

differs from the latter. The pseudoperistome is conspicuous and very large extending  $3/4$  of the body length and has deeply stained lips but in known forms they are very short. Endosprits are definite in number whereas the other species have 9–15 endosprits. The cilia in *C. pyriforme* are very long and extend throughout the anterior half of the body and in *C. cunhai* they are arranged in two rows while in new form they are in 3 or 4 rows. In both the known species the round macronucleus is central in position but in the new species it is oval and is at the posterior end of the body. The contractile vacuole is at the posterior tip of the body in new species whereas in the known forms it is at the posterior dorsal region of the body, but not at the tip.

In view of these differences the present species is a new one, so it is designated as *Cyathodinium indiae* n. sp.

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#### ACANTHOSQUILLA TIGRINA (NOBILI) (CRUSTACEA : STOMATOPODA) FROM THE INSHORE WATERS OF PORTO NOVO

FIVE species of *Acanthosquilla* have been reported to occur in the Indo-West Pacific Region (Manning<sup>1</sup>) of which *Acanthosquilla acanthocarpus* and *Acanthosquilla multifaciata* are known from the coastal waters of India (Kemp<sup>2</sup>, Chhapgar and Sane<sup>3</sup> and Shanbhogue<sup>4</sup>) while Alikunhi<sup>5, 6, 7</sup>, collected two larvae of *Acanthosquilla tigrina* from the plankton of the Madras coast and reared one of them in the laboratory to the adult stage.

The type specimen of *Acanthosquilla tigrina* (Nobili) lodged in Sarawak Museum (Kemp<sup>2</sup>), is a male measuring 45 mm and was found in Santubong, Borneo. Kemp<sup>2</sup> re-examined the specimen and supplemented Nobili's description with additional notes. Alikunhi<sup>7</sup> reared a 9.00 mm larva of *A. tigrina* in the laboratory. The larva lived for 81 days and underwent 8 moults before it

metamorphosed into a young measuring 29.7 mm in length.

A male specimen of *Acanthosquilla tigrina* measuring 60 mm with right raptorial leg missing was collected on 4–8–1971 from the trawl catches of the inshore waters at Porto Novo (11° 29' N–79° 48' E). *Acanthosquilla tigrina* collected from Porto Novo waters agrees with the description by Kemp<sup>2</sup> especially in the presence of four pairs of fixed marginal teeth on the telson and with a posterior on the ventral margin of sixth abdominal somite but there are some differences. Firstly on the raptorial leg, the dactylus has 15 teeth (two terminal are partly damaged) but Kemp<sup>2</sup>, mentions that in the type species the raptorial dactylus has 11 teeth and Alikunhi<sup>7</sup> found 13 teeth on the raptorial dactylus of the metamorphosed specimen. In *Acanthosquilla tigrina*, probably the number of teeth on the raptorial dactylus varies between 11 and 15 as seen in some other stomatopods, *Lysiosquilla maculata* and *Lysiosquilla spinosa* (Kemp<sup>2</sup>).

Secondly, on the telson, there are 15 denticles instead of 12 small denticles in between submedians as indicated by Kemp<sup>2</sup>. According to Alikunhi<sup>7</sup> there are 18 denticles in between submedians. The specimen is colourless in formalin. The differences noted above are minor variations from the previous descriptions of *A. tigrina* by Kemp<sup>2</sup> and Alikunhi<sup>5, 6, 7</sup>.

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#### FLUORESCENT ANTIBODY STAINING FOR DETECTION OF COCONUT ROOT WILT ANTIGEN

FLUORESCENT antibody (FA) technique serves as a useful tool for detecting *viral antigens* and their localization *in situ*. Coons *et al.*<sup>1</sup> developed this technique. Since then the technique has been widely employed for both plants and animals, infected with fungi, bacteria, viruses and mycoplasma<sup>2–5</sup>. Among plants consi-

derable work has been done with atleast five viruses, namely, tobacco mosaic<sup>6</sup>, wound tumor<sup>7</sup>, southern bean mosaic<sup>8</sup>, cauliflower mosaic<sup>9</sup> and narcissus yellow stripe<sup>10</sup>.

The present report describes the use of FA technique in the detection of coconut root wilt antigen in the epidermal cells of tobacco. The coconut root wilt virus<sup>11</sup> was transmitted and maintained on *Nicotiana tabacum* cv. White Burley. The antigen was purified after Summanwar *et al.*<sup>11</sup>. The concentrated antigen was administered to rabbits weekly by four intravenous injections and two intramuscular injections (with Freund's adjuvant). The blood sample collected by bleeding the rabbits after 15 days following the last injection, was allowed to clot at room temperature for four hours and stored overnight in a refrigerator for clot to shrink. The serum was clarified by low speed centrifugation at 4,000 rpm for 15 minutes. The antiserum reacted specifically with the coconut root wilt antigen and had a titre of 1:1024.

