OBSERVATIONS ON THE DEVELOPMENT OF ALLOLOBOPHORA DUBIOSA (ÖRLEY) 1880 AND ITS PARASITE ZYGOCYSTIS ASTER N. SP.

by

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Received on December 10th, 1966

According to the literary data, this species can be found in Czechoslovakia (Černosvitov, 1935), in Yugoslavia (Černosvitov, 1931), in Roumania (Pop, 1949), in the Soviet-Union (Malevitsch, 1955) and in Hungary (Zicsi, 1963). Zicsi in his study mentioned above, has stated that this species belongs to those few earthworms, whose undisturbed life — activity needs a soil flooded with water. These animals can reach sexual maturity only under such conditions. The maturing and the greatest activity of individuals can be observed from May to August, but in the laboratory they can be kept in an active state the whole year around (Zicsi, 1963).

The first specimens of *A. dubiosa* were collected in March, 1963, from a meadow often flooded with water, near Dunaföldvár. The first specimens of *Zygocystis aster* n. sp. were also found in these animals. These individuals were kept under laboratory conditions and examined throughout the year. During this time their habits and life-activity have been observed.

The stages of development of the host were compared with those of the parasite because, according to P. Grasse (1953) "There is a harmony, a time-coincidence between the development of Gregarines and the host, which is favourable for the parasite to finish its developmental cycle. The sporocysts and the gametes of an earthworm get free in the same time, respectively sporocysts are produced when the host's maturity comes to pass"

Hentschel (1930) thinks it probable, that "one of the endocrine glands of the earthworm, or some kind of material of a gonad causes the gamogony and the sporogenesis". During the examinations both mature animals and juveniles were dissected. It was found that sporogenesis could proceed also in the coelom as it did always contain cysts and spores.

It is remarkable that cysts gradually draw off towards the tail and that regenerated tails are more frequent here than among other Lumbricides which were examined.

K e i l i n (1925) has described similiar phenomena and explained the autotomy of the tail with the presence of numerous Gregarines and considered it as a mode of reinfection. Earlier several authors dealt with the modes of infection. It was generally assumed that by the death and decay of the host the parasite gets out of the infected animal, or rather already the cocoon becomes infected by the sexual product of the host. K e i l i n considers the regeneration of the tail as a new mode of transmission. He observed that the regeneration of *A. terrestris longa* (U d e), *A. chlorotica* (S a v.) and *A. caliginosa caliginosa* (S a v.) was most frequent. According to him, the autotomy of the tail proceeds in such a way that Gregarines surround themselves with lymphocytes, they form cysts and accumulate as "brown bodies" in the posterior segments of the coelom. In some infected tails he observed more than a hundred cysts. He found also a few Nematode grubs among the cysts.

He explains the autotomy of the tail by saying that this great mass exerts pressure on the blood-vessel system and the gut, so autotomy takes place and later on the tail regenerates. Parasites escape from the separated part of the tail.

Z i c s i (1963) in his study of *A. dubiosa*, pays a great attention to the regeneration of the tail. He explains it with the peculiarity of the animal's way of life. The animal is known to live in the soil suffused with water. It respires by its tail floating in the water and so beasts of prey can easily mutilate it.

To test the probable cause of autotomy nearly a hundred animals were opened. Then the cysts, accumulated in the posterior segments were weighed to determine, whether this mass could exert so great a pressure as to cause autotomy. (See the table 1).

Table 1.

Data of cysts air dry found in the tail of Allolobophora dubiosa (Örley) 1880

Rate of maturity	Number of specimens examined	Normal tail	Without spore	With spore	Regenerated tail	Without spore	With spore	Greatest weight	Smallest weight	Average weight
Juvenile	25	22	6	16	3	—	3	0,5 mg	0,1 mg	0,2 mg
Transitional state .	35	26	6	20	9	—	9	11,7 mg	0,2 mg	2,9 mg
Mature	41	34	6	25	7	—	7	16,7 mg	0,2 mg	3,5 mg

From these examinations the following conclusions can be drawn.

1. The individuals examined were at different stages of maturity. Regeneration of the tail was found to occur with young individuals too.

2. The weight of the cysts (in air-dry state) varied from 16,7 mg to 0,1 mg. The last 30 segments of some more medium-infected worms were dissected and weighed. The weight of the cysts which were contained in these segments was 10 per cent of the total weight.

