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STUDIES ON ECHINOCOCCOSIS

III. ON EXPERIMENTAL INFECTION IN DOGS, ESPECIALLY ON THE DEVELOPMENT OF *ECHINOCOCCUS GRANULOSUS* (BATSCH, 1786)

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Cestodes of the genus *Echinococcus* have been much studied in the past. At present 8 species of *Echinococcus* are considered valid: *E. granulosus* (BATSCH, 1786); *E. oligarthrus* (DIESING, 1863); *E. longimanubris* CAMERON, 1926; *E. minimus* CAMERON, 1926; *E. cameroni* ORTLEPP, 1934; *E. lycaontis* ORTLEPP, 1934; *E. felidis* ORTLEPP, 1937; *E. sibiricensis* RAUSCH et SCHILLER, 1954. However the present authors have been somewhat doubtful of the identification of these species. Recently RAUSCH (1953) has pointed out that of these species *E. minimus*, *E. longimanubris* and *E. cameroni* are synonyms of *E. granulosus*. There is need for an evaluation of morphological characters to be used as the basis for species differentiation and for obtaining further knowledge of morphological changes in the development of these tapeworms, especially *E. granulosus*.

For the purposes of such work the most important material has been obtained through the experimental infection of dogs, as in such experimental infection the duration and the actual age of the cestodes can be controlled.

MATERIAL AND METHODS

On October 25th, 1954, a large hydatid cyst was found in the liver of a Corriedale ewe which was born in 1951 and imported from Australia to Hokkaido in April, 1953, as described in the authors' first report.[5)]

On the next day numerous scolices (so-called hydatid sands) from the fluid of this cyst were collected by using a centrifugal machine, and they were washed 3 times repeatedly with physiological saline solution. About 1000 individuals were administered to 5 dogs per os respectively.

The size of scolices used as the material to produce infection are shown in table 1.

These 5 dogs were dissected on the 16th, 17th, 35th, 135th and 375th day after infection respectively, and pathological observations of the intestinal wall were carried out. The cestodes were removed from the intestine immediately after the death of the dog and were allowed to relax in the physiological saline solution. They were pressed between

TABLE 1. *Size of Scolex in Hydatid Cyst* (μ)

NO.	SCOLEX	INVAGINATED PART
1	140×108	100×76
2	156×116	120×92
3	148×116	116×92
4	140×108	100×80
5	148×108	116×80
Average	146.4×111.2	110.4×84.0

two slide glasses and fixed in 70% alcohol for 4 or 5 days. Then they were stained with borax carmine and DELAFIELD's hematoxylin and permanently mounted. Accurate observations on rostellar hooks were made by applying adequate pressure and by mounting detached scolices.

The authors examined the feces of the dogs by means of YAOITA's antiformin ether method, a month after the infection, in order to discover the first occurrence of eggs in dog feces after infection.

The feces of dog No. 2 were examined for a long period of time to ascertain the fluctuation of the occurrence of eggs.

RESULTS

1. The Cestodes in Each Stage of Infection

On the 16th and 17th day after infection Two dogs, No. 3 and No. 5, died on the 16th and 17th day after infection respectively. Numerous small young cestodes were found in the duodenum and anterior part of the jejunum extending over about 60 cm. The intestine showed acute catarrh. The most of cestodes were buried in the mucus. They could be easily removed from the intestinal wall. The cestode of this stage consists of a scolex and 2 proglottids. However, in a few specimens which were younger the borderline between the 1st and 2nd proglottid was not clear.

The development of the sexual organ of cestode is not distinct but traceable. The rostellar hooks are 32 to 40 in number, and large hooks and small ones are alternately arranged in 2 rows. Their shape is similar in appearance to that of the scolices within hydatid cyst as shown in fig. 6. The suckers are also similar to those of adult worms, excepting only the difference of size. The appearance of the excretory canal is clear as in adult worm.

