Bull. Inst. r. Sci. nat. Belg. Bull. K. Belg. Inst. Nat. Wet.	Bruxelles Brussel	31-XII-1974
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SCANNING ELECTRON MICROSCOPE AND OPTICAL MICROSCOPE OBSERVATIONS ON UROCYCLID LAND SNAIL RADULAE (MOLLUSCA, PULMONATA, UROCYCLIDAE)

BY

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(With 4 plates)

INTRODUCTION

For more than a century, optical microscope study of molluscan radulae has involved staining and mounting the flattened radula between glass slide and cover slip. Observations have been made at 100-1.000X magnification, with results communicated to others by means of photographs or drawings. In the last few years, use of the scanning electron microscope has provided an alternative means of observing and illustrating radular structures (see references in A. Solem, 1972, 1973; A. J. Kohn, J. W. Nybakken, and J.-J. Van Mol, 1972). The scanning electron microscope provides about 500 times the depth of field obtainable with a compound optical microscope at the same magnification, noticeably better resolution, and permits examining the same individual teeth from a variety of angles and at many different magnifications.

If the primary interest in the observations concerns the basal plate, supporting structures, and fine details of the teeth, then the scanning electron microscope is essential. If, however, it is sufficient to determine tooth numbers and the patterns of cutting cusp structure, then optical microscope study of larger pulmonate radulae remains satisfactory and permits easy comparison with previously published work. The present report came about because one of us (A. S.) is working on a broad comparative study of tooth support structure in pulmonate snails using the scanning electron microscope, while the other (J. V. G.) has completed a monographic review of the *Urocyclinae* incorporating extensive optical observations on radulae. A. S. wanted to study urocyclid support mechanisms, while J. V. G. wanted to compare the data obtainable from the two techniques. By presenting scanning microscope photographs and optical microscope drawings of the same species, readers will have, for the first time, direct visual comparison of the results. They can better assess the suitability of each procedure in relation to their own objectives.

MATERIALS AND METHODS

Intact buccal masses of two urocyclid species were sent to A. S. by J. V. G. :

— *Atoxon pallens* (SIMROTH, 1895) from Zaïre, Parc National des Virunga (ex Parc National Albert), Kabalwa River, right tributary of Talya River (approximately 0° 20' N, 29° 45' E), collected 11-I-1955 by P. VANSCHUYTBROECK in tree savanna at 1.130 meters elevation dissection nr. 3693, 3694),

— *Dendrolimax osborni* PILSBRY, 1919, from Zaïre, Parc National des Virunga (ex Parc National Albert), Imamene River, tributary of Ngite River, near the new Watalinga road (approximately 0° 40' N, 29° 37' E), collected 15-III-1955 by P. VANSCHUYTBROECK in ombrophilous forest at 1.000 m elevation (dissection nr. 635); Balombi River, right tributary of Lume River, near the Beni-Kasindi road (approximately 0° 17' N, 29° 40' E), collected 7-II-1955 by P. VANSCHUYTBROECK in tree savanna at 1.000 m elevation (dissection nr. 2409).

These specimens are part of the material used by J. V. G. during the preparation of his monograph (J. VAN GOETHEM, in press).

Two buccal masses of *A. pallens* and two of *D. osborni* were macerated in KOH. One radula of each species was prepared for scanning microscope study according to the technique outlined by A. SOLEM (1972, p. 329). The other radula was prepared by « critical point drying » before mounting on the scanning electron microscope specimen stub. This involves gradual transfer from 70 % alcohol to absolute alcohol, then through several mixed solutions to pure amyl acetate. The radula, in a drop of amyl acetate, is placed in the critical point dryer. The chamber is filled with liquid CO_2. This displaces the acetate, which then is flushed out. The chamber of the critical point dryer is then heated to the critical temperature at which CO_2 goes directly to vapor. This procedure dries a specimen without membrane shrinkage and the distorsions produced by such shrinking. After critical point drying, the specimen was mounted on the specimen stub and coated as outlined by A. SOLEM (1972, p. 330).

