

Table 3. Effect of 4-AP on K⁺ uptake in *S. cerevisiae*

Concentration of 4-AP (mM)	Time (min)	Intracellular K ⁺ content (μmole/mg dry dw)
0	0	0.504 ± 0.201
0	30	0.656 ± 0.280
0	60	0.679 ± 0.199
10	30	0.665 ± 0.150
10	60	0.623 ± 0.188

Results are mean ± SD of three independent experiments. Method: ref. 8.

concentration of TEA and 4-AP, was restored on supplementing the plates with 0.2 M KCl (Table 2). The presence of 0.2 M KCl in the medium might be helping to overcome the lethal effect of these drugs. Furthermore, intracellular K⁺ content of the untreated (control) as well as 4-AP treated yeast cells was of the same order (Table 3). These results suggest that 4-AP might not be inhibiting K⁺ uptake by blocking K⁺ transporters, but it may be causing lethality in *S. cerevisiae* by acting at some other level. The restoration of growth on supplementation of KCl (0.2 M) to the synthetic complete medium agar plates suggested another possibility that 4-AP and TEA may be causing some osmotic stability defect and the presence of 0.2 M KCl has osmotic stabilizing effect. The growth of *S. cerevisiae* was also restored when the plates containing inhibitory concentration of TEA and 4-AP were supplemented with NaCl (0.2 M) or mannitol (0.4 M). Hence, the lethality conferred by TEA and 4-AP to *S. cerevisiae* cells may be attributed to the osmotic stability defect caused by these drugs.

Mutants with the osmotic stability defect have been reported in *S. cerevisiae*⁵. These mutants showed normal growth on the medium containing osmotic stabilizer, i.e. 10% sorbitol or mannitol and 1.6% NaCl, but lysed on transfer to hypotonic solution. These mutants had defects in both cell wall and cell membrane^{6,7}. So, it is possible that TEA and 4-AP may be causing cell lysis in *S. cerevisiae* by inducing some alterations either in cell wall or cell membrane.

4-aminopyridine caused lysis of *S. cerevisiae* cells (Table 4). It may be possible that 4-AP interacts with some protein involved in the maintenance of cell integrity, thereby inducing some osmotic stability defect. In the presence of osmotic stabilizer (Table 2), this defect might be restored. It may be noted that in the presence of normal saline (control), some material absorbing at 280 nm radiations is released. During normal growth, yeast cells release a number of metabolites in the medium. Some of these might be absorbing at 280 nm and it may not be the result of cell lysis.

On the other hand, TEA-treated yeast cells did not show any lysis (Table 4), indicating that it may be causing lethality by resulting in some other defects in

Table 4. Effect of TEA and 4-AP on membrane integrity of *S. cerevisiae*

Time (hours)	Extinction at 280 nm		
	Normal saline	4-AP (2 mM)	TEA (0.2 M)
1	0.166 ± 0.012	0.542 ± 0.060	0.122 ± 0.003
2	0.306 ± 0.019	1.012 ± 0.042	0.172 ± 0.002

Results are mean ± SD of three independent experiments. Method: ref. 9.

the plasma membrane. TEA having both polar as well as non-polar groups might have replaced amphipathic membrane lipids, thereby affecting the functioning of membrane proteins, which led to the lethality of yeast cells. The restoration of growth of *S. cerevisiae* cells on addition of KCl, NaCl or mannitol in the synthetic complete medium agar plates containing TEA can be explained by considering that the presence of osmotic stabilizer somehow inhibited the incorporation of the drug in the membrane, but this proposal needs further investigation.

1. Serrano, R., *Curr. Top. Cell. Regul.*, 1984, **23**, 87-126.
2. Rothsten, A., in *Ciba Foundation Study Group No. 6*, J. and A. Churchill Ltd., London, 1960, pp. 53-68.
3. Hille, B., *Prog. Biophys. Mol. Biol.*, 1970, **21**, 3-32.
4. Pelhate, M. and Pichon, Y., *J. Physiol.*, 1974, **242**, 90.
5. Venkov, P. V., Hodjiolov, A. A., Battaner, E. and Schlessinger, D., *Biochem. Biophys. Res. Commun.*, 1974, **56**, 599-604.
6. Kozhina, T., Stateva, L. I. and Venkov, P., *Mol. Gen. Genet.*, 1979, **170**, 351-354.
7. Maerkisch, U., Reuter, G., Stateva, L. I. and Venkov, P., *Int. J. Biochem.*, 1983, **15**, 1373-1377.
8. Camacho, M., Ramos, J. and Rodriguez-Navarro, A., *Curr. Microbiol.*, 1981, **6**, 295-299.
9. Stateva, L. I., Oliver, S. G., Trueman, L. J. and Venkov, P. V., *Mol. Cell. Biol.*, 1991, **11**, 4235-4243.

