospore from a few-spored sporangium showing appendages at either end. 6. Two few-spored sporangiola. 7. A sporangiophore with one monosporous and two few-spored sporangiola borne on primary vesicle. 8. Two few-spored sporangia. 9. A number of columellae showing the range in size and shape. 10. Four sporangiospores from a multi-spored sporangium showing appendages at either end. 11. An enlarged sporangiospore from a multi-spored sporangium showing appendages at either end. 12. Hyphae showing chlamydo-spores.

Plate V. 13—27. *Blakeslea circinans*. 13. A sporangiophore with a vesicle. 14 & 15. Two sporangiophores each with a bifurcated primary vesicle, secondary vesicles borne on long stalks. 16—19. Four 4 to 8 spored sporangiola from the sporangioliferous head. 20. One-spored sporangium. 21. One sporangiospore from a few-spored sporangium showing striations and appendages. 22. A multi-spored sporangium borne on the circinate sporangiophore. 23. Columellae showing the range in size and shape. 24. A sporangiophore showing swelling. 25. Four few-spored sporangia. 26. Three sporangiospores from a sporangium showing striations and appendages. 27. Hyphae showing chlamydo-spores.

Plate VI. 28—41. *Blakeslea tandonii*. A part of the sporangioliferous head showing attachment of sporangiola. 29. A part of the sporangioliferous head showing irregular growths on the vesicles. 30. A vesicle showing elongated beaded structures. 31. Vesicles showing sterigmata like out growths. 32. A young sporangium with its long stalk. 33. A vesicle showing two young sporangiola borne on long stalks. 34. Two matured sporangiola borne on long stalks arising from the vesicle. 35. A breaking sporangium with the spores and the pieces of its wall. 36. A part of the wall of the sporangium and three sporangiospores. 37. A number of the sporangiospores from a few-spored sporangium showing the range in size and shape. 38. Three sporangiospores from a few-spored sporangium showing hyaline structure at one end. 39. A sporangiospore showing appendages at either end. 40. Two sporangiospores showing appendages at several points on the surface. 41. A reduced sporangium on the circinate sporangiophore. Note the absence of columella.

Plate VII. 42—45. *Blakeslea circinans*. 42. Photograph showing the growth on SMA. 43. Photomicrograph showing a sporangiophore with sporangioliferous head and the sporangiospores. 44. Photomicrograph showing sporangiospores from a multi-spored sporangium,  $\times 600$ . 45. Two few-spored sporangiola from a sporangioliferous head. Note the presence of 7 to 8 spores in the sporangiola,  $\times 500$ .

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