Further Studies on the Symbiotes of Scale Insects.

By

S. Mahdihassan.

(With 14 figures in the text and plate 3.)

From previous communications, mentioned in the Bibliography, two main conclusions have been drawn: 1. Blood smears of different insects examined for their symbiotic microorganisms show morphologically distinct forms, thus indirectly enabling the host coccids to be identified. 2. The presence of yeast like forms of symbiotes, as opposed to bacterial forms, is physiologically associated with the excessive formation of sugars. Such insects may either excrete the sugars as honey dew or further transform them into waxes or into lac, likewise with meltable properties, as opposed to pseudo-lac. That sugars can be transformed into waxes has been experimentally recognised in the case of the honey bee, a fact familiar to every bee-keeper. The same is supposed to be true in the case of scale insects. At any rate, those coccids, which are constantly visited by ants on account of the honey dew, or those which secrete either wax copiously or genuine shellac, never possess bacterial forms of symbiotes. Although the synthesis of fats from sugars in the animal body is even better known than the transformation of sugars into wax by the honey bee, the reverse process in animal metabolism, the formation of sugars from the glycerine component of fats, is relatively less familiar, while the investigations hitherto show that fatty acids are not easily transformed into carbohydrates and the insects get rid of them through the formation of secretion products. In other words, sugars are excreted as such or secreted as waxes and waxes are polymerised fatty acids; thus sugars as well as wax originate from body fat.

These are concluded from the hypothesis that the symbiotic yeasts attack the fatty tissue of the scale insects. It is the very reverse of the theory that the yeasts are in the insects because the insects otherwise contain plenty of sugar where the cause of the origin of these sugars in the bodies of scale insects is not known. If we may imagine these insects to be suffering from Diabetis then, according to the opinion of others, the presence of yeasts in the body of the scale insects is a secondary infection of a saprophytic kind. According to the present hypothesis the cause of sugar excretion is to be found in the nature of attack by the micro-organisms themselves; the symbiotes are the causal agents of the origin of sugars. The present article is restricted to the micro-biological side of the phenomenon. At the present state of our knowledge observations must be further increased preparatory to the problem being attacked biochemically.

The common bacteriological routine of preparing blood smears involving the heating of a slide over a flame and the subsequent post mortem staining of microorganisms, is very drastic for the study of large sized yeast cells. The technique introduced by the late Prof. UNNA of Hamburg and further enlarged by his School, represented by Drs. SCHUMACHER and GUTSTEIN of Berlin, involve the use of mordants as well as of heating. It must, however, be admitted that it has many benificial features; but it is sometimes necessary for the yeast cells to be examined as transparent objects and particularly searched for the cell contents which this technique does not permit. The microorganisms are best studied in the living condition, perhaps better still with the help of such mild vital stains as Neutral Red. On the contrary, the very fine cytological method employed for the study of Bacteria by Prof. KUHN of Gießen and for the study of yeasts by Prof. HENNEBERG of Kiel, although perfect in themselves, are superfluous for the present purpose. After many trials the following method has been found to give the best results.

A mixture is made in equal parts of glycerine, lactic acid and water. Glycerine alone causes strong plasmolysis so that the yeasts look as if heated; even dilute glycerine has the same end effect. Addition of lactic acid prevents this shrinkage of protoplasm while a futher dilution with water facilitates an easy operation with the mixture. A drop of this mixture is placed on a slide and the scale insect, after being previously cleaned by means of a fine brush to remove dust and waxy powder which always accompany coccids, is

