

Additional Notes on *Tubiluchus corallicola* (Priapulida), Based on Scanning Electron Microscope Observations*

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Abstract

Cuticular differentiations on the body surface and the pharyngeal armature of adult *Tubiluchus corallicola* van der Land were examined with the scanning electron microscope. The morphology of abdominal tumuli, flosculi and tubuli, and of pectinate teeth and fimbrillae on the pharynx is described, and an attempt is made to interpret their functional properties. Taking into account the structural details, relative size, and topographic arrangement in relevance to interaction with the interstitial environment, we assume that tumuli are protective, flosculi chemoreceptive, and tubuli adhesive, and that the pharyngeal armature is a scraping mechanism for feeding on epigrowth on sand grains.

Introduction

Tubiluchus corallicola is hitherto the only known meiofaunal representative of the phylum Priapulida. Discovered by van der Land (1968) in sand samples from Curaçao, Dutch W. Indies, the species was afterwards reported from Bermuda (Coull, 1970), Bonaire, Dutch W. Indies (van der Land, 1970) and Barbados, W. Indies (Kirsteuer and van der Land, 1970). In addition to its small body size and comparatively long tail, *T. corallicola* shows a number of morphological and biological characteristics indicative of an interstitial mode of life. The species, however, is not confined to subtidal sand, and was also found on mud bottoms and on a subtidal stromatolite.

A comprehensive account of the morphology of preserved specimens was given by van der Land (1970), and further information derived from studies of living animals was presented by Kirsteuer and van der Land (1970). Except for two low-magnification scanning electron micrographs of tumuli (van der Land, 1970), to date all published observations pertaining to *Tubiluchus corallicola* were made with the light microscope. Due to the size of the animals (abdomen 1 to 2 mm in length), however, details of cuticular differentiations on the body surface, as well as of the

various pharyngeal structures are extremely difficult to discern with this technique, even when phase contrast is employed, and minor inconsistencies in descriptions have already emerged (Kirsteuer and van der Land, 1970; van der Land, 1970). A more detailed examination with the scanning electron microscope seemed desirable, not only for purely didactic reasons, but also in view of the possibility that the fine structure of tumuli, flosculi, tubuli, and of pharyngeal teeth and fimbrillae may provide useful diagnostic characteristics should more tubiluchids be found in the future.

Material and Methods

For the present study, 2 mature specimens of *Tubiluchus corallicola* from the Barbados collection (Kirsteuer and van der Land, 1970), deposited at the American Museum of Natural History, New York, USA, were used. One specimen (Figs. 1—3) was an ethanol-preserved female which, although having been narcotized ($MgCl_2$) prior to fixation, had the introvert and neck retracted, but retained tumuli, flosculi and tubuli in good condition. The other specimen (Figs. 4—6) was kept as a whole mount, squeezed preparation in formalin-glycerin (1:1). Preceding fixation, the pressure on the narcotized animal had been increased until the pharynx became completely evaginated. The latter never occurs in living specimens under normal circumstances (Kirsteuer and van der Land, 1970), and has to be achieved artificially to make the whole complement of pharyngeal differentiations accessible for observation. After removal from the whole mount preparation, the specimen was transferred to 70% ethanol.

To avoid further distortion of surface structures, we prepared both specimens for scanning electron microscopic examination using the critical-point drying method of Anderson (1951). They were carefully cleaned of adherent particles by treatment in an ultrasonic cleaner, using changes of 70% ethanol as medium. Dehydration followed in a series of ethanol

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(80, 90, 96, $3 \times 100\%$), 10 min each, under constant gentle agitation. The absolute ethanol was displaced by amyl acetate in three steps (3:1, 1:1, 1:3) and the amyl acetate was changed twice (5 min each step). For better protection, the specimens were isolated in small glass tubes (15 mm long, 4 mm inner diameter), with nylon bolting cloth epoxy-cemented to each end. Quickly, to avoid evaporation of the amyl acetate, they were then placed inside a pressure chamber under a small but constant flow of liquid carbon dioxide (10 min at room temperature, ca. 21 °C). By warming the chamber to 50 °C, the liquid carbon dioxide that had now displaced the amyl acetate passed its critical point, and the gas could be slowly released.

The dried specimens were mounted onto a small circular coverslip with traces of a diluted household glue, and kept in a desiccator. Shortly before examination, the coverslips with the specimens were mounted on metal stubs with carbon ink, and coated with approximately 20 nm of gold. The observations were made with a Cambridge Stereoscan Mark IIA. Primary magnifications used were $50 \times$ to $15,000 \times$.

Results and Discussion

Tumuli

Tumuli are small protuberances on the surface of abdomen and tail (Fig. 1:1). They are regularly arranged in 40 (van der Land, 1968) to 45 distinct longitudinal rows on the abdomen (Fig. 1:2), but show a less regular distribution along the tail. The tumuli are quite flexible, and their height as well as shape changes considerably with varying states of contraction of the body (Figs. 1:2, 3, 4; 3:10, 11, 15). Being rather flat cones in expanded individuals (Kirsteuer and van der Land, 1970), they become higher with increasing contraction, and are concomitantly moved closer together (Figs. 1:4; 3:10). In areas of extreme contraction, the tumuli are sideways compressed, and so densely positioned that continuous chains of either longitudinally (Fig. 1:2, 3) or transversely (Fig. 3:11, 15) oriented crests are formed. Each tumulus contains 10 to 13 cuticular rods (Kirsteuer and van der Land, 1970; Fig. 16), which in the steep-conical stage of the tumulus are indicated by the radial ridges (Figs. 1:4; 3:10). These rods evidently unite centrally at the apex of the tumulus and, depending on the direction (relevant to the body) in which contraction proceeds, two opposing ridges become more pronounced and ultimately form an uninterrupted longitudinal or transverse crest supported vertically by the remaining rods.

