MORPHOLOGY AND PHYLOGENY OF THE ELAPHOSTRONGYLINAE (NEMATODA: PROTOSTRONGYLIDAE)

BY

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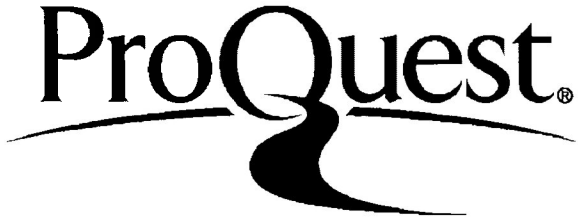

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ABSTRACT

The morphology of elaphostrongyline nematodes parasitizing North American cervids was studied using light and scanning electron microscopy in order to improve previous descriptions that were based exclusively on light microscopical studies. Structures including the spicules, gubernaculum, bursa, cephalic region, sensory structures, and female tails of *Parelaphostrongylus tenuis*, *P. odocoilei, P. andersoni*, and *Elaphostrongylus cervi rangiferi* are described. The morphology of *E. c . rangiferi* as described in this study is similar to previous light microscopical descriptions. The spicules of all three *Parelaphostrongylus* spp. were alike but differed from those of *E. c. rangiferi* by having a dorsal branch of the spicule shaft in the distal region that extends to the tip. A membrane spans the area between the branch and the shaft. The corpus of the gubernaculum of *P. odocoilei* is not split distally as has been described by previous authors, but instead has a longitudinal groove in the distal region. Ventral protuberances associated with the cloaca are present in *Parelaphostrongylus* spp. but absent in *E. c. rangiferi*. Sensory papillae and pores associated with the cloaca and the bursal rays are described. In dorsal view, the base of the dorsal ray has a prominent edge of thickened cuticle in *P. odocoilei* and *P. andersoni*, whereas in *E. c. rangiferi* and *P. tenuis* it is smooth. The female tails of *Parelaphostrongylus* spp. have a papilla-like projection at their tips.

The phylogeny of the Elaphostrongylinae was reconstructed using cladistic techniques. Morphological characters described in this study provide strong evidence

for the monophyly of *Parelaphostrongylus*. The genus is made up of two clades, one containing *P. odocoilei* and *P. andersoni*, the other being *P. tenuis*. The biogeography of the Cervidae of North America in relation to the phylogeny of the Elaphostrongylinae is discussed. The genus *Parelaphostrongylus* probably coevolved with the genus *Odocoileus* in the Nearctic, while *Elaphostrongylus* spp. most likely had a Palearctic origin.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Nematodes belonging to the subfamily Elaphostrongylinae Boev and Schulz, 1950 are parasites of the central nervous system and skeletal muscles of cervids. They are of particular interest in wildlife management. Of the four species known to occur in North American cervids, two can cause a neurologic disease in their hosts. *Parelaphostrongylus tenuis* (Dougherty, 1945) occurs in the subdural space of the brain and spinal cord of white-tailed deer, usually without causing significant pathology. However, in most other North American cervids including moose *(Alces alces* Linnaeus), woodland caribou (*Rangifer tarandus caribou* Linnaeus), wapiti (*Cervus elaphus canadensis* Linnaeus), and mule deer (*Odocoileus hemionus* Rafinesque), *P. tenuis* infection may be fatal (Anderson and Prestwood 1981). *Elaphostrongylus cervi* Cameron, 1931 causes neurologic disease in caribou when young animals are heavily infected (Lankester and Northcott 1979). *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier, 1934) and *P. andersoni* Prestwood, 1972 inhabit the skeletal muscles of the back and the limbs of cervids and do not cause clinical disease. Each of the four species produces dorsal-spined larvae which are morphologically indistinguishable.