3. Autotomy takes place probably over the weight of 16,7 mg.

4. The regenerated tail always contains sporocysts even in juvenile state. Consequently, the host does not have to be mature for spore formation in coelomic Gregarines. It may further be concluded that presence of Gregarines helps the autotomy of the tail of the worm, and this may be a defence of the host against hyperinfection. The separated part of the tail will be the source of a new infection.

Zygocystis aster n. sp.

Host: *Allolobophora dubiosa* (Ö r l e y) 1880.

Site of infection: coelom.

This Lumbricide has not been examined for parasites, therefor the observations were made with special care. A very interesting Monocystid form was found at first sight. The detailed examinations showed that this form is not similiar to any parasite described so far. Its habit and way of life is different from other Monocystides. It was identified as belonging to the subfamily *Monocystinae* and to the genus *Zygocystis*. Up to the present this subfamily has contained nearly without exception species described as parasites of *Allolobophoras*.

The most common species found in the seminal vesicle was *Z. cometa* (S t e i n). So, the spread area of this species can be supplemented by the Törekymoore.

Characteristics of *Zygocystis aster* n. sp.

Length:	$470-855$ μ
Width:	$168-371$ μ (maximum)
	$50-185$ μ (minimum)
Nucleus:	$13{,}5-20{,}2$ μ, ovoid, with $1-3$ nucleoli
Spore:	$19-21\times9-9{,}7$ μ, ovoid, boat-shaped
Cyst:	2 mm $\times$ 1,7 mm, ovoid

Upon opening the host a regular star-shaped trophozoite is visible to the naked eye. In the formation of the starshaped trophozoite 3,4 or 6 individuals may take part. They stick mostly to the stomach wall but occur also ventrally. It is rather difficult to remove them without causing injury as they stick tight with their mucros. Most *Zygocystis* species described so far, consists of two unicellar individuals, it is rare to find specimens of 3 individuals. Up to the present no forms consisting of four or six individuals have been described. *Zygocystis aster*, however, consists exceptionally of four, sometimes of six individuals. This trophozoite is pear-shaped, may reach a length of $470-855$ μ, which is a considerable size among unicellar organisms. Because of its being pear-shaped two values of width were given, a greater and a smaller.

The ectosarc is well developed. The external layer is the epicyte which can be $3-4$ μ thick. The epicyte is striated, which can be seen mainly in young individuals at the slendering end of the body. The ectocyte is rather thick. At the slendering end of the body the mucro is to be found. In its vicinity dense myoneme threads are visible which perhaps are in connection with the energetic motion. Around the mucro there is a zone of attachment.

The endosarc is filled with paramylum granules. These are somewhat ovoid, they measure about 3 – 8μ, but there are larger ones too, mainly at the thinner end of the body. Adults are vacuolated mostly along the absorbed sticking lines.

The nucleus is somewhat ovoid, contains 1 – 3 micronuclei (indicating perhaps sexual dimorphy ?). The size of the nucleus is 13,5 – 20,2 μ. It is located nearly at the middle of the body and is surrounded by an area of a faint colour. The nucleus grows as the animals grow older, then the micronuclei seem to swell.

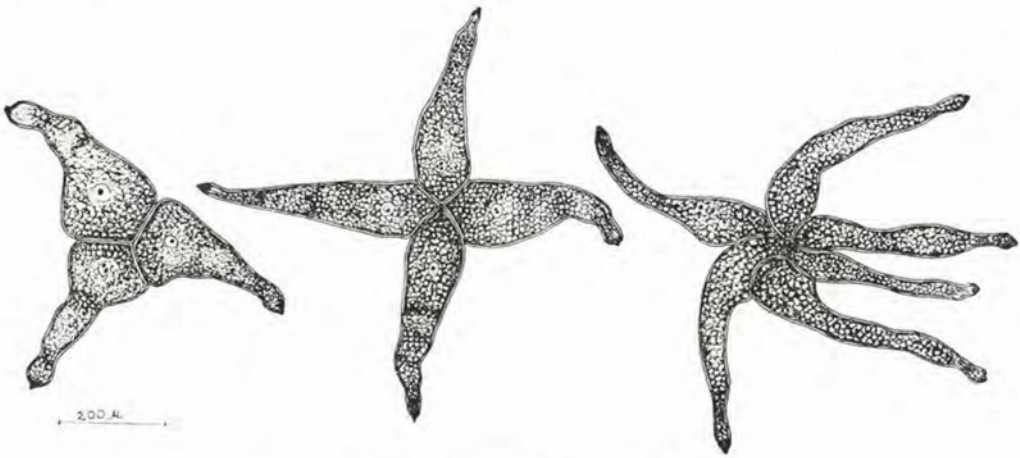

Fig. 1. Different forms of *Zygocystis aster* n. sp.