No particular function has hitherto been ascribed to the tumuli, but their gradual transformation caused by contraction of the body, thus allowing for their dense arrangement leading to what may be called a secondary body surface, suggests strongly that they are protective structures. The absence of tumuli on the neck region, which together with the introvert is

retracted into the abdomen during adverse conditions, also points in this direction. Reinforcement of the body wall is a widespread feature in interstitial fauna, and is achieved in various ways (Swedmark, 1964). In *Tubiluchus corallicola*, a high capacity to contract (by itself an effective means of protection) is evidently combined with a strengthening of the cuticle, i.e., the tumuli. The latter are flexible, and arranged in such a way that the body retains pliability for peristaltic movement in locomotion (Kirsteuer and van der Land, 1970), whereas during contraction of irritated animals they can form a rigid armor.

Flosculi

Flosculi do not have a regular distribution on the body surface, but they are always more numerous on the neck and in the anterior abdominal region (Fig. 1:3) than on the remainder of the abdomen. They are lacking on the introvert, and only a few are present on the proximal portion of the tail. Van der Land (1970) aptly described these organs as having the shape of a flower. Each flosculus consists of a calyx resting on an elevated base on which also an accessory seta is inserted (Fig. 2:9). The petals, of which 10 to 11 were previously thought to be present (van der Land, 1970), are prominent bulges, which run vertically on the outside of the calyx and continue as short projections above its rim (Fig. 2:6—9). In all flosculi examined with the scanning electron microscope, only 7 or 8 petals per calyx were found. Calyces with 7 petals (Fig. 2:6) are less common than those with 8 (Fig. 2:7—9). In the octo-petalous flosculi, however, the two petals closest to the accessory seta are smaller, and not as strongly separated as the others (Fig. 2:8), thus giving the impression that one of seven original petals is secondarily divided by a notch; one is inclined to imagine that the intrinsic structural pattern of all flosculi is heptaradial. The calyx is closed on top, except for a central porus (Fig. 2:6, 8). The accessory setae are slender conical to club-shaped (Fig. 2:6—9), and supersede the calyces in height. Frequently, they lean on the calyx along the notch between the two smaller petals (Fig. 2:7—9). On several setae (Fig. 2:7—9), three tiny knobs were observed on the distal end, but it was not possible to ascertain whether the dark spot which they surround (Fig. 2:7, 8) represents an opening, or is just caused by shadow.

In non or only slightly contracted areas of the abdomen, the flosculi are distinctly higher than the tumuli and the calyx extends freely from the base (Fig. 2:5). In strongly contracted regions, the flosculi usually do not reach above the level of the crested tumuli in their vicinity, and the calyx is partly retracted into the base, which forms a collar around the proximal portion of calyx and seta (Fig. 2:6, 7, 9).

A comparison of flosculi (Fig. 2:6—9) shows slight differences in the prominence of the petals and in the angle in which they ascend, as well as in the shape of the

