Rhabditis adenobia sp. n. (Nematoda: Rhabditidae) from the Colleterial Glands of *Oryctes monoceros* L. and Other Tropical Dynastid Beetles (Coleoptera: Scarabaeidae)

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During investigations of the nematode associates of *Oryctes rhinoceros* L. and other tropical dynastid beetles, a study was conducted on the nematodes inhabiting the colleterial glands of these coleoptera.

Hoyt (1962a, b) was one of the first to record nematodes from the colleterial glands of dynastid beetles in Africa and New Guinea (Table 1) and his and subsequent reports led to the present investigation on the taxonomy and biology of these nematodes and their effect on the host.

In attempts to determine the source of nourishment of nematodes developing in the colleterial glands, a detailed study of the glands was undertaken to better understand their function.

Materials and Methods

Nematodes were removed from the colleterial glands and aedeagi of living *Oryctes rhinoceros* L. from Malaysia; *O. monoceros* Ol., *O. boas* F., and *O. owariensis* Beauv. from West Africa and *Xylotrupes gideon* L. and *Scapanes australis grossepunctatus* Endrödi from the territory of Papua and New Guinea, and placed directly on sterile artificial media. This medium was made by mixing 150 grams of ground Gaines Gravy Train dog food briquets with 7.5 grams of water agar and 500 ml water. This mixture was then placed in screw cap test tubes, autoclaved and could be used after several months storage. Each tube supported heavy nematode populations for one month at which time transfers could be made. The nematodes fed on assorted bacteria introduced with them during the transfer.

For detailed studies of certain aspects of the relationship between these nematodes and tropical beetles, *O. monoceros* in the Ivory Coast was selected because of its abundance and high incidence of nematode infestation.

The colleterial glands of *O. monoceros* with the associated nematodes were removed entire and fixed in cold Bouin's for histological sections. After embedding, sections were cut at 7 μ and stained with crystal violet and Heidenhain's iron hematoxylin with 1% eosin in 90% alcohol.

For ultrastructural investigations of the colleterial glands of *O. monoceros*, the entire gland was fixed for 2 hr in 1% osmium tetroxide, buffered with 0.1 M phosphate buffer, then dehydrated and embedded in araldite. Sections were cut with a Porter-Blum MT-2 microtome and stained with uranyl acetate and lead citrate. They were examined with an RCA-3F electron microscope.

For crossing experiments between nematodes from different sexes and species of beetles, individual juvenile nematodes were placed in isolated depression cells with a small amount of dog food medium. After reaching the adult stage, pairs were placed together and observations made during the following five days.

Results

Besides occurring in the colleterial glands of female beetles, nematodes were also found in the passages of the endophallic tube of male beetles. Nematodes removed from both sexes of the 6 species of dynastid beetles developed well on artificial media. Specimens from the colleterial glands and aedeagi of *O. monoceros* were found to be the same species after morphological examinations and interbreeding tests were conducted. This species was considered new to science and a description follows below. All measurements are given in microns.

Rhabditis (*Rhabditis*) *adenobia* sp. n. (Figs. 1 and 2)

Rhabditoidea (Örley, 1880) Travassos, 1920; Rhabditidae Örley, 1880; Rhabditis Dujardin, 1845 as defined by Goodey (1963).

GENERAL CHARACTERISTICS: Lips closed,

Table 1. A list of dynastid beetles reported to contain nematodes in their colleterial glands.

Beetle	Locality	Reference
Oryctes blucheaui Frm.	Madagascar	Bedford (1968b)
O. boas F.	Ivory Coast	Poinar (present study)
O. centaurus Sternb.	Papua and New Guinea	Hoyt (1962a) Paine (1966)
O. gigas Cast.	Madagascar	Bedford (1968b)
O. gnu Mohn	Malaysia, Borneo	Paine (1966)
O. insularis Coq.	Madagascar	Bedford (1968b)
O. monoceros Ol.	East Africa	Hoyt (1962b)
"	Ivory Coast	Mariau (1967)
"	Ivory Coast	Poinar (present study)
O. owariensis Beauv.	Ivory Coast	Poinar (present study)
O. pyrrhus Burm.	Madagascar	Bedford (1968b)
O. ranavalo Coq.	Madagascar	Bedford (1968b)
O. rhinoceros L.	India and Ceylon	Paine (1966)
"	Maldive Islands	Paine (1966)
"	Malaysia	Paine (1966), Poinar (present study)
"	Borneo and Timor	Paine (1966)
"	Papua and New Guinea	Bedford (1968a)
"	Mauritius	Bedford (1968b)
O. simiar Coq.	Madagascar	Bedford (1968b)
Scapanes australis grossepunctatus Endrödi	Papua and New Guinea	Bedford (1968a)
"	Papua and New Guinea	Poinar (present study)
Xylotrupes gideon L.	Papua and New Guinea	Bedford (1968a)
"	Papua and New Guinea	Poinar (present study)
X. lorquini Schauf.	Papua and New Guinea	Hoyt (1962a)

