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A STUDY OF THE EPIDERMAL STRUCTURES OF THE MIRACIDIA OF *CALICOPHORON CALICOPHORUM* (FISCHOEDER, 1901) NÄSMARK, 1937 AND *PARAMPHISTOMUM MICROBOTHRIUM* (FISCHOEDER, 1901)

P. J. SWART, Veterinary Research Institute, Onderstepoort

Introduction

The terebratorium, i.e. apex of the miracidia of various trematodes, has been described by various authors but no detailed analysis of its papilla-like structures has been attempted (Lynch, 1933; Dutt & Srivastava, 1961; Dönges, 1964). A comparative study of the terebratorium of *Calicophoron calicophorum* and of *Paramphistomum microbothrium* will be made and reasons given for its use as a valid criterion for morphological differentiation.

Materials and Methods

The silver impregnation technique of Lynch (1933) was modified in that miracidia were centrifuged during each stage and mounted in canada balsam. The advantage of centrifuging is that there is a break-down of some of the miracidia which facilitates *en face* examination; break-down by pressure on the cover slip proved inferior to centrifuging.

Description of Miracidium

The miracidia of *C. calicophorum* and of *P. microbothrium* are alike in that each has papilla-like structures on its terebratorium; each shows twenty epidermal cells arranged to the same formula of 6: 8: 4: 2 and of the same approximate proportions. The nature of the papilla-like structures is unknown, whether sense organs or openings of glands; for convenience they will be referred to as " papillae ".

I. Papillae situated on the terebratorium

The papillae are arranged in a bilaterally symmetrical fashion; to facilitate comparison and enumeration, arbitrary lines independent of dorso-ventral orientation have been drawn across " more or less " related groups of papillae, which groups are designated as Group 1, 2, 3 and 4 respectively. Along each line, aggregations of papillae are identified alphabetically (Fig. 1).

