

Description and Observations on a Cuticular Infection of *Thelastoma pterygoton* sp. n. (Thelastomatidae: Nematoda) from *Oryctes* spp. (Scarabaeidae: Coleoptera)

GEORGE O. POINAR, JR.

Division of Entomology, University of California, Berkeley 94720

ABSTRACT: *Thelastoma pterygoton* sp. n. is described from larvae of *Oryctes monoceros* Ol. from Abidjan, Ivory Coast, West Africa. Distinguishing characters of *T. pterygoton* are the female lip cone distinctly set off from the rest of the body, the excretory pore located behind the basal bulb in both sexes, the presence of large lateral alae in the male, and the third pair of male anal papillae not fused as in other species in the genus.

T. pterygoton also occurs in *Oryctes boas* F., but in this host the adult nematodes often have a cuticular infection caused by bacteriallike microorganisms closely appressed to the cuticle. Since all studied material was fixed, the microorganisms were not cultured, but resembled both bacteria and mycoplasma-like bodies. They appeared to dissolve the outer portion of the nematode's cuticle.

During a study on the nematode parasites and associates of members of the beetle genus *Oryctes* attacking coconut palms, thelastomid nematodes were recovered from the intestine of third-stage larvae of *Oryctes rhinoceros* L. in Western Samoa, Malaysia, and New Guinea and from *O. boas* F., *O. monoceros* Ol., and *O. owariensis* Beauv. from West Africa.

The larvae of *Oryctes* feed on decaying organic matter. The developmental biology of *O. rhinoceros* has been intensively studied and is probably more or less similar to that of other *Oryctes* species collected here. The larval period of *O. rhinoceros* lasts from 3 to 6 months and most of this time is spent in the third or last larval instar. The larvae occur in a variety of habitats, including decaying coconut trunks and other logs, sawdust, compost, and cattle dung. While the adult beetles cause considerable damage to palms, the larvae are innocuous except occasionally damaging timber posts and stumps set in the ground (Catley, 1969).

Because of the abundance of material, the nematodes found in the larvae of *Oryctes monoceros* and *O. boas* in West Africa were extensively studied and found to be undescribed. The present paper describes this species, discusses its morphology in comparison with other members of the genus, and describes a cuticular infection that occurs on this nematode.

Materials and Methods

Nematodes were removed from the intestine of third-stage field-collected larvae of *Oryctes monoceros* Ol. and *O. boas* F. from Port Bouet, Ivory Coast, West Africa. They were heat-killed, fixed in TAF (triethanolamine, formalin, and water), and processed to glycerin.

For electron micrograph studies of the cuticular region, specimens of *T. pterygoton* were prefixed in 1.5% glutaraldehyde for 2 hr, then washed in 0.05 M phosphate buffer and fixed in 1% osmium phosphate buffer (pH 7) for 1 hr. After dehydration in a graded ethanol series, the specimens were embedded in Araldite 6005 and sectioned with glass knives in a Porter-Blum microtome (MT-2). The sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Phillips EM-300 electron microscope.

Results and Discussion

Nematodes removed from the third-stage larvae of both *O. monoceros* and *O. boas* belong to the same species, which was undescribed. In the quantitative portion of the following description of this species, the first figure after the character represents the average value for that character, while the numbers in parentheses represent the range. All measurements are in millimeters.

Thelastoma pterygoton sp. n.

(Thelastomatidae: Oxyuroidea) (Figs. 1–2)

ADULTS: Head with eight distinct labial papillae and paired amphids; stoma simple, pharynx composed of a corpus, isthmus, and valvated bulb; tail of both sexes filiform, vulva near middle of body; ovaries paired; spicules present; male with four pairs of caudal papillae, well-developed lateral alae, and genital cone.

