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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 5 WASHINGTON, D. C., JULY, 1938 NUMBER 2

On the occurrence of *Gongylonema verrucosum* in sheep and cattle in the United States. DALE A. PORTER, U. S. Bureau of Animal Industry.

Gongylonema verrucosum was first described from sheep and the zebu, *Bos indicus*, in India by Giles (1892, Sci. Mem. Officers India, pt. 7, pp. 45-49); Railliet and Henry (1918, Bull. Soc. Path. Exot. 11:82-86) next reported this parasite from the goat in Belgian Congo; Sugimoto (1925, Dept. Agr. Govt. Research Inst. Formosa, pp. 93-137) reported it from Formosan sheep and cattle; Baylis (1926, J. Comp. Path. and Ther. 39:134-137) writes that in a letter sent to him, Mönnig stated that *G. verrucosum* occurred fairly frequently in cattle, sheep, and goats at Pretoria, but specimens sent to Baylis by Mönnig from South African sheep were found by the former to be a new species, *G. mönnigi*, whereas those sent to him from cattle were *G. verrucosum;* thus, Mönnig (1928, Union So. Africa Dept. Agr., 13-14 Rpt. Dir. Vet. Research, pt. 2, pp. 801-837) lists only South African cattle as hosts of *G. verrucosum*. However, Le Roux (1932, Ann. Bull. Dept. Animal Health N. Rhodesia (1931) pp. 9-24) recorded *G. verrucosum* from sheep and cattle in Northern Rhodesia. Bhalerao (1933, Indian J. Vet. Sci. and Animal Husbandry 3:163-165) reported this parasite from the hill goat, *Capra sibirica*, in India, and Pillers (1933, Vet. Rec. (n. s.) 13:964-966) reported it from the goat in Cyprus.

G. verrucosum has been reported from ruminants 3 times in the United States. The first report was by Price (1927, J. Parasitol. 14:54) from Texas goats and the second report also by Price (1928, J. Parasitol. 14:201-202), was on the occurrence of this species in Texas sheep and goats. Dikmans and Lucker (1935, Proc. Helminth. Soc. Wash. 2:83) reported *G. verrucosum* from the rumen of a deer, *Odocoileus virginianus*, from the Ocala National Forest, Florida. This parasite first came to the writer's attention when specimens were collected from 2 of 4 sheep shipped from Defuniak Springs, Florida, and slaughtered at a packing plant in Moultrie, Georgia. A week later the rumens of 20 sheep from the same locality were examined and approximately 200 specimens of *G. verrucosum* were observed in the rumen of one animal. As this parasite had apparently not been reported from cattle in this country, the rumens of 4 groups of cattle from markets in Georgia, Florida, Mississippi, and Alabama were examined at intervals during July and August, 1937, at the packing plant in Moultrie, Georgia. Five more sheep were also examined in September. The results of these examinations are given in table 1. It will be seen that 4 of 29 sheep (13.7 per cent) and 8 of 86 cattle (9.3 per cent) examined were infested with *G. verrucosum*. The rumen appears to be the normal habitat of this parasite, but worms were found occasionally in the omasum and reticulum when complete examinations were made. For the most part fairly heavy infestations were encountered, the worms being clearly visible on the mucous membrane of the rumen, threaded in and out among the papillae. Tissue penetration or tunneling such as observed in the case of several other species of *Gonyglonema* was not seen; however, in the cases of heavy infestation the worms in some areas appeared to be packed into the mucosa by the force of the food material present.

Although its incidence is comparatively low, *G. verrucosum* appears to be fairly well distributed in the southeastern States as it is encountered in sheep from Florida, and cattle from Georgia, Florida, Mississippi, and Alabama. Price (1927, loc. cit.) has suggested that *G. verrucosum* may have been introduced into Texas through importations of Brahman cattle from India. These cattle were apparently distributed throughout the South during the latter part of the last century as Camp (1932, Univ. Fla. Expt. Sta. Bull. 248) states that Brahman cattle were introduced into Florida from Nashville, Tennessee, in 1880.

TABLE 1.—Incidence of *Gongylonema verrucosum* in cattle and sheep examined in 1937

Host	Locality	Date examined	No. examined	No. positive	Number and location of worms		
					Rumen	Omasum	Reticulum
Sheep	Defuniak Springs, Florida	June 10	4	2	115 725	14 17	0 3
do	do	June 17	20	1	200[1]	not examined	not examined
do	Quincy, Florida	Sept. 21	5	1	2	0	0
Cattle	Ocala, Florida	July 29	20	3	100 to 200[2]	not examined	not examined
do	Jackson, Mississippi	August 26	27	2	300[2]	do	do
do	Montgomery, Alabama	August 26	26	1	298	do	do
do	Moultrie, Georgia	August 27	13	2	125 3	do	do

[1]Estimated number.
[2]Estimated number of worms per rumen.

Two new trematode parasites of the genus *Styphlodora* (Plagiorchiidae: Styphlodorinae) from the gall bladder of a water-snake, with a discussion on the systematics of the subfamily. ELON E. BYRD and J. FRED DENTON,* University of Georgia, Athens, Georgia.

During the past 8 years specimens of what now appear to be 2 species of very characteristic trematode parasites have been encountered from the gall bladder of the water-snake, *Natrix sipedon sipedon* (L.), a single specimen of these parasites having been recovered from each of 3 host specimens. The snakes from which the material has been obtained were collected from widely separated areas, as follows: Columbus, Mississippi (1930); New Orleans, Louisiana (1933); and Athens, Georgia (1936). At the time the worms were collected they were identified as belonging to the same species and this was assigned tentatively to the subgenus *Allopharynx* Strom (1928, Zool. Anz. 79: 167-172) of the genus *Xenopharynx* Nicoll (1921, Proc. Zool. Soc. London, pp. 851-856). This tentative assignment was made because of the striking resemblance between our material and *Distomum tropidonoti* MacCallum (1918, Zoopathologica, p. 96) and the similarity of both of these worms to Strom's species, *X. (Allopharynx) amudariensis.* However, in reviewing the literature treating on these and related flukes we have been convinced our material belongs to

*The writers wish to express their appreciation to Drs. E. W. Price and A. McIntosh, U. S. Dept. of Agriculture, Bureau of Animal Industry, Washington, D. C., for their helpful suggestions on points of taxonomy.

the genus *Styphlodora* Looss (1899, Zool. Jahrb., Abt. System 12: 521-784). For the 2 species in the present collection we propose the names *Styphlodora magna*, n. sp. and *Styphlodora natricis*, n. sp.

Styphlodora magna, n. sp. (Fig. 15)

Description.—Body lanceolate, very thin and transparent, very weakly muscular, more pointed anteriorly than posteriorly, and with almost parallel sides, 8.40 mm long by a maximum width of 2.90 mm at testes; width at ventral sucker 1.84 mm. Cuticula thin, without spines. Oral sucker subterminal, 0.56 mm long by 0.47 mm wide. Ventral sucker smaller, 0.47 mm in diameter, located 1.50 mm from anterior end. Prepharynx short. Pharynx at caudal boundary of oral sucker, 0.16 mm long by 0.18 mm wide, without gland cells. Esophagus short, 0.36 mm long, bifurcating 0.34 mm in front of ventral sucker. Caeca long, more or less regular tubes reaching to beginning of last body fifth, *i.e.*, to level 1.75 mm in front of posterior end. Testes oval to ovoid in outline, testis on ovarian side of body about half its diameter more posterior in position; left testis 0.47 mm long by 0.38 mm wide, lies 0.97 mm behind ovary; right testis 0.38 mm long by 0.41 mm wide, one-half its diameter in advance of left. Vasa efferentia with dilated proximal portion, uniting just before entering cirrus sac. Cirrus sac non-muscular, 0.77 mm long by a maximum width of 0.24 mm, wider anteriorly than posteriorly, extending from genital pore around left side of ventral sucker to about its equatorial plane, containing tube-like vesicula seminalis that fills posterior third of sac, a weakly developed pars prostatica, and weakly muscular ductus ejaculatorius of about same length as vesicula seminalis. Genital pore submedian, just right of midline, ventral to right caecum at bifurcation. Excretory bladder bifurcates in region of ovary; cornus short, not reaching acetabulum. Ovary close (0.11 mm) behind ventral sucker, left of midline, 0.32 mm in diameter. Oviduct short. Oötype surrounded by conspicuous shell gland. Laurer's canal present. Receptaculum seminis large, 0.40 mm long by 0.34 mm wide, in midline posterior to ovary. Uterus slender tube descending and ascending between testes, descending to within 0.34 mm of posterior end of body, coils of uterus not separating ovary and ventral sucker. Metraterm weakly developed, about 2/3 length of cirrus sac. Vitellaria follicular, follicles dendritic, in clusters, from 6 to 11 clusters on each side, more extensive on one side than on other, extending from level of ovary to level 0.68 mm behind posterior testis, ending well in front of ends of caeca. Single transverse vitelline duct on each side. Yolk reservoir prominent, between ovary and receptaculum seminis. Ova numerous, operculated, from 16 to 21μ wide by 26 to 36μ long.

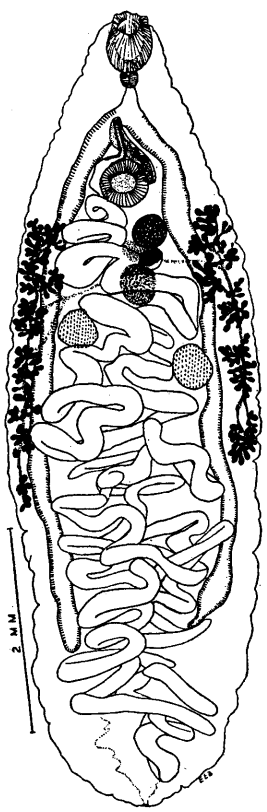

FIG. 15
Styphlodora magna, n. sp., ventral view. Original.

Host.—*Natrix sipedon sipedon* (L.).

Habitat.—Gall bladder.

Locality.—Athens, Georgia, and Columbus, Mississippi, U. S. A.

Styphlodora magna is described from a single complete specimen taken from

the gall bladder of *Natrix sipedon sipedon* collected from Athens, Georgia. The posterior portion (posterior four-fifths) of a second specimen from the gall bladder of a second host specimen from Columbus, Mississippi, is available for study. The species appears to be distinct from previously described members of the genus due to its large size, the shape of the body, the extended distribution of the vitellaria, and the position of the genital pore. In appearance it is more closely related to *S. solitaria* Looss than to other members of the genus. From this species *S. magna* differs in its larger body size, the position of the gonads, the length of the cirrus sac, the position of the ventral sucker, the length of the caeca, and the distribution of the vitellaria.

Styphlodora natricis, n. sp. (Fig. 16)

Description.—Body thin and transparent, weakly muscular, more pointed anteriorly than posteriorly, 6.35 mm long by 1.67 mm wide at testes, 1.10 mm wide at bifurcation of caeca. Cuticula thin, without spines. Oral sucker subterminal, 0.30 mm long by 0.40 mm wide. Ventral sucker larger, 0.36 mm long by 0.40 mm wide, located 1.20 mm from anterior end of body. Prepharynx short. Pharynx muscular, 0.11 mm in diameter. Esophagus short, 0.23 mm long, bifurcating 0.52 mm in front on acetabulum. Caeca slender tubes with few undulations, reaching to within 0.74 mm of posterior end, near posterior limits of uterus. Testes slightly irregular in outline, left testis a little more advanced than right, right testis situated 0.50 mm behind ovary; testes located in anterior half of body: right testis 0.34 mm long by 0.38 mm wide; left testis 0.38 mm long by 0.27 mm wide. Vasa efferentia uniting just before entering cirrus sac. Cirrus sac slender, non-muscular, lying on right side of acetabulum, 0.63 mm long, reaching to about equatorial plane of ventral sucker, containing tubular vesicula seminalis that occupies about one-half length of cirrus sac, a short pars prostatica, and slightly muscular ductus ejaculatorius; no eversible cirrus observed. Excretory system similar to that described for *Styphlodora magna*. Genital pore ventral, on midline, 0.18 mm behind fork of caeca and 0.34 mm in front of acetabulum. Ovary small, 0.20 mm long by 0.27 mm wide, situated to right of midline, 0.32 mm behind acetabulum. Receptaculum seminis large, 0.36 mm long by 0.43 mm wide, just posterior to conspicuous shell gland. Oviduct short. Laurer's canal present. Vitellaria follicular, follicles very small, mainly lateral to caeca, from level of caudal boundary of acetabulum to positions 0.18 mm behind right testis on right side and 0.85 mm behind left testis on left side; follicles arranged in clusters. Single transverse yolk duct on each side. Yolk reservoir prominent, located immediately behind ovary. Uterus slender tube descending and ascending between testes, descending to within 0.55 mm of posterior end of body, much coiled. Metraterm weakly developed, about ½ length of cirrus sac. Ova numerous, operculated, 18μ wide by 30μ long.

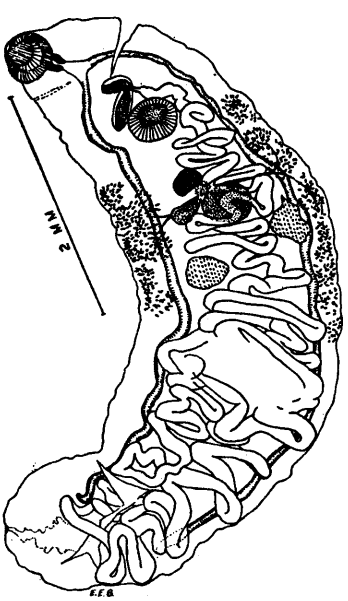

FIG. 16
Styphlodora natricis, n. sp., ventral view. Original.

Host.—*Natrix sipedon sipedon* (L.).

Habitat.—Gall bladder.

Locality.—New Orleans, Louisiana, U. S. A.

Styphlodora natricis is described from a single specimen taken from the gall bladder of *Natrix sipedon sipedon* collected from New Orleans, Louisiana. The species appears to be more closely related to *Styphlodora magna*, n. sp. and *S. solitaria* Looss than to the other members of the genus. From *S. magna* it can be distinguished by its smaller body size, the greater length of the caeca, the position of the genital pore, the relative distance between the bifurcation of the caeca and acetabulum, the position of the ovary and the tendency of the uterus to separate the ovary from the acetabulum. From *S. solitaria* the present species can be distinguished by its greater body size, the more forward position of the acetabulum, the position of the ovary, testes, genital pore and bifurcation of the caeca, the length of the cirrus sac, and caeca, and the distribution of the vitellaria.

