

Figures 1 and 2. 1. Development of the spore (SP) and multiple crystals (MC) in crystalliferous bacillus. 2. Vegetative cells (VC), liberated spores (S) and multiple crystals belonging to same vegetative cell are shown.

(ISPC-4) produces 2-5 crystals per cell, it would be worth exploring its performance in biocontrol programmes.

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SEED SURFACE STUDY OF POSSIBLE HYBRID BETWEEN C_3 AND C_4 SPECIES OF *CLEOME* (CAPPARIDACEAE)

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ATTEMPTS have been made to produce hybrids between C_3 and C_4 plants through breeding programme in the genera *Panicum*, *Zygophyllum*, *Euphorbia*, and *Atriplex*. However, such hybridisations have met with success only with *Atriplex* species¹. This report presents the results of interspecific hybridisation between C_3 and C_4 species in the genus *Cleome* using conventional breeding techniques.

Two species of *Cleome*, *Cleome viscosa* L. and *Cleome gynandra* L. (*Gynandropsis gynandra* L.) representing C_3 and C_4 plants respectively were selected for the present study. The plants were raised in beds (4 × 3 m) under field conditions during November 1980-February 1981. The beds were initially over-seeded and the 2-3 week old seedlings were thinned to 10-15 plants/m². Plants in the border rows were not used for artificial cross-pollinations. The usual cultural operations were provided and the plots irrigated whenever necessary.

Both the species commenced flowering after 40-45 days after sowing. Flower buds appeared initially in leaf axils singly in *C. viscosa* and in terminal corymbs in *C. gynandra*. The flowers in both species develop and open in an acropetal succession and opened early in the morning. Flower buds borne at the lower leaf axils of the stem were chosen for emasculation. Flowers were emasculated in the evening preceding anthesis and covered with a paper bag to prevent natural cross-pollination. All untreated flower buds very close to artificially pollinating flowers were removed in each branch. Emasculated flowers were artificially cross-pollinated approximately at the time of anthesis. Sufficient care was taken to avoid physical injury during the process of hybridisation. The fruits of cross pollinated flowers were harvested at maturity and drying.

Mature seeds of freshly dehusked and dry fruits were prepared for scanning electron microscopy. Seeds were mounted on specimen stubs with silver conducting paste and gold coated in a sputter coater. Gold-coated specimens were stored in a desiccator until examination. Observations were made at 15 kV with a JEOL JSM-35 SEM and recording images with INDU 120 mm, ASA 125 film at the University of Hyderabad.

The genus *Cleome* was selected since it contains species with C_3 (*C. viscosa*) and C_4 (*C. gynandra*) plants (Rajendrudu and Das, unpublished work)². The primary objective of this study was to achieve interspecific hybrids between C_3 and C_4 species in *Cleome* using conventional breeding techniques. Data on the number of flowers crossed and fruits realised are furnished in table 1. The success in artificial cross-pollination was 5.05%. In the present study the fruits with either empty or with shrivelled seeds was more pronounced. Further it was evident that the emasculated flowers of *C. viscosa* failed to develop into fruits without artificial pollination.