The mounted specimens were studied with a Cambridge Stereoscan instrument at the Electron Optics Laboratory of the American Dental

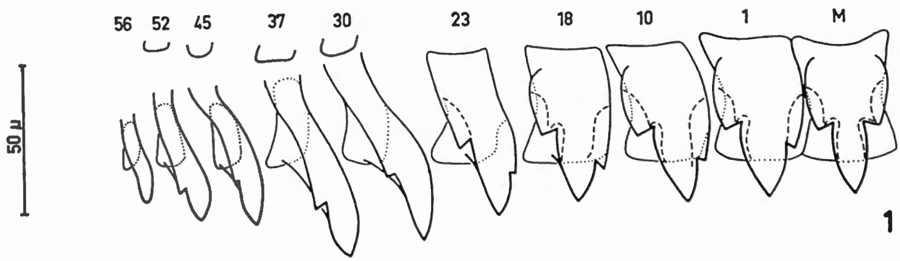
Association Research Institute. The assistance of Mr. John LENKE and Mr. George NAJARIAN from the Electron Optics staff is gratefully acknowledged.

OBSERVATIONS

Each species is discussed separately, then a few comparative remarks are appended.

Atoxon pallens SIMROTH, 1895

Radulae studied by J. V. G. ranged in length from 3.7 to 5.1 mm and in breadth from 1.7 to 2.3 mm. Drawings of teeth prepared by J. V. G. from slide mounts (fig. 1) show the pattern of cusp outlines and degree of tooth



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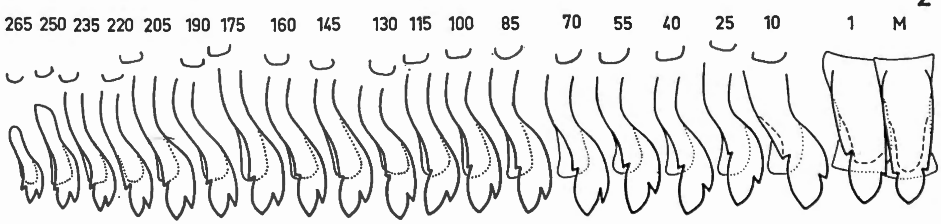

Fig. 1. — *Atoxon pallens* SIMROTH, 1895. Dissection nr. 3687; Zaïre, Parc National des Virunga. Teeth drawn from left side of radula.

Fig. 2. — *Dendrolimax osborni* PILSBRY, 1919. Dissection nr. 643; Zaïre, Parc National des Virunga. Teeth drawn from left side of radula.

slant towards the middle of the radula, but contain no data concerning the anterior tooth margin. The figured radula (dissection nr. 3687) had 126 rows of teeth with the half-row formula : 28-27 + 3-4 + 21-20 + M. Pl. I, fig. 1 and pl. III, fig. 9 come the closest of the scanning microscope photographs to having the same viewing angle as the optical illustration. The other scanning electron microscope photographs were taken at different angles to study the basal plate and cusp support structures. Pl. I, fig. 2, and pl. II, fig. 6-7, were made from dissection nr. 3693, which had been critical point dried, while pl. I, fig. 1, 3-4 and pl. II, fig. 5 were made from dissection nr. 3694, which had been given standard treatment.

The greater quantity of white fibrous material visible in the latter illustrations reflects a less complete job of sonic cleaning (see A. SOLEM, 1972, p. 330) before mounting. Probably these particles are a combination of macerated tissue and KOH crystals.

