

THE FUNGI CULTIVATED BY THE TERMITES OF BARKUDA.

By S. R. BOSE, M.A., Ph.D., F.L.S., Professor of Botany,
Carmichael Medical College, Belgachia.

(Plate VII.)

A good number of combs of termite-nests from Barkuda I., Chilka Lake, were very kindly presented to me by Dr. N. Annandale, Director of the Zoological Survey of India. He has made an elaborate study of the habits of the termites in those nests, which has been recorded in the foregoing paper; the termites themselves have been identified by Prof. Silvestri of Portici. I am specially indebted to Dr. Annandale for the critical study of the combs and the nests and for the names of the termites used in this paper and other zoological details in it. During the latter part of July, 1922 I accompanied Dr. Annandale to the island of Barkuda and stayed there for about a week in order to study the fungi of the termite nests.

In the island of Barkuda, as Dr. Annandale observes, there are at least four forms of termites which cultivate fungi for food.

Mound-builders :—

1. *Odontotermes (Cyclotermes) obesus* (Ramb).
2. *O. (C). obesus* var. *oculatus* Silvestri.

Burrowers :—

3. *Eurytermes assmuthi* Wassmann.
4. *Microtermes anandi* Holmgren.

As Dr. Annandale has described in detail the structure of the combs of the different species of termites and of the chambers in which they are situated, in his part of the report, I will only allude to them briefly here.

Dr. Annandale notes that the four forms of combs are interesting as illustrating progressive development from those of *Microtermes anandi* to those of *Odontotermes obesus*. *Microtermes anandi* does little more than cultivate its mushrooms on a manure bed just as we do; there are hardly any definite cells on the comb, the irregular holes on the surface of the mass representing the rudiments of the cells. In the case of *Eurytermes assmuthi* the combs are a little more complex in structure, the masses of excrement are a little more compact and less fragile than those of *Microtermes* and they have the form of a comb in a pectinate sense. *Odontotermes obesus* var. *oculatus* forms elongated irregular vertical cells in a massive comb and there is one large fungus-garden contained in a single chamber. Finally the typical form of *O. obesus* adopts a more orderly arrangement of cells in the comb. There is a wide gap in the complexity of fungus-gardens between those of *Eurytermes assmuthi* and of *Odontotermes obesus* var. *oculatus*.

A comb consists entirely of excreta of termites. Under the microscope wood-vessels of vegetable substance which have been eaten and have

passed almost unaffected through the digestive tract can be detected. It shows also spores of fungi and in one case at least I could find one distinct teleutospore. The comb is probably partially sterilized by its passage through the alimentary canal of the termites. Outwardly the more elaborate combs resemble a coarse bath sponge, and the colour varies from yellow-brown to dark brown. The upper part of the comb is often of a harder substance and of a darker colour than the lower.

Some fresh combs of *Odontotermes obesus* from red soil were cleared of termites by putting them in the track of a colony of black ants for some time. They were then wetted with water and on 16th September, 1920 three or four of the wet combs were placed upon water-soaked blotting-paper under the cover of a bell-jar. The next day the combs, which were almost bare before, were fully covered by a thin layer of dense white cottony mycelium. On 18th September there arose from the mycelium a large number of white upright stalks from 10 to 20 cm. long—the stromata of *Xylaria nigripes* (a photograph taken on the 2nd day of growth is shown in pl. VII, fig. 1). Some became attached to the sides of the bell-jar and some almost reached the top. These stalks of *Xylaria* are strongly heliotropic. They were at first white and looked like loosely-twisted strands of wool. In the course of two days, *i.e.*, on the 20th, the lower parts of the stalks turned black, and this black colour travelled from the base upwards, till in the course of a week or so everything turned black except the white apices and the stalks became solid with a white core of parallel hyphae enclosed by a black epidermal layer. The elongated stromata anastomosed in various ways in contact with the walls of the jar (a photo taken on the 12th day of growth is shown in pl. VII, fig. 2).

Some of the stalks had a white coating of conidia towards the lower parts. Water was added to the comb almost every day. Most of the stromata grew thicker, showed dichotomous branching (sometimes 4 to 6 divisions were found at the apices) and became conidioferous. A section of the apex under the microscope showed the conidial layer on the outside, but no perithecia were seen except in the case of *Xylaria* from *O. obesus* var. *oculatus* which was grown for a very long period. Most of the cultures of *Xylaria nigripes* were kept under observation for more than two and a half months.

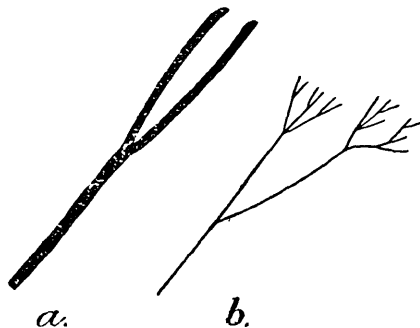
In the course of 12 to 14 days, the long stalks began to collapse and the majority of the looser stromata drooped down on the combs, which had by this time lost the regular character of their cells and had dissolved into an almost homogeneous black mass as shown in fig. 2, pl. VII; only a few remained attached to the sides of the bell-jar. During this time the bell-jar was opened for about a minute and this hastened the collapse of the stromata.