On the 35th day after infection Dog No. 4 was killed and dissected. Many cestodes were found in the duodenum and the anterior part of jejunum extending over about 30 cm. Catarrh was found in the intestine. The strobila consists of a scolex and 3 proglottids as that of adult worm. The last proglottid has grown larger and it shows the typical form of *Echinococcus* tapeworm. However, the development of the egg is not completed. The uterus has fairly well developed, and it harbors many immatured eggs.

The characteristic chitinous shell or embryophore of egg is not visible. The oncosphere is not completed.

The present authors have found only a specimen in which, although the number of proglottids is the same as in the other specimens, the development of the sexual system of its last or 3rd proglottid is the same as in the 2nd proglottid of the other specimens. The arrangement and number of the rostellar hooks and suckers are not different from those of the other stages. The hooks are larger and stronger, especially their posterior part developing well; the posterior edge of some hooks has one shallow indentation. The size of hook is twice or thrice as great as that of specimens on the 16th day after infection.

On the 135th day after infection On the 10th of March, 1955, dog No. 1 was killed and dissected. Many cestodes were found in the intestine, extending over about 60 cm in the duodenum and anterior part of jejunum. It is easy to remove them from the intestinal wall. About 100 gravid proglottids among these cestodes were used for experimental infection of sheep. The last proglottid showed the gravid structure, and the 2nd proglottid showed the mature type. That is to say, these cestodes showed the typical forms of the adult worms of genus *Echinococcus*. The number of testes ranges from 45 to 65 (about 56 in average), and the testes are about as numerous anterior to the genital pore as posterior to it. Rostellar hooks are 32 to 40 (32 is common) in number, being somewhat variable in shape. Gravid uterus is sac-like or showing lateral sacculations. Eggs are highly variable in size. The size of the embryophore is from 29.6 to 44 μ by 27 to 42.5 μ (36.5 by 32.2 μ in average) in the specimens fixed with 70% alcohol or 10% formalin and stained with DELAFIELD's hematoxylin. Its thickness is 3.15 to 4.81 μ (3.48 μ in average). The size of the oncosphere is 28 to 37.2 μ by 24 to 29.2 μ (31.5 by 26.5 μ in average). Its hooklets are 6 in number and 11.5 to 13.2 μ (11.9 μ in average) in size.

On the 375th day after infection Dog No. 2 died and was dissected on the 5th of November, 1955. About 800 individual cestodes were found in the duodenum and anterior part of jejunum. The strobila has become larger being about twice the size of the specimen on the 135th day after infection. The appearance of the sexual organ is the same as in the above. Gravid uterus shows sac-like form having lateral sacculations or lateral branches.

The number of lateral sacculations on the gravid uterus of *Echinococcus* spp. has been accorded value as a specific character by some workers. On the basis of the present results, however, the authors have come to the conclusion that the forms of gravid uterus cannot be relied upon as a specific character in this genus. This is in agreement with the opinion of RAUSCH (1953).

2. The Occurrence of Eggs in Dog Feces

By the fecal examination of 2 dogs, Nos. 1 and 2, the eggs of cestodes were first discovered on the 48th and 61st day after infection respectively. The true shell of the egg had been dissolved off in the intestine of the dog. It consists of a thick chitinous shell (so-called embryophore), with radial striations, in which occur six hooklets characteristic of all cestodes as its name implies. The dimensions of eggs within the uterus of the fixed specimens are as noted above: however there are found many eggs belonging to the

group of comparatively larger dimensions.

It is well known that this species of cestode belongs to the Taenia type. So in this species it has been considered an implied fundamental rule that the eggs are excreted in the removed gravid proglottid. The eggs are found in the feces only by destruction of proglottids under mechanical impetus in the host-intestine or after having been excreted with the feces.

As above noted, in the uterus of the specimens on the 35th day after infection the eggs showed immature forms. Accordingly it is supposed that the completion of eggs may be observed after 40 to 50 days of infection in dogs.

