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CONTENTS

A Key to Species in the Genus *Bursaphelenchus* with a Description of *Bursaphelenchus hunanensis* sp. n. (Nematoda: Aphelenchoididae) Found in Pine Wood in Hunan Province, China[1]

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ABSTRACT: *Bursaphelenchus hunanensis* sp. n. is described and illustrated. It has a distinct stylet longer than other species in the genus and has no basal thickenings. The female lacks a vulval flap and has a short postuterine branch. The male spicules do not have a cucullus and terminate posteriad in a blunt rounded tip. The rostrum is digitate. An alphabetical list of all 49 nominal species in the genus, a key to 44 valid species, and tables of diagnostic data of males and females are also included.

KEY WORDS: Nematoda, Aphelenchoididae, *Bursaphelenchus hunanensis* sp. n., nematode taxonomy and systematics, *Pinus massoniana*, key to species.

Species within the genus *Bursaphelenchus* Fuchs, 1937, were regarded only as associates of bark beetles (Ipidae) until the insidious pinewood nematode *B. xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970, started to devastate entire forests in Japan (Mamiya, 1972). Current concern on chance introduction of this species into new areas has renewed interest in the genus and its species.

A *Bursaphelenchus* species was first found in October 1984 in the wood of dead pine trees, *Pinus massoniana* Lamb, in Huaihua County, Hunan Province, China, during a survey for the pine wilt disease caused by *Bursaphelenchus xylophilus*. Detailed studies of the nematode revealed that it differed from the 43 species listed in the generic review by Tarjan and Baeza-Aragon (1982) and 5 additional nominal species (Kakuliya and Devdariani, 1965; Devdariani et al., 1980; Giblin and Kaya, 1983; Thong and Webster, 1983; Giblin et al., 1984).

The purpose of this paper, in addition to reporting a new species, is to provide a key and a table of essential measurements and ratios of nominal species within the genus.

Materials and Methods

Bursaphelenchus specimens were extracted by the Baermann funnel method from wood fragments cut from wilted trees. Studies were made on living as well as fixed specimens. Measurements and drawings were made from specimens fixed in TAF (triethanolamine,

formaldehyde, and water) and stained with polychrome blue. Spicule lengths were measured along the median line of the arc of the spicule. The specimens were mounted in glycerin. Measurements are in micrometers unless stated otherwise.

Information on other *Bursaphelenchus* species was obtained solely from perusal of pertinent references. Drawings of spicules of each species were made from the original publication, and then either enlarged or reduced to a standard size. The majority of the spicule drawings shown in Figure 13 came from Tarjan and Baeza-Aragon (1982).

Generic Diagnosis

There have been no emendations proposed by others to the generic diagnosis of the genus given by Nickle (1970) which is presented below:

Bursaphelenchus Fuchs, 1937
Syn. *Aphelenchoides* (*Bursaphelenchus*) (Fuchs, 1937) Rühm, 1956

DEFINITION: Aphelenchoididae. Usually long. Lips high, offset. Stylet with small rounded basal knobs. Excretory pore usually behind median bulb. Vulval lips sometimes protruding; postuterine sac usually long. Female tail rounded, conoid, or sharply pointed. *Spicules large, narrow, usually with prominent rostrum. Male tail strongly arcuate, variously pointed, with short, terminal caudal alae.* Usually 2 pairs of caudal papillae, 1 adanal, the other postanal. Gubernaculum absent.

BIONOMICS: Mycophagous, insect associate.

TYPE SPECIES: *Bursaphelenchus piniperdae* Fuchs, 1937.

1
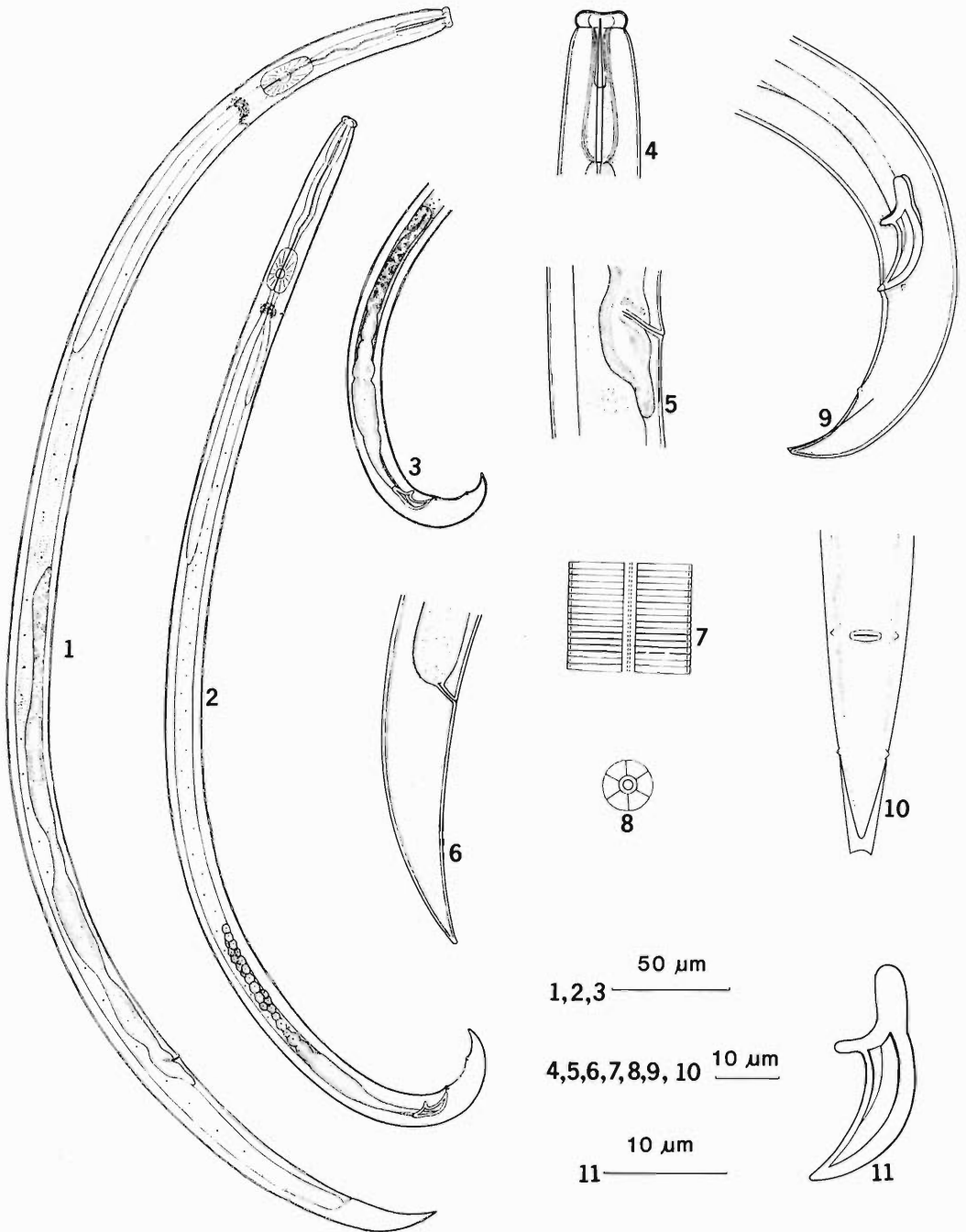
Figures 1–11. *Bursaphelenchus hunanensis* sp. n. 1. Female. 2. Male. 3. Posterior part of male showing flexure of the anterior end of the testis. 4. Female anterior end. 5. Vulva region. 6. Female tail. 7. Lateral view of cuticle in midbody region. 8. En face view of head. 9. Lateral view of male tail. 10. Ventral view of male tail. 11. Male spicule.

Results and Discussion

Bursaphelenchus hunanensis sp. n.
(Figs. 1–11)

FEMALES (30) (paratypes):   L = 0.67 (0.58–0.79) mm; a = 35.5 (31.1–41.5); b = 8.4 (7.3–9.6); c = 15.9 (13.6–18.6); V = 77.9 (75.7–79.5)%; stylet = 22 (20–26).

MALES (15) (paratypes):   L = 0.53 (0.48–0.59) mm; a = 40.9 (37.6–45.3); b = 7.1 (6.5–8.8); c = 16.1 (15–19.1); T = 26.1 (16.4–31.7)%; stylet = 20 (19–21); spicule = 15 (13–20).

FEMALE (holotype):   L = 0.67 mm; a = 36.4; b = 7.7; c = 17; V = 79.2%; stylet = 23.

MALE (allotype):   L = 0.52 mm; a = 39.4; b = 6.6; c = 16.4; T = 26.3%; stylet = 19; spicule = 15.

FEMALE: Body ventrally curved when killed with heat, tapering gradually near extremities. Cuticle marked by fine annules, about 1 μm wide in midbody region, interrupted by a narrow lateral field which is about $\frac{1}{10}$ of the maximum body width and has 2 longitudinal, refractive lines on either side of a narrow refractive band (Fig. 1). Lip flattened at oral opening, set off by a constriction. En face view of head showing 6 equal lips (Fig. 8). Anterior stylet cone less than ½ total stylet length, posterior shaft without basal thickening (Fig. 4). Procorpus cylindrical, twice as long as vulval body width. Median bulb elongate-oval, with conspicuous valve just posterior to center. Excretory pore ½ to 1 length of median bulb behind the bulb on a level with, or just behind, the nerve ring. Esophageal glands dorsally overlapping intestine, 9 times as long as vulval body width. Reproductive system prodelphic, gonad outstretched, occupying ⅓ the body length. Oocytes arranged in single file except at the anterior part of the ovary. Vagina an anteriorly inclined tube, without vulval flap. Postuterine branch usually shorter than vulva body diameter (Fig. 5). Vulva–anus distance equal to 2.5 times tail length. Tail conoid with slight ventral curvature, 3 times longer than anal body width, with a finely rounded terminus (Fig. 6).

MALE: Body J-shaped when killed with heat. Anterior body region similar to female. Reproductive system monorchic, testis usually outstretched, extending ¼ of body length. Approximately ⅛ of the testis reflexed anteriorly (Fig. 3). Spermatogonia arranged in several rows and spermatocytes in single file. Seminal vesicle oval. Vas deferens composed of anterior cylindrical part and posterior slender duct. Spicules paired,
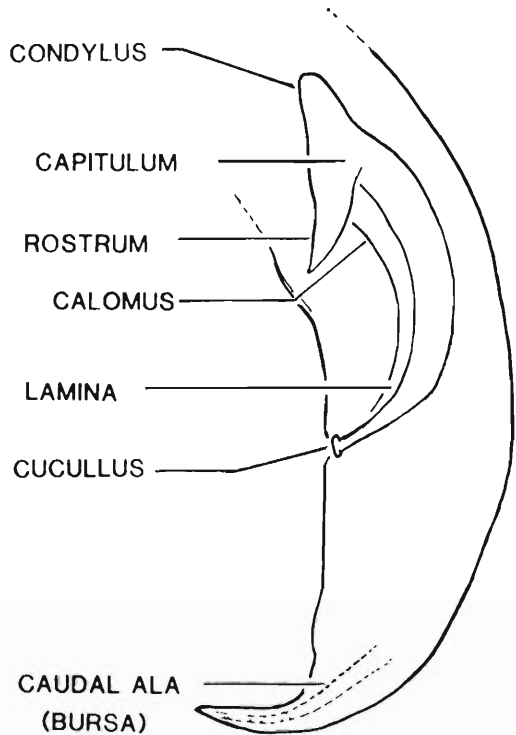
Figure 12. Lateral view of the *Bursaphelenchus* spicules (after Tarjan and Baeza-Aragon, 1982).

arcuate, with blunt prominent rostrum; distal end of spicule obtuse, without cucullus (cf. Fig. 2). Center of capitulum depressed, condylus hemispherically rounded. Tail ventrally arcuate, terminus finely rounded, with short terminally truncated bursa (Fig. 10). Two pairs of caudal papillae present; 1 adanal pair, 1 pair about ⅓ tail length from tail terminus.

DIAGNOSIS: *Bursaphelenchus hunanensis* sp. n. differs from all other species in the genus by the length of female stylet, 20–26 μm. Among those species that lack a vulval flap, *B. hunanensis* appears to be closest in spicule shape to *B. eggersi,* from which it differs due to shorter female body length (580–790 vs. 990–1,122 μm) and more posteriorly placed vulva (76–80 vs. 69–70%). The spicules of *B. hunanensis* also resemble those of *B. talonus, B. tritrunculus,* and *B. teratospicularis.* The new species differs markedly from the first 2 species by its greater stylet length as well as the more posteriorly positioned vulva (71–73 vs. 76–80%). *Bursaphelenchus hunanensis* appears close to *B. teratospicularis* in all essential measurements except "c" ratio.

The 2 species can be further differentiated by variances in the spicules. In *B. hunanensis,* the rostrum is more elongate-digitate and the lamina is more symmetrical rounded than for *B. teratospicularis.*

TYPE DESIGNATIONS: HOLOTYPE: Female, collected by Mrs. Qiu Shouwei, October 1984. Specimen number: *Bursaphelenchus hunanensis* holotype 1, Nematode Collection, Department of Plant Protection, South China Agricultural University, Guangzhou, China.

ALLOTYPE: Male, same data as holotype. Specimen number: *Bursaphelenchus hunanensis* allotype 1.

PARATYPES: Same data as holotype and allotype. One male and female paratype, slide T-3794p, USDA Nematode Collection, Beltsville, Maryland, U.S.A.; and slide 86c/8/1, Nematode Collection, Rothamsted Experimental Station, Harpenden, Herts, England. Five female and 4 male paratypes in Nematode Collection, Department of Plant Protection, South China Agricultural University, Guangzhou, China.

TYPE HABITAT: Dead wood of *Pinus massoniana* Lamb.

TYPE LOCALITY: Hongjiang, Huaihua County, Hunan Province, China.

Definition and Current Status of the Genus *Bursaphelenchus*

Since the review of the genus *Bursaphelenchus* by Tarjan and Baeza-Aragon (1982), 6 new species have been described and 1 species has been transferred into the genus, making a total of 49 recognized species (Table 1).

Kakuliya et al. (1980) referred to *Bursaphelenchus populneus* and *B. tbilisensis* and Vosilite (1980) to *B. sexdentai* which, to the best of our knowledge, are nomina nuda and are not included in the key or in Tables 1 and 2.

The following key to valid *Bursaphelenchus* spp. lists synonymies which were proposed by Tarjan and Baeza-Aragon (1982). It does not list *B. conjunctus* (Fuchs, 1930) and *B. conurus* (Steiner, 1932) which were deemed species incertae sedis, *B. cryphali* (Fuchs, 1930) placed in species inquirendae, and *B. ruehmi* Baker, 1962 which was regarded as species indeterminata.

Key to *Bursaphelenchus* Species

1. Spicule with cucullus (cf. Fig. 12) 2
   Spicule without cucullus 6
2(1). Female tail always with digitate or pointed terminus 3

Female tail terminus mostly broadly rounded, rarely with digitate terminus ...... *xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970[4] (syn.: *lignicolus* Mamiya and Kiyohara, 1972)
3(2). Female tail tapering to a pointed terminus, female "c" ratio 12–17 ...... *borealis* Korentchenko, 1980
Female tail definitely mucronate, female "c" ratio 20–30 4
4(3). Male spicule 17–21 μm long, vulva 68–75% ...... *kolymensis* Korentchenko, 1980
Male spicule 21–29 μm long, vulva 73–77% 5
5(4). Female stylet 12–14 μm long, male spicule 21–22 μm long ...... *fraudulentus* Rühm, 1956
Female stylet 14–16 μm long, male spicule 23–29 μm long ...... *mucronatus* Mamiya and Enda, 1979[5]
6(1). Female stylet 20–26 μm long ...... *hunanensis* sp. n.
Female stylet shorter than 20 μm (*teratospicularis* female stylet 18–22 μm) 7
7(6). Spicular lamina with a blunt-round tip 8
Tip of spicular lamina more acute to finely rounded 11
8(7). Spicular rostrum with acute tip; male "a" ratio 19–28; female length 390–830 μm ...... *hunti* (Steiner, 1935) Giblin and Kaya, 1983
Tip of spicular rostrum digitate to almost flattened; male "a" ratio 27–45; female length 690–1,350 μm 9
9(8). Spicule 13 μm long, spicular rostrum almost flattened ...... *gonzalezi* Loof, 1964
Spicule 18–27 μm long, spicular rostrum digitate 10

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[4] Wingfield et al. (1983) reported finding *B. xylophilus* in balsam fir which had digitate tails and which would mate with *B. xylophilus* from pine, but not with *B. mucronatus* Mamiya and Enda, 1979.
[5] Baujard (1980) considered *B. mucronatus* a synonym of *B. lignicolus* Mamiya and Kiyohara, 1972 (=*B. xylophilus*), but de Guiran et al. (1985) demonstrated distinct differences on the basis of enzyme electrophoresis.

Table 1.　Diagnostic data on *Bursaphelenchus* females: essential measurements and ratios.

Bursaphelenchus species	Body length (μm)	"a" ratio	"c" ratio	Stylet length (μm)	Vulval %	Vulval flap
bakeri	924–1,232	38–41	26	18–19	73–75	?
bestiolus	830	32	17	13–14	74	+
borealis	507–1,018	26–38	12–17	14–16	71–77	+
chitwoodi	619	21	16	12	69	?
corneolus	650–700	29	18	12*	73	+
crenati	825–1,125	39–54	26–32	11	72–74	?
cryphali	447	19	19	12	71	−
digitulus	790–1,260	31–44	13–20	11–14	84–88	−
eggersi	990–1,122	37–39	20	16–18	69–70	−
eidmanni	795–990	25–32	70–73†	14–18	72–78	−
elytrus	890–960	35–41	17–21	15	73	−
eremus	858–990	41	23	13	69–73	?
eucarpus	825–1,275	39–46	20–26	12–14	68–76	?
fraudulentus	675–900	32	21–26	12–14	74–75	+
fungivorus	770–1,160	28–43	10–15	14–16	67–73	−
georgicus	656	33	20	12	82	−
gonzalezi	690–1,100	25–36	12–19	13–16	71–76	−
hunanensis sp. n.	580–790	31–41	14–19	20–26	76–80	−
hunti	390–830‡	21–29	9–13	12–14	67–83	−
idius	610–885	32–34	23–25	11–14	76–80	?
incurvus	660–840	24–25	19–22	15–19	72–73	−
kevini	810–980	26–29	17–19	15–17	76–81	−
kolymensis	473–716	22–36	17–24	12–15	68–75	+
leoni	580–860	33–44	10–15	12–17	69–74	+
lignicolus	710–1,010	33–46	23–32	14–18	67–78	+
lignophilus	992–1,257	46–55	20–25	11–14	70–73	?
mucronatus	700–980	36–46	20–30	14–16	73–77	+
naujaci	730–1,300	35–51	19–30	13–18	71–76	?
newmexicanus	1,500	40	28	15	73	+
nuesslini	660–1,020	22–36	24–29	12–15	71–76	?
pinasteri	550–650	35–47	19–25	11–13	69–73	+
piniperdae	551§	40	27	12	76	−
pityogeni	940	30	26	14–15	73	+
poligraphi	605–914§	41–57	45	9	76	?
ratzeburgii	720–825	39–41	21–22	11	71–74	−
sachsi	660–763	34	14–15	12–14	71–72	?
scolyti	840	40	20	11	72	−
seani	770–1,350	25–32	14–19	15–19	76–82	−
silvestris	720–1,220	35–47	11–15	12–16	72–76	+
steineri	456–475	22–26	9	12–14	68–73	?
sutoricus	930	44	25	13	64	−
sychnus	634–662	23–29	11–12	12–13	73–74	?
talonus	800	33	25	11	73	?
teratospicularis	580–675	29–34	22–23	18–22	75–79	?
tritrunculus	650	29	11	13	71	?
varicauda	710–890	26–36	15–25	12–17	73–82	+
wilfordi	900	60	27	14*	70*	−
xerokarterus	600–930	26–38	20–30	12–15	73–80	?
xylophilus	447–609‖	37–48	23–31	13	73–77	+

* Measured from illustration.
† An obvious error.
‡ Measurements of lectotype material by Nickle, 1970.
§ Measurements from Fuchs, 1937.
‖ Measurements of lectotype material by Nickle et al., 1981.

Table 2. **Diagnostic data on *Bursaphelenchus* males: essential measurements and ratios.**

Bursaphelenchus species	Body length (μm)	"a" ratio	"c" ratio	Stylet length (μm)	Spicule length (μm)
bakeri	858–990	38–45	25–26	18	19–22
bestiolus	670	32	18	13–14	18*
borealis	548–813	25–43	16–29	12–16	16–20
chitwoodi	547	34	24	12	16
conjunctus	455	31	15	16.5	14.5
corneolus	570–700	35	18	12*	13*
crenati	825–1,080	39–51	29–42	11	17–18
cryphali	885–945†	51–54	25–37	12–14	14–15
digitulus	530–710	33–41	15–19	11–12	16*
eggersi	450–990	21–31	19–28	14–16	18–24
eidmanni	675–855	32–39	19–23	14–18	18–21
elytrus	830–1,040	41–52	21–26	15	28*
eremus	782–840	40–49	31–36	11–12	14–19
eucarpus	750–1,020	43–49	33–45	12–14	15–16
fraudulentus	645–685	33–37	20–27	12–15	21–22
fungivorus	570–1,030	31–45	18–25	14–15	18*
georgicus	720	39	24	?	19
gonzalezi	580–970	30–38	21–29	12–15	13*
hunanensis sp. n.	480–590	38–45	15–19	19–21	13–20
hunti	360–740‡	19–28	18–21	12–13	16–17
idius	619–643	34	33	11	14
incurvus	600–645	21–24	24–26	12–17	17–18
kevini	890–1,120	27–36	15–19	14–16	18–22
kolymensis	423–706	24–46	18–29	12–15	17–21
leoni	510–1,060	36–56	16–26	13–17	10–20
lignicolus	590–820	36–47	21–31	14–17	25–30
lignophilus	912–1,147	48–64	18–23	11–14	21–23
mucronatus	640–970	39–51	26–36	14–16	23–29
naujaci	630–1,200	32–52	20–27	13–18	13–20
newmexicanus	1,250	47	26	15	24*
nuesslini	780–900	43–45	28–32	15	15–16
pinasteri	500–620	42–51	20–25	11–13	12–14
piniperdae	570†	38	24	12	14
pityogeni	820	31	22	14–15	17*
poligraphi	589–785†	35–65	34	10	13–14
ratzeburgii	630–675	38–39	28	9–11	11–12
sachsi	662–691	26–29	25–27	12–14	17–18
scolyti	800	38	25	11	7*
seani	660–1,290	27–33	18–27	13–18	18–27
silvestris	730–895	37–44	18–22	12–15	28–31
steineri	476–480	23–26	21	12	17
sutoricus	740	34	14	14	22
sychnus	490–547	24–30	22–24	12–14	19–23
talonus	800	47	25	11	15*
teratospicularis	450–490	28–30	23–33	18–22	15–18
tritrunculus	650	29	23	13	14*
varicauda	540–840	30–41	15–23	15–17	13–17
wilfordi	670–750	61	26	14*	10*
xerokarterus	680–825	31–41	24–34	12–18	14–15
xylophilus	520–601§	35–45	21–29	13–14	19–23

* Measured from illustration.
† Measurements from Fuchs, 1937.
‡ Measurements of lectotype material by Nickle, 1970.
§ Measurements of lectotype material by Nickle et al., 1981.

10(9). Vulva 67–73%, male "a" ratio 31–
45 .... *fungivorus* Franklin and
Hooper, 1962
Vulva 76–82%, male "a" ratio 27–
33 .... *seani* Giblin and Kaya, 1983

11(7). Female length 456–475 μm, "c" mea-
surement 8.6–8.8, spicule thin-
elongate .... *steineri* (Rühm, 1956)
Goodey, 1960
Female length 580 μm and greater,
"c" measurement 10 or more,
spicule more stout 12

12(11). Male spicule 26–31 μm long 13
Male spicule 24 μm or less in length
.... 15

13(12). Female without vulva flap, "c" ra-
tio = 17–21 ...... *elytrus* Massey, 1960
Female with vulval flap, "c" ratio
either more than 21 or less than
17 14

14(13). Condylus (cf. Fig. 12) of spicular ca-
pitulum with a conspicuous pos-
terior flexure, male length 730–895
μm .... *silvestris* (Lieutier and
Laumond, 1978) Baujard, 1980
Condylus of spicule without poste-
rior flexure, male length 1,250 μm
.... *newmexicanus* Massey, 1974

15(12). Vulva 82–88% 16
Vulva 78% or less (*varicauda* vulva
73–82%) 17

16(15). Spicule relatively slender in width,
condylus digitate, male "c" ratio
11–12 .... *digitulus* Loof, 1964
Spicule relatively broader in width,
condylus broadly conical, male "c"
ratio 24
.... *georgicus* Devdariani, Kakuliya,
and Khavtasi, 1980

17(15). Condylus of spicular capitulum with
a posterior flexure ....
.... *leoni* Baujard, 1980
Condylus of spicule without poste-
rior flexure 18

18(17). Spicule length (measured along arc)
11–13 μm or less 19
Spicule length 14 μm or greater (*var-
icauda* spicule 13–17 μm) 22

19(18). Female with vulva flap, length 650–
700 μm long; "c" value of male
18.5 .... *corneolus* Massey, 1966
Female without vulva flap, length

720 μm or greater; "c" value of
males 21–29 20

20(19). Female stylet 12–16 μm long, spic-
ular laminae strikingly widened or
"mitten-shaped" at midpoint ....
.... *wilfordi* Massey, 1964
Female stylet 9–11 μm long, spicular
laminae not excessively widened
.... 21

21(20). Male length 800 μm, spicule 7 μm
long .... *scolyti* Massey, 1964
Male length 630–675 μm, spicule 11–
12 μm long
.... *ratzeburgii* (Rühm, 1956)
Goodey, 1960

22(18). Females with vulval flap 23
Females without vulval flap, or
presence of flap not determined 25

23(22). Male stylet 15–17 μm long, spicule
length 13–17 μm
.... *varicauda* Thong and
Webster, 1983
Male stylet 13–15 μm long, spicule
length 17–18 μm 24

24(21). Lateral incisures present, male length
820 μm, female length 940 μm ....
.... *pityogeni* Massey, 1974
Lateral incisures absent, male length
670 μm, female length 830 μm ....
.... *bestiolus* Massey, 1974

25(22). Male length 450–547 μm (*eggersi*
male length 450–990 μm) 26
Male length 547 μm or more 27

26(25). Male stylet 12–14 μm long, vulva
73–74%, female "c" ratio 11–12
sychnus (Rühm, 1956)
Goodey, 1960
Male stylet 18–22 μm long, vulva
75–79%, female "c" ratio 22–23
teratospicularis Kakuliya and
Devdariani, 1965

27(25). Male stylet 18–19 μm 28
Male stylet 18 μm or less, average
length 16 μm or less 29

28(27). Female stylet 18–19 μm, vulva 72–
74% .... *bakeri* Rühm, 1964
(syn.: *naujaci* Baujard, 1980)
Female stylet 16–18 μm vulva 75–
76% .... *piniperdae* Fuchs, 1937

29(27). Spicule length 18–24 μm 30
Spicule length no greater than 18 μm
.... 33

30(29). Stylet length of both sexes 11–14 μm,
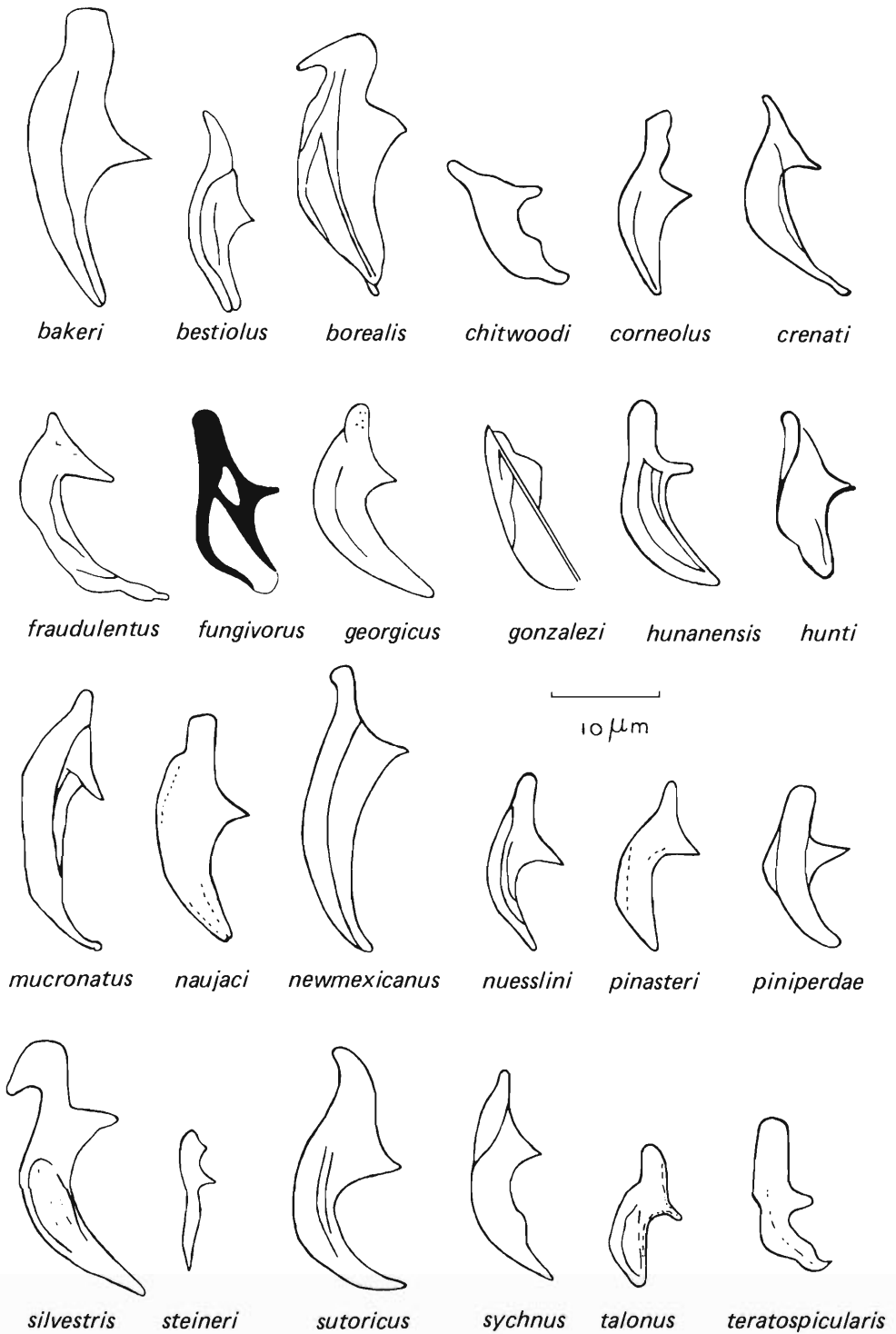
Figure 13. Spicules of *Bursaphelenchus* spp. (partially after Tarjan and Baeza-Aragon, 1982).

digitulus   eggersi   eidmanni   elytrus   eremus   eucarpus

idius   incurvus   kevini   kolymensis   leoni   lignophilus

10 μm

pityogeni   polygraphi   ratzeburgii   sachsi   scolyti   seani

tritrunculus   varicauda   wilfordi   xerokarterus   xylophilus
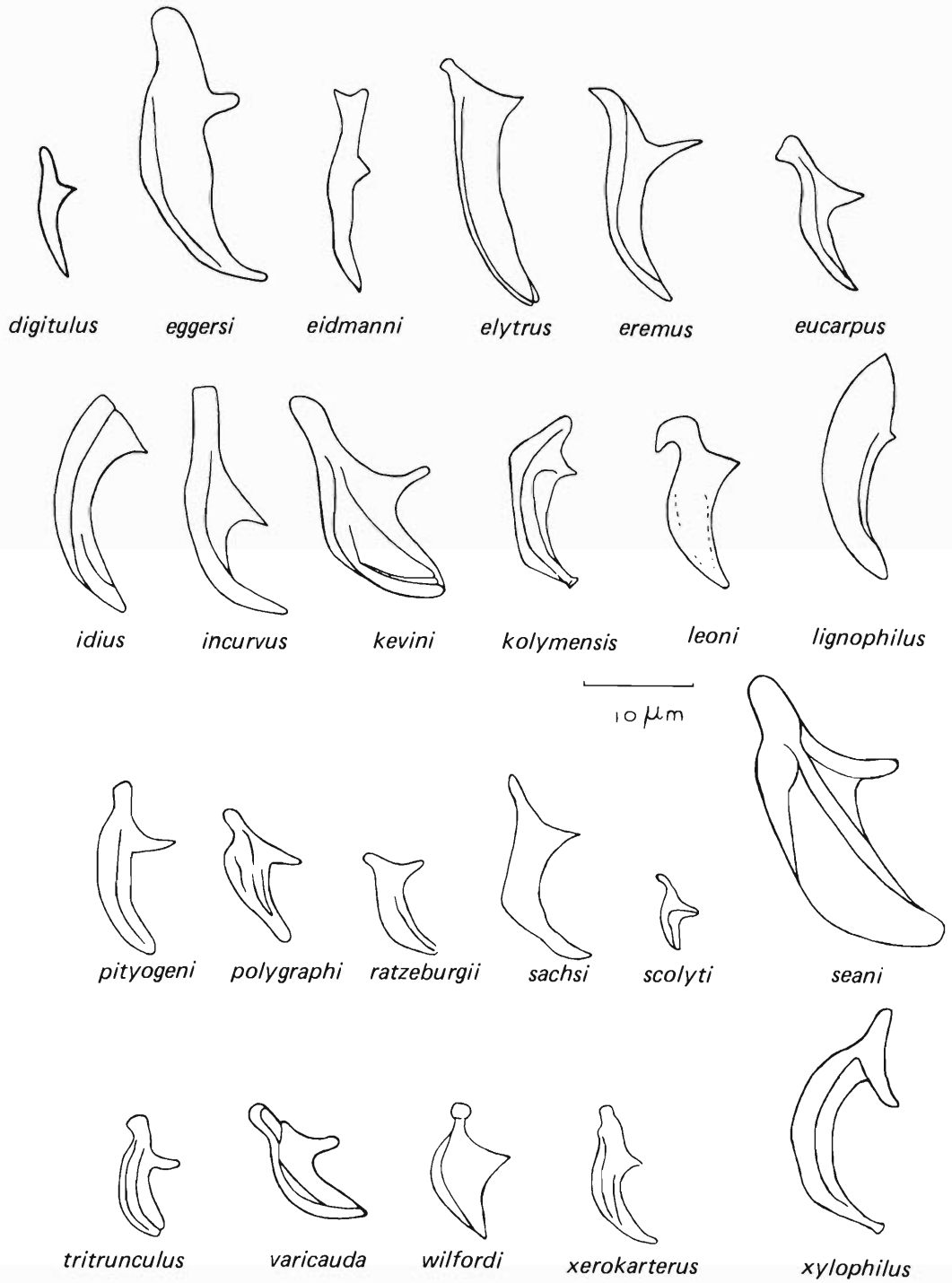
Figure 13.   Continued.

"a" ratio 48 or more .... *lignophilus* (Körner, 1954) Meyl, 1960

Stylet length of both sexes 14 μm or greater, "a" ratio 39 or less 31

31(30). Female length 991–1,122 μm, vulva 69–70%, female "a" ratio 37–39 .... *eggersi* (Rühm, 1956) Goodey, 1960

Female length 990 μm or less, vulva 72–81%, female "a" ratio 26–32 32

32(31). Male length 675–855 μm; spicule narrow with thornlike pointed rostrum .... *eidmanni* (Rühm, 1956) Goodey, 1956

Male length 890–1,120 μm, spicule broad with narrow digitate rostrum .... *kevini* Giblin, Swan, and Kaya, 1984

33(29). Male length 547 μm, female length 619 μm .... *chitwoodi* (Rühm, 1956) Goodey, 1960 (syn.: *pinasteri* Baujard, 1980)

Male length 570 μm and greater, female length 650 μm or greater (*xerokarterus* range 600–903 μm, *idius* range 610–885 μm) 34

34(33). Spicule length 14–15 μm (exc. *poligraphi* spicule 13–18 μm) 35

Spicule length 15 μm or more (*eremus* range 14–19 μm) 38

35(34). Male length 650 μm, vulva 71% .... *tritrunculus* Massey, 1974

Male length greater than 680 μm or less than 643 μm, vulva 73% or more 36

36(35). Condylus of spicule a reduced protuberance, male length 619–643 μm .... *idius* (Rühm, 1956) Goodey, 1960

Condylus of spicule prominent, male length 680–825 μm 37

37(36). Vulva 73–80%, stylet length both sexes 12–18 μm .... *xerokarterus* (Rühm, 1956) Goodey, 1960 (syn.: *sutoricus* Devdariani, 1974)

Vulva 73%, stylet length both sexes 11 μm .... *talonus* (Thorne, 1935) Goodey, 1960

38(34). Rostrum of capitulum sharply pointed 39

Rostrum of capitulum digitate 42

39(38). Condylus rectangular to rectangular-elongate 40

Condylus digitate to broadly digitate 41

40(39). Female stylet 12–14 μm long, male length 750–1,020 μm, male "c" value 33–45 .... *eucarpus* (Rühm, 1956) Goodey, 1960

Female stylet 15–19 μm long, male length 600–645 μm, male "c" value 24–26 .... *incurvus* (Rühm, 1956) Goodey, 1960

41(39). Male length 780–900 μm, "c" value of female 24–29, spicular laminae with symmetrically curved dorsal surface .... *nuesslini* (Rühm, 1956) Goodey, 1960

Male length 662–691 μm, "c" value of female 14–15, spicular laminae with dorsal surface forming an 80° angle .... *sachsi* (Rühm, 1956) Goodey, 1960

42(38). Female stylet 9–11 μm long, female "c" value at least 26, spicular capitulum slightly depressed 43

Female stylet 13 μm long, female "c" value 23, spicular capitulum moderately depressed .... *eremus* (Rühm, 1956) Goodey, 1960

43. Female stylet 9 μm long, male length 589–785 μm, spicule length 13–14 μm .... *poligraphi* Fuchs, 1937

Female stylet 11 μm long, male length 825–1,080 μm, spicule length 17–18 μm .... *crenati* (Rühm, 1956) Goodey, 1960

The essential measurements and ratios (Tables 1, 2) and spicule shapes found within the species (Fig. 13) which follow are an amplification of those presented in Tarjan and Baeza-Aragon (1982), which are brought up to date with the inclusion of the 6 recently described species and additional data on the older species. It is incumbent upon the authors to caution potential users that several of the species are poorly described and lacking essential data. Almost no mention of nematode variation is made, hence the key and diagnostic data must be relied upon only with adequate reservation. Spicule shape has been *assumed* to exhibit little, if any, variation. Be-

cause of the paucity of differentiating morphological characters between species, the shape of the spicule probably is the most visible feature characterizing species. It is unfortunate that variation in spicule shape and other morphological features have not yet been extensively investigated, except for some of the studies conducted by Giblin and Kaya (1983) and Giblin et al. (1984). When adult variation is intensively studied, the genus may possibly be reduced to half its present species.

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The Effect of *Thelastoma bulhoesi* and *Hammerschmidtiella diesingi* (Nematoda: Oxyurata) on Host Size and Physiology in *Periplaneta americana* (Arthropoda: Blattidae)

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ABSTRACT: Three thousand *Periplaneta americana* were sacrificed and the following data collected: number parasitized by oxyurid nematodes, age, sex, weight, length, specific gravity of hemolymph, differential cell counts of hemocytes, and the number of each nematode species present. The mean number of nematodes per host for adults and nymphs, and males and females were compared using the Student's *t*-test. The mean number of nematodes is significantly greater in adults and females. Therefore parasitized hosts are generally longer and heavier. The presence of oxyurid parasites did not appear to affect differential hemocyte counts or hemolymph specific gravity.

KEY WORDS: *Thelastoma bulhoesi,* Oxyurata, Nematoda, *Periplaneta americana,* Blattidae, Arthropoda, cockroach, host–parasite relationships, *Hammerschmidtiella diesingi.*

Oxyurid nematodes are ubiquitous and abundant in *Periplaneta americana,* often occurring in 80% of laboratory populations (Tsai and Cahill, 1970). Undoubtedly, they are present in many research colonies where they are unrecognized or ignored. The effect these worms may have upon the host is not well documented. Lethal effects occur, but are apparently rare. More subtle effects such as sterilization, weight loss, or interference with normal metabolism are difficult to identify. Barlow (1962) investigated the effect of parasitism on hemolymph electropherograms. Taylor (1968) described tissue damage by *Leidynema,* although this occurred in an abnormal host and may have been a secondary invasion. Interference with host lipid metabolism has been proposed by Majumbar (1970) for *Leidynema appendiculata.* Massey (1974) cited host–parasite relationships in nematode–bark beetle systems as the most pressing need in that field. This report describes the effect of oxyurid nematodes on host weight, length, hemolymph specific gravity, and hemolymph cell counts, and the effect of host sex and age on nematode populations.

Materials and Methods

In the course of research on the biology of *Thelastoma bulhoesi* over the past 10 yr, the following data have been collected for 3,721 *Periplaneta americana* as they have been sacrificed: number parasitized; the age, sex, weight, and length of each host; the specific gravity of the hemolymph of each host; a differential cell count for the number of prohemocytes, transitional hemocytes, and large hemocytes in the hemolymph; and the numbers of *T. bulhoesi* and *Hammerschmidtiella diesingi* in each host.

Means for the following variables were calculated:

host weight, host length, specific gravity of the hemolymph, and the proportion of pro-, transitional, and large hemocytes in hemolymph smears. These means for both parasitized and nonparasitized cockroaches were compared by a Student's *t*-test of unrelated measures (Table 1).

As cockroaches studied herein were infected with 2 species of nematodes, the mean number per host of each species as well as the mean total number of nematodes per host was compared by means of a Student's *t*-test for adults vs. nymphs (Table 2) and for males vs. females (Table 3). All tests of significance were determined at the probability level of $P \leq 0.05$ levels.

Results

The mean weights and lengths of parasitized hosts were generally greater for parasitized hosts than for nonparasitized hosts. The relative numbers of hemolymph cells and hemolymph specific gravity were not significantly different in these same 2 groups (Table 1). In Table 2, the mean numbers of nematodes per host in adults and nymphs are compared. The mean number of nematodes was significantly greater in adults and in females (Table 3).

Discussion

It is not uncommon for there to be a difference in the rate of infection of different sexes or ages. The older the host the longer it has had to be in contact with the parasite or parasites. Dogiel (1961) suggests that in fish both numbers and variety of parasites increase with age. In contrast, animals with a stronger immune response are generally thought to be more susceptible to infections in early life before the immune system can develop.

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Table 1. Mean weights, lengths, hemolymph specific gravity, and hemolymph cell counts for both parasitized and nonparasitized *Periplaneta americana.*

Cockroach	Para-sitized: $\bar{x}$ (SD)	Non-para-sitized: $\bar{x}$ (SD)	Significance: t value at 0.05 level
Weight (g)	0.97 (0.28)	0.86 (0.28)	2.915 (+)*
Length (mm)	32.65 (3.94)	30.93 (4.88)	2.792 (+)
Hemolymph specific gravity	1.21 (1.30)	1.18 (1.16)	0.157 (−)†
Prohemocytes	30.38 (11.03)	30.07 (8.17)	0.148 (−)
Transitional hemocytes	61.12 (10.31)	59.63 (8.48)	0.740 (−)
Large hemocytes	8.56 (4.96)	10.68 (6.56)	−1.758 (−)

* (+) significant.
† (−) not significant.

Differences in infection rate in different sexes are also well documented. *Polystoma* (Trematoda) numbers in frogs appear to be higher in males just before the reproductive season and it is thought that the female hormones increase host resistance (Kennedy, 1974). In *Discocotyle* (Trematoda), the oncomiricidia appear to be attracted to male mucus more than to that of females (Paling, 1965). In *Mesocoelium* (Cestoda), a parasite of lizards, the elevated infection in females was believed to be due to a larger appetite and consequent ingestion of more intermediate hosts (Thomas, 1965).

The mean weight and length of parasitized cockroaches were significantly different from

Table 2. Mean numbers of *Thelastoma bulhoesi* and *Hammerschimidtiella diesingi* parasitizing adult and nymphal *Periplaneta americana.*

Nematode	Adults $\bar{x}$ (SD)	Nymphs $\bar{x}$ (SD)	Significance: t value at 0.05 level
T. bulhoesi	6.21 (6.33)	1.97 (1.51)	3.801 (+)*
H. diesingi	2.22 (2.31)	0.36 (0.65)	4.551 (+)
Total	8.40 (7.95)	2.33 (1.38)	4.349 (+)

* (+) significant.

Table 3. Mean numbers of *Thelastoma bulhoesi* and *Hammerschmidtiella diesingi* parasitizing cockroaches, *Periplaneta americana,* by host sex.

| Nematode | Host sex | | Significance: t value at 0.05 level |
	Male $\bar{x}$ (SD)	Female $\bar{x}$ (SD)	
T. bulhoesi	2.16 (2.088)	8.24 (6.78)	−6.839 (+)*
H. diesingi	0.81 (1.08)	2.89 (2.58)	−5.326 (+)
Total	2.95 (2.40)	10.92 (8.58)	−7.134 (−)†

* (+) significant.
† (−) not significant.

those of nonparasitized hosts (Table 1). This is probably only a reflection of the larger number of worms in adult female *Periplaneta* (Tables 2, 3), adults being larger than nymphs and females being generally larger than males. There may be behavioral and dietary differences between nymphs and adults or molting may play a part in resistance even though Lee (1960) has shown that the thelastomatids can survive this process. The reasons for differences in infection rates between the sexes are unexplained.

Patton (1962) discussed the potential for hemolymph specific gravity differences in parasitized and nonparasitized insects. He concluded that differences exist when parasites of the hemocoel were present but not with intestinal parasites. The results of this study are consistent with his suggestion. Also, there appears to be no significant change in all differential counts due to the presence of nematodes.

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Interference in the lipid metabolism of the cockroach, *Periplaneta americana,* following infection of a thelastomatid nematode, *Leidynema appendiculata.* Proceedings of the Zoological Society of Calcutta 23:161–168.

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A previous background in laboratory methods is recommended.

In Vitro Culture of *Baylisascaris procyonis* and Initial Analysis of Larval Excretory–Secretory Antigens

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ABSTRACT: Larvae of *Baylisascaris procyonis* hatched from embryonated eggs were cultured in vitro for 6 wk. Larvae molted once during culture, and increased in length from $\bar{x} = 0.278$ to 0.513 mm. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of excretory–secretory (ES) materials collected from these cultures demonstrated a heterogeneous group of antigenic proteins ranging from 10 to 70 kd. Immunoblots using these ES antigens allowed for the differential diagnosis of *B. procyonis* from *Toxocara canis* infections in rabbits, and they may prove useful in the immunodiagnosis of *B. procyonis* infections in other species, including humans.

KEY WORDS: nematode, in vitro culture, *Baylisascaris procyonis, Toxocara canis,* excretory–secretory (ES) antigens, SDS-PAGE, immunoblotting, immunodiagnosis.

Baylisascaris procyonis, the common ascarid of raccoons (*Procyon lotor*), has recently emerged as an important cause of larva migrans in animals and humans (Kazacos, 1986). Currently, definitive diagnosis of larva migrans due to *B. procyonis* requires microscopic identification of larvae either within or recovered from host tissues (Huff et al., 1984; Fox et al., 1985). A specific immunoassay for *B. procyonis* infections is therefore highly desirable. Excretory–secretory (ES) materials released by parasitic nematodes during in vitro culture represent a potential source of useful diagnostic antigens. This report describes an in vitro culture technique for larvae of *B. procyonis,* and the initial analysis of ES antigens collected from this system.