placed on the drop. With fine lancet shaped needles the edge of the body is ruptured in places enabling the body fluid to exude, leaving the internal organs as little uninjured as possible. The exuding fluid teams with hundreds of symbiotic cells and when sufficient liquid has been extracted out of the body the skin of the scale insect is lifted away and a cover glass placed on the drop. The edges are cleaned with pieces of blotting paper and a fine layer of quick drying oil varnish applied to the edges. The slide is left for a couple of days and when the oil varnish is hard and dry the edge is repainted with Canada Balsam, over the oil varnish. This double sealing has given every staisfactaction. Altogether the time required for making a dozen preparations of symbiotic microorganisms from each species of insect according to this method is not more than what is necessary for blood smears of the same number of slides after the usual bacteriological technique. As a rule one insect was used in preparing each slide but where material was scarce several slides were made from a single insect and the body of the insect pressed to squeese out the contents. Preparations were labelled atonce and examined at convenience. Slides examined after a week of their being sealed and again after nine years have shown no difference. In a few cases where Cotton Blue was used as a stain the colour maintained its original state of preservation.

The symbiotes were also isolated on artificial media and studied in single cell cultures after the method of Prof. Lindner. They proved one and all to be very polymorphic; so much so that cultures sent to other specialists invariably brought the objection that they were presumably impure. Prof. Přibram was able to convince himself of the polymorphic nature of such a microorganism and has given a beautiful chart to show the divergent shapes this fungus may give rise to and thereby emphacised the extreme nature of variation. The potentiality of the microorganism to be polymorphic, is not exhibited as long as the symbiote is living within the body of the host insect; on isolation however it shows an unlimited rage of variability. The restricted range of variation shown by blood smear preparations of symbiotes enables a much easier identification than is the case with their single cell cultures or when grown on artificial media. This remark, however, is only relatively true, for cases have been already recorded in this Journal, where the symbiote showed perplexingly divergent forms. To contrast with this defect in the method, it may be said, that two new insects have been discovered

by their offering entirely new pictures of symbiotic microorganisms viz. *Tachardina Silvestrii* (1928) and *Ceroplastodes Guilliermondi* (1933).

The abnormalities that sometimes occur among blood smears have been shown to be invariably confined to the adult. Mother insects under exceptional conditions would give a pure picture with rod shaped forms while the young larvae, issuing from the body of such a mother insect, would exhibit the normal yeast shapes forms of symbiote. Under such circumstances, not only greater weight was attached to the blood smears of young larval insects but, it was imperative to study them in the first instance and rather to supplement this by the examination of preparations delivered by the adult insects. A drop of liquid from the body of an adult scale insect is easily obtained; a similar attempt to extract a drop from the body of a young larva, which is very tiny indeed, offers great technical difficulties. A new method was developed to show the entire microflora of a young insect body. The larvae are fixed in a modified CARNOY's Fixative: Absolute Alcohol 60 Parts, Chloroform 30, Glacial Acetic Acid 5, Formol (40 % Strength) 10 Parts. The larvae were fixed in the above mixture for ten minutes and were gradually removed to water and finally treated with a 5 % Solution of Potassium Hydroxide in the cold until the skins were quite transparent. It was noticed that a long treatment with this reagent was very injurious in so far as the subsequent staining of the symbiote gave very poor results. It was found better to treat the objects rather less than allow them to remain in the solution too long in order to get well stained forms of symbiotic cells. The clear and transparent skins were washed in water, treated with dilute acetic acid and ultimately transferred to alcohol and stained in Acid Fuchsin or Alcoholic-Eosin. They were later treated with xylol containing a trace of Picric Acid and the objects observed under a microscope until the skins just began to become yellowish. They were washed with pure xylol and mounted in Canada Balsam. In good preparations the microflora stands out bright red against a pale orange colour of the skin. Lac insects were kindly sent to me through the courtesy of the Director of Agriculture in Tonkin. Some cells showed dead larvae which would have otherwise swarmed out but were left there in a mummied condition. Most lac incrustations show the presence of such larvae. These dead larvae were treated as above described and the stained preparations showed the specific symbiote of *Lakshadia chinensis*, an insect found in South China, Bhutan, Assam, Siam, Burma and Indochina.

