

Volume 33

July 1966

Number 2

PROCEEDINGS

of

# The Helminthological Society of Washington

**A semiannual journal of research devoted to  
Helminthology and all branches of Parasitology**

Supported in part by the  
Brayton H. Ransom Memorial Trust Fund

Subscription \$7.00 a Volume; Foreign, \$7.50

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# Proceedings of the Helminthological Society of Washington

VOLUME 33

JULY 1966

NUMBER 2

## A Compendium of the Genus *Criconemoides* (Criconematidae: Nemata)<sup>1</sup>

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### SUMMARY

A key and pertinent morphological data derived from the original publications are presented on 89 nominal species of *Criconemoides*. *Criconemoides anura*, *C. demani*, *C. heideri*, *C. hispalensis*, *C. hygrophilum*, *C. peruense*, *C. rusticum*, and *C. simile* are transferred to *species inquirendae*.

### INTRODUCTION

*Criconemoides* contains more described species than any other genus of plant-parasitic nematodes. Yet, despite its taxonomic popularity it is one of the most difficult genera in which to work. Numerous authors have proposed species which are supposedly distinctive mainly by certain morphological characteristics lacking for closely related species. In certain cases, morphological variability has not been considered. Neither has the possibility of the existence of artifacts due to fixation and preservation when only one or a few specimens are at hand on which to base the species. Differences in interpretation of characters, separation on dubious or indistinct morphological structures, unfamiliarity with the nominal species, and inaccurate measurements have very probably contributed to the mass of species currently in the genus. It is felt that biometrical data must be relied upon as a sensible approach to species separation and that taxonomy of such a large genus must be on an objective rather than subjective basis.

In the present study, number and ornamentation of body annules, length of stylet, and number of annules between the vulva and the tail terminus, on the tail, and between the labial disc and excretory pore were considered

primary differentiating characters. Range of body length, vulva percentage, tail and tail terminus shape, ratio of the stylet divided by the body length, and the ratio of vulva-terminus length divided by the width of the body at the vulva were regarded as secondary characters. The Demanian ratios a, b, and c were not considered as usable diagnostic characters. When possible, other important data such as presence of sublateral lobes or unusual labial annules were used in constructing the key.

### DISCUSSION OF RECENT PROPOSED CHANGES

#### *The classification of de Grisse and Loof (1965)*

These workers have recently divided *Criconemoides* into six genera, based primarily on presence or absence of sublateral lobes, female labium shape, stylet length, body length, and annule shape. They resurrected *Macroposthonia* de Man, 1880 and placed 39 species in the genus on the basis of their finding males of *M. annulata* de Man, 1880 in Belgium, Germany, and Bergen op Zoom, The Netherlands, associated with females of *C. kirjanovae* Andrassy, 1962. The criteria which they felt were important in deciding that their males were *M. annulata* were narrow head, ventrally bent pointed tail, and various measurements. However, a comparison of their drawings with that of de Man's shows their specimens to have a broader anterior region, while the measurements of their three populations show differences from those of de Man's in body length, number of annules to excretory pore, and number of tail annules. Then, too, the only population they collected in The Netherlands was about 75 km from the type locality of Leiden, and about 30 km from Breda where de Man (1921) reported the species as occurring a

<sup>1</sup>University of Florida Citrus Experiment Station, Lake Alfred, Florida. Florida Agricultural Experiment Stations Journal Series No. 2286. The assistance of R. L. Ward, who aided in the compilation of data, is appreciated.

second time. There have been about 11 species of *Criconemoides* reported from the area occupied by The Netherlands and Belgium; this raises the question whether the males and females found in the population belonged to the same species.

De Grisse and Loof have suggested a modification of the accepted concept of *Criconema* in that they propose species having "females with scales, spines, or fringe on posterior margins of annules. Submedian lobes absent" be placed in *Criconema* while species with "posterior margins of annules smooth in adult females, or slightly ornamented, but then submedian lobes present" be placed in one of the six genera which they establish. One species, *Criconema pruni* Siddiqi, 1961, which was originally described as having definite annular fringes appearing as "continuous membranous flaps marked by deep longitudinal lines" was placed in *Macroposthonia*. Three species were placed in *Lobocriconema*. Of these three species, *Criconema laterale* Khan and Siddiqi, 1964 and *C. serratum* Khan and Siddiqi, 1963 have definite spines while *C. sulcatum* Golden and Friedman, 1964 has noticeable scales. All four species thus exhibit annules which deviate from the "slightly ornamented" category in which they are placed. Then, too, it would be questionable to base such a generic separation on labial projections which very often are quite indefinite, probably subject to malformation due to fixation, and whose reported presence can often be dependent on the observer's interpretation. Hence, it is felt that the separation between *Criconema* and *Criconemoides* should remain as before, determined mainly by the presence of spines, scales, or fringes on females of *Criconema*, even though crenation of the posterior edge of the annules can occur in both genera.

In their generic description of and keys to *Macroposthonia*, the authors use dubious differences between species such as lateral contour of annules, size of sublateral lobes, inclination of tail tip, and width of vulva opening. They claim that *C. basili* Jairajpuri, 1964 has three to four annules between the vulva and tail terminus, when it was reported as having four to five; that the posterior edges of annules of *C. crenatus* Loof, 1964 and *C. pseudosolvagum* de Grisse, 1964 are conspic-

uously or strongly crenate, yet these species are placed in a genus that is only supposed to have slight, fine ornamentation on the posterior edge of annules; and that *C. irregulare* de Grisse, 1964 has body annules with irregular margins (No. 32, in their key) but placed the species in a group having smooth posterior margin of annules (No. 12, in their key). Their generic diagnosis for *Macroposthonia* definitely characterizes the males with bursa and the juveniles without scales when, actually, males and juveniles were not described for several of the species included in the genus.

For these reasons, transfer of the 38 species listed by de Grisse and Loof to *Macroposthonia* is rejected and the species are all regarded in the genus *Criconemoides* Taylor, 1936, as is *C. kirjanovae* Andr assy, 1962. *Macroposthonia annulata* de Man, 1880 remains a problematic species especially in the light of the rather striking disparity between de Man's 1880-1884 and 1921 specimens. Goodey (1951) and Tarjan (1960) regarded this species as *incertae sedis*, but it now seems more logical to place *M. annulata* in *species indeterminata*.

The genus *Nothocriconema* de Grisse and Loof, 1965 is characterized by the absence of sublateral lobes, posterior margin of annules smooth in females and with scales on juveniles, and an offset or modified female head. Perhaps the greatest shortcoming of this genus is that only one (*Criconemoides mutabilis* Taylor, 1936) of the 13 species included fulfills the generic requirement of "Juveniles with annules showing smooth-edged or dentate scales . . ." Juveniles were not reported for 11 of the species and nothing was mentioned or illustrated for the juveniles found for the remaining species (*C. annuliferum* de Man, 1921). Whether or not sublateral lobes exist when the original authors did not make specific reference to these structures remains a matter of personal interpretation. In one case (*C. princeps* Andr assy, 1962), the species was originally described as having nonprojecting sublateral lobes, but it is nevertheless included in *Nothocriconema*. In another case, a species without lip region annule modification was included (*C. longulum* (Gunhold, 1953)).

The genus *Lobocriconema* is characterized as having sublateral lobes and offset labium, smooth or slightly ornamented annules, less

than 50 body annules, and juveniles with longitudinal rows of scales on the posterior edge of annules. Of five species included, three were transferred from the genus *Criconema*, viz. *C. laterale*, *C. serratum*, and *C. sulcatum*. It previously has been stated, in the present paper, that these species should remain in their original genus. With their withdrawal from *Lobocriconema*, only two species remain, viz. *C. crassianulatum* (de Guiran, 1963) and *C. aberrans* Jairajpuri and Siddiqi, 1963 for which juveniles have not been described, necessitating rejection of that part of the generic diagnosis dealing with juveniles. The diagnosis thus mainly calls for the presence of an offset lip region, sublateral lobes, and less than 50 body annules. Depending on the observer's interpretation of what constitutes an offset lip region and sublateral lobes, four additional species, viz. *C. axeste* Fassuliotis and Williamson, 1959, *C. neoaxeste* Jairajpuri and Siddiqi, 1963, *C. petasus* Wu, 1965, and *C. pseudosolvagum* de Grisse, 1964, could possibly meet the requirements for inclusion in this genus. Hence, it is apparent that the genus is not sufficiently clearly defined and without adequate justification for its existence.

The more important diagnostic characters of *Criconemella* are body length (0.18–0.32 mm), tail markedly trapezoid, posterior margin of annules smooth or finely crenate, and excretory pore located more than 30 annules from anterior end. Included in this genus were *C. parvum* Raski, 1952, *C. goodeyi* de Guiran, 1963, *C. parvulum* Siddiqi, 1961, and *C. zavadskii* (Tulaganov, 1941). Inconsistencies appear with *C. goodeyi* which is described as having heavily crenate annules in the specific key while being included in a genus with smooth to finely crenate annules, and with *C. zavadskii* for which an excretory pore was not mentioned in the original description but nonetheless is included in the genus that, by definition, has to have a posteriorly located excretory pore. There are 15 other species that have been described with specimens in the length range of this genus. Likewise, determination of a tail as being "markedly trapezoid" is definitely a matter of personal opinion and hardly of concrete taxonomic value.

*Discocriconemella* is mainly characterized as having a saucer-shaped labial annule, distinctly

crenated annules, with many anastomoses, and with certain male characters. De Grisse and Loof have examined paratypes of *C. mauritiense* Williams, 1960, one of the two species placed in the genus, and report that "the annules are crenate and numerous anastomoses occur." Since Williams (1960) did not mention or depict the crenation or frequent anastomosing in his original description, it could be assumed that these characters are not readily apparent and accordingly are hard to determine.

*Xenocriconemella* is distinguished mainly by a long flexible stylet measuring about 40% of the total body length, finely crenate annules, and male characters. A number of other species possess long flexible stylets. Although the two species included in this genus, viz. *C. macrodorum* Taylor, 1936 and *C. juniperi* Edward and Misra, 1964, were originally described as having smooth annules, de Grisse (1964) depicts the latter species as having a very fine annule crenation whereas Corbett (1962), who also found the species, does not mention annule crenation as existing. Hence, the genus is characterized by questionable criteria.

#### *The Genus Neocriconema Diab and Jenkins, 1965*

The diagnosis for this genus was published immediately after the work by de Grisse and Loof appeared, strongly indicating that Diab and Jenkins were unaware of the earlier work. Essentially the genus was created for those species "having crenations along the posterior edges of annules in the adult female . . ." The account gave the description of a new species, *Neocriconema adamsi*, and included ten other nominal species in the genus.

There are two principal objections to this genus as it now stands: (1) species with annules having posterior margins that are not typically crenate but irregular, e.g., *Criconemoides axeste*, *C. crassianulatum*, *C. maritimum* de Grisse, 1964, and *C. neoaxeste* create a problem, as do (2) species that have annules with posterior margins bearing extremely fine crenations which are on the border of visibility, e.g., *C. rosae* Loof, 1964. Hence, the usefulness of this genus is negated because of indecision as to what constitutes crenation. It is

felt that acceptance of this genus will further confuse the *Criconemoides* situation; accordingly, the new species is designated as *Criconemoides adamsi* n. comb.\*

#### CONCLUSIONS ON RECENT PROPOSED CHANGES

Although the systematic arrangements of de Grisse and Loof (1965) and Diab and Jenkins (1965) are herein disputed, this writer nevertheless is wholly in accord with the objectives of these workers—to clarify the confusion that exists in the genus *Criconemoides* and to simplify the current classification. Yet, any proposed classification must be justifiable, accurate, and useful. The work of de Grisse and Loof (1965), although particularly impressive, only partially fulfills these requirements. It is hoped that these workers will amplify their studies to eventually furnish a usable classification for the genus. Until that time, however, it is best to continue regarding *Criconemoides* as a valid genus, albeit necessarily bulky and unmanageable.

#### SYNONYMIES

*Criconemoides citri* Steiner, 1949 was considered a synonym of *C. sphaerocephalus* Taylor, 1936 by Loof (1964). De Grisse (1964) synonymized *C. deconincki* de Grisse, 1963 to *C. crassianulatum* while Jairajpuri (1964) re-

\* I recently learned of a publication by T. Yokoo (1964. On a new species of ring nematode from Japan. II., Agr. Bull. Saga Univ. 20: 63-65) which describes *Criconemoides sagaensis*. This new species is reputed to be close to *C. rusticum* and *C. xenoplax* but differs mainly in stylet length.

A paper appearing after this compendium was submitted for publication is that by Raski, D. J., and A. M. Golden (1966. Studies on the genus *Criconemoides* Taylor, 1936 with descriptions of 11 new species and *Bakernema variabilis* n. sp. (Criconematidae: Nematoda). *Nematologica* 11(4) (1965): in press.) These authors propose 11 new species of *Criconemoides*. They regard *C. juniperi* a synonym of *C. macrodorum*, *C. rotundicaudatus* and *C. hemisphaericaudatus* synonyms of *C. annulatum*, *C. siddiqii* a synonym of *C. magnoliae*, *C. naitalense* a synonym of *C. curvatum*, and *C. hygrophilum* a synonym of *C. annulifer*. They also propose that *Criconema laterale*, *C. serratum*, *C. sulcatum*, *C. tenuiannulatum*, *C. pruni*, and *C. brevicaudatum* be transferred to *Criconemoides*. The first three *Criconema* species have been discussed earlier in this paper, the fourth species has already been synonymized to *C. simile*, while the change in genus for the last two species appears justified even though, as already stated, the annule ornamentation of *C. pruni* does not fit the generic criteria of *Macroposthonia* (sensu de Grisse and Loof) and warrant that proposed change in genus name. Finally, *C. hispalensis* Arias Delgado *et al.*, 1963 is placed in *species inquirendae*.

Taking into consideration this additional information and based on the arguments presented herein, the genus can be regarded as comprised of 72 species. In addition, there are 16 species that have been transferred to *species inquirendae* and 13 species that have been placed in synonymy.

named his *C. goodeyi* of 1964 as *C. basili*, since the former name was preoccupied.

De Grisse and Loof (1965) synonymized *C. kirjanovae* to *Macroposthonia annulata* (this synonymy was rejected earlier in the present paper) and also proposed the following: *Criconemoides obtusicaudatum* Heyns, 1962 as a synonym of *C. ferniae* Luc, 1959; *C. anura* (Kirjanova, 1948) and *C. flandriensis* de Grisse, 1963 as synonyms of *C. informe* (Micoletzky, 1922); *C. lobatum* Raski, 1952 as a synonym of *C. rusticum* (Micoletzky, 1922) (which is rejected since the latter species is too poorly defined); *C. beljaevae* (Kirjanova, 1948), *C. cylindricum* (Kirjanova, 1948), *Criconema tenuiannulata* Tulaganov, 1949, and *Criconemoides xenoplax* Raski, 1952 as synonyms of *C. simile* (Cobb, 1918); *C. raskii* Goodey, 1963 and *C. magnoliae* Edward and Misra, 1964 as synonyms of *C. mutabilis*; *C. elegantula* (Gunhold, 1953) as a synonym of *C. longulum*; and *C. goffarti* (Volz, 1951) as a synonym of *C. macrodorum*.

Despite certain differences in the descriptions and measurements of species which are above regarded as being similar, the following synonymies are accepted thus decreasing the size of the genus, which undoubtedly can be further reduced pending further studies: *Criconemoides obtusicaudatum* as a synonym of *C. ferniae*; *C. flandriensis* as a synonym of *C. informe*; *C. xenoplax* and *Criconema tenuiannulata* as synonyms of *Criconemoides beljaevae*; *C. magnoliae* and *C. raskii* as synonyms of *C. mutabilis*; and *C. elegantula* as a synonym of *C. longulum*.

#### TRANSFERS TO OTHER GENERA

*Hemicriconemoides obtusus* Colbran, 1962 was placed in *Criconemoides* by Siddiqi and Goodey (1964) because the thick cuticle seemed "not to be formed of two separate parts, and because some annules are slightly retrose . . ." The present writer concurs with the judgment of de Grisse and Loof (1965), based on Colbran's view (*in litt.*), that the species belongs in *Hemicriconemoides*.

#### TRANSFERS TO SPECIES INQUIRENDAE

The first transferal to the *species inquirendae* category was made by Andrassy (1960) who placed *C. sinensis* Rahm, 1937 in this category.

Siddiqi and Goodey (1964) then transferred *C. boettgeri* Meyl, 1954, *C. congolense* (Schuermans-Stekhoven and Teunissen, 1938), and *C. goffarti* into *species inquirendae*. *Criconemoides boettgeri* was described from only one specimen and lacked essential information necessary for its proper characterization. De Grisse and Loof (1965) report examining many specimens of this species and determining that it is a *Criconema* but give no drawings and necessary essential information justifying the action or otherwise permitting the others to logically arrive at the same conclusion. Until such information appears, the species should remain in the *inquirendae* category.

The judgment of de Grisse and Loof is correct that *Criconemoides morgense* (Hofmänner in Hofmänner and Menzel, 1914) should be placed in *species inquirendae*. Yet, the description and figure of this species, which is the type for *Criconemoides*, is generally consistent with the generic requirements. Figure 20, Table 6 in Hofmänner and Menzel (1914) corroborates this view and seemingly does not offer any evidence that the species might belong to *Hemicriconemoides* as suggested by de Grisse and Loof. There is nothing in the 1964 International Code of Zoological Nomenclature to nullify the acceptability of a nominal genus whose type species is regarded *species inquirenda*, this term being defined on page 152 as "A doubtfully identified species needing further investigation." Accordingly, *Criconemoides* remains a valid genus with a type species bearing the generic characters but without sufficient characterization to permit reliable placement among other species in the genus.

*Criconemoides congolense*, *C. heideri* (Stefánski, 1916), *C. hercyniensis* (Kischke, 1956), and *C. komabaensis* (Imamura, 1931) were insufficiently characterized and are questionable as de Grisse and Loof have suggested, but it is felt that these species are *Criconemoides* and hence should be placed in *species inquirendae*, and not the *incertae sedis* category.

The author has critically inspected the original publications of all of the species in the genus. Those descriptions that are based upon one specimen and, in addition, omit essential information for the proper characterization of the species are transferred to *species inquirendae*. Such species are *C. anura* (Kirjanova,

1948) and *C. cylindricum* (Kirjanova, 1948) for which tail characteristics were not shown; *C. hispalensis* Arias, López, and Jiménez for which there were no drawings and the vulva position was not determined; and *C. hygrophilus* Goodey, 1963 (Andrássy, 1952) for which there were no figures given.

*Criconemoides demani* (Micoletzky, 1925) is placed in *species inquirendae*. It was based on a single gravid female from Denmark. Although Micoletzky gave pertinent data on annules and body measurements, he omitted necessary figures of the anterior and posterior ends of the body and presented only a drawing of the annules at the middle of the body. Taylor describes 12 specimens collected from the United States (South Carolina) which he identified as this species. His measurements and ratios decidedly vary with those of Micoletzky, and the figures which he presents as "*C. demani*" apply only to the South Carolina specimens. This leaves the true identity of the species in doubt justifying the present transfer.

*Criconemoides peruense* (Steiner, 1920) is also placed in *species inquirendae*. Steiner (1920) described one juvenile specimen under the name *Hoplolaimus rusticus*, var. *peruensis* as having the second labial annule directed forward. Cobb (1924) apparently redrew the same specimen but found the vulva, as well as the anus, thus indicating that Steiner's specimen was an adult. Cobb, however, showed the second labial annule to be directed laterally, not forward. This discrepancy, coupled with the fact that only one female was at hand, justifies the new category for this species.

*Criconemoides rusticum* (Micoletzky, 1915) is placed in *species inquirendae*. It was originally proposed on the basis of one female specimen. It is apparent that Micoletzky (1915, 1917) and Menzel (1917) did not interpret the vulva correctly since they refer to it as being in the middle of the body and mistook it as the anus. Yet, both quote the body length as 0.44 mm and the stylet as 58  $\mu$  long. Seidenschwarz (1923), working under Micoletzky, correctly quoted the vulva as being at 90 to 96% but failed to find the anus in the numerous specimens he collected. He corrected the range of body length to 0.29 to 0.42 mm. Micoletzky (1925) further corrected the range of body length to 0.29 to 0.64 mm

and quoted the vulva percentage at 94%. Taylor (1936) did not give a complete formula on the nematodes he studied but adjudged that he had *C. rusticum* and quoted the body length as 0.6 mm and the stylet as 75  $\mu$  long. Goodey (1951) gave the body length as 0.41 to 0.46 mm and the stylet length as 50  $\mu$ . Raski (1952) admitted that the identity of *C. rusticum* was in question but accepted the length 0.6 mm as being representative for the species in his key. Timm (1956) gave the length as being 0.489 to 0.639 mm long and the stylet as 57  $\mu$  long. In view of these obvious discrepancies, this species very logically can be put in the *inquirendae* status.

*Criconemoides simile* Cobb, 1918 is also regarded as *species inquirenda*. Although Cobb (1918) referred to the species as occurring in Washington, D.C., filter beds and in grape roots from Missouri, his description presumably was on one specimen. No illustrations were given by him or by Chitwood (1949), who described a population from peach roots in Salisbury, Maryland. Chitwood's account conforms well with Cobb's rather brief description except for one major discrepancy. Cobb claimed "tail conoid to the blunt terminus" while Chitwood stated "tail a rounded button." This variance in description as well as the absence of figures suggests the *inquirendae* status for this species.

KEY TO FEMALES OF *Criconemoides*

The following key and table of diagnostic data have been prepared using the original publications on each species. The listing of some species in the key is not an indication of their validity, but only that these names are not invalid until proven so by subsequent workers. Due to the paucity of apparent measurable differences between certain species, it has been deemed advisable to follow the suggestions of de Grisse (1964) and use two of his ratios as diagnostic characters. These are the length of the stylet times 100 divided by body length (St%L) and length of the body from the vulva to the tail terminus divided by the width of the body at the vulva (VL/VB). The latter ratio certainly is subject to criticism since pressure on the body due to improper mounting in the slide can result in a lower (VL/VB) ratio. Yet, such ratios were used

only when more substantial criteria were found lacking.

The key is, as always, only an expedient to tentatively identify a nematode. Precise identification must be made using a number of specimens and by reference to Table 1 and/or the original publication. Those species that were erected on only one female specimen are designated with an asterisk (\*).

*Criconemoides* Taylor, 1936. Criconematinae.  
Adult females without sheath. Posterior margins of cuticular annules smooth or crenated, but never with scales, spines, plates, or stalked appendages.  
Type species: *Criconemoides morgense* (Hofmänner in Hofmänner and Menzel, 1914) Taylor, 1936.

1. Stylet more than 100  $\mu$  long ..... 2  
Stylet less than 100  $\mu$  long ..... 7
2. Stylet 122  $\mu$  long, number of annules vulva to tail terminus 13 to 17 .....  
..... *sphagni* (Micoletzky, 1925)  
Stylet 100 to 112  $\mu$  long, number of annules vulva to tail terminus 6 to 12 ..... 3
3. Body annules more than 100 ..... 4  
Body annules less than 100 ..... 5
4. Body annules about 140, tail annules 6 ..... *annulatum* Cobb in Taylor, 1936  
Body annules 106 to 110, tail annules 10 ..... *macrodonum* Taylor, 1936
5. Body annules 70 to 79, tail terminus bilobed to knob-like ... *bakeri* Wu, 1965  
Body annules 53 to 65, tail terminus digitate or spicate ..... 6
6. Tail annules 3 to 4 .....  
..... *annulifer* (de Man, 1921)  
Tail annules 8 ... *stygia* (Schneider, 1940)
7. Average number of body annules more than 135 ..... 8  
Average number of body annules less than 135 ..... 13
8. Body annules 165 or more ..... 9  
Body annules less than 155 ..... 11
9. Body annules 200, number of annules vulva to tail terminus 7 to 8 .....  
..... *zavadskii* (Tulaganov, 1941)  
Body annules 168 to 194, number of annules vulva to tail terminus 10 to 15 ..... 10
10. Stylet 30 to 34  $\mu$  long, excretory pore



- 44 annules from anterior end .....  
 ..... *parvulum* Siddiqi, 1961
- Stylet 40 to 52  $\mu$  long, excretory pore  
 60 to 68 annules from anterior end  
 ..... *adamsi* (Diab and Jenkins, 1965)
11. Stylet length 85 to 103  $\mu$ , body length  
 0.53 to 0.78 mm .....  
 ..... *rotundicaudatus* Wu, 1965
- Stylet length about 36 to 41  $\mu$ , body  
 length 0.44 mm or less ..... 12
12. Terminal tail annule truncate without  
 projections; first labial annule not  
 disc-like ..... *parvum* Raski, 1952
- Terminal tail annule with 2 to 3 pos-  
 terior lobe-like projections; first  
 labial annule disc-like .....  
 ..... *mauritiense* Williams, 1960
13. Average stylet length 70 to 100  $\mu$  ..... 14
- Average stylet length less than 70  $\mu$  ..... 31
14. Body annules less than 80 ..... 15
- Body annules greater than 80 ..... 24
15. Body annules 50 or less ..... 16
- Body annules greater than 55 ..... 18
16. Body annules 38 to 43, number of an-  
 nules vulva to tail terminus 4 to 5  
 ..... *aberrans* Jairajpuri and  
 Siddiqi, 1963
- Body annules 45 to 50, number of an-  
 nules vulva to tail terminus 7 to 12 ..... 17
17. Vulva at 89 to 90% of body length,  
 number of annules vulva to tail ter-  
 minus 7 to 8 .... *neoaxeste* Jairajpuri  
 and Siddiqi, 1963
- Vulva at 82% of body length, number  
 of annules vulva to tail terminus 12  
 ..... *petasus* Wu, 1965\*
18. Stylet length 87  $\mu$ , St%L 22.9 .....  
 ..... *princeps* Andrassy, 1962\*
- Stylet length 83  $\mu$  or less, St%L less  
 than 20 ..... 19
19. St%L 12.6 or less ..... 20
- St%L 15.7 or more ..... 21
20. Number of annules vulva to tail ter-  
 minus 4 to 5, St%L 12.3 to 12.6,  
 tail hemispherical .....  
 ..... *basili* Jairajpuri, 1964
- Number of annules vulva to tail ter-  
 minus 7 to 14, St%L 8.9 to 10.9,  
 tail bluntly to sharply conical ..... 20a
- 20a. Number of annules vulva to tail ter-  
 minus 13 to 14, tail sharply conical  
 ..... *crotaloides* (Cobb, 1924)
- Number of annules vulva to tail ter-  
 minus 7 to 8, tail bluntly conical  
 ..... *montserratii* Arias Delgado,  
 Jiménez Millán, and López  
 Pedregal, 1965
21. Vulva at 90% or less of body length,  
 VL/VB ratio 1.24 or greater ..... 22
- Vulva at 91 to 95% of body length,  
 VL/VB ratio 0.97 or less ..... 23
22. Tail terminus bilobed, St%L 18.0 to  
 19.4 .... *duplicivestitum* Andrassy, 1963
- Tail terminus digitate, St%L 16.2 to  
 18.4 ..... *mongolense* Andrassy, 1964
23. Body annules 56 to 62 .....  
 ..... *informe* (Micoletzky, 1922)
- Body annules 68 to 73 .....  
 ..... *maritimum* de Grisse, 1964
24. Vulva at 85% of body length .....  
 ..... *longulum* (Gunhold, 1953)\*
- Vulva at 90% or more of body length  
 ..... 25
25. St%L 18 or higher, VL/VB less than  
 0.9 ..... 26
- St%L less than 18, VL/VB more than  
 0.9 ..... 28
26. Body annules averaging more than 115  
 ..... *hemisphaericaudatus* Wu, 1965
- Body annules averaging less than 115  
 ..... 27
27. St%L 18 to 22, tail annules 5 to 6 ....  
 ..... *teres* Raski, 1952
- St%L 36 to 38, tail annules 8 to 9 .....  
 ..... *juniperi* Edward and Misra, 1964
28. Average St%L greater than 16, aver-  
 age body length less than 0.45 mm  
 ..... *antipolitanum* de Guiran, 1963
- Average St%L less than 16, average  
 body length more than 0.45 mm .... 29
29. Body annules averaging 88 to 109 ....  
 ..... *quadricorne* (Kirjanova, 1948)
- Body annules averaging more than 110  
 or less than 87 ..... 30
30. Body annules 81 to 86, excretory pore  
 23 to 24 annules from anterior end  
 ..... *macrolobatus* Jairajpuri and  
 Siddiqi, 1963
- Body annules 108 to 133, excretory  
 pore 31 to 39 annules from anterior  
 end .... *pseudohercyniensis* de Grisse  
 and Koen, 1964

\* Description based on one specimen.

31. Labium resembling inverted trapezoid with concave base, maximum body length 0.23 mm .. *limitaneum* Luc, 1959  
Labium not a trapezoid, maximum body length at least 0.27 mm or greater ..... 32
32. Body annules averaging over 100 ..... 33  
Body annules averaging under 100 .... 39
33. Body annules 128 to 136 .....  
..... *onoensis* Luc, 1959  
Body annules 100 to 119 ..... 34
34. Stylet 33 to 38  $\mu$  long .....  
..... *goodeyi* de Guiran, 1963  
Stylet 46 to 71  $\mu$  long ..... 35
35. Tail annules 7 to 9, VL/VB 1.30 or more ..... 36  
Tail annules 5 to 6, VL/VB 1.02 or less ..... 37
36. VL/VB 1.34, St%L 11.6 .....  
..... *kovacsi* Andr ssy, 1963\*  
VL/VB 1.69 St%L 15.4 to 21.3 .....  
..... *siddiqii* Khan, 1964
37. Number of annules vulva to tail terminus 9 to 10, St%L 10.8 or less ...  
..... *mutabilis* Taylor, 1936  
Number of annules vulva to tail terminus 7 to 8, St%L 11.5 or greater .. 38
38. Stylet 51 to 55  $\mu$ , St%L 11.5 to 12.8  
..... *lobatum* Raski, 1952  
Stylet 55 to 71  $\mu$ , St%L 13.4 to 13.7  
... *nainitalense* Edward and Misra, 1963
39. Stylet 25 to 26  $\mu$ , St%L 6.1 .....  
..... *microdorum* (de Grisse, 1964)  
Stylet 37  $\mu$  or greater, St%L 9.6 or greater ..... 40
40. Body length 0.72 mm, VL/VB 0.91  
..... *beljaevae* (Kirjanova, 1948)\*  
Body length 0.62 mm or less, VL/VB ratio 0.68 or greater ..... 41
41. Number of annules vulva to tail terminus 14 to 16, VL/VB ratio 2.39  
..... *quasidemani* Wu, 1965  
Number of annules vulva to tail terminus 12 or less, VL/VB ratio 1.62 or less ..... 42
42. Average number body annules 60 or less ..... 43  
Average number body annules 61 or more ..... 45
43. Body annules 33, VL/VB 0.91 .....  
..... *crassianulatum* de Guiran, 1963  
Body annules 42 to 60, VL/VB 1.1 to 1.3 ..... 44
44. Tail annules 2 to 3, excretory pore 12 to 15 annules from anterior end .....  
..... *axeste* Fassuliotis and Williamson, 1959  
Tail annules 3 to 5, excretory pore 15 to 20 annules from anterior end .....  
..... *pseudosolivagum* de Grisse, 1964
45. Stylet length averaging 37 to 46  $\mu$  ..... 46  
Stylet length averaging 47 to 70  $\mu$  ..... 48
46. Body length 0.37 mm, St%L 12.2 .....  
..... *ferniae* Luc, 1959  
Body length 0.21 to 0.32 mm, St%L 12.8 to 18.1 ..... 47
47. Body annules 75 to 84, excretory pore 22 to 24 annules from anterior end .....  
..... *crenatus* Loof, 1964  
Body annules 91 to 96, excretory pore 26 to 28 annules from anterior end .....  
..... *tescorum* de Guiran, 1963
48. Average number body annules 63 or less ..... 49  
Average number body annules 64 or more ..... 50
49. Stylet 66  $\mu$  long, tail annules 6 .....  
..... *solivagum* Andr ssy, 1962  
Stylet 48 to 53  $\mu$  long, tail annules 4 to 5 ..... *irregulare* de Grisse, 1964
50. St%L 11.0 to 11.1, VL/VB 1.62 .....  
..... *raskiense* de Grisse, 1964  
St%L 12.1 or more, VL/VB 1.58 or less ..... 51
51. Average number body annules 80 or more ..... 52  
Average number body annules less than 80 ..... 57
52. Tail annules 3 to 4, VL/VB 0.83 or less ..... 53  
Tail annules 5 to 8, VL/VB 0.98 or more ..... 54
53. Number of annules vulva to tail terminus 8, body length 0.4 mm .....  
..... *tenuicute* (Kirjanova, 1948)  
Number of annules vulva to tail terminus 5 to 6, body length 0.28 to 0.36 ..... *pullum* (Kirjanova, 1948)
54. Posterior edge of body annules crenate, VL/VB 1.47 to 1.58 ..... 55  
Posterior edge of body annules smooth, VL/VB 0.98 to 1.18 ..... 56
55. Tail annules 8, stylet 51 to 54  $\mu$  long  
..... *kirjanovae* Andr ssy, 1962

- Tail annules 5, stylet 62 to 70  $\mu$  long  
 ----- *oostenbrinki* Loof, 1964
56. St%L 14.9 to 15.3, tail annules 5 to 6  
 ----- *curvatum* Raski, 1952  
 St%L 12.7 to 13.3, tail annules 6 to 8  
 ----- *ornatus* Raski, 1958
57. Number of annules vulva to tail terminus 4 to 5, VL/VB 0.73 to 0.77 .. 58  
 Number of annules vulva to tail terminus 6 to 9, VL/VB 0.88 to 1.35 .. 59
58. Tail terminus truncate, St%L 15 to 19  
 ----- *sphaerocephalus* Taylor, 1936  
 Tail terminus three-lobed, St%L 13.3  
 ----- *tulaganovi* (Kirjanova, 1948)
59. Average stylet length less than 60  $\mu$  .... 60  
 Average stylet length greater than 60  $\mu$  ----- 61
60. Body annules 61 to 73, St%L 12.6 to 15.6 ----- *rotundicauda* Loof, 1964  
 Body annules 73 to 78, St%L 19.6 to 23.7 ----- *citricola* Siddiqi, 1965
61. Average number body annules less than 70, tail annules 4 to 5 ----- 62  
 Average number body annules more than 70, tail annules 5 to 6 ----- 63
62. Sublateral lobes large -----  
 ----- *complexus* Jairajpuri, 1964  
 Sublateral lobes indistinct -----  
 ----- *insigne* Siddiqi, 1961
63. Average number body annules 75, anterior lip of vulva not pointed -----  
 ----- *vadensis* Loof, 1964  
 Average number body annules 72, anterior lip of vulva with two points ..  
 ----- *rosae* Loof, 1964

Table I is a synopsis of body measurements, ratios, and other information of diagnostic value on the species listed in the key, as well as those in *species inquirendae* or in synonymy. The data are based on females and extracted wholly from the original publications dealing with the species. When certain information necessary for the table was not presented in the publications, it was obtained from the accompanying drawings, if possible. If information was omitted from the original account, a dash (-) appears in Table I. A question mark (?) means that the accompanying information is questionable.

An explanation of the code symbols used and illustrations of the basic tail-tail terminus shapes are presented at the end of the table.

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TABLE 1. Diagnostic data in *Criconeimoides* spp.

<i>Criconeimoides</i>	Status*	Body length in mm	a	b	c	V%	Stylet length in $\mu$				Annules*				First body annule	Tail* Shape	Terminus	St%L*	VL/VB*	No.* Juv. $\frac{\sigma}{\phi}$ PMA
							RB	Ran	RV-T	R-Exp	PM	SL*	SB	SB						
<i>abereans</i> Jairajpuri and Siddiqui, 1963	K 16	0.45-0.54	7-11	3-5	-	92-95	68-78	38-43	-	4-5	15	C	S	S	Saucer-shaped	BC	R	14.4-15.1	0.86	20
<i>adamsi</i> (Diab and Jenkins, 1965)	K 10	0.27-0.30	12-15	3-4	27-32	90-92	40-52	170-194	6-9	10-14	60-68	C	No	No	---	HMI	R	13.3-19.3	0.78	12
<i>annulatum</i> Cobb in Taylor, 1936	K 4	0.88-1.0	13-14	-	-	95?	105	140	6	9	-	S	-	-	---	HM	2L?	10.5-11.9	1.21	-
<i>annulifer</i> de Man (de Man, 1921)	K 6	0.47-0.55	10-11	-	9	89	100-108	58-61	3-4	6-9	-	S	-	Thin small	BC	D	19.6-21.3	1.50	4	
<i>antipolitanum</i> de Guiran, 1963	K 28	0.37-0.47	11-12	3-4	29-37	93-94	66-75	85-90	5-6	7-8	23	-	L	---	HMI	T	16.0-17.8	1.04	4	
<i>anara</i> (Kirjanova, 1948)	s.l.	0.48	11	5	-	97	75	60	?	6	-	S	-	Directed forward	BC	R	15.6	0.95	1	
<i>avete</i> Fassaliotis and Williamson, 1959	K 44	0.32-0.53	8-12	4	21-31	90-96	51-60	42-54	2-3	4-5	12-15	I	S	Not retrose	SC	R	11.3-15.9	1.08	140	
<i>bakeri</i> Wu, 1965	K 5	0.56-0.75	11-13	3-5	19-29	89-92	102-112	70-79	5-6	8-10	22-25	S	?	Not retrose	BC	1-2L	14.9-18.2	1.39	8	
<i>basili</i> Jairajpuri, 1964	K 20	0.54-0.60	12-13	4-5	-	94-96	68-74	71-74	2-3	4-5	20-23	S	L	Directed forward	HM	T	12.3-12.6	1.00	10	
<i>belphege</i> (Kirjanova, 1948)	K 40	0.72	14	6	18	92	75	84	4	7	-	S	?	---	BC	L?	9.6	0.91	1	
<i>boetgeri</i> Meyl, 1954	s.l.	0.44	6	5	14	?	85	40	5-6	-	-	-	-	Cup-shaped	HM	T	19.3	0.80	1	
<i>citri</i> (Steiner, 1949)	syn.	0.25	8	2	32	91	50	70	4	6	-	-	-	Not retrose	HM	R	14.2-17.5	0.84	-	
<i>citricida</i> Siddiqui, 1965	K 60	0.38-0.47	9-11	4-6	22-32	90-92	48-51	73-78	4-5	8-9	23-26	C	L	Directed forward	SC	D-L	19.6-23.7	1.07	15	
<i>complexus</i> Jairajpuri, 1964	K 62	0.45-0.53	10-12	4-5	-	92-94	60-70	63-70	4-5	7-8	22	S	L	Retrose	BC	2-L	13.2-13.3	1.07	3	
<i>congolense</i> (Schuurmans-Stekhoven and Teunissen, 1938)	s.l.	0.53	12	4	14	89-90	70	73	-	7	-	S	-	Not retrose	BC	R	13.2	1.23	1	
<i>crassanulatum</i> de Guiran, 1963	K 43	0.32-0.35	7-8	3	-	93-95	51-52	33	-	6-7	11-12	I	S	Slightly retrose	BC	T	14.9-15.9	0.91	2	
<i>erematus</i> Loof, 1964	K 47	0.21-0.32	7-10	3-4	27-56	91-94	38-46	75-81	1	6-8	22-24	C	L	---	HMI	D	14.4-18.1	0.98	14	

<i>erosoloides</i> (Cobb, 1921)	K 20 a	0.70	10	-	17.1	86	70-75	72	7	13-14	22	-	-	Wide	SC	R	10.0-10.7	1.92	13?	-
<i>curvatum</i> Raski, 1952	K 56	0.30-0.45	9-13	3-5	22	91-96	47-67	78-101	5-6	6-10	21-29	S	S	Directed forward	BC	R	14.9-15.3	0.98	37	S
<i>cylindricum</i> (Kirjanova, 1948)	s.i.	0.50	15	3	41	93	85	89	2-3	6	-	S	?	-	HM	R	16.7	0.92	1	-
<i>deconincki</i> de Grisse, 1963	syn.	0.24-0.36	6-8	2-3	33-100	91-94	56-70	35-41	2-4	5-7	12-13	I	-	Disc-like smooth	BC	L	19.4-23.3	0.97	20	-
<i>denanti</i> (Micolozky, 1925)	s.i.	0.49	11	5	13	85	66	70	7	12	-	-	-	-	-	-	13.5	-	1	-
<i>duplicivestitum</i> Andrássy, 1963	K 22	0.31-0.40	10	4	17-21	88-90	66-72	55-60	4-5	7-8	18	S	No	-	BC	1-2L	18.0-19.4	1.34	-	-
<i>elegantula</i> (Camboid, 1953)	syn.	0.21-0.25	6	3	12	83	70	90	-	-	-	-	-	-	EL	S	29.2	-	3	-
<i>ferninae</i> Lue, 1959	K 16	0.37	11	4	18	92	45	93	5	8	27	S	L	Flap-like	HM	R	12.2	0.96	1	-
<i>flandriensis</i> de Grisse, 1963	syn.	0.33-0.48	7-11	3-4	18-31	86-92	58-64	51-56	3-5	6-9	15-19	S	L	Irregular	BC	T-2L	13.6-16.6	1.29	21	C
<i>goffardi</i> (Volk, 1951)	s.i.	0.19-0.24	7-9	2	?	87-91?	63-92	120-125	?	11-14	-	-	-	-	HM	-	?	32.6-38.3	-	-
<i>goodeyi</i> de Guiran, 1963	K 31	0.20-0.27	10-13	3-4	14-19	90-94	33-38	108-119	7-9	9-11	36-39	C	S	-	SC	3L	14.1-16.5	1.22	8	-
<i>heidleri</i> (Stefánski, 1916)	s.i.	0.89	15	8	35	-	90	65	3	-	-	-	-	Directed forward	SC	D	10.1	?	1	-
<i>hemisphaeritendulatus</i> Wu, 1965	K 26	0.32-0.49	10-14	3-4	23-32	90-96	68-92	113-137	5-8	7-12	31-40	S	?	Directed forward	HMI	R	18.7-21.1	0.79	35	C
<i>hercyniense</i> (Kischke, 1956)	s.i.	0.77-0.82	17-21	5	-	92-93	87-90	113-128	4	8	-	-	-	-	BC	T	11.0-11.3	1.38	5	-
<i>hispanensis</i> Arias, López, and Jimenez, 1963	s.i.	0.22	6	14	13	-	35	57	3-4	-	-	-	No	-	BC	?	16.1	-	1	-
<i>hygraphitum</i> Goodey, 1963	s.i.	0.39	10	-	10?	84	92	63	7	10	-	-	-	-	-	-	23.9	1.50	1	-
<i>informe</i> (Micolozky, 1922)	K 23	0.44-0.53	10-11	-	?	91-92	73-81	56-62	?	6-7	-	-	?	Not retrose	HM	T	16.2-16.5	0.97	4	-
<i>insigne</i> Stidliqi, 1961	K 62	0.40-0.53	11-13	4-5	30-35	92-93	60-64	65-69	4	6-7	22-23	S	No	Directed forward	BC	D	12.1-15.0	1.15	5	C
<i>irregularis</i> de Grisse, 1964	K 49	0.33-0.43	9-12	3-4	19-28	91-94	48-53	58-64	4-5	5-7	18-21	C	S	Directed forward	BC	R	12.3-14.6	1.09	20	S-I
<i>juniperi</i> Elward and Misra, 1964	K 27	0.20-0.25	9-10	2-3	12-14	91-92	75-91	105-115	8-9	11-12	37-38	-	S	-	HM	R?	36.4-37.5	0.83	5	-
<i>kirjanovae</i> Andrássy, 1962	K 55	0.38-0.41	9-10	4	12-13	88-90	51-54	79-89	8	10-12	26-27	C	S	Directed forward	SC	D	13.2-13.4	1.58	5	-

TABLE 1. Continued.