DISCUSSION

The genus *Styphlodora* was proposed by Looss (1899, loc. cit.) for the reception of his species *S. serrata* and *S. solitaria*. Odhner (1911, Results Swedish Zool. Exped. Egypt 4 (23A): 1-166) has suggested that *Distomum horridum* Leidy, 1850 and *Distomum similis* Sonsino, 1890 be placed in the genus. The following additional species have been added: *S. condita* De Faria (1911, Mem. Inst. Oswaldo Cruz 3: 40-45), *S. bascaniensis* Goldberger (1911, Proc. U. S. Natl. Mus. 40: 233-239), *S. najae* Nicoll (1912, Proc. Zool. Soc. London, pp 851-856), *S. persimilis* Nicoll (1914, Proc. Zool. Soc. London, pp. 139-154), *S. lachesidis* MacCallum (1921, Zoopathologica, pp. 137-204), *S. renalis* Tubangui (1933, Philippine J. Sci. 52: 167-197), and *S. nicolli* Bhalerao (1936, J. Helminth. 14: 181-206). The present paper adds 2 additional species, *S. magna*, n. sp. and *S. natricis*, n. sp. So far as the writers are able to determine only 4 of the above listed species are recorded from North America as follows: *S. horridum* Leidy, from the excretory ducts of *Boa constrictor*, *S. bascaniensis* Goldberger, from the liver of *Coluber constrictor* (= *Bascanion constrictor*), *S. magna*, n. sp. and *S. natricis*, n. sp., from the gall bladder of *Natrix sipedon sipedon*. All the other members of the genus are reported from various habitats in reptiles from South America, Europe, Africa, and the Orient.

In creating the genus *Styphlodora*, Looss assigned it to the subfamily Plagiorchiinae (=Lepodermatinae) and compared it with the genera *Astiotrema* Looss (=*Astia* Looss), *Piagiorchis* Lühe (=*Lepoderma* Looss), *Opisthioglyphe* Looss, and *Glossidium* Looss. Pratt (1902, Amer. Nat. 36: 887-910) included the genus *Styphlodora* with the genera *Ochetosoma* Braun, *Renifer* Pratt, *Oistosomum* Odhner, and *Astiotrema* Looss in his subfamily Reniferinae. Baer (1924, Parasitol. 16: 22-31) excluded the genus *Styphlodora* from the family Plagiorchiidae because of the abarrent type of excretory vesicle it exhibited, but created the subfamily Styphlotrematinae for the genera *Styphlotrema* Looss and *Pachypsolus* Looss and placed the group as a subfamily under the family Reniferidae Baer. Mehra (1931, Parasitol. 23: 157-178) depressed the family Reniferidae Baer, but accepted the subfamily Styphlotrematinae as a valid subfamily of the family Plagiorchiidae. To the subfamily Styphlotrematinae Mehra added the genera *Styphlodora* Looss, *Glossidium* Looss, *Glossidiella* Travassos, *Spinometra* Mehra, and *Aptorchis* Nicoll. In the same publication Mehra considered *Styphlodora bascaniensis* Goldberger as being too aberrant to be included in the genus to which it was assigned, and created a new genus, *Platymetra*, for the reception of this species; the new genus was assigned to the subfamily Reniferinae. Later Mehra (1937, Ztschr. Parasitenk. 9: 429-469) added the genus *Glossimetra* Mehra to the subfamily Styphlotrematinae, but removed the genus *Aptorchis* Nicoll to the subfamily Reniferinae. Dollfus (1937, Bull. Comité Études Hist. et Sci. Afrique Occid. Franc. 19: 397-519), in commenting on the subfamily Styphlotrematinae Baer, states it is *Styphlotrema* and not *Styphlodora* which possesses the aberrant type of excretory vesicle, and further suggests (p. 506) "C'est une sous-famille des *Styphlodorinae* qui aurait

pu être proposée (pour *Styphlodora* et *Pachypsolus*) et non pas une sous-famille des *Styphlotrematinae* (pour *Styphlotrema* et *Pachypsolus*).'' On the next page (p. 507) we find the same author saying ''Si je laisse aujourd'hui, très provisoirement, *Pachypsolus* dans la sous-famille de Jean G. Baer, je tiens à dire que ce n'est pas selon non opinion personnelle, mais parce que les matériaux me manquent présentement pour étude comparée des genres réunis dans cette sous-famille.''

The writers are of the opinion that the genera *Pachypsolus* Looss and *Styphlotrema* Looss should be removed from the subfamily Styphlotrematinae Baer of Mehra and be placed in the subfamily Enodiotrematinae Baer due to the striking similarity between the body form and the type of excretory vesicle exhibited by these 2 genera and the members of the subfamily Enodiotrematinae. We cannot accept the genus *Platymetra* Mehra (1931, loc. cit.), created for the reception of *Styphlodora bascaniensis* Goldberger, since the point of differences between *S. bascaniensis* and the other members of the genus, as indicated by Mehra, are in our opinion specific rather than generic. It is to be suggested also that the genus *Glossimetra* Mehra (1937, loc. cit.) differs from the genus *Spinometra* Mehra only in the absence of a well defined receptaculum seminis. When more material is available for study it is quite possible the former genus will be considered synonymous with the latter.

The genus *Allopharynx* Strom (1928, loc. cit.) shows a remarkable similarity in body form, arrangement of internal structures, and hosts to the genus *Styphlodora*. We find the 2 genera to differ in 3 characters as follows: (1) The reproductive organs are more posteriorly placed (in the middle of the body) in *Allopharynx* whereas these organs are anterior to the body middle in *Styphlodora*. (2) The vitellaria are more extensive, beginning at about the level of the genital pore and extending to or beyond the level of the posterior testis in *Allopharynx* while these glands are postacetabular and are usually confined to the region of the reproductive organs in *Styphlodora*. (3) The uterus makes a variable number of transverse loops across the body in front of the ovary in *Allopharynx* whereas the uterus is comparatively straight from the level of the anterior testis to the genital pore, sometimes with only an indication of a loop in the region of the ovary in *Styphlodora*. In considering *Styphlodora magna*, n. sp. and *S. natricis*, n. sp. we find the differences between *Styphlodora* and *Allopharynx* less marked since the characters of these 2 species are somewhat intermediate in that the ovary and testes are more posterior, the vitellaria more extensive, and the uterus shows a definite tendency to form transverse loops across the side of the body opposite the ovary than is the case with the typical styphlodorid fluke. There is a decided tendency for the uterus to separate the ovary from the ventral sucker in *S. natricis*, a character which is definitely like the members of the genus *Allopharynx*. We consider, however, we have insufficient evidence to consider *Allopharynx* synonymous with *Styphlodora* since our material consists of only 2 specimens for the 2 species.

In the light of the above we consider the subfamily *Styphlodorinae* Dollfus to be composed of the following genera: *Styphlodora* Looss, *Glossidium* Looss, *Aptorchis* Nicoll, *Glossidiella* Travassos, *Allopharynx* Strom, *Spinometra* Mehra, and *Glossimetra* Mehra.

A new philophthalmid trematode of the spotted sandpiper from Michigan and of the black-necked stilt from Florida. ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

In this paper is described a new species of trematode belonging to the genus *Cloacitrema* Yamaguti, 1934. This species is based on 3 specimens, 2 from spotted sandpipers collected June 26 and July 3, 1928, in the vicinity of the University of Michigan Biological Station, Douglas Lake, Michigan, and 1 specimen from a black-necked stilt collected April 26, 1930, in the vicinity of the University of Miami, Coral Gables, Florida.

Cloacitrema michiganensis, n. sp.

Description.—Body approximately oval in outline, 2.67 mm long by 1.2 mm wide, with tapering anterior extremity; cuticula without spines. Oral sucker subterminal, 225μ by 240μ; acetabulum equatorial, 720μ by 80μ; pharynx oval, 170μ by 140μ; esophagus 130μ long by 100μ wide, somewhat contracted; intestinal crura ending in testicular zone, about 400μ from the posterior end of the body. Excretory system similar to that of genotype. Testes oval, 160 to 170μ by 90 to 110μ, zones coinciding, separated by a distance approximately equal to the diagonal length of a testis. Vasa efferentia only slightly discernible, apparently uniting near posterior border of acetabulum to form the vas deferens; the vas deferens on its way to the cirrus sac passes dorsad usually along the left margin of the acetabulum; anterior portion of vas deferens, from anterior region of acetabulum to the cirrus sac, functioning as a vesicula seminalis. Cirrus sac elongate, 250μ by 70μ, situated for the greater part of its length ventral to esophagus; genital pore median, ventral to posterior portion of pharynx.

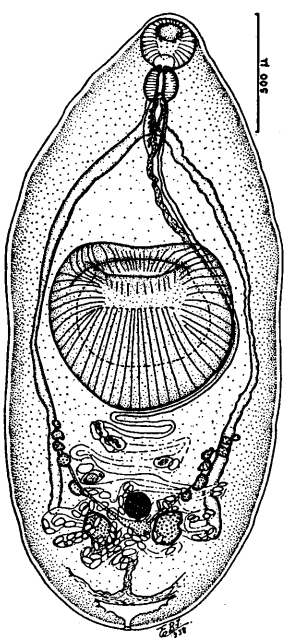

FIG. 17
Cloacitrema michiganensis, n. sp., ventral aspect.

Ovary spherical, 100μ in diameter, median, slightly anterior to equatorial zone of testes; ovicapt distinct, projecting from postero-ventral surface of the ovary; oviduct short, leading into a small fecundarium lying dorsal to ovarian complex and situated immediately posterior to ovary. Mehlis' gland immediately ventral to fecundarium; vitelline reservoir ventral to Mehlis' gland. Seminal receptacle small, lateral to fecundarium (left in the specimen in which a detail examination was made). Laurer's canal long, convoluted, extending posteriorly from ovarian complex, making a reflexed loop, then continuing anteriorly and opening on dorsal surface near level of posterior border of ovary and to right of ovicapt. Eggs light yellow, somewhat variable in size, typical specimens measuring 65μ by 29μ, those in the portion of uterus lying anterior to ovary embryonated and probably hatching before or soon after deposition, as oculated miracidia (95μ by 53μ) free of their shells were present in uterus of one (type) specimen.

Habitat.—Cloaca of spotted sandpiper, *Actitis macularia* (Linnaeus), type host, and black-necked stilt, *Himantopus mexicanus* (Müller).

Distribution.—Michigan, Douglas Lake (Monroe Lake) and Florida, Coral Gables (Tamiami Trail).

Specimens.—U. S. N. M. Helm. Coll. Nos. 42980 (type; fig. 17) and 42981 (paratype).

The genus to which the new species is assigned was proposed by Yamaguti (1934, Japan J. Zool. 6:161-163) for a single related species, *Cloacitrema ovatum* Yamaguti, 1934, from the cloaca of *Bucephala clangula clangula* (Linn.). The 2 species of the genus *Cloacitrema* may be separated by the following key:

Acetabulum postequatorial; uterus with coils both posterior and anterior to acetabular zone ..... *C. ovatum* Yamaguti

Acetabulum equatorial; uterus with no coils in preacetabular zone ..... *C. michiganensis*, n. sp.

A new species of *Dactylogyrus* (Monogenea: Dactylogyridae), with the proposal of a new genus. EMMETT W. PRICE, U. S. Bureau of Animal Industry.

An examination of the gills from two species of fresh-water fishes, collected in India by Dr. V. N. Moorthy, revealed the presence of a light infestation with a monogenetic trematode belonging to the genus *Dactylogyrus* Diesing. Unfortunately the specimens were not well preserved and much of the internal structure could not be made out; however, the morphology of the heavily cuticularized structures shows the species to be new and for it the name *Dactylogyrus moorthyi* is proposed.

Dactylogyrus moorthyi, n. sp.

Description.—Body elongate, 165 to 266μ long by 37 to 75μ wide. Haptor 22 to 44μ wide, armed with 1 pair of large hooks (anchors) 18 to 30μ long and supported by a single ox-yoke-shaped bar about 18μ long, and with 14 marginal hooklets 15 to 18μ long. Eyes present. Oral aperture about 35 to 45μ from anterior end of body; pharynx globular, 15 to 18μ in diameter; remainder of digestive tract not observed. Genital aperture about 55 to 65μ from anterior end of body. Male copulatory apparatus consisting of a long slender cirrus describing a complete loop at its proximal end and an accessory piece consisting of 2 parts, one portion finger-shaped, about 28μ long, and a crescentic portion about 20μ long. Vagina present, opening on right side of body. Gonads and vitellaria not distinctive. Eggs not observed.

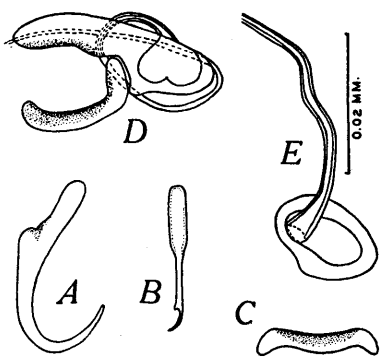

FIG. 18
Dactylogyrus moorthyi. A—Large haptoral hook. B—Marginal hooklet. C—Haptoral bar. D—Male copulatory apparatus. E—Vagina.

Hosts.—*Puntius puckelli* and *P. ticto.*

Location.—Gills.

Distribution. — India (Chitaldrug District, Mysore State).

Specimens.—U. S. N. M. Helm. Coll. Nos. 41144 (type) and 41145 (paratypes).

This species differs from all other species of the genus *Dactylogyrus* in the morphology of the male copulatory apparatus and in the shape of the haptoral bar.

Up to the present time the genus *Dactylogyrus* Diesing contains about 72 species which, on the basis of the number of haptoral bars, fall into 2 distinct groups. It is proposed to regard each of these groups as distinct genera, retaining for those forms having a single haptoral bar the genus *Dactylogyrus* and proposing for those forms having 2 haptoral bars the new genus *Neodactylogyrus.*

Genus *Dactylogyrus* Diesing, 1850

Diagnosis.—Haptor moderately developed, with 1 pair of large hooks (anchors) supported by a single bar, and with 14 marginal hooklets. Vagina present, with or without cuticular supporting structures.

Type species.—*Dactylogyrus auriculatus* (Nordmann, 1832) Diesing, 1850.

Additional species.—*Dactylogyrus amphibothrium* Wagener, 1857; *D. anchoratus* (Dujardin, 1845); *D. atromaculatus* Mizelle, 1938; *D. bini* Kikuchi, 1929; *D. bychowskyi* Mizelle, 1937; *D. cordus* Nybelin, 1937; *D. cyprini* Buschkiel, 1930; *D. dujardinianus* (Diesing, 1850); *D. extensus* Mueller and Van Cleave, 1932; *D. falcatus* (Wedl, 1857); *D. fallax* Wagener, 1857; *D. formosus*

Kulwiec, 1927; *D. intermedius* Wegener, 1910; *D. inversus* Goto and Kikuchi, 1917; *D. microphallus* Mueller, 1938; *D. minutus* Kulwiec, 1927; *D. puntii* Buschkiel, 1930; (?) *D. siluri* Wagener, 1857; *D. similis* Wegener, 1910; *D. sphyrna* Linstow, 1878; *D. tenax* Mueller, 1938; *D. tuba* Linstow, 1878; (?) *D. uncinatus* Wagener, 1857; *D. vastator* Nybelin, 1924 (syn. *D. crassus* Kulwiec, 1927); *D. wegeneri* Kulwiec, 1927; and *D. moorthyi*, n. sp.