In 1931, specimens of a nematode were found in the skeletal muscles of red deer (*Cervus elaphus elaphus* Linnaeus) in Europe, and T. W. Cameron proposed the genus *Elaphostrongylus* for the new species *E. cervi.* Three years later a similar species was discovered in the skeletal muscles of black-tailed deer (*Odocoileus*

hemionus columbianus) in North America and named *Elaphostrongylus odocoilei* by Hobmaier and Hobmaier (1934). The third related species, first discovered in the lung of a white-tailed deer (*Odocoileus virginianus* Zimmermann), was named *Pneumostrongylus tenuis* by Dougherty (1945). Subsequently, it was discovered that most adults of this species inhabit the central nervous system of their hosts (Anderson 1956; Whitlock 1959; Degiusti 1955; Anderson 1963).

The genus *Parelaphostrongylus* was erected by Boev and Schulz (1950), with *P. odocoilei* as the type species. The diagnostic features of this genus included the bifid dorsal ray of the male bursa, a complex gubernaculum with a corpus that was separated into two cords, and the presence of crurae (Skrjabin *et al.* 1952). A redescription of *P. odocoilei* by Brunetti (1969) validated the genus *Parelaphostrongylus*, and the species first described by Dougherty as *Pneumostrongylus tenuis* was subsequently transferred to *Parelaphostrongylus* by Pryadko and Boev (1971). These authors characterized the genus as having a gubernaculum composed of a single corpus with paired crurae and spicules that "may have a longitudinal cleft towards the end". Members of the genus *Elaphostrongylus* were described as having a gubernaculum without crurae and spicules with a shaft that is not cleft (Pryadko and Boev 1971).

The most recently discovered species to be placed in the genus was *P. andersoni*. It was first discovered in the skeletal muscles of white-tailed deer in the southeastern United states (Prestwood 1972) and was later found to be widespread in North American caribou (Lankester and Hauta 1989). This species differs from

others in the genus by having reduced crurae and shorter spicules (Prestwood 1972).

Members of the genus *Elaphostrongylus* occur in a variety of Eurasian cervids including reindeer *(Rangifer tarandus tarandus)*, red deer, sika deer (*Cervus nippon* Gervais, 1841), roe deer (*Capreolus capreolus* Linnaeus) and moose. In addition to *E. cervi*, three other species have been assigned to the genus. One of these, *E. panticola* Liubimov, 1945, was described from *C. elaphus sibiricus* and was said to be distinct from *E. cervi* on the basis of an additional bursal ray present between the dorsal and externodorsal rays (Boev 1957). Another species, *E. rangiferi*, was described by Mitskevich (1958) from reindeer and was said to have a shorter and differently - shaped gubernaculum than that of *E. cervi* and *E. panticola*. Also, the bursal rays were said to have a heavier structure and additional branches unlike those of *E. cervi* and *E. panticola* (Mitskevich 1958). No clear morphological characters were presented, however, that could enable one to accurately distinguish between the three species. Kutzer and Prosl (1975) considered *E. panticola* and *E. rangiferi* to be synonymous with *E. cervi*, and Kontrimavichus *et al.* (1976) classified the former two as subspecies of *E. cervi*.

More recently, several authors have given species status to the several forms of *Elaphostrongylus* spp., citing morphological differences and host specificity as species criteria (Steen *et al.* 1989; Steen and Johansson 1990; Gibbons *et al.* 1991; Steen 1991). These authors support the recognition of *E. cervi* and *E. rangiferi*. In addition, *E. alces* Steen *et al.*, 1989 was described as a distinct species on the basis of morphometric differences, a bottle - shaped esophagus, and an oval - shaped bursa

(the bursa was said to be circular in *E. rangiferi* and *E. cervi*). Also, the epidural location of adult *E. alces* in the nervous system of moose differed from the subdural and subarachnoidal location of *E. rangiferi*, *E. panticola*, and *E. cervi* (Steen 1991). Furthermore, in a revision of the genus *Elaphostrongylus* by Gibbons *et al.* (1991), characters including the shape of the anterior end of the body, morphology of the cephalic region, the length of the bursal lobes, and the shape of the female tail were also used to distinguish the species. Although subtle morphological differences appear to exist between forms from different hosts, there do not seem to be solid criteria for distinguishing them. Morphological descriptions of *Elaphostrongylus* spp. show that the bursa is intraspecifically variable, and the ranges of morphometric measurements of structures such as the gubernaculum show considerable overlap. Therefore, for the purpose of this study, all of the various forms of the genus *Elaphostrongylus* will be regarded as subspecies of *E. cervi* as proposed by Kontrimavichus *et al.* (1976). Thus, the form of *E. cervi* occurring in North America (Newfoundland) (Lankester and Northcott 1979) will be referred to as *E. c. rangiferi*.