metarhabdions isomorphic, each bearing 5 tubercles. Esophagus without a median bulb, the corpus gradually widening just before the isthmus. Hemizonid present. Lips without setae or bristles, but small sensorial papillae are visible under high magnification. Amphids pore-like on lateral lips.

MALE (n = 15). Length 926 (768–1,248); greatest width 45 (31–77); length of esophagus 203 (177–233); length stoma 18 (17–21); length esophageal collar 6 (4–9); length free portion of lips 3 (2–4); length head to hemizonid 166 (139–200); length head to excretory pore 173 (150–205). Tail leptoderan, 46 (40–52) long and 27 (23–38) wide. Spicules paired, separate, with well pronounced head and slight arch, 47 (40–53) long and 9 (6–13) wide at the widest portion. Length gubernaculum 21 (20–26); length bursa 75 (65–98), open, with 9 bursal papillae, 2 of which are preanal, 1 adanal, and the rest postanal. The last pair is greatly reduced and the 2nd, 5th, 7th, and 9th pair do not meet the rim of the bursa. Anal papillae (tubercles, knobs) rudimentary. a = 16.2–24.8; b = 4.3–5.6; c = 19.2–24.0.

FEMALE (n = 15). Length 1,200 (1,056–1,296); greatest width 55 (46–69); length of esophagus 223 (208–239); length stoma 21 (18–23); length esophageal collar 6 (5–8); length head to hemizonid 185 (169–208); length head to excretory pore 195 (177–211); length free portion of lips 3 (2–4); vulva median 53 (49–56)% of body length from head; reproductive system amphidelphic, ovaries reflexed, distance from extended portion of anterior ovary to vulva 369 (267–423); distance from extended portion of posterior ovary to vulva 377 (285–447); tail conical, tapering to a fine point, length 102 (93–115); tail width 29 (25–31). a = 18.8–22.9; b = 5.1–5.4; c = 11.3–11.8.

TYPE LOCALITY: Abidjan, Ivory Coast, West Africa.

TYPE HOST AND LOCATION: Found in the colleterial glands of the adult female and the aedeagi of the adult male of *Oryctes monoceros* Ol.

TYPE SPECIMEN: Deposited in the U. S. Department of Agriculture Nematode Collection, Beltsville, Maryland. Holotype (♀) T-187t; allotype (♂) T-188t, and paratypes, T-847p.

Diagnosis

The characters of *Rhabditis adenobia* place this species in the subgenus *Rhabditis* of the genus *Rhabditis*. Besides having the characters of this subgenus, *R. adenobia* possesses a long, narrow stoma, never more than half enclosed by the esophageal collar, and an esophagus lacking a median bulb. *R. adenobia* reproduces amphimictically and the males possess an open, leptoderan bursa with 9 pairs of bursal papillae. This combination of characters separates it from most of the described species in the genus *Rhabditis*.

R. adenobia can be separated from *R. korneri* Osche, 1952 which has distinct anal tubercles, anisomorphic metarhabdions and a longer male tail.

The species most similar to *R. adenobia* are *R. lucianii* Maupas, 1919 and *R. terrestris* Stephenson, 1942. However both of the latter species have a fully developed ninth bursal papilla and *R. terrestris* possesses a slight median

bulb while *R. lucianii* has a proportionally longer esophageal collar and the female tail is not as drawn out as in *R. adenobia*. Also, the rectum of *R. lucianii* is ⅔ the length of the tail, which is not the case with the species described here.

Biology and Host Relationship

Approximately 70% of the female beetles examined contained *Rhabditis adenobia* in their colleterial glands while 50% of the male beetles contained this nematode in their endophallic tubes.