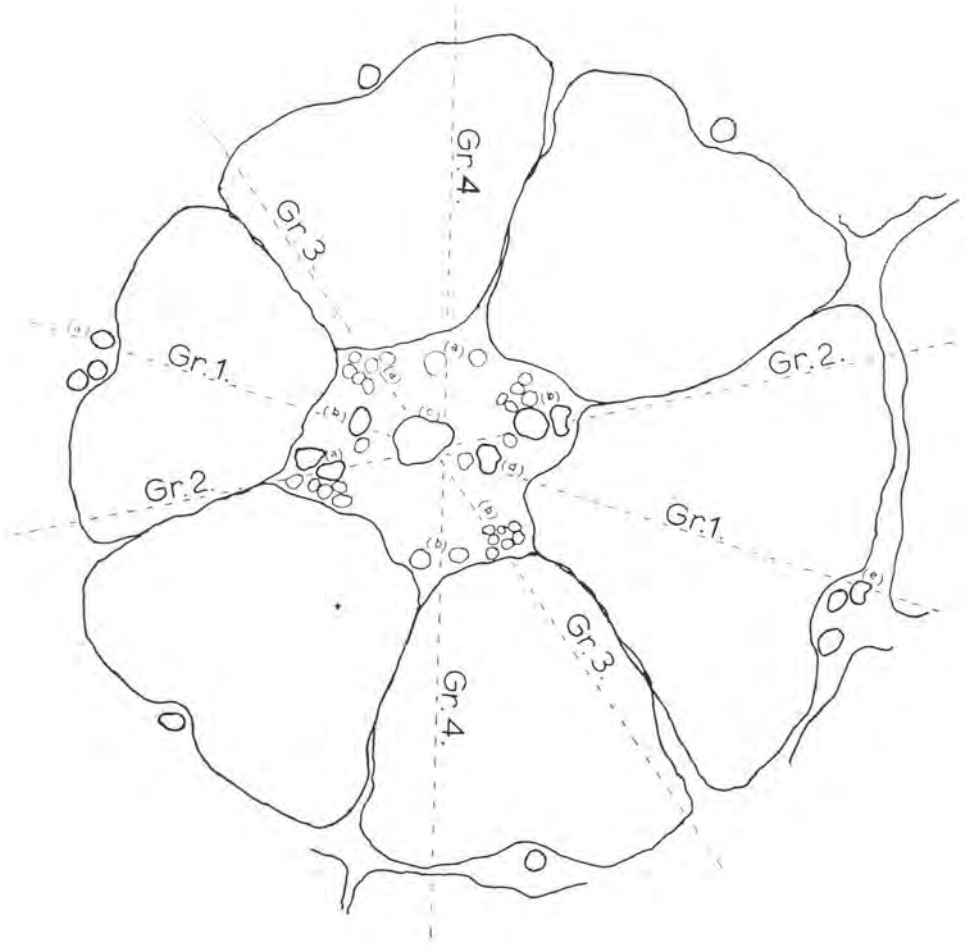

FIG. 1.—The terebratorium and tier No. I of epidermal cells of *C. calicophorum*, with arbitrary lines drawn through the groups of "papillae"

In Tables 1 and 2 a comparison is made between the numbers of papillae found in each aggregation in each of the four groups. Aggregations (*a*) and (*e*) of Group 1 are excluded because they are situated not on the terebratorium but between epidermal cell tiers No. I and II.

TABLE 1.—*The numbers and arrangement of papillae on the terebratorium of ten* C. calicophorum *Miracidia*

No.	Group 1			Group 2		Group 3		Group 4		Total
	b	c	d	a	b	a	b	a	b	
1.....	2	1	2	8	8	6	7	3	3	40
2.....	2	1	2	8	8	6	7	4	4	42
3.....	2	1	2	8	9	6	7	2	4	41
4.....	2	1	2	9	8	6	7	3	3	41
5.....	2	1	2	8	8	6	7	3	4	41
6.....	2	1	2	8	8	7	7	3	2	40
7.....	2	1	2	9	8	6	6	3	2	39
8.....	2	1	2	9	8	6	8	3	3	42
9.....	2	1	2	7	8	7	6	4	4	41
10.....	2	1	2	7	8	6	7	3	3	39

TABLE 2.—*The numbers and arrangement of papillae on the terebratorium of ten* P. microbothrium *Miracidia*

No.	Group 1			Group 2		Group 3		Group 4		Total
	b	c	d	a	b	a	b	a	b	
1.....	2	1	2	7	7	6	6	—	—	31
2.....	2	1	2	7	5	6	6	—	—	29
3.....	2	1	2	9	8	6	6	—	—	34
4.....	2	1	2	7	7	6	6	—	—	31
5.....	2	1	2	7	7	6	5	—	—	30
6.....	2	1	2	8	7	6	6	—	—	32
7.....	2	1	2	7	8	7	6	—	—	33
8.....	2	1	2	7	8	6	7	—	—	33
9.....	2	1	2	7	7	6	6	—	—	31
10.....	2	1	2	9	8	6	6	—	—	34

The number of papillae per aggregation varies considerably, and the number per aggregation is hence of no value as a characteristic for differentiation. However, in *C. calicophorum*, the total number of papillae varies from 39 to 42 whereas in *P. microbothrium* it varies from 29 to 34 (Tables 1 & 2). This means that the former has at least 5 and can have up to 13 more than the latter.

The most significant morphological difference, however, lies in aggregations (*a*) and (*b*) of papillae in Group 4, which are present in *C. calicophorum* (Fig. 1 & 2 and Table 1) but absent in *P. microbothrium* (Fig. 3; and Table 2). This difference between the species is so marked that it is obvious even in either a dorsal or a ventral view. This obviates the necessity of preparing *en face* mounts of the terebratorium.

A further point of difference between the species is that the larger of the two papillae found in aggregations (*b*) and (*d*) of Group 1, is larger than (*c*) of Group 1 in *P. microbothrium* (Fig. 3) and smaller in the case of *C. calicophorum* (Fig. 2).