There has also been disagreement in morphological descriptions of the North American *Parelaphostrongylus* spp. In particular, various investigators have interpreted the structure of the spicules differently based on light microscopical observations. In the initial description of *P. tenuis*, for example, the spicules are illustrated as having a longitudinal slit in the distal portion of an otherwise solid shaft (Dougherty 1945). This slit has also been described as a foramen by Platt (1978). Whitlock (1959), however, stated that the *P. tenuis* spicule is not really split, but that

it only appears to be split when the spicules are not protruded through the cloaca.

Similarly, the spicules of *P. andersoni* have been depicted as being longitudinally split in the distal quarter of the shaft (Pybus and Samuel 1981). More recently, Lankester and Hauta (1989) concluded that a split does not exist in this part of the spicule, and that it is instead a lightly sclerotized region of the spicule shaft. It is evident, therefore, that certain aspects of the morphology of the Elaphostrongylinae have been difficult to interpret and require re-examination.

The use of scanning electron microscopy (SEM) can aid in providing more accurate morphological descriptions of nematodes (Hirschmann 1983; Gibbons and Khalil 1990). Recently, SEM has been used to study the morphology of *Elaphostrongylus* spp. (Steen and Johansson 1990; Gibbons *et al.* 1991). SEM would thus be a useful tool for studying the more problematic aspects of the morphology of North American elaphostrongyline nematodes with the advantages of providing higher resolution and emphasizing a surface rather than a transparent view of structures which are difficult to discern by light microscopy.

In recent years, phylogenetic systematics, or cladistics (Hennig 1966; Wiley 1981; Wiley *et al.* 1991) has been used increasingly in phylogenetic studies of parasitic helminths (eg. Brooks and Glen 1982; Glen and Brooks 1985; Platt 1988; Hoberg and Adams 1992; Carney and Brooks 1991). Cladistic techniques have also been used to reconstruct the phylogeny of North American elaphostrongyline nematodes (Platt 1978; Platt 1984). However, the morphological characters used in the latter two studies were based on light microscopical observations that have been variously

interpreted by different authors.

The purpose of this study was threefold. First, SEM and light microscopy were used to elucidate the detailed morphology of *P. tenuis, P. andersoni, P. odocoilei,* and *E. cervi*. In particular, the spicules and the gubernaculum were examined, but other aspects of the morphology, such as the female tail, male bursa, and the cephalic region of the worms were included. Secondly, characters derived from this study were used to reconstruct the phylogeny of these nematodes using cladistics to show that numerous homologies found in *Parelaphostrongylus* spp. provide strong evidence for the monophyly of this genus. Thirdly, based on the findings of this study, the functional morphology of the structures examined was discussed.

MATERIALS AND METHODS

Collection of specimens

Specimens of *P. tenuis* were collected from white-tailed deer (*O. virginianus*) that were killed by vehicles near Grand Marais, Minnesota, USA, in the winter of 1990. The crania of the deer were necropsied as follows: a portion of the dorsal part of the skull was removed by making a series of incisions forming a rectangle on the dorsal surface using a saw. The rectangular piece was removed and the brain was allowed to drop through this opening for examination. The cranial cavity was searched for nematodes. The dura was removed from the cranium, placed in saline solution, and searched for worms. Tissue from the intercavernous sinus was also removed from the cranial cavity and examined in saline solution. After its surface was examined, the tissue from the intercavernous sinus was cut into smaller pieces to detect nematodes hidden within. Specimens of *P. tenuis* were recovered from the intercavernous sinus, subdural space, epidural space, and the dorsal longitudinal sinus.

