

TAXONOMY AND BIONOMICS OF THE
NEMATODE GENUS *Butlerius*

By
CHARLES CLAYTON RUSSELL

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

August, 1967

AGRI-
CULTURAL
LIBRARY

UNIVERSITY OF FLORIDA



3 1262 08552 2810

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to his committee: Dr. V. G. Perry, Chairman, Dr. J. T. Creighton, Professor R. D. Dickey, Dr. D. A. Roberts and Dr. G. C. Smart for their assistance in the preparation of this manuscript; to Dr. V. G. Perry especially for his invaluable suggestions, encouragement and criticism throughout the writer's graduate study; to Professor W. T. Calaway for provision of facilities and financial assistance through National Institute of Health grant W. P. 00508; to Mr. K. A. Noegel and Dr. H. N. Miller for assistance in the reproduction of the plates; and finally, to his wife for her understanding and assistance during this period of graduate study.

TABLE OF CONTENTS

Acknowledgments	ii
List of Tables	v
List of Plates	vi
List of Figures	vii
Introduction	1
Materials and Methods	
General Techniques	9
Morphology and Taxonomy	11
Bionomics	13
Embryology	13
Larval development	14
Molting	16
Reproduction	16
Feeding	17
Results and Discussion	
<u><i>Eutletius lacinius</i></u>	19
Dimensions	19
Description	19
Diagnosis	21
Holotype	24
Paratypes	24
Bionomics	24
General	24
Embryology	25
Life cycle	29
Molting	30
Reproduction	30
Feeding	32
<u><i>Eutletius rasilus</i></u>	38
Dimensions	38
Description	38
Diagnosis	40
Holotype	43
Paratypes	43
Bionomics	43
General	43
Embryology	44
Life cycle	49

Molting	49
Reproduction	50
Feeding	51
<u>Putleila barospicus</u>	60
Dimensions	60
Description	61
Diagnosis	62
Holotype	63
Paratype.....	63
Bionomics	63
General.....	63
Embryology.....	63
Life cycle.....	69
Molting	69
Reproduction	69
Feeding.....	70
Summary	75
Literature Cited	77
Biographical Sketch	79

LIST OF TABLES

Table 1.	Larval Stage Duration and Body Lengths of <u>Butlerius</u> <u>lucius</u>	27
Table 2.	Larval Stage Duration and Body Lengths of <u>Butlerius</u> <u>racialis</u>	49
Table 3.	Larval Stage Duration and Body Lengths of <u>Butlerius</u> <u>hospitius</u>	69
Table 4.	Average Time Lapse of the Developmental Stages of <u>Butlerius</u> spp. (in hrs from oviposition at 27°	75

LIST OF PLATES

Plate I. <u>Butlerius</u> <u>lucinus</u> n. sp.	23
Plate II. <u>Butlerius</u> <u>recaulus</u> n. sp.	42
Plate III. <u>Butlerius</u> <u>hispidus</u> n. sp.	64

LIST OF FIGURES

Fig. 1.	Embryonic development of <u>Butlerius lacinus</u>	28
Fig. 2.	Copulation of <u>Butlerius lacinus</u>	34
Fig. 3.	Feeding of <u>Butlerius lacinus</u>	37
Fig. 4.	Embryonic development of <u>Butlerius passalus</u> to the morula stage	46
Fig. 5.	Embryonic development of <u>Butlerius passalus</u> from blastula to eclosion	48
Fig. 6.	Molting of <u>Butlerius passalus</u>	52
Fig. 7.	Molting of <u>Butlerius passalus</u>	54
Fig. 8.	Copulation of <u>Butlerius passalus</u>	57
Fig. 9.	Feeding of <u>Butlerius passalus</u>	59
Fig. 10.	Embryonic development of <u>Butlerius hamospiens</u>	68
Fig. 11.	Copulation of <u>Butlerius hamospiens</u>	72
Fig. 12.	Cannibalism by <u>Butlerius hamospiens</u>	74

INTRODUCTION

The genus Butlerius T. Goodey, 1929, was erected as a monospecific genus with the type species, B. butleri, named after Dr. E. J. Butler, the collector. The description was based on nine specimens extracted from rotted banana roots preserved in "strong alcohol." The stomal morphology of Butlerius butleri was originally described by T. Goodey (5) as follows:

The head is rather broad and anteriorly flattened. In the center it is raised up in the form of a truncated cone with the mouth aperture in front. There do not appear to be any distinct lips and the walls of the conical region seem rather thin and membranous. There are six cephalic papillae equally spaced round the head end behind the conical mouth region. Each has a fairly wide basal part which carries a moderately long seta. Amprids were not observed.

As seen in optical section (fig. 20), the buccal capsule is made up of two principal parts; an anterior buccal and a posterior pharyngeal region, a faint line of demarcation between the two being seen running across the capsule. The forward part of the buccal walls form one truncated cone of the mouth. They are relatively thin and hyaline but gradually increase in thickness posteriorly where they articulate with the pharyngeal part. The latter is in the form of a hollow ring with convex walls which are thicker posteriorly than anteriorly. The base of the ring on each side is expanded and is attached to the anterior end of the oesophagus where it curves outward. The dorsal side is a little in advance of the ventral. The cuticle covering the anterior end of the oesophagus is relatively thick and is produced into two inwardly pointing teeth; one dorsal and the other ventral, the former a little in front of the latter. These two teeth are found on either side of an almost cylindrical hollow let into the anterior face of the oesophagus, a little deeper than wide and having the lumen of the oesophagus leading out of its floor.

Goodey further stated:

Another difference shown by Butlerius is in the buccal capsule, the great size and structure of which separate it from

Dinlogaster. The two fixed teeth guarding the entrance to the hollow cavity in the anterior end of the oesophagus are again different from the movable teeth found in some species of Dinlogaster. The cavity just mentioned is another distinctive feature of the new genus. The blunt anterior end with the mouth on the summit of the truncated cone, the sides of which are without radially segmented leaf-crown elements and the absence of lips mark it off from Dinlogaster.

The females of this species were described as possessing a vulva at 58 to 64 per cent of the body length and didelphic reflexed ovaries. The male had a single reflexed testis, well-developed spicules and, according to Goodey, "a remarkably large gubernaculum." Goodey further stated: "The gubernaculum is more than two-thirds the length of the spicule; its head end lies close to the dorsal side of the body wall and is curved inwards towards the spicules in a small expansion or hook. The body of the gubernaculum is hollow and in the distal third of its length it seems to enclose the tapering spicules which pass completely through it. On each side of its ventral end there is a small lateral prominence."

No mention was made of the life history or food habits of B. butleri in this paper.

In 1930, Adam (1) described B. filicostatus from specimens extracted from a compost heap. Adam's description was based on both living and heat-relaxed specimens (number unknown).

Butlerius filicostatus exhibited the following distinctive morphological characters: six movable lips faced on their inner surface with the anteriormost portion of the cheilognathion, the lips slightly overlap and unite to form a somewhat flattened cone; six labial papillae each bearing two unequal setae or bristles; a flexible ring connecting the anterior and posterior portions of the stoma; a large dorsal tooth which is perforated with a canal; and, finally,

the smaller subventral teeth. The position of the vulva was described at 45 per cent and the distal ends of the two reflexed ovaries extended back to the vulva. The testis was reflexed and simply constructed. Spicules were heavily sclerotized and elongated.

Adam (1) stated (translation from German):

I believe, after studying my living material as closely as possible, that a more precise study of Goodey's material would show that:

1. The cone described by Goodey in B. butleri consisted of six movable lips. Therefore the lips are not missing as he indicates in his descriptive

2. The cephalic papillae of B. butleri perhaps consist of two setae and not one.

3. The two chitinized stomal parts are separated by an expanding membranous ring, and not, as Goodey said, by means of a 'faint line of demarcation.' The latter is probably no longer to be seen on fixed material as it is severely contracted on the death of the animal.

4. Finally there are probably two ventral teeth in the case of B. butleri also as they scarcely can be seen without ventral observation.

In 1938, B. okai and B. brevianiculatus Schuurmans-Stekhoven and Teunissen were described. The description of B. okai (11) was based on thirty-nine specimens extracted from soil moss in Hainan. According to Rahm, a precise description was not possible because of (translation from German) "post-mortem alteration (particularly in the position of the sexual organs)."

Rahm describes four teeth in the stoma of B. okai; the two sub-ventrals as described by Adam, a large dorsal and a smaller dorsal tooth, apparently in tandem with the larger one. He was unable to corroborate either the canal in the dorsal tooth or the anterior "chitinized ring" described by Adam. Rahm stated that he was unable

to observe the arphids, spinneret, excretory pore or the reproductive organs, except for the vulva (which was at 48 to 51 per cent) and the heavily cuticularized spicules (not reported to be accompanied by a gubernaculum).

Rahm supplied no biological information pertaining to B. okai other than to hypothesize that the elastic ring, observed by both Adam and him, might make possible the functioning of the entire stoma as a sucking device.

Schuermans-Stekhoven and Teunissen (13) described B. brevispiculatus from a single male collected from a bamboo forest in the Congo. They were unable to give much detail on the structure of the stoma because of its state of contraction. They did state, however, that the cylindrical stoma was armed with two curved pointed teeth. The robust heavily sclerotized, noncephalated spicules were equipped with a distinct longitudinal ridge which extended their entire length. The gubernaculum was robust and its length was one-half that of the spicules. No mention was made of the bionomics of this species.

In his book, Soil and Freshwater Nematodes, P. Goodey (6) agreed with Adam's interpretation of the lip structure of Butlerius and, based on stomal morphology, suggested that this genus might have a predaceous habit.

Meyl (9) described B. perlechi, using twenty-two female and three male specimens collected from the water reserve of the bromeliad, Arnselia arvensis (Vell.) Mez. in Brazil. His description of the stoma is in complete agreement with that of Goodey (5). The female specimens were described as having almost symmetrical arphidelphic reflexed ovaries reaching almost halfway to the vulva which was located

at 45.2 to 47.5 per cent. The cuticle was finely annulated and had ten longitudinal striae. The spicules were equipped with knobbed heads followed by an expanded section which tapered to sharp tips. The gubernaculum was large, blade-like, elaborated proximally, and formed a cuff at the cloacal end inside of which the tapered ends of the spicules glide.

Meyl listed the food habits of B. gerlachi as unknown, but suggested that the species probably feeds on protozoa.

The second Brazilian species, B. singularis, was collected by Lordello and Zamith (♂) from soil around the roots of cultivated Carica papaya L. The description of this species was based on both fresh and fixed material.

The description of the stoma of B. singularis is similar to that of B. butleri. Each of the six setose papillae terminate in a rounded body of variable size. The vulva is located at 44.3 per cent and the two reflexed ovaries are of unequal length with either ovary being the longer. The single testis of the male is reflexed; the cephalated spicules are strong, curved and their tips are surrounded by the gubernaculum. According to Lordello and Zamith, the most distinctive morphological character of this species is an unpaired phasmid-like organ for which they proposed the name paraphasmid.

Lordello and Zamith studied the food habits of B. singularis by mounting specimens in water between a slide and a coverglass. They determined that B. singularis was predaceous in habit. They were unable to recognize the victims, but stated that, with little doubt, some of the victims were other specimens of B. singularis.