In successive fecal examinations of the infected dogs, Nos. 1 and 2, the authors have found that there is a period which interrupts the occurrence of eggs in dog feces. The period covers about 1 or 1.5 months. Perhaps the period lasts from the first removement to the next one of a gravid proglottid.

3. Comparative Dimensions of the Developing Cestodes

Length of strobila The length of strobilae are 0.72 to 1.20 mm (0.98 mm in average), 2 to 3.35 mm (2.76 mm in average), 3.20 to 4 mm (3.69 mm in average) and 6.40 to 9.20 mm (7.76 mm in average) on the 16th, 35th, 135th and 375th day after infection respectively. In other words these dimensions are in the proportion of 1, 3, 4 and 8. The last proglottid swells, but it is not long. In the specimens on the 35th, 135th and 375th day, the last proglottid is the longest and corresponds to about a half of the whole strobila, while on the 16th day the scolex is generally longer than the other proglottids.

TABLE 2. *Length of Strobila of Young and Adult Worm (mm)*

NO.	16 DAYS AFTER INFECTION	35 DAYS AFTER INFECTION	135 DAYS AFTER INFECTION	375 DAYS AFTER INFECTION
1	0.72	3.20	3.60	6.80
2	1.04	2.00	3.40	7.60
3	1.12	2.80	3.60	8.80
4	1.16	2.40	3.20	9.20
5	0.88	2.80	3.60	6.80
6	1.04	3.35	3.80	6.40
7	1.20	3.30	4.00	6.80
8	0.88	2.40	3.80	8.90
9	0.76	2.75	3.90	8.80
10	1.00	2.60	4.00	7.50
Average	0.98	2.76	3.69	7.76

Size of rostellum The diameters of rostella are 68 to 88 μ (76.8 μ in average), 96 to 116 μ (108.4 μ in average), 100 to 120 μ (112 μ in average) and 152 to 180 μ (168.4 μ in average) at each stage respectively; namely they are in proportion of 1, 1.4, 1.5 and 2. The rostellum of the scolex or hydatid sand within the cyst collected from sheep-liver is

52 to 64 μ (56 μ in average), namely 5/7 of the diameter of the rostellum of the 16th day specimens from dog.

TABLE 3. *Diameter of Rostellum* (μ)

NO.	HYDATID SAND FROM SHEEP	YOUNG AND ADULT WORM FROM DOG INFECTED EXPERIMENTALLY			
		16 Days after Infection	35 Days after Infection	135 Days after Infection	375 Days after Infection
1	52	72	96	100	180
2	56	68	108	112	180
3	56	76	116	120	152
4	52	80	110	120	168
5	64	88	112	108	162
Average	56	76.8	108.4	112	168.4

Size of hook The large hooks are 24.9 μ, 33 μ, 36.9 μ and 39.9 μ on the average, on the 16th, 35th, 135th and 375th day after infection respectively. The proportion of these size is 1, 1.3, 1.5 and 1.6. The small hooks are 20.8 μ, 22.4 μ, 25.7 μ and 27.9 μ on the average respectively being in the proportion of 1, 1.1, 1.2 and 1.3. According to these dimensions the growth of the large hook is faster than that of the small hook.

The size and number of hooks of young worm on the 16th day are almost the same as corresponding values of scolex within the hydatid cyst. Age of the individual cestode must be considered to affect the hook size; subsequent growth in the handler and guard occurs after ingestion of the larval cestode.