Specimens of *P. odocoilei* were obtained from mule deer (*O. h. hemionus)* from both Vancouver Island and Penticton, British Columbia, Canada, in 1989 and 1990. The worms occurred deep within the longissimus dorsi muscles. Thin, transverse sections through the longissimus dorsi were cut and examined under a stereomicroscope. The muscle tissue was teased apart using forceps to search for worms located among the muscle fibres.

The above method was also used to obtain specimens of *P. andersoni.* White-tailed deer from Penticton and from Grand Forks, British Columbia, were searched for this muscleworm, and barren ground caribou (*R. t. tarandus*) from Fort Smith, Northwest Territories, Canada, were also searched. Additional specimens of *P. andersoni* and *P. odocoilei* were kindly provided from experimental infections of white-tailed deer and mule deer, respectively, by Dr. M. Pybus, Alberta Department of Energy and Natural Resources, Fish and Wildlife Division.

Specimens of *E. c. rangiferi* were obtained from moose (*A. alces*) which had been naturally infected on range shared with woodland caribou at Middle Ridge, Newfoundland, Canada, in May, 1990. Other specimens of *E. c. rangiferi* were obtained from the cranium of a caribou collected in Newfoundland in March, 1985.

Specimens of elaphostrongyline nematodes from several hosts and locations that had been collected in the years preceding this study were fixed in glycerine alcohol (95 mL 70% ethanol : 5 mL glycerine). These specimens were subsequently removed from glycerine alcohol and cleared in lactophenol. Standard measurements were taken using a light microscope and a drawing tube. Specimens that were obtained fresh at necropsy were placed in saline solution and frozen. For light microscopical measurements, these specimens were thawed and measurements taken without clearing the worms in lactophenol.

Light microscopy and histology

Light microscopical observations were made by clearing specimens in lactophenol and observing them under a Zeiss standard photomicroscope using bright

field, phase, and differential interference contrast. Light micrographs were taken using Kodak Technical Pan film.

Scanning electron microscopy

Male specimens examined included 20 *P. tenuis*, 10 *P. odocoilei*, 20 *P. andersoni*, and 10 *E. c. rangiferi*. Two or 3 females specimens of each of the four species were also examined.

Two methods were used to remove tissue surrounding the spicules and gubernaculum of male nematodes, depending on whether specimens were fresh or previously fixed. The tails of specimens that had been stored frozen in saline solution were immersed in a pepsin digest (1g pepsin, 1.3mL conc. HCl, 166mL distilled water) until tissues surrounding the spicules and gubernaculum had dissolved. Thirty minutes to one hour were required to isolate the male copulatory structures. The pepsin digest could not be used to free the spicules and gubernaculum from specimens that had been stored in glycerine alcohol. Instead, pre-fixed worms were placed in a 5% javex bleach solution until tissue surrounding the copulatory structures had dissolved (5-15 minutes).

Once isolated, the spicules and gubernaculum were rinsed in Sorensen's phosphate buffered saline solution (pH 7.2). They were subsequently fixed in 2.5% glutaraldehyde for 1-3 hours and post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series and sublimation dried using Peldri 2 (Pelco International). The spicules and gubernaculum were mounted on minuten pins which were, in turn, glued to SEM stubs, sputter coated with gold, and viewed in a Hitachi S570 scanning

electron microscope. Intact posterior ends of male and female specimens were post-fixed and dehydrated as described above. Most of these specimens were prepared by critical point drying using liquid carbon dioxide in a Sorvall critical point drying system. The tails were then mounted on stubs and sputter coated with gold.

Phylogenetic analysis

Morphological results were used to infer phylogenetic relationships among *Parelaphostrongylus* spp. using the principles of phylogenetic systematics (Hennig 1966; Wiley 1981; Wiley *et al.* 1991). An outgroup comparison was conducted using *Elaphostrongylus cervi rangiferi* as the outgroup. Details of characters and state information are provided in a later section.