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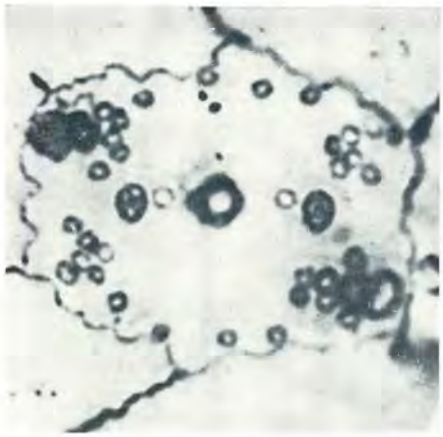

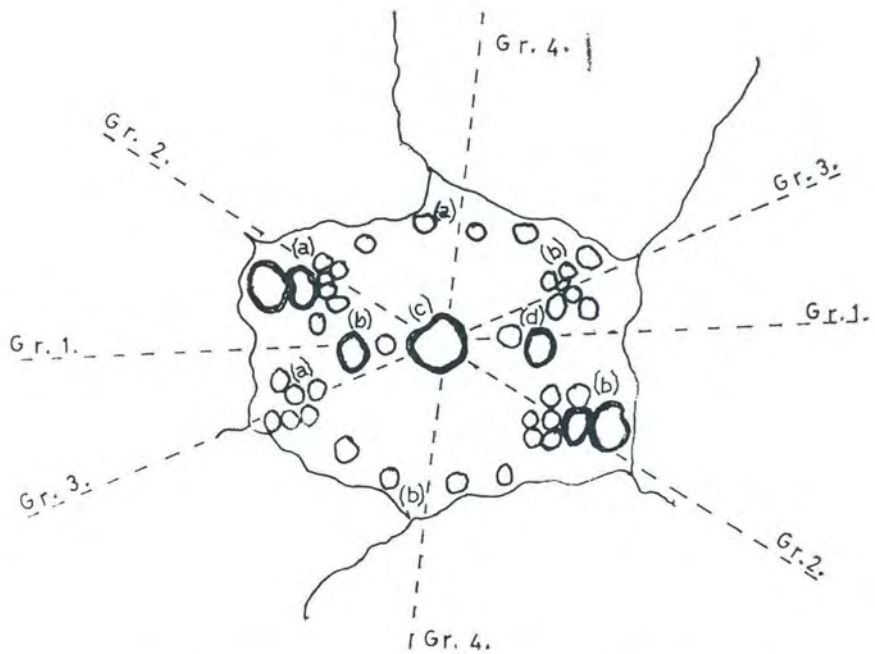

FIG. 2.—A micro-photo of the terebratorium of *C. calicophorum*

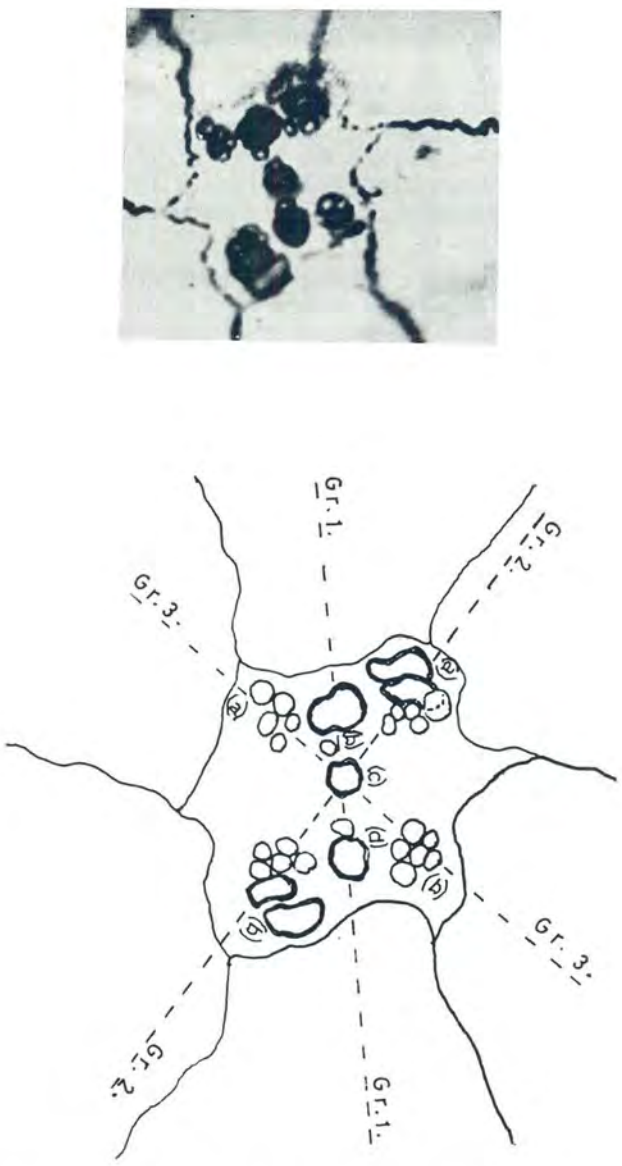

FIG. 3.—A micro-photo of the terebratorium of *P. microbothrium*

II. Papillae not situated on the terebratorium

The following description is included since the author could find no reference in the literature to papillae situated in the inter-cellular spaces of the epidermal cells on the rest of the body of miracidia of the Paramphistomidae. No morphological differences, however, could be found between the two species of miracidia examined.

Anteriorly there are ten papillae situated between the first and second tier of epidermal cells, arranged as follows: Three papillae are grouped together in a small indentation at the base of each of the two lateral epidermal cells, i.e. Group 1 (*a*) and (*e*) (Fig. 1). A single papilla at the base of each of the two dorsal and of the two ventral cells completes the number of papillae situated between the first and second tiers of epidermal cells (Fig. 1).

