Eulimdana florencae n.sp. (Nematoda: Filarioidea) from Micropalama himantopus (Aves: Charadriiformes): evidence for neonatal transmission, ephemeral adults, and long-lived microfilariae among filarioids of shorebirds

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Eulimdana florencae n.sp. from Stilt Sandpipers (Micropalama himantopus (Bonaparte)) (Charadriiformes: Scolopacidae) at Little Quill Lake, Saskatchewan, and Gem, Alberta, Canada, differs from its closest congener (Eulimdana pseudolari) by the lack of a cleft at the posterior extremity of the female. In addition, the microfilaria of E. florencae has a long, attenuated tail unlike that of any previously described species. Adult worms occurred in neck tissues and microfilariae in skin. Seven of 17 juvenile (less than 2 months old) Stilt Sandpipers were infected; four contained mixed sex infections and in two of these birds microfilariae were present in both the female worms and the host's skin. The remaining three infected juvenile birds contained only adult male or only adult female worms. Eight of 12 adult birds were infected; six contained only skin-inhabiting microfilariae, one contained microfilariae plus a single adult female (probably senescent), and one contained a single male. Support is provided, therefore, for a recent hypothesis predicting that Eulimdana spp. are often acquired early in life by scolopacid shorebirds, that postreproductive adult worms are ephemeral, and that the skin-inhabiting microfilariae are long-lived.

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Eulimdana florencae n.sp., trouvé chez des Bécasseaux à échasses (Micropalama himantopus (Bonaparte)) (Charadriiformes: Scolopacidae) au lac Little Quill, Saskatchewan, et à Gem, Alberta, Canada, diffère de l'espèce la plus apparentée (Eulimdana pseudolari) par l'absence d'échancrure à l'extrémité postérieure de la femelle. De plus, la microfilaire d'E. florencae a une longue queue amenuisée très différente de celle des autres espèces connues. Les vers adultes parasitent les tissus du cou et les microfilaires se retrouvent dans la peau. Sur 17 oiseaux juvéviles (âgés de moins de 2 mois), sept étaient parasités; quatre étaient infectés par des mâles et des femelles et deux de ceux-là contenaient des microfilaires présentes à la fois dans les vers femelles et dans la peau des hôtes. Les trois autres oiseaux infectés contenaient seulement des vers adultes mâles ou des vers adultes femelles. Huit des 12 oiseaux adultes étaient infectés; six contenaient seulement des microfilaires dans la peau, un contenait des microfilaires et une seule femelle adulte (probablement sénescente), et un contenait un seul mâle. Ces données corroborent l'hypothèse récente selon laquelle les espèces d'Eulimdana sont souvent acquises tôt au cours du cycle par des scolopacidés, les vers adultes sont éphémères après la reproduction, et les microfilaires qui vivent dans la peau ont longue vie.

[Traduit par la revue]

Introduction

Species of Eulimdana are the most common filarioid nematode parasites of birds in the order Charadriiformes. In general, adult worms inhabit connective tissues in the head and neck and microfilariae occur in the skin. However, Bartlett et al. (1989) were unable to find adult worms in the vast majority of charadriiforms in which they found microfilariae. Consequently, they suggested that adult worms are short-lived (i.e., ephemeral) and, upon death, are quickly resorbed by the host whereas microfilariae persist for prolonged periods in the skin. They also suggested that infections would generally be acquired early in life through the agency of louse vectors and predicted that juvenile birds would offer the best prospect in which to find adult worms. Their hypothesis was based on Eulimdana infections in juvenile and adult Willets (Catoptrophorus semipalmatus (Gmelin)), adult Red-necked Phalaropes (Phalaropus lobatus (L.)), adult Wilson's Phalaropes (Phalaropus tricolor (Vieillot)), and adults of six other species in the family Scolopacidae. Through the cooperation of the Canadian Wildlife Service, we have recently had the opportunity to examine juveniles of one of these six species, namely the Stilt Sandpiper (Micropalama himantopus (Bonaparte)), and to test the above hypothesis. The present paper describes Eulimdana florencae n.sp. on the basis of worms found in these juveniles and provides new evidence for the hypothesis that adult worms are ephemeral and microfilariae long-lived.