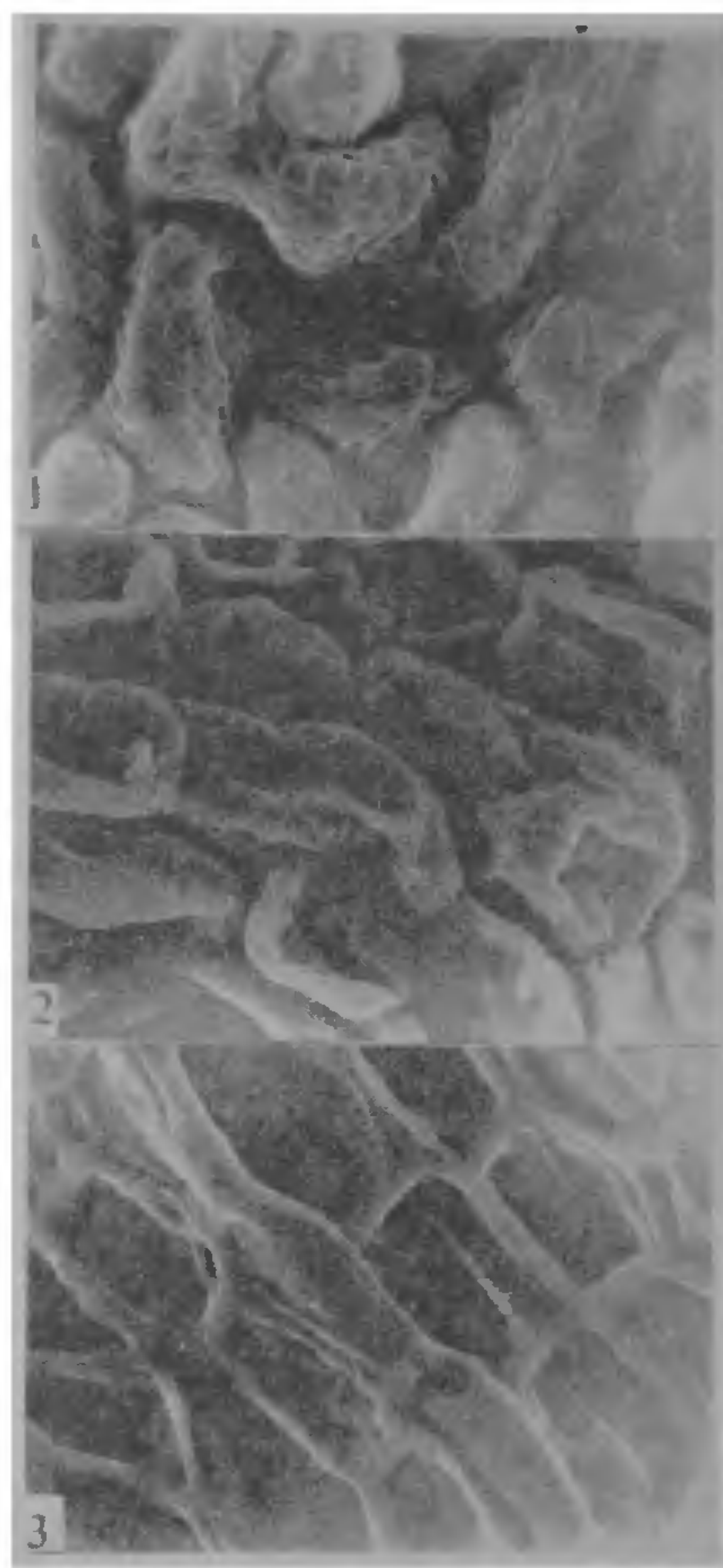
TABLE 1

Number of pollinations made, number of fruits obtained and percentge of crossability among two species of Cleome

Female parent/ Male parent	Number flowers crossed	Number of fruits obtained		% of cross-ability
		Empty/ with shrivelled seed	With a few developed seed	
<i>C. viscosa</i> / <i>C. gynandra</i>	218	207	11	5.50
<i>C. gynandra</i> / <i>C. viscosa</i>	94	0	0	0

The seeds of *C. viscosa* were dark brown in colour and those of *C. gynandra* black in colour. The seeds of both species differ considerably in their characteristic seed surface reticulation. The seed surface in *C. viscosa* was wavy with prominent outgrowths. Moreover, the individual reticulation on the outgrowths was prominent in *C. viscosa* (figure 1). The pattern of reticulation in *C. gynandra* was cup-shaped with striking sunken floor (figure 2).