An overall scanning electron microscope view of the rhachidian and early lateral teeth at 540X in near vertical view (pl. I, fig. 1) shows the basic cusp and anterior tooth margin structure. The rhachidian tooth (right side of photograph) has prominent ectoconal cusps slightly in front of the tooth middle, while the anterior tooth margin is bilobed with a central indentation. The whole tooth at the lower right corner of the photograph shows the relation between mesoconal cusp extension and posterior basal plate termination, with the mesocone extending far beyond the basal plate termination. Trends in the first few lateral teeth include the ectocone becoming stubbier and wider, the endocone reduced in prominence, and for the anterior margin contour to broaden proportionately. The endocone is always smaller and sits slightly posterior of the ectocone. The anterior margin of the lateral teeth is sinuated with a central depression and indentation matched by elevated ridges that are roughly in line with the ectocone and endocone.

When viewed from a lateral angle (pl. I, fig. 2) at higher magnification (1.625X), both rhachidian and lateral teeth are seen to have the mesocone supported by a thick buttress angling up from the basal ridge to its tip. Under cutting stress (or as a result of membrane shrinkage in these mounts), the support ridge of the mesocone will fit into the central depression on the anterior margin of the tooth in the next posterior row. As pressure elevates the tooth in the latter row, its anterior margin will be forced back and downwards. The central depression will fit against the mesoconal support ridge of the next anterior tooth. This will guide the two lateral flanges as the tooth under stress continues to be elevated. Under extreme stress and elevation, the lateral flanges will rest against the basal plate of the tooth in the next row. If this tooth is poised on the odontophoral tip, then the resistance to cutting by one tooth will be transferred to the basal plate of the next tooth to meet the object, thus aiding its cut into the surface. A similar lateral view to the left of the rhachidian tooth at slightly lower magnification (pl. I, fig. 3, at 1.100X) shows a « resting phase » position of the teeth. This photograph demonstrates much more clearly the shape of the supporting ridge. A low (570X) magnification view of several lateral teeth (pl. I, fig. 4) was taken at a place where a fold had been induced in the mounted radula. This demonstrates several of the stages in tooth elevation. In a very oblique view (pl. II, fig. 5) of teeth in this region at 1.985X, the nature of the support system becomes even clearer. There are weak elevated ridges on the outer margin of the basal plate (see upper left) that would aid the central ridge in supporting a tooth under extreme stress and help prevent any lateral twisting of the tooth.

Marginal teeth were photographed from the right side of the radula. The early marginals (pl. II, fig. 6, at 1.585X) have lost the endocone, the mesocone is smaller (blunted from wear in this photograph), while the ectocone is much larger in size. The mesoconal supporting ridge has become greatly reduced in size, the posterior section of the basal plate is shortened, and lacks the distinct elevated ridges found on the lateral teeth basal plates. The greatest changes have occurred in the anterior region of the tooth. The free anterior basal plate margin has disappeared, with all the basal plate being attached to the membrane. The mesocone curves regularly anteriorly for a short distance. The basal plate is truncated both anteriorly and posteriorly.

Middle to late-middle marginal teeth at 2.200X (pl. II, fig. 7) show a reduction in the size of the ectocone, development of a small accessory ectoconal spur on the side of the tooth, continued posterior shortening of the basal plate, and the anterior basal plate margin becoming gradually tapered and rounded. The ectoconal spur is a highly variable feature in many taxa and has no taxonomic significance in this species.

In a more vertical view (lower right of photograph) the teeth are seen to be quite narrow, and the mesocone extends much higher above the basal plate than the ectoconal cusp or spur. On the outermost marginals (pl. II, fig. 8), seen at 2.200X, the ectoconal cusp and spur are absent, resulting in a unicuspid, very small tooth. Its tapered basal plate extends a fair distance anteriorly.

Dendrolimax osborni Pilsbry, 1919

Radulae studied by J. V. G. ranged from 5.7 to 7.8 mm in length and 4.1 to 6.7 mm in breadth. Drawings of glass slide mounted teeth by J. V. G. (fig. 2) show cusp structure and partial pattern of tooth inclination. Pl. III, fig. 9 is taken from approximately the same angle of view as the drawings of the rhachidian and first lateral teeth. Pl. III, fig. 12, and pl. IV, fig. 13, were made from dissection nr. 635, which had been critically point dried, and pl. III, fig. 9-11 and pl. IV, fig. 14 were made from dissection nr. 2409, which had been given standard treatment.

