Radular Tooth Structure in Three Species of Terebridae

(Mollusca : Toxoglossa)

BY

PATRICIA M. MILLS

Department of Zoology, University of Queensland, St. Lucia, 4067, Queensland, Australia

(3 Plates; 1 Text figure)

INTRODUCTION

RADULAR DENTITION of the three major toxoglossan families Terebridae, Turridae and Conidae shows a great range of variation. Details of tooth structure are best known in the Conidae for which data exist establishing correlations of radular tooth structure of some *Conus* with prey type (ENDEAN & RUDKIN, 1965; NYBAKKEN, 1970a; FREEMAN & SILVA, 1973), and indicating the possible use of tooth structure in systematics and taxonomy (NYBAKKEN, 1970b). Similar studies on other toxoglossan families could furnish further information of this nature and may contribute to an understanding of evolutionary relationships among Turridae, Terebridae and Conidae, since tooth structure, along with development of the venom apparatus, has been used as evidence of their affinities (RUDMAN, 1969; PONDER, 1973).

In the Conidae, only the marginal teeth remain in the radula (PONDER, 1973), each tooth consisting of a sheet of chitin rolled to form a hollow tube (PEILE, 1939; KOHN, NYBAKKEN & VAN MOL, 1972). This is the instrument for the introduction of venom into the prey (KLINE, 1956; HINEGARDNER, 1958; KOHN, 1956, 1963).

Radular form in the Turridae varies from the prototypic, *i. e.* possessing all tooth types, to a form possessing only marginal teeth as in the Conidae (POWELL, 1964; RUDMAN, 1969), while one genus (*Cenodagreutes*) lacks a radula (SMITH, 1967b). In those turrid species with only marginal teeth, two structural types are found. One type is a rolled hollow structure similar to that of the Conidae (POWELL, 1964; RUDMAN, 1969), while the other type does not form a tube but is deeply grooved (POWELL, op. cit., SMITH, 1967a).

Many terebrid species possess no radula, but there are several species with a radula consisting of marginal teeth only (RUDMAN, 1969; MILLER, 1970, 1971). In the latter species, the radular teeth are similar to those of the Conidae in consisting of a rolled sheet of chitin (RISBEC, 1953; MARCUS & MARCUS, 1960; RUDMAN, op. cit.; MILLER, opera cit.).

Traditionally, elucidation of radular tooth structure has relied on line drawings from light microscope observations (e.g., TROSCHEL, 1866; TRYON, 1885; BERGH, 1896; Peile, 1939; MARCUS & MARCUS, 1960; SMITH, 1967a; SONGDAHL, 1973). Because the teeth are transparent and often complex, the surface relief of teeth is difficult to discern using transmitted light. The scanning electron microscope (hereafter SEM) has been used in studies of various mollusc radulae (e.g., SOLEM, 1972, 1975; FERREIRA & BERTSCH, 1975; MARDINLY & MAR-DINLY, 1975). The advantages of such SEM studies were outlined by SOLEM (1972). Although the SEM has clearly elucidated surface features of some cone radular teeth (KOHN, NYBAKKEN & VAN MOL, 1972; FREEMAN & SILVA, 1973) scanning techniques have not yet been applied to the teeth of toxoglossan genera other than Conus.

This study reports on the structure of the radular teeth of *Terebra subulata* (Linnaeus, 1767), *T. guttata* (Röding, 1798) and *T. succinea* Hinds, 1844 as elucidated by optical microscopy and SEM.

MATERIALS AND METHODS

TERMINOLOGY

Following recent toxinological conventions (RUSSELL & BRODIE, 1974), I have referred to the venom apparatus as the whole structure involved in production and introduction of venom, whereas previous authors (e. g., RUD-MAN, 1969; MILLER, 1970, 1971; PONDER, 1973) have referred to the toxin producing structure as the poison gland.