A female crawling larva of the Mysore lac insect was treated as above and its symbiotic microflora is shown in Text-Fig. 1: the symbiotic cells are indicated in two places with S. Some yeast cells are hidden from view on account of the thick chitinous parts composing the head of the insect. This difficulty is met with only in photographing the preparation; by focussing up and down it is otherwise possible to trace the presence of yeast cells otherwise hidden by the chitinous portions of the body. In Text-Fig. 1 there are other parts of the body which also bear markings. In a previous communication it has been shown that the crawling larva of a lac insect is accompanied by a wax shield which is divisible in 11 segments. The female larva after treatment with caustic solution and its symbiote stained is seen lying on its back in Text-Fig. 1. The body segments are not all visible but most of the folds are indicated by numbers. On the margin, particularly, where the folds of segments with the numbers 6 and 7 are indicated, fine dots are visible resembling spiracles. These are pores of glands which secrete hard wax in the form of pencils; the secretion product issuing from the body of such a larva has been already illustrated (1930, Text-Fig. 7). Segment 11 is indicated with the corresponding number and shows a structure first brought into prominence in CHAMBERLIN'S Monograph on Lac Insects (1923) where on p. 162 he calls it Pseudocerarius. These are, in the commercial species of insects found in India, twin spines arising from a flat plate of chitin. There is a distinct plate which CHAMBERLIN in his description nor in his illustrations brings out clearly. A. B. MISRA (1931) looks upon it likewise as simple spines and moreover does not use the special terminology of CHAMBERLIN so that one is not sure if he even understood their specific nature. The pseudo-cerarii are better seen from the side and have been already illustrated in a pen and ink drawing (1930, Text-Fig. 1, p c) but unfortunately the word is misprinted as Pseudo-carius which must be corrected. The body ends in the Analring bearing ten Analring Hairs, A. R. H. which have, on either side, a long Major Apical Hair, of which only one is seen in Text-Fig. 1 here. The anterior portion of the body shows the Brachial Plate, B. P. on the margin of the body and the associated Spiracle, S r.

When such an object, as Text-Fig. 1 represents, is examined with an oil immersion the shape of the symbiotic cells can be specifically identified. The picture here reproduced does not give the necessary enlargement for it is intended to show the entire microflora within the body of a young larva. Text-Fig. 2 gives the

picture of symbiotic cells from *Lakshadia mysorensis* experimentall cultivated on *Acacia farnesiana* in a Zinc pot and daily watere