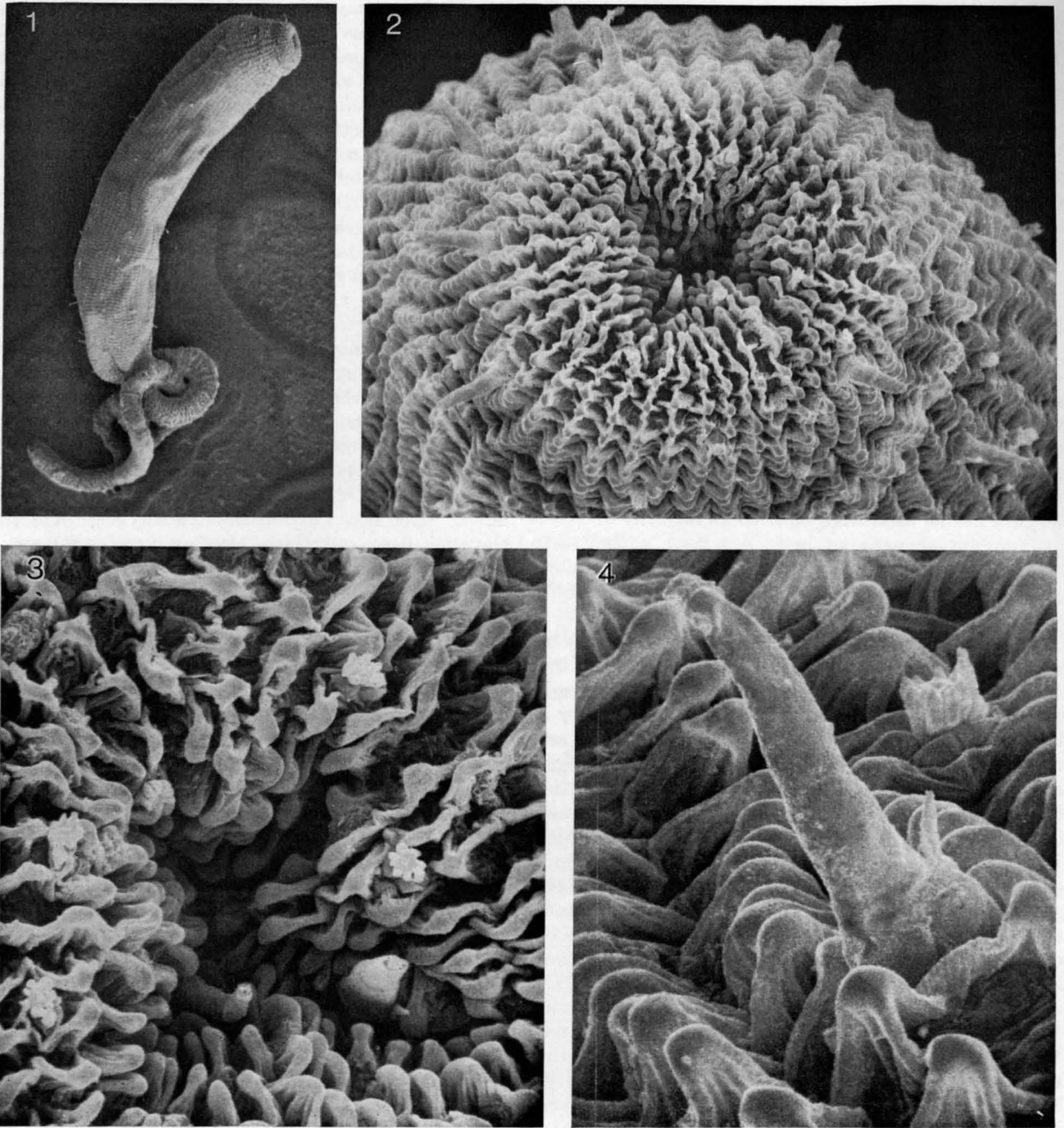


Fig. 1. *Tubiluchus corallicola*. 1: Total view of female specimen with introvert and neck region invaginated (50 \times); 2: contracted anterior end of abdomen with rows of crested tumuli (600 \times); 3: flosculi between crested tumuli on anterior end of abdomen (1,500 \times); 4: surface of abdomen with tubulus, flosculus, and tumuli showing supporting rods (2,800 \times)

petal tips, which are bluntly rounded or conically pointed. Furthermore, the top of the calyx with the central porus is concave in some flosculi and convex in others. Together, these differences could be interpreted as changes of a relatively soft and flexible organ which lacks the strong cuticular support present in other

surface structures. The latter is perhaps also indicated by the fact that calyces (but also setae) respond with a different signal during scanning examination than do adjacent areas of the body surface; particularly under high magnification, they produce lighter (more exposed) images (Fig. 2:7–9).