Materials and methods

Seventeen frozen juvenile and two frozen adult Stilt Sandpipers were provided by the Canadian Wildlife Service, Saskatoon, Sask.; these birds had been collected at Little Quill Lake (51°55′N, 104°05′W), Sask., Canada, on 17–20 August 1988. Seven adult Stilt Sandpipers were shot by the authors near Gem (50°57′N, 112°11′W), Alta., Canada, on 17 May 1987, and three adults at Tilley "B" Reservoir (50°35′N, 111°38′W) near Tilley, Alta., Canada, on 27 May 1988. Birds collected by the authors were immediately placed in plastic bags and later frozen.

Thawed carcasses were examined for adult filarioid worms and microfilariae, as described in Bartlett *et al.* (1989). Adult worms were fixed in a hot solution of 5% glycerin in 70% alcohol and studied in glycerin. Microfilariae from the skin were studied in a solution of equal volumes of 5% formalin and saline. Microfilariae dissected from the uteri of female worms were studied in glycerin.

Specimens were deposited in the parasite collection of the United States National Museum (USNM) in Beltsville, Maryland. The different types of infections (i.e., sterile, mixed sex, patent) referred to herein are defined in Bartlett *et al.* (1989).

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Results

Adult filarioids, representing the new species of *Eulimdana* described below, were found in connective tissues of the head and neck; microfilariae were found in skin.

Seven of 17 juvenile Stilt Sandpipers were infected (Table 1). Two birds contained adult male worms, adult female worms with microfilariae, and skin-inhabiting microfilariae. Two other birds also contained adult male worms and adult females, but these females contained only ova; in one (No. C-59) of these birds, the single male worm was found ventrally at the root of the free neck, whereas females were at the chin. In the other bird (No. C-66), spicules were not visible in the single male. The remaining three infected juvenile birds contained sterile infections.

Eight of 12 adult Stilt Sandpipers were infected (Table 1). One contained skin-inhabiting microfilariae plus a single female with only ova, six contained only skin-inhabiting microfilariae, and one contained a sterile infection.

The description below is based on adult worms and microfilariae from patent infections in juvenile sandpipers. Specimens from adult birds were morphologically similar to those from juveniles.

Eulimdana florencae n.sp. (Figs. 1–39; Tables 2, 3)

GENERAL: Filarioidea, Onchocercidae, Lemdaninae, Eulimdana Founikoff, 1934 (sensu Bartlett et al. 1985). Short nematodes with bluntly rounded extremities. Cuticle with delicate transverse striations; individual striations not extending completely around body. Cuticle also with numerous slight, irregularly round to fusiform elevations distributed irregularly over body with orientation at mid-body tending to be transverse over muscle fields and longitudinal over lateral fields; elevations readily apparent in transverse section of body (Figs. 6, 7, 29, 30), but difficult to discern in surface view unless glycerin has been allowed to drain away (Figs. 8, 28). Cuticular striations and elevations together imparting a delicate bumpy appearance to cuticle in side view (Figs. 9a, 31a, 31b); bumpiness varying considerably within the same and among different individuals (owing to irregular distribution of elevations), generally more pronounced in females than males. Cuticle minutely thicker in lateral than in dorsal or ventral fields (Figs. 6, 29). Cephalic extremity with small, oval oral opening, four pairs of papillae, and two lateral amphids (Figs. 5, 27); papillae generally symmetically arranged. Supra and suboral cuticular inflations present, well defined with (in lateral view: Figs. 2, 3, 21, 26) small base and high profile; inflations readily apparent in both sexes. Cuticular oral ring delicate. Oesophagus stout, club shaped, muscular, clearly demarcated from intestine (Figs. 2, 21). Excretory pore not observed. Phasmids terminal.