Neodactylcgyrus, new genus

Diagnosis.—Haptoral hooks (anchors) supported by 2 similar or dis similar bars. Other characters as in *Dactylogyrus.*

Type species.—*Neodactylogyrus megastoma* (Wagener, 1857), n. comb.

Additional species.—*Neodactylogyrus acus* (Mueller, 1938); *N. affinis* (Bychowsky, 1933); *N. alatus* (Linstow, 1878); *N. amblops* (Mueller, 1938); *N. apos* (Mueller, 1938); *N. bifurcatus* (Mizelle, 1937); *N. borealis* (Nybelin, 1937); *N. bulbus* (Mueller, 1938); *N. chranilowi* (Bychowsky, 1933); *N. confusus* (Mueller, 1938); *N. cornu* (Linstow, 1878); *N. cornutus* (Mueller, 1938); *N. crucifer* (Wagener, 1857); *N. cryptomeres* (Bychowsky, 1934); *N. distinguendus* (Nybelin, 1937); *N. duquesni* (Mueller, 1938); *N. difformis* (Wagener, 1857); *N. fraternus* (Wegener, 1910); *N. frisii* (Bychowsky, 1933); *N. fulcrum* (Mueller, 1938); *N. gemellus* (Nybelin, 1937); *N. gracilis* (Wedl, 1861); *N. haplogonus* (Bychowsky, 1933); *N. kulwieci* (Bychowsky, 1931); *N. macracanthus* (Wegener, 1910); *N. malleus* (Linstow, 1877); *N. micracanthus* (Nybelin, 1937); *N. minor* (Wagener, 1857); *N. mollis* (Wedl, 1857); *N. nybelini* (Markevich, 1933); *N. orchis* (Mueller, 1938); *N. parvus* (Wegener, 1910); *N. perlus* (Mueller, 1938); *N. photogenis* (Mueller, 1938); *N. propinquus* (Bychowsky, 1931); *N. rubellus* (Mueller, 1938); *N. scutatus* (Mueller, 1938); *N. simplex* (Mizelle, 1937); *N. suecicus* (Nybelin, 1937); *N. simplicimalleata* (Bychowsky, 1931); *N. tenuis* (Wedl, 1857); *N. urus* (Mueller, 1938); *N. wunderi* (Bychowsky, 1931); and *N. zandti* (Bychowsky, 1933).

Egg output of the root-knot nematode. JOCELYN TYLER, U. S. Bureau of Plant Industry.

A commonly quoted estimate of the number of eggs laid by one female of the root-knot nematode, *Heterodera marioni*, is from 300 to 500. These figures may legitimately be used as a conservative average for purposes of computing soil populations, but they do not give a true conception of the normal egg output of this nematode.

LITERATURE

Higher egg counts have been published. Godfrey (1931) mentions a single cowpea root with "approximately 4,000 egg masses attached, each containing between 500 and 1,000 eggs. (As many as 1,300 were found in one egg mass in this laboratory.)" Godfrey and Oliveira (1932) state that "many egg masses were counted with between 600 and 800 eggs. As many as 1,200 eggs were counted from one mass, showing evidence of eggs that had already hatched and, likewise, evidence that new eggs were still being deposited at the time the egg mass was removed. Such large egg masses were to be found in both pineapple and cowpea roots." These high counts were intended to indicate "the very rapid rate of reproduction under Hawaiian conditions."

The highest count so far published, a total of 1,998 eggs laid by one female, was given by the writer (Tyler, 1933a) as evidence that isolated nematodes developing in tomato-root cultures could be as healthy as nematodes under natural conditions. This count was more complete than counts from ordinary field material merely because the larvae remained in the test tube after hatching. Larvae and unhatched eggs were counted. The subject was not pursued farther with culture material.

PRELIMINARY OBSERVATIONS

A few counts made from galls of unknown age, but higher than the 500 eggs formerly considered the "maximum" number, are given in table 1. No significant host relationship can be deduced from these random selections. They should be considered as minimum counts, and are included merely to indicate the possibility of high egg output from a wider range of host plants than those considered in table 2.

METHODS

Since larvae normally leave the egg mass soon after hatching, the only accurate count must include the numerous empty egg shells rather than the few available larvae. When the "gelatinous" material surrounding the egg mass—its true composition has been analyzed by Chitwood (1938)—is macerated for about 15 minutes in a 10 or 12 per cent solution of clorox (procedure adapted from Lee, 1921, p. 250. Clorox contains 5.25 per cent sodium hypochlorite by weight) the empty shells as well as the developing eggs fall apart. Ordinarily the empty shells, crushed flat by pressure from the constantly increasing mass of eggs, form a layer surrounding the more recently deposited eggs. They are seen clearly in reflected light. As soon as sufficient maceration has taken place, the clorox solution must be further diluted with several volumes of water; otherwise it dissolves the chitin of the egg shells and renders the counting of empty shells exceedingly difficult. Eggs in early stages of development may also be destroyed by this solution.

Seeds were planted on April 14, 1938, in greenhouse flats heavily inoculated with infested soil and with galls. Several days later the seedlings were rinsed in water and potted in noninfested soil, immediately surrounded by white sand to distinguish the infested region of the root from later growth (method originated by J. R. Christie). Squash, cucumber, and soybean seedlings were transplanted in this way on April 20, pea on April 23, wheat April 25, tomato April 27, and Sudan grass April 28. Thus practically all the nematodes in the first lot of roots entered between April 15 or 16 and April 20, and there is only a possible 4 or 5 days' difference in the amount of development of the various nematodes in these plants. No plants were kept for more than one examination, but all plants used were started from seed at the same time.

TABLE 1.—*Egg counts made from field or greenhouse galls of unknown age. Empty shells were not counted except in the two 1938 records.*

Host plant	*Date*	*Locality*	*Number of eggs*
Begonia semperflorens, Vernon type hybrid	1938	U. S. Dept. Agr. greenhouse	1,640
Fragaria sp., strawberry	1929	California	980
Nicotiana tabacum, tobacco	1938	U. S. Dept. Agr. greenhouse	1,296
Oxalis sp., sorrel	1937	U. S. Dept. Agr. greenhouse	791
Polianthes tuberosa, tuberose (root)	1937	North Carolina	661
Solanum tuberosum, potato	1929	California	1,322
Tagetes patula, French marigold, var. Dwarf Royal Scot	1937	U. S. Dept. Agr. greenhouse (soil inoculated)	669
Zinnia elegans, Giant Dahlia-Flowered zinnia, var. Meteor	1937	U. S. Dept. Agr. greenhouse (soil inoculated)	1,344

TABLE 2.—*Numbers of eggs laid by individual females of Heterodera marioni. All host plants were started from seed in heavily inoculated flats on April 14, 1938. The period of infestation was limited, from 3 to 5 days in cucumber, squash, and soybean, and from 7 to 12 days in the other plants. A separate root was used at each examination.*

Host plant	May 11	May 16	June 1	June 7	June 8	June 9	June 17	June 20	June 22	June 24	June 25	June 26
Cucumis sativus, Chicago Pickling cucumber	8[1]	-------	405[4]	-------	-------	-------	1849[8]	1408	-------	-------	-------	2567[14]
	-------	-------	599	-------	-------	-------	1966	1623	-------	-------	-------	-------
	-------	-------	685	-------	-------	-------	2041	2377	-------	-------	-------	-------
	-------	-------	697	-------	-------	-------	-------	-------	-------	-------	-------	-------
	-------	-------	739	-------	-------	-------	-------	-------	-------	-------	-------	-------
Cucurbita sp., Early Yellow Summer Crookneck squash	3[2]	-------	883[4]	-------	-------	-------	1269	2197	-------	1506[10]	-------	-------
	-------	-------	-------	-------	-------	-------	-------	2475	-------	-------	-------	-------
	-------	-------	-------	-------	-------	-------	-------	2513	-------	-------	-------	-------
Holcus sorghum sudanensis, Sudan grass	-------	-------	-------	-------	576[7]	-------	-------	-------	-------	-------	-------	-------
Lycopersicum esculentum, tomato	-------	-------	-------	-------	-------	779[7]	-------	-------	-------	-------	922[13]	1409
	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	1592[15]
Pisum sativum, Canada field pea	-------	1	-------	1069[5]	-------	-------	-------	-------	1910	-------	2802[11]	-------
	-------	-------	-------	-------	-------	-------	-------	-------	2882[9]	-------	-------	-------
Soja max, Virginia soybean	0[3]	-------	416	-------	-------	-------	-------	-------	-------	2153[11,12]	-------	-------
	-------	-------	432	-------	-------	-------	-------	-------	-------	-------	-------	1945
	-------	-------	707	-------	-------	-------	-------	-------	-------	-------	-------	-------
	-------	-------	913[4]	-------	-------	-------	-------	-------	-------	-------	-------	-------
Triticum aestivum, wheat	-------	-------	-------	760[6]	-------	-------	-------	-------	-------	-------	-------	-------

[1]Egg masses in each of 3 roots.
[2]Only 1 egg mass; 5 roots examined.
[3]"Gelatinous" matrix extruded by several females in 2 roots; no eggs laid; 5 roots examined.
[4]Embryonated eggs but no empty shells observed.
[5]Many empty egg shells.
[6]Two empty egg shells.
[7]Hatching had begun.
[8]Many secondary galls.
[9]Female not exhausted.
[10]662 eggs in a secondary gall; 6 hatched.
[11]Female exhausted.
[12]Egg mass distended.
[13]Female not quite exhausted.
[14]123 eggs in secondary gall.
[15]93 eggs in secondary gall, developed to 8-cell stage.

TABLE 3.—*Computations from the data of Table 2, indicating the possible average rate of egg-laying during various periods. The egg counts used are the highest recorded on the given dates.*

	Entire egg-laying period		Analysis of different intervals during the egg-laying period	
Host plant	Dates	Average number of eggs per day	Dates	Average number of eggs per day
Cucumis sativus	May 11-June 26	55.6	May 11-June 1	34.8
			May 11-June 17	54.9
			May 11-June 20	59.2
			June 1-17	81.4
			June 1-20	86.2
			June 1-26	73.1
			June 17-20	112.0
			June 17-26	58.4
			June 20-26	31.7
Cucurbita sp.	May 11-June 20	62.5	May 11-June 1	41.9
	May 11-June 24	34.2	May 11-June 17	34.2
			June 1-17	24.1
			June 1-20	85.8
			June 1-24	27.1
			June 17-24	33.9
Lycopersicum esculentum	May 11-June 26	34.6	May11-June 9	26.9
			June 9-26	47.8
Pisum sativum	May 11-June 22	68.6	May 11-June 7	39.6
	May 11-June 25	62.3	May 16-June 7	48.5
	May 16-June 22	78.1	June 7-22	120.9
	May 16-June 25	70.0	June 7-25	96.3
Soja max	May 11-June 24	48.9	May 11-June 1	43.5
	May 11-June 26	42.3	June 1-24	53.9
			June 1-26	41.3

CONSIDERATION OF A POSSIBLE INFLUENCE BY THE HOST PLANT ON THE RATE OF NEMATODE DEVELOPMENT

Observations on 5 host plants are discussed as though they were one series. The detailed records are given in table 2. Two additional plants—wheat, which has considerable resistance to root-knot, and Sudan grass, which has not previously been reported as a host of this nematode, are being studied in an experiment on resistance to root-knot. The egg counts from these plants are included here for their interest in that connection. Development would appear to be slowest in Sudan grass, slightly better in tomato, and most rapid and vigorous in pea. These differences are not conspicous as are the differences demonstrated by Godfrey and Oliveira (1932) between cowpea and pineapple. None of the data here given are sufficiently extensive to warrant any conclusions on the relative rates of nematode development in these different host plants or on the relation between host and maximum number of eggs laid. The root that gave the highest count (Canada field pea, 2,882 eggs on June 22) gave another count of 1,910 eggs on the same day.

Undoubtedly there is much individual variation in rate of development, in rate of egg-laying, and in number of eggs laid, and also variation owing to the season or to the vigor of the particular host plant. The longer exposure of pea and tomato seedlings to infestation allows a wider age difference among the nematodes in these roots, but the slower germination of the tomato seeds must also be taken into account. For some reason, the infested taproots of the soybean were unhealthy.

Temperature data are unfortunately not available. The records from tomato are therefore given for comparison, since controlled temperature experiments on root-knot nematodes developing in tomato roots have already been reported (Tyler, 1933b). The early summer in Washington has been moderate, and development of nematodes in indicator roots of other greenhouse experiments has been less rapid than is usual at this time of year.

OBSERVATIONS ON EGG-LAYING

Examination of roots on May 11 (see table 2) showed that egg-laying was just beginning in squash and cucumber and had not yet begun in soybean. On June 1, embryonated eggs were found in 3 hosts. No empty egg shells were observed in the masses counted, but hatching should have occurred before this time, since empty shells have been found in egg masses smaller than these (note Sudan grass in table 2; and squash in table 2, footnote 10). Also, the period from June 2 to 24 was almost too brief to allow the second generation to develop from larva to larva. Plenty of empty shells were found on June 7. By June 17 egg counts had increased strikingly, there were hundreds of empty shells in each egg mass, and second-generation galls were numerous. On June 24 the third generation was beginning to hatch. One of the masses in a secondary gall on squash (table 2, footnote 10) contained 662 eggs, including 6 empty shells. Three other secondary galls on the same squash root, evidently formed later than the advanced one, had masses of fewer than 100 eggs. At this time, but not sooner, many of the original females were reaching exhaustion. All plants examined on June 24, 25, and 26 contained females more or less exhausted but other females still opaque. Godfrey and Oliveira (1932) found exhausted females in cowpea on the 43rd day after inoculation, and after at least 16 days of egg-laying at summer temperatures.