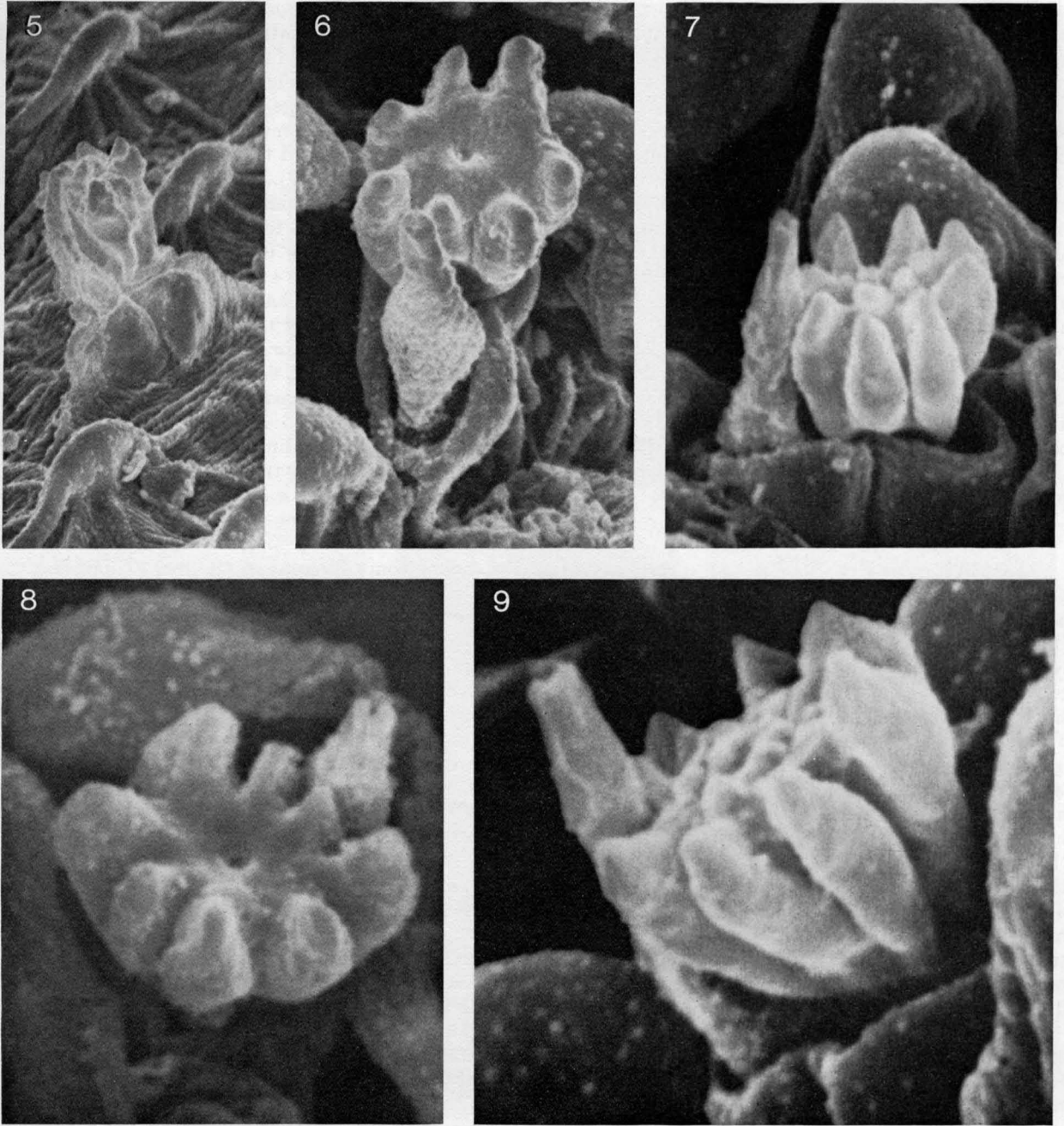


Fig. 2. *Tubiluchus corallicola*. 5: Flosculus extending freely from base and above the tumuli (5,000 \times); 6: flosculus with heptaradial calyx and club-shaped seta (10,000 \times); 7: partly retracted flosculus with base forming a collar around the octo-petalous calyx and the seta (10,000 \times); 8: calyx with 8 petals, of which 2 are smaller, and seta with knobs on distal end (14,000 \times); 9: same as 7 (15,000 \times)

The present observations do not provide conclusive evidence as to the functional properties of flosculi, but do lend further support to van der Land's (1970) presumption that they serve as chemoreceptors. The tendency to protect the flosculi during disturbance by

partly retracting the calyces into the base and by screening them off on all sides by the rising tumuli on the contracting abdomen, fits well into this concept. Conversely, the presence of flosculi on the neck, which is invaginated when animals are disturbed, and the

sheltered position of flosculi on the contracted abdomen exclude the possibility that they are adhesive structures. The central porus on the calyx is, thus, presumably not the opening of a secretory efferent duct, but rather a means for communication with the surrounding medium, and as such would be most likely to occur in a sensory structure of chemoreceptive nature. Examination of flosculi with the transmission electron microscope will certainly produce more information to settle this question, and also to elucidate the interrelation of calyx and accessory seta. At present, attention is only directed to the striking similarity expressed in the hepta-radial morphology of calyces and the ultrastructural organization of epidermal receptors in *Priapulius caudatus*, which latter were studied by Moritz and Storch (1971; Fig. 1), and show in cross-section 7 microvilli regularly arranged around a seven-times indented cilium. The diameter of this cilium-microvilli rosette and the width of calyces in their proximal portion (e. g., Fig. 2:9) are in approximately the same order of magnitude.

Tubuli

Tubuli are confined to the abdomen, where they are irregularly distributed over the whole surface (Fig. 1:1). Of the three surface differentiations presently discussed, the tubuli are the most conspicuous and extend even in contracted areas of the abdomen considerably above the crested tumuli (Figs. 1: 2, 4; 3: 11, 15). The slender, distally tapering, stiff tubes have a cup-shaped end demarcated by a circular constriction (Fig. 3:10, 13, 14), and are proximally jointed into a base. The transition from tube to base is obscured by irregular folds, and it is difficult to decide whether the accessory seta, which is always present, inserts on the tube or on the base (Figs. 1:4; 3:10—13). No distinct opening was found at the end of the tubuli but, in a frontal view into the cup (Fig. 3:15), roundish structures are discernible, which could be either pores or granules. On several other tubuli (Fig. 3:10, 13, 14), droplets of secretion extrude distally.

Tubuli also occur on the larva of *Tubiluchus corallicola*, and on larvae but not on adults within the family Priapulidae. In general, their function is enigmatic, and only for those on the introvert of *Hali-cryptus spinulosus* larvae was an adhesive property ascertained (van der Land, 1970). The tubuli of *T. corallicola* were originally assumed to be tactile organs (van der Land, 1968), because preliminary histological examination did not reveal gland cells connected to them. Observation of living larvae and adults (Kirsteuer and van der Land, 1970), however, showed that sand grains are often attached to the tubuli, and that secretion exudes from their tips. The latter is now again convincingly demonstrated in some of the scanning electron micrographs, and there can be no doubt that the tubuli of *T. corallicola* are adhesive organs.

Pharyngeal Armature

The pharyngeal armature consists of pectinate teeth in the anterior portion of the pharynx and of fimbrillae in its posterior part (Fig. 4:16). Both structures exhibit a remarkable range of variance, which is particularly evident in the middle of the pharynx (Figs. 4:17; 5:19) and toward the posterior end of the fimbrillar region (Fig. 5:21). Nowhere, however, does this modification lead to spines, and the previously described prickles between teeth and fimbrillae (Kirsteuer and van der Land, 1970; van der Land, 1970) are definitely lacking (Figs. 4:17; 5:19). The impression of their existence must have been caused by higher optical density of either the median fold on the posterior teeth (Fig. 6:24), or of overlapping hairs on the anterior fimbrillae (Fig. 6:25).