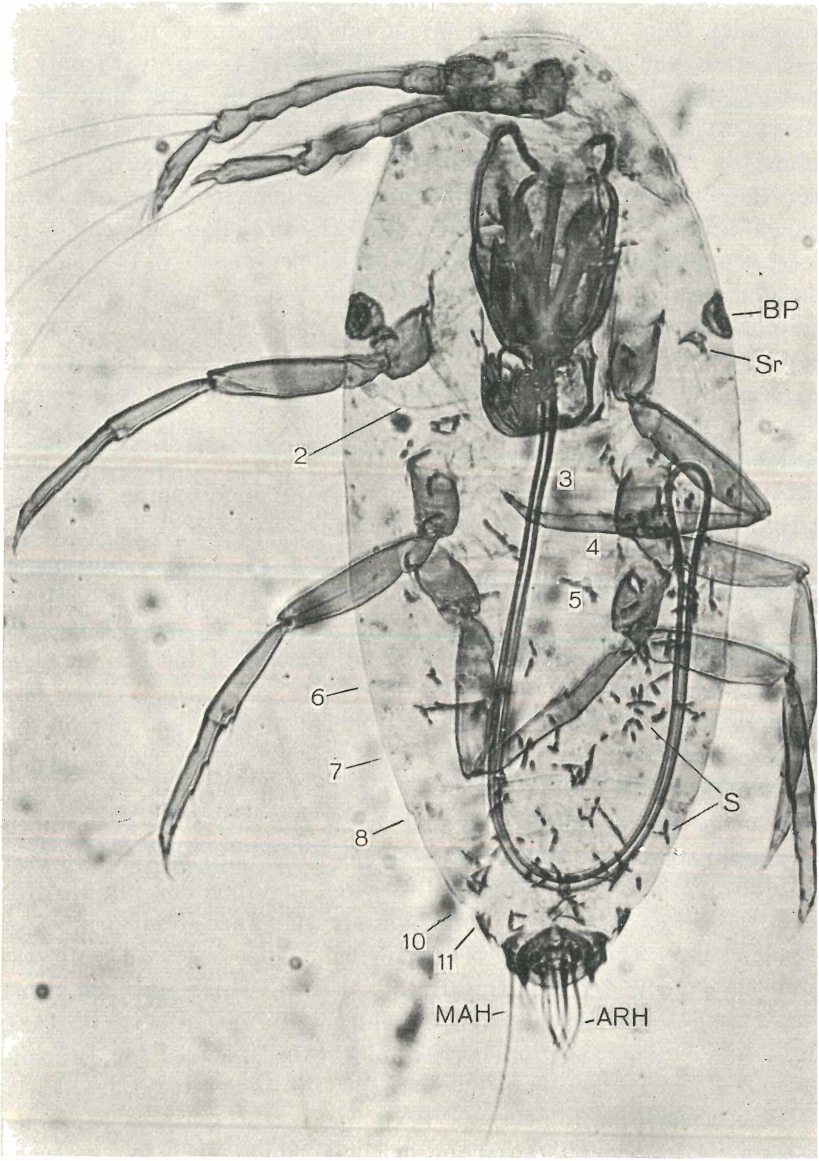

Fig. 1. The entire symbiotic microflora of a female crawling larva of *Lakshadi mysorensis* stained with Alcoholic-Eosin after the insect was treated with alka solution.

The larvae were introduced on the plant on 2. September 1924 and the picture was made from a female insect on 2. January 1925 at the Indian Institute of Science, Bangalore. This picture may be compared with Text-Fig. 3 of the same insect on *Shorea talura* to show the specific nature of the symbiote and its independence of the nature of the host plant upon which the insect has been feeding.

In a previous communication (1929) it has been particularly mentioned that there are specialists who believe the colour of lac insects is attributable to the activity ot the symbiotic microorganisms. The encrustations of the species *Lakshadia communis* have been copiously illustrated in this journal (1932). On the contrary, the encrustations of the species *Lakshadia mysorensis* have not been figured so far and may therefore be illustrated here. The tree, *Nephelium litchi*, in the Botanical Garden, Bangalore, was infected with *Lakshadia mysorensis* and a living colony collected iu the middle of April 1922 is shown on Pl. 3 Fig. 1. At the top the twig shows fine red dots being insects which died as larvae. The encrustation itself shows that the tree has not been ideal for the growth of this insect. It grows best only on its favourite host, *Shorea talura*, from which an encrustation collected dry during November 1921 is reproduced in Pl. 3 Fig. 3. It shows the twig was growing horizontal-vertical, more horizontal than vertical and is seen from below. The colony shows the typical appearance in so far as the insects build a thicker encrustation at the base of the twig than at its top end. Pl. 3 Fig. 2 gives the encrustation from the same source collected in July 1921.

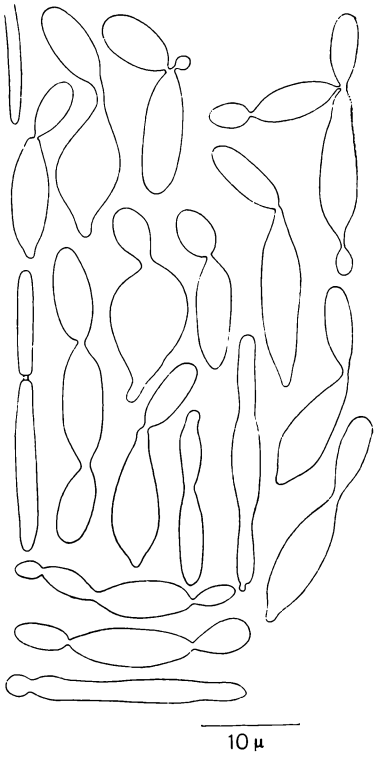

Fig. 2. The symbiote of *L. mysorensis* on *Acacia farnesiana*, 2 Sept. 1924.