TABLE 4. *Size of Hook* (μ)

NO.	HYDATID SAND FROM SHEEP		YOUNG AND ADULT WORM FROM DOG INFECTED EXPERIMENTALLY							
			16 Days after Infection		35 Days after Infection		135 Days after Infection		375 Days after Infection	
	Large H.	Small H.	Large H.	Small H.	Large H.	Small H.	Large H.	Small H.	Large H.	Small H.
1	25.0	20.0	21.3	17.5	32.5	20.0	35.0	23.8	37.5	25.0
2	25.0	20.0	22.5	18.8	35.0	22.5	35.0	23.8	38.8	26.3
3	23.8	21.3	25.0	20.0	33.8	21.3	36.3	26.3	40.0	25.5
4	25.0	21.3	23.8	20.0	32.5	20.0	35.0	23.8	40.0	26.3
5	22.5	20.0	27.5	21.3	33.8	21.3	37.5	27.5	38.8	27.5
6	26.3	20.0	26.3	22.5	32.5	23.8	37.5	25.0	40.0	27.5
7	25.0	20.0	27.5	22.5	32.5	24.5	37.5	26.3	41.3	30.0
8	25.0	20.0	25.0	21.3	32.5	23.8	37.5	27.5	38.8	32.5
9	25.0	20.0	25.0	22.5	32.5	22.5	40.0	26.3	41.3	28.8
10	25.0	20.0	25.0	21.3	32.5	23.8	37.5	26.3	42.5	30.0
Average	24.7	20.8	24.9	20.8	33.0	22.4	36.9	25.7	39.9	27.9

Size of sucker The sucker is nearly round and 92 μ, 101.2 μ, 118.8 μ and 140.6 μ in average longitudinal diameter respectively. The proportion of these is 1, 1.1, 1.3 and 1.5. This proportion is applicable to the growth of transverse diameter of the sucker.

TABLE 5. *Size of Sucker* (μ)

NO.	HYDATID SAND FROM SHEEP	YOUNG AND ADULT WORM FROM DOG INFECTED EXPERIMENTALLY			
		16 Days after Infection	35 Days after Infection	135 Days after Infection	375 Days after Infection
1	52.5×37.5	88×80	104×100	120×120	140×132
2	55.0×37.5	100×92	96×104	120×108	128×140
3	52.5×37.5	88×92	104×100	112×104	144×120
4	52.5×40.0	88×92	104×100	124×108	144×124
5	52.5×42.5	92×84	100×88	112×100	152×152
6	51.3×42.5	92×88	100×92	116×100	140×136
7	53.8×45.0	100×92	100×104	120×120	140×140
8	51.3×45.0	92×88	100×104	120×120	156×144
9	52.5×47.5	88×88	100×100	124×120	152×144
10	52.5×45.0	92×84	104×104	120×124	160×140
Average	52.6×42.0	92.0×88.0	101.2×91.0	118.8×112.4	140.6×137.2

Size of cirrus sac In the specimen on the 16th day after infection, the cirrus sac does not yet occur, while in the specimen on the 35th day the cirrus sac of the last proglottid appears with the typical form. On the 135th and 375th days, it shows a typical form not only in the last proglottid or gravid one, but also in the pre-gravid proglottid or mature one. The sizes of cirrus sac of the last proglottid on the 35th, 135th and 375th day are respectively 224 μ, 240 μ and 317 μ in average, that is, in the proportion of 1, 1.1 and 1.4.

The size of cirrus sac of the mature proglottid on the 375th day is only slightly larger than that of the gravid proglottid on the 135th day, while the swollen part of the former is very much larger than that of the latter.

DISCUSSION

According to the above described results, it is clear that this species is *Echinococcus granulosus* (BATSCH, 1786) and that there are variations in size and shape of the various organs not only at the same stage after infection but also with the development of the cestodes. Consequently it is very necessary for the differentiation of *Echinococcus* spp., to understand such variations sufficiently and to carry out further work, on the basis of experimental infections of other species.

