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Ananeosis, a New Phenomenon in the Life-History of the Enchytraeids (Oligochaeta)

By

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Since 1971, I took regularly soil samples in a sparse Quercetum petraeae-cerris wood at Julianna-major, near Budapest. The dominant Enchytraeid species is *Stercutus niveus* in this habitat during the winter and early spring months. I propose to discuss the detailed results obtained during this study (horizontal and vertical distribution, seasonal fluctuation, etc.), with respect to this animal, in a future paper, the more so as my work is still in process. The present contribution concerns only a revision of *Stercutus niveus* as well as my observations and the results of experiments concerning its life-history.

A revision of Stercutus niveus Mich., 1888

MICHAELSEN based the genus *Stercutus* on a single species, *niveus*, in 1888 (4), this latter being its sole representative even today. Although BELL described in 1954 (1) another species under the name *S. ugandensis*, as both NIELSEN and CHRISTENSEN (3) have clearly shown, this latter cannot belong to the same genus. The difference with respect to the fundamental features is so great between the two species (head pore, setae, dorsal vessel, nephridia) that its relegation to the genus *Stercutus* is wholly incomprehensible. According to NIELSEN and CHRISTENSEN, BELL's species is probably assignable to the *Haemienchytraeus* alliance.

The establishment of a genus on a single species is rather difficult. Also MICHAELSEN gives but a short description, remarking that he considers the erection of a new genus necessary in view of some characteristics of the species which preclude its relegation to any known genus. In my opinion this assertion still holds, and thus, together with the revision of the species, I submit here also a brief redescription of the genus, with the reservation that the generic characteristics are to a certain extent subject to modification in the case of the description of new species assignable to it.

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Stercutus MICHAELSEN, 1888

Setae sigmoid, with nodulus. Head pore and dorsal pores absent. Oesophagus and intestine gradually fusing. Peptonephridia, oesophageal appendages and intestinal diverticula absent. Dorsal vessel originating anteclitellarily. Blood colourless. Nephridia with well developed interstitial tissue, anteseptale consisting of a distinct funnel and some coils of nephridial canal; postseptal elongate, efferent duct originating antero-ventrally, near septum. — According to MICHAELSEN's description the anus is closed. This must be modified, because the anus is open (see detailed description in the discussion of the species). — Spermatheca without diverticula, free, unconnected with oesophagus. Vas deferens thin, long. No true penial bulb present.

The genus is doubtless nearest to *Cognettia* NIELSEN and CHRISTENSEN 1959, owing to the antero-ventral origin of the efferent duct of the nephridium, the free spermatheca, the gradual transition between the oesophagus and the intestine, the absence of the peptonephridia, the oesophageal appendage and the intestinal diverticula, and the posteriorly incised brain. At the same time, there are features also more primitive than in *Cognettia*, indicating a relationship probably towards *Cernosvitoviella* NIELSEN and CHRISTENSEN 1959; such are the still discernible (weakly developed) nodules on the sigmoid setae and the fact that there is no true penial bulb but minute atrial glands around the male pore. On the other hand, a characteristic more advanced than in *Cognettia* is the fact that the anteseptal part of the nephridia in *Stercutus* does not consist of merely a funnel but exhibits some small coils of the nephridial ducts. I cannot therefore concur with CERNOSVITOV's (2) statement that the genus *Stercutus* stands between the genera *Achaeta* and *Mesenchytraeus*.

Stercutus niveus MICHAELSEN, 1888

A small species, 3–5 mm long and 0.4–0.5 mm wide, tapering anteriorly and posteriorly. Number of segments 19–21 (according to MICHAELSEN, 6 mm long and 0.6 mm wide, with 22–28 segments). Head pore and dorsal pores absent. Color vivid white, owing to chloragogeneous cells filling entire body cavity.

Setae: sigmoid, with weakly developed nodulus (Fig. 1 *a;* Fig. 2 *A*), 2(3)–2 : 4(3)–(4)3. MICHAELSEN makes no mention of the nodulus; it has probably escaped his attention, since it is not too conspicuous, yet a stained preparation shows it clearly. Length of setae 50–60 μ. The large and robust setae, especially the 4 ventro-lateral groups each, are highly characteristic of the species. Already MICHAELSEN remarked that the bristles are occasionally arranged in double rows, a phenomenon observable at the time of setal change, when the new setae are already evolved but the old ones have not yet fallen out.

Clitellum well developed at XII–1/2 XIII; glandular cells situated irregularly (Fig. 3 *A*). — According to CERNOSVITOV's measurements (2), the epidermal + subcutaneous layers are generally 12 μ deep, attaining, however, 50–60 μ at the clitellum.

Brain (Fig. 1 *b*) $1^1/_2$–2 times longer than wide, deeply excised posteriorly. Peptonephridia, oesophageal and intestinal diverticula absent. Oesophagus gradually transitional into intestine.