LOSS OF EGGS FROM LARGER MASSES

Egg counts were much lower on June 24 than on previous days. The search for complete egg masses on that and succeeding days became increasingly difficult. At the outer surfaces, formerly protected by the thick "gelatinous" covering, embryonated eggs were practically exposed. The actual sloughing off

of a layer of crushed empty egg shells was seen in at least one case. Evidence of its having occurred was seen many times. In other masses, pressure from the more recently laid eggs had pushed aside the "gelatinous" cover at its base near the egg-laying female, and eggs were protruding with little or no covering material to hold them in place. In some cases the entire "gelatinous" matrix appeared distended. At last an apparently complete egg mass was found on a pea root on June 25, but the count was slightly lower than one made 3 days previously on the same host, from an opaque female that still contained eggs *in utero*, and had already deposited 2,882 eggs.

RATE OF EGG-LAYING

A computation of various possible average rates of egg-laying during different periods is given in table 3. For each host, the average rate of output between each 2 dates has been calculated on the basis of the highest egg counts recorded on those dates. These calculations give only a rough indication of what may actually occur, since each egg mass counted was produced by a different nematode, and not all of these nematodes started their development at the same time. Where the record is too obviously out of line, e.g., the count from tomato on June 25, it is omitted from the calculations.

The rate computed—from insufficient data—in an earlier paper (Tyler, 1933b) was only 36 eggs per day at the optimum temperature. The situation is apparently less simple than was then assumed. Godfrey and Oliveira (1932) give egg-laying records which suggest a slow start, a peak of rapid laying, and a gradual decline. Since their females, in excised roots, had presumably laid eggs prior to the observations cited, the curve of their egg counts may not be significant, but it does present a hypothesis which helps to explain the present data and which would also help to explain the rate of hatching sometimes noted from egg masses *in vitro*. On the other hand, the varying rates of egg-laying on different days must be influenced, primarily or secondarily, by temperature. No attempt is made here to explain the changes in rate of output.

SUMMARY

Females of the root-knot nematode, *Heterodera marioni*, in a greenhouse experiment, continued egg-laying throughout the development of a second generation and showed no signs of exhaustion until the time the third-generation larvae were beginning to hatch. Counts of more than 500 eggs, the number commonly quoted as a maximum, are frequent; the highest count obtained was 2,882 eggs laid by one nematode, and this particular female showed no signs of approaching exhaustion. Counts of more than 500 eggs already produced by young females are reported also from 2 resistant plants: wheat and Sudan grass. Reasons are given for the rare finding of complete egg masses. The average rate of egg-laying varied widely during different periods.

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A consideration of the nematode genus *Citellinema* with description of a new species, *Citellinema columbianum*. GERARD DIKMANS, U. S. Bureau of Animal Industry.

The nematode genus *Citellinema* was established by Hall (1916, Proc. U. S. Natl. Mus. 50:1-258) on the basis of one male specimen from *Citellus elegans* obtained from alcoholic material collected by Messrs. N. R. Warren and H. R. Durand. In the same paper, Hall established a second new genus, *Warrenius*, for some nematodes obtained from *Eutamias quadrivittatus*. Cameron (1923, J. Helminth. 1(2):71-76) quotes the opinion of Travassos to the effect that *Warrenius* is a synonym of *Heligmosomum*, and *Citellinema* a synonym of *Vianella*. Sleggs (1925, Parasitol. 17(4):410-416) described a nematode from *Citellus richardsonii*, which he named *Warrenius bifurcatus*. He described and figured the female, showing it to belong to the family Trichostrongylidae, contrary to Travassos' opinion. Manter (1930, Trans. Amer. Micros. Soc. 49(1): 26-33) makes the genera *Citellinema* and *Warrenius* synonymous and since *Citellinema* has page priority he places the genus *Warrenius* in synonymy. As this would change the name of Sleggs' form from *Warrenius bifurcatus* to *Citellinema bifurcatum*, a homonym of *Citellinema bifurcatum* Hall, he proposed for Sleggs' species the name of *Citellinema sleggsi*. He also proposes the name *Citellinema monacis* for a nematode obtained by him from the woodchuck, *Marmota monax canadensis*, making the known species of the genus *Citellinema* as follows: *Citellinema bifurcatum* Hall, 1916 (type species); *C. quadrivittati* (Hall, 1916) Manter, 1930; *C. sleggsi* Manter, 1930 (Syn., *Warrenius bifurcatus* Sleggs, 1925); and *C. monacis* Manter, 1930.

In the dicussion following the description of *C. monacis*, Manter states, "*C. monacis* differs from *C. bifurcatum* in possessing much larger ventral rays and shorter spicules. The relative sizes of the rays, in fact, are exactly the reverse of those shown in Hall's drawing of *C. bifurcatum*. Since he (Hall) did not identify the dorsal ray, it is possible he confused dorsal and ventral surfaces. If so, then *C. monacis* and *C. bifurcatum* are very similar except for spicule measurements of 280 and 360 microns respectively. The number of longitudinal striae is not known for *C. bifurcatum*."

The present writer has had opportunity to examine all of the material on which the descriptions of the various species of *Citellinema* are based, namely, U. S. N. M. No. 16176, type material of *Citellinema bifurcatum;* U. S. N. M. No. 16185, type material of *Warrenius quadrivittati;* U. S. N. M. No. 26067, cotypes of *Warrenius bifurcatus* Sleggs, 1925, (=*Citellinema sleggsi* Manter, 1930); U. S. N. M. No. 8082, type specimens of *Citellinema monacis* Manter, 1930. As a result of this examination, the dorsal and externodorsal rays of *Citellinema bifurcatum* have been identified from the type specimen and it has been definitely ascertained that in Hall's original drawings, the rays of the bursa are reversed. The rays labeled ventroventral and lateroventral are the posterolateral and mediolateral. The externolateral is correctly labeled. The rays labeled mediolateral and posterolateral are ventrolateral and ventroventral respectively. The lobes or the bursa are asymmetrical, and the rays of the bursa are identical with those figured for *Warrenius quadrivittati*. The only difference noted is in the length of the spicules. Having established that Manter's surmise as to the reversal of the bursal rays in Hall's drawing of *Citellinema bifurcatum* is correct, the only difference remaining between *Citellinema bifurcatum* and *Citellinema monacis* is the length of the spicules. Hall states that the spicules of *C. bifurcatum* are 360μ long, and Manter gives the length of the spicules of *C. monacis* as 280μ. An examination of the 2 male specimens deposited in the U. S. National Museum as type specimens of *C. monacis* shows that the length of the spicules varies from 310 to 330μ. Since this difference can hardly be considered sufficient grounds for the erection of a new species, *Citellinema monacis* is regarded as a synonym of *Citellinema bifurcatum*.

In adition to this material, there were available for study the following specimens in the U. S. National Museum Helminthological Collection: Nos. 2341, 18064, 18238, 27851, 27862, and 28916.

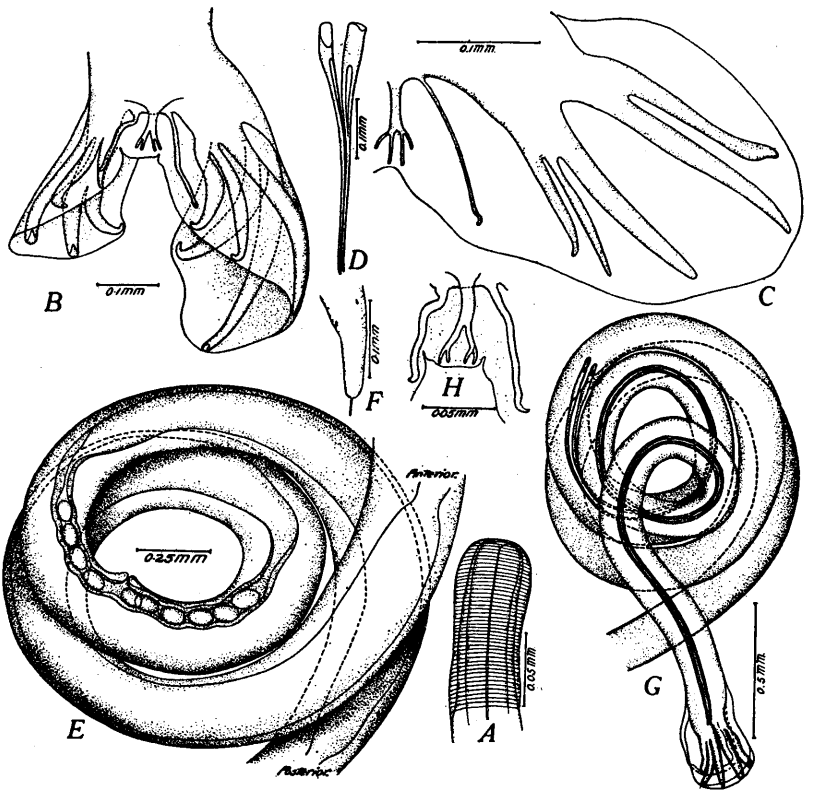

FIG. 19

A-F—*Citellinema bifurcatum*. A—Anterior end [from one of Sleggs' cotypes of *Warrenius bifurcatus* (=*C. bifurcatum*)]; B—Bursa, dorsal view; C—Bursa, lateral view (from type specimen); D—Spicules; E—Genital region of female; F—Tail of female. G & H—*Citellinema columbianum*. G—Posterior end of male showing bursa and spicules; H—Dorsal rays of bursa.

No. 2341 consists of 2 specimens, 1 male and 1 female, collected from *Tamias striatus* by Hassall July 23, 1891. These nematodes have been identified as belonging to the genus *Citellinema*. They correspond to *C. bifurcatum* except that the spicules of the male are 375μ long and the branching of the dorsal ray occurs at a slightly higher level than in *C. bifurcatum*. These specimens are regarded as *C. bifurcatum*, the slight difference not being considered sufficient to establish a new species and only requiring a slight modification of the diagnosis of *C. bifurcatum*.

No. 18064 consists of some nematodes collected by H. Douthitt from the duodenum of *Sciurus hudsonicus* at Bemidji, Minnesota, September, 1911. They were provisionally identified as *Nematodirus* sp. Examination of these nematodes shows them to belong in the genus *Citellinema* and they have been identified as *C. bifurcatum*.

No. 18328 consists of 2 entire specimens, 1 male and 1 female, and 1 piece of a nematode. They were collected from the duodenum of *Marmota monax rufescens* by Dr. W. A. Riley, at Ithaca, New York, in August, 1916. They had been provisionally identified as *Heligmosomum* sp. An examination of this material shows that they do not belong in the genus *Heligmosomum* but in the genus *Citellinema*. They agree with Manter's description of *C. monacis*, and since *C. monacis* has been made a synonym of *C. bifurcatum*, these nematodes are regarded as *Citellinema bifurcatum*.

Nos. 27851 and 27862 consist of specimens collected from the small intestine of *Sciurus carolinensis* by Drs. Hassall and Price. These had been identified as *Warrenius bifurcatus*. Since *Warrenius* has become a synonym of *Citellinema*, these nematodes now become *Citellinema bifurcatum*. The spicules in these specimens are 420 to 425μ long.

No. 28916 consists of 2 slides, I showing a male and 1 containing a part of a female nematode collected from the small intestine of a flying squirrel, *Glaucomys sabrinus macrotis* at Douglas Lake, Michigan, by A. McIntosh. These also have been identified as *Citellinema bifurcatum*. The spicules in this male are 415 to 420μ long.

Through the courtesy of the Bureau of Biological Survey, the Zoological Division received the viscera of several specimens of *Citellus elegans* and *Citellus armatus* from Jackson, Wyoming, and Soda Springs, Idaho. The examination of the anterior part of the small intestine of these hosts yielded several nematodes which proved to belong to the genus *Citellinema*. It may be noted that the host from which Hall's original type material of *Citellinema bifurcatum* was collected, was *Citellus elegans* from Walden, Colorado. The male specimens in the material from *Citellus elegans* from Jackson, Wyoming, are 10 to 12 mm long and 170 to 175μ wide just anterior to the bursa; there are 16 to 20 longitudinal striae; the spicules are 340 to 360μ long; the bursa is asymmetrical and the course of the rays is similar to that figured for *Citellinema quadrivittati* (Hall, 1916) Manter, 1930 and *Citellinema sleggsi* Manter, 1930. The female is 18 to 20 mm long. The width of the head is 42 to 50μ, and the esophagus is 700μ long. The ovaries, uteri and ovejectors are double; the uteri end in small, muscular ovejectors, which are in turn followed by a comparatively long, thick-walled, nonmuscular vagina. The vulva is located 3.4 to 3.8 mm from the tail end, and the distance from the anus to the tip of the tail is 120 to 140μ. The eggs are 73 to 77μ long by 38 to 40μ wide.

Since these specimens agree in all essential features with the type material of *C. bifurcatum* as determined by reexamination, and since the material was collected from the same host and from an adjoining state, it is reasonable to conclude that they are identical.

A comparison of the cotypes of *Citellinema sleggsi* Manter, 1930 (*Warrenius bifurcatus* Sleggs, 1925) collected from *Citellus richardsonii* with *Citellinema bifurcatum* from *Citellus elegans*, from Jackson, Wyoming, shows them to be identical. The unilateral cervical wing mentioned by Sleggs as a reason for placing his form in the genus *Warrenius* is not a constant feature, as may be seen from figure 19,A which was drawn from a specimen selected at random from his cotypes. There appears to be a slight difference in the distance from the vulva to the tail between his specimens and those of the writer; this may be due, however, to differences in length of the specimens examined. Therefore, *Citellinema sleggsi* is a synonym of *Citellinema bifurcatum* as shown by a comparison of specimens from *Citellus elegans* from Jackson, Wyoming, and from *Citellus elegans* from Waldon, Colorado, with the types of *C. sleggsi*.

In the course of the examination of rodents for nematode parasites, there were collected by the writer from the fox-squirrel, *Sciurus niger neglectus*, from Priest's Bridge, Maryland, some nematodes which are similar in all respects, except one, to *Citellinema bifurcatum*. This one exception consists in the presence of a pair of prominent prebursal papillae. These papillae are about

45μ long. No prebursal papillae have been, so far, either figured or mentioned for representatives of this genus. Thinking, therefore, that this feature had been merely overlooked, specimens from various hosts were reexamined, but in no case could the presence of prebursal paplillae be established. The original generic diagnosis of *Citellinema* is, therefore, amended as follows:

Genus *Citellinema* Hall, 1916

Diagnosis.—Trichostrongylidae: Head surrounded by a cuticular inflation marked by annular striations. Males with 2 spicules, each bifurcating near the proximal, wide, tubular end to form 2 long, filiform processes. Bursa asymmetrical, the right lobe of bursa much larger than the left lobe. Ventral rays arising from a common stem, lateroventral ray largest of the rays of the bursa. Lateral rays arising from a common stem, separate from the stem of the ventral rays. Externodorsal rays slender. Dorsal ray bifurcated near the distal end, each bifurcation dividing into 2 terminal branches. Prebursal papillae present or absent. Females with uteri terminating in short, muscular ovejectors. Vagina long, with thick, nonmuscular walls. Tip of tail surmounted by a terminal spine-like process.