In the same paper Lordello and Zamith erected the genus

Butlerius, having B. okai Rahm the type species based on the lack of a gubernaculum. They also transferred B. brevispiculatus Schuurmans-Stekhoven and Teunissen to the genus Butlerius Schultze (in Jaros), 1957.

Meyl (10) erected the genus Butlerioides and named B. filicardatus, Adam as the type species. Butlerioides was characterized as having: the dorsal transition area with a large hollow dorsal tooth; telosten as an oesophageal cylinder; six cephalic papillae equipped with paired bristles which are approximately 30 per cent of the head width; and six movable, anteriorly protruding lips.

In the revised second edition of Soil and Freshwater Nematodes, J. B. Goodey (7) rejected Lordello and Zamith's transfer of B. brevispiculatus Schuurmans-Stekhoven and Teunissen to Diplosas er and the erection of Butlerioides to contain B. okai Rahm as well as Meyl's transfer to B. filicardatus to the newly erected Butlerioides. He stated that, "the descriptions of B. brevispiculatus and B. okai approach the category of species inquirenda and on these grounds the actions of Meyl, 1960 and Lordello and Zamith, 1959 are not accepted." On these bases Goodey synonymized all three new combinations with the originally proposed names, resulting in a total of six species in the genus Butlerius.

The seventh species of Butlerius described was B. longicauda Taylor, 1964 (14). This is a monodelphic species with the vulva located at 48 to 50 per cent. The testis is single reflexed, and the spicules are ventrally arcuate. Taylor described the gubernaculum as complex and illustrated it as being more than half as deep as long.

The morphology of the first two chambers of the stoma corresponds

very closely to that described by previous authors. Comparisons of the posterior chamber of this species with others is impossible since Taylor's description stated, "Posterior chamber contains a large dorsal tooth and other smaller teeth and rasping structures as shown in Fig. 1B."

Taylor's extensive observations of the feeding habits of B. monhystera are quoted in their entirety below.

The feeding of B. monhystera has been observed, and this species is predaceous upon other nematodes, as has been reported by Lordello and Zamith (1959) for B. singularis. Most specimens observed attacked nematodes having a body diameter smaller than themselves. The prey was usually ingested tail, or less commonly, head-first. When attacking its prey, the predator's stoma became shortened, caused by a contraction of the non-sclerotized middle chamber, and teeth in the basal chamber were thus able to puncture the prey's body wall. Body contents of the prey were ingested and moved posteriorly in the lumen of the predator's procorpus. Passage of food was accompanied by extreme dilation of the lumen. Food flow was less rapid in the posterior portion of the oesophagus and was not accompanied by an expansion of the lumen, indicating the probable valvular function of the sclerotized plates in the metacorpus. Failure of this species to become established on a culture of Chelonus average Bastian, 1865, prevented additional observation.

Taylor supported Goodey's synonymization of Butleriellus filicardis (Adam, 1930) Meyl, 1960; Butlerioides akai (Rahm, 1938), Lordello and Zamith, 1959, and Diplogaster brevispiculatus (Schuurmans-Stekhoven and Teunissen, 1938) Lordello and Zamith, 1959. He presented further arguments for the synonymization of the latter two combinations, but like Goodey, presented no justification for synonymization of Butleriellus filicardis (Adam, 1930) Meyl, 1960. At present the genus contains:

B. butleri T. Goodey, 1929; type species

B. brevispiculatus Schuurmans-Stekhoven and Teunissen, 1938
syn. Diplogaster brevispiculatus (Schuurmans-Stekhoven and Teunissen, 1938) Lordello and Zamith, 1959

- B. filicaudatus Adam, 1930
syn. Butleriellus filicaudatus (Adam, 1930) Meyl, 1960
- B. scutellari Meyl, 1957
- B. mathyotana Taylor, 1964
- B. okai Rahm, 1938
syn. Butlerioides okai (Rahm, 1938) Lordello and Zamith, 1959
- B. singularis Lordello and Zamith, 1959

It is apparent from the descriptions that the above species exhibit an unusual degree of morphological variation for a genus, and that the information on their bionomics is meager at best. Therefore, upon the recovery of three nematode populations which were identified as undescribed species of the genus Butlerius, a program of research was initiated to (1) prepare descriptions of the new species and to make detailed studies of the: (2) embryology; (3) larval development; (4) molting process; (5) reproductive and (6) feeding habits of each species.

MATERIALS AND METHODS

General Techniques

Field samples of varying volumes were obtained and transported to the laboratory in plastic bags; nematodes were extracted by a modification of the Seinhorst sedimentation process described by Goodey (4). Specimens of each species of Butlerius were then hand-picked to 1 per cent water agar in separate Petri dishes, and several hundred specimens of Panagrellus redivivus (Linn., 1767) Goodey, 1945 were introduced into each dish as a food source. After the colonies of Butlerius became established, each species was subcolonized by transferring ten males and a single gravid female of each species to an agar plate. All individuals used in the following studies were obtained from stock colonies established from the progeny of these females.

Stock colonies were maintained in 90-mm Petri dishes filled to a depth of approximately 5 mm with a 0.75 per cent water agar. Pyrex and polystyrene dishes were used. The dishes were labeled with an indelible marker on the side of the bottom section rather than on the space provided on the cover. Observations of the nematodes were not hampered by the label in this way and the colony remained properly labeled, even though the covers might have been switched during routine colony maintenance.

Colonies of Aerobeloines sp., Brevibucca sp., Grindrocerus sp., Panagrellus redivivus, Panagrolaimus sp. and Rhabditis spp. were

maintained for use as prey in studying life histories and food habits of Eutlenius.

Colonies of Panagrellus redivivus were established on cooked oatmeal placed in the centers of 1.5-l wide-mouth fruit jars and covered with circular panes of glass. After the colonies were well established, the nematodes were removed from the sides of a jar with a scraper constructed by inserting half of a double-edged razor blade in a wooden handle. The nematodes then were washed to a 325-mesh screen and rinsed with running water. The specimens were then washed into a beaker with a surplus of water, and allowed to settle. Then they were drawn into a 50-ml pipette fitted with a rubber bulb and allowed to concentrate in the finely drawn tip of the pipette. This facilitated their transfer directly to the colonies of Eutlenius with a minimum of water. The repeated washing and settling eliminated most of the bacteria and yeasts that would otherwise contaminate and discolor the agar.

The other genera listed above were reared on 1 per cent water agar, with Metrecal as the source of nutrient (12). When a high population of nematodes had been reached, the surface of the agar was gently flooded with water. Five to 10 min later, the water containing the prey nematodes was poured onto a 325-mesh screen and the washing and settling procedure outlined for P. redivivus was followed.

Prey nematodes that had been concentrated in the dropper were transferred to a small Syracuse (Bureau of Plant Industry type) watch glass and were hand-picked with a dental pulp canal file when small numbers of prey were required. Stock colonies of Monopyleptis sp. and Dorydaimys subtilis, were maintained in the same manner as Eutlenius colonies. All studies were conducted at a room temperature of approximately 27°C.

Morphology and Taxonomy

The morphology of living specimens of the three undescribed species of Eulerius was studied at magnifications up to 1000x. Specimens were mounted in egg albumen, on compress mounts (described below), or on agar slide preparations to slow down their movements.

Compress mounts were prepared by placing living nematodes in a drop of water on a clean microscope slide. A glass rod with a diameter very slightly less than the greatest body diameter of the specimen was transferred to the drop. The rod was broken into four small pieces and arranged so as to come to rest near the corners of a 24 x 24 mm coverglass which was then placed on the water droplet. The coverglass floated on the excess water and could be centered on the slide without rolling the specimens. The excess water then was removed and simultaneously the specimens were drawn to the center of the mount by application of filter paper points to appropriate edges of the coverglass. When the coverglass had come to rest on the glass rods, two of its diagonally opposing corners were fastened with wax droplets from a small candle. Only two corners of the coverglass so fastened proved sufficient to prevent rolling of the specimens, yet allowed the coverglass to bend. By this technique, the observer was able to control the amount of pressure brought to bear on the specimens by addition or withdrawal of water from under the coverglass; thus, control was maintained over the degree of freedom of movement allowed the specimen. Staining in vivo was greatly facilitated because dilute stains could be added to the edge of the coverglass.

Agar slide mounts were prepared by stirring autoclaved 2 per cent

water agar with a magnetic stirrer until a temperature of 40°C was reached. A drop of agar was then transferred to the surface of a clean microscope slide and quickly spread to a thickness of approximately 1 mm. After the agar had solidified, the specimens to be studied were transferred to a very small droplet of water on the surface of the agar and a 24 x 48 mm coverglass was applied. The mount was then sealed completely with wax from a small candle. The final agar concentration obtained by this method was somewhat greater than the initial 2 per cent and the nematodes' movements were considerably impeded, thus affording observations into the bodies of the specimens from all directions.

Several accepted methods for killing nematodes for microscopic examination were used, but the technique described below proved the most satisfactory in reducing post-mortem distortion. Specimens from the stock colonies were washed by picking them first into a watch glass containing water and then into a 35-mm polystyrene Petri dish half-filled with water. The Petri dish was then floated in a covered insulated container three-fourths filled with water held at a temperature of 50°C and stirred slowly on a magnetic stirrer to maintain constant water temperature throughout the bath. The dish remained in the bath for 10 min, was then removed and the nematodes were mounted on a clean glass slide following standard techniques for temporary water mounts (4).

Specimens to be studied after fixation or processed to glycerine for permanent mounts were relaxed by the above method; but upon transfer to the hot water bath, a second 35-mm polystyrene Petri dish containing a quantity of TAF fixative equal to the quantity of water in the first dish also was placed in the bath. After 10 min, the fixative was added to the dish which contained the nematodes. The dish was then placed in a moisture chamber for a minimum of 12 hrs for fixation. Following

fixation, the specimens were mounted temporarily in T.F. solution and studied or allowed to remain in the moisture chamber until needed for mounting.

Specimens to be permanently mounted were fixed for a least 24 hrs and then processed to desiccated glycerine following the Seinhorst method (4). Specimens were then mounted in desiccated glycerine between two coverglasses on Cobb aluminum slides following the methods of Thorne (15).

Descriptions were prepared using the morphological terminology of Chitwood (3). Illustrations were prepared using the methods described by Thorne (15). Photomicrographic techniques were also developed for this purpose.

Photomicrographs of fixed and permanently mounted nematodes were made on 35-mm Kodak Fine Grain Positive film. Higher quality prints were obtained at greater enlargements with this film than with other films used. Observation of film during development by use of a safe-light made it possible to control development of the negative so that the desired structures could be accentuated. Fine Grain Positive is a slow film (ASA 3) that requires exposures of up to 40 sec. It cannot be used, therefore, on living material or in laboratories where microscope vibration occurs.

Bionomics

Embryology

All eggs were obtained from well-fed females. Ten to fifteen gravid females were transferred from the colony to a watch glass

containing deionized water. They were then transferred to a droplet of deionized water in the center of a 24 x 24 mm square coverglass which was then inverted on a hanging drop microculture slide without ringing. The females were observed with the aid of dissecting microscope until three or more eggs had been laid; at this time, the coverglass was turned over, the females removed, and the eggs positioned in the center of the droplet free from surface tension of the water. A droplet of 2 per cent water agar that had been cooled with agitation (37°C) was quickly added to the water droplet. When the agar had solidified, the coverglass was inverted onto a hanging drop microculture slide, previously ringed with petroleum jelly, so that the agar drop was placed in the polished 13 x 1.75 mm straight-walled concavity. The coverglass was then depressed and slightly rotated until a complete seal was obtained. The slide was constantly observed throughout the embryonic development of the eggs, and photomicrographs were taken at magnifications up to 1000x at intervals dictated by the degree of change observed in the eggs. The lapsed time was recorded for each photomicrograph.