These papillae agree with the " *anterior papillae* " of Dutt & Srivastava (1961). The extra two papillae of (*a*) and (*e*) giving a total of ten instead of the six for the " *anterior papillae* ", would agree with their four "*lateral papillae* ". In the author's opinion these four " *lateral papillae* " in the instance of the two Paramphistomidae miracidia examined, belong to the " *anterior papillae* ", because they are situated in the indentations at the base of the two relevant epidermal cells of the first tier. In the Schistosomatidae, however, the " *lateral papillae* " are distributed at random according to the illustrations of Dutt & Srivastava (1961).

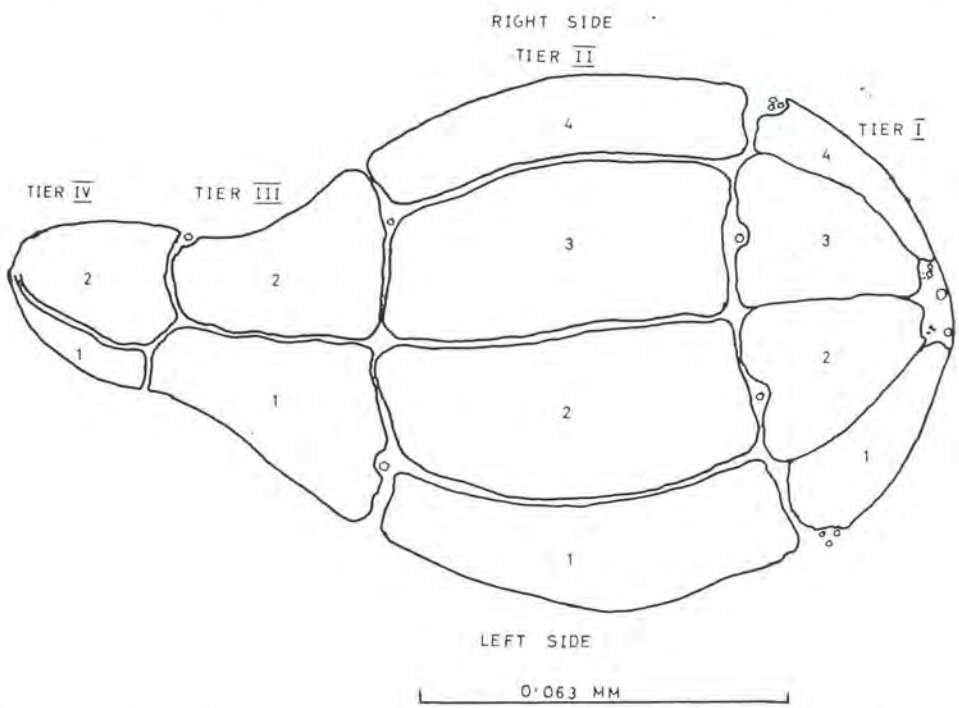

FIG. 4.—The ventral surface of the miracidium of *P. microbothrium* showing the two ventral papillae between tiers II and III of epidermal cells

A further two papillae were found between tiers II and III of epidermal cells (Fig. 4). In relation to the two flame cells and the excretory pores, these two papillae are situated on the ventral surface of the miracidia. They are usually situated in line with the spaces between epidermal cells No. 1 & 2 and 3 & 4 of the second tier. These two papillae are in all probability synonymous with the "*papillae of unknown nature*" of Dutt & Srivastava (1961), and the "*Ventralpapillen*" of Dönges (1964).