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Terminology relevant to the structure containing the radular teeth has not been consistently applied to different taxa within the Toxoglossa. HINEGARDNER (1958) referred to the whole structure in *Conus* as the radular sheath. This consisted of 3 parts: the long arm, the short arm, and the ligament sac. ENDEAN & DUCHEMIN (1967) referred also to these 3 parts but termed the entire structure to the radular sac. In the turrids, SMITH (1967a) referred to a radular sac and a radular caecum, the latter being situated near the junction of the radular sac with the buccal sac. MILLER (1970) followed this convention for the Terebridae and I have retained that convention for this discussion, but for brevity, have referred to the whole structure (*i. e.*, radular sac plus radular caecum) as the radular sheath.

PROCEDURES

Specimens of *Terebra subulata*, *T. guttata* and *T. succinea* were collected on the Great Barrier Reef from reefs between 23°50' S and 15°45' S. Data were collected from 8 male and 4 female *T. subulata*, 4 male and 2 female *T. guttata* and 1 male *T. succinea*. The animals were maintained unfed in aquaria for up to 3 weeks before use. The shells were measured, cracked in a vise and the animals removed.

The tips of the shells are often eroded by boring algae and are commonly broken. To estimate the maximum linear dimension of the unbroken shell, the shell was pressed horizontally into a flattened block of plasticine to about half way up the side of the shell (Figure 1a). The shell was removed and the angle and direction of each side of the impression projected beyond the broken tip. The length from the intersection of these lines to the base of the shell I have termed the projected length (Figure 1b). To test this method, intact shells were measured, the tips broken and the projected length determined. Two specimens of *Terebra subulata* and 1 *T. guttata* were tested in this way as these were the only individuals received with intact shells. In the 2 T. subulata, the projected length overestimated real length by 0.3 cm in each case, while in T. guttata the overestimation was 0.5 cm. Since the average discrepancy between broken length and projected length in 24 specimens of T. subulata was 0.9



Plan of the plasticine impression after removal of the shell showing projection of sides to give projected shell length

cm \pm 0.07 (standard error) and 1.4 cm \pm 0.36 in 6 T. guttata, I think projected length gives a reasonable estimation of real shell length for my purposes, although tests using such small sample sizes cannot be expected to give an accurate estimation of the error involved.

For routine light microscope and SEM preparations, the animal was dissected under sea water, the venom apparatus removed and fixed for times ranging from 18 to

Explanation of Figures 2 to 8

Terebra subulata radular tooth

Figure 2: Whole tooth. Phase Contrast

Figure 3: Whole tooth. SEM

- Figure 4: Tip of tooth showing slight swelling. Phase Contrast.
- Figure 5: Tip showing opening of central canal. SEM
- Figure 6: Base of tooth showing concavity to one side and basal rim. Phase Contrast
- Figure 7: Base showing basal swelling and concavity to one side. SEM
- Figure 8: Base showing hook. Phase Contrast
- The measured bars in Figures 2, 3, 4, 6, 8 represent $100 \,\mu\text{m}$ in Figure 5 - $10 \,\mu\text{m}$ and in Figure 7 - $40 \,\mu\text{m}$

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[MILLS] Figures 2 to 8



Figure 2



Figure 4



Figure 6



Figure 8



Figure 3



Figure 5



Figure 7



24 hours in either Susa's fixative or Baker's formol calcium prepared according to CULLING (1963). Specimens fixed in Susa were transferred to 95% alcohol and those fixed in formol calcium were washed overnight in running water before being brought to 70% alcohol. Specimens were stored in 70% alcohol until needed. The radular sheath was subsequently removed from the venom apparatus and the teeth prepared by a number of methods.

1. Following KOHN, NYBAKKEN & VAN MOL (1972), teeth were dissected from the radular sheath, rinsed in 1% sodium hypochlorite followed by 2 changes in distilled water.

2. Using FREEMAN & SILVA'S (1973) method, teeth were dissected out, rinsed in 0.9% saline and soaked overnight in distilled water.

3. The radular sheath was placed in concentrated NaOH (10g NaOH in 10 ml distilled water) as used by SONGDAHL (1973) until the teeth were freed from other tissue (*i. e.*, 24 - 30 hours for *Terebra subulata*). The teeth were removed to distilled water.