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Comparative studies on the radula teeth of two species of *Conus* from the Indian coast

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The scanning electron microscope (SEM) has been used to determine the surface morphology of the radula teeth of the two species of marine gastropod of the genus *Conus*. The teeth are adapted to the cap-

ture of prey and show modelling of the pointed apex and surrounding barbs. There is considerable variation in the radular morphology of the two species as differences in their prey predict different hunting strategies. The shafts of the teeth are formed from a rolled sheet of apparently chitinous material and are thickened and expanded at the base to form a butt and basal spur.

THE genus *Conus* comprises a large family of predatory marine gastropods that have caused injury and occasionally death to humans¹. Adhering to the general trend, teeth of toxoglossa radula show a reduction in the number of teeth and their specialization for venom injection. The teeth must be adapted to perform three functions²: (i) it must pierce the body; (ii) firmly lodge itself and (iii) inject venom into the prey. The general

features of the radular tooth have been known for many years and were used for establishing correlation of tooth structure with prey type as an indicator of diet³⁻⁵, or for taxonomic and phylogenetic affinities⁴.

The genus *Conus* is divided into three groups on the basis of feeding habits. Vermivorous cones typically prey on polychaets while molluscivorous and piscivorous species paralyse and engulf other molluscs and small fish respectively⁶, and the venoms secreted by the three groups are appropriate to their specific prey^{5,7,8}. The morphological features of the radular tooth have been used⁹ to classify the genus *Conus* and also, to relate tooth type to the prey hunted³. All the previous work reveals that although the individual radula could be differentiated, there is a clear demarcation between the three predatory modes.