Scanning electron microscope examination of the rhachidian and lateral teeth in near vertical view at 835X (pl. III, fig. 9) showed weakly irregular side cusping on the rhachidian tooth, which often has an interior terminal papilla on the mesoconal edge. Such terminal papillae have been seen on taxa with broadened cusps in several families (A. S., unpublished). Their presence and degree of prominence is highly variable within species and is not a significant specific character. Lateral teeth have a weak posterior endocone and a more prominent ectocone that is located slightly in back of the tooth middle. Both side cusps become stronger on early lateral teeth that are at a greater distance from the rhachidian tooth. The upper side is much flatter than in *Atoxon* and the anterior margin (see lower right of pl. III, fig. 9) is less strongly sinuated. Unworn lateral teeth tend to have the same terminal papilla on the mesocone as do the median teeth. Late lateral teeth at 1.675X (pl. III, fig. 10) from the left side of the radula are altered in form. The entire tooth is slanted towards the middle of the radula, the endocone is far more prominent than the ectocone, while the mesocone is more nearly triangular and shows no obvious trace of the central terminal papilla. There is a long anterior basal plate extension (see upper right), while the cutting blade is angled from the plane of the basal membrane at about a 45° angle (see upper left). Each endocone and part of the mesocone extends laterally above the basal plate of the next tooth towards the middle of the radula.

Marginal teeth (pl. III, fig. 11 at 1.625X; pl. III, fig. 12 at 2.040 X; pl. IV, fig. 13 at 2.000X) show several trends. First, a reduction in mesoconal size and more clearly triangular shape (pl. III, fig. 11), with increasing size for both ectocone and endocone. Second, there is continued greater inward (towards middle) slant for the elevated cusps. Pl. III, fig. 11 is of early marginals, where the ectocone is enlarged, the mesocone reduced and somewhat papillate, and the inner margin of the tooth is sharply curved. Pl. III, fig. 12 and pl. IV, fig. 13 are nearer the outer marginal field. These teeth show a smoother curve to the inner margin and there is a marked tendency for small accessory ectoconal denticles to form on the outer edge of the tooth margin (pl. III, fig. 12). Occasionally the main ectocone will become split (lower right of pl. III, fig. 12).

Finally, worn early marginals from the right anterior portion of the radula at 1.775X (pl. IV, fig. 14) show that all cusps have been worn to a single nub that extends barely posterior of the basal plate, whereas normally (pl. III, fig. 11) the cusps extend well posterior of the basal plate margin.

COMPARATIVE RESULTS

Atoxon and *Dendrolimax* differ significantly in the total number of teeth on their radula, with *Atoxon* having about 14,000 and *Dendrolimax* an estimated 85,000 denticles. The size of the adult animals, measured by preserved length, overlaps significantly. Adult specimens of *Atoxon* measure 36-63 mm, and those of *Dendrolimax* 47-72 mm in length. The radular lengths, when flattened in a slide mount, are similar in proportion to total body length, approximately 0.09 for both taxa, but the radular widths differ in both actual measurements and proportion of length to width. *Dendrolimax* has a radula whose flattened width is 0.71-0.86 times its flattened length, while in *Atoxon* the same ratio is 0.42-0.50. We have no information as to differences of radular configuration within the buccal mass itself.

The radula of *Dendrolimax* is significantly wider and has many more teeth than *Atoxon*. There also are significant differences in tooth shape and form. *Dendrolimax* teeth are smaller, more flattened above and noticeably more strongly tricuspid, except for the teeth nearest the middle of the radula, which have the side cusps greatly reduced. In general form, the teeth of *Dendrolimax* are most similar to the early marginal teeth of *Atoxon* (especially from young individuals, see J. VAN GOETHEM, in press, fig. 266), although obviously narrower, flattened above, more strongly curved, and with tricuspid, instead of bicuspid tips.