Materials and Methods

Adult *B. procyonis* were obtained from the intestines of naturally infected raccoons, and eggs were collected and embryonated as described by Kazacos et al. (1981). Embryonated eggs were washed 3 times in 0.85% NaCl and decoated for 30–90 min (without agitation) at 37°C in a 1:1 mixture of 5.25% sodium hypochlorite and 1% NaOH. Decoating was stopped when the outer layers of the egg shells had been removed and the remaining vitelline membranes became distorted by the movements of the enclosed larvae. Following decoating, eggs were washed 4 times in 0.85% NaCl and hatched in Earle's balanced salt solution (EBSS, Grand Island Biological Co. [GIBCO], Grand Island, New York) as described for *Ascaris suum* by Urban et al. (1981). The resulting larval suspension was washed 2 times in EBSS and layered over sterile cotton gauze in a glass funnel and maintained overnight at 37°C. The following morning, larvae were collected aseptically in 10 ml aliquots and washed 6 times in warm, sterile Dulbecco's phosphate buffered saline (DPBS, GIBCO). Larvae were dispensed into Dulbecco's modified Eagle's medium (DMEM, GIBCO), containing 100 units/ml penicillin and 100 μg/ml streptomycin, in 24-well polystyrene tissue culture plates (Corning Glass Works, Corning, New York). Each well contained 2 ml DMEM with a larval concentration of 10,000/ml. Larvae were maintained for 6 wk at 37°C in 5% CO_2 and compressed air and the media changed weekly. Larval mortality was assessed on a weekly basis by examining 100 larvae stained with 1:10,000 new methylene blue. Dead larvae rapidly absorbed the dye along their cuticles while live larvae remained largely unstained and were motile. Samples of larvae were also collected at regular intervals and fixed in hot alcohol–formalin–acetic acid (AFA) for morphometric and developmental analyses.

Culture medium collected at weekly intervals was dialyzed against DPBS and concentrated by diafiltration using spiral cartridge and stir cell apparatuses with molecular weight exclusion limits of 10,000 (Amicon Corp., Danvers, Massachusetts). Protein concentration determinations, SDS-PAGE, and immunoblotting were performed according to manufacturer's instructions, utilizing equipment and reagents obtained from Bio-Rad (Richmond, California). SDS-PAGE was performed under reducing conditions in a discontinuous system utilizing 4% stacking and either 12% or 15% separating gels. Following completion of the SDS-PAGE run, gels were either silver stained or the proteins were electrophoretically transferred from the gels onto nitrocellulose using a Bio-Rad transblot apparatus. SDS-PAGE and silver staining were performed with between 3.5 and 4.5 μg of protein collected from each of the 6 wk of culture. Immunoblotting was performed with pooled protein samples collected from the 1st 3 wk of culture, utilizing 200 μg of protein per 16 × 11 cm gel.

Following protein transfer, the portion of the nitrocellulose containing molecular weight standards was cut off and stained with 0.1% amido black. Reactive sites were blocked with 3% gelatin and the membranes incubated for 3 hr in a 1:100 dilution of antisera. Antisera were obtained from New Zealand White (NZW) rabbits orally inoculated with 10,000 embryonated eggs of *B. procyonis* or *T. canis.* Serum was collected from
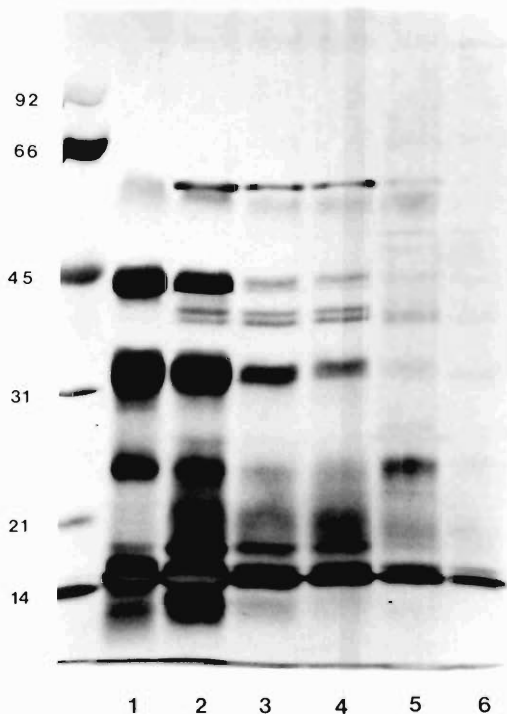
Figure 1. Silver-stained SDS-PAGE gel (15%) of *B. procyonis* ES proteins collected at weekly intervals (1–6). Molecular weight standards (in kilodaltons) are in far left lane.

these rabbits at 14 and 56 days postinfection, respectively, as well as from uninfected control rabbits. Serum was also collected from 2 pet rabbits naturally infected with *B. procyonis* following accidental exposure to *B. procyonis* eggs from a raccoon previously housed in the same cage. The size of the infective dose and the duration of infection could not be positively determined for these rabbits. However, based on previous observations they had probably ingested large numbers of eggs within the previous 3–6 wk (Kazacos, 1986). Following incubation in rabbit antisera, nitrocellulose membranes were incubated for 1 hr in a 1:3,000 dilution of goat anti-rabbit IgG (H and L chains) conjugated with horseradish-peroxidase. Color development was visualized after the addition of 4-chloro-1-naphthol.

Results and Discussion

Initially, decoating of embryonated eggs of *B. procyonis* required periods of time varying from 30 to 90 min. However, subsequent trials revealed that decoating could routinely be accomplished in 30 min when 10 ml of eggs were gently agitated with 2 changes of 5.25% sodium hypochlorite (without 1% NaOH) on a shaker platform. Larvae either expressed from embryonated
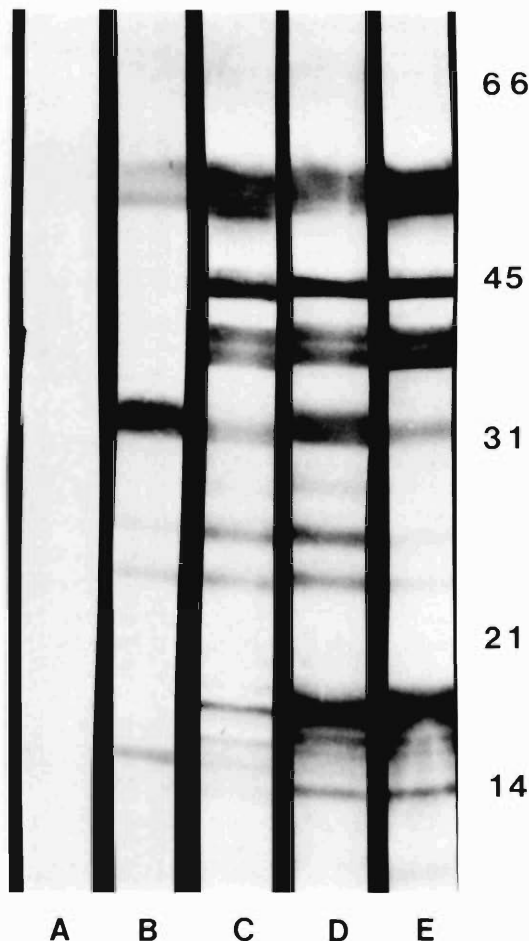
Figure 2. Immunoblots of *B. procyonis* ES antigens transferred from 12% SDS-PAGE gels. Lane A = Normal rabbit sera. Lane B = *Toxocara canis* antisera, rabbit, 56 days PI. Lane C = *Baylisascaris procyonis* antisera, rabbit, 14 days PI. Lanes D and E = Sera from 2 rabbits naturally infected with *Baylisascaris procyonis*. Approximate molecular weights (in kilodaltons) are shown on the right.

eggs, or recovered following hatching and Baermannization, were loosely enclosed in the cuticle of the previous stage. Within 24 hr 8% of the larvae had shed this cuticle, and by 72 hr >90% of larvae had completed this molt. Larvae continued to grow throughout the culture period, but no further molts were detected. *Ascaris suum* larvae have also been shown to grow and develop when cultured in DMEM (Urban and Tromba, 1982), whereas *T. canis* larvae did not molt in DMEM when maintained for periods up to 18 mo (de Savigny, 1975).

In the present study it is unclear whether the

cuticle shed during the first few days of culture represents the 1st or 2nd molt. Sprent et al. (1973) stated that the 2nd molt took place 1–2 wk after infection of mice with eggs of either *B. devosi* or *B. tasmaniensis,* and Douvres et al. (1969) stated that the 2nd molt of *A. suum* occurred in the liver within 1 wk. In contrast, Maung (1978) reported that the 1st 2 molts of *A. suum* occur within the egg, and that 3rd-stage larvae may hatch from the egg still enclosed within the 2nd-stage cuticle. Further work is needed to determine which larval stage of *B. procyonis,* as well as other ascarids, hatches from the embryonated egg.

After hatching and Baermannization, *B. procyonis* larvae measured an average of 0.278 mm in length ($N = 25$). The larvae continued to grow averaging 0.333 mm at 1 wk, 0.409 mm at 2 wk, 0.451 mm at 3 wk, and 0.513 mm at 6 wk ($N = 25$ at each wk). Larval mortality progressively increased from 5% (wk 1), to 9% (wk 3), and finally to near 50% by 6 wk when the cultures were terminated. During the 1st 3 wk of culture, each larva produced about 1–3 ng of protein per day. Protein production was not determined beyond 3 wk because of the large number of dead and dying larvae which were present in the cultures at this time.

SDS-PAGE analysis of ES proteins collected at each week of culture revealed a heterogeneous mixture with molecular weights ranging from 10 to 70 kd (Fig. 1). Quantitative and qualitative variations in ES components occurred between weeks as indicated by the relative intensity of staining and the presence or absence of certain bands. These variations tended to correlate with the age of larvae as well as the degree of larval mortality. The relative intensity of staining of major components at 35 and 43 kd decreased with time, and by 5 and 6 wk these bands were barely discernible. A more diffuse banding pattern was detected after wk 4 with the appearance of new bands. This diffuse pattern is also seen with silver-stained gels of homogenized larvae (data not shown), and the presence of new bands after wk 4 may be due to the release of proteins from dead and dying larvae. Other ES components (doublet at 39 kd and band at 60 kd) were only apparent after wk 1. However, SDS-PAGE analyses of numerous other cultures have shown that these components are sometimes present in material collected from the 1st week of culture, and they are always present by the 2nd week. Qualitative differences have also been detected

in *T. canis* ES components from different culture batches (Speiser and Gottstein, 1984; Badley et al., 1987).

Equal amounts of ES proteins were pooled from the 1st 3 wk of culture for use in immunoblotting, since larval mortality was > 10% after 3 wk. The major ES proteins identified by silver staining of polyacrylamide gels were recognized by immunoblot analysis with sera from rabbits infected with *B. procyonis* (Fig. 2). Serum from the *T. canis* infected rabbit cross-reacted with several *B. procyonis* ES antigens, with the notable exceptions of those having apparent molecular weights of 18, 39 (doublet), and 43 kd. These results have been substantiated by immunoblot analysis using sera from several other *T. canis-*infected rabbits, and immunoblotting using ES antigens has consistently allowed us to differentiate *B. procyonis* infections from *T. canis* infections in rabbits.

Toxocara canis larval ES antigens are of proven value in the serodiagnosis of toxocaral visceral larva migrans (Glickman and Schantz, 1981). However, cross-reactions have been detected between *T. canis* ES antigens and antisera against various other ascarid nematodes (Nicholas et al., 1984). In the present study, cross-reactions were detected between *T. canis* antisera and *B. procyonis* ES antigens, but several ES antigens appeared to be specific for *B. procyonis*. Further studies are indicated to determine the usefulness of these ES antigens in differentiating *B. procyonis* from other ascarid infections in a variety of host species, including humans.

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An In Vitro Test for Drug Resistance in *Haemonchus contortus*

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ABSTRACT: A cambendazole-resistant (CR) strain of *Haemonchus contortus,* the large stomach nematode of sheep, had similar in vitro growth characteristics compared to the parent strain (BPL). When the in vitro cultivation system was modified to detect drug resistance: (1) exsheathed infective larvae of the BPL strain did not develop in a culture medium with a concentration of 2.5 μg/ml thiabendazole (TBZ), but the CR strain developed to the mid-4th larval stage in 6 days at the same concentration of TBZ; and, (2) larvae of both strains of *H. contortus* were killed prior to or in the 3rd molt in a culture medium with 2.5 μg/ml levamisole.

KEY WORDS: Nematoda, Trichostrongyloidea, *Haemonchus contortus,* bioassay, in vitro cultivation, drug resistance, levamisole, thiabendazole.

The most objective criteria for defining a drug-resistant population of a species is its ability to grow and develop in the presence of a drug concentration that either kills a susceptible population or inhibits its growth and development. Some methods previously used for detecting drug resistance in parasitic nematodes include: drug efficacy studies, egg hatching, larval motility assays, tubulin binding, and aggregation assays. These methods are discussed later in this paper.

Recently, Stringfellow (1984, 1986) cultured *Haemonchus contortus* to egg-laying and sperm-producing adults in vitro. The use of in vitro methods to test the effects of anthelmintics on *Haemonchus contortus,* the large stomach nematode of sheep, goats, and other ruminants, is not new. Stringfellow and Fetterer (unpubl. data) used these in vitro culture methods to study development of drug resistance in this parasite. Douvres et al. (1980) used in vitro methods to study the effects of drugs on *Ostertagia ostertagi* grown in vitro, and Rew et al. (1982) studied the effects of drugs on adult *H. contortus* maintained in vitro. However, studies of the effects of drugs on the developmental stages of susceptible and drug-resistant strains of *Haemonchus contortus* are rare. The cambendazole-resistant strain (CR) used in the present study was selected from the susceptible strain (BPL) by Kates et al. (1973). The objective of the present study was to determine if in vitro methods could be adapted for use as an assay for detecting thiabendazole resistance in *H. contortus.*

Materials and Methods

Experimental animals and nematodes

Neutered Polled Dorset sheep, raised helminth free except for minimal infection with *Strongyloides pap-illosus,* were used as hosts for the nematodes. They were maintained in individual concrete-floored pens and fed a pelleted feed (mixture of alfalfa meal, barley, bran, corn, meal, oats, and salt).

Susceptible (BPL) and the cambendazole-resistant (CR) strains of *H. contortus* were maintained in the sheep. The cambendazole-resistant strain was originally isolated from the susceptible parent strain by Kates et al. (1973). Infective larvae were recovered from fecal cultures, cleaned with sterile 0.85% saline, and exsheathed by treatment with 1.25% sodium hypochlorite in saline. They were then washed with sterile saline and Earle's balanced salt solution containing antibiotics (penicillin: 5 million units; streptomycin: 5 g; fungazone: 5 mg/5.1 liters) (Douvres, 1983).

Culture procedures

The 1st series of experiments compared the growth and development of the BPL and the CR strains of *H. contortus* in vitro. Techniques for the cultivation of this worm from infective larvae to advanced stages have been previously described (Stringfellow, 1984, 1986). Preparation of medium API-1 was described previously by Douvres and Malakatis (1977) and Fildes' reagent (a peptic digest of defibrinated bovine blood) was described by Douvres (1983). The ovine gastric contents (OGC) was prepared fresh. It was first cleared of ingesta by straining it through cheese cloth, then centrifuged at 3,900 g and 2,400 g on a Damon Cu5000 and Sorvall RC5C for 30 min each. It was then filtered through an AP25 clarifying pad at 40 psi. After clarifying the OGC, it was Millipore filtered and separated by Dacron separators. The top filter was the AP25 clarifying pad followed by the 8, 1.2, 0.45, and 0.22 μm final filter. The OGC was recovered sterile and used as a supplement. The experimental design as well as specific details can be found in Tables 1 and 2.

The 2nd series of experiments compared the growth and development of each strain of *H. contortus* in API-1 culture medium supplemented with 1.38 mM ascorbic acid and 3.2 mM cysteine. The gas phase for the 100 ml culture vessels was 85% N_2 : 5% O_2 : 10% CO_2 at pH 6.4 for the 6 days of incubation (DIC) (39°C). It was important that the culture vessels be sealed tightly. This 2nd series of experiments compared the growth

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Table 1. *Haemonchus contortus*—development of susceptible (BPL) and drug-resistant (CR) strains from artificially exsheathed larvae to advanced stages in API-1 culture medium supplemented with Fildes' reagent and ovine gastric contents (OGC).

Strain of *H. contortus*	Time (days) for development to stage							
	Parasitic 3rd	3rd molt	Early 4th	4th molt	Young adult		Mature adult	
					Male	Female	Male	Female
In vitro*								
BPL	0	3–5	3–5	19	21	25	28	35
CR	0	2–5	3–5	14	21	25	28	35

* Two trials, each trial consisting of 2 culture vessels.

and development of the 2 strains in: (1) the presence of a graded concentration of a drug (TBZ) to which they had developed resistance (Tables 3, 4); and, (2) the presence of a graded concentration of levamisole (LV) to which they had not developed resistance. Control cultures did not have the drugs. The cultures were diagnosed at 6 DIC because the growth characteristics of these 2 strains are similar up to about 6 DIC when the nematodes are in the mid-4th larval stage.

The methods of Douvres et al. (1966) were used to test sterility of all stocks of media and to examine and evaluate the development of the nematodes. Cultures were judged free of contamination if there was no visible sign of fungi and bacteria. The larval stages, adults, and eggs were identified according to Veglia (1915) and from original observations.

Drug preparation and delivery

Both thiabendazole-hydrochloride (Merck and Co., Rahway, NJ) and levamisole (American Cyanamid, Princeton, NJ) were dissolved in deionized water at concentrations ranging from 10 to 0.01 μg/ml of the drug when 0.1 ml of the drug was added to 9.9 ml of the culture medium at the beginning of each experiment. One-tenth ml of deionized water was added to each control culture. The amount of drug available to the worms in the culture medium was then about 1% less than that added because of the dilution factor when 0.1 ml of the larvae were added to the culture vessel. Only data for concentrations of the drug ranging from 2.5 to 0.1 μg/ml are presented in Tables 3 and 4. Where pilot studies were run at 10, 5, and 0.01 μg/ml to work out the most useful concentrations of the drug, those data are reported in the text where they provide useful information.

Results and Discussion

Growth and development of BPL and CR strains of *H. contortus* in vitro

In general, growth and development of BPL and CR strains in vitro and in vivo were similar to those results reported previously by Stringfellow (1986). Both strains of *H. contortus* developed from infective larvae to mature egg-laying females in 35 days (Table 1) in API-1 supplemented with Fildes' reagent and ovine gastric contents (OGC). There was no apparent difference in the rate of development of males and females of both strains up to about the early to mid-4th stage; however, beyond that point the CR strain grew better than the BPL strain at least to the 4th molt and young adult stage. In general, males of both strains underwent the 4th molt a few days before the females. The largest mature adult male and female BPL strain worms grown in vitro were 9 mm and 12–14 mm long, and the adults of the CR strain measured 10 mm and 15 mm, respectively.

Data on survival and yields of advanced stages of both strains of *H. contortus* are given in Table 2. The cultures were terminated at 42 DIC. There was an average of 29 males (0.07%) and 29 egg-laying and egg-producing females (0.07%). All worms of both strains were alive at 14 DIC and were at the 4th stage. At 21 DIC, 14% of the larvae of the CR strain and 0% of the larvae of the BPL strain had reached the young adult stage. By 28 DIC, 5% of the males and females of the BPL strain had reached the young adult stage and only 1% of the CR strain had reached the adult stage. In general, the results obtained here for both strains of *H. contortus* were similar to those previously reported for the BPL strain (Stringfellow, 1986).

Growth and development with and without drugs

There was no noticeable effect on the larvae of either the BPL or the CR strains when 0.1 ml of deionized water was added to the control cultures. Larval growth in API-1 medium plus reducing agents was similar to growth obtained in the "optimal" system (Table 1); however, both BPL and CR strains developed slightly faster in the 100-ml vessels (Table 3). Larvae of the BPL strain gradually stopped moving when concentrations of thiabendazole (TBZ) were added to the cultures. Exsheathed BPL larvae did not develop beyond the parasitic 3rd larval stage when

Table 2. *Haemonchus contortus*—survival and yields of advanced stages that developed from artificially ex-sheathed infective larvae* in roller culture bottles consisting of API-1 culture medium supplemented with Fildes' reagent and ovine gastric contents.

Days in culture	Strain of *H. contortus*	Total inocu-lum alive (%)	Live (%) worms in stage					
			Parasitic 3rd	3rd molt	4th stage	4th molt	Young adult	
							Male	Female
7	BPL	100	8	1	91	0	0	0
	CR	100	6	3	91	0	0	0
14	BPL	100	0	0	100	0	0	0
	CR	100	0	0	100	0	0	0
21	BPL	74	0	0	91	7	0	0
	CR	90	15	0	47	24	6	8
28	BPL	82	0	0	65	31	3	2
	CR	61	0	0	64	33	1	0

* Forty thousand larvae per culture.

TBZ was added at 2.5 μg/ml (Fig. 1). Some BPL larvae did reach the 3rd molt and early 4th stage when concentrations of TBZ were 1.0 and 0.1 μg/ml; larvae reaching early 4th stage were in poor condition. BPL larvae grew like the controls at 0.01 μg/ml concentrations of TBZ. The CR strain developed to the mid-4th larval stage in concentrations of TBZ from 0.01 to 2.5 μg/ml.

They remained at the parasitic 3rd larval stage at 5 μg/ml. Survival and yields of both strains of larvae are presented in Table 4.

In general, although the BPL larvae did not develop and remained at the parasitic 3rd larval stage, they were viable; thus the high rate of survival at 2.5 μg/ml. The survival and yields of the CR strain were similar to the controls when the concentration of TBZ ranged from 0.01 to 2.5 μg/ml.

Table 3. *Haemonchus contortus*—development of susceptible (BPL) and drug-resistant (CR) strains from artificially exsheathed larvae to mid-4th stage in API-1 culture medium with and without thiabendazole (TBZ) or levamisole (LV).

Strain of *H. contortus*	Drug concen-tration (μg/ml)	Time (days) for development to mid-4th stage*			
		Para-sitic 3rd	3rd molt	Early 4th	Mid-4th
BPL					
	0	0	3	3	6
TBZ	2.5	0	0	0	0
	1.0	0	2	3	6
	0.1	0	2	3	6
LV	2.5	0	0	0	0
	1.0	0	2	3	6
	0.1	0	2	3	6
CR					
	0	0	2	3	6
TBZ	2.5	0	2	3	6
	1.0	0	2	3	6
	0.1	0	2	3	6
LV	2.5	0	0	0	0
	1.0	0	2	3	6
	0.1	0	2	3	6

* Six DIC, 4 trials of 1 culture each.

Table 4. *Haemonchus contortus*—survival and yields of larval stages of a susceptible (BPL) and drug-resistant (CR) strain with and without thiabendazole (TBZ) or levamisole (LV).

Strain of *H. contortus*	Drug con-cen-tration (μg/ml)	Total inoc-ulum alive (%)	Live (%) worms in stage			
			Para-sitic 3rd	3rd molt	Early 4th	Mid-4th
BPL						
	0	100	75	11	7	8
TBZ	2.5	100	100	0	0	0
	1.0	86	48	6	42	3
	0.1	88	44	2	47	6
LV	2.5	84	100	0	0	0
	1.0	100	91	0	5	5
	0.1	100	83	0	8	8
CR						
	0	100	6	0	12	81
TBZ	2.5	100	27	1	40	32
	1.0	100	22	17	43	18
	0.1	100	24	10	38	28
LV	2.5	70	96	4	0	0
	1.0	100	49	0	22	30
	0.1	100	50	0	23	26

* Six DIC, 4 trials of 1 culture each.

was active against both the BPL and the CR strains. The CR larvae did not develop beyond the 3rd molt (frequently aborted the 3rd molt) remaining as parasitic 3rd-stage larvae at LV concentrations of 2.5 μg/ml (70% survival) (Fig. 2). At LV concentrations of 1.0 and 0.1 μg/ml a slightly greater percentage of CR larvae developed to mid-4th stage than in the control group, reflecting the fact that CR larvae develop better in 100-ml vessels. Survival and yields of both strains of larvae are presented in Table 4.

Conclusions

Thiabendazole, the imidazole used in the present study to test for drug resistance, interferes with energy generation (Rew, 1978). It was used in the present study in place of cambendazole because it is more water soluble, and there were no synergistic effects of the solvent and the drug on the nematode. Levamisole, which interferes with neuromuscular transmission (Rew, 1978), was used in the present study as a positive control. The older methods for detecting drug resistance using animals are very effective (Kates et al., 1973; Colglazier et al., 1974); however, they are also very expensive. In those tests the nematode was grown to the adult stage in the host; the hosts were treated with the drug and the efficacy of the drug was determined by comparing the number of worms recovered from treated and control groups. In recent years several methods have been developed to detect resistance of nematode parasites to drugs. Some of these methods are economically appealing. Le Jambre (1976) and Dobson et al. (1986) found that thiabendazole- and levamisole-resistant strains of *H. contortus* hatched at higher concentrations of the drugs than susceptible strains. Gerald C. Coles (pers. comm., July, 1985) and coworkers, in a test of the growth and development of larvae of cambendazole-resistant and susceptible strains of *H. contortus*, found that their larval test was more sensitive than the egg-hatch test. More recently, Sangster et al. (1985) reported that benzimidazoles affected microtubule-dependent acetylcholine esterase secretion, the formation of microtubules in intestinal cells, and colchicine binding in susceptible versus resistant strains of *Trichostrongylus colubriformis*. Also, Jenkins et al. (1986) have reported that the aggregation response of *T. colubriformis* was useful for screening anthelmintics.

The results presented herein indicate that in

Figures 1, 2. 1. BPL strain of *Haemonchus contortus* parasitic 3rd-stage larvae (arrow) exposed to 2.5 μg/ml thiabendazole. Larvae are viable. ×125. 2. CR strain of *Haemonchus contortus* parasitic 3rd-stage larvae (arrow) exposed to 2.5 μg/ml levamisole. Larvae abort the 3rd molt. ×125.

When levamisole (LV) was added to the culture medium, the larvae of both BPL and CR strains tended to round up and stop moving although they appeared to be alive. The exsheathed BPL larvae did not develop beyond the parasitic 3rd stage when LV was added at 2.5 μg/ml (Tables 3, 4). The larvae developed beyond the 3rd molt to the mid-4th stage when the concentrations of LV were 1.0 and 0.1 μg/ml, similar to the controls (Table 4). Similar results were obtained with the CR larvae indicating that the LV

vitro procedures can be used as an assay for detecting thiabendazole resistance in *H. contortus.* The methods described are a modification of in vitro methods originally used to culture this nematode to the mature adult stages (Stringfellow, 1984, 1986). The use of in vitro culture methods provides a useful investigative tool to separate resistant from sensitive populations of this nematode. These 2 strains were easily identified with the in vitro culture techniques described in the present paper.

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Observations on the Ability of Larval *Ostertagia ostertagi* to Migrate through Pasture Soil

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ABSTRACT: The ability of larval *Ostertagia ostertagi* to migrate through soil was investigated. In the fall of 1986, bovine fecal pats containing approximately 200,000 eggs were placed on pasture with soil composed of sand (72%), silt (20%), and clay (8%). Pats were enclosed within open-ended galvanized steel cylinders sunk to depths of 5, 10, and 15 cm. Over the ensuing 9 wk, samples of herbage within and surrounding the steel cylinders were analyzed for number of *O. ostertagi* L_3 per kg dry herbage. The results showed that *O. ostertagi* migrated down into the soil to at least 15 cm, and returned to the surface grass within 5 wk after the start of the experiment; larvae from nonenclosed control fecal pats appeared on grass within 2 wk. It was concluded that *O. ostertagi* larvae are capable of vertical soil migration and eventual return to the surface grass in at least this soil type. Further investigations are required to assess the significance of the soil sequestration as a means for allowing survival over periods of environmental stress.

KEY WORDS: Nematoda, Trichostrongyloidea, *Ostertagia ostertagi* L_3, epidemiology, ecology, soil migration, cattle, behavior.

The survival of *Ostertagia ostertagi* free-living stages on pasture in regions experiencing cold winters is generally believed to be due to the sequestering of larvae and eggs in the dung pat (Michel, 1969). However, the ability of *O. ostertagi* larvae to survive long periods on ungrazed pastures (Bairden et al., 1985), through prolonged drought (Barger et al., 1984), and through severely cold winter conditions (Gibbs, 1979) has raised the question of whether the soil might, to some extent, act as a reservoir during hazardous periods.

The ability of trichostrongyle larvae to migrate into soil has been examined by several investigators, but their results have not been consistent. Al Saqur et al. (1982) reported that in Scotland free-living 3rd-stage larvae (L_3) of *Ostertagia ostertagi* could migrate down to 7–15 cm in medium loam soil, and considered such migration to be of epidemiological importance. In contrast, Borgsteede and Boogaard (1983) concluded that survival of *O. ostertagi* L_3 in soil in the Netherlands was epidemiologically unimportant. Similarly, Rose and Small (1985) concluded that the vertical soil migration of *Trichostrongylus vitrinus* L_3 was not sufficient in southern England to be considered important to larval overwintering. Only a few experimental studies on the

soil migration behavior of trichostrongyle larvae have been reported (Fincher and Stewart, 1979; Grønvold, 1979; Gruner et al., 1982; Callinan and Westcott, 1986), therefore, further investigation of this aspect of *O. ostertagi* ecology and its relevance to the epidemiology of ostertagiasis in cattle is warranted. We had an opportunity to carry out an experiment in the fall of 1986 designed to learn whether *O. ostertagi* larvae which migrate downward from the cow pat to various soil depths could return to the surface herbage, an important consideration if such behavior is to have any epidemiological importance. Previous studies had not clearly resolved the issue of whether larvae that had migrated into soil could return.

Materials and Methods

Three groups of 3 open-ended 18-gauge galvanized steel cylinders (30 cm in diameter) with lengths of 35, 40, and 45 cm were inserted into the ground at a distance 1 m apart to depths of 5, 10, and 15 cm, respectively. Care was taken to water the soil thoroughly at the point where the cylinders were to be inserted into the earth; they were driven in by a forceful twisting action. Once the cylinder was pushed into the earth to the 5-, 10-, or 15-cm depth the soil was tamped firmly at the inner and outer junction of ground with cylinder to eliminate hollow spaces. The cylinders were then lined on the outside with fiber glass building insulation (8.8 cm thick) and polyurethane sheeting to prevent extreme fluctuations in air temperature inside the cylinder; the cylinder top was left open.

Feces containing approximately 200 eggs/g were collected from *O. ostertagi*-infected donor calves, shaped into pats (1,000 g), and placed inside the 9 cylinders.

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Table 1. Number of larvae recovered from control pats and pats placed inside cylinders buried at 5-cm, 10-cm, and 15-cm depths.

Week no.	Control pats	Mean number of larvae per kg dry herbage at cylinder wall depths					
		5 cm		10 cm		15 cm	
		Inside	Outside	Inside	Outside	Inside	Outside
1	—	—	—	—	—	—	—
2	106	—	—	—	—	—	—
3	64	46	0	33	0	11	1
4	38	333	13	55	36	135	0
5	161	269	33	1,213	10	2,140	38
6	1,038	742	19	1,223	348	1,096	77
7	211	768	9	759	6	1,324	8
8	153	1,046	9	674	45	2,000	88
9	56	1,000	8	712	30	19	1,202

Additionally, 3 pats with no cylinders surrounding them served as controls and were placed 1 m from the nearest cylinders.

When regular sampling of herbage adjacent to the control pats revealed that 3rd-stage larvae had developed and migrated onto the vegetation, weekly collections were initiated. Samples of 25–50 g of herbage were snipped from the area surrounding the control pats and from inside and outside (adjacent to) all of the cylinders. Data were pooled for each of the 3 depth groups. Throughout the trial the herbage was regularly trimmed to maintain a height of 6–10 cm. Larvae were recovered from herbage samples with a modified Baermann apparatus. This consisted of paper facial cloths (Scotties®)[2] held in rectangular stainless steel baskets constructed with type SS304 4 × 4 wire mesh (diameter 1.2 cm), measuring 12.5 × 9.5 × 2.5 cm. These apparatuses were placed in 17.5-cm-diameter plastic plant pot liners and flooded with water for 24 hr. The washings were sieved over a 25-μm aperture Endicott sieve, and sedimented. The *O. ostertagi* larvae recovered were identified and counted with the aid of a compound microscope. The herbage was then oven dried, weighed, and the total number of larvae per kg of dry herbage (KDH) computed.

The study pasture was a mixed-grass plot, predominantly Kentucky bluegrass, *Poa pratensis.* Since the pasture had not previously been grazed by cattle it was not considered a source for *O. ostertagi* larvae. Herbage samples, collected and examined the week before the trial commenced, confirmed this.

The texture of the pasture soil was considered sandy loam and was composed of 72% sand, 20% silt, and 8% clay and had a pH of 6.7. Further analyses revealed a high concentration of magnesium (148.5 kg/hectare), low phosphate (42.8 kg/hectare), and high potash (261 kg/hectare).

This study was carried out from 3 October to 5 December 1986. During this period the average minimum and maximum ambient air temperatures were 9.0 and 20.2°C, respectively, for October, and 11.9 and 21.0°C,

respectively, for November. The total rainfall was 47.2 mm for October and 151.4 mm for November. The 30-yr means for rainfall for these 2 months are 81.0 mm and 74.3 mm, respectively.

Results and Discussion

Ostertagia ostertagi larvae were first recovered on herbage from the control pats by wk 2 and from the enclosed pats, both inside and outside the cylinders, beginning on wk 3 (Table 1). Larval collections continued throughout the 9-wk sampling period. Although the number of larvae/ KDH recovered from inside the cylinder was comparable to that of the controls, the larval counts outside the cylinder were considerably less, although larvae were consistently present after wk 4 to 5. The number of larvae/KDH recovered outside the 15-cm-deep cylinder did not exceed 10 until wk 5; in contrast, the recoveries for the 2 shallower depths exceeded this by wk 4. This suggested that the speed of larval migration in the soil was retarded somewhat by the deeper barrier. Between wk 5 and 8, recoveries were comparable for all soil depths. The final samples, taken at wk 9, revealed an overall decline of larvae on herbage outside the cylinders except for the 15-cm depth, which exhibited a large increase.

Our results from this experiment demonstrate that in this soil type, *O. ostertagi* L_3 can penetrate soil to a 15-cm depth, move laterally, and migrate upwards onto herbage in a relatively brief period, a total distance well over 30 cm. The delay, compared to nonbarrier controls, in appearance of larvae migrating to 15 cm was about 3 wk, and 2 wk for those larvae migrating to 5 and 10 cm. These results do not suggest the exact mechanism responsible for this movement. Al-

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[2] ®Scotties tissues are 24.1 × 19.5 cm. Scott Paper Co., Scott Plaza, Philadelphia, Pennsylvania 19113.

though soil and plant nematodes are well known to move purposefully and by direct body action through soil, in this case the role of other invertebrates must be seriously considered. Bryan (1976) and Fincher (1973) have shown that dung beetles can incidently move larvae vertically into soil. Further, studies by Grønvold (1979) have shown that earthworm activity may also facilitate larval dispersal into soil. However, the large number of larvae recovered from outside herbage in this experiment casts some doubt that movement was entirely due to an invertebrate vehicle.

Gruner et al. (1982) studied the migration of four ovine trichostrongylid L_3 species in soil. Their studies employed narrow plastic cylinders filled with soil which were inoculated with L_3 and downward movement of these larvae was noted. Our studies, however, were aimed at maintaining the intact natural state of the soil and herbage where possible. Except for the insertion of the steel cylinder into the earth, the conditions of our study did not disturb the earth and herbage. Our objective was to observe the migration of L_3 from the dung pat, downwards through the soil, around a barrier, upwards to the surface, and then on to herbage.

The implication of our findings for the epidemiology of *O. ostertagi* is problematical. No attempt was made to determine the total number of larvae emerging outside the cylinders. Larvae were collected from herbage only and not from the soil. The number of L_3/KDH recovered from herbage outside the barriers was far less than that obtained from herbage inside the cylinder except for 1 sample taken outside the 15-cm cylinder at wk 9. Perhaps of greater significance, in the case of the 5- and 10-cm cylinders, is the observation that the number of larvae recovered over the 9-wk sampling period decreased sharply after wk 7, implying that the soil may not have served as a continuing reservoir for recruitment of L_3 to herbage. The variation reported in herbage recovery methods makes consistent assessment of larval transmission potential difficult at best. It is possible, however, that under more severe climatic stress, the emergence of L_3 from the soil is retarded and only resumes with the onset of mild weather, such as that experienced during the last weeks of this experiment. The November rainfall was twice the normal amount and the temperature ranged from 0.6 to 3.3°C above normal during the final week. Further, on 3 December, 2 days prior to the last collection day, the temperature was 6.6°C above normal and 31 mm of rain fell between 1 and 5 December. These conditions may be responsible for the sudden increase in larvae recovered outside the 15-cm cylinder at wk 9, although it is not obvious why these same climatic conditions did not lead to increases in larvae outside the 5- and 10-cm cylinders. If emergence is arrested during cold weather, larvae sequestered in soil could contribute to the contamination of pasture in spring (Gibbs, 1979). Because of unavoidable personnel changes, we were not able to extend the experiment further. However, it is hoped that these results will stimulate more extensive studies on larval soil migration, and its dependence upon soil type and climate, to provide the data necessary to assess the overall importance of the soil to the epidemiology of trichostrongylosis.

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Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a Related Mycelial and Endospore-forming Bacterial Parasite from *Pratylenchus brachyurus*[1]

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ABSTRACT: Major distinctions exist between members of the *Pasteuria penetrans* group occurring on the root-lesion nematode, *Pratylenchus brachyurus*, and the root-knot nematode, *Meloidogyne incognita*. These dissimilarities include highly restricted nematode host ranges, differences in the bacterial life stages in relation to those of the host nematodes, and disparities in ultrastructural morphology of their mature sporangia and endospores. The several stages of the bacterial parasite of the root-lesion nematode (RLP) occur in all the nematode's larval stages. The mature RLP sporangia are rhomboidal, and average 2.40 ± 0.24 μm in diameter and 2.15 ± 0.19 μm in height. The endospores within the RLP sporangia are ellipsoidal or almost spherical, narrowly elliptic in section, having axes averaging 1.08 ± 0.21 by 1.29 ± 0.14 μm. By contrast, the mature bacterial stages from the root-knot nematode (RKP) occur only in the adult female nematode and not in the larvae. The RKP sporangia are cup-shaped, and average 3.42 ± 0.18 μm in diameter and 2.48 ± 0.22 μm in height. The RKP endospores, also ellipsoidal but broadly elliptic in section, have axes averaging 1.10 ± 0.11 by 1.42 ± 0.12 μm. These several differences strongly suggest that these 2 members of the *P. penetrans* group belong in separate taxa. Considerable morphological diversity was also evident from light-microscopic examination of members of the *P. penetrans* group occurring in archival nematode material (genera *Dolichodorus*, *Heterodera*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Tylenchorhynchus*, and *Xiphinema*) from the USDA Nematode Collection.
KEY WORDS: *Pasteuria penetrans* group, bacterial parasites, morphology, SEM, TEM, plant-parasitic nematodes, root-knot nematodes, *Meloidogyne incognita*, root-lesion nematodes, *Pratylenchus brachyurus*.

The mycelial and endospore-forming bacterial parasites of plant-parasitic nematodes, presently assigned to the species *Pasteuria penetrans* (ex Thorne, 1940) Sayre and Starr, 1985, are indeed widespread. These bacteria parasitize some 175 different nematode species belonging to about 70 genera, and occur in at least 12 of the United States as well as in about 40 other countries or other political units on 5 continents and on various islands in the Atlantic, Pacific, and Indian oceans (Sayre and Starr, 1988). Albeit the literature is scanty on this point, each preparation so far examined exhibited a rather limited host range and considerable morphological diversity, par-

ticularly in size and shape of sporangia and endospores (Sayre and Starr, 1985, 1988). These pathotypes and morphotypes of diverse geographical origins probably comprise a multiplicity of taxa (Sayre and Starr, 1985, 1988).

The intention of Sayre and Starr (1985) in establishing the species *Pasteuria penetrans* was to limit this taxon to bacteria of this sort with relatively small endospores, such as those found parasitizing *Pratylenchus* and *Meloidogyne* species. Although the first clear description of a member of this group (Thorne, 1940) dealt with a parasite of the root-lesion nematode, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, ensuing investigations on this bacterial group have concentrated largely on bacteria parasitizing economically important root-knot nematodes of the genus *Meloidogyne*. For this reason, contemporary knowledge about *P. penetrans* pertains mainly to

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bacteria from root-knot nematodes. Moreover, the descriptive type material (Sayre and Starr, 1985) of *P. penetrans* is exclusively based on the bacteria from *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. According to the nomenclatural code used for bacteria (Lapage et al., 1975), the name is fixed by the type material. Hence, we have recommended, during the current period of taxonomic uncertainty, that the name *Pasteuria penetrans* (or its earlier synonyms, *Duboscqia penetrans* Thorne, 1940, or *Bacillus penetrans* Mankau, 1975) mean "member(s) of the *Pasteuria penetrans* group" (Sayre and Starr, 1988).

One objective of this study was to compare members of the *Pasteuria penetrans* group occurring in *Pratylenchus brachyurus* and *Meloidogyne incognita*. This comparison was facilitated by availability of Thorne's (1940) original material of *Duboscqia penetrans* on a root-lesion nematode deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland. In addition, fresh material from *Pratylenchus* and *Meloidogyne* was examined by light and electron microscopy. A second objective was to establish classificatory criteria to enable better definitions of the new taxa that will surely be delineated in the assemblage now constituting the *P. penetrans* group. Nomenclatural issues are treated separately (Starr and Sayre, 1988). To avoid cumbersome locutions and premature nomenclatural commitment, we refer here to the members of the *P. penetrans* group from *M. incognita* as the root-knot *Pasteuria* (abbreviated RKP) and to those from *P. brachyurus* as the root-lesion *Pasteuria* (abbreviated RLP).

Materials and Methods

Archival material

An important asset for this study was the availability of the preserved specimens studied by Thorne (1940) of the root-lesion nematode *Pratylenchus brachyurus* parasitized by the protozoan *Duboscqia penetrans*. Thorne had originally labeled the nematode in this archival material as *Pratylenchus pratensis*, but later relabeled it when he realized its correct identity. In current nomenclature, Thorne's protozoal name is equivalent to "member of the *Pasteuria penetrans* group." These specimens were originally found by Thorne (1940) in Florence, South Carolina in 1938 and in Perry, Georgia in 1939. This South Carolina RLP material, which was later deposited in the USDA Nematode Collection (slide numbers G-9031 to G-9039), was examined in detail in the present study. Those of Thorne's preparations found in good condition were remounted individually in glycerine on glass

slides and examined for the stages of the bacterium on and within the bodies of nematodes. Other specimens were selected from material submitted for identification to the USDA Nematode Collection by various colleagues over a period of years, or collected by one of the present authors (Table 1). Morphometric data were obtained by use of an eyepiece micrometer on a light microscope at highest magnification ($\times 1,300$) and then subjected to analysis of variance and Duncan's Multiple Range Tests (LeClerq, 1957). Photomicrographs of the stages of the bacterium were taken with an automatic 35-mm camera attached to a compound microscope fitted with an interference contrast system.

Fresh material

Pratylenchus brachyurus larvae and adults reared on the roots of peanut plants in greenhouse pot cultures were extracted from the roots using the method suggested by Chapman (1957). Nematodes parasitized or encumbered with the bacterial endospores of RLP were prepared for scanning electron microscopy by chemical fixation with 3.0% glutaraldehyde in 0.05 M phosphate buffer for 1.5 hr, followed by dehydration in an ethanol series. Thereafter, the preparations were subjected to critical-point drying and placed onto the surface of an aluminum stub. Stubs containing the dried specimens were coated with gold-palladium and then examined with a Hitachi HHS-2R, S430, or S530 scanning electron microscope operating at 15 or 20 kV.

Measurements of endospores from scanning electron micrographs were made either from anaglyphs of digitized images that were collected and viewed with a Kevex 8000 energy dispersive X-ray analyzer equipped with an image-analysis software program or from stereographic images that were viewed with a 4-mirror stereoscope having a floating light-point attachment. A 10° tilt was used to record the stereo images. Either the image-analysis program or the formula $Z = P/2M$ $\sin(Q/2)$, which is described in an earlier study (Wergin, 1985), was used to obtain the Z measurements.

For transmission electron microscopy, single nematodes parasitized by RLP were handpicked into a small droplet of water and placed in the wells of a microtitration plate. The wells were then filled with molten 3.0% water–agar at 50°C. When the agar solidified, the cores containing a larva were removed from the plate, placed in 3.0% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for 3 hr, washed in several changes of fresh buffer, postfixed in 2.0% osmium tetroxide for 2 hr, and dehydrated in an acetone series. Finally, the cores were infiltrated with a low-viscosity medium (Spurr, 1969). Silver-grey sections of selected nematodes were cut on a Sorvall model MT2 ultramicrotome using a diamond knife, and then mounted on uncoated copper grids (75 by 100 mesh). The sections were stained for 10 min with 2.0% aqueous uranyl acetate, and then for 5 min with 2.66% lead citrate. The stained thin sections were viewed with a Philips model 200 transmission electron microscope operating at 60 kV with 20-μm apertures.

No new material was prepared for RKP. Instead, the extensive archival collection of photomicrographic negatives of RKP, some of which have figured in our other publications (Sayre et al., 1983; Sayre and Starr, 1985), was drawn upon for comparison with the new

Table 1. Dimensions* and other information about specimens of members of the *Pasteuria penetrans* group, arranged in order of increasing sporangial size, and their host nematodes deposited in the USDA Nematode Collection, Beltsville, Maryland.

Nematode host	Sporangial diameter, μm (A) [STAT SIG]†	Central endospore diameter, μm (B)	Ratio A/B	Comments	Place and date of collection; USDA NC slide number	Reference or collector
Pratylenchus scribneri	2.8 ± 0.2 [a]	1.2 ± 0.2	2.3	Small sporangia; small endospores; *Meloidogyne javanica* also a host	Benton Co., AR 10-23-86 G-8956	E. J. Wehunt, first report this paper, Fig. 6
Pratylenchus brachyurus	3.1 ± 0.2 [a b]	1.5 ± 0.3	2.1	Thorne's (1940) original material of RLP	Florence, SC 8-10-38 G-9031– 9039	Thorne (1940), this paper, Figs. 2–4
Pratylenchus brachyurus	3.3 ± 0.2 [b c]	1.8 ± 0.1	1.8	Common reference standard for RLP	Jay, FL 1977, G-6882	E. Dutky (1978); R. A. Kinlock, this paper, Fig. 5
Pratylenchus scribneri	3.5 ± 0.2 [c]	1.7 ± 0.2	2.0	Fairly small sporangia; small endospores; *Meloidogyne javanica* also a host	Beltsville, MD 10-27-80 C-80-17; 8-20-85 G-8480	A. M. Golden, this paper, Fig. 7
Tylenchorhynchus maximus	4.2 ± 0.4 [d]	2.1 ± 0.3	2.0	Relatively large endospores in medium sporangia; large nematode host	Beltsville, MD C-75-2 6-6-79; G-5938	A. M. Golden, this paper, Figs. 12–15
Heterodera goettingiana	4.2 ± 0.5 [d]	1.8 ± 0.2	2.3	Sporangia in cysts; may be attracted to adult males	Germany 11-21-83 G-7799	Sturhan (1985)
Xiphinema americanum	4.2 ± 0.4 [d]	1.6 ± 0.2	2.6	Small endospores in medium sporangia	Ithaca, NY 12-10-85 G-8848	Jaffee et al. (1985)
Xiphinema rivesi	4.3 ± 0.4 [d e]	1.4 ± 0.3	3.0	Small endospores in medium sporangia	Crawford Co., AR 10-31-86 G-8977	E. J. Wehunt, this paper, Fig. 21
Meloidogyne incognita	4.4 ± 0.6 [e f]	1.8 ± 0.1	2.4	Common reference standard for RKP	Beltsville, MD 1977	E. Dutky (1978)
Dolichodorus obtusus	4.6 ± 0.2 [e f]	2.4 ± 0.1	2.0	Relatively large endospores in fairly large sporangia	Napa Co., CA 3-15-51 T-258p	Allen (1957), this paper, Figs. 16–19
Xiphinema americanum	4.7 ± 0.3 [f]	1.4 ± 0.3	3.4	Small endospores in fairly large sporangia	Crawford Co., AR 10-31-86 G-8978	E. J. Wehunt, this paper, Fig. 20
Hoplolaimus galeatus	6.5 ± 0.1 [g]	3.0 ± 0.1	2.2	Very large endospores (encircled by "double rings") in very large sporangia	Biglerville, PA 1985; G-7834	Jaffee et al. (1985)
Hoplolaimus galeatus	7.0 ± 0.3 [g]	3.2 ± 0.3	2.2	Very large endospores (encircled by "double rings") in very large sporangia	Florida 7-22-69 G-2540	Jaffee et al. (1985), this paper, Figs. 8–11

* Dimensions are based on examination by interference-contrast light microscopy of specimens mounted in glycerol. Other methods of specimen preparation, such as those used for scanning and transmission electron microscopy, yield different apparent dimensions (see Starr and Sayre, 1988). Each value is the mean of 15 measurements ± 1 standard deviation.