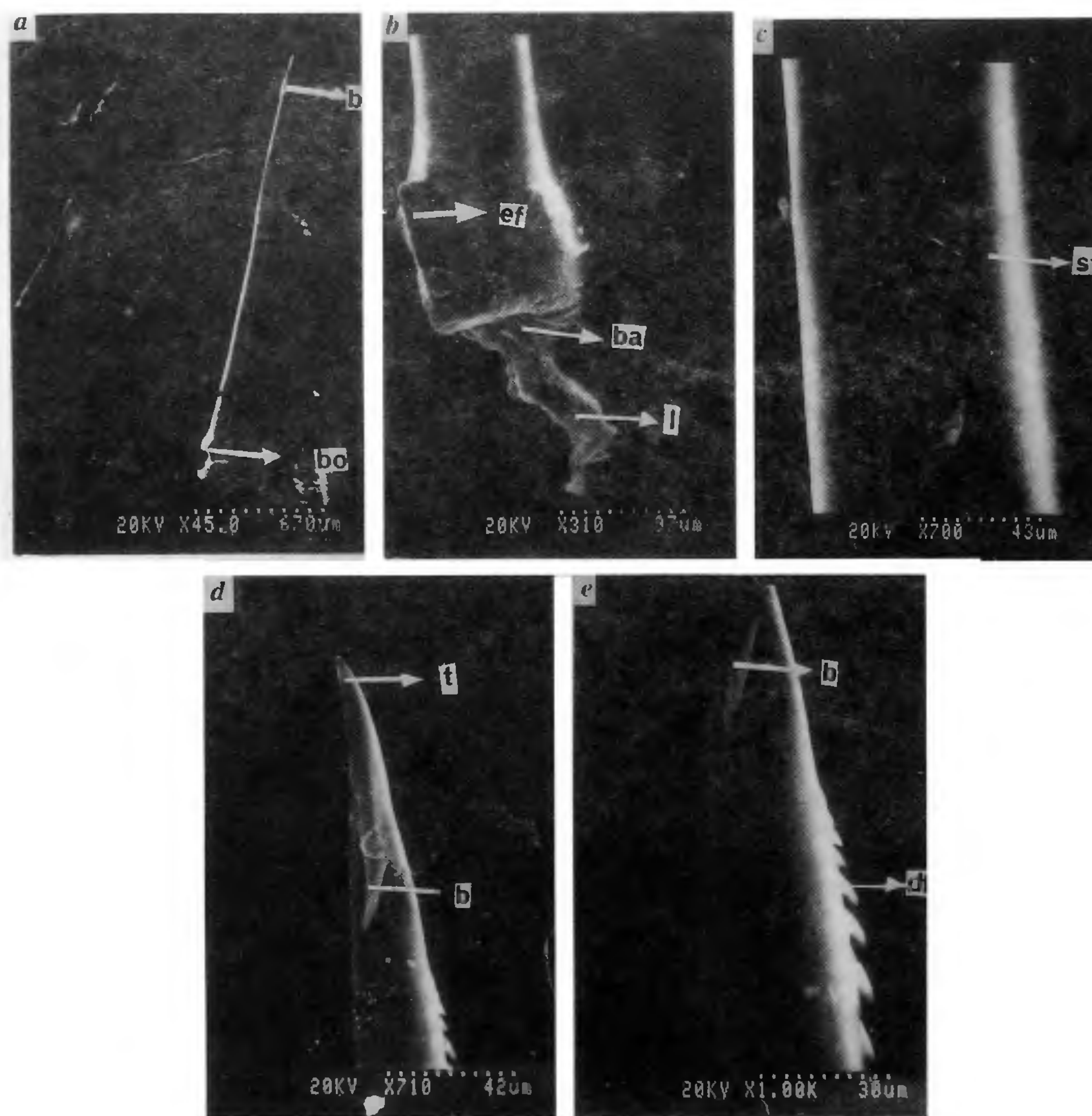


Figure 1. Scanning electron micrographs of *C. monile* single radular teeth. Magnifications as shown in the pictograph. b, barb; bo, basal opening; ef, external fold; ba, base; l, ligament; st, shaft; t, tip; dt, denticles.

The present study attempts to elucidate by SEM, the radula structure of *C. amadis* Gmelin, a molluscivorous snail and *C. monile* Hwass, a vermivorous snail⁹, and also to confirm the differences between the molluscivorous and vermivorous feeding types.

Specimens of *C. amadis* and *C. monile* were collected from the coast of Parangipettai (Lat. 11°29'N; Long. 79°46'E), South India. The venom apparatus was dissected out of the animals and the radula teeth were removed from the proximal arm of the radula sheath. The teeth were rinsed in 0.9% saline solution and then soaked overnight in distilled water. They were then dehydrated in increasing concentrations of ethanol, left for one hour in absolute ethanol, and air-dried overnight in a desiccator containing silica gel to prevent rehydration. Minute entomological pins mounted on wooden sticks

with epoxy glue provided an effective tool for manipulating the radular teeth under the microscope. With this aid individual air-dried teeth were oriented and pressed down firmly on the double sided adhesive tape which was previously mounted on a specimen stub. The coating was earthed to the specimen stub with Dag 915 (colloidal silver) solution. The mounted specimen were coated with a thin layer of 40/60 gold/palladium in a Hitachi Hus-4 vacuum evaporator, by direct evaporation of the alloy at 20 volts. The thickness of the metal coating was calculated to be 20 nm. The preparations were examined on a Hitachi (S-2-20) SEM at an accelerated potential of 20 kV and the magnifications as shown in the scans. The length of the tooth was measured on a vernier microscope, and the length was taken as a straight line between the tip and the base of tooth.