Moreover, from the authors' observations which extended over about 1 year, it is clear that the strobila of this species increases in size continuously for a

TABLE 6. *Size of Cirrus Sac of Young and Adult Worm* (μ)

NO.	35 DAYS AFTER INFECTION			135 DAYS AFTER INFECTION			375 DAYS AFTER INFECTION					
	In Last Segment			In Gravid Segment			In Mature Segment			In Gravid Segment		
	Total Length	Tubular Part	Swollen Part	Total Length	Tubular Part	Swollen Part	Total Length	Tubular Part	Swollen Part	Total Length	Tubular Part	Swollen Part
1	224	112	112×64	240	128	112×60	240	80	160×80	288	96	192×64
2	224	104	120×64	240	112	128×48	288	128	160×96	288	128	160×96
3	224	112	112×72	240	96	144×64	288	112	176×80	320	160	160×96
4	208	80	128×72	240	128	112×64	240	64	176×64	320	160	160×80
5	240	96	144×80	240	128	112×60	256	96	160×96	368	160	208×112
Aver.	224	101	123×70	240	118	122×59	262	96	166×83	317	141	176×90

long period of growth; the variance between the size of specimens on the 135th day and those of the 375th day after infection is very large. This fact indicates that a long period of experimental infection is needed in order to obtain effective data on the differentiation of *Echinococcus* spp.

SUMMARY

1. Experimental infections of *Echinococcus* in dogs were carried out by using the Australian sheep strain of hydatid cyst.

2. The morphological observations and comparative dimensions of the cestodes on the 16th, 17th, 35th, 135th and 375th day after ingestion of the larval cestode were described respectively.

3. This species is *Echinococcus granulosus* (BATSCH, 1786).

4. The cestodes occur in the duodenum and anterior part of jejunum with limits of about 40 to 60 cm.

5. In young specimens on the 35th day after infection, the strobila shows the adult form already, while the embryophore of egg is out of sight and the oncosphere is not completed.

6. The first occurrence of the egg in the feces from 2 dogs was on the 48th and 61st day after ingestion of the larval cestode respectively.

7. There is a period in which the occurrence of the egg is interrupted; this period is about 1 or 1.5 months. Perhaps this is the period from the first removement to the next one of gravid proglottid.

8. The proportions of the development of the cestodes depend upon the average dimensions on the 16th, 35th, 135th and 375th day after ingestion of the larval cestode. Those proportion are as follows:

(1) Length of strobila, 1 : 3 : 4 : 8
(2) Size of rostellum, 1 : 1.4 : 1.5 : 2
(3) Size of hook;
Large hook, 1 : 1.3 : 1.5 : 1.6
Small hook, 1 : 1.1 : 1.2 : 1.3
(4) Size of sucker, 1 : 1.1 : 1.3 : 1.5

The proportions of the development of cirrus sac in the last proglottid of cestodes on the 35th, 135th and 375th day show a very good development.

10. The form of uterus in the gravid proglottid of this species is widely variable. The lateral succulations of the uterus become more in number and their shape changes into lateral branch with the development of cestodes.

11. From this work the present authors are quite convinced that further work, based on experimental infections, is needed to clarify important relationships involving the species of *Echinococcus* and then the intermediate and final hosts.

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EXPLANATION OF PLATES

PLATE I.

Figs. 1~4. Comparison of the size and structure of strobila on each day after ingestion of the larval cestode. The drawing of the excretory canal is omitted. 1, on the 16th day; 2, on the 35th day; 3, on the 135th day; 4, on the 375th day after infection.