† STAT SIG means statistical significance. For example, a mean value followed by the letter "a" in square brackets is significantly different from a mean value not having the "a"; those mean values having the letter "g" are significantly different from those not having the "g" after the mean value, and so on.

RLP preparations. The preparative methods for these RKP specimens were identical to the ones described here for RLP.

Results

Light microscopy of archival specimens

Specimens of various plant-parasitic nematodes parasitized by members of the *Patseuria penetrans* group have been deposited in the USDA Nematode Collection in Beltsville, Maryland (Table 1). Fortuitously, some of these specimens, including Thorne's (1940) archival material from *Pratylenchus* (Figs. 1–4), were retrievable for examination by light microscopy, even after decades of storage. Unfortunately, none provided samples suitable for examination by electron microscopy.

Distinct morphological differences exist among the members of the *Pasteuria penetrans* group found parasitizing the 13 nematode specimens from the Beltsville collection (Table 1). In particular, the morphometric data on the average mean diameters of the bacterial sporangia were statistically different by the F-test of the analysis of variance. Application of Duncan's Multiple Range Test (LeClerq, 1957) separated the 13 specimens into 3 distinct subgroups (Table 1). The smallest sporangial-size subgroup (2.8–3.5 μm in diameter) encompassed the 4 *Pratylenchus* specimens (Table 1; Figs. 2–7). The largest sporangial-size subgroup (6.5–7.0 μm in diameter) is represented by the 2 specimens of *Hoplolaimus galeatus* (Figs. 8–10). An intermediate sporangial-size subgroup (4.2–4.7 μm in diameter) contained 7 nematode specimens that differ widely in their taxonomy and their relationships to host plants.

No consistent correlation exists between size of the bacterial sporangia and the size of the host nematode. For example, *Tylenchorhynchus maximus* is an exceptionally large plant-parasitic nematode, but the bacterial sporangia found in it were not particularly large (Figs. 12–15). Nor does the size of the central endospore correlate consistently with the size of the sporangium. For example, the bacterial parasite of *Dolichodorus obtusus* has a relatively large central endospore (2.4 μm) within a moderately sized sporangium; this feature separated this specimen from the others in the intermediate sporangial-size subgroup (Figs. 16–19). Another example is the case of 3 specimens in the intermediate sporan-
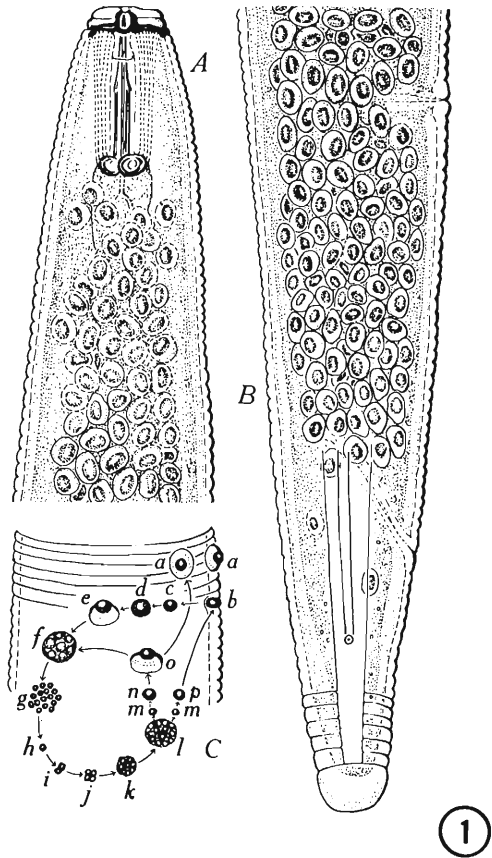

Figure 1. Drawing of anterior (A) and posterior (B) portions of *Pratylenchus pratensis* parasitized by *Duboscqia penetrans*. (C.) Drawing of probable life cycle of *Duboscqia penetrans* (labeled in Thorne's protozoological terminology). Labels: a, spores attached to cuticle; b, sporoblast passing through the cuticle; c, d, maturing sporoblast; e, mature spore; f, schizogony of spore; g, 16 sporonts formed during schizogony; h–l, 1 of the 16 sporonts undergoing repeated binary fission to form 16 sporoblasts; m, 2 of the 16 small sporoblasts; n, o, development of sporoblast into spore from which the life cycle is repeated within the nematode body but if liberated into soil it attaches to body as in a; p, developing sporoblast liberated into soil where it will attack and enter into another nematode as shown in b. ×1,200. (After Thorne, 1940. Courtesy of the Proceedings of the Helminthological Society of Washington.) Notes: The nematode host species was later identified by Thorne as *Pratylenchus brachyurus* rather than *P. pratensis*. As summarized in the text, the presumptive protozoan *Duboscqia penetrans* is actually a member of the *Pasteuria penetrans* group of mycelial and endospore-forming bacteria.

gial-size subgroup that parasitize *Xiphinema* spp.; the medium-sized bacterial sporangia in these hosts have relatively small endospores with a diameter of 1.4–1.6 μm (Figs. 20, 21). This general lack of correlation is clearly demonstrated in Table 1; note particularly the column headed "Ratio A/B" (the ratio of sporangial diameter over endosporal diameter), which shows that the mean diameters of the central endospores varied independently of the size or diameter of its sporangia. This ratio ranged from 1.8 in the small bacterial parasite of *Pratylenchus* to 3.4 in the medium-sized sporangia found in *Xiphinema* spp.

The distribution of sporangia tended to be similar within all the nematodes examined. Generally, sporangia were not abundant in the anterior or posterior regions of the nematode hosts until very late in the nematodes' developmental cycles. Often, esophageal structures were visible (Figs. 1, 6), and the musculature surrounding anus and vulva persisted, even though sporangia had already filled the abdominal region of the nematode (Figs. 1, 4, 9).

Attachments were observed of the endospores to the cuticular surfaces of the juvenile stages of several nematodes (Figs. 4, 7, 10–15, 17–19) and in adult males of *Heterodera goettingiana* (not

figured here). Penetration of the nematode cuticle by the germinating bacterial endospore was observed in *Dolichodorus obtusus* (Fig. 18).
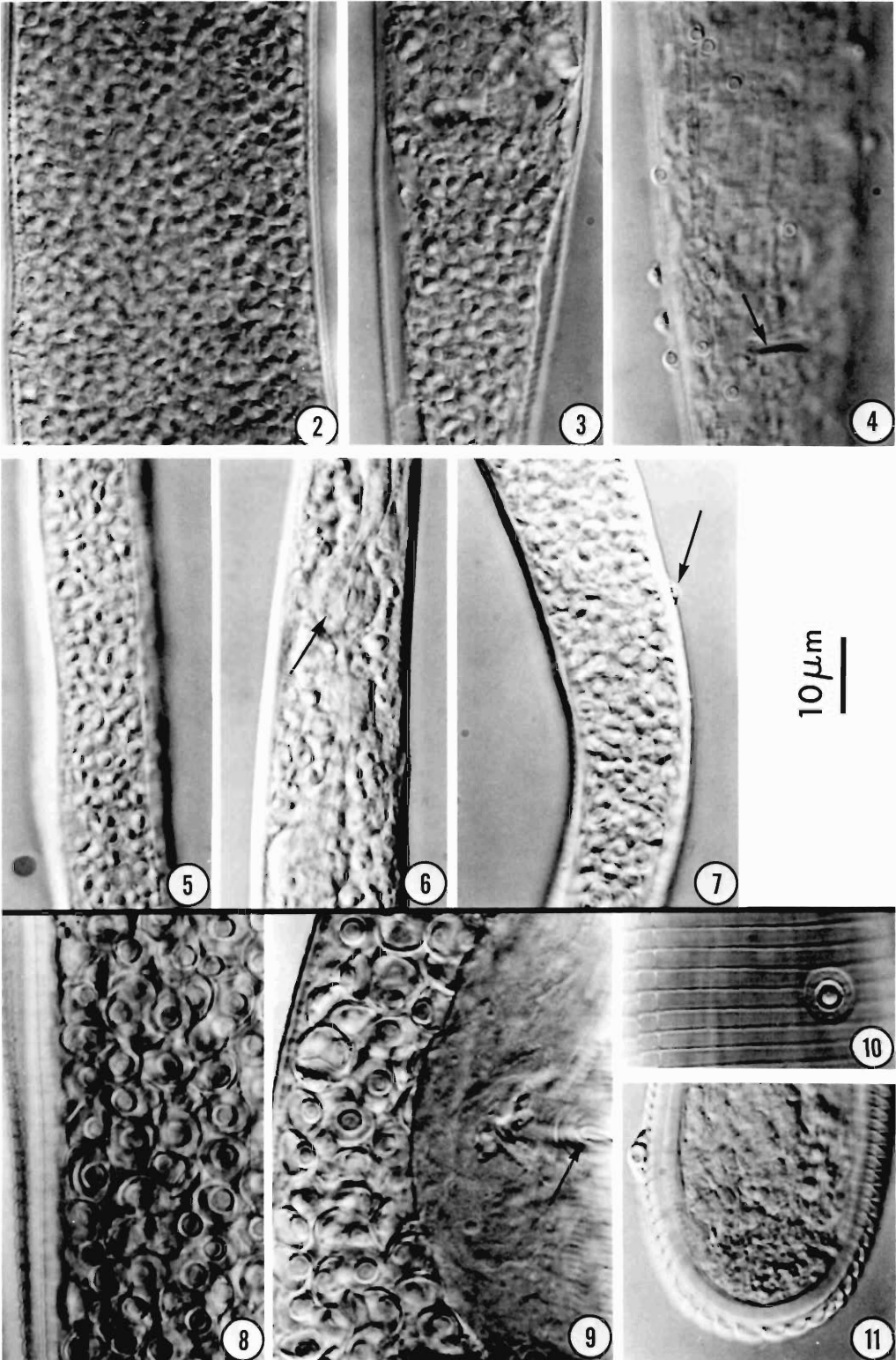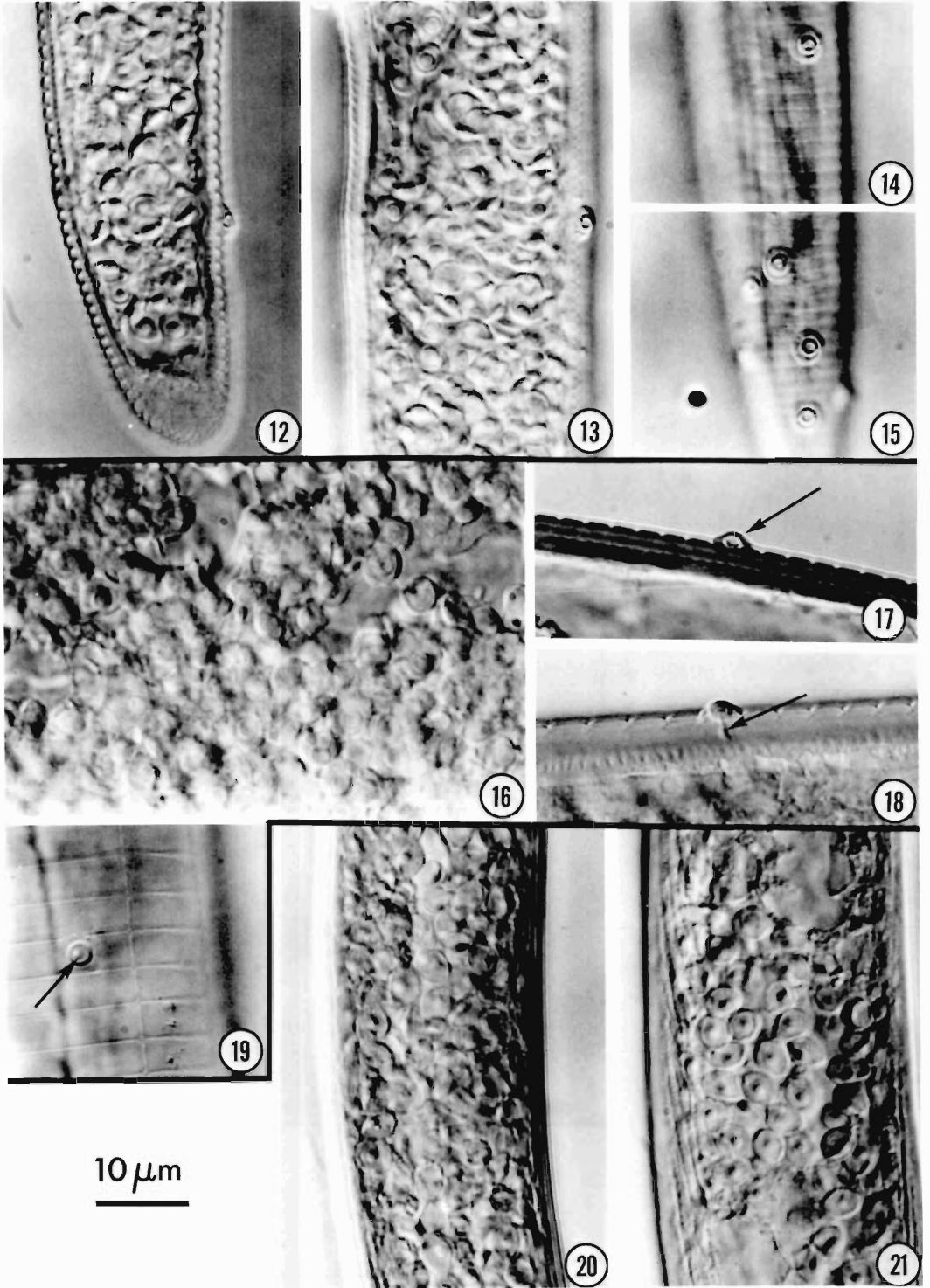
Scanning electron microscopy

Mature endospores with associated parasporal structures and sporangial remnants of members of the *Pasteuria penetrans* group from root-knot nematodes (RKP) were found adhering to the surfaces of *Meloidogyne incognita* (Fig. 22); the entire sporal unit measured about 3.5 μm in diameter in these scanning electron micrographs. Two distinct forms of these RKP endospores have been observed (Sayre and Starr, 1985). In one form, the surface appeared to be covered by a wrinkled membrane, presumably the remnants of the sporangium, which encompassed the entire endospore but could become sloughed. When sloughing occurred, the exposed surface can be resolved into 2 distinct components: a central body, the endospore itself, averaging 1.42 μm in diameter; and a peripheral matrix (parasporal fibers and remnants of sporangium), 1.0 μm wide, that surrounds the endospore proper (Fig. 22). In the case of members of the *P. penetrans* group from the root-lesion nematodes (RLP), the endospore and related structures, after attachment

$\longrightarrow$

Figures 2–11. Photomicrographs of specimens belonging to different nematode genera and species infected with sporangia or encumbered with endospores of members of the *Pasteuria penetrans* group. All figures at ×1,000. 2. Sporangia fill the intestinal region of *Pratylenchus brachyurus* in a specimen collected from peanuts, South Carolina. 3. Sporangia fill the vulvar region of this specimen of *P. brachyurus* collected by Thorne. 4. Endospores encumbering the cuticular area and surrounding the anus (arrow) in a specimen of *P. brachyurus* collected by Thorne, South Carolina. 5. Sporangia in *P. brachyurus* collected from peanuts, Maryland. 6. A few sporangia about the median bulb (arrow) in the esophageal region of *Pratylenchus scribneri* from peach roots, Arkansas. 7. Attached endospore (arrow) and sporangia in the intestinal region of *P. scribneri* collected from roots of river birch, Beltsville, Maryland. 8. Numerous sporangia in the midregion of *Hoplolaimus galeatus* from peach, Florida. 9. Numerous sporangia near vulva (arrow), but not attacking vaginal muscle tissues, of *H. galeatus*. 10. Polar view of a single circular endospore, showing a double-ring configuration, attached to *H. galeatus*. 11. Endospore attached to the posterior of *H. galeatus*, showing also a few internal sporangia.

Figures 12–21. Photomicrographs of specimens belonging to different nematode genera and species infected with sporangia or encumbered with endospores of members of the *Pasteuria penetrans* group. All figures at ×1,000. 12. Endospore attached to posterior of section of *Tylenchorhynchus maximus*, showing also numerous internal sporangia. 13. Midbody of *T. maximus* with an attached endospore and numerous internal sporangia. 14. Polar view of a single attached endospore on the cuticular surface of *T. maximus*. 15. Multiple attachment of endospores to *T. maximus*. 16. Numerous sporangia in the midbody region of *Dolichodorus obtusus* (type specimen) from manzanita, California. 17. Lateral view of an endospore (arrow) attached to *D. obtusus*. 18. Penetration structure of the germinating endospore (arrow) entering the cuticular layers of *D. obtusus*. 19. Polar view of an endospore, with an eccentric spore configuration, attached to the cuticular surface of *D. obtusus*. 20. Many medium-sized sporangia enclosing relatively small endospores in *Xiphinema americanum* from peach, New York. 21. Numerous medium-sized sporangia, with small internal endospores, in *Xiphinema rivesi* from river birch.

Figures 22, 23. Scanning electron micrographs of endospores of RKP and RLP members of the *Pasteuria penetrans* group. 22. An endospore of RKP attached near the lateral field (LF) of a larva of *Meloidogyne incognita*. 23. An endospore of RLP attached to the lateral field (LF) of *Pratylenchus brachyurus*. Bars = 0.5 μm.

10 μm
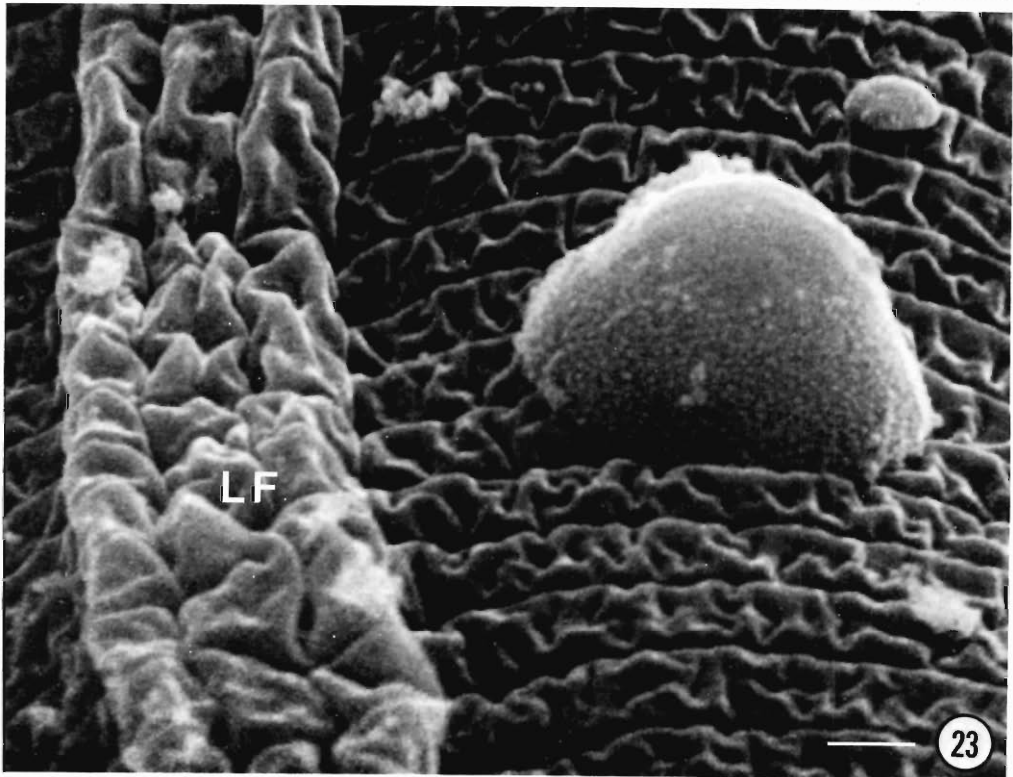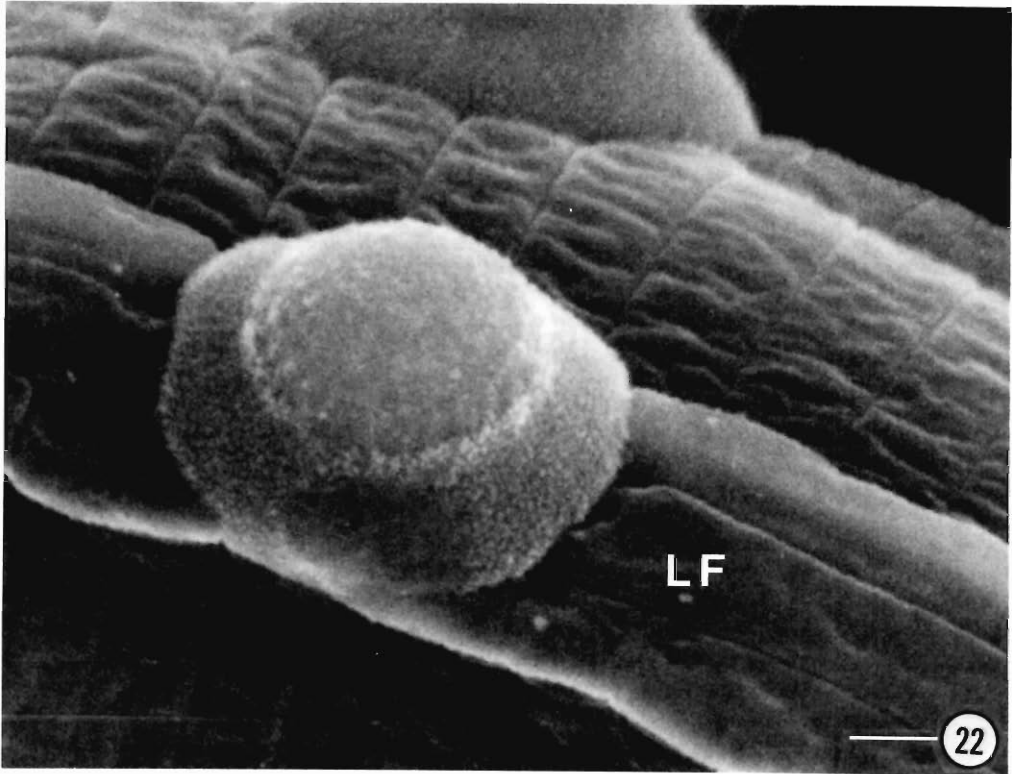
to the surface of a *Pratylenchus* larva, measured 2.27 μm in diameter and approximately 1.63 μm in height in such scanning electron micrographs (Fig. 23). Wrinkled exosporial coverings were not observed on RLP endospores. The central body, the endospore proper, in RLP was 1.6 μm in diameter; the peripheral matrix measured approximately 0.6 μm in width (Fig. 23).

Transmission electron microscopy—vegetative growth

Germination of the RLP endospore and penetration of the nematode cuticle have not yet been observed; therefore, comparison is not yet possible with the early stages of development in RKP (Sayre and Starr, 1985, 1988). Hyphal colonies were the first observed vegetative growth stages of RLP in the pseudocoelom of *Pratylenchus brachyurus* (Figs. 24, 26). Several developmental stages (vegetative mycelium, early and late stages of endospore formation, and mature sporangia) of RLP occurred simultaneously in the thin sections of this nematode host. The comparable RKP material is depicted in Figure 25.

The dichotomously branching hyphal colonies of RLP were septate, bounded by a compound wall 0.041 ± 0.006 μm in thickness (mean of 20 measurements, ± one standard deviation), and composed of outer and inner membranes (Fig. 26). The inner membrane entered into formation of septations and delineated individual cells. Unlike the situation in RKP (Fig. 25), where mesosomes were frequently found associated with the septa, mesosomes were not observed in the thin sections of RLP vegetative growth. Under the conditions of our study, it was impossible to determine the size of the RLP microcolonies or their general shape.

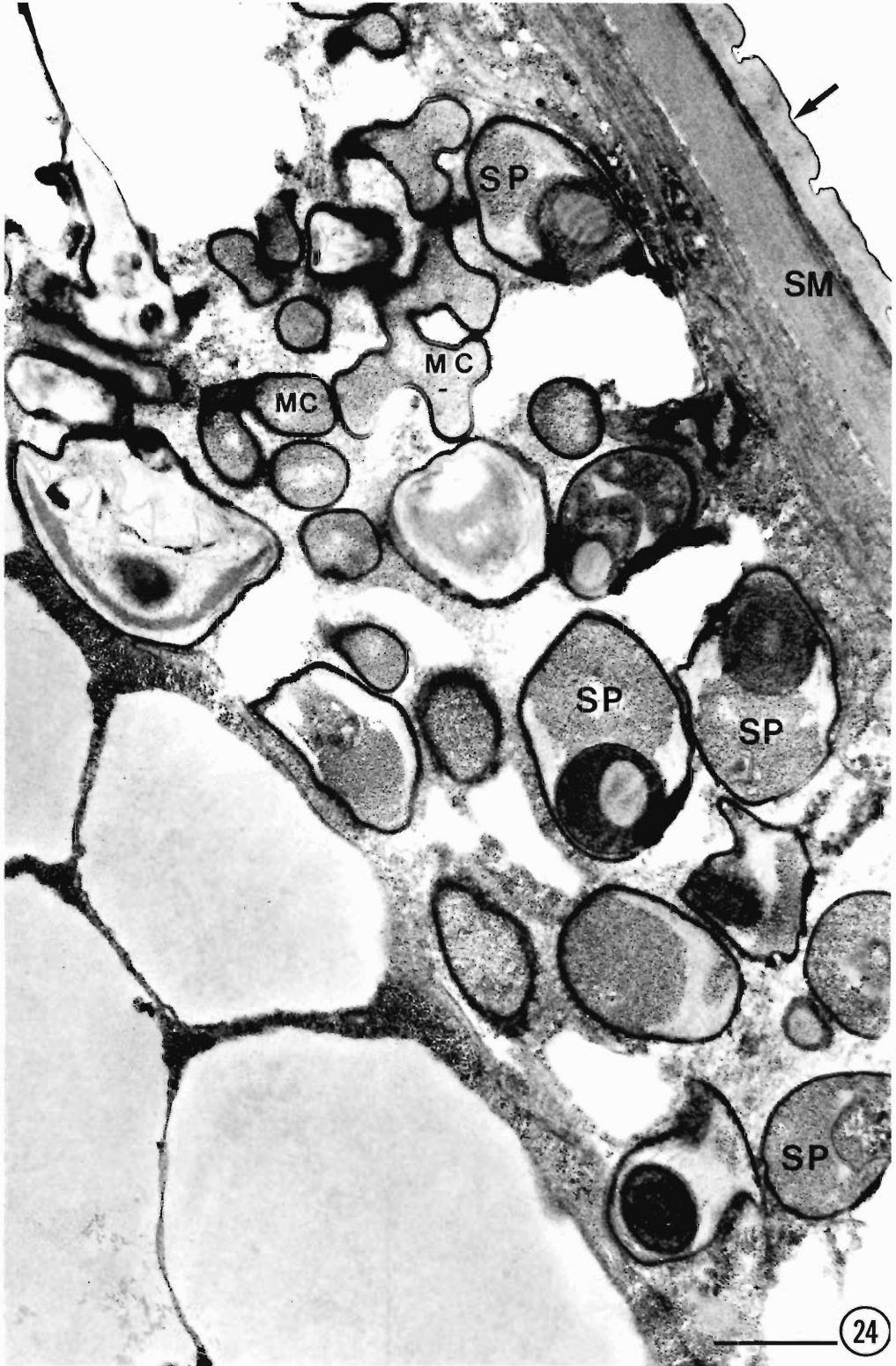## Transmission electron microscopy—endospore formation

The sporulation process in both RKP and RLP involved the terminal filamentous cells of the microcolonies. In both organisms, these terminal cells enlarged and became more ovate (Figs. 27, 28). The cytoplasts of the ovate cells changed from a granular matrix of many ribosomes to cytoplasts having well-defined organelles. During the early alterations, transverse membranes formed within the developing sporangia. These membranes separated the upper ⅓ of a sporangium, the developing forespore, from the lower parasporal portion (Figs. 29, 30). In both RKP and RLP, the upper portion condensed into an electron-dense central core that became encircled by multilayered walls and eventually formed the mature endospore (Figs. 31, 32).

The most noticeable morphological differences between RKP and RLP in the later stages of endosporogenesis were the thickness of the multilayered walls and the general shape of the central bodies or developing endospores. In RKP, the developing endospore was ellipsoidal (broadly elliptic in section) with the major axis horizontal to the base of the sporangium and measuring 1.30–1.54 μm; the minor axis measures 0.99–1.21 μm; the endospore has a relatively thick wall measuring about 0.28–0.34 μm in width. In RLP, the developing endospore is ellipsoidal or nearly spherical (narrowly elliptic in section), with axes of 0.96–1.20 by 1.15–1.43 μm; the wall is much thinner than in RKP, measuring about

→

Figure 24. Transmission electron micrograph of a longitudinal section of *Pratylenchus brachyurus*, showing the cuticle (arrow) and somatic muscles (SM) of the nematode and mycelial colonies (MC) and sporangial stages (SP) of an RLP member of the *Pasteuria penetrans* group. Note the simultaneous occurrence of all life stages of the bacterium in the nematode. Bar = 1.0 μm.

Figures 25, 26. Transmission electron micrographs of vegetative stages of RKP and RLP members of the *Pasteuria penetrans* group. 25. In this RKP from *Meloidogyne incognita*, septa (S) delineate cells and mesosomes (M) are often associated with the cell division membranes. A "sacrificial" intercalary cell (arrow) has lysed, permitting daughter colonies to be formed. 26. Vegetative stage of an RLP from *Pratylenchus brachyurus*. Both septa and mesosomes were absent from this bifurcating (arrow) mycelial colony. Bars = 0.5 μm.

Figures 27, 28. Transmission electron micrograph of the swollen hyphal tips of microcolonies of RKP and RLP members of the *Pasteuria penetrans* group. 27. A "sacrificial" intercalary cell (arrow) of RKP from *Meloidogyne incognita* lyses allowing for eventual separation of the swollen mycelial tips that become sporangia. 28. A swollen hyphal tip in a microcolony of an RLP member of the *Pasteuria penetrans* group from *Pratylenchus brachyurus*. A septum (S) separates this terminal cell that will become a sporangium. Bars = 0.5 μm.
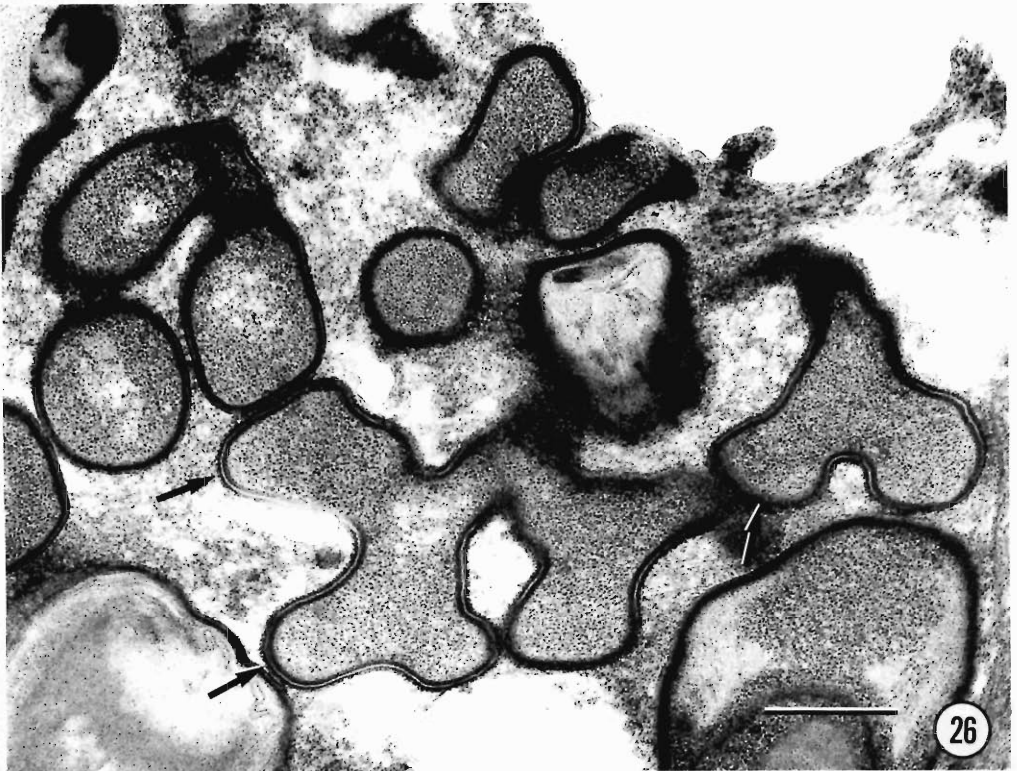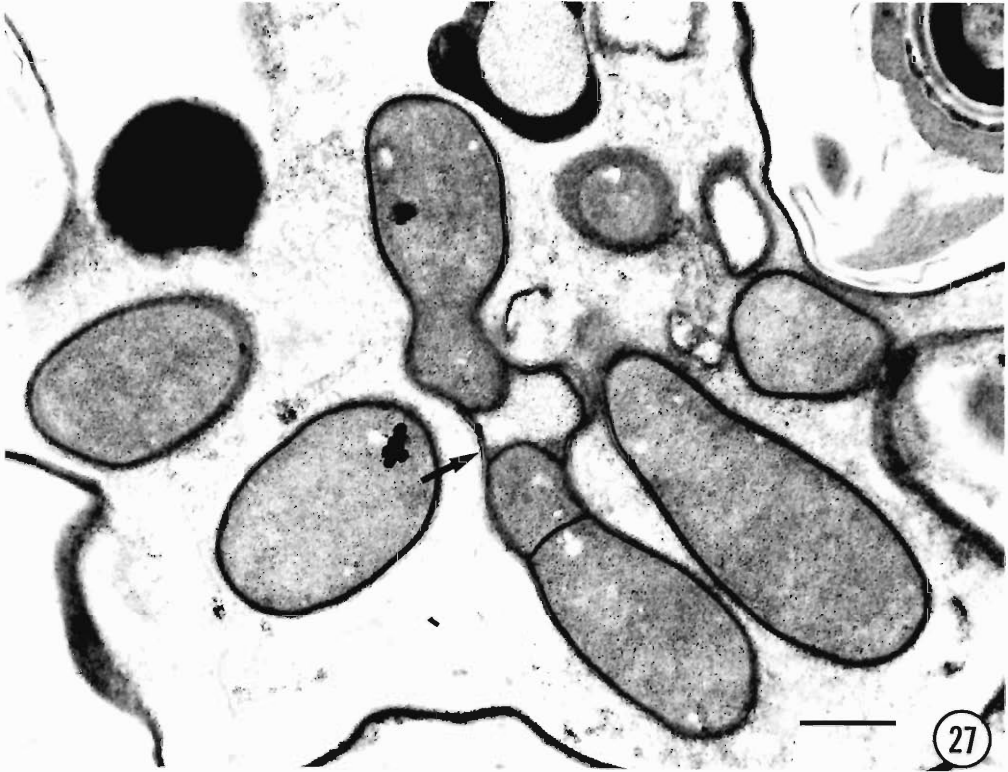
0.22–0.26 μm in width. These and other differences become more pronounced in the mature sporangia and endospores (Figs. 33–36).

Transmission electron microscopy — mature sporangia

The sporangial walls, double membranes about 0.042 ± 0.0055 μm thick (mean of 20 measurements, ±1 standard deviation), appear to be similar in dimensions in RKP and RLP. In RKP, the basal portion of the sporangium became largely devoid of its granular matrix and the sporangial wall collapsed upward toward the base of the mature endospore. In the basal or parasporal region of RLP, the differentiated fibrous strands intermingle with the electron-dense granular materials. Consequently, the sporangia of RLP retained their rhomboidal shape (Figs. 37–40).

The parasporal fibers of RKP and RLP, attached to the cortical walls of the developing endospores, were similar in thickness. However, fewer fibers occurred in RLP than in RKP, and these were attached at a much sharper downward angle with respect to the wall of its endospore. The mature RKP endospore is ellipsoidal (broadly elliptic in section), with axes measuring 0.99–1.21 by 1.30–1.54 μm. Its wall was 0.28–0.34 μm thick, with a pore at the base having a diameter of 0.28 ± 0.11 μm. In RLP, the outer cortical wall at the equator of the endospore measured 0.22–0.26 μm in thickness; it became progressively thinner until, at the base of the endospore, it tapered to an opening about 0.13 ± 0.01 μm in diameter. The axes of the ellipsoidal to almost spherical mature RLP endospore were 0.96–1.20 by 1.15–1.43 μm.

In RKP, there is a more electron-dense inner cortical wall of irregular thickness that surrounds the endospores only laterally (Fig. 16). In RLP (Fig. 17), the inner cortical wall surrounds the endospore only at the sublateral positions; con-

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Figures 29–32. Transmission electron micrographs of cross sections of early sporangia in RKP and RLP members of the *Pasteuria penetrans* group. 29. In this early RKP sporangium from *Meloidogyne incognita,* a septum (S) divides the upper portion that will ultimately contain the endospore from the lower portion that will become vacuolated and collapse upon the developing endospore. 30. In an early sporangium of RLP from *Pratylenchus brachyurus,* a septum (S) delineates the apical portion, which condenses into the endospore, from the parasporal portion, which develops into the adhesive attachment fibrils. 31. In a more mature RKP sporangium from *M. incognita,* the apical portion shows the condensed forespore surrounded by a membrane. The less dense region (arrows) will become the parasporal attachment fibers. 32. In this RLP from *P. brachyurus,* the apical portion, which contains the condensed forespore, is surrounded by a membrane. The less dense areas will become the parasporal attachment fibers. Bars = 0.5 μm.

Figures 33–36. Transmission electron micrographs of late sporangial stages of RKP and RLP members of the *Pasteuria penetrans* group. 33. In this RKP from *Meloidogyne incognita,* the developing endospore is surrounded by thin cortical walls. The area of parasporal fiber formation has expanded downward into the basal portion of the sporangium. The basal portion is a matrix of fine granules. 34. RLP sporangium from *Pratylenchus brachyurus* with developing endospore surrounded by a thin cortical wall (C). The parasporal fibers extend into the basal portion, which is largely filled by a fine granular matrix. A mesosome (M) persists at the point of separation of the sporangium from the microcolony. 35. In the development of an RKP sporangium from *M. incognita,* the old sporangial wall is loosened from the almost mature endospore. The basal portion is made up of large granules and widening spaces. The sporangial wall has begun to fold. 36. In the development of an RLP sporangium from *P. brachyurus,* the endospore is surrounded by thin cortical walls. Individual parasporal fibers are evident. The parasporal basal matrix remains as fine granules; the sporangial wall is rigid. Bars = 0.5 μm.

Figures 37–40. Cross sections showing lateral and polar views of the mature endospores of RKP and RLP members of the *Pasteuria penetrans* group. 37. In this lateral view of RKP from *Meloidogyne incognita,* the endospore is surrounded externally by a loose and collapsing sporangial wall. Between the sporangial wall and the endospore is a very thin inner exosporium (arrow) covering the parasporal fibers and the endospore. A basal pore has been formed by the cortical wall. 38. Lateral view of a mature RLP sporangium from *Pratylenchus brachyurus,* showing inner sublateral cortical wall (arrows) that gives the endospore a triangular appearance. The basal cortical wall of the endospore thins to provide a germinal pore. The basal portion of the sporangium contains an irregular granular matrix intermingled with fibrillar strands. 39. Polar view of RKP from *M. incognita.* The old sporangial wall (SP) is convoluted and has collapsed inward. The central endospore is surrounded by its cortical walls (CW) and parasporal attachment fibers (arrow). 40. Polar view of RLP from *P. brachyurus.* The central endospore is surrounded by a thin cortical wall (CW) and fine parasporal attachment fibers (arrow). Bars = 0.5 μm.
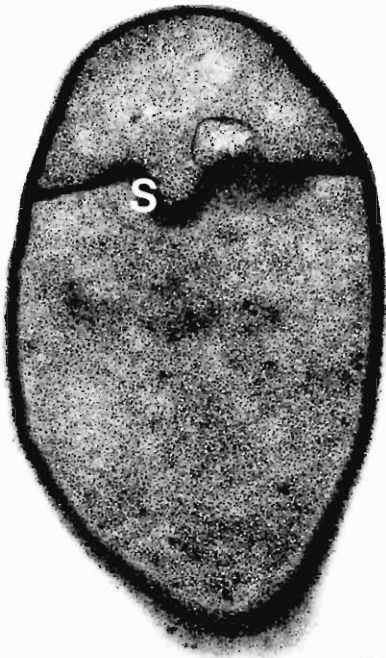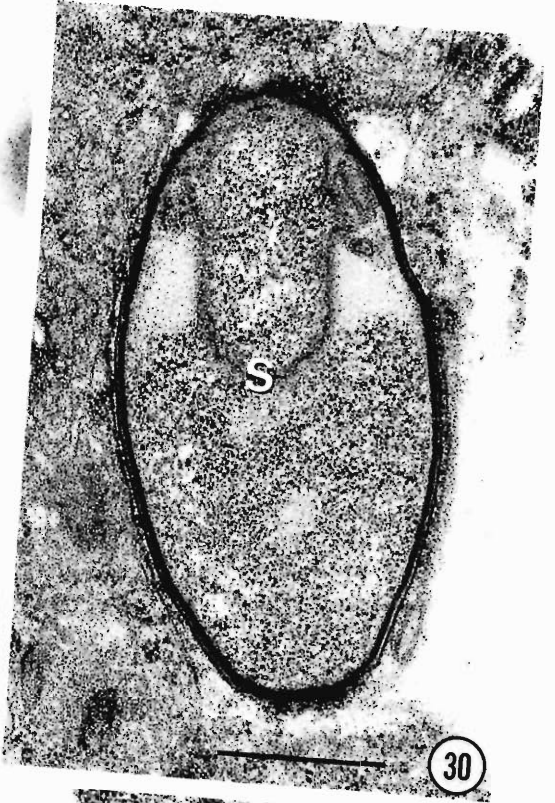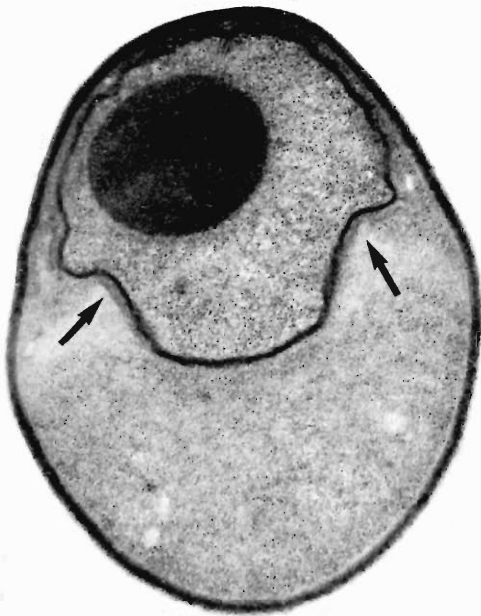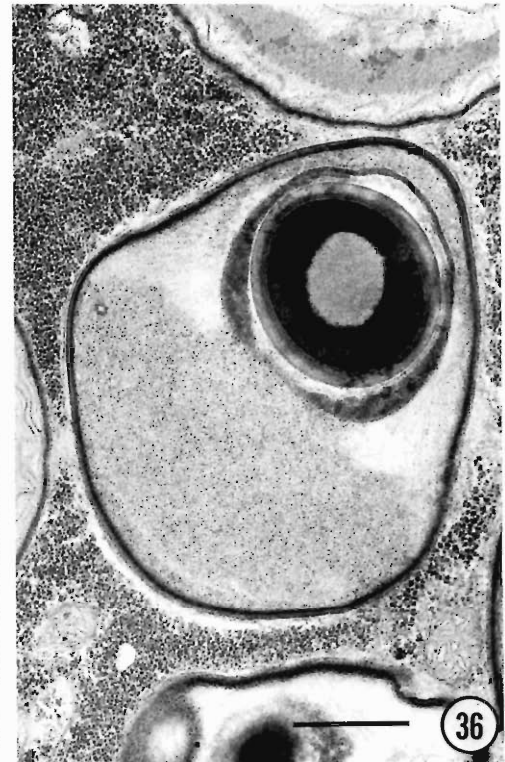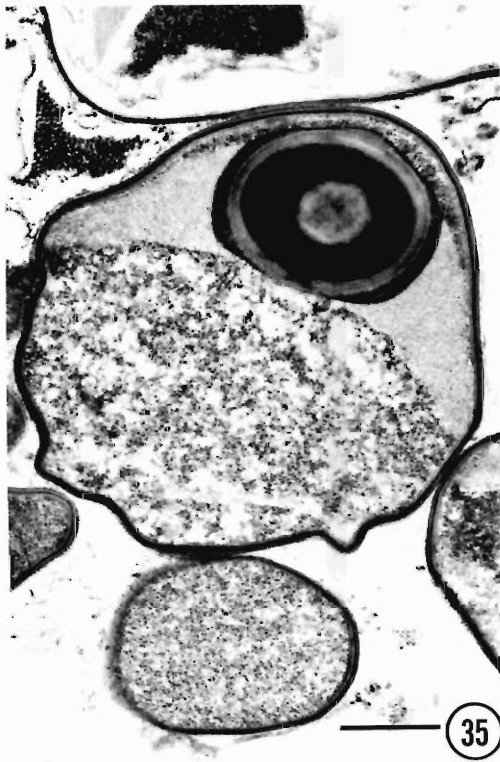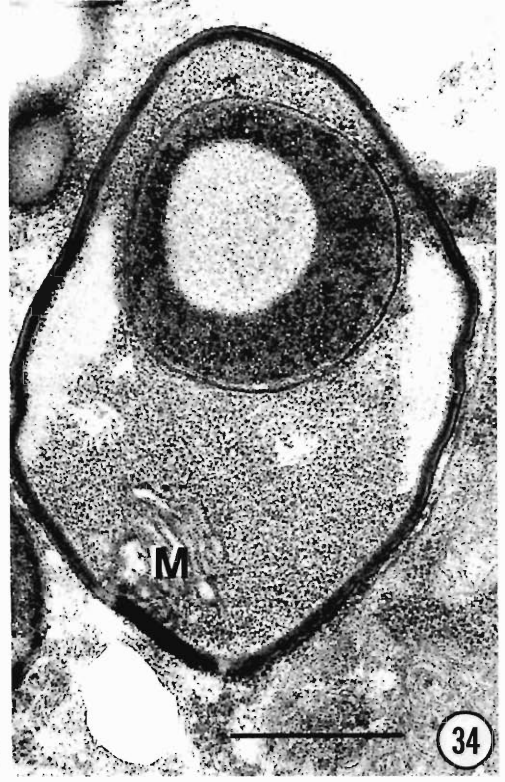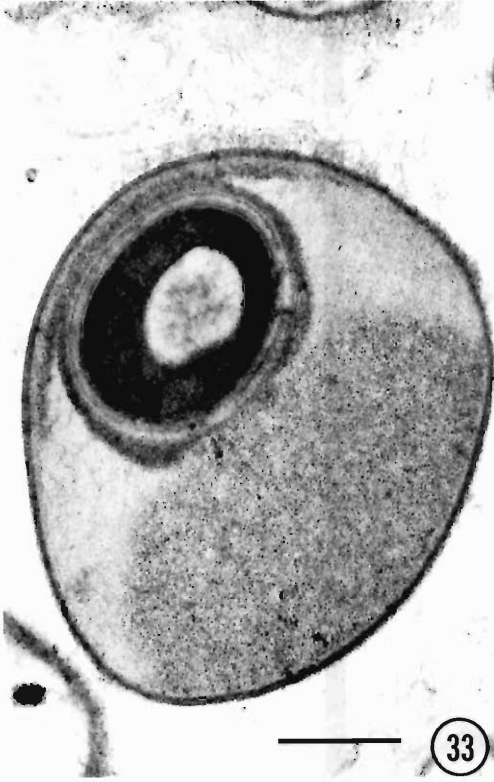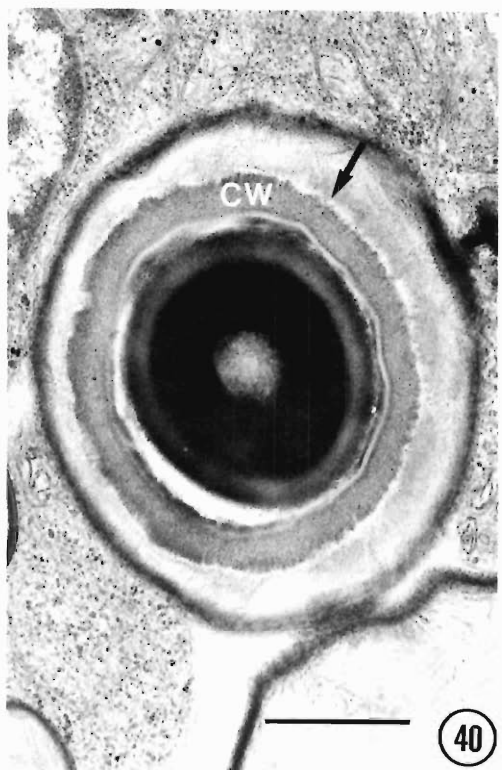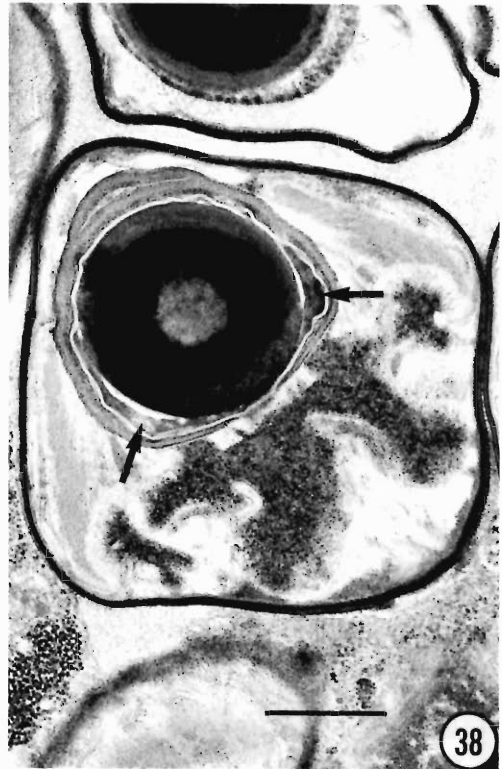
Table 2. Comparative characteristics of RLP and RKP members of the *Pasteuria penetrans* group occurring on the root-lesion nematode, *Pratylenchus brachyurus,* and on the root-knot nematode, *Meloidogyne incognita.* The given dimensions are based on electron micrographs; somewhat different, usually larger values might be obtained from light-microscopic examination.