Exactly analogous changes have occurred in Indian and Southeast Asian Helicarionidae. For example, *Cryptaustenia* and *Durgella* are widely distributed in this region and very similar in external appearance, pallial structures, and genitalia, but differ in radular tooth structures and numbers (see A. SOLEM, 1966, p. 64, figs. 13b-c). While the details of the changes are different, the pattern of tooth homogenization accompanied by great increase in tooth numbers and development of accessory ectoconal cusps in *Durgella* parallels exactly the shift from an *Atoxon*-like pattern to *Dendrolimax*. Intermediate conditions are seen in such genera as *Sitala* and *Cryptosoma* (see W. T. BLANFORD and H. H. GODWIN-AUSTEN, 1908, p. 210, fig. 74 D and p. 226, fig. 78 D) from Burma and India.

Presumably this change in radular width and tooth structure relates to an alteration in diet, but we don't know exactly what is the nature of the change.

In terms of basic shape and support structure, the teeth of *Atoxon* agree exactly with those of the Limacidae and Ariophantinae (A. SOLEM, unpublished). The Urocyclidae, Limacidae and Ariophantinae have evolved independently into slugs or slug-like taxa that differ greatly in general comparative morphology. The continued basic similarity of the radular structure, however, suggests that they share common ancestry and generally have diversified in respect to other environmental factors than diet. An exception would be in the parallel evolution to many relatively uniform teeth in genera such as *Dendrolimax* and *Durgella*.

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Accepted 28 March 1973.

EXPLANATION OF PLATES

PLATE I

Fig. 1-4. — *Atoxon pallens* SIMROTH, 1895. Radula.

Fig. 1 : Rhachidian (right) and left lateral teeth in near vertical view at 540X. — Fig. 2 : Rhachidian, 1st and 2nd right lateral teeth viewed at a low angle from the posterior at 1.625 X. — Fig. 3 : Rhachidian and 1st lateral teeth viewed from a low right side angle at 1.100X. — Fig. 4 : Right side lateral teeth on a fold in the radula at 570X.

PLATE II

Fig. 5-8. — *Atoxon pallens* SIMROTH, 1895. Radula.

Fig. 5 : Oblique view from anterior low angle of lateral teeth at 1.985X. — Fig. 6 : Marginal teeth from right side of radula at a place where the membrane was torn to permit seeing basal plate structure, viewed from a high outside angle at 1.585X. — Fig. 7 : Mid-marginal teeth from right side of radula seen from a high outside angle at 2.200X. — Fig. 8 : Outermost marginal teeth from right side of radula at 2.200X. The white flecks on the teeth are bits of macerated tissue that were not washed off during preparation.

PLATE III

Fig. 9-12. — *Dendrolimax osborni* PILSBRY, 1919. Radula.

Fig. 9 : Rhachidian and lateral teeth in vertical view at 835X. — Fig. 10 : Late lateral teeth from left side of radula on a sharp fold, seen from a low posterior angle at 1.675X. — Fig. 11 : Early marginal teeth from left side of radula at 1.625X. — Fig. 12 : Mid-marginal teeth from left side of radula seen from a low central angle at 2.040X.

PLATE IV

Fig. 13-14. — *Dendrolimax osborni* PILSBRY, 1919. Radula.

Fig. 13 : Mid-marginal teeth from left side of radula at 2.000X. — Fig. 14 : Worn marginal teeth from anterior right portion of radula viewed from a low outside angle at 1.775X.