The teeth are arranged in longitudinal and diagonal rows (Figs. 4:16—17; 5:18). Seven or eight teeth are present in each longitudinal row. The majority of teeth (Figs. 5:18; 6:22) show the typical pectinate structure (van der Land, 1970), i. e., a slender manubrium carrying a semicircularly bent comb on its distal portion. In addition, however, there is always a much smaller secondary comb in front of the main comb (Figs. 5:18; 6:22). It is just a fringed crest, probably resting on the manubrium, but certainly constituting an integral part of the tooth. This becomes obvious by looking at the gradual transformation on the posterior teeth in each row (Figs. 4:17; 5:19), where in the first stage following a normal tooth (compare Fig. 6:22 and 23), the secondary comb increases in width and spans laterally much farther than the main comb, which itself is reduced in size. In the second and final stage (Fig. 6:24), the secondary comb has turned into the prominent component of the tooth. It is now a wide, winglike structure with a median fold, covering partly the "main" comb, which has a roundish, spatulate shape, and only about half the size of the main comb on anterior teeth. The tiny, hairlike projections on the secondary comb do not change significantly in length (Fig. 6:22—24). The spinules of the main comb (Fig. 6:22), however, fuse proximally and decrease in length (Fig. 6:23), so that finally (Fig. 6:24) the rim configuration of "main" and secondary comb is nearly identical.

The fimbrillae exhibit the same longitudinal and diagonal pattern of arrangement as the teeth, and follow in direct continuation of the rows of teeth (Figs. 4:16, 17; 5:19). Structural changes on the fimbrillae are less profound but, nevertheless, distinct. All fimbrillae consist of a ridgelike base with a fringe of hairs, which usually point posteriad. On the most anterior fimbrillae (Figs. 4:17; 5:20), the hairs are comparatively long and flexible (as indicated by the bent and irregular course of extension), and the base resembles a quarter of a sphere protruding from the pharynx surface (Fig. 6:25). Progressing backward (compare Fig. 5:20 and 21), the hairs become shorter

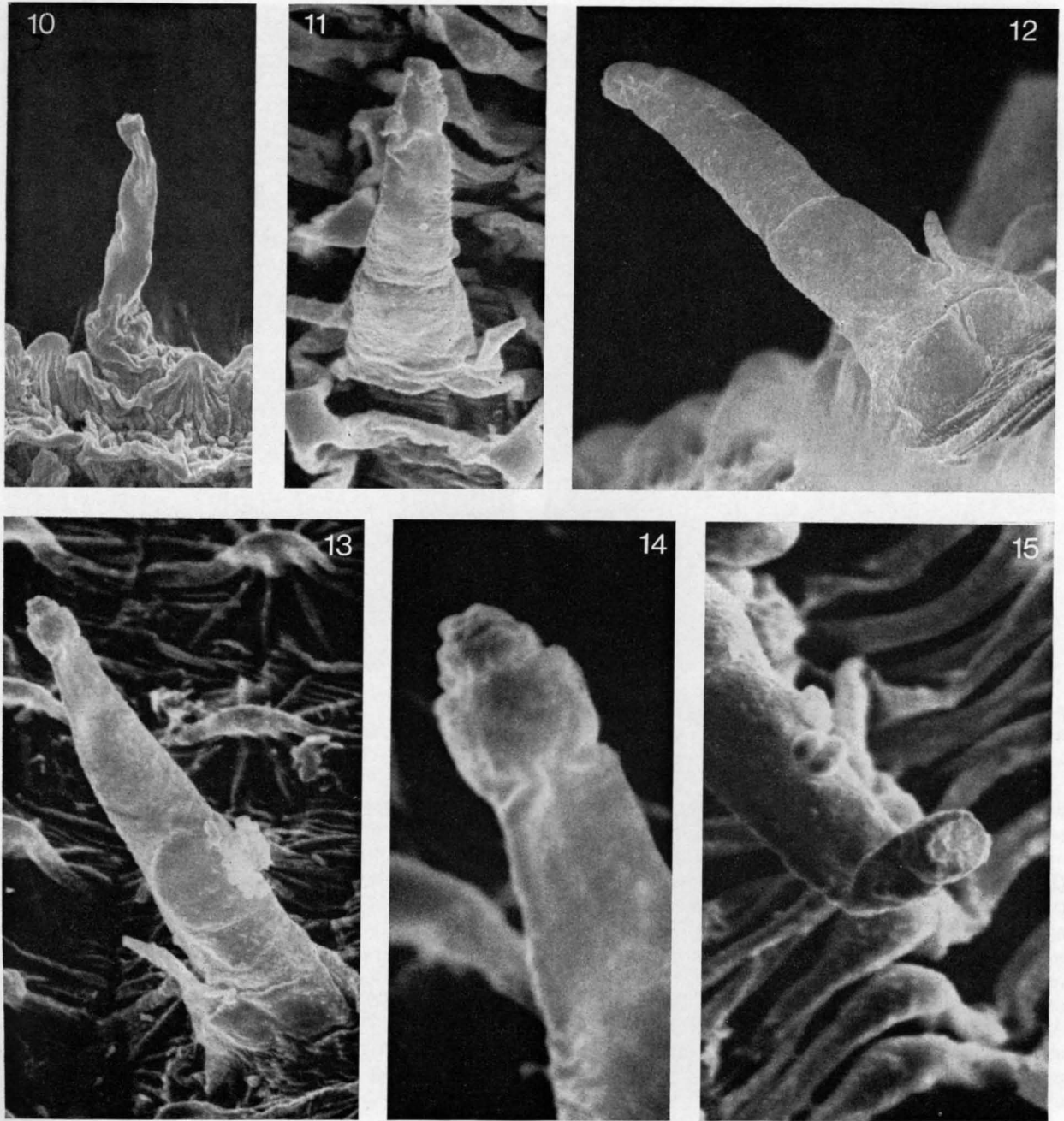


Fig. 3. *Tubiluchus corallicola*. 10: Tubulus with secretion on distal end (2,000 \times); 11 and 12: tubuli with setae in different views (3,150 \times , 3,400 \times , respectively); 13: tubulus with cup-shaped distal end and secretion protruding from it (3,150 \times); 14: same as 13 (10,500 \times); 15: view into cup-shaped distal portion of tubulus (5,600 \times)

and the base wider and less convex, finally leading to flattened, semi-disc-shaped fimbriae (Fig. 6:26) with hairs extremely reduced in length. Each row of 8 or 9 fimbriae terminates posteriorly in an elongate, cuticular plate, on which a transverse band of minute hairs is recognizable (Fig. 5:21).