Trait	Bacterium (RLP) from *Pratylenchus*	Bacterium (RKP) from *Meloidogyne*
Microcolonies	Elongate clusters; few mycelial strands	Spherical, to clusters of elongated grapes
Sporangium		
Shape	Rhomboid	Cup-shaped
Diameter		
Range (μm)	2.22–2.70	3.0–3.9
Mean (μm)	2.40 ± 0.24*	3.42 ± 0.18*
Height		
Range (μm)	1.96–2.34	2.26–2.60
Mean (μm)	2.15 ± 0.19*	2.48 ± 0.22*
Endospore		
Shape	Ellipsoidal or almost spherical, narrowly elliptic in section, with long axis horizontal	Ellipsoidal, broadly elliptic in section, with long axis horizontal
Size		
Range (μm)	0.96–1.20 by 1.15–1.43	0.99–1.21 by 1.30–1.54
Mean (μm)	1.08 ± 0.21 by 1.29 ± 0.14*	1.10 ± 0.11 by 1.42 ± 0.12*
Spore pore		
Formation	Basal cortical wall thins to expose inner spore walls	Basal annual opening formed from thickened outer cortical walls
Diameter (μm)	0.13 ± 0.01†	0.28 ± 0.11*
Spore cortical walls		
Thickness (μm)	0.25 ± 0.035‡	0.31 ± 0.32‡
Inner wall	Partial wall; only sublaterally surrounds endospore	Wall irregular in thickness; only laterally surrounds endospore; absent at poles
Host nematode	*Pratylenchus brachyurus*	*Meloidogyne incognita*

* Mean of 50 measurements ± 1 standard deviation.
† Mean of 12 measurements ± 1 standard deviation.
‡ Mean of 20 measurements ± 1 standard deviation.

sequently, the RLP endospores with walls appeared triangular in cross section.

Discussion

During the past 4 decades, members of the *Pasteuria penetrans* group of endospore-forming and mycelial bacteria have been reported as parasites of many kinds of nematodes from widely separated geographical areas (Sayre and Starr, 1985, 1988). Until recently, these bacteria were known by several other names, among them *Duboscqia penetrans* and *Bacillus penetrans.* We have examined representative archival material from the USDA Nematode Collection, consisting of 13 nematode specimens bearing such bacterial parasites. Fortunately, these specimens included the parasitized *Pratylenchus brachyurus* material used by Thorne (1940) in preparing the first thorough description of this nematode parasite. This preserved material was similar in all observable traits to the populations of these bacteria currently found as parasites of *P. brachyurus* in Florida and Maryland. Examination of the archival material by light microscopy revealed 3 sporangial-size subgroups, confirming the inherent diversity in this assemblage of bacteria. In general, sporangial size varies independently of endospore size and size of the host nematode.

Until very late in the interaction, the bacterial sporangia do not overrun the anterior feeding apparatus of the nematode nor do they destroy the musculature about the anal and vulvar openings. This localization might spare the nematode from a premature death; perhaps the reprieve is long enough in some cases to allow the nematode to complete its life cycle.

Table 3. Reported "host specificity" of members of the *Pasteuria penetrans* group, generally as scored by attachment of bacterial endospores to cuticles of nematode larvae or, sometimes, by observation of numerous endospores within the nematode's body. Associations with the homologous nematode species or variety were sometimes more frequent than with heterologous nematodes.

Nematode from which *Pasteuria penetrans* endospores originated	Attempted attachments or infections by endospores with these nematodes	Endo-spores observed (+) or not (−)*	Authority and reference
Meloidogyne javanica	*Aphelenchoides* sp.	−	Mankau and Prasad (1977)
	Aphelenchus avaenae	−	
	Aporcelaimus sp.	−	
	Ditylenchus dipsaci	−	
	Heterodera schachtii	−	
	Meloidogyne arenaria	+	
	Meloidogyne hapla	+	
	Meloidogyne incognita	+	
	Meloidogyne javanica	+	
	Pratylenchus brachyurus	−	
	Pratylenchus scribneri	+	
	Pratylenchus vulnus	−?	
	Trichodorus christiei	−	
	Tylenchorhynchus claytoni	−	
	Xiphinema index	−	
Meloidogyne incognita	*Aphelenchoides ritzemabosi*	−	Dutky and Sayre (1978)
	Ditylenchus dipsaci	−	
	Ditylenchus triformis	−	
	Meloidogyne hapla	+	
	Meloidogyne incognita	+	
	Meloidogyne javanica	+	
	Meloidoderita sp.	−	
	Pratylenchus brachyurus	−	
	Pratylenchus penetrans	−	
	Tylenchorhynchus claytoni	−	
Pratylenchus brachyurus	*Aphelenchoides ritzemabosi*	−	Dutky and Sayre (1978)
	Ditylenchus dipsaci	−	
	Ditylenchus triformis	−	
	Meloidogyne hapla	−	
	Meloidogyne incognita	−	
	Meloidogyne javanica	−	
	Meloidoderita sp.	−	
	Pratylenchus brachyurus	+	
	Pratylenchus penetrans	−	
	Tylenchorhynchus claytoni	−	
Meloidogyne incognita acrita	*Meloidogyne arenaria*	+	Slana and Sayre (1981)
	Meloidogyne grahami	+	
	Meloidogyne hapla	+	
	Meloidogyne incognita acrita	+	
	Meloidogyne incognita incognita	+	
	Meloidogyne javanica		
Meloidogyne incognita	*Meloidogyne arenaria*	+	Brown and Smart (1985)
	Meloidogyne incognita	+	
	Meloidogyne javanica	+	
Meloidogyne javanica	*Meloidogyne hapla*	−?	Stirling (1985)
	Meloidogyne incognita	−?	
	Meloidogyne javanica	+	
Meloidogyne incognita	*Aphelenchoides* sp.	−	Nishizawa (1984, 1986)
	Aphelenchus sp.	−	
	Helicotylenchus sp.	−	

Table 3. Continued.

Nematode from which *Pasteuria penetrans* endospores originated	Attempted attachments or infections by endospores with these nematodes	Endo-spores observed (+) or not (−)*	Authority and reference
	Heterodera elachista	−	
	Heterodera glycines	−	
	Meloidogyne hapla	+	
	Meloidogyne incognita	+	
	Meloidogyne javanica	+	
	Paratrichodorus porosus	−	
	Pratylenchus coffeae	−	
	Pratylenchus penetrans	−?	
	Pratylenchus vulnus	−	
	Tylenchulus semipenetrans	−	
	Tylenchus sp.	−	
	Various saprophagous nematodes	−	

* Symbols: + = associations of the nematodes with the typical endospores of members of the *Pasteuria penetrans* group were observed; in the heterologous systems, the associations were as frequent or almost as frequent as in the homologous system. − = associations of the nematodes with the bacterial endospores definitely did not occur. −? = associations of the nematodes with the bacterial endospores were rare, barely perceptible, and/or questionable.

The highly refractile nature of the bacterial endospores and sporangia may contribute to overestimating the sizes of their diameters when viewed with the light microscope. This observational imprecision should be taken into account in interpreting differing dimensions recorded here and elsewhere based on measurements made by light microscopy and those made on electron micrographs of thin sections. A similar caveat applies to measurements stemming from scanning electron microscopy; meaningful dimensions must include specification of all methodological details!

Our electron-microscopic examination of members of the *Pasteuria penetrans* group from root-knot nematodes (RKP) and root-lesion nematodes (RLP) identified several morphological, ultrastructural, and developmental differences between the bacteria from the 2 kinds of nematodes. One striking difference is the simultaneous occurrence in individual *Pratylenchus brachyurus* larvae of all developmental stages of RLP, from mycelial microcolonies to mature endospores. Unlike the development of RKP in *Meloidogyne incognita*—where the parasite and host develop synchronously, beginning with the bacterial vegetative stage in the early molts of the host nematode and ending with the sporangial stages of RKP in the adult female (Sayre and Starr, 1985)—there appeared to be no such synchrony among the various stages of RLP. Nor

was RLP development related to the development of its nematode host. The intermingled vegetative and sporangial material, rarely seen in thin sections of RKP-infected *M. incognita* adults, might result simply from arrested development of vegetative cells.

Meaningful comparisons of the shape and sizes of microcolonies of RLP and RKP were not feasible, since space limitations in the confining pseudocoelom of *P. brachyurus* precluded our making margin-to-margin measurements of microcolonies of RLP. In contrast, microcolonies of RKP were easily visualized in the swollen sedentary forms of *M. incognita*.

The thin sporangial membrane, which surrounds the endospore of RKP, was not observed about the endospores of RLP. The membrane may indeed be absent or it might be an ephemeral structure missed as a result of methods for preparing and handling specimens of RLP. Our failure to observe in RLP the mesosomes—which often were associated with vegetative cell division in RKP—may also have resulted from sampling at inopportune times. Mesosomes are probably more abundant during rapid vegetative growth. Because sporulation of RLP had begun in all our samples of *P. brachyurus,* vegetative growth may have been curtailed at sampling. Consequently, with only a few cells dividing at the time of sampling, the chance to find a mesosome was diminished.

Table 4. Nematode hosts and geographical distribution of members of the *Pasteuria penetrans* group occurring in the genera *Meloidogyne* and *Pratylenchus*.

Nematode	Location	Authority and reference
Meloidogyne ardenensis	Germany (BRD)	Sturhan (1985)
Meloidogyne arenaria	Netherlands	Kuiper (1958)
Meloidogyne arenaria	U.S.A.: California	Mankau and Prasad (1977)
Meloidogyne arenaria	U.S.A.: Florida	Esser (1980)
Meloidogyne coffeicola	Brazil	Sturhan*
Meloidogyne exigua	Colombia	Baeza-Aragon (1978)
Meloidogyne graminis	Germany (BRD)	Sturhan (1985)
Meloidogyne hapla	Japan	Nishizawa (1984, 1986)
Meloidogyne hapla	U.S.A.: California	Mankau and Prasad (1977)
Meloidogyne hapla	U.S.A.: Maryland	Dutky and Sayre (1978)
Meloidogyne incognita	Japan	Nishizawa (1984, 1986)
Meloidogyne incognita	Mauritius	Williams (1967)
Meloidogyne incognita	South Africa	Spaull (1981)
Meloidogyne incognita	Togo	Sturhan (1985)
Meloidogyne incognita	U.S.A.: California	Prasad and Mankau (1969)
Meloidogyne incognita	U.S.A.: Florida	Esser (1980)
Meloidogyne incognita	U.S.A.: Louisiana	Birchfield and Antonopoulos (1964)
Meloidogyne incognita	U.S.A.: Maryland	Dutky and Sayre (1978)
Meloidogyne javanica	Australia	Stirling and White (1982)
Meloidogyne javanica	Brazil	Lordello (1966)
Meloidogyne javanica	India	Dutky (1978)
Meloidogyne javanica	Japan	Allen (1957)
Meloidogyne javanica	Mauritius	Williams (1967)
Meloidogyne javanica	U.S.A.: California	Boosalis and Mankau (1965)
Meloidogyne javanica	U.S.A.: Florida	Esser (1980)
Meloidogyne javanica	U.S.A.: Maryland	Dutky (1978)
Meloidogyne naasi	Finland	Sturhan (1985)
Meloidogyne naasi	Germany (BRD)	Sturhan (1985)
Meloidogyne sp.	Germany (BRD)	Sturhan (1985)
Meloidogyne sp.	Nicaragua	Sturhan (1985)
Pratylenchus brachyurus	U.S.A.: Florida	Dutky (1978)
Pratylenchus brachyurus	U.S.A.: Georgia	Thorne (1940)
Pratylenchus brachyurus	U.S.A.: Maryland	Dutky and Sayre (1978)
Pratylenchus brachyurus	U.S.A.: South Carolina	Thorne (1940)
Pratylenchus convallariae	Germany (BRD)	Sturhan*
Pratylenchus crenatus	Germany (BRD)	Sturhan (1985)
Pratylenchus fallax	Germany (BRD)	Sturhan (1985)
Pratylenchus flakkensis	Germany (BRD)	Sturhan (1985)
Pratylenchus neglectus	Austria	Sturhan*
Pratylenchus neglectus	Germany (BRD)	Sturhan (1985)
Pratylenchus penetrans	Germany (BRD)	Sturhan (1985)
Pratylenchus penetrans	Netherlands	Kuiper (1958)
Pratylenchus penetrans	U.S.A.: Florida	Esser (1980)
Pratylenchus pratensis	Germany (BRD)	Sturhan (1985)
Pratylenchus pratensis	Netherlands	Kuiper (1958)
Pratylenchus scribneri	U.S.A.: California	Prasad and Mankau (1969)
Pratylenchus sp.	Germany (BRD)	Boosalis and Mankau (1965)
Pratylenchus sp.	Greece	Sturhan (1985)
Pratylenchus sp.	U.S.A.: Florida	Esser and Sobers (1964)
Pratylenchus sp.	U.S.A.: Oregon	Prasad (1971)
Pratylenchus spp.	U.S.A.: Illinois	Boosalis and Mankau (1965)
Pratylenchus spp.	U.S.A.: Maryland	Dutky (1978)
Pratylenchus thornei	Germany (BRD)	Sturhan (1985)
Pratylenchus zeae	Dominican Republic	Sturhan (1985)
Pratylenchus zeae	Mozambique	Sturhan*
Pratylenchus zeae	South Africa	Spaull (1981)
Pratylenchus zeae	U.S.A.: Florida	Esser (1980)

* Personal communication from Dieter Sturhan, November, 1986.

One of the first obvious indicators that endospore formation had begun in both RKP and RLP was the swelling of terminal cells in the microcolonies. During the subsequent formation of the single endospore within each sporangium, there was little difference in the development and sequence of events to differentiate between RKP and RLP. However, the final mature sporangia that are released from the 2 host nematodes are distinctly different from each other in several respects. RLP sporangia are smaller in diameter than those of RKP and slightly less in height (Table 2). These dimensions reflect the respective shapes; RLP sporangia are rhomboidal and RKP sporangia are cup-shaped. The endospores of the 2 also are different in size and shape (Table 2). The central endospore of RLP is nearly spherical; that of RKP is ellipsoidal, broadly elliptic in section. Compared with RKP, the RLP endospore is surrounded by thinner cortical walls, and it has a smaller basal germinal pore not clearly set off by the cortical walls as in RKP. The parasporal fibers surrounding the endospore provide further morphological features to separate RLP from RKP. The number of fibers radiating from the cortical walls appeared to be fewer in number in RLP; in transverse sections, their angle of attachment gave the RLP endospore the appearance of an arrowhead. This arrangement is markedly different from the saucer-shaped transverse sections of RKP endospores.

The relationships of RLP and RKP with particular nematodes are quite specific. Host specificity of members of the *Pasteuria penetrans* group is usually scored by quantifying attachments of bacterial endospores to nematode cuticles. Based on such methods, RKP has been reported to parasitize only *Meloidogyne* and RLP to parasitize only *Pratylenchus*; often only one species of the indicated genus is parasitized. In fact, the only exception to this hard-and-fast rule in the literature (Tables 3 amd 4; excerpted from more comprehensive presentations in Sayre and Starr, 1988) is the single case of a bacterial preparation from *Meloidogyne javanica*, the endospores of which were reported (Mankau and Prasad, 1977) on one occasion to attach to *Pratylenchus scribneri*. Examination of published transmission electron micrographs (Mankau, 1975; Imbriani and Mankau, 1977; Mankau and Prasad, 1977) of the member of the *P. penetrans* group parasitizing *M. javanica* suggests this bacterium may be different from both RLP

and RKP in the following respects: the several stages of vegetative growth and sporulation could be observed in a single thin section of *M. javanica* material (the nonsynchronous RLP pattern); the mesosomes of the bacteria from the *M. javanica* material appeared to be more laminar in structure, unlike the fine network in RKP; the central dome of the mature endospore is more pointed than RKP's; and there is no well-defined basal pore as in the RKP endospore, rather the basal cortical walls taper to a opening similar to that in the RLP endospore. Unfortunately, further details are not currently available to permit a firm decision to be made about the relationship of the bacterium from *M. javanica* to either RKP or RLP; perhaps it represents a taxon different from both RKP and RLP.

The several morphological and developmental differences between RLP and RKP, coupled with the clear host specificity, strongly suggest that these 2 members of the *Pasteuria penetrans* group belong in separate taxa. Formal taxonomic and nomenclatural recommendations regarding appropriate taxa appear elsewhere (Starr and Sayre, 1988).

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Effects of Coccidiosis on Activities of Glycogen Phosphorylase and Glycogen Synthase in Livers from 2- to 3-Week-Old Broiler Chicks

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ABSTRACT: Glycogen and activities of glycogen phosphorylase and glycogen synthase were measured in liver homogenates of 2- to 3-wk-old broiler chicks infected with either *Eimeria acervulina* or *E. tenella* during the period 4 to 8 days after inoculation (DAI). At 5 and 7 DAI, significant differences between infected groups and controls occurred in the activities of the 2 active forms of the enzymes, synthase I and phosphorylase *a,* and the ratio synthase I/phosphorylase *a.* However, these changes did not correlate with changes in liver glycogen levels. No differences were seen between the enzyme activities in control chicks and those starved for 24 and 48 hr and subsequently refed, in spite of large fluctuation in liver glycogen.

These experiments suggest that in healthy young broiler chicks, starvation and refeeding may cause sufficient allosteric modulation of the glycogen synthase–phosphorylase complex to significantly affect liver glycogen levels without altering actual enzyme activities as measured in vitro. Further, because enzyme activities were changed, but glycogen levels were not correlated, allosteric mechanisms may also control liver glycogen levels in coccidia-infected chicks.

KEY WORDS: coccidiosis, *Eimeria acervulina, Eimeria tenella,* glycogen phosphorylase, glycogen synthase, broiler chicks.

Liver glycogen is a major carbohydrate reserve in chicks, and is readily mobilized in response to short term food deprivation and stress. Previous investigations (Ruff and Allen, 1982) have shown that there are different liver glycogen dynamics in young broiler chicks infected with *Eimeria acervulina,* a duodenal parasite, as compared to those infected with *Eimeria tenella,* a cecal parasite. Infection with *E. acervulina* causes significant diminutions in liver glycogen between 4 and 5 days after inoculation (DAI) (time corresponding to maximum oocyst production and gross intestinal lesions) and significant elevations with respect to controls at about 7 DAI. On the other hand, infection with *E. tenella* may be associated with either no change, or with significant diminutions in liver glycogen at about 5 DAI (time corresponding to maximal cecal lesions), but no significant elevation at 7 DAI.

Liver glycogen is synthesized and broken down by glycogen synthase (E.C. 2.4.1.11) and glycogen phosphorylase (E.C. 2.4.1.1), respectively (Lehninger, 1970). A number of workers have tried to correlate levels of liver glycogen with activities in the liver of one or both of these enzymes (Hue et al., 1975; Rosebrough et al., 1977). In particular, Watts and Gain (1976) postulated that in the neonatal rat, a close relationship exists between liver glycogen levels and the ratio of the active forms of the 2 enzymes (glycogen synthase I and phosphorylase *a,* respec-

tively). Therefore, a study was undertaken to determine if coccidial infections influence in any way the levels of liver glycogen synthase or glycogen phosphorylase. Since anorexia is a part of the disease syndrome of almost all coccidial infections in chickens, glycogen levels and enzyme activities were also assayed in chicks subject to short term starvation and chicks subsequently refed in order to isolate any effects of reduced feed intake.

Materials and Methods

Animals and housing

Broiler chicks, 2–3 wk old, were distributed in experimental groups of 6 birds/cage on the basis of weight (Gardiner and Wehr, 1950) and were housed under constant lighting at 28°C. They were fed a standard starter ration without medication and provided water ad lib.

Coccidia and inoculum

The strains of *E. acervulina* (#12) and *E. tenella* (#10) used were those originally isolated by Dr. S. A. Edgar (Auburn University) and were reisolated and maintained at the Animal Parasitology Institute. The standard inoculum of sporulated oocysts/bird was 2 × 10^6 for *E. acervulina* or 2 × 10^5 for *E. tenella.* These doses routinely yielded individual lesion scores of about +4 (Johnson and Reid, 1970).

Protocols

EXPERIMENT 1: Groups of infected chicks along with control groups were analyzed at 5 and 7 DAI.

EXPERIMENT 2: Groups of infected birds along with

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Table 1. Effect of coccidial infections on liver glycogen and percent active glycogen phosphorylase.

Days after inoculation	Group	N	Liver glycogen (mg/g fresh weight)	% active phosphorylase
5	Control	22	10.5 ± 0.6*	68 ± 3
	E. acervulina	22	6.9 ± 0.6†	71 ± 4
	E. tenella	21	9.2 ± 0.8	66 ± 4
7	Control	22	12.2 ± 0.7	75 ± 3
	E. acervulina	22	16.7 ± 1.5†	69 ± 3
	E. tenella	20	13.2 ± 1.3	68 ± 4

* Values are means ± SEM.

† Denotes mean that is significantly different from its control mean ($P < 0.05$).

a control group were analyzed on each day from 4 to 8 DAI.

EXPERIMENT 3: Groups of birds were analyzed 24–48 hr after removal of feed and again 24 hr after refeeding starved birds. A full-fed control group was analyzed along with each treatment group.

Collection and storage of tissue samples

In Experiment 1, chicks were killed by cervical dislocation, and the livers were immediately removed and frozen on dry ice. These tissues were analyzed for glycogen and glycogen phosphorylase only. In experiments 2 and 3, chicks were anesthetized with Na pentobarbital (V-Pento, A. J. Buck, 65 mg/ml) at a dose of 0.5 ml/kg body weight. Portions of the liver were freeze clamped at liquid nitrogen temperature (Wollenberger et al., 1960) and used for glycogen phosphorylase, glycogen synthase, and glycogen assays. Tissues obtained for enzyme analyses were processed and assayed directly. Tissues for glycogen analyses were stored at −20°C until assayed.

Analyses

In Experiment 1, glycogen phosphorylase (active *a* form and total) was measured in the direction of glycogen synthesis in dry ice-frozen samples by the method of Hue et al. (1975). In Experiments 2 and 3, glycogen phosphorylase (both active *a* form and total) was measured in the direction of glycogen synthesis using U-(^{14}C)-glucose-1-phosphate as a substrate (Gilboe et al., 1972). The ratio of fresh tissue weight to homog-

enizing medium was adjusted to at least 1:200. At this dilution, treatment with dowex 1 resin to get rid of endogenous AMP (Gilboe et al., 1972) was unnecessary.

Both the I and D forms of glycogen synthase were measured in the direction of glycogen synthesis using UDP-U-(^{14}C)-glucose as a substrate (Thomas et al., 1968). Protein in both the glycogen phosphorylase and glycogen synthase preparations was measured according to Lowry et al. (1951) and the enzyme activities were expressed as mU/mg protein, where 1 mU equals micromoles substrate incorporated into glycogen/min. The activity of phosphorylase *a* was also expressed as a percentage of total phosphorylase activity (% phosphorylase *a*).

In all 3 experiments glycogen in homogenates of liver tissue (0.1 M acetate buffer, pH 4.5) was degraded to glucose with amyloglucosidase (Keppler and Decker, 1974). The resultant glucose as well as free glucose in undigested homogenates was assayed by a glucose oxidase method according to Mattenheimer (1970). The glycogen content was expressed as mg net glucose/g fresh weight of tissue.

Statistical analyses

Data were examined by analysis of variance, and significances of differences between control and experimental means determined by the Wilcoxon 2-sample test (Siegel, 1956).

Results

In Experiments 1 and 2, liver glycogen in *E. acervulina*-infected chicks was significantly decreased at 5 DAI and significantly increased at 7 DAI with respect to control chicks (Tables 1, 2). The mean glycogen levels did not vary significantly from control levels in *E. tenella*-infected chicks (Tables 1, 2).

In Experiment 1, *E. acervulina* and *E. tenella* infections did not significantly change the percent active phosphorylase activities in chick liver (Table 1). In Experiment 2, glycogen phosphorylase *a* activity significantly increased at 7 DAI in *E. acervulina*-infected chicks and at 5 and 7 DAI in *E. tenella*-infected chicks. However, when this activity was expressed as percent of total phosphorylase, no differences in activities were seen between infected and control samples (Ta-

Table 2. Effect of coccidiosis on chick liver glycogen with time after infection.

Group	Glycogen (mg net glucose/g fresh weight) at days after infection				
	4	5	6	7	8
Control	12.7 ± 2.5*	17.3 ± 2.0	31.0 ± 7.4	20.5 ± 1.9	18.2 ± 2.1
E. acervulina	9.6 ± 2.2	8.8 ± 1.4†	24.3 ± 3.4	32.7 ± 5.1†	19.0 ± 3.4
E. tenella	13.7 ± 2.0	11.7 ± 3.4	27.8 ± 5.0	22.8 ± 3.7	16.0 ± 3.9

* Values are means ± SEM.

† Denotes means which are significantly different from the appropriate control means ($P < 0.05$).

Table 3. **Effect of coccidiosis on glycogen phosphorylase *a* with time after infection.**

Activity	Group	Days after infection				
		4	5	6	7	8
Phosphorylase *a*	Control	60.7 ± 7.5*	46.7 ± 5.6	63.8 ± 12.6	45.8 ± 7.7	74.3 ± 8.2
(mU/mg protein)	E. acervulina	43.6 ± 6.7	55.5 ± 10.2	44.7 ± 4.1	109.5 ± 8.7†	76.7 ± 10.1
	E. tenella	71.0 ± 6.7	103.5 ± 8.3†	52.4 ± 3.6	98.5 ± 5.0†	75.7 ± 5.4
% phosphorylase *a*	Control	80.8 ± 4.4	76.7 ± 6.2	72.3 ± 3.4	69.5 ± 8.7	67.7 ± 8.1
(mU/mg protein)	E. acervulina	68.6 ± 7.5	67.2 ± 6.5	66.6 ± 5.8	76.5 ± 4.5	69.7 ± 9.0
	E. tenella	78.6 ± 4.7	86.2 ± 1.5	79.0 ± 5.1	80.8 ± 2.4	78.1 ± 5.0

* Values are means ± SEM.

† Denotes means which are significantly different from the appropriate control means ($P < 0.05$).

ble 3). Synthase I activity increased in the control group between 6 and 7 DAI, but not in either of the infected groups. Consequently, in both infected groups at 7 DAI (Table 4) synthase I activity was significantly lower than controls. Similarly, the mean ratios of synthase I : phosphorylase *a* were significantly lower in both infected groups than in the control at 7 DAI (Table 4).

Starvation for 24 hr (Experiment 3) caused about a 72% decrease in mean liver glycogen, although the level rebounded somewhat after 48 hr starvation. However, after 24 hr refeeding the mean glycogen level was about 300% higher than the control level (Table 5). Nevertheless, starvation and refeeding had no significant effects on the activities of phosphorylase *a* or synthase I, or on the ratio of synthase I : phosphorylase *a*. A significant decrease in percent phosphorylase *a* activity was seen at 48 hr starvation (Table 5).

Discussion

In Experiment 1, it was thought that the methods of killing the chicks and collecting the liver tissues might have influenced the activities of glycogen phosphorylase *a*. Therefore, pentobarbital anesthetization of the chicks and freeze clamping of the liver in situ as described by Stalmans et al. (1974) were subsequently used to minimize adrenergic stimulation of liver phosphorylase *a* activity. In all 3 experiments the percentages of glycogen phosphorylase *a* in the control groups were rather high. However, they are consistent with reports that liver glycogen decreases after hatching, and is lower in young chicks as compared to adults (Pearce and Brown, 1971).

Although *E. acervulina* and *E. tenella* infections elicited significantly different changes in liver glycogen with time after infection as compared to controls, only 1 significant difference in effects on enzyme activities was noted, i.e., an increase in phosphorylase *a* and consequential decrease in the synthase I : phosphorylase *a* ratio at 5 DAI in the *E. tenella*-infected chicks.

In both the infection and starvation and refeeding experiments there were apparently no correlations between phosphorylase *a* activities and liver glycogen levels. These results are consistent with the findings of Watts and Gain (1976)

Table 4. **Effect of coccidiosis on glycogen synthase I and the ratio of synthase I : phosphorylase *a*.**

Activity	Group	Days after infection				
		4	5	6	7	8
Glycogen synthase I	Control	3.7 ± 0.7*	1.9 ± 0.4	2.3 ± 0.5	5.0 ± 1.0	4.5 ± 0.8
(mU/mg protein)	E. acervulina	3.0 ± 0.8	1.8 ± 0.3	1.3 ± 0.2	1.7 ± 0.3†	3.6 ± 1.0
	E. tenella	2.5 ± 0.5	1.9 ± 0.2	2.2 ± 0.3	1.9 ± 0.3†	6.0 ± 1.4
Synthase I : phosphorylase *a*	Control	0.07 ± 0.02	0.04 ± 0.0005	0.04 ± 0.008	0.11 ± 0.02	0.06 ± 0.01
	E. acervulina	0.08 ± 0.03	0.04 ± 0.0008	0.03 ± 0.006	0.02 ± 0.003†	0.03 ± 0.01
	E. tenella	0.04 ± 0.01	0.02 ± 0.004†	0.04 ± 0.004	0.02 ± 0.003†	0.08 ± 0.02

* Values are means ± SEM.

† Denotes means which are significantly different from the appropriate control means ($P < 0.05$).

Table 5. Effect of starvation and refeeding on chick liver glycogen, glycogen synthase I, and glycogen phosphorylase *a*.

	Glycogen (mg net glucose/g fresh weight liver)	Glycogen synthase I (mU/mg protein)	Glycogen phosphorylase *a* (mU/mg protein)	Synthase I : phosphorylase *a*	% phosphorylase *a*
Control	7.9 ± 0.6*	10.4 ± 3.0	58.3 ± 5.0	0.18 ± 0.05	58.0 ± 6.4
24 hr starved	2.2 ± 0.6†	9.3 ± 2.6	59.8 ± 8.9	0.16 ± 0.04	55.0 ± 5.9
Control	5.2 ± 0.6	6.3 ± 1.6	97.0 ± 3.5	0.07 ± 0.02	71.6 ± 4.4
48 hr starved	4.0 ± 0.5	10.2 ± 3.4	73.8 ± 13.9	0.15 ± 0.04	50.4 ± 8.6†
Control	10.0 ± 1.9	6.1 ± 1.5	78.5 ± 11.0	0.08 ± 0.02	56.8 ± 5.3
24 hr refed	27.8 ± 3.3†	4.3 ± 0.7	79.5 ± 9.0	0.06 ± 0.01	61.7 ± 5.7

* Values are means ± SEM.

† Denotes means that are significantly different from appropriate control means ($P < 0.05$).

for fetal and neonatal rats and Rosebrough et al. (1977) for poults. However, in contrast to the results of Watts and Gain (1976) no direct correlations were found between the liver glycogen levels and the activity ratios of synthase I : phosphorylase *a* in groups of infected chicks, starved and/or refed chicks, or controls. In fact, in *E. acervulina*-infected chicks, opposite trends in data were observed at 7 DAI.

Forty-eight hours of starvation followed by 24 hr of refeeding had no significant effects on the synthase I : phosphorylase *a* ratio despite the fact that the glycogen levels changed dramatically (Table 5). It seems clear, therefore, that the significant changes in the ratio seen at 7 DAI in both infected groups are not associated with resumption of feeding after a period of anorexia.

It is apparent from these experiments that the fluctuations in liver glycogen seen in coccidial infections cannot be explained by the effects of infection on activities of glycogen synthase I and phosphorylase *a* as measured in vitro. Infection does affect activities, but the changes themselves apparently do not directly affect liver glycogen levels. It is known that these active enzyme forms are interconvertable with their inactive forms through phosphorylation and dephosphorylation by kinases and phosphatases, respectively. These latter regulating enzymes are themselves subject to control by hormones and metabolites (Lehninger, 1970). Furthermore, these same enzymes are not controlled at the same rates (Stadtman, 1970; Lin et al., 1972; Watts and Gain, 1976). In the young broiler chick host, infection and/or starvation and refeeding may cause sufficient allosteric modulation of the synthase–phosphorylase complex, perhaps through changes of blood levels of pancreatic and gut hormones (Allen and

McMurtry, 1984), to significantly affect glycogen levels of liver without altering the actual levels of enzymes.

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Electron Microscopic Observations of *Theileria cervi* in Salivary Glands of Male *Amblyomma americanum*

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ABSTRACT: Sporogony of *Theileria cervi* was studied in salivary glands of unfed and feeding male *Amblyomma americanum*. Parasites were observed only in complex granular cells (E) of type III acini. Parasite development was more rapid in some males than females and mature sporozoites were present as soon as 3 days of tick feeding. The morphology of residual bodies with formed sporozoites in males varied from those described in similar cells in females.

KEY WORDS: protozoa, *Theileria cervi, Amblyomma americanum*, male tick, sporogony, ultrastructure, white-tailed deer.

The development of *Theileria* spp. in tick salivary glands has been limited to studies in female ticks. Electron microscopic observations of sporogony in *T. parva parva, T. parva lawrencei,* and *T. taurotragi* in salivary glands of unfed and feeding female *Rhipicephalus appendiculatus* have extended our understanding of this phase of parasite development (Fawcett et al., 1985). Sporogony was observed to be coordinated with tick feeding. As the parasite developed, it increased in size and complexity, eventually occupying most of the hypertrophied host cell. Sporozoites were formed by segmental fission along complex projections of parasite cytoplasm. Recent studies on *T. cervi* in female *Amblyomma americanum* have described a similar but not identical sequence of development (Hazen-Karr et al., 1988). The present study was initiated to determine the developmental sequence of *T. cervi* in unfed and feeding male *A. americanum*. These studies were initiated because feeding activities and cellular morphology of salivary glands of male ticks differ markedly from females.

Materials and Methods

Amblyomma americanum nymphs (approximately 2,000) were allowed to feed on a splenectomized white-tailed deer that was naturally infected with *T. cervi*. Nymphs were placed in a closed wooden box (0.6 m × 0.6 m) with screen ventilation holes along with the splenectomized deer that had a 25% parasitemia of *T. cervi*. After 12 hr, the deer was moved to an elevated cage; the engorged nymphs were collected when replete and placed in a humidity chamber (90–98% relative humidity) at 25°C with a 14-hr photophase. Ticks were maintained under these conditions until they molted to the adult stage. At 50 days postrepletion, molted adults were divided into 2 groups and used for morphology and transmission studies.

To document the infectivity of the ticks being studied, 25 pairs of *A. americanum* adults exposed to *T. cervi* as nymphs were allowed to feed on an intact noninfected fawn. The fawn was determined to be free of infection with *T. cervi* because it was born and raised in isolation and piroplasms were not seen in repeated examinations of peripheral blood films. Blood films were examined daily after tick attachment until piroplasms were detected.

For morphologic studies, adult *A. americanum* infected with *T. cervi* (150 pairs) and uninfected controls (100 pairs) were placed in separate stockinettes attached to closely shorn sheep. Ten infected pairs (male/female) along with 10 pairs of controls were removed from the sheep beginning with unfed ticks and continuing through each day of feeding (days 1–6). The dorsal exoskeleton of the ticks was removed with a razor blade and salivary glands were removed with fine forceps.

Salivary glands were placed immediately into cold 2% glutaraldehyde (pH 7.4) in 0.2 M sodium cacodylate buffer and allowed to fix at 4°C for 48 hr. Tissues were washed in buffer, postfixed in 2% osmium tetroxide, dehydrated in a graded series of alcohol, infiltrated with a mixture of 1:1 propylene oxide/DER 732 for 12 hr and embedded in DER 732. Thick sections (1 μm) were prepared, stained with Mallory's stain (Richardson et al., 1960) for 2 min at 60°C, and examined with a light microscope. Fine sections (silver-reflective) were prepared with a Dupont diamond knife and a Sorvall MT 5000 ultramicrotome. The sections were stained with 5% uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and examined and photographed on a JEOL 100 CX II TMSCAN electron microscope at 100 kV.

Results

Piroplasms were detected in blood of the susceptible fawn at 11 d after attachment of the adult
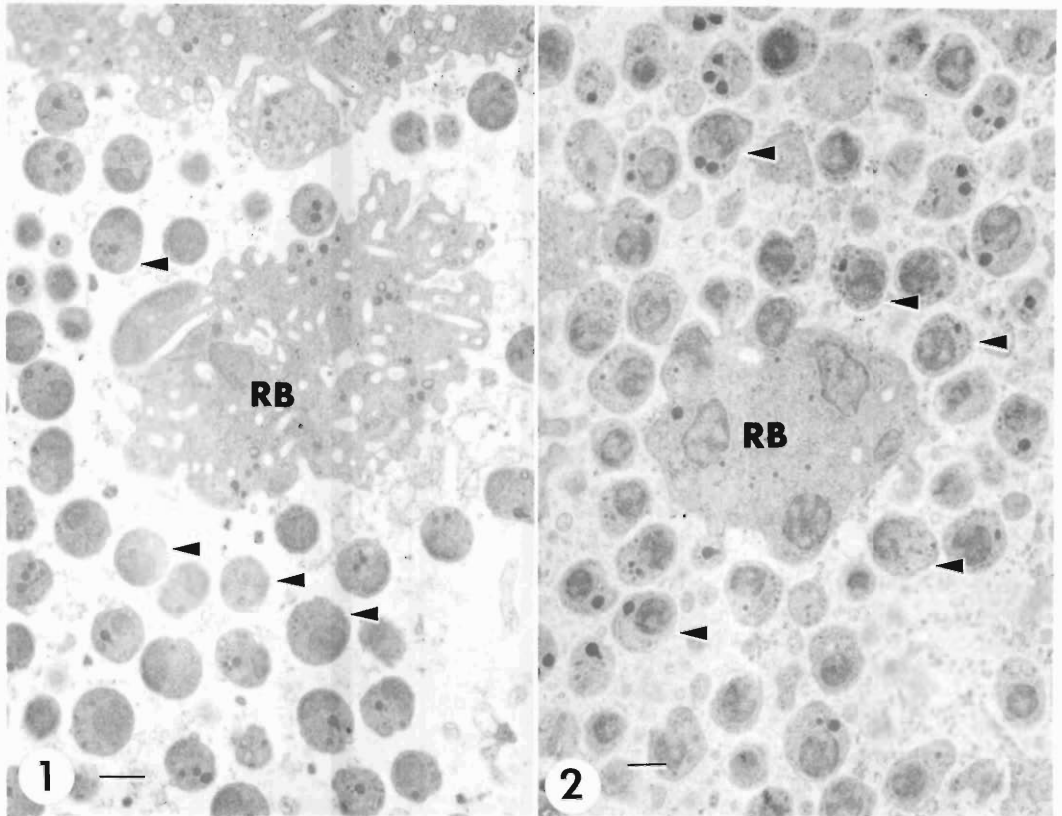
Figures 1, 2. Electron micrographs of developing *Theileria cervi* in salivary glands of *Amblyomma americanum*. 1. Irregularly shaped residual bodies (RB) in male *A. americanum* containing numerous cellular organelles. Arrows indicate some of the numerous sporozoites present at 3 days post tick attachment. Bar = 1 μm. 2. Rounded residual body (RB) with smooth surface contour from female *A. americanum*. Arrows indicate some of the numerous sporozoites present at 6 days post tick attachment. Bar = 500 nm.

A. americanum that were exposed to *T. cervi* as nymphs.

Electron microscopy

Infections with *T. cervi* in male *A. americanum* were observed only in complex granular cells of type III acini as reported by Krolak et al. (1982). The sequential development of the parasite syncytium was similar to that observed in feeding females (Hazen-Karr et al., 1988) but the feeding period necessary for development of sporozoites in some males was shorter. Sporozoites were observed in males as early as 3 days after tick attachment while females required 5 days of feeding for development of sporozoites. Considerable variation was observed among males in the feeding time required for sporozoite production. Light microscope evaluation of 1-μm sections of salivary glands from both male and females at 6 days of feeding revealed that 100% of the ticks

were infected with an average of 3.7 infected acini per tick. Males averaged 3.3 (range: 1–5) infected acini per tick while females averaged 4.8 infected acini. At 3 days after tick attachment, 3 of 10 males (range: 1–9) had infected acini with mature sporozoites while none of the parasites in acini of females had developed to the point of sporozoite formation. In male *A. americanum*, in which *T. cervi* sporozoites were observed after 3 or 4 days of tick feeding, residual bodies were more irregular in shape (Fig. 1) than those in either females (Fig. 2) or males in which sporozoites were not evident until the ticks had fed for 5 or 6 days. Residual bodies contained parasite organelles including nuclei, mitochondria, micronemes, and rhoptries.

Discussion

Development of *T. cervi* sporoblasts in unfed and feeding male *A. americanum* was similar to

that reported for females exposed and dissected concurrently. Notable differences were observed however, in the time required for development of sporozoites. These differences are probably due to the feeding pattern of males being markedly different than that of females. For example, males are intermittent feeders and do not require large quantities of blood, as do females. Male *Dermacentor variabilis* are reported to feed for only 2 or 3 days when females are present (Sonenshine et al., 1982). Males that attach and feed may detach, presumably in response to a sex pheromone produced by the attached females. In the absence of females, attached males either do not feed or will remain attached and feed slowly (Sonenshine et al., 1982). In addition, the size of the blood meal taken by ticks appears to vary considerably even within species of the same genus. In *A. americanum,* the size of the blood meal has been shown to vary with the sex of the tick; adult males take considerably smaller volumes than females although males ingest whole blood much earlier after attachment than do females (Sauer and Hair, 1972).

Differences in the sporogonous cycle of *T. cervi* in adult male and female *A. americanum* were noted in the morphology and composition of the residual bodies present following sporozoite production. With the exception of *T. taurotragi,* residual bodies of most species of *Theileria* have been noted to be round in shape with a smooth surface contour (Fawcett et al., 1985). The large, irregularly shaped *T. cervi* residual bodies seen in male *A. americanum* varied from those seen in similarly infected female ticks by having an irregular surface contour and numerous cellular organelles, probably due to the more rapid sporogonous process in these acini.

The findings of this study document the first report of sporogony in male *A. americanum* infected with *T. cervi.* The more rapid development of sporogony in some males suggests that they may be important in transmission of *T. cervi* and warrant further study.

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A New Species of *Hysterolecitha* (Trematoda: Hemiuridae) from the Mullet, *Mugil liza,* in the State of Rio de Janeiro, Brasil

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ABSTRACT: *Hysterolecitha brasiliensis* sp. n. is described from the stomach and occasionally the intestine of *Mugil liza* Valenciennes, 1836, from Rio da Guarda and the coast of the state of Rio de Janeiro. This species is characterized by the relatively thick tegument; presence of tegumental plications; rounded and well-developed pars prostatica, surrounded by numerous prostatic cells; true seminal receptacle; uterine seminal receptacle; and a conspicuous genital atrium. It is distinguished from *H. elongata* Manter, 1931, by the shorter hindbody, the longer forebody, the well-developed pars prostatica, larger eggs, and the true seminal receptacle. Of the more than 18 species described known to date, it is most similar to *H. pseudorosea* (Bravo-Hollis, 1956) Yamaguti, 1971, because of the possession of a true and a uterine seminal receptacle but differs by the shape of the body, position of genital pore which is immediately posterior to pharynx, the larger true seminal receptacle, and egg size (24–43 [mean = 36] by 16–22 [mean = 19] μm).

KEY WORDS: *Hysterolecitha brasiliensis* sp. n., Hemiuridae, digenetic trematodes, *Mugil liza,* mullet, Rio de Janeiro, Brasil.

The fishes of the family Mugilidae have broad geographic distribution, occurring in the tropical and subtropical waters of the world, mainly restricted to the coastal estuarine waters. They are hosts of a large number of trematode species, and due to the constant migration between marine and freshwaters, were attributed by Manter (1957), as being ecological bridges between trematodes of freshwater and those of coastal marine waters.

In the present work we describe Brasilian specimens from 2 different environments in the state of Rio de Janeiro as a new species of *Hysterolecitha.*

Material and Methods

The fishes from Rio da Guarda (RG) watershed (22°45′S and 23°00′S, 43°50′W) were caught by the first author and professional fishermen, employees of the W. O. Neitz Parasitological Research Station, Universidade Federal Rural do Rio de Janeiro. The fishes from the coast of the state of Rio de Janeiro (CRJ) (22°00′S, 44°00′W) were caught by commercial trawlers. The trematodes were collected following the general technique described by Amato (1985), fixed in alcohol–formalin–acetic acid (AFA) under slight coverslip pressure, stained with Mayer's Carmalum or Delafield's Hematoxylin, and cleared with beechwood creosote. The prevalence and the intensity of infection are indicated as follows, for each station: no. of hosts positive for *H. brasiliensis*/no. of hosts examined: size of each infrapopulation. The drawings were made with a drawing tube and a Leitz Dialux 20 EB microscope. All measurements are in micrometers unless otherwise

stated; ranges of measurements are followed by the mean values within parentheses. Type specimens were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro (IOC/FIO-CRUZ) and at the Harold W. Manter Laboratory, University of Nebraska State Museum, The University of Nebraska–Lincoln, Lincoln, Nebraska, U.S.A. (HWML).