Bull. Inst. r. Sci. nat. Belg. — T. 50, N° 7 Biologie, 1974.
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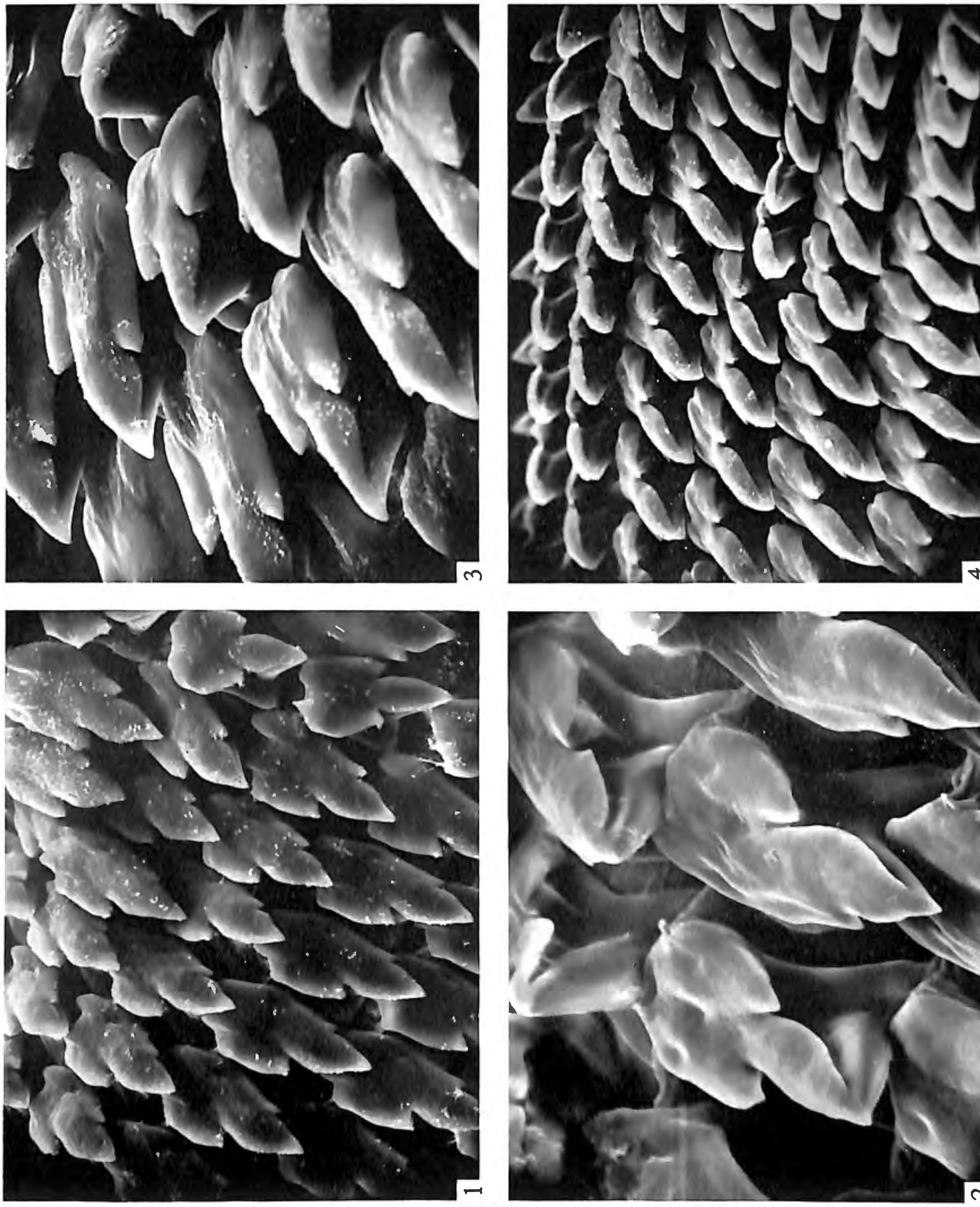