Criconemoides	Status*	Body length in mm	a	b	c	V%	Styilet length in $\mu$	Annules*			PM	SL*	First body annule	Tail*		No.* Juv. $\frac{\sigma}{\varnothing}$ PMA			
								RB	Ran	RV-T				R-Exp	Shape		minus	Shape	VL/VB*
<i>komabensis</i> (Imamura, 1931)	s.i.	0.43-0.44	13-14	4	14-16?	86-90	50?	140	10?	16-17	-	-	-	SC	R	8.6-8.8	2.33	2	-
<i>korvaci</i> Andrassy, 1963	K 36	0.51	15	5	19	90	59	117	9	14	31	-	No	SC	R	11.6	1.34	1	-
<i>limitaneum</i> Luc, 1959	K 31	0.21-0.23	6-8	3	16	87-89	52-53	90-111	7	11	34	C	?	BC	D-2L	23.3-25.1	0.89	?	-
<i>lobatum</i> Raski, 1952	K 38	0.40-0.48	11-14	4-5	25	94-95	51-55	99-107	5-6	7-8	31	S	L	HM	2-3L	11.5-12.8	0.86	14	C
<i>longatum</i> (Cnuthold, 1953)	K 24	0.43	9	3	10	85	76	88	10	18	-	S	-	EL	S	17.7	2.25	1	-
<i>macrodonum</i> Taylor, 1936	K 4	0.27-0.30	10-11	-	-	90	110	106-110	10	11	40	S	-	BC	R	36.6-40.7	1.12	60	-
<i>macrolobatus</i> Jaisripuri and Siddiqui, 1963	K 30	0.54-0.80	13-18	4-6	-	93-96	71-75	81-86	-	6-7	23-24	-	L	HM	R	9.4-13.2	0.93	10	I
<i>magnolae</i> Edward and Misra, 1964	syn.	0.29-0.41	11-12	4	16-22	92-94	46-52	100-110	7-8	10-11	28-29	?	L	HM	T	12.7-15.9	1.26	30	-
<i>maritimum</i> de Grisse, 1964	K 23	0.36-0.46	11-14	3-4	35-65	93-95	68-72	68-73	3-5	6-7	19-24	S-I	S	BC	T-L	15.7-18.9	0.75	6	C
<i>maritense</i> Williams, 1950	K 12	0.28-0.39	9-10	4	-	93-95	36	140	-	7-9	42-45	-	No	HM	2-3L	9.5-12.9	0.76	5	-
<i>microdonum</i> (de Grisse, 1964)	K 39	0.36-0.43	8-9	5-6	19-24	93	25-26	83-87	5-9	7-10	22-24	C	S	BC	L-R	6.1	0.91	3	C
<i>mongolense</i> Andrassy, 1964	K 22	0.38-0.47	9-10	4	-	87-89	70-76	57-61	5	8	18	S	S	SC	D	16.2-18.4	1.62	2	-
<i>monstrata</i> Arias Dolgado et al., 1965	K 20a	0.65-0.74	9-11	4-5	10-15	90-92	78-83	69-73	5-6	7-8	-	S	-	BC	2-L	8.9-10.9	1.38	6	-
<i>morgense</i> (Hofmanner in Hofmanner and Menzel, 1914)	s.i.	0.55-0.59	11-12	-	20?	94	80-85	110-115	-	9	-	S	-	SC	S	14.4-14.5	1.23	?	-
<i>mutabilis</i> Taylor, 1936	K 37	0.48-0.58	14-15	-	-	94-95?	52	100	7	9-10	20	S	-	BC	L?	9.0-10.8	0.93	100+	I
<i>maintidense</i> Edward and Misra, 1963	K 38	0.41-0.52	8-11	3-6	21-31	90-93	55-71	100-110	5	7	32-33	S	S	HM	2-L	13.4-13.7	1.02	25	-
<i>neoxeste</i> Jaisripuri and Siddiqui, 1963	K 17	0.48-0.60	10-11	5	-	89-90	65-75	45-49	-	7-8	15	I	?	BC	R	12.5-13.5	1.37	3	I
<i>obtusicaudatum</i> Heyes, 1962	syn.	0.35-0.43	8-12	4	-	92-96	45-57	72-84	3	7-8	20-24	S	S	HM	R	12.6-13.6	0.91	10	-
<i>oncensis</i> Late, 1959	K 33	0.39-0.49	11-15	4-5	19-20	91-94	40-45	128-136	8	8-11	31	S	S	BC	T	9.2-10.3	1.24	?	-



<i>oostenbrinkii</i> Loof, 1964	K 55	0.38-0.51	10-16	3-4	-	80-94	62-70	85-94	5	8-10	24-26	C	L	Saucer-shaped	SC	2-L	13.7-16.3	1.47	22	C
<i>ornatus</i> Raski, 1958	K 56	0.36-0.44	10-13	4	16	90-94	48-56	87-92	6-8	7-9	25-27	S	S	Directed forward	HM	2-L	12.7-13.3	1.18	19	C
<i>parvum</i> Siddiqi, 1961	K 10	0.27-0.32	11-14	4-5	-	94-95	30-34	168-104	6	12-15	44	-	No	-	HM	?	10.6-11.1	0.83	20	S
<i>parvum</i> Raski, 1952	K 12	0.26-0.30	12-15	3	-	93-96	38-41	142-156	3	11-12	46-49	S	S	Lobed	HM	T	13.7-14.6	1.33	13	S
<i>petraense</i> (Cobb, 1924)	s.i.	0.67	10	-	7	93	75	79	-	7	-	-	L	Directed forward	SC	S	11.2	1.24	1	-
<i>petasus</i> Wu, 1965	K 17	0.55	9	4	25	82	75	50	5	12	13	S	-	Saucer-shaped	SC	D	13.7	1.78	1	-
<i>princeps</i> Andrassy, 1962	K 18	0.38	10	3	19	88	87	63	6	10	22	-	S	Directed forward	SC	L	22.9	1.64	1	-
<i>pseudohercyniensis</i> de Grisse and Koen, 1964	K 30	0.56-0.68	10-17	4-5	21-36	92-94	79-91	108-133	4-8	7-13	31-39	C	S	Not retrose	HM	R	12.6-16.0	1.15	20	C
<i>pseudosolitum</i> de Grisse, 1961	K 41	0.29-0.47	8-11	3-4	14-30	88-92	50-57	47-60	3-5	5-8	15-20	C	S	Smaller	BC	2-3L	12.1-17.2	1.33	22	C
<i>pullum</i> (Kirjanova, 1948)	K 53	0.28-0.36	10-11	3	29-36	92-95	55-61	80-85	3-4	5-6	-	S	-	-	HM	2L	16.1-20.7	0.62	20	-
<i>quadriforce</i> (Kirjanova, 1948)	K 29	0.57-0.66	12	4-5	30-31	95	80-85	90-93	4-5	8	-	S	?	-	BC	R	13.0-15.1	1.10	5	-
<i>quasidemani</i> Wu, 1965	K 41	0.38-0.49	10-13	4-5	8-11	82-86	62-70	73-87	10-12	11-16	21-26	S	-	Directed forward	EL	D	14.4-16.3	2.39	15	-
<i>raskiense</i> de Grisse, 1964	K 50	0.45-0.51	9-12	4-5	10-16	85-90	50-56	62-72	6-9	9-12	19-22	C	S	Smaller	SC	S-T	11.0-11.1	1.62	-	C
<i>raskii</i> Gooley, 1963	syn.	0.27-0.41	10-15	3-5	20-34	90-93	50-58	101-113	7-8	10-12	25-32	-	No	Not retrose	HM	4-L	13.8-17.3	0.93	-	C
<i>rosae</i> Loof, 1964	K 63	0.33-0.43	8-11	3-4	15-24	90-93	58-68	66-77	5-6	7-9	10-23	S	L	Directed forward	BC	2L	13.5-17.0	1.12	23	C
<i>rotundicauda</i> Loof, 1964	K 60	0.32-0.47	8-11	3-4	21-31	92-94	50-59	61-73	3-4	6-7	19-22	-	L	-	HM	L	12.6-15.6	0.88	13	S-1
<i>rotundicaudatus</i> Wu, 1965	K 11	0.53-0.78	13-19	3-5	27-38	94-95	85-103	132-153	6-8	9-11	38-44	S	?	Not retrose	HM	R	13.2-15.9	1.03	15	C
<i>rusticum</i> (Micolatzky, 1915)	s.i.	0.44	11	6	?	?	75	99	?	6	-	-	-	-	HM	T	12.5	0.92	1	-
<i>siddiqii</i> Khan, 1963	K 36	0.30-0.39	10-16	3-4	20-26	90-93	60-61	112-118	7-8	12-14	32-33	S	-	Not retrose	BC	R	15.4-21.3	1.69	12	C
<i>simile</i> (Cobb, 1918)	s.i.	0.6	10	4	20	93	75	91-101	4-5?	-	26	-	-	-	BC	T?	12.5	-	?	-
<i>sinensis</i> (Rahm, 1937)	s.i.	1.3	21-26	7-8	6	47	-	38	-	-	-	-	-	-	-	-	-	-	3	-
<i>solitum</i> Andrassy, 1962	K 49	0.46	10	3	16	90	66	61	6	8	19	I	No	Not retrose	BC	T	14.4	1.31	1	-
<i>sphaeroccephalus</i> Taylor, 1936	K 58	0.3	9-10	-	-	94	57	68-72	2	4-5	20-24	S	-	-	HM	T	15.0-19.0	0.73	20	-
<i>sphagni</i> Micolatzky, 1925)	K 2	0.39-0.54	9-13	3	12-15	84-88	122	95-103	8-10	13-17	31-32	S	-	Directed forward	SC	S	22.6-31.9	1.68	10	-

TABLE 1. Continued.

<i>Criconemoides</i>	Status*	Body length in mm	a	b	c	V%	Stylet length in $\mu$	Annules*			First body annule	Tail*		No.* Juv. $\frac{N}{Q}$ PMA			
								RB	Ran	RV-T		R-Exp	PM		SL*	Shape	Terminus
<i>stegia</i> (Schneider, 1940)	K 6	0.38-0.44	9-11	-	-	-	110	54-65	8	10	-	-	EL	S	25.0-29.0	1.60	40
<i>tenacata</i> (Kifjanova, 1948)	K 53	0.40-0.41	11-12	3	32	93	60-62	87	3-4	8	-	S	HM	T	15.9-16.3	0.83	2
<i>terres</i> Raski, 1952	K 27	0.31-0.42	12-13	3-4	-	92-95	75-76	106-113	5-6	8-9	28-30	S	HM	R	18.1-22.1	0.88	6
<i>tescorum</i> de Guirán, 1963	K 47	0.23-0.32	9-11	3-4	20-33	92-94	37-41	91-96	5-6	8-10	26-28	S	HM	2L	12.8-16.1	0.97	10
<i>tuiganovi</i> (Kifjanova, 1948)	K 58	0.40	9	4	33	92	53	70	2-3	4-5	-	S	BC	3L	13.3	0.77	1
<i>vadensis</i> Loof, 1964	K 63	0.36-0.52	9-14	4-5	17-28	91-93	57-65	70-81	5-6	7-9	20-26	S	BC	D-2L	12.5-15.8	1.35	16
<i>xenoplas</i> Raski, 1952	syn.	0.40-0.62	8-14	3-5	23-56	90-95	71-86	87-114	4-8	6-11	25-35	S	BC	R-L	13.9-17.8	0.92	88
<i>zavadskiti</i> (Tuliganov, 1941)	K 9	0.22-0.32	8-16	4-6	15-26	94-95?	25-40	200	5-6	7-8	-	S	HM	T	11.6-12.7	0.73	?

\* CODE.

Status:

K (number) = appears in the preceding key under the number indicated.  
 s.i. = placed in *species inquirendae*.  
 syn. = placed in synonymy.

Annules:

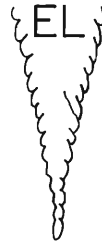
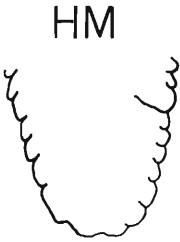
RB = Total number of body annules.  
 Ran = Annules from anus to tail terminus.  
 RV-T = Annules from vulva to tail terminus.  
 R-Exp = Annules from excretory pore to anterior end.  
 PM = Posterior margin of annules.  
 C = crenate.  
 I = irregular.  
 S = smooth.

SL: Sublateral lobes.

No = none observed.  
 ? = obscure.  
 S = small.  
 L = large.

Tail Shape:

HM = hemispherical or rounded.  
 BC = bluntly conical.  
 SC = sharply conical.  
 EL = elongate.



**HEMISPHAERICAUDATUS**  
**WU, 1965**

**ONOENSE**  
**LUC, 1959**

**CITRICOLA**  
**SIDDIQI, 1965**

**LONGULUM**  
**(GUNHOLD, 1953)**

Tail Terminus:

T = truncate.  
 R = rounded.  
 L = (number of) -lobes.  
 D = digitate.  
 S = spicate.



**SOLIVAGUM**  
**ANDRÁSSY,**  
**1962**

**NEOAXESTE**  
**JAIRAJPURI &**  
**SIDDIQI, 1963**

**TULAGANOVI**  
**(KIRJANOVA,**  
**1948)**

**VADENSIS**  
**LOOF, 1964**

**STYGIA**  
**(SCHNEIDER,**  
**1940)**

St%L: The ratio of stylet length multiplied by 100 and divided by the body length. Where a range is given, the shortest stylet length is divided by the greatest body length and the greatest stylet length is divided by the shortest body length.

VL/VB: Ratio of length of body from vulva to tail terminus divided by width of body at vulva as determined from illustrations.

No. ♀: Number of females on which the original description was based.

Juv. PMA: Posterior margin of annules on juveniles.

C = crenate.  
 I = irregular.  
 S = smooth.

## Effects of Soil Temperature and Moisture on the Survival and Activity of *Xiphinema americanum*<sup>1</sup>

G. D. GRIFFIN AND K. R. BARKER<sup>2</sup>

### SUMMARY

Populations of *Xiphinema americanum* from Colorado blue spruce soil and jack pine soil differed in their response to soil temperature in the absence of host plants. The spruce population showed a corresponding increase in maturation with an increase in temperature from 5 to 20 C. However, the nematodes matured more slowly at 28 C than at 20 C. There was an increase in nematode maturation with an increase in temperature from 5 C to 32 C in the jack pine population. Eggs hatched at all temperatures above freezing while both nematode populations failed to survive in frozen soil. Only four gravid females were found in a total of 60,000 g of spruce soil and 64,000 g of pine soil. The optimum temperature for reproduction of *X. americanum* on tomato and strawberry plants was 20 and 24 C, respectively. The optimum temperature for root growth of both hosts was 20 C. *X. americanum* failed to survive at moisture levels of 10, 20, 90, and 100% field capacity or in potted soil, but decreased very little at intermediate moisture levels during a 14-week period.

### INTRODUCTION

There have been few successful demonstrations of the pathogenicity of the American dagger nematode, *Xiphinema americanum* Cobb, in the greenhouse. White (1955, 1959) showed this nematode to be pathogenic on pine, strawberry, corn, and apple. Perry (1958) also found *X. americanum* responsible for poor growth of strawberry. There have been many reported instances of unsuccessful pathogenicity experiments because of the inability of workers to maintain or increase it in potted soil under

greenhouse conditions (Tarjan, 1956; Hansbrough and Hollis, 1957). Griffin and Epstein (1964) observed the association of *X. americanum* with poor growth and winterkill of spruce but failed to obtain an increase of nematodes added to soil.

Many factors affect the survival of the American dagger nematode in the greenhouse. Van Gundy *et al.* (1962) found that the concentration of oxygen and its ability to diffuse through soil pores was important for survival. Lownsbery and Maggenti (1963) demonstrated the importance of temperature and moisture on population levels on cherry and strawberry and were able to increase the population in a 5-month period.

The present study was initiated to determine effects of temperature and moisture on survival and activity of *X. americanum* under controlled laboratory and greenhouse conditions.

### MATERIALS AND METHODS

An experiment was designed to determine the effects of temperature on survival of *X. americanum* in the absence of a host plant. Miami loam soil infested with eggs and third- and fourth-stage *X. americanum* was collected from the roots of Colorado blue spruce, *Picea pungens* Engelm., in an ornamental nursery in Wisconsin 2 weeks after the soil had thawed in the early spring (soil temperature 7 C). The soil was thoroughly mixed and divided into 500-g lots and sealed individually in polyethylene bags in pint containers. The original nematode population and soil moisture content were determined (moisture content was 66% of field capacity; field capacity was 29%). Containers were stored in constant temperature rooms at 0, 5, 10, 15, 20, and 28 C. Soil from each temperature room was processed weekly by a sifting and gravity and modified Baermann funnel method over a 20-week period, and the number and maturation stages of the nematodes present were determined.

A similar study on a population of *X. americanum* in Plainfield sand was initiated in the

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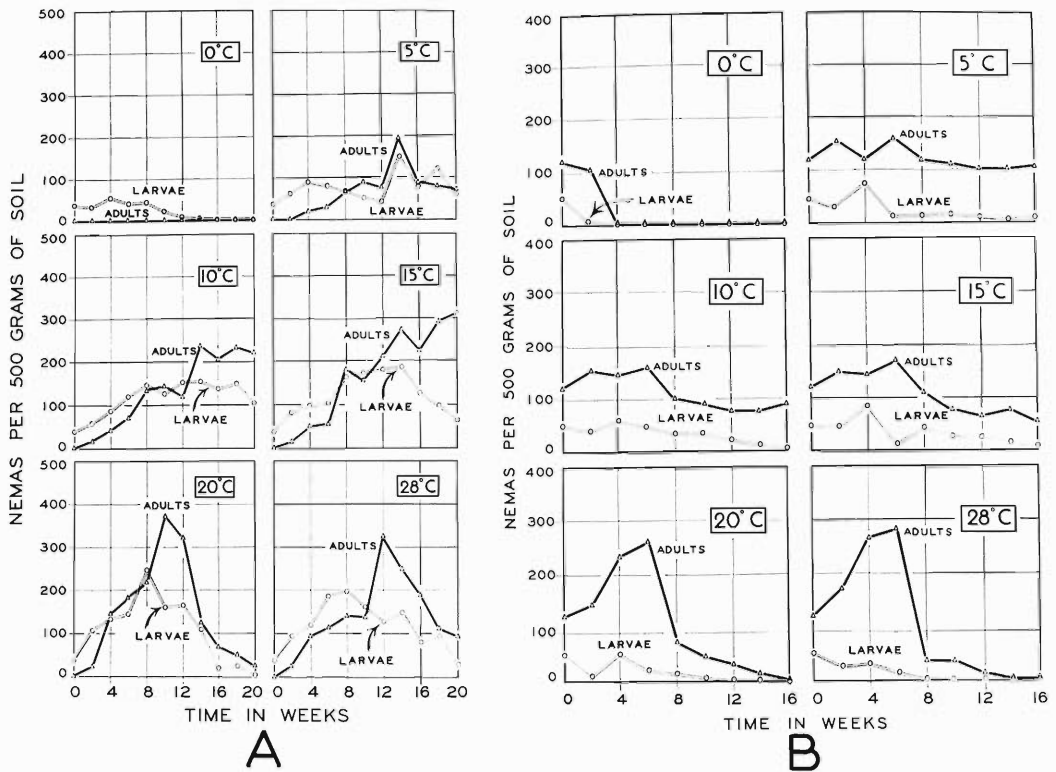


Fig. 1. Effect of soil temperature on hatching and survival of *X. americanum*: (A) blue spruce population; (B) jack pine population.

late spring using soil collected from around the roots of jack pine, *Pinus banksiana* Lamb. Soil temperature was 18 C, and the soil moisture was 47% of field capacity (field capacity was 17%). Containers were stored at temperatures of 0, 5, 10, 15, 20, 24, 28, and 32 C. Duplicate lots of soil were processed at intervals of 1 week, as previously described, for 16 weeks, and the nematode number and maturation were determined.

A third study was made to determine effect of different moisture levels on the survival of *X. americanum*. Soil containing *X. americanum* was collected from roots of jack pine in central Wisconsin. It was thoroughly mixed, screened, and divided into 11 separate lots. Some lots were air dried and distilled water was added to others to give a moisture range of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% field capacity. Soil was dried to desired moisture

levels by continuous rotation in air-exposed containers. Each of the 10 lots of soil was divided into 500-g lots and sealed in polyethylene bags as described in the first experiment and maintained in a temperature room at 20 C. The 11th lot was maintained in 6-inch clay pots on a greenhouse bench at a temperature of 20 to 25 C and distilled water added to approximate normal greenhouse watering procedures. Representative samples were processed biweekly as previously described.

Reproduction of the blue spruce population of *X. americanum* as effected by soil temperature was investigated in greenhouse temperature tanks. A mixture of Miami loam soil and quartz sand (2:1) was used for growing the host plants. Six tomato seedlings, *Lycopersicon esculentum* Mill. var. Bonny Best, or two strawberry plants were transplanted to each 6-inch crock. One hundred handpicked nematodes

Table 1. Effect of soil moisture on survival of *X. americanum* in absence of host plants.<sup>a</sup>

Weeks	Per cent Field Capacity										Pots <sup>b</sup>
	10	20	30	40	50	60	70	80	90	100	
0	157	157	157	157	157	157	157	157	157	157	157 <sup>c</sup>
2	158	269	237	163	120	111	133	194	173	96	119
4	163	230	247	176	198	179	186	102	87	69	111
6	146	257	398	284	244	176	193	107	113	59	60
8	136	288	329	396	294	293	209	273	224	56	75
10	8	198	233	440	273	172	164	212	136	52	111
12	0	56	223	265	234	240	206	163	103	40	15
14	0	29	230	239	270	216	193	184	92	32	0

<sup>a</sup> Nematodes per 500 g infested Plainfield sand.

<sup>b</sup> Six-inch clay pots of soil maintained on greenhouse bench and distilled water added to approximate normal greenhouse watering procedures.

<sup>c</sup> This figure is probably inaccurate due to the effect of adding or deleting water from soil on nematode population.

extracted from the soil by the combination of the sieving and Baermann funnel techniques were added to each crock at transplanting within 24 hr after extraction.

### RESULTS

The Colorado blue spruce population of *X. americanum* disappeared after 16 weeks at 0 C. There was a direct relation between an increase in temperature and an increase in the maturation of the nematodes between 5 and 20 C (Fig. 1A). Most adults were seen after 10 weeks at 20 C, 12 weeks at 28 C, 20 weeks at 15 and 10 C, and 14 weeks at 5 C. The intestines of adults became tessellated and transparent at all temperatures above freezing within 4 to 6 weeks after becoming mature. Adults in this condition were partly or completely devoid of granular material and usually only an outline of the internal body structures could be seen. Transparent nematodes died within 1 to 3 weeks. A greater percentage of nematodes matured simultaneously at 20 C and 28 C than at the other temperatures resulting in 75 to 80% of the adults being transparent at 20 C on the 11th week, but most of these transparent adults died before the 20th week. At 10 and 15 C the number of adults was near its peak at the end of the 20th week.

Only four gravid females were observed in the total population from 60,000 g of soil processed during the study. There were two gravid females from the Miami loam soil stored at 20 C on the 6th week and two from Miami loam soil stored at 15 C on the 9th week. Adults were first to become transparent. Transparent larvae never exceeded 1% of the total population at any temperature above freezing.

Eggs in the soil hatched at all temperatures

above freezing throughout the experiment. The number of newly hatched second-stage larvae was small in relation to the total population increase at any given temperature. This number varied from week to week and was not consistent with temperature and time. The largest number of second-stage larvae was observed on the 8th week at 20 C.

The jack pine population of *X. americanum* also failed to survive at 0 C. There was an increase in nematode maturation at 6 weeks with increases in temperature from 5 to 28 C (Fig. 1B). Data obtained at 24 and 32 C (not given in Fig. 1B) also supported this relationship. Approximately half of the adults became transparent after 4 weeks at 20, 28, and 32 C. Transparent adults were observed first after 6 weeks at 15 C and 8 weeks at 5 and 10 C. Gravid females were not seen at any temperature, and newly hatched nematodes were seen for only 3 weeks at 24, 28, and 32 C; for 6 weeks at 20 C; for 8 weeks at 10 and 15 C; and 9 weeks at 5 C. The greatest number of newly hatched nematodes was observed after 1 week at 32 C.

Survival of *X. americanum* in the absence of a host plant was adversely effected at high and low moisture levels and in potted soil at a temperature of 20 to 25 C (Table 1). Transparent nematodes which died within 1 to 3 weeks were seen after the first week at the 10%, 90%, 100% levels and in potted soil, and after 5 weeks at the 20% moisture level. Transparent nematodes appeared at the 7th and 8th week in smaller numbers in the intermediate moisture levels, and the total population decreased little during 14 weeks. The largest number of eggs hatched at 20% and 30% levels and the smallest number hatched

Table 2. Effect of soil temperature on the reproduction and pathogenicity of *X. americanum* on tomatoes and strawberries.

Temperature (C)	Tomato				Strawberry			
	Fresh root weight/crock (g)		Nemas per crock		Fresh root weight/crock (g)		Nemas per crock	
	Inoculated	Control	a	b	Inoculated	Control	a	b
16	44	54	24	40	33	30	24	40
20	63	75	106	177	39	47	44	73
24	57	57	93	155	27	28	76	127
28	18	13	28	47	13	12	7	12

<sup>a</sup> Nematodes recovered with sieving and Baermann funnel technique.

<sup>b</sup> Nematode population per crock considering only 60% of nematodes are recovered.

at 90% and 100% levels and in potted soil as indicated by presence of second-stage larvae. Few or no eggs hatched at the 10% moisture level.

The optimum temperature for reproduction of the blue spruce nematode population on tomato was 20 C and 24 C on strawberry (Table 2). In contrast, the optimum temperature for root growth of both hosts was 20 C. Nematode reproduction occurred on both hosts although there was no increase in the initial population of nematodes from strawberry and only a slight increase from tomato. However, considering approximately 40% of *X. americanum* are lost during recovery procedures (Griffin and Epstein, 1964) there probably was an increase in nematodes on both strawberry and tomato roots (Table 2). Lower numbers of nematodes were recovered at 16 and 28 C regardless of the host. Inoculation with *X. americanum* resulted in a slight reduction of fresh root weight of tomato at 16 and 20 C and of strawberry at 20 C.

#### DISCUSSION

The surprising finding of nematodes maturing and surviving in the absence of a host plant may be due to one of several things. *X. americanum* has been observed attacking a species of *Rhabditis* in soil washings by the authors and may be predacious as well as plant parasitic. Observations of competition among nematodes such as those of Griffin and Darling (1964), where a decrease in a population of *Criconemoides xenoplax* Raski was associated with an increase in *X. americanum* in a Colorado blue spruce planting, have been made by many investigators. Maturation may also be due to the nematodes feeding on small root pieces, mycorrhizae, algae, fungi, or any other microorganisms or plant tissues in the

soil. Populations of *Xiphinema index* Thorne and Allen have been observed to decrease very little 2 years after removal of host crops (Raski and Hewitt, 1963). Further study is necessary to clarify this interesting phenomenon.

The amount of available food was not sufficient for the nemas to complete their life cycles since only four gravid females were found in the total number of nematodes extracted from 60,000 g of Miami loam soil and 64,000 g of Plainfield sand. The Colorado blue spruce population disappeared after 12 weeks and the jack pine population disappeared after 3 weeks in frozen soil. This time difference may be attributed to the greater change in temperature to which nematodes in Plainfield sand were subjected (18 to 0 C for Plainfield sand; 7 to 0 C for Miami loam soil). The inability of nematodes to survive in frozen soil in the laboratory agrees with data obtained in field studies where *X. americanum* was unable to survive long periods in frozen soil (Griffin and Darling, 1964; Norton, 1963).

There was decline in the number of nematodes at high and low moisture levels (10, 20, 90, and 100% field capacity) and in potted soil. Van Gundy *et al.* (1962) found that the concentration of oxygen and its ability to diffuse through soil pores was important to the survival of *X. americanum*. Oxygen diffusion is undoubtedly involved in soil at 90% and 100% field capacity and in potted soil. The inability of these nematodes to withstand continual water fluctuations in potted soil may be partially responsible for their failure to survive in most greenhouse experiments.

The optimum temperature for reproduction of the Colorado blue spruce population of *X. americanum* was 20 to 24 C, depending on the host, which agrees closely with Lownsbery and

Maggenti (1963). The optimum for root growth for both hosts, tomato and strawberry, was 20 C. In many cases the optimum temperature for nematode reproduction corresponds to that for growth of the host plant.

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### Survival of Encysted Larvae of *Trichinella spiralis*: Effect of Exposure to 0 F, Using Precooled and Fresh Ground Pork

CHARLES H. HILL<sup>1</sup>

The exposure of encysted trichina larvae in pork to a temperature of about 0 F (-17.8 C) has been shown to have lethal effects by Gibier and Bouley (1882), Ransom (1914, 1915, 1916), Blair and Lang (1934), and by others.

The amount of trichinous pork exposed is important. According to Ransom (1916), a higher death rate of larvae occurs in small amounts, less than half a pound, frozen for a fairly long time. Moreover, in the latter case, differences in amounts used and subsequent freezing and thawing rates make no appreciable difference in the death rate of the larvae.

No investigations have been published on the results from using pork precooled at 35 F for several weeks and then frozen at 0 F, insofar as is known to the author. This paper describes such tests and the subsequent effect on the encysted trichina larvae.

#### MATERIALS AND METHODS

Trichinous pork was provided by infecting hogs with 500 larvae per pound of live weight; the hogs were killed from 2 to 7 months later.

**PRECOOLED PORK.** Seven series of tests were carried out using precooled pork. Hams and shoulders weighing 25 to 45 pounds were stored at 35 F for 104 to 171 days. Periodically, some were boned, ground, mixed, wrapped in butcher's paper in 1-pound lots, and placed in a freezer at 0 F.

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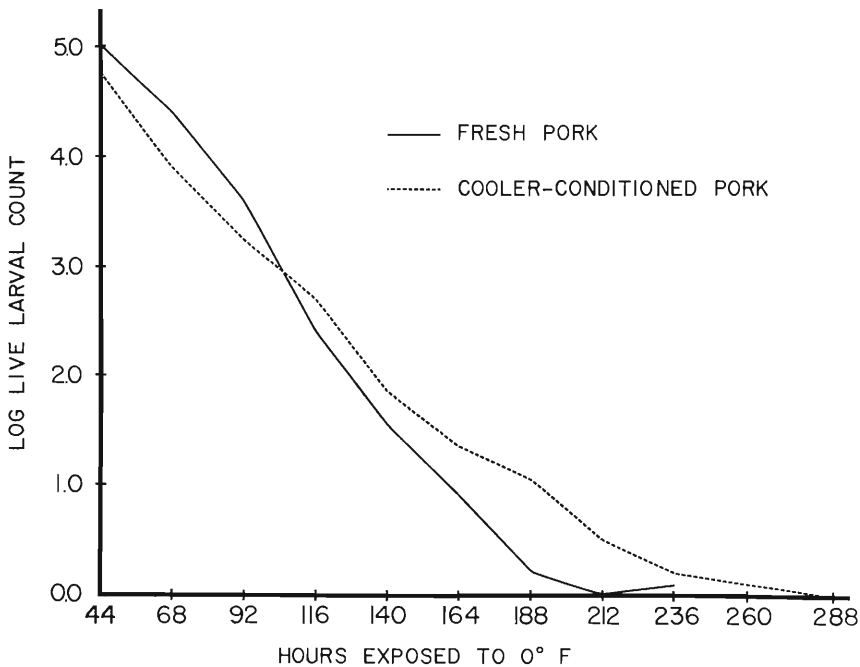


Fig. 1. Survival of trichina larvae in ground pork at a temperature of 0 F: Logarithm of the number of surviving larvae against freezer time for precooled and fresh pork.

**FRESH PORK.** Ten series of tests were carried out using pork from freshly killed hogs. The pork was ground, mixed, wrapped in butcher's paper in 1-pound lots, and placed in the freezer at 0 F.

**PREFREEZING TESTS.** Before freezing, the ground pork was tested for thoroughness of mixing. Three random samples of 400 g each were artificially digested and the larvae estimated by dilution counts; the numbers in all samples ranged within 10%.

Unfrozen controls were made in 1-pound lots from both the precooled and the fresh pork; the packages were artificially digested and the larvae estimated by dilution counts. Percentage determinations were made, based on the ratio between larvae in these samples and larvae surviving the tests.

**FREEZER TEMPERATURES.** Temperature changes in packaged pork and the surrounding air were followed by a recording potentiometer and thermocouples. The thermocouples were either inserted into the center of the packages

or attached to the freezer shelf and exposed to air at different levels.

After 2 hours in the freezer, temperatures in some test packages had dropped to freezing (32 F). The temperature curve then leveled. After 10 hours in the freezer, all packages were at freezing temperature. After this time, the temperature dropped rapidly and reached 0 F about 14 hours later, or 24 hours after the start of the test.

Air temperatures varied until 0 F was reached. Variation between the highest and lowest was as much as 10 F, although the variation was usually about 5 F. The temperature decreased during the first 4 hours to 13 to 18 F; within the next 12 hours the range was 4 to 11 F; within the next 4 hours the range was 2 and 3 F. An air temperature of 0 F was reached in the next 2 hours or 22 hours after the beginning of the tests. After 0 F was reached, the temperature was maintained until the end of the test.

**TEST DESIGN.** The chest-type freezer was

TABLE 1. Survival of *Trichinella spiralis* larvae in ground pork at 0 F.\*

Time in freezer (hours)	Precooled pork			Fresh pork		
	Range	Average	Percent	Range	Average	Percent
0 (Controls)	76,666-270,000	129,000		68,666-160,000	120,466	
40	56,000-100,000	71,333	55.2968	5,000-176,000	76,150	63.2128
48	22,000-73,000	47,500	36.8217	4,500-180,000	70,250	58.3152
64	1,500-32,000	13,333	10.3356	14-70,000	24,053	19.9666
72	2,000-16,000	7,500	5.8139	150-70,000	18,948	15.7289
88	1,400-10,000	5,450	4.2248	0-22,000	5,165	4.2875
96	250-7,000	3,733	2.8937	5-7,500	2,255	1.8718
112	200-2,000	1,275	0.9883	0-2,250	553	0.4590
120	39-5,000	1,034	0.8015	1-2,800	381	0.3162
136	15-500	193	0.1496	0-500	81	0.0672
144	4-300	106	0.0821	0-267	53	0.043995
160	4-255	86	0.066666	0-82	20	0.016602
168	0-200	42	0.032558	0-49	14	0.011621
184	1-187	44	0.034108	0-12	2.62	0.002490
192	0-150	33	0.025581	0-1	0.12	
208	0-24	7.71	0.006201	0-0		
216	0-5	4.16	0.003100	0-0		
232	1-20	1.15	0.000775	0-12	1.75	0.001660
240	0-3	1.0	0.000775	0-0		
256	0-2	0.80	0.000775	0-0		
264	0-0			0-0		
280	0-3	0.60	0.000775	0-0		
288	0-0			0-0		
304	0-0			0-0		
312	0-0			0-0		

\* Seven tests used pork precooled at 35 F for 104-171 days before freezing; ten tests used pork from freshly killed pigs.

fitted with a wire mesh shelf placed midway between the freezer floor and the lid. This shelf and the freezer floor were filled with 1-pound packages of pork in such a way that four series of tests could be made at one time. Packages were randomly placed on the floor and shelf. The same number of packages of any one series was placed on the floor and shelf. During each test period, a random package of the test series was removed twice daily, 8 a.m. and 4 p.m., and thawed at room temperature. The contents were artificially digested for 18 hours and processed for recovery of live larvae as described by Hill (1957). The viability of larvae was judged by standards outlined by Ransom (1916).

Maximum freezer time was 312 hours.

### RESULTS

The test results are shown in Table 1 and Figure 1. Table 1 gives the range in numbers, the average number of larvae recovered in both test and control packages, and the percentage of surviving larvae (ratio between numbers in test and control packages). In Figure 1 the logarithm of the number of surviving larvae is plotted against freezer time for precooled and fresh pork.

The data show that larvae in precooled pork

were able to resist the lethal effects of a temperature of 0 F significantly longer than larvae in fresh pork. For the first 72 hours in the freezer, the death rate of larvae was about the same in both precooled and fresh pork (Fig. 1). After this time the death rate in fresh pork was much more rapid. The death rate in precooled pork was relatively consistent for about 264 hours.

Table 1 shows that live larvae were recovered for as long as 280 hours in precooled pork but only for 232 hours in fresh pork. After the first 72 hours the death rate for fresh pork was 0.737 logarithms per 24 hours whereas that for precooled pork was 0.525 per 24 hours. The difference between these two death rates was statistically significant at the 95% level.

Packages placed next to the freezer walls and those centrally located were compared to ascertain if there was any difference in number of surviving larvae. It was concluded that the position within the freezer did not significantly affect the death rate.

### DISCUSSION

Although trichinous pork became contaminated with bacterial and fungal growths after several weeks in the cooler, the larvae digested

therefrom were not unlike those obtained from fresh pork. The explanation for the enhanced resistance in precooled pork may be similar to that of Crofton (1948), who found that the eggs of a parasite of rabbits die if placed on outside plots in the winter but survive if placed there in the fall. He offered the explanation of gradual acclimatization. The age of the infection in fresh and precooled pork of any one test was obviously different; however, no pattern of difference that was caused by such a factor throughout the limited tests could be discerned. Enzyme action in the aging pork, action of bacteria and fungi, either on the cyst wall or encysted larvae, and the gradual, if slight, desiccation in the large cuts of pork are also obvious differences between fresh and precooled pork used in these experiments.

#### SUMMARY

Tests were made on the survival of encysted larvae of *Trichinella spiralis* in 1-pound, ground pork packages at a temperature of 0 F. The study used pork that had first been precooled at 35 F for 104 to 171 days and pork from freshly killed hogs. Larvae in precooled pork survived for as long as 280 hours but only for 232 hours in fresh pork. These results indicated that precooling or "preconditioning" in-

creases the resistance of larvae to the lethal effects of a temperature of 0 F.

#### ACKNOWLEDGMENT

The author is indebted to Dr. Richard P. Lehmann for the statistical analysis of the data.

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### Report of the Brayton H. Ransom Memorial Trust Fund

FUNDS ON HAND, 1 January 1965 .....	\$2,321.12
RECEIPTS: Interest received in 1965 .....	96.37
DISBURSEMENTS: Grant to Helminthological Society of Washington .....	10.00
BALANCE ON HAND, 31 December 1965 .....	\$2,407.49

A. O. FOSTER  
Secretary-Treasurer

## Cellulytic and Pectolytic Enzymes in the Nematode, *Aphelenchus avenae*<sup>1</sup>

K. R. BARKER

Cellulytic and pectolytic enzymes have been found in several plant-parasitic nematodes whereas little or none of these enzymes have been recovered from saprophagous forms and only cellulase has been found in mycophagous forms. The limited research on cellulases from nematodes was recently reviewed by Dropkin (1963). Tracey (1958) found high cellulase activity in homogenates of *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, and less activity in *Ditylenchus myceliophagous* J. B. Goodey, 1958. Homogenates of *Ditylenchus triformis* Hirschmann and Sasser, 1955, *Pratylenchus zaeae* Graham, 1951, and *D. dipsaci* were found by Krusberg (1960) to exhibit cellulase activity. He also found pectinmethylesterase activity in *D. triformis* and *D. dipsaci* and later (1964) reported *D. dipsaci* to have polygalacturonase activity, whereas *D. triformis* did not. Morgan and McAllan (1963) found cellulase and pectinase activity in *Pratylenchus penetrans* (Cobb, 1917) Chitwood and Oteifa, 1952 and *Heterodera trifolii* Goffart, 1932. They confirmed Tracey's (1958) earlier report that *Turbatrix aceti* (Müller, 1783) Peters, 1927 had no cellulase. Dropkin (1963) similarly found the plant-parasitic nematodes, *Tylenchulus semipenetrans* Cobb, 1913, *D. dipsaci*, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 as well as *D. myceliophagous* J. B. Goodey, 1958 to produce cellulase but none was found in *Panagrellus* sp., *Neodiplogaster*, or *Trichinella spirallis* Owen, 1835.

Most investigators studying these hydrolytic enzymes employ various viscometric or reducing group assays. One disadvantage common to both techniques in studying nematodes is the relatively large quantity of enzyme required. Sherwood and Kelman (1965) and Durbin and Huppler (1965) have described two instruments not previously used for visco-

metric enzyme assays. The rotating spindle instrument described by Sherwood and Kelman requires large volumes and would be of limited use in many studies. The Brookfield cone-plate viscometer mentioned by Durbin and Huppler requires only 1 ml of reaction mixture and should be ideally suited for investigations where the source of enzyme is limited.

This work was initiated to determine (1) the types of pectolytic and cellulytic enzymes in the mycophagous nematode, *Aphelenchus avenae* Bastian, 1865 and (2) the suitability of the Brookfield cone-plate viscometer for hydrolytic enzyme assays.

### MATERIALS AND METHODS

Nematodes for enzyme assays were increased monoxenically on *Rhizoctonia solani* Kühn, 1858 grown on potato dextrose agar at 23 C  $\pm$  1. Established *R. solani* cultures were mass-inoculated with *A. avenae*. The nematodes were allowed to reproduce for 4 weeks. They were then extracted by the Baermann funnel technique (Thorne, 1961). The nematodes were collected 10 to 20 hr later and washed by eight to ten centrifugations of 1 to 2 min each at 3,300 g.

The nematodes were homogenized in 0.25 M sucrose in Ten-Broeck homogenizers which were powered by a variable speed Tri-R Laboratory motor (Tri-R Instruments, Jamaica, New York). This type of homogenizer was found to be much more efficient than the available sonic oscillator (Model S75, Branson Instruments Incorporated, Stamford, Connecticut). The nematodes, one g of fresh weight/19 ml sucrose solution, were kept in ice water during homogenization.

After homogenizing the suspension was centrifuged at 3,300 g at 4 C for 30 min and the liquid decanted. The liquid was centrifuged again at 54,450 g for 1 hr. Fatty substances that accumulated at the top of the centrifuge tubes were decanted, and the remaining liquid was then filtered and assayed for enzyme activity or dialyzed against distilled water for 24 hr and then assayed.

<sup>1</sup> Research supported in part by funds from research grant AI-04259-04 from the National Institutes of Health, United States Public Health Service, and by Hatch funds from Project 904. Published with the approval of the Director, Wisconsin Agricultural Experiment Station, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

Several procedures were employed in assaying for the various pectolytic enzymes. The method of Smith (1958) was used in determining pectinmethylesterase activity. One ml of homogenate was added to 9.0 ml of substrate. Autoclaved homogenates were used in the controls. Reaction mixtures were incubated for 24 hr at 30 C and then titrated with 0.05 N NaOH to their original blue color. Rates of change in optical density at 230 m $\mu$  in a Beckman DB spectrophotometer as described by Albersheim (1963) were used in measuring pectin transesterase activity.

Polygalacturonase activity was determined by viscometric, colorimetric, and chromatographic procedures. Reducing groups liberated from sodium polypectate (Nutritional Biochemicals Corp., Cleveland, Ohio) were measured by Miller's (1959) dinitrosalicylic acid method as modified by Wycoff and Stahmann (Deese and Stahmann, 1962). Reducing groups were expressed as  $\mu$ eq/ml homogenate. Changes in viscosity of pectin (Exchange brand, Sunkist Growers, Inc., Ontario, California) and sodium polypectate solutions due to polygalacturonase activity were measured with 5-ml Ostwald pipettes at 30 C and with the Brookfield cone-plate viscometer (Model LVT, Brookfield Engineering Laboratories, Stoughton, Massachusetts) at 27.5 C. Viscosity assays were carried out on 0.5% sodium polypectate or 1.0% pectin with citrate-phosphate buffer at pH 5.0 (0.1 M citric acid + 0.2 M dibasic potassium phosphate). This same buffer was used for pH values of 3.0 to 7.0. A borate buffer (0.2 M boric acid + 0.5 M sodium borate) was used for pH levels of 7.0 to 9.0. One ml of enzyme preparation was added to 4 ml of substrate prepared in buffer. Five ml of reaction mixture were used in Ostwald pipettes, whereas 1 ml was used in the cone-plate viscometer. The reaction time for all enzyme assays was 15 min unless indicated otherwise. The Brookfield viscometer was run at 30 rpm in most assays. Chromatographic procedures described by Echandi and Walker (1957) as well as an ethyl acetate, acetic acid, and water (2:1:2) solvent, were employed in testing for the appearance of galacturonic acid. Chromatographs irrigated with the latter solvent were sprayed with anisidine phosphate reagent (Smith, 1960).

Cellulase (Cx) activity was determined viscometrically and by measurements of reducing groups liberated. The modified dinitrosalicylic method mentioned earlier was used in following the release of reducing groups from 0.5% carboxymethylcellulose (Cellulose gum, CMC 7MP, Hercules Powder Co.). Chromatographic tests for the breakdown of carboxymethylcellulose to cellobiose and glucose were made with the ethyl acetate solvent system and anisidine phosphate reagent referred to earlier. The aniline reagent described by Smith (1960) was also used.

## RESULTS

Homogenates of *A. avenae* were found to exhibit slight to moderate polygalacturonase activity (Fig. 1A). The flow rate of 0.5% sodium polypectate in Ostwald pipettes was slowly reduced by 35 to 60% during 1 hr, whereas that of pectin was reduced by only 10 to 15%. The optimum pH for this enzyme on pectate as determined viscometrically was pH 5.0.

Reducing group measurements by the dinitrosalicylic acid colorimetric method also showed moderate polygalacturonase activity. The number of  $\mu$ eq/ml homogenate vs. time gave a straight-line relationship during a 60-min period (Fig. 2A). Four to 6  $\mu$ eq reducing groups were liberated/ml homogenate/hr. Although considerable reducing groups were liberated by the polygalacturonase, no galacturonic acid was detected chromatographically when dialyzed enzyme preparations were used. Small quantities were found when nondialyzed preparations were used.

No pectin transesterase or pectinmethylesterase activity was found in any homogenates of *A. avenae*. Supernatants obtained by keeping the nematodes in water at 24 C failed to exhibit any pectolytic or cellulolytic enzyme activity.

*A. avenae* homogenates had very high cellulase (Cx) activity. The most active dialyzed preparations reduced the viscosity of 0.5% carboxymethylcellulose by 5 to 6 cps during 60 min (Fig. 1B). This same preparation released 9 to 10  $\mu$ eq reducing groups/ml homogenate during the same period. The greatest cellulase activity occurred at pH 6.0 to 7.0; little activity was detected at pH 3.0.

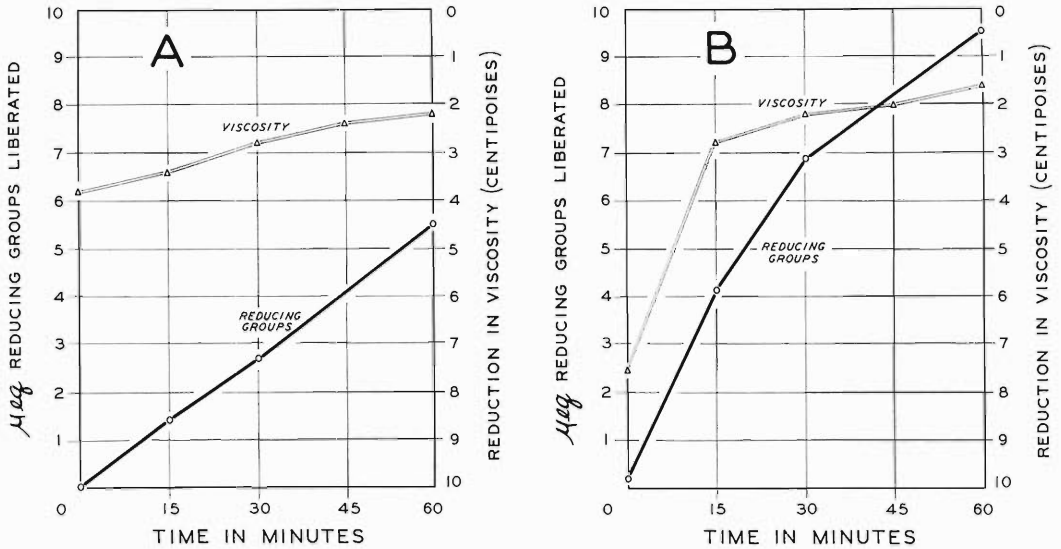


Fig. 1. Polygalacturonase and cellulase activity in homogenates of *A. avenae*: (A) Reduction in apparent viscosity of 0.5% sodium polypectate and reducing groups liberated by polygalacturonase; (B) Reduction in apparent viscosity of 0.5% carboxymethylcellulose and reducing groups liberated by cellulase.

Reductions in flow time of the above substrate as determined with Ostwald pipettes were 5% at pH 3.0 compared to 70% at pH 7.0. No glucose or cellibiose was liberated when dialyzed homogenates were used. Glucose was present in nondialyzed homogenate-carboxymethylcellulose reaction mixtures, but it was in the nematode homogenate rather than being released from the substrate.

The practical suitability of the cone-plate viscometer was determined in comparative assays of cellulase and polygalacturonase with the Ostwald pipette. Curves of apparent reductions in viscosity of sodium polypectate and carboxymethylcellulose for the two methods were similar. If readings obtained by the Brookfield viscometer were converted to percentages, the results were very similar to those obtained with Ostwald pipettes. The pipettes gave a greater decrease in apparent viscosity during the first 15 min than did the cone-plate viscometer (Fig. 2). The concentration of substrate and the speed at which the cone-plate viscometer was run affected the apparent viscosity reductions considerably.

#### DISCUSSION

Although *A. avenae* apparently has no pectinmethylesterase or pectin transesterase, moderate polygalacturonase activity was present in its homogenates. The polygalacturonase from *A. avenae* apparently was of the endo-type as no galacturonic acid was detected when dialyzed homogenates were used. It is possible that the host fungus, *R. solani*, may have produced enough polygalacturonase constitutively for the nematodes to retain small quantities of the enzyme while feeding. The type of fungus upon which mycophagous nematodes feed may influence their secretion of this enzyme. Krusberg (1964) found another mycophagous nematode, *D. triformis*, had no polygalacturonase whereas *D. dipsaci*, a closely related plant parasite, did. Morgan and McAllan (1962) found pectinases in *H. trifolii* and *P. penetrans*, so these enzymes are more prevalent in plant-parasitic nematodes.

The lack of a highly active polygalacturonase in nematodes, such as *A. avenae* or *D. triformis*, that feed primarily on fungi may be responsible for their limited parasitism of

higher plants. The findings of Goffart (1962) and Krusberg (1964) that several plant-parasitic nematodes, such as *D. dipsaci*, release pectin-splitting enzymes in cell-free substrates indicate this type of nematode has very different digestive and feeding mechanisms compared to the few mycophagous nematodes studied. The failure of *A. avenae* to release pectinases and cellulases in living plant cells may be one of the major reasons that its feeding on roots of higher plants is very limited, although it reproduces very rapidly on callus tissues (Barker and Darling, 1965). The same may be true for *D. trifurmis* and other mycophagous nematodes.

The cellulase from *A. avenae* was very active on carboxymethylcellulose. The optimum pH of 6.0 to 7.0 is similar to other cellulases described from nematodes. Dropkin (1963) obtained enzymes from *D. myceliophagous* and *M. incognita* that differed in their activity on different substrates with only the former releasing reducing groups from cotton. Although Tracey (1958) found more cellulase in homogenates of *D. dipsaci* than *D. myceliophagous*, one would expect mycophagous nematodes to secrete highly active cellulases.