Results

Hysterolecitha brasiliensis sp. n.
(Figs. 1–3)

DESCRIPTION (based on 11 specimens from Rio da Guarda): Body elongated, with anterior extremity attenuated, without ecsoma, 1.8–6 (4.3) mm long by 399–1,015 (774) wide at acetabulum level. Tegument relatively thick, with tegumental plications at the anterior extremity. Acetabulum larger than oral sucker, at the end of the anterior ⅓ of body, 281–696 (527) long by 318–733 (529) wide, with 3 pairs of large papillae distributed on the acetabular border. Oral sucker subterminal, 177–395 (309) long by 185–414 (304) wide. Prepharynx absent. Pharynx well developed, rounded, 74–222 (158) long by 44–207 (139) wide. Esophagus absent. Ceca long, sinuous, but not reaching the posterior extremity. Sucker width ratio 1:1.7–2.0. Forebody (sensu Yamaguti, 1971), 658–1,504 (1,133). Hindbody 1.1–4.7 (3.2) mm. Testes rounded, smooth, diagonal, together or separated, in middle ⅓ of body. Anterior testis 185–357 (279) long by 118–318 (241) wide. Pos-

58
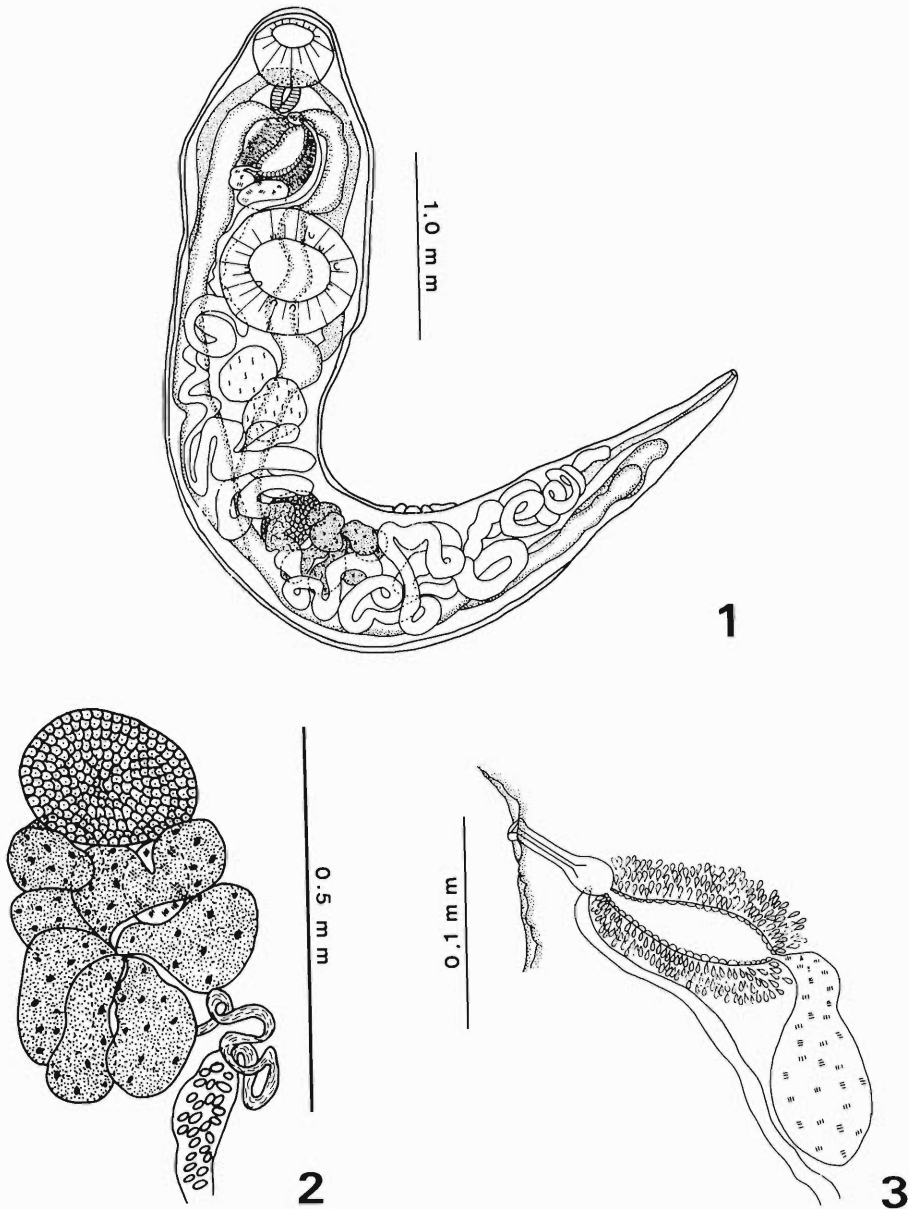
Figures 1–3. *Hysterolecitha brasiliensis* sp. n. 1. Adult, ventral view. 2. Ovarian complex. 3. Terminal genitalia, lateral view.

terior testis 192–303 (269) long by 148–406 (249) wide. Seminal vesicle saccular, 185–717 (384) long by 111–303 (191) wide. Pars prostatica spherical, well developed, surrounded by numerous prostatic cells, 233–733 (462) long by 163–376 (306) wide. Ovary rounded, posttesticular, in the middle ⅓ of body, 185–288 (230)

long by 142–322 (233) wide. Mehlis' gland between ovary and vitelline glands. Vitelline glands postovarian, disposed in 2 groups of 3 and 4 digitiform lobes, forming a rosette. True seminal receptacle rounded, immediately postovarian, 111–148 (133) long by 111–133 (118) wide. Laurer's canal not observed. Uterus occupying

entire hindbody. Proximal portion of uterus functioning as uterine seminal receptacle. Metraterm opening at base of hermaphroditic sac. Hermaphroditic sac pyriform, weakly developed, containing only the hermaphroditic duct. Hermaphroditic duct narrow and short. Genital atrium rounded with thin walls. Genital pore ventral, immediately posterior to pharynx, near cecal bifurcation. Eggs operculate, without polar filaments, 24–43 (36) long by 16–22 (19) wide. Excretory pore terminal, with excretory arms uniting dorsal to oral sucker.

(Morphometric data based on 5 specimens collected from the coast of the state of Rio de Janeiro): Body 4.8–5.7 (5.5) mm long by 752–1,316 (985) wide. Acetabulum 564–846 (665) long by 526–658 (650) wide. Oral sucker 320–470 (402) long by 457–508 (421) wide. Pharynx 126–834 (397) long by 148–423 (230) wide. Sucker width ratio 1:1.3–1.7. Forebody 1.1–1.3 (1.2) mm. Hindbody 3.5–4.7 (4.2) mm. Anterior testis 185–395 (289) long by 281–588 (371) wide. Posterior testis 322–432 (393) long by 319–320 (319) wide. Seminal vesicle 296–564 (396) long by 133–301 (225) wide. Pars prostatica 273–480 (378) long by 259–414 (312) wide. Ovary 259–414 (336) long by 199–376 (279) wide. True seminal receptacle 111 long by 111 wide. Eggs 33–41 (37) long by 14–22 (17) wide.

SYNONYM: *Hysterolecitha elongata* Manter, 1931, of Travassos et al. (1967).

TYPE HOST: *Mugil liza,* Mugilidae, tainha.

SITE OF INFECTION: Stomach and occasionally intestine.

PREVALENCE AND INTENSITY OF INFECTION: (RG 10/141: 3, 1, 1, 1, 1, 9, 15, 6, 2, 3); (CRJ 17/74: 2, 2, 1, 8, 8, 3, 4, 2, 3, 5, 1, 3, 8, 1, 2, 3, 27).

ETYMOLOGY: The specific name refers to the type locality, Brasil.

SPECIMENS EXAMINED: IOC/FIOCRUZ Nos. 30035, 30036, and 30037 (voucher) of *H. elongata* of Travassos et al. (1967).

SPECIMENS DEPOSITED: IOC/FIOCRUZ Nos. 32287 (holotype), and 32288 a, b (paratypes); HWML No. 20772 (paratype).

Discussion

Overstreet (1973), comparing material from the Gulf of Mexico with that of Manter (1931) from Beaufort, North Carolina and that of Travassos et al. (1967), discussed fully the characters of *H. elongata*. He mentioned the differences observed between his material and Manter's. Overstreet suggested that the Brasilian material, hesitantly described by Travassos et al. (1967) as *H. elongata* probably could be considered a different and new species in relation to that described from North America.

According to Overstreet (1973) the Brasilian specimens differ from *H. elongata* by having larger suckers, a longer forebody, larger eggs, numerous prostatic cells within the hermaphroditic sac, a genital atrium, and a true seminal receptacle. We agree with Overstreet (1973), with the exception of the prostatic cells inside the hermaphroditic sac which we were not able to see, and in relation to the uterine seminal receptacle which he might have not seen because of the large quantity of eggs sometimes present. This also happens in our material.

Hysterolecitha brasiliensis sp. n. is characterized by having tegumental plications; well-developed, rounded pars prostatica surrounded by numerous prostatic cells; a true seminal receptacle which is rounded and well developed; the proximal portion of the uterus containing a large quantity of spermatozoa, characterizing the uterine seminal receptacle; and finally, a conspicuous genital atrium. The seminal vesicle is not bipartite although it shows in Figure 1 as if it were. This is due to the position in which the specimen was killed. The drawing in Figure 3, made from another specimen, clearly shows that the seminal vesicle is saccular.

According to King and Noble (1961) the true seminal receptacle is a structure which is lacking in the majority of the species of *Hysterolecitha* with the exception of *H. pseudorosea* (Bravo-Holis, 1956) Yamaguti, 1971, even though Yamaguti (1971) did not include the character in his generic diagnosis.

Hysterolecitha brasiliensis differs from *H. elongata* mainly by the possession of a longer forebody and a shorter hindbody, by the well-developed and rounded pars prostatica surrounded by numerous prostatic cells, by having both rounded and well-developed seminal receptacle and uterine seminal receptacle, and by the conspicuous genital atrium.

Among the more than 18 species so far described in the genus, *H. brasiliensis* is more similar to *H. pseudorosea* due to the presence of a true seminal receptacle and a uterine seminal receptacle. It differs in the shape of the body, the presence of tegumental plications, the position

of the genital pore, the larger size of the eggs, and the presence of a true seminal receptacle.

There is a possibility that the host of this species referred to by Travassos et al. (1967) as *M. platanus* Günther, 1880, is incorrect. According to Menezes (1983), *M. platanus* occurs from Rio de Janeiro to Argentina and has a color pattern identical to *M. liza*. If this is true, *H. brasiliensis,* so far, has been collected from only 1 host species, *M. liza*.

Acknowledgments

We thank Dr. Delir Corrêa Gomes for the loan of specimens deposited at the Helminthological Collection of the Fundação Instituto Oswaldo Cruz, Rio de Janeiro and Dr. Naércio Menezes, of the Universidade de São Paulo, for the identification of the fish hosts. Thanks are also due to SEMA (Secretaria Especial do Meio Ambiente) and to CNPq. (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for partially supporting the authors.

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Revision of the Multicalycidae (Aspidocotylea) with Comments on Postlarval Development

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ABSTRACT: Based upon examination of a large number of specimens from both the Pacific and Atlantic oceans, *Taeniocotyle* Stunkard, 1962, and *Trigonostoma* Szidat, 1966, are considered junior synonyms of *Multicalyx* Faust and Tang, 1936. *Trigonostoma callorhynchi* Szidat, 1966, is considered a synonym of *Multicalyx elegans* (Olsson, 1869). The family Multicalycidae consists of a single genus, *Multicalyx,* and 2 species, *M. elegans* from holocephalans, and *M. cristata* from elasmobranchs. *Multicalyx elegans* matures at a much smaller size than *M. cristata.*

KEY WORDS: *Multicalyx, Taeniocotyle, Trigonostoma,* Platyhelminthes, Multicalycidae, Aspidocotylea, taxonomy, gall bladder, elasmobranch, holocephalan, postlarval development, synonyms.

The study of aspidogastrids in the gall bladder of chondrichthian fishes has a long, confused history (Brinkmann, 1957; Stunkard, 1962; Bray, 1984) that has resulted in the establishment of 3 monotypic genera. Olsson (1869) established the genus *Macraspis* for *M. elegans,* a gall bladder parasite of the holocephalan *Chimaera monstrosa* from the North Sea. Faust and Tang (1936) described *Stichocotyle cristata* from a single specimen collected from the spiral valve of the cownose ray, *Rhinopterus bonasus,* in Biloxi Bay, Mississippi. In order to accommodate their new species into *Stichocotyle* Cunningham, 1884, Faust and Tang (1936) emended the generic description and subdivided the genus into 2 subgenera; *Stichocotyle,* to include *S. (Stichocotyle) nephropis* Cunningham, 1884, and *Multicalyx,* to include *S. (Multicalyx) cristata* Faust and Tang, 1936. Manter (1954) transferred *S. cristata* to *Macraspis* Olsson, 1869. Stunkard (1962), noting that *Macraspis* Olsson, 1869, was preoccupied by *Macraspis* MacLeay, 1819, proposed the new genus *Taeniocotyle* to replace *Macraspis,* and elevated *Multicalyx* to generic rank for *M. cristata,* which he considered generically different. Szidat (1966) established a new genus and species, *Trigonostoma callorhynchi,* for worms from *Callorhynchus callorhynchus* collected off Argentina. He separated his new genus from *Taeniocotyle elegans* (although Szidat did not reference Stunkard [1962] and called it *Macraspis elegans*) based on the presence of a triangular cephalic shield at the anterior end of the worms. The validity of some of the existing genera has been questioned in subsequent papers. Hendrix and Overstreet (1977) stated that *T. elegans* and *M. cristata*

probably belonged to the same genus, and Gibson and Chinabut (1984) based a new family on *Multicalyx* because the validity of *Taeniocotyle* and *Trigonostoma* was open to question.

Our review of the literature in conjunction with our previous studies (Thoney and Burreson, 1986, 1987) indicated that an examination of a large number of specimens was needed to determine if the 3 existing genera, *Multicalyx, Taeniocotyle,* and *Trigonostoma,* were valid. Consequently, a careful review of pertinent literature, and an examination of available deposited specimens and recently collected material was conducted. The review clearly established the need for a revision of the family Multicalycidae.

Materials and Methods

Most of the individuals examined were stained and mounted museum specimens, but some specimens of *M. cristata* from our collections were studied in temporary glycerin mounts. In all, 47 mature specimens, 16 immature specimens, and the relevant literature form the basis of this revision. Important morphological characters were photographed and measurements were made with the aid of an ocular micrometer. Specimens examined are listed below.

Multicalyx cristata

Two mature specimens (1981.11.23.40–45, 1981.11.23.46–48) from the British Museum (Natural History) deposited by R. van der Elst and G. J. B. Ross, respectively; 2 mature (USNM 74467) and 2 immature specimens (USNM 74468, 74469) in the U.S. National Museum Helminth Collection deposited by S. S. Hendrix; 2 immature specimens (USNM 51694) deposited by H. W. Manter; 1 immature specimen (HWML 22977) in the Harold W. Manter Laboratory, University of Nebraska State Museum deposited by H. W. Manter; 3 mature specimens (USNM 79412, 79413) deposited by D. A. Thoney; 13 mature specimens from

Table 1. Meristics and morphometrics of *Multicalyx elegans* (mean with range in parentheses, in millimeters).

	Collection				
	BM (NH)*	MACN	HWML, USNM	USNM	ZMUB
Location	NE Atlantic	SW Atlantic	SW Pacific	NE Pacific	NE Atlantic
Host	*Chimaera monstrosa*	*Callorhynchus callorhynchus*	*Callorhynchus milii*	*Hydrolagus colliei*	*Chimaera monstrosa*
Site	Gall bladder	Gall bladder	Gall bladder	Gall bladder	Gall bladder
No. examined	1	4	8	6	17
Alveoli anterior to ovary	17	12 (10–14)	13 (12–13)	18 (15–22)	14 (13–18)
Alveoli anterior to testis	91	15 (11–26)	40 (31–54)	84 (77–96)	41 (11–77)
Total alveoli	99	21 (11–37)	55 (27–75)	101 (89–113)	49 (11–100)
Total length	15.1	3.6 (2.3–6.5)	11.7 (5.3–15.5)	18.3 (14.8–21.3)	9.0 (2.5–22.6)
% length anterior to ovary	21	52 (46–58)	47 (32–63)	32 (23–37)	48 (24–81)
% length anterior to testis	94	78 (71–85)	81 (65–92)	89 (80–92)	85 (72–90)
Testis L × W	0.70 × 0.62	0.39 (0.14–0.72) × 0.25 (0.14–0.40)	0.80 (0.31–1.40) × 0.54 (0.18–0.95)	0.76 (0.40–1.25) × 0.58 (0.40–1.00)	0.64 (0.08–1.28) × 0.56 (0.10–1.35)
Ovary L × W	0.55 × 0.39	0.30 × 0.15	0.40 (0.17–0.63) × 0.45 (0.15–0.90)	0.61 (0.20–0.80) × 0.40 (0.15–0.60)	0.32 (0.10–0.67) × 0.23 (0.08–0.50)
Egg length	0.12 (0.11–0.13)	0.14 (0.13–0.16)	0.12 (0.10–0.15)	0.12 (0.09–0.14)	0.12 (0.10–0.13)
Author		Szidat, 1966	Manter, 1954	Henrix and Overstreet, 1977	Brinkmann, 1957

* BM (NH) = British Museum (Natural History), MACN = Museo Argentino de Ciencias Naturales, HWML = H. W. Manter Laboratory, USNM = U.S. National Museum, ZMUB = Zoological Museum, University of Bergen.

our own collection; and 2 mature specimens (35 mm slides) loaned by R. A. Campbell, Southeastern Massachusetts University.

Taeniocotyle elegans

One mature specimen (1976.4.8.155–156) from the British Museum (Natural History), deposited by G. Rees; 6 mature specimens (USNM 70861, 70862, 70863) in the U.S. National Museum Helminth Collection, deposited by J. E. Lynch; 1 mature specimen (USNM 49154) deposited by H. W. Manter; 1 immature and 6 mature specimens (HWML 22978) in the Harold W. Manter Laboratory, University of Nebraska State Museum, deposited by H. Manter; and 7 immature and 10 mature specimens (43700, 43701, 43702, 43703, 43704, 43708, 44230) in the Zoological Museum, University of Bergen, Norway, deposited by A. Brinkmann, Jr.

Trigonostoma callorhynchi

One mature and 3 immature specimens (27.949b, 27.950) in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" e Instituto Nacional de Investigacion de las Ciencias Naturales, deposited by L. Szidat.

Results

A revision of the family Multicalycidae is presented below. Comparative meristics and morphometrics of *M. elegans* from various parts of its geographic range are listed in Table 1.

Family Multicalycidae
Gibson and Chinabut, 1984

Multicalyx Faust and Tang, 1936

=*Macraspis* Olsson, 1869; *Taeniocotyle* Stunkard, 1962; *Trigonostoma* Szidat, 1966.

DIAGNOSIS: Body greatly elongated, cylindrical. Ventral haptor extending nearly entire length of body, consisting of a well defined single, continuous row of alveoli, subdivided by transverse septa. Marginal organs present laterally on each transverse septum. Mouth terminal, funnel-shaped; pharynx muscular; intestine simple, terminating blindly at posterior end. Testis single, situated at posterior extremity of uterus. Vas deferens extending anteriorly ventral or lateral to intestine, forming seminal vesicle distally. Cirrus pouch claviform, enclosing seminal vesicle, prostate complex, and ejaculatory duct; protrusible cirrus thick walled, muscular. Genital pore ventral, anterior to haptor. Ovary anterior to testis; uterus descending from ovary posteriorly to level of testis, then turning and ascending anteriorly to genital pore. Eggs operculate, oval in shape, usually filling uterus in mature specimens. Vitellaria consisting of 2 laterally situated bands

of follicles extending from near posterior end anteriorly to level between ovary and genital pore. Two lateral excretory ducts descending posteriorly from level of pharynx to end of vitellaria where ducts fuse to form excretory vesicle and exit through dorso-terminal pore. Parasitic, usually in gall bladder of Chondrichthyes.

TYPE SPECIES: *Multicalyx cristata* Faust and Tang, 1936.

Multicalyx cristata Faust and Tang, 1936
(Figs. 1–3)

=*Stichocotyle* (*Multicalyx*) *cristata* Faust and Tang, 1936; *Macraspis cristata* (Faust and Tang, 1936) Manter, 1954; *Macraspis* sp. of Manter (1954); *Multicalyx multicristata* Parukhin and Tkachuk, 1980.

DIAGNOSIS: Testis elongate, not located at posterior end of worm except in young individuals. Ovary located posterior to 60th alveolus in mature individuals. Marginal organs only slightly protrusible. Testis and ovary not apparent at 16-alveoli stage of development. Pharynx round. Mature individuals may reach 60 cm in length with over 1,500 alveoli. Parasites of gall bladder of elasmobranchs; immature individuals found in littoral teleosts.

Multicalyx elegans (Olsson, 1869) comb. n.
(Figs. 4–10)

=*Macraspis elegans* Olsson, 1869; *Taeniocotyle elegans* (Olsson, 1869) Stunkard, 1962; *Trigonostoma callorhynchi* Szidat, 1966.

DIAGNOSIS: Testis round to slightly oval, located near posterior end of body. Ovary located anterior to 25th alveolus. Testis and ovary apparent when 11 alveoli have developed. Marginal organs highly protrusible. Pharynx elongate. Mature individuals reach 23 mm in length with as many as 113 alveoli. Parasites in gall bladder of holocephalans.

POSTLARVAL DEVELOPMENT: Testis and ovary visible in specimen with 11 alveoli; gonads small, positioned adjacent to each other at posterior end of worm (Fig. 6). Gonads have increased in size when 14 alveoli have developed (Fig. 7), and by 25 alveoli gonads have grown apart by apposition and subsequent elongation of body (Fig. 8). Alveoli develop at posterior end of body and migrate anteriorly between ovary and testis as body elongates. Ovary remains at approximately level of 10th to 22nd alveolus, testis remains at

posterior end of body. This pattern can be seen by examination of Table 1 and Figures 6–8. Number of alveoli anterior to testis is greater in larger, and presumably older, individuals. Specimens from *Hydrolagus collei,* which were all mature, have many more alveoli anterior to testis than those from *Callorhynchus callorhynchus,* which were mostly juveniles. The same trend is seen in percentage of length anterior to ovary, where higher percentages are found in juvenile worms. Worms become mature during the time required for development of 27 to 37 alveoli.

Discussion

Taxonomy

Our examination of the specimens of *Multicalyx cristata, Taeniocotyle elegans,* and *Trigonostoma callorhynchi* indicates that there is insufficient morphological difference to warrant separate genera. Differences in position of testis and ovary, number of alveoli, and shape of testis, pharynx, and marginal organs are considered by us to be specific characters only. *Multicalyx* Faust and Tang, 1936, is the oldest valid nominal genus and has priority; *Taeniocotyle* Stunkard, 1962, and *Trigonostoma* Szidat, 1966, are regarded as junior synonyms.

Szidat (1966) established a new genus and species for his specimens based primarily on the triangular shape of the buccal funnel (Fig. 9). After examination of Szidat's specimens and of many specimens of *M. elegans* (Table 1), we have concluded that the shape of the buccal funnel varies among specimens and probably depends on its state of contraction at the moment of fixation. A triangular buccal funnel was also observed in specimens of *M. elegans* collected from *C. milii* from the southwest Pacific (Fig. 10) and several other characters listed in Table 1 also overlap between these species. In addition, reexamination of Szidat's specimens revealed that the eggs are operculate, as in *M. elegans*. Also, Szidat identified a juvenile specimen of *M. elegans* in the same host as *T. callorhynchi,* indicating that there is no host specificity or geographical boundary between *M. elegans* and *T. callorhynchi.* For these reasons, we consider *T. callorhynchi* a junior synonym of *M. elegans.* *Multicalyx elegans* has been collected from the Pacific and Atlantic oceans in both the Northern and Southern hemispheres.

As indicated in the diagnoses, several significant characters separate *M. cristata* and *M. ele-*
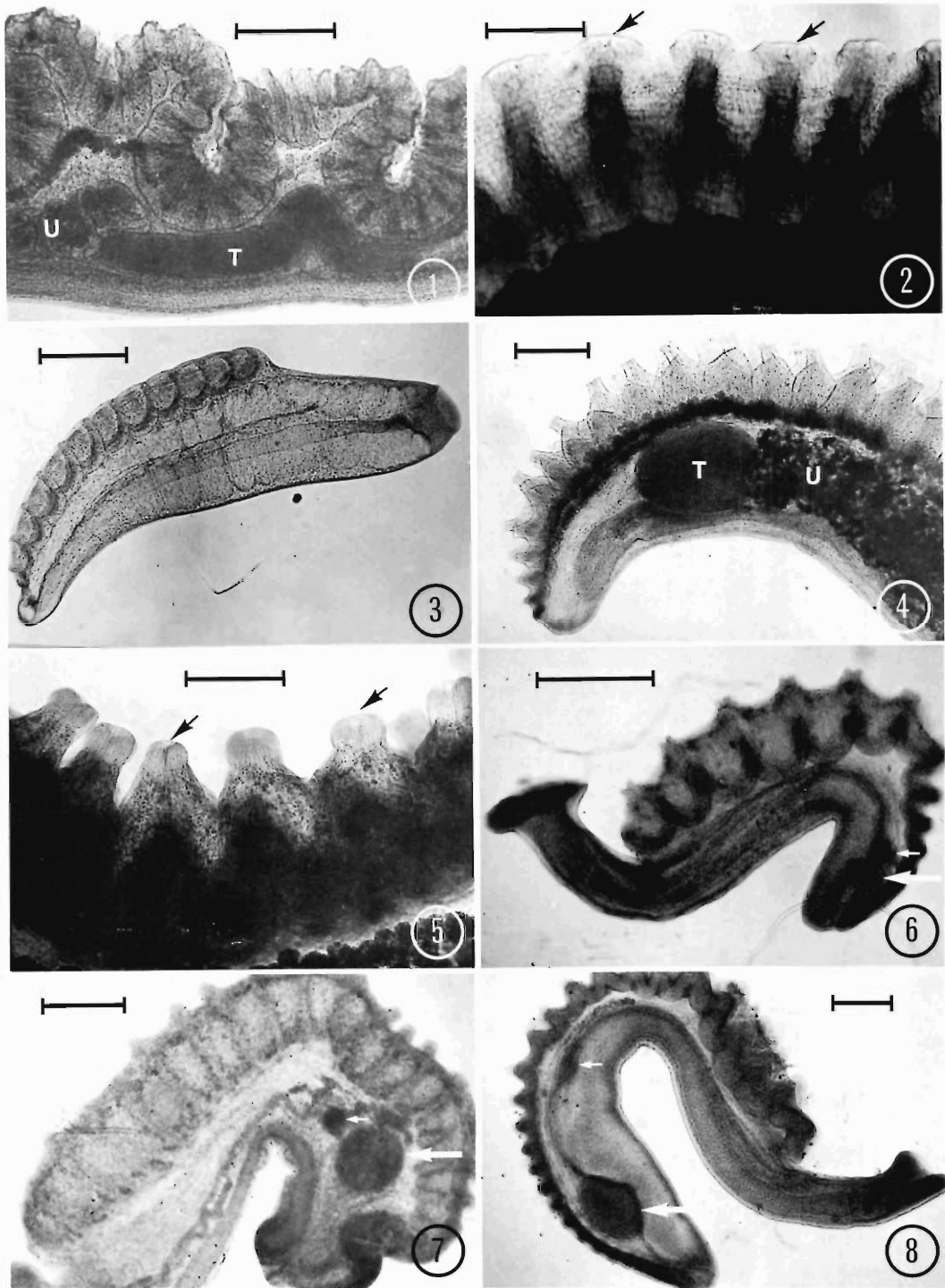
Figures 1–8. Morphology of the genus *Multicalyx*. 1. Midbody view of *M. cristata* showing testis (T) and uterus (U), bar = 0.5 mm. 2. Marginal organs (arrows) of *M. cristata*, bar = 0.15 mm. 3. Juvenile *M. cristata* with 12 alveoli, bar = 0.5 mm. 4. Posterior end of *M. elegans* showing testis (T) and uterus (U), bar = 0.5 mm. 5. Marginal organs (arrows) of *M. elegans*, bar = 0.25 mm. 6. Juvenile *M. elegans* with 11 alveoli showing developing testis (large arrow) and ovary (small arrow), bar = 0.5 mm. 7. Juvenile *M. elegans* with 20 alveoli, arrows as in Figure 6, bar = 0.5 mm. 8. Juvenile *M. elegans* with 26 alveoli, arrows as in Figure 6, bar = 0.5 mm.
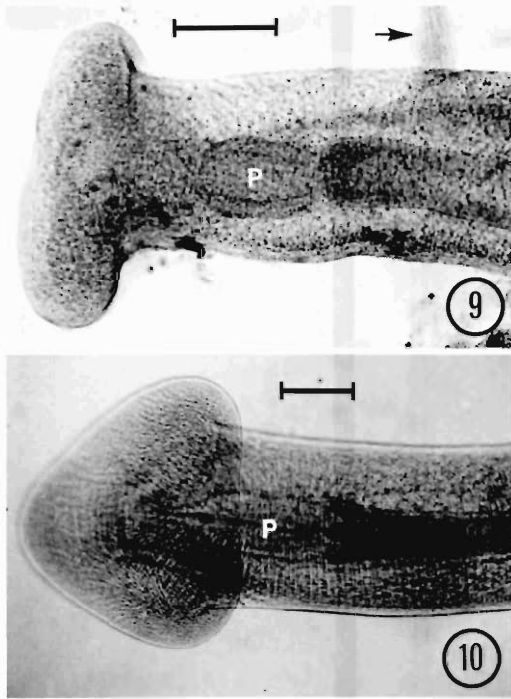
Figures 9, 10. Morphology of the anterior end of
M. elegans. 9. Specimen collected by L. Szidat from
Callorhynchus callorhynchus that he called *Trigono-
stoma callorhynchi,* p = pharynx, arrow = cirrus,
bar = 0.025 mm. 10. Specimen of *M. elegans* collected
by H. Manter from *C. milii* showing obvious triangular
cephalic shield, p = pharynx, bar = 0.25 mm.

gans. Other characters, such as egg size, overlap
greatly (Thoney and Burreson, 1987). The host
specificity of *M. cristata* in elasmobranchs and
M. elegans in holocephalans suggests that these
2 species may have diverged at an early period
of chondrichthyan evolution.

Postlarval development

The pattern of development of *M. elegans* is
very similar to that suggested for immature *M.
cristata* by Thoney and Burreson (1987), where
alveoli produced at the posterior end of the body
continue development anterior to the testis until
some period near maturity. Subsequently, alveo-
li develop posterior to the testis as the body elon-
gates and this development causes the testis to
be positioned relatively more anteriorly in the
body until there may be only 28% of the body
anterior to the testis in *M. cristata,* compared
with greater than 65%, usually 80%, in *M. ele-
gans.* In the specimens of *M. elegans* examined,

only a few alveoli ever occurred posterior to the
testis (Fig. 4).

Specimens collected from littoral teleosts are
thought to be immature *M. cristata* because: (1)
their alveoli resemble the anterior alveoli of adult
specimens (Thoney and Burreson, 1987), (2) they
occur in fishes that are prey of elasmobranchs
(Thoney and Burreson, 1986), (3) they lack pro-
trusible marginal organs, and (4) their gonads are
not as well developed as those of juvenile *M.
elegans* (Fig. 3, cf. 6). The position of the ovary
posterior to the 60th alveolus in adult *M. cristata*
suggests that development of gonads in *M. cris-
tata* may occur at a later stage than in *M. elegans.*
Unfortunately, immature individuals of *M. cris-
tata* have not been collected from elasmobranchs
so it is difficult to identify the specimens from
teleosts with certainty. They are clearly not *M.
elegans,* but they may represent an undescribed
species.

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Helminth Parasites of Ribbon Seals, *Phoca fasciata,* in the Bering Sea and Their Intermediate Hosts

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ABSTRACT: Sixty-one ribbon seals from the spring pack ice of the Bering Sea were examined for helminth parasites. Thriteen species were found: *Anophryocephalus ochotensis, Diphyllobothrium cordatum, D. lanceolatum, Orthosplanchnus fraterculus, Contracaecum osculatum, Pseudoterranova decipiens, Dipetalonema spirocauda, Corynosoma semerme, C. strumosum, C. validum, C. villosum, C. hadweni,* and *Bolbosoma* sp. Four of these (*D. cordatum, D. lanceolatum, O. fraterculus,* and *C. hadweni*) are new host records. Eighteen species of fishes were examined as possible intermediate hosts. All 10 taxa of larval helminths identified from those fishes have previously been found as adults in ribbon seals of the Bering or Okhotsk seas.

KEY WORDS: helminth parasites, *Phoca fasciata,* ribbon seal, *Anophryocephalus ochotensis, Diphyllobothrium cordatum, Diphyllobothrium lanceolatum, Orthosplanchnus fraterculus, Contracaecum osculatum, Pseudoterranova decipiens, Dipetalonema spirocauda, Corynosoma semerme, Corynosoma strumosum, Corynosoma validum, Corynosoma villosum, Corynosoma hadweni, Bolbosoma* sp., new host records, intermediate hosts, fishes.

The ribbon seal, *Phoca fasciata,* is a pelagic pinniped of the Bering and Okhotsk seas that utilizes drifting sea ice as a platform on which to haul out to bear young and to molt. Because ribbon seals are not commonly present in coastal areas of North America and are seldom harvested by Eskimo hunters, specimen material with which to study their parasites has not been readily available. Until recently, specimens for study have been available only in connection with Soviet commercial sealing ventures (Shustov, 1965, 1969; Popov, 1975). From 1976 to 1979, 61 ribbon seals were obtained for study as part of the Alaskan Outer Continental Shelf Environmental Assessment Program (OCSEAP). Seals were collected in the southern ice front of the Bering Sea pack ice from March to June. The results of helminthological examinations of these seals are presented in this paper.

In conjunction with ribbon seal collections, otter trawls were conducted in areas of collections to determine what fishes or invertebrates served as food items for these seals. Fishes identified as food items for ribbon seals were routinely examined for the presence of larval stages of ribbon seal helminths.

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Materials and Methods

Collection locations were: Area I, between the Pribilof Islands and St. Matthew Island; Area II, between St. Matthew and St. Lawrence islands; Area III, north of St. Lawrence Island (Fig. 1). Seals were collected on the pack ice by shooting, returned to the research vessel, and necropsied within approximately 6 hr.

From each animal, the lungs, heart, and liver were excised and examined externally and internally for the presence of helminths. The stomach was excised, opened, and the ingesta removed. The entire intestinal tract was opened and the contents were diluted with tap water, decanted, and examined both macroscopically and under ×10 magnification. Helminths were relaxed in tap water containing a few drops of pentabarbitol sodium (Nembutal) and then fixed in hot 10% formalin. Cestodes, trematodes, and acanthocephalans were stained in Semichon's acetic carmine, dehydrated in ethanol, cleared in terpineol, and mounted in permount. Nematodes were similarly stained, destained in 70% ethanol, and mounted directly in Hoyer's solution, thus greatly enhancing the visibility of intestinal diverticula needed for specific identification. Musculature was not examined for *Trichinella spiralis.*

Fishing with otter trawls was conducted in areas where seals were collected to identify the intermediate hosts of helminths found in ribbon seals. A semiballoon otter trawl constructed of 2.5-cm mesh net with a 0.6-cm mesh cod end liner and headrope length of 5.8 m was used to make tows of 20 min duration at a speed of 2–4 knots (1 knot = 1.852 km/hr). These trawls were conducted on the continental shelf at depths varying from 42 to 214 m. Contents of trawls were sorted and identified using appropriate taxonomic keys (Margolis, 1956; Hartwich, 1974). Fishes were measured and nec-
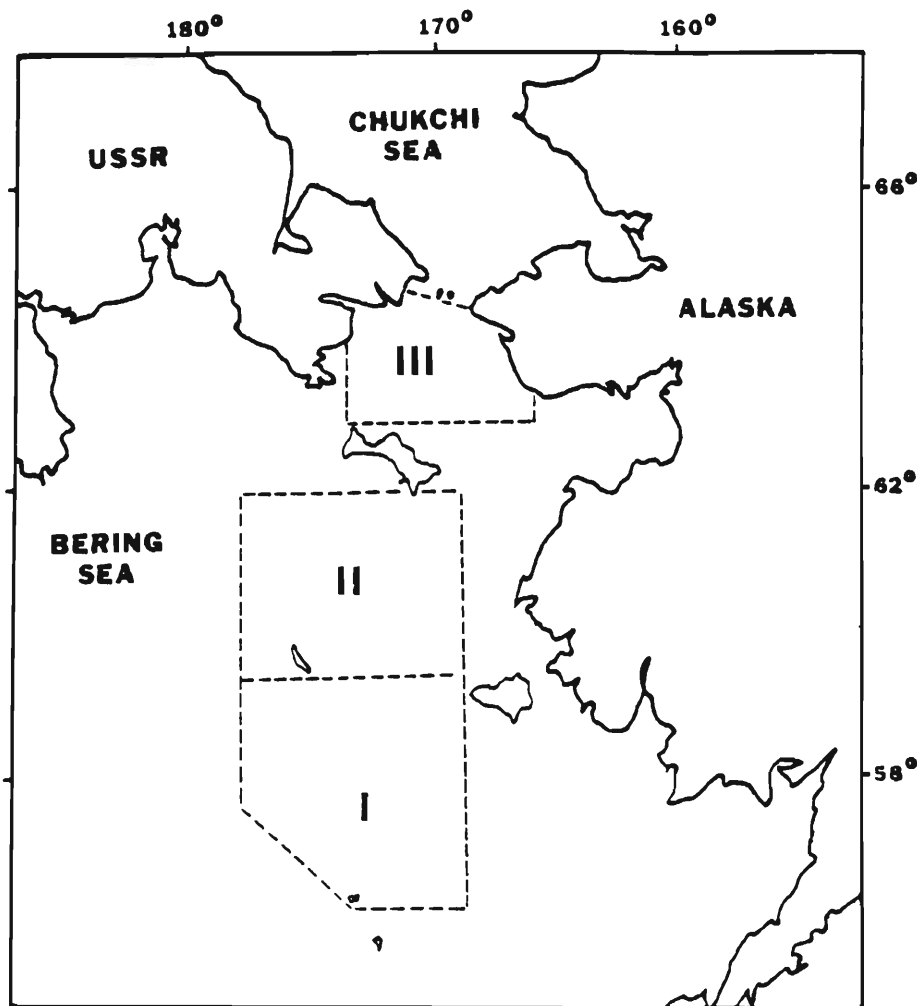
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Figure 1. Geographical locations of ribbon seal collections.

ropsied; recovered helminths were treated in the manner described previously. Representative specimens of each adult helminth have been deposited in the National Parasite Collection, Agricultural Research Service, Beltsville, Maryland (USNM Helm. Coll. Nos. 75940–75952).

Results

Definitive hosts

Helminths were identified from ribbon seals in each of the 3 collection areas (Table 1). Two genera of cestodes (*Diphyllobothrium* and *Anophryocephalus*) were found in the small intestine. Specimens of *A. ochotensis* were present only as immature individuals. Specimens of *D. cordatum* and *D. lanceolatum,* reported for the first time from this host, were mature adults. A single species of trematode, *Orthosplanchnus fraterculus,* also was found in the intestines although this could have been due to postmortem migration from the bile ducts. This is the first time this trematode, a common inhabitant of the bile ducts and gall bladder of bearded seals (*Erignathus barbatus*), sea otters (*Enhydra lutra*), and walruses (*Odobenus rosmarus divergens*), has been reported from ribbon seals. Three species of nematodes were identified: *Contracaecum osculatum* in the stomach and small intestine, *Pseudoterranova decipiens* in clusters attached to the stomach wall, and *Dipetalonema spirocauda* in the heart and testicular sheath. The most commonly encountered helminths were Acanthocephala of 2 genera, including *Bolbosoma* sp. and

Table 1. Prevalence (%) of helminth parasites found in ribbon seals from the Bering Sea in relation to area and time of collection (numbers in parentheses indicate number of seals infected).

Helminth species	USNM #	Area I: March, April 1976, 1977 ($N = 13$)	Area II: April, May 1978, 1979 ($N = 31$)	Area III: May, June 1978 ($N = 17$)
Cestodes				
Anophryocephalus ochotensis	75942	8 (1)	6 (2)	12 (2)
Diphyllobothrium cordatum	75940	—	6 (2)	—
D. lanceolatum	75941	—	3 (1)	—
Trematodes				
Orthosplanchnus fraterculus	75943	8 (1)	—	6 (1)
Nematodes				
Contracaecum osculatum	75944	85 (11)	98 (30)	100 (17)
Pseudoterranova decipiens	75945	31 (4)	16 (5)	6 (1)
Dipetalonema spirocauda	75946	—	3 (1)	—
Acanthocephala				
Corynosoma semerme	75947	23 (3)	87 (27)	94 (16)
C. strumosum	75948	92 (12)	81 (25)	76 (13)
C. validum	75949	—	45 (14)	18 (3)
C. villosum	75950	31 (4)	—	—
C. hadweni	75951	—	13 (4)	6 (1)
Bolbosoma sp.	75952	8 (1)	16 (5)	6 (1)

5 species of *Corynosoma.* All were restricted to the small intestine except *C. semerme* which was found at the ileo–caecal junction.

Three species were found only in seals from Area II and 1 only in Area I. *Contracaecum osculatum* and *Corynosoma semerme* increased in prevalence from south to north (Area I to Area III), whereas *Pseudoterranova decipiens* and *Corynosoma strumosum* decreased. The observed trend was significant by the *G*-test (Sokal and Rohlf, 1969) only for *C. semerme* (Area I vs. Area II, $G = 14.216$, alpha $= 1$, $P < 0.005$; Area I vs. Area III, $G = 14.138$, alpha $= 1$, $P < 0.005$). All seals from Area I were collected in 1976–1977 whereas those from Areas II and III were collected in 1978–1979. Since these samples were all obtained from the same migrant ribbon seal population (Burns, 1981), the observed differences and suggested trends probably can be attributed to small sample size rather than to any real differences between areas.

Intermediate hosts

Nine taxa of larval helminths were found in the 18 species of fishes we examined (Table 2). Nematodes, particularly *Pseudoterranova decipiens* were the most common, occurring in 13 of 18 (72%) of the species. Diphyllobothriid plerocercoids occurred in 50% of the fish host species

and Acanthocephala, represented by 4 species of the genus *Corynosoma,* in 61%.

Because fishes were opportunistically examined and samples were small and nonsystematic, it was not possible to determine prevalence of infection.

Discussion

Four of the species identified from ribbon seals in this study were new host records (Table 3). They included the cestodes *Diphyllobothrium cordatum* and *D. lanceolatum,* which are common in bearded seals and not present in other Bering Sea phocids; the trematode *Orthosplanchnus fraterculus,* also found in bearded seals; and an acanthocephalan, *Corynosoma hadweni,* which has been reported from ringed seals (*Phoca hispida*) (Margolis and Dailey, 1972).

All other helminths identified in this study, with the exception of *Dipetalonema spirocauda,* have been previously identified from Bering Sea ribbon seals (Table 3; Shustov, 1965; Yurakhno et al., 1968). The 3 species we found to be most common, *Contracaecum osculatum, Corynosoma semerme,* and *C. strumosum,* were also the most common in other western and northern Bering Sea samples. In the northern Bering Sea ($N = 1,207$), Shustov (1965) found *Contracae-*

Table 2. Intermediate hosts of ribbon seal helminths from the Bering Sea. Only fishes known to be ribbon seal prey are included. Numbers following helminth species correspond to fish hosts listed in column 1.

Fish host	Helminth species	Infection site
Clupeidae		
1. *Clupea harengus*	*Anisakis* sp.	Intestinal mesentery
Osmeridae		
1. *Mallotus villosus*	*Contracaecum osculatum*	Intestinal mesentery
Gadidae		
1. *Boreogadus saida*	*Contracaecum osculatum* 1, 4	Intestinal mesentery
2. *Eleginus gracilis*	*Pseudoterranova decipiens* 1, 4	Intestinal mesentery
3. *Gadus macrocephalus*	Diphyllobothriidae 1, 3, 4	Cyst in stomach wall
4. *Theragra chalcogramma*	*Pyramicocephalus phocarum* 2	Cyst in stomach wall
	Corynosoma strumosum 3	Intestinal mesentery
	C. villosum 3	Intestinal mesentery
	Anisakis sp. 3, 4	Intestinal mesentery
Stichaeidae		
1. *Lumpenus maculatus*	Diphyllobothriidae	Cyst in stomach wall
Zoarcidae		
1. *Lycodes palearis*	*Pseudoterranova decipiens* 1, 2	Intestinal mesentery
2. *L. raridens*	Diphyllobothriidae 1, 2	Cyst in stomach wall
	Contracaecum osculatum 2	Intestinal mesentery
	Corynosoma strumosum 1	Intestinal mesentery
	C. validum 1, 2	Intestinal mesentery
	C. semerme 2	Intestinal mesentery
Cottidae		
1. *Dasycottus setiger*	*Corynosoma semerme* 3, 6, 7	Intestinal mesentery
2. *Gymnocanthus galeatus*	*C. strumosum* 3, 6, 7	Intestinal mesentery
3. *Hemilepidotus jordani*	*C. validum* 5, 6, 7	Intestinal mesentery
4. *Icelus spiniger*	*Contracaecum osculatum* 1, 7	Intestinal mesentery
5. *Myoxocephalus jaok*	*Anisakis* sp. 2, 3, 7	Intestinal mesentery
6. *M. polyacanthocephalus*	*Pseudoterranova decipiens* 2, 3, 4, 5, 7	Intestinal mesentery
7. *M. scorpius*	Diphyllobothriidae 3, 6	Cyst in stomach wall
Cyclopteridae		
1. *Liparis* sp.	Diphyllobothriidae	Cyst in stomach wall
	Corynosoma validum	Intestinal mesentery
Pleuronectidae		
1. *Hippoglossoides elassodon*	*Corynosoma* sp.	Intestinal mesentery

cum to be present in the stomachs of all ribbon seals older than 1 yr.

The helminth fauna reported from ribbon seals from the Okhotsk Sea (Popov, 1975, 1976) is similar to that in Bering Sea seals. Of the 13 species identified from Okhotsk Sea seals, only 3 (*Anophryocephalus skrjabini, Anisakis simplex,* and *Otostrongylus circumlitus*) have not also been found in Bering Sea ribbon seals (Table 3). As in the Bering Sea, *Contracaecum osculatum* and *Corynosoma strumosum* were 2 of the most common helminths, occurring in over 50% of the seals examined. *Corynosoma semerme* was considerably less common and *Anisakis* sp. far more common in Okhotsk Sea seals.