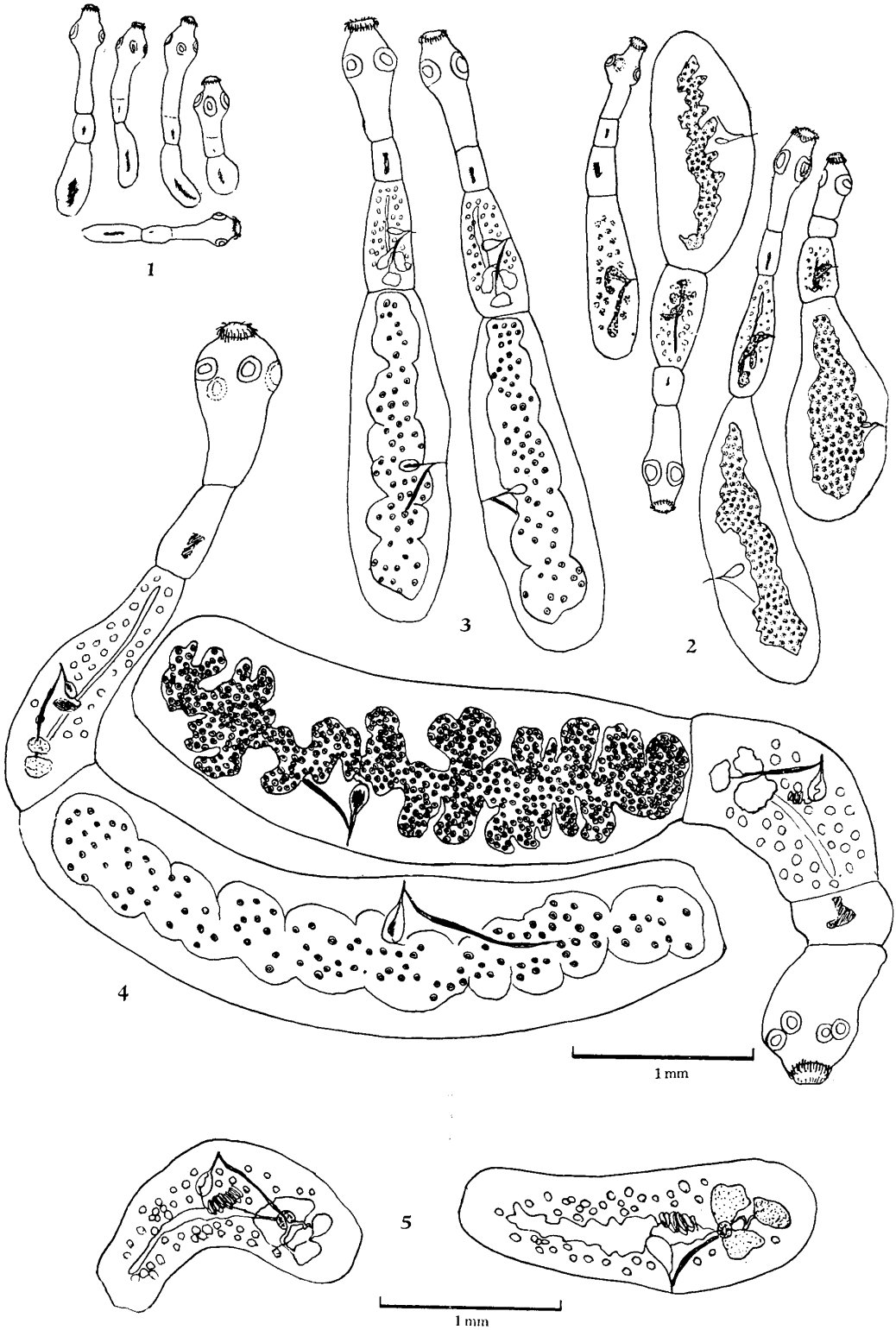
Fig. 5. Sexual system of the mature proglottids on the 375th day after infection. Left, ventrolateral view; Right, ventral view. The uterus has lateral indentations already.

PLATE II.

Figs. 6~10. Comparison of the size of hooks at each above stage. 6, scolex (hydatid sand) within the cyst; 7, on the 16th day; 8, on the 35th day; 9, on the 135th day; 10, on the 375th day after infection.

Figs. 11~15. Comparison of the size of suckers at each stage. 11, scolex within the cyst; 12, on the 16th day; 13, on the 35th day; 14, on the 135th day; 15, on the 375th day after infection.