One to three similarly prepared hanging drop slides, containing from three to ten eggs of one nematode species, were observed at half-hour intervals throughout development. These observations were conducted using a separate research microscope adjacent to the one used for the complete embryology study and photomicrographic series. Stage of development at each observation period was recorded for the second series of eggs, but no photomicrographs were taken.

Larval development

Preliminary studies of larval development were conducted by

observing the development of individual larvae within thin layers of water agar held in polystyrene Petri dishes of either a 90-mm, 55-mm, or 35-mm size. Use of the two larger dishes proved impracticable as excessive time was required to locate active Butlerius larvae following egress from the eggs. The 35-mm dishes proved unsatisfactory because the thin layer of agar dried out before the nematode reached the adult stage. The technique described below was developed to allow observation of large numbers of developing larvae and minimize expenditure of time and agar desiccation.

Petri dishes of a 90-mm diameter were filled with 0.75 per cent water agar of approximately 2 mm by swirling the agar in the dish until it had become viscous. After the agar had solidified, seven disks, 20 mm in diameter, were cut with a cork borer. One disk was cut in the center of the plate and the other six were spaced equidistant around the center. The plate was then inverted and the agar between the disks was removed and discarded. A single egg of Butlerius in a larval stage of development was transferred from the stock colony to the center of each agar disk. Specimens of Panagrellus redivivus were introduced, and each disk was covered with a disk which previously had been cut with a sharp cork borer from a thin plastic film. The plates were then inverted onto the stage of a research microscope and observed at magnifications up to 200x. The larvae were observed at 2- to 4-hr intervals throughout development. Between observations the plates were kept in inverted metal 10-cm diameter cans equipped with plastic self-sealing lids; these served as moisture chambers.

Method

Although molting was observed in all larval stages, detailed studies were conducted on freshly fed fourth-stage larvae. Fourth-stage larvae were selected for study because they initiated molting soon after feeding, and their larger size facilitated detailed observations of rhabdional migration and formation.

Two techniques were used to study the molting process. In the first, a thin plastic film was placed in the inverted cover of a 90-mm polystyrene Petri dish; a small quantity of 1 per cent water agar was poured onto the plastic film. The bottom of the Petri dish was pressed into the cover and held by pressure until the agar had solidified. The cover was then removed leaving a 1-mm layer of water agar between the dish bottom and the plastic film. A small "V" was cut in the plastic film and the apex pulled back to expose a section of the agar. Three to five larvae were transferred to the exposed agar surface and the plastic was replaced. The molting process was observed through the plastic film with the aid of a microscope equipped with an oil-immersion objective. This technique allowed ample oxygen to reach the agar through the plastic film and the low profile of the mount allowed easy access to the specimens.

The second technique, used only for obtaining photomicrographs, involved the use of the agar slide preparations described above.

Reproduction

Specimens of Butlerius s. were frequently observed in copulatrix in stock colonies, and more detailed studies were made of virgin females

obtained from larval development studies or other individuals especially reared from larvae. Males that had not mated were obtained in the same manner as the females; males that presumably had mated were obtained from the stock colonies. Detailed observations were made at magnification up to 1000x by both techniques described above under "Molting." Observations of behavioral responses by males to the introduction of virgin females into heavily populated stock colonies and responses to single virgin females in agar plates were made at magnifications up to 200x.

Interspecific mating experiments among the three species of Butlerius were conducted in 55-mm polystyrene Petri dishes containing 0.75 per cent water agar at a depth of 4 mm. Nine plates containing ten males each (three plates per species) were prepared and supplied with specimens of Panagrellus redivivus to serve as prey. Three plates containing ten larval females each (one plate per species) were prepared and fed as above. After the females in each plate molted to the adult stage, they were transferred in groups of three to a plate containing the males of their own species, the males of the second species, or the males of the third species. The plates were alternately observed for 12 hrs, after which the females were transferred to individual plates and observed periodically for 8 days to determine if any eggs were produced. Subsequent to the removal of the females to individual plates, three virgin females of the same species as the males were placed in each plate with the males to assure that the males in each dish were capable of reproduction.

Feeding

Feeding was observed in stock colonies throughout the above studies

by introducing various prey nematodes with specimens of each of the three species of Butlerius under conditions provided by the above techniques. Data on competition of each species of Butlerius with Mononchoides sp. and Dorylaimus subtilis were recorded by inoculating plates of agar with equal numbers of the two species under study at any one time. All possible combinations of the three species of Butlerius with each other and with the other two genera were thus tested.

RESULTS AND DISCUSSION

Butlerius lacinus n. sp. Plate I

A population of nematodes, determined to be an undescribed species of the genus Butlerius Goodey, 1929, was recovered from a wet mixture of soil, rotting hay and sheep manure. The sample was taken July 6, 1963 from the edge of an inundated area in a holding pen of the Department of Veterinary Science, University of Florida, Gainesville. The specific name lacinus [L. lacini = flap] was given to denote the papillate flap covering the cloacal opening of the male.

The caudal filament is frequently bitten or broken off, thus each of the lengths were measured from the lips to the base of the filament.

Dimensions

Female: (N = 10) L = 1.6 mm (1.3 - 1.8); a = 32.1 (27.5 - 36.0);
b = 5.17 (4.3 - 6.0); c = 15.58 (11.0 - 17.75); V = $\frac{17.9}{54.5} \frac{17.9}{17.9}$
(47 - 56).

Male: (N = 10) L = 1.46 mm (1.35 - 1.47); a = 34.7 (30.0 - 42.1);
b = 5.22 (4.90 - 5.60); c = 14.06 (13.5 - 14.75); T = 59.2%
(50.9 - 66.6)

Description

Body tapering slightly anteriorly and considerably posteriorly,

the tail of both sexes with long caudal filament. Cuticle with fine transverse striation, lateral field indistinct but with three lines. Subcuticle marked with transverse rows of conspicuous punctations in the head and neck region changing to a rosette arrangement in the body region, back to transverse rows postanally, and ending abruptly at the base of the caudal filament. Amphid opening a transverse oval located at the level of the apex of the dorsal tooth. Lip region not offset, flattened anteriorly but with 6 membranous lips partially innerfaced with the flexible cheilorhabdions arching up and inward to form a cone around the oral aperture. Six setose cephalic papillae surrounding the lip region, male with doubled papillae in subdorsal and subventral position for total of 10. Male stoma 3 times as long as wide, female stoma slightly broader. Cheilostom comprising anterior 1/3 of anterior chamber, cheilorhabdions fusing with pronhabdions at the level of the bases of the papilla. Pronhabdions encircle the posterior 2/3 of the anterior chamber, base of pronhabdions attached to mesorhabdions by a hyaline, flexible ring of variable length. Base of mesorhabdions resting on broad metarhabdions which dorsally bear a large cupped tooth, which is perforated postapically by dorsal gland orifice. Both subventral metarhabdions bearing a smaller cupped tooth. Telorhabdions forming basal plate of teeth. A highly muscular oesophageal collar, enlarged dorsally, surrounding stoma to the base of mesorhabdions. Procorpus swollen anteriorly, then becoming cylindrical and swelling again to form the valvulated metacarpus. Entire corpus strongly muscular. Isthmus broad, enlarges gradually to form the broader terminus which is not distinctly bulbous. Tissue of posterior oesophagus largely glandular but interspersed with weak musculature. Nerve

ring crosses isthmus just posterior to metacarpus. Excretory pore ventral at level of rive ring. Phasmids prominent in both sexes.

Female. Gonads paired, opposed and reflexed to near the level of the vagina. Ovaries usually equal in length, only one egg at any given time in each of the uteri.

Male. Testis single, reflexed 1-1.5 body diameters. Rudimentary bursa discernible on some specimens. 12 pairs of variably positioned caudal papillae (3 pair preanal, 1 pair of very small papillae on cuticular flap covering cloacal opening and 1 pair on posterior lip of cloacal opening or slightly lateral to that position). Remaining papillae in typical diplogasteroid arrangement. Spicules paired, ventrally arcuate, capitulum cephalated, calomus narrow near capitulum then broadening and narrowing to the long lamina which makes up nearly $1/2$ the total length of the spicules. Gubernaculum $3/4$ as long as spicules and 7 times as long as deep. Dorsal end bearing a scoop-like projection separated from the remainder of the gubernaculum by a septum and directed toward the underside of spicules. Ventral $1/3$ of gubernaculum enfolding spicule lamina and bearing two pairs of sclerotized hooks.

Diagnosis

The females of Butlerius lacinus are distinguished by the paired subventral teeth and perforated dorsal tooth which it has in common only with P. filiceudatus. The cephalic papillae of B. lacinus lack the paired bristles borne on the cephalic papillae of P. filiceudatus.

Plate I.. Buller's Wren n. sp.

- A. Male head, lateral. B. Female, in front.
C. Female head, lateral. D. Male. E. Female.
F. Male tail, lateral. G. Female, in front.
H. Female head, ventral.



Males of B. lacinus are distinguished by the papillate cuticular flap which covers the cloacal opening, by the gubernaculum which bears a ventrally pointed scoop on the dorsal end and 2 pairs of hooks on the ventral end, and by the 12 caudal papillae.

Holotype

Single females on slide no. 1 labeled "Butlerius lacinus" (holotype), in collection of the Department of Entomology, University of Florida, Gainesville, Florida.

Paratypes

Deposited in collection of the Department of Entomology, University of Florida and others in the United States Department of Agriculture Nematode Collection, Nematology Investigations, Agricultural Research Service, Beltsville, Maryland.

Bionomics

General. Butlerius lacinus was recovered most frequently from semiaquatic habitats and appeared to be well adapted to this type of environment. Unlike most soil nematodes which sink rapidly in water, B. lacinus was repeatedly observed in water of a depth of 2 to 3 cm to swim from the bottom to the surface and remain near the surface, swimming strongly for periods in excess of 15 min. Other individuals were observed swimming just beneath the surface of the water in a 10 gal aquarium 3 days after introduction. The specimens were then

eaten by the fish. Colonies were maintained for a year in Petri dishes filled with water, after which time the technique was abandoned in favor of a standardized agar medium. Swimming was accomplished with a whipping motion of the entire body. Swimming was frequently interrupted by a pause of 1 to 2 sec. During these pauses the head, with lips extended similar to the attitude illustrated by Cobb (2) for Monophasis, was moved from side to side in a jerking or groping motion. Pauses in swimming became longer and more frequent when the specimens were stimulated by introduction of Panagrellus redivivus or other prey nematodes. Once swimming activities were resumed, the lips usually resumed a closed conical position. While swimming, E. lacinus exhibited an apparent greater degree of control of both muscular activity and direction than is seen in the rather frantic flailing of the body by such aquatic nematodes as species of Prismatolaimus.

Numerous samples containing specimens of E. lacinus were obtained from Mr. R. P. Esser, Division of Plant Industry, Florida State Department of Agriculture, Gainesville. The samples were collected from a variety of locations, including nursery soil and commercial earthworm beds. They provided for frequent comparisons of wild populations and the stock colonies.