Following Spendlove<sup>12</sup>, the  $\gamma$ -globulins were precipitated with equal volume of 3.2N ammonium sulphate. The precipitated globulins were recovered by centrifugation at 1,000 g for 30 minutes. The precipitate was dissolved in distilled water equal to the original volume of the serum and reprecipitated with ammonium sulphate as before. Residual sulphate was removed by overnight dialysis using distilled water. The pH of the globulin solution was raised to 9.0 using 0.5 M carbonate-bicarbonate buffer. 50 mg of fluorescein isothiocyanate (FITC) was added per gram protein in cold (4°C) and kept for 18 hours. The unconjugated dye was removed by overnight dialysis against buffer.

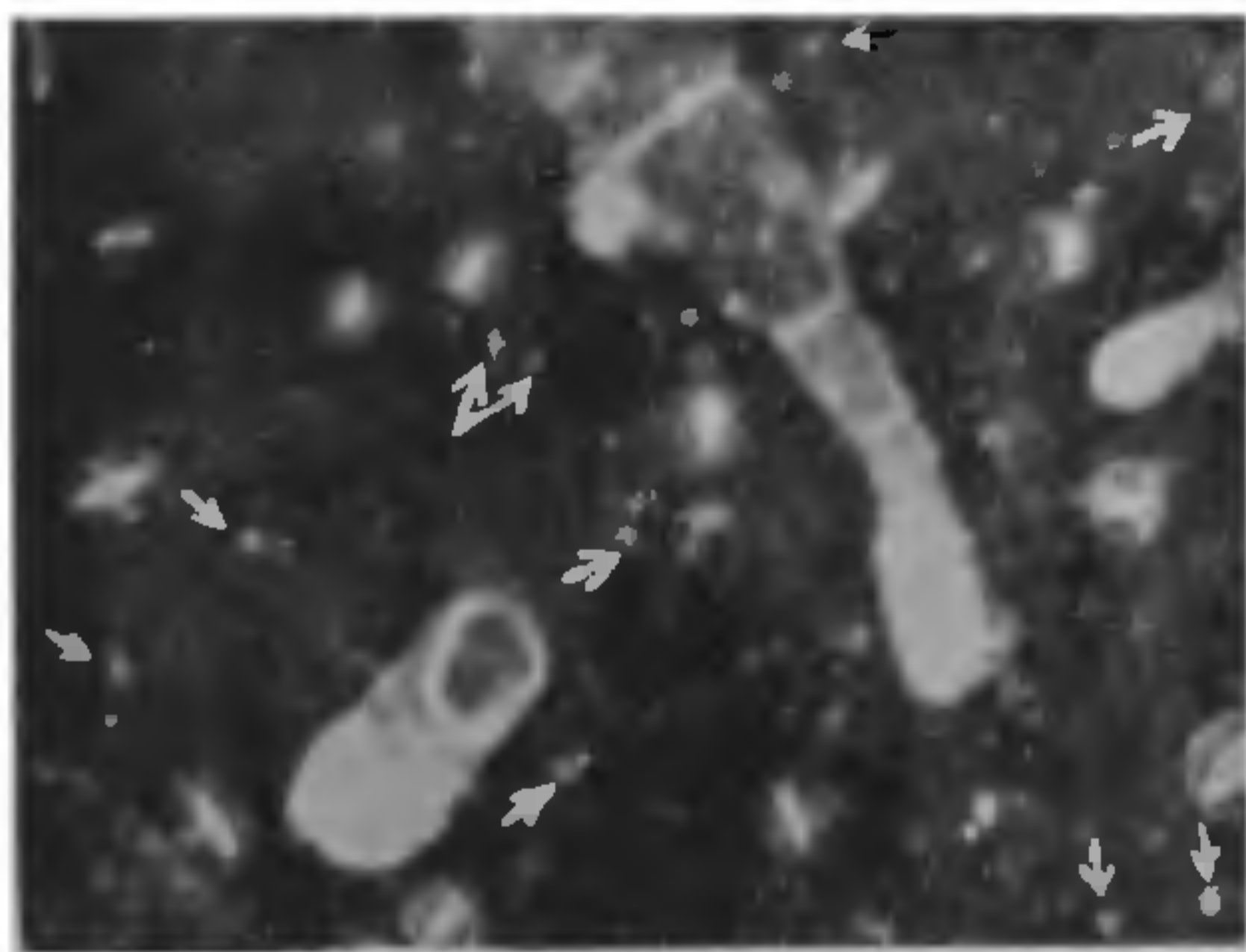


FIG 1. Diseased epidermal peel of *N. tabacum* cv. White Burley. Arrows indicate labelling of the coconut root wilt antigen with FITC.

Epidermal peels were taken from healthy and diseased tobacco leaves infiltrated in acetone,

The peels were mounted on glass slides and allowed to almost dry. Labelled FITC was added to the glass slides and allowed to remain for 4-6 hours. The peels were then washed with buffered saline pH 7.5 (0.01 M  $\text{PO}_4$  and 0.15 M NaCl) and mounted in the same buffer.

Observations were made using super-high-pressure mercury bulb HBO 200 with BG 3 and BG 12 light filters. Pictures were taken using suppression OG 1 filters. Healthy peels showed a uniformly dull orange fluorescence. On the other hand, the diseased peels showed an overall orange fluorescence, interspersed with deep apple green spots in the cytoplasm of the cells (Fig. 1, arrow marked—white spots). The deep apple green spots marked the labelling of the coconut root wilt antigen in the systemically infected tobacco epidermal cells.

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