A few words should be said about the nature of the colleterial glands in these beetles in order to shed light on how the secretions might be used as a source of nourishment by the nematodes. Unfortunately very little is known about colleterial glands of insects in general, and those of *Oryctes* have never been investigated. Snodgrass (1935) considers colleterial glands similar to accessory glands and cites their function as secreting an adhesive substance that attaches the egg to a substrate. Since *Oryctes* spp. deposit their eggs singly in soil or debris, these glands may have yet another function. In all species of *Oryctes* reported here, the females contained 2 pairs of colleterial glands situated on opposite sides of the vaginal wall (Fig. 2, E). The distal member of each pair (designated as gland A) was crescent shaped and varied in color from light to dark brown in *O. monoceros*. The outer surface of gland A was nearly smooth and covered with an apparent sclerotized layer. Histological sections showed tubular extensions of the hypodermal cells extending to the surface of the gland, suggesting a possible deposition route of the sclerotized material (Fig. 7). The function of these glands is unknown.

The proximal gland of each pair (designated as gland B) was mushroom or doughnut-shaped and varied from white to light brown in *O. monoceros*. Because of their color, they were sometimes difficult to distinguish from the vaginal wall. Large tracheal trunks entered these glands through a central cavity (Fig. 3). The outer surface of gland B was composed of numerous vesicles containing a viscous-like material (Figs. 5, 6). Beneath the vesicles lay several rows of glandular cells which in turn surrounded an inner lumen. Electron micrographs showed the vesicle walls to be lined