Three pairs of primary, dorsally free, and two pairs of small secondary, septal glands present. MICHAELSEN mentions only the primary septal glands. Septal glands exhibiting, especially in older specimens, a loose, spumous struc-

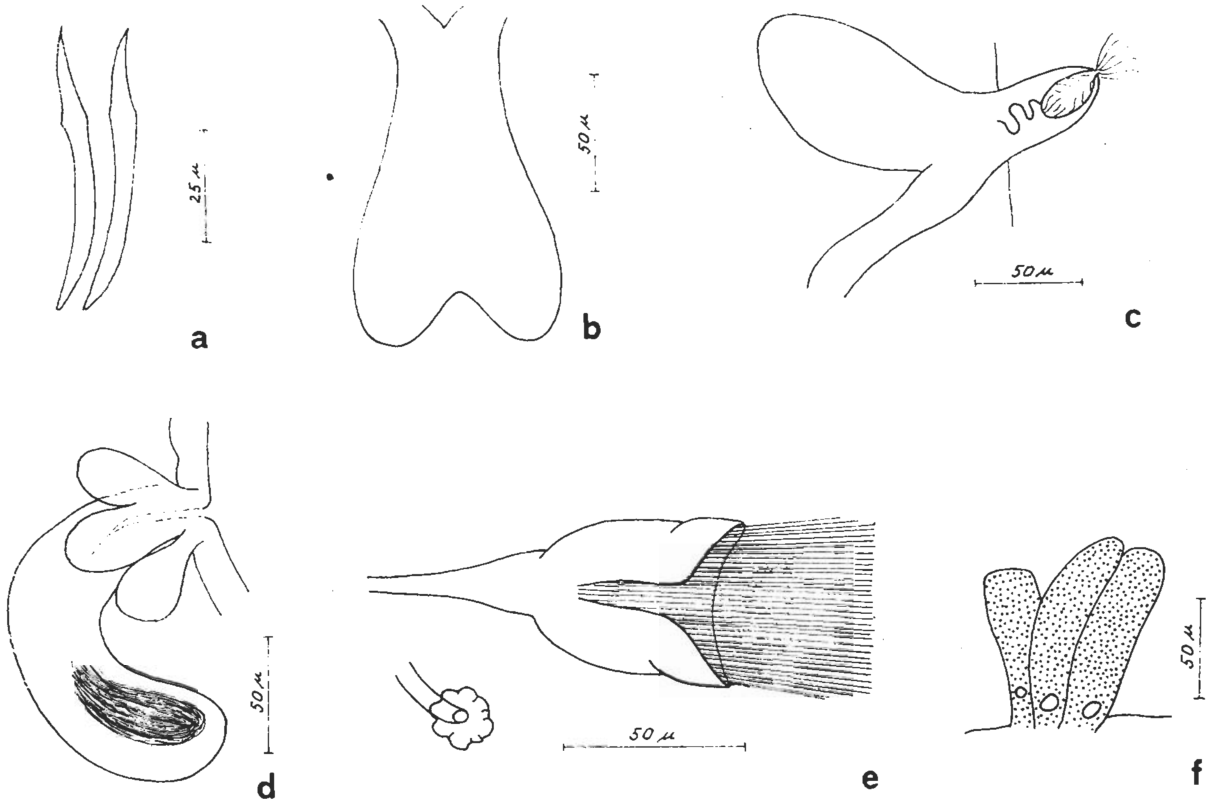

Fig. 1. *a:* setae; *b:* brain; *c:* nephridium; *d:* chloragogenous cells; *e:* sparmatheca; *f:* sperm funnel; *g:* male pore

ture (according to MICHAELSEN's observations, occasionally emptying their contents into the oesophagus and the intestine).

Lymphocytes occurring in very small numbers only; of irregular shape, rather oval, very finely granulate; usually, however, wholly absent. Enormous chloragogeneous cells, highly characteristic of the species filling entire body cavity. In some cases, detached and disintegrating chloragogeneous cells observable in body cavity. Even by most careful observations, MICHAELSEN also failed to find lymphocytes in the body cavity.

Chloragogeneous cells present beginning with segment IV, 70–100 μ long and 15–25 μ wide, full with shining oil drops, brown in transmitted light, and with a well discernible nucleus. These enormous cells, hanging from the intestinal wall, fill entire body cavity (Fig. 2 *D*), thus coloring the whole animal intensely white. — The extremely large cells, differing from the chloragogeneous cells of all heretofore described Enchytraeid species, undergo an interesting development or process of changes, during the life of the animal. They play an undisputable role in reproduction. MICHAELSEN had also observed that the chloragogeneous cells become disattached in the clitellar region when the animals reach maturity, and thus inferred that the vitellar substance of the eggs form from the material of the chloragogeneous cells. This assumption, however, needs further investigations for substantiation. In any case, the chloragogeneous cells of this species are subject to unusual changes, to be discussed in detail in the chapter on life-history.

Dorsal vessel originating in segment IX. Blood colourless. Nephridia with well developed interstitial tissue. Anteseptal part of nephridia consisting of a large funnel and some coils of nephridial duct; postseptal part extensive, efferent duct originating antero-ventrally, near septum.

