

seeds were coated with conidia.

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## Surface morphology of some articulated corallines from India

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Surface structures of seven species belonging to four genera of subfamily Corallinoidea (Fly: Corallinaceae) were microscopically investigated. Two distinct surface morphologies were revealed namely a 'Corallina type' (c-type) with round to irregular cell outlines and round trichocyte bases and another 'Jania type' with elongate polygonal cell outlines and round trichocyte bases. Trichocytes were observed in all species except in *Amphiroa anceps* and *Arthrocardia capensis*.

CALCAREOUS algae are scattered in all major divisions of algae. The method of lime deposition, type and amount of calcification vary from group to group and even from genus to genus<sup>1</sup>. Corallinean forms deposit calcite which is rich in magnesium<sup>2</sup>. Calcite crystals are deposited among the fibrils of cell wall and show distinct organization<sup>3</sup> which is of great taxonomic significance<sup>4</sup>. Corallinoidea is further divided into two tribes on the basis of the reproductive structures. These are Janiæ and Corallinae<sup>5</sup>. Vegetative distinction between the members of Janiæ and Corallinae has been hardly studied.

All the calcified forms along the Indian coast have not been worked out for many aspects like mineralogy, taxonomy, surface morphology, etc. Since surface morphology provides additional taxonomic characters, an attempt has been made to study the structure of CaCO<sub>3</sub> of articulated corallines.

To study the surface morphological features, air-dried specimens of *Amphiroa* (*A. anastomosans*, *A. anceps*, *A. foliacea* and *A. fragilissima*), *Arthrocardia capensis*, *Cheilosporum spectabile* and *Jania rubens* were coated with gold-palladium<sup>4</sup>. The observations were made using an scanning electron microscope (camera Camebax

model 571).

Intergenicular surfaces of *Amphiroa anastomosans*, *A. anceps*, *A. foliacea*, *Arthrocardia capensis*, *Cheilosporum spectabile*, *Jania rubens* under SEM revealed two distinct surface morphologies (Figures 1a-h). All species show C-type surface morphologies except *Jania rubens* which shows J-type surface features (Figure 1h). Cell outlines of the species of *Amphiroa*, *Arthrocardia* and *Cheilosporum* vary from round to irregular and these species show roundish trichocyte base (Figure 1a, d, e, g). Conspicuous concavities were also observed in these species (Figures 1a to g). In *A. fragilissima* and *A. foliacea* (Figure 1a) trichocytes were scattered randomly and quite conspicuous in *A. anastomosans* and *A. fragilissima* (Figures 1a, c). Trichocytes in all these species show a slightly raised doughnut-shaped base with a pore lacking a flange and appeared slightly smaller than the surrounding concavities. In the case of *A. foliacea*, there appeared to be additional cells produced at the juncture of other cells (Figure 1e). *Amphiroa anceps* and *Arthrocardia capensis* show absence of trichocytes (Figures 1b, f). Cells of reproductive region in *A. fragilissima* (Figure 1d) were smaller than those of the vegetative region. Clear termination of surface feature next to the adjoining walls was not observed in species of *Arthrocardia* and *Cheilosporum*. In *Jania rubens* regular rows of depression and a terminal ridge around the distal and proximal ends adjacent to the contiguous genicula were observed (Figure 1h).

Trichocytes in *J. rubens* showed a single pore with an expanded flange and trichocyte bases could be easily distinguished from the surrounding epithelial cells (Figure 1h).

Garbary and Johansen<sup>4</sup> showed vegetative distinction between Janiæ and Corallinae. Corallina type surfaces occur with several modifications in all genera of the Corallinae while *Jania* type surface characters are limited to tribe Janiæ<sup>6</sup>. We have observed that all species of *Amphiroa*, *Cheilosporum*, *Arthrocardia* show C-type surface morphology. All C-type surface cells are characterized by round to irregular cell outlines and with round trichocyte bases. All the studied species except *Amphiroa anceps* and *Arthrocardia capensis* show the presence of trichocytes and this should be considered as an additional taxonomic character for these species. Production of a single hair by trichocytes, i.e. cell and cell complexes was so far reported in *Jania rubens* and in some crustose coralline forms<sup>7,8</sup>. To the best of our knowledge except for *Yamadea*, *Jania* and *Haliptilon* trichocytes have not been reported in the other genera of Corallinoidea<sup>4,9</sup>.

Our results show that C-type and J-type of surface morphological features are common in members of Corallinae and Jamiæ tribes respectively. Smaller cells in the conceptacular areas in *A. fragilissima* may be

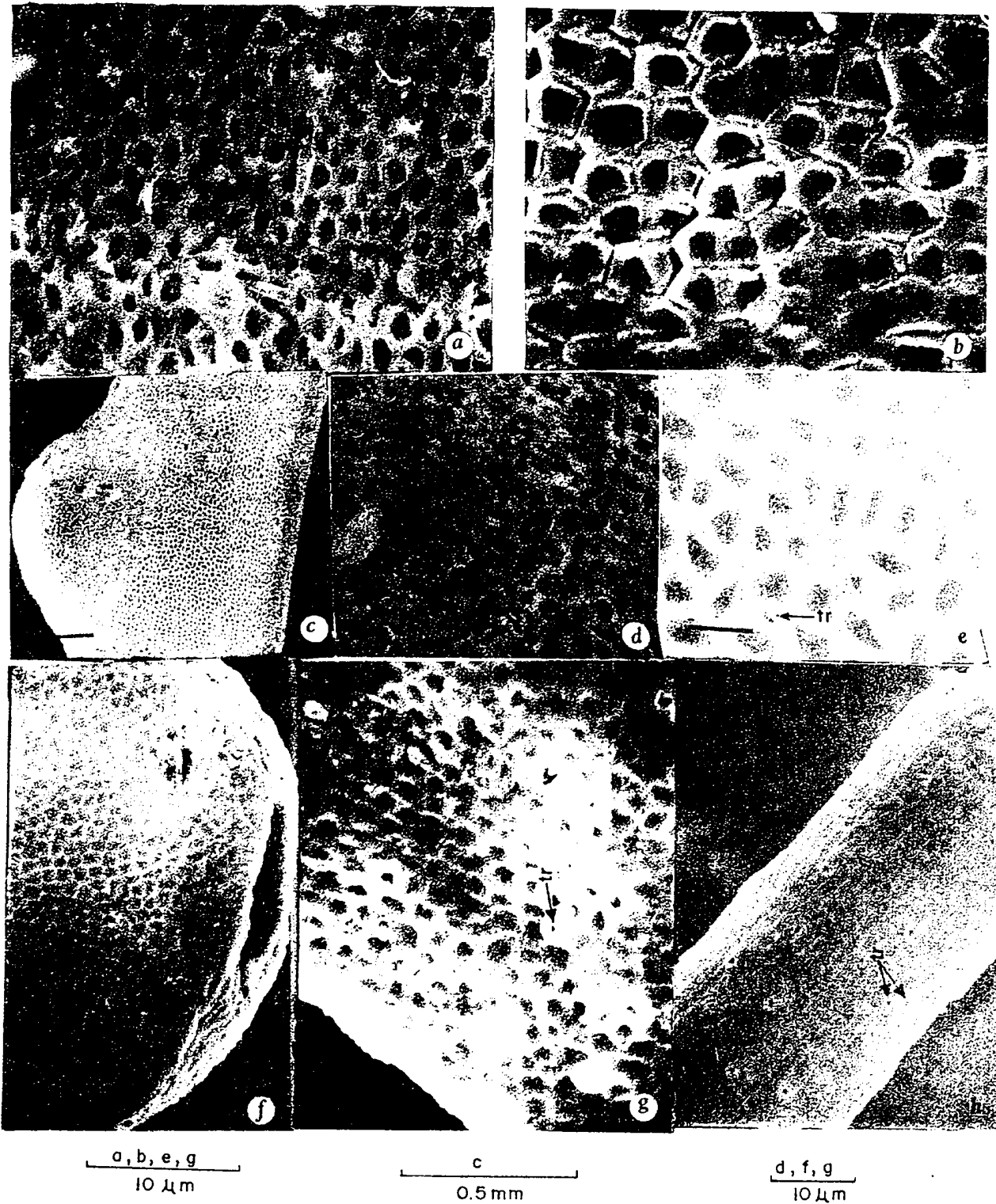


Figure 1. Surface morphology of *a*, *Amphiroa anastomosans* W. V. bosse; *b*, *Amphiroa anceps* (Lamk) Decaisne; *c*, *Amphiroa fragilissima* (L) Lam. bearing tetrasporic conceptacles (showing small reproductive cells compared to the vegetative cells); *d*, 'c' enlarged; *e*, *Amphiroa foliacea* Lamouroux; *f*, *Arthrocardia capensis* Areschoey; *g*, *Chelosporum spectabile* Harvey; *h*, *Jania rubens* (L) Lamk. Tr. trichocyte.

due to the state of the division at the reproductive portion.

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## Protein polymorphism in muscle myogens of *Heteropneustes fossilis*

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To score protein loci for identifying subpopulations by simple electrophoresis, muscle myogens from the catfish, *Heteropneustes fossilis* were subjected to starch and polyacrylamide gel electrophoresis employing different protocols. Best results leading to detection of polymorphism based on a normal locus and two isoloci, were obtained by Laemmli's protocol on 8.5% gels with 0.5 M urea, provided the ratio of stacking to separating gel was 1 : 4. At least four subpopulations of the catfish around Aligarh could be identified by improved resolutions.

ELECTROPHORETIC patterns of muscle sarcoplasmic proteins or myogens which contain glycolytic enzymes, in addition to a few other proteins soluble in low ionic strength buffers<sup>1</sup>, have been conveniently used to address problems of biological inter-relationship<sup>2</sup>. Although only a few proteins occur in concentrations sufficient to take protein-stain, electropherograms of muscle myogens have shown great constancy against variables such as physiological conditions, sex size and differences in habitats<sup>2</sup>. More recently, isoelectric focussing has been used to demonstrate considerably higher degree of heterogeneity of fish tissue extracts including muscle myogens<sup>3,4</sup>. The information, however, has largely supplemented morphological inter-relationship between various taxa, whereas during the last few years the emphasis is to

search for easy-to-monitor biochemical markers for identifying fish stocks and lineages<sup>5</sup>. The applied value of the approach in aquaculture practices as a method of routine analysis of large number of samples will rate higher if it is relatively simple and inexpensive. With these objectives in view, we have screened muscle myogen from a large number of *Heteropneustes fossilis* using different methods and have succeeded in detecting polymorphism in it with only minor modifications in widely adopted method of Laemmli<sup>6</sup>. To our knowledge, there is no report of isoloci polymorphism in myogens of Indian fish species, and even in other instances such polymorphism has only been demonstrated by specific histochemical staining<sup>7</sup>.

Fish samples were obtained from the local fish market at Aligarh where *H. fossilis* is brought alive. Over 300 samples procured during 1990-1993 were analysed. Dorsal white muscle tissue samples of anterior part of the body were homogenized in 4-5 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.5 and cleared of insoluble material by centrifugation at 10,000 rpm and 4°C for 30 minutes. Clear supernatants were electrophoresed in starch<sup>1</sup> or polyacrylamide gels using Laemmli's<sup>6</sup> and other buffer systems<sup>7</sup>. Best resolutions leading to detection of polymorphism were obtained in 8.5% polyacrylamide gels with Laemmli's protocol, provided the stacking gel was 1/4th the height of separating gel and all buffers contained 0.5 M urea. Though 0.5 mm thick gels were generally used by us, this dimension is not of any consequence to the quality of resolution. Electrophoresis was continued till bromophenol blue line reaches the very end of the 10-cm long separating gels. Protein bands were visualized by staining with Coomassie brilliant blue.

Several electrophoresis buffer systems<sup>7</sup> with different concentrations of acrylamide were employed, but the selected patterns shown in Figure 1 demonstrate that the best resolutions were obtained by the method of Laemmli<sup>6</sup>. For practical reasons polyacrylamide gel was preferred over even Scopes<sup>1</sup> method of starch gel electrophoresis which gives highly improved resolutions of myogens in general. The method did not help in resolving the thick band designated *pmb* which was eventually found polymorphic. High concentrations of acrylamide retarded the resolution of this band while the gels of lower concentration were equally unsuitable for its desired separation (Figure 1a). Some improvement was observed in the resolution of *pmb* in 8.5% gels (Figure 1b) even in the absence of urea; but a clear resolution of this band into 1, 2 and 3 components was achieved only in 0.5 M urea and the increased height of stacking gel as stated earlier. To avoid dissociation of multimetric proteins, urea concentration had to be kept minimal and no significant perturbation was observed even in comparatively unstable proteins of temperate fish species<sup>8</sup>.