Embryology. A total of 19 eggs of E. lacinus was used in two different embryological studies. The eggs ranged from 68 to 81 μ in length and 37 to 43 μ in width. Average time lapse from oviposition (ranges included parenthetically) to each developmental stage appears in summary form in the legend of Fig. 1.

Eggs were deposited singly while in the one-cell stage. Eggs in

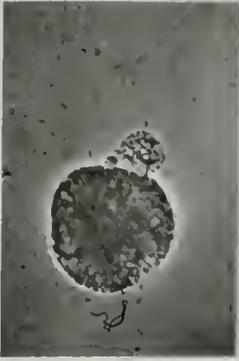
the two-cell or later stages were observed in the uterus of senile females and those that had been injured or had experienced a mechanical blockage of the vagina or vulva. Such females died prior to advanced development of the egg and embryonic development was never observed for this species. On two occasions, very old females were observed laying eggs without a chorion (Fig. 1a).

Cellular division proceeded rapidly, following the same general pattern described for embryonic development of other nematodes. A notable aspect of the intrachorionic development of B. latipes was the low level of development of the stomal rhabdions in the first stage larva at the initiation of the first molt (Fig. 1m). At this developmental stage the rhabdions were so poorly developed as to be indefinable within the chorion and were resolved with difficulty even on larvae which had been removed from the chorion. Thus it was necessary to use the separation of the labial cuticle (Fig. 1n), actually an indicator of advanced stages of molting in later larval stages, as indicative of the initiation of the intrachorionic molt.

Larval egress from the egg was accomplished by most larvae apparently solely through repeated stretching of the chorion. All observed larvae repeatedly pressed their lip regions against the chorion; this resulted in the chorion becoming extremely flexible just prior to egress. At this point, two of the larvae were observed to draw the chorion into their stoma while vigorously moving their dorsal tooth; no contact could be observed, however, between the tooth and chorion. The 33- to 60-hr period from the first larval molt to egress proved to be the most variable period in the development of the egg of B. latipes (Fig. 1p). The molted cuticle of the first stage larva could not be observed in any empty chorions.

Fig. 1. Embryonic development of Butlerius lacinus.

- a. Egg deposited without chorion. b. 1-cell stage immediately following deposition. c. Initiation of cleavage. d. 2-cell stage (.5 - 1 hr). e. 4-cell stage (1 - 2 hr). f. 8-cell stage (2 - 5 hr). g. 16-cell stage (6 - 7 hr). h. 32-cell stage (7 - 8 hr). i. Morula (8 - 10 hr). j. Blastula (11 - 15 hr). k. Gastrula (14 - 16 hr). l. Tadpole (15 - 17 hr), note poorly developed rhabdions. n. Molt (25 - 30 hr), note loose labial cuticle. o. 2nd larval stage (30 - 38 hr), note rhabdion development. p. Eclosion (38 - 60 hr).



a



b



c



d



e



f



g



h



i



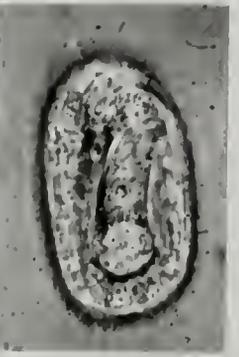
j



k



l



m



n



o



p

Life cycle. The duration of the various larval stages of B. lacinus was, for the purposes of this study, considered to be the time lapse from the initiation of one molt to the next initiation of molting. Larval lengths were later calculated using specimens from stock colonies, the stages of development of which were determined on the basis of degree of genital primordia development. Sex of the larvae can be determined with certainty in the fourth stage by the elongate posteriorly directed enlargement of the male primordium as compared with the balanced, more robust primordium of the female. The larval stage durations, reported in Table 1, are averages of 19 individuals for the first and second stage (to egress from egg) larvae and 17 each for the male and female larvae of the post-egress second, third and fourth stages. Lengths given are averages of 45 individuals with ranges included parenthetically.

Table 1. Larval Stage Duration and Body Lengths of Butlerius lacinus

Stage	Duration in hrs	Body Length in mm		
		Average	Range	
1st	19	0.30	(0.30 - 0.31)	
2nd	39	0.50	(0.35 - 0.63)	
3rd	17	0.76	(0.68 - 0.85)	
4th	♂	21	1.05	(1.00 - 1.07)
	♀	50	1.03	(0.95 - 1.50)

Following oviposition, the adult stage was reached in an average of 114 hrs by females and 102 hrs by males. Longevity studies per se were not conducted, but maximum adult longevity was determined to be in excess of 6 wks for virgin individuals. The recorded time schedules

probably approach the minimum since the duration of each larval stage is dependent to a large degree upon the availability and quality of food. The body length measurements, however, probably are similar to specimens produced in nature, since the larvae that were measured had developed under the competitive environment of a heavily populated stock colony.

Molting. Preliminary studies of the molting process in B. lacinus indicated that the sequence of rhabdion migration and formation is identical to that of B. passalus, given later.

Reproduction. Males of B. lacinus were found capable of locating virgin females at distances up to 80 mm while in 0.75 per cent water agar plates. Immediately following their introduction to a plate containing virgin females, the males appeared to undergo a period of disorientation ranging from a few seconds to, in a single instance, 10 min. During this period they moved aimlessly about, moving their cephalic region from side to side in a rapid, jerking motion. This motion was originally thought to be a response to the proximity of prey; however, it became quite apparent during the reproductive studies that this behavior is a positive tactic response to any attractive stimulus. This behavior pattern is herein referred to as "casting."

The period of disorientation was terminated when the male moved rapidly in the direction of the female with the casting being somewhat subdued during periods of rapid forward motion. The female was approached in an approximately straight line, even in instances where masses of prey nematodes were between the male and female. Such obstructions rarely resulted in any appreciable delay or deviation

from the line of approach. The attractant appeared to originate at the vulvar region, since initial contact was made there if the female did not move during the approach of the male. In instances where the female had vacated a location immediately prior to his arrival, the male would initiate a very rapid casting and then follow the path of departure of the female. Under conditions of continual movement by the female following the introduction of the male, the line of approach formed a curve to intersect the path of the female, and ultimately, the male followed the female until contact was made.

Upon contact with the female, the anterior part of the male moved beyond the vulva until the caudal region of the male contacted the body of the female. The caudal region curled rapidly around the body of the female at the first point of contact. Considerable variation occurred following the initial approach and contact. Most males initiated a thrusting of the spicules while coiling and uncoiling around the female's body. Other males did not thrust their spicules but lay loosely coiled until movements by the female brought her vulva into close proximity to the male's cloacal opening, at which time the male would tighten the coil usually with sufficient force to constrict the body of the female (Fig. 2a).

When alignment of the vulva and cloacal opening was achieved, the long spicules were inserted rapidly, frequently to a depth exceeding that of the vagina (Fig. 2b). The end of the gubernaculum was also inserted into the vagina (Fig. 2c). The duration of the union was exceedingly variable, ranging from 2 to 30 min, during which time the vagina and uteri underwent a muscular activity which resulted in an observable movement of sperm into both uteri. This muscular action was,

in most cases, sufficiently forceful to bend the thin laminae of the spicules (Fig. 2c).

Subsequent to copulation, the female moved away, leaving the male loosely coiled. Frequently, if the female moved away before the spicules and gubernaculum were withdrawn or if the female was disturbed during copulation, the male was dragged by the female. Apparently, this resulted in the inability to disengage the sclerotized gubernacular hooks from the vagina. Females in liquid media frequently were observed swimming vigorously with one or more males coiled tightly around their midsections.

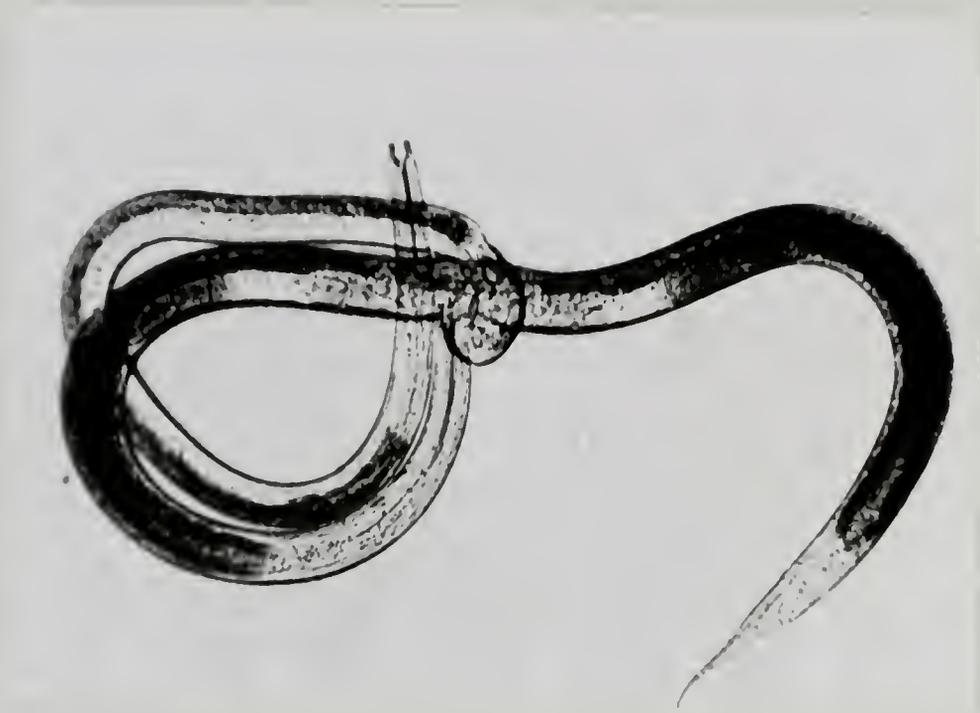
Each virgin female of B. lacinus copulated and produced viable eggs when introduced to plates containing males of this species. No attempt was made by the males of the other two Eutlerius spp. to copulate with females of B. lacinus, nor were the B. lacinus males observed to attempt copulation with females of the two monodelphic species. Upon contact with them, they either withdrew rapidly or killed and ate them. The females of the monodelphic species deposited no eggs within 8 days following separation from the males of B. lacinus.

Feeding. Specimens of B. lacinus usually feed by ingesting small prey nematodes in toto and by breaking the cuticle of larger prey nematodes and ingesting the body contents. Small nematodes, such as Cylindrocorypus and Microheloides, when caught in the midsection, were consumed by breaking the body wall and then ingesting the entire body.