FEMALE ($n = 10$). Body covered with cuticular annulations approximately 8μ apart; width of head at first annule 0.046 (0.037 – 0.054); distance of first annule from head 0.026 (0.024 – 0.031); total length 2.16 (1.41 – 2.95); width near vulva 0.18 (0.11 – 0.27); lip cone [width 0.025 (0.021 – 0.028); height 0.010 (0.009 – 0.012)] set off from remainder of body by a deep constriction; lip region slightly eight-lobed, bearing a row of eight terminal papillae and two emphids located laterally; an inner row of six papillae is also present. The sclerotized stomatal ring (= mouth cylinder = buccal cavity = vestibule wall) was 0.011 (0.010 – 0.012) long and 0.010 (0.009 – 0.011) wide. It is lined by an internal layer of pharyngeal tissue which is expanded at the base into three roughened areas. A faint ring of tissue just above the stomatal ring may represent the cheilorhabdions. Pharynx slender 0.39 (0.32 – 0.44) long with a constriction just above the basal bulb; nerve ring 0.20 (0.17 – 0.21) from head, located approximately in the center of the pharynx; body width at nerve ring 0.10 (0.08 – 0.14); excretory pore 0.44 (0.37 – 0.54) from head, approximately 0.04 behind the basal bulb; width of body at excretory pore 0.15 (0.10 – 0.24). Ventricular portion of intestine expanded and containing bacterial cells; % vulva 51 (48 – 54); length vagina 0.170 (0.125 – 0.229); spermatheca present at tip of uterine branches; ovary tips outstretched or reflexed; uterus containing eggs in various stages of division; tail length 0.49 (0.32 – 0.62); width

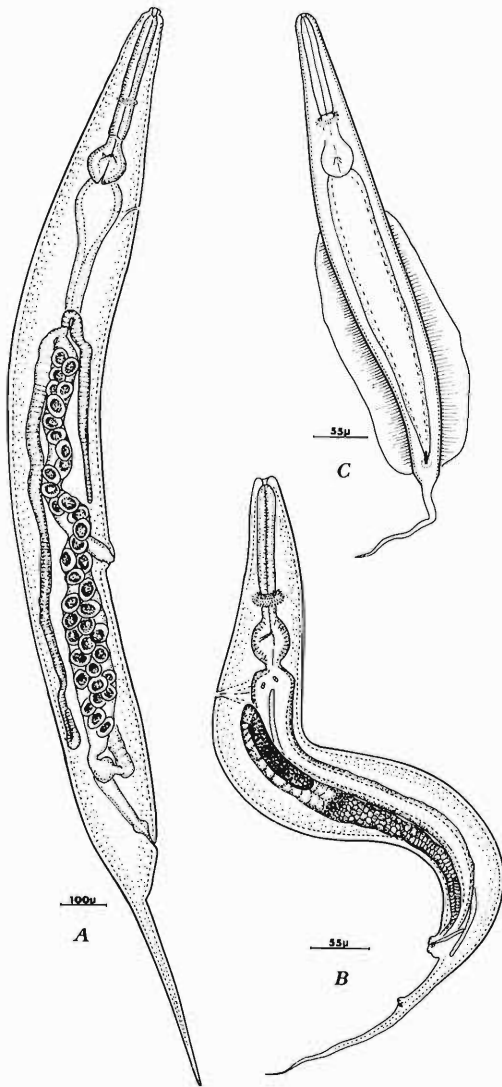
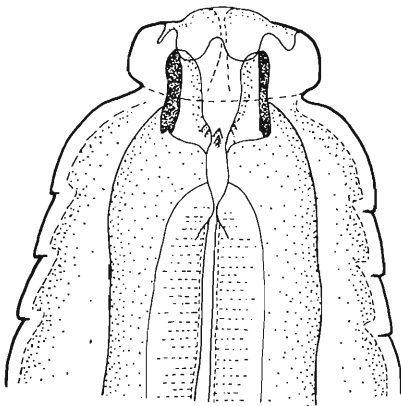
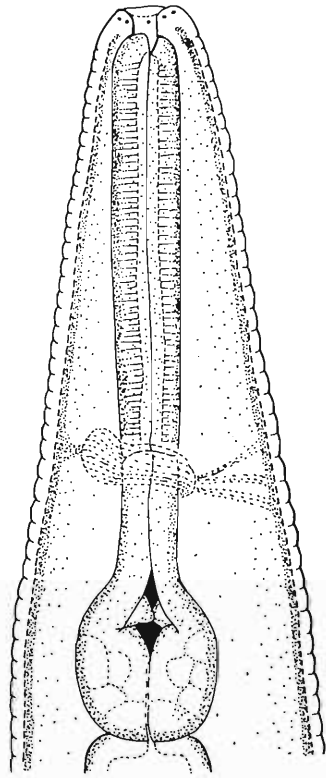


Figure 1. *Thelastoma pterygoton* sp. n. A. Lateral view of adult female. B. Lateral view of adult male. C. Ventral view of adult male showing enlarged lateral alae.