When a twig is quite vertical the young insects settle all around it so that a circular crust encloses the twig. This happens

best in the case of *Lakshadia nagoliensis* which secrets lac very copiously. Pl. 3 Fig. 4 represents an encrustation of *Lakshadia mysorensis* on *Shorea talura* collected, from an almost vertical twig, on 13. February 1923. The encrustation shows a backbone like central partition where the insects have settled relatively poorly, being the side away from gravity. This narrow partition is indicative of a poorer secretion of lac and would never be found in encrustation of *Lakshadia nagoliensis.*

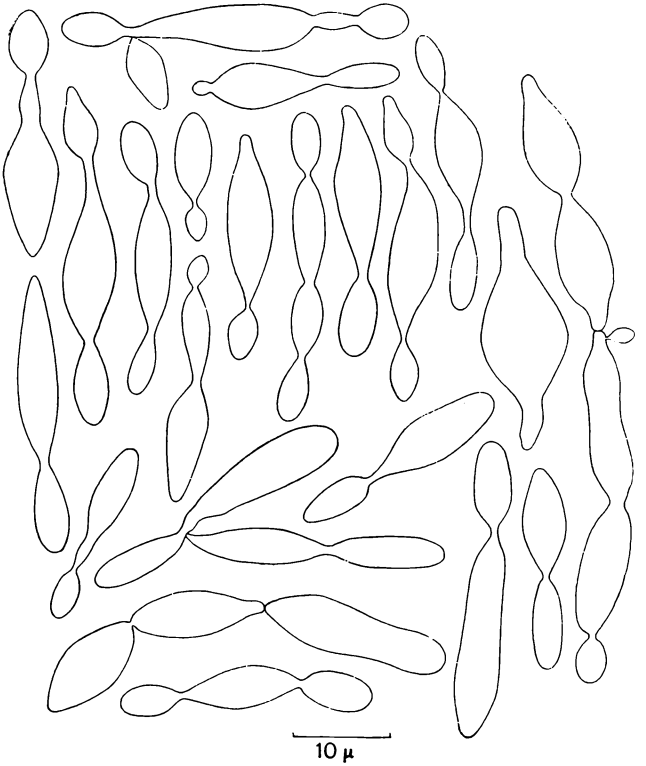

Fig. 3. The symbiote of *L. mysorensis* on *Shorea talura,* 15. Aug. 1925.

The species *Lakshadia mysorensis* gives three crops during 13 Lunar months; Figs. 2 to 4 belong to the same season of two different years, Fig. 4 was rawn without being stored while Fig. 2 was drawn after two years of its being collected.

Late in May 1921 a small plant of *Butea frondosa* was infected with *Lakshadia mysorensis* and after the larvae had swarmed away from the colony the empty encrustation of *Lakshadia mysorensis* on

Butea frondosa was illustrated in Fig. 5 without loss of time, late in October. The fine dust of wax prevents the natural colour of encrustation from appearing. Another portion of the same encrustation was brushed with a hard tooth brush and is shown in Fig. 6. Comparing Fig. 3 with Fig. 6 it is apparent that there is hardly any difference in colour although the host plants are different. The plant *Butea frondosa* was growing in a garden where water supply was better than the forest with *Shorea talura* trees, which explains why the specimen shown in Figs. 5 and 6 was collected earlier than the specimen in Fig. 3. At any rate the nature of host plant as well as the season show no effect on the morphology of the symbiotic organism nor on the colour of the encrustation. In the latter case external impurities like wax or leached out colour spoiling the external appearance of the encrustation must be carefully taken into account.