It should be pointed out that unless the nematodes are grown monoxenically and extracted under sterile conditions, small numbers of contaminating bacteria may be the organisms actually secreting the hydrolytic enzymes. Although *A. avenae* was grown monoxenically, it is possible that a few bacteria introduced in the extraction and homogenization procedures could have secreted the polygalacturonase. This possibility seems unlikely since supernatants from large quantities gave no enzyme activity. However, more critical experiments with various types of nematodes should be done in which they are reared and extracted under gnotobiotic conditions similar to those used by Tiner (1961).

The cone-plate viscometer described was found to be suitable for viscometric assays of polygalacturonase and cellulase activity. This instrument overcomes the disadvantage of the large volume required by the rotating spindle viscometer used by Sherwood and Kelman (1964). Although the cone-plate viscometer has many good features, it also presents some problems that may not be so

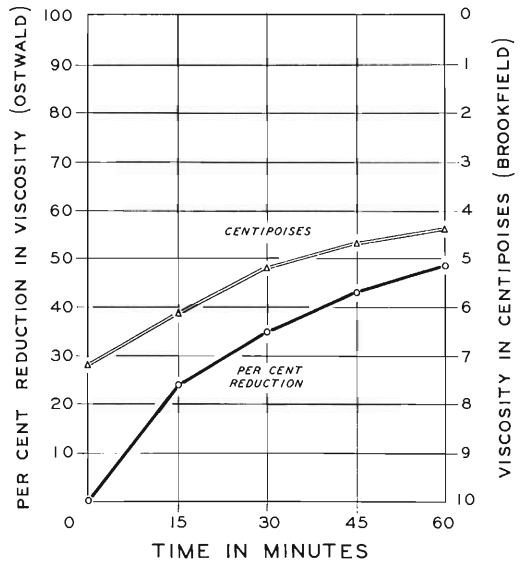


Fig. 2. Comparison of apparent reductions in viscosity of 0.5% carboxymethylcellulose as determined with the cone-plate viscometer and Ostwald pipettes.

evident in instruments such as Ostwald pipettes. Sherwood and Kelman (1964) concluded that pectin and carboxymethylcellulose solutions were near Newtonian at the shear rates they used. Durbin and Huppler (1965) found these compounds exhibited non-Newtonian behavior at rather low shear rates which may occur in capillary pipette viscometers. Although the purpose of this present study was not to investigate the rheological properties of these materials, the apparent viscosity of sodium polypectate and carboxymethylcellulose solutions used in these experiments decreased as the shear rate was increased. Thus, it is important that the Brookfield viscometer be used at low shear rates as Durbin and Huppler (1965) stated. Another problem with this instrument is the effect of any minute amount of debris on viscosity readings. If the cone and plate are not washed thoroughly and air dried and the solutions completely free of debris, apparent increases in viscosity may be obtained as the instrument rotates. Although enzyme assay by viscometry has many limitations, this type of viscometer should be espe-

cially valuable in studying the release of hydrolytic enzymes in plant tissues or solutions since small quantities of enzymes could be assayed.

#### SUMMARY

Viscometric and colorimetric assays of dialyzed homogenates of the nematode, *Aphelenchus avenae*, grown on *Rhizoctonia solani* gave moderate polygalacturonase (PG) activity and high cellulase (Cx) activity. A temperature-controlled cone-plate viscometer manufactured by Brookfield Engineering Laboratories, Stoughton, Massachusetts, was found to be suitable for viscometric assays of enzyme activity. This instrument requires only 1.0-ml samples. The polygalacturonase was more active on 0.5% sodium polypectate than on 1.0% pectin but failed to hydrolyze either substrate to galacturonic acid at 32 C during 24-hr incubation. The optimum pH for this enzyme was 5.0 whereas the optimum for the cellulase was 6.0 to 7.0. Colorimetric assays with dinitrosalicylic acid reagent showed both enzymes to liberate reducing groups rapidly. Water in which several grams of nematodes had remained for 24 to 48 hr at 24 C gave no cellulase or polygalacturonase activity. The nematodes apparently do not release these enzymes in such a liquid. No pectinmethylesterase or pectin transeliminase activity was detected in the nematode homogenates.

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## Studies on Species of Belondiroidea (Nematoda : Dorylaimida) from India<sup>1</sup>

M. RAFIQ SIDDIQI

Some of the species of the superfamily Belondiroidea (Thorne, 1939) Thorne, 1964, of India have been reported by Siddiqi (1964a, 1964b, 1964c) and Jairajpuri (1964, 1965). The nematodes collected by the author from soil around the roots of plants in various localities in India contained eight more belondiroid species, five of which are new to science. These are: *Belondira ujjanica* n. sp.; *B. bulbosa* n. sp.; *Dorylaimellus dorylaimoidurus* n. sp., *D. salimi* n. sp.; *D. andrassyi* Heyns, 1963; *D. directus* Heyns, 1963; *D. vexator* Heyns, 1963; and *Oxydirus gangeticus* n. sp. The new forms are here described and differential keys to the nominal species provided for the genera concerned.

### Family Belondiridae Thorne, 1939

#### Genus *Belondira* Thorne, 1939

#### *Belondira ujjanica* n. sp. (Fig. 1, A-F)

MEASUREMENTS: Holotype (female): L = 1.2 mm; a = 40; b = 5.2; c = 29; V = 34.

DESCRIPTION: Body almost straight, cuticle double-layered, thickened at tail end. Amphid stirrup-shaped, with aperture measuring a little less than corresponding body width. Lip region broadly rounded, continuous with body contour, with inner sclerotization (Fig. 1A). Spear thin, 5  $\mu$  or two-thirds as long as head width, with minute aperture. Spear extension simple not distinguishable into two parts, followed by a spindle-shaped esophageal swelling. Esophagus narrowing as it passes through nerve ring, enlarging near its middle to form a cylindrical bulb enveloped by spirally arranged muscle bundles (Fig. 1B). Esophago-intestinal valve well developed, rounded. Intestine with fine refractive granules and wide lumen.

Vulva a depressed, transverse slit with wide gaping opening, one-fifth body width (Fig. 1F). Vagina extending halfway into body, wide, +-shaped, with thick walls enveloped

by sphincter muscles which give it a bulboid appearance; its anterior end semisclerotized (Fig. 1C, E). Anterior uterine sac packed with sperms, 2.2 times as long as body width. Ovary posterior. Uterus in two parts, proximal muscular and distal glandular.

Prerectum about five times anal body width. Rectum longer than anal body width. Tail lozenge-shaped, inner core with conoid terminus; both layers of tail cuticle almost equally swollen; 1.8 times anal body width (Fig. 1D). Male not found.

TYPE HABITAT AND LOCALITY: Collected from cultivated soil near Ujjain, Madhya Pradesh, India.

TYPE MATERIAL: Holotype female in the Nematode Collection of Plant Pathology Section, Aligarh Muslim University, Aligarh, India.

RELATIONSHIP: In having a long tail, *Belondira ujjanica* n. sp. resembles *B. bulbosa* n. sp., *B. parva* Thorne, 1964, and *B. sacca* Thorne, 1964. From *B. bulbosa* it differs in having a broadly rounded lip region, spear extension not differentiated into two parts, more anterior vulva, and outer layer of tail cuticle not abnormally swollen.

*B. parva* has a longer body, shorter anterior uterine sac, shorter esophagus-vulva distance, and shorter tail which has rounded inner mass.

*B. sacca* has a pointed-truncated lip region, esophagus enlarged in its basal two-fifths, and a postrectal blind sac.

#### *Belondira bulbosa* n. sp. (Fig. 2, G-M)

MEASUREMENTS: Holotype (female): L = 0.93 mm; a = 44; b = 4.2; c = 24; V = 43.

Paratype (female): L = 0.99 mm; a = 47; b = 4.7; c = 23; V = 43.

Juvenile (1): L = 0.8 mm; a = 40; b = 4.2; c = 25.

DESCRIPTION: Female: Body almost straight when relaxed by gentle heat. Cuticle thin over body but abnormally thickened at tail. Amphids elongate, aperture curved, three-fourths body width. Lateral hypodermal chords two-

<sup>1</sup> Contribution from the Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh (U.P.), India.

The author is thankful to Dr. Abrar Mustafa Khan, Incharge, Section of Plant Pathology, for encouragement and providing facilities.

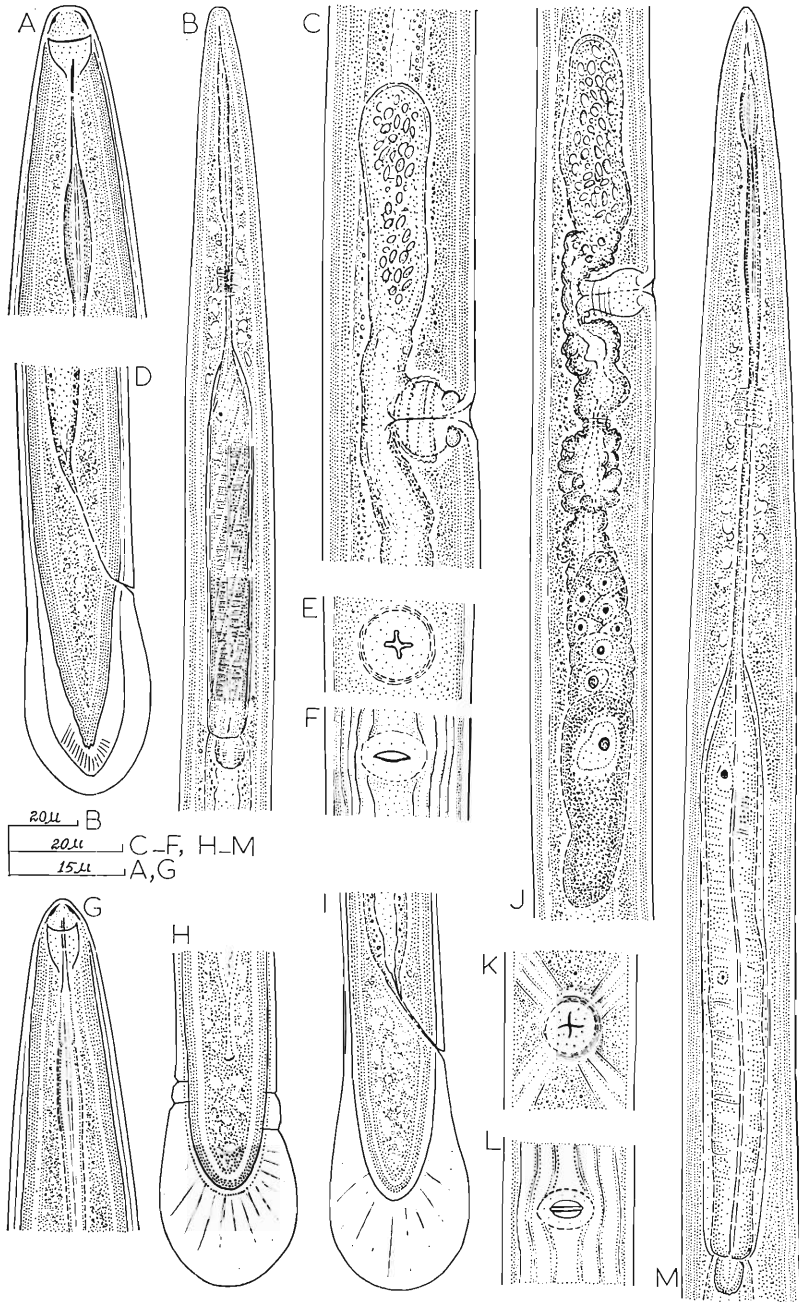


Fig. 1, A-F. *Belondira ujanica* n. sp. A. Head end of female; B. Esophagus of female; C. Vulva and anterior uterine sac, lateral; D. Tail end of female; E. Vagina, ventral; F. Vulva, ventral. G-M. *B. bulbosa* n. sp. G. Head end of female; H. Tail end of female, ventral; I. Tail end of female, lateral; J. Reproductive organs of female; K. Vagina, ventral; L. Vulva, ventral; M. Esophagus of female.

ninths body width. Lip region anteriorly conoid, not offset, with internal sclerotization. Spear thin, 4.5  $\mu$  or about two-thirds head width, with minute aperture. Spear extension 14  $\mu$  long, in two parts, reminiscent of that in *Dorylaimellus*; anterior part sclerotized, posterior slightly swollen.

Esophagus a slender tube narrowing as it passes through nerve ring, enlarging near its middle to a cylindroid bulb enveloped by spirally arranged muscles; its tissues with a few muscle fibers and glandular areas; gland nuclei as depicted (Fig. 1M). Esophago-intestinal valve large, rounded. Vulva-anus distance 8-10 times body width.

Vulva a fine transverse slit, about one-fourth body width. Vagina extending more than half-way into body, +-shaped, enveloped by sphincter muscles (Fig. 1K). Anterior uterine sac packed with sperms, 2.3 times body width. Posterior reproductive branch normal, reflexed at oviduct. Uterus with a distinct swelling distally, joined to the glandular chamber of oviduct through a narrow muscular region (Fig. 1J).

Prerectum 3.6 times and rectum a little longer than anal body width. Tail bulboid mostly due to the swelling of its outer layer of cuticle, with cylindroid, slightly tapering core; 2.3 times anal body width. Outer layer of cuticle at tail terminus about as wide as anal body width, with riblike, radially arranged cuticular strands (Fig. 1H, I).

Male not found.

**TYPE HABITAT AND LOCALITY:** Collected from soil around roots of banana plants (*Musa paradisiaca*) at Sitapur (District headquarter), U.P., India.

**TYPE MATERIAL:** Holotype female in the Nematode Collection of Plant Pathology Section, Aligarh Muslim University, Aligarh, India. A paratype female and juvenile in the author's personal collection.

**RELATIONSHIP:** *Belondira bulbosa* n. sp. is most closely related to *B. clavicaudata* (Williams, 1958) Andrassy, 1963; *B. caudata* Thorne, 1939; and *B. parva* Thorne, 1964. *B. clavicaudata* has more anterior vulva ( $V = 35$ ), shorter anterior uterine sac, and shorter tail ( $c = 42$ ). *B. caudata* has shorter esophagus which is enlarged only in its posterior two-fifths, more anterior vulva, anterior uterine

sac measuring about one body width, and outer layer of caudal cuticle not abnormally swollen. *B. parva* has anterior vulva ( $V = 35$ ), shorter anterior uterine sac, and shorter vulva-esophagus distance.

KEY TO SPECIES OF *Belondira*  
(based on females)

1. Esophagus enlarged at or anterior to its middle ..... 2  
Esophagus enlarged posterior to its middle ..... 10
2. Body length about 2.0 mm ..... 3  
Body length about 1.5 mm or less ..... 4
3. Posterior three-fifths of esophagus enlarged ..... *apitica* Thorne, 1939  
Posterior half of esophagus enlarged ..... *porta* Thorne, 1964
4. Esophagus enlarged at its middle ..... 5  
Esophagus enlarged considerably anterior to its middle ..... 9
5. Body length 1.4 mm; spear 9  $\mu$  long ..... *cylindrica* Thorne, 1964  
Body length less than 1 mm; spear less than 7  $\mu$  long ..... 6
6. Spear extension in two parts ..... 7  
Spear extension not differentiated into two parts ..... 8
7. Tail about 1.2 times anal body width,  $V = 35$  ..... *clavicaudata* (Williams, 1958) Andrassy, 1963  
Tail about 2.3 times anal body width,  $V = 43$  ..... *bulbosa* n. sp.
8. Anterior uterine sac 2.2 times body width ..... *ujjanica* n. sp.  
Anterior uterine sac one body width or less ..... *parva* Thorne, 1964
9. Posterior two-thirds of esophagus enlarged ..... *ortha* Thorne, 1939  
Posterior three-fifths of esophagus enlarged ..... *neortha* Siddiqi, 1964
10. Postrectal intestinal sac present ..... *sacca* Thorne, 1964  
Postrectal intestinal sac absent ..... 11
11. Anterior uterine sac shorter than body width ..... 12  
Anterior uterine sac not shorter than body width ..... 13
12. Spear very slender, aperture obscure ..... *tenuidens* Thorne, 1964  
Spear not very slender, aperture distinct ..... *clava* Thorne, 1964

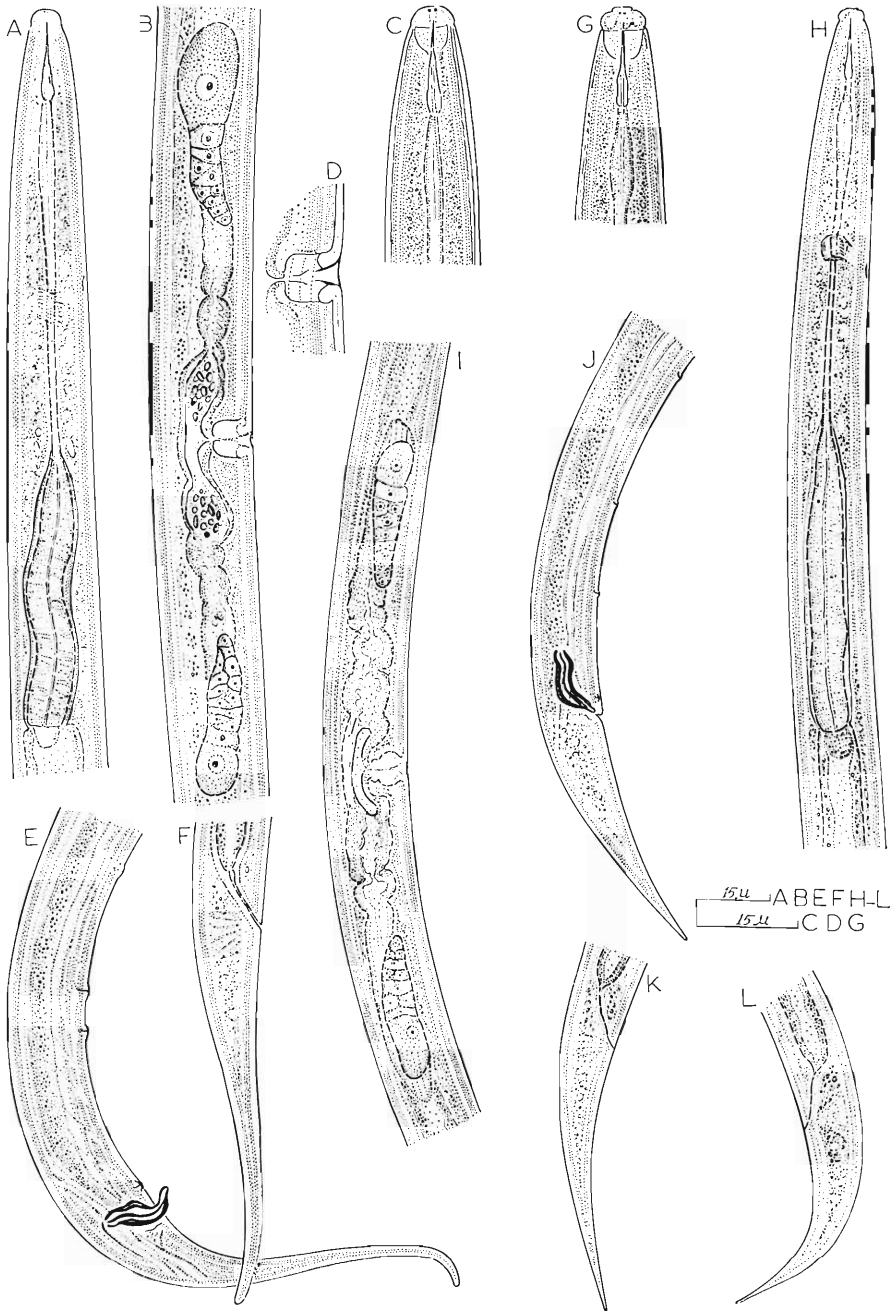


Fig. 2, A-F. *Dorylaimellus dorylaimoidurus* n. sp. A. Esophagus of female; B. Reproductive organs of female; C. Head end of female; D. Vulva and vagina, lateral; E. Tail end of male, lateral; F. Tail end of female, lateral. G-L. *D. salimi* n. sp. G. Head end of female; H. Esophagus of female; I. Reproductive organs of female; J. Tail end of male, lateral; K. Tail end of female, lateral; L. Tail of larva.

13. Body length 0.89 mm; tail short,  $c = 56$ ;  $V = 33.6$  ..... *nepalensis* Siddiqi, 1964  
 Body length 1.24 mm; tail long,  $c = 41$ ;  
 $V = 36$  .... *caudata* Thorne, 1939 (Syn.  
*paraclava* Jairajpuri, 1964)

Genus *Dorylaimellus* Cobb, 1913  
*Dorylaimellus dorylaimoidurus* n. sp.  
 (Fig. 2, A-F)

MEASUREMENTS: Holotype (female):  $L = 0.84$  mm;  $a = 40$ ;  $b = 5.6$ ;  $c = 10.5$ ;  $V = 42.6$ .

Male:  $L = 0.84$  mm;  $a = 44$ ;  $b = 5.7$ ;  $c = 11.6$ ;  $T = 38$ .

DESCRIPTION: Female: Body only slightly ventrally arcuate, anterior end a little twisted. Cuticle apparently smooth, subcuticle marked by fine, transverse striac. Lateral hypodermal chords narrow, one-fourth to one-fifth body width, glandular bodies obscure. Lip region smoothly rounded, papillae not elevated. Amphids large, stirrup-shaped. Sclerotized platelets around vestibule distinct. Spear very slender, its lumen difficult to observe,  $6.5 \mu$  long, shorter than head width. Spear extension flanged at base,  $12 \mu$  long. Esophagus enlarged in its posterior two-fifths. Nerve ring a little behind middle of anterior slender portion of esophagus; hemizonid near anterior edge of nerve ring. Esophago-intestinal valve small, rounded.

Vulva apparently longitudinal, ventral view not possible. Vagina extending two-fifths way into body, with thick walls appearing squarish in lateral view (Fig. 2D). Reproductive organs paired, symmetrical, opposed, reflexed (Fig. 2B). Uteri with oval sperms. Prerectum not definable, rectum about anal body width long. Tail elongate conoid to blunt terminus, with distal portion dorsally arcuate, reminiscent of elongate tails of *Dorylaimoides*, five and two-thirds times anal body width long (Fig. 2F).

Male: Body straight anteriorly, ventrally curved posteriorly in the form of an angling hook. Head smoothly rounded; refractive platelets distinct. Spear, esophagus, and tail as in female. Testes paired, dorylaimoid, each about  $40 \mu$  long; behind a few large-sized spermatocytes each testis packed with elongate, round-ended sperms. Spicula paired, heavy, sharply bent at their distal third,  $17 \mu$  long. Lateral sides of rectum slightly sclerotized dis-

tally to form guiding pieces of spicules. Supplements in the form of an adanal pair and three ventromedian papillae arranged as illustrated (Fig. 2E).

TYPE HABITAT AND LOCALITY: Collected from soil around roots of sal trees (*Shorea robusta*) in Jaulasal Forest Range (Haldwani Forest Division), 25 km on Tanakpur-Haldwani Road, Nainital District, U.P., India.

TYPE MATERIAL: Holotype and a male in the Nematode Collection of Plant Pathology Section, Aligarh Muslim University, Aligarh, India.

RELATIONSHIP: *Dorylaimellus dorylaimoidurus* n. sp. comes close to *D. filiformis* Jairajpuri, 1964 and *D. spicatus* Loof, 1964. From the former species it differs in having a smoothly rounded head, more slender spear with obscure lumen, vaginal wall thicker and appearing squarish in lateral view, and less attenuated, dorsally bent, round-ended tail measuring less than 6 times anal body width. From the latter it can be differentiated in having shorter enlargement of esophagus, shorter neck, more anterior vulva, and dorsally bent tail ( $b = 2.7-3.2$ ;  $V = 51-56$  and straight tail in *D. spicatus*).

*Dorylaimellus salimi* n. sp.\*  
 (Fig. 2, G-L)

MEASUREMENTS: Holotype (female):  $L = 0.7$  mm;  $a = 53$ ;  $b = 4.7$ ;  $c = 12.7$ ;  $V = 54$ .

Males (3):  $L = 0.74-0.80$  mm;  $a = 43-45$ ;  $b = 4.9-5.4$ ;  $c = 14-17$ ;  $T = 36-47$ .

Juvenile (1):  $L = 0.57$  mm;  $a = 36$ ;  $b = 4.4$ ;  $c = 12$ .

DESCRIPTION: Female: Body in an open C form when relaxed. Outer and inner layers of cuticle marked by fine transverse striations. Lateral hypodermal chords wide, about one-third body width; glandular bodies obscure. Lip region offset, lips distinctly marked, inner margins of lips forming a distinct labial disc. Amphid large, cup-shaped, practically enveloping body at that region (Fig. 2G). Sensillar sac  $10 \mu$  behind amphidial pouch. Sclerotized platelets around vestibule present. Spear very slender,  $5 \mu$  long, its aperture obscure. Spear extension slightly swollen at base, not distinctly flanged,  $7.5 \mu$  long.

\* Named in honor of my father, Mr. Mohammad Salim Siddiqi, Advocate of Karwi (Banda District), U.P.

Esophagus slightly swollen behind spear extension, narrowed as it passes through nerve ring, expanding at its posterior two-fifths. Enlarged part with distinct radial muscles, enveloped by a muscular sheath. Nerve ring a little behind middle of anterior slender part of esophagus. Hemizonid near posterior edge of nerve ring. Esophago-intestinal valve conoid-rounded.

Vulva depressed. Vagina extending halfway into body. Reproductive organs paired, reflexed at oviduct. Uteri with a proximal muscular portion forming ovejector and a distal glandular part. Latter joined to a spherical chamber at the proximal end of the oviduct through a narrow passage (Fig. 2I). Pre-rectum not definite. Rectum about anal body width long. Tail regularly tapering to a fine terminus, a little bent ventrad, 4.7 times anal body width (Fig. 2K).

Male: Body ventrally arcuate, more so in posterior region. Head, spear, and esophagus as described for female. Intestine with fine, refractive granules. Testes paired, dorylaimoid; sperms elongate-oval, 5  $\mu$  long. Spicules paired, thick, ventrally bent near middle, 17  $\mu$  long. Lateral guiding pieces present. Supplements an adanal pair and a ventromedian series of three papillae situated 27, 44-46, and 74  $\mu$  anterior to anus. Tail regularly tapering to a pointed terminus, 4-4.5 times anal body width long.

Juvenile: Labial disc prominent. Esophagus enlarged in its basal two-fifths. Spear as described for female. Tail regularly tapering to a pointed terminus, ventrally arcuate, 4 times anal body width long.

**TYPE HABITAT AND LOCALITY:** Collected from soil around roots of jungle trees in Dewangan Valley, 6 km from Karwi (Banda District), U.P., India. Male worms from mango soil, Bahraich, U.P.

**TYPE MATERIAL:** Holotype and a male in the Nematode Collection of Plant Pathology Section, Aligarh Muslim University, Aligarh, India; two males and a juvenile in author's personal collection.

**RELATIONSHIP:** In having an elongate, pointed tail, *Dorylaimellus salimi* n. sp. comes close to *D. filiformis* Jairajpuri, 1964 and *D. spicatus* Loof, 1964. From the former it differs in having a distinct labial disc, more

slender spear with obscure aperture, a posterior vulva, and a shorter tail ( $V = 43$ , tail 8 anal body widths long in *D. filiformis*). It differs from *D. spicatus* in having shorter neck, enlarged part of esophagus measuring only two-fifths of its length, and regularly tapering, ventrally arcuate tail.

#### KEY TO SPECIES OF *Dorylaimellus*

Jairajpuri (1964) gave a key to the species of the genus *Dorylaimellus*. Unfortunately, he included in his key some species which belong to the other genera outside Belondiroidea. These are: *Dorylaimus heterurus* (Schuurmans-Stekhoven and Teunissen, 1938) Heyns, 1963; *Enchodelus hedickei* (Paesler, 1941) Andrassy, 1960; *Pungentus engadinensis* (Altherr, 1950) Altherr, 1952; and *Tylencholaimus brevicaudatus* (Tarjan, 1953) Tarjan, 1956 (= *Dorylaimellus mirabilis* (de Man, 1884) Thorne, 1939 (vide Loof, 1961). *Dorylaimellus multipapillatus* Schuurmans-Stekhoven and Teunissen, 1938 and *Dorylaimellus fuorni* (Altherr, 1950) Altherr, 1950 are species of doubtful position (vide Heyns, 1963 and Altherr, 1952, respectively). Furthermore, *Dorylaimellus clavicaudatus* Williams, 1958 had already been shifted to *Belondira* by Andrassy (1963). Since Jairajpuri (1964), as many as 25 more species have been added to this genus. Heyns (1963) gave a satisfactory key to *Dorylaimellus* species, but to cover a large number of the lately described species, the following key is provided.

It is noted that *Dorylaimellus labiatus* Thorne, 1964 is remarkably similar to *D. vexator* Heyns, 1963, but the tail sketched by Thorne (1964) is different in being more pointed. A comparative study of these species may show more morphological differences. Great similarity also exists between *D. directus* Heyns, 1963 and *D. monticolus* Clark, 1963 but the total length of spear and its extension is greater in the former species. The author has failed to find any consistent difference between *D. discocephalus* Siddiqi, 1964 and *D. cephalus* Jairajpuri, 1964 and also between *D. indicus* Siddiqi, 1964 and *D. curvatus* Jairajpuri, 1964 although specimens from a number of localities in India have been compared. *D. cephalus* and *D. curvatus*, therefore, have not been considered valid species.

1. Ovary single .....	2	18. Posterior three-fifths of esophagus enlarged .....	<i>spicatus</i> Loof, 1964
Ovaries paired .....	3	Posterior two-fifths of esophagus enlarged .....	<i>dorylaimoidurus</i> n. sp.
2. Ovary prodelphic ... <i>aequalis</i> (Cobb, 1918) Thorne, 1939		19. Tail hemispherical .....	20
Ovary opisthodelphic .....		Tail bluntly conoid, elongate conoid, or subdigitate .....	24
..... <i>porosus</i> Thorne, 1939		20. Length over 0.8 mm .....	21
3. Esophagus short, b = 6 or more .....	4	Length under 0.6 mm .....	22
Esophagus long, b = less than 6 .....	9	21. Labial disc present .....	
4. Tail elongate-conoid, ventrally curved ..	5	..... <i>projectus</i> Heyns, 1962	
Tail bluntly conoid to hemispheroid ..	6	Labial disc absent .....	
5. Tail about twice anal body width long .....		..... <i>longicollis</i> Loof, 1964	
..... <i>andrassyi</i> Heyns, 1963		22. Lateral chords with 15 pairs of glandular bodies or more .....	23
Tail about five times anal body width long .....		Lateral chords with 11 pairs of glandular bodies .....	
..... <i>basiri</i> Jairajpuri, 1964		..... <i>caffrae</i> Kruger, 1965	
6. Spear with well-developed flanges .....	7	23. Small labial disc present .....	
Spear without well-developed flanges ..	8	..... <i>parvulus</i> Thorne, 1939	
7. Spear about head width, small sclerotized bodies present in vaginal wall ..		Small labial disc absent .....	
..... <i>imitator</i> Heyns, 1963		..... <i>yangambiensis</i> Geraert, 1962	
Spear three-fourths head width, small sclerotized bodies not present in vaginal wall ..		24. Tail subdigitate .....	25
..... <i>indicus</i> Siddiqi, 1964		Tail bluntly conoid or elongate-conoid ..	26
8. Length 1.69-1.83 mm .....		25. Length about 1.4 mm .....	
..... <i>bambesae</i> de Coninck, 1962		..... <i>virginianus</i> Cobb, 1913	
Length 0.67 mm .. <i>digitatus</i> Siddiqi, 1964		Length about 0.8 mm .....	
9. Esophagus not constricted while passing through nerve ring .....		..... <i>demani</i> Goodey, 1963	
..... <i>nodochordus</i> Cobb, in Thorne, 1939		26. Spear about three-fourths head width long .....	27
Esophagus constricted while passing through nerve ring .....	10	Spear about one head width or longer ..	31
10. Cuticle with prominent coarse striations .....		27. Lip region continuous with body contour .....	<i>capitatus</i> Siddiqi, 1964
..... <i>striatus</i> Cobb, in Thorne, 1939		Lip region offset .....	28
Cuticle without prominent coarse striations .....	11	28. Length about 0.8 mm or more, c = 27 or more .....	29
11. Tail 4 times anal body width or more ..	12	Length about 0.7 mm or less; c = 20-24 .....	<i>parvus</i> Jairajpuri, 1965
Tail less than 4 times anal body width ..	19	29. Tail tip narrowly rounded .....	
12. Tail clavate .....	13	..... <i>labiatus</i> Thorne, 1964	
Tail not clavate .....	14	Tail tip broadly rounded .....	30
13. Spear 4-5 $\mu$ long .. <i>clavatus</i> Thorne, 1964		30. Tail ventrally convex, b = 4.0-5.5 ..	
Spear 7 $\mu$ long ... <i>nygellurus</i> Loof, 1964		..... <i>discocephalus</i> Siddiqi, 1964	
14. Length 1 mm or more, tail very long (c = 5) .....		Tail ventrally straight to concave, b = 2.9-3.6 .....	<i>voxator</i> Heyns, 1963
..... <i>filicaudatus</i> Thorne, 1964		31. Tail bluntly rounded, about 1.5 anal body widths .....	32
Length less than 1 mm, tail not very long (c = 9 or more) .....	15	Tail elongate-conoid, about two or more body widths .....	33
15. Tail 8 times anal body width .....		32. Length about 0.6 mm; prerectum less than two body widths .....	
..... <i>filiformis</i> Jairajpuri, 1964		..... <i>tenuidens</i> Thorne, 1939	
Tail less than 6 times anal body width ..	16		
16. Labial disc present .....	<i>salimi</i> n. sp.		
Labial disc absent .....	17		
17. Tail not attenuated, broadly rounded ..			
..... <i>longicaudatus</i> Jairajpuri, 1964			
Tail attenuated .....	18		

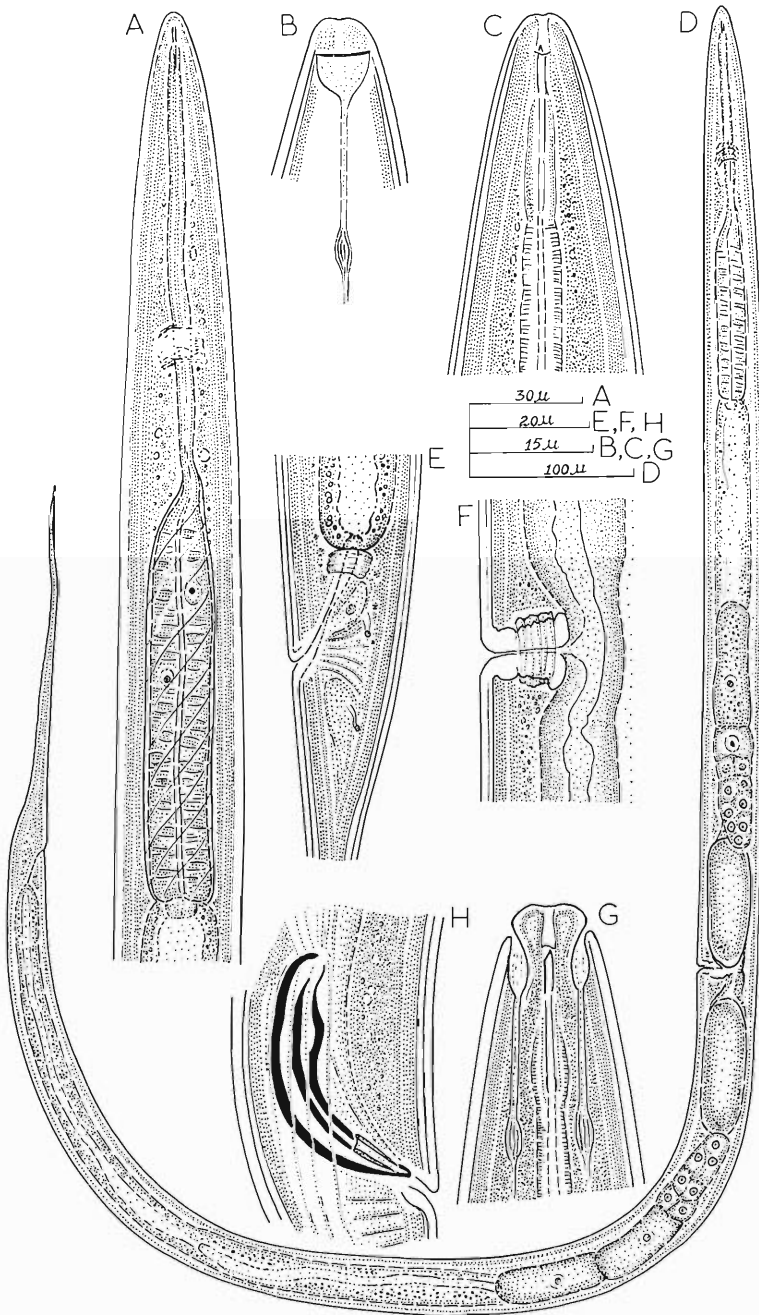


Fig. 3, A-F. *Oxydirus gangeticus* n. sp. A. Esophagus of female; B. Amphidial system; C. Head end of female; D. Female; E. Anal region of female; F. Vulva and vagina, lateral. G, H. *O. magnus*. G. Head end of male, ventral; H. Spicule and lateral guiding piece.



- Length about 0.9 mm; preectum about five body widths .....  
 ----- *occidentalis* Thorne, 1939
33. Length over 0.8 mm .....  
 ----- *graminis* Kruger, 1965
- Length less than 0.8 mm ..... 34
34. Esophagus expanded in its basal half ..  
 ----- *montenegricus* Andrassy, 1959
- Esophagus expanded in its basal two-thirds ..... 35
35. Spear and extension 20.5–22.3  $\mu$  long  
 ----- *directus* Heyns, 1963
- Spear and extension about 17  $\mu$  long ..  
 ----- *monticolus* Clark, 1963

Family Oxydiridae (Jairajpuri, 1964)  
 Thorne, 1964

Genus *Oxydirus* Thorne, 1939  
*Oxydirus gangeticus* n. sp.  
 (Fig. 3, A–F)

MEASUREMENTS: Females (3): L = 1.34–1.66 mm; a = 41–45; b = 6.0–6.4; c = 6–7; V = 35–37.

Holotype (female): L = 1.57 mm; a = 42; b = 6.4; c = 7; V = 37.

DESCRIPTION: Female: Body almost straight. Cuticle smooth, apparently three-layered. Amphids stirrup-shaped, two-thirds head width. Sensillar sac 15  $\mu$  behind amphidial pouch. Head narrow, smoothly rounded, continuous with body contour. Spear 6–7  $\mu$  long, slightly asymmetrical, aperture one-fourth its length; spear extension thrice as long as spear. Spear guiding ring simple, near apex of spear. Esophagus a slender tube, enlarging a little behind its middle to form a strongly muscular cylindroid bulb, 115 by 19  $\mu$ ; basal part enveloped by broad, spirally arranged muscular bands (Fig. 3A). Nerve ring a little behind middle of anterior part of esophagus. Esophago-intestinal valve broadly rounded.

Vulva–anus distance 2.2–2.5 times that from vulva to base of esophagus which itself is 70–100  $\mu$  longer than neck length. Reproductive organs paired, opposed, reflexed at oviduct. Two uterine eggs, 85 by 30  $\mu$  and 87 by 30  $\mu$ . Preectum about 7.5 times body width. Rectum lined with thick cuticle, a little longer than anal body width; preectum–rectum junction controlled by sphincter muscles; rectal glands as illustrated (Fig. 3E). Tail elongate, filiform,

12–13 anal body widths or a little less than neck length (Fig. 3D).

Male not found, no spermatozoa in uteri of females.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of grass, *Eleocharis* sp., from knee-deep water at the bank of the river Ganges near the southern end of the road bridge on Moradabad–Delhi Road, about 43 miles from Moradabad, U.P., India.

TYPE MATERIAL: Holotype in the Nematode Collection of Plant Pathology Section, Aligarh Muslim University, Aligarh, India; two females in author's personal collection.

RELATIONSHIP: *Oxydirus gangeticus* n. sp. comes close to *O. oxycephalus* (de Man, 1885) Thorne, 1939; *O. oxycephaloides* (de Man, 1921) Thorne, 1939; and *O. tropicus* Thorne, 1964.

From *O. oxycephalus* it differs in having a shorter esophageal enlargement, absence of vaginal sclerotization, and paired ovaries. It can be differentiated from *O. oxycephaloides* in having a shorter body, shorter and more slender spear, vulva–anus distance 2.2–2.5 times vulva–esophagus distance (1.5–1.66 times in *O. oxycephaloides*), and a shorter tail. From *O. tropicus* it can be distinguished in having a shorter body, smaller and weaker spear, and shorter tail.

*Oxydirus magnus* Timm, 1964  
 (Fig. 3G, H)

Syn. (?) *Oxydirus gigus* Jairajpuri, 1964

A single male specimen of this species was collected around grass roots at Kareli (District Narsinghpur), Madhya Pradesh, Central India. Measurements of the worm are as follows:

L = 3.7 mm; a = 88; b = 13; c = 14; T = 53; spear = 6  $\mu$ ; spear extension = 11  $\mu$ ; spicula = 49  $\mu$ ; lateral guiding pieces of spicules = 10  $\mu$ . There are 15 ventromedian supplementary papillae and 7 pairs of preanal subventral papillae, the first of which is located 17  $\mu$  anterior to anus.

*O. gigus* Jairajpuri, 1964 is very similar to *O. magnus* Timm, 1964 and Timm has expressed the view (*in litt.*) that it is a synonym of *O. magnus* as the latter has page priority. However, a comparative study of the type materials of the two species is needed to confirm this view. Till such time, the author prefers to regard the two as synonymous.

KEY TO SPECIES OF *Oxydirus*  
(based on females)

1. Ovary single ..... 2  
Ovaries paired ..... 3
2. V = 37, bisexual ..... *oxycephalus*  
(de Man, 1885) Thorne, 1939  
V = 30, monosexual .....  
..... *tenuicaudatus* Thorne, 1964
3. Body length under 2.5 mm ..... 4  
Body length over 3 mm ..... 6
4. Body length 1.34–1.66 mm; tail short,  
c = 6–7 ..... *gangeticus* n. sp.  
Body length 2 mm or more; tail long,  
c = less than 6 ..... 5
5. Tail abruptly tapering a little behind  
anal region .... *oxycephaloides* (de Man,  
1921) Thorne, 1939  
Tail not abruptly tapering behind anal  
region ..... *tropicus* Thorne, 1964
6. Esophagus long, b = 8.3 .....  
..... *elongatus* Altherr, 1963  
Esophagus short, b = over 10 ..... 7
7. Tail very long, c = 4–6 .....  
..... *novus* Jairajpuri, 1965  
Tail not very long, c = 10–15 .....  
..... *magnus* Timm, 1964

SPECIES INQUIRENDAE: As it is not definite if the following species have a muscular sheath around the basal enlargement of the esophagus, they are here regarded as *species inquirendae*.

- (1) *Oxydirus denticaudatus* (Imamura, 1931) Andrassy, 1960
- (2) *O. japonicus* (Cobb, in Thorne and Swanger, 1936) Andrassy, 1960
- (3) *O. leptus* (Cobb, in Thorne and Swanger, 1936) Andrassy, 1960
- (4) *O. tambo* (Imamura, 1931) Andrassy, 1960

SUMMARY

*Belondira ujanica* n. sp.; *B. bulbosa* n. sp.; *Dorylaimellus dorylaimoidurus* n. sp.; *D. salimi* n. sp.; *D. andrassyi* Heyns, 1963; *D. directus* Heyns, 1963; *D. vexator* Heyns, 1963; and *Oxydirus gangeticus* n. sp. are reported from soil around plant roots in India. *Belondira paraclava* Jairajpuri, 1964 is listed as a synonym of *B. caudata* Thorne, 1939. *Dorylaimellus cephalus* Jairajpuri, 1964 and *D. curvatus* Jairajpuri, 1964 are regarded synonyms of *D. discocephalus* Siddiqi, 1964 and *D. indicus*

Siddiqi, 1964, respectively. It is pointed out that *D. labiatus* Thorne, 1964 and *D. directus* Heyns, 1963 are very similar to *D. vexator* Heyns, 1963 and *D. monticolus* Clark, 1963. *Oxydirus gigus* Jairajpuri, 1964 has doubtfully been listed a synonym of *O. magnus* Timm, 1964. Differential keys to the species of the genera *Belondira*, *Dorylaimellus*, and *Oxydirus* have been provided. *O. denticaudatus* (Imamura, 1931) Andrassy, 1960; *O. japonicus* (Cobb, in Thorne and Swanger, 1936) Andrassy, 1960; *O. leptus* (Cobb, in Thorne and Swanger, 1936) Andrassy, 1960; and *O. tambo* (Imamura, 1931) Andrassy, 1960 have been regarded *species inquirendae*.

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### Phospholipid and Long-chain Fatty Acid Composition of the Nematode *Panagrellus redivivus*<sup>1</sup>

P. SIVAPALAN AND W. R. JENKINS<sup>2</sup>

Relatively few studies have been published on the lipids of free-living and plant-parasitic nematodes. Preliminary studies of three species of the plant-parasitic nematode, *Ditylenchus* (Filipjev), and the free-living nematode, *Turbatrix aceti* (Muller), indicated that the composition of total lipids in the former averaged 33% (Tracey, 1958) and in the latter 41% (Comenga, 1955). Both determinations were made on the basis of the total dry weight. The percentages of lipids in these forms are much higher than those determined for all the animal-parasitic forms investigated, which ranged from 1 to 8% of the total dry weight. Except for a determination of total lipid composition, no information has yet been published on the different lipid components in the free-living and plant-parasitic nematodes. It was therefore considered desirable to investigate, as an initial phase, the major phospholipid fractions and the different long-chain fatty acids present in a typical free-living nematode, *Panagrellus redivivus*.

#### MATERIALS AND METHODS

The free-living nematode, *Panagrellus redivivus*, used in this investigation was cultured in the laboratory on oatmeal as follows: Boiling distilled water was added to oatmeal (Mothers Oats, Quaker Oat Co.) spread evenly in 150-mm Petri dishes. When cooled to room temperature, the oatmeal was inoculated with 2 ml of a diluted suspension of nematodes and associated microorganisms, predominantly yeasts, from a stock culture and then left in the dark at about 25 C. After 10 to 12 days, large numbers of nematodes were washed with sterile, distilled water from the underside of the Petri dish cover where they had congregated. These nematodes were suspended in 10 volumes of the lipid solvent, consisting of chloroform and methanol (2:1 v/v) and homogenized for 15 min using a Mullard Ultrasonic Tissue Homogenizer. To insure complete extraction, the homogenized suspension was kept stirred for a minimum period of 3 hours at room temperature. After evaporating the solvent at 40 C under reduced pressure in a flash evaporator, the residue was reextracted with redistilled chloroform. The filtered chloroform extract was washed twice with a weak salt solution consisting of an equal mixture of 0.01 M MgCl<sub>2</sub> and NaCl. The chloroform layer

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey. This research was supported in part from regional research funds and is a contribution to NE-34, Physiology and Biochemistry of Nematodes and Nematode-Host Relationships.

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containing the total lipids was then carried over for further analysis.