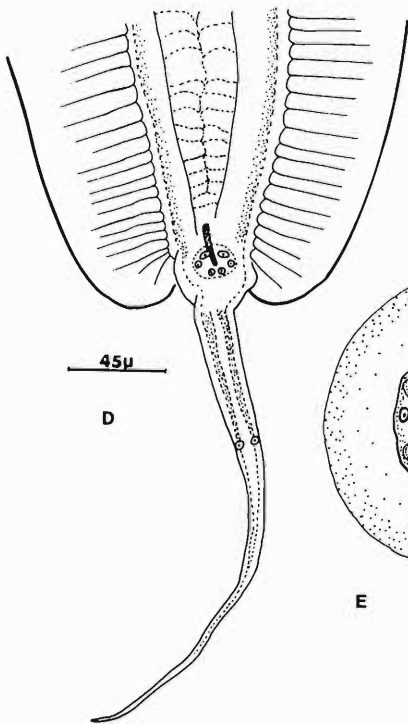
Figure 2. *Thelastoma pterygoton* sp. n. A. Lateral view of female head. B. Lateral view of pharyngeal region of male. C. Lateral view of tail region of male. D. Ventral view of tail region of male. E. "En face" view of female.



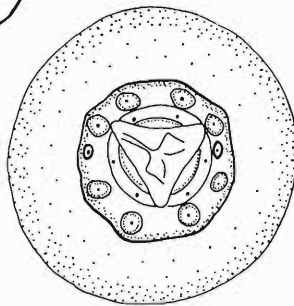
10 μ



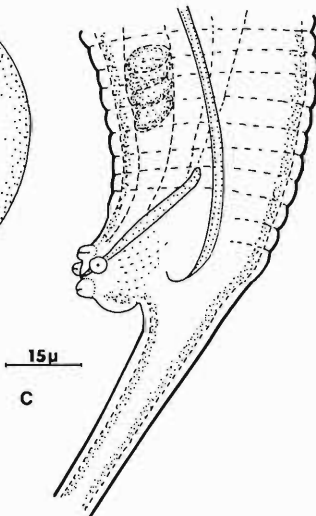
15 μ



45 μ



13 μ



15 μ

at tail 0.10 (0.08–0.13); tail extended into a narrow spine 0.39 (0.27–0.51) long.

MALE ($n = 8$): Body covered with cuticular annulations approximately 4–5 μ apart; total length 0.80 (0.69–0.98); greatest width 0.08 (0.07–0.11); lips fused but not set off from rest of body as a cone; eight terminal papillae—inner papillae not seen; length stoma 0.004 (0.003–0.005); width stoma 0.004 (0.003–0.006); pharynx slender, 0.15 (0.14–0.16) long, with constriction (isthmus) located farther above the basal bulb than in the female; nerve ring located in the posterior half of the pharynx, 0.09 (0.08–0.10) from the head; width of body at nerve ring 0.06 (0.05–0.07); excretory pore 0.20 (0.17–0.25) from head, approximately 0.03 behind the basal bulb; body width at excretory pore 0.08 (0.06–0.10); testis single, almost extending to excretory pore, reflexed at tip; spicule single, 0.034 (0.031–0.035) long, 0.002 (0.002–0.003) wide; anal area forming a genital cone [length 0.011 (0.010–0.014)] containing three pairs of papillae. The postanal pair are separate—not fused as in other thelastomids; the fourth pair of papillae are located at $\frac{1}{3}$ of the length of the tail spine; distance from the base of the spine to the fourth pair of papillae 0.050 (0.041–0.058); distance from the fourth pair of papillae to the tip of the tail spine 0.141 (0.129–0.166); length total tail spine 0.20 (0.18–0.23); length tail 0.21 (0.19–0.24); body width at tail 0.04 (0.03–0.05); lateral alae from 0.028–0.050 wide, begin at the level of the ventricular portion of the intestine and extend to just above the base of the tail spine.

TYPE HOST: *Oryctes monoceros* (Ol.) (Coleoptera: Scarabaeidae).

TYPE LOCALITY: Abidjan, Ivory Coast, West Africa.

TYPE SPECIMEN: Deposited in the USDA Nematode collection, Beltsville, Maryland [Holotype (male), T-211t; Allotype (female), T-212t].

Diagnosis

The female of *Thelastoma pterygoton* possesses a distinct lip cone well set off from the body, the excretory pore behind the basal bulb, the nerve ring situated in the middle of the pharynx, and the tail less than one-quarter

the total body length. The male contains an excretory pore also behind the basal bulb and possesses large lateral alae. The third pair of anal papillae just beneath the anus are separate. These latter two characters separate *T. pterygoton* from other males in the genus. Aside from being twice the size, *T. robustum* Christie (1938) possesses an eight-lobed lip cone that is not so distinctly separated from the rest of the body as in the present species. Also, the tail and pharynx of the male *T. pterygoton* are proportionally longer than in *T. robustum*.