No seminal vesicle. Sperm funnel (Fig. 1 *f;* Fig. 3 *C*) as long as wide, funnel-shaped, very small (50 μ), about one-tenth as long as diameter of body, best observable in already oviposited animals. Vas deferens about 600–900 μ long and very narrow (7 μ). No true penial bulb present, male pore being surrounded by minute glands (Fig. 1 *g*). Spermatheca (Fig. 1 *e;* Fig. 4 *Aa*) free, unconnected with oesophagus, consisting of an oval or spherical ampulla and a thick, robust ectal duct; this latter 2–3 times longer than ampulla. Ectal orifice of spermatheca surrounded in a circle by large, pyriform, hyaline (usually 6) glands. The canal of ectal duct discernible only at orifice. 1–2 till 10–15 large eggs at a time, filling entire body cavity, arranged beside one another or longitudinally (Fig. 4 *B–D*). Dimensions of one egg: 360–560 μ long and 240–420 μ wide, oval; if many present then rather spherical and also smaller, about 280–300 μ. Eggs laid in cocoons. Cocoons lemon-shaped (Fig. 3 *D*), with a pale brownish cover, one minute tip invariably pointed, other one obtuse. Cocoon externally not covered with plant fragments, allowing snow-white eggs well observable. Usually several eggs are oviposited in one cocoon.

MICHAELSEN's description contained a feature unusual in the Enchytraeids, a closed anus, as well as the lemon yellow mass of cells present in the rectum and the beginning of the mid intestine. MICHAELSEN explained the closed anus by the animals ingesting probably liquid food only, since he never found plant remnants or mineral granules in the intestine of his research material. On the other hand, I was able to observe well discernible plant fragments in the intestine of worms (kept in the laboratory or found in the samples) in certain times, indeed, also the process of emptying of these bits from the intestine (Fig. 5 *A*, *B*). I succeeded to clarify the problem by a series of cross-section

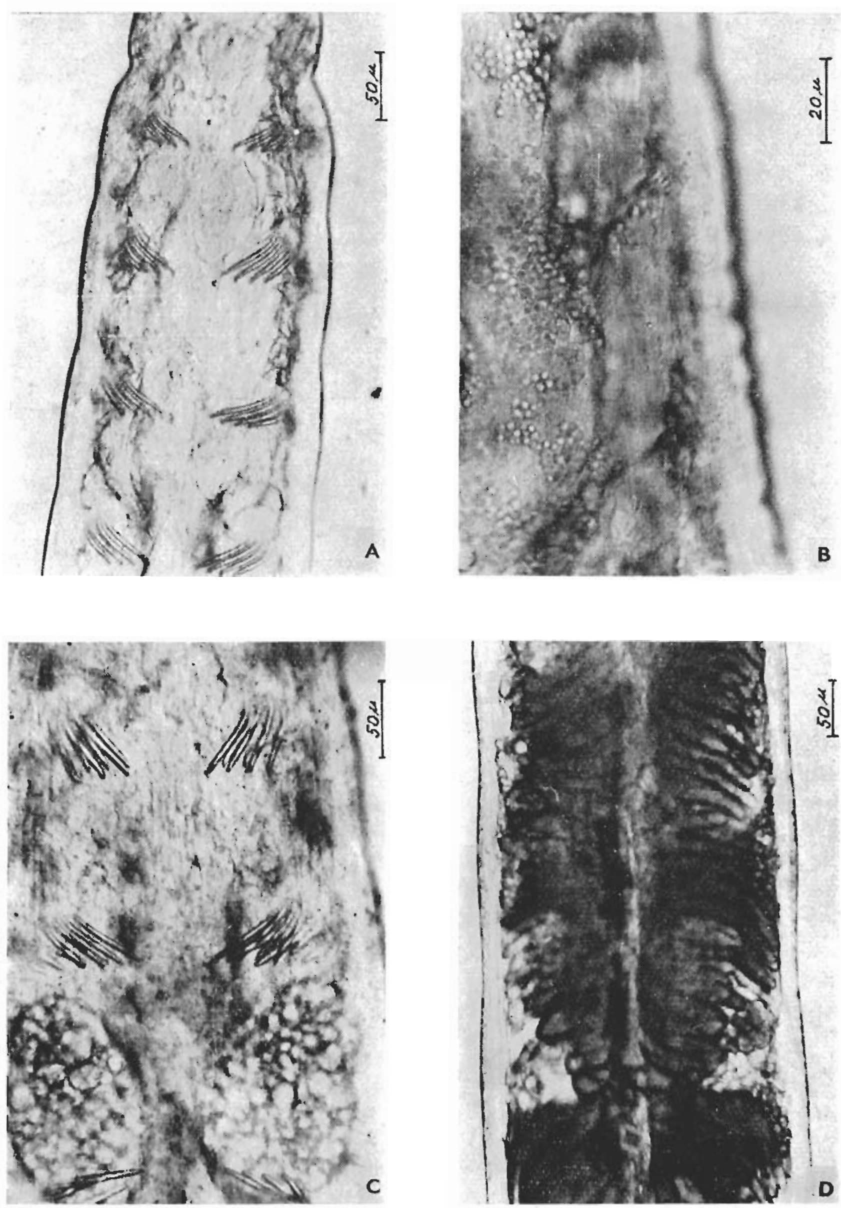

Fig. 2. *A:* ventral setae; *B:* chloragogenous cells of juvenile animal emerged from **cocon;** *C:* double setae at time of setal change; *D:* chloragogenous cells of adult worms