### I. Phospholipid analysis

The phospholipids were precipitated from the total lipids by adding cold acetone and leaving the tightly closed tube overnight at  $-20^{\circ}\text{C}$ . The clear supernatant containing the neutral lipids was saved and stored at  $-10^{\circ}\text{C}$  for further analysis. Isolated phospholipids were washed repeatedly with cold acetone and then dissolved in chloroform.

Analysis of the individual classes of phospholipids was done by thin-layer chromatography. Silica gel G (Research Specialties Co.) was applied to a thickness of  $250\ \mu$  on  $5 \times 20$ -cm glass plates, according to the method of Stahl (1961). Chromatography was performed using a solvent system consisting of  $\text{CHCl}_3:\text{MeOH}:7\text{N NH}_4\text{OH}$  (60:35:5). Tentative identification of the different fractions was made by observing color responses to specific chromogenic reagents, according to the method of Skidmore and Entenman (1962).

Confirmation of the tentatively identified phospholipid fractions was performed by eluting the fractions from the chromatoplates and hydrolyzing the elutriate with  $6\ \text{N HCl}$ . The hydrolysis products were dissolved in distilled water and rechromatographed alongside authentic reference samples, using a solvent system consisting of  $\text{MeOH}:\text{H}_2\text{O}:7\text{N NH}_4\text{OH}$  (6:3:1).

Myoinositol was confirmed from the hydrolysis products of the tentatively identified inositol phosphatide fraction by converting it to a trimethyl silyl (TMS) ether and analyzing this derivative by gas-liquid chromatography, according to the method of Roberts *et al.* (1965).

### II. Long-chain fatty acid analysis

Long-chain fatty acid analysis was performed on the total lipids as well as on the separated phospholipid and neutral lipid fractions by gas-liquid chromatography.

#### Saponification

Lipids were saponified with an alcoholic solution of  $\text{NaOH}$  (RAPSAP—Applied Science Laboratories, Inc.) and by heating for 3 min

at  $85^{\circ}\text{C}$ . Two volumes of hot ( $70^{\circ}\text{C}$ ) distilled water were added to the mixture and the system was allowed to cool to room temperature. On cooling, the solution was neutralized with  $1\ \text{N HCl}$ , and the system made slightly acidic. The fatty acids were extracted with redistilled petroleum ether.

#### Methylation

The fatty acids were methylated by the technique of Metcalfe and Schmitz (1961) with slight modification. The fatty acid solution was transferred to a pretreated anion exchange resin (Amberlite 400—Rohm and Haas, Pa.) and the resin was subsequently washed repeatedly with petroleum ether. The fatty acids were methylated while remaining attached to the resin with boron trifluoromethanol (Applied Science Laboratory Inc.) over a steam bath for 3 min.

#### Fractionation of the fatty acid esters

The fatty acid esters were fractionated on the basis of the degree of unsaturation with a slightly modified method of De Vries (1963). The esters were eluted from a column of silicic acid impregnated with silver nitrate, using varying proportions of a mixture of redistilled diethyl ether and petroleum ether (b.p.  $30$  to  $40^{\circ}\text{C}$ ). The saturated esters were eluted with petroleum ether, and the different unsaturated fractions were eluted with a mixture of diethyl ether in petroleum ether with increasing proportions of diethyl ether.

The solvent in each eluted fraction was evaporated under a slow stream of nitrogen, and the fatty acid methyl esters were dissolved in chloroform and analyzed by gas-liquid chromatography.

#### Hydrogenation of unsaturated fatty acid esters

The fatty acid esters were dissolved in iso-octane and hydrogenated at  $65^{\circ}\text{C}$  with purified hydrogen, in the presence of a 10% palladium catalyst, according to the method of Low *et al.* (1964).

#### Gas-liquid chromatography of the fatty acid esters

Gas-liquid chromatography of the long-

TABLE 1.  $R_f$  values of the different phospholipid fractions of *Panagrellus redivivus* fractionated by thin-layer chromatography,\* and the color reactions of the individual fractions with specific spray reagents.\*\*

Phospholipid	$R_f$ value	Color reactions				
		I <sub>2</sub>	Mo	Nin	Bi	Ag
Phosphatidic acid	0.75	+	+	-	-	+
Phosphatidyl ethanolamine	0.51	+	+	+	-	-
Phosphatidyl choline	0.37	+	+	-	+	-
Phosphatidyl inositol	0.31	+	+	-	-	+
5***	0.22	+	+	-	-	+
6	0.17	+	+	-	-	-
7	0.13	+	+	-	-	-

\* Chromatograms were developed with a solvent system consisting of chloroform:methanol:7N ammonium hydroxide mixed in the ratio of 60:35:5.

\*\* I<sub>2</sub> = iodine fumes; Mo = molybdc acid; Nin = ninhydrin; Bi = Dragendorf reagent; Ag = ammoniacal silver nitrate.

\*\*\* 5 to 7 represent unidentified fractions.

chain fatty acid methyl esters was performed on U-shaped columns (6 ft × 2.5 mm ID) packed with 20% diethylene glycol succinate (DEGS) on 80- to 90-mesh Anakrom ABS, at either 180 C or 200 C and an input pressure of 12 psi of nitrogen corresponding to an output flow rate of 50 ml/min. A column of similar size, packed with 15% Apiezon L Grease, was used as a cross-check for certain long-chain fatty acids. A Research Specialities Gas Chromatograph of the 600 series with an H<sub>2</sub> flame-ionization detector was used in this investigation.

*Calculation of the percent composition of fatty acids*

The percent composition of the individual fatty acid esters in the sample was calculated on the basis of the area of the representative peaks in the chromatogram. The area of each peak was determined as a percentage of the total area of all peaks.

RESULTS

The total weight of nematodes after oven-drying at 80 C for 24 hours averaged 20.8% of the total wet weight. On a dry-weight basis, the composition of the total lipids in *P. redivivus* averaged 24% and the phospholipids 32.8% of the total lipids.

From the thin-layer chromatographic stud-

TABLE 2.  $R_f$  values of standards and of the hydrolysis products of the phospholipid fractions of *Panagrellus redivivus* analyzed by thin-layer chromatography\* and the color reactions of the compounds with specific spray reagents.\*\*

Hydrolysis products and standards	$R_f$ value	Color reaction	
		Nin	Bi
Phosphatidyl ethanolamine	0.51	+	-
Ethanolamine (standard)	0.52	+	-
Phosphatidyl choline	0.14	-	+
Choline (standard)	0.16	-	+

\* Chromatograms were developed with a solvent system consisting of methanol:water:7N ammonium hydroxide mixed in the ratio of 6:3:1.

\*\* Nin = Ninhydrin; Bi = Dragendorf reagent.

ies, seven iodine-positive spots were observed, all of which reacted positively with molybdc acid. On the basis of color reactions, spots 1, 2, 3, and 4 were tentatively identified as phosphatidic acid, phosphatidyl ethanolamine, phosphatidyl choline, and phosphatidyl inositol, respectively (Table 1). Spot 5 was very weak and was not seen consistently in all the chromatograms tested. The  $R_f$  value of spot 6 compared closely to that of an authentic sample of sphingomyelin; however, this spot reacted negatively to Dragendorf reagent, which specifically detects choline-containing compounds. All test reagents reacted negatively with spot 7 and no specific observations were made.

On the basis that spot 1 had a high  $R_f$  value (0.75) and reacted positively with ammoniacal silver nitrate, it was tentatively identified as phosphatidic acid. No other conclusive tests were made on this compound. The  $R_f$  values of spots 2 and 3 were found to agree well with that of authentic samples of phosphatidyl ethanolamine and phosphatidyl choline, respectively.

The hydrolysis products of the elutriate of spots 2 and 3 were rechromatographed on thin-layer plates using the second solvent system (solvent II). The hydrolysis products of the tentatively identified phosphatidyl ethanolamine was found to contain free ethanolamine, which reacted positively with ninhydrin. The hydrolysis products of the tentatively identified phosphatidyl choline was found to contain free choline which developed a positive color with Dragendorf reagent. The  $R_f$  values were in close agreement with those of authentic sam-

TABLE 3. Long-chain fatty acids of the total lipids of *Panagrellus redivivus*, analyzed by gas-liquid chromatography on a polar DEGS column at 180 C and an input pressure of 12 psi.

Peak No.	Percent of total	Relative retention time*	Fatty acid
1	0.1	0.152	12:0
2	0.2	0.178	12:1
3	0.5	0.281	14:0
4	4.0	0.334	14:1
a <sup>†</sup>	0.4	0.433	—
5	3.9	0.533	16:0
6	3.7	0.652	16:1
b	0.9	0.834	—
7	0.6	1.000	18:0
8	16.3	1.218	18:1
9	20.7	1.562	18:2

\* Retention time measured relative to methyl stearate (18:0).

<sup>†</sup> a and b represent unidentified peaks.

ples of ethanolamine and choline, chromatographed alongside the hydrolysis products of phosphatidyl ethanolamine and phosphatidyl choline, respectively (Table 2).

Confirmation of the spot tentatively identified as phosphatidyl inositol was made by identifying myoinositol from the hydrolysis products. The trimethyl silyl (TMS) ether was obtained from the hydrolysis products, and analyzed by gas-liquid chromatography; the retention time was found to agree well with that of the TMS of an authentic sample of myoinositol.

## II. Long-chain fatty acid analysis

Hydrogenation of the complete mixture of fatty acids produced straight-chain fatty acids of even carbon number, ranging from 12 to 20. There were no straight-chain fatty acids of chain length greater than 20. Fatty acids of odd carbon number were not detected. The peaks 1 to 14 and their hydrogenation products were all identified on the basis of comparisons of retention times with those of authentic samples of pure fatty acids. The possibility of the presence of branched-chain isomers among the unidentified peaks was not investigated. These results are summarized in Tables 3 and 4.

The findings on the polar DEGS column were supported by the results obtained from the analysis using the relatively nonpolar Apiezon L column. There was complete agreement between the retention times of these peaks and those of the reference samples.

TABLE 4. Long-chain fatty acids of the total lipids of *Panagrellus redivivus*, analyzed by gas-liquid chromatography on a polar DEGS column at 200 C and an input pressure of 12 psi.

Peak No.	Percent of total	Relative retention time*	Fatty acid
1	0.1	0.181	12:0
2	0.2	0.227	12:1
3	0.5	0.318	14:0
4	4.0	0.378	14:1
a <sup>†</sup>	0.4	0.484	—
5	3.9	0.560	16:0
6	3.7	0.692	16:1
b	0.9	0.840	—
7	0.6	1.000	18:0
8	16.3	1.174	18:1
9	20.7	1.515	18:2
10	1.5	1.931	18:3
c	2.2	2.219	—
11	0.6	2.651	20:2
12	4.7	2.954	20:3
13	5.2	3.371	20:4
14	4.5	4.545	20:5
d	4.6	5.189	—
e	1.6	7.386	—
f	11.3	8.636	—
g	12.4	10.909	—

\* Retention time measured relative to methyl stearate (18:0).

<sup>†</sup> a to g represent unidentified peaks.

## Saturated fatty acids

The identification of the fatty acids eluted from the silicic acid column are summarized in Table 5. The carbon chain lengths of the saturated fatty acids ranged from 12 to 18 in even numbers. Saturated fatty acids of chain lengths of more than 18 carbon atoms were not found, and chain lengths of less than 12 carbon atoms were not investigated. Peaks adjacent to 3 and 5 corresponding to the positions of peaks 4 and 6 in the total mixture were not identified.

## Mono-unsaturated fatty acids

The identifications of the mono-unsaturated acid eluted from the silicic acid column are summarized in Table 6 (Fraction II). As seen from this table, mono-unsaturated fatty acids of even carbon number and carbon chain lengths ranging from 12 to 18 were found. Assuming complete recovery from the silicic acid column, the ratio of the area of peak 4 to that of 8, and the ratio of the area of peak 6 to that of 8, were found to be less than the corresponding ratios in the total mixture prior to fractionation on the silicic acid column. This decrease in the area of peaks 4 and 6 was found to be in agreement with the proportion

TABLE 5. Gas-liquid chromatographic analysis\* of the saturated fatty acid fraction (Fraction No. I)\*\* of the total lipids of *Panagrellus redivivus*.

Peak No.	Percent of total	Fatty acid
1	0.1	12:0
3	0.5	14:0
4	1.6	—
5	3.9	16:0
6	1.3	—
7	0.6	18:0

\* Gas-liquid chromatographic analysis was performed on a polar DEGS column at 180 C and an input pressure of 12 psi.

\*\* Fraction eluted from a column of silicic acid impregnated with silver nitrate.

of the area of the peaks adjacent to 3 and 5 recovered in the saturated fatty acid fraction (Table 5). From calculations of the relative areas, it was found that 59.4% of the total area of peak 4 and 63.2% of the total area of peak 6 appeared with the mono-unsaturated fraction. On this proportionate basis, the percent compositions of C-14:1 and C-16:1 were calculated (Table 6).

*Di-unsaturated fatty acids*

The identifications of the di-unsaturated fatty acids are summarized in Table 6 (Fraction III). Only two di-unsaturated fatty acids of chain lengths of 18 and 20 carbon atoms were found to be present.

*Tri-unsaturated fatty acids*

Identifications of the tri-unsaturated fatty acids are summarized in Table 6 (Fraction IV). Only two tri-unsaturated fatty acids of chain lengths 18 and 20 were identified.

*Tetra- and penta-unsaturated fatty acids*

The identifications of the tetra-unsaturated fatty acids and the mixed fraction of tetra- and penta-unsaturated fatty acids eluted from the silicic acid column are summarized in Table 6 (Fraction V).

As seen from the above results, peaks 1 to 14, except 4 and 6, were each representative of a single fatty acid.

*Fatty acids of the phospholipid fraction*

The long-chain fatty acids of the phospholipid fraction are summarized in Table 7.

TABLE 6. Gas-liquid chromatographic analysis\* of the unsaturated fatty acid fractions (Fractions II, III, IV, and V)\*\* of the total lipids of *Panagrellus redivivus*.

Fraction and peak Nos.	Percent of total	Hydrogenation product	Fatty acid
Fraction II			
2	0.2	12:0	12:1
4	2.4	14:0	14:1
6	2.4	16:0	16:1
8	16.3	18:0	18:1
Fraction III			
9	20.7	18:0	18:2
11	0.6	20:0	20:2
Fraction IV			
10	1.5	18:0	18:3
12	4.7	20:0	20:3
f	11.3	—	—
Fraction V			
13	5.2	20:0	20:4
14	4.5	20:0	20:5
g	12.4	—	—

\* Gas-liquid chromatographic analysis was performed on a polar DEGS column at 200 C and an input pressure of 12 psi.

\*\* Fractions eluted from a column of silicic acid impregnated with silver nitrate.

Fatty acids of carbon chain length ranging from 12 to 20 were found to be present. The saturated C-12 fatty acid was not found in this fraction. Of the total identified fatty acids, 87% were unsaturated. The C-18 fatty acids accounted for 50% and the C-20 fatty acids for 42% of the total phospholipid fatty acids. The remaining 8% consisted of C-12, C-14, and C-16 fatty acids.

*Fatty acids of the neutral lipid fraction*

The long-chain fatty acids of the neutral lipid fraction are summarized in Table 8. Of the total fatty acids in the neutral lipid fraction, 96% were unsaturated. The C-18 fatty acids accounted for 75% of the total fatty acids, and C-20 fatty acids accounted for only 11%. The remaining 14% consisted of C-12, C-14, and C-16 fatty acids.

DISCUSSION

The percent composition of the total lipids in *Panagrellus redivivus* was found to account for 24% of the total dry weight. In comparison to the higher values of 33% in *Ditylenchus* sp. (Tracey, 1958) and 41% in *Turbatrix acetii* (Comenga, 1955), the amount of lipids in *P. redivivus* thus appears to be relatively low. Yet this value of 24% is relatively much higher than the values obtained for all animal-para-

TABLE 7. Gas-liquid chromatographic analysis\* of the long-chain fatty acids of the phospholipid fraction of *Panagrellus redivivus*.

Peak No.	Percent of total	Relative retention time**	Fatty acid
2	0.3	0.231	12:1
3	0.1	0.335	14:0
4	2.6	0.385	14:1
5	2.2	0.578	16:0
6	1.3	0.687	16:1
b***	1.9	0.846	—
7	6.5	1.000	18:0
8	10.3	1.197	18:1
9	17.7	1.501	18:2
10	0.1	1.970	18:3
c	12.3	2.190	—
11	0.6	2.549	20:2
12	9.5	2.991	20:3
13	15.1	3.435	20:4
14	3.9	4.522	20:5
e	3.5	7.585	—
f	4.5	9.129	—
g	7.5	11.622	—

\* Gas-liquid chromatographic analysis was performed on a polar DEGS column at 200 C and an input pressure of 12 psi.

\*\* Retention time measured relative to methyl stearate (18:0).

\*\*\* Peaks b to g represent unidentified peaks.

TABLE 8. Gas-liquid chromatographic analysis\* of the long-chain fatty acids of the neutral lipid fraction of *Panagrellus redivivus*.

Peak No.	Percent of total	Relative retention time**	Fatty acid
1	0.1	0.186	12:0
2	0.4	0.231	12:1
3	0.5	0.335	14:0
4	3.1	0.385	14:1
a***	0.2	0.479	—
5	1.5	0.578	16:0
6	3.3	0.687	16:1
b	0.3	0.846	—
7	0.2	1.000	18:0
8	15.3	1.197	18:1
9	32.9	1.501	18:2
10	0.2	1.970	18:3
c	3.0	2.190	—
11	0.4	2.549	20:2
12	2.7	2.991	20:3
13	1.9	3.435	20:4
14	2.3	4.522	20:5
d	2.2	5.455	—
f	13.2	9.129	—
g	16.3	11.622	—

\* Gas-liquid chromatographic analysis was performed on a polar DEGS column at 200 C and an input pressure of 12 psi.

\*\* Retention time measured relative to methyl stearate (18:0).

\*\*\* a to g represent unidentified peaks.

sitic nematodes investigated, the lipid composition of which ranged from 1 to 8% of the total dry weight.

The phospholipids in *P. redivivus* were found to be 32.8% of the total lipids. This value is higher than that observed for the two species of animal-parasitic nematodes, which included males of *Ascaris lumbricoides* containing 12.8%, and larvae of *Eustrongylides ignotus* containing 25.8% of the total lipids as phospholipids (von Brand and Winkeljohn, 1945). From the quantity of phospholipids present in *P. redivivus*, which accounts for about 8% of the total dry weight, it is probable that these lipids play a significant role in the biology and physiology of this nematode.

The results of the qualitative analysis of the total phospholipids in *P. redivivus* by thin-layer chromatography appear to agree in general with the observations made on the animal-parasitic nematode, *Ascaris lumbricoides*, by Rogers and Lazarus (1949), Fairbairn (1956), and Beames (1964). The major phospholipid fractions were determined as phosphatidyl ethanolamine and phosphatidyl choline. As no phosphatidyl serine was detected, the cephalin fractions appeared to be largely composed of phosphatidyl ethanolamine. Further, by visual inspection of the thin-layer chromato-

grams, phosphatidyl ethanolamine appeared to be the major fraction of the total phospholipids. Even in the larger animal-parasitic nematode, *A. lumbricoides*, phosphatidyl serine was reported only in traces (Rogers and Lazarus, 1949; Fairbairn, 1956; and Beames, 1964). It is quite possible that the failure to detect phosphatidyl serine in *P. redivivus* may have been due to the smaller size of sample used in this investigation, a size too small to detect trace amounts.

No sphingomyelin was detected in *P. redivivus*, yet it is possible that trace quantities of this compound may be present in this nematode as reported in the case of the animal-parasitic nematode *A. lumbricoides*. Traces of sphingomyelin have been found in this animal in the perienteric fluid and intestinal tissues, although the bulk of the tissues, such as the reproductive organs and body wall, lack this compound (Rogers and Lazarus, 1949; Fairbairn, 1956).

The detection of phosphatidyl inositol in *P. redivivus* appears to be the first recorded incidence of this compound in nematode tissues. In his recent investigation, Beames (1964) fractionated the phospholipids of *Ascaris* by silicic acid column chromatography and failed to detect inositol phosphatides.



The failure to detect this compound was attributed by him to possible losses during the extraction and fractionation procedure. In the present investigation, the use of gas-liquid chromatographic analysis to detect trace amount of myoinositol from the hydrolysis products of the phosphatidyl inositol fraction has rendered possible the confirmation of the presence of this compound among the phospholipid fractions of *P. redivivus*.

Although a relatively large amount of phosphatidic acid was tentatively identified, this was not characterized definitely. No attempt was made to identify the corresponding plasmalogen fractions of the different phospholipid fractions of *P. redivivus*.

In an attempt to study the nature of the long-chain fatty acids of the phospholipids and neutral lipids, the nature of the long-chain fatty acids of the total lipids were first investigated in detail. Fatty acids of carbon chain length 18 to 20 constituted more than 80% of the total. The C-18 fatty acids accounted for 59% and the C-20 fatty acids for 23% of the total. The C-16 fatty acids accounted for only 11%, while the remaining 7% comprised the C-12 and C-14 fatty acids. All fatty acids detected were of even carbon number, and fatty acids of carbon chain length greater than 20 were not detected. Approximately 90% of the total fatty acids were unsaturated. In addition to the saturated fatty acids the C-12, C-14, and C-16 fatty acids were each found to contain mono-unsaturated fatty acids. In addition to the saturated and mono-unsaturated acids, the C-18 fatty acids contained the poly-unsaturated fatty acids with 2 and 3 units of unsaturation. The C-20 fatty acids were not found to contain any saturated acids, but included acids with 2, 3, 4, and 5 units of unsaturation.

The phospholipid fraction contained a high proportion of C-20 fatty acids, which accounted for 42% of the total long-chain fatty acids of this fraction. This proportion of the C-20 fatty acids in the phospholipid fraction of *P. redivivus* is much higher relatively than the amount determined in the animal-parasitic nematode *A. lumbricoides*, in which the phospholipid C-20 fatty acids accounted for only 10 to 20% of the total (Beames, 1964). The C-18 fatty acids in *P. redivivus*, which account

for 50% of the total, are less than the amount of these fatty acids in the phospholipid fraction of *A. lumbricoides*, in which these account for 60 to 80% of the total (Beames, 1964). It appears that the C-20 fatty acids play a predominant role in the phospholipid fraction of *P. redivivus*. Arachidonic acid (20:4) accounts for about 50% of the total C-20 fatty acids, and eicosatrienoic acid (20:3) accounts for about 30%, the remaining 20% being mainly the penta-unsaturated acid, eicosapentaenoic acid (20:5), with the dienoic acid (20:2) being present only in trace amounts. Of the C-18 fatty acids, about 80% were unsaturated, and thus of the unsaturated fatty acids of the phospholipid fraction of *P. redivivus*, the C-18 and C-20 fatty acids were found in almost equal amounts. The major saturated fatty acid is the C-18 acid, stearic acid (18:0). Among the total identified long-chain fatty acids of the phospholipid fraction, 87% were determined to be unsaturated.

A greater proportion of unsaturated fatty acids was found in the neutral lipid fraction of *P. redivivus*, in which 96% of the total identified long-chain fatty acids were unsaturated. This unusually high proportion of unsaturated fatty acids was found to consist mostly of the C-18 acids, 18:1 (oleic acid) and 18:2 (linoleic acid), and these accounted for 75% of the total. The C-20 fatty acids accounted for only 11% of the total; the remaining 14% were found to consist of C-12, C-14, and C-16 fatty acids. Unlike the phospholipid fraction, the C-18 saturated fatty acid C-18:0 (stearic acid) was found in traces, the major saturated fatty acid being palmitic acid (C-16:0). Among the C-20 fatty acids of the neutral lipid fraction, which account for 11% of the total, arachidonic acid accounts for only 25%, thus being relatively very much less than the amount in the phospholipid fraction. Eicosatrienoic acid (20:3) and eicosapentaenoic acid (20:5) account for 70% of the C-20 fatty acids, these acids being present in about equal amounts, and the dienoic acid (20:2) accounts for the remaining 5%.

The results of the long-chain fatty acid analysis of *P. redivivus* indicate that the greatest concentration of the C-20 fatty acids occurs in the phospholipid fraction, in which arachidonic acid (20:4) appears to play a prominent

role. The C-20 fatty acids, along with the C-18 fatty acids, constitute the major proportion of the phospholipid long-chain fatty acids; the C-12, C-14, and C-16 fatty acids constitute only a small proportion. The greatest concentration of the C-18 fatty acids occurs in the neutral lipid fraction, composed almost entirely of oleic acid (18:1) and linoleic acid (18:2). The C-20 fatty acids, along with the C-12, C-14, and C-16 fatty acids, occur in smaller amounts.

*P. redivivus* was cultured on oatmeal and was found to feed mainly upon the yeast growing on this medium. It is possible that this nematode feeds both on the yeast and on the breakdown products of oats. To ascertain the origin of the long-chain fatty acids in *P. redivivus*, the fatty acids of oats and yeast were examined.

As no C-20 fatty acids were found either in the oats or in the yeast, *P. redivivus* probably has the ability to synthesize the C-20 fatty acids, possibly from the C-18 fatty acids, oleic (18:1) or linoleic acid (18:2).

The long-chain fatty acids, linoleic acid (18:2), linolenic acid (18:3), and arachidonic acid (20:4), are considered to be essential fatty acids in most vertebrate animals. For normal growth of mammals, at least one of these fatty acids has to be supplied with the diet (Deuel and Reiser, 1955). Lack of knowledge on the metabolism of these fatty acids among invertebrates prevents generalization, but it is known that, under certain conditions, even some bacteria require a source of unsaturated fatty acids such as oleic acid (18:1) in the culture medium for normal growth and multiplication. It would be interesting to investigate further to see if *P. redivivus* has the ability to synthesize, in addition to the C-20 fatty acids, the C-18 fatty acids, or whether these nematodes have to be supplied with at least one of the C-18 fatty acids in their diet for normal growth and multiplication. Such findings may eventually lead to clues that will help solve the problems of developing chemically defined media for culturing these and other nematodes.

In the gas-liquid chromatogram of the long-chain fatty acids of *P. redivivus*, a few peaks were left unidentified and characterized as "a" through "g." The retention times of these

unidentified peaks did not compare with any of the known samples of normal (straight-chain) long-chain fatty acids, ranging from 12 carbon atoms to 23 carbon atoms. When the long-chain fatty acids of yeast were analyzed, the unidentified peaks c, d, and e were also present. It is possible that some of these peaks correspond to branched-chain fatty acids, or possibly to polar hydroxy or keto acids of some of the short-chain fatty acids which are retained on the polar DEGS column and subsequently are eluted with the long-chain fatty acids. These possibilities are evident from the fact that, on using the relatively nonpolar Apiezon column, some of these peaks (a to c) did not appear at all, while the others appeared very much earlier than on the polar DEGS column. The possibility that these peaks may not correspond to fatty acids cannot, however, be ruled out. They may well be contaminating aldehydes, dimethylacetals, or fatty alcohols. On the other hand, Beames (1965) has analyzed *Ascaris lumbricoides* and determined the presence of a branched 15 C compound in its neutral lipid fraction. This same author also reported the presence of odd-numbered C-chain compounds in *Ascaris*. Since parasitic habits and food sources markedly differ, further investigations of these possibilities are necessary.

#### SUMMARY

Total lipids in *Panagrellus redivivus* were 24% of the total dry weight, and of this amount about 33% were phospholipids. Thin-layer chromatographic analysis of the phospholipids showed that the major phospholipid fractions were phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol, and phosphatidic acid. Phosphatidyl serine and sphingomyelin were not detected.

The results of the long-chain fatty acid analysis of *P. redivivus* indicate that the fatty acids of carbon length 18 and 20 constituted more than 80% of the total. Approximately 90% of the total fatty acids were unsaturated. The greatest concentration of the C-20 fatty acids occur in the phospholipid fraction, and these acids, along with the C-18 fatty acids, constitute the major proportion of the phospholipid long-chain fatty acids; the C-12, C-14, and C-16 fatty acids constitute only a small

proportion. The greatest concentration of C-18 fatty acids occur in the neutral lipid fraction; the C-20 fatty acids along with the C-12, C-14, and C-16 acids occurred in smaller amounts.

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**Studies on the Genera *Calolaimus* Timm, *Galophonema* Siddiqi, *Qudsianema* Jairajpuri, and *Utahnema* Thorne (Nematoda: Leptonchidae), with Description of *U. gracile* n. sp.<sup>1</sup>**

M. RAFIQ SIDDIQI

The genus *Calolaimus* Timm, 1964 was proposed as a new genus to accommodate *C. papillatus* Timm, 1964 while *Galophonema* Siddiqi, 1965 was proposed a few months later to contain *G. lenorum* Siddiqi, 1965. *Galophonema* is very similar to *Calolaimus* differing in the shape of the spear including extension, the vulva, and the occurrence of vaginal sclerotization. *Galophonema* has a tiny spear and sclerotized, rodlike spear extension not immediately differentiated from the spear; circular vulva

and unsclerotized vagina. Examinations of specimens of *C. papillatus* kindly supplied by Timm have shown that the spear in this species is small and that the spear extension is sclerotized (Fig. 1E). As the shape of the vulva and the sclerotization of the vagina are not to be relied upon as of generic diagnostic value in Dorylaimoidea, *Galophonema* Siddiqi, 1965 is here regarded as a synonym of *Calolaimus* Timm, 1964.

DIAGNOSIS OF *Calolaimus* (AMENDED): Leptonchidae: Large-sized worms. Amphids cup- or stirrup-shaped, with elongate-ellipsoidal ap-

<sup>1</sup> Contribution from the Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

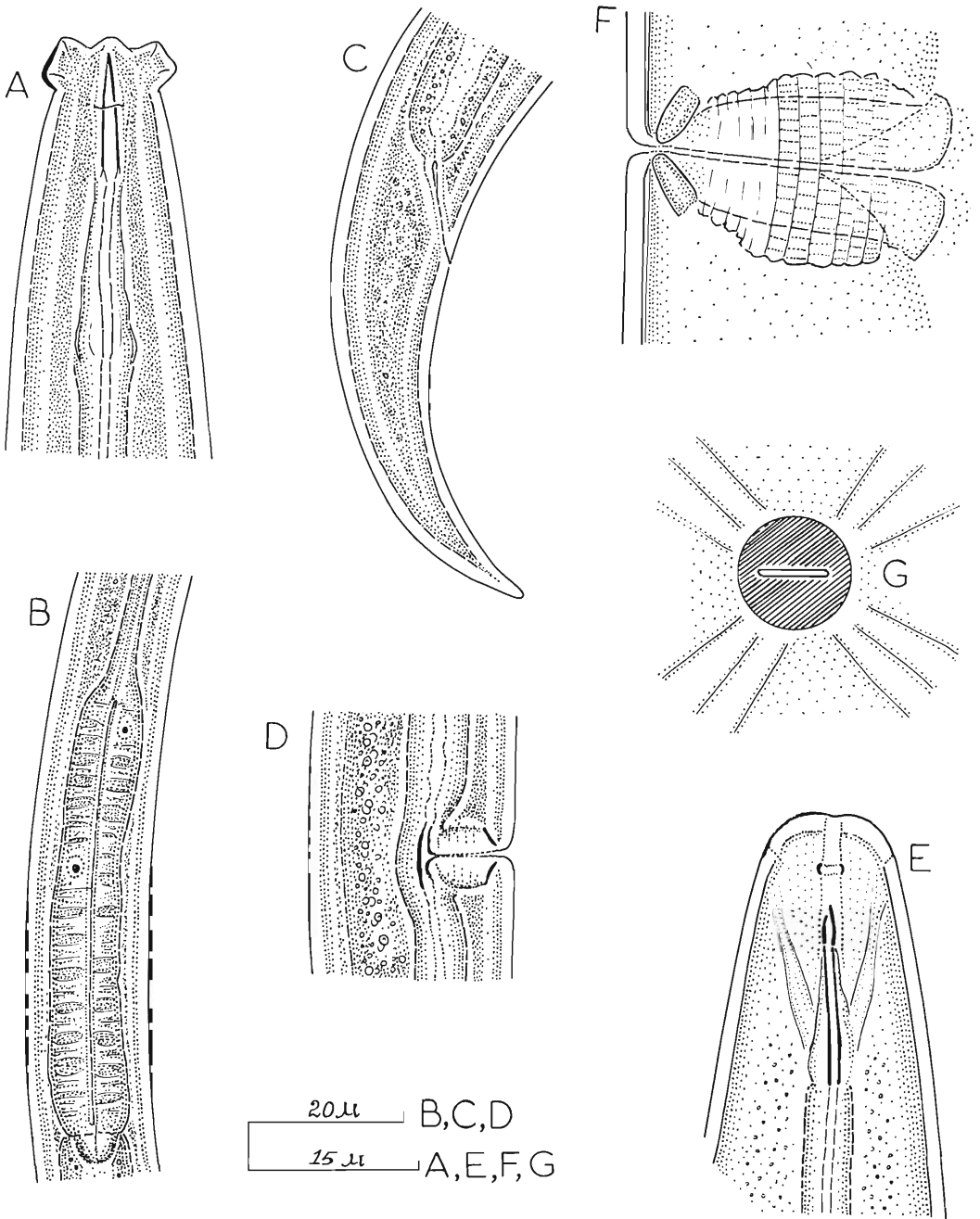


Fig. 1. A-D. *Eudorylaimus amabilis* (Jairajpuri, 1965). A. Head end of female. B. Enlarged part of esophagus. C. Tail of female. D. Vulval region. E-G. *Calolaimus papillatus* Timm, 1964. E. Head end of female. F. Vulva and vagina. G. Vulva, ventral.

erture. Spear guiding ring large, simple, belt-like in appearance, situated near base of lip region. Spear small, irregular in outline, with minute aperture; its extension simple, sclerotized, somewhat flanged at base. Enlarged part of esophagus occupying about  $\frac{1}{3}$  or less the length of neck, with prominently thickened inner cuticle in anterior region (also in posterior region but less pronounced).

Female reproductive system paired, opposed; vulva circular or transverse slit, with or without sclerotization. Testes, spicules, and supplements dorylaimoid. Paired submedian papillae present on male tail. Lateral guiding pieces of spicules appearing constricted. Tail in the two sexes similar, elongate-tapering. Occurring in soil about plant roots in freshwater habitat.

TYPE SPECIES: *Calolaimus papillatus* Timm, 1964.

OTHER SPECIES: *C. ditlevseni* (Micoletzky, 1922) Timm, 1964 (Syn. *Dorylaimus ditlevseni* Micoletzky, 1922; *Dorylaimus tenuis* of Ditlevsen, 1912, nec Linstow, 1879; *Dorylaimoides ditlevseni* (Micoletzky, 1922) Thorne and Swanger, 1936); *C. lenorus* (Siddiqi, 1965) n. comb. (Syn. *Galophinema lenorum* Siddiqi, 1965).

Jairajpuri (1965) proposed the genus *Qudisianema* in the family Leptonchidae to contain *Q. amabile* (specific name amended from *amabilis*), a nematode collected around the roots of "various plants" at Nainital (U.P.), India. The chief characters of the genus are the bibulbar appearance of the enlarged part of the esophagus and the spear extension being "strongly flanged appearing more muscular than cuticularized." From the type locality of *Q. amabile*, the present author collected a few female individuals of a nematode identified as *Q. amabile*. However, in the present specimens the esophageal bulb is typically dorylaimid and the basal swellings of the spear extension are also dorylaimid, appearing more muscular than cuticularized (Fig. 1A, B). Dr. Andr ssy of Budapest examined these specimens and identified them as belonging to the genus *Eudorylaimus*.

The bibulbar appearance of the enlarged part of the esophagus is often met with in occasional specimens of the Dorylaimidae. *Discolaimus discocephalus* Tulaganov, 1949 carries a prom-

inent isthmus in the middle of the enlarged part of the esophagus (Tulaganov, 1949). In Jairajpuri (1965), Figure 1B shows a median depression (not constriction) in the enlarged part of the esophagus, while in Figure 1E this depression is not as marked and the overall picture is typically dorylaimid. Such a condition of the esophagus may be attributed either to the faulty techniques of killing, fixing or dehydrating the specimens, or to the destruction of the muscular elements at a particular region resulting in the collapse of the boundary tissues. Hence it is concluded that this character does not carry any diagnostic value. *Qudisianema* Jairajpuri, 1965 and *Qudsiannematinae* Jairajpuri, 1965 are, therefore, proposed as synonyms of *Eudorylaimus* Andr ssy, 1959 and Dorylaiminae (de Man, 1876) Filipjev, 1918 respectively. *Qudisianema amabile* Jairajpuri, 1965 thus becomes *Eudorylaimus amabilis* (Jairajpuri, 1965) n. comb.

The position of the genus *Utahnema* Thorne, 1939, has been uncertain since its inception. Thorne (1939) regarded it a genus of uncertain position under Dorylaimidae. Baker (1962) followed Thorne (*loc. cit.*) while Clark (1961) and Goodey (1963) placed it along with *Xiphinema* and *Longidorus* under Tylencholaiminae of Dorylaimidae.

In a collection made in Tunisia by Mr. K. F. Brown of the Shell International Chemical Co., London, were a few female and juvenile individuals almost identical with *Utahnema tenuidens* Thorne, 1939, the type and the only species of the genus. Nonetheless, the differently shaped head and esophageal enlargement help in differentiating this species from *U. tenuidens*. A detailed examination of these specimens revealed that the species belonged to the family Leptonchidae Thorne, 1935 in being meromyarian and having undulated loose cuticle, fewer lateral body pores, small esophageal enlargement, oligocytous intestine, and small offset head. Dr. Juan Heyns of Pretoria, South Africa also examined these specimens and found them to be true leptonchids. The present species is very similar to but differs from *U. tenuidens*, and is described here as a new species of *Utahnema* and the genus placed under Leptonchidae.

The position of the genus *Utahnema* within the family Leptonchidae merits some com-

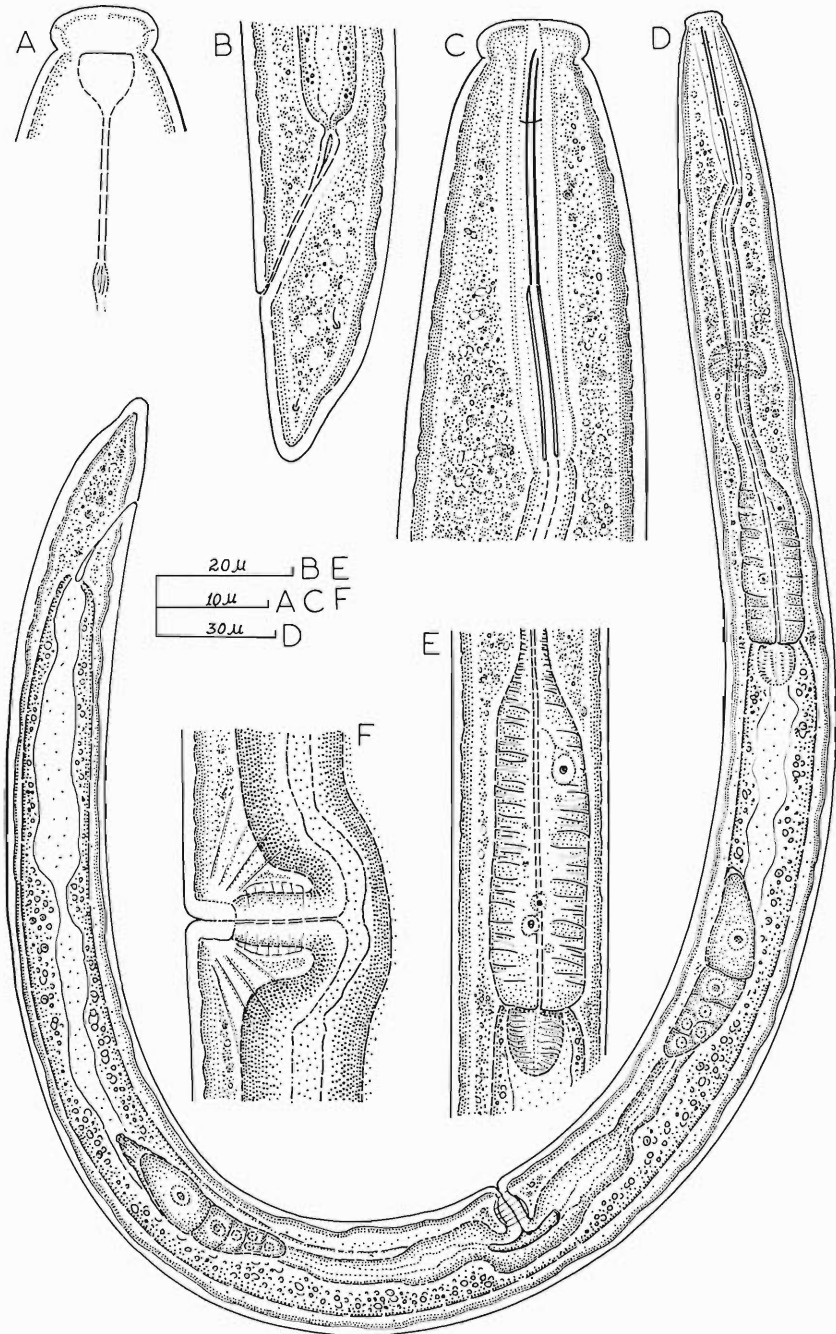


Fig. 2. A-F. *Utahnema gracile* n. sp. A. Amphidial system. B. Tail end of female. C. Head end of female. D. Female. E. Esophageal enlargement and esophago-intestinal valve. F. Vulva and vagina.

ments. In this family, the genera also with an attenuated spear are *Leptonchus* Cobb, 1920; *Proleptonchus* Lordello, 1955; *Doryschota* Thorne, 1964; *Oostenbrinkella* Jairajpuri, 1965 and *Xiphinemella* Loos, 1950 (Syn. *Botalium* Heyns, 1963, n. syn. This conclusion is drawn after comparing the specimens of the type species, *Botalium eversum*, with a *Xiphinemella* species from South India. *Botalium eversum*, therefore, is redesignated as *Xiphinemella eversa* (Heyns, 1963) n. comb.). According to the classification proposed by Jairajpuri (1964, 1964a), the first three of these belong to the subfamily Leptonchinae, the fourth to the Tylencholaimellinae, while the last one to the Xiphinemellinae. This scheme is based on the structure of the spear extension—simple in Leptonchinae, knobbed in Tylencholaimellinae, and flanged at base in Xiphinemellinae.

*Utahnema*, a relative of *Xiphinemella* by virtue of possessing elongate, rodlike spear extension cannot justifiably be put within Xiphinemellinae as it lacks flanges at the base of the spear extension. The present author feels that both these genera are not far removed from *Leptonchus* to permit the erection of a separate subfamily as the basic character of the spear extension is very divergent in the leptonchid genera (other characters of the subfamily, presence of a labial disc and more numerous supplementary papillae in male are also shared by the members of the Leptonchinae). It is, therefore, proposed that Xiphinemellinae be regarded a synonym of Leptonchinae and *Utahnema* be placed near *Xiphinemella* within Leptonchinae.

*Utahnema gracile* n. sp.  
(Fig. 2, A–F)

MEASUREMENTS: Females (4): L = 0.60–0.72 mm; a = 24–30; b = 3.7–4.0; c = 25–33; V = 50–54%; spear = 21–23  $\mu$ ; spear extension = 15–18  $\mu$ .

Holotype (Female): L = 0.72 mm; a = 30; b = 4; c = 33; V = 52.5%.

DESCRIPTION: Body almost straight when relaxed. Cuticle apparently in two layers, marked by fine transverse striae, wavy as in leptonchids. Amphids stirrup-shaped, about half as wide as head. Head set off from body by a constriction, 10  $\mu$  wide and about half

as much high; six amalgamated lips give the head a slightly hexagonal outlook. Spear elongate-cylindrical, with a small aperture, about twice head width. Spear extension simple, rodlike, shorter than spear. Spear guiding ring single, about a head width from anterior end. Esophagus a cylindrical muscular tube, expanding in its posterior  $\frac{2}{7}$  to form a cylindroid bulb, about  $\frac{3}{8}$  as wide as body; three gland nuclei seen (Fig. 2E). Esophago-intestinal valve large, rounded,  $\frac{1}{2}$  as wide as body. Nerve ring a little behind middle of esophagus. Intestine oligocytous, with wide lumen and few granules in its cells.

Vulva a transverse slit, with slightly raised lips; its dilator muscles forming an X-shaped pattern when seen from ventral side. Vagina extending about halfway into body, not sclerotized (Fig. 2F). Gonads paired, symmetrically opposed, with flexure at oviduct, each ovary with a few oocytes (Fig. 2D). Pre-rectum about three body widths long. Rectum about as long as tail. Tail dorsally convex-conoid to a rounded terminus, about  $1\frac{1}{3}$  times anal body width, slightly subdigitate (Fig. 2B). Two pairs of caudal pores present.

Male not found.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of almond trees near Tunis-Sausse Road, about 76 km from Tunis, Tunisia.

TYPE MATERIAL: Holotype deposited in the nematode collection of the Plant Pathology Section, Aligarh Muslim University, Aligarh, U.P., India; three females in author's personal collection.

RELATIONSHIP: *Utahnema gracile* n. sp. differs from *U. tenuidens* Thorne, 1939, the only species in the genus, in having a more slender body (a = 17 in *U. tenuidens*), a differently shaped head (lips projecting forward to form a dome-shaped elevation bearing oral opening in *U. tenuidens*), a less attenuated front end of esophagus, a longer and uncontracted esophageal enlargement, a larger cardia, a shorter neck, and vulva more anterior.

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### *Xiphinema macrostylum* n. sp. (Nematoda: Longidoridae)

R. P. ESSER<sup>1</sup>

In April 1960, root samples from banana (*Musa* × *paradisica* L. var. *sapientum*, Kuntze) and associated soil from Guayaquil, Ecuador, were examined for nematodes. Banana production in the sample site had been sharply reduced. Plants in the affected area were stunted or killed. Nonmarketable or no banana bunches at all comprised production from the affected area. Division of Plant Industry plant pathologists isolated *Fusarium oxysporum* Schlecht. ex Fr. from the rhizome, fruit stalk, and leaf sheath tissue of affected plants taken from the site of the malady.

Nematode examination revealed *Helicotylenchus microlobus*, Perry, 1959, in very large numbers; *Meloidogyne* sp., *Trichodorus nanus*, Allen, 1957, in moderate numbers; and a few *Radopholus similis* (Cobb, 1893) Thorne, 1949. In addition a moderate number of a previously undescribed species of *Xiphinema* were found the description of which follows.

*Xiphinema macrostylum* n. sp. (Fig. 1,

A-H). 16 ♀♀: Length 2.34 (2.15-2.48) mm; a = 28 (24-33); b = 4.6 (4-6); c = 48 (41-71); V = 43 (39-51). Total stylet length 278 μ (257-294).

FEMALE (HOLOTYPE): Length 2.36 mm; a = 27; b = 4.2; c = 46; V = 44. Total stylet length 294 μ.

FEMALE DESCRIPTION: Body elongate, obese tail bluntly conoid 35-55 μ long, tail/anal body diameter 0.6-0.7 μ. Cervical region tapered, lip region not set off. Amphids distinct laterally (Fig. 1, A). Minute fibrils seen in the sensillae pouches. In an *en face* view the oral aperture is ragged in appearance, roughly rectangular in shape. A slightly raised hexagon comprises the circumoral elevation. Six lips present, one papilla present on the apical portion of each lip in the internal circle. Two papillae like structures present on the basal portion of the subventral and subdorsal lips in the external circle. A previously undescribed flaplike structure extends over the amphid on either side of the head. This structure, herein named "amphidial shield," arises near the basal part of the lateral lips extending to the

<sup>1</sup>Nematologist, Florida Department of Agriculture, Division of Plant Industry, Gainesville, Florida. Acknowledgment is made to F. S. Donaldson who prepared the *en face* section and first noted the amphidial shield.