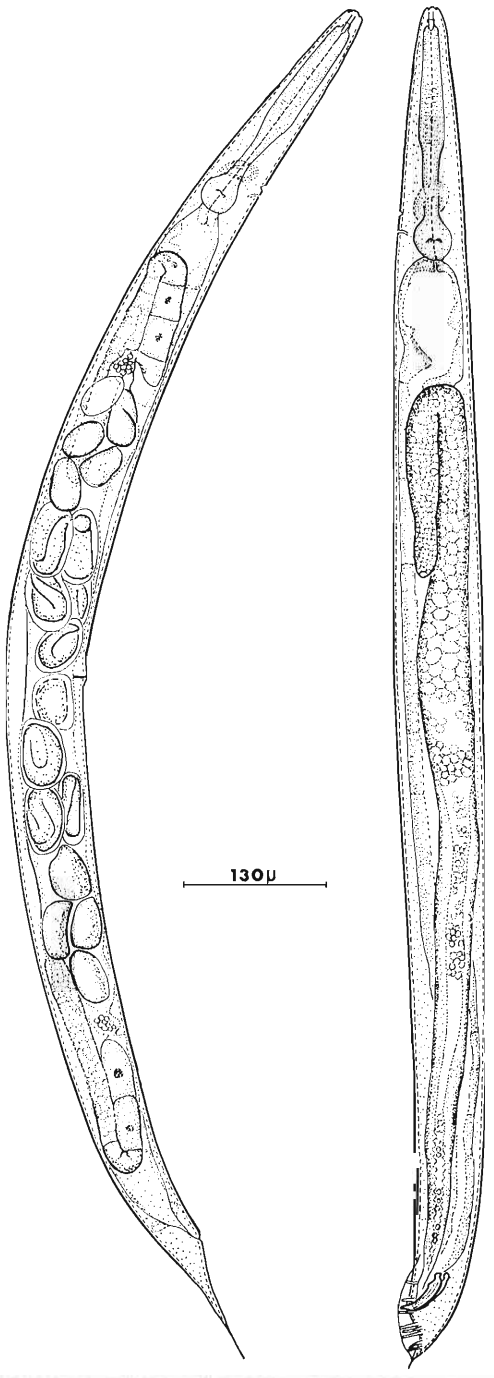
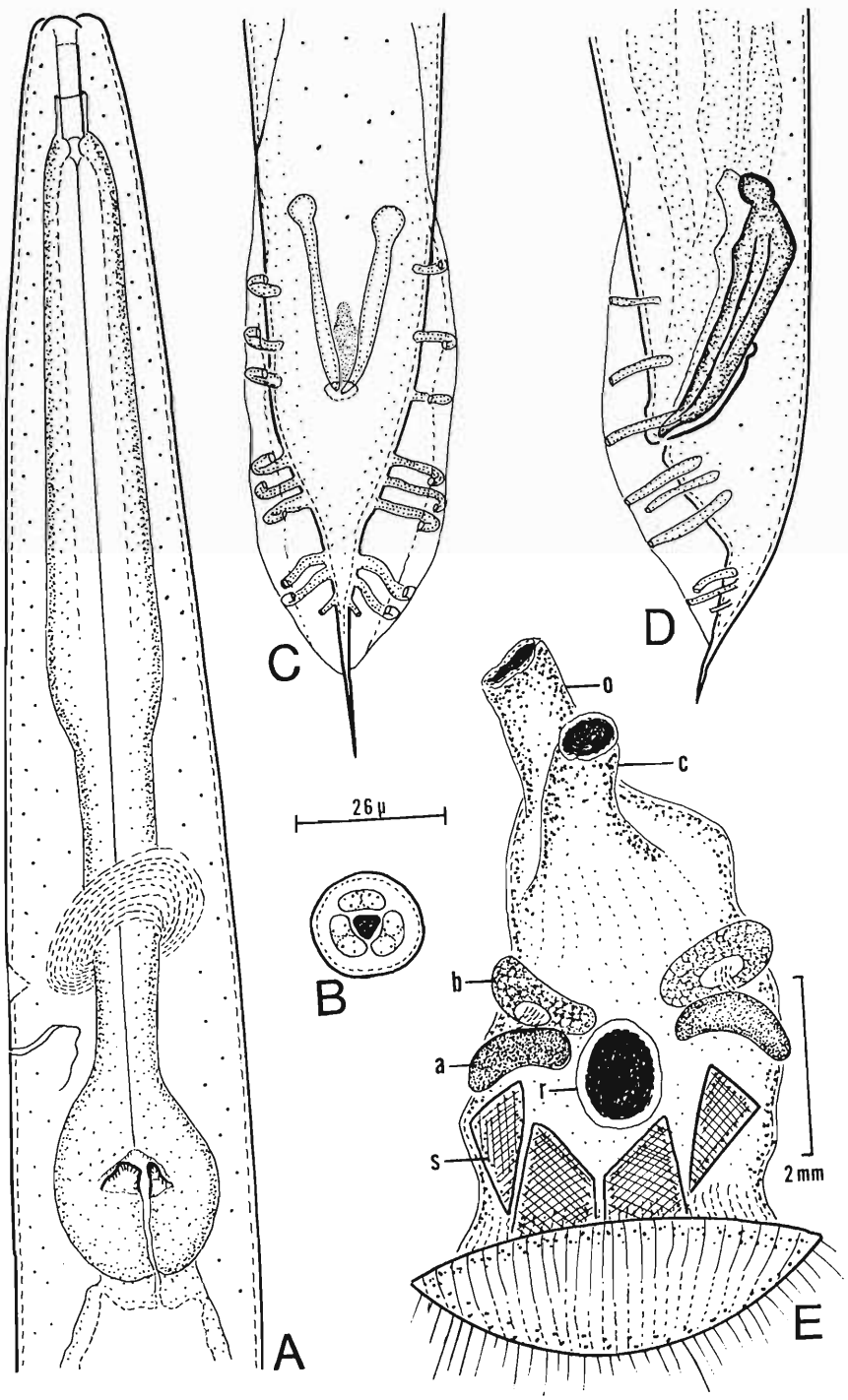


Figure 1. Male and female of *Rhabditis adenobia* sp. n.