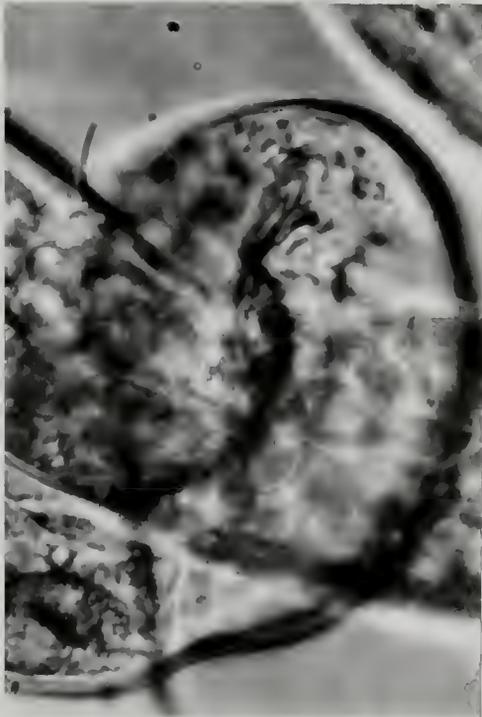
Ingestion of whole prey specimens is accomplished by retracting the dorsal tooth (and presumably the subventrals), dilating the tri-radiate lumen of the oesophagus and moving the entire prey body through

Fig. 2. Copulation of Butlerius lacinus.

a. Copulatory posture, note constriction of female's body in vulvar region. b. Spicule penetration. c. Bending of spicules due to female muscular action, note depth to which gubernaculum is inserted.



a



b



c

the oesophagus by a peristalsis-like muscular action. During this activity, secretions moved in the dorsal oesophageal gland duct, located dorsally in the radiating musculature of the oesophagus, and into the posterior portion of the dorsal tooth (Fig. 3a). Secretion of the dorsal oesophageal enzymes through the tooth orifice was not observed during feeding, but secretions did move through this orifice accompanying dorsal tooth movement in compress slides. Sclerotized structures, such as the rhabdions, of larvae of Butlerius were frequently observed in the intestines of adult specimens (Fig. 3b).

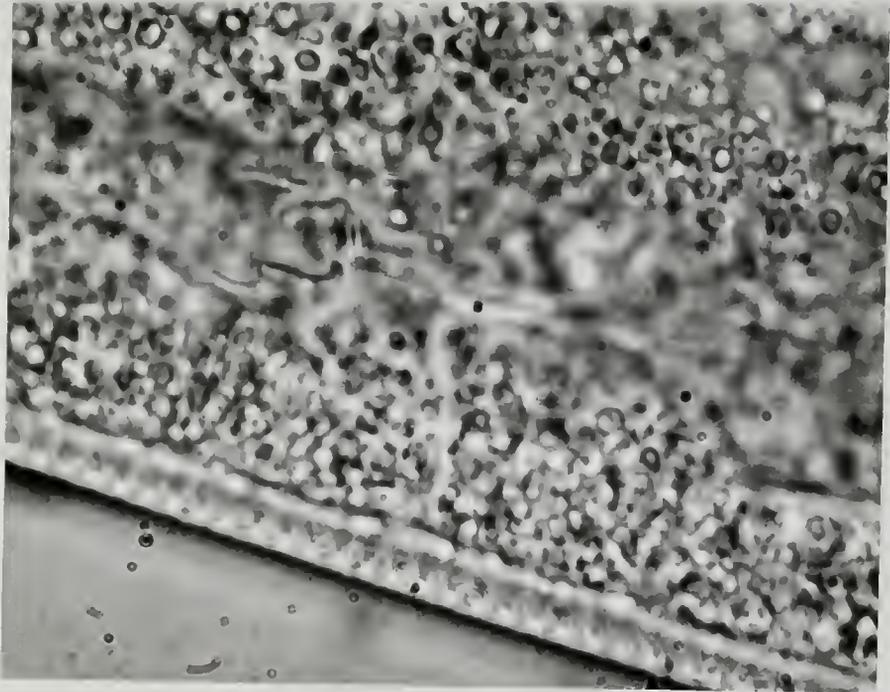
The body wall of prey such as Parasrellus redivivus is ruptured by B. lacinus by the use of the dorsal tooth. The membranous lips are pressed against the body wall of the prey and the stoma elongates convulsively pulling the cuticle (usually by multiple pumpings) to the level of the dorsal tooth.

Under in vitro conditions the prey of B. lacinus includes: Acroteloides sp., Previbucca sp., Helicicarpus sp., Parasrellus redivivus, Paragrolsirus sp., and Rhabditis spp. The incidence of cannibalism in B. lacinus was low in well-fed colonies, but in underfed or overpopulated colonies cannibalism occurred frequently.

Survival ability of B. lacinus in competition with the other two Butlerius spp., Monochoides sp., and Dorclairus subtilis in both agar and water colonies was determined by colonizing B. lacinus with each of the others. B. lacinus, probably due to its greater degree of activity and larger size, destroyed the populations of the other two Butlerius spp. in both water and agar colonies. In water colonies, probably because of its ability to swim, B. lacinus maintained population levels when colonized with either Monochoides sp., or Dorclairus subtilis, and was

Fig. 3. Feeding of Entlerius lacinus.

a. Portion of the intestine of an adult containing part of the stoma and oesophagus of a larva. b. Adult head of female, note dorsal gland orifice and articulated cheilorhabdions.



a



b

observed to prey on the larvae of each. Under conditions of agar colonization, both Dorylaimus subtilis and Mononchoides sp. destroyed the populations of B. lacinus.

Butlerius passalus n. sp. Plate II

A population of nematodes, determined to be an undescribed monodelphic species of the genus Butlerius Goodey, 1929, was recovered from a sample of moist frass of the horned passalus beetle (Popilius disjunctus (Illiger)) in an oak (Quercus sp.) log. The sample was obtained June 11, 1963 from "College Park," University of Florida campus. The specific name passalus is given to the nematode to denote the type locality. The caudal filament is frequently missing, thus all measurements were based upon length from the lips to the base of the filament.

Dimensions

Female: (N = 10) L = 1.08 mm (1.0 - 1.25); a = 22.04 (22.0 - 22.7); b = 4.28 (4.0 - 4.5); c = 10.8 (10.0 - 11.25); V = 23.05 68.3% (66.6 - 10.45).

Male: (N = 10) L = .84 mm (.70 - .95); a = 19.5 (18.35 - 22.50); c = 8.40 (7.0 - 9.0); T = 48.64%.

Description

Body tapering anteriorly and posteriorly, greatest body diameter at ovary flexure, both sexes bear an unusually long caudal filament.

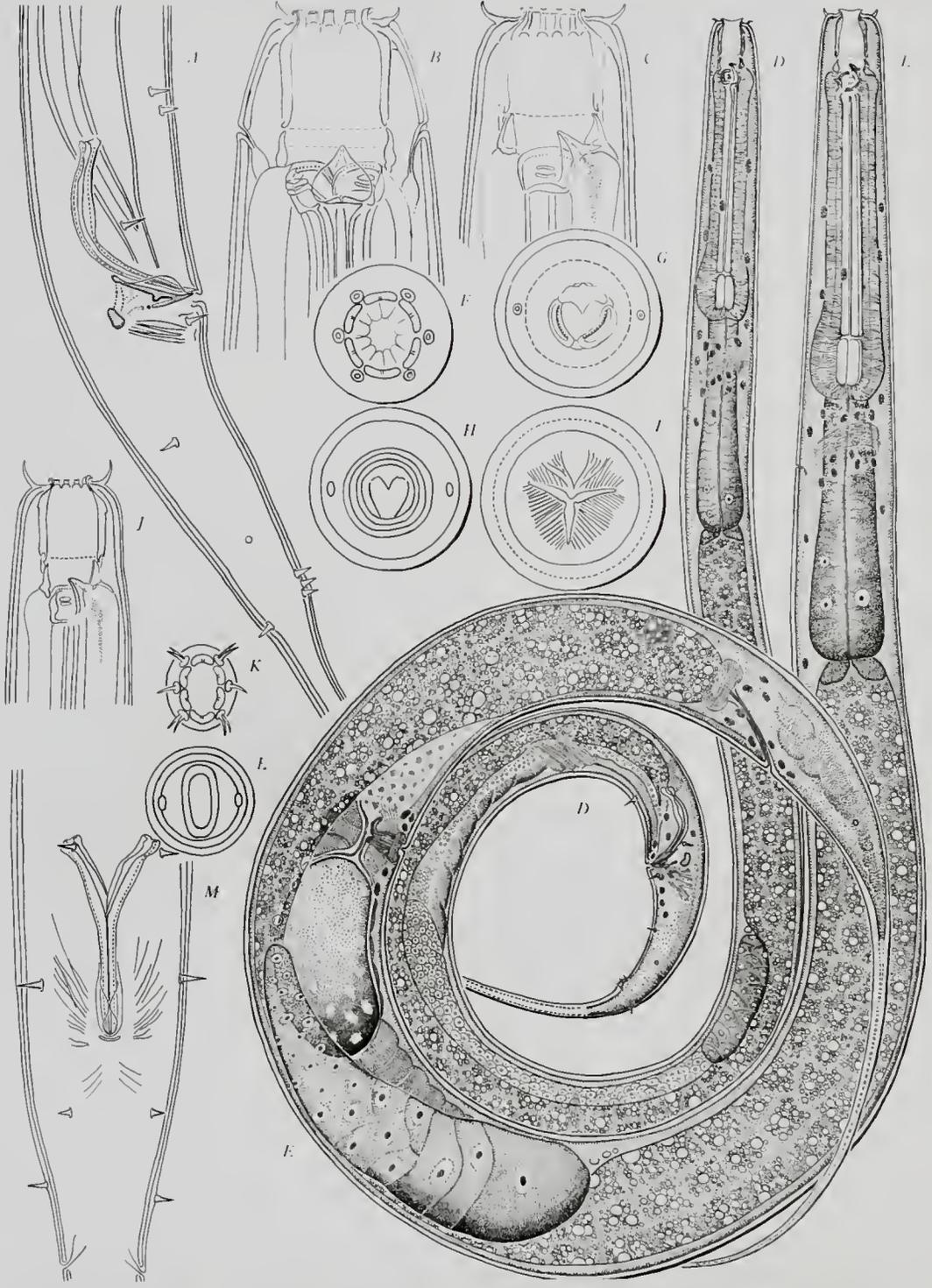
Cuticle of both sexes bearing longitudinal ridges broken by fine transverse striae resulting in a weakly beaded appearance. Subcuticle exhibiting punctations between striations in transverse rows, punctations occupy alternate positions longitudinally, a character observed with difficulty in the males where the beaded longitudinal ridges are more pronounced. Amphid opening transversely oval, located slightly anterior to the apex of the dorsal tooth. Head not offset, somewhat blunt anteriorly with corona of 12 recurved, membranous lips. Lips innerfaced by cheilorhabdions to point of recurvature. Cheilorhabdions articulated on anterior prorhabdions. Prorhabdions joined, by a flexible membrane of variable length, to the outside of the mesorhabdions. Mesorhabdions broadly based on the metarhabdions. A large tooth is present on the dorsal metarhabdion and a cup-shaped rasping plate is present on each subventral metarhabdion. Dorsal tooth perforated subapically by dorsal gland orifice. Rasping plates each bear a single semicircular row of 12 or more denticles. Female stoma twice as deep as wide, male stoma 3 times as deep as wide. Female with 6 heavy cephalic setae, male with 4 additional setae located in subdorsal and subventral positions. A swollen oesophageal collar surrounding stoma as far as base of the mesorhabdions. Procorpus narrows posterior to base of stoma. Metacarpus swelling to form valvulate median bulb. Entire corpal region strongly muscular. Isthmus rather broad, gently expanding posteriorly without forming a distinct basal bulb. Isthmus and posterior region of oesophagus predominantly glandular but interspersed with weak musculature. Nerve ring crosses isthmus just posterior to the metacarpus. Excretory pore ventral, equidistant between base of the metacarpus and cardia.

Female. Monodelphic reflexed, lining of vagina uterina peculiarly refractive. Post-uterine branch usually filled with sperm.