Geographic trends in frequency of occurrence were not apparent for the most common species in all areas, *Contracaecum osculatum*. Two *Corynosoma* species did suggest west to east or south to north trends. In our samples the prevalence of *C. semerme* was greatest in the northernmost Areas II and III (87% and 94%) and lowest in the southernmost Area I (23%) (Table 1). The prevalence for Okhotsk Sea seals, 14% for northern and southern samples combined (Popov, 1975, 1976), was lower still, suggesting that the north-to-south trend was real rather than an artifact of sample size. *Corynosoma strumosum* was equally common in Okhotsk Sea and western Bering Sea seals (51% and 50%, respectively) and

Table 3. **Comparative helminth faunas of ribbon seals of the Bering and Okhotsk seas expressed as percent infected.**

Helminth species	Popov (1975): S Okhotsk (N = 230)	Popov (1976): N Okhotsk (N = 80)	Shustov (1969): W Bering (N = 17)	Delyamure and Yu-rakhno (1974): NW Bering (N = 221)	Delyamure et al. (1976): W Bering (N = 9)	This study: Central Bering (N = 61)
Cestodes						
Anophryocephalus ochotensis	—	—	—	1	—	8
A. skrjabini	1	—	—	—	—	—
A. pacificus	—	0	0	1	—	—
Diplogonoporus tetrapterus	12	—	—	3	—	—
Diplogonoporus sp.	—	—	12	1	—	—
Diphyllobothrium cordatum	—	—	—	—	—	5
D. lanceolatum	—	—	—	1	—	2
Diphyllobothrium sp.	—	—	17	—	—	—
Diphyllobothriidae gen. sp.	15	6	—	—	—	—
Pyramicocephalus phocarum	7*	1	—	1	—	—
Trematodes						
Orthosplanchnus fraterculus	—	—	—	—	—	3
O. arcticus	—	—	—	8	—	—
Nematodes						
Contracaecum osculatum	82	88	71	68	44	95
Pseudoterranova decipiens	1	—	6	2	—	16
Phocascaris cystophorae	55*	65*	—	63	56	—
P. phocae	—	—	59	—	—	—
Anisakis simplex	1	—	—	—	11	—
A. pacificus	46	—	—	—	—	—
Anisakis sp.	32*	11	—	—	—	—
Anisakidae gen. sp.	46*	4*	53	—	33	—
Terranova azarasi	1	—	—	11	33	—
T. decipiens	1	—	—	—	—	—
Terranova sp.	1	1	—	—	—	—
Otostrongylus circumlitus	1	—	—	—	—	—
Dipetalonema spirocauda	1	—	—	—	—	2
Acanthocephala						
Corynosoma semerme	10	24	12	56	44	75
C. strumosum	46	64	47	51	44	82
C. validum	2	8	—	14	11	28
C. villosum	1	—	—	—	11	7
C. hadweni	—	—	—	5	—	10
C. ventronudum	—	—	—	20	—	—
Bolbosoma nipponicum	12	3	29	1	11	—
Bolsosoma sp.	—	—	—	—	—	11

* Denotes maximum value—sites of infection listed separately.

most common in our northcentral samples (82%). One other species, *C. validum,* also showed an apparent geographic trend. It was least common in Okhotsk Sea seals (3%), somewhat more common in western Bering Sea seals (13%), and most common in northcentral samples (28%).

Soviet investigators have regarded the observed differences in helminth faunas between regional samples as indicative of localized populations of ribbon seals (Shustov, 1965). With the present state of knowledge of these seals and their parasites, this is highly speculative. Differences between samples could be due to annual, seasonal, or regional differences in the diet of the seals, or to changes in availability of infected intermediate hosts.

The effects on ribbon seals of infection with helminths are largely unknown. Yurakhno (1971) speculated that helminths are responsible for the high mortality rate in both young and old seals. He stated that *Orthosplanchnus arcticus* causes severe growth of connective tissue around ducts and vessels of the liver as well as between and within the liver lobules; *Bolbosoma nipponicum*

frequently perforates the intestinal wall; and *Phocascaris cystophorae* seriously affects the alimentary tract. He reported that a 3-yr-old female with 372 helminths present (species unspecified) gave birth to a dead pup, and from that concluded that helminths do particular harm to pregnant females.

Shustov (1969) reported that nematode infestations in ribbon seals caused damage to mucosa membranes of the stomach and small intestine. Ulcers were sometimes present with damage extending into submucosal and muscular layers. Popov (1975) considered *B. nipponicum* and the nematodes *C. osculatum* and *D. spirocauda* to be the most pathological helminths of Okhotsk Sea ribbon seals. None of the ribbon seals examined in the present study exhibited any of the pathological conditions described above and we suggest that helminths play a limited role in the mortality of ribbon seals, at least in the population from which our specimens were collected.

Ribbon seals in the Bering Sea eat primarily fishes, and, to a lesser extent, invertebrates such as octopus and shrimps (Shustov, 1965; Frost and Lowry, 1980; Burns, 1981). All of the fishes which we examined for helminth parasites (Table 2) have been reported as ribbon seal prey. The major prey of the 61 seals considered in this study were pollock, arctic cod, and eelpout (Frost and Lowry, 1980).

Of the 13 taxa of helminths we identified from ribbon seals, only 4 (*Orthosplanchnus, Dipetalonema, Corynosoma hadweni,* and *Bolbosoma*) were not found in the fishes we examined. Conversely, all taxa of larval helminths identified from fishes in this study have been previously found as adults in ribbon seals of the Bering or Okhotsk Seas (Table 3). Only 2, *Pyramicocephalus phocarum* and *Anisakis* sp., were not found in the 61 ribbon seals examined by us.

Detailed life history studies of these helminths and their hosts are needed before helminth faunas can be utilized as indicators of population discreteness for ribbon seals in the Bering Sea.

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Some Helminth Parasites of Chipmunks, *Eutamias* spp. (Sciuridae) in Southern Nevada

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ABSTRACT: One hundred seventy-five chipmunks, *Eutamias panamintinus* and *E. palmeri,* collected from 4 counties in southern Nevada were necropsied for helminth parasites. Four species of worms were recovered including 1 of Acanthocephala, *Moniliformis moniliformis,* and 3 of Nematoda, *Syphacia eutamii, Heteroxynema cucullatum,* and *Pterygodermatites coloradensis.* A discussion of each of these parasites is presented.

KEY WORDS: *Moniliformis moniliformis, Syphacia eutamii, Heteroxynema cucullatum, Pterygodermatites coloradensis,* survey, pathogenicity, *Eutamias panamintinus, Eutamias palmeri,* chipmunks, helminths, Nematoda, Acanthocephala.

Nevada is one of the few states in which extensive parasitological studies of vertebrates have not been undertaken, although it is recognized that certain lower animals may play an important role in the transmission of certain parasitic diseases to man. The present investigation is concerned with a helminthological survey of 2 species of chipmunks of the genus *Eutamias.* Hall (1946) and Blair et al. (1957) list 6 species of the genus as occurring in Nevada—*E. palmeri, E. quadrivittatus, E. dorsalis, E. panamintinus, E. amoenus,* and *E. speciosus.* However, according to Dr. W. G. Bradley, Mammalogist, of this Department only the first 4 of these species are found in the southern part of the state. Two species, *E. palmeri* and *E. panamintinus,* were included in this survey.

Materials and Methods

Despite extensive trapping efforts by the writers within areas of southern Nevada where 4 species of chipmunks are known to occur, only 2 species of *Eutamias* were collected—*E. panamintinus* (7 animals) and *E. palmeri* (168 animals). The hosts were taken from 4 counties in southern Nevada—Nye, Esmeralda, Lincoln, and Clark. Collections were made with Museum Specials, Victor rat traps, and Sherman live traps. A mixture of peanut butter and oatmeal was used as bait. Dead chipmunks were placed in plastic bags, brought to the laboratory, sexed, and weighed prior to necropsy. Live animals were kept in cages until sacrificed. Most animals were examined within 24 hr after collection. Necropsies were performed in accordance with routine parasitological procedures. Specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland under USNM Helm. Coll. Nos. 79865–79870.

Results and Discussion

Of the 175 chipmunks collected, only 4 species of helminths were recovered including 1 of Acan-thocephala and 3 of Nematoda. A discussion of each of these parasites is presented.

Acanthocephala

Moniliformis moniliformis (Ward, 1917)

Of the 175 chipmunks necropsied, 5 animals were infected with this species; the range of infection being from 1 to 3 specimens per host. The parasites were located in the small intestine wherein most had their proboscises embedded within the intestinal mucosa. Morphological study of specimens showed that they fit the description of the species as presented by Ward (1917). *Moniliformis moniliformis* appears to infect a variety of rodent hosts (Van Cleave, 1953; Yamaguti, 1963). Study of the life cycle of the species was undertaken by several investigators including Vitale (1935), Sita (1949), Coronel Guevara (1953), and Nazarova (1959). Oshima (1953) experimentally showed that the species could develop in the roach, *Blattella americanus.* Several other investigators found *Periplaneta americana* to be the 1st intermediate host for the parasite (Von Ihering, 1902; Seurat, 1912; Southwell, 1922; Bacigalupo, 1927). Yamaguti (1963) stated toads, frogs, and lizards may serve as paratenic hosts for helminths. Beck (1959) reported an infection in humans.

In the present study, lesions due to the attachment of the parasite's proboscis to the intestinal wall were evident. Moore (1946) reported fatal peritonitis in flying squirrels, *Glaucomys volans,* due to heavy infections with *M. dubius.* It is conceivable that *M. moniliformis,* likewise, could initiate such a pathological condition. Because of its large size, the species possibly could occlude the intestinal lumen if present in large numbers. The pathogenic potential of *M. moniliformis* is

offset by its infrequent and localized occurrence (Parker, 1971; Welborn, 1975).

Nematoda

Genus *Syphacia* Seurat, 1916

Syphacia eutamii Tiner, 1948

Worms of the genus *Syphacia* are cosmopolitan in distribution, being found in the cecum and large intestine of rodents throughout much of the world. Yamaguti (1961) listed 23 species as comprising the genus, although a number of additional species from rodents have since been described. Ogden (1971) redescribed and compared 11 species of *Syphacia* using mean-value data, charts, and graphs, based upon mathematical computations. A review of the genus *Syphacia* is presented by Quentin (1971), in which he distributed the species of the genus into 10 groups based upon morphological similarities. In his first 3 groups, he placed some species of *Syphacia* parasitizing Sciuridae.

Syphacia eutamii was collected from both *Eutamias panamintinus* and *E. palmeri* living in localities of Pahrump (Nye County) and higher elevations of Mt. Charleston (Lincoln County) in southern Nevada. The parasites were free within the cecum and large intestine and no recognizable lesions could be attributable to the nematodes. The range of intensity of infection was 2–9 worms per host, with a mean of 5. *Syphacia eutamii* was described by Tiner (1948) from the cecum and large intestine of the chipmunk, *Eutamias minimus,* collected in Minnesota. The species is readily recognizable since, according to Tiner, it is the only North American member of the genus possessing 2 rather than 3 mammelons and with a poorly chitinized gubernaculum.

Genus *Heteroxynema* Hall, 1916

Heteroxynema cucullatum Hall, 1916

This genus was established by Hall (1916) with *H. cucullatum* as genotype, being collected in Colorado from *Eutamias amoenus amoenus.* Frandsen and Grundman (1959) later collected the nematode from 4 species of *Eutamias* in Utah. From 175 chipmunks collected, *H. cucullatum* was recovered 109 times (62%), being taken from the lumen of the cecum and large intestine. The number of specimens collected ranged from 3 to 15 per host. Most worms were females. A morphological comparison was made between the Nevada *Heteroxynema* and the original description as presented by Hall (1916) and the one later given by Yamaguti (1961). The Nevada specimens seem to resemble closer the description of *H. cucullatum* as presented by Hall, although the females appear to be somewhat larger, ranging in length from 5 to 12 mm in comparison to the 7.39–7.90 mm as cited by Hall. There were also several other variations shown by the Nevada specimens—i.e., a somewhat longer esophagus with a smaller bulb, and a slightly different egg size range, being 0.09–0.19 mm long by 0.03 mm wide for *H. cucullatum* and 0.09–0.10 mm by 0.03–0.04 mm for the Nevada specimens. These variations, however, were considered by the writers to be only minor differences within the species. Lesions associated with *Heteroxynema* infections were not observed in this study.

Genus *Pterygodermatites* Quentin, 1969

Pterygodermatites coloradensis (Hall, 1916)

This species was collected in the Mt. Charleston area of Lincoln County from 2 chipmunks, *E. palmeri,* which indicate a new host and locality record. The Nevada nematode agrees closely with the description of *P. coloradensis* described from *Eutamias quadrivittatus* by Hall (1916) and Lichtenfels (1970). Two specimens (male and female) were recovered from the Nevada chipmunks. Morphology of the cuticle of *Pterygodermatites* in possessing spines and comb plates suggests that the parasite could cause pathological damage.

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Cuticular Ridge Patterns of Seven Species of Ostertagiinae (Nematoda) Parasitic in Domestic Ruminants

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ABSTRACT: The cuticular ridge patterns (synlophes) of 7 species of *Ostertagia* and *Teladorsagia* were studied. Different synlophes were found for *Ostertagia ostertagi, Ostertagia leptospicularis,* and *Teladorsagia circumcincta.* The other 4 species had synlophes identical to 1 of the 3 different patterns. The synlophe of *Ostertagia lyrata* was identical to that of *O. ostertagi,* that of *Ostertagia kolchida* was identical to that of *O. leptospicularis,* and both *Teladorsagia trifurcata* and *Teladorsagia davtiani* had synlophes identical to *T. circumcincta.* Previous studies with other trichostrongyloid nematodes indicated the synlophe to be useful for separating species. Thus, the absence of differences in the synlophes within these 3 groups of species provides new evidence to support the proposal for polymorphism in the Ostertagiinae. *Ostertagia leptospicularis* and *O. kolchida* could be separated from the other species by an elongated esophageal valve.

KEY WORDS: *Ostertagia ostertagi, Ostertagia lyrata, Ostertagia leptospicularis, Ostertagia kolchida, Teladorsagia circumcincta, Teladorsagia trifurcata, Teladorsagia davtiani,* synlophes, nematode morphology, Nematoda, cuticle, Ostertagiinae, Trichostrongyloidea, ruminants, SEM.

The Ostertagiinae are among the most severe pathogens of domestic ruminants, especially cattle (American Association of Veterinary Parasitologists, 1983). Proper management of ostertagiasis is hampered because the systematics of the Ostertagiinae are unsettled with no agreement on how many genera and species are present in domestic ruminants (Jansen and Gibbons, 1981). The systematics of this group were plunged into additional controversy when Lancaster and Hong (1981) and Lancaster et al. (1983) proposed that the 7 species listed in Table 1 are polymorphs of only 3 species. The polymorphism proposal was based on: (1) observations that some species always were found together, with 1 species constituting a major proportion of a population and another species a minor proportion; (2) observations of consistent morphological features in all the minor species—stout spicules and enlarged genital cone dorsally; and (3) cross-breeding studies in which 1 male morphotype was mated with unidentified females to produce males of more than 1 morphotype.

The present study was undertaken to determine whether characteristics of the synlophe (the pattern of longitudinal cuticular ridges on the surface of the nematode) differed among the 7 male morphotypes (or species) included in the polymorphism proposal (Lancaster and Hong, 1981; Lancaster et al., 1983), and if so whether females could be matched with the males. The synlophe has been found to be useful for identifying both males and females of many species of trichostrongyloid nematode parasites of ruminants (Lichtenfels, 1983; Lichtenfels and Pilitt, 1983a, b; Lichtenfels et al., 1986).

Materials and Methods

Nematodes

Six experimental strains of nematodes, including 6 presumptive species, were provided by the Central Veterinary Laboratory, Weybridge, England. These strains were described by Lancaster et al. (1983). Species included in the Weybridge strains were *Ostertagia ostertagi, O. lyrata, O. leptospicularis, O. kolchida, Teladorsagia circumcincta,* and *T. trifurcata.* In these strains selective mating was used to increase the proportion of the minor species *O. lyrata, O. kolchida,* and *T. trifurcata* in the populations to more than 50% (Lancaster et al., 1983). Selection for *T. trifurcata* did not increase the proportion of *T. davtiani* in the population. Instead, *T. davtiani* disappeared from the selected population by the 4th generation (Lancaster et al., 1983). Additional specimens of the 7 species (including *T. davtiani*), mostly from naturally occurring infections (Table 1), were obtained from the National Parasite Collection, Agricultural Research Service, USDA, Beltsville, Maryland. The specimens from Weybridge, England were accessioned as USNM Helminthological Collection Numbers 79692–79696.

Microscopy

Specimens were studied either as: (1) temporary whole mounts cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) and examined with regular light microscopy or interference–contrast light microscopy at a magnification of 400; or

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Table 1. Specimens of Ostertagiinae studied, by host and locality.

Species and synonyms	Number of lots/number of specimens by host, locality, and sex
Ostertagia ostertagi (Stiles, 1892) Ransom, 1907. Syn. (according to Ransom, 1911): *Strongylus convolutus* Ostertag, 1890 nec Kuhn, 1829; *S. ostertagi* Stiles, 1892; *Strongylus* sp. Harker, 1893 (in part); *S. cervicornis* Gilruth, 1899; *S. harkeri* Stoeder, 1901 (in part); *Ostertagia caprae* Andreeva and Nikolsky, 1957	*Bison bison,* buffalo New York, 1/2 ♂ Wyoming, 1/5 ♂ *Bos taurus,* cattle England, 1/9 ♂ Louisiana, 1/6 ♂ Maryland, 4/8 ♂, 4 ♀ *Odocoileus virginianus,* white-tailed deer Georgia, 1/2 ♂ *Oreamnos americanus,* mountain goat South Dakota, 4/9 ♂
Ostertagia lyrata Sjöberg, 1926 nec *O. lyrata* of Bye and Halvorsen, 1983. Syn.: *Sjobergia lyrata* (Sjöberg, 1926) Sarwar, 1956; *Skrjabinagia lyrata* (Sjöberg, 1926) Andreeva, 1957; *Grosspiculagia lyrata* (Sjöberg, 1926) Jansen, 1958; (according to Travassos, 1937) *Ostertagia occidentalis* of Gebauer, 1932 (nec Ransom, 1907)	*Bison bison,* buffalo New York, 1/1 ♂ *Bos taurus,* cattle England, 1/12 ♂ Louisiana, 1/1 ♂ Maryland, 2/6 ♂ Mississippi, 1/5 ♂
Ostertagia leptospicularis Assadov, 1953. Syn.: *Capreolagia skrjabini* Schulz, Andreeva, and Kadenazii, 1954; *Ostertagia capreolagi* Jansen, 1958; *O. capreoli* Andreeva, 1957; *O. taurica* Kadenazii and Andreeva, 1958; *O. crimensis* Kadenazii and Andreeva, 1958; *Capreolagia antipini* Kadenazii, 1957; *C. paraskrjabini* Kadenazii, 1957; *O. paracapreoli* Kadenazii and Andreeva, 1957	*Bos taurus,* cattle California, 1/9 ♂ *Ovis aries,* sheep England, 1/21 ♂, 14 ♀
Ostertagia kolchida Popova, 1937. Syn.: *Sjobergia kolchida* (Popova, 1937) Sarwar, 1956; *Skrjabinagia kolchida* (Popova, 1937) Andreeva, 1956; *G. kolchida* (Popova, 1937) Jansen, 1958; *O. popovi* Kassimov, 1942; *Skrjabinagia popovi* (Kassimov, 1942) Altaev, 1952; *Grosspiculagia popovi* (Kassimov, 1942) Jansen, 1958; *O. lasensis* Assadov, 1953; *G. lasensis* (Assadov, 1953) Jansen, 1958; *S. lasensis* (Assadov, 1953) Andreeva, 1957; *Muflonagia podjapolski* Schulz, Andreeva, and Kadenazii, 1954; *S. podjapolski* of Andreeva, 1957; *G. podjapolski* of Jansen, 1958	*Bos taurus,* cattle Oregon, 1/2 ♂ *Capra hircus,* goat England, 1/14 ♂
Teladorsagia circumcincta (Stadelmann, 1894), Dróżdż, 1965. Syn.: *Strongylus circumcinctus* Stadelmann, 1894; *S. vicarius* Stadelmann, 1893; *S. cervicornis* McFadyean, 1897; *S. instabilis* Julien, 1897; *Ostertagia (O.) circumcincta* (Stadelmann, 1894) Ransom, 1907; *O. turkestanica* Petrov and Shakhovtseva, 1926; *Stadelmannia circumcincta* (Stadelmann, 1894) Sarwar, 1956; *Ostertagiella circumcincta* (Stadelmann, 1894) Andreeva, 1957	*Antilocapra americana,* pronghorn South Dakota, 1/1 ♂ *Bos taurus,* cattle California, 1/9 ♂ *Capra hircus,* goat England, 1/17 ♂, 14 ♀ *Oreamnos americanus,* mountain goat Canada, 2/8 ♂ South Dakota, 4/25 ♂ *Ovibos moschatus,* muskox Alaska, 1/1 ♂ *Ovis aries,* sheep Mississippi, 1/8 ♂ Missouri, 1/4 ♂ Nebraska, 1/2 ♂ *Ovis dalli,* Dall sheep Alaska, 1/2 ♂
Teladorsagia trifurcata (Ransom, 1907) Dróżdż, 1965. Syn.: *Ostertagia trifurcata* (Ransom, 1907; *Ostertagiella trifurcata* (Ransom, 1907) Andreeva, 1957; *Stadelmannia trifurcata* (Ransom, 1907) Sarwar, 1956; *Teladorsagia davtiani* Andreeva and Satubaldin, 1954; *Ostertagia pinnata* Daubney, 1933; *Stadelmannia pinnata* (Daubney, 1933) Sarwar, 1956; *T. pinnata* (Daubney, 1933) Dróżdż, 1965; (according to Yen, 1963) *Ostertagia erschowi* Hsu, Ling, and Liang, 1957	*Oreamnos americanus,* mountain goat Canada, 1/1 ♂ South Dakota, 2/2 ♂ *Ovis aries,* sheep England, 1/19 ♂, 4 ♀ California, 1/1 ♂ Maryland, 3/6 ♂ Indiana, 1/5 ♂ Wyoming, 1/1 ♂

Table 1.   Continued.

Species and synonyms	Number of lots/number of specimens by host, locality, and sex
Teladorsagia davtiani Andreeva and Satubaldin, 1954, *in* Skrjabin et al. (1954) (Considered herein to be a synonym of *T. trifurcata,* but listed separately to allow listing of specimens studied)	*Oreamnos americanus,* mountain goat Canada, 2/6 ♂ *Ovis aries,* sheep England, 1/2 ♂ Scotland, 1/1 ♂ Maryland, 2/12 ♂ Missouri, 1/2 ♂ Vermont, 1/2 ♂ Washington, 1/1 ♂ West Virginia, 1/6 ♂

(2) critical point dried, coated with gold palladium, and viewed at 5–20 kV with scanning electron microscopy (SEM) (Madden and Tromba, 1976).

Characters studied

Male specimens were identified to species on the basis of the morphology of the spicules and genital cone (Andreeva, 1958; Rose, 1960; Dróżdż, 1965; Becklund and Walker, 1971) prior to study of the synlophe and esophagus. Bursal ray patterns were determined and described using the system of Durette-Desset and Chabaud (1981). Cross sections were studied in either freehand cuts made with a cataract knife or in paraffin-embedded sections. The position of the subventral esophageal gland orifices (SVGO) (Caveness, 1964) relative to the cervical papillae was determined. The distance of both the SVGO and the cervical papillae from the anterior end was measured (Table 2). The lengths of the esophageal valves (determined to extend from the posterior end of the 3 cuticular bands that line the triradiate lumen of the esophagus to the posterior end of the esophagus (Figs. 13, 15, 17) were measured (Table 2). Measurements are in micrometers unless indicated otherwise.

Synonymies in Table 1 follow Dróżdż (1965) and Skrjabin et al. (1954) unless noted otherwise.

Results

Among the 7 species studied, previously unknown characteristics of the synlophe (Figs. 1–11) and esophagus (Figs. 12–17) were discovered that were used to group the 7 species into 3 groups.
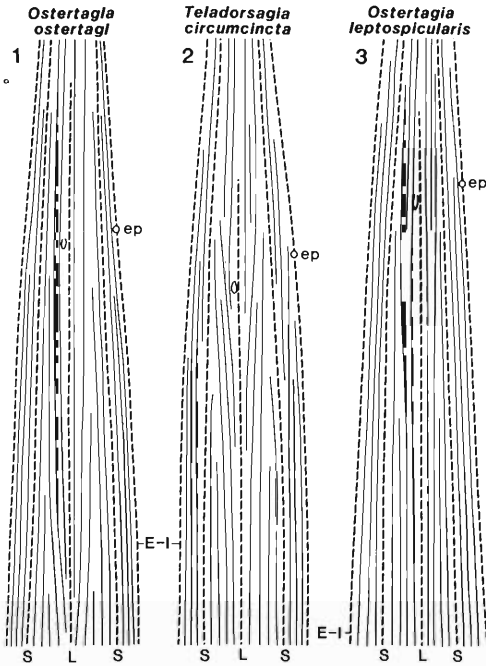
Synlophes

All species studied had a similar number of longitudinal ridges over much of their body. About 36 ridges were grouped in 2 lateral fields of 9 ridges each and dorsal and ventral fields of 9 ridges each (Figs. 1–6) at the level of the esophageal–intestinal junction. The ventral and dorsal fields were bordered laterally by continuous ridges (dash lines "S" in Figs. 1–6). A ventral ridge, on which the excretory pore was located, and a mid-dorsal ridge were also continuous for most of the

length of the nematode. These continuous ridges delimited 8 longitudinal fields in which shorter ridges appeared in a somewhat variable pattern resulting in a variable number of ridges when counted in cross sections. Considerable variation occurred in the number and position of ridges in individual specimens, but it was possible to recognize patterns that were typical of species or groups of species. No differences in pattern were observed between males and females.

Two basically different lateral ridge patterns were discovered. One group of 5 species including *O. ostertagi, O. lyrata, T. circumcincta, T. trifurcata,* and *T. davtiani* had only 1 continuous ridge in each lateral field (Figs. 1, 2). The other ridges in the lateral field angled toward the lateral ridge posteriorly and ended adjacent to it. This pattern, named Type 1 lateral synlophe, was present in the anterior 35–50% of the length of the nematode after which the lateral ridges were parallel to each other. The 5 species with the Type 1 lateral synlophe were separated into 2 groups by differences in the diagonal ridges of the lateral fields. In *O. ostertagi* and *O. lyrata* 1 pair of ridges adjacent to the lateral ridge (1 ridge ventral to and 1 ridge dorsal to the lateral ridge) were angled diagonally toward the lateral ridge and ended near, or posterior to, the esophageal–intestinal junction (Fig. 1). In contrast, the other 3 species with a Type 1 lateral synlophe, *T. circumcincta, T. trifurcata,* and *T. davtiani,* had 2 or 3 pairs of ridges that ended next to the lateral ridge in the region of the esophagus (Fig. 2). In other words, in the latter 3 species the lateral synlophe was telescoped anteriorly compared to the more elongated synlophe observed in *O. ostertagi* and *O. lyrata.*

A 2nd pattern of lateral ridges was observed in the species *O. leptospicularis* and *O. kolchida.* In this pattern, named Type 2 lateral synlophe,

Figures 1–3. Diagrammatic drawings of synlophes, right lateral view, showing lateral ridges (L), subventral or subdorsal ridges (S), excretory pore (ep), and base of the esophagus (esophageal–intestinal junction) (E–I). 1. *Ostertagia ostertagi*. 2. *Teladorsagia circumcincta*. 3. *Ostertagia leptospicularis*. Note: dashed lines for emphasis only, ridges not interrupted.
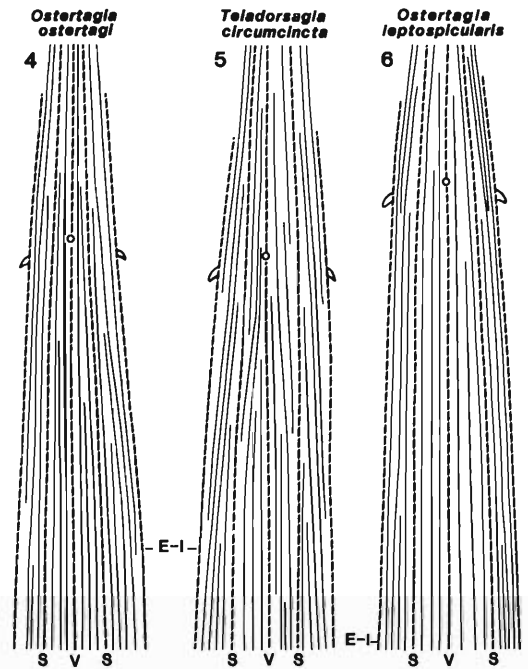
Figures 4–6. Diagrammatic drawings of synlophes, ventral view, showing ventral ridges (V), subventral ridges (S), and base of esophagus (esophageal–intestinal junction) (E–I). 4. *Ostertagia ostertagi*. 5. *Teladorsagia circumcincta*. 6. *Ostertagia leptospicularis*. Note: dashed lines for emphasis only, ridges not interrupted.

3 continuous lateral ridges were parallel and closer together than the other ridges (Figs. 3, 11).

Two different patterns were observed in the ventral and dorsal fields that can be used to separate species within the 2 groups defined by the type of lateral ridge pattern. In each species the pattern observed in the dorsal field was usually very similar to that in the ventral field. It was easier to determine the pattern in the ventral field than in the dorsal field, however, because the excretory pore can be used to identify the ventral ridge. In Type A ventral synlophe, the ventral ridge and 1 ridge on each side of it were continuous and parallel to each other, and the shorter ridges in the ventral field were lateral to the 3 ventral ridges (Figs. 4, 7). The Type A pattern was usually found in *O. ostertagi, O. lyrata, O. leptospicularis,* and *O. kolchida*. In the latter 2 species the ridges on each side of the ventral ridge extended anteriorly only 150–200 μm past the cervical papillae (Fig. 6) instead of all the way to the cephalic expansion as in the former 2 species (Fig. 4). In Type B ventral synlophe, the

shorter ventral ridges were next to the single continuous ventral ridge (Figs. 5, 9). Type B ventral synlophes were usually found in *T. circumcincta, T. trifurcata,* and *T. davtiani*. There was considerably more variation in the ventral ridge pattern than in the lateral ridge pattern, thus, the ventral pattern should only be used in combination with other characteristics of the synlophe to identify species.

The pattern of ridges near the copulatory bursa of the males was somewhat similar in all species as the lateral ridges reached the level of the prebursal papillae and the dorsal and ventral ridges ended more anteriorly and were spaced further apart than the lateral ridges in all species. Despite the overall similarities, however, differences were observed among the species in the synlophes in the preburmal region of the males. *Ostertagia leptospicularis* and *O. kolchida* had dorsal and ventral ridges that extended closer to the prebursal papillae (within about 100 μm) than in the other species. The 3 species of *Teladorsagia* had a narrow band of lateral ridges that extended to the
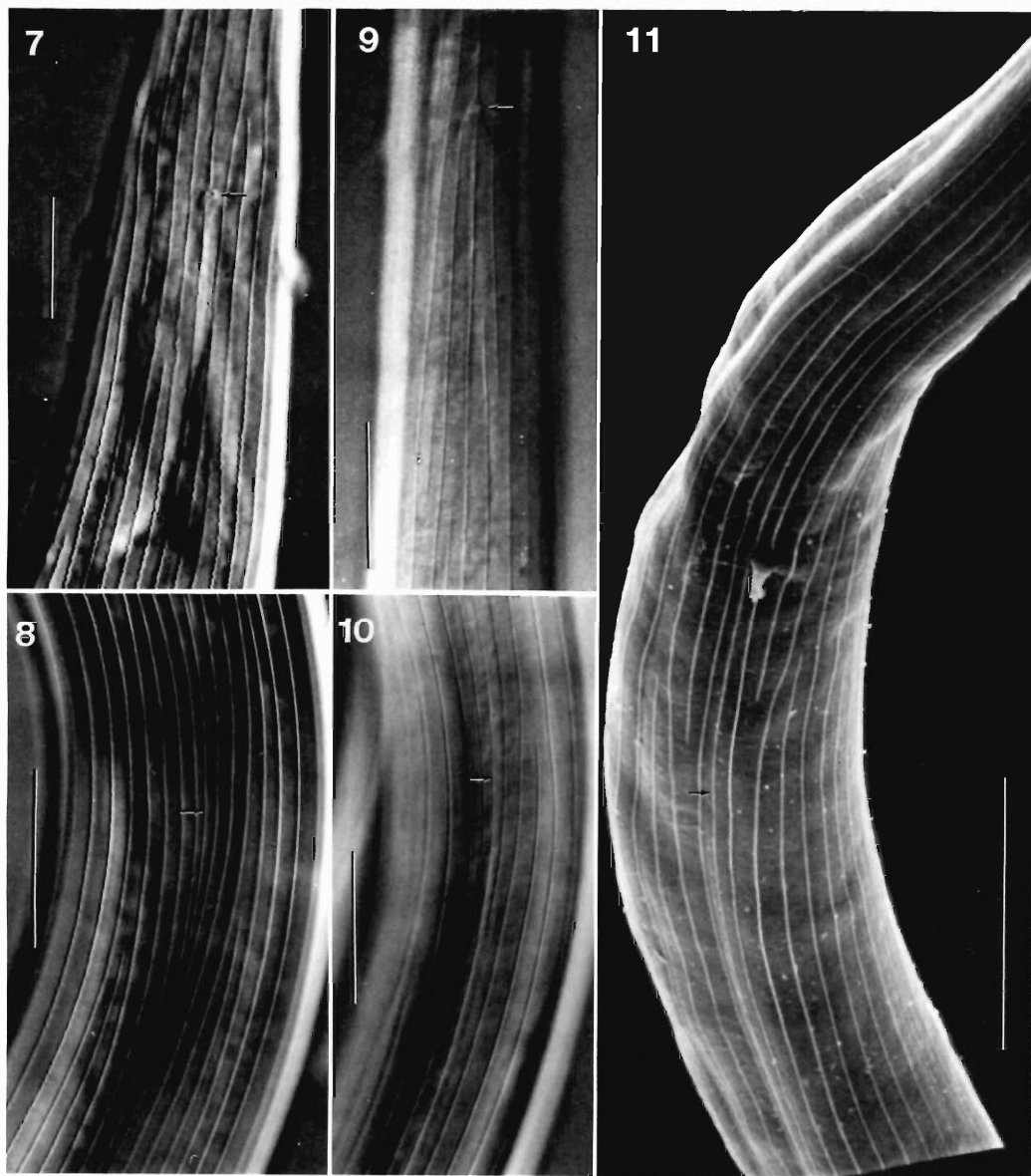
Figures 7–11. Cuticular ridge patterns, ventral and lateral views (7–10 are light micrographs, 11 is a scanning electron micrograph). 7, 8. *Ostertagia ostertagi.* 7. Arrow at execretory pore. 8. Ridges just posterior to esophageal–intestinal junction (arrow indicates lateral ridge). 9, 10. *Teladorsagia circumcincta.* 11. *Ostertagia leptospicularis* (arrow indicates lateral ridge). Scale bars = 50 μm.

preburbal papillae with ventral and dorsal ridges that ended about 300 μm anterior to the pre-bursal papillae. *Ostertagia ostertagi* and *O. lyrata* were closer to *Teladorsagia* spp. in this characteristic of the synlophe, but differed in having broader lateral bands of ridges and dorsal and ventral ridges that extended slightly closer to the prebursal papillae.

Esophagus

Two characteristics of the esophagus were found to differ among the 7 species. Differences in the length of the esophageal valve were correlated with differences observed in the lateral synlophe. The 2 species with a Type 2 lateral synlophe (*O. leptospicularis* and *O. kolchida*) both had a long esophageal valve (120–140 μm; Table
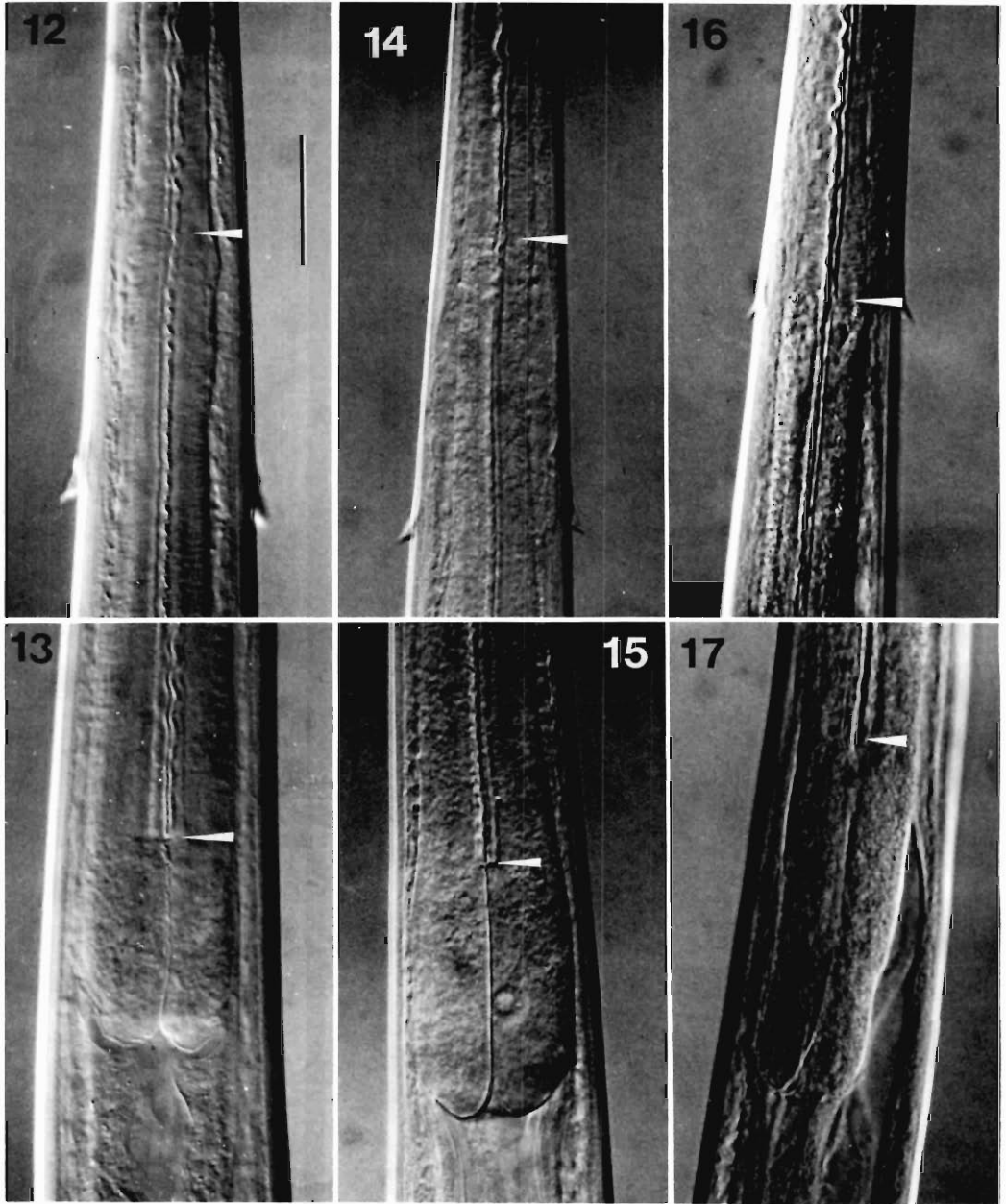
Figures 12–17. Subventral gland orifices (arrows) and esophageal valves (arrows at end of the cuticular lining of the esophagus). 12, 13. *Ostertagia ostertagi*. 14, 15. *Teladorsagia circumcincta*. 16, 17. *Ostertagia leptospicularis*. Scale bar = 50 μm.

2; Fig. 17). All 5 species with a Type 1 lateral synlophe had a short esophageal valve (55–75 μm; Table 2; Figs. 13, 15). The position of the openings of the subventral esophageal gland ducts (SVGO) in relation to the position of the cervical papillae was so variable that its value for identifying species was limited. However, the position of the SVGO was closer to the cervical papillae in *O. leptospicularis* and *O. kolchida* than in the other species (Table 2; Figs. 12, 14, 16),

Table 2. Morphometrics (in micrometers; range with mean in parentheses) of males* of 7 species of Ostertagiinae in domestic ruminants.

Characteristics	Species						
	Ostertagia ostertagi (*N* = 18)	*Ostertagia lyrata* (*N* = 16)	*Ostertagia leptospicularis* (*N* = 17)	*Ostertagia kolchida* (*N* = 10)	*Teladorsagia circumcincta* (*N* = 24)	*Teladorsagia trifurcata* (*N* = 24)	*Teladorsagia davtiani* (*N* = 23)
Body length	5,400–7,440 (6,121)	5,040–7,320 (6,232)	4,440–7,220 (4,962)	4,480–7,200 (5,080)	5,400–10,080 (7,792)	6,240–10,680 (8,351)	4,920–9,120 (7,518)
Esophagus length	566–744 (672)	525–740 (665)	507–832 (696)	644–856 (728)	502–752 (601)	528–684 (617)	516–666 (574)
Esophageal–intestinal valve length	51–87 (75)	50–77 (62)	102–140 (115)	97–156 (111)	53–94 (78)	51–99 (79)	51–97 (71)
Subventral gland orifices, length from anterior end	204–280† (248)	224–293 (260)	255–340 (280)	192–332‡ (271)	188–256 (219)	188–280 (236)	196–320§ (228)
Cephalic papillae, length from anterior end	296–372 (339)	278–392 (344)	260–332 (285)	178–336‡ (287)	264–408 (332)	296–396‖ (352)	292–412 (331)
Spicule length	212–264 (224)	229–268 (241)	168–217 (183)	188–229 (202)	242–408 (306)	188–139 (248)	173–260§ (221)
Sjöberg's organ	absent	present	absent	present	absent	present	present
Bursal ray pattern¶	2-1-2	2-1-2	2-1-2	2-1-2	2-2-1	2-2-1	2-2-1

* Females not measured as only 3 different females could be identified.

† *N* = 16.

‡ *N* = 9.

§ *N* = 22.

‖ *N* = 23.

¶ Pattern of rays in lateral lobes of bursa following system of Durette-Desset and Chabaud (1981).

but this was due to the more anterior position of the cervical papillae in *O. leptospicularis* and *O. kolchida* than in the other 5 species.

Bursal rays

The bursal ray formulae proposed by Durette-Desset and Chabaud (1981) were found to differ only between the 3 *Teladorsagia* spp. and the 5 *Ostertagia* spp. as reported earlier by those authors and Durette-Desset (1983) (Table 2). This character was therefore not useful in separating species of the proposed species pairs.

Discussion

The use of the previously unknown characteristics of the synlophe and esophageal–intestinal valve length separated the 7 species of Ostertagiinae into only 3 groups. Within the 3 groups the synlophes, esophageal–intestinal valves, and other characters listed in Table 2 were identical to the 3 polymorphic species proposed by Lancaster and Hong (1981) and Lancaster et al. (1983). Two of the 7 species studied, *O. leptospicularis* and *O. kolchida,* normally parasites of cervids, differed in several ways from the other 5 species, normally parasites of bovids. *Ostertagia leptospicularis* and *O. kolchida* differed from the other 5 species in the shorter distance from the cervical papillae to the anterior end (Table 2). This was previously reported by Bisset et al. (1984) as a significant difference between *O. leptospicularis* and *O. ostertagi.* Those authors also observed that the SVGO was closer to the cervical papillae in *O. leptospicularis* because the cervical papillae were closer to the anterior end of the nematode.

The SVGO position and short length of the esophageal–intestinal valve were identical within the 3 species of *Teladorsagia* and in *O. ostertagi* and *O. lyrata,* but differed from the more posterior SVGO position and long esophageal–intestinal valves found in *O. leptospicularis* and *O. kolchida.* Of the 2 esophageal characters, valve length is easier to observe and is less variable than the SVGO position, which because it is expressed relative to a surface cuticular structure, may vary significantly in contracted specimens.

The differences in the synlophe, length of esophageal valve, and position of the cervical papillae described herein may indicate that *O. leptospicularis* and *O. kolchida* belong to a different group of the Ostertagiinae than do the other 5 species studied here. The bursal ray formula,

however, groups *O. leptospicularis* and *O. kolchida* with *O. ostertagi* and *O. lyrata* and separates these species from the 3 species of *Teladorsagia.* It is apparent that more characters and more species must be studied before any attempt is made at sorting out the generic groups of the Ostertagiinae.

A common feature of the species pairs is that the major and minor species occur together. Exceptions to this have been reported, however. *Ostertagia kolchida* was recently discovered in an Oregon calf, *Bos taurus,* by Rickard and Zimmerman (1986) in the absence of its major species, *O. leptospicularis.* Another report of a minor species, *O. lyrata,* occurring independently from a major species, *O. ostertagi,* was that of Bye and Halvorsen (1983) who reported *O. lyrata* from reindeer, *Rangifer tarandus platyrhynchus,* in Norway. It was determined that this report was a misidentification of *O. arctica* (Karstein Bye, pers. comm., 25 March 1986).

Previous reports of differences in host specificity between members of a species pair were reported by Borgsteede (1981) who reported that *O. lyrata* disappeared from a mixed population of *O. ostertagi* and *O. lyrata* when the population was transferred from cattle to sheep. However, *O. ostertagi* also developed poorly and in lower numbers in sheep and the disappearance of *O. lyrata* may have been an accident of sampling.

Possible differences in susceptibility to an anthelmintic between major and minor species has been reported by Le Jambre et al. (1977) in which *T. trifurcata* increased proportionally in a mixed population of that species and *T. circumcincta* after anthelmintic treatment.

At the beginning of this study the first author expected to find differences in the synlophes that would permit the identification of males and females of the 7 species that were studied. Earlier studies of trichostrongylid nematodes from several subfamilies had indicated that the synlophe usually differed markedly among species identified on the basis of differences in spicules and bursal characteristics (Lichtenfels, 1977, 1983; Lichtenfels and Pilitt, 1983a, b; Lichtenfels et al., 1986). However, in this study differences in the synlophe, esophagus, and other characters were found only among the 3 groups of species and not within any of the 3 groups.

The only characters that differed between or among members of the proposed morphotypes (within the species pairs) were the well-known

differences in spicules and genital cones. As reported by Lancaster and Hong (1981), all minor species had thick spicules and an enlarged sclerotized dorsal part of the genital cone (Sjöberg's organ), and all major species had slender spicules and a smaller unsclerotized dorsal part of the genital cone.

As pointed out by Lancaster and Hong (1981) additional evidence supporting the polymorphism proposal is the lack of evidence of hybrids. Hybrids between nematode species have been found to have intermediate characteristics. Examples of intermediate characteristics for hybrids have been reported for the synlophes of *Haemonchus contortus* × *H. placei* by Lichtenfels et al. (1986) and for spicules of *Cooperia oncophora* × *C. pectinata* by Isenstein (1971). No evidence of hybrids among members of the species pairs of *Ostertagia* have been seen in this study or by Lancaster and Hong (1981).

It is premature to conclude that the polymorphism proposal of Lancaster and Hong (1981) is correct, however, the results of this study are consistent with that proposal. Studies of additional species of the Ostertagiinae are in progress. Any revision of the systematics of this subfamily must await the results of these and other studies.

Acknowledgments

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Rhinergasilus piranhus gen. et sp. n. (Copepoda, Poecilostomatoida, Ergasilidae) from the Nasal Cavities of Piranha Cajú, *Serrasalmus nattereri,* in the Central Amazon

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ABSTRACT: The female of *Rhinergasilus piranhus* gen. et sp. n. (Copepoda, Poecilostomatoida, Ergasilidae) is described from the nasal cavity of *Serrasalmus nattereri* (Kner) from the central Amazon. The new species resembles members of *Ergasilus* from which it differs by having both thoracic segment V and leg 4 reduced, and the terminal segment of the 1st endopod lacking spines and bearing 2 plumose setae. *R. piranhus* is the 1st species of Ergasilidae reported from the nasal cavity of a Neotropical fish.

KEY WORDS: Copepoda, Poecilostomatoida, *Rhinergasilus piranhus* gen. et sp. n., Ergasilidae, *Serrasalmus nattereri,* piranha, piranha cajú, nasal cavity, Amazon, Brazil.

Recent efforts to determine the fauna of parasitic copepods of Amazon fishes have resulted in discovery of several species inhabiting the nasal cavities of their hosts (Thatcher and Boeger, 1984a, b, c; Thatcher and Paredes, 1985). With the exception of *Perulernaea gamitanae* Thatcher and Paredes, 1985, a lerneaeid, all other species described from the nares of Neotropical fishes are members of the Vaigamidae Thatcher and Robertson, 1984.

During an investigation on the ectoparasites of the piranha cajú, *Serrasalmus nattereri* (Kner), specimens of 2 undescribed species of a new genus of Ergasilidae Nordmann, 1832, were recovered from the nasal cavities of fish captured near Manaus in the central Amazon. The present paper includes the description of 1 of these species, the 1st ergasilid reported from the nasal cavities of Neotropical fishes.