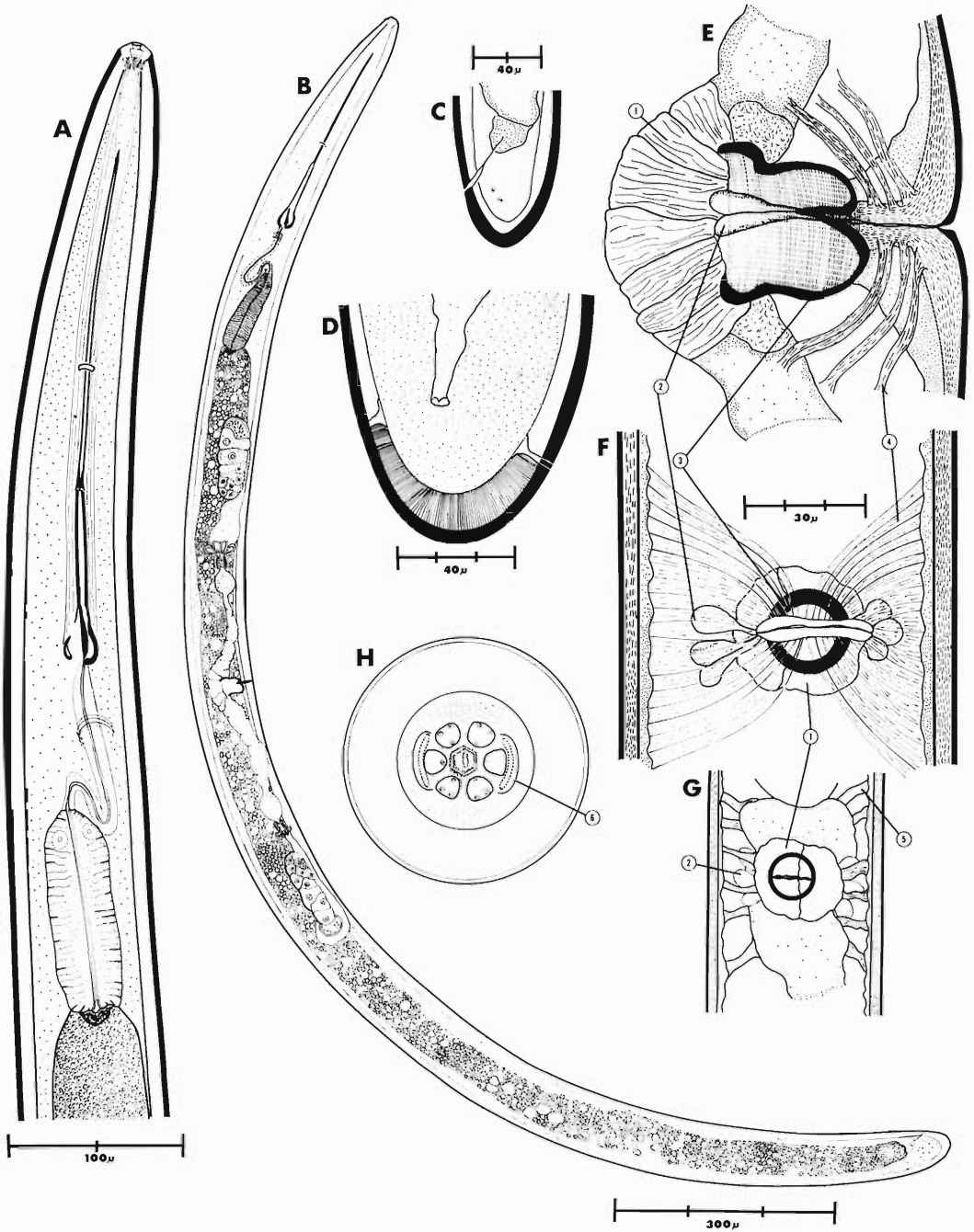


Fig. 1. *Xiphinema macrostylum* n. sp.: A, anterior view, right lateral; B, female, right lateral; C, posterior view, right lateral; D, posterior view, ventral; E, vagina region, right lateral; F, female genital region, ventral; G, female genital region, dorsal; H, en face view. (1) vagina (2) vaginal epithelial cells (3) vagina (4) dilator vulvae muscles (5) dorsal uterine muscles (6) amphidial shields.

nearest edge of the subdorsal and subventral lips (Fig. 1, H-6). Crescent-shaped prominent amphid apertures are located adjacent to the basal edge of the lateral lips.

Odontostyle typical of the genus, 164–200  $\mu$ ; basal portion 80–100  $\mu$ ; stylet flange width 18–27  $\mu$ . Guide ring single (appeared double in some specimens after fixation). Nerve ring indistinct about halfway between flange base and anterior tip of the muscular esophagus. Muscular esophagus 110–160  $\mu$  by 28–40  $\mu$ , one nucleus in the dorsal side, one ventral. Nuclei at the same level, but in some specimens nuclei were anterior, in others at the mid portion of the muscular esophagus. Distance from esophagus base to oral aperture 400–560  $\mu$ . Esophago-intestinal valve wider than long, irregular in shape. Intestine eight cells in circumference, filled with irregular shaped globules. Prerectum and rectum distinct. Anterior lip of the anus indented. Gonads amphidelphic, reflexed; anterior gonad 325  $\mu$ , posterior gonad 351  $\mu$ . Sphincters present between the oviduct and uterus, Z-organ not observed. Caudal pores, two to three on each side of the tail, the first near the level of the anus. In one specimen the lumens of the caudal pores were seen expanding posteriorly from the pore and laterally extending into the lateral chord, the pores being the caudal limits of the lateral chord (Fig. 2). Lateral chords  $\frac{1}{6}$ – $\frac{1}{4}$  body width. Gland cells of the lateral chord as described for *X. citri* Siddiqi, 1959, were present. A single egg measured 209  $\mu$  long by 63.5  $\mu$  wide. Hemizonid and lateral pores not observed.

**ANATOMY OF THE VAGINAL REGION** (Fig. 1, E, F, and G): Two distinct vulvar lips are situated on the ventral side of the body (Fig. 1, F). Unsclerotized vulvar lips extend about 15  $\mu$  from the outer lips to the heavily sclerotized vagina. The *dilator vulvae* muscles appear at three levels in a lateral view (Fig. 1, E 4) extending from the walls of the internal vulvar lips to an insertion ventrolaterally in the hypodermis. In a ventral view the *dilator vulvae* appear as 19 adjacent muscle bands arising from each side of the internal vulvar lips (Fig. 1, F 4). The vagina appears basket-shaped in a lateral view (Fig. 1, E) and as a circular tube in a dorsal (Fig. 1, F) or ventral view (Fig. 1, G). Three vaginal epi-

thelial cells (Fig. 1, E, F and G 2) are situated on each lateral side of the vagina in a dorsal or ventral view and extended to the base of the vagina in a lateral view. The sclerotized vagina is surrounded by indistinct bands of circular muscle (Fig. 1, E).

Posterior to the vagina lies the large sac-like *vagina uterina* (Fig. 1, E, F and G 1). In lateral view this structure appears as seven large folds spreading fanlike over the vagina. In a dorsal or ventral view it appears as a large irregular sac, cleft its width at the vaginal juncture in line with the longitudinal axis of the body. In a dorsal view (Fig. 1, G) two muscle bands are attached to the *vagina uterina* on either side of the body. Just anterior and posterior to the *vagina uterina* two muscle bands attach to the uterus on either side of the body. A total of 12 muscles are situated dorsally in the vagina uterine area. Juveniles resembled females in appearance. Males were not found.

**TYPE HABITAT:** Soil associated with the roots of *Musa*  $\times$  *paradisiaca* L. var. *sapientum*, Kuntze.

**TYPE LOCALITY:** Guayaquil, Ecuador.

**HOLOTYPE:** Collected by J. R. Peavy, Production Manager, Ecuador Banana Plantation, at type locality. Slide T-76t. U. S. Dept. Agr., Nematode collection, Beltsville, Maryland.

**PARATYPES:** Females and larvae: U. S. Dept. Agr., Beltsville, Maryland, and Division of Plant Industry Laboratory, Gainesville, Florida.

**DIAGNOSIS:** *X. macrostylum* n. sp. differs from the 15 amphidelphic species of the genus, having rounded or hemispherical tails, in that *X. macrostylum* n. sp. has a long stylet (257–294  $\mu$ ) in comparison to a relatively short body (2.15–2.48 mm), and a small alpha (24–33). Of the 63 species of *Xiphinema* (including valid species, synonyms, and departures) compared from the literature, only *X. macrostylum* n. sp. possessed amphidial shields, and vaginal glands. The upper range of the stylet length, 294  $\mu$ , is the longest for any described species in the genus.

**FIXATION:** Almost all measurements and drawings were made within 24 hours after death in 2% cold formalin. Half of the specimens then were fixed in lacto-phenol acid

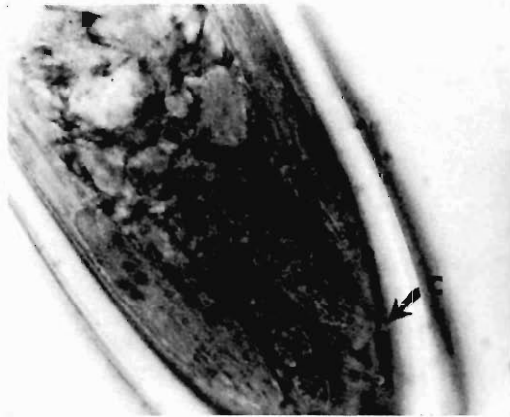


Fig. 2. Tail showing A. caudal pore lumen, B. tissue differentiation, C. caudal pore.

fuscin; the remainder, in glycerine using "Baker's rapid method technique."

#### DISCUSSION

*X. macrostylum* n. sp. possesses several morphological attributes peculiar to itself and worthy of mention. The amphidial shields are so named because they extend over the amphid aperture and possibly serve as protection for the amphids as the nematode moves through its substratum. The shields are very thin and were not detected in lateral or ventral views of the head. Although several authors have extended the caudal pore lumen (without a connection) into the lateral chord in their

drawings, the relationship has not been clarified. Possibly some special physiological condition in the specimen must be present at time of fixation to show the caudal pore lumen extending into the lateral chord because only one of 16 females observed showed this connection. Tissue differentiation was noted in the pore lumen center a short distance from the pore (Fig. 2, B).

The six vaginal epithelial cells are demonstrated for the first time in *Xiphinema*. Similar cells have been shown in *Hystriognathus rigidus* (8 cells) and *Trilobus* (4 cells), Chitwood and Chitwood, 1937.

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### *Chitwoodius* nom. nov. for *Chitwoodia* Furstenberg and Heyns, 1966

J. P. FURSTENBERG AND JUAN HEYNS

It has been pointed out to us by Dr. A. D. Baker that the name *Chitwoodia*, which we proposed for a new nematode genus (Furstenberg and Heyns, 1966) is preoccupied by *Chitwoodia* Gerlach, 1956. We therefore propose the name *Chitwoodius* nom. nov. in its place, and the name of the only species becomes *Chitwoodius transvaalensis* (Furstenberg and Heyns, 1966) n. comb.

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## Two New Species of Digenetic Trematodes from Venezuelan Amphibians

P. NASIR<sup>1</sup>

Lutz (1928) recorded *Glyphelmims palmipedis* (Lutz, 1928) Travassos (1930) from the lungs of *Rana palmipes* Spix, in Caracas, Venezuela and also found some cercariae in *Planorbis cultratus* as well as in *P. anatinus* and these cercariae were thought to develop into *Glyphelmims repandum* (Rudolphi, 1819) Travassos (1924) but no experimental evidence was given. The present author while dissecting some frogs of the species *Pseudis paradoxa* (L.), brought from Valle de la Pascua, Edo. Guarico, recovered four trematodes from the small intestine of one specimen and two from the lungs of another individual. One of those trematodes encountered from the lungs was partly mutilated and, therefore, was employed for histological sectioning. As a result of comparative study to be discussed below these trematodes have proved to be new species and named respectively as *Glyphelmims incurvatum* and *G. ramitesticularis*. As the names of these species suggest *G. incurvatum* has the preacetabular region of its body characteristically incurved ventrally whereas *G. ramitesticularis* is the only other species in the genus *Glyphelmims* in which the testes are deeply branched.

Living flukes on removing from their hosts were washed several times in normal saline and then relaxed by gradual addition of urethane crystals prior to fixation with hot Gilson's fluid. Permanent preparations have been deposited in the USNM Helm. Coll., Beltsville, Maryland and their code numbers are cited below.

All measurements are given in mm.

### *Glyphelmims incurvatum* n. sp. (Fig. 1)

Body uniformly spinose. Lateral margins of body in preacetabular region incurved ventrally so as to form a longitudinal depression. This incurving constituting a characteristic feature of this species. Oral sucker larger than ventral sucker. Ventral sucker preequatorial.

Prepharynx present. Pharynx subspherical. Esophagus not extending to ventral sucker. Intestinal ceca not extending to posterior end of body. Testes oval, diagonally placed, slightly anteroposteriorly elongated, intercecal, postacetabular. Cirrus sac median or slightly submedian, overlapping central part of ventral sucker, about one-third of length of cirrus sac extending anterior to anterior border of ventral sucker. Cirrus sac enclosing a coiled seminal vesicle and prostate glands. Ovary globular, larger than testes, on one side of median longitudinal line, partly overlying posterolateral aspect of ventral sucker. Receptaculum seminis and Laurer's canal present. Metratrem opening independently in a common genital pore. Common genital pore anterior to ventral sucker, submedian, not bifurcal. Vitelline follicles in two lateral fields, almost entirely extracecal. Anterior limits of vitelline follicles prebifurcal, postbifurcal but never pharyngeal or acetabular. Posterior limits of vitelline follicles terminating with almost posterior terminations of intestinal ceca, not extending to extreme posterior border of body. Transverse vitelline ducts between ovary and anterior testis. Mehlis' gland complex posterior to ovary. Uterus characteristically coiled, extending to posterior end of body, mostly intercecal with numerous loops in both descending and ascending limbs. Excretory vesicle Y-shaped, extending anterior to anterior border of anterior testis. Arms of Y running laterally around receptaculum seminis. Intrauterine eggs nonembryonated. Measurements of four specimens: body 1.728–2.196 by 0.540–0.612; oral sucker 0.193–0.206 in diameter; ventral sucker 0.131–0.165 in diameter; prepharynx 0.030–0.037 long; pharynx 0.081–0.131 in diameter; esophagus 0.075–0.112 long; ovary 0.100–0.131 in diameter; anterior testis 0.075–0.100 by 0.050–0.087; posterior testis 0.087–0.100 by 0.062–0.087; cirrus sac 0.150–0.175 by 0.062–0.075; eggs 0.025–0.033 by 0.014–0.016; preacetabular extent 0.625–0.737; postacetabular extent 0.762–1.300; post-intestinal extent 0.162–0.337; distance of ovary from anterior end of body 0.687–0.850; dis-

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tance of posterior testis from posterior end of body 0.737–1.050; distance of anterior testis from anterior end of body 0.850–0.887; pre-vitelline extent 0.350–0.662; postvitelline extent 0.300–0.650.

TYPE HOST: *Pseudis paradoxa* (L.).

HABITAT: Intestine.

TYPE LOCALITY: Valle de la Pascua, Edo. Guarico, Venezuela.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 60735—*Glypthelmins incurvatum* (holotype).

*Glypthelmins ramitesticularis* n. sp.

(Fig. 2)

Body uniformly spinose. Preacetabular region of body not incurved ventrally. Oral sucker larger than ventral sucker. Ventral sucker preequatorial. Prepharynx transversely distended. Pharynx muscular. Esophagus bulbous. Ceca not extending to extreme posterior border of body. Testes branched, constituting diagnostic character of this species, not tandem one obliquely behind the other. Cirrus sac not strongly developed, obliquely placed dorsally over central preequatorial region of ventral sucker, partly extending beyond anterior border of ventral sucker. Cirrus sac enclosing a long coiled seminal vesicle and prostate glands. Ovary anteroposteriorly elongated, partly overlying posterolateral border of ventral sucker, not extending anterior to anterior border of ventral sucker. Receptaculum seminis present. Uterus so completely filled with eggs that its real pattern cannot be determined. Metraterm opening independently in a common genital pore. Common genital pore slightly submedian, immediately anterior to anterior border of ventral sucker. Intrauterine eggs non-embryonated. Vitelline follicles in two lateral fields, almost entirely extracecal, not extending beyond posterior terminations of intestinal ceca, anteriorly not extending anterior to middle region of pharynx. Excretory vesicle Y-shaped, stem of Y extending as far as preequatorial region of anterior testis. Measurements based on a single specimen: body 2.520 by 0.936; oral sucker 0.350 by 0.400; ventral sucker 0.300 by 0.325; prepharynx 0.012 in length; pharynx 0.162 by 0.200; esophagus 0.050 in length; ovary 0.275 by 0.187; anterior testis 0.387 by 0.437; posterior testis 0.375

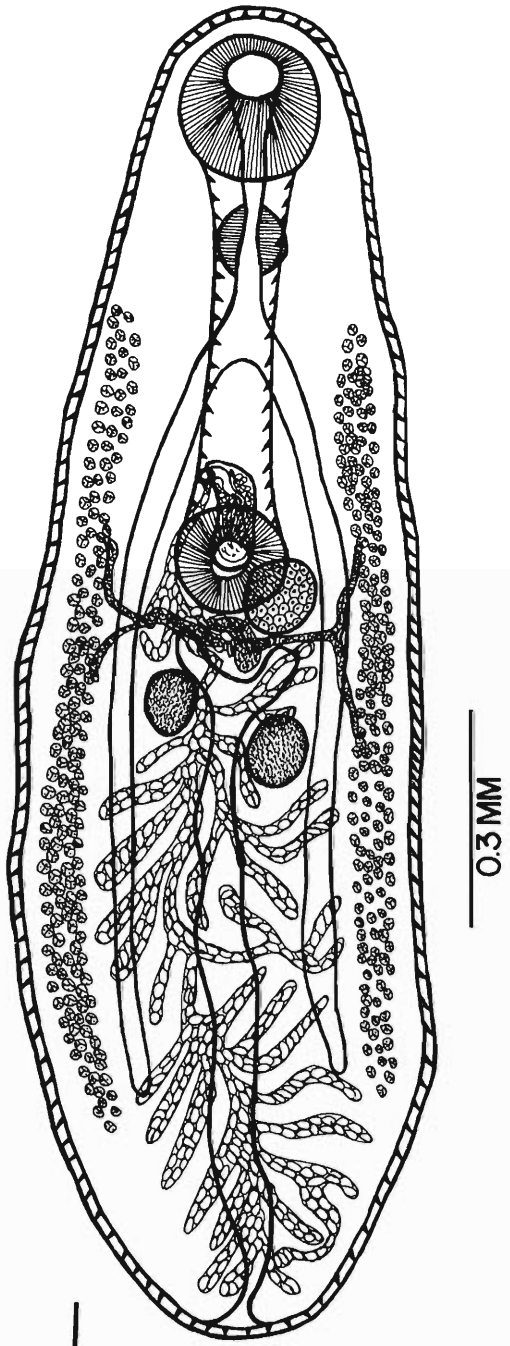


Fig. 1. *Glypthelmins incurvatum* n. sp., ventral view.

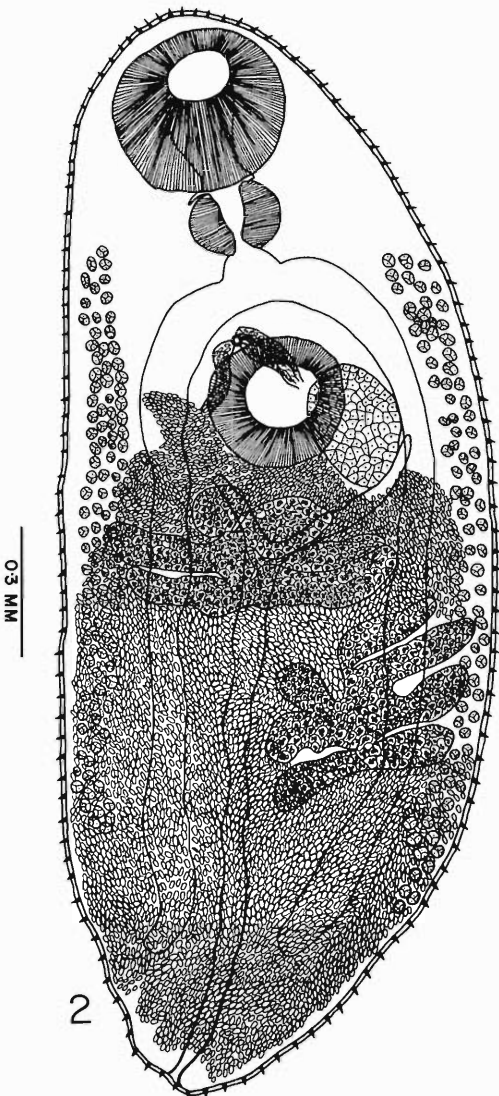


Fig. 2. *Glypthelmins ramitesticularis* n. sp., ventral view.

by 0.650; eggs 0.033 by 0.016; preacetabular extent 0.750; postacetabular extent 1.531; cirrus sac 0.187 by 0.062; previtelline extent 0.692; postvitelline extent 0.612; postintestinal extent 0.357; posttesticular extent 0.925.

TYPE HOST: *Pseudis paradoxa* (L.).

HABITAT: Lungs.

TYPE LOCALITY: Valle de la Pascua, Edo. Guarico, Venezuela.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 60736—*Glypthelmins ramitesticularis* (holotype).

#### DISCUSSION

Stafford (1905) erected *Glypthelmins* for the reception of *G. quieta* (Stafford, 1905). Cort (1919) created another genus *Margeana* for *M. californiensis*. Miller (1930) considered *Glypthelmins* and *Margeana* as synonyms and removed *M. californiensis* to *Glypthelmins*. Thus, *M. californiensis* became *Glypthelmins californiensis* (Cort, 1919). Pereira and Cuocolo (1941) established another genus *Choledocystus* for *C. eucharis*. Ruiz (1949) regarded *Choledocystus eucharis* as synonym of *Glypthelmins elegans* Travassos (1926) but retained the genus *Choledocystus* for *Choledocystus elegans*. Skrjabin (1958) considered *Glypthelmins* and *Margeana* as congeneric. Yamaguti (1958) reduced *Margeana* and *Choledocystus* to the synonymy of *Glypthelmins*. Cheng (1959) divided *Glypthelmins* and *Margeana* principally in the presence or absence of pharyngeal glands and brought forth a new genus *Reynoldstrema* to accommodate *Reynoldstrema africana* (Dollfus, 1950), syn. *Glypthelmins africana* Dollfus (1950). Byrd and Maples (1963) not only reestablished *Choledocystus* but also added a new genus *Repandum* for *R. repandum* (Rudolphi, 1819) Travassos (1924), *R. palmipedis* (Lutz, 1928) Travassos (1930), and *R. sera* (Cordero, 1944) syn. *Glypthelmins sera* Cordero (1944). Thus, there have appeared the "so called" five closely related genera, namely, *Glypthelmins*, *Margeana*, *Choledocystus*, *Reynoldstrema*, and *Repandum*. Accordingly, a great deal of assignment and reassignment of species to the corresponding genera has taken place.

As already pointed out by Byrd and Maples (1963) the splitting of *Glypthelmins* into *Glypthelmins* for those forms possessing pharyngeal glands and *Margeana*, the members of which are lacking in pharyngeal glands, does not seem to be justified as the detection of these glands depends upon the methods employed for preparation of whole mounts. Furthermore, Byrd and Maples remarked that "in many of our specimens the glands stain

so poorly, or not at all, that their taxonomic value becomes worthless." During the course of the present investigation both the whole mounts and the sectioned material were studied but it was rather difficult to arrive at a definite conclusion as to the presence or otherwise of these peripharyngeal glands. In some of the whole mounts and the sectioned specimens deeply stained structures were observed in the peripharyngeal region. A character which is too difficult to be worked out with a certain amount of certainty, does not seem to provide a sound basis for the generic separation.

The characters employed by Pereira and Cuocolo (1941), Ruiz (1949), Cheng (1959), and Byrd and Maples (1963), like more diffuse and extensive coiling of uterus near the gonads and the ventral sucker in *Choledocystus*, posteriorly located uterus and posteriorly situated testes in *Reynoldstrema*, diagonal position of testes, distribution of vitellaria, location of the ventral sucker, larger size of ovary in relation to that of testes, development of uterus in the area of and just in front of the testes in *Repandum*, are all subject to considerable variations, even in the individuals of the same species, and depend upon methods of fixation and state of maturity of worms. Moreover, none of these characters is strictly applicable to any one of the above cited genera. Thus, in the author's opinion *Margeana*, *Choledocystus*, *Reynoldstrema*, and *Repandum* should be reduced to the synonymy of *Glyphelminis* and the species scattered under these genera be transferred to *Glyphelminis*.

The genus *Glyphelminis*, as it stands now, includes the following valid species:

- G. africana* Dollfus, 1950;
- G. californiensis* (Cort, 1919);
- G. diana* Belouss in Skrjabin and Antipin (1959);
- G. elegans* Travassos, 1926;
- G. facioi* Brenes Madrigal; Arroyo Sancho; Jimenez-Quiros; and Delgado Flores, 1959;
- G. festina* Cordero, 1944;
- G. intermedia* Caballero; Bravo; and Cerecero, 1944;
- G. linguatula* (Rudolphi, 1819);
- G. parva* Travassos, 1924;

- G. pennsylvaniensis* Cheng, 1961;
  - G. proxima* Freitas, 1941;
  - G. quieta* (Stafford, 1900);
  - G. rugocaudata* (Yoshida, 1916);
  - G. repanda* (Rudolphi, 1819);
  - G. shastai* Ingles, 1936;
  - G. staffordi* Tubanguui, 1928;
  - G. vesicalis* (Ruiz and Leão, 1942);
  - G. vitellinophila* Dobbin, 1958;
- plus the two new species described in this paper.

The following species are regarded as synonyms:

- G. palmipedis* (Lutz, 1928) (= *G. linguatula*);
- G. sera* Cordero, 1944 (= *G. linguatula*);
- G. simulans* Freits, 1941 (= *G. linguatula*);
- G. subtropica* Harwood, 1932 (= *G. quieta*);
- Choledocystus eucharis* Pereira and Cuocolo, 1941 (= *G. elegans*).

As far as the identification of *Glyphelminis incurvatum* and *G. ramitesticularis* is concerned, these two species are readily separated from all other species in the genus by the fact that *G. incurvatum* has a characteristic incurving of body in preacetabular region, while *G. ramitesticularis* is the only other species in the genus *Glyphelminis* in which the testes are deeply branched. Apart from these main diagnostic features, *G. incurvatum* and *G. ramitesticularis* differ from other species in one or more of the following characters: extent of cuticular spination, extent of cirrus sac in relation to ventral sucker, postintestinal extent, relative size of suckers, pharynx, ovary, testes, cirrus sac, and finally eggs.

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### *Brachylaima degiustii* n. sp. from *Columba livia* in Venezuela<sup>1</sup>

PIR NASIR AND LORENZA RODRIGUEZ M.<sup>2</sup>

While studying trematode infestations of birds around the Universidad de Oriente, where the present investigation has been carried out, 31 specimens of a new species, *Brachylaima degiustii*, were recovered from the small intestine of a pigeon *Columba livia*.

Living trematodes were washed several times in normal saline and then fixed by plunging into hot Zenker's fixative. Whole mounts were stained with Semichon's carmine, while for histological sectioning Mallory's triple staining technique was employed.

All measurements are given in mm.

#### *Brachylaima degiustii* n. sp. (Figs. 1-3)

Body spinose, posterior to second testis spination reduced, or may be absent altogether. Oral sucker subterminal. Ventral sucker smaller than oral sucker. Prepharynx absent. Pharynx muscular, smaller than ventral sucker. In sectioned material a very short esophagus present. Ceca not extending to extreme posterior end of body. Testes entire, tandem, equal in transverse diameter but in anteroposterior diameter posterior testis slightly larger than anterior testis. Ovary spherical, between testes or its anterior region partly overlapped by posterior region of anterior testis. Transverse vitelline ducts,

common vitelline reservoir and ootype complex posterior to ovary. Seminal receptacle present. Laurer's canal opening dorsally on body surface in anterior region of posterior testis. A reconstruction of ootype complex shown in Figure 2. Uterus thrown into several transverse loops in both ascending and descending limbs, packed with operculate eggs. Vitelline follicles in two lateral fields, variable in distribution even in same specimen, mostly extracecal. Anterior limits of vitelline follicles vary from behind posterior border of ventral sucker to halfway in preacetabular region of body. Posteriorly vitelline follicles may extend posterior to anterior testis, but in most specimens limited slightly posterior to anterior border of anterior testis. Common genital pore pretesticular, ventral, submedian. Cirrus pouch and metraterm opening independently in common genital pore. Cirrus pouch enclosing ductus ejaculatorius. Prostatic glands and cirrus absent. Seminal vesicle considerably dilated, narrowing before joining cirrus sac. Terminal genitalia shown in Figure 3. Excretory vesicle tubular to saccate. Measurements of 20 specimens: body 4.104-4.860 × 0.360-0.540; oral sucker 0.212-0.240 in diameter; ventral sucker 0.156-0.225 in diameter; pharynx 0.135-0.155 × 0.123-0.144; anterior testis 0.175-0.237 × 0.175-0.250; posterior testis 0.237-0.300 × 0.175-0.250; ovary 0.187-0.237 × 0.187-0.262; eggs 0.018-0.024 × 0.012-0.015; postintestinal extent 0.100-0.137; preacetabular extent 0.650-0.750; post-

<sup>1</sup>Named in the honor of Dr. Dominic L. DeGiusti with whom the senior author had an opportunity to work while as a Post-Doctoral Research Fellow in Wayne State University, Detroit, Michigan, U.S.A.

<sup>2</sup>Parasitological Laboratory, Universidad de Oriente, Nucleo de Sucre, Cerro Colorado, Cumaná, Venezuela.



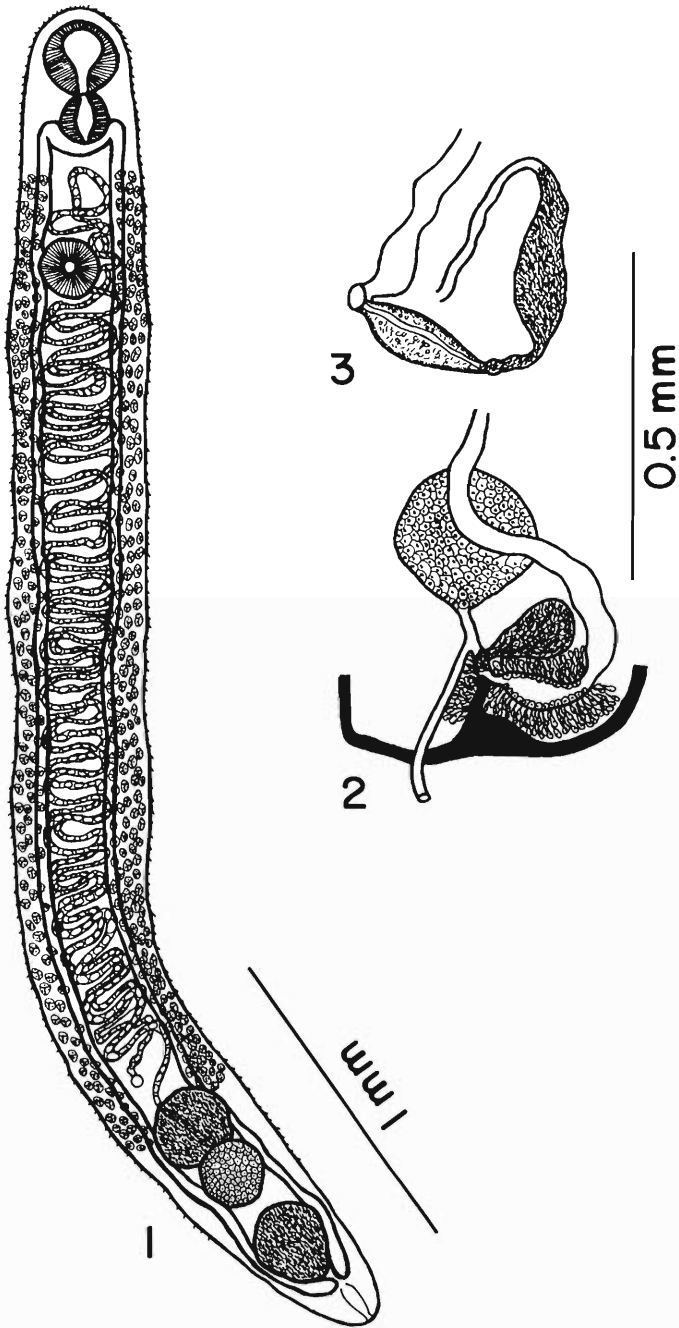


Fig. 1. *Brachylaima degiustii* n. sp., ventral view.

Fig. 2. Reconstruction of ootype complex, showing seminal receptacle, Laurer's canal, ootype, uterus, transverse vitelline ducts, common vitelline reservoir, median vitelline duct, and Melnik's gland.

Fig. 3. Terminal genitalia, showing cirrus sac with the enclosed ductus ejaculatorius, enlarged seminal vesicle, and metraterm.

testicular extent 0.137–0.200; distance of anterior testis from anterior end of body 3.387–3.862.

TYPE HOST: *Columba livia*.

HABITAT: Small intestine.

TYPE LOCALITY: La Llanada de San Juan, about 4 kilometers south of Universidad de Oriente, Cumaná, Venezuela.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 60737—*Brachylaima degiustii* (holotype).

#### DISCUSSION

There are several species in the genus *Brachylaima* (Dujardin, 1843) Kruidenier and Gallicchio (1959) including *Brachylaima* sp. (Dollfus, 1935), *Brachylaima* sp. (Gnedina and Potekhina, 1950), *B. mesostomus* (Rudolphi, 1803) as described by Dollfus (1935), *B. columbae* (Mazzanti, 1889) as described by Dollfus (1935) sp. inq., *B. nicolli* (Witenberg, 1925), and *B. mazzanti* (Travassos, 1927) from the pigeons *Columba livia*, *C. livia domesticata*, and *C. palumbus*. All of these species can be distinguished from *Brachylaima degiustii* by the varying combinations of following characters: the presence or absence of cuticular spination; the location of ventral sucker in relation to anterior end of body; the sizes of suckers, pharynx, testes, ovary, and eggs; the ratio of suckers; and finally the distribution of vitelline glands.

Heyneman, Brenes, and Diaz-Ungria (1960) described *Brachylaima* sp. from the intestine of *Tyrannus melancholicus chloronotus* in Cabure, Edo. Falcón, Venezuela but their observations are based only on one specimen. *Brachylaima degiustii* resembles very closely *Brachylaima* sp. in the distribution of cuticular

spination, the approximate shapes of the testes and ovary, the position of genital pore, the pattern and extent of the uterus, the size of eggs, and in having operculated eggs. However, *B. degiustii* differs from *Brachylaima* sp. in having smaller suckers, smaller pharynx, smaller testes, and smaller ovary. The ratio of the suckers in *B. degiustii* is greater than their counterparts met within *Brachylaima* sp. Moreover, in *Brachylaima* sp. the vitellaria extend from posterior margin of its ventral sucker to a considerable distance anterior to the anterior testis whereas in *B. degiustii* although the anterior limits of the vitellaria are variable even in the same individual the posterior limits never terminate a considerable distance anterior to the anterior testis but always approach the anterior testis.

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***Hirschmanniella nana* n. sp. and *H. magna* n. sp. (Nematoda: Pratylenchidae) from India<sup>1</sup>**

M. RAFIQ SIDDIQI

The plant-parasitic nematodes of the genus *Hirschmanniella* Luc and Goodey, 1963 collected in India included two new forms which are named and described here as *Hirschmanniella nana* n. sp. and *H. magna* n. sp.

*Hirschmanniella nana* n. sp.  
(Fig. 1, A-H)

MEASUREMENTS: Ten females (in glycerine): Length = 1.00–1.13 mm; a = 48–56; b = 9–11; c<sup>2</sup> = 15–20; V = 52–58%; spear = 14–16  $\mu$ .

Six males (in glycerine): Length = 0.98–1.03 mm; a = 47–55; b = 8.5–10.0; c = 15–17; T = 32–38%; spear = 14–16  $\mu$ ; spicules = 21–23  $\mu$ ; gubernaculum = 10–11  $\mu$ .

Holotype female: Length = 1.05 mm; a = 51; b = 9.2; c = 18; V = 32–54–29%; spear = 15.5  $\mu$ .

DESCRIPTION: FEMALE: Body cylindrical with tapering ends, slightly arcuate ventrally; cuticle marked by transverse striae spaced at an average interval of 1.4  $\mu$  near mid-body. Lateral fields not aerolated, marked by four incisures,  $\frac{1}{10}$ – $\frac{3}{8}$  as wide as body, extending beyond phasmids to near tail terminus; outer incisures crenate. Phasmids opposite each other or a little displaced, 18–28  $\mu$  from tail tip, usually at  $\frac{2}{3}$  the distance down the tail. Anterior and posterior cephalids as shown in Figure 1A. Hemizonid faint, three body annules long, two body annules to just anterior to excretory pore; hemizonion not seen.

Lip region round, continuous, bearing 3–4 annules; labial framework moderately sclerotized, outer margins extending 2–3 annules into body, inner margins forming a spear guide (Fig. 1A). Spear slender, weak; tip measuring a little less than half its length, terminating to a sharp point with obscured aperture; knobs small rounded, a little flattened anteriorly, about 3  $\mu$  across. Orifice of dorsal esophageal gland 2–3  $\mu$  behind spear base. Procorpus broad; metacarpus round,

$\frac{1}{2}$  as wide as corresponding body; distance from anterior end to base of metacarpus 70–72  $\mu$ . Esophageal glands forming a long ventral overlap over intestine.

Nerve ring just following metacarpal swelling. Excretory pore 93–102 (avg 95)  $\mu$  from anterior end of body, a little above the level of esophago-intestinal junction. Vulva a prominent transverse slit; reproductive organs as illustrated (Fig. 1B). Spermatheca in mature specimens double. Rectum longer than anal body width, not overlapped by intestine. Tail conoid, 55–70  $\mu$  long; terminus conoid, with a finely pointed mucro.

MALE: Similar to female in many details. Excretory pore 88–102  $\mu$  from anterior end of body. Testis single, outstretched. Bursa crenate, arising from near level of head of spicules and terminating at or posterior to phasmids. Spicules short, stout, cephalated, averaging 22  $\mu$  long; gubernaculum lineate, trough-shaped, half the length of spicules. Tail 58–67  $\mu$  long; terminus conoid, with a sharply pointed spine.

TYPE MATERIAL: Holotype (♀) and a pair of paratypes (1♀, 1♂) at the Crops Research Branch, USDA, Beltsville, Maryland, USA. A pair of paratypes (1♀, 1♂) will be deposited at each of the following centers: Rothamsted Experimental Station, Harpenden, England; Plantenziektenkundige, Dienst, Wageningen, Holland; Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, India. Two males and five females in author's possession.

TYPE HOST AND LOCALITY: Collected from soil and roots of paddy plants, *Oryza sativa* L., at Avadi near Madras, South India. Also collected from grass soil at Madras, South India.

DIAGNOSIS AND RELATIONSHIP: *Hirschmanniella nana* n. sp. is differentiated by its small body size, spear, and spicules; weakly built spear, excretory pore lying 93–102  $\mu$  from anterior end of body, lateral fields extending to near tail terminus, and a conoid tail terminus bearing a sharply pointed mucro. It

<sup>1</sup> From Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.  
<sup>2</sup> Tail length taken after excluding the terminal spine.

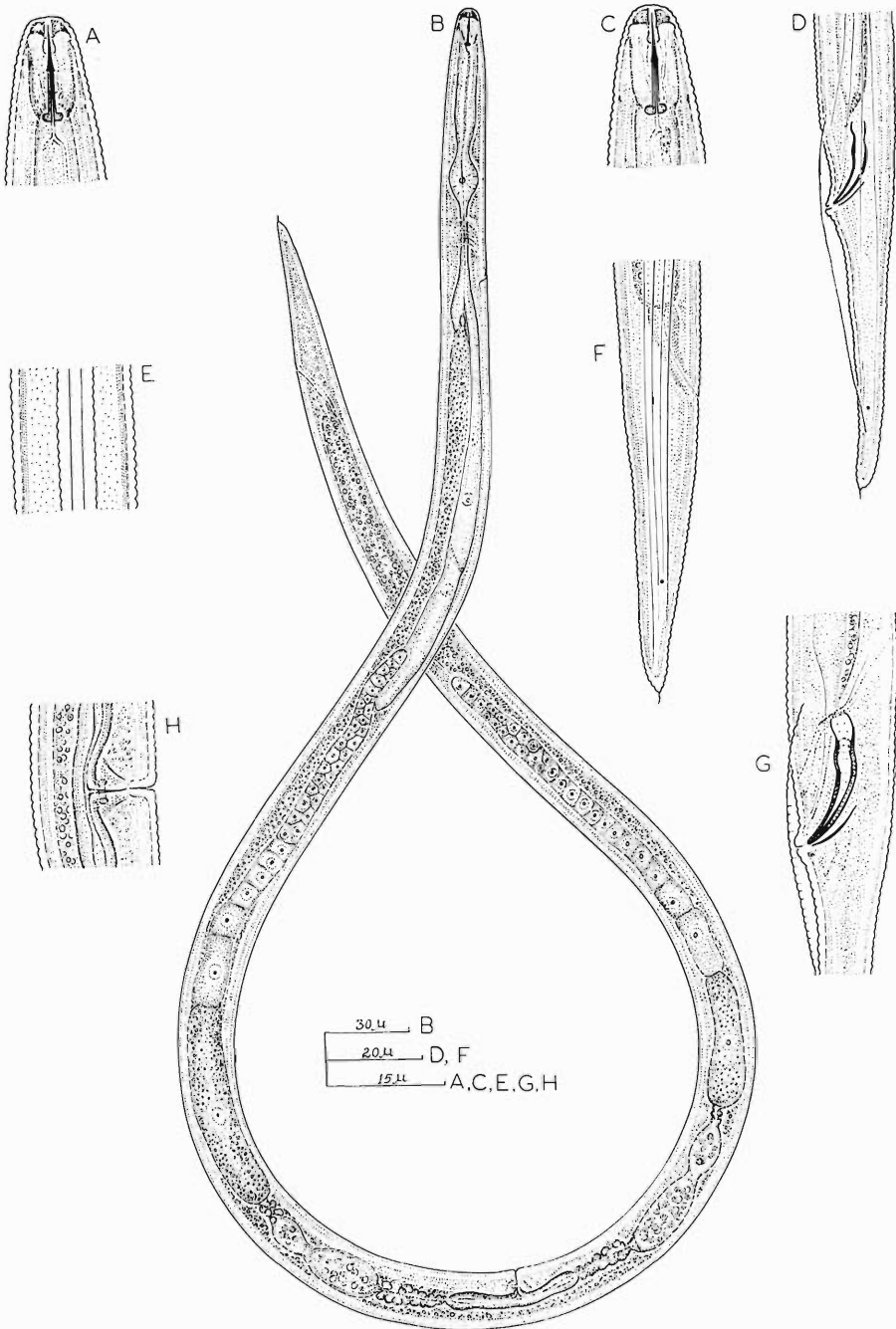


Fig. 1, A-H. *Hirschmanniella nana* n. sp. A. Head end of female. B. Female (holotype). C. Head end of male. D. Tail end of male. E. Lateral field. F. Tail end of female. G. Anal region of male showing spicule, gubernaculum, and bursa. H. Vulval region.

comes close to *H. oryzae* (v. Breda de Haan, 1902) Luc and Goodey, 1963 and *H. gracilis* (de Man, 1880) Luc and Goodey, 1963. From both of these it differs in having a smaller body size, spear, and spicules. As redescribed by Thorne (1961), *H. oryzae* has lateral fields ending anterior to phasmids, a strong buccal spear and bursa not reaching phasmids; and *H. gracilis* has phasmids near middle of tail and intestine extending over rectum.

*Hirschmanniella magna* n. sp.

(Fig. 2, A-M)

MEASUREMENTS: Nine females (in glycerine): Length = 2.4–2.7 mm; a = 65–72; b = 14–16; c = 17–20; V = 50–52%; spear = 28–29  $\mu$ .

Five males (in glycerine): Length = 2.2–2.6 mm; a = 65–70; b = 13–15; c = 17–20; T = 30–36%; spear = 28–29  $\mu$ ; spicules = 33–35  $\mu$ ; gubernaculum = 17  $\mu$ .

Holotype female: Length = 2.7 mm; a = 70; b = 14.5; c = 20; V = <sup>20</sup>51–<sup>19</sup>%; spear = 28  $\mu$ .

DESCRIPTION: FEMALE: Body ventrally arcuate. Cuticle bearing distinct transverse striae averaging 1.8  $\mu$  apart on mid-body. Lateral fields marked by four incisures, outer ones being crenate, interrupting transverse striae, about  $\frac{3}{10}$  as wide as body, terminating on tail near phasmids. Cephalids as illustrated in Figure 2A. Hemizonid not prominent, three body annules long, at level of excretory pore, or just anterior to it; hemizonion 12–14 annules below hemizonid. Excretory pore 150–160  $\mu$  from front end of body. Phasmids opposite each other or a little displaced, 40–62  $\mu$  from tail tip.

Lip region conoid–rounded, continuous with body contour, with five to six annules; labial framework highly sclerotized, its outer margins extending three annules into body. Spear robust, divisible into equal parts; knobs large, rounded, compact, 6  $\mu$  across. Orifice of dorsal esophageal gland about 5  $\mu$  behind spear base. Procorpus broad; median esophageal bulb well developed, oval; anterior end of body to base of median esophageal bulb 112–118  $\mu$ . Esophageal glands with an overlap of 220–240  $\mu$  over intestine, dorsal gland occupying half this distance.

Vulva a transverse slit, about  $\frac{2}{3}$  the body width; vagina extending  $\frac{1}{4}$  into body, vagina vera almost equal to vagina uterina. Reproductive organs paired, symmetrical, opposed; spermatheca single, oval; ovaries with a single row of oocytes except for a few in region of multiplication. Intestine partially extending over rectum, more on its dorsal side. Tail elongate–conoid; terminus ventrally drawn into a claw-like spine; tail length 135–160  $\mu$ .

MALE: Similar to female in many respects. Head with 5–6 annules. Spear strong, as in female. Testis single, outstretched. Bursa crenate, adanal, extending 38  $\mu$  anterior and 43  $\mu$  posterior to cloaca, not reaching phasmids. Spicules strong, avg. 34  $\mu$ . Gubernaculum half the length of spicule, distal half thickened appearing keel-shaped in lateral view. Lateral fields disappearing a little behind middle of tail, anterior to phasmids. Phasmids 40–60  $\mu$  from tail tip.

TYPE MATERIAL: Holotype ( $\varnothing$ ) and a pair of paratypes (1  $\varnothing$ , 1  $\delta$ ) at the Crops Research Branch, USDA, Beltsville, Maryland, USA. A pair of paratypes (1  $\varnothing$ , 1  $\delta$ ) will be deposited at each of the following centers: Rothamsted Experimental Station, Harpenden, England; Plantenziektenkundige, Dienst, Wageningen, Holland; Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, India. Four females and one male in author's possession.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of grasses (dominant among them being *Eleocharis* sp.) in knee-deep water at the southern end of the bridge over the river Ganga on Moradabad-Delhi Road, about 43 miles from Moradabad, U.P., India.