Type species.—*Citellinema bifurcatum* Hall, 1916.

Other species:

Citellinema quadrivitatti.

Host.—Western chipmunk (*Eutamias quadrivittatus*).

Citellinema bifurcatum.

Hosts.—Ground squirels (*Citellus elegans*), *Citellus armatus*, and *C. richardsonii;* red squirrel (*Sciurus hudsonicus*); grey squirrel (*Sciurus carolinensis*); fox squirrel (*Sciurus niger neglectus*); flying squirrel (*Glaucomys sabrinus macrotis*); woodchucks or groundhogs (*Marmota monax canadensis, Marmota monax rufescens*).

During the course of the examination of the viscera of ground squirrels received through the courtesy of the Bureau of Biological Survey, some menatodes were recovered from the small intestine of *Citellus columbianus* from Soda Springs, Idaho. These nematodes are regarded as representing a new species of *Citellinema* which is described as follows:

Citellinema columbianum, n. sp.

Description.—Male unknown. The portion of the male available for study shows a pair of spicules decidedly longer than any spicules so far recorded for any member of this genus. These spicules are 3.6 mm long; proximal portions expanded. They bifurcate about 150μ from the proximal end. The bursa is asymmetrical as in other members of this genus, and presents nothing unusual.

Female 17.2 mm long and about 160μ wide in the region of the vulva. Esophagus, in this specimen, 650μ long. Posterior ovejector about 2.2 mm from tail end; vulva about 4.2 mm from tail end. Position of anterior ovejector could not be determined with certainty. Anus 100 to 120μ from tip of tail. Tip of tail terminates in a "spike" about 20μ long.

Only one female and the posterior portion of a male were available for examination.

Host.—Citellus columbianus.

Location.—Small intestine.

Locality.—Soda Springs, Idaho, U. S. A.

Type specimen.—U. S. N. M. Helm. Coll., No. 30466.

This nematode is described as a new species because, while possessing the characters of the genus, it differs from *Citellinema bifurcatum*, the only other member of the genus, in the markedly greater length of the spicules.

New genera and species of the nematode superfamily Filarioidea. I. *Serratospiculum amaculata*, n. sp. E. E. WEHR, U. S. Bureau of Animal Industry.

Several years ago a portion of the thoracic region of a prairie falcon, *Falco mexicanus mexicanus*, containing a large number of nematodes was sent to the writer at Miles City, Montana, by Mr. E. C. Cates of the Bureau of Biological Survey. The nematodes were so entwined in the connective tissue and twisted among themselves that it was impossible to remove more than 2 or 3 complete specimens. More recently, specimens of this nematode have been identified from the prairie falcon in North Dakota, Oregon and California, and from the duck hawk, *Falco peregrinus anatum*, in Pennsylvania. These nematodes belong to the subfamily Dicheilonematinae Wehr, 1935, and to the genus *Serratospiculum* Skrjabin, 1916; they are regarded as representing a new species for which the name *Serratospiculum amaculata* is proposed.

Serratospiculum amaculata, n. sp.

Description.—Body elongated, stout, attenuated at extremities. Cuticle without small papillae or tubercles, finely striated transversely, not elevated in form of teeth-like processes at sides or oral opening. Oral opening more or less circular, surrounded by 2 lateral, trilobed, cuticular, bean-shaped areas which appear porous in *en face* view. Cephalic papillae consisting of 4 pairs of large submedians of the external circle; internal circle of papillae absent. Esophagus distinctly divided externally into 2 parts.

Male 65 to 88 mm long by 394μ wide. Anterior portion of esophagus 295μ long, gradually merging into a wider and much longer posterior portion, 8.9 mm long. Nerve ring surrounding first division of esophagus about 166μ from anterior end of body. Intestine of about the same diameter as esophagus at point of union with the latter, gradually narrowing toward posterior end of body. Long spicule 2.1 mm in length, relatively narrow, slightly curved, and provided with lateral wings or alae for the posterior ⅔ of its length; short spicule 720μ long, arcuate, alate almost its entire length, with its tip rounded. Tail rounded, with short broad alae meeting behind tip; each ala about 202μ long and 14μ wide. Twelve pairs of pedunculated caudal papillae present: 4 pairs preanal, 1 pair adanal, and 7 pairs postanal. Cloacal aperture about 79μ from posterior end of body.

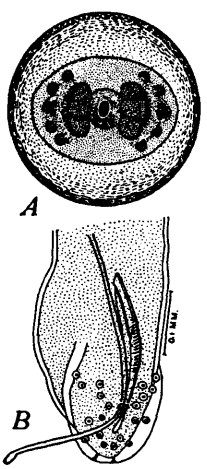

FIG. 20 *Serratospiculum amaculata*, n. sp. A—Head of female, *en face* view. B—Tail of male, lateral view.

Female 200 to 225 mm long by 600μ wide. Anterior portion of esophagus 495μ long by 102μ wide; length of posterior portion not ascertainable, since the posterior end was obscured by the egg-filled uteri. Nerve ring 195μ from head end. Vulva 657μ from anterior end of body, or at a level slightly posterior to union of the 2 divisions of the esophagus. Vagina 5.1 mm long, straight, directed posteriorly; uteri parallel. Posterior extremity rounded; anus terminal. Eggs 54μ long by 29μ wide, with thick shells, containing embryos at time of deposition.

Hosts.—*Falco mexicanus mexicanus* and *F. peregrinus anatum*.

Location.—Connective tissue of thoracic and abdominal cavities.

Distribution.—Montana, Oregon, North Dakota, and California, U. S. A.

Specimens.—U. S. N. M. Helm. Coll. No. 32332 (type, male and female).

The following key will aid in the differentiation of the species now included in the genus *Serratospiculum*.

1. Body covered with numerous conical papillae 2
   Body not covered with conical papillae 3
2. Male with 4 pairs of preanal papillae
   S. guttata (Schneider, 1866) Skrjabin, 1915
   Male with 6 pairs of preanal papillae ..... *S. turkestanicum* Skrjabin, 1915
3. Long spicule more than 1 mm in length 4
   Long spicule less than 1 mm in length 6
4. Long spicule about 2.1 mm in length ..... *S. amaculata*, n. sp.
   Long spicule not over 1.44 mm in length 5
5. Vulva 1 to 1.12 mm from anterior end of body ..... *S. thoracis* Tubangui, 1934
   Vulva 2.5 mm from anterior end of body
   S. tendo (Nitzsch in Giebel, 1837) Railliet, 1918
6. Short spicule 100μ long, with definite distal sickle-like hook
   S. helicinum (Molin, 1857) Walton, 1927
   Short spicule 340μ long, without distal sickle-like hook
   S. chungi Hoeppli and Hsü, 1929

The influence of infections with the tapeworm, *Raillietina cesticillus*, on the growth of chickens. PAUL D. HARWOOD and GEORGE W. LUTTERMOSER, U. S. Bureau of Animal Industry.

INTRODUCTION

There is very little experimental evidence available by which one can determine whether the ubiquitous tapeworm parasites of poultry and other domestic animals are more or less harmless inhabitants of the intestine, or whether they are injurious to the host. Certainly tapeworm infestations do not ordinarily lead to acute manifestations of disease, but as these infestations are usually of long duration (Harwood, 1938, Livro Jubilar do Professcr Lauro Travassos, p. 213) even slight injury by tapeworms may lead ultimately to extensive losses. The latter possibility is questioned by Taylor (1933, Atti 5. Cong. Mond. Pollicolt. Roma, 3:219) who studied experimental infections of *Davainea proglottina* in chickens.

In the present paper the writers report the results of experimental infections with *Raillietina cesticillus* in laboratory-raised chickens and compare the growth rate of these birds with that of uninfested controls.

EXPERIMENTAL METHODS

White Leghorn or Rhode Island Red birds, 2 to 4 weeks old, were paired off by weight, and one bird of each pair was given various numbers of infective cysticercoids of *R. cesticillus* which had been reared experimentally in beetles, *Aphodius* spp. or *Tribolium* spp. The other chick of each pair served as a control. Experiment 2 is an exception as only 2 controls and 4 experimentally infested birds were used in this case. Following infection, the birds were maintained under conditions which were sufficient to prevent extraneous infestation with helminths. Two diets were fed in this series of experiments. The diet given to the chicks in experiment 1 consisted of yellow corn meal, 40 pounds; corn gluten meal, 10 pounds; ground wheat, 22 pounds; dried buttermilk, 10 pounds; desiccated meat meal, 10 pounds; steamed bone meal, 3 pounds; alfalfa leaf meal, 2.5 pounds; yeast, 2 pounds; salt, 0.5 pounds, and cod-liver oil, 1.5 pounds. As this diet was low in available manganese and resulted in one case of perosis, the birds in the remaining experiments were given a diet consisting of corn meal, 36 pounds; wheat middlings, 30 pounds; rolled oats, 10 pounds; buttermilk, 10 pounds; soy bean meal, 5 pounds; alfalfa leaf meal, 5 pounds; ground limestone, 2 pounds; steamed bone meal, 1 pound; salt, 0.5 pound; and cod-liver oil, 2 pounds. The weights of the control and experimental birds were recorded at weekly intervals, and the effects of the

TABLE 1.—Effect of infection with *Raillietina cesticillus* on the growth of chickens

Experiment number	*Breed*	*Number*		*Age when infected*	*Date infected*	*Mean number cysts administered*	*Mean gain in weight 4 weeks after infection*		*Difference in mean gain*
		Infected	*Controls*				*Infected group*	*Control group*	
				(Weeks)			(Grams)	(Grams)	(Per cent)
1	Rhode Island Red	3	3	2	5/9/35	Unknown	184.7	275	32.8
2	Rhode Island Red	4	2	2	8/3/35	40	229.7	235	2.3
3	White Leghorn	7	7	2	11/2/37	300	216	233.8	7.6
4	Rhode Island Red	22	22	2	3/15/38	364	187	205.4	8.6
5	White Leghorn	5	5	4	1/11/38	840	278.4	306.6	9.2

tapeworm infestation on the growth rate was determined by an analysis of these data.

EXPERIMENTAL DATA

As may be seen in table 1 the controls in each experiment gained more than the infested birds. Since the greatest difference in mean gain occurred in experiment 1, it seems possible that the shortage of manganese in the diet may have aggravated the tapeworm injury. One of the control birds in this series developed symptoms of perosis at the end of the second week after infection, and was badly crippled by the end of the fourth week. However, in spite of the affliction this bird continued to gain faster than its infested mate. Five weeks after the date of infection the birds in experiment 1 were killed and examined for tapeworms. The infested birds contained 52, 21, and 13 tapeworms, respectively; judging from data obtained in other experiments in which a known number of cysts were fed it appears likely that these birds had received fewer than 200 cysts.

The number of cysts fed to each individual bird in the remaining experiments varied from 40 to 1,000; this is not excessive since Reid, Ackert and Case (1938, Trans. Amer. Micros. Soc. 57:65) reported that 626 cysts of *Raillietina cesticillus* have been found in one beetle. The chickens used in experiment 4 were examined for tapeworms 6 weeks after infection. The highest number of tapeworms found in any one bird was 155, the host in question having received 1,000 cysts.

DISCUSSION

The difference between the means in experiment 1 was analyzed by the ordinary statistical methods and it was found that there was less than 1 chance in 50 that the difference could be due to chance. The difference between the means in not one of the remaining experiments approaches significance, but in experiment 4 a comparison between the birds receiving 400 or more cysts each and all the controls shows that there was 1 possibility in 20 of the difference being due to chance. When the percentage differences in the mean gains in weight of experiments 3 to 5 were submitted to statistical analysis it was found that there was less than 1 possibility in 1,000 of the difference being due to chance. Therefore, these experiments indicate that infection with *Raillietina cesticillus* produced a definitely injurious effect on the growth weight of chicks fed adequate rations and kept under optimum conditions, but when the diet was deficient in manganese the degree of injury was apparently more severe. Experiment 5 was continued until the infestation was 9 weeks old. From the fourth to the ninth week the difference between the mean gains in weights of the controls and the infested chickens increased slightly, but as the individual variations also increased, the significance of this difference between the means either decreased slightly or remained approximately constant.

Examination of the data presented by Taylor (*vide supra*) shows that only 4 of the birds in his experiments carried heavy infestations of *Davainea proglottina* (from 1,900 to 3,900 worms). The mean gain in weight of these 4 birds was several ounces less than the mean gain in weight of the remaining birds that were either lightly infected or not infected at all. This difference between the means suggests that Taylor's data and that obtained by the writers are comparable.

SUMMARY AND CONCLUSIONS

1. Forty-one chicks were infected experimentally with *Raillietina cesticillus*, and their growth rates compared with those of uninfested controls.
2. The growth rate of chicks on a diet short in manganese seemed to be markedly retarded by a light infestation.
3. The growth rate of chicks on an adequate diet seemed to be slightly retarded by only a very light infestation and definitely retarded by a moderate infestation.

Persistence of swine lungworm larvae in earthworms. L. A. SPINDLER, U. S. Bureau of Animal Industry.

Lungworms are common parasites of swine, and when present in large numbers may cause general unthriftiness which results in considerable economic loss (Schwartz, 1936, U. S. Dept. Agr. Leaflet 118, pp. 1-5). The wide prevalence of these parasites is attested to by the fact that 69 per cent of 348 swine from the southeastern part of the United States examined by the writer from September, 1929, to August, 1931, were infested with lungworms (Spindler, 1934, Proc. Helminth. Soc. Wash. 1(2):40-42). Lungworms are known to be widespread in swine in other sections of the United States.

Earthworms serve as intermediate hosts of lungworms, these annelids becoming infected as a result of ingesting the eggs that are eliminated with the feces of infested swine. Swine become infected with lungworms as a result of swallowing infested earthworms which they bring to the surface of the soil by rooting. Consequently, as pointed out by Schwartz (1938, U. S. Dept. Agr. Farmers' Bull. 1787, pp. 1-45) effective control of lungworm infestations in swine involves the use of the sanitation system of swine management; under this system hogs are raised on well-drained temporary pastures at some distance away from wet areas, old hog lots and permanent pastures; earthworms abound in the places mentioned. In order to control successfully lungworms by such management practices, it is essential to know how long the lungworm larvae survive in infested earthworms. Schwartz and Alicata (1934, U. S. Dept. Agr. Tech. Bull. 456, pp. 1-41) reported that individual earthworms collected from areas to which swine had not had access for a number of years harbored slight infestations with lungworm larvae. This observation indicates that lungworm larvae probably survive in the intermediate hosts over considerable periods.