MALE: N=5, including holotype and one paratype from bird No. C-76 and three vouchers from bird No. C-67; all specimens mature adults. Measurements given in Table 2. Body width increasing gradually over anterior third of body, approximately uniform over middle third, decreasing gradually over posterior third (Fig. 1). Anterior end of body U-shaped between cephalic extremity and nerve ring (Fig. 2). Tail generally in slight ventrad curve (Fig. 1), lacking ala-like structures. Anus subterminal. Terminal end of rectum spherical, with thick cuticular walls and appearing in ventral view as well-defined ring around anus (Figs. 15–17). Single, laterally elongate papilla present immediately anterior to anus. Additional papil-

TABLE 1. Microfilariae and adult Eulimdana florencae n.sp. in infected juvenile and adult Stilt Sandpipers (Micropalama himantopus) from Little Quill Lake, Saskatchewan (S), and Gem, Alberta (A)

Bird No.	Location	Microfilariae ^a	No. of ∂ worms	No. of ♀ worms	
Juveniles					
C-49	S	_	1	0	
C-59	S	_	1	2^b	
C-61	S	_	0	1^b	
C-64	S	_	0	1	
C-66	S	_	1	2^b	
C-67	S	+	3	1^c	
$C-76^d$	S	+	2	7^c	
Adults					
C-7	S	20	0	0	
C-63	S	. —	1	0	
G-62	Α	8	0	0	
G-79	Α	26	0	0	
G-80	Α	2	0	0	
G-81	Α	1	0	0	
G-82	Α	70	0	1^b	
G-83	Α	11	0	0	

^aSkin-inhabiting microfilariae reported as present (+), absent (-), or number found in a $2-3 \times 2-3$ mm sample of cheek skin that had been teased in three or four drops of saline on a microscope slide.

lae present on tail (Figs. 12–20), sessile with central stalk, distributed in two groups (Fig. 16) as follows: (I) in two rows, one on each side of anus, with three in both rows of holotype (Fig. 17) and generally three, but occasionally two, in one or both rows of other specimens; papillae variable in size and position; and (II) as an adanal pair in area of spherical rectum (note: papillae pattern most apparent in ventral view and central stalk of papillae apparent only if specimen appropriately oriented as, for example, in Fig. 14). Phasmids near posteriormost papillae (Figs. 14, 16, 17), difficult to discern in lateral view. Two small sessile postdeirids present, one in each lateral field at level of, or slightly anterior to, anus (Figs. 12, 13, 15). Spicules subequal in length, dissimilar in morphology (Figs. 10, 11), capitula enlarged. Distal end of left spicule pointed, point sharp or slightly blunt. Distal end of right spicule rounded, with cap of clear cuticle.

FEMALE: N=8, including allotype and six paratypes from bird No. C-76 and one voucher from bird No. C-67; all specimens containing microfilariae. Measurements given in Table 3. Slender worms with body width increasing gradually over anterior fifth of body, approximately uniform over middle three fifths, decreasing gradually over posterior fifth. Anterior end of body U-shaped between cephalic extremity and nerve ring (Figs. 21–24). Slight dilation in diameter of body generally (Figs. 21–23), but not always (Fig. 24), present in oesophageal region; dilation not imparting bulbous appearance, occurring in specimens with (Fig. 23) and without (Figs. 21, 22) uterine loops extending to nerve ring. Vulva postoesophageal, lips slightly salient (Fig. 37). Complex ovijector absent. Vagina with one or two gradual turns, length obscured by uteri. Uteri convoluted, in some specimens loops extending as far as nerve ring and posterior extremity. Germinative ends of ovaries in posterior two thirds of body, generally obscured by uteri. Posterior end of body U-shaped (Fig. 33), generally slightly

^bFemale(s) not containing microfilariae.

^{&#}x27;Female(s) containing microfilariae

^dHost from which the type specimens of E. florencae were obtained.