Materials and Methods

Specimens of *Serrasalmus nattereri* were captured during November 1984, with gill nets from 2 localities near Manaus, Amazonas, Brazil: Ilha de Marchantaria in the Solimões-Amazonas River and Furo do Catalão at the mouth of the Rio Negro. Nasal cavities of fish were washed with a solution of 1:4,000 formalin. The wash containing the parasites was vigorously shaken to free the copepods from the host mucous after which the formalin concentration was then increased to 5%. Some specimens were dissected using glass microprobes in Grey and Wess' mounting medium. Others were stained with acid Fuchsin or Fast Green, cleared in phenol and creosote, and mounted in Permount. Drawings were made with the use of a camera lucida. Measurements were obtained with an ocular micrometer and are given in micrometers; the average is followed by the range in parentheses.

Ergasilidae Nordmann, 1832
Ergasilinae Thatcher and Boeger, 1983
Rhinergasilus gen. n.

DIAGNOSIS: Female: Antennule 6-segmented. Antenna 4-segmented. Thoracic segments V and VI greatly reduced. Five pairs of legs; legs 4, 5 reduced to single seta. Parasites in nasal cavity of fish. Male unknown.

Rhinergasilus piranhus sp. n.
(Figs. 1–10)

TYPE LOCALITY: Ilha da Marchantaria, Solimões-Amazonas River near Manaus, Amazonas, Brazil.

OTHER LOCALITIES: Furo do Catalão near Manaus, Amazonas, Brazil.

TYPE SPECIMENS: Holotype, INPA PA 309-1; paratypes, INPA PA 309-2 to 309-5, USNM 79850, 79851, HWML 20767, 20768.

MALE: Unknown.

ETYMOLOGY: The generic name is derived from Greek (*rhinos* = a nose) and refers to the infestation site in the host. The specific name is adapted from the common name of the host.

DESCRIPTION (based on 15 females; measurements in Table 1): Cephalothorax bullet-shaped; head fused with 1st 2 thoracic segments. Eyes not visible. Blue pigment distributed in 2 longitudinal bands anteriorly fused, extending from cephalothorax to segments IV and V; pigmentation often radiating into legs. Thorax with 5 free segments; segments III and IV with row of ventral spinules on posterior margin; segments V and VI reduced. Genital segment subrectan-
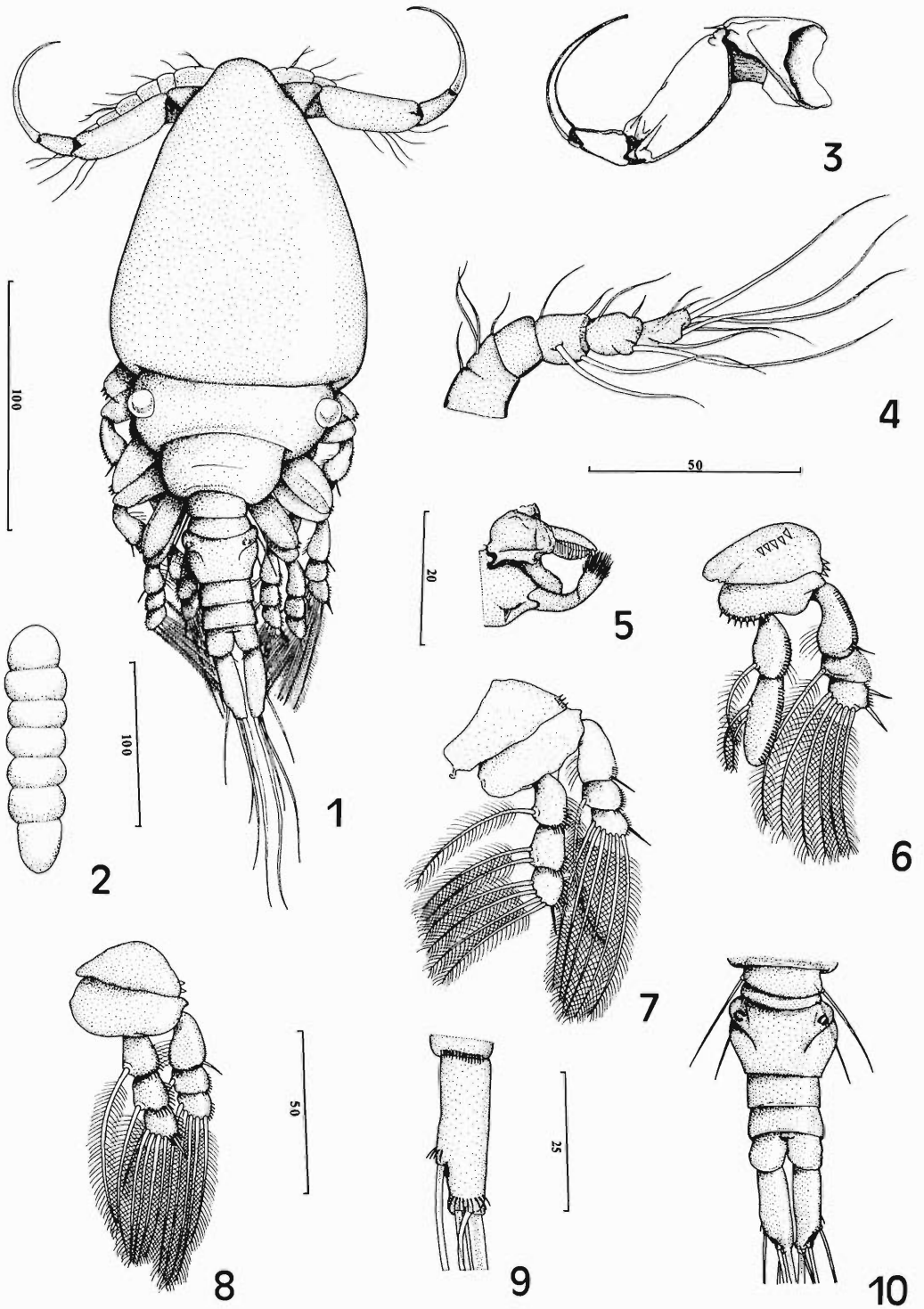
Figures 1–10. *Rhinergasilus piranhus* gen. et sp. n. 1. Composite drawing, whole mount (dorsal). 2. Egg sac. 3. Antenna. 4. Antennule. 5. Mouthparts. 6. Leg 1. 7. Leg 2. 8. Leg 3. 9. Uropod (ventral view). 10. Posterior end of a specimen showing legs 4 and 5, genital segment, abdominal segments, and uropod (dorsal).

gular. Abdomen 3-segmented (Fig. 10); each segment with row of ventral spinules along posterior margin; segment III invaginated posteriorly. Uropod (Fig. 9) with 3 terminal, 1 subterminal lateral setae, and 2 ventral rows of spinules (1 row at base of subterminal seta, other at bases of terminal setae). Antennule (Fig. 4) comprising 6 segments, each bearing simple setae; segments 1 and 2 incompletely fused; setal formula: 1–3–1–4–4–5 (total = 18). Second antenna 4-segmented (Fig. 3); basal segment with distal spine-like sensillum; segments 2, 3, and 4 lacking conspicuous sensilla; ratio of segmental length 1:1.3: 0.5:1.2. Mouthparts (Fig. 5): mandible with bristled tip, palp apparently 2-segmented; maxilule vestigial; maxilla with terminal bristle, 1 subterminal spine. Leg 1 (Fig. 6): endopod 2-segmented, exopod 3-segmented; 1st endopodal segment laterally plumose with lateral row of long spinules, 1 medial plumose seta; terminal segment with lateral row of spinules, 2 short plumose setae medially; 1st exopodal segment medially plumose with row of lateral spinules, 1 posterolateral spine; 2nd exopodal segment laterally covered with very small spinules, 1 plumose seta medially; terminal exopodal segment with lateral row of spinules, 1 lateral, 1 posterolateral spine, 4 terminal plumose setae; basipod with medioposterior row of spines; coxopod with 3 lateral spines, anterior row of 5 spines. Leg 2 (Fig. 7): both rami 3-segmented; 1st endopodal segment laterally plumose with posterolateral row of long spinules, 1 medial plumose seta; 2nd endopodal segment laterally plumose with posterolateral row of long spinules, 2 medial plumose setae; terminal endopodal segment laterally plumose with lateral row of long spinules, 1 long terminal spine, 4 plumose setae; 1st exopodal segment medially plumose with small row of posterolateral spinules, 1 long posterolateral spine; 2nd exopodal segment with lateral row of spinules, 1 medial plumose seta; terminal exopodal segment with lateral row of spinules, 1 long posterolateral spine, 6 terminal plumose setae; coxopod with 3 lateral spines. Leg 3 (Fig. 8): both rami 3-segmented; 1st endopodal segment laterally plumose with posterolateral row of long spinules, 1 medial plumose seta; 2nd endopodal segment with posterolateral row of long spinules, 1 medial plumose seta; terminal endopodal segment with lateral row of long spinules, 1 long terminal spine, 4 terminal plumose setae; 1st exopodal segment medially plumose with posterolateral row of spinules, 1 posterolateral long spine; 2nd exopodal

Table 1. Measurements (μm) of adult females of *Rhinergasilus piranhus* gen. et sp. n.

	Length	Width
Body (less caudal setae)	263 (237–282)	98 (95–102)
Cephalothorax	124 (110–141)	98 (95–102)
Free thoracic segments		
III	26 (18–33)	84 (75–96)
IV	25 (23–27)	50 (44–55)
V	8 (7–10)	27 (25–30)
VI	4 (3–6)	29 (26–30)
VII (genital)	26 (23–29)	33 (30–37)
Abdominal segments		
I	9 (7–11)	24 (21–28)
II	9 (7–11)	23 (22–25)
III	10 (8–11)	22 (21–23)
Uropod	27 (25–28)	10 (9–11)
Egg sac	117 (96–138)	32 (31–34)
Antennule	58 (55–60)	12 (11–14)
Antenna segments		
1	38 (34–42)	26 (24–30)
2	48 (45–51)	18 (15–22)
3	21 (19–23)	11 (9–13)
4	46 (37–52)	6 (5–7)

segment with posterolateral row of spinules, 1 medial plumose seta; terminal exopodal segment with lateral row of spinules, 5 terminal plumose setae; coxopod with 2 lateral spines. Legs 4 and 5 (Fig. 10) each reduced to single seta. Egg sac with row of up to 7 eggs (Fig. 2).

Discussion

Thatcher and Boeger (1983) created Abergasilinae (Ergasilidae) to contain (*Abergasilus* Hewitt, 1978, and *Brasergasilus* Thatcher and Boeger, 1983, both characterized by species bearing 3 pairs of legs and 3-segmented antennae. *Rhinergasilus* gen. n. is superficially similar to these genera in that its members also have 3 pairs of nonreduced legs. However, the presence of legs 4 and 5 (both reduced to a single seta) and a 4-segmented antenna requires the assignment of the new genus to the Ergasilinae Thatcher and Boeger, 1983.

Rhinergasilus piranhus resembles species of *Ergasilus,* from which it differs by having thoracic segment V and leg pair 4 reduced, and the terminal segment of the 1st endopodite lacking spines and bearing 2 plumose setae.

Two other ergasiloid species were concomitantly found with *R. piranhus: Gamidactylus jaraguensis* Thatcher and Boeger, 1984 (USNM 79849 and HWML 20769), and an undescribed species of *Rhinergasilus* (USNM 79848 and HWML 20771). The latter was not described

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herein due to the lack of an adequate number of specimens.

Previous to the present study, 3 species of parasitic crustaceans have been reported from *Serrasalmus nattereri,* all argulids: *Argulus multicolor* Stekhoven, 1937 (Malta, 1983), *Dolops bidentata* Bouvier, 1899 (Malta, 1982), and *D. carvalhoi* Castro, 1949 (Malta and Varella, 1983).

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Research Note

Anisakis simplex (Nematoda: Ascaridoidea): Formation of Immunogenic Attachment Caps in Pigs

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ABSTRACT: *Anisakis simplex* larvae maintained in simple salt solutions were observed attached to the surface of a petri dish in vitro by a translucent cap. Previous reports attributed the formation of these caps to contributions by human serum. Dissolved caps reacted in Western blot with the sera of rabbits sensitized with *A. simplex* excretory–secretory products. Caps were demonstrated in vivo in the mucosae of experimentally infected miniature swine; they were partially composed of larval excretory–secretory products and remained embedded in stomach mucosae after removal of the invasive larvae.

KEY WORDS: Nematoda, Ascaridoidea, anisakiasis, *Anisakis simplex,* nematode larvae, attachment cap, calotte, excretory–secretory products, immunogenic, miniature swine, *Sus scrofa,* stomach mucosae.

Formation of a calotte or cap by approximately 16% of *Anisakis simplex* (=*A. marina*) larvae isolated from herring and incubated in tubes of human serum at 37°C was described by van Thiel (1967). On the basis of acrolein–Schiff staining, the clear cap was identified as consisting of albumin (P. van Duyn *in* van Theil, 1967). The cap was found with sera from persons unlikely to have experienced any exposure to anisakine nematodes and with the sera of anisakiasis patients. Noting that parasite excretory products were granular, van Thiel postulated that the clear cap was at least partly of host origin. We have frequently observed the formation of similar structures in vitro in simple salt solutions such as phosphate-buffered saline (PBS) or Ringer's solution and in more complex media such as Medium 199 (with and without 10% fetal bovine serum). This observation suggested that structures similar to van Thiel's "calotte" could be formed by parasite products alone and raised the question as to whether formation of these structures accompanied attachment to and invasion of stomach mucosae by *A. simplex* larvae.

In this report, culture conditions were the same as those described previously (Raybourne et al., 1986), in which PBS (pH 7.2) with 1% glucose was used. For experimental exposure, Hormel–Hanford miniature pigs (*Sus scrofa*) were maintained in masonry kennels with indoor–outdoor runs (indoor winter heat), fed USDA ration 1160 at 25 g/kg/day, and allowed water ad libitum. The pigs were treated with the anthelmintic Atgard (Fermenta Animal Health Products, St. Louis, Missouri) at least 2 wk before experimental exposure. Third-stage larvae of *A. simplex* were isolated from the viscera of bocaccio (*Sebastes paucispinis*) by digestion with 2% pepsin-HCl (pH 2) at 35°C. Larvae were maintained at 2–6°C before pigs were experimentally infected. Food was withheld from the pigs for 24 hr before exposure. Each pig was then fed 100 larvae by stomach tube. Pigs were necropsied 24 hr after exposure; tissues were fixed in Bouin's fluid, dehydrated, and sectioned by the method of Hinton et al. (1987).

Rabbit antiserum to larval excretory–secretory (ES) proteins was produced by subcutaneous injection of 60 µg ES protein in Freund's complete adjuvant, followed in 2 wk by 60 µg ES protein in Freund's incomplete adjuvant. Control serum was obtained from the rabbits before immunization. ES proteins used for immunization were collected as described previously (Raybourne et al., 1983).

Sections cut at 6–10 µm thickness were rehydrated in PBS and incubated on glass slides with a 1:40 dilution of rabbit anti-ES serum or control serum for 1 hr at room temperature. The sections were washed for 30 min in 3 changes of PBS and incubated with a 1:40 dilution of fluorescein-labeled goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, Missouri) for 1 hr. The sections were rinsed in PBS, mounted in 90% glycerol–10% PBS, and examined with a Leitz fluorescence microscope. Photographs were taken with an automatic metering device designed to provide a uniform level of exposure (Wild, Heerbrugg, Switzerland).

Attachment caps adhering to the bottom of a petri dish were rinsed 3 times with PBS and made soluble by heating in PBS with 10% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol. The samples were separated on a 12% SDS-PAGE

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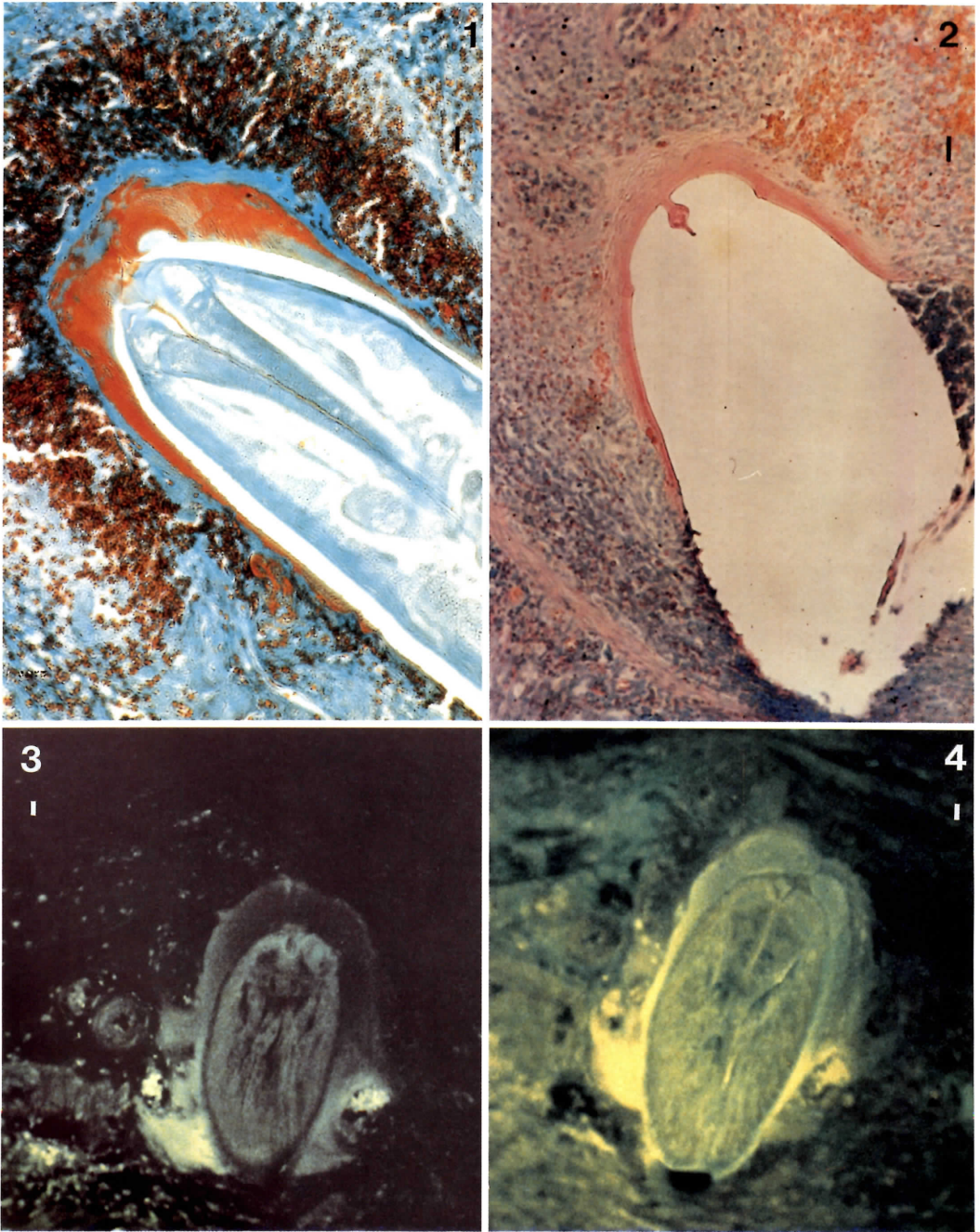
Figures 1–4. Longitudinal sections through attachment caps of *Anisakis simplex* larvae in fundus of pig stomach. Scale bars = 0.1 mm. 1. Anterior end, 3rd-stage larva stained by modified Shorr's stain after Vetterling and Thompson (1972). Note that cationic sites in attachment cap are deeply stained by Biebrich scarlet stain (bright orange area surrounding anterior end) and differentiated from the adjacent red blood cells (rust colored) stained by orange G. 2. Lesion showing attachment cap from which nematode was physically removed. Similar structures are encountered in mucosae of infected pigs. Azure A–Eosin B stain after Lillie (1965). 3. IFA staining of attachment site with rabbit antiserum to *Anisakis simplex* and fluoresceinated goat antibody to rabbit serum. Exposure time 30 sec. 4. Same attachment site as in Figure 3 (5 intervening sections) treated with presensitization serum and goat fluoresceinated antibody to rabbit antibody. Exposure time 2 min 30 sec. Exposure demonstrates yellow autofluorescent material and background green fluorescene associated with IFA staining.

gel, electroblotted onto a nitrocellulose mem-
brane, and reacted with a 1:50 dilution of anti-
ES serum or control serum (Raybourne et al.,
1986). Bands were developed with protein A per-
oxidase (Boehringer Mannheim, Indianapolis,
Indiana) diluted 1:1,000. One lane of the gel/blot
contained 10 µg ES material.

When crowded (>100 larvae per 75-mm petri
dish), a greater proportion of the worms ap-
peared to form caps than when fewer organisms
were present (≤50 larvae per petri dish). Grossly,
all the larvae in a culture appeared to be attached
to the petri dish by their anterior ends. Individual
nematodes were extracted by pulling with for-
ceps, leaving the cap attached to the dish. Caps
of nematodes that broke were not used. The caps,
or groups of caps, were translucent and refractile,
and could be scraped from the petri dish. They
appeared to be similar to the structures described
by van Thiel (1967) which formed in the pres-
ence of, and were thought to be partially com-
posed of, normal human serum. The formation
of caps by *A. simplex* larvae in media without
serum or other protein additives suggests that
caps may be formed entirely from material of
parasite origin.

Similar structures apparently form in the
stomach mucosae of experimentally infected pigs.
In vivo, the cap surrounds the rudimentary lips
and anterior end of the stage 3 larvae (Fig. 1).
Caps were also observed around the lips and
anterior ends of *A. simplex* stage 4 larvae in pigs.
The flaring shape and knoblike handle at the apex
suggested that the cap aids the parasite in main-
taining attachment to the stomach mucosae.
Some components of these structures remained
at attachment sites that the worms vacated or
from which they were removed mechanically (Fig.
2). Caps with nematodes are frequently observed
in tissue sections of infected pigs. Caps were ob-
served in more than 25 lesions with worms that
were sectioned, stained, and examined in detail.

At least some of the components of the cap
were of parasite origin, as demonstrated by in-
direct fluorescent antibody (IFA) staining of the
cap in vivo with anti-ES serum (Figs. 3, 4). Al-
though the cap proteins could not be resolved on
SDS gels as clearly as were the ES proteins, some
of the components of the caps formed in vitro
appeared to be equivalent to parasite ES pro-
teins, based on their reactivity in Western blots
(Fig. 5). These ES proteins, present in the su-
pernatant fluid of larval maintenance cultures,
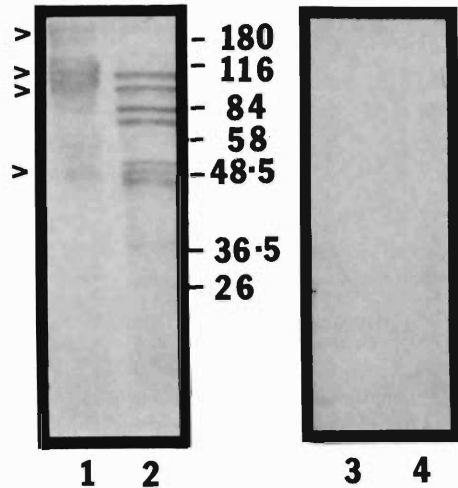are important immunogens in human anisakiasis

Figure 5. Comparison by Western blot of antigens
from solubilized attachment caps and ES products col-
lected from in vitro cultures of *Anisakis simplex*. Lanes:
1 and 3, solubilized cap; 2 and 4, ES proteins. Lanes 1
and 2 were incubated with anti-ES serum; lanes 3 and
4 were incubated with control serum. Arrows indicate
antigens of similar molecular weight present in solu-
bilized caps and ES proteins.

(Raybourne et al., 1986). These caps may pro-
vide a residual of antigenic material that may
prolong the inflammatory response observed af-
ter nematodes can no longer be detected in chronic
human anisakiasis, or after surgical removal of
the larva (Oshima, 1972).

The mechanism by which the caps are formed
is not known. Proteolytic enzymes present in the
anterior secretory structures and ES products of
A. simplex larvae (Ruitenberg and Londersloot,
1971; Mathews, 1982, 1984) may be involved.
These proteases may contribute to the formation
of attachment caps by a mechanism similar to
that of the proteases which function to form a
gel in the limulus amoebocyte lysate assay (Shi-
shikura et al., 1983).

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Research Note

Binding of Concanavalin A to Areas Compatible with the Locations of the Amphids and Phasmids of Larvae of *Dirofilaria immitis* (Nematoda: Filarioidea) and *Toxocara canis* (Nematoda: Ascaridoidea)

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ABSTRACT: Third-stage and 4th-stage larvae of *Dirofilaria immitis* and infective-stage larvae of *Toxocara canis* were fixed in 10% formalin and incubated with the fluorescein-labeled lectin, concanavalin A. The larvae were then washed, mounted on slides, and examined using an epifluorescent microscope. Upon examination, each larva was found to have 2 bright areas of fluorescence on both the anterior and posterior ends; these areas of fluorescence were not present on worms that had been incubated with fluorescein-labeled concanavalin A that had been preincubated with methyl α-D-mannopyranoside. The locations of the areas of fluorescence on these worms were found to be consistent with the described locations of the amphids and phasmids of these larval stages.

KEY WORDS: concanavalin A, amphids, phasmids, *Dirofilaria immitis, Toxocara canis,* Nematoda, Filarioidea, Ascaridoidea, lectins, chemoreception, cuticle, morphology.

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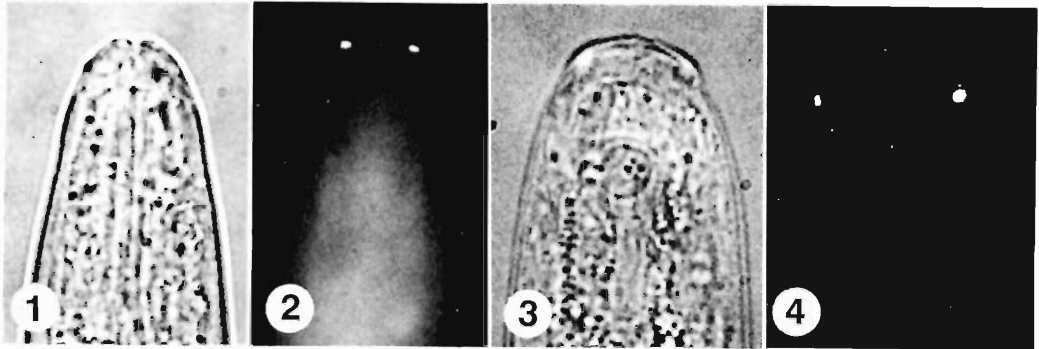[4] To whom correspondence should be addressed.

As part of a study on various surface characteristics of larval nematodes, 3rd- and 4th-stage larvae of *Dirofilaria immitis* and infective larvae of *Toxocara canis* were incubated with the fluo-

Figures 1–4.   Specific binding of fluoresceinated concanavalin A to areas compatible with the locations of the amphids and phasmids of 3rd-stage larvae of *Dirofilaria immitis*. 1. Dorsal view of anterior end of larva (×2,000). 2. Dorsal view of anterior end of larva showing fluorescence in the area of the amphids (×2,000). 3. Ventral view of posterior end of larva (×2,000). 4. Ventral view of posterior end of larva showing fluorescence in the area of the phasmids (×2,000).

rescein-labeled lectin, concanavalin A. Third-stage larvae of *D. immitis* were obtained from infected mosquitoes, and 4th-stage larvae were recovered from in vitro culture (both methods as described by Lok et al., 1984). Larvae of *T. canis* were obtained from an in vitro maintenance system (as described by de Savigny, 1975). Suspensions of larvae, 1 ml each, were placed in separate tubes and centrifuged for 1 min at 7,000 *g*. The supernatant was decanted from the tubes, and the larvae were fixed overnight at 4°C in 1 ml of a 4% solution of 37% formaldehyde (10% methanol added as a preservative by J. T. Baker Chemical Co., Phillipsburg, New Jersey) in 0.01 M phosphate buffered saline, pH 7.2 (PBS). Prior to adding the lectin, larvae were washed once, using centrifugation, with 1 ml of PBS. The pellet of larvae was then resuspended in 0.05 ml of fluorescein-isothiocyanate-conjugated concanavalin A (FITC–con A) (Vector Laboratories, Inc., Burlingame, California) at a concentration of 200 μg/ml of PBS and incubated at 37°C for 1 hr. To test the specificity of the reaction, a volume of the FITC–con A was incubated for 30 min at 37°C with an equal volume of its complementary sugar, methyl α-D-mannopyranoside (Sigma Chemical Co., St. Louis, Missouri), at a concentration of 0.04 M in PBS. Larvae were then incubated in this mixture for 1 hr. After incubation with the lectin or the lectin–sugar mixture, the larvae were washed using centrifugation 3 times with PBS, mounted in 25% glycerol in PBS with 0.1% p-phenylenediamine (Sigma), and examined using an epifluorescent microscope.

When the worms were examined, 2 very bright areas of fluorescence were seen on their anterior and posterior ends. The locations of these areas are consistent with the locations of the amphids and phasmids of these worms (Nichols, 1956; Lichtenfels et al., 1985). The areas of fluorescence on each 3rd-stage larvae of *D. immitis* appeared as discrete spots on the anterolateral surfaces of the anterior end (Figs. 1, 2) and laterally on the posterior end (Figs. 3, 4). The fluorescent areas on the 4th-stage larvae of *D. immitis* were very similar in appearance and location to those on the 3rd-stage larvae. The areas of fluorescence on the anterior end of the larval *T. canis* were on the anterolateral surfaces of the worm, appeared larger than those of *D. immitis*, and were funnel-shaped in appearance. However, the anteriorly projecting cuticle on the ventral surface of the anterior end and the large buccal capsule made these amphids more difficult to visualize. The areas of fluorescence on the posterior end of the larval *T. canis* were subterminal and lateral in position making it difficult to view both areas in the same focal plane. In all cases, fluorescence of these areas on the anterior and posterior ends was not seen after the larvae had been incubated with the FITC–con A that had been preincubated with methyl α-D-mannopyranoside.

All assays in the present study were performed on formalin-fixed worms, and therefore, it is not known if concanavalin A would bind to the amphids of living worms. Other workers (McClure and Zuckerman, 1982) have shown that the amphids of living adult hermaphrodites of *Caenorhabditis elegans* and living 2nd-stage larvae of *Meloidogyne incognita* do not bind concanavalin

A. These workers first placed the living worms in the presence of the lectin and secondly in the presence of a hemocyanin probe for concanavalin A. The binding sites of the hemocyanin were observed by examining the worms with scanning electron microscopy, and although they did find binding to the anterior end of the worm just posterior to the area of the cephalic papillae, they found no marked binding at the site of the amphids. Thus, until living larvae of *T. canis* and *D. immitis* are examined in a similar manner, it will not be possible to determine if the binding sites for concanavalin A are present or exposed on worms that are not formalin fixed.

It has been suggested that the morphology of the amphids and phasmids of nematodes is that of a chemoreceptive organ (Ward et al., 1975). Also, Bone and Bottjer (1985) have demonstrated that the binding of a lectin, *Lens culinaris* agglutinin, to the surfaces of adult males of *Trichostrongylus colubriformis* significantly decreased their responses to an attraction pheromone collected from cultures of female worms. This behavioral intervention would argue that exposed sugars on the surfaces of these living worms somehow function as part of a chemoreceptive system. Thus, if the sugars found on the amphids and phasmids of the formlin-fixed larvae of *D. immitis* and *T. canis* are exposed on living worms, studies can be undertaken to determine what role, if any, these sugars and the organs on which they are located play in the sensory perception of these larval nematodes.

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Research Note

Spicule Lengths of the Ruminant Stomach Nematodes *Haemonchus contortus, Haemonchus placei,* and Their Hybrids

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ABSTRACT: The value of spicule lengths for identifying *Haemonchus contortus* and *Haemonchus placei* was assessed. Spicule lengths were determined for populations identified to species on the basis of length of the synlophe, and for experimentally produced hybrids. Hybrids had spicules intermediate in length between the shorter spicules of *H. contortus* and the longer spicules of *H. placei.* The means of relatively large samples of spicule lengths of the 2 species and their hybrids were significantly different from each other, but the ranges overlapped considerably. Mean spicule lengths of small samples (10–12 specimens) from individual hosts were relatively homogeneous, with a small standard error, and were distributed above 450 μm for *H. placei* and below 450 μm for *H. contortus.* It was concluded that mean spicule length of small samples of 10–12 specimens was a useful character for identifying most populations.

KEY WORDS: Nematoda, *Haemonchus contortus, Haemonchus placei,* ruminants, hybrid nematodes, stomach nematodes, abomasal nematodes, Australia, North America, South America, China, Hawaii, cattle, *Bos taurus,* sheep, *Ovis aries.*

Recent studies by Le Jambre (1979) demonstrated that *Haemonchus contortus* of sheep and *Haemonchus placei* of cattle will produce hybrids although they are sterile by the F_1 or F_2 generation. Lichtenfels et al. (1986) reported that *H. contortus* and *H. placei* could be identified on the basis of differences in the length of their synlophes (surface longitudinal cuticular ridges) and that experimentally derived hybrids had a synlophe intermediate in length between the 2 species. Earlier workers (Roberts et al., 1954; Herlich et al., 1958) reported differences in spicule lengths between *Haemonchus* from cattle and those from sheep, although there was considerable overlap in the spicule lengths between populations from the 2 hosts. Furthermore, Herlich et al. (1958) reported that host species had no significant effect on spicule length. The present report describes spicule lengths of specimens raised under experimental conditions in Australia and compares them with spicule lengths of specimens from naturally occurring infections from North America, South America, China, and Hawaii (Fig. 1). The objectives of this study were: (1) to determine the variability of spicule lengths in experimentally derived populations of known species composition; (2) to determine the spicule lengths of experimentally derived hybrids; (3) to determine if spicule lengths of experimental populations provide reliable estimates of variation in natural infections; and finally, (4) to reassess the value of spicule lengths as a character for identifying naturally occurring populations of *H. contortus* and *H. placei.*

All Australian nematodes were from experimental infections using strains originally isolated from natural infections. Specimens from experimental infections in Australia included *H. contortus* from sheep (30 males) and cattle (30 males), *H. placei* from sheep (60 males), and their hybrids (20 males each of: Hc ♀ × Hp ♂ F_1; Hp ♀ × Hc ♂ F_1; and Hc ♀ × Hp ♂ F_2) all from sheep (total 60 males) (Fig. 1). For localities and hosts of naturally occurring specimens see Lichtenfels et al. (1986). Specimens were studied in temporary mounts cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute alcohol). Measurements of spicules were made with a calibrated ocular micrometer. The calculation of 2 standard deviations on each side of the means provided theoretical confidence limits within which 95% of the individuals should fall. Two standard errors of the means provided 95% confidence limits of the sample means (Steel and Torrie, 1960).

As expected from earlier reports on spicule lengths of *H. contortus* and *H. placei* in Australia (Roberts et al., 1954) and in North America (Herlich et al., 1958), spicules of most specimens of *H. placei* were longer than those of most *H. contortus* (Fig. 1). As in the earlier studies, the ranges of spicule lengths of the 2 species over-
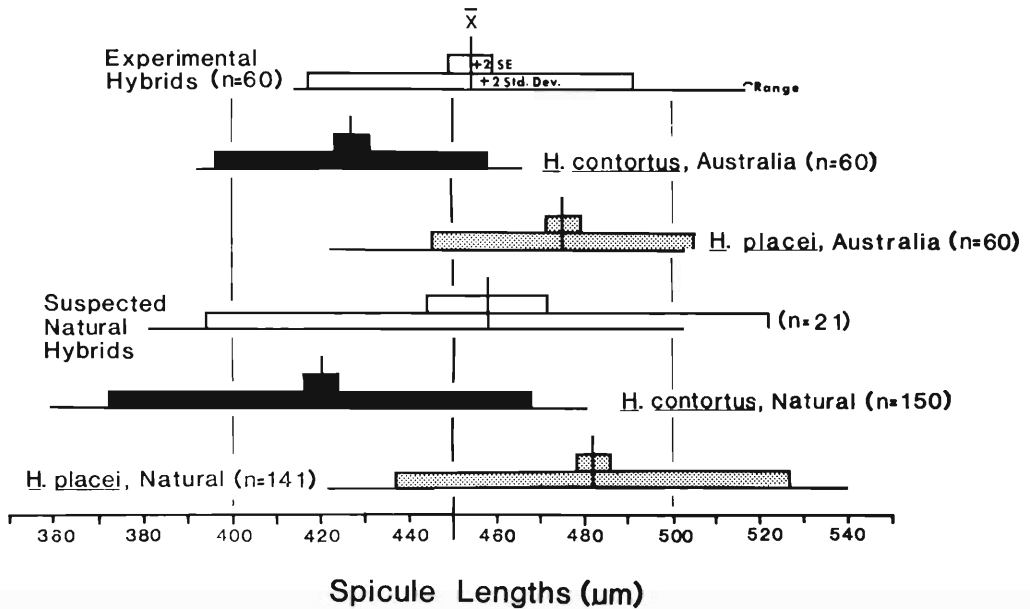
Figure 1. Spicule lengths of *Haemonchus contortus, H. placei,* and their experimentally derived hybrids from Australia; and *H. contortus, H. placei,* and suspected hybrids from naturally occurring infections in North America, South America, Hawaii, and China.

lapped considerably, but the means were significantly different (Fig. 1). There was no significant difference between the mean spicule lengths of specimens from Australian experimental infections and those from the natural infections of the same species from other parts of the world (Fig. 1). Thus, the experimental infections provided a good estimate of the means and ranges of variation of the natural infections even though they came from different continents. The apparent reliability of the estimates may be due in part to the large sample sizes in the study.

One would not routinely measure spicules of 60–150 specimens; therefore, we examined the variability among small samples of 10 specimens each to determine whether such small samples would provide reliable estimates of mean spicule lengths for these species. Means were calculated for all lots for which at least 10 specimens were available (Table 1). The means of samples of 10–12 specimens from individual hosts were distributed in 2 nonoverlapping groups with all *H. contortus* mean spicule lengths shorter than 450 μm, and all those of *H. placei* longer than 450 μm (Fig. 2). The wide variability among the means (with relatively small standard errors) of the small samples of 10–12 specimens indicated that spicule lengths of the specimens from individual hosts

Table 1. Spicule lengths in μm (with estimates of central tendency) of selected populations of *Haemonchus placei* and/or *H. contortus* from individual hosts.*

Species (lot no.)		Range	Mean (N)	Two standard errors of mean	Two standard deviations of sample mean
H. placei	(1)	436–503	480 (10)	12.4	39.1
	(5)	490–525	514 (12)	7.0	23.1
	(6)	444–510	463 (10)	13.0	41.0
	(7)	438–496	465 (11)	10.8	35.9
	(8)	421–488	463 (10)	11.9	37.6
	(10)	448–481	466 (10)	6.7	21.3
	(11)	451–503	474 (12)	8.0	27.7
	(12)	466–540	509 (10)	12.7	40.1
	(16)	466–500	482 (10)	7.6	24.1
H. contortus	(18)	403–455	439 (10)	9.0	28.4
	(19)	407–459	428 (10)	10.2	32.3
	(20)	392–429	410 (12)	7.4	25.5
	(21)	370–422	405 (10)	11.7	37.0
	(22)	385–429	406 (10)	8.7	27.4
	(26)	407–463	426 (12)	9.6	31.7
	(31)	359–439	400 (10)	17.7	55.8
	(32)	392–427	410 (10)	7.7	24.2
	(25)	377–433	401 (10)	11.3	35.8
Mixed or hybrid	(23)	381–496	414 (10)	28.3	89.3

* Localities, hosts, and descriptions of synlophes of these lots are given in Lichtenfels et al. (1986).
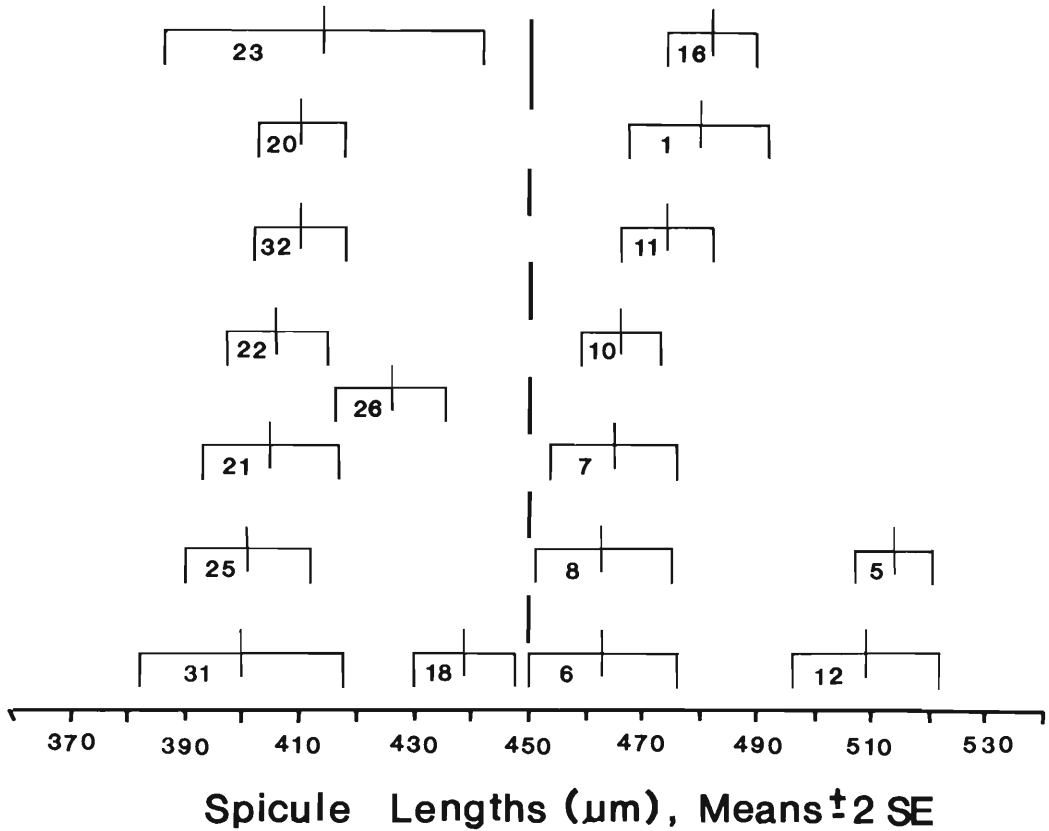
Figure 2.   Mean spicule lengths (+2 SE) of small samples, by lot number (Table 1), of *Haemonchus contortus* and *H. placei*. Mean spicule lengths of *H. contortus* were all shorter than 450 μm; those of *H. placei* were all longer than 450 μm.

were fairly uniform. Thus, we concluded that samples from a number of individual hosts are needed to provide an estimate of spicule length for the species.

The apparent low variability in lots of 1 species from individual hosts provided a way of identifying lots that include hybrids and/or more than 1 species. In samples such as lot number 23 (Table 1), the extremely large range of spicule lengths provided a quick indication of the presence of a mixed species or a hybrid population. The intermediate spicule lengths of experimentally derived hybrids observed in this study were in agreement with previous observations of hybrid nematodes (Isenstein, 1971). Because of the occurrence of hybrids with spicules of intermediate (but wide ranging) lengths (Fig. 1), the use of spicule lengths of individual specimens and/or of mean lengths of small samples must be regarded as of value only as a supplementary char-

acter for identifying species. For example, in lot number 23 (Lichtenfels et al., 1986, Table 1), 8 of 10 males had synlophe lengths typical of *H. contortus* and spicule lengths from 374 to 420 μm, well within the range for this species. However, 1 male from population 23 had a synlophe length typical of *H. placei,* 1 male had a synlophe of intermediate length, and both had spicules longer than 490 μm. In population 23, therefore, spicule lengths supported the data on the synlophe (Lichtenfels et al., 1986) and indicated that either a mixed species or hybrid population was probably present. In another example, population number 25 (Lichtenfels et al., 1986, Table 1) consisted of 9 males with synlophes and spicule lengths typical of *H. contortus,* but 1 male had an intermediate synlophe. The latter male had a spicule length of only 418 μm, however, which leads one to suspect that the intermediate male is probably one of the 5% of *H. contortus*

expected to fall outside the 95% confidence limits for the species.

As a result of this study several conclusions were made: (1) the experimental populations were somewhat less variable in spicule length than the naturally occurring populations, but the difference may have been due to the smaller sample size and fewer hosts in the experimental populations; (2) hybrids have intermediate spicule lengths; (3) experimental infections derived from natural infections in Australia provided accurate estimates of spicule lengths for populations from other parts of the world; and, (4) the most discriminating morphological character for species identification is the synlophe (Lichtenfels et al., 1986), but spicule length is an easily observed character useful for screening populations to be identified to species. Only populations not clearly identifiable on the basis of spicule length (because of intermediate or wide ranging lengths) need to be examined more closely to determine synlophe length.

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Research Note

Occurrence of the Trematodes *Uvulifer ambloplitis* and *Posthodiplostomum minimum* in Juvenile *Lepomis macrochirus* from Northeastern Illinois

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ABSTRACT: Bluegill from a northeastern Illinois slough were examined for *Uvulifer ambloplitis* and *Posthodiplostomum minimum*. Ninety-five bluegill were collected during October and November 1981. Of these, 93 (98%) were infected with *U. ambloplitis* and 34 (36%) were infected with *P. minimum*. The mean intensities of infection were 29.7 and 1.7 for *U. ambloplitis* and *P. minimum,* respectively. The prevalence and mean intensity of *U. ambloplitis* were higher than values reported by most investigators while the values for *P. minimum* were lower than those usually found.

KEY WORDS: *Uvulifer ambloplitis,* black-spot, *Posthodiplostomum minimum,* white grub, *Lepomis macrochirus,* bluegill, prevalence, mean intensity, northeastern Illinois.

The occurrence of *Uvulifer ambloplitis* (black-spot) and/or *Posthodiplostomum minimum* (white grub) in juvenile bluegill has been studied by Colley and Olson (1963) in California, Spall and Summerfelt (1970) and McDaniel and Bailey (1974) in Oklahoma, Amin (1982) in Wisconsin, and Lemly and Esch (1983, 1984a, b, 1985) in North Carolina. This report presents data on the infection of bluegill, *Lepomis macrochirus,* with larval *U. ambloplitis* and *P. minimum* in northeastern Illinois and compares the findings to those of other studies.

The study site was the eutrophic slough on the campus of Illinois Benedictine College (IBC) in Lisle, DuPage County, Illinois. Juvenile bluegill were collected during October and November 1981. Following the guidelines of Lemly (1980), bluegill ≤70 mm in length were considered to be juveniles. Fish were collected with a 4- × 1.5-m minnow seine (mesh size 0.5 cm²). Fish were taken alive to the laboratory where they were preserved in 10% formalin. The total length of each fish was measured after which it was dissected with the aid of a binocular dissecting microscope to enumerate the cysts of both parasite species. The terms prevalence and mean intensity follow definitions given by Margolis et al. (1982). Voucher specimens of each parasite have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland, Nos. 79943 and 79944).

A total of 95 bluegill were examined. The mean length of the fish was 33.1 mm (range 24–59 mm). Cysts of *U. ambloplitis* were recovered from the skin, muscles, and fins of bluegill while *P. minimum* cysts were recovered from the viscera and serous membranes of the peritoneal cavity. Ninety-three (98%) of the bluegill were infected with *U. ambloplitis* (Table 1), but only 34 (36%) were infected with *P. minimum* (Table 1). The mean intensity for *U. ambloplitis* was 29.7 ± 5.3 while that for *P. minimum* was 1.7 ± 0.3 (Table 1).

The prevalence of *U. ambloplitis* in bluegill was higher than that observed by McDaniel and Bailey (1974) and comparable to that observed by Lemly and Esch (1984a). McDaniel and Bailey (1974) did not report mean intensities for their study. However, the mean intensity in the current study was equivalent to that reported by Lemly and Esch (1984a). These investigators stated that the values they observed for prevalence and mean intensity were ". . . exceptionally high in bluegill from Reed's Pond" as compared with other locations in North America (Dechtiar, 1972; Cone and Anderson, 1977; Berra and Au, 1978). Lemly and Esch (1984a) attributed the high prevalence and intensity to a congregating of kingfishers resulting in high parasite egg input at the same time that snail density was at its greatest. These factors eventually resulted in high cercariae production and heavy infection of bluegill. Unfortunately, I did not gather quantitative data on the occurrence of *Helisoma* sp. and kingfishers, so it is not known if factors similar to those reported by Lemly and Esch (1984a) could

Table 1. Infection of juvenile *Lepomis macrochirus* with *Uvulifer ambloplitis* and *Posthodiplostomum minimum* from the Illinois Benedictine College slough.