Except for their smaller size, the secondary combs on the anterior teeth are very similar to the most posterior fimbriae (Figs. 5:21; 6:22, 26). The "hairs" on many of the more anteriorly placed fimbriae (Fig. 5:20) have the same dimensions as the spinules on the main comb of anterior teeth (Fig. 5:18),

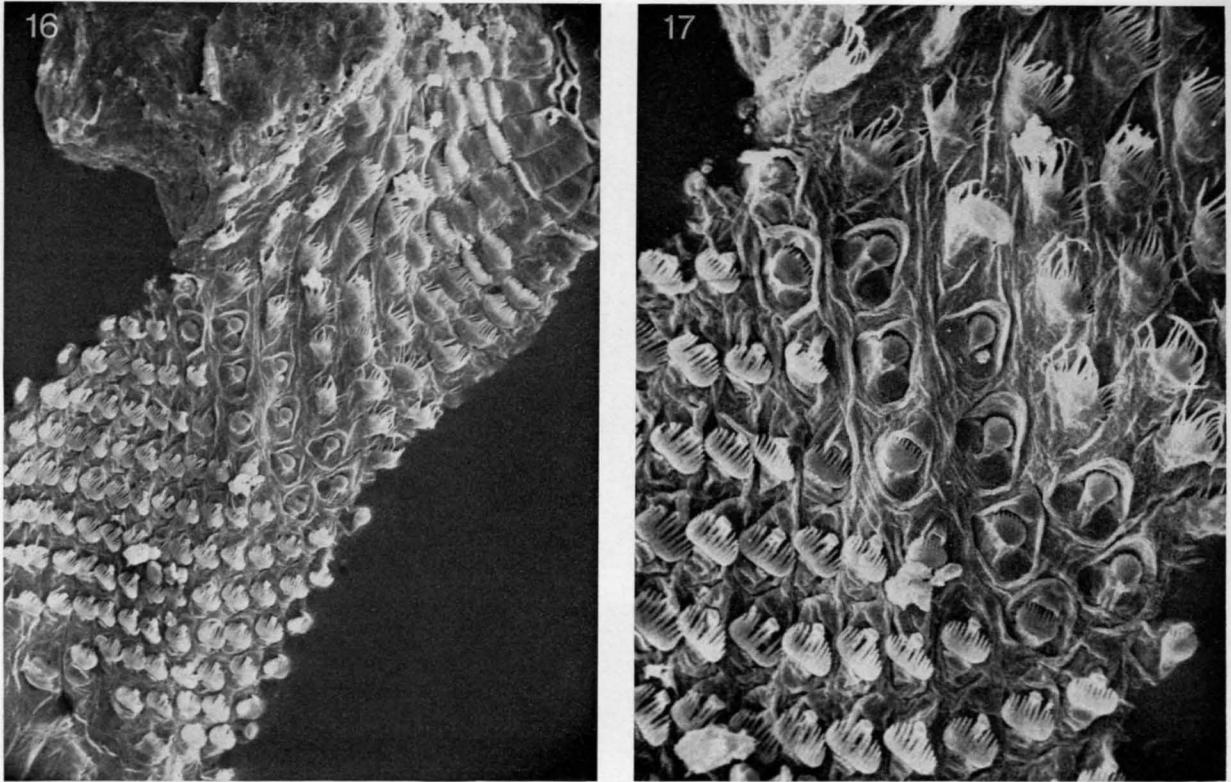


Fig. 4. *Tubiluchus corallicola*. 16: Completely evaginated pharynx (posterior end in upper right corner), showing whole complement of surface differentiations (500 \times); 17: transition of posterior tooth region to anterior fimbrillar region of pharynx (1,000 \times)

whereas the "spinules" on the "main" comb of posterior teeth (Fig. 6:23, 24) are reduced to short projections as they are also found on the posterior fimbrillae (Figs. 5:21; 6:26). A distinct transitional stage between teeth and fimbrillae is not present, yet it is easily conceived by elongating the projections on the secondary comb and reducing the main comb of the teeth, i.e., by continuing the transformation already initiated in the posterior tooth region (Fig. 6:23, 24). As, furthermore, teeth and fimbrillae are also arranged in an identical, uninterrupted pattern over the whole pharynx, it stands to reason that they are homonomous structures.

Life observations in regard to the feeding habit of *Tubiluchus corallicola* have hitherto not been made, but because of the unique pharyngeal armature, van der Land (1970) concluded that it must be different from the predaceous mode prevailing in priapulids with cuspidate teeth. From the two alternatives suggested by van der Land, i.e., detritus feeding or scraping, the latter merits more consideration. The dense and regular arrangement of many uniform, comb-shaped teeth gives the anterior part of the pharynx a rasplike appearance, which by itself is suggestive of a scraping function. Scraping could also account for the

presence of the manubrium, which probably serves as mechanical support of the comb when horizontal stress occurs during backward movement of the tooth along a hard surface (comparable to the rectangularly attached handle on many scraping tools). Food particles obtained by scraping are of microscopic dimensions, and the fringed ridges of the fimbrillae seem well suited to gather such particles and, combined with muscular contractions on the posterior part of the pharynx (compare Figs. 1, 3, 4, in Kirsteuer and van der Land, 1970), they probably also aid in the transport of food toward the polydiridium.