In this connection, the present writer collected and examined earthworms from a farm near Westminster, Md., where no hogs had been kept for approximately 4 years; the location of the hog lot where the worms were collected precluded the possibility of fecal contamination from hogs from adjoining farms. The soil of this lot had apparently remained undisturbed since last used. Of 75 sexually-mature earthworms examined, 35 (46.6 per cent) were infested with lungworm larvae. The infestations ranged from 1 to about 100 larvae per annelid; the larvae were located in the region of the calciferous glands. None of 25 young earthworms examined were found infested. A number of the larvae recovered, together with 25 unexamined mature earthworms from the same lot, were fed to a susceptible pig to test the viability of the larvae. The pig became infested, as adjudged by the presence of lungworm eggs in the feces of this host animal 31 days after infection. Two control pigs from the same litter, kept under conditions similar to those of the test animal, did not become infested.

In view of the above findings, any system of management designed to control lungworm infestation in swine must be so planned as to keep susceptible animals away from infested areas for periods longer than 4 years, or to eradicate the intermediate host of the parasite from the areas involved. In this connection, Schwartz (1936, U. S. Dept. Agr. Leaflet 118, pp. 1-5) observed that temporary pastures harbor comparatively few earthworms and he attributed this to the frequent plowing to which temporary pastures are subjected. In light of this observation the findings herein reported further emphasize the need of raising swine on frequently plowed pastures as a practical procedure for the control of lungworms in swine. This is in agreement with procedures recommended by the U. S. Bureau of Animal Industry for the control of parasites of swine in general.

Notes on free-living and plant-parasitic nematodes. IV. GERALD THORNE, U. S. Bureau of Plant Industry, Salt Lake City, Utah.

(1) PANAGRELLUS PYCNUS, N. G. N. SP. (CEPHALOBIDAE, PANAGROLAIMINAE)

Diagnosis:—♀: 1.0-1.4 mm; $a = 18$; $\beta = 8.0$; $\gamma = 8.5$; V—[38] 73.[31]
♂: 0.8-1.2 mm; $a = 21$; $\beta = 6.8$; $\gamma = 9.0$; T—63

Body obese, tapering anteriorly to the narrow lip region which is not set off, while posteriorly it ends in a uniformly conoid, pointed tail. Wings appearing as 4 bright lines which frequently are obscure. Lips low, rounded, bearing an inner circlet of 6 easily visible papillae and an outer circlet of very obscure ones, probably 10 in number. Amphid apertures minute, pore-like. Pharynx panagrolaimoid; *en face* view the cheilostom is a broad, symmetrical, escutcheon-shaped chamber with moderately sclerotized walls followed by a shallow, triquetrous prostom with much heavier refractive walls. Meso-meta- and telostoms fused, connecting directly with the esophageal lumen. Esophagus panagrolaimoid, the corpus broadly cylindrical, isthmus about as long as neck width, cardiac bulb with a conspicuous valvular apparatus. Esophagus enveloping the pharynx only to the base of the prostom. Vulva located at a ventral contraction of the body. Vagina extending forward to join the long uterus which serves as a spermatheca. Ovary frequently extending past the anus into the tail cavity. Eggs ovate, sometimes deposited but more frequently hatching within the body. Testis single, reflexed. Spicula elongate-linear, arcuate, with striking cephalation and furcate terminus. Gubernaculum lineate. Ventral preanal supplement present. Four pairs of ventrosubmedian and 1 pair of dorsosubmedian caudal papillae. Tails of both sexes conoid acute.

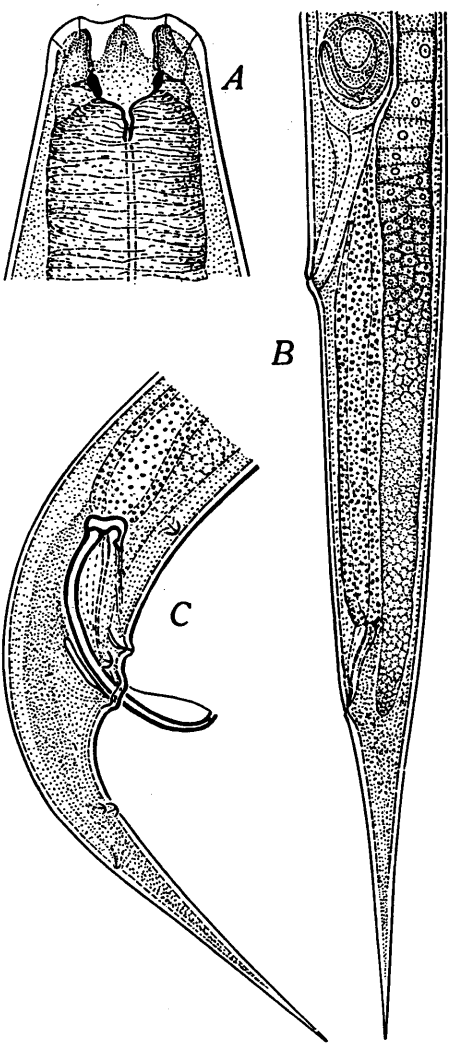

FIG. 21

Panagrellus pycnus.
A—Head, lateral view; X 1500. B—Posterior portion of female; X 250. C—Posterior portion of male; X 500.

This genus is distinctive because of its characteristic pharynx, ventromedian supplement and lineate, cephalated spicula. It presents an interesting combination

of the characters of *Panagrolaimus* and *Neocephalobus* combined with the lineate, cephalated spicula resembling those of some Diplogasters.

Habitat.—Slime from unidentified disease of the Great Plains cottonwood, *Populus sargentii* Dode, collected near Magna, Utah, U. S. A.

(2) RENAMING OF HOMONYMS

Dr. Oehser has called the writer's attention to the fact that *Stegella* Thorne, 1937 (Cephalobidae) was preoccupied by *Stegella* E. Stechow, 1919, hydroid (1919, München Med. Wchnschr. (30), p. 852). Dr. Schwartz has also written that *Dorylaimus truncatus* N. A. Cobb, 1936 in Thorne and Swanger (1936, Capita Zool. 6:88) was preoccupied by *D. truncatus* (Cobb, 1913) Micoletzky, 1922 (Synonym: *Antholaimus truncatus* Cobb, 1913) (1922, Arch. Naturgesch., Abt. A, 87 (8-9):454). These homonyms are renamed as follows:

Stegella incisa Thorne, 1937, becomes *Stegelleta incisa* (Thorne, 1937), n. comb.

Dorylaimus truncatus N. A. Cobb, 1936, becomes *Dorylaimus cobbi*, nom. nov.

Aspiculuris caviellae, a new name for _Aspiculuris schulzi_ Freitas, Lent and Almeida, 1937, preoccupied. T. F. TEIXEIRA DE FREITAS, HERMAN LENT and J. LINS DE ALMEIDA, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

We have recently received a communication from Dr. Benjamin Schwartz, U. S. Bureau of Animal Industry, informing us that the name *Aspiculuris schulzi* Freitas, Lent and Almeida, 1937 (Mem. Instit. Oswaldo Cruz 32(2): 195-209) was preoccupied by *Aspiculuris schulzi* Popov and Nazarova, 1930 (Vestnik Mikrob. Epidem. i Parazitol., Saratow, 9 (1): 105-108) which appeared in a paper unknown to us. We propose, therefore, for our species the name *Aspiculuris caviellae*, n. nom., and thank Dr. Benjamin Schwartz for the kindness of his communication.

A redescription of _Thelastoma robustum_ Leidy with comments on other species of the nematode family Thelastomatidae. J. R. CHRISTIE, U. S. Bureau of Plant Industry.

In 1850 Leidy (Proc. Acad. Nat. Sci. Phila. 5:101-102) described under the name *Thelastoma robustum* a nematode found in the intestine of "a lamellicorn insect" presumably collected near Philadelphia, Pennsylvania. So far as the writer is aware this nematode has never been reported by subsequent investigators and Leidy's brief description without figures constitutes our total information regarding it. While examining larvae of the scarabaeid beetle *Osmoderma scabra* Beau. collected at New Boston, New Hampshire, the writer removed from the posterior end of the alimentary tract nematodes that apparently are identical with Leidy's *T. robustum*. Larvae of *Xyloryctes satyrus* Fab. collected at Falls Church, Virginia, harbored specimens of the same species in the same location. The following description is based on material from these 2 collections.

Thelastoma robustum Leidy, 1850

Synonyms.—*Anguillula* (*Thelastoma*) *robusta* (Leidy) Diesing, 1861. *Aoroorus* (*Thelastoma*) *robustus* (Leidy, 1850) Walton, 1927.

Diagnosis.—Male 1.3 mm long by 85μ wide. Body distinctly annulated throughout, annules about 5μ wide near head increasing in width to 10 or 12μ at middle of body. Tail 130μ long, moderately slender. Alae conspicuous, extending from region of esophageal bulb to opposite proximal end of spicule, 20μ wide near middle of body (much wider in larval stages). Esophagus about 160μ long; corpus nearly cylindrical, about 110μ long by 14μ wide; isthmus about 19μ long by 12μ wide; bulb about 30μ wide with distinct valve. Intestine

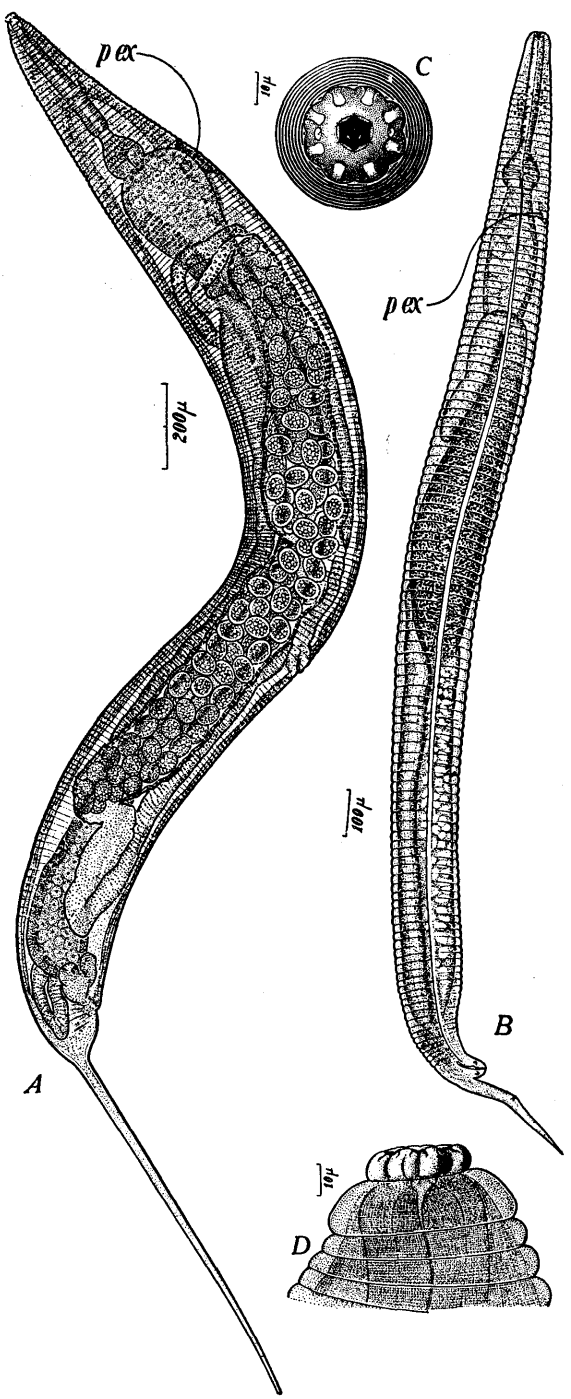

FIG. 22
Thelastoma robustum
A—Female. B—Male. C—Head of female, *en face* view.
D—Head of female, lateral view.

with anterior end moderately dilated. Nerve ring about 75μ from anterior end. Excretory pore about 200μ from anterior end or slightly posterior to base of esophagus. Testis wide, reflexed. Spicule 32 to 40μ long, slightly curved, bearing an enlargement on ventral side; distal end pointed. Anus situated on rounded elevation that bears 3 pairs of papillae, one pair slightly preanal, one pair slightly postanal and a fused pair, ventromedian and slightly postanal. A pair of papillae occurs on the tail about 50μ from the anus.

Female 3.9 to 4.4 mm long by 700 to 800μ wide. Cuticular annules vary in width from 8μ near the head to 20μ at middle of body. First annule back of head 19μ wide. Tail slender, spicate, 1.1 to 1.25 mm long. Alae inconspicuous. Head about 10μ long by 36μ wide, distinctly set off, 8-lobed when seen *en face*, each lobe bearing a labiopapilla about 3.5μ wide. Amphids distinct. Stoma about 10μ deep by 8μ wide, armed at base with 3 more or less tooth-like projections. Esophagus about 460μ long; corpus nearly cylindrical, about 340μ long by 40μ wide; isthmus about 30μ long by 30μ wide; bulb about 100μ wide, provided with distinct valve. Intestine pronouncedly dilated at its anterior end. Nerve ring about 250μ from anterior end. Excretory pore about 500μ from anterior end or slightly posterior to base of esophagus. Reproductive system amphidelphic; vulva not salient, slightly posterior to middle of body

when not including tail; vagina about 300μ long, directed anteriad. Egg ellipsoidal, 75μ long by 50μ wide, deposited before segmentation.

Hosts.—Larvae of *Osmoderma scabra* Beau and of *Xyloryctes satyrus* Fab.

Location.—Posterior end of alimentary tract.

Localities.—New Boston, New Hampshire, and Falls Church, Virginia, U.S.A.

The 8-lobed head of the female differentiates *Thelastoma robustum* from all other known species of *Thelastoma* except *T. labiatum* Leidy, 1850. In the latter species, however, the head is relatively larger and more distinctly set off and is divided into sectors by deep clefts. Females of *T. robustum* are larger and more robust than those of *T. labiatum*. The male of the latter has not been described. In the writer's opinion *T. myolabiatum* Cobb, 1929, is a synonym of *T. labiatum*.

Discrepancies between measurements noted in the above description and those given by Leidy appear to be within the limits of variation that one may expect for a species of this family. In a redescription (Leidy, 1853, Smithsn. Misc. Collect. 5:48) the tail is said to be "little more than one-eighth the length of the body" but the measurement given is 1/22 of an inch (1.15 mm). In the material at hand the tail, measured from the anus, is from 1.13 to 1.25 mm long. A marked variation was noted in the width of the alae on the male. In some specimens each of these structures had a width equal to the diameter of the body while in others the width did not exceed 20μ. Specimens possessing wide alae were apparently young individuals that had not undergone the final molt. In the Thelastomatidae larval stages frequently possess wide alae while on adults of the same species these structures are much narrower.