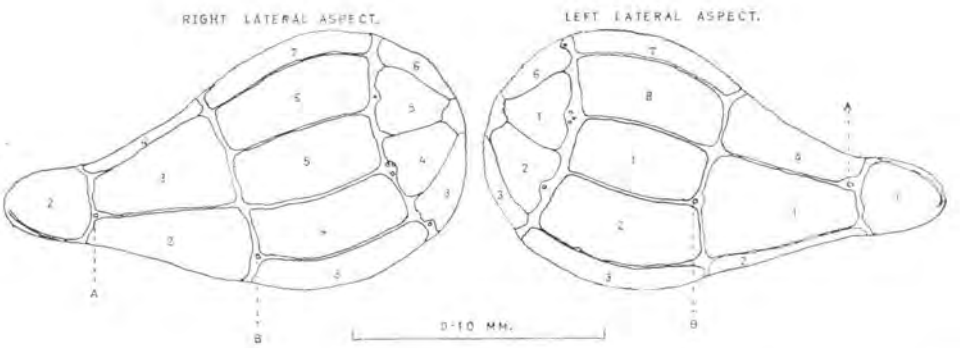

FIG. 5.—The lateral aspects of the miracidium of *P. microbothrium*. A = excretory pore, B = ventral papillae

As eye-spots are absent the orientation of the miracidia is difficult. In the previous paragraph reasons are given for assigning the two papillae between tiers II and III of epidermal cells, to the ventral surface. During the study of the miracidia it became apparent that excellent lines exist which divide the miracidium on a frontal plane. This line runs along the dotted line indicated as Group 1 in Fig. 1; it is continued via aggregations (*a*) and (*e*) of Group 1, at the lateral aspects of the miracidia as more-or-less straight lines indicated by the inter-cellular spaces between the epidermal cells of tiers II and III (Fig. 5). These lines end at the excretory pores (Fig. 5) situated between tiers III and IV (Fig. 4) of epidermal cells. The numbering of epidermal cells of tiers II and III was started at the lower epidermal cells next to the lateral line. Thus, on the left lateral aspect of a miracidium the lateral line will be between epidermal cells No. 8 and 1 of the second tier (Fig. 5). The counting was done in an anti-clockwise direction, thus the lateral line on the right side lies between epidermal cells No. 4 and 5 of the second tier (Fig. 5). In the third tier of epidermal cells the lateral line is situated between epidermal cells No. 4 and 1 on the left-hand side and between 2 and 3 on the right-hand side (Fig. 5). It will be apparent from Fig. 1 that the two epidermal cells of the first tier situated anterior to aggregations (*a*) and (*e*) of Group 1 consisting of three papillae each, should be indicated as No. 1 at the left and No. 4 at the right-hand side.

Discussion

It was found impossible to compare the groups of terebratorium—papillae of the miracidia of the two paramphistomes under discussion—with those of other species even where drawings are available, in that the aggregations have never been systematically described or represented. Miracidia of the two species can be differentiated as follows:—

1. The presence of aggregations (*a*) and (*b*) of Group 4 in *C. calicophorum* and their absence in *P. microbothrium.*

2. The total number of papillae (39 to 42) on the terebratorium of the miracidium of *C. calicophorum*, always exceeds that (29 to 34) of *P. microbothrium* by at least five.

3. In *C. calicophorum* the two larger papillae in aggregations (*b*) and (*d*) (Group 1), are smaller than the central papilla (*c*); in *P. microbothrium* Group 1 (*b*) and (*d*) are larger than the central (*c*).

Summary

A detailed study of the number and arrangement of papilla-like structures on the terebratorium was carried out; it is found that these can be used to differentiate between the miracidia of the two related species of Paramphistomidae, *C. calicophorum* and *P. microbothrium.*

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