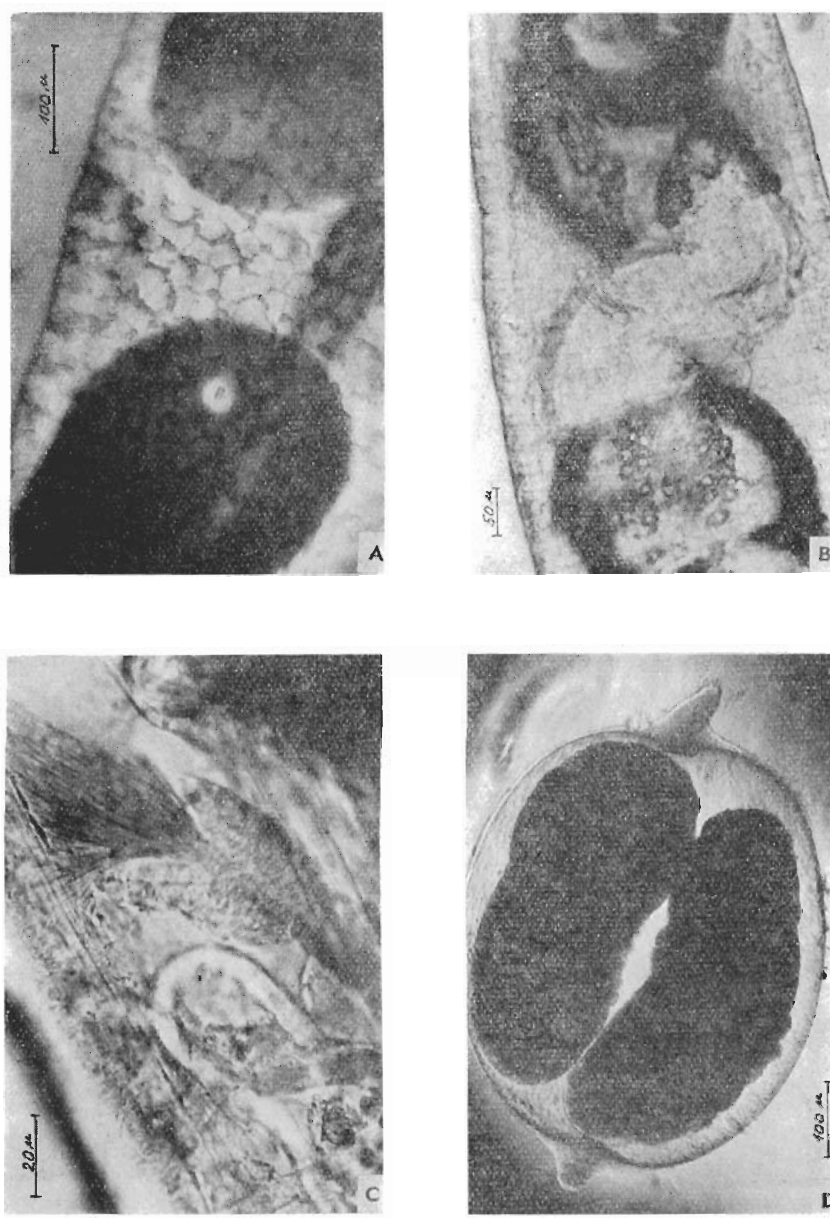

Fig. 3. *A:* glandular cells of clitellum; *B:* segments XII–XIII of oviposited and retrojuvenated animal; *C:* sperm funnel; *D:* cocoon