RESULTS

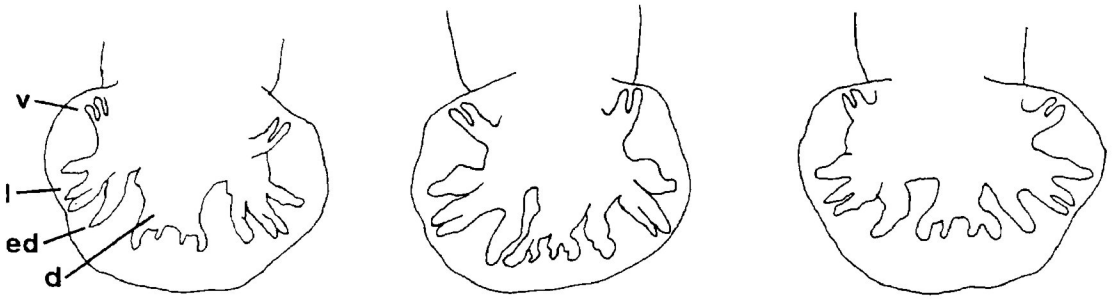
Light microscopical observations

Elaphostrongyline nematodes are long, slender worms, generally light brown in colour. Intestinal contents may make some specimens appear darker. Females are larger than males. The longest is *P. tenuis*, and *E. c. rangiferi* is somewhat shorter but wider. The muscleworms are the smallest, with *P. andersoni*, for example, being about one-half the length and width of *P. tenuis* (Table 1). Males are bursate, and females have a slender, tapering tail.

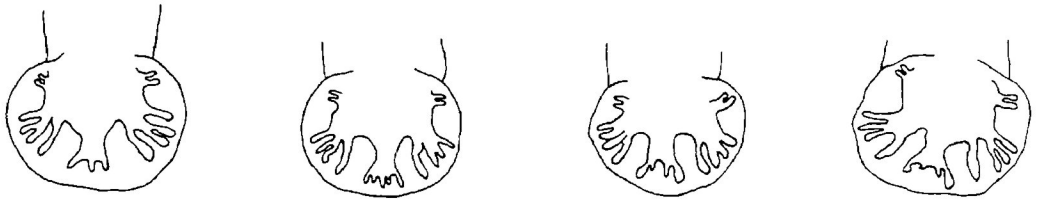
The bursa of *E. c. rangiferi* is larger than that of *P. tenuis*; *P. andersoni* has the smallest bursa (Fig. 1). In each species, the ventral rays are shorter than the other rays. The externodorsal rays of *Parelaphostrongylus* spp. are more closely associated with the lateral rays than in *E. c. rangiferi*, where they are closer to the dorsal ray.

Considerable variation was seen in the dorsal ray of the four species studied (Fig. 1). The ray becomes bifid distal to its narrow base, and the two main branches arising from the base may have smaller rays arising between them. In most specimens of *P. tenuis* and *P. andersoni*, the dorsal ray has two large outer branches with two smaller rays between them. The dorsal ray of most *P. odocoilei* specimens has three branches which are similar in size; in *E. c. rangiferi*, two branches are

Fig. 1. Line drawings of outlines of the bursae of North American elaphostrongyline nematodes. v = ventral rays, l = lateral rays, ed = externodorsal, and d = dorsal ray.



P. tenuis



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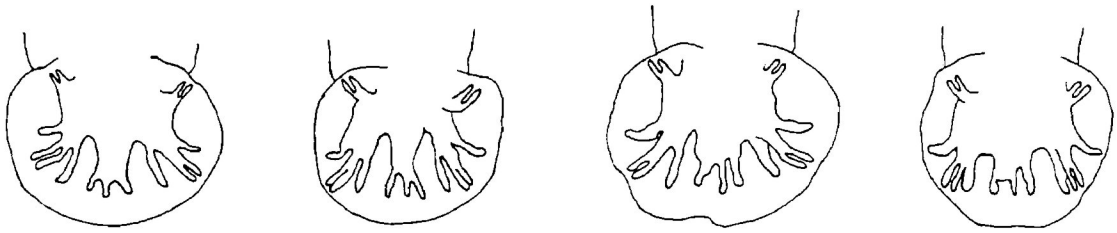