	Uvulifer ambloplitis	*Posthodiplostomum minimum*
Prevalence*	93/95 (98)	34/95 (36)
No. of larvae recovered (range)	2,760 (1–386)	57 (1–11)
Mean intensity ± 1 SE	29.7 ± 5.3	1.7 ± 0.3

* No. infected/no. examined (%).

be responsible for the high prevalence and mean intensities observed in my study.

Posthodiplostomum minimum prevalence and mean intensity were much lower than those seen for *U. ambloplitis* (Table 1). The values in this study were also lower than those observed by other investigators who studied *P. minimum* infections of young bluegill (Colley and Olson, 1963; Spall and Summerfelt, 1970; Amin, 1982). The low values in this study might have been caused by low densities of definitive hosts and/or 1st intermediate hosts. However, lacking quantitative data on these hosts, this is a tenuous conclusion.

I thank Dr. A. Dennis Lemly for identifying *U. ambloplitis,* Dr. John Mickus for providing laboratory space and equipment, and Drs. Patrick M. Muzzall and Dennis J. Minchella for reading and commenting on this manuscript.

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Research Note

The Taxonomic Status of *Alaria marcianae* (Trematoda: Diplostomidae)

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ABSTRACT: *Alaria marcianae* and *A. americana* are similar but distinct species. *Alaria canis* is retained as a junior synonym of *A. americana.*

KEY WORDS: Trematoda, Diplostomidae, mesocercariae, adults, *Alaria marcianae, Alaria americana, Alaria canis,* taxonomic status, synonymy.

La Rue (1917) described a mesocercaria from the gartersnake, *Thamnophis marcianus,* and named it *Cercaria marcianae.* Though Cuckler (1940) showed that it belonged in the genus *Alaria,* Walton (1949) first formally proposed the combination *Alaria marcianae.* Dubois (1970) compared descriptions of the mesocercaria of *A. marcianae* by La Rue (1917), Cort (1918), and Cort and Brooks (1928), but not Johnson (1968), with that of the mesocercaria of *A. canis* by Pearson (1956) and decided that they referred to a single species.

Cort and Brooks (1928) identified as *Agamodistomum marcianae* (=mesocercaria of *A. marcianae*) mesocercariae recovered from tadpoles (species not given) exposed to cercariae from naturally infected *Planorbis trivolvis* (=*Helisoma trivolvis*) and *Planorbis campanulatus* (=*Helisoma*

campanulatum) from Douglas Lake, Michigan. Examination of their experimental mesocercariae (USNM Helm. Coll. No. 55122) revealed that the dorsal spination was the same as Pearson (1956) described in *Alaria canis* (=*A. americana*) and not the same as Johnson (1968) found in *A. marcianae.* Cort and Brooks (1928) described the dorsal spination of the cercaria as extending back to the level of the ventral sucker, then absent medially over the posterior portion, a condition differing from that in the cercariae of both *A. americana* and *A. marcianae* and so presumably in error. Cort's (1918) description of the spination of *Agamodistomum marcianae* from Douglas Lake, Michigan, agrees with that of *A. marcianae* as seen by us. Thus, Cort (1918) and Cort and Brooks (1928) found both *A. americana* and *A. marcianae* in Michigan. This was confirmed by studying wholemounts of mesocercariae from Michigan made by K. Wu (USNM Helm. Coll. No. 79097) that revealed a mixture of *A. americana* and *A. marcianae* under a single coverslip.

As Dubois (1963) had earlier proposed *A. canis* as a synonym of *A. americana* based on adult

characters, he (1970) then proposed *A. americana* as a junior synonym of *A. marcianae*. We, too, consider *A. canis* a synonym of *A. americana,* as comparison of the holotype of *A. americana* with experimental adults of Pearson (1956) showed in both a thick-walled ejaculatory pouch (see below), and, as pointed out by Johnson (1968), both were described from the dog. However, we do not agree that *A. americana* is a synonym of *A. marcianae*.

The type specimens of *A. marcianae* are mesocercariae, hence recourse to the mesocercaria is the ultimate arbiter in deciding the taxonomic status of this species. Pearson (1956) distinguished between the mesocercaria of *A. americana* with uniform dorsal spination and that of *A. marcianae* with incomplete dorsal spination (implied but not explicitly stated), but failed to point out that he had confirmed this difference from some of La Rue's original material of *Cercaria marcianae*. Johnson (1968) also found incomplete dorsal spination in the mesocercaria of *A. marcianae* and later (1970) used this character to separate *A. marcianae* from *A. americana* in a key to mesocercariae. Johnson (1968) considered the difference in spination more significant than differences in size of mother sporocysts, metacercariae, and adults, in position of furcal excretory pores in cercariae, and in thickness of ejaculatory pouch wall in adults. A further difference is seen in definitive hosts; *A. marcianae* is primarily a parasite of felids and mustelids and *A. americana* of canids.

In brief, *A. marcianae* differs from *A. americana* in having a larger mother sporocyst (>14 mm cf. <10 mm), a cercaria with marginal furcal excretory pores (cf. submarginal), a mesocercaria with spination dorsally interrupted between pharynx and level of ventral sucker (cf. spination dorsally uninterrupted), a smaller metacercaria (656 μm av. cf. 1,250 μm av.), a smaller adult (<2 mm av. cf. >2 mm av.) with a thinner ejaculatory pouch wall (<20 μm), and mustelids and felids as common definitive hosts (cf. canids). We are first reporting here measurements of mature adult worms of *A. americana* (see Pearson, 1956) and *A. marcianae* (see Bhatti and Johnson, 1972) from experimentally infected dogs. Adult worms of the former species measured 2.47–2.71

mm long with an ejaculatory pouch wall 33 (23–39) μm thick, while those of the latter measured 1.66–2.06 mm long with the pouch wall always less than 20 μm.

If, then, there are two similar species, *A. marcianae* and *A. americana,* care must be taken in differentiating them, especially when setting up experimental lines from natural infections with the most readily available stage, the mesocercaria.

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Research Note

Occurrence of a Metacercaria (Trematoda: Gymnophallidae) in Acmaeid Gastropods, *Lottia digitalis* and *"Collisella" scabra*

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ABSTRACT: Two species of gastropod molluscs (*Lottia digitalis* and *"Collisella" scabra*) were found to be 2nd intermediate hosts for *Parvatrema obscurus* (Ching, 1960). The metacercariae were found unencysted in the digestive gland of their acmaeid hosts. Infected limpets were found in 5 rocky, high intertidal sites in California.

KEY WORDS: parasite, metacercaria, gymnophallid trematode, digestive gland, molluscan hosts, *Lottia digitalis*, *"Collisella" scabra*, California.

Infections with the metacercaria of a gymno-phallid trematode were found in 2 species of gastropod molluscs. Only 4 gastropod hosts in contrast to 44 bivalve hosts have been reported for the metacercariae of 68 species of gymnophallid trematodes (Holliman, 1961; Loos-Frank, 1971). Furthermore, the metacercariae were found in the digestive gland, and the extrapallial area or the mantle are the usual sites for gymnophallid metacercariae in gastropod hosts. The digestive gland and gonads were examined for parasites from a total of 1,042 gastropods and the metacercariae were identified to be that of *Parvatrema obscurus* (Ching, 1960) James, 1964.

HOSTS: *Lottia digitalis* (Rathke, 1833) and *"Collisella" scabra* (Gould, 1846) were sampled in 5 and 3 localities, respectively, in California. (The names of acmaeid gastropods including *"C." scabra* are undergoing change [Lindberg, 1986].) The localities, dates of collection, number of limpets sampled, and percent prevalence are: *Site 1:* Año Nuevo Island, San Mateo County, California; 24-5-1985; *L. digitalis* 161, 63%; *"C." scabra* 91, 56%. *Site 2:* University of California Landels Hill–Bigcreek Reserve, Monterey County, California; 21-6-1985; *L. digitalis* 258, 29%; *"C." scabra* 22, 31%. *Site 3:* Año Nuevo State Park, San Mateo County, California; 22-6-1985; *L. digitalis* 355, 18%; *"C." scabra* 19, 0%. *Site 4:* Bodega Marine Laboratory, Sonoma County, California; 14-7-1985; *L. digitalis* 44, 34%. *Site 5:* San Nicholas Island, Santa Barbara County, California; 29-8-1985; *L. digitalis* 92, 4%.

Sites 3 and 5 are exposed rocky coasts while the other 3 sites are somewhat more protected.

Samples were collected during the day at low tide from large numbers of limpets present in the high intertidal zone. The limpets were kept at 13°C in the laboratory and the digestive gland and gonads of the limpets were examined within 2 wk.

PARASITES: Only metacercariae were found in the digestive gland and they occurred free, not enclosed within membranes or visible tissue. No other larval digenetic trematode forms such as sporocysts, germinal sacs, or cercariae were encountered. The range of intensity was 1–25 in *L. digitalis* and 1–15 in *"C." scabra*. The metacercariae were studied alive using neutral red as a vital stain. For permanent slides, the metacercariae were killed in hot sea water, fixed in alcohol–formalin–acetic acid, stained with Semichon's acetocarmine, and mounted in permount. Representative specimens of the metacercaria have been deposited in USNM Helminthological Collection, No. 79923.

Measurements are given for 18 specimens, with the mean followed by the range in micrometers in parentheses. Body transversely spined, length 181 (137–215); body width 113 (91–137); oral sucker surrounded by unicellular glands, with lateral papillae, length and width 61 × 59 (50–70 × 50–66). Ventral sucker length and width 25 × 27 (19–30 × 23–32). Sucker ratio 1:0.45 (1:0.39–1:0.53). Pharynx 19 × 18 (13–30 × 12–30). Genital pore wide, immediately anterior to or short distance anterior to ventral sucker; testes nearly symmetrical; ovary anterodextral to testis. Vitellaria 2 compact lobes dorsal to ventral sucker. Excretory bladder V-shaped, lobes reaching to level of oral sucker. Flame cell pattern not determined.

The metacercaria was identified as that of *Parvatrema obscurus* and was similar to the description of the adults (Ching, 1960). The specimens were smaller than adults from the original collection, but the gonads were discrete. The placement of internal organs was variable due to the extension of the excretory bladder, and the body

shape differed depending on the extent of contraction upon preservation. Seven kinds of gymnophallid metacercariae have been observed from the Pacific coast of North America by the first author and are different from *P. obscurus.*

Other molluscs in the high intertidal zone were not examined as possible hosts for *P. obscurus.* The 2nd intermediate hosts of *P. obscurus* are abundantly located on rock surfaces subjected to heavy wave action. Oystercatchers, *Haematopus bachmani,* commonly prey on limpets and the single bird host examined in 1960 in Washington had *L. digitalis* in its stomach contents and over 100 adult worms in the intestine (Ching, 1960).

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Research Note

Prevalence of Larval *Echinococcus multilocularis* in Native Montana Small Mammals and Susceptibility of Laboratory Rodents to a Montana Isolate

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ABSTRACT: A field survey to determine the intermediate host(s) of *Echinococcus multilocularis* in southwestern Montana involved the examination of 1,271 small mammals representing 17 species. Two naturally infected muskrats (*Ondatra zibethicus*) were collected from the East Gallatin River in Gallatin County during the winter of 1980. No other species were found to be infected with this larval cestode. Fifty-eight experimental inoculations of 5 species of rodents and 1 lagomorph by feeding ova induced fertile cyst development in 1 deer mouse (*Peromyscus maniculatus*) and 1 muskrat. Successful culturing of the Montana isolate by intraperitoneal inoculation of cyst material occurred in 4 of 11 cotton rats (*Sigmodon hispidus*). Nineteen deer mice were refractory to infection via intraperitoneal injection.

KEY WORDS: Cestoda, *Echinococcus multilocularis,* experimental infections, hydatid disease, muskrat, *Ondatra zibethicus.*

The occurrence of the cestode *Echinococcus multilocularis* in Montana was first demonstrated by Leiby et al. (1970). Subsequent records of the distribution of the cestode in Montana (Seesee and Worley, 1976; Eastman and Worley, 1979) suggest that it is enzootic throughout the state east of the Continental Divide.

In Montana, adult *E. multilocularis* has been found to occur in red foxes (*Vulpes vulpes*) (Seesee and Worley, 1976) and coyotes (*Canis latrans*) (Seesee et al., 1983). The larval stage typically occurs in cricetine and microtine rodents. The deer mouse (*Peromyscus maniculatus*) and meadow vole (*Microtus pennsylvanicus*) appear to be the most important intermediate hosts in the contiguous United States (Leiby et al., 1970; Rausch and Richards, 1971). Naturally infected deer mice have been reported from eastern Montana (Leiby et al., 1970). Eastman and Worley (1979) reported the occurrence of *E. multilocularis* in the muskrat (*Ondatra zibethicus*) in southwestern Montana, a new host record in North America.

The purpose of this study was to determine

Table 1. Prevalence of larval *Echinococcus multilocularis* in 1,271 Montana small mammals from November 1979 to May 1981.

Species	No. infected/ no. examined
Order Insectivora	
Sorex cinereus	0/2
S. palustris	0/5
S. vagrans	0/100
Order Rodentia	
Castor canadensis	0/86
Clethrionomys gapperi	0/23
Eutamias amoenus	0/8
Microtus longicaudus	0/22
M. montanus	0/1
M. pennsylvanicus	0/89
M. richardsoni	0/2
Mus musculus	0/11
Ondatra zibethicus	2/657
Peromyscus maniculatus	0/208
Spermophilus richardsoni	0/33
Thomomys talpoides	0/1
Zapus princeps	0/16
Order Lagomorpha	
Lepus townsendii	0/7
	2/1,271 (0.16%)

the extent of involvement of native small mammals in maintaining the life cycle of *E. multilocularis* in southwestern and central Montana. In addition, experimental inoculations of rodents and lagomorphs with a local isolate of *E. multilocularis* were performed in order to culture the isolate.

From fall 1979 to spring 1981, 1,271 small mammals, representing 3 orders and 17 species, were collected from 7 Montana counties (Broadwater, Fergus, Gallatin, Jefferson, Madison, Park, and Phillips) and examined for *E. multilocularis* cysts. Internal organs and associated mesenteries were examined macroscopically. All tissues with suspected lesions were excised and fixed in 10% buffered formalin, embedded in Paraplast, sectioned at 5 µm, and stained with hematoxylin and eosin. In cases where obvious cysts of *E. multilocularis* were found, the tissue was placed in 0.85% saline solution and used for experimental purposes. A portion of the cyst and adjacent host tissue was also fixed in 10% buffered formalin and sectioned as mentioned above.

Experimental inoculations of rodents and lagomorphs with the Montana isolate of *E. multilocularis* were performed by 1 of 3 methods:

(1) feeding gravid proglottids to rodents and lagomorphs via a stomach tube; (2) surgical implantation of a small intact cyst into the peritoneal cavity of rodents; or (3) intraperitoneal injection of either cyst fluid containing protoscolices (Hinz, 1972), or 0.5 g of diced cyst material, including cyst wall (Marchiondo and Andersen, 1985). Gravid proglottids were obtained from fox carcasses. Cyst material was obtained from 2 naturally infected muskrats and 1 experimentally infected deer mouse. Inoculated animals were laboratory reared except for the muskrats, which were trapped from a natural population. Deer mice (*P. maniculatus osgoodi*) used in experimental inoculations were derived from breeding stock from Lewistown, Montana.

Of the 1,271 small mammals examined, larvae of *E. multilocularis* occurred in only 2 muskrats (*O. zibethicus oboyoosensis*) (Table 1). The 2 infected muskrats were collected from the East Gallatin River, 15 km northwest of Bozeman, Gallatin County, Montana. All other small mammals surveyed were negative for *E. multilocularis* infection. Eight hundred fifteen of the small mammals examined were collected from 2 counties where foxes infected with adult *E. multilocularis* had previously been collected (Fergus and Gallatin counties). Also, 190 insectivores and rodents (other than muskrats and beavers) were collected within 0.5 km of the location where 2 naturally infected muskrats were collected. The failure to collect infected animals other than muskrats suggests that the muskrat may be important in maintaining the life cycle of *E. multilocularis* in southwestern Montana. Studies of the ecology of *E. multilocularis* in the Balkash territory (USSR) indicate the prevalence of larval infection in the muskrat may be related to the extent of irrigation (Shiryaev, 1983). Much of southwestern Montana consists of irrigated agricultural land which may promote a fox–muskrat life cycle. Also, the low number of infected animals (0.16%) indicates that the introduction of *E. multilocularis* to southwestern Montana may have been recent.

The results from the experimental inoculations are presented in Table 2. One deer mouse and 1 muskrat developed multilocular cysts after being fed 3 and 5 gravid proglottids, respectively. The infected deer mouse was exposed to proglottids at the same time and from the same fox as 9 other deer mice and 4 gerbils. Four muskrats and 6 cotton rats received inocula obtained from

another fox. The observed low infectivity of *E. multilocularis* may suggest that the proglottids contained nonviable eggs. Although we do not know if the eggs were indeed viable, 24 animals were exposed to proglottids that came from common sources and yielded 2 infections. Since the inoculated muskrats were trapped from a natural population there was no assurance that they had not been previously exposed to *E. multilocularis* ova. However, during the field survey, 203 muskrats from the same location showed no evidence of infection. Therefore, the chance of previous exposure of the experimental animals was minimal.

Intraperitoneal injection of cyst fluid containing protoscolices failed to produce infection in 19 deer mice and 3 cotton rats. Infections were obtained in 4 cotton rats which received either diced cyst material containing portions of the cyst wall or a surgically implanted cyst. Deer mice were relatively resistant to infection by direct feeding of gravid proglottids and lateral (cyst fluid) transmission.

The deer mouse is an important intermediate host of *E. multilocularis* in other areas of the United States and Canada (Leiby et al., 1970; Rausch and Richards, 1971; Chalmers and Barrett, 1974). The difficulty in experimentally infecting deer mice with *E. multilocularis* is consistent with our inability to find naturally infected deer mice. This apparent resistance of deer mice to infection may be attributable to differences in subspecies of intermediate host (Ohbayashi et al., 1971). Alternatively, the resistance of deer mice to infection may be indicative of strain differences of the Montana isolate. Although muskrats were fed more proglottids than the deer mice and the data are limited ($N = 4$), infection was more easily induced in the muskrat. These data also support the importance of a fox–muskrat life cycle in southwestern Montana, a geographic area where the red fox is not indigenous. Records indicate that the first foxes colonized the area in question during the late 1950's (Hoffmann et al., 1969). This, as well as the low prevalence noted in this study, seems consistent with the hypothesis that the parasite was introduced into the region recently and hence has not yet become widely established in a variety of intermediate hosts. The role of the muskrat as an intermediate host of *E. multilocularis* has not been reported elsewhere in North America.

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Table 2. Experimental inoculations of rodents and lagomorphs with *Echinococcus multilocularis* eggs or cyst material.

Route of inoculation	Species	Dosage	Positive cases/ no. inoculated
Oral	Deer mouse	1 proglottid	0/10
		3 proglottids	1/21
	Gerbil	3 proglottids	0/8
	Cotton rat	3 proglottids	0/6
	Muskrat	5 proglottids	1/4
	European rabbit	5 proglottids	0/5
I.P.*	Deer mouse	900 protoscolices	0/4
		8,700 protoscolices	0/6
		12,000 protoscolices	0/3
		14,000 protoscolices	0/2
		17,000 protoscolices	0/4
	Cotton rat	900 protoscolices	0/3
		0.5 g cyst material	2/8
S.I.†	Cotton rat	1 intact cyst	2/3

* I.P. = intraperitoneal inoculation.
† S.I. = surgical implantation.

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Research Note

Ancyrocephalids (Monogenea) of Redbreast Sunfish, Bluegill, and Their Hybrids from Lake Norman, North Carolina: Remarks on Monogeneans as Indicators of Parent Species of Hybrids

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ABSTRACT: Five ancyrocephalid species were found on redbreast sunfish, *Lepomis auritus,* 7 on bluegill, *L. macrochirus,* and 8 on redbreast sunfish × bluegill hybrids from Lake Norman, North Carolina. All 8 species infesting the hybrids were present on one or both of the parent species. The hybrids inherited the ability to harbor monoxenous parasites from their parent species. Investigators may be able to use monogeneans, especially monoxenous species, to identify parent species of hybrids, which are often difficult to identify. *Urocleidus tuberculatus* Allison and Rogers, 1970, is transferred to *Onchocleidus* as *Onchocleidus tuberculatus* (Allison and Rogers, 1970) comb. n.

KEY WORDS: Monogenea, Ancyrocephalidae, *Actinocleidus bennetti, Actinocleidus fergusoni, Anchoradiscus anchoradiscus, Cleidodiscus robustus, Haplocleidus dispar, Onchocleidus ferox, Onchocleidus tuberculatus* comb. n., *Pterocleidus acer,* bluegill, redbreast sunfish, hybrids, *Lepomis auritus, Lepomis macrochirus.*

Nybelin (1937) reported that only those dactylogyrid monogeneans parasitizing the parent host species were found on 3 cyprinid hybrid combinations. These data indicate that monogeneans may be useful in determining parent species of hybrids that are difficult, if not impossible, to identify through morphometric characters.

Ancyrocephalid monogeneans have been reported from all 11 species of the sunfish genus *Lepomis* (see Hoffman, 1967; Beverley-Burton, 1984). However, only 1 species of ancyrocephalid from 2 *Lepomis* hybrid combinations has been reported (Mizelle, 1936), although *Lepomis* hybrids are common in nature (Childers, 1967). Mizelle (1936) listed *Onchocleidus ferox* (Mueller, 1934) as *O. mucronatus* Mizelle, 1936, from bluegill (*L. macrochirus* Rafinesque) × pumpkinseed (*L. gibbosus* (Linnaeus)), orangespotted sunfish (*L. humilis* (Girard)) × pumpkinseed, and all 3 parent species. This paper reports on the species and abundance of ancyrocephalids parasitizing redbreast sunfish (*L. auritus* (Linnaeus)) × bluegill and their parent species from Lake Norman, North Carolina, and further investigates the use of monogeneans to identify parent species of hybrid fishes.

Table 1. Relative density (total number of parasites/total number of hosts), range, and prevalence (% infestation) of ancyrocephalids infesting redbreast sunfish, bluegill, and their hybrids from Lake Norman, North Carolina.

Parasite	Redbreast sunfish			Bluegill			Hybrids		
	Relative density	Range	Prevalence	Relative density	Range	Prevalence	Relative density	Range	Prevalence
Actinocleidus bennetti Allison and Rogers, 1970	3.1	0–8	88.9	0.0	0	0.0	1.0	0–5	55.6
Actinocleidus fergusoni Mizelle, 1938	0.0	0	0.0	16.2	1–36	100.0	1.3	0–8	44.4
Actinocleidus sp.	0.1	0–1	11.1	0.0	0	0.0	0.2	0–1	22.2
Anchoradiscus anchoradiscus Mizelle, 1941	0.0	0	0.0	0.3	0–1	33.3	0.0	0	0.0
Cleidodiscus robustus Mueller, 1934	0.0	0	0.0	0.3	0–1	33.3	0.0	0	0.0
Haplocleidus dispar (Mueller, 1936)	6.3	3–20	100.0	7.2	0–21	88.9	16.8	2–37	100.0
Onchocleidus ferox (Mueller, 1934)	0.0	0	0.0	7.3	0–19	88.9	2.9	0–6	88.9
Onchocleidus tuberculatus (Allison and Rogers, 1970) comb. n.	4.0	0–10	77.8	0.0	0	0.0	1.4	0–6	44.4
Onchocleidus sp.	0.0	0	0.0	0.2	0–2	11.1	0.2	0–1	22.2
Pterocleidus acer (Mueller, 1936)	0.1	0–1	11.1	2.0	0–4	77.8	2.0	0–5	77.8
Total	13.6	3–25	100.0	33.5	2–61	100.0	25.8	4–57	100.0

Nine specimens each of redbreast sunfish, bluegill, and redbreast sunfish × bluegill hybrids ranging from 117 to 137 mm (total length) were caught by angling in a single cove on Lake Norman, Lincoln County, North Carolina, during 12–14 April 1985. Although some *Lepomis* hybrid combinations are difficult or seemingly impossible to identify, these redbreast sunfish × bluegill hybrids were easily identified because of their intermediate color patterns and morphometric characters. The gills were excised and frozen immediately after the fish were caught. After being frozen overnight, the gills were preserved in 10% formalin. The ancyrocephalids were picked from the gills under a dissecting microscope, mounted in glycerin jelly, and identified under a phase contrast microscope.

Ten species of ancyrocephalids representing 6 genera (sensu Beverley-Burton, 1984, 1986) were found during this study (Table 1). *Urocleidus tuberculatus* Allison and Rogers, 1970, is transferred to *Onchocleidus* as *Onchocleidus tuberculatus* (Allison and Rogers, 1970) comb. n., based on the presence of a type 2 penis, accessory piece with proximal aperture through which distal tip of penis protrudes, 2 pairs of approximately equal-sized hamuli lacking a projection on inner curve of blades, transverse bars not articulating with each other, marginal hooks dissimilar in shape and size, and other characters of the genus *Onchocleidus* Mueller, 1936 (see Beverley-Burton, 1984).

All sunfish were infested with ancyrocephalids (Table 1). Five ancyrocephalid species were found on redbreast sunfish, 7 on bluegill, and 8 on the hybrids. Redbreast sunfish harbored 3 species not found on bluegill, whereas bluegill harbored 5 species not found on redbreast sunfish. *Haplocleidus dispar* (Mueller, 1936) and *Pterocleidus acer* (Mueller, 1936) were the only ancyrocephalids found on both redbreast sunfish and bluegill. All 8 species infesting the hybrids were present on one or both of the parent species. The lack of *Anchoradiscus anchoradiscus* Mizelle, 1941, and *Cleidodiscus robustus* Mueller, 1934, on the hybrids was probably due to the small sample size of hosts and rarity of the parasites (lacking on redbreast sunfish and 0.3 parasites per host on bluegill) rather than inability of the hybrids to harbor these species. The data show that the hybrids inherited the ability to harbor monoxenous parasites from their parent species. For example, *Actinocleidus bennetti* Allison and Rogers, 1970, *Actinocleidus* sp., and *O. tuberculatus,* known only from redbreast sunfish, and *Onchocleidus* sp., known only from bluegill, were found also on the hybrids. It is also important to note that species of ancyrocephalids found only on other species of *Lepomis* inhabiting Lake Norman (*Actinocleidus oculatus* (Mueller, 1934), *Actinocleidus recurvatus* Mizelle and Donahue, 1944, and *O. similus* Mueller, 1936, on pumpkinseed; *Actinocleidus flagellatus* Mizelle and Seamster, 1939, *Clavunculus okeechobeensis* (Mizelle and Seamster, 1939), and *O. chaenobryttus* (Mizelle and Seamster, 1939) on war-

mouth, *L. gulosus* (Cuvier); *Actinocleidus bifidus* Mizelle and Cronin, 1943, *Clavunculus bifurcatus* (Mizelle, 1941), and *O. variabilis* (Mizelle and Cronin, 1943) on redear sunfish, *L. microlophus* (Gunther)) were not found on the hybrids (unpubl. data).

The 2 ancyrocephalids that occurred on both parent species had either equal (*P. acer*) or greater (*H. dispar*) relative densities on the hybrids (Table 1). However, the ability of the hybrids to harbor an ancyrocephalid found on only one of the parent species appears to be moderated. Relative densities of *A. bennetti, A. fergusoni* Mizelle, 1938, *O. ferox,* and *O. tuberculatus* on the hybrids were less than those on the infested parent host species. The 2 exceptions to this trend were *Actinocleidus* sp. and *Onchocleidus* sp., which were rare on the parent host species and hybrids. If 1 or 2 more individuals of these parasites had occurred in the parent host samples, these parasites also would have displayed smaller relative densities on the hybrids than on the parent species.

This sample of redbreast sunfish × bluegill hybrids from Lake Norman could have been identified by analysis of ancyrocephalid parasites because the specimens harbored only those species of ancyrocephalids found on the parent species. These data, in corroboration with Nybelin (1937), suggest that monogeneans, especially monoxenous species, may be used widely to identify or verify identifications of parent species of hybrid fishes. A limitation, as was the case here, is that an individual specimen of a hybrid may harbor species of parasites from only 1 parent species, or no parasites. Therefore, a sample of several individuals may be necessary to determine parent species.

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Obituary Notices

NORMAN A. MEINKOTH

Born: 29 January 1913
Elected Member: 14 January 1948
Deceased: Spring 1987
18th Vice President of the Society in 1952

LLOYD ANCIL SPINDLER

Born: 1900
Deceased: 16 November 1987
Elected Member: 10 November 1927
Offices Held: 9th Recording Secretary, 1933
18th President, 1935

Research Note

Brugia sp. from a Domestic Cat in California

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1430 Tulane Avenue, New Orleans, Louisiana 70112 and
[2] Department of Microbiology and Immunology, School of Veterinary Medicine,
University of California, Davis, California 95616

ABSTRACT: A mature female filaria in a cervical lymph node from a domestic cat in California is described and identified as a species of *Brugia*. The presence of microfilariae in the tissues and the absence of associated focal inflammation suggest that the domestic cat is a natural host of the species and may be a reservoir of a human infection acquired in the same region.

KEY WORDS: *Brugia,* domestic cat, California.

In November 1976 a slide bearing 2 sections of a lymph node containing a mature female filaria in a lymph vessel of the capsule, and several microfilariae in the cortical and medullary tissue of the node, was received for identification from Dr. David Gribble of Davis, California. He had received it from a pathologist in Castro Valley who in turn had received the lymph node biopsy from a clinician at Forestville Veterinary Hospital, Sebastopol, in the north central part of the state. The node had been taken from a chain of masses along the left jugular vein of a 2-yr-old domestic cat that was destroyed following a diagnosis of lymphoma.

The worm apparently was alive when fixed. The wall of the vessel containing it was not appreciably thickened and no focal inflammation was evident around the worm or microfilariae. Both longitudinal and transverse sections, representing posterior and midbody levels of the worm, were present (Fig. 1). The following morphological features were evident: diameter of most sections approximately 50 µm, maximum 60 µm. Cuticle finely striated transversely, with smooth outer surface, thickness about 1 µm, slightly increased over the lateral cord areas. Lateral cords inconspicuous, compressed between uteri and cuticle. Somatic muscles coelomyarian with 2–4 cells per quadrant. Dorsal and ventral cords unremarkable. Intestine relatively small, about 10 µm in outside diameter, with 1–3 nuclei per section. Paired uteri, sparsely filled with degenerating ova, together with the intestine occupying most of the pseudocoel space (Fig. 2).

Microfilariae sheathed (Fig. 3), about 4.5 µm in maximum diameter, with the cephalic space 8 µm long in a truncated section (Fig. 4). As the observed adult female was infertile the presence of microfilariae indicated that at least 1 other female and a male were present elsewhere in the body.

The worm's morphology and its location in a capsular vessel of a lymph node, and the presence of sheathed microfilariae with a long cephalic space, clearly identify the infection as that of a *Brugia* species indistinguishable from sections of *Brugia beaveri* Ash and Little, 1964, described from raccoons and experimentally infected cats by Harbut (1975), and from those of *Brugia* species from humans reported by Baird et al. in 1986 and by others earlier. *Brugia beaveri* has been reported from naturally infected raccoons and a bobcat in Louisiana and Florida, respectively. A second North American *Brugia* species is *B. leporis* Eberhard, 1984, whose known hosts are rabbits (*Sylvilagus aquaticus* and *S. floridans*) in Louisiana (Eberhard, 1984, 1986).

The diagnostic features of *B. leporis* in tissue sections are as yet undescribed. Therefore, the worm described in the present report cannot be compared with the known North American species. It can only be identified as a species of *Brugia*, which is true also for the 16 *Brugia* infections observed thus far in tissues from humans in the United States (Baird et al., 1986). Interest in the *Brugia* infection in a native California cat was stimulated by the report of a nonpatent *Brugia* infection in a California man, a resident of Fresno (Baird et al., 1986). The presence of microfilariae in the tissues of the cat and the absence of focal inflammation associated with either the microfilariae or the adult worm suggest that cats are natural hosts of this species of *Brugia* in California, and may be a reservoir of the human infection acquired there.
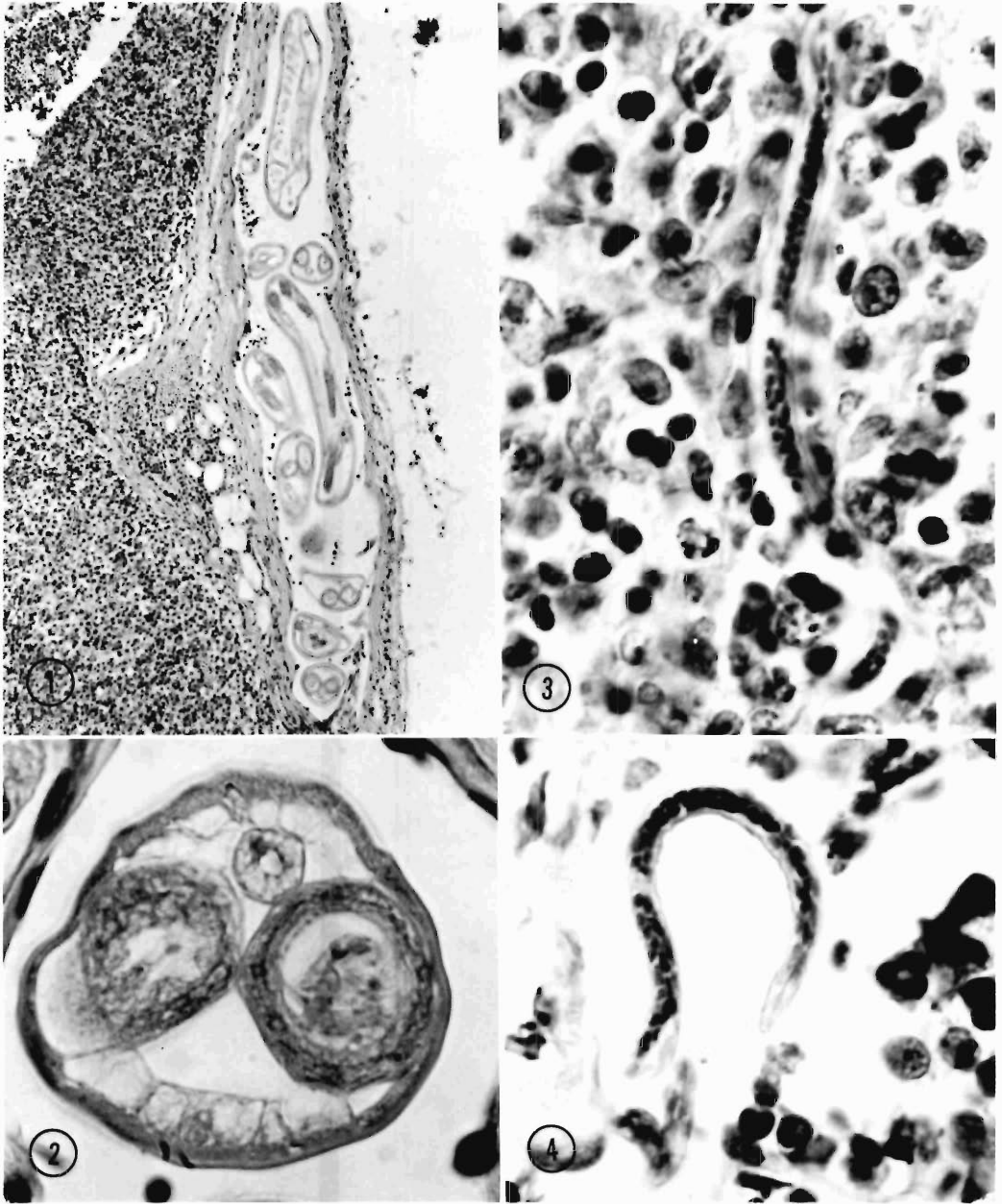
Figures 1–4. *Brugia* sp. in sections of a cervical lymph node from a cat in California. 1. Mature, infertile female in a lymph vessel of the node capsule (×100). 2. Transverse section of the worm shown in Figure 1 (×1,000). 3. Microfilaria, longitudinal section including the nerve ring and showing the presence of a sheath (×1,000). 4. Microfilaria, anterior end showing the nerve ring and incomplete (truncated) cephalic space (×1,000).

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PRESENTATION OF THE 1987 ANNIVERSARY AWARD TO EVERETT LYLE SCHILLER
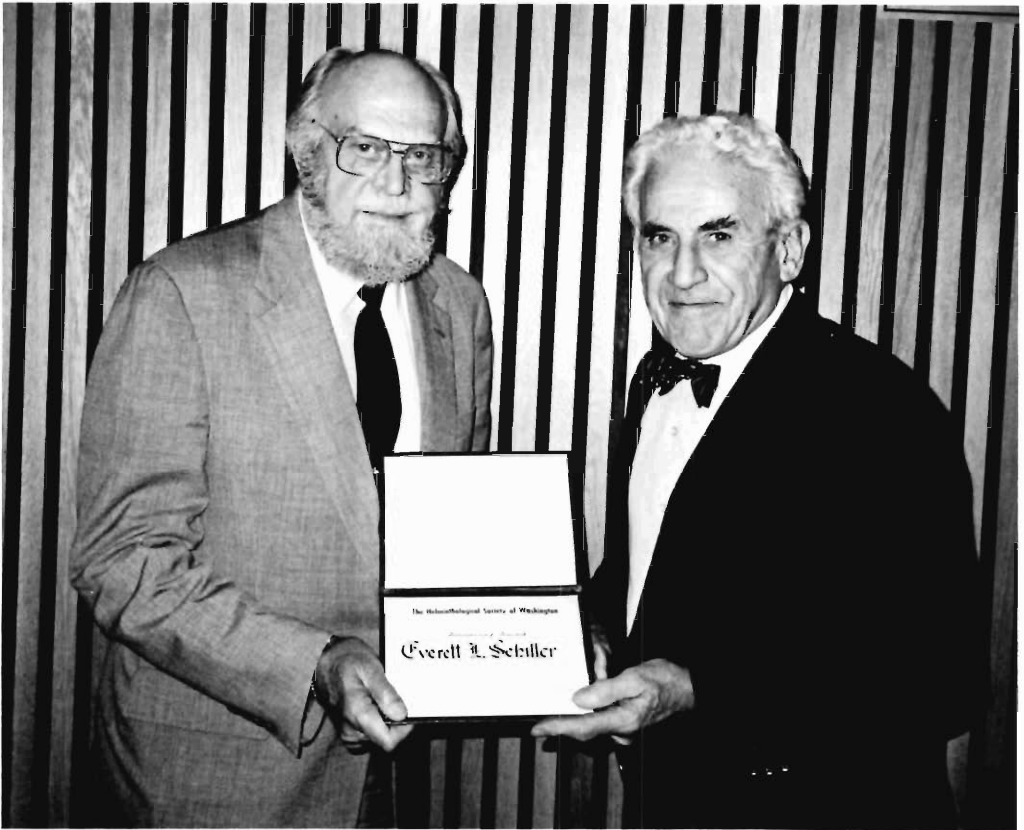
Dr. Leon Jacobs (right) presenting the Anniversary Award to Dr. Everett Schiller.

When Dr. Stirewalt telephoned me recently to make this presentation in her stead, I accepted readily because of my admiration for the recipient. I will now give her prepared talk.

The Anniversary Awards Committee of the Helminthological Society of Washington has had a very enjoyable task; namely that of selecting for the 1987 Anniversary Award a recipient who, according to the Bylaws of the Society, has achieved one or more of the following:

1) made outstanding contributions to parasitology or related sciences which bring honor and credit to the Society, or
2) read an exceptional paper at a Society meeting or published such a one in its *Proceedings,* or
3) given outstanding service to the Society, or

4) made some other contribution warranting highest and special recognition.

Parenthetically, it might interest you to know that there has been a total of 28 recipients with Edna Buhrer in 1960 as the first.

This year the Awards Committee unanimously recommended to the Executive Committee Dr. Everett Lyle Schiller receive the Award. He is a Badger, born in Marshfield, Wisconsin; and his predoctoral education was in Wisconsin. He says he had his introduction to the odors and customs peculiar to parasitology laboratories at the Veterinary Science Department of the University of Wisconsin where he began his training as a lab assistant to Dr. Banner Bill Morgan. His first task there was to take care of a sinkful of

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dirty lab glassware. The decor was enlivened by some very odoriferous mammals whose worm burdens graduate student Robert Rausch was studying. Upon his timid complaint about the terrible smell, he was told by the graduate student that the "quality of an odor was merely relative" and that he "had committed the unforgivable error of making a subjective judgement." This, he says, was his introduction to parasitology, his first lesson in scientific logic, and the beginning of a long friendship with Dr. Rausch. This story shines a little light on Dr. Schiller's early experience at the University of Wisconsin and also on his own sense of humor, for I have lifted it from his acceptance speech upon receiving the Henry Baldwin Ward Medal given by the Society of Parasitologists in 1964 (*Journal of Parasitology* 51:484–486, 1965).

The orderly progress of his life was interrupted twice: first by World War II from 1941 to 1946 when he fought with the U.S. Army and the U.S. Air Force; and second, during his graduate studies, between receiving the M.Sc. degree at Wisconsin in 1950 and beginning work on his Doctorate at the Johns Hopkins University with Dr. Clark Read. This 5½ year interlude with Dr. Rausch provided rare opportunities to expand his experience in helminth taxonomy through identification of parasites of various birds and mammals of the area, and to develop a philosophy of the modification and adaptation of these parasites. It also provided a foundation for research at Hopkins and made it possible for him later, with his own students to return to field studies in the Arctic, this time in the Mackenzie delta region of the Northwest Territories of Canada studying parasite transmission among nesting birds.

Geographically, Dr. Schiller's fieldwork has not been limited to the northland. Turning his attention to human parasitology, he spent a rewarding year in India through the Johns Hopkins Program for Medical Research in association with the well known parasitologists at the Calcutta School of Tropical Medicine. He returned to Calcutta in the early 1960's as Director of the Parasitology Program of the Johns Hopkins Center for Medical Research and Training.

Otherwise in the world, Dr. Schiller has been an invited speaker at conferences and seminars, a site visitor, a leader of field teams, and a consultant in various countries: Japan, England, Guatemala, Panama, Venezuela, Liberia, Kenya, Cameroon, and Nigeria.

For all of his professional life, he has had teaching responsibilities: at Johns Hopkins, of course, where he has been Professor in the School of Hygiene and Public Health since 1965 and Professor Emeritus since 1983; and as Special Lecturer or Visiting Professor at the Uniformed Services University of the Health Sciences, Bethesda, Maryland; The Washington University School of Medicine, St. Louis, Missouri; WRAIR, Washington, D.C.; and Rutgers State University, New Brunswick, New Jersey.

In various advisory capacities he has served such organizations as AID; the Gorgas Memorial Institute, for which he was elected Secretary of the Executive Committee in 1987; WRAIR; Wake Forest University; WHO; NIH; the Pan American Health Organization; ILRAD; and such journals as *The Journal of Parasitology, American Journal of Epidemiology, Experimental Parasitology, Transactions of the American Microscopical Society,* and *Journal of Chemical Ecology.*

Concurrently with all these activities, Dr. Schiller has managed to do research. He has described new species of the genus *Hymenolopis* and 2 new species of trematodes; worked on life cycles and morphology of cestodes of rats, voles, Alaskan sea otters, gulls, geese, kittiwakes, and eider ducks; done cryopreservation and in vitro cultivation studies on *Onchocerca volvulus*; and studied the problems and progress of immunodiagnosis of various helminthic infections.

Now, believe me, I have not exhausted my material, but I have exhausted my time. Let me say just a word about Dr. Schiller's contributions to the Helminthological Society. He was elected to membership 11 October 1950. He has published in the Society's *Proceedings,* served as a member of the Executive Committee and as Recording Secretary, and arranged and hosted the Baltimore meetings at Johns Hopkins for years.

I want to add one thing to this presentation. When I reread Dr. Schiller's speech of acceptance of the Henry Baldwin Ward Medal, I was impressed with one paragraph, part of which I want to read here:

> My father was a carpenter. I envy him because of the tangible nature of his accomplishments. Even now, in the later years of his retirement, when driving through the Wisconsin countryside, he takes great pride in pointing out this or that barn which he built. In reviewing my own work, I find little having sub-

stance such as this. During a brief visit to my old hometown this summer, I told him about this Award. He said, "Bury your medal, son, so that you will not be tempted to take it as a sign that you have arrived. It is better that you should regard it as a mark of a good beginning.

Obviously, Everett took his dad's advice about continuing to work.

As you see, Dr. Schiller qualifies for the Anniversary Award in all the categories which I mentioned in the beginning. On behalf of the Helminthological Society of Washington and the members of the Awards Committee (Peg Stirewalt and Sherman Hendrix), I am happy to present the 1987 Anniversary Award to Dr. Everett Schiller.

Leon Jacobs
substituting for
M. A. Stirewalt, Chair
Awards Committee

ANNIVERSARY AWARD RECIPIENTS

Edna M. Buhrer	1960	David R. Lincicome	1975
Mildred A. Doss	1961	Margaret A. Stirewalt	1975
* Allen McIntosh	1962	* Leo A. Jachowski, Jr.	1976
* Jesse R. Christie	1964	Horace W. Stunkard	1977
Gilbert F. Otto	1965	Kenneth C. Kates	1978
* George R. LaRue	1966	* Everett E. Wehr	1979
* William W. Cort	1966	O. Wilford Olsen	1980
* Gerard Dikmans	1967	Frank D. Enzie	1981
* Benjamin Schwartz	1969	Lloyd E. Rozeboom	1982
* Willard H. Wright	1969	Leon Jacobs	1983
Aurel O. Foster	1970	Harley G. Sheffield	1984
Carlton M. Herman	1971	A. Morgan Golden	1985
May Belle Chitwood	1972	Louis S. Diamond	1986
* Elvio H. Sadun	1973	Everett L. Schiller	1987
E. J. Lawson Soulsby	1974		

HONORARY MEMBERS

* George R. LaRue	1959	Justus F. Mueller	1978
Vladimir S. Ershov	1962	John F. A. Sprent	1979
* Norman R. Stoll	1976	Bernard Bezubik	1980
Horace W. Stunkard	1977	Hugh M. Gordon	1981

CHARTER MEMBERS 1910

* W. E. Chambers	* Philip E. Garrison	* Maurice C. Hall	* Charles A. Pfender
* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	Lloyd E. Rozeboom	1975
* Albert Hassall	1931	Albert L. Taylor	1975
* Charles W. Stiles	1931	David R. Lincicome	1976
* Paul Bartsch	1937	Margaret A. Stirewalt	1976
* Henry E. Ewing	1945	* Willard H. Wright	1976
* William W. Cort	1952	* Benjamin Schwartz	1976
* Gerard Dikmans	1953	Mildred A. Doss	1977
* Jesse R. Christie	1956	* Everett E. Wehr	1977
* Gotthold Steiner	1956	Marion M. Farr	1979
* Emmett W. Price	1956	John T. Lucker, Jr.	1979
* Eloise B. Cram	1956	George W. Luttermoser	1979
* Gerald Thorne	1961	John S. Andrews	1980
* Allen McIntosh	1963	* Leo A. Jachowski, Jr.	1981
Edna M. Buhrer	1963	Kenneth C. Kates	1981
* Benjamin G. Chitwood	1968	Francis G. Tromba	1983
Aurel O. Foster	1972	A. James Haley	1984
Gilbert F. Otto	1972	Paul C. Beaver	1986
* Theodor von Brand	1975	Raymond M. Cable	1986
May Belle Chitwood	1975	Harry Herlich	1987
Carlton M. Herman	1975		

* Deceased.

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(Continued from Front Cover)