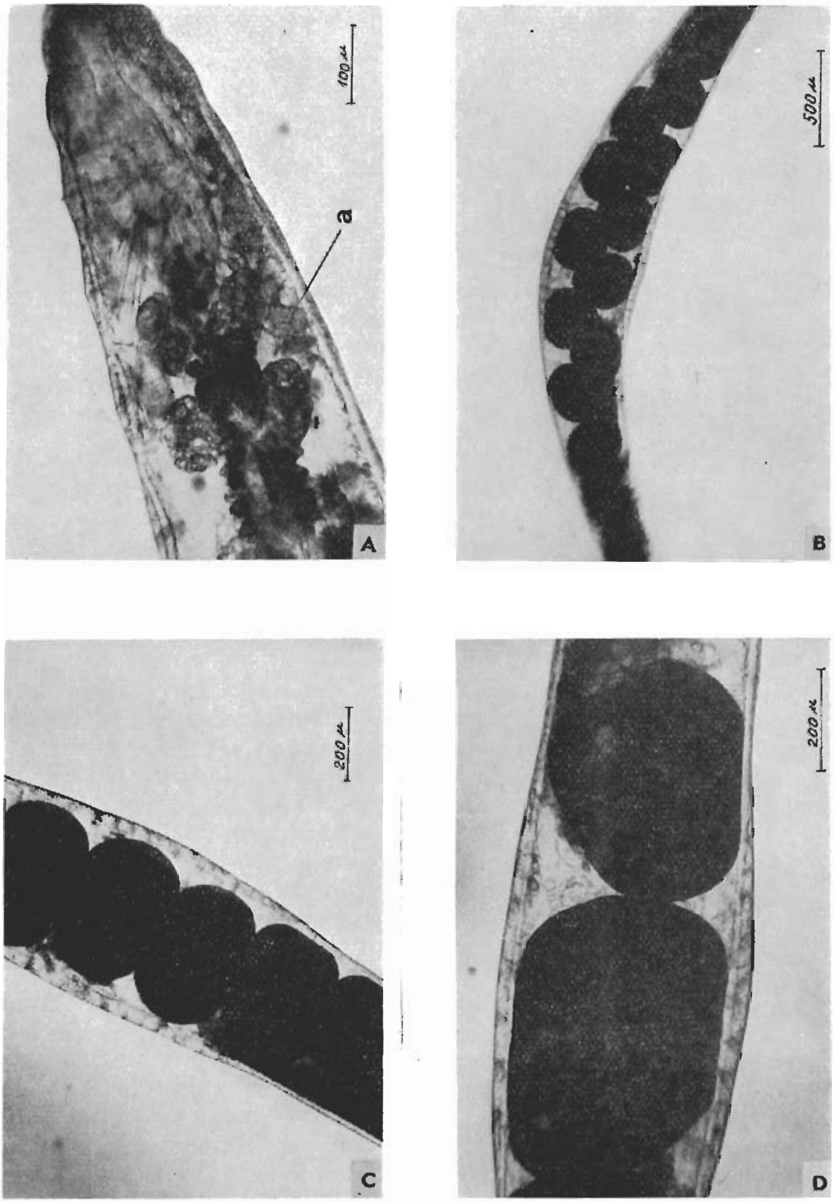

Fig. 4. *A:* front of body segment VI (*a:* spermatheca); *B–D:* eggs in various arrangements

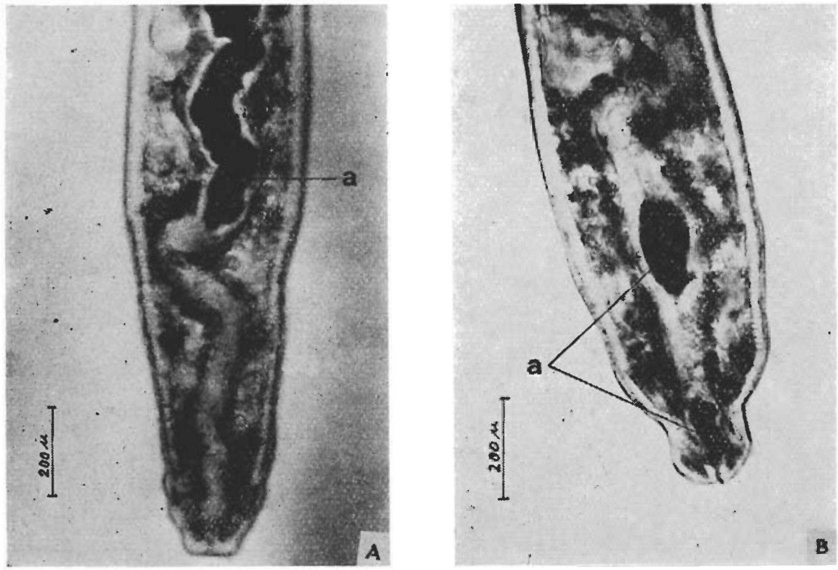

Fig. 5. *A–B:* ends of body, evacuation of plant fragments *(a)* from intestine

slides. I could establish that although a yellow mass of cells (Fig. 6 *a*) intrudes deeply into the intestine and thus considerably restricts its cavity, the rectum is still open (Fig. 6 [17]). The series of cross-sections was made by the usual method, fixing the animal in bouen, carrying it through alcohol and toluol and imbedding it in parafin, with sectioning at 10 μ, stained in haematoxylene and eosin, and a permanent preparation made in Canada balsam. It should be noted that the series of cross-sections was made of animals with an empty intestine, therefore probably in the state in which MICHAELSEN observed them. The contradiction between MICHAELSEN's and my own observations is solved in an interesting manner by a knowledge of the ecology of the species, to be taken up in that chapter.

Chromosome number was established by the help of Professor B. CHRISTENSEN, both for the eggs prepared from the animals and for those oviposited in the cocoons. Accordingly, n : 50, 2n : 100.

I wish to express my gratitude for Professor CHRISTENSEN's help also in this place.