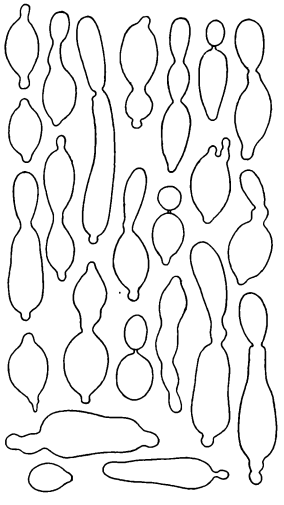

Fig. 4. The symbiote of *Saissetia oleae* Bern.

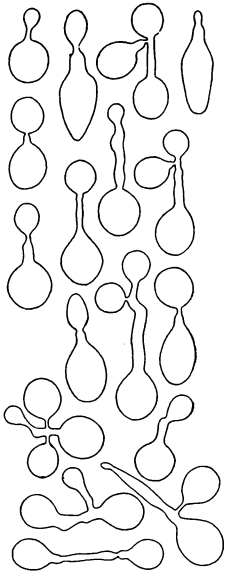

Fig. 5. The symbiote of *Saissetia Ramakrishnae* Green.

Saissetia olae is found in Europe as well as on many foreign and Indian plants in Bangalore. At the last named locality it is a serious pest in many a nursery and has been also found in the field particularly on a species of *Breynia euphorbiacae*, locally called *Soolie*. This plant furnished specimens of the insect whose symbiote is shown in Text-Fig. 4.

The Mango plant is liable to the attack of many coccids. From a tree badly infected from more than one scale insect specimens were collected resembling *Saissetia nigrum*. Blood smears revealed an entirely different symbiote shown here in Text-Fig. 5. This ultimately led to the insect being identified as *Saissetia Ramakrishnae*, Green and apart from its mention by its discoverer, Dr. Ramakrishna, this is the first time it is recorded. It is interesting to mention

the way in which the insect was identified, viz. through its specific symbiote.

On a large species of *Ficus*, probably *Cunnighami*, a scale insect was identified as *Lecanium geometricum*, GREEN. It has been searched for elsewhere but only one tree was found to be infected with this species. Its symbiote is illustrated in Text-Fig. 6.

The locality which supplied *Saissetia Ramaskrishnae* contained other mango trees infected with *Lecanium mangiferae*, GREEN, whose symbiote is shown in Text-Fig. 7. Along with *Saisettia Ramaskrishnae* a mixed infection with *Lecanium piperis*, GREEN was found. The symbiote of *Lecanium piperis* is illustrated in Text-Fig. 8.

Lecanium piperis grows on the upper surface of the leaf facing the scorching sun. *Vinsonia stellifera*, on the contrary, is always found on the under surface of a mango leaf. It is interesting to note that this insect was once considered a species of *Ceroplastes*. It has been remarked by an authority on Coccids that the *Ceroplastes* insects secrete wax much as is known to be the case with the honey bee where no special wax secreting pores are present. The *Ceroplastes* insects possess special glands and pores and not only this but that they secrete two kinds of waxes which have not been hitherto recognised. There is a hard wax hidden from view which acts as a frame work supporting the mass of secretion appearing as a coat of soft wax. *Vinsonia stellifera* produces also two kinds of waxes whose nature is much easier to recognise as the soft wax is somewhat transparent and permits the inner hard wax to glimmer through the semitransparent coat. The symbiote of *Vinsonia stellifera* is shown in Text-Fig. 9 and distinct from those of *Ceroplastes* insects which have been previously illustrated (1928). On *Cajanus indicus*, along with lac insects as a result of natural infection, *Ceroplastodes cajani*, was accidentally found. This insect was most frequently found in Bangalore on *Tephrosia purpurea*, also a leguminous plant. Insects growing on the latter plant supplied the picture of its symbiote in Text-Fig. 10. The species *Ceroplastodes chiton* is bigger and is found very frequently on *Pongamia glabra* from where individuals collected gave the picture shown in Text-Fig. 11.