The drooping stalks were now attacked externally by white and yellowish rounded patches of parasitic *Mucor* and by yellow tufts of some *Discomycetes*, while small white sluggish mites also appeared on the tips of the stromata. The tips of some of the stromata still showed signs of branching and became flat and ultimately conidioferous. Fresh loose white stromata again developed here and there from the black stalks on 11th October (*i.e.*, on the 25th day), and ultimately became black and

firm like the original ones. Their white tips likewise became branched and conidioferous and these scanty fresh growths with single and branched conidioferous tips were observed on dying stromata on 20th October, 31st October, and finally on 20th November and following days. The more complex much-branched stalks ultimately assumed the form called *furcata* by some mycologists.

During this period, the maximum temperature of the room in which the jar was kept ranged from 81° to 88° F., and the minimum from 68° to 74° F. About half a dozen cultures of *Xylaria* from different termite-combs were grown, and the ultimate results were almost the same in all cases whether the termites were removed or not.

In two cases after about two weeks there was a sudden growth from the side of the jar of a very short-lived Agaric—*Coprinus niveus*, which is very common in Bengal in dung and heaps of rotten straw. A full description with a plate has been published in the Proceedings of the Science Convention of the Indian Association for the Cultivation of Science for 1918.



a. Branching of typical *Xylaria nigripes*.
b. Branching of the form *furcata*.

Possibly the spores of *Coprinus* had somehow travelled with the combs after their removal from the nest, though ordinary precautions were taken. In one of the two cases one of the combs did not produce *Xylaria* stromata; but was first of all fully covered with a thick white mycelial mass of sessile spheres which, in the course of a week, turned green like green moulds on decomposed wood. The mass remained the same colour throughout, and eventually produced a number of rounded sporangia consisting of green spores.

The following notes show the minor differences observed in cultures of *Xylaria* from different combs. In the case of typical *O. obesus* from red soil, the *Xylaria* stromata were as long as 20 cm. and many of them were rather thick. In the case of a comb of *O. obesus* var. *oculatus* which contained one large fungus-garden in a single chamber, the stromata on the other hand were shorter (4-5 cm.) and the majority of them much thicker. Subsequent cultures of *Xylaria* from the combs of *O. obesus* and *O. obesus* var. *oculatus* in July, 1922 did not show any difference in the length or thickness of the stromata. In the case of *Microtermes anandi* the growth of *Xylaria* stromata was very scanty and the stalks long, about 10-14 cm., and very thin. These *Xylaria* from the different combs are the same species, all representing forms of *Xylaria nigripes*, which is exclusively confined to abandoned termite-

nects (*cf.* *Xylaria* Notes, no. 1, by C. G. Lloyd, September, 1918, pp. 10-11). If cultivated sufficiently long in a very moist atmosphere they assume, as I have stated, the form *furcata* (see text-fig.). These *Xylaria* have been kindly identified by Mr. C. G. Lloyd and Mr. T. Petch of the Peradeniya Botanical Gardens.

When the combs of *Eurytermes assmuthi* were put under bell-jars with living termites in them, the stalks of *Xylaria* grew up after a long time—5 or 6 days after the first day of culture. During this time most of the termites had died under the bell-jar. The growth was not abundant, and the stalks were very feeble and short, only about 3 cm. long. Some few remained unbranched, and most of them showed after a month regular but feeble dichotomous branching and in the form *furcata*, feebler sub-branching. Most of the *Xylaria* artificially grown in cultures were in conidial stages, but in the case of *Xylaria nigripes* from the comb of *O. obesus* var. *oculatus* in sandy soil, which was kept for a longer time, I succeeded in getting a number of rounded perithecia with asci and black oval spores, about $4 \times 2 \mu$ in longer and shorter diameter.

The nests of *O. obesus* form continuous series on the island of Barkuda, as can be seen from text-fig. 2, p. 247. They are mostly under-ground, throwing up a mound here and there above the surface.

From the part of the nest of *O. obesus* above ground were collected on 23rd June, 1920 during the rainy season a number of edible mushrooms (*Collybia albuminosa* (Berk) Petch),—some fully developed and others in a state of bud. In this fungus the stalk is about 16 cm. long with a spreading umbonate cap; its spores are pink, oval, and in measurement $8 \times 4 \mu$. The specimens had long rooting stalks going down to the combs. The caps of some were partly eaten by beetles. These white *Collybia* were the only fungi which appeared, though only for a short time, from the mounds, which were full of living termites (*O. obesus*). These Agarics, however, appear but rarely at Barkuda. None were observed in 1922, which was a comparatively dry year, although Dr. Annandale made a careful search for them on many occasions.