The infection of the host may come about in either of two ways, by food or by evacuating the sexual products. So already the cocoon can be infected. Perhaps this is the reason for the fact that earlier developmental stages can be found in the coelom or under the peritoneum parietalis.

In order to establish the next developmental stage, additional sections were prepared. When infection takes place through the mouth, then probably under the effect of intestinal secretions the sporocyst opens and the sporozoite becomes free. It penetrates the wall of the bowels, grows through it and in fully developed stage gets into the coelom. When the sporozoite has become free in the coelom it penetrates the peritoneum parietalis, and the trophozoite develops there. Consequently the initial developmental stage is intracellular. When the individual reaches suitable size, the host cell, it had developed in, will be absorbed, it decays and the young trophozoite will be liberated. Being *Zygocystis* they form syzygy immediately, stick together by their wider ends, and paraglycogen accumulates intensively. In this stage they spend a long time, then the absorbtion of the connecting cell walls begins. The animal gradually loses its starshape and becomes more and more similiar to the cyst state in which the gametes are formed, then it secretes mucus with which it will be surrounded. At such times there is a change in its location in the host. While the

star-shaped individuals are located in front of the clitellum or just behind it, the round forms accumulate gradually in the tail. There they will be surrounded by degenerate nephrides and get a characteristic brownish colour. No copulation of gametes could be observed so far.

Out of the zygote develops the spore which has biconical, boatlike form. The spore measures $19-21 \times 9-9{,}7$ μ. Inside the relatively thick hyalin envelope there are eight sporozoites.

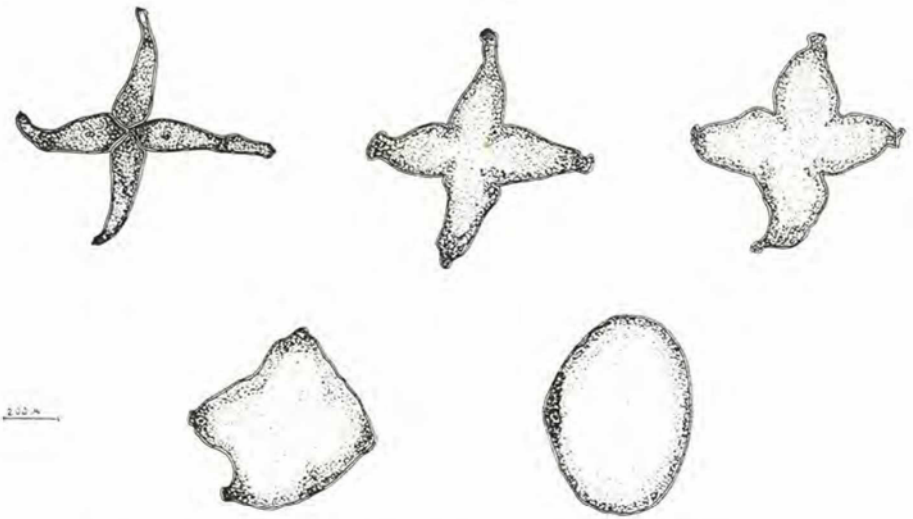

Fig. 2. Scheme of the cyst-forming process of *Zygocystis aster* n. sp.

Summary

1. In contrast with other species *Zygocystis aster* was not found in the seminal vesicle but in the coelom.

2. Among the *Zygocystis* species known so far, the most general is *Z. cometa*, which was also found in *A. dubiosa*, but it differed from *Z. aster* n. sp. both in habits and size. While the average length of the individuals of *Z. cometa* was found to be 143,7 μ, whereas *Z. aster* n. sp. can be as long as 850 μ.

3. In the literature usually specimens consisting of two individuals have benn reported. Only M. M e i e r (1956) has mentioned a triplicate form of *Z. cometa*. On the contrary I have found *Z. aster* n. sp. to consist almost exlusively of $4-6$ individuals.

4. Not the whole body is striated like that of the *Z. legeri* (H e s s e), but only the area surrounding the mucro.

5. With the exception of *Z. legeri* all *Zygocystis* species have hair-formations on the epicyte. No such formations were found on *Z. aster*.

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