Insects of the genus *Cardiococcus* secrete a horn like opaque hard wax that dissolves in xylol only on warming. Insects of this genus were formerly classified as species of *Inglisia*. The physical nature of the wax in this case is so different that I was convinced the insects should be generically different. I was proposing to create a new genus for these insects when Mr. GREEN kindly wrote to say

this had meanwhile been done. This is mentioned to emphacise the utility of consideriing physiological properties which are sometimes very striking, more so than the purely morphological ones. In

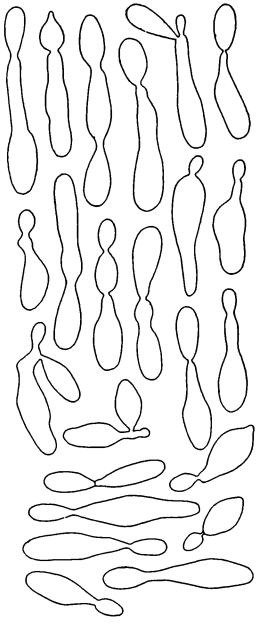

Fig. 6.

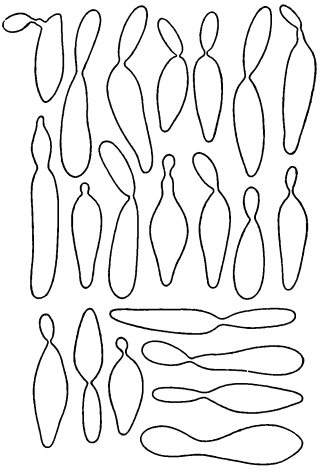

Fig. 7.

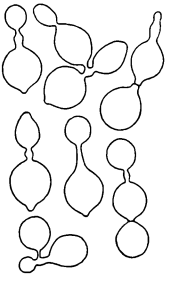

Fig. 8.

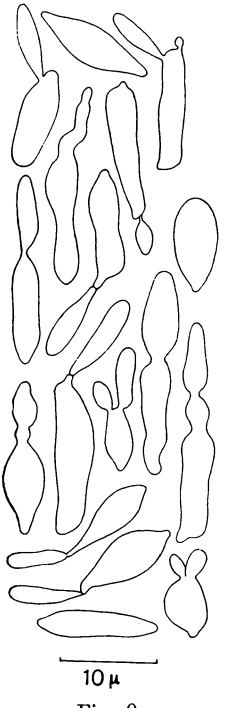

Fig. 9.

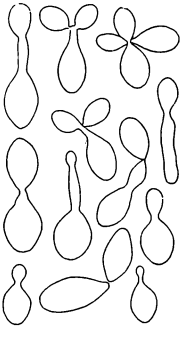

Fig. 10.

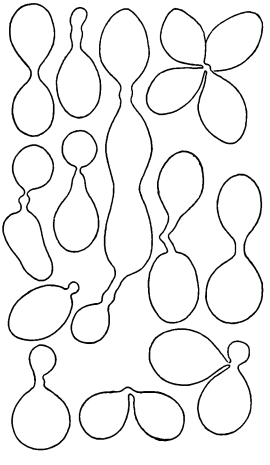

Fig. 11.

Fig. 6. The symbiote of *Lecanium geometricum* Green. — Fig. 7. The symbiote of *Lecanium mangiferae* Green. — Fig. 8. The symbiote of *Lecanium piperis* Green. — Fig. 9. The symbiote of *Vinsonia stellifera* Westw. — Fig. 10. The symbiote of *Ceroplastodes cajani* Mask. — Fig. 11. The symbiote of *Ceroplastodes chiton* Green.