The seed surface of *C. viscosa* following the artificial cross pollination was reticulated with broad sunken floor as in the male parent, *C. gynandra* (figure 3). Therefore, artificial cross-pollination of *C. viscosa* presented cross-pollination with *C. gynandra* as a male parent. Subsequent trials with a reciprocal cross, indicated cross-incompatability. The success in cross-pollination was evaluated in terms of the number of fruits containing a few developed seeds obtained with reference to the number of flowers crossed. The results have shown that the artificial hybridisation between *C. viscosa* (a C_3 plant) and *C. gynandra* (a C_4 plant) can yield hybrid seeds. Further studies related to the present report are under progress to raise the hybrid plants.



Figures 1-3 Scanning electron micrographs of seed surfaces in (1) *Cleome viscosa* ($\times 1,320$), (2) *Cleome gynandra* ($\times 1,320$) and (3) *Cleome viscosa* \times *Cleome gynandra* hybrid ($\times 1,320$). Note the hybrid seed surface resemble more the male parent (*C. gynandra*) than the female parent (*C. viscosa*).

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CRYPTOBIA INDICA N. SP. (PROTOZOA: KINETOPLASTIDA) ECTOPARASITE ON THE GILLS OF PUNTIUS SARANA (HAM)

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STUDIES on flagellates that parasitise fish have gained importance in recent years, as a result of an intensified effort to cultivate a wide variety of freshwater and marine fish under semi artificial conditions. Some species are injurious to gill-breathing vertebrates. During the examination of parasitic protozoa of freshwater fish of Lake Parvatsar, Rajasthan, an ectoparasite flagellate was found infecting the gills of *Puntius sarana*. The parasite was indentified as belonging to the genus *Cryptobia*, and it is the first report from Rajasthan, India. Even on a global basis very few new species of *Cryptobia* have been reported. Becker¹ clarified the different habitats of the genera *Trypanoplasma* and *Cryptobia*. Cryptobiid biflagellates occupy blood of either freshwater or marine fish and are designated as *Trypanoplasma* while *Cryptobia* occur as ectoparasites on gills or skin or endoparasites in digestive tracts of fish. He further stated that for *Cryptobia*, the undulating membrane is either "vestigial" or "absent". No mention of undulating membrane is given by Levine *et al.*² in their newly revised classification of Protozoa, and they placed the genus under the order Kinetoplastida: sub-order Bodonina.