The foregoing interpretation does not, of course, exclude the possibility that detritus is occasionally ingested or that it may constitute the main diet of specimens inhabiting mud bottoms. Detritus feeding, however, can also be accomplished with cuspidate teeth, viz., larvae of *Priapulius caudatus* (Lang, 1939), and it is, therefore, reasonable to assume that the peculiar pharyngeal armature in the only known interstitial species of the phylum is primarily adapted for a new mode of feeding (as far as priapulids are concerned), which most probably is scraping off epigrowth from sand grains (see also Remane, 1952; Wieser, 1953; Boaden, 1964).

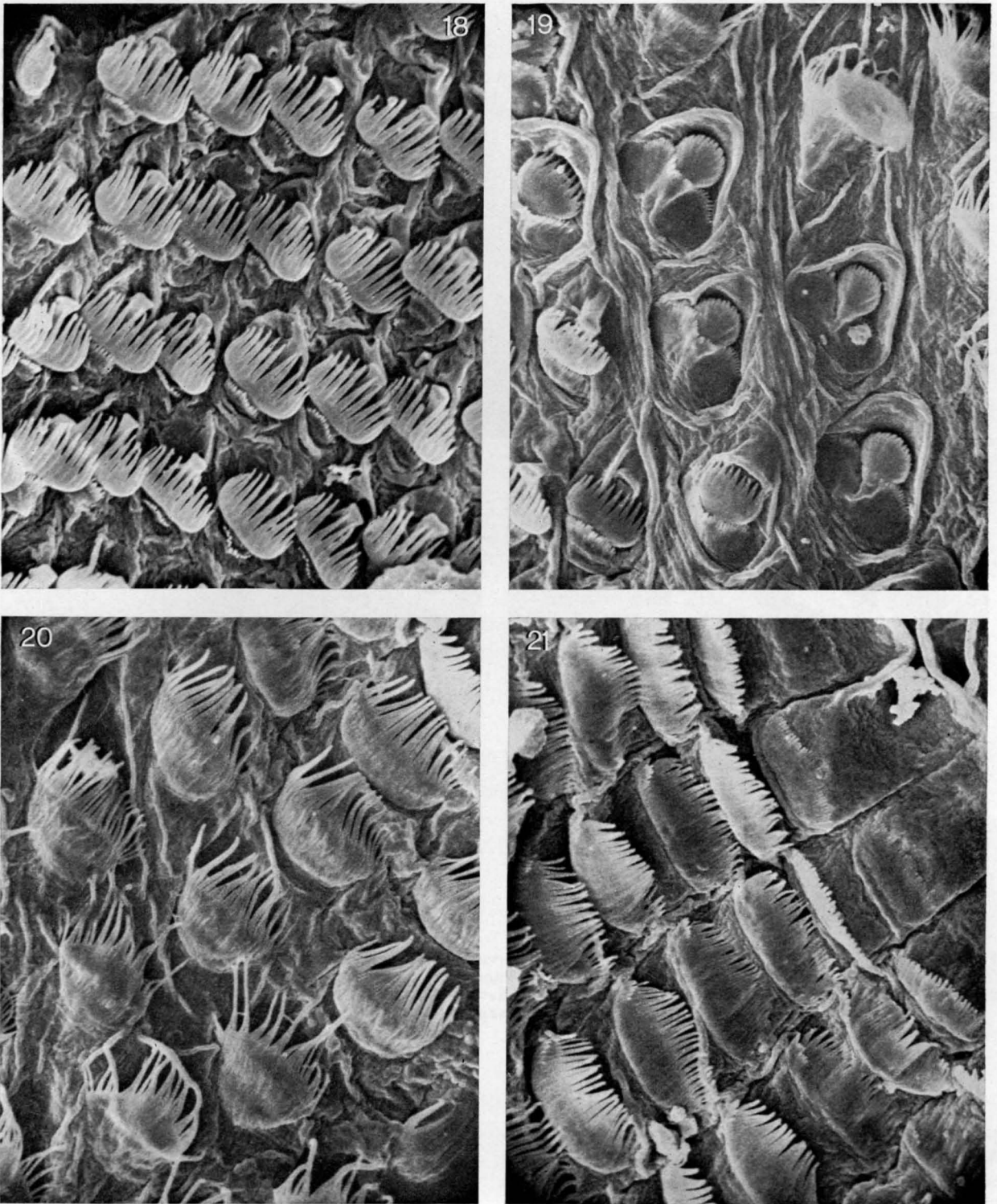


Fig. 5. *Tubiluchus corallicola*. 18: Pectinate teeth in anterior portion of pharynx; 19: pectinate teeth in various stages of transformation in posterior tooth region; 20: anterior fimbrillae with long hairs; 21: rows of posterior fimbrillae terminating in cuticular plates. (All 2,000 \times ; further explanation in text)