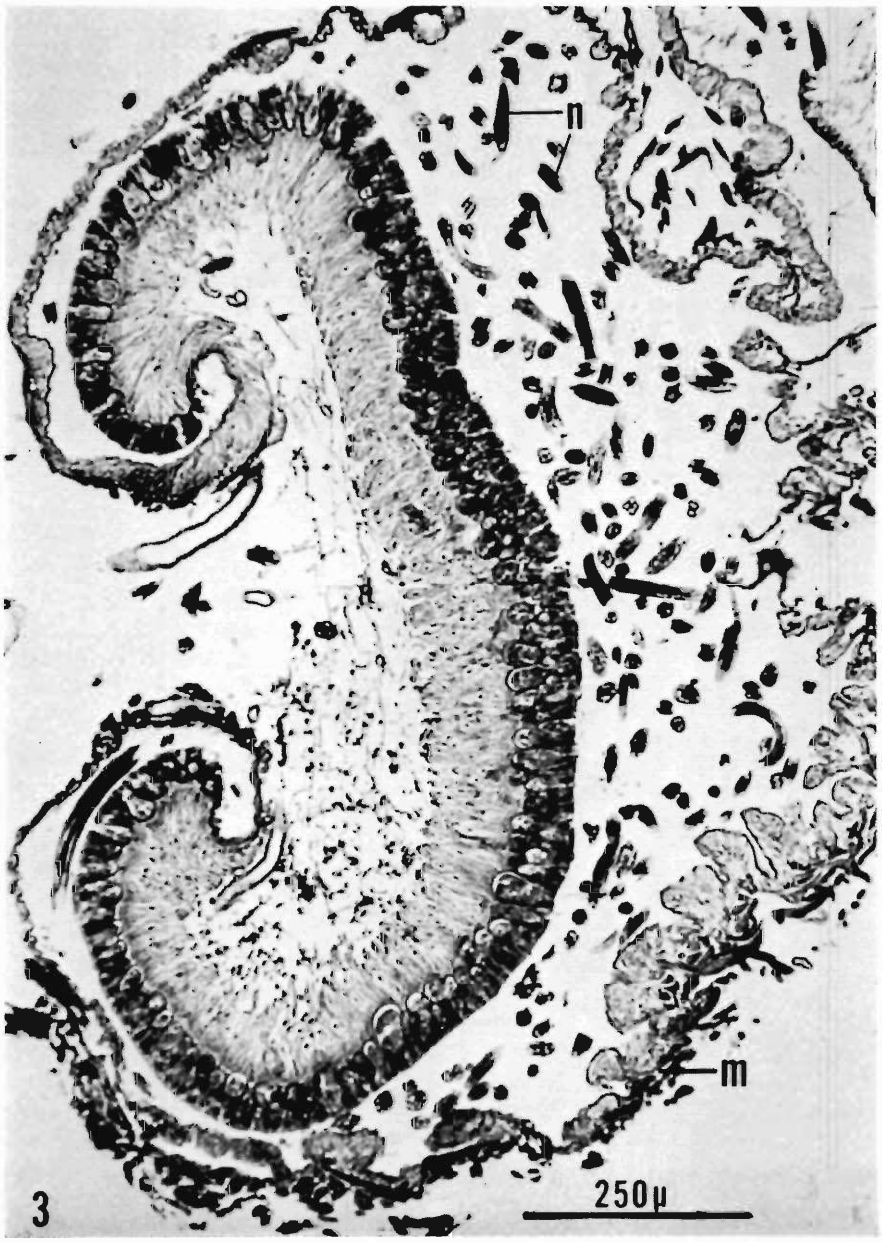


Figure 3. Histological section of gland B of *O. monoceros* showing populations of *R. adenobia* within the surrounding membrane (m); (n = nematodes).

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Figure 2. *R. adenobia* sp. n. A. Anterior portion of male. B. En face view of male. C. Ventral view of male tail. D. Lateral view of male tail. E. Internal genital area of a female of *O. monoceros*. (o = common oviduct; c = stalk of bursa copulatrix; a = gland A; b = gland B; r = rectum; s = sclerite.)

with droplets, similar in appearance to mucous secretions (Fawcett, 1966) (Figs. 8, 9). These droplets probably coalesce and coat the egg as it passes through the vagina. Bacteria were also found in the vesicles (Fig. 9) and it is not known if these represent some type of symbiont which is important for larval nutrition or if these are simply "opportunists" that came in independently or with the nematodes.

A common membrane surrounded each gland pair and the secretions of both glands reached the vagina through a common duct. Most of the nematodes occurred within the membrane adjacent to gland B (Fig. 3). However, when numbers were high they sometimes occurred within gland B (Fig. 5) and were also found in the proximity of gland A (Fig. 4).

In *O. monoceros*, reproducing nematodes, as well as normal juveniles or dauer stages, were frequently encountered in the colleterial glands, although the latter stages were more common. Within the glands, the nematodes probably fed on both the glandular secretions, especially from gland B, and the bacteria that were associated with these glands. When reproducing forms were present, nematodes could sometimes be found in the vagina and even in the bursa copulatrix. It is not known why some beetles contained adult nematode populations and others only dauer or normal juveniles. When the female beetles died of natural causes, the nematodes were sometimes able to reproduce in the cadaver; yet conditions were not always favorable, since in many instances, the nematodes died within the glands. In the male beetles, only dauer or non-dauer juveniles were found in the aedeagal glands and in *O. monoceros*, these nematodes were usually associated with another nematode, *Oryctonema genitalis* Poinar, 1970.