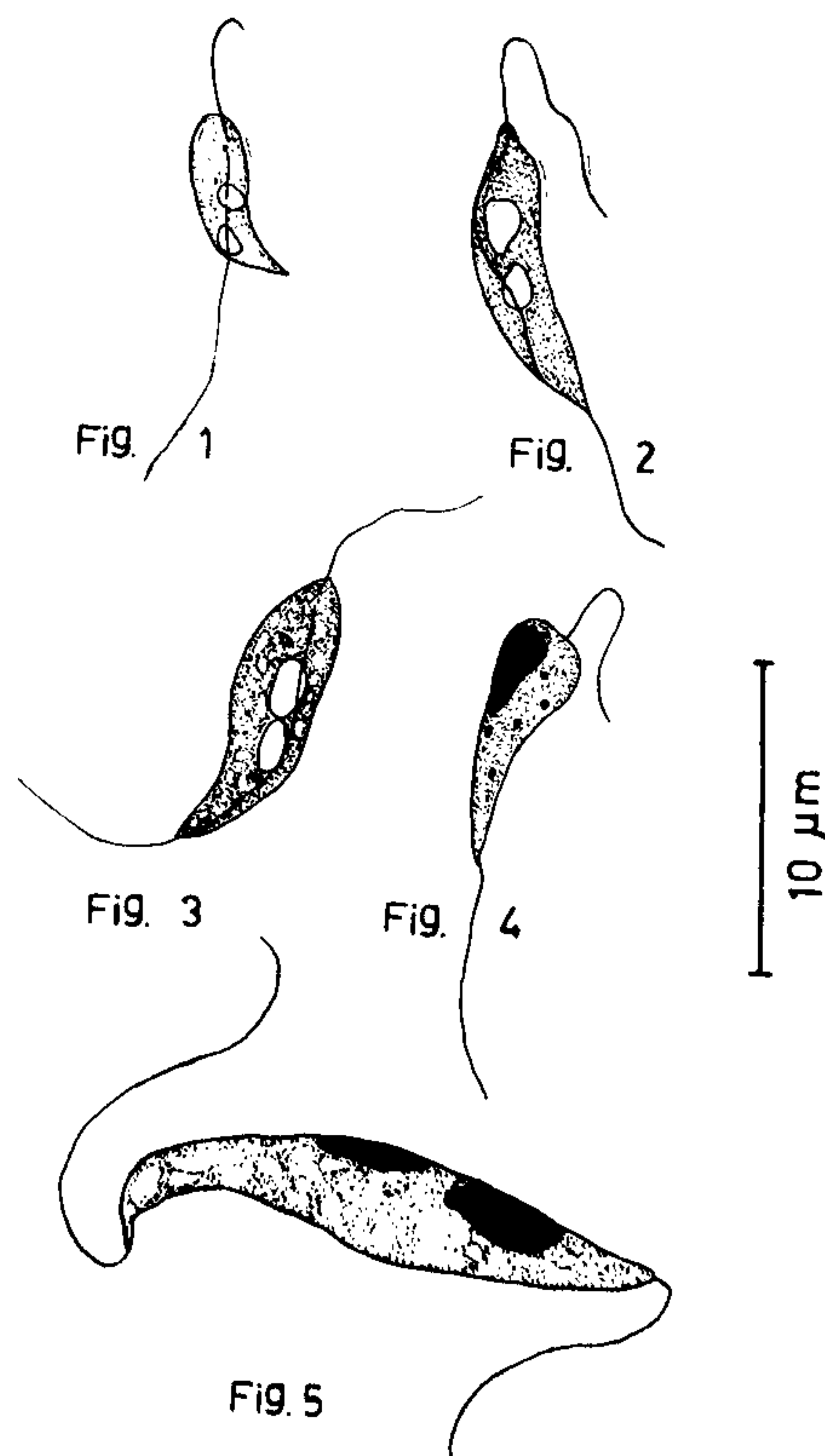
Smears were prepared from the gill material, fixed in methyl alcohol or Schaudinn's fluid and stained with Giemsa's or Heidenhain's iron haematoxylin respectively. Sections of the gills did not show the infection. Thus they are ectoparasitic in nature. Measurements of 112 flagellates were taken. The mean and range are given in micrometers. Drawings were made with camera lucida.

Characteristic features of *Cryptobia*

Cryptobia from the gills and skin of freshwater fish usually have a triangular body, with a bluntly rounded anterior and tapered or pointed posterior. One flagellum extends in front of the body and the other runs backward along the body surface, and then extends free posteriorly. An undulating membrane is vestigial

or absent in most species of *Cryptobia*. The kinetoplast is usually in an anterior-lateral position. They are obligate parasites attach themselves by means of their posterior flagellum.

The stained biflagellate reveal the following morphological characters. They are usually triangular or elongate with anterior ends drawn into knobs or points. The cytoplasm is homogenous, sometimes highly vacuolated (figure 5). Occasionally darkly stained granules are seen in the cytoplasm (figure 4). The kinetoplast ($3.2 \times 2.2 \mu\text{m}$) which lies anterior to the nucleus is oval or round, and is always bigger than the nucleus. Nucleus ($2.5 \times 1.6 \mu\text{m}$) is situated centrally or posteriorly. The two flagella originate separately, one running anteriorly as a free flagellum, and



Figures 1-5. Camera lucida diagrams of *Cryptobia Indica* N. Sp. 1. Kinetosomes are clear, 2. Elongate form with homogenous cytoplasm. 3. Form with vacuolated cytoplasm. 4. Cytoplasm with darkly stained granules. 5. Largest form observed with a highly vacuolated cytoplasm.