TABLE 2. Major dimensions (in µm, unless otherwise indicated) of male *Eulimdana florencae* n.sp. from juvenile and adult Stilt Sandpipers (*Micropalama himantopus*)

	From juvenile birds				From adult bird	
	Holotype ^a	Paratype ^a	Vouchers ^a	Others ^b	Other ^c	Other
N	1	1	3	2	1	1
Total length (mm)	5.0	5.1	5.2,5.8,5.9	2.7,4.8	4.6	4.6
Maximum width	200	185	185,185,170	-d,175	152	165
Nerve ring from anterior	80	75	70,95,75	80,90	80	100
Body width at nerve ring	135	140	145,155,135	-d,138	135	140
Oesophagus length	205	190	190,225,210	205,220	195	240
Maximum width of oesophagus	42	35	35,38,35	32,32	35	42
Body width at end of oesophagus	135	150	150,155,145	-d,130	135	140
Anus from posterior	40	37	28,30,28	13,35	25	20
Left spicule	135	135	123,127,118	140, -e	128	120
Right spicule	120	120	123,110,116	120, -e	125	115

Note: Measurements of each specimen listed.

bulbous (Fig. 34), containing nerve strands. Anus subterminal (Figs. 32, 35), tiny, difficult to discern. Posterior extremity of body lacking cleft. Postdeirids not observed. Phasmids extremely difficult to discern (Fig. 32).

MICROFILARIA: (1) N=20, from skin adjacent to adultworms in juvenile bird No. C-76, mean followed by range. Length 115, 106-126 μm. Maximum width 7, 6-8 μm, attained near mid-body. Anterior end bluntly rounded, with (Fig. 38) or without slight constriction. Tiny, cephalic cuticular structures present. Cuticle with fine transverse striations. Inner body not observed; other internal structures not visible. Tail pointed, long, and markedly attenuated. Loose, oval sheath present (Fig. 38); maximum width 16, 10–22 μ m. (2) N=8, from cheek skin of adult bird No. G-62, mean followed by range. Length 122, 110-130 µm. Maximum width 7, 6-8 µm. Morphologically similar to microfilariae from skin of juvenile bird. (3) N=25, from uteri of paratype No. 2, mean followed by range. Length 100, 91-113 μm. Maximum width 8, 7-9 μm, attained near mid-body. Body shape similar to microfilariae from skin. Sheath visible as wrinkled envelope around microfilarial body (Fig. 39).

HOST TYPE: Stilt Sandpiper, Micropalama himantopus (Bonaparte) (Charadriiformes: Scolopacidae).

SITE IN HOST: Adult worms in connective tissues of head and neck, most commonly in interclavicular region between fat lobules or under skin. Microfilariae in skin, particularly that of head, neck, and wings.

LOCALITIES: Type locality: Little Quill Lake (51°55'N,

104°05′W), Sask., Canada. Other locality: near Gem (50°57′N, 112°11′W), Alta., Canada.

SPECIMENS: Type specimens from bird No. C-76, USNM No. 80899 (holotype), USNM No. 80900 (allotype), and USNM No. 80901 (paratypes). Voucher specimens from bird No. C-67, USNM No. 80902. Specimens from birds Nos. C-59 and C-66, USNM No. 80903; from birds Nos. C-49, C-61, C-63, and C-64, USNM No. 80904; and from bird No. G-82, USNM No. 80905.

ETYMOLOGY: florencae, in honour of Mrs. Florence Bartlett of Brooks, Alta., Canada.

DIAGNOSIS: Eulimdana florencae n.sp. is distinguished by the lack of a cleft at the posterior end of the body of the female, which is present in its morphologically closest congener, E. pseudolari Bartlett, Anderson, and Bush, 1989 (see Fig. 93) in Bartlett et al., 1989). In addition, the vulva of E. florencae is 680-965 µm from the anterior extremity in specimens containing microfilariae (N=8), whereas that of E. pseudolari is $2800-3100 \mu m$ from the extremity (N=2). Furthermore, the microfilaria of E. florencae has a long, attenuated tail, whereas the microfilaria of E. pseudolari has a short tail. Eulimdana asperum Bartlett, Anderson, and Bush, 1989 and E. juventarum Bartlett, Anderson, and Bush, 1989 are the only other species described from North American birds. Eulimdana florencae has a delicately bumpy cuticle, whereas that of E. asperum is markedly wrinkled; E. florencae has supra and suboral cuticular inflations, which are absent in E. juventarum.