Transmission of *R. adenobia* from host to host, as with the bursa-inhabiting nematode, *O. genitalis*, was accomplished during mating of the beetles. A male beetle picks up the nematodes in the aedeagal passages after mating with an infected female and then deposits the nematodes in the vagina or bursa copulatrix of a non-infested female. The nematodes then make their way into the colleterial glands. It is possible that the dauer stage (non-feeding 3rd stage juvenile) is able to enter the colleterial glands by invading the vagina directly from the environment; however, this was never observed and *R. adenobia* was never collected from the beetle environment. Although these nematodes could develop well on artificial media, they were not found in grass, water agar or artificial media that contained adult females and males of *O. monoceros* for 1 week, even though some of the beetles died after 3 days and there was ample opportunity for the nematodes to leave the beetle and enter the substrate.

Breeding tests conducted between nematodes taken from the colleterial glands of *O. monoceros* and *O. owariensis* were successful and since nematodes from both sources were similar morphologically, they are regarded as the same species. Although *R. adenobia* was morphologically similar to nematodes occurring in the colleterial glands of *O. boas*, crossing attempts were unsuccessful and the identity of the forms in *O. boas* remains unclear at this time.

No injurious effect on either sex of *O. monoceros* could be determined due to the presence of *R. adenobia* during the course of this investigation. Although the function of the colleterial glands may be affected when nematode populations are high, it is not known what significance this may have on the development of the beetle.

Discussion

Members of the family Rhabditidae generally have a simple life cycle, surviving in the

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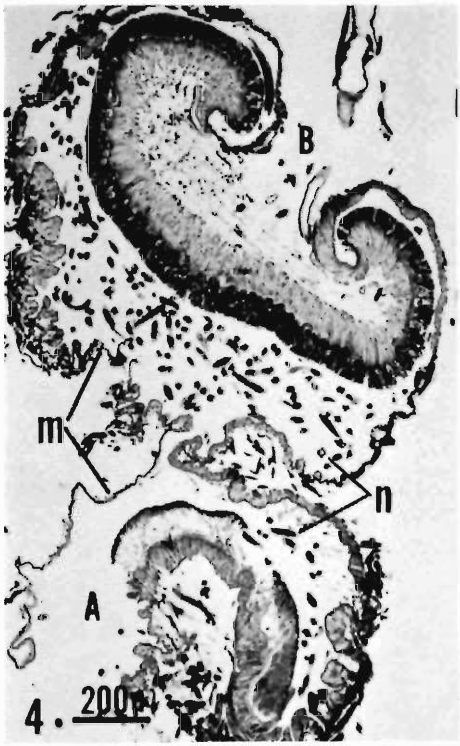
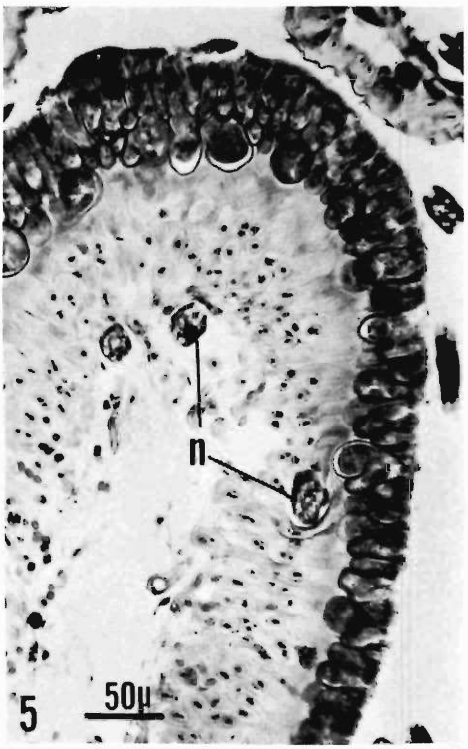
Figure 4. Histological section showing the common membrane (m) surrounding colleterial glands A and B in *O. monoceros*; (n = nematodes).