DIAGNOSIS AND RELATIONSHIP: *Hirschmanniella magna* n. sp. is recognized by its large body size (over 2 mm), 28–29  $\mu$  long spear, lateral fields terminating on tail near phasmids, excretory pore lying 150–160  $\mu$  from anterior end of body, equatorial vulva, intestine partially extending over rectum, and bursa in male not reaching phasmids.

This species is close to *H. spinicaudata* (Schoorman-Stekhoven, 1944) Luc and Goodey, 1963 and *H. mucronata* (Das, 1960) Timm, 1965. From the former it differs in having a smaller spear (40  $\mu$  long in *H. spinicaudata*),

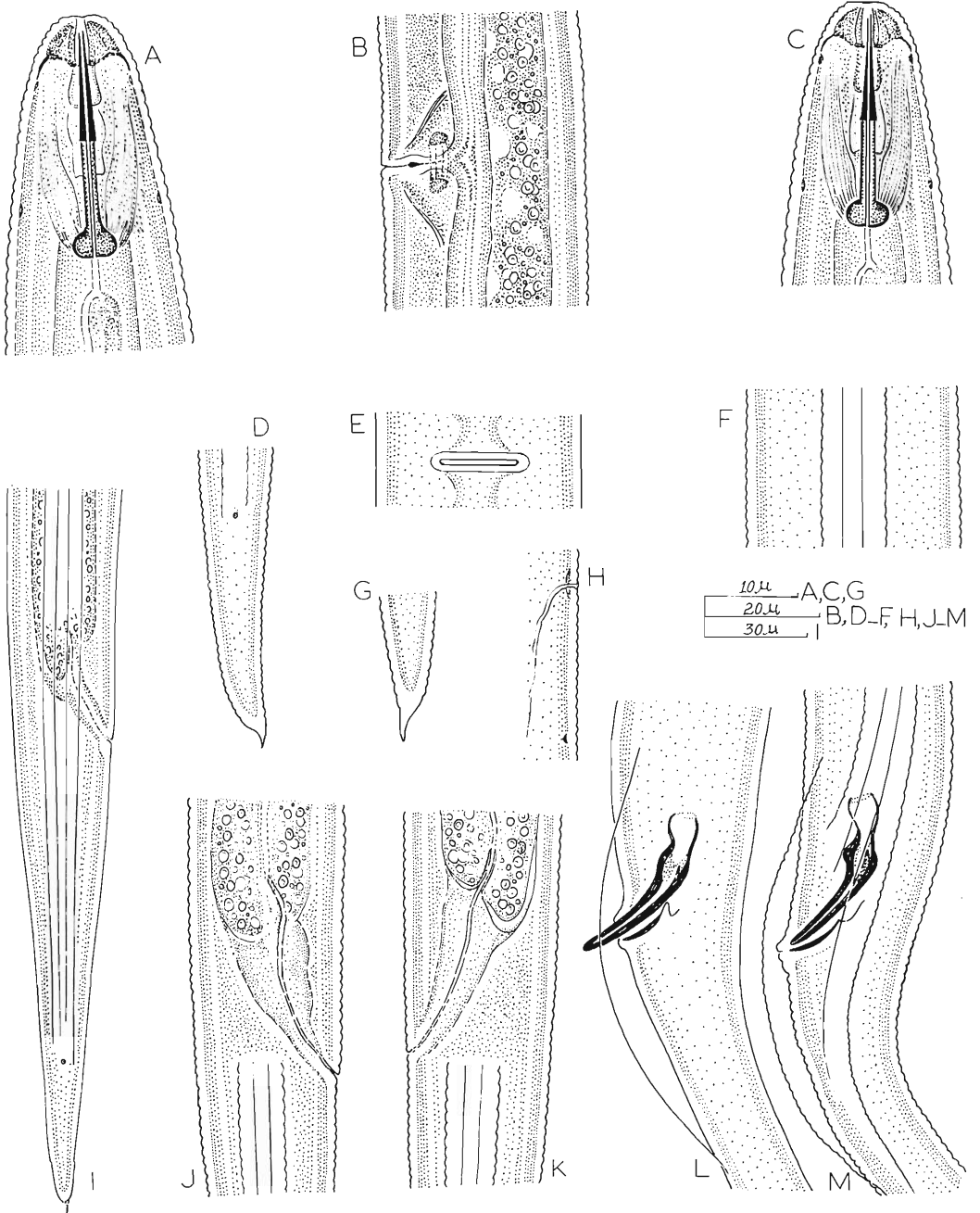


Fig. 2, A-M. *Hirschmanniella magna* n. sp. A. Head end of female. B. Vulval region. C. Head end of male. D. Tail end of female. E. Vulva, ventral. F. Lateral field. G. Tail end of male. H. Excretory pore, hemizonid, and hemizonion. I. Tail end of female. J and K. Rectal region of female. L and M. Spicule, gubernaculum, and bursa.

un-aerolated lateral fields and shorter spicules ( $43 \mu$  long in *H. spinicaudata*). From the latter it can be differentiated in having larger body size, longer spear, and bursa not reaching phasmids (body size = 1.76–1.90 mm; spear =  $24 \mu$  long in *H. mucronata*).

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## Nematode Parasites of the Coelomic Cavity of Earthworms.

V. *Plutellonema*, *Iponema*, and *Filiponema*, New Genera (Drilonematidae)R. W. TIMM AND A. R. MAGGENTI<sup>1</sup>

Notre Dame College, Dacca, East Pakistan

A large collection of earthworm nematode parasites sent to the United States Department of Agriculture, Beltsville, Maryland, between 1928 and 1933 by Dr. G. E. Gates has been made available for study through the courtesy of the Nematology Section, U.S.D.A. Two new genera and three new species from this collection are herein described. All specimens have been remounted in glycerin from the original glycerin slides; at least 10% shrinkage occurred in all specimens in the process of remounting. The slides will be redeposited in the U.S.D.A. collection under their original slide numbers. Dr. Gates has kindly informed us that some of the collection dates and collection areas given on the slides are obviously wrong; corrected information will be indicated. The third new genus described in this paper, containing a single species, was collected in the Philippines by the first author during the tenure of a South-East Asia Treaty Organization Research Fellowship.

*Plutellonema* new genus

DIAGNOSIS: Drilonematidae. Nonungellate. Amphids and symmetrical caudal suckers elliptical. Nerve ring posterior to esophagus.

<sup>1</sup> On leave as a Fulbright Research Scholar from the Department of Nematology, University of California, Davis, California.

Clitellum-like bulge around vulva. Postvulvar uterine sac. Male possessing copulatory apparatus and delicate genital bursa.

TYPE SPECIES: *Plutellonema clitellatum* n. sp.

*Plutellonema* is most closely allied to *Filiponema* n. g., which lacks the bulge around the vulva of the female and differs in cephalic structure. Among the previously described genera of the Drilonematidae it is closest to *Perodira* Baylis, 1943, but the latter lacks a copulatory apparatus.

*Plutellonema clitellatum* new species  
(Figs. 1, A–E)

FEMALE (10): Length = 1.63 mm (1.56–1.74); esophagus = 128 microns (118–138); esophagus to vulva = 0.81 mm (0.72–0.86); vulva to anus = 0.61 mm (0.57–0.67); tail = 108 microns (101–122); maximum body diameter (at bulge) = 81 microns (70–90).

MALE (4): Length = 1.42 mm (1.3–1.57); esophagus = 119 microns (112–125); esophagus to anus = 1.2 mm (1.08–1.34); tail = 102 microns (96–108); maximum body diameter = 35 microns (32–38).

HOLOTYPE FEMALE: Length = 1.56 mm; esophagus = 134 microns; esophagus to vulva = 0.77 mm; vulva to anus = 0.59 mm; tail = 108 microns; maximum body diameter = 80 microns.

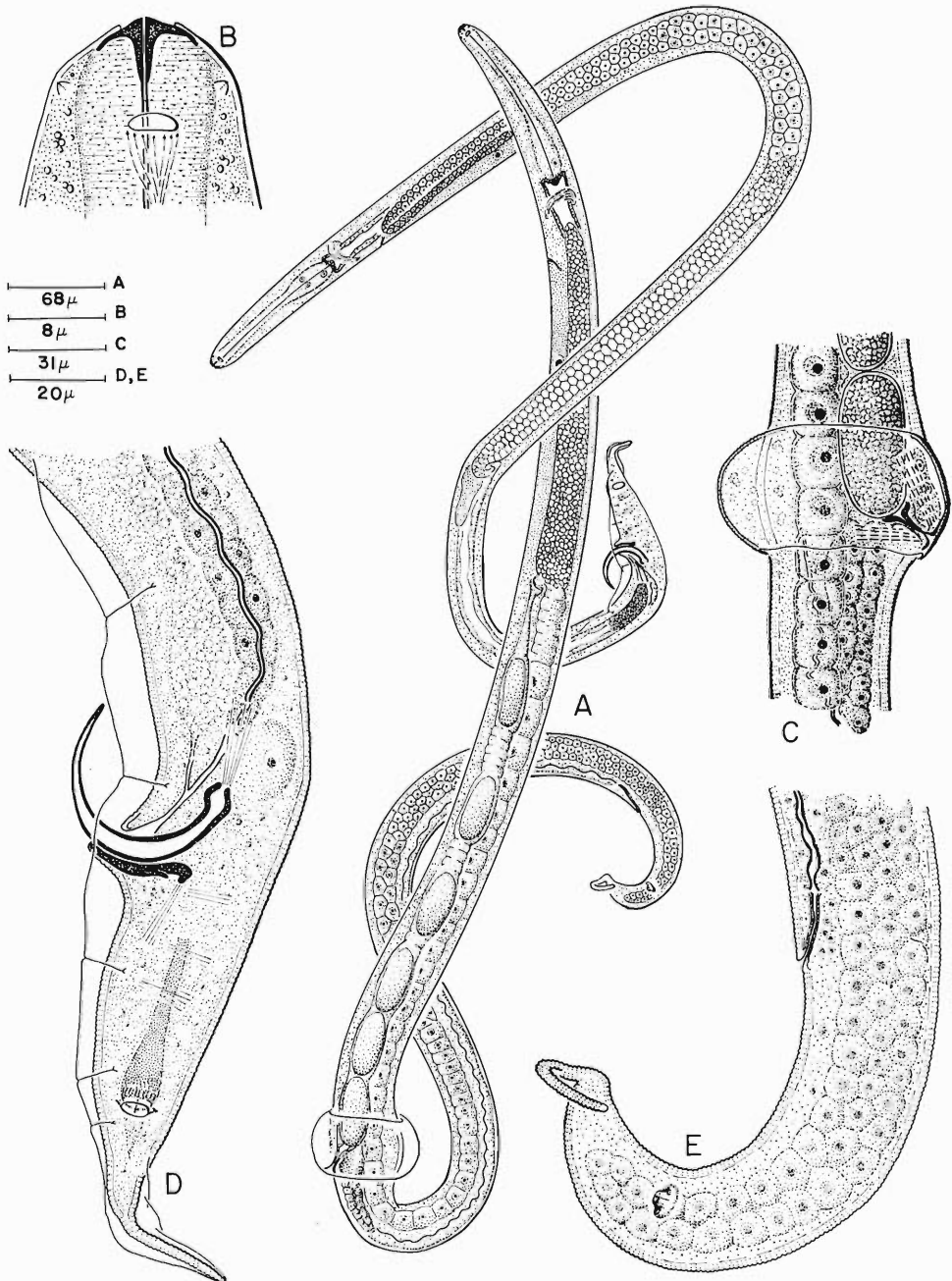


Fig. 1. *Plutellonema clitellatum* n. sp. A. Entire male and female; B. Head; C. Vulvar region; D. Male tail; E. Female tail.



ALLOTYPE MALE: Length = 1.57 mm; esophagus = 125 microns; esophagus to anus = 1.34 mm; tail = 102 microns; maximum body diameter = 38 microns.

DESCRIPTION: Cuticle thin, striated; striae about two microns wide, coarsely punctate, with punctations raised above surface of cuticle (Fig. 1, C). Head rounded, continuous with body contour; head diameter 20–25 microns in female, 16–20 microns in male; cephalic hooks lacking; stoma lacking but esophageal lining slightly thickened and protruding at anterior end. Faint elliptical amphids, about one-fourth head diameter wide, with broad pouch and prominent sensilla. Esophagus clavate, slightly narrower at isthmus; swollen posterior portion about 40 microns long; short conical cardia. Nerve ring at base of esophagus or slightly posterior. Excretory pore inconspicuous, about 1.5 body diameters posterior to esophagus, with short, lightly sclerotized duct; sublateral excretory gland cells with prominent nuclei and wavy canals. Intestine ventral to ovary behind vulva. Ovary begins in tail posterior to caudal suckers or between suckers and anus, extends forward to long elliptical spermatheca 3 body diameters posterior to esophagus; vulva surrounded by large clitellum-like bulge, more prominent on dorsal side; vulva opens at posterior of bulge. Ova elliptical, with unornamented shell,  $56 \times 24$  microns, one to six in number. Short postvulvar uterine sac. Testis extending to within 2 body diameters of esophagus, reflexed ventrally 4–5 body diameters. Two equal spicules, 31 microns across arc, very arcuate, with large capitulum. Gubernaculum parallel to spicules, 15 microns long, with proximal posterior knob. Delicate, striated, irregular caudal alae; up to five fine genital rays supporting alae. Caudal suckers broadly elliptical to circular, situated near posterior of conical part of tail. Tail in both sexes conical, with long digitate tip; female tail ventrally curved.

TYPE HOST: *Plutellus* sp.

TYPE LOCALITY: Nepal.

TYPE HABITAT: Coelom.

HOLOTYPE: Female on Slide 1L<sub>1a</sub>, collected by Dr. G. E. Gates on 14 March 1933.

ALLOTYPE: Male on Slide 1L<sub>1b</sub>; same data as holotype.

PARATYPES: Males and females on Slides 1L<sub>1</sub>–1L<sub>5</sub>.

#### *Iponema* new genus

DIAGNOSIS: Nonungellate. Head slightly swollen. Amphids faint, broadly elliptical to circular. Nerve ring crossing isthmus. Copulatory apparatus present; genital bursa absent.

TYPE SPECIES: *Iponema major* n. sp.

This genus is most closely related to *Plutellonema* n. g. and *Filiponema* n. g., but differs chiefly in the absence of a male genital bursa.

#### *Iponema major* new species

(Fig. 2, A–D)

FEMALE (10): Length = 2.94 mm (2.44–3.65); esophagus = 172 microns (154–192); esophagus to vulva = 1.06 mm (0.96–1.34); vulva to anus = 1.45 mm (1.26–1.86); tail = 258 microns (243–282); maximum body diameter = 43 microns (35–58).

MALE (10): Length = 1.83 mm (1.64–2.15); esophagus = 164 microns (138–179); esophagus to anus = 1.56 mm (1.41–1.86); tail = 109 microns (90–124); maximum body diameter = 31 microns (26–33).

HOLOTYPE FEMALE: Length = 2.64 mm; esophagus = 176 microns; esophagus to vulva = 0.9 mm; vulva to anus = 1.31 mm; tail = 256 microns; maximum body diameter = 35 microns.

ALLOTYPE MALE: Length = 1.8 mm; esophagus = 176 microns; esophagus to anus = 1.54 mm; tail = 118 microns; maximum body diameter = 32 microns.

DESCRIPTION: Body finely striated. Head slightly swollen. Stoma lacking; lips often slightly protruding. Amphids faint, broadly elliptical to circular. Esophagus long, clavate; cardia broad and flat. Nerve ring surrounding isthmus. Excretory pore 1–2.5 body diameters posterior to esophagus. Ovary begins in tail at anterior sucker; spermatheca distinctly offset; ova 1–17 in number,  $61\text{--}64 \times 32\text{--}35$  microns, without ornamentation; postvulvar uterine sac present. Testis reflexed ventrally at anterior. Spicules 40–54 microns long across arc, distinctly cephalated; gubernaculum 30 microns long, parallel to spicules. Female tail narrow and tapering to acute tip; 9–12.1 anal body diameters long. Male tail conical in an-

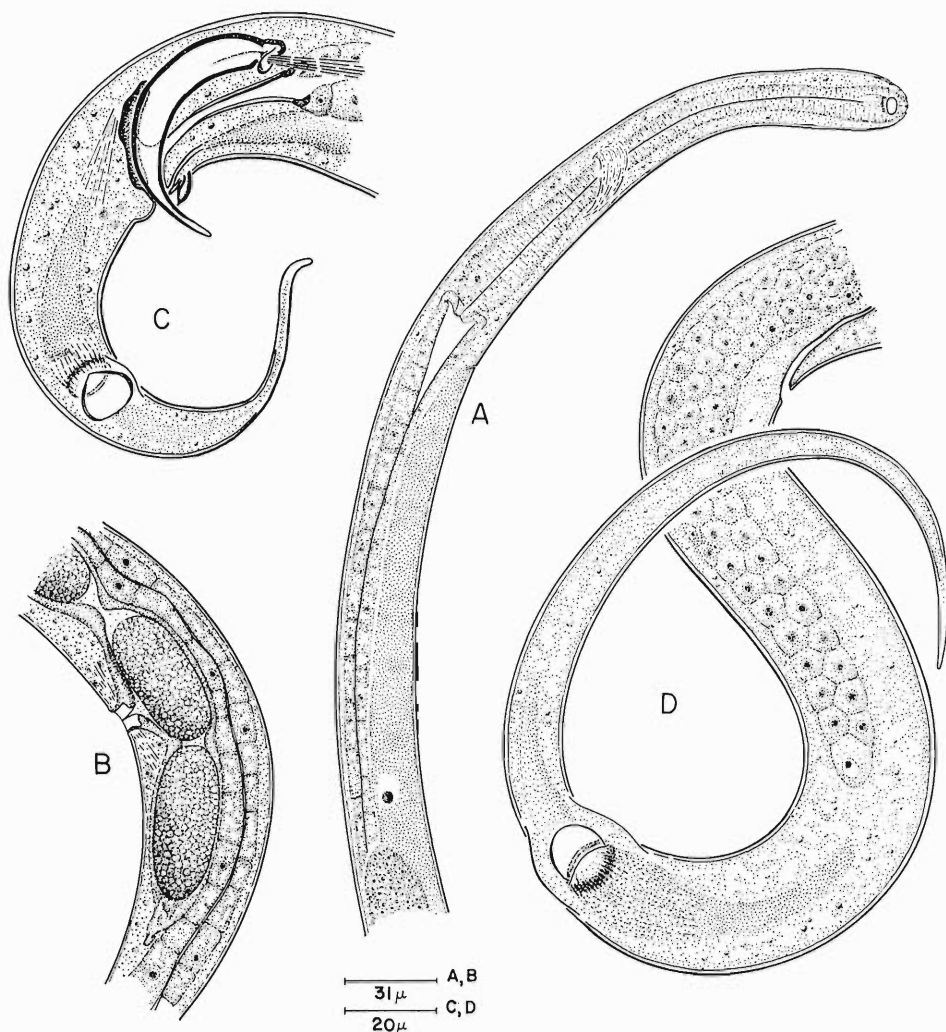


Fig. 2. *Iponema major* n. sp. A. Esophageal region; B. Vulvar region; C. Male tail; D. Female tail.

terior portion and spicate in posterior portion. Caudal suckers large, elliptical, asymmetrically disposed, with a large pocket beneath the surface anterior and posterior to each sucker.

TYPE HOST: *Eutyphoeus planatus* Gates, 1929.

TYPE LOCALITY: Prome, Burma.

TYPE HABITAT: Coelom.

HOLOTYPE: Female on Slide 12N<sub>1a</sub>; collected by Dr. G. E. Gates on 9 October 1932.

ALLOTYPE: Male on Slide 12N<sub>2</sub>; same data as holotype.

PARATYPES: Males and females on Slides 12N<sub>1</sub>–12N<sub>4</sub>.

DISCUSSION: This species differs from *Iponema minor* n. sp. by its larger size and by the different construction of the gubernaculum.

*Iponema minor* new species  
(Fig. 3, A–C)

FEMALE (8): Length = 1.92 mm (1.6–2.14); esophagus = 148 microns (118–163); esophagus to vulva = 0.73 mm (0.62–0.83); vulva to anus = 0.8 mm (0.74–0.93); tail =

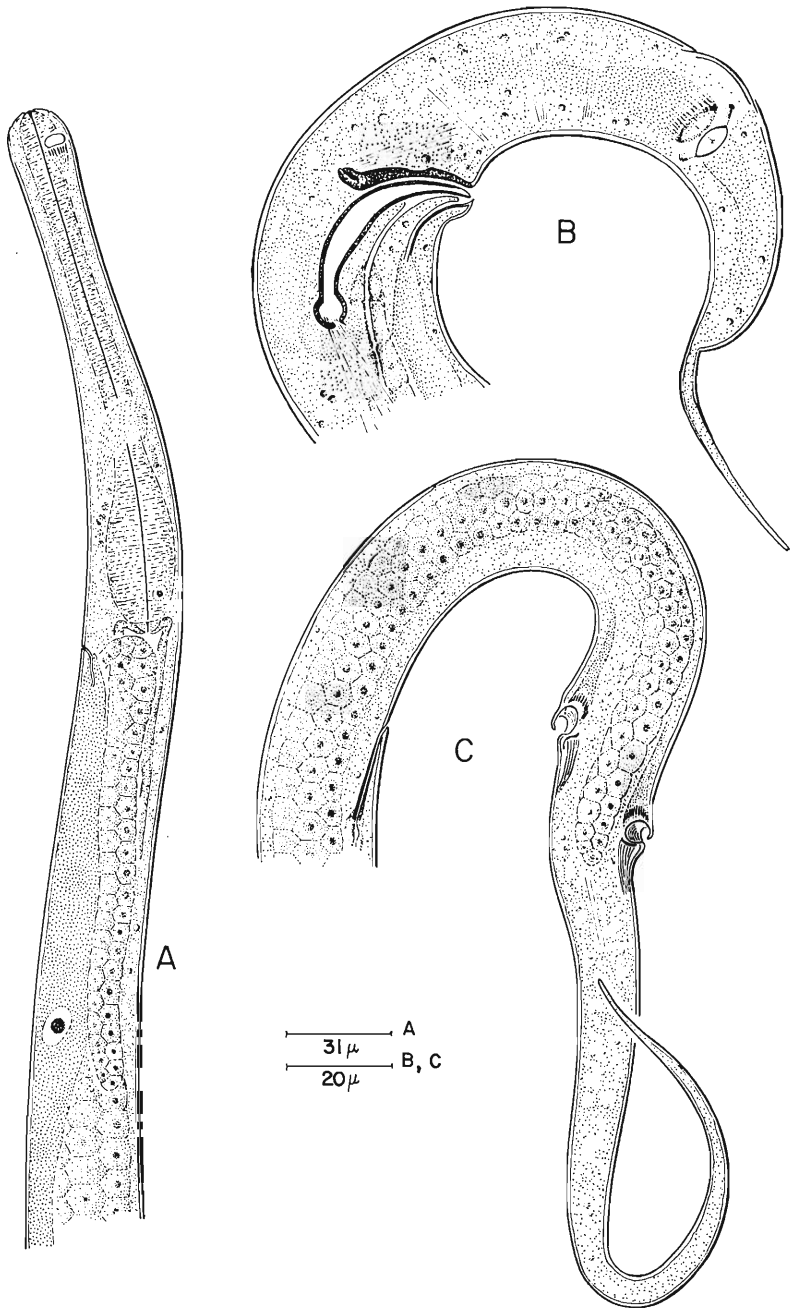


Fig. 3. *Iponema minor* n. sp. A. Esophageal region; B. Male tail; C. Female tail.

245 (211–272); maximum body diameter = 42 microns (36–51).

MALE (10): Length = 1.65 mm (1.33–1.92); esophagus = 147 microns (118–163); esophagus to anus = 1.41 mm (1.09–1.66); tail = 101 microns (90–115); maximum body diameter = 31 microns (26–38).

HOLOTYPE FEMALE: Length = 2.14 mm; esophagus = 145 microns; esophagus to vulva = 0.83 mm; vulva to anus = 0.93 mm; tail = 234 microns; maximum body diameter = 42 microns.

ALLOTYPE MALE: Length = 1.74 mm; esophagus = 146 microns; esophagus to anus = 1.47 mm; tail = 115 microns; maximum body diameter = 29 microns.

DESCRIPTION: Body finely striated, barely resolvable as composed of fine punctations. Head usually swollen slightly, with four clear sublateral areas bearing a central innervation. Stoma lacking, but lips often protruding. Amphids faint, broadly elliptical in female, more circular in male, with central innervation and broad sensilla. Esophagus long, clavate; isthmus not narrower than corpus; cardia short and flat. Nerve ring surrounding isthmus. Excretory pore at base of esophagus to one body diameter posterior; lightly sclerotized terminal excretory duct. Ovary single, anterior, beginning in tail; ova 6–11 in number, broadly elliptical, 65–75 × 25–32 microns, without ornamentation; postvulvar uterine sac present. Testis single, extending anteriorly from within one body diameter of esophageal base to one body diameter anterior to esophageal base, reflexed ventrally. Spicules long and thin, 39 microns long across arc, distinctly cephalated, with capitulum turned half-ventrally. Gubernaculum 25 microns long, thin and parallel to spicules, with proximal posterior knob. Bursa and genital papillae lacking. Female tail 9–9.7 anal body diameters long, uniformly tapering to acute tip. Male tail conical in anterior portion, 90–110 microns long; bluntly spicate in posterior portion, 22–42 microns long; tail region doubly coiled ventrally. Caudal suckers broadly elliptical, asymmetrical, 14 microns apart or less than one tail diameter, with prominent pouch beneath the surface.

TYPE HOST: *Eutyphoeus bullatus* Gates, 1933.

TYPE LOCALITY: Tiddim, Chin Hills, Burma (corrected locality).

TYPE HABITAT: Coelom.

HOLOTYPE: Female on Slide 3K<sub>3</sub>; collected by Dr. G. E. Gates in September 1932 (corrected date).

ALLOTYPE: Male on Slide 3K<sub>4</sub>; same data as holotype.

#### *Filiponema* new genus

The nematodes were found in both pre- and postclitellar segments of six out of ten specimens of *Pheretima benguetensis* examined. The largest number from one individual was eight females and four males. Mixed infections with a species of *Synoeocnema* occurred.

DIAGNOSIS: Drilonematidae. Nonungellate. Ten short cephalic setae supporting a raised membrane. Amphids and symmetrical caudal suckers circular, conspicuous. Nerve ring posterior to esophagus. Copulatory apparatus present in male; delicate genital bursa supported by hairlike papillae.

TYPE SPECIES: *Filiponema philippinense* n. sp.

#### *Filiponema philippinense* n. sp. (Fig. 4, A–D)

FEMALE (7): Length = 2.72 mm (1.65–2.96); esophagus = 161 microns (138–172); esophagus to vulva = 1.33 mm (0.88–1.52); vulva to anus = 0.92 mm (0.63–1.04); tail = 295 microns (210–340); maximum body diameter = 51 microns (35–68).

MALE (4): Length = 1.85 mm (1.36–2.21); esophagus = 149 microns (138–160); tail = 110 microns (79–127); maximum body diameter = 42 microns (35–48).

HOLOTYPE FEMALE: Length = 2.71 mm; esophagus = 163 microns; esophagus to vulva = 1.32 mm; vulva to anus = 0.96 mm; tail = 275 microns; maximum body diameter = 51 microns.

ALLOTYPE MALE: Length = 2.14 mm; esophagus = 160 microns; esophagus to testis = 512 microns; testis = 1.36 mm; tail = 112 microns; maximum body diameter = 35 microns.

DESCRIPTION: Cuticle finely striated. Lateral field prominent, consisting of two rows of cells. Head not set off; head diameter 24 microns; thin raised cuticular membrane around anterior, supported by six setose papil-

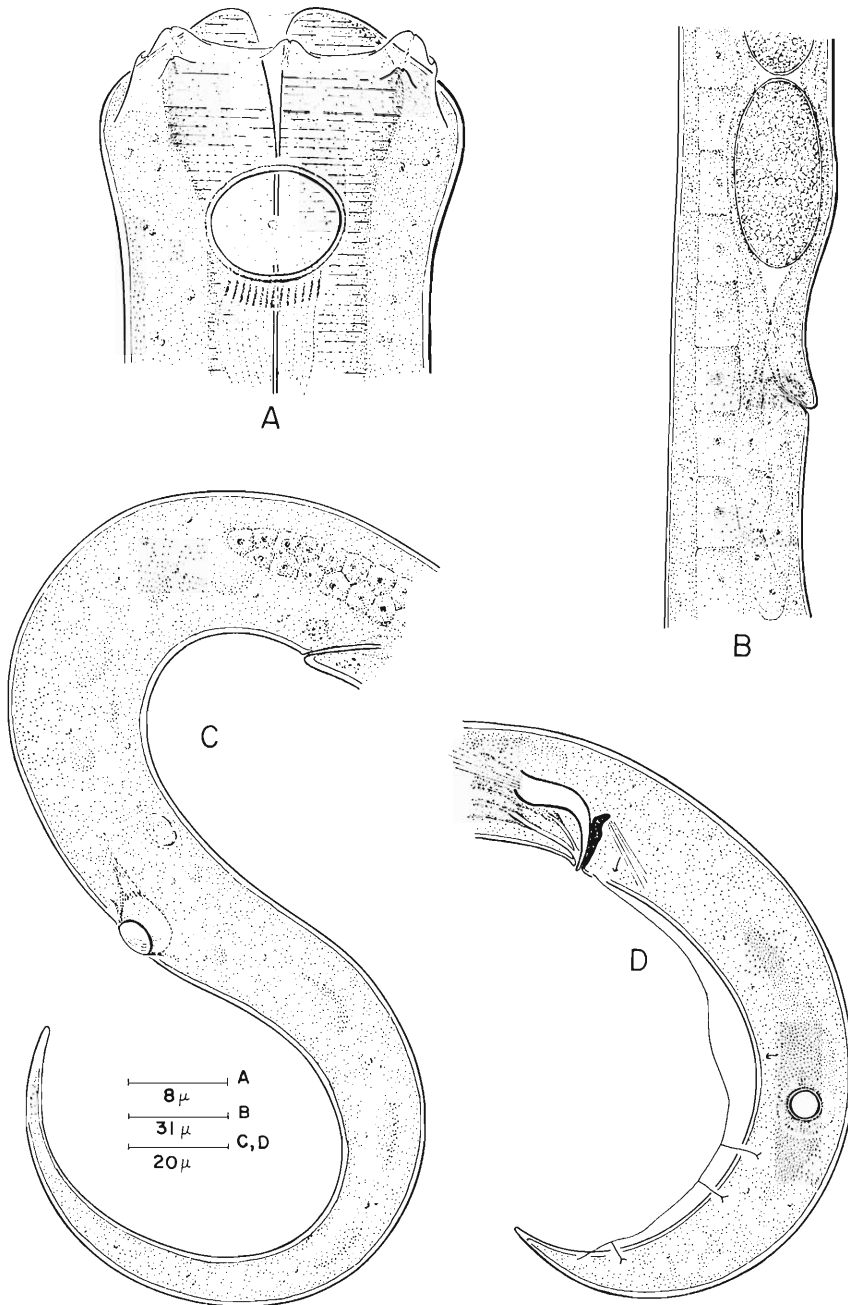


Fig. 4. *Filiponema philippinense* n. sp. A. Head; B. Vulvar region; C. Female tail; D. Male tail.

lae of inner circle; four smaller setose papillae in outer circle. Large prominent broadly elliptical to circular amphids, 9 microns in diameter or about 50% of head diameter in both sexes. Esophagus clavate. Nerve ring just behind esophageal base. Excretory pore inconspicuous, two body diameters anterior to spermatheca. Subventral excretory gland cells prominent. Ovary begins between anus and caudal suckers; elliptical spermatheca 2.5 body diameters long, five body diameters posterior to esophagus. Seven ova (6–11) in uterus,  $60 \times 30$  microns, with clear shell; postvulvar uterine sac present. Testis dorsal to intestine at anterior, reflexed 190–290 microns ventrolaterally. Sperm spherical. Spicules 21 microns long across arc, cephalated, not internally divided. Gubernaculum 9–14 microns long, with dorsoposterior knob. Irregular delicate caudal alae, supported by wavy hairlike genital papillae; other short papillae present,

not extending to margin of alae. Female tail uniformly tapering to fine tip, 8.2–11 anal body diameters long. Male tail ventrally curved in a half-circle, 5 anal body diameters long. Opposite circular caudal suckers at about 40% of tail length, about 50% of corresponding tail diameter in diameter.

TYPE HOST: *Pheretima benguetensis* Bedard, 1912.

TYPE LOCALITY: Ateneo de Manila University, Quezon City, Philippines.

TYPE HABITAT: Coelom.

HOLOTYPE: Female, from collection of Biology Department, Ateneo de Manila University; dissected out by R. W. Timm on 9 January 1964; slide will be deposited with the Gates–U.S.D.A. collection.

ALLOTYPE: Male; same data as holotype.

PARATYPES: Males and females on Slide A65, Notre Dame College, Dacca; specimens also deposited in Department of Nematology, Davis, California.

### On the Trematode genera *Lutztrema* Travassos, 1941 and *Anchitrema* Looss, 1899 from Malayan Bats, with a Discussion of Allometric Growth in Helminths

KLAUS ROHDE<sup>1</sup>

Malaya has an extremely rich bat fauna which represents over one-third of the total mammalian fauna (nearly 200 species) of this area. Since the helminth fauna of bats had not yet been studied, a survey was conducted from July 1961 to October 1964. The first results of this survey have already been published and include records of the following species of trematodes: *Postorchigenes duboisi* Rohde, 1963, *Prosthodendrium longiforme* (Bhalerao, 1926), *Lecithodendrium linstowi* Dollfus, 1931,

*Prosthodendrium parvouterus* (Bhalerao, 1926), *Prosthodendrium swansoni* (?) Macy, 1936, *Odeningotrema bivesicularis* Rohde, 1962 (originally described from *Nycticebus coucang*, comp. Rohde 1962), small unidentified Lecithodendriidae, *Renschetrema malayi* Rohde, 1964, *Renschetrema sandoshami* Rohde, 1964, *Renschetrema* sp., and *Maxbraunium baeri* Rohde, 1964 (see Rohde 1963, 1964a and b). Altogether, 393 bats belonging to eight species of Megachiroptera (Pteropidae) and at least 28 species of Microchiroptera were dissected. Members of six of the seven families of Chiroptera which, according to Chasen (1940), occur in Malaya were examined. Only the Megadermidae are not represented. The localities at which the bats were caught are spread over a large part of Malaya (see map) and include a variety of different habitats. A brief character-

I wish to thank Dr. Allen McIntosh and Mr. W. W. Becklund, Beltsville Parasitological Laboratory, for the loan of *Anchitrema sanguineum* from Chamaeleo, Egypt; Mr. S. Prudhoe, British Museum (Natural History) for the loan of *A. sanguineum* from *Taphozous perforatus* and *Chamaeleo*, Egypt; Lord Medway, Department of Zoology, University of Malaya, for identifying the bats; and Miss Lilian Lim for technical assistance.

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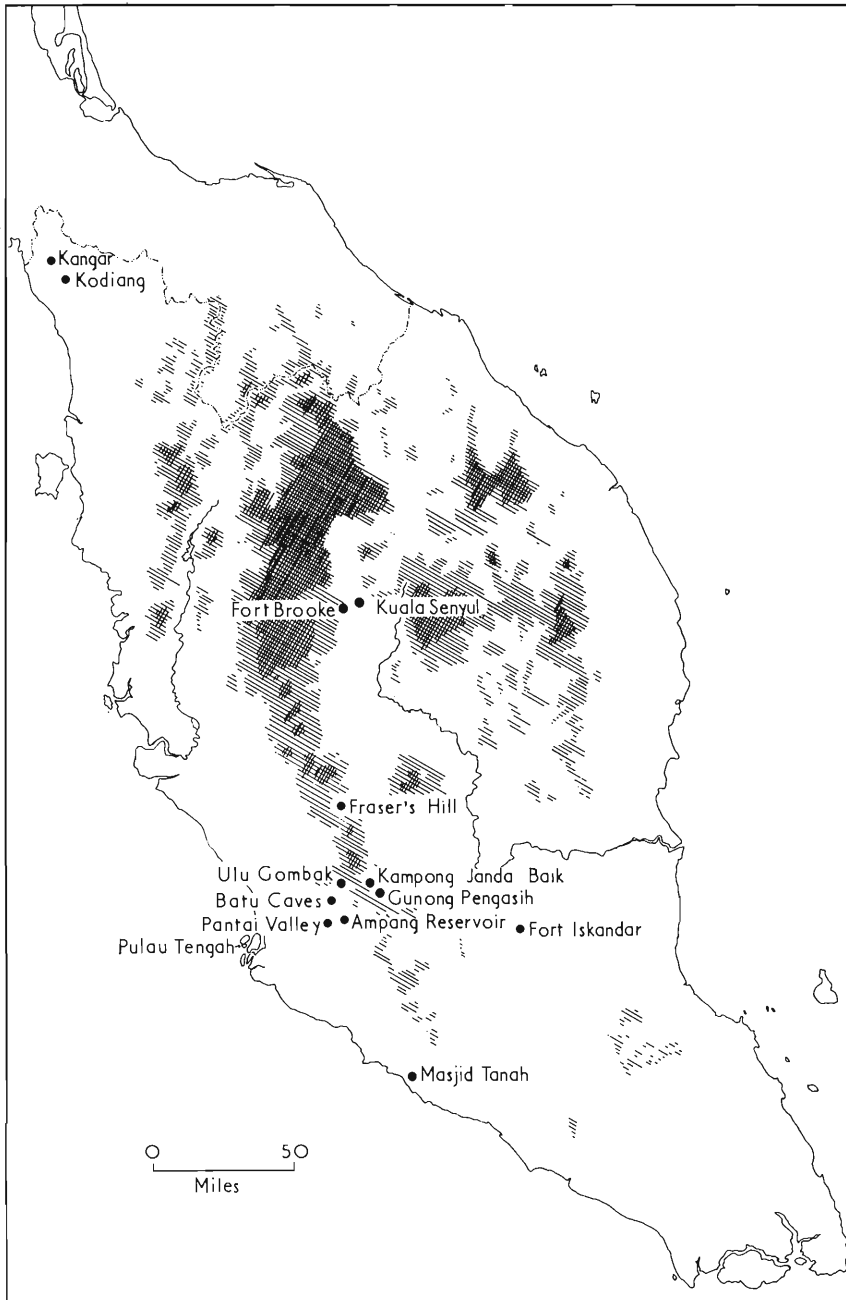


Fig. 1. Map of Malaya with mountain ranges and localities at which bats were caught.

ization of the various localities is given in the following:

- Pantai Valley, Kuala Lumpur. 101°40' E, 3°7' N, 90 m above sea level. Open country with buildings. *Kerivoula* Gray, 1842, caught in buildings, *Cynopterus* Cuvier, 1824 in open secondary scrub.
- Ulu Gombak, 16th mile. Near Kuala Lumpur. 101°46' E, 3°20' N, 260 m above sea level. Tall secondary forest.
- Batu Caves, near Kuala Lumpur. 101°41' E, 3°15' N, about 150 m above sea level. Large caves in limestone hill, surrounded by open culture landscape. Bats caught at entrance of caves.
- Ampang Reservoir, outskirts of Kuala Lumpur. 101°47' E, 3°10' N, 150 m above sea level. Bats caught at entrance into large water culvert in water reservoir, about 10 m from bank, connected to it by narrow foot bridge. Dense forest and large clearance nearby.
- Pulau Tengah, 101°15' E, 3° N. Small island off Port Swettenham, Selangor. Little above sea level. Mangrove forest and tidal mud flats.
- Gunong Pengasih, near Gunong Nuang, Ulu Langat District, Selangor. 101°53' E, 3°21' N, 600 m above sea level. Hill forest.
- Fraser's Hill, Perak. 101°43' E, 3°44' N, 1,200 m above sea level. Mountain forest.
- Fort Iskandar, Tasek Bera, Pahang. Approximately 102°39' E, 3°2' N. Swampy open country and disturbed forest.
- Masjid Tanah, northwest of Malacca. 102°7' E, 2°22' N, 30 m above sea level, about 6 km from coast. Rubber plantation. Bats caught under roof of estate building.
- Fort Brooke, Ulu Kelantan District, Kelantan, 101°29' E, 4°41' N, altitude not available, probably about 600 m above sea level. Secondary jungle with much bamboo.
- Kuala Senyul, Ulu Kelantan District, Kelantan. 101°41' E, 4°33' N. Altitude not available, probably about 600 m above sea level. Aboriginal settlement in clearance surrounded by dense secondary jungle. *Myotis* Kaup, 1829, caught in banana trees.
- Kangar, Perlis. 100°12' E, 6°27' N. Bats

caught in Istana (Palace) of the Raja of Perlis.

- Kodiang, near southern border of Perlis. 100°18' E, 6°24' N. Bats caught in caves (about 150 m above sea level?).

The Megachiroptera examined belong to the following species:

- Pteropus vampyrus* (Linn.), *Macroglossus lagochilus* Matsch., *Aethalops alecto* Thos., *Penthetor lucasi* (Dobs.), *Balionycteris maculata* (Thos.), *Cynopterus brachyotis* (S. Müll.), *C. horsfieldi* Gray, *Eonycteris spelaea* (Dobs.).

The Microchiroptera examined belong to the following families and species:

- Emballonuridae: *Taphozous melanopogon* Temm., *T. saccolaimus* Temm.
- Nycteridae: *Nycteris javanica* Geoff.
- Rhinolophidae: *Rhinolophus stheno* And., *R. luctus* Temm., *R. trifolius* Temm., *R. affinis* Horsf., *R. sedulus* And., *R. sp.*, *Hipposideros bicolor* (Temm.) = *H. pomona* And., *H. galeritus* Cant., *H. diadema* (Geoff.), *H. armiger* (Hodgs.), *H. cineraceus* Blyth.
- Molossidae: *Cheiromeles torquatus* Horsf., *Tadarida mops* (de Blainv.).
- Vespertilionidae: *Tylonycteris robustula* Thos., *T. pachypus* (Temm.), *T. sp.*, *Kerivoula pusilla* Thos., *K. hardwickii* (Horsf.), *K. papillosa* (Temm.), *K. sp.*, *Murina suilla* (Temm.), *M. cyclotis* Dobs., *M. aenea* Hill., *Nyctalus stenopterus* (Dobs.), *Glischropus tylopus* (Dobs.), *Pipistrellus sp.* (or *Glischropus sp.*), *Myotis mystacinus* (Kuhl), *M. hasseltii* (Temm.), *Scotophilus temminckii* Leach.

In some cases, diagnosis down to species level was impossible, because the specimens were lost in the field.

In the tables below, the numbers of specimens from the various localities of all species examined are given. Also given is the frequency of infection with the major helminth groups infecting the stomach and intestine. In addition to the bats listed, the intestines and stomachs of an unknown number of *Taphozous melanopogon* from Kodiang, Kedah, were examined. The specimens were fixed in formalin



TABLE 1. Megachiroptera (Pteropidae). Frequency of infection with helminths of the intestine and stomach.

Genus	Species	Locality	Number of specimens examined	Number of specimens infected with:		
				Trematodes	Cestodes	Nematodes
<i>Cynopterus</i>	<i>brachyotis</i>	Pantai Valley	6	1 ( <i>Paralecithodendrium</i> sp.)		
		Janda Baik	3			
		Fraser's Hill	1			
	<i>horsfieldi</i>	Janda Baik	1			
<i>Macroglossus</i>	<i>lagochilus</i>	Fraser's Hill	1			
<i>Balionycteris</i>	<i>maculata</i>	Kuala Senyul	1			
<i>Aethalops</i>	<i>alecto</i>	Fraser's Hill	1			
<i>Eonycteris</i>	<i>spelaea</i>	Batu Caves	1			
<i>Penthetor</i>	<i>lucasi</i>	Kuala Senyul	1			
<i>Pteropus</i>	<i>campyrus</i>	Ulu Gombak	2		1	
			18	1	1	

and given to us by the Institute for Medical Research, Kuala Lumpur.

While the stomach and intestine were examined in all specimens, other organs, i.e., liver, lungs, pancreas, and esophagus, were examined only in part of the specimens. Helminths from the body cavity were collected only when they were visible during removal of the digestive tract and the internal organs. Helminths from organs other than the digestive tract were found in the following specimens:

One specimen of *Scotophilus temminckii* from Masjid Tanah had nematodes in the liver and lungs. It was also infected with nematodes of the stomach and intestine. Another specimen belonging to the same species had, besides nematodes of the stomach and intestine, nematodes of the liver.

One specimen of *Hipposideros pomona* Andersen, 1918, from Ampang Reservoir had nematodes in its body cavity, another nematodes in the lungs.

Trematodes were recovered from the gall bladder of one *Myotis mystacinus* from Janda Baik. It was also parasitized by trematodes in the intestine and stomach and nematodes in the intestine.

One *Glischropus tylopus* from Kuala Senyul had nematode cysts in its liver and another from the same locality nematodes in the abdominal cavity. Both specimens were also infected with nematodes and trematodes of the digestive tract.

Of *Tylonycteris robustula*, only one speci-

men from Kuala Senyul had nematodes in its body cavity, while one *Rhinolophus affinis* from Janda Baik contained nematode larvae in its body cavity. The latter specimen was also infected with intestinal trematodes.

Only once were trematodes found in the gall bladder. The 13 specimens collected proved to belong to a new species of *Lutztrema*; this genus was previously known only from the bile ducts and bladder of birds, with one exception, i.e., *L. callosciuri* Fischthal and Kuntz (1965) from a squirrel in Borneo.

Dicrocoeliidae Odhner, 1911.

Dicrocoeliinae Looss, 1899.

Lutztrematini Yamaguti, 1958.

*Lutztrema* Travassos, 1941.

*Lutziella* n. subg.

*Lutztrema (Lutziella) microacetabulare* n. sp

DESCRIPTION (based on whole mounts of ten and sections of three specimens): Delicate, flattened dorsoventrally. Longer than broad, maximum width approximately at level of ovary. Gradually tapering posteriad. Surface of body smooth. Oral sucker with weak musculature, subterminal, pharynx small. Digestive tract behind pharynx much narrower than pharynx, gradually becoming wider, branching directly in front of ovary. Two lateral branches of digestive tract short and wide, terminating at level or in front of ovary. Digestive tract more or less filled with large crystals. Acetabulum with weak musculature, smaller than oral sucker, at anterior boundary of second

TABLE 2. Microchiroptera. Frequency of infection with helminths of the intestine and stomach.