COMMENTS: Adult E. florencae from thawed birds were in

Figs. 1–20. Eulimdana florencae n.sp., mature adult &; Figs. 2, 4, 17, and 20 of holotype; Fig. 3 of paratype; Figs. 1, 5–16, 18, and 19 of vouchers from bird No. C-67. Fig. 1. Outline of body; arrow indicates direction of view of Fig. 20. Fig. 2. Anterior end, lateral view. Figs. 3–5. Anterior extremity, lateral, ventral, and enface views, respectively. Fig. 6. Transverse section at mid-body. Fig. 7. Detail of body wall over part of dorsal, lateral, and ventral fields in Fig. 6; note slight, irregular elevations in cuticle. Fig. 8. Cuticle, surface view at mid-body showing irregular cuticular elevations; arrow indicates junction of lateral and dorsal fields, long vertical lines outline underlying muscles. Fig. 9. Cuticle, edge view at mid-body of whole mount; Fig. 9a showing greater degree of irregular bumpiness than Fig. 9b. Figs. 10, 11. Right and left spicules, respectively. Figs. 12–14. Posterior extremity, right lateral views. Figs. 15–17. Posterior extremity, ventral views; note papilla Groups I and II and single preanal papillae (P) as indicated in Fig. 16. Figs. 18, 19. Posterior extremity, left lateral views. Fig. 20. Posterior extremity, semi-apical view as indicated by arrow in Fig. 1. Postdeirids, large arrows in Figs. 12, 13, and 15. Phasmids, small arrows in Figs. 14, 16, and 17.

[&]quot;From mixed sex infections in which female worms contained microfilariae.

^bFrom mixed sex infections in which female worms contained only ova.

^cFrom sterile infections.

^dSpecimen damaged.

^{&#}x27;Spicules absent.

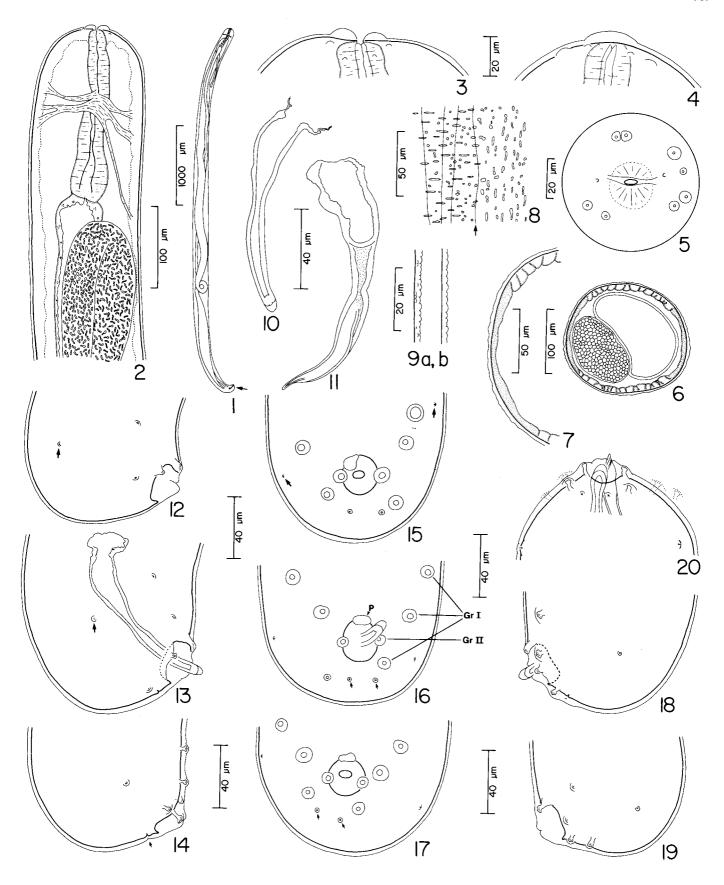


Table 3. Major dimensions (in µm, unless otherwise indicated) of female Eulimdana florencae n.sp. from juvenile and adult Stilt Sandpipers (Micropalama himantopus)