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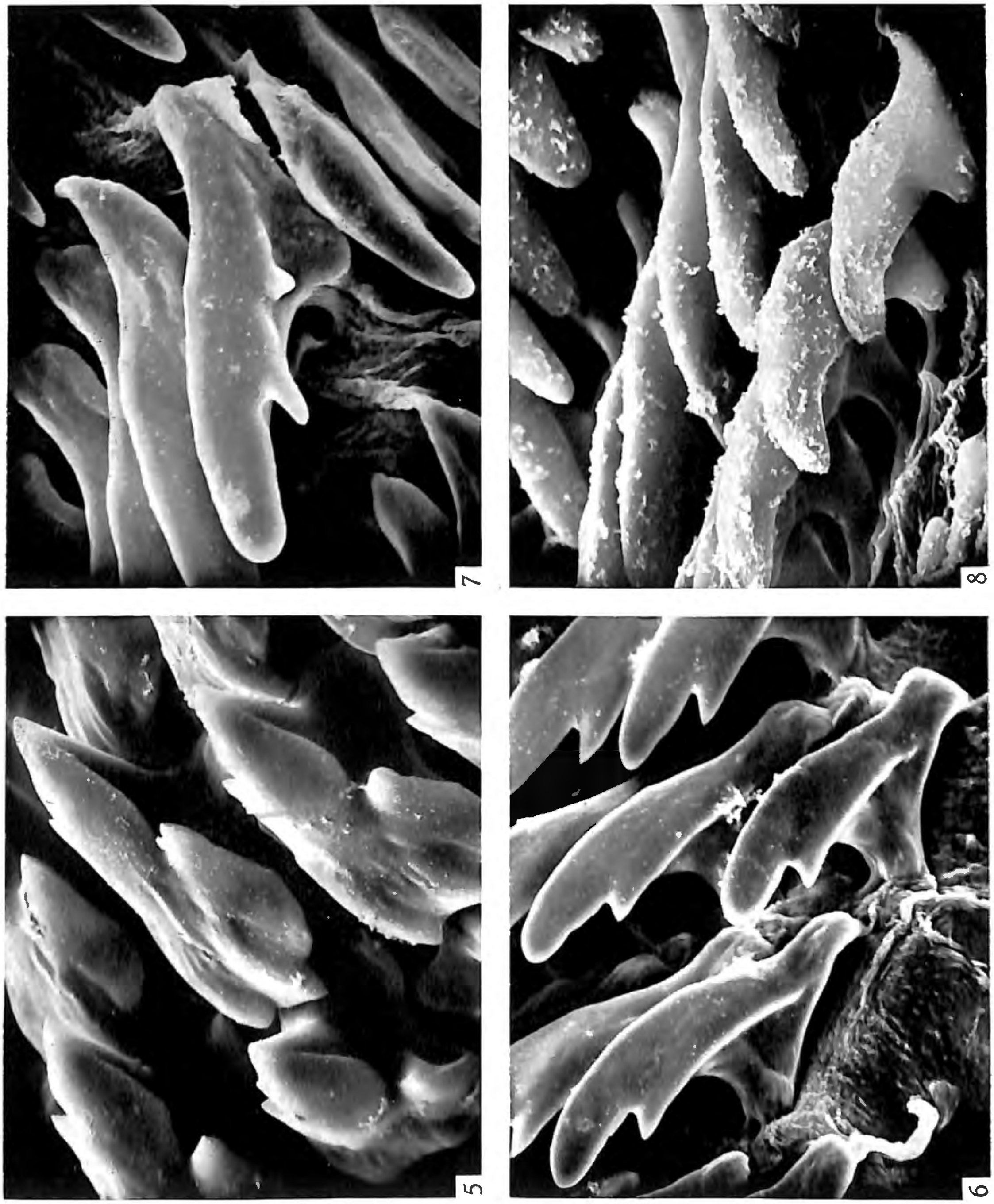