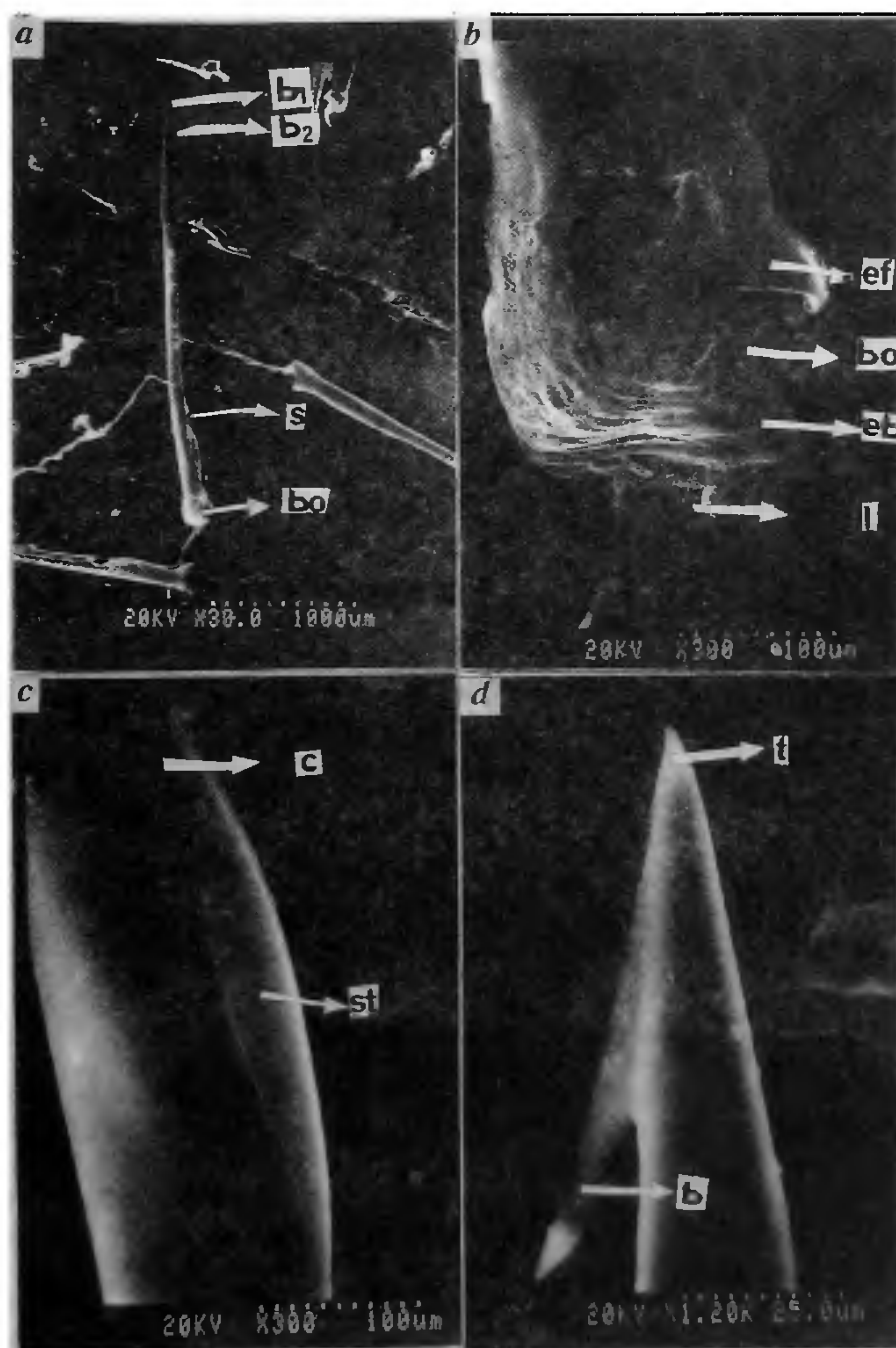


Figure 2. Scanning electron micrographs of *C. amadis* single radular teeth. Magnifications as shown in pictograph. b₁ and b₂, barbs 1 and 2; s, spur; bo, basal opening; ef, external fold; eb, elevated base, l, ligament; c, constriction; st, shaft.

Details of the structures of the radula of both the species are shown in Figures 1 and 2 respectively. The teeth of *C. monile* (Figure 1a) averaged 1–1.2 mm in length compared to the teeth of *C. amadis* which has a length of 1.5 mm (Figure 2a). The teeth of both the species consist of chitinous material rolled into a tube, which is sharpened to a point at the apex and has adapical barbs (b) (Figures 1a and 2a). Both the species observed show 2 barbs and 2 cutting edges with the base (ba) of the tooth of both the species starting at the basal opening (bo). The basal opening with an elevated base (eb) and external fold (ef) is broader in *C. amadis* when compared to *C. monile*. A constriction (c) just before the expansion of the tooth base is seen (Figure 2b), and shows the basal opening open into the lumen and attachment ligament (l) which was also observed for *C. monile*. The teeth of *C. amadis* has a basal spur (s) at its base which is lacking in the teeth of *C. monile*.