-	From juvenile birds				From adult birds	
	Allotype ^a	Paratypes ^a	Voucher ^a	Others ^b	Others ^c	Other ^d
N	1	6	1	4 ^e	2^f	1
Total length (mm)	17.2	12.4,13.4,15.0,15.0,15.6,15.6	19.0	9.4,12.0,9.0,13.6	6.6,7.4	13.0
Maximum width	340	325,310,340,340,355,325	360	310,320,225,280	170,180	260
Nerve ring from anterior	100	80,125,95,100,85,110	100	80,75,75,100	80,75	100
Body width at nerve ring	240	250,220,275,270,280,230	275	240,260,210,225	165,160	195
Oesophagus length	290	270,255,270,275,250,275	265	200,190,200,240	210,220	200
Maximum width of oesophagus	41	42,50,50,41,39,45	48	40,48,45,35	35,35	38
Body width at end of oesophagus	280	220,250,300,280,300,250	320	260,300,220,235	180,155	210
Vulva from anterior	680	860,880,940,750,925,775	965	815,590,650,625	525,375	730
Body width at vulva	260	270,250,270,270,280,250	305	260,260,200,230	160,150	190
Anus from posterior extremity	15	20,25,10,10,20,40	15	30,10,10,40	30,20	20

Note: Measurements of each specimen listed.

^cFrom sterile infections.

excellent condition. We wish to emphasize that these specimens were fixed in hot glycerin—alcohol, since we have found that morphologic detail is not equally apparent in specimens similarly obtained from thawed carcasses, but fixed in cold glycerin—alcohol. Microfilariae were easily detected in thawed skin.

Worms from mixed sex infections in which female worms contained only ova were morphologically similar to worms on which the taxonomic description is based, with the exception noted previously (i.e., the absence of spicules in the male in bird No. C-66). Males and females (Fig. 25) from sterile infections and the single female in a patent infection were also morphologically similar to worms on which the taxonomic description is based. However, some of these additional worms were smaller (Tables 2 and 3).

Discussion

The prediction that adults of *Eulimdana* spp. will most likely be found in juvenile shorebirds if infections are commonly acquired early in life (Bartlett *et al.* 1989) was borne out by the present study. Stilt Sandpipers winter in South America and breed in northern Canada and Alaska. Near Churchill, Manitoba, birds nest in June and incubation lasts 19.5–21 days (Jehl 1973). Thus, juveniles examined herein were probably less than 2 months old.

Ephemerality apparently characterizes postreproductive adults of *E. florencae*, otherwise one would find adult worms in all birds with skin-inhabiting microfilariae rather than mainly in the recently infected juveniles. Ephemerality of adults is evidently

accompanied by longevity of the skin-inhabiting microfilariae, since only the latter were found in most adult birds. Bartlett *et al.* (1989) lacked data about patent infections in both juvenile and adult birds of the same species, but suggested that adult worms are short-lived whereas skin-inhabiting microfilariae persist. They outlined how adult ephemerality might be evolutionarily advantageous if it enhanced microfilarial survival. We wish to add that ephemerality might also be advantageous if it curtails production of microfilariae, thus limiting the numbers of microfilariae in the skin and decreasing the probability that vectors ingest lethal numbers.

In the two juvenile Stilt Sandpipers that contained mixed sex infections but not microfilariae, the female worms probably had not been fertilized. In one of these birds, the male was far from the females, whereas in the other, the male appeared malformed. The three remaining juvenile birds contained sterile infections, as did one adult. Sterile infections are not unusual among other scolopacids (Bartlett *et al.* 1989), but it is not known if individuals with such infections can be reinfected.

One adult bird contained both skin-inhabiting microfilariae and a single adult female worm with ova. This female was similar in size to females containing microfilariae (Table 3) and may have been a senescent worm from the original infection that had not yet been resorbed by the host. Alternatively, this female may have resulted from a reinfection, but we consider this unlikely; Bartlett *et al.* (1989) suggested that protective immunity develops in individuals with patent infections and that reinfection is rare.