D i s t r i b u t i o n : Hungary: Julianna-major, Hársbokor-hegy: Querceetum petraeae-cerris and Querceto luzuletum woods; Szendehely: Quercetum petraeae-cerris and Querceto carpinetum woods; Lajos-forrás, Mts Pilis: Querceto carpinetum, Querceto luzuletum, and Fagetum silvaticae woods; Mts Vértes: Querceto carpinetum. – Germany: in "Fischdünger", in the fauna of the Lower Elba (4).

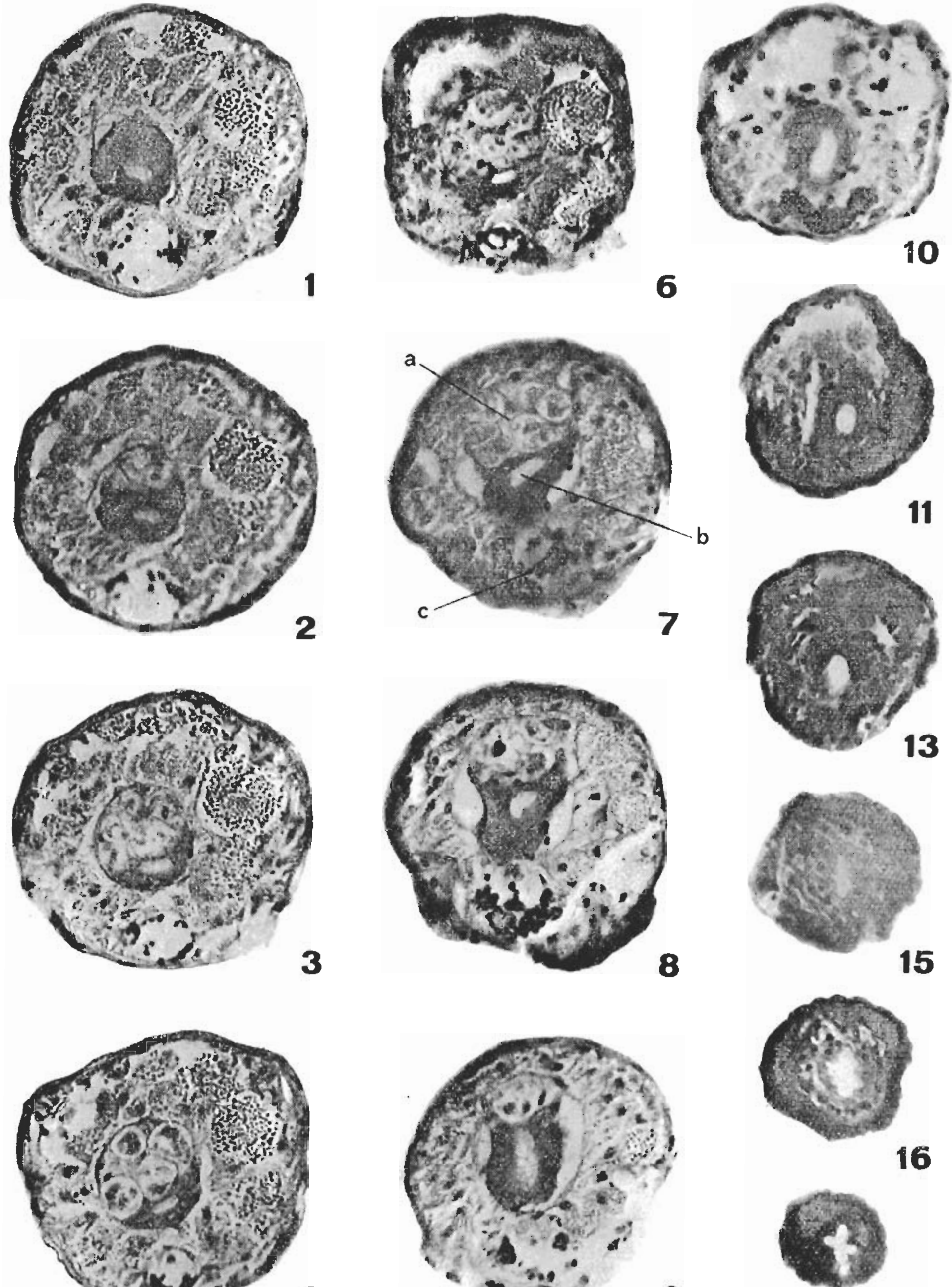

Fig. 6. Cross-section series from end of body of a *Stercutus niveus* specimen; thickness of sections 10 μ. *1:* prior to intrusion of yellow cell mass into intestine; *2–9:* cell mass *(a)* intruding into intestine and restricting its cavity; *10–17:* section after intruding cell mass; intestinal lumen well expressed, anus open at end of body *(17)*; *b:* intestinal lumen; *c:* ventral ganglion