Figs. 16~18. Comparison of the size of rostellum at each stage. 16, on the 16th day; 17, the 35th day; 18, the 135th day; 19, the 375th day after infection.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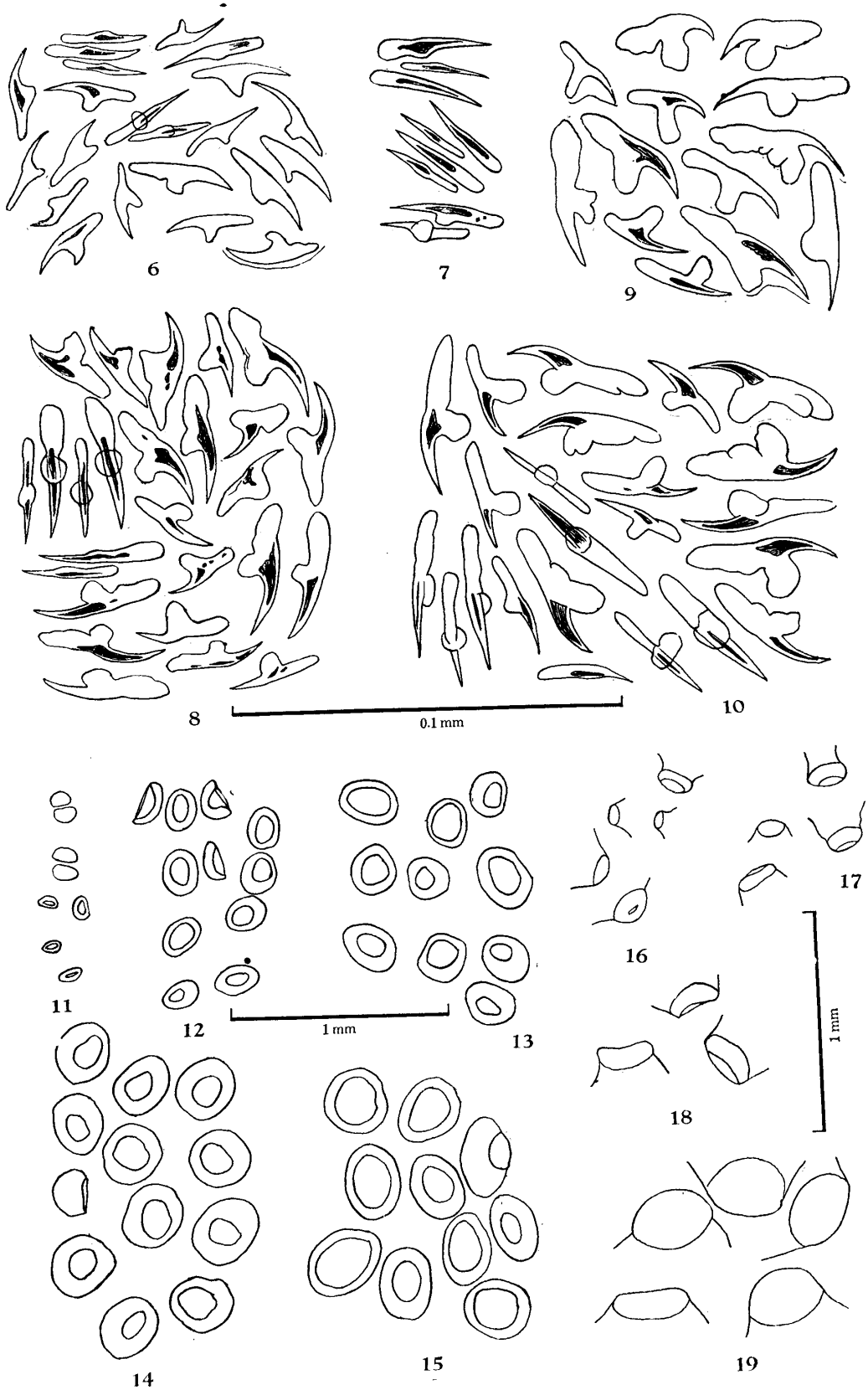
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