The anteriorly placed excretory pore and nerve ring and the protruding vulva in the female of *T. alatum* Johnston (1914) separate it from the present species. The shape of the lateral alae and proportionally smaller tail in the male of *T. alatum* also separate it from *T. pterygoton*.

The larger size of the female of *T. toxi* van Waerebeke (1970) as well as its small lip cone and the presence of cuticular protuberances on the male distinguish it from *T. pterygoton*.

The excretory pore in the female of *T. macramphidum* Christie (1931) lies anterior to the basal bulb and the lip cone is not set off as in *T. pterygoton*. The head region of the male of *T. macramphidum* is set off by a constriction and contains narrower alae (4 μ) and a longer spicule (40–55) than *T. pterygoton*.

The female of the variety *gallica* Théodoridès (1955) of *T. macramphidum* possesses the lip cone less separated from the body than *T. pterygoton*. The male of variety *gallica* Théodoridès and three forms of this variety were described by Jarry (1964). The lateral alae of var. *gallica* extend to the nerve ring and the male tail is proportionally smaller than in *T. pterygoton*. The junction of the corpus and isthmus in the male *gallica* is further anterior than in *T. pterygoton*. However, the variety *gallica* itself differs from the typical *T. macramphidum* by having larger lateral alae (15–25 μ), a smaller spicule (28–30 μ), and the excretory pore in the female opening behind the basal pharyngeal bulb. Thus, *T. macramphidum*, as defined by Jarry (1964), is a variable species. Jarry and Jarry (1968)