The juvenile animals appear at the beginning of November. They are 1.5–1.8 mm long and 0.18–0.20 mm wide, reaching their complete size in about 4–5 months. The attainment of sexual maturity, however, requires a lot more time. The great majority of the specimens stays in the litter or in the uppermost soil layer until March; their intestines are full with the ingested decaying leaf remnants. Beginning with April–May, most of them retreat deeper into the substrate. Sexually mature exemplars can be found only from the end of July till October. The experimental (laboratory) specimens reveal what happens with the full-grown worms until attaining also their sexual maturity. The 113 specimens, collected in February, 1971, were placed in a plastic container among a deposit of decaying leaves. By the end of April and the first days of May, the animals congregated into 3–4 groups, lying motionless and closely packed near one another. They did not feed, or at least their intestines were completely empty. This cannot be but an inactive stage—yet not too deep either, because if I touched them, or moved the leaves, they immediately began moving vividly for a time. Left to themselves, however, they never moved or changed places during the entire resting period. Since temperature in the chamber of experiment was not high (13–18 C°) and their environment humid, I cannot but regard this state as a reproduction of the accustomed inactive phase of their life-history by which they tide over the usually arid and hot conditions of the summer months in nature. As corroborated by my samples, they retreat in the field during this period into the deeper soil layers and congregate there into smaller to larger groups. The reproduction of this inactivity in the experimental conditions outlined above is highly interesting, the more so as it does not concerns a direct reaction against dryness. Although there is no doubt that an original adaptation to survive the unfavourable external conditions manifests itself in inactivity also here, we have to do with an inheritable, rhythmic diapause, since the experimental animals failed to reproduce even in conditions optimal for their proliferation and retained their natural, seasonal life rhythm.

I observed the first sexually mature specimens by the end of June. Some shift showed, however, against the phenomena observed in the field, because my samples contained sexually mature specimens only at the end of July, and egg-bearing ones only by the end of August. This is probably ascribable to differences in humidity. The first signs of sexual maturity consist in the disattaching of the chloragogeneous cells from the intestinal wall in the clitellar region, settling in the body cavity in this area. Soon the sexual organs can also be detected (spermatheca, sperm funnel), and after one or two days the eggs also appear. Concerning the distribution of sexual maturity and egg numbers, the experimental animals showed the following picture.

On the first date therefore 68.2% of the worms were sexually mature, with 44.6% of egg-bearing exemplars having 1–2 eggs. The maximum number of eggs was 15. In the course of experiments this became slightly modified, some of the animals forming additional eggs to the already present ones, so that of the group bearing 1–2 eggs, one worm was found to have 3 eggs, one other to have 4 eggs, and another one 7 eggs; of the group carrying originally 3 eggs, one each was found to have 4 and 6 eggs, respectively, etc. At the same time, of the individuals sexually mature but still having no eggs seven were found to carry one or two eggs. The above data represent the two pictures formed on

	30 June, 1972	16 July, 1972
Number of eggs	number of specimens	number of specimens
1–2	25	29
3	5	4
4	7	8
5–6	8	11
7–8	3	4
9	3	3
10	2	2
12	2	2
15	1	1
sexually mature but without eggs	21	13
sexually immature	36	

the respective dates. I was unable to follow the subsequent development of specimens still sexually immature at the beginning of observations, because, though the sexual organs appeared, the majority of the animals perished.

The first cocoon, containing two eggs, was laid on 15 July (Fig. 3 *D*); the majority of cocoons were formed at the end of July and some in August. The measurements of the cocoons varied to some extent, between 0.71–1.35 mm in length and 0.49–0.64 mm in width. Their smaller, obtuse apex was 70–80 μ long, the longer, pointed one 100–120 μ long. According to my observations, a worm lays its entire egg store into one cocoon. I found only very few exceptions, namely when one or two eggs remained in the body cavity after the laying of the cocoon; these eggs disappeared after some days without the formation of any fresh cocoon. In spite of this, however, I think it probable that in a small percentage of cases the animals divide their egg stores between two cocoons. Unfortunately, I did not succeed to have the cocoons hibernate, because the ones laid in the laboratory were attacked by some fungus and they perished. However, I found a similarly destroyed cocoon in a sample taken in October, 1970 (the distance between its two small apices was 689 μ, its width 639 μ). Through its external cover the small, already developed worms were clearly visible, crawling round and round within the cocoon. By the characteristic ventral setae I could identify them at once as *Stercutus niveus*. I kept the cocoon in some water in a Petri dish for three days, but it failed to burst even though the worms were animatedly moving around in it. Applying some pressure by a covering glass on the cocoon placed on a slide I cracked its wall carefully. At once six minute *Stercutus niveus* exemplars emerged. A year earlier (October, 1969), I also found a similar cocoon, releasing 4 juvenile *Stercutus niveus* exemplars.

The young animals obtained from the cocoon found in 1970 were examined under the microscope. I took exact measurements which agreed with those taken from the smallest juvenile specimens found in the field about the same time of year. All six specimens had 20 segments, and this number did not change during their life in the laboratory (three months). The length of their characteristic ventral setae is hardly less than those of completely grown worms (40–50 μ), appearing therefore even bigger and more conspicuous. The formation of the chloragogeneous cells is also interesting. In the freshly emerged specimens they are round (diameter 10–14 μ) and finely granulate (Fig. 2 *B*). The granules are still greenish-transparent in transmitted light at that time. The chloragogeneous cells of juvenile animals emerging from cocoons kept in the laboratory attained a length of 35–45 μ by two and a half months, filled by that time with dark brown, untransparent granules in transmitted light.