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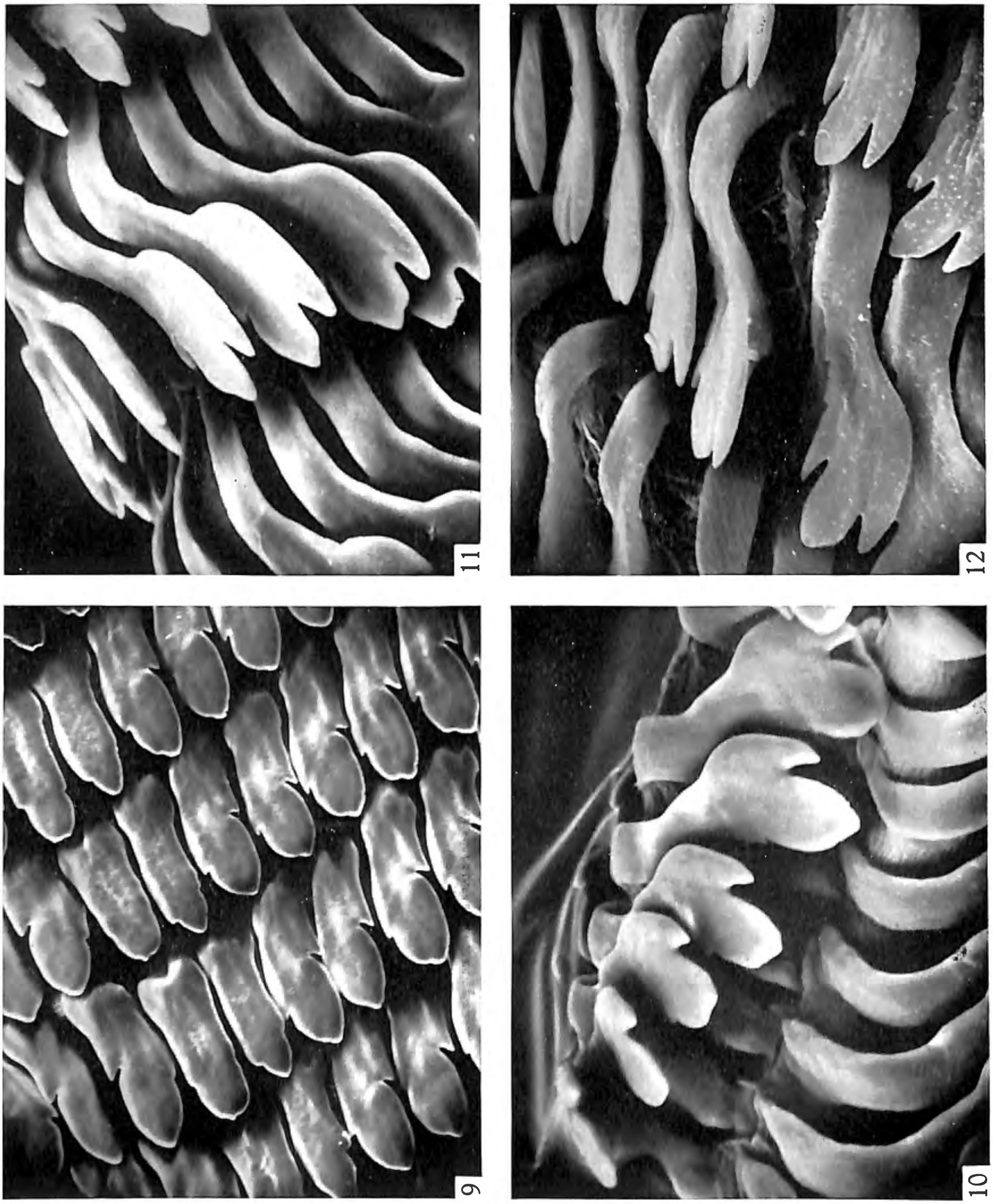

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