In a previous paper (Christie, 1931, J. Agr. Research 42(8):463-482) 4 species of thelastomatids were described as new. That one of these, *Scarabanema cylindricum*, is a synonym of *Cephalobellus papilliger* Cobb, 1920, has already been pointed out (Christie, 1933, J. Wash. Acad. Sci. 23(7):358). The writer wishes to call attention to certain other errors that occurred in this same paper (Christie, 1931, loc. cit.).

(a) *Thelastoma papilliferum* Christie, 1931, is a synonym of *T. macramphidum* Christie, 1931. A study of additional material has demonstrated very convincingly that the characters used to differentiate these 2 alleged species were due to age differences. For example in young females that are beginning to deposit eggs the vulva does not protrude appreciably but as the individual grows older the vulva becomes more salient.

It also should be mentioned that *Thelastoma macramphidum* very closely resembles *T. alatum* Johnston (1914, Proc. Roy. Soc. Queensland, 26:76-84). Describing the male of the latter species Johnston writes, "At each side of the hind portion of body is a prominent ala, which, just in front of the anal region, is somewhat arched and expanded. It becomes narrowed and then again widened to form a rather thin rounded lobe lying above the anus and terminating at the base of the tail." This structure is shown in figure 7 of Johnston's paper. Similar alae were not observed on the male of *T. macramphidum* and this appears to be the only difference between the 2 species.

(b) *Aorurus subcloatus* Christie, 1931 is a synonym of *A. agile* (Leidy, 1849) Baylis and Daubney, 1926. In his original description Leidy (1849, Proc. Acad. Nat. Sci. Phila. 4:230-231) states, "Generative aperture situated about twenty-four rings above the anal aperture, which latter is placed between the last two annuli of the body." Through the courtesy of Dr. J. Percey Moore, University of Pennsylvania, the writer had the privilege of examining the material from the bottles in the Leidy Collection that contain the nematodes from millipeds. All specimens belonging to the genus *Aorurus* were of one species and were identical with the material on which the present writer based his *A. subcloatus*. Leidy was evidently in error regarding the position of the vulva and *A. subcloatus* must fall as a synonym of *A. agile* as Chitwood and Chitwood (1934, Philippine J. Sci. 52(4):387) already have indicated.

Further studies on nemic skeletoids and their significance in the chemical control of nemic pests. B. G. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.

Recently the writer (1936) described a series of chemical experiments on the external cuticle of *Ascaris lumbricoides,* finding it to be composed of several substances including a collagen, a fibroid, and a keratin. Solubility in alkalis precluded the possibility of chitin in the external cuticle. Further data regarding the external cuticle and other hard parts of the nemic body have been accumulated during the past two years. Limitations of material or time have prevented a more thorough study in many cases and interesting tests were found to be useful after much of the work had been completed. During the preliminary work the writer was working in the U. S. Bureau of Animal Industry. He also received considerable assistance from Mr. Leon Jacobs, then of the U. S. Bureau of Plant Industry.

1. *Toxocara canis.* The esophageal lining is not digested by artificial gastric juice; it dissolves in boiling 10 per cent KOH; is pale yellowish in iodine-1 per cent H_2SO_4; gives strong xanthoproteic and sulphide reactions. These reactions are presumptive evidence of keratin.

2. *Trichuris vulpis.* The spicule is digested by artificial gastric juice; softened but not dissolved by Fairchild's trypsin; dissolved by hot 10 per cent KOH; gives positive xanthoproteic and mercuric nitrite tests; gives negative or very faint sulphide test; and is colored deep orange by iodine-H_2SO_4. These tests eliminate chitin and keratin. Negative nitrite, xanthoproteic and iodine-sulphuric tests after Fairchild's trypsin indicate that the spicule is a mixture, possibly containing collagen and a glucoprotein. The external layer is physically different from the internal prismoid layer. The cloacal lining in the same tests behaves throughout as keratin.

3. *Spironoura affine.* Spicules (formalin fixed) are not digested by gastric juice or Fairchild's trypsin; dissolve in hot 10 per cent KOH; give positive xanthoproteic and nitrite reactions; become deep orange in iodine-H_2SO_4; and give a negative sulphide reaction. Solubility in KOH eliminates chitin; insolubility in gastric and tryptic solutions would supposedly indicate keratin but that substance is apparently precluded by the negative sulphide reaction. Since the xanthoproteic reaction became negative after exposure to trypsin, it seems possible that we are dealing here with a collagen-glucoprotein mixture, the collagen being somewhat protected from gastric digestion. Results dubious.

4. *Ascaris lumbricoides.* The shell of ascarid eggs (including *Ascaris lumbricoides, Parascaris equorum, Toxocara canis, T. cati,* etc.) has been studied by several workers including Fauré-Fremiet (1912-1913), Yoshida and Takano (1923), Zawadowsky (1914, 1928), Kosmin (1928), Schulze (1924) and Schmidt (1936).

It is now generally recognized that the so-called shell consists of 3 layers of different chemical composition, these being known as (1) the albuminous layer, (2) the shell proper (described as 3 layers by Zawadowsky) and (3) the fibrous layer (vitelline membrane).

(1) *Albuminous layer.* Apparently this layer has been studied only by Yoshida and Takano (1923) and Kosmin (1928). Present observations confirm and extend their results. This layer is dissolved by artificial gastric juice, Fairchild's trypsin, 0.2 per cent HCl, 1 per cent acetic acid, 1 per cent KOH, picric acid, and picric acid-alcohol at room temperature; is insoluble in water and does not coagulate upon heating in acidified solution. Upon the basis of these tests the albuminous layer is certainly not an albumin, collagen, fibroid or keratin. It is, however, almost certainly a protein. One would presume it to belong to the conjugated proteins such as mucoids, which form a similar covering of the egg in other animals (e.g. the "gelatinous envelope" of the frog egg is a glucoprotein). Ordinarily this layer is formed after the shell and all of the evidence indicates that it is a secretion product of the uterus; it is formed on both fertile and infertile eggs.

(2) *Shell proper (chitinous layers, refractive layers, birefringent layers).* Fauré-Fremiet obviously must have had chemical proof of the constitution of the egg shell since he described the mode of its origin from glycogen. However, the evidence has only been presented by Schulze and Schmidt (Zawadowsky's papers inadequately read by the present writer). Insolubility in hot KOH and optical characteristics were the only forms of evidence presented by these authors. Following the technic given by Campbell (1929) further evidence is presented by the present writer.

The egg shell may be characterized as follows: It is not digested by artificial gastric or pancreatic juices, is insoluble in acetic acid in all concentrations and temperatures, insoluble in dilute mineral acids, soluble in 5 per cent NaOCl at room temperature, and birefringent. However, all of these descriptions might well be applied to keratin. Keratin is often soluble with difficulty in alkalis and may easily resist boiling in saturated KOH where dilute KOH will cause it to swell and rapidly dissolve. However, keratin cannot withstand superheating in KOH. This was done by placing material in a vial, adding saturated KOH and a small glass rod to avoid boiling over; a rubber nipple with a fine cut at the tip was attached to the end of the flanged test tube forming a bunsen valve. The test tube was placed in a glycerin bath and heated at 160 to 170° C. under pressure for 1 hour. Thereafter water was added, and the substance washed by centrifuging several times. Only the hard egg shell remained after such treatment. It retains its appearance but its chemical properties are altered. It is immediately soluble in 3 per cent acetic acid, reprecipitated by 1 per cent H_2SO_4 as chitosan sulphate, turns brown in iodine-potassium iodide and purple in 1 per cent H_2SO_4. Shells may be dissolved in 75 per cent H_2SO_4 and chitosan sulphate reprecipitated through imbibition of water in a moist chamber (24 hours). Chitosan sulphate so formed is minutely sphaerocrystallin, said sphaerocrystals staining in 0.1 per cent Rose Bengal. These tests, involving the transformation first to chitosan then to chitosan sulphate, are supposedly conclusive proof of chitin.

(3) *Vitelline membrane (fibrous membrane, lipoid layers).* Fauré-Fremiet and Zawadowsky have identified the internal layer or semi-permeable membrane, as lipoid. It is apparently soluble in absolute alcohol, ether, chloroform, etc. Fauré-Fremiet first called it coprosterol but later named the substance "ascarylique acid" giving the formula $C_{20}H_{40}O_3$. Flury (1912) named the insaponifiable extract of ascarids ascaryl alcohol with the formula $C_{32}H_{64}O_4$. Differences in the melting point and staining properties of "ascarylique" as present in the ovum permit the suggestion that the extracts studied by Fauré-Fremiet and Flury were possibly of 2 coexisting interrelated substances. The membrane itself seems to behave as a sterol.

As observed by Fauré-Fremiet, both the true egg shell and the vitelline membrane are apparently formed by the ovum itself in the ectoplasm of the egg as differentiations when stimulated by entrance of a sperm.

5. *Dioctophyma renale.* The eggs of this species have been previously studied by Lukasiak (1930) who found that the shell withstands strong H_2SO_4, HNO_3, NaOH and KOH, while in 5 per cent KOCl the outer layers are dissolved first, finally leaving a thin inner membrane. He termed the shell pseudochitin. The eggs used in the following tests were from a formol preserved specimen some 20 years old. For that reason negative tests may be considered as questionable.

According to the writer's observations there are at least 4 distinct compounds forming the "shell" of *Dioctophyma,* namely: (1) the operculae or terminal plugs; (2) the exterior rugose or cortical layer comprising the bulk of the "shell"; (3) the internal refractive layer or shell proper; and (4) the vitelline membrane. None of the 4 substances are digested in artificial gastric juice or Fairchild's trypsin.

(1) *Operculae.* These structures are readily soluble in 10 per cent KOH, 10 per cent H_2SO_4, 5 per cent NaOCl and conc. HNO_3, but are not soluble in

10 per cent acetic acid even on boiling for extended periods. The solubility precluded microchemical color tests for proteins. Failure to digest in gastric juice or to dissolve in acetic acid may be due to the formation of a formate. If this is true, the operculae are probably mucoid.

(2) *Cortical layer.* This layer is extremely resistant to both acids and alkalis and may withstand boiling in KOH. However, it is dissolved by superheating in saturated KOH (see Campbell technic under *Ascaris*) or by autoclaving 4 hours at 20 pounds pressure in the same solution. It is also slowly soluble in NaOCl, boiling conc. HNO_3, and boiling 75 per cent H_2SO_4 (incompletely dissolved in 10 per cent H_2SO_4 on boiling). Xanthoproteic and nitrite reactions (latter sometimes dubious) are positive while the sulphide reaction is negative; reaction of vaseline heated, ninhydrin presoaked cortical layer is positive. [The colored product of the ninhydrin reaction is water soluble. In order to use this reaction as a microchemical test for protein (free carboxyl and free amino group) the tissue may be soaked in 0.2 per cent solution of ninhydrin; then the solution drawn off until the tissue becomes "just dry" under a binocular. Covered with a drop of vaseline and a cover slip, the object is then warmed. A blue color is obtained. Prior washing in absolute alcohol or heating may be desirable to permit entrance of ninhydrin through a thermolabile (sterol) membrane]. The cortical layer swells tremendously before dissolving in acids. Solubility in NaOCl cannot be regarded as evidence of chitin since the writer found that the following substances are acted upon and wholly or partially dissolved in this substance at room temperature in 18 hours: Hair (quickly), finger nail (slowly), wing of cockroach (slowly), tendon collagen (incomplete), ligament elastin (incompete).

Insolubility in water following Na_2S seems to exclude keratin since the following comparable results were obtained: Cockroach wing and elastin not affected, collagen very little affected, boiled white of egg, hair and finger nail completely dissolved.

Conclusion dubious; possibly due to formalin fixation.

(3) *Shell proper.* This layer apparently dissolves in HNO_3 and H_2SO_4 more readily than does the cortical layer. It is not dissolved on superheating in KOH, is not affected by Na_2S and is less readily soluble in NaOCl than is the cortical layer, but it eventually dissolves. Iodine-potassium iodide on superheated KOH material gives a deep purple coloration and such shells are also dissolved by 3 per cent acetic acid. Xanthoproteic and nitrite reactions are uniformly negative. Shells dissolved in 3 per cent acetic acid give no sulphide reaction. Material prepared by autoclaving in saturated KOH instead of using the Campbell technic, gives the same results except that the violet color in 1 per cent H_2SO_4 following iodine-potassium iodide rapidly disappears and in such material a thin membrane representing the *external* surface of the cortical layer often persists, this membrane being shrunken up against the shell proper as though a matrix had dissolved.

Autoclaved shells dissolved by heating in 50 per cent HNO_3, recrystallize as minute sphaerocrystals and true crystals insoluble in water and selective to Rose Bengal. Similar sphaerocrystals were also obtained by recrystallizing in 75 per cent H_2SO_4. All of the evidence seems to indicate that the true egg shell is chitin.

(4) *Vitelline membrane.* This membrane (probably the inner membrane referred to by Lukasiak) is insoluble in NaOCl; it is absent in KOH heated material.

6. *Strongylus equinus.* (1) External cuticle. Bondouy (1910) published a statement relative to the skeletoids of the external cuticle of *Strongylus.* He found it to be soluble in KOH, to be digested by trypsin (not by gastric juice), and to have the following reactions: Biuret, positive; xanthoproteic, positive; Adamkiewicz, positive.

Observations by the present writer are very incomplete owing to lack of material but appear to be substantially similar to those previously obtained

(Chitwood 1936) for *Ascaris*. The possibility of the presence of a sterol was not thought of at the time the work was done. The cuticle is subdivisible into layers and Bondouy's observations apply to the cortical layer. This latter is soluble in hot 10 per cent NaOH, is not digested by gastric juice, and gives very strong positive xanthoproteic and sulphide reactions. It is apparently a keratin. The fiber layers are digested by gastric juice and, therefore, are presumably related to ascarocollagen which forms the corresponding layers in *Ascaris*.

(2) *Stomatal lining and esophagus.* Immink (1924) characterized the stomatal and esophageal linings of *Strongylus* as a chitinoid containing protein, the mixture having the following reactions: Millon's, positive; xanthoproteic, positive; Biuret, positive; iodine-H_2SO_4, negative; insoluble in KOH.

The writer has found that boiling in 10 per cent NaOH first dissolves the musculature and body wall of *S. equinus* then that part of the stoma between the internal corona radiata and the base, excluding the dorsal gutter and esophageal lining. Further vigorous boiling with the addition of 10 per cent NaOH at intervals appears eventually to dissolve the entire esophageal and stomatal lining. As previously shown (Chitwood 1936), keratin in nematodes may be very resistant to alkalis and its hydrolysis may be incomplete or slow if the alkali is either too strong or too weak. Solubility in alkali under any condition eliminates chitin from consideration.