The oviposited animals undergo a most interesting and hitherto unknown change in the Enchytraeids, detected during the observation of the experimental specimens. After the laying of the eggs, the worms dispose of a much depleted chloragogeneous cell mass, and then even the rest of the cells becomes disattached and swims freely in the body cavity. After about two weeks, the chloragogeneous stock becomes, as it were, regenerated, so that the intestine is covered by cells of similar size and granulation as in the juvenile animals. I was most astonished to observe that this "rejuvenation" is wellnigh literal, and that it sweeps over almost the entire organism of the worm. First the spermatheca degenerates, then also the sperm funnels, so that in the course of one or two months an apparently juvenile animal, completely lacking sexual organs, comes into being. Its whilom sexual character is indicated now by nothing more than a lighter intestinal section covered by small chloragogeneous cells in the clitellar region; in all probability, the chloragogeneous cells regenerate here at the lates (Fig. 3 *B*). Finally, also this difference disappears.

I propose to designate this hitherto unknown phenomenon in the life-history of the Enchytraeids as a n a n e o s i s*.

While the animals do not feed during the wholly inactive then sexually mature and ovipositing stages, they begin feeding in this rejuvenating phase on decaying plant fragments. This can be clearly observed if some desiccated and then moistened elder leaves are given to the experimental animals: the green leaf bits show conspicuously though the intestinal wall. This observation also helped in solving the problem of the closed or unclosed state of the anus (cf. MICHAELSEN). MICHAELSEN observed the animals probably during the long stage of inactivity and the sexually mature phase (April, May–September, October) when they do not feed, and therefore assumed the ingestion of merely liquid food. The role of the lemon-coloured cells intruding into the intestine, observed and believed to be bits of excrement by MICHAELSEN, is still obscure. I, too, think it probable that they represent some sort of final product, accumulating in the course of the long period of inactivity. This latter assumption is the more probable as the cell mass is absent in juvenile animals, whereas it is rather bulky and conspicuous in old and sexually mature worms; in the rejuvenating old specimens it becomes disattached from the intestinal wall and arrives in the body cavity and then after some time disappears entirely—being possibly evacuated from the organism.

Retrojuvenation extends even to the setae. In 80% of the animals collected in the field in October, I found double setae per bundle, therefore 4–4 : 8(6)–6 (Fig. 2 *C*); by the falling out of the old setae, the normal number of bristles again reappeared in the experimental animals in the course of a single week.

Accordingly, the life cycle of the individuals of the species extends to more than a year; an exact determination of the period in question needs further investigation.

VEJDOVSKY (5.) mentions the degeneration of the reproduction organs of an Oligochaeta living in water, *Chaetogaster diphanus*, proceeding similarly to the above written. Thus it seems to be probable that this phenomenon is much more general among the Oligochaeta and so with the Enchytraeids too, as we have actually knowledge of it.

* ανανεωσις = retrojuvenation.

SUMMARY

Author revises the species *Stercutus niveus* MICH., 1888, and discusses the development of this curious Enchytraeid. It was found that the animal is sexually mature only from the end of July to October, ovipositing (1–15 eggs per specimen) in cocoons, but usually laying all eggs in one cocoon during this period. The juvenile animals appear in November. A special feature of the life-history is the prolongated, inactive state of the worms from April or May until July or August, when, without feeding, they retreat to the deeper layers of the soil and agregate into smaller groups. The most intresting characteristic, never before observed in Enchytraeids, is the gradual reduction of the sexual organs after oviposition and rejuvenating process, a phenomenon defined here as a n a n e o s i s.

ZUSAMMENFASSUNG

Ananeose, eine neue Erscheinung im Leben der Enchytraeiden (Oligochaeta)

Die Autorin gibt in diesem Aufsatz einerseits die Revision der Art *Stercutus niveus* MICH.' 1888 an, andrerseits beschreibt sie den Entwicklungsgang dieser eigenartigen Enchytraeidenart' woraus es hervorgeht, daß die Art nur vom Ende Juli bis Oktober geschlechtsreif ist, als sie nämlich ihre Eier (1—15 je Tier) in Kokons legt. In den meisten Fällen legt eine jedes Tier seinen ganzen Eibestand in ein Kokon. Die juvenilen Tiere erscheinen dann im November. Eine interessante Eigenschaft dieser Art bildet, daß diese Würmer von April—Mai bis Juli—August in einen langen inaktiven Zustand verharren, wobei sie keine Nahrung zu sich nehmen und sich in die tieferen Schichten des Bodens zurückgezogen in kleinere Gruppen zusammentun. Der interessanteste Zug der Ökologie der Art, der bei den Enchytraeiden noch niemals beobachtet wurde, ist daß bei dem Tier allmählich, nachdem es seine Eier gelegt hat, eine Regression der Geschlechts, organe erfolgt und ein Verjüngerungsprozeß einsetzt, den die Autorin „ A n a n e o s e “ (Ananeosis) nennt.

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