4. The sheath was placed in 1% sodium hypochlorite and observed until the teeth were freed of surrounding tissue. The time taken varied with the species, being approximately $\frac{1}{2} - 1\frac{1}{2}$ hours for *T. subulata*, $1 - 2\frac{1}{2}$ hours for *T. guttata*, and $\frac{1}{2} - \frac{3}{4}$ hours for *T. succinea*. Teeth were rinsed in 2 changes of distilled water.

Following the method devised by D. C. McColm (personal communication) for mounting *Conus* teeth for light microscopy, the teeth were transferred from distilled water and mounted in Womersley's Mounting Medium. The length and width of the teeth were measured using an eyepiece micrometer. I considered length as the maximum distance in a straight line from tip to base and made no allowance for curvature of the tooth. I considered width as the maximum width at the basal rim.

For electron microscopy, teeth were dehydrated by transfer through a graded series of ethyl alcohols to absolute alcohol. One of 2 methods was then followed.

1. Teeth were affixed to a $1\frac{1}{4}$ cm diameter coverslip by an adhesive removed from adhesive tape with chloroform and painted onto the coverslip as suggested by the Electron Microscope Unit of the University of Queensland.

2. Teeth were transferred through amyl acetate and dried onto the coverslip in a critical point drier. This

technique was carried out by the Electron Microscope Unit of the University of Queensland.

Following either of these treatments, teeth were coated under vacuum with aluminum and examined and photographed with either a Cambridge Stereoscan 2A (*Tereb*ra subulata and *T. guttata*) or a Cambridge Stereoscan 600 (*T. succinea*).

In one specimen each of *Terebra subulata* and *T. guttata*, the radular sheath was dissected unfixed and dyed with 0.001% aqueous methylene blue, and another *T. subulata* radular sheath was dissected and dyed with 0.5%aqueous acid fuchsin, to try to detect a ligament from the tooth base to the sheath wall. The latter dye was one used by FREEMAN & SILVA (1973), who reported that the ligaments of 2 *Conus* species colored feebly with the dye but that the teeth colored strongly.

RESULTS

GENERAL OBSERVATIONS

In all 3 species, the teeth are secreted in 2 rows with the bases of the teeth lying along the posterior margin of the radular sheath. Early stages in tooth development were commonly found in the radular sac while only mature teeth were obtained from the radular caecum. Two specimens of *Terebra subulata* were found with no teeth in the radular sac, but this was probably a dissection artifact.

MARCUS & MARCUS (1960) reported a ligament attaching the base of the tooth to the wall of the radular sheath in the terebrid *Hastula cinerea* (Born, 1780) and similar structures are known in Conidae (BERGH, 1896; HINE-GARDNER, 1958; ENDEAN & DUCHEMIN, 1967; SONG-DAHL, 1973). Such a ligament was not visible in dissection of the radular sheath of *Terebra subulata* or *T. guttata* and was not detected by staining with either methylene blue or acid fuchsin. Paraffin sections of the whole radular sheath of all 3 species have revealed some connective tissue elements around the teeth (unpublished data), but no discrete ligament to each tooth has been detected.

TOOTH SIZE AND NUMBER

Methods 2 and 4 (see 'Materials and Methods') were used for light microscope preparations from which tooth measurements were made. Method 4 without subsequent critical point drying was used for the material presented