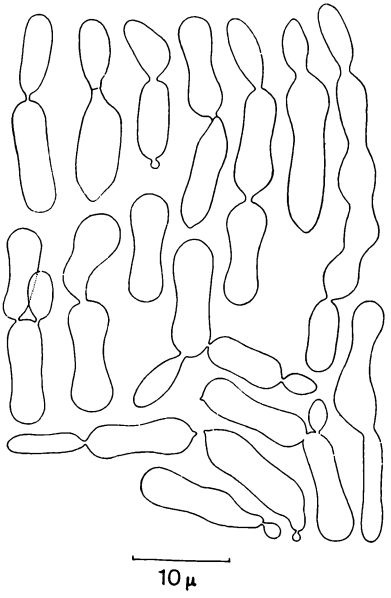

Fig. 12. The symbiote of *Cardiococcus bivalvata* GREEN.

Bangalore there are two species of this genus. *Cardiococcus bivalvata*, GREEN is so criptic on *Pongamia glabra*, where it resembles the broken end of a leaf stalk, that I have no hesitation in looking upon the tree as the most favourite host of this insect. Text-Fig. 12 gives a picture of its symbiote which recalls a photograph reproduced by Dr. BRAIN, who illustrates the symbiote of *Cardiococcus* (*Inglisia*) *geranii*, BRAIN. It seems necessary to study the symbiote of BRAIN'S species in greater detail to establish its specific nature if the insect is correctly identified. *Cardiococcus castillae*, GREEN, was found on *Solanum verbascifolia* and is much rearer than the

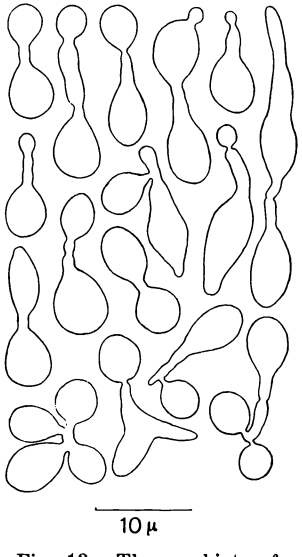

Fig. 13. The symbiote of *Cardiococcus castilloae* GREEN.

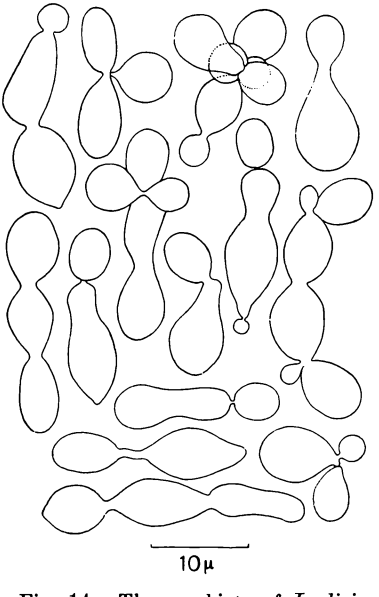

Fig. 14. The symbiote of *Inglisia chelonioides* GREEN.

other species. Its symbiote is illustrated in Text-Fig. 13 wich easily separates it from that of the sister insect, *Cardiococcus bivalvata.*

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Explanation to plate.

Fig. 1. A twig of *Nephelium litchi,* showing a not fully developed living colony of *Lakshadia mysorensis,* Bangalore, April 1922.

Fig. 2. A twig of *Shorea talura* with the encrustation of *Lakshadia mysorensis,* dry encrustation after larval swarming, July 1921.

Fig. 3. *L. mysorensis* encrustation on *S. talura,* Nov. 1921.

Fig. 4. *L. mysorensis* on *S. talura* July 1923.

Fig. 5. *L. mysorensis* on *Butea frondosa* experimentally inoculated. The encrustation was free from living larvae and the surface was smeared with a powdery wax.

Fig. 6. *L. mysorensis* encrustation, same as in Fig. 5, another portion brushed off to show the real colour of the encrustation.


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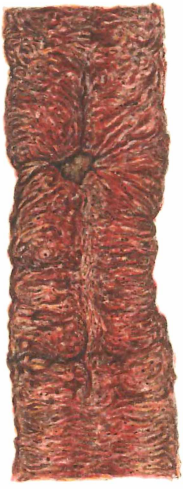
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