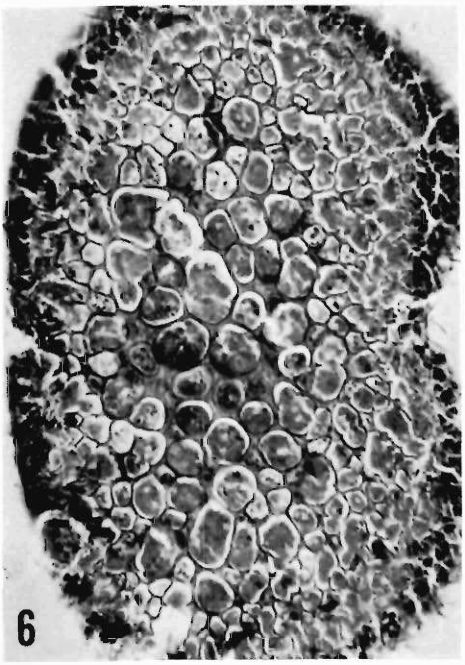
Figure 5. Histological section of gland B in *O. monoceros* showing nematodes (n) within the glandular cells.

Figure 6. Histological section showing the surface of gland B covered with minute vesicles; (magnification same as Fig. 5).

Figure 7. Histological section of gland A in *O. monoceros* showing tubular extensions of the hypodermal cells (e).







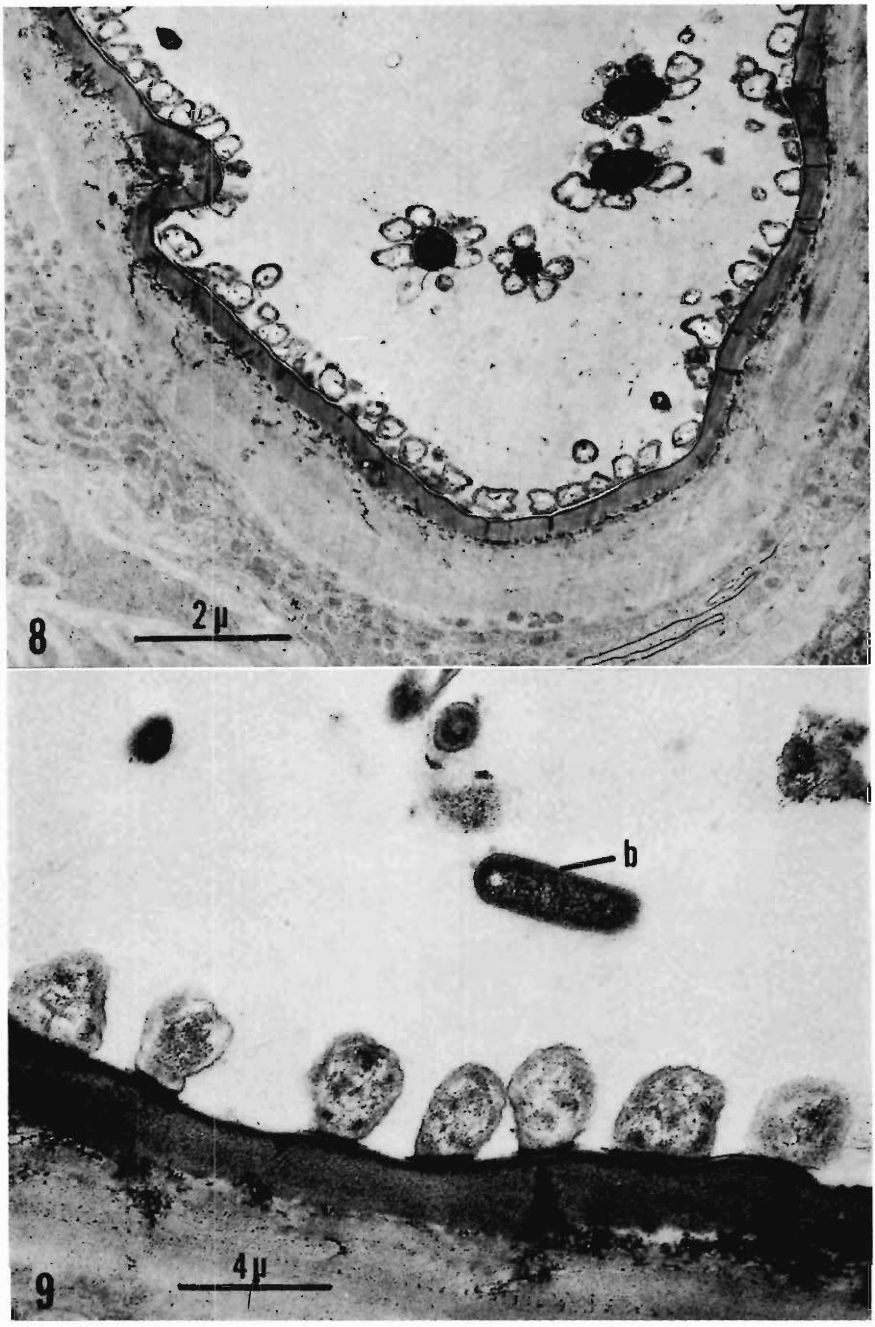


Figure 8. Electron micrograph showing droplets lining the surface of a vesicle wall of gland B in *O. monoceros*.