The microfilaria of E. florencae has a much longer and more

Figs. 21–39. *Eulimdana florencae* n.sp., young adult ♀ (Fig. 25 only), mature adult ♀, and microfilaria; Figs. 21, 22, 32–34, and 37 of allotype; Figs. 26–31, 35, and 36 of paratype No. 2; Fig. 23 of paratype No. 5; Fig. 24 of paratype No. 3; Fig. 25 of specimen from bird No. C-64. Figs. 21–25. Anterior end, lateral views. Figs. 26, 27. Anterior extremity, lateral and *enface* views, respectively. Fig. 28. Cuticle, surface view at mid-body showing irregular cuticular elevations; arrow indicates junction of lateral and dorsal fields, long vertical lines outline underlying muscles. Fig. 29. Transverse section at mid-body. Fig. 30. Detail of body wall over part of dorsal and lateral fields in Fig. 29; note slight, irregular elevations in cuticle. Fig. 31. Cuticle, edge view at mid-body of whole mount; Fig. 31 a showing greater degree of irregular bumpiness than Fig. 31 b or 31 c. Fig. 32. Posterior extremity, lateral view; P, phasmid; A, anus. Figs. 33, 34. Posterior end, lateral views. Fig. 35. Posterior extremity, ventral view; A, anus. Fig. 36. Posterior end, ventral view. Fig. 37. Vulva, lateral view. Figs. 38, 39. Microfilaria, from skin of host and uteri of paratype, respectively.

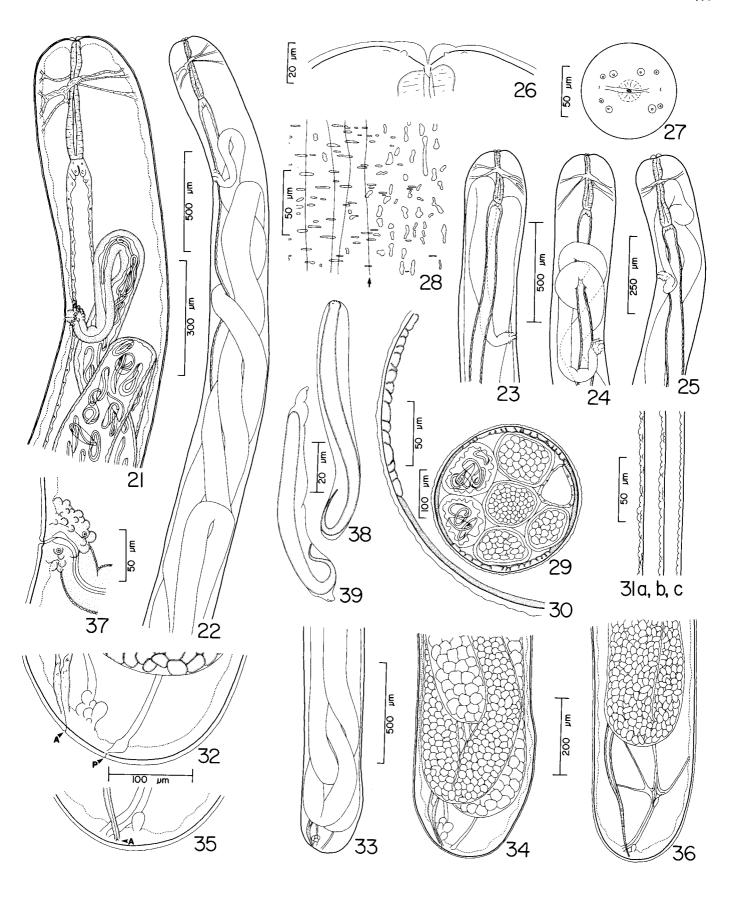
^aFrom mixed sex infections, females containing microfilariae.

^bFrom mixed sex infections, females containing only ova.

^dFrom patent infection, single adult female containing only ova present.

First two specimens (both damaged) are from bird No. C-59, second two from No. C-66.

First specimen is from bird No. C-61, second from No. C-64.



attenuated tail than that of any other described species of *Eulimdana*. We have, however, observed a morphologically similar microfilaria in five other species of birds in the family Scolopacidae and one in Recurvirostridae and it might not be diagnostic (C. M. Bartlett and R. C. Anderson, unpublished data). The species of *Eulimdana* present in those birds have not been identified.

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