The stomatal and esophageal linings of dissected specimens have the following characteristics: (a) Corona, teeth, dorsal gutter and esophageal lining turn rich orange-brown in iodine-1 per cent H_2SO_4; give strong xanthoproteic and mercuric nitrite reactions (indicating a benzene ring and tyrosine); give a very strong sulphide reaction (indicating cystine); are not digested by artificial gastric juice or Fairchild's trypsin; and are insoluble in boiling 10 per cent acetic acid. These tests all indicate keratin, possibly infiltrated with a mucoid. (b) The chief part of the stomatal lining of dissected specimens, i.e., that between the internal corona radiata and the basal teeth, exclusive of the dorsal gutter, has the following characteristics: Colors rich orange-brown in iodine-1 per cent H_2SO_4; gives strong xanthoproteic and mercuric nitrite reactions; is digested in artificial gastric juice but not in Fairchild's trypsin; gives negative sulphide test; dissolves slowly in boiling 10 per cent acetic acid. These characteristics positively eliminate keratin and fibroids. They leave two possibilities: a mucoid or a mucoid-collagen mixture. Before being digested in gastric juice the stomatal lining first becomes soft, retaining its gross structure. At this time xanthoproteic and iodine-1 per cent H_2SO_4 tests are weak or negative. This is regarded as evidence that the wall itself is collagen from which mucoids are dissolved before actual digestion takes place.

7. *Miscellaneous observations.* (1) The esophageal lining of fresh *Agamermis decaudata* is not digested by gastric juice but is soluble in hot 10 per cent KOH and gives a positive xanthoproteic reaction.

(2) The spicules of *Theristus setosus* give positive xanthoproteic and ninhydrin reactions, a negative iodine-H_2SO_4 reaction and are not digested by gastric juice.

(3) The external cuticle of *Rhabditis strongyloides* and of *Oncholaimium oxyuris* behaves like that of *Ditylenchus dipsaci* (see below) in gastric juice and trypsin and also in sodium hypochlorite followed by alcohol. There seem to be 4 types of compounds involved: (1) A "lipoid" or sterol (thermolabile membrane), (2) a keratoid (cortical layer), (3) a fibroid (matrix) and (4) a collagen (fiber=basal layers).

8. *Heterodera marioni.* The eggs of *H. marioni* are deposited in a "gelatinous" substance. In common with other eggs, they have a vitelline membrane and an egg shell.

(1) *"Gelatinous" substance.* This material is minutely fibrous, gives positive ninhydrin and xanthoproteic reactions, indicating protein; it is insoluble in a saturated solution of picric acid, alcohol-picric acid and acid mercuric

nitrate. It is rapidly soluble in 5 per cent NaOCl and in 30 per cent Scott's reagent (Na_2CO_3-CaOCl); is slowly and decreasingly soluble in 10 per cent NaOH, 10 per cent acetic acid, 2 per cent NaOH, 0.1 per cent HCl, sat. $Ca(OH)_2$ and 0.2 per cent NaOH in the order named. It is apparently insoluble in 0.2 per cent HCl and sat. Na_2S. It is digested partially, freeing many eggs after 24 hours at 38° C. in either artificial gastric juice or Fairchild's trypsin. The extract of egg masses formed by boiling in 10 per cent acetic acid gives a strong Molisch test but it is not proven that this carbohydrate test actually came from the "gelatinous" mass. However, on the basis of circumstantial evidence it is presumed to be a mucoid. The xanthoproteic and ninhydrin tests together show it to be a protein containing a benzene ring, a free amino group and a free carboxyl group. The very slow solubility in dilute alkalis or saturated lime water differentiates it from the more common mucoids. The function of this jelly seems to be protective in the following manner: Carbon disulphide, being relatively insoluble in water, is prevented from reaching the vitelline membrane; similarly sodium sulphide reaches the vitelline membrane and embryo much more slowly when the eggs are enclosed in jelly.

(2) *Shell proper.* The egg shell of this species like those of other nematodes withstands autoclaving in 10 per cent NaOH or heating to 160° C. for 15 minutes in sat. KOH. After the former treatment the egg shell turns lavender to violet in zinc-chlor-iodide. After the latter treatment it is soluble in 3 per cent acetic acid and gives positive iodine-1 per cent H_2SO_4 tests. It is fairly rapidly (30 minutes) dissolved in 5 per cent NaOCl and eventually dissolves in 30 per cent Scott's reagent. Evidently it is chitin.

(3) *Vitelline membrane.* This structure may best be studied by removing the gelatinous mass and egg shell in NaOCl. At room temperature it is insoluble in this solution and slowly permeable to it. It is dissolved immediately by absolute alcohol, acetone, or glacial acetic acid; is more slowly dissolved by sat. Na_2S; is insoluble in 10 per cent NaOH, sat. KOH, 10 per cent HCl, and 10 per cent acetic acid but is eventually (24 hours) penetrated by these substances. Artificial gastric juice and Fairchild's trypsin do not penetrate nor dissolve the membrane. Raising the temperature to 48° C. makes the membrane permeable to sodium hypochlorite causing the egg contents to be dissolved. The membrane melts at approximately 70° C. (73+, 65—, 70±). It is not stained by osmic acid or Sudan III, and gives a negative ninhydrin reaction. When dealing with whole eggs (with shell) gentian violet is useful as a criterion of permeability and membrane presence. Ordinarily eggs cannot be stained with gentian violet but after heating to 70° C. or washing in absolute alcohol the stain readily penetrates. This membrane is presumably a sterol such as cholesterol.

9. *Ditylenchus dipsaci.* (1) *Egg shell.* The egg shell of *D. dipsaci* is apparently devoid of mucoids, there being 2 layers,—the shell proper and the vitelline membrane.

a. *Egg shell.* This structure is soluble in sodium hypochlorite; is insoluble at room temperature in alcohol, acetone, 10 per cent or concentrated glacial acetic acid, 10 per cent HCl, 10 per cent NaOH and sat. KOH; is insoluble in boiling 10 per cent NaOH, superheated (160° C.) sat. KOH, and boiling acetic acid. Other solvents were not tested. Presumably it is chitin but eggs in sufficient quantity for establishing this statement have not been obtained.

b. *Vitelline membrane.* This membrane is soluble in alcohol, acetone and glacial acetic acid; is insoluble in 10 per cent acetic acid, 5 per cent sodium hypochlorite, 10 per cent NaOH and 10 per cent HCl; is melted by heat (the exact temperature not as yet determined); and is not stained by osmic acid or Sudan III. This substance is apparently a sterol.

(2) *External cuticle.* The cuticle of preadults is quite resistant to reagents and impermeable as evidenced by lack of penetration by 0.25 per cent gentian violet (the stain enters very slowly at the stoma, amphidial and excretory pores). Investigations come under 2 headings: (a) A study of cut specimens wherein

all the various layers are exposed and (b) a study of uncut specimens, which in reality amounts to a study of the superficial layer. No notable difference between the cuticle of adults and preadults has been observed but the open, functional, normal body openings of adults (i.e., anus, vulva) make them much less resistant to chemical reagents.

a. *Cut specimens.* The cuticle is not digested in gastric juice, Fairchild's trypsin, papain, or ficin but is split into 2 distinct (thick) layers in Fairchild's trypsin. It is soluble in 10 per cent NaOH (external layer slowly); soluble (24 hours) in 1 per cent NaOH; and vaseline heated presoaked ninhydrin specimens give a positive ninhydrin reaction; "dry" xanthoproteic reaction is positive; and the sulphide reaction is apparently negative (? Cuticle dissolves too readily). Sodium hypochlorite dissolves all except the outer part of the exterior layer; the latter is an exceedingly delicate membrane which dissolves in acetone, alcohol, and 10 per cent NaOH (24 hours) but does not stain in osmic acid, scharlach R or Nile blue sulphate. It is absent after specimens have been heated to 65° C. The evidence, as it stands, indicates that the external cuticle is a complex made up of several layers as follows: The exterior *thermolabile membrane,* possibly a wax or sterol (but differing from the vitelline membrane in being soluble in 10 per cent NaOH); the chief exterior layer (cortical layer), apparently a keratoid (insoluble in gastric juice; soluble in water after prolonged exposure to Na_2S); and, underneath, a fibroid matrix layer (soluble in trypsin) and collagenous fiber layers (insoluble in trypsin).

b. *Uncut specimens.* Preadults exposed to papain for 7.5 hours at 40° C. revived but did not revive after 24 hours' exposure; similar specimens revived after 24 hours' exposure to ficin at 40° C.; specimens exposed to NaOCl may survive 30 minutes or more but eventually the solution enters at the normal body openings and thereafter the specimen is immediately killed. Living specimens after being placed in water at 50° C. for 10 minutes revive and are not stained by gentian violet but if the stain is put in the heating solution it enters the specimen and kills it; if such specimens are afterwards treated with NaOCl one finds the *thermolabile membrane* has persisted. Specimens heated to 65° C. for 10 minutes are killed and rendered permeable to gentian violet; when such specimens are treated in NaOCl one finds that the thermolabile membrane has been destroyed. From this evidence it seems assured that ordinarily the thermolabile membrane governs permeability. Heating to a sufficiently high temperature or dissolving in alcohol, acetone, or acetic acid will remove this membrane and render the organism permeable; at such a time it is dead. Heating to lower temperatures (i.e., 50° C.) increases the permeability of the membrane temporarily, and during such a temporarily permeable period if a substance which otherwise could not enter (i.e., a non-sterol permeable substance) is present in solution it may enter and cause death.

c. *Specialized cuticular structures.* The spicules, stylet and esophageal lining all come under this general heading. These structures, for the most part, give identical reactions, staining deeply in Nile blue sulphate, or gentian violet, becoming orange-brown in iodine-1 per cent H_2SO_4, and giving strong ninhydrin and xanthoproteic reactions. The outer part of the stylet shaft and the knobs (mesorhabdions-telorhabdions) are digested by gastric juice and pancreatic juice while the other structures are not so digested. Since the corresponding morphological regions in *Rhabditis* are not digested, we must regard the knobs of the *Ditylenchus* stylet as specializations (a fibroid?) for the attachment of muscles and not as the original telorhabdions. The remaining structures are probably keratoid with possible mucoid infiltration.

General Considerations. On the basis of the assembled data it appears that there is little if any important difference in the nemic skeletoids in different types of nematodes. In so far as protection from environmental conditions is concerned, the functional membrane of egg, larva, or adult is apparently a sterol or related substance. Zawadowsky (1928) brought attention to the fact that such a "lipoidal" substance controlled permeability in ascarid eggs. This

is also true for eggs of *Heterodera* and *Ditylenchus* and *a similar substance* forms a thermolabile membrane on the surface of both adults and larvae of *Ditylenchus* and *Rhabditis*.

The thermolabile membrane was discovered after its existence was predicted through the effect of reagents on *Ditylenchus*. (Data, to be published elsewhere, include the effect of varied concentrations of chlorinated hydrocarbons, organic acids, alcohols, aldehydes and sulphides). The relative lack of penetration of nonfat solvent substances at room temperature, the increased lethality of the same concentrations of substances at higher temperatures, and the chief action of fat solvents within the effective range of their dissolving concentrations all pointed to some protection of the larva other than protein. The resistance of preadults or third-stage larvae of other nematodes can be explained since the partial closing of normal body openings requires substances to enter through this membrane. In conformity with these points it has been found that the preadult of *Ditylenchus dipsaci* (so-called resistant stage) is not the stage most resistant to chemicals. The egg is the most resistant stage since it is completely covered by the vitelline membrane whereas the thermolabile membrane covering the larva is broken at the mouth where substances such as NaOCl may slowly penetrate.

Furthermore, it may be noted that except in cases where a nematocide is supposed to be taken by the nema *per orem* or through other normal body openings, successful nematocides are fat solvents or react with fats (NaOH, etc.). The assumption of a sterol membrane would explain this. It would also explain the well known impermeability of the nemic cuticle.

If, as we believe, such a membrane may be general in nemas, then the study of nematocides essentially involves the permeability of this membrane. Soil nematocides effective against *Heterodera marioni* and *Ditylenchus dipsaci* should be just as successful in the sterilization of manure or fox runs for nemic eggs and larvae.

In the study of nematocides of plant parasitic nematodes where eggs and preadults coexist in the host (such as *Ditylenchus dipsaci*) effort should be made to study the egg as well as the larva since it is the egg that is most completely protected. By dissolving the egg shell in NaOCl the effect of reagents on the vitelline membrane can be easily demonstrated. Stains such as gentian violet also provide a useful index to permeability.

SUMMARY

The observations described in this paper demonstrate the following points:

(1) There is little, if any, difference in the chemical reactions of the same morphologic layer in different nemas or their eggs.

(2) The only truly chitinous structure in nemas is the egg shell proper. Other hard parts (supporting or skeletal in function) are scleroproteins or mixtures of scleroproteins and mucoids.

(3) Probably the function of the scleroproteins and chitin is chiefly or wholly supportive. Regulation of the environmental contact with the nema or nemic embryo is apparently governed by a "lipoidal" membrane which behaves as a sterol. This membrane takes the form of a vitelline membrane in the egg and a thermolabile membrane in the larva or adult.

(4) Nematocides, unless designed to enter the nema *per orem*, should be soluble in, dissolve, or be dissolved by lipoids.

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MINUTES

One hundred ninety-third to one hundred ninety-sixth meetings

The 193rd meeting was held February 15, 1938. Dr. Bartsch moved that the Society extend to Dr. Yoshida the best wishes of the Society in commemoration of his 25th year as professor of zoology. Papers were presented by Ewing, Dikmans, Shorb, Steiner, and Harwood.

The 194th meeting was held March 15, 1938. Dr. Jerstad announced that an index of the old number of the Proceedings was being prepared for publication. Papers were presented by Hall, Dikmans, and Andrews.

The 195th meeting was held April 19, 1938. Papers were read by Cushing, Cram, and Christensen.

The 196th meeting was held on May 17, 1938. Dr. Paul Bartsch moved that the Society go on record by expressing, with suitable resolutions, the loss sustained in the death of Dr. Maurice C. Hall, one of the founders of the Society; that a copy of the resolutions be sent to Dr. Hall's family and a copy be spread on the minutes of the Society. The Chair appointed Drs. Wright, Bartsch, Christie, and Price as a Committee to draw up the resolutions.

Dr. Dikmans announced to the members of the Society that Dr. Bartsch had expressed a desire to have the members over to his home on some meeting date. After thanking Dr. Bartsch for his invitation it was decided that the President and the corresponding secretary, after conferring with Dr. Bartsch, should select a suitable time for the meeting and notify the members as to the date. Papers were presented by Dinaberg, Spindler, Luttermoser, and Harwood.

ALLEN MCINTOSH, *Recording Secretary.*

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