Genus	Species	Locality	No. of specimens examined	Number of specimens infected with			
				Trematodes	Cestodes	Nematodes	Acanthocephala
<i>Taphozous</i>	<i>saccolaimus</i>	Masjid Tanah	3	1		1	
	<i>melanopogon</i>	Batu Caves	14	10			
<i>Nycteris</i>	<i>javanica</i>	Alor Star	5				
	<i>stheno</i>	Janda Baik	3				
<i>Rhinolophus</i>	<i>stheno</i>	G. Pengasih	1	1			
	<i>luctus</i>	Janda Baik	1	1			
	<i>luctus</i>	Janda Baik	3	3	1		1
	<i>luctus</i>	Fraser's Hill	1	1			
	<i>trifoliatus</i>	Kuala Senyul	1				
	<i>affinis</i>	Janda Baik	3	2			1
	<i>sedulus</i>	Ulu Gombak	2	2			
	sp.	Janda Baik	3	2			
<i>Hipposideros</i>	<i>bicolor</i> =	Ampang Resv.	194	18	22		27
	<i>pomona</i>	Janda Baik	1				1
	<i>galeritus</i>	Ulu Gombak	1				
	<i>diadema</i>	Janda Baik	7	4			1
	<i>armiger</i>	Batu Caves	2				1
	<i>armiger</i>	Janda Baik	1	1			
	<i>cineraceus</i>	Fraser's Hill	1	1	1		
	<i>cineraceus</i>	Ampang Resv.	17		2		2
	<i>cineraceus</i>	Kuala Senyul	1		1		
<i>Cheiromeles</i>	<i>torquatus</i>	Fort Iskandar	4	2			
	<i>torquatus</i>	Janda Baik	1	1			
<i>Tadarida</i>	<i>mops</i>	Fort Iskandar	6				
<i>Tylonycteris</i>	<i>robustula</i>	Janda Baik	12	3			
	<i>robustula</i>	Kuala Senyul	15	2	3		3
	<i>robustula</i>	Fort Brooke	5	3	3		
	<i>pachyypus</i>	Janda Baik	1				
	sp.	Kuala Senyul	4	3	1		1
	<i>pachyypus</i>	Janda Baik	1	1	1		
<i>Kerivoula</i>	<i>pusilla</i>	Pantai Valley	1	1			
	<i>hardwicckii</i>	Janda Baik	2	1			
	<i>papillosa</i>	Janda Baik	2				
	sp.	Kuala Senyul	1	1	1		
	<i>papillosa</i>	Fort Brooke	2	2	1		1
<i>Murina</i>	<i>suilla</i>	Janda Baik	1				
	<i>cyclotis</i>	Janda Baik	2				
	<i>cyclotis</i>	Fraser's Hill	1				
	<i>aenea</i>	Janda Baik	1				
<i>Nyctalus</i>	<i>stenopterus</i>	Janda Baik	1				
<i>Glischropus</i>	<i>tylopus</i>	Kuala Senyul	7	7			4
<i>Pipistrellus</i>	sp.	Ulu Gombak	1		1		
(or <i>Glischropus</i> )	sp.	Ulu Gombak	1				
<i>Myotis</i>	<i>mystacinus</i>	Janda Baik	10	6			2
	<i>mystacinus</i>	Kuala Senyul	2	2			1
	<i>mystacinus</i>	Pulau Tengah	1				1
<i>Scotophilus</i>	<i>hasselti</i>	Masjid Tanah	26	2			1
	<i>temmincki</i>	Masjid Tanah	26		1 (larva)		22
			375	89 (23.7%)	39 (10.4%)	70 (18.7%)	1

fourth of body. Testes symmetrical at level of posterior margin of acetabulum, straight-margined, round or oval. Ovary submedian, posterior to testes, straight-margined, round or oval, as large as or larger than testes. Seminal receptacle much larger than ovary, behind ovary, lateral to it, and overlapping it; its wall invaginated. Vitellaria lateral, from level of testes to short distance behind middle of body, that on side of ovary always extending over shorter distance than that on opposite side. Transverse yolk duct behind seminal receptacle, small yolk reservoir and Laurer's canal present. Mehlis' gland near yolk reservoir. Uterus fills most of body from level of

cirrus pouch to short distance in front of posterior end of body, overlapping vitellaria, seminal receptacle, and acetabulum, and in some specimens parts of ovary and testes. Cirrus pouch more or less median, in first fourth of body, with coiled seminal vesicle, ejaculatory duct, and prostatic glands. Genital opening median, some distance behind pharynx. Excretory opening terminal, excretory bladder tubular. Mature eggs brown, small, oval, and operculated.

HOST: *Myotis mystacinus* (Kuhl).

LOCALIZATION: Gall bladder.

INTENSITY OF INFECTION: 13.

LOCALITY: Janda Baik, Pahang, Malaya.

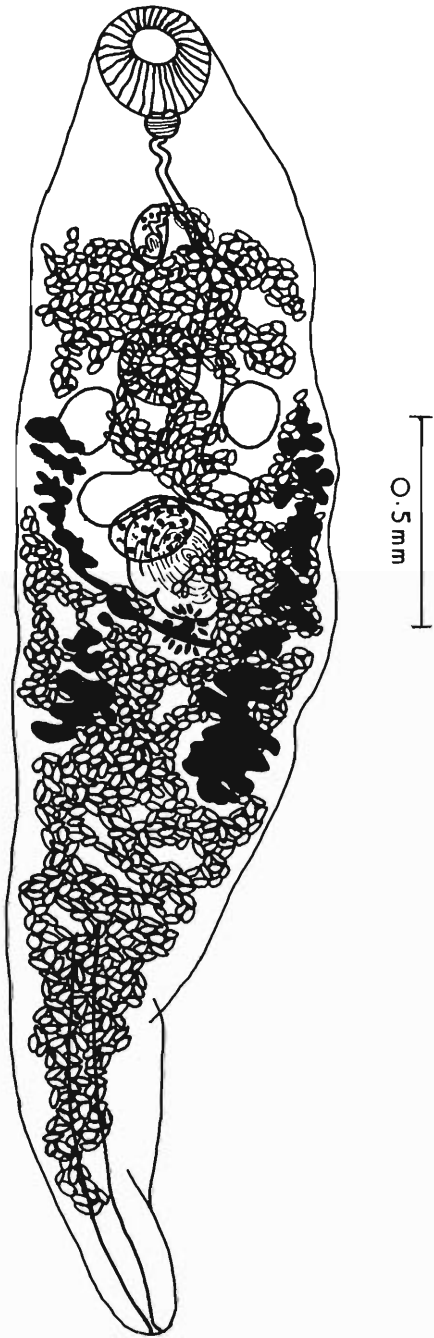


Fig. 2. *Lutztrema (Lutziella) microacetabulare* n. sp.

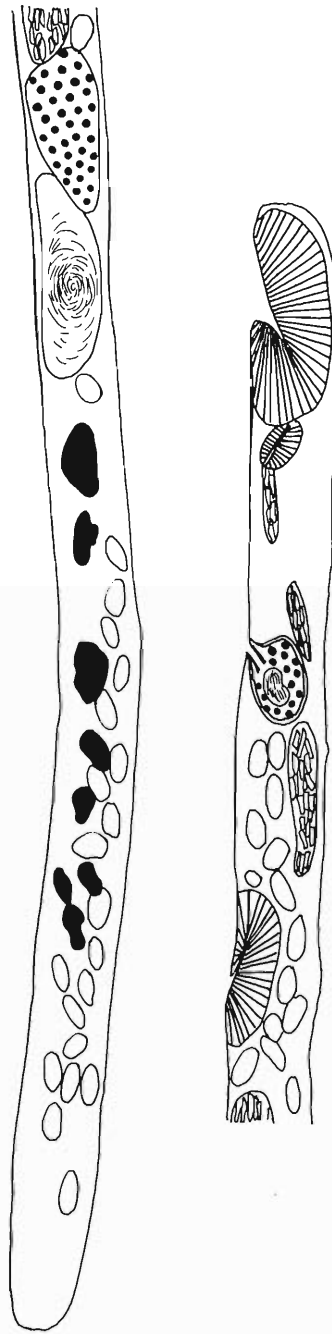


Fig. 3. *Lutztrema (Lutziella) microacetabulare*. Diagrammatic sagittal section. Note: Vitellaria drawn though they do not extend into median field.

TABLE 3. *Lutztrema* (*Lutziella*) *microacetabulare*: Measurements of nine specimens (in millimeters, longitudinal diameter of organs first).

Specimen No.:	1	2	3	4	5	6	7	8	9
Length	2.8	2.9	3.1	3.1	3.2	3.2	3.7	3.7	4.0
Maximum width	0.83	0.86	0.74	0.87	0.83	0.83	0.87	0.93	0.96
Oral sucker	0.22×0.27	0.24×0.24	0.24×0.27	0.22×0.25	0.27×0.24	0.22×0.27	0.27×0.30	0.27×0.30	0.27×0.31
Pharynx	—	0.06×0.06	0.06×0.05	0.05×0.05	0.05×0.06	—	—	—	0.04×0.07
Acetabulum (diameter)	0.17	—	0.19	0.24	0.21	0.19	0.23	0.22	0.23
Testis (ovarial)	0.16×0.18	0.22×0.15	0.15×0.15	0.18×0.15	0.18×0.13	0.15×0.10	0.16×0.16	0.17×0.17	0.25×0.21
Testis	0.16×0.15	0.22×0.18	0.18×0.09	0.17×0.15	0.16×0.15	0.18×0.13	0.13×0.15	0.21×0.17	0.22×0.19
Ovary	0.19×0.18	0.19×0.24	0.17×0.17	0.16×0.22	0.18×0.19	0.18×0.16	0.14×0.21	0.18×0.19	0.21×0.22
Vitellarium (ovarial)	0.85	0.64	0.64	0.80	0.81	0.69	0.81	0.91	0.84
Vitellarium	0.91	0.76	0.90	0.96	1.01	1.15	1.03	1.11	1.29
Eggs	0.033–0.045×0.019–0.024 (average 0.037×0.021)								

HOLOTYPE: British Mus. Nat. Hist. Helm. Coll.

PARATYPES: U. S. Nat. Mus. Helm. Coll. No. 57498; Dept. Zool. Univ. Malaya; British Mus. Nat. Hist. Helm. Coll.; and Helm. Coll. Humboldt–Universität Berlin.

#### DISCUSSION

As indicated by the structure of its digestive tract, the new form is, among all Dicrocoeliidae, most closely related to the species of *Lutztrema* Travassos, 1941 which are the only dicrocoeliids with a single cecum or rudimentary double ceca. The Malayan material differs from the described species of this genus mainly in the following characteristics: its acetabulum is smaller than the oral sucker, while in *Lutztrema* it is always larger than the oral sucker; the testes are symmetrical, while in *Lutztrema* they are always oblique or tandem; the vitellaria extend into the space in front of the ovary, while in *Lutztrema* they are postovarian; and it is a parasite of Chiroptera, while most other described members of the genus *Lutztrema* are parasitic in birds, only one species occurring in rodents.

The symmetry of the testes cannot be used as a generic characteristic. Apparently, it depends on the relative size of the testes as compared with the width of the body at the testicular level.\* Thus, in the related genus *Lyperosomum* Looss, 1899, the testes are tandem or oblique if the body is relatively narrow (for instance in *L. longicauda* (Ru-

dolphi, 1809)) and symmetrical, if the body is relatively broad (for instance in *L. alectoris* (Nöller et Enigk, 1933)). Similarly, the relative sizes of the suckers do not justify separation at the generic level, because they vary considerably in various representatives belonging to one genus of the Dicrocoeliinae. Thus, in *Eurytrema alveyi* Martin et Gee, 1949, the acetabulum is much larger than the oral sucker, while in *E. pancreaticum* (Janson, 1889), it is much smaller.

The remaining difference, i.e., extension of the vitellaria into the preovarian space, distinguishes the Malayan form so clearly from all other species of the genus *Lutztrema* that the establishment of a new subgenus for it appears to be justified.

The diagnosis of this subgenus is as follows:

#### *Lutziella* n. subgen.

Dicrocoeliidae, Dicrocoeliinae, LutztreMATINI: body long, slender. Acetabulum smaller than oral sucker, in anterior third of body. Oral sucker subterminal. Pharynx small, followed by long digestive tract which becomes gradually wider and bifurcates into two rudimentary ceca immediately in front of ovary. Testes symmetrical, at level of posterior margin of acetabulum, separated by uterine coils. Cirrus pouch median, preacetabular, genital opening some distance behind pharynx. Ovary submedian, posttesticular. Receptaculum seminis overlapping ovary and behind it. Laurer's canal present. Vitellaria lateral, from level of testes to short distance behind middle of body. Vitellarium on side of ovary shorter than that on opposite side. Uterus occupies most of hindbody, passing between testes and filling

\* I wish to thank Dr. Georges Dubois, Neuchâtel, for pointing this out to me and for his other valuable advice regarding *Lutztrema microacetabulare*.

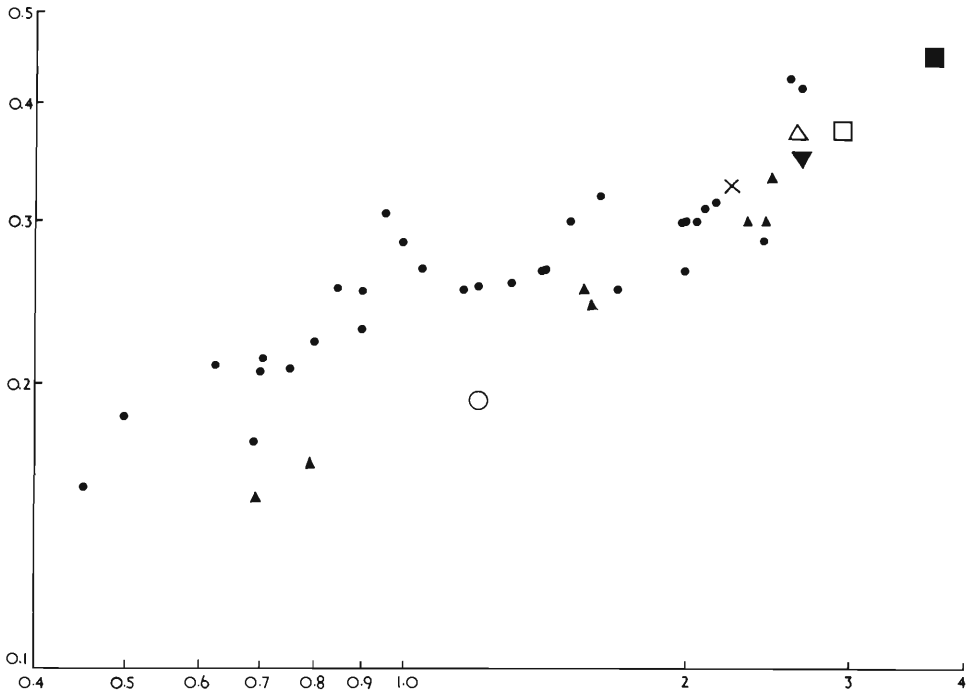


Fig. 4. *Anchitrema sanguineum*. Size of oral sucker in specimens of different body size. Abscissa: size of body (length + maximum width)/2 in mm. Ordinate: average diameter of organ in mm.

- *A. sanguineum* from Malayan bats.
- ▲ *A. sanguineum* from *Chamaeleo*, Egypt.
- ▼ *A. sanguineum* from *Taphozous perforatus*, Egypt.
- *A. sanguineum* after Looss (1899, average data).
- *A. sanguineum* after Odhner (1911, maximum data).
- △ *A. sanguineum* after Pande (1935, average data).
- × *A. "congolense"* after Sandground (1937).
- *A. "philippinorum"* after Tubangui (1928, average data).

space between level of posterior margin of cirrus pouch and testes. Excretory vessel tubular. Parasitic in gall bladder of Chiroptera.

TYPE SPECIES: *Lutztrema (Lutziella) microacetabulare*.

The generic diagnosis, as given by Yamaguti (1958) has to be revised as follows:

*Lutztrema*

Dicrocoeliidae, Dicrocoeliinae, Lutztremani: body long, slender. Acetabulum smaller or larger than oral sucker, in anterior third of body. Oral sucker subterminal, followed by pharynx. Esophagus continued into a single,

comparatively long, median cecum or rudimentary ceca. Testes symmetrical, tandem or diagonal, postacetabular, may or may not be separated from the other by uterine coils. Cirrus pouch claviform, preacetabular, containing winding seminal vesicle, pars prostatica, and cirrus. Genital pore about halfway between two suckers, or nearer to pharynx. Ovary median or submedian, immediately post-testicular. Receptaculum seminis and Laurer's canal present. Vitelline follicles large, few in number, postovarian or beginning at the testicular level. Uterus occupying most of hind-body, passing between testes or between ovary

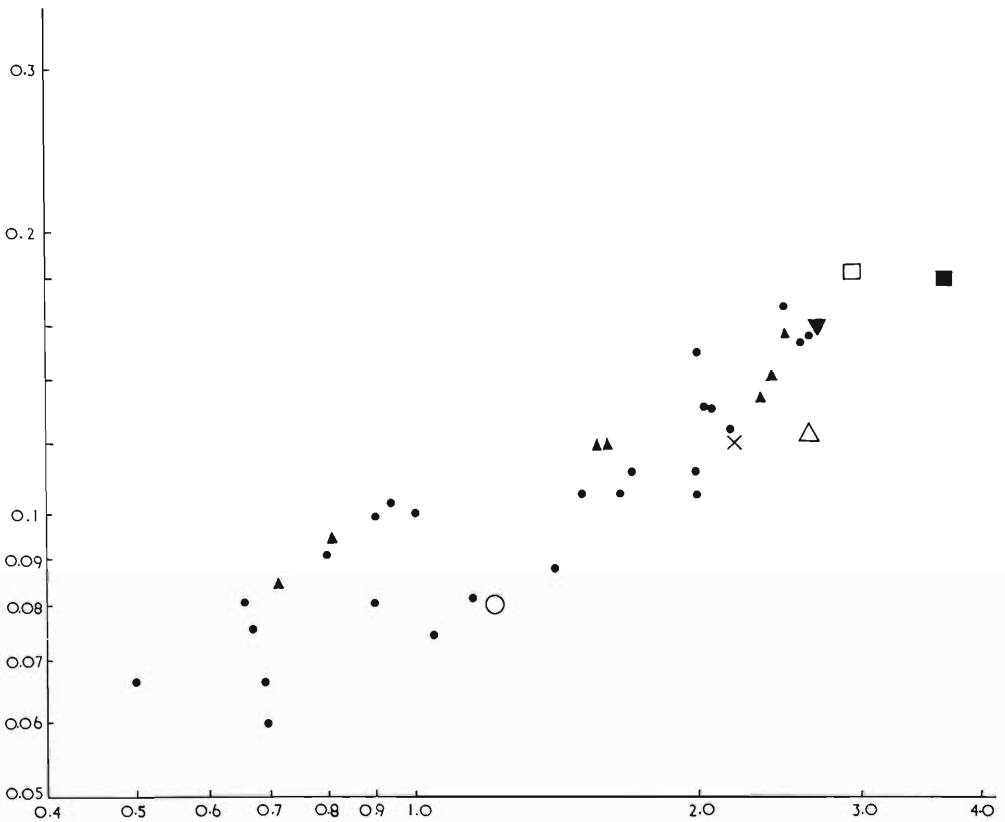


Fig. 5. *A. sanguineum*. Size of pharynx in specimens of different body size. Coordinates and symbols as in Figure 4.

and posterior testis, may fill most of space from level of cirrus pouch to level of testes. Excretory vessel tubular. Parasitic in bile ducts and bladder of birds and bats.

Genotype *L. obliquum* (Travassos, 1917) Trav., 1941.

*Anchitrema sanguineum* (Sonsino, 1894)  
Looss, 1899

The only other representative of the Dicrocoeliidae, found in Malayan bats, is *Anchitrema*, an intestinal parasite. This worm is sometimes put in a separate family, i.e., Anchitremidae Caballero, 1960 (compare Caballero, 1960) or included in the family Lecithodendriidae (comp. Skarbilovich, 1948).

Specimens, belonging to the genus *Anchitrema*, were found in the following hosts:

*Glischropus tylopus*, Kuala Senyul, 1-8-63, infected with 14 specimens.

*Glischropus tylopus*, Kuala Senyul, 1-8-63, infected with 2 specimens.

*Rhinolophus sedulus*, Ulu Gombak, 27-6-63, 1 specimen.

*R. luctus*, Janda Baik, 3-9-63, 1 specimen.

*Taphozous melanopogon*, Kodiang, 9-4-63, 1 specimen.

*T. saccolaimus*, Masjid Tanah, 4-9-64, 6 specimens.

*Hipposideros pomona*, Ampang Reservoir, 3-3-64, 1 specimen.

*Hipposideros pomona*, Ampang Reservoir, 3-3-64, 1 specimen.

*Hipposideros pomona*, Ampang Reservoir, 13-3-64, 1 specimen.

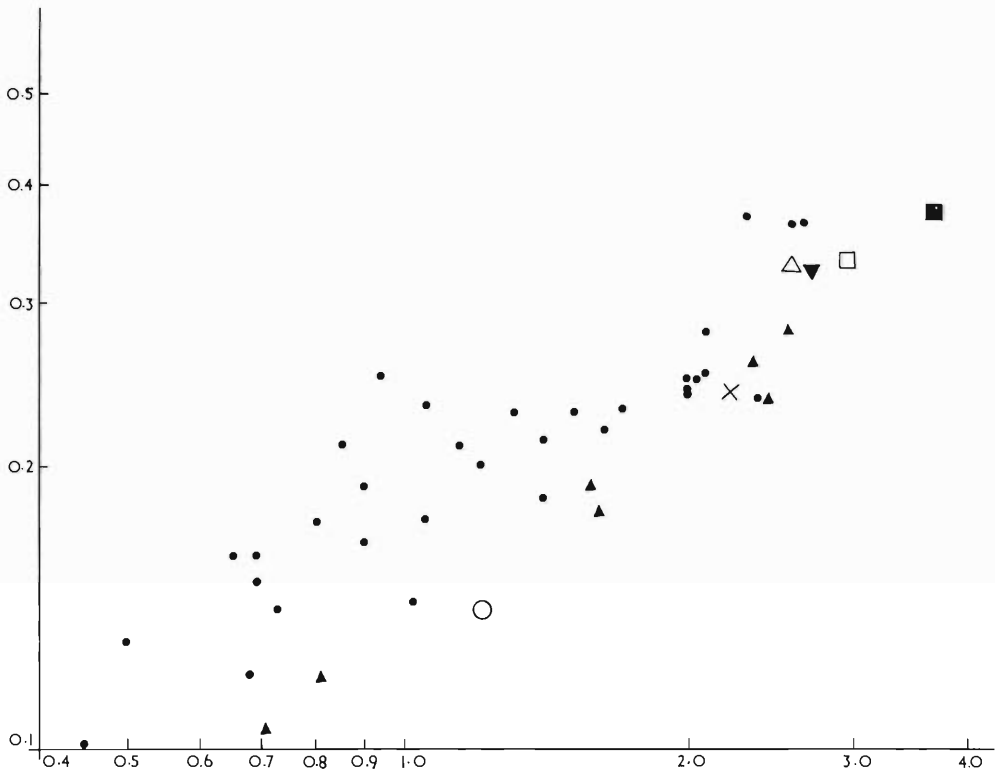


Fig. 6. *A. sanguineum*. Size of acetabulum in specimens of different body size. Coordinates and symbols as in Figure 4.

- Hipposideros pomona*, Ampang Reservoir, 15-4-64, 2 specimens.
- Hipposideros pomona*, Ampang Reservoir, 15-4-64, 1 specimen.
- Hipposideros pomona*, Ampang Reservoir, 9-7-64, 1 specimen.
- Hipposideros pomona*, Ampang Reservoir, 17-9-64, 1 specimen.

All specimens were collected during the months March-September. Of 94 specimens of Microchiroptera from all over Malaya, dissected between October and January, none harbored *Anchitrema*. Of 280 Microchiroptera, dissected between March and September, 13 (= 5%) harbored altogether 33 specimens of *Anchitrema*. In February no bats were examined.

The only locality at which bats were caught over a long period is Ampang Reservoir. The two species *Hipposideros pomona* and *H.*

*cineraceus* were collected every month with the exception of February. The infection with *Anchitrema* in bats from this locality shows the same fluctuation as above.

While 55 *Hipposideros pomona* and 2 *H. cineraceus*, dissected between October and January, did not yield any *Anchitrema*, 7 (= 5%) *H. pomona* out of 138 *H. pomona* and 15 *H. cineraceus*, examined between March and September, were parasitized by altogether eight specimens. These findings suggest a seasonal fluctuation of the infection with this trematode. However, more data are necessary to confirm the results. A seasonal fluctuation with parasites in Malaya is interesting, because Malaya has an extremely constant climate throughout the year, with only minor fluctuations in temperature, moisture, etc.

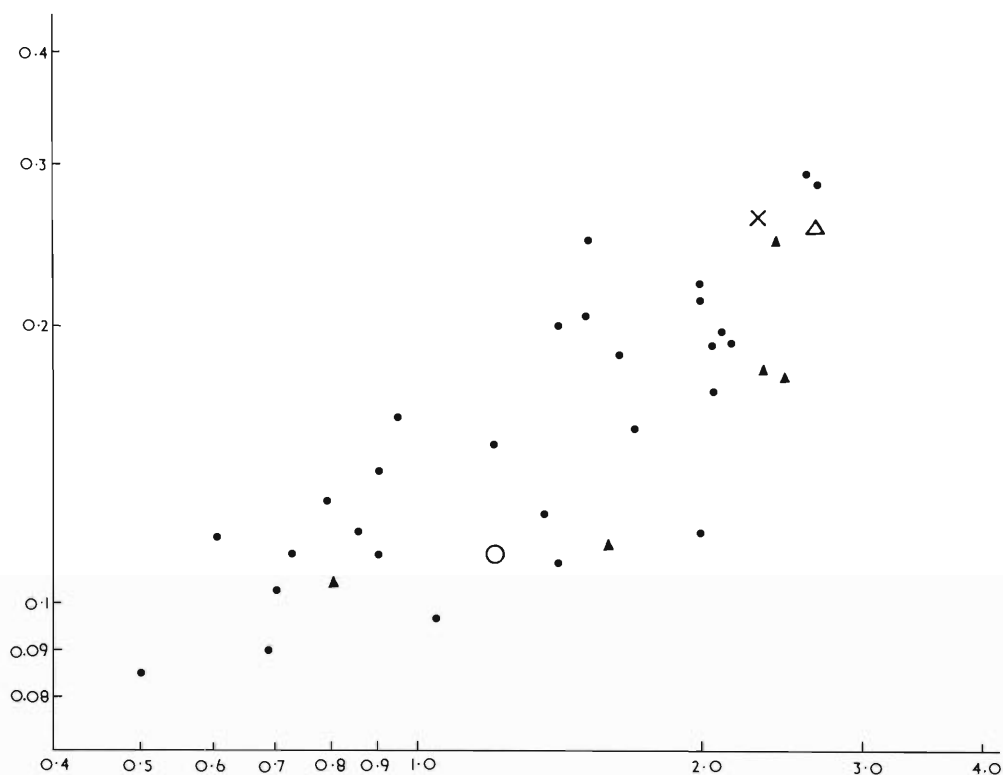


Fig. 7. *A. sanguineum*. Size of ovary in specimens of different body size. Coordinates and symbols as in Figure 4.

The Malayan specimens show a great degree of variation in their body size and, correspondingly, in the relative sizes of various organs and in the proportions of the body. While the small specimens are similar to the description for *A. philippinorum* (Tubangui, 1928) Skarbilovich, 1947, the medium-sized ones correspond to *A. congolense* (Sandground, 1937) Yamaguti, 1958, and the largest ones to *A. sanguineum* (Sonsino, 1894) Looss, 1899.

According to the descriptions, the three species differ in the size of the body (*A. philippinorum* 1.93 mm long, *A. congolense* 3.4 mm long, *A. sanguineum* 3.23–5.15 mm long), in the relative size of various organs, and in the proportions of the body, i.e., there is a relatively longer hindbody in the larger species.

Comparisons between Malayan specimens of different body size and with the three described species show that the Malayan speci-

mens belong to the species *A. sanguineum* and that *A. philippinorum* and *A. congolense* are synonyms of *A. sanguineum*. The differences, mentioned above, are due to allometric growth of various organs and parts of the body.

Since many species descriptions of closely related forms of different size are based on such differences in relative organ sizes and body proportions without taking into account that these may be due to allometric growth, the data for *Anchitrema* are discussed in detail and some considerations concerning the allometric growth in other helminths are given.

In most animals, the growth of body and organs is three-dimensional and, therefore, can be expressed by the function formula  $y = b \cdot x^\alpha$ , where  $y$  = organ size,  $x$  = body size,  $\alpha$  = allometric exponent,  $b$  = constant. This formula can be converted into  $\log y = \log b + \alpha \cdot \log x$  which corresponds graphically, to a straight



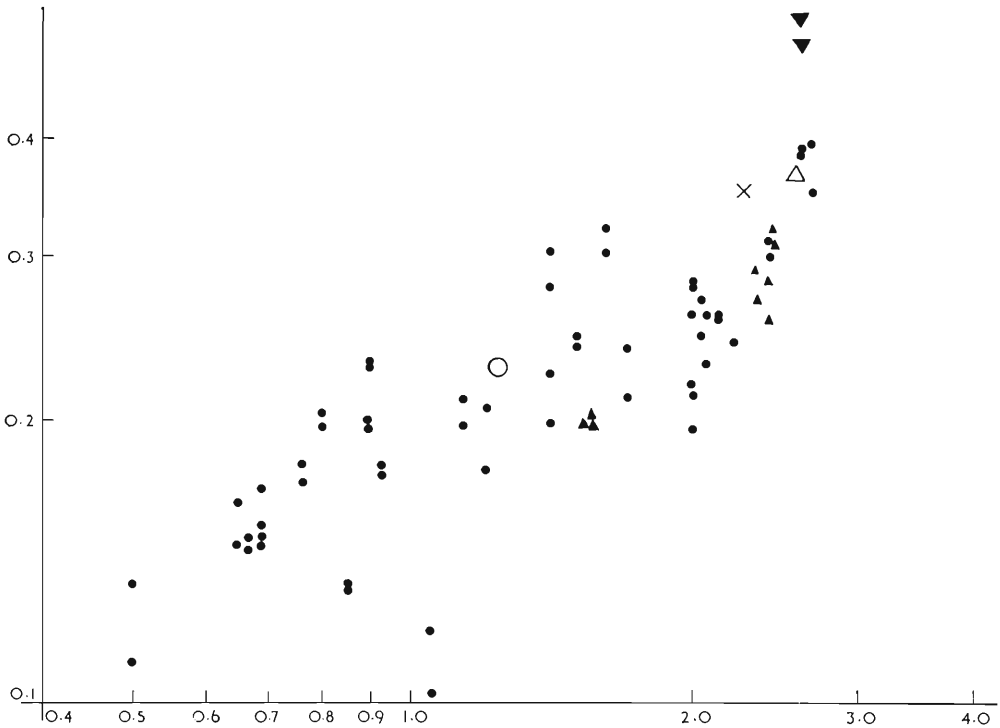


Fig. 8. *A. sanguineum*. Size of testes in specimens of different body size. Coordinates and symbols as in Figure 4.

line in a double-logarithmic system of coordinates. In order to get a linear relationship, this system was chosen for the graphical representation of the data for *Anchitrema*, i.e., for each specimen, the organ sizes are plotted against the body size in a system of double-logarithmic coordinates.

As shown by Rohde (1961), the nematodes *Ancylostoma tubaeforme* (Zedcr, 1800) and *A. caninum* (Ercolani, 1859) also grow at first three-dimensionally, while they grow predominantly in one direction during the last period of growth; in *Ancylostoma* this begins when the worms are 4 mm in length. This part of their growth can be represented as a straight line in a system of linear coordinates.

The size of the specimens of *Anchitrema* and their organs is given as average diameter, calculated from two-dimensional measurements  $(\text{length} + \text{maximum width})/2$ . This is justified, because *Anchitrema*, like most trematodes,

is rather flat. Therefore, the thickness of the worms and organs can, for practical purposes, be neglected. For thick forms like, for instance, amphistomes, the size of body and organs should be given as average diameter calculated from three-dimensional measurements.

The graphs (Figs. 4–8) show that the data for the specimens of *Anchitrema* from all hosts belonging to all three “species” are arranged around continuous lines, thus indicating that the specimens belong to one species. The greatest variability is shown by the genital organs. The allometric exponents for the various organs which can be considered approximate only because of the relatively small number of specimens and the relatively great variability in the organ sizes, are 0.51 for the oral sucker, 0.55 for the pharynx, 0.60 for the acetabulum, 0.62 for the testes, and 0.67 for the ovary. All organs have a strongly negative allometric growth, i.e., they grow much

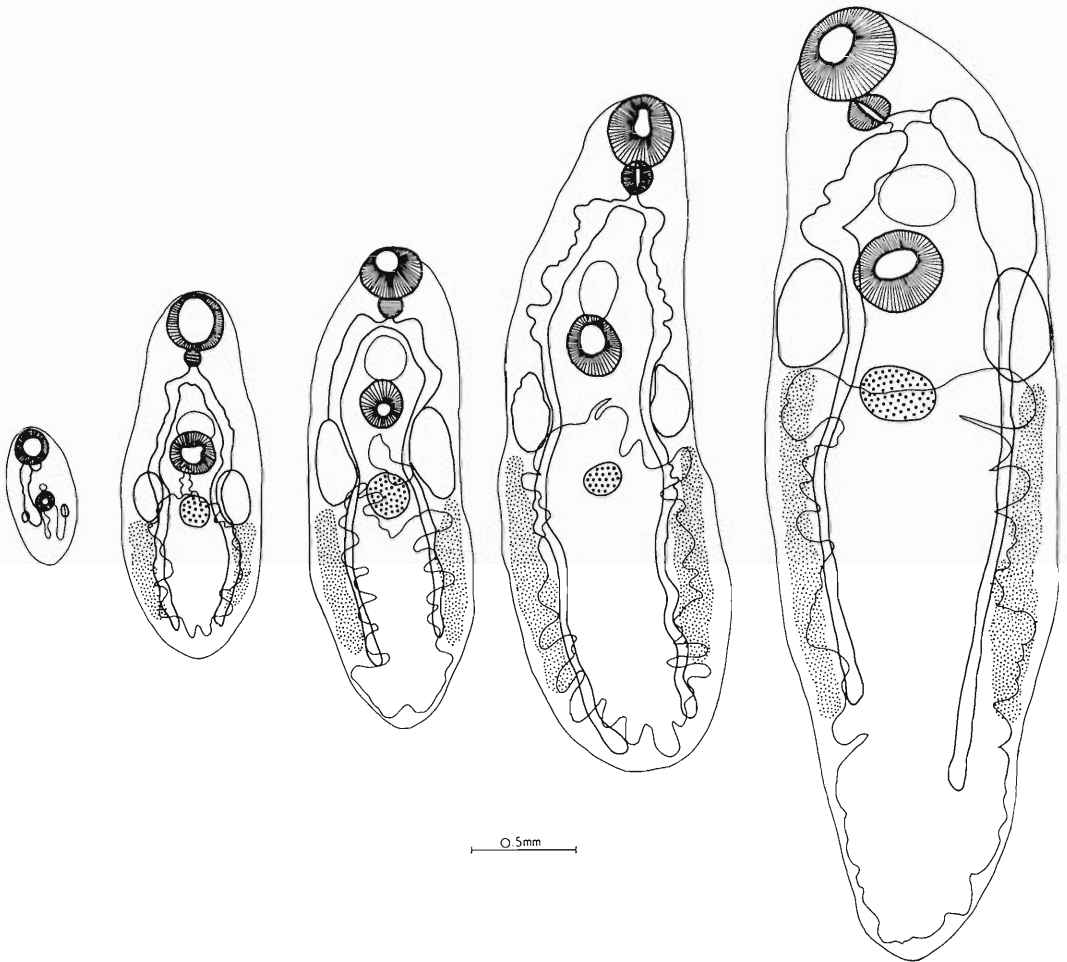


Fig. 9. *A. sanguineum* from Malayan bats. Specimens of different body size. Note: Relatively longer hindbody in larger specimens; suckers and pharynx relatively much larger in small specimens.

more slowly than the whole body (allometric exponent smaller than 1).

Figure 9 shows that the other difference among the three "species," i.e., a relatively longer hindbody in the larger forms, is due to positive allometric growth of the hindbody. In specimens of about 2 mm length, the accumbulum is located at the end of the anterior half of the body (as in *A. philippinorum*), while in larger specimens it is found in the anterior third of the body (as in *A. congo-lense* and *A. sanguineum*). There are intermediate stages between the various forms.

It can, of course, not be completely excluded that certain species differ *only* in their body size. This, however, can be expected only in a very small number of cases. The establishment of a species should be based on different body size alone only, if infection and cross-breeding experiments show that it is reproductively isolated from the related species (compare Rohde, 1959).

If a population is different in size to a described species and if intermediate forms are missing, it is at present difficult to decide whether it is conspecific with the known

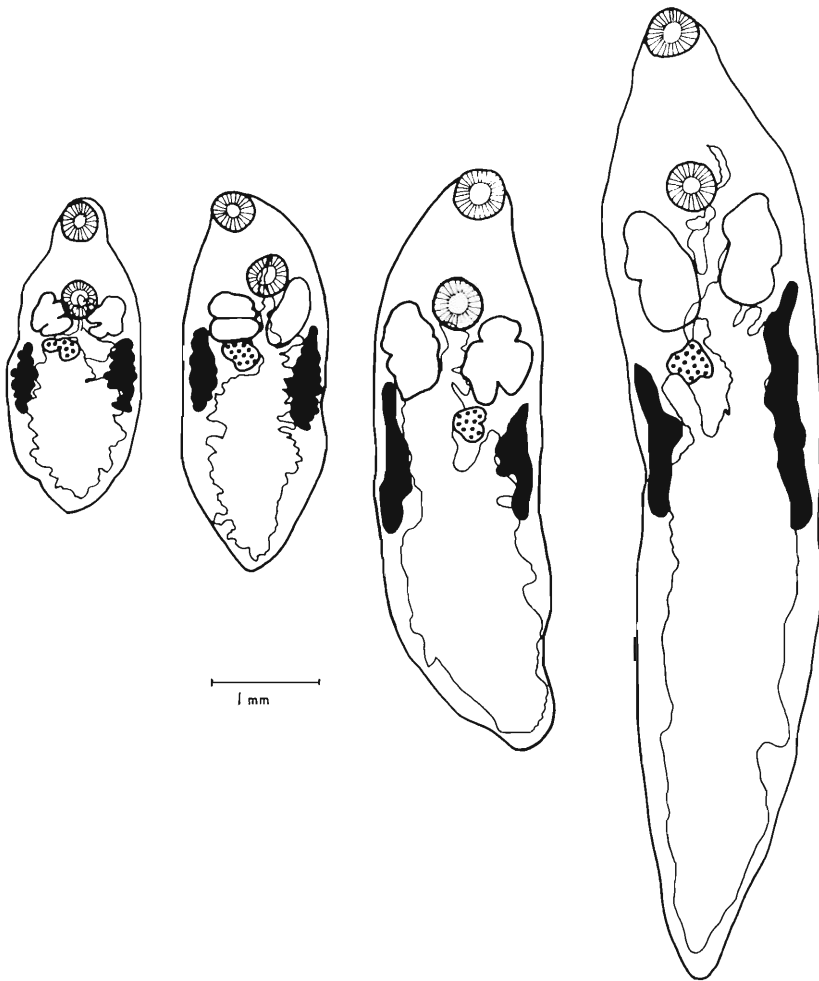


Fig. 10. *Platynosomum fastosum* from Malayan cats. Specimens of different body size. Note: Relatively longer hindbody in larger specimens; suckers and pharynx relatively much larger in small specimens.

species, especially if there are considerable differences in the proportions of the body and the relative size of various organs. To make such a decision possible, it would be useful to know the allometric exponents for many organs in many species. Using these, allometric trends in various groups of helminths could be formulated quantitatively. Extrapolation would show whether a population belongs to a known species or not.

The examination of 13 species of trematodes, belonging to nine monogenetic and digenetic families, showed that allometric trends can actually be demonstrated. Thus, in all species examined (*Platynosomum fastosum* Kossack, 1910 (see Fig. 10), *Zonorchis* sp. (Dicrocoeliidae), *Mesocoelium* sp. (Mesocoeliidae), *Diaschistorchis multitesticularis* Rohde, 1962 (Pronocephalidae), *Maxbraunium haeri* Rohde, 1964, *Odeningotrema hypergenitalis* Rohde,

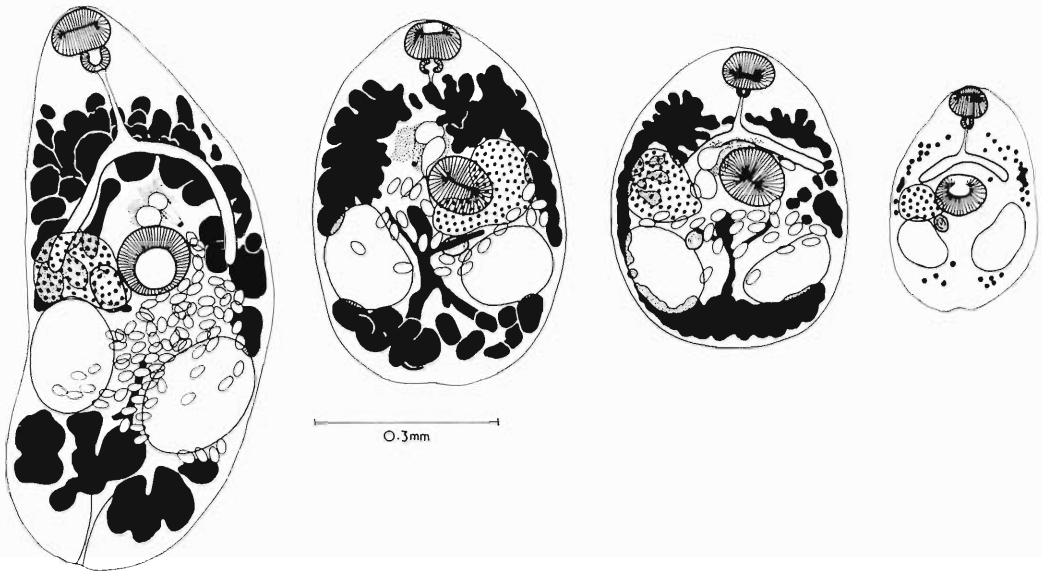


Fig. 11. *Odeningotrema hypergenitalis* from Malayan bats. Specimens of different body size. Note: Suckers and pharynx relatively much larger in small specimens; hindbody in large specimens only slightly longer than in small specimens.

1962 (see Fig. 11). *Novotrema nycticebi* Rohde, 1962 (Lecithodendriidae), *Renschotrema malayi* Rohde, 1964 (Microphallidae), *Kaurma intermedia* Rohde, 1963 (Plagiorchiidae), *Parorientodiscus magnus* Rohde, 1962 (Paramphistomidae), *Opisthorchis viverini* Poirier, 1886 (Opisthorchidae), *Polystomoides malayi* Rohde, 1963, and *P. renschi* Rohde, 1965 (Polystomatidae) the suckers and pharynx (if present) have a strongly negative allometric growth. In the first three species which are characterized by the presence of a very well developed uterus in the posterior part of the body, the hindbody has a strongly positive allometric growth.

In descriptions, measurements of specimens of different body size should be given separately, in order to render possible the calculation of allometric exponents. It is not sufficient to give average sizes and the range of measurements only. These data would also be of great value in studies of speciation and evolution of helminths.

It should also be noted that for diagnostic purposes, data like ratios of sucker/body size

or size of suckers and pharynx can be used only in connection with the absolute body size.

#### SUMMARY

Three hundred ninety-three Malayan bats belonging to 36 species of Mega- and Microchiroptera were examined for helminths. Data on the localities and the frequency of infection with helminths are given. In Megachiroptera (*Cynopterus brachyotis*), trematodes (one *Paralecithodendrium* sp.) were found only once. Only one specimen of acanthocephalan was found.

A new trematode subgenus with one species, *Lutztrema* (*Lutziella*) *microacetabulare*, from the gall bladder of *Myotis mystacinus*, is described. It differs from all other species of the genus *Lutztrema* in the following characteristics: acetabulum smaller than oral sucker, testes symmetrical, vitellaria extend into pre-ovarian space. The only other discocoeleiid found is *Anchitrema sanguineum* from the intestine of several species of Microchiroptera. *A. congolense* and *A. philippinorum* are synonymized with *A. sanguineum*.

Allometric growth of helminths in general and of *Anchitrema sanguineum* in particular is discussed. It is suggested that, in species descriptions, measurements for specimens of different body size should be given separately. This will facilitate the calculation of the allometric exponents for many organs and species. Using these, a decision will be possible as to whether populations which differ from known species in body size, proportions of body, and relative size of organs, are conspecific with them or not. For diagnostic purposes, data like ratios of sucker/body length or size of suckers and pharynx can be used only in connection with the absolute body size.

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**Suppression of *Nematodirus rufaevastitatis* Durbin and Honess, 1951, a Nematode Described from *Ovis aries*, as a Synonym of *Nematodirus davtiani* Grigorian, 1949**

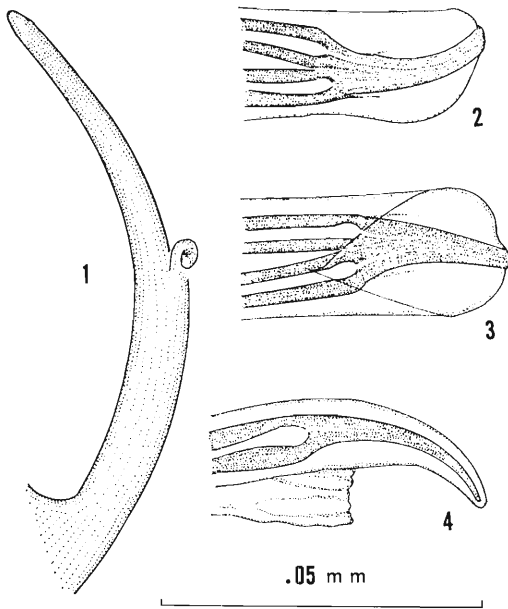
WILLARD W. BECKLUND<sup>1</sup>

*Nematodirus rufaevastitatis* Durbin and Honess, 1951, was described from male specimens collected from *Ovis aries* at Red Desert, Wyoming. To the writer's knowledge, this species has not been reported elsewhere. One holotype (USNM Helm. Coll. 42922) and four paratype specimens (USNM Helm. Coll. 46921) have been studied. A brief redescription based on the type specimens of *N. rufae-*

*vastitatis*, with all measurements in mm, is as follows:

Body length 12.9-14.2 (one male with shriveled cuticle 10.5). Esophagus 0.4-0.486 long. Lateral lobes of bursa large, approximately 0.27 long, with moderate number of bosses. Dorsal lobe not distinct from lateral lobes; lobules minute and not separated by pronounced median notch between the rays. Ventral, lateral, and externodorsal rays similar to figures in original description. Dorsal rays (Fig. 1) approximately 0.09 long, not bifid at distal end, with 0.01 branch near middle.

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Figs. 1-4. Drawings made with the aid of the camera lucida of parts of the holotype specimen of *Nematodirus rufaevastitatis*. 1. Dorsal ray. 2-4. Dorsal, ventral, and lateral aspects, respectively, of the distal end of the spicules.

Spicules 0.941-1.02 long. Proximal part of spicule shafts separate almost to middle, parallel and united by membrane in most of distal half; at distal tenth, shafts diverge laterally and at distal end converge and adjoin. Spicule tip with two large ventrally bent processes; holotype specimen with two additional fine, short, inconspicuous lateral processes (Figs. 2-4). Membranous expansion at spicule tip broad, moderately sharp when spicule tip is flattened dorsoventrally under pressure.

*Nematodirus davtiani* Grigorian, 1949, was described from males collected from the bezoar goat, *Capra aegagrus*, in Armenia. Skrjabin *et al.* (1954) reported it from *Ovis ophion armeniana* in Armenia and according to the Index-Catalogue of Medical and Veterinary Zoology this nematode has also been found in *Rupicapra rupicapra caucasica* in Azerbaïdzhân, and sheep and goats in the Aktyubinsk Region of Kazakhstan. An English translation of the original description is available in

Skrjabin *et al.* (1960); however, the figures in the translation are reduced in size and fine details are obscure. These details are shown by illustrations with the original description of the species and in Skrjabin *et al.* (1954).