Figure 9. Electron micrograph showing the similarity of droplets on the vesicle wall of gland B to mucous secretions; (b = bacterium).

soil where they are frequently associated with decomposing plant and animal matter. Many possess the ability to survive under a wide range of physical and chemical conditions.

Occasionally, representatives are found that have or are in the process of adapting at least a portion of their life to an invertebrate or vertebrate. In many instances, they are not able to take full nutritional advantage of the new host and must eventually return to the soil to complete their life cycle.

Some rhabditids e.g. *R. pellio*, exploit earthworms as a source of nourishment, yet, at their present evolutionary state, only one or two larval stages occur in the body cavity or excretory system of the living annelid. They are unable to complete further development until the earthworm dies and conditions become similar to their original habitat. It is obvious that these nematodes have not yet evolved the resources for developing within living annelids, yet we know this is possible, since adults of another group of nematodes, the Drilonematoidea, do occur in the body cavity of living earthworms and probably are able to obtain their complete nourishment from this habitat.

An association with a vertebrate host has occurred with *Pelodera strongyloides* (Schneider). The dauer and post dauer stages of this nematode occur in the orbits of murid rodents (Poinar, 1965) where some growth occurs, although the nematode must enter the soil again before reaching maturity. Similarly, it is a case of not being able to completely exploit the environment, rather than the environment being nutritionally deficient, since eye worms of the genus *Thelazia*, etc. are able to mature and reproduce in this specialized habitat.

In contrast, however, all stages of the rhabditid, *Oryctonema genitalis* occur in the bursa copulatrix of the coconut beetle, *Oryctes monoceros*, and have adapted so completely to this environment that they no longer are able to revert back to their original free-living habits (Poinar, 1970).

With *R. adenobia* and similar forms, complete dependence on conditions within the colleterial glands has not yet occurred, although the association appears to be heading in this direction. This hypothesis is based on the following observations. First, reproducing colonies of *R. adenobia* occasionally could be found within the membrane surrounding the colleterial glands—indicating that nutrients in this locality were being utilized by the nematodes. Secondly, *R. adenobia* was never found apart from the beetle and colonies within the glands often died after the beetle succumbed, indicating their inability to withstand the altered conditions of the dead beetle. Thirdly, the dauer stage of *R. adenobia* was nonspecialized in comparison with many free-living rhabditids. It was not surrounded by an ensheathing cuticle, nor was the mouth always completely closed. Since the normal functions of the dauer stage are to carry the species over periods when the food supply is limited or facilitate the invasion of hosts, either for transport or nourishment, we can better understand this lack of specialization here. Indeed, the conditions within the colleterial glands are constant—there would be a continuous supply of secretions as long as the beetle survived and the natural copulatory habits of the beetles have replaced the need for an invasive stage. As the normal functions of the dauer no longer become necessary, it seems logical that factors involving their formation would become lost from the population.

For these reasons, it is suspected that *R. adenobia* is in an evolutionary stage between a free-living form and a species obligately associated with the colleterial glands of dynastid beetles. Its ability to survive on specially prepared media with bacteria illustrates its close ties with the free-living nematodes. Such an obligate association already has occurred with the rhabditid, *Oryctonema genitalis*, which multiplies in the bursa copulatrix of certain dynastid beetles (Poinar, 1970). These examples point out the adaptive ability of rhabditid nematodes.

It is possible that when nematode populations within the colleterial glands become large, some damage to these organs may occur. This could result in a reduction of secretions from gland B and if the secretions play an important role in egg development (perhaps preventing infection by soil organisms) then this could have an influence on general viability. Again, if the bacteria surrounding the glands are some type of symbionts (although there is no proof of this now) which eventually end up in gastric caeca of the larval midgut, they could play an important role in larval nutrition. If the nematodes, by feeding,

interfere with the supply of bacteria, larval development could be affected. However before either of these hypotheses can be proven, the exact function of the colleterial glands in these beetles should be thoroughly elucidated.

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