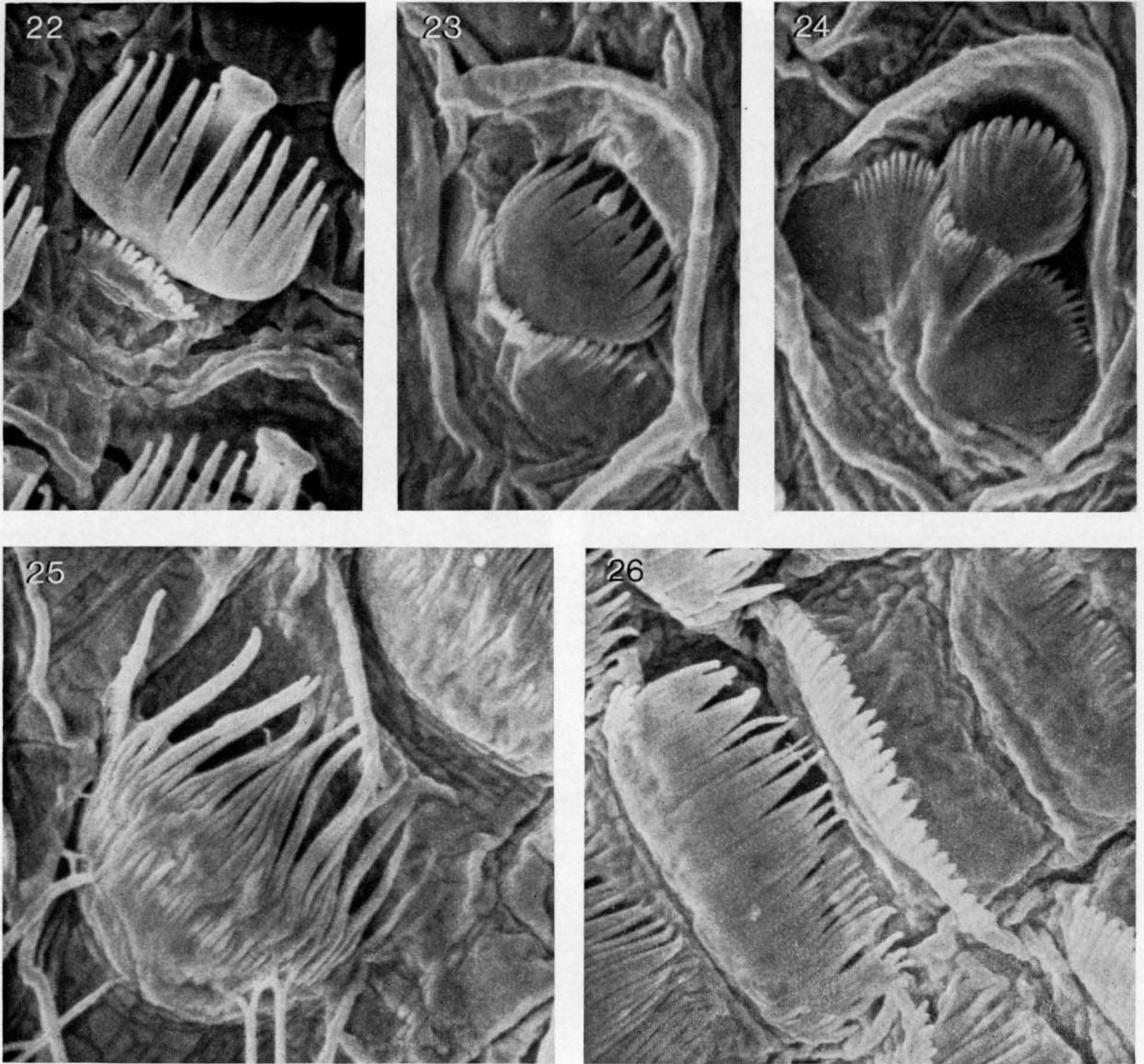


Fig. 6. *Tubiluchus corallicola*. 22: Pectinate tooth with manubrium, long spinules on main comb, and small secondary comb in front of it; 23: posterior pectinate tooth in first stage of change; 24: posterior pectinate tooth in second stage of change; 25: strongly convex, anterior fimbriilla with long hairs; 26: flattened, posterior fimbriillae with hairs reduced in length. (All 5,000 \times ; further explanation in text)

Summary

1. Tumuli, flosculi and tubuli on the abdomen, and pectinate teeth and fimbriillae on the pharynx of *Tubiluchus corallicola* van der Land, the only known interstitial species within the phylum Priapulida, were examined with the scanning electron microscope. Morphological details are described, and the probable function of the structures studied is discussed.

2. Tumuli are flexible surface protuberances, which change from flat cones to prominent ridges with in-

creasing contraction of the animal. The ridges consist of two of the 10 to 13 centrally united, cuticular, supporting rods present in each tumulus. In strongly contracted areas of the abdomen, the ridges are so close together that a protective cover is formed.

3. The calyx of flosculi has only 7 or 8 petals. It is closed on top, except for a central porus. In contracted areas, the calyces are partly retracted into their bases and the flosculi do not extend above the crested tumuli in their vicinity. An adhesive property can thus be excluded, and it is presumed that flosculi are sense

organs which, because of the opening on the calyx, are most likely to serve for chemoreception.

4. Tubuli are stiff tubes, jointed proximally into an elevated base and ending distally in a cup-shaped differentiation. Inside this cup, pores or tiny granules are discernible and, on some tubuli, droplets of secretion are protruding from it. An adhesive function of tubuli is, thus, quite certain.

5. The pharyngeal armature consists of pectinate teeth and of fimbriellae; prickles are definitely lacking. Because of morphological changes on teeth and fimbriellae, resulting in very similar differentiations, and because of the identical pattern of arrangement of teeth and fimbriellae, it is concluded that they are homonomous structures. The functional significance of pectinate teeth with a manubrium and of the fringed fimbriellar ridges is explained as scraping and particle gathering, respectively, in a feeding mechanism adapted for grazing the epigrowth on sand grains.

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