TADIC 1									
Species	Shell Length (cm)	Total no. of teeth	Tooth length				Tooth width		
			Range (mm)	Mean (mm) ± S.E.	No. measured	Tooth length Shell length	Range (mm)	Mean (mm) ± S.E.	No. measured
Terebra	6.5	16	0.52 - 0.56	0.55 ± 0.012	14	1:118	0.06-0.08	0.07 ± 0.005	14
subulata	12.0	24	0.77 - 0.88	0.80 ± 0.005	19	1:150	0.10 - 0.12	0.11 ± 0.007	19
	12.2	19	0.62 - 0.66	0.65 ± 0.003	16	1:188	0.08 - 0.11	0.10 ± 0.003	18
	12.6	19	0.71 - 0.74	0.74 ± 0.004	17	1:170	0.07-0.11	0.10 ± 0.003	17
	12.7	20	0.80 - 0.84	0.83 ± 0.003	18	1:153	0.10 - 0.11	0.10 ± 0.001	19
	13.9	20	0.64 - 0.78	0.75 ± 0.013	10	1:185	0.08 - 0.12	0.10 ± 0.002	10
	13.9	20	0.66 - 0.71	0.69 ± 0.004	16	1:202	0.08 - 0.11	0.09 ± 0.003	19
	16.1	30	0.70 - 0.77	0.75 ± 0.003	27	1:215	0.10 - 0.13	0.11 ± 0.002	26
Terebra	11.2	21	1.63 - 1.70	1.67 ± 0.006	14	1:67	0.15 - 0.19	0.17 ± 0.012	16
guttata	13.1	22	1.22 - 1.30	1.26 ± 0.012	8	1:104	0.11 - 0.19	0.15 ± 0.008	8
	15.1	21	1.26 - 1.31	1.29 ± 0.006	10	1:117	0.17 - 0.20	0.19 ± 0.002	10
	16.1	21	1.44 - 1.65	1.56 ± 0.016	15	1:103	0.19 - 0.25	0.22 ± 0.006	15
	17.1	20	1.30 - 1.48	1.41 ± 0.021	11	1:121	0.15 - 0.22	0.19 ± 0.005	14
	19.0	23	1.96 - 2.07	2.03 ± 0.008	20	1:94	0.22 - 0.33	0.31 ± 0.001	20
Terebra succinea	9.6	11	-	0.28	1	1:343	-	0.07	1

Table 1

in the electron micrographs. Details of the number of teeth and their dimensions are presented in Table 1. Owing to the small size and delicate nature of the teeth, inevitably some were damaged in preparation so that not every tooth furnished a measure of each dimension.

TOOTH STRUCTURE

In all 3 species each tooth consists of a sheet of chitin rolled to form a hollow, slightly curved tube that tapers to a point. The so-called "bridges" (MARCUS & MARCUS, 1960: 39) observed in *Hastula cinerea* were not observed in any of the teeth examined. Details of tooth structure of each species will be described separately.

Terebra subulata

The tooth of this species is simple in having no blades, barbs or serrations. There is a slight swelling near the tip (Figure 4) in some preparations, but it is likely that this is an artifact of unrolling of the chitin sheet during preparation. The opening of the central tube is slightly proximal to the pointed tip of the tooth (Figure 5). The shaft is simple and smooth (Figures 2 and 3) with a slight swelling towards the base (Figures 3 and 7). Immediately basal to this swelling the shaft is twisted and concave on one side (Figures 6 and 7). This concavity appears in teeth prepared using all methods and I therefore believe it to be real rather than artifact. Basally, the tooth ter-

Explanation of Figures 9 to 15

Terebra guttata radular tooth

Figure 9: Whole tooth. Optical microscopeFigure 10: Whole tooth (shows unrolling of chitin sheet). SEMFigure 11: Upper half of tooth showing tip with barb and sloping blade with barb. Phase Contrast

Figure 12: As for Figure 11, but using SEM (shows unrolling of chitin sheet)
Figure 13: Tip showing opening and barb. SEM

Figure 14: Base of tooth showing rim and hook. Phase Contrast

Figure 15: Base showing distinct opening on one side, basal rim and hook, SEM

The measured bars in Figures 11, 12, 14, 15 represent $100 \,\mu\text{m}$ in Figures 9 - $500 \,\mu\text{m}$; 10 - $200 \,\mu\text{m}$; and 13 - $10 \,\mu\text{m}$ The Veliger, Vol. 19, No. 3

[MILLS] Figures 9 to 15



Figure 9



Figure 11



Figure 13



Figure 14



Figure 10



Figure 12



Figure 15



Mills, P M. 1977. "RADULAR TOOTH STRUCTURE IN 3 SPECIES OF TEREBRIDAE MOLLUSCA TOXOGLOSSA." *The veliger* 19, 259–265.

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