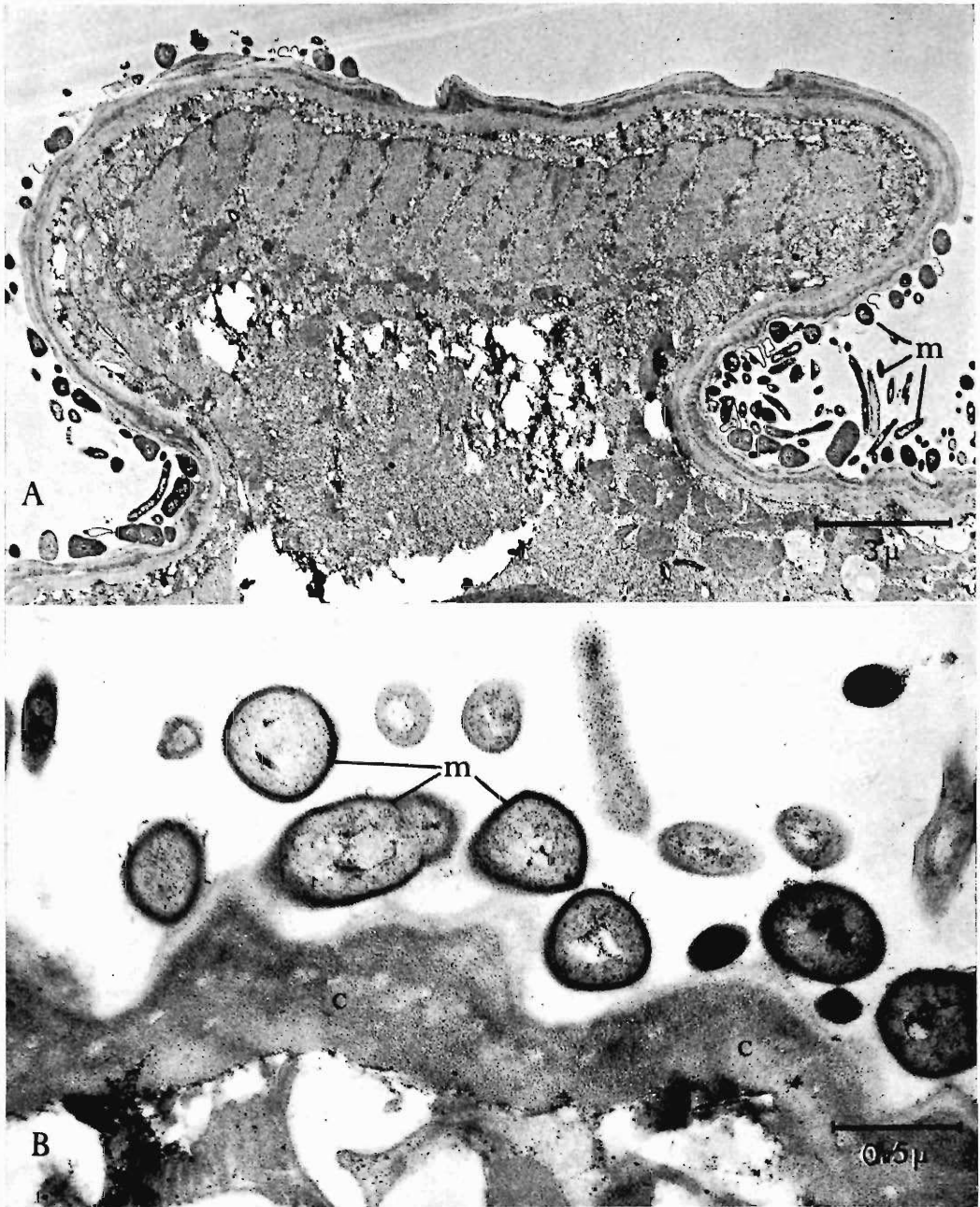


Figure 3. Cuticular infection of *Thelastoma pterygoton* sp. n. A. A portion of the body partially constricted from the remainder with associated microorganisms (m). B. Irregular grooves in the cuticle (c) and associated microorganisms (m).

feel that most of the described species in the genus *Thelastoma* are only varieties and have reduced all described species to three, *T. macramphidum*, *T. robustum*, and *T. attenuatum*, based on the position of the excretory pore in the female and the position of the genital papillae in the male. This can be determined with certainty only after additional studies have been conducted. Investigations on the morphological variation and interbreeding of thelastomatids is a challenging goal for the future. The separated pair of postanal papillae in the male of *T. pterygoton* is a diagnostic character and with the wide lateral alae and offset mouth cone, separates this species from previously described thelastomatids.

The presence of *T. pterygoton* in the larvae of *O. monoceros* and *O. boas* did not appear to have any effect on the hosts. However, adults of *Thelastoma pterygoton* removed from third-stage larvae of *O. boas* frequently exhibited rough areas on their cuticle that were visible under the light microscope. Portions of the body were sometimes partially constricted off from the remainder (Fig. 3A). Electron micrographs of the affected areas showed numerous microorganisms closely appressed to the cuticular surface. A close examination of the cuticle near these microorganisms showed the presence of irregular grooves and depressions often matching the shape of the associated cells (Fig. 3B). These results suggest that the microorganisms are capable of dissolving at least part of the nematode's cuticle and establishing colonies on the surface of the nematode.

Unfortunately, only fixed material was available; thus the cells were not cultured and their identification is not known. They exhibited a great irregularity in size and shape, many were empty, and the outer surface could not be definitely matched with any known organism. They may be some type of bacteria or mycoplasma-like body. Why these cuticular irregularities were never observed on *T. pter-*

ygoton from the type host, *O. monoceros*, is not known.

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Literature Cited

- Catley, A. 1969. The coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae: Dynastinae). PANS 15: 18-30.
- Christie, J. R. 1931. Some nemic parasites (Oxyuridae) of coleopterous larvae. J. Agr. Res. 42: 463-482.
- . 1938. A redescription of *Thelastoma robustum* Leidy with comments on other species of the nematode family Thelastomatidae. Proc. Helm. Soc. Wash. 5: 65-67.
- Jarry, Denise-T. 1964. Les Oxyuroïdes de quelques Arthropodes dans le midi de la France. Ann. Parasit. Hum. Comp. 39: 381-508.
- Jarry, D.-M., and Denise-T. Jarry. 1968. Tentative de clarification à propos de 60 espèces des genres *Cephalobellus* et *Thelastoma* (Nematoda—Oxyuroidea). Ann. Parasit. Hum. Comp. 43: 339-352.
- Johnston, T. H. 1914. Some new Queensland endoparasites. Proc. Roy. Soc. Queensland 26: 76-84.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.
- Théodoridès, J. 1955. Contribution à l'étude des parasites et phorétiques de Coleoptères terrestres. Vie et Milieu Suppl. No. 4, 310 p.
- van Waerebeke, D. 1970. Deux oxyures parasites de larves de Lucanidae a Madagascar. Entomophaga 15: 5-13.