In no case observed were *Xylaria* stromata produced from combs *in situ* within the mounds. Some short (about 3 cm. long) firm black stalks of *Xylaria aemulans* Starb. (determined by Mr. Lloyd) were, however, collected from a piece of dead wood sticking to the outer earthen wall of the tall cylindrical mound of *O. obesus* var. *oculatus*, and from the dead prostrate trunks of *Ficus bengalensis* and from logs here and there close to these mounds. *Xylaria aemulans* is very common at Barkuda on prostrate logs.

Another Agaric, *Entoloma microcarpum* B. S. Br., was found in a big mound deserted by *O. obesus* but occupied by small colonies of *Microtermes anandi*. It had no connection with the comb of *Microtermes anandi*, but was simply growing out of the moist wall of the mound. The species was also found growing in clusters in the red soil covering the old roots of a *Ficus* tree. It is commonly eaten by the villagers in Bengal. I have collected it also from Hughli District, growing in close proximity to a termite-nest, in fact from the external surface of it, as well as from various places on the roadside and cultivated fields, and grassy walks, quite independent of termite-nests.

A number of sections of intestines of different species of fresh termites and of their different forms as workers, soldiers, sexual nymphs, larvae, etc., were made in the months of July and August to examine their contents. In the case of *O. obesus* and *O. obesus* var. *oculatus*, sections of the intestines of workers and soldiers showed the presence of fragments of fungal hyphae and some oval black spores, whereas in the case of *Microtermes anandi*, sections of intestines of workers and soldiers showed the presence of oval spores, fragments of some fungal hyphae and some wood vessels (spiral, reticulate, etc). But when I examined a number of sections of intestines of workers and soldiers of *Odontotermes obesus* during the months of May and June, I could not find any fungal spores in any of them ; this might be due to the fact that fungi are scarce during the extreme hot weather. One of the sections showed a white pollen grain with a number of small warts on the upper surface. From the spores and fragmentary hyphae it cannot be determined exactly if they belong to the *Collybia* or to the *Xylaria*, because most fungi have oval spores. They only show how both or either fungus may be propagated from nest to nest.

Mr. Petch, the distinguished worker of Ceylon fungi, has contributed a masterly and elaborate account of the fungi of termite-nests in Ceylon to the *Ann. Roy. Bot. Gardens, Ceylon*, Vol. III, page 185 to 270 with seventeen plates. In this he states that the combs ultimately produce an Agaric—which has been assigned by various mycologists to *Lentinus*, *Collybia*, *Pluteus*, *Pholiota*, *Flammula* and *Armillaria* (now settled as *Collybia albuminosa*) which forms the chief edible mushroom amongst the Cingalese. The termites, he notes, regularly cultivate this agaric for food ; they eat its mycelium and spheres and though the growth of *Xylaria nigripes* has never been found on combs *in situ*, it takes place regularly in deserted combs after continued rains and after two days in bell-glass cultivations of termite-free combs. Later on, it is succeeded by patches of *Mucor*, *Aspergillus*, yellow and red moulds, and some yellow *Discomycetes* (*Peziza*). From this, Prof. Petch concludes that the termites maintain a pure culture, that they weed out all foreign fungi except the edible one, but that *Xylaria nigripes* defies their efforts to exterminate it, that it is only kept under, that its mycelium somehow vegetates in the comb-substance and that all the other fungi which develop in cultures are probably due to infection after removal from the nest.

Later on, in 1913, in vol. V of the *Ann. Roy. Bot. Gardens, Ceylon*, pp. 303—341, Mr. Petch has brought out a complete and excellent summary of all that is known on the fungus-cultivation by termites in different parts of the world.

The long-stalked white mushrooms (*Collybia albuminosa*) from the termite nests are edible ; they are found in different parts of Bengal, often associated with termite nests : some I got from the Holkar State through the divisional forest officer, with a note that it always grows in connection with ant-hills there. I have published a description of this *Collybia* with a plate in *Journ. As. Soc. Bengal*, Vol. XVI, no. 8, 1920. The fungus has been carefully analysed by Mr. C. B. Roy, B.Sc., M.B., of the Chemical Physiology Department of the Calcutta Medical

College, to whom my sincere thanks are due. It is found to contain 12·8% proteids, 14·8% carbohydrates, and a trace of fats (ether extractives) almost in the same proportion as in case of the local edible mushrooms. This edible mushroom is collected and sometimes sold during the rainy season in Bengal, and sometimes at the end of the rains. The rainfall¹ at Barkuda island is small, much smaller than that recorded for Peradeniya (Ceylon) by Mr. Petch, or than that of most parts of Bengal. Moreover, it is practically confined to a few months in the year.

¹ See Annandale, *Mem. Asiatic Soc., Bengal*, Vol. VII, No. 4, pp. 259—260.