The morphologic characteristics of the type specimens of *N. rufaevastitatis* conform closely to the description of *N. davtiani*, as well as to that of a second species, *N. dogieli* Sokolova, 1948. An English translation of the description of the latter species is also available in Skrjabin *et al.* (1960). *Nematodirus davtiani* and *N. dogieli* are apparently almost identical except for small differences in their dorsal rays and dorsal lobes. *Nematodirus dogieli* is described as having dorsal rays which bifurcate at their distal ends with one branch being considerably shorter than the other, and a dorsal lobe which subdivides into two lobes (lobules) with a median notch. The illustration of the bursa of this species indicates that the lateral and dorsal lobes are distinctly separated by deep indentations in the bursal margin lateral to the dorsal rays. *Nematodirus davtiani* is described as having dorsal rays without bifurcations at their distal ends and a short branch near the middle of each ray. The illustrations of the bursa indicate that the dorsal lobe is not distinct from the lateral lobes, lobules are minute, and a median notch is not apparent between the dorsal rays. Thus, the type specimens of *N. rufaevastitatis* are more similar to *N. davtiani* than to *N. dogieli*.

Comparison of the holotype specimen of *N. rufaevastitatis* with the description and seven figures of *N. davtiani* indicates that the two species differ morphologically only in the presence of a pair of fine, short, inconspicuous lateral processes at the tip of the spicule of the holotype specimen of *N. rufaevastitatis* (Figs. 2-3). These processes are not mentioned or illustrated in the description of *N. davtiani*. Because they are inconspicuous in the holotype and were not observed in all the paratype specimens, the writer does not consider them a diagnostic characteristic; therefore, the name *N. rufaevastitatis* is suppressed as a synonym of *N. davtiani*. Hence, *N. davtiani* is a parasite of domestic sheep in Wyoming.

*Nematodirus davtiani* males were also found among nematodes collected from *Ovis cana-*

*densis* on Wildhorse Island, Flathead Lake, Lake County, Montana. The body length of four males ranged from 10.3–12.0 mm, their spicule lengths ranged from 0.845–0.986 mm, and the inconspicuous lateral processes heretofore mentioned were not observed at the spicule tips.

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\_\_\_\_\_, \_\_\_\_\_, AND \_\_\_\_\_. 1960. Essentials of nematodology. Vol. 3. Trichostrongylids of animals and man. English translation. U.S. Dept. Commerce, Washington, D.C. 704 p.

***Trianchoratus*, a New Genus of Monogenea<sup>1</sup>**C. E. PRICE AND W. S. BERRY<sup>2</sup>

## INTRODUCTION

Three host specimens of the kissing gourami, *Helostoma rudolfi* (Machan), were frozen for 24 hours. The recovered gill parasites were treated as described by Price and Mizelle (1964) and measurements made as outlined by Mizelle and Klucka (1953). Appropriate measurements and illustrations were made microscopically with the aid of a filar micrometer ocular and a camera lucida, respectively. All measurements are given in microns.

*Trianchoratus* n. gen.

GENERIC DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. A moderate-sized form provided with a smooth cuticle. Two pairs of eyespots, members of anterior pair larger. Head organs three to five pairs, not well developed. Haptor well set off from body proper; three anchors, a symmetrical pair posterolateral and one located median. Haptor hooks 16 (8 pairs). Ovary pretesticular. Cirrus tubular, with basally articulated accessory piece. Vagina opens on right body margin; seminal receptacle present. Vitellaria moderately well developed, forming two lateral bands. Intestinal crura not observed throughout entire length, but convergence of vitellarial bands strongly indicate a posterior confluency.

TYPE SPECIES: *Trianchoratus acleithrium*.

TYPE HOST: *Helostoma rudolfi* (Machan).

*Trianchoratus acleithrium* sp. n., gen. n.

HOST AND LOCALITY: *Helostoma rudolfi* (Machan), the kissing gourami; Sumatra, Borneo, Java, Malaya, Thailand. (Host obtained from Cordell Farm Supply Co., Milledgeville, Georgia.)

BODY REGION OCCUPIED BY PARASITE: Gills.

SPECIMENS STUDIED: Ten.

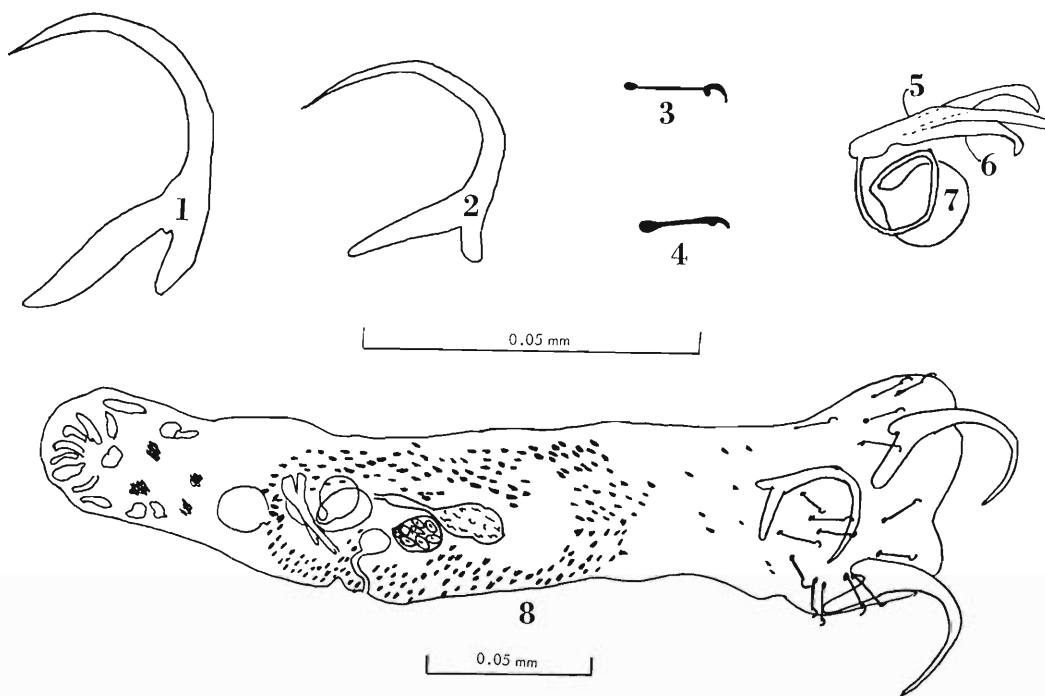
TYPE: Holotype deposited in USNM Helm. Coll. No. 60893, Washington, D.C. Paratypes in authors' collections.

DESCRIPTION: Dactylogyridae, Ancyrocephalinae. A dactylogyrid of moderate size, provided with a thin, smooth cuticle devoid of scales or spines; body length 285 (278 to 300), greatest width of body 57 (52 to 62). Essentially no tendency to form either anterior or lateral cephalic lobes (Fig. 8). Eyespots four in number, members of anterior pair larger and slightly farther apart. Eyespots exhibit a slight tendency to dissociate (probably due to coverslip pressure). Head organs three to five pairs, not well developed. Peduncle in most short and stout; in two specimens it tends to become more elongate. Haptor subpentagonal in outline, well set off from body proper; haptor length 45 (41 to 49), width 73 (70 to 75). Pharynx subspherical, somewhat elongate longitudinally in most; transverse diameter of pharynx 16 (14 to 18) (Fig. 8).

anchors three in number, a symmetrical pair posterolateral, with remaining anchor

<sup>1</sup>This work was supported by the Faculty Research Fund of The Woman's College of Georgia, Milledgeville, Georgia.

<sup>2</sup>The Woman's College of Georgia, Milledgeville, Georgia.



### TRIANCHORATUS ACLEITHRIUM GEN. N. SP. N.

*Trianchoratus acleithrium* n. gen., n. sp. 1, lateral anchor; 2, median anchor; 3, 4, haptoral hooks; 5, cirrus; 6, accessory piece; 7, prostate; 8, entire worm (ventral view).

medial (Figs. 1, 2). Length of lateral anchors 39 (36 to 42), width of base 30 (28 to 32); length of medial anchor 30 (28 to 32), width of base 28 (25 to 31). Each anchor composed of (1) a solid base equipped with well-defined deep and superficial roots, (2) a solid shaft, and (3) a solid point. Anchor wings not observed. Haptoral bars lacking.

Hooks 16 in number (8 pairs), similar in morphology and subequal in size (Figs. 3, 4). Hooks arranged atypically for the Ancyrocephalinae. Each hook composed of (1) a small elliptical-to-ovoid base, (2) a solid shaft, and (3) a sickle-shaped termination provided with an opposable piece. Hook lengths: Posterior-most members, 13 (12 to 14); all others, 15 (14 to 16).

Copulatory complex composed of a cirrus and accessory piece (Figs. 5, 6). Cirrus

tubular, arising from an expanded base, the cirrus tube opening ventrally; length of cirrus 24 (21 to 26). Accessory piece articulated to cirrus base; arises as a sclerotized rod bifurcate into two distinct rami, one of which is recurved distally; length of accessory piece 19 (17 to 21). Prostate single, subcircular, filled with a yellowish, granular substance; the duct of the prostate forms a circle over the glandular portion before entering cirrus base (Fig. 7). Seminal vesicle thin-walled. Vagina not heavily sclerotized, but what is apparently a vaginal atrium opens on or near right body margin just posterior to copulatory complex; a short lightly sclerotized vaginal tube seen in association with a seminal receptacle.

Vitellaria dense in most forms, moderately dense in three; vitellarial granules form into two lateral bands. Intestinal crura not ob-



served throughout their entire length, but vitellaria converge prominently just anterior to haptor, indicating a strong possibility of confluency of crura.

#### DISCUSSION

The posterolateral anchors are attached to cusps of tissue, and can apparently be shifted from a lateral to a posterior position and vice versa. When these anchors are extended laterally, the median anchor moves to a posterior position, and when the laterals are shifted posteriorly, the median anchor moves forward a corresponding distance. Both conditions were observed during the course of the study.

The only other genus of the subfamily Ancyrocephalinae possessing three anchors is *Heteroncoeleidus* Bychowski (1957), which contains but the type species, *H. buschkieli*. Bychowski's genus possesses three median "Dactylogyrus-type" anchors plus the rudiment (point and part of shaft) of a fourth anchor. The haptor armament also includes two bars. Examination of figures given for this parasite in Yamaguti (1963) indicates that the accessory piece is not attached to the cirrus base.

*Trianchoratus* also possesses three anchors, but lacks both the rudimentary fourth anchor and haptor bars. Additionally, the accessory piece is basally attached to the cirrus. It thus appears that *Trianchoratus* is readily established as a new genus of the Ancyrocephalinae.

#### SUMMARY

Three host specimens of the kissing gourami, *Helostoma rudolfi* (Machan), were examined

for gill parasites.<sup>3</sup> Ten parasite specimens of a new genus belonging to the subfamily Ancyrocephalinae were recovered. This new genus, *Trianchoratus*, is unusual in that it possesses three anchors, two posterolateral and one median. This parasite is most closely related to *Heteroncoeleidus* Bychowski, 1957, the members of which also possess three anchors. The basic differentiating factors between these two genera are: (1) *Heteroncoeleidus* possesses the rudiments of a fourth anchor, missing in *Trianchoratus*, (2) the haptor bars present in *Heteroncoeleidus* are lacking in this new form, and (3) the accessory piece is basally articulated in *Trianchoratus*, whereas it is nonarticulate in *Heteroncoeleidus*. The type species, *Trianchoratus acleithrium*, is morphologically described.

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<sup>3</sup> Host specimens were obtained from Cordell's Farm Supply, Milledgeville, Georgia, to which firm the authors wish to express their thanks.

**On a New Nematode, *Procamallanus muelleri* n. sp. from the  
Stomach of a Freshwater Fish, *Heteropneustes fossilis*<sup>1</sup>**

VINOD ACRAWAL<sup>2</sup>

INTRODUCTION

Baylis (1923) created the genus *Procamallanus* with *P. laeviconchus* (Wedl, 1862) as its type. Since then a large number of species have been reported from various parts of the world. To date the genus *Procamallanus* contains some 41 species. Olsen (1952) created a new genus *Spirocamallanus* to accommodate species of *Procamallanus* possessing spiral thickenings on the buccal capsule. Ali (1956) divided the genus *Procamallanus* into three subgenera on the basis of differences in the characters of spicules. He established the subgenera *Procamallanus*, *Monospiculus*, and *Isospiculus*. The subgenus *Procamallanus* is characterized in having unequal spicules, the subgenus *Isospiculus* in having two equal spicules, and the subgenus *Monospiculus* in having a single spicule. Ali (1960) added a fourth subgenus *Aspiculus* for *P. aspiculus* Khera, 1955 for the absence of spicules. Ali (1960) considered *Spirocamallanus* to be a synonym of *Procamallanus* due to large variations in the markings on the buccal capsule and in having certain other structures in addition to spiral thickenings on the wall of the buccal capsule. He also gave a key to the species of the genus *Procamallanus* in which there are certain discrepancies, and it appears that Ali is at variance with the original literature. He placed *P. planoratus* in the group of species having buccal capsule with spiral thickenings while they are absent in the original description.

Campana-Rouget (1961) did not agree with Ali and suppressed the subgenera *Monospiculus*, *Isospiculus*, and *Procamallanus*. He also considered *P. aspiculus* Khera, 1955 to be a synonym of *P. bagarii* Karve et Naik, 1951. Pande, Bhatia, and Rai (1963) and Fernando and Furtado (1963) are also against dividing the genus on the basis of differences in the

characters of the spicules. I agree with these authors because the spicules are weakly chitinized in camallanids and are therefore of little systematic importance. I disagree with Campana-Rouget (1961) who considers *P. aspiculus* to be a synonym of *P. bagarii*. *P. aspiculus* is distinct from *P. bagarii* in not having spicules instead of being present. Ali (1956, 1960) described seven species of the genus *Procamallanus* from freshwater fishes of India. In the opinion of the author *P. hyderabadensis* and *P. viviparus* should be considered synonyms of *P. singhi*, *P. clarius* a synonym of *P. heteropneustus*, and *P. ophicephalus* a synonym of *P. globoconchus*. Ali distinguished *P. viviparus* from *P. singhi* and *P. hyderabadensis* in the possession of wide alae which meet in front of the ventral surface, in possessing unequal dissimilar spicules, and in the number and arrangement of anal papillae. He also distinguished *P. hyderabadensis* from *P. singhi* in having 11 pairs of caudal papillae instead of 10, in having relatively shorter caudal alae, and in the absence of two short processes at the tip of female tail. In *P. hyderabadensis* the spicules measure (right 0.18–0.20; left 0.042–0.05 mm) while in *P. viviparus* (right 0.186; left 0.069 mm) and in *P. singhi* (right 0.20–0.205; left 0.042–0.044 mm) in length. In *P. hyderabadensis* there are six preanal, four postanal; in *P. viviparus* six preanal, four postanal; and in *P. singhi* seven preanal and four postanal papillae. The above forms are identical in all respects except for minor differences in the nature of caudal alae and in the possession of two short processes at the tip of female tail. These characters are insignificant and of little systematic importance. All these forms are therefore conspecific. Ali distinguished *P. clarius* from *P. heteropneustus* in having well-developed caudal alae, in the number of anal papillae, and in the ratio of spicule length. In *P. heteropneustus* the spicule ratio is 7:4 while in *P. clarius* it is 3:1; the caudal alae in *P. heteropneustus* are prominent while in *P. clarius* they are feebly developed; and there are 13 pairs of caudal

<sup>1</sup> Part of thesis accepted for the degree of Doctor of Philosophy at the University of Lucknow, Lucknow.

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The work has been carried out under the guidance of Dr. S. P. Gupta, to whom the author is indebted for invaluable help and encouragement.

papillae present in *P. heteropneustus* while there are 14 pairs in *P. clarius*. The differences pointed out by Ali are specific variations and hence *P. clarius* falls as a synonym of *P. heteropneustus*. Ali distinguished *P. ophicephalus* from *P. globoconchus* in the shape and symmetry of caudal alae, the arrangement of the caudal papillae, and in the length of spicules. In *P. ophicephalus* there are eight pairs of preanal, five pairs of postanal, and two pairs of circumanal papillae while in *P. globoconchus* there are eight pairs of preanal and five postanals. In *P. ophicephalus* the right spicule measures 0.15 mm, left 0.034 mm, while in *P. globoconchus* (right 0.15–0.18; left 0.03–0.038 mm) in length. In *P. ophicephalus* the caudal alae are broad while in *P. globoconchus* caudal alae are broad and asymmetrical. *P. ophicephalus* agrees in all respects to *P. globoconchus* except in the possession of two pairs of circumanal papillae and in the nature of caudal alae. In the opinion of the author the characters used by Ali are not valid and hence both are identical.

Family Camallanidae Railliet and Henry, 1915

*Procamallanus muelleri* n. sp.  
(Figs. 1–5)

Only two males, one of which is damaged, and a large number of females were collected from the stomach of a freshwater fish, *Heteropneustes fossilis* (Bloch.) at Lucknow. All measurements are in mm.

**DESCRIPTION:** Worms slender, slight difference in size of sexes. Mouth surrounded by two lateral and four submedian papillae. Buccal capsule barrel-shaped, with smooth lining without ridges or leaf crowns. Basal part of buccal capsule thickened to form a rim articulating with anterior end of esophagus. Cuticle more striated in female than in male specimens. Striations, 0.012–0.015 apart in male and 0.015–0.018 apart in female.

**MALE:** Body 4.64 long, 0.11 wide. Head 0.05 in diameter. Buccal capsule 0.07 × 0.04 in size. Esophagus divided into two parts, an anterior muscular club-shaped part measuring 0.27 × 0.04 and a posterior glandular part, 0.24 × 0.03. Entire esophagus 0.51 long. Nerve ring 0.12 and excretory pore 0.18 from anterior end. Tail bluntly rounded at tip,

0.13–0.22 long. Caudal end of male curled ventrally and forms a single turn of a spiral. Caudal alae well developed, extends to tip of tail, 0.35–0.51 long. Fourteen to 18 pairs of anal papillae of which eight to nine pairs preanal, one pair adanal, and five to seven pairs postanal. Preanal papillae pedunculated, situated almost at regular intervals. One pair pedunculated adanal papillae just close to anus. Two pairs of very small sessile papillae surround anal aperture, regarded as circumanal. Three to five postanal papillae long, pedunculated, and close together in a group and most posterior one very small, sessile, and isolated. A pair of phasmids also observed near tip of tail. Spicules tubular, equal, similar, broader at anterior end and sharply pointed at posterior end, 1.40–1.41 long. Gubernaculum absent.

**FEMALE:** Body 3.56–6.54 long, 0.11–0.17 wide. Head 0.05–0.06 in diameter. Buccal capsule 0.057–0.07 × 0.04–0.05 in size. Anterior muscular esophagus measuring 0.28–0.31 × 0.04–0.06; posterior glandular esophagus 0.31–0.39 × 0.05–0.07. Entire esophagus 0.59–0.69 long. Nerve ring 0.13–0.16 and excretory pore 0.17–0.21 from anterior end. Tail short, bluntly rounded at tip terminating in two small spinous processes, 0.10–0.15 long. Vulva postequatorial at 1.98–3.61 from anterior end. Uterus filled with a large number of larvae and eggs.

**HOST:** *Heteropneustes fossilis* (Bloch.).

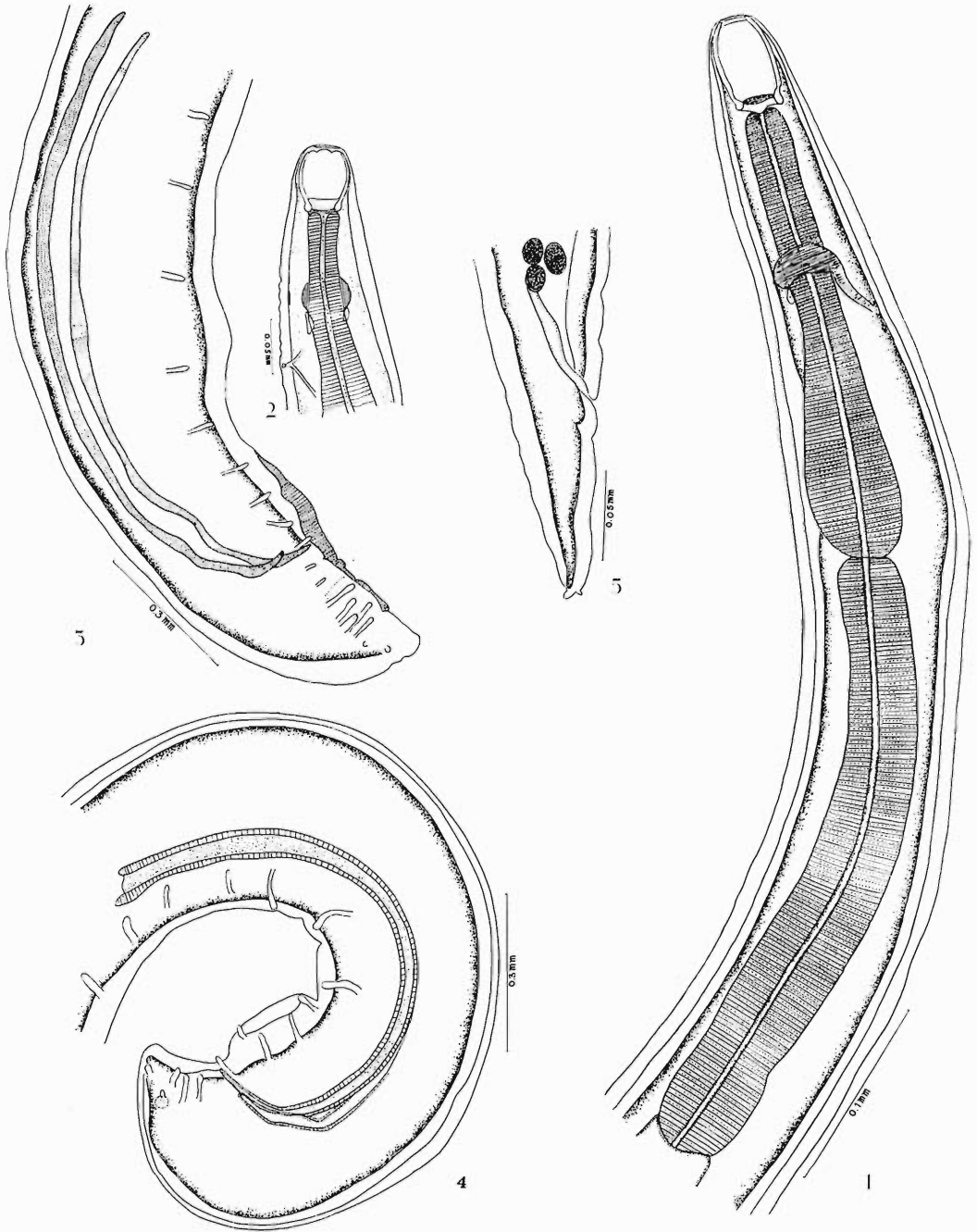
**LOCATION:** Stomach.

**LOCALITY:** Lucknow.

**TYPE SPECIMENS:** Paratype and holotype specimens will be deposited in Dr. G. S. Thapar's Helminthological Collection, Lucknow, U.P., India.

#### DISCUSSION

The following species of the genus *Procamallanus* Baylis, 1923 with smooth buccal capsule without transverse or spiral thickenings and no leaf crown have been described so far from fishes, namely *P. laeviconchus* (Wedl, 1862) Baylis, 1923; *P. signai* Yamaguti, 1935; *P. murrayensis* Johnson et Mawson, 1940; *P. lonis* Yamaguti, 1941; *P. annulatus* Yamaguti, 1954; *P. aspiculus* Khera, 1955; *P. heteropneustus* Ali, 1956 (syn. *P. clarius* Ali, 1956); *P. spiculogubernaculus*



Figs. 1-5. *Procammallanus muelleri* n. sp. 1. Anterior region of male. Lateral view. 2. Anterior region of male showing excretory pore. Lateral view. 3. Male tail. Lateral view. 4. Male tail. Lateral view. 5. Female tail. Lateral view.

Agrawal, 1958; *P. daccal* Gupta, 1959; *P. mathurai* Pande, Bhatia, and Rai, 1963; and *P. confusus* Fernando and Furtado, 1963. *Procamallanus muelleri* n. sp. differs from *P. aspiculus* in the possession of two equal spicules instead of being absent. *P. muelleri* can also be distinguished from *P. laeviconchus*, *P. signai*, *P. murrayensis*, *P. lonis*, *P. annulatus*, *P. heteropneustus* (syn. *P. clarius* Ali, 1956), *P. spiculogubernaculus*, and *P. confusus* in having equal spicules instead of unequal. *P. muelleri* can further be distinguished from *P. laeviconchus* in having vulva postequatorial instead of preequatorial. *P. muelleri* differs from *P. daccal* and *P. mathurai* in having two equal spicules instead of one. *P. muelleri* is closely allied to *P. planoratus* in having equal spicules but differs from it in having eight to ten pairs preanal, one pair adanal, two pairs circumanal, and five to seven pairs postanal papillae.

The following four species *P. heteropneustus* (syn. *P. clarius* Ali, 1956), *P. spiculogubernaculus*, *P. mathurai*, and *P. confusus* are known from the same host *Heteropneustes fossilis*. *P. muelleri* differs from *P. spiculogubernaculus*, *P. heteropneustus*, and *P. confusus* in having equal spicules instead of unequal, in the number and arrangement of anal papillae, and in having two small spinous processes at the tip of female tail. Further it can be distinguished from *P. spiculogubernaculus* and *P. confusus* in the absence of a gubernaculum. The new form can also be distinguished from *P. heteropneustus* in having glandular esophagus nearly equal to muscular esophagus instead of being glandular esophagus nearly twice the length of muscular esophagus. *P. muelleri* differs from *P. mathurai* in having two equal spicules, in the absence of gubernaculum, in the number and arrangement of anal papillae, and in having two small spinous processes at the tip of female tail. It is possible that they all belong to the same species but on the basis of the descriptions and of the present status of camallanid taxonomy each of these species is quite different. On the basis of the above mentioned differences, the present specimens are considered here to be a new species to which the name *Procamallanus muelleri* is given. The new species is named in honor of Justus F. Mueller, State

University of New York, Upstate Medical Center, New York, U.S.A.

Key to the species of the genus  
*Procamallanus* Baylis, 1923 from freshwater fishes of India, Pakistan, and Ceylon

1. Buccal capsule with spiral thickenings 2  
Buccal capsule without spiral thickenings ..... 7
2. Spicules equal ..... *P. mehrii*  
Spicules unequal ..... 3
3. Gubernaculum present ..... 4  
Gubernaculum absent ..... 5
4. Five pairs of preanal papillae; female tail without caudal spines .....  
..... *P. gubernaculus*  
Seven pairs of preanal papillae; female tail with three spines at its tip .....  
..... *P. bagarii*
5. Three pairs of circumanal papillae present ..... *P. globoconchus*  
Circumanal papillae absent ..... 6
6. Fifteen pairs of caudal papillae .... *P. attui*  
Eleven pairs of caudal papillae .. *P. singhi*
7. Forms without spicules ..... *P. aspiculus*  
Forms with spicules ..... 8
8. Gubernaculum present ..... 9  
Gubernaculum absent ..... 12
9. Spicule single ..... 10  
Spicules two ..... 11
10. Twelve pairs of caudal papillae .....  
..... *P. mathurai*  
Seventeen to eighteen pairs of caudal papillae ..... *P. daccal*
11. Seven pairs of caudal papillae and gubernaculum incompletely fused with small spicule .....  
..... *P. spiculogubernaculus*  
Eighteen pairs of caudal papillae and gubernaculum do not fuse with the spicule ..... *P. confusus*
12. Unequal spicules ..... *P. heteropneustus*  
Equal spicules ..... *P. muelleri* n. sp.

SUMMARY

*Procamallanus muelleri* n. sp. has been described from a freshwater fish, *Heteropneustes fossilis*. A key to the species of the genus *Procamallanus* from freshwater fishes of India, Pakistan, and Ceylon is given. *P. hyderabadensis* and *P. viviparus* are considered to be synonyms of *P. singhi*, *P. clarius* a synonym

of *P. heteropneustus*, and *P. ophicephalus* a synonym of *P. globoconchus*.

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### Presentation

## 1965 ANNIVERSARY AWARD OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON 415th Meeting, 16 December 1965

Gilbert Fred Otto, whom we honor this day with the Anniversary Award of the Helminthological Society of Washington, has a long, varied, and full career in parasitological and other endeavors.

The man of whom we speak is commonly known as "Otto" to those who have known him longest; perhaps today more commonly called "Gil" or "Gilbert" by former students and those who have recently come to know him, but he is also one who is seldom called "Dr. Otto"; and it will be recalled by those who knew him as a professor that his students seldom referred to him as "Professor." We commonly heard reference to him by certain well-known, compound, graphic appellations with one or more epithetical modifiers. I do not know the names that I have applied to this man, but I recall having referred to him in public as a "parasitological sputnik" and also as "the iron man." It is probably these appellations that I am now to defend.

In his parasitological sorties, Otto has not only circled the globe but has well identified himself all around the circuit. You will recall that he was a professor, researcher, teacher, and administrator for 27 years at Johns Hopkins University School of Hygiene and Public

Health after having been a graduate student there for something over 2 years; during this time he was also public health worker, developer of graduate students, and prolific writer. During the years that he served as assistant dean of the school (1940-1947) he left indelible impressions not only on faculty and students but also on the dean, not to mention the president of the university. After this experience, which was primarily in fields where parasitology was oriented to human medicine, he went to the Abbott Laboratories where he was made director of veterinary parasitology and, as though that were not challenge enough, was soon made director of agricultural and veterinary research. During these careers in which he also became involved in all manner of problems relating to human and animal sciences, theoretical and applied, he found it possible to teach protozoology and helminthology at Douglas Lake Biological Station.

A great amount of time meanwhile was given to serving on Evaluation Committees for NIH, as consultant to the Army and Public Health Service, as responsible officer and committeeman in many professional organizations, and even as adviser to university presidents



Dr. Gilbert Fred Otto (left) receiving the 1965 Anniversary Award of the Helminthological Society of Washington (presented by A. O. Foster).

who were looking for higher competence in key positions on their staffs.

It is, of course, known to all who know Otto well that his involvements with Boy Scouts, PTA's, local academies of science, public health committees, and even as long-term head man of the Lake Bluff Mosquito Eradication Committee are incredible, and nearly so are his routine, sometimes unmentionable, exploits. He is at home with anybody, anywhere; and he has never lost respect for the man who works with his hands; indeed, he does a great deal of it himself with skill, flourish, and aplomb!

I need not state that he decided to go for an education—of sorts, anyway—when he went to Kalamazoo College. Also, before he

went to Baltimore, he spent 2 years under the paternal sympathetic tutelage of Dr. James E. (Uncle Jimmie) Ackert at Kansas State University in Manhattan.

His published titles, beginning in 1927, number more than 100. In the last edition of *American Men of Science*, Otto listed his special interests as: Epidemiology of parasitic diseases; immunity to animal parasites; chemotherapy of parasitic diseases; pharmacology of arsenic and antimony; ascariasis; trichinosis; hookworm disease; filariasis; amebiasis; trypanosomiasis; malaria; leucocytozoon; and coccidiosis. He could have listed other subjects, e.g., capillariasis, echinococcosis, to each of which he made contributions of note. To recount specific research accomplishments

would be a long undertaking; suffice it to mention that on many occasions over the years I have myself felt constrained to comment upon what he has presented at scientific meetings!

In the American Society of Parasitologists, Otto has served in about every office and has been at one time or another a member of just about every important committee. He was its president in 1957. He has been a veritable mentor in innumerable other societies, not the least of which is the Helminthological Society of Washington.

In this organization, he has been a member since 17 September 1927. He was a founder of the Brayton H. Ransom Memorial Trust Fund in 1936, and has been a trustee of the fund continuously since that date and chairman of the board since 1956. He is also a benefactor of the fund not only by direct contribution to it but through the good use that he has made for so many years of interest-bearing capital. It would be too long a list to cite the innumerable committees of "Helm Soc" on which he has diligently worked and overwhelmingly contributed, but we should perhaps note that he was president of the Society in 1935-1936. He has been a member of the Editorial Committee of the *Proceedings* since their independent publication in 1934, and editor of the *Proceedings* for the past 14 years, beginning in 1952.

I want to say of this man that I have found it possible, despite what has been said, to get him to give long hours to discussion, much of it simply "drifting on the billows of whatnot." I have never known him to be tired. I have known him to go through two or three extremely difficult situations without even being discouraged. He is probably not gifted today with the glowing health that he enjoyed only a decade or so ago, but he is certainly the same indefatigable man.

Although there are a few matters on which Otto has neither convinced me nor I him, they are inconsequential by comparison with the many and larger ones on which we see "eye to eye." It has indeed meant much to me to have had Otto as a friend and colleague for 35 years. I know whereof I speak regarding this tireless "iron man" and this redoubtable, indomitable "parasitological sputnik."

The Awards Committee and the Society, while deserving congratulations on their choice, found this one easy, direct, and natural. In deference to my friend, yet with sincere thanks to Dr. "Peg" Stirewalt, Chairman of the Awards Committee, who asked me to make this presentation in behalf of the Society, I not only congratulate the 1965 recipient of the Anniversary Award of the Helminthological Society of Washington, Dr. Gilbert Fred Otto, but have kept my remarks, as seemed fitting, to those that were easy, direct, and natural.—A. O. Foster.

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## MINUTES

### Four Hundred Thirteenth—Through Four Hundred Twentieth Meetings

413th Meeting: Armed Forces Institute of Pathology, Walter Reed Army Medical Center, Washington, D. C., October 13, 1965. Fifty-fifth Anniversary meeting. Nominations of Dr. Francis G. Tromba as Editor of the *Proceedings*, starting in January 1966, and Dr. Dewey Raski to the Editorial Committee, from January 1966 to December 1966 to 1970, were approved by the Society. Papers presented: Mendelian Genetics after a Century,

by John W. Crenshaw; and The inheritance of X-irradiation-induced effects in the rat tapeworm, *Hymenolepis diminuta* by E. L. Schiller. A film on "Medical Genetics" was also shown.

414th Meeting: Beltsville Parasitological Laboratory, Beltsville, Maryland, November 19, 1965. Officers elected: D. B. McMullen, President; M. B. Chitwood, Vice-President; W. B. DeWitt, Recording Secretary; E. M. Buhner, Corresponding Secretary-Treasurer.



Papers presented: Immunizing action of *in vitro* attenuated *Histomonas meleagridis* in chickens and turkeys, by E. E. Lund, P. C. Augustine, D. J. Ellis; *Sarcocystis*, by L. A. Spindler; Influence of NCTC 109, serum, and swine kidney tissue cultures on development of *Stephanurus dentatus* to fourth stage, by F. W. Douvres, F. G. Tromba, and D. J. Doran; The endogenous stages of the swine coccidium *Eimeria deblicieki* Douwes, 1921, by J. M. Vetterling; Leishmaniasis infections involving army personnel in Panama, by B. Walton.

415th Meeting: Walter Reed Army Institute of Research, Washington, D. C., December 16, 1965. The 1965 Anniversary Award of the Helminthological Society of Washington was presented to Dr. Gilbert Otto. Appointments made: H. H. Vegors, Assistant Corresponding Secretary-Treasurer; J. Humphrey, Librarian; D. Shorb, Archivist; A. O. Foster, Representative to Washington Academy of Sciences; C. G. Durbin, Representative to ASP; E. Sadun, Anniversary Award Committee; L. Diamond and D. Lincicome, Members-at-Large of the Executive Committee. A motion was approved to send a message of condolence on behalf of the Society to the family of Dr. Emmett Price. Papers presented: Studies on susceptibility of splenectomized chimpanzees to *Plasmodium falciparum*, by R. L. Hickman, W. S. Gochenour, Jr., J. D. Marshall, Jr., and N. B. Guilloud; Susceptibility of the gibbon, *Hylobates lar*, to *Falciparum malaria*, by R. A. Ward; Studies on the mechanism of primaquine action: A metabolic requirement for *in vitro* erythrocyte toxicity, by J. N. George, R. L. O'Brien, S. Pollack, and D. J. Wicker; Parasitological, clinical, hematological, pathological, and serological studies of chimpanzees infected with *Schistosoma mansoni*, by E. H. Sadun, F. von Lichtenberg, J. I. Bruce, R. L. Hickman, and J. Smith; Murine angiostrongylosis in the Pacific and in the Indian Oceans, by J. E. Alicata (U. of Hawaii).

416th Meeting: National Institutes of Health, Bethesda, Maryland, January 17, 1966. The membership voted to contribute \$25 to the Joint Board on Science Education to support education activities in science and engineering for the youth of the area. Papers presented: Collection of cercariae from large

volumes of water by continuous-flow centrifugation, by L. Olivier; Studies on the transmission of toxoplasmosis, by L. Jacobs and M. Melton; Studies on the tissue stages of simian malarias, by J. Held; Fine structure of the first generation merozoites of *Eimeria bovis*, by H. G. Sheffield and D. M. Hammond; Studies on the relation of Ferrihemic Acid to drug-resistance in *Plasmodium berghei*, by K. O. Phifer, F. L. Yielding, and S. N. Cohen.

417th Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, February 18, 1966. Auditor's report was read and approved. The membership voted to continue through the coming year the same financial and page authorization policies for the Proceedings that were adhered to during 1965. A committee appointed by the President presented an up-to-date compilation of the Society's Constitution and Bylaws. Copies are to be prepared and circulated to the membership. Papers presented: Observations on the life history and pathogenicity of a sporozoan parasite of Chesapeake Bay oysters, by C. J. Sindermann; Fluorescent antibody studies of haplosporidian parasites of oysters, by J. H. Barrow; Observations on some unsettled problems in the life history of aquatic amoebae, by T. K. Sawyer; Host range of *Plasmodium circumflexum*, by C. M. Herman; Trichinosis in a Maryland raccoon, by D. J. Winslow; Problems and progress in the establishment of a blackfly colony, by I. B. Tarshis.

418th Meeting: Department of Agriculture, Plant Industry Station, Beltsville, Maryland, March 16, 1966. Papers presented: Morphological aspects of *Panagrellus radivivus*, by E. Powers; The taxonomic research program in nematology investigations, by A. M. Golden; A turbellarian preying on nematodes, by R. Sayre; On the classification of the insect parasitic family Allantonematidae, by W. R. Nickle.

419th Meeting: Adult Education Center, University of Maryland, College Park, Maryland, April 20, 1966. It was decided that the benefits and drawbacks of the proposed affiliation with AIBS will be studied further and brought to vote at a later meeting. A film, "Control of sub-periodic filariasis," by J. F. Kessel was shown. Papers presented: Etiology and pathology of tropical pulmonary eosino-

philia, by W. Pacheco; Problems in prepatent filariasis, by D. L. Price; Field studies of filariasis in monkeys in Taiwan, by J. F. Bergner, Jr.; Recent international development in filariasis, by L. A. Jachowski, Jr.

420th Meeting: New Bolton Center, University of Pennsylvania, Kennet Square, Pennsylvania, May 14, 1966. Papers presented: *Theileria parva* studies in the tick vector, H. M. Martin; Development and growth of *Echinostoma revolutum* in natural and ectopic sites, B. Fried; Species specific antigens of *Toxocara canis*, E. Jeska; Gel diffusion studies of *Ascaris suum* larval antigens, J. F. Williams; Cultural and antigen studies of *Trypanosoma theileri*, E. J. Splitter; Interactions between ascaris larvae and lymphocytes, E. J. L. Solusby.

The following were elected to membership at the meetings indicated: **413th**—L. M. Wiest, Jr., D. A. Dean, R. R. Calhoun, I. A. Siddiqui; **414th**—W. D. Hope, J. B. Poole, W. G. Dyer, J. A. Ansari, R. S. Isenstein, D. Teliz; **415th**—R. L. Beaudoin, L. M. Howard, L. K. Martin, D. M. McKinstry, B. C. Redington, W. A. Reid, Jr.; **416th**—M. T. Franklin, F. Jimenez-Millan, W. A. Rogers; **417th**—L. I. Miller, M. S. Ferguson, Devandra Nath Das, Lie Kian Joe, B. McDaniel, Jr., Songul Aytan, R. M. Sayre; **418th**—G. Pacheco, C. E. Price, A. L. Ager, Jr.; **419th**—C. M. Schneider, V. Sprague, J. M. Kinsella, W. P. Carney; **420th**—J. A. Poiley, C. Sindermann.

WILLIAM B. DEWITT  
Recording Secretary

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### New Books

GOODEY, J. BASIL, MARY T. FRANKLIN, AND DAVID J. HOPPER: **T. Goodey's The Nematode Parasites of Plants Catalogued Under Their Hosts.** The style of the body of the book is one of convenience. On the left side of the page is an alphabetical catalogue of the scientific and common names of plants which have been reported as hosts of nematodes. On the right side of the page, corresponding to each host, are the names of the nematodes, arranged alphabetically. There are 35 pages of references. Commonwealth Agricultural Bureau, Central Sales Branch, Farnham House, Farnham Royal, Bucks, England. Published 31 December, 1965, 214 + IV pp. 60s. Od. (\$9.00).

PEACHEY, J. E., AND MARGARET R. CHAPMAN: **Chemical Control of Plant Nematodes.** This book gives a short introduction to Plant Nematology. Several important crops and their nematode parasites are mentioned. There is a table of some of the important species that attack stems, leaves, buds, and flowers, including the most important species that feed on roots. Since all plant nematodes spend some time in the soil, most attempts to control them have been by soil treatments. The author discusses the more general problems of using chemicals against plant nematodes, citing only recent general or fundamental references. This is followed with 91 pages of bibliography of the most relevant literature since 1932. Commonwealth Agricultural Bureau, Central Sales Branch, Farnham House, Farnham Royal, Bucks, England. Published 28 March, 1966, 119 pp. 12s. 6d.

## EMMETT WILLIAM PRICE

1896-1965

Dr. Emmett W. Price, an outstanding member of "Helm. Soc." since 1926, and a helminthologist and veterinary parasitologist of distinguished international reputation, died after a long illness in Gadsden, Alabama, 10 December 1965. For nearly four decades, he was very active in the affairs of the Society. He was elected president in 1932, and for many years served on the executive committee and as the Society's representative to the Washington Academy of Sciences. Beginning in 1926, he presented many interesting notes on helminths at the Society's monthly meetings, and gave active support to the establishment of separate publication of the Society's *Proceedings* in 1934. The first paper in Vol. 1, No. 1 of the *Proceedings* is a description of *Stephanoproraoides lawi*, n. g., n. sp., a trematode from a beaver by Dr. Price. Thereafter, he published more than two dozen papers in the *Proceedings*, which materially aided in the secure establishment of this new publication.

Born in Clover Lick, West Virginia, in 1896, Dr. Price came to Washington, D.C. in his teens and began his scientific career by earning a D.V.M. degree from George Washington University in 1918 at the age of 20. The same year, he served for a short period in the Army Veterinary Corps. Shortly thereafter (1919-1926), he held the position of associate professor of pathology, School of Veterinary Medicine, Texas A & M College, and at that post rapidly developed an interest in animal parasitology. During this period, the late Dr. M. C. Hall, then chief of the Zoological Division, Bureau of Animal Industry, USDA, visited Texas A & M, and, after noting the young professor's interest and capability in parasitology, invited him to apply for a position with the Zoological Division in Washington, D.C. When Dr. Price joined the Division in 1926, it already consisted of a group of outstanding animal parasitologists, namely, Drs. M. C. Hall, E. B. Cram, G. Dikmans, A. Hassall, B. Schwartz, and W. H. Wright. Thus began a long productive career with the USDA in the nation's capitol and in later years at the Agricultural Research Center, Beltsville,



Md. After returning to Washington, D.C., Dr. Price also continued his academic training, and earned the M.S. degree from American University in 1931 and the Ph.D. degree from George Washington University in 1935. At the Zoological Division Laboratory, he rapidly became widely known as an authority on parasitic helminths of domestic and wild animals. In 1936, he became assistant chief of the Zoological Division, and later, when the laboratory staff was transferred to Beltsville, Md., he was placed in charge of the laboratory there. After a reorganization in the early 1950's, he became agricultural administrator in charge of the Helminth Parasite Section of the Animal Disease and Parasite Research Division, ARS, USDA, a position he held when he retired from federal service because of poor health in 1956.

After retirement, Dr. and Mrs. Price moved to Gadsden, Alabama, so they could be near a married daughter and their grandchildren. There he partially regained his health after a serious operation, and accepted a position

teaching bacteriology, histology, and parasitology at nearby Jacksonville State College, a position he held with distinction until January 1965, when he retired for the second time. He was honored in Alabama by appointments to the State Board of Medical Examiners and as an honorary member of the Alabama Academy of Sciences. For several years while teaching in Alabama he continued his studies on monogenetic trematodes with the assistance of a National Science Foundation research grant and returned to the Beltsville Parasitological Laboratory for several summers while pursuing this activity. Dr. Price published about a dozen papers during this period.

The development of parasitology as a recognized professional specialty in the twentieth century paralleled closely the professional career of Dr. Price. Because of his varied academic background, official position, and expertise, he exerted a not inconsiderable influence on the progress of parasitology in this country. His first technical paper in this field was published in 1925. That same year the American Society of Parasitologists was organized; he became a charter member and immediately took an active interest in its affairs. He served on the Society's council for 11 years and was elected president in 1952. He was also influential in veterinary affairs, and served from 1942–1947 as a member of the Research Council for Parasitology of the American Veterinary Medical Association.

Dr. Price's major research interest was in the taxonomy of parasitic helminths of vertebrates, especially, but not limited to, trematodes. All who knew him were impressed by his ability to identify numerous miscellaneous helminths with little apparent effort. His wide knowledge of helminths was based upon years of intense study of both the literature and the parasites, and was aided by an unusually retentive memory.

Because Dr. Price was first trained as a veterinarian, he had a lasting interest in helminthic diseases of domestic animals and their control, and in animal pathology. His general proficiency in veterinary helminthology was demonstrated in his Presidential Address before the American Society of Parasitologists,

"The Fluke Situation in American Ruminants," and in many other publications.

Another contribution he made to parasitology of considerable international significance was his personal and administrative support over many years for the work on, and the publication of, the USDA Index-Catalogue of Medical and Veterinary Zoology. He was a great admirer of Dr. Albert Hassall, and fully realized the importance to parasitological science, and to human and veterinary medicine as well, of thorough indexing of world literature on parasites, hosts, vectors, and antiparasitic medications. Furthermore, over many years he was largely responsible for influencing higher administrative authority to make adequate provision for the maintenance of the United States National Museum Collection of helminths and related parasite collections at the Beltsville facility for the use and benefit of professional parasitologists everywhere.

The publications of Dr. Price number more than 150 items, and consist of scores of original papers on helminthology and many special articles for encyclopedias, agricultural yearbooks, and other similar publications. There is little doubt, however, that he took the greatest pleasure in the work that resulted in a series of 11 papers published from 1937 to 1962 on the taxonomy of North American monogenetic trematodes. On this subject he had no peer. His domestic and foreign colleagues paid him honor on numerous occasions by naming 22 new species of helminths after him, including one new genus, *Pricetrema*.

Dr. Price was a kindly man who had a sincere affection for most segments of the human race, but especially for parasitologists. Visitors to his laboratory and home were always treated with the utmost courtesy. Many remember his kindness and his characteristically pungent remarks on parasites, parasitological research, and parasitologists. He was one of the giants of parasitology in the twentieth century, and will long be remembered for his substantial and enduring contributions to science.—K. C. KATES, *Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.*

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