Male. Stoma laterally depressed when viewed en face. Testis single, anterior end reflexed from 1 to 2 body diameters. Nonbursate. 9 pairs of caudal papillae in typical diplogasteroid arrangement, variable in position, 2 to 3 pairs preanal. Spicules heavy, paired, ventrally arcuate. Capitulum cephalated, calomus broad and narrowing to the long laminae which comprise more than half the total length of the spicules. Tips of laminae gently arcuate enfolded 25 per cent of their length by the gubernaculum. Gubernaculum keel-like, 40 per cent the length of spicules and half as deep as long; tip and subventral margins heavily sclerotized; dorsal margin collapsed and very lightly sclerotized. Bottom of gubernaculum bearing large lobe of relatively dense material.

Diagnosis

Females of Butlerius passalus are distinguished from all didelphic species by the single ovary. The single large dorsal tooth which is perforated subapically by the dorsal oesophageal gland orifice and the rasping plates located on each subventral metarhabdion separates B. passalis from B. monhystera for which Taylor (14) illustrates one large and one small dorsal tooth and a single, semicircular rasping plate. Males of B. passalus are distinguished from all other males of the genus Butlerius on the basis of the unique, keel-like gubernaculum.



Holotype

Single female on slide no. 1 labeled "Eutlerius passalidus" (holotype), in collection of the Department of Entomology, University of Florida, Gainesville, Florida.

Paratypes

Deposited in collection of the Department of Entomology, University of Florida and in the United States Department of Agriculture Nematode Collection, Nematology Investigations, Agricultural Research Service, Beltsville, Maryland.

Bionomics

General. Specimens of B. passalidus were recovered only from the frass of the horned passalid, in the type locality and two other locations in Alachua County, Florida. No specimens were recovered from numerous other organic substrates; a definite habitat specificity, therefore, must be attributed to this species, even though relatively few samples of passalid frass were processed. Eutlerius passalidus, although capable of swimming, lacks the obvious adaptation to aquatic conditions noted for B. lacinus. Although specimens of both species employ the same basic movement in swimming, B. passalidus swims with a much slower and more exaggerated rhythmic movement of the body and does not swim above the bottom for extended periods of time, as does B. lacinus.

Embryology. The 10 eggs of B. passalus ranged from 50 to 60 μ in length and 37 to 43 μ in width. The chorion of the eggs exhibited hyaline, adhesive protuberances (Fig. 4k and m), the function of which was not determined. Average time lapse (ranges included parenthetically) from oviposition to each developmental stage is summarized in the legend of Figs. 4 and 5.

Eggs were deposited singly while in the one-cell stage. Eggs in the two-cell stage, and those in more advanced stages of development, were not observed in the uteri of healthy females. Egg deposition was not observed for females bearing more than one egg or an egg developed beyond the two-cell stage in the uterus. Such females often lived for considerable periods of time, however, and epitokia uterica was observed occasionally.

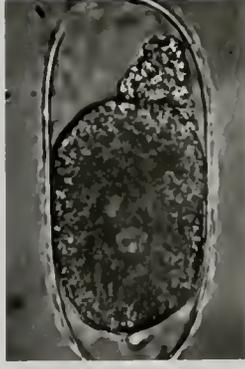
The embryonic development from oviposition to eclosion of B. passalus required nearly twice the time determined for B. lacinus. Egg development in B. passalus followed the same general pattern as the embryonic development described for other nematodes. As noted for B. lacinus, the stomata of first-stage larvae of B. passalus exhibited a very limited degree of rhabdional formation. The intrachorionic molting process was more easily observed in B. passalus because of relatively greater degree of inflation of the separating cephalic cuticle (Figs. 5h, i, and p).

Larval egress from the egg was apparently accomplished in the same manner as described for B. lacinus, but the 70 to 78 hr variation in time from the first larval molt of B. passalus to egress was less than that reported for B. lacinus.

Newly emerged larvae were observed in two cases to re-enter the



a



b



c



d



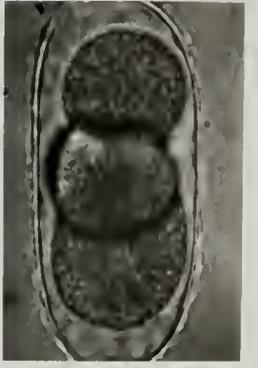
e



f



g



h



i



j



k



l



m



n



o



p

Fig. 5. Embryonic development of Butlerius passalus from blastula to eclosion.

a. Blastula (19 - 24 hr). b., c. Gastrula (22 - 26 hr). d., e. Tadpole stage (28 - 31.5 hr). f. 1st larval stage (32 - 40 hr). g-l. Molting (38 - 49 hr), note separation of cephalic cuticle and degree of rhabdion development. j. 2nd larval stage (47 - 60 hr). k. 2nd larval stage, note ventral view of stoma exhibiting dorsal tooth and subventral rasping plates. l. 2nd larval stage pressing against chorion. m., n. Egress (70 - 78 hr). o. Chorion after egress, note molted cuticle in lower right corner. p. Molting larva which has been removed from the e g, note separated cephalic cuticle with dot-like molted rhabdions.



a



b



c



d



e



f



g



h



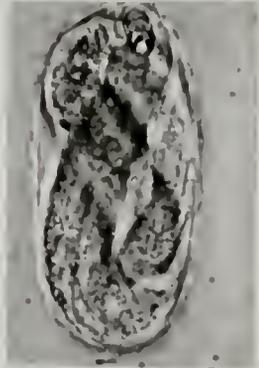
i



j



k



l



m



n



o



p

chorion and maul the molted first stage larval cuticle, which remained in the egg following egress (Fig. 5o).

Life cycle. The duration of a larval stage was considered to be the time from initiation of one molt to initiation of the next. Larval measurements, obtained in the manner described for B. lacinus are included with larval stage durations in Table 2.

Table 2. Larval Stage Duration and Body Lengths of Butlerius passalus

Stage	Duration in hrs	Body Length in mm	
		Average	Range
1st	11	0.30	(0.28 - 0.32)
2nd	68	0.44	(0.38 - 0.58)
3rd	45	0.50	(0.50 - 0.60)
4th ♂	49	0.68	(0.60 - 0.72)
♀	53	0.70	(0.60 - 0.80)

The adult stage was reached by females in an average of 212 hrs and by males in an average of 203 hrs.

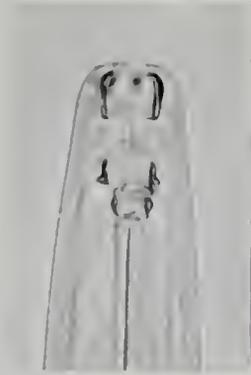
Molting. The molting process was initiated with a quiescent period of variable duration. During this time the walls of the lumen of the oesophagus cleared and the procorpus appeared to withdraw pulling with it the posterior chambers of the stoma (Fig. 7a). This resulted in the separation of the prorhabdions from the mesorhabdions and the stretching of the elastic connection membrane (Fig. 6a). Following separation of the pro- and mesorhabdions, a gentle side to side movement of the posterior stoma was initiated and the cephalic tissues were slowly withdrawn (Figs. 6b-d). Simultaneously, the tissues surrounding

the posterior stoma became hyaline (Fig. 6c). The posterior stoma ceased movement and contracted slightly as faint outlines of the new rhabdions formed outside and slightly posterior to the old ones. At this time the new lip region appeared (Figs. 6d-f). As the armature of the stoma became easily discernible and the cheilo- and pro-rhabdions formed, the elements of the larval stoma and oesophageal lining migrated almost imperceptibly anteriorly and the lining of the oesophageal lumen regained its normal appearance (Figs. 6d-g). Movement was resumed upon the sclerotization of the new rhabdions, which were continually flexed for the remainder of ecdysis. The body cuticle began to stretch and loosen with increased motion by the larva. As the mass of larval rhabdions and oesophageal lining were ejected from the newly formed stoma, the linings of the amphidial pockets were withdrawn (Figs. 6h-j). At this stage, the lips of the vulva were discernible (Fig. 6k). As the nematode pushed and mauled the ejected rhabdions (Figs. 6j and l; Fig. 7b), the linings of the excretory pore (Fig. 6m) and rectum, which were the only attachment points for the loose larval cuticle, were molted by rolling the body within the larval cuticle (Fig. 6n). Once all attachment to the larval cuticle was broken, the young adult repeatedly pressed against it in the cervical region (Fig. 6o) until ecdysis was completed 24 to 35 hrs after the first signs of molting (Fig. 6p; Fig. 7c). Newly molted individuals were occasionally seen to return and maul the molted cuticle.

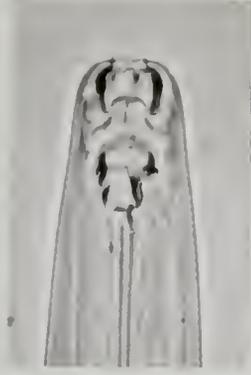
Reproduction. Males of E. passalus located virgin females in 0.75 per cent water agar plates at distances up to 80 mm. The males appeared to undergo a period of disorientation upon introduction to a

Fig. 6. Molting of Dutlerius passalus.

a. Initiation of molt, note separation of pro- and mesorhabdions. b. Withdrawal of cephalic tissues. c. Hyaline rhabdion formation area developing in procorpus. d-g. Dorsal rhabdion formation. h. Collapse of larval stoma. i, j. Molting amphidial lining and withdrawal of larval stoma. k. Vulvar development. l-n. Mauling larval rhabdions. m. Flexing of larval cuticle, note larval anus located dorsally. n. Molt of excretory pore lining. o. Pressing against stretched cuticle. p. Completion of ecdysis, note complete larval stoma at right.



a



b



c



d



e



f



g



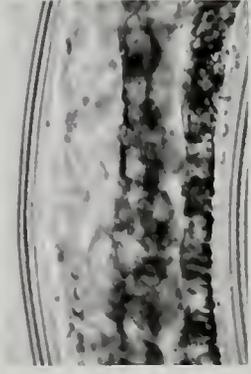
h



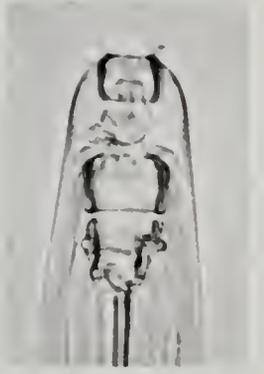
i



j



k



l



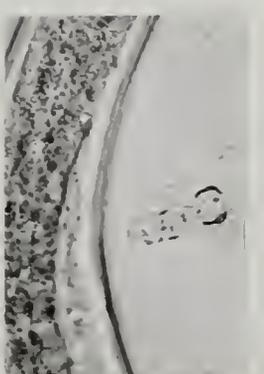
m



n



o



p

Fig. 7. Molting of Butlerius passalus.

a. Initiation of molt. b. Pressing against larval cuticle prior to egress, note rectal lining appressed to cuticle. c. Completion of ecdysis, note larval cuticle and molted rhabdions.



a



b



c

plate containing a virgin female. Following the period of disorientation, the males initiated a casting behavior which was much more subdued than that observed for males of B. lacinus. The approach, though less rapid than that of B. lacinus, was equally direct, and apparently no more difficulty was encountered by obstacles such as masses of prey nematodes.

Observations indicated a conditioning of mated males, which resulted in their achieving union with the female more rapidly than non-mated males. Upon contact with the female, mated males most frequently proceeded beyond the vulva and assumed a copulatory coil only after the caudal region made contact with the vulvar region (Fig. 8a). The non-mated males, however, more frequently assumed a copulatory coil immediately upon encountering the vulvar region, resulting in initial copulatory attempts in the cervical or anal region of the female (Fig. 8b).

The gubernaculum was not observed to enter the vulva, and the laminae of the spicules were not observed to penetrate more than 1/2 the depth of the vagina (Fig. 8c). Following copulation, a gelatinous appearing plug was observed covering the vulva (Fig. 8d). The function of the plug was not determined, but it could function as an adhesive during copulation or as a sealant to prevent sperm loss immediately following copulation. The plug was usually lost within 5 min after copulation.

The interspecific mating tests yielded no copulatory contact of males and females of B. passalus with the other two species of Butlerius. Conversely, all intraspecific contacts resulted in production of eggs.

Feeding. Specimens of B. passalus fed readily upon the same prey reported for B. lacinus. The cuticle of large prey nematodes was drawn

Fig. 8. Copulation of Patlerius nasutus.

- a. Initial copulation attempt by previously mated males, note proximity to vulva.
- b. Initial copulation attempt by non-mated males.
- c. Spicule penetration, note position of gubernaculum.
- d. Vulvar plug.



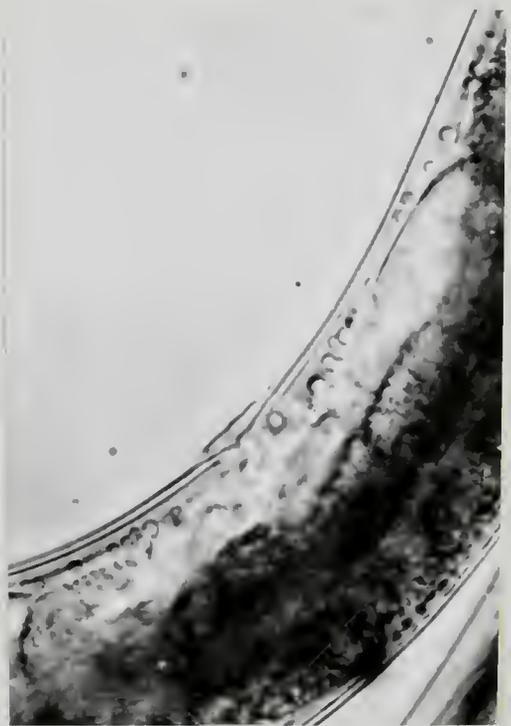
a



b



c



d

Fig. 9. Feeding of Cutlerius rassalus.

a. Larva attacking Panagrellus redivivus larva, note rupture in cuticle resulting from previous attack by same larva. b. As a, note, P. redivivus cuticle drawn to point of dorsal tooth. c. Ingestion of prey in toto, note dorsal tooth withdrawn into wall of stoma.



a



b



c

into the stoma and ruptured with the dorsal tooth (Figs. 9a and b). Smaller prey nematodes were ingested in toto by folding the dorsal tooth into the wall of the stoma (Fig. 9c).

Competition studies indicated that B. passalus is the least competitive of the three species of Butlerius under study. Butlerius passalus failed to become established in mixed colonies with B. lacinus, Mononchoides sp. or Dorylairus subtilis. In mixed colonies with the other monodelphic Butlerius sp. they became established, but, after a period of inadequate food supply, they were dominated by that species.

Butlerius hamospicus n. sp. Plate III

A population of nematodes, determined to be an undescribed monodelphic species of the genus Butlerius Goodey, 1929, was recovered from a sample of wet organic soil near the overflow drain of a spring house. The sample was taken July 13, 1963 from the property of Mr. J. C. Russell, Sanford, Florida. The specific name hamospicus [L. hamo = hook - L. spic = spicule] is given to denote the ventrally arcuate spicules of the male. All measurements are based on length from lips to the base of the caudal filament since the filament is frequently bitten or broken off.

Dimensions

Female: (N = 10) L = .93 mm (.85 - .97); a = 18.6 (17.0 - 19.5);
b = 4.4 (4.3 - 4.8); c = 11.3 (9.7 - 13.0); V = ^{22.8}67.2% (64.7 - 68.4).

Male: (N = 10) L = .77 mm (.67 - .85); a = 31.1 (23.9 - 38.8);

b = 4.2 (3.9 - 4.8); c = 8.7 (7.8 - 9.7); T = 39.7%.

Description

Body tapering anteriorly and posteriorly, greatest body diameter at ovary flexure, both sexes bear an unusually long caudal filament. Cuticle of both sexes bearing longitudinal ridges broken by fine transverse striae resulting in a weakly beaded appearance. Subcuticle exhibiting punctations which are evenly spaced in either longitudinal or transverse rows. Amphid opening large, transversely oval, (larger in males where they occupy up to $1/3$ of the diameter of the head), located anterior to the apex of the dorsal tooth in females and at the level of the dorsal tooth in males. Head not offset, bluntly rounded anteriorly with six membranous lips which are partially innerfaced by cheilorhabdions. Cheilorhabdions broadly articulated and overlapping the anterior prorhabdions which are enlarged near the point of articulation. Prorhabdions joined, by a flexible membrane of variable length, to the outside of the mesorhabdions. Mesorhabdions also heavy and broadly based on the metarhabdions. A large tooth is present on the dorsal metarhabdion and a cup-shaped rasping plate is present on each subventral metarhabdion. Dorsal tooth perforated subapically by dorsal gland orifice. Rasping plates each bear an imperfect row of small denticles reaching only half the distance across the plate. Female stoma 2.5 times as deep as wide, male stoma 4 times as deep as wide. Female with 6 heavy cephalic setae, male with 4 additional setae located in subdorsal and subventral positions. Procorpus forming a slightly enlarged collar surrounding the stoma as far as the base of

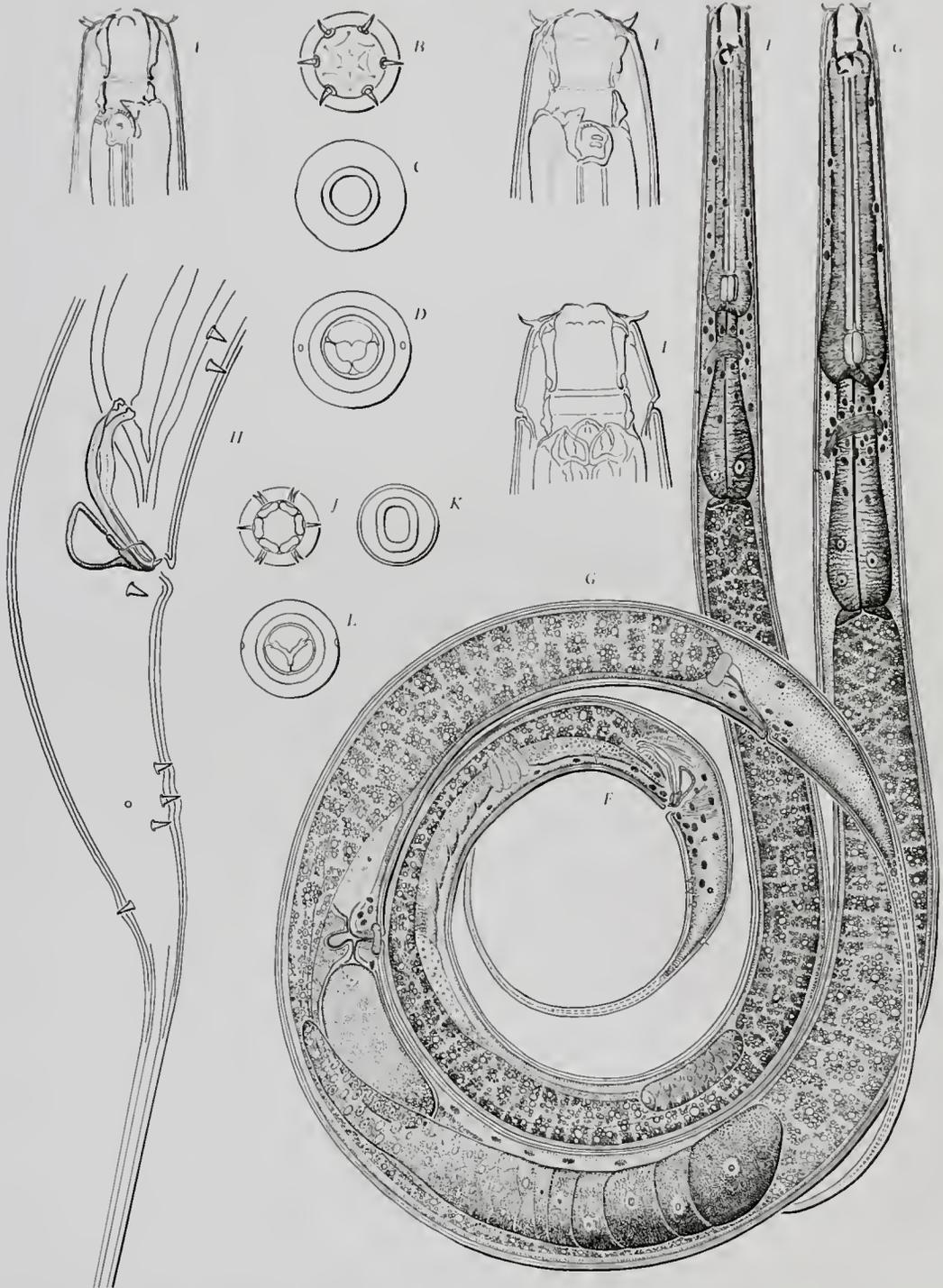
the metarhabdions. Procorpus narrows slightly posterior to the stoma. Metacorpus enlarging to form a valvulate median bulb. Entire corpus thick and heavily muscular. Isthmus rather broad, gradually expanding posteriorly without forming a distinct basal bulb. Isthmus and posterior region of oesophagus predominantly glandular but interspersed with weak musculature. Nerve ring crossing isthmus just posterior to the metacorpus. Excretory pore ventral, just posterior to nerve ring.

Female. Monodelphic reflexed. Lining of the vagina uterina peculiarly refractive and exhibiting a small anteriorly directed pouch, function unknown. Post-uterine branch usually filled with sperm.

Male. Prostom laterally depressed when viewed en face. Testis single, anterior end reflexed only slightly more than one body diameter. Nonbursate. 9 pairs of caudal papillae (3 pair preanal) in typical diplogasteroid arrangement, variable in position. Spicules heavy, paired, ventrally arcuate, capitulum cephalated, calomus broad and narrowing to the long heavy laminae which are enfolded $1/3$ of their length by the gubernaculum. Tips of laminae strongly arcuate. Gubernaculum keel-like, $1/2$ the length of the spicules and $1/2$ as deep as long. Gubernaculum heavily sclerotized in the section where it enfolds the spicules. The remainder of the keel margin is complete and equally sclerotized.

Diagnosis

Females of Butlerius hamaxicus are distinguished from didelphic species of Butlerius by the single ovary. The single large dorsal



tooth which is perforated subapically by the dorsal oesophageal gland orifice and the rasping plates located on each subventral metarhabdion distinguishes it from B. monhystrax. Separation from B. passalus is by the half-row of denticles on each rasping plate, and by the six lips. Males of B. hamospicus are distinguished from all other species by the arcuate laminae and the completely sclerotized border on the keel-shaped gubernaculum.

Holotype

Single female on slide no. 1 labeled "Butlerius hamospicus" (holotype), in collection of the Department of Entomology, University of Florida, Gainesville, Florida.

Paratypes

Deposited in collection of the Department of Entomology, University of Florida and others in the United States Department of Agriculture, Nematode Collection, Nematology Investigations, Agricultural Research Service, Beltsville, Maryland.

Bionomics

General. Butlerius hamospicus is closer to B. passalus in ethology and morphology than to B. lacinus. The movements during swimming as well as the behavioral responses exhibited in the reproduction studies and feeding habits are of a relatively sluggish nature when compared to those of B. lacinus.

The author is indebted to Mr. R. P. Escher for supplying samples containing natural populations of B. ~~lacinus~~ recovered from the same types of habitats as B. lacinus. These populations provided for continual comparison of wild and laboratory populations.

Embryology. The 20 eggs used in this study measured from 68 to 87 μ in length and 31 to 37 μ in width. The chorion, as did that of B. passalus, exhibited a large number of relatively large hyaline, adhesive protuberances. Average time lapse from oviposition (ranges included parenthetically) to each developmental stage is summarized in the legend of Fig. 10.

Eggs were laid singly while in the one-cell stage and, as reported for B. passalus, two-cell or more advanced stages of development were not observed in the uteri of healthy females. Oviposition was not observed for females bearing more than one egg, or eggs developed to or beyond the two-cell stage, in their uteri; but the latter females did live for considerable periods of time.

The embryonic development of B. ~~lacinus~~ was of only slightly shorter duration than that of B. passalus and development was generally the same. The first stage larvae exhibited the same limited rhabdial development and the intrachorionic molting process was difficult to observe.

Larval egress from the egg was accomplished apparently in the same manner as described for the other two species of Butlerius, although the interval between intrachorionic molting and egress was not so variable as that reported for B. lacinus.

Fig. 10. Embryonic development of Putlerius hamospicus.

a. 1-cell stage. b. Initiation of cleavage. c. 2-cell stage (1.5 - 2.5 hr). d. Division toward 4-cell stage. e. 4-cell stage (2.5 - 3.5 hr). f. 8-cell stage (3.5 - 8.5 hr). g. 16-cell stage (12 - 15.5 hr). h. 32-cell stage (14.5 - 17.5 hr). i. Morula (16.5 - 19 hr). j. Blastula (19.5 - 24 hr). k. Gastrula (26.5 - 30.5 hr). l. Tadpole (29.5 - 35 hr). m. 1st larval stage, note degree of rhabdion development. n. Molting (44 - 49 hr), note separation of cephalic cuticle and rhabdion development. o. 2nd stage larva. p. Egress.



a



b



c



d



e



f



g



h



i



j



k



l



m



n



o



p

Life cycle. The duration of each larval stage and measurements of body length were calculated in the manner previously discussed for the other two species and are included in Table 3.

Table 3. Larval Stage Duration and Body Lengths of Butlerius hamospicus

Stage	Duration in hrs	Body Length in mm	
		Average	Range
1st	10	0.25	(no variations)
2nd	61	0.36	(0.27 - 0.45)
3rd	35	0.50	(0.43 - 0.60)
4th ♂	40	0.58	(0.48 - 0.65)
♀	40	0.66	(0.60 - 0.70)

The adult stage was reached by the females in an average of 190 hrs and by the males in an average of 175 hrs.

Molting. The molting process of B. hamospicus was essentially identical to that described for B. passalus.

Reproduction. The behavioral responses of B. hamospicus males were observed to be almost identical to those exhibited by males of B. passalus. Virgin females were approached directly even when obstacles intervened and in several instances B. hamospicus males penetrated large masses of P. redivivus specimens in locating and copulating with conspecific females (Fig. 11a).

The process of copulation was intermediate between that described for the two other species. The spicules were thrust deeply into the female, penetrating the entire depth of the vagina (Fig. 11b). The gubernaculum was not inserted into the vagina and the spicules were forced

into the enfolding ring of the gubernaculum to the expanded upper portion of the calomus (Fig. 11b). During copulation, muscular action of the female genitals were observed similar to that described for B. lacinus. The spicules were pulled toward the single ovary, not toward the post-uterine sack in which sperm were later observed. The laminae of the spicules of this species are much more stoutly constructed than those of B. lacinus, however, and did not bend. The cephalated heads of the spicules and gubernaculum turned within the male's body (Fig. 11c). Reproductive isolation of B. hamospicus was confirmed by the reproductive isolation tests.

Feeding. The predaceous habits of B. hamospicus were similar to those described for the other two species. Figure 12 shows an example of cannibalism in an underfed, overpopulated colony, under which conditions cannibalism was commonly observed. The list of prey for the two preceding species is identical with that of B. hamospicus.

Competition studies resulted in the eventual annihilation of B. hamospicus populations under all mixed colony situations except in colonization with B. passalus in which case the smaller species, B. hamospicus, prevailed.

Fig. 11. Copulation of Eutlerius hamospicus.

- a. The male penetrated this mass of prey nematodes in order to mate (arrow).
- b. Spicule penetration, note position of gubernaculum and capitulum. c. Rotation of spicules and gubernaculum due to female's muscular action.



a



b



c

Fig. 12. Cannibalism by Eutlerius hamospicus.

Note material in lumen of the oesophagus of attacking specimens and dilated oesophageal lumen of the attacked male.



SUMMARY

Three new species of the genus Butlerius T. Goodey, 1929, were found in Florida and the taxonomic descriptions were prepared. The names Butlerius lacinus, B. passalus and B. hamoniscus were assigned. The embryonic and larval development of each species was studied. The average time lapse per developmental stage for each species is given in Table 4.

Table 4. Average Time Lapse of the Developmental Stages of Butlerius spp. (in hrs from oviposition at 27°C)

Stage	<u>B. lacinus</u>	<u>B. passalus</u>	<u>B. hamoniscus</u>
Oviposition in 1-cell	0	0	0
2-cell	.5	1.0	1.5
4-cell	1.5	3.0	3.0
8-cell	3.0	6.0	5.5
16-cell	6.5	12.0	13.0
32-cell	7.5	15.0	15.0
Morula	9.0	18.0	17.25
Blastula	13.0	21.0	21.25
Gastrula	15.0	26.0	28.75
Tadpole	16.0	30.0	30.75
1st Larval	18.0	34.0	30.0
Intrachorionic Molt	27.0	45.0	46.0
2nd Larval	42.0	74.0	68.0
Molt - 3rd Larval	64.0	109.0	105.0
	67.0	115.0	109.0
Molt - 4th Larval	81.0	154.0	135.0
	84.0	159.0	150.0
Molt - Adult	102.0	203.0	175.0
	114.0	212.0	190.0

Studies on reproductive habits of each of the three species of Butlerius demonstrated that the males can locate females of their own

species from distances of up to 80 mm under in vitro conditions, despite intermediate masses of prey nematodes and other individuals of their own species. None mated with individuals of the other two species. An indication of accumulation of experience was exhibited in the copulatory responses of previously mated males.

The predaceous habits of the three species were similar. The cuticle of large prey nematodes was drawn into the stoma of specimens of Butlerius by suction. This suction resulted from the repeated, rapid elongation of the stoma. The cuticle was then ruptured by the dorsal tooth and the body contents ingested. Small prey nematodes were ingested in toto. During the latter type of feeding the dorsal tooth was withdrawn into the wall of the stoma.

None of the three species of Butlerius described was as competitive as Nogorchoides sp. or Dorylaimus subtilis. Butlerius lacinus was more competitive than the other two Butlerius spp., whereas B. passalus was the least competitive.

LITERATURE CITED

1. Adam, W. 1930. Ein neuer freilebender Nematode aus der Erde: Butlerius filicaudatus n. spec. Zool. Anz. Leipzig. 91:139-142.
2. Cobb, N. A. 1917. The Mononchs. Soil Science. 3(5):1-124.
3. Chitwood, B. G., and M. B. Chitwood 1938. Introduction to nematology. Baltimore 213 pp.
4. Goodey, J. B. 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bull. Minist. Agr., Lond. 2.
5. Goodey, T. 1929. On some new and little-known free-living nematodes. J. Helminthol. 7:27-62.
6. Goodey, T. 1951. Soil and freshwater nematodes. Methuen, London. 390 pp.
7. Goodey, T. 1963. Soil and freshwater nematodes. 2nd Ed. Edited by J. B. Goodey. Methuen, London. 544 pp.
8. Lordello, L. G. D., and A. P. L. Zamith 1959. Observações sobre o gênero "Butlerius" de nematódeos de vida livre. Rev. Bras. Biol. 19:177-182.
9. Meyl, A. H. 1957. Two new free-living nematodes, found in the rain-water reserve of Quesnelia grandis (Vell.) Mez. (Bromeliaceae) from Brasil. Proc. Helminthol. Soc. Wash. 24:62-66.
10. Meyl, A. H. 1960. "Freilebenden Nematoden" In: Die Tierwelt Mitteleuropas, edited by P. Brohmer, P. Ehrmann, and G. Ullner 1(5a):164 pp. Leipzig, Quelle & Meyer.
11. Rahm, G. 1938. Freilebende und saprophytische Nematoden der Insel Hainan. Anot. Zool. Jap. 17:646-667.
12. Russell, C. C. 1964. A rearing medium for microphagous nematodes. The Florida Entomologist. 47(4):263.
13. Schuurmans-Stekhoven, J. H., and R. J. H. Teunissen 1938. Nematodes libres terrestres. Explor. Parc. Nat. Albert (Mission 1933-35), Brussels 229 pp.
14. Taylor, D. P. 1964. Butlerius manhusana (Nematoda, Diplogasteridae) a new species of predaceous nematode from Illinois. Proc. Helminthol. Soc. Wash. 31(2):129-132.

15. Thorne, G., 1961. Principles of nematology. McGraw-Hill, New York. 553 pp.

BIOGRAPHICAL SKETCH

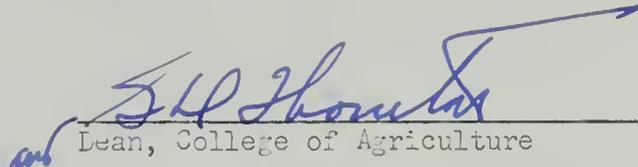
Charles Clayton Russell was born October 9, 1937, at Key West, Florida. He attended public schools in Sanford, Florida and was graduated in 1955 from Seminole High School in Sanford, Florida. He attended Piedmont College from 1955 until 1957 when he transferred to the University of Florida. He received the Bachelor of Science in Agriculture degree in June, 1960 and the Master of Science in Agriculture degree in December, 1962.

He is a member of: Sigma Xi, Phi Sigma, Gamma Sigma Delta, Alpha Zeta, Society of Nematologists, Florida Nematology Forum, Newell Entomological Society and Florida Entomological Society.

He is married to the former Carole Ann Bridges and they have a son, John Charles, and a daughter, Cynthia Ann.

This dissertaion was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

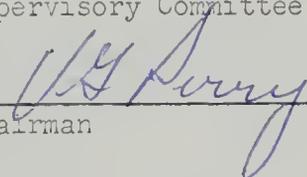
August 12, 1967



Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:



Chairman