The middle portion of the teeth of both the species shows considerable variations (Figures 1c and 2c). The shafts (st) of *C. monile* teeth are tubular (Figure 1c) whereas those of *C. amadis* are constricted and uneven, with a thick swelling before joining with the tip of the radula (Figure 2c). Moreover, the *C. amadis* radula clearly lack denticles (dt) which are seen on the *C. monile* teeth. The tips (t) of the teeth of both the species are elaborated (Figures 1d and 2d). Simple modifications of the apex and the prominent denticles are adequate for *C. monile* tooth to penetrate and hold on to the body of the worm. Conduction of the venom from the venom gland to the victim is via the basal opening, the lumen and through to the adapical opening.

The structure of the radula by SEM confirms *C. amadis* to be molluscivorous and *C. monile* as vermivorous and is in agreement with previous reports⁹ based on feeding behaviour. The radular teeth are highly modified, consisting of a sheet of chitin rolled into a tube^{2,9} and not arranged in rows, but attached to a common basal membrane. They are secreted individually in two files with each individual tooth having its own ligament. They are stored in the short arm of the radular apparatus and are always kept in readiness for use. The ligaments which are remnants of the basal membrane function to move the teeth into the short arm.

Members of the worm feeding cones feed on polychaete worms that have a soft body wall and dwell in tubes or burrows. These cones possess an additional requirement, the denticles and basal spur in their teeth to pull the worm out of its habitat. Also the spur and the denticles serve to retain the tooth in the proboscis and also probably serves to provide a better grip for the proboscis when it attempts to pull out the worm⁹.

Radular morphology of *Conus* shows considerable interspecific variation with respect to the number of barbs, cutting edges and length of the teeth. Mollusc-hunting cones (to which *C. amadis* belongs) have 2

barbs and 2 cutting edges with the presence of denticles along the shaft. *C. textile* a mollusc-hunting cone, is unique in this group in that it does not possess any denticles on the shaft and, now, *C. amadis* is another which has a non-denticular shaft. The spur at the middle of the shaft in *C. amadis* ensures that the tooth is not pushed back into the proboscis when it attacks the prey. The presence of spur and decrease in barb number show that the prey of *C. amadis* are slow responding organisms like their own kind and also the absence of a basal spur suggests that it does not retain the teeth after attacking, unlike *C. monile* which retains it. The radula teeth of worm-hunting cones like *C. imperialis* and *C. pulicarius* are typified by the presence of a long cutting edge.

From the above studies it can be concluded that *C. amadis* is molluscivorous and *C. monile* vermivorous. Based on this and the previous studies the teeth of *Conus* can be classified into three main predatory groups based on their structure². Although the three feeding groups can be distinctly differentiated, there are differences within each feeding group.

1. Cleland, J. B. and Southcott, R. V., *Commonwealth of Australia*, 1965, 196–207.
2. Kohn, A. J., Nybakken, J. W. and Van Mol, J. J., *Science*, 1972, 176, 49–51.
3. Endean, R. and Rudkin, C., *Toxicon*, 1965, 2, 225–249.
4. Nybakken, J. W., *Am. Mus. Novit.*, 1970, 2414, 1–29.
5. Freeman, S. E. and Silva, S. R., *Micron*, 1973, 4, 247–255.
6. Kohn, A. J., *Ecology*, 1966, 47, 1041–1043.
7. Freeman, S. E. and Turner, R. J., *Br. J. Pharmacol.*, 1972, 46, 329–343.
8. Freeman, S. E., Turner, R. J. and Silva, S. R., *Toxicon*, 1974, 12, 587–592.
9. Peile, A. J., *Proc. Malaco. Soc. London*, 1939, 23, 348–355.

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Visitation patterns of birds and butterflies at a *Helicteres isora* Linn. (Sterculiaceae) clump

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There have been very few studies on pollination by birds in India. In this study, animal species visiting a flowering clump of *Helicteres isora*, their feeding behaviour and visitation patterns are recorded. A total of 20 species (11 birds, 8 butterflies, and a mammal) were recorded during the observation period of 36 hours. Birds accounted for the majority of visits, followed by butterflies. Only four species (birds) were pollinators and these visited 45.5% of the total flowers visited by all species, 12 were 'thieves' and four were 'robbers'. Among the birds, Jungle Babblers (*Turdoides striatus*) visited the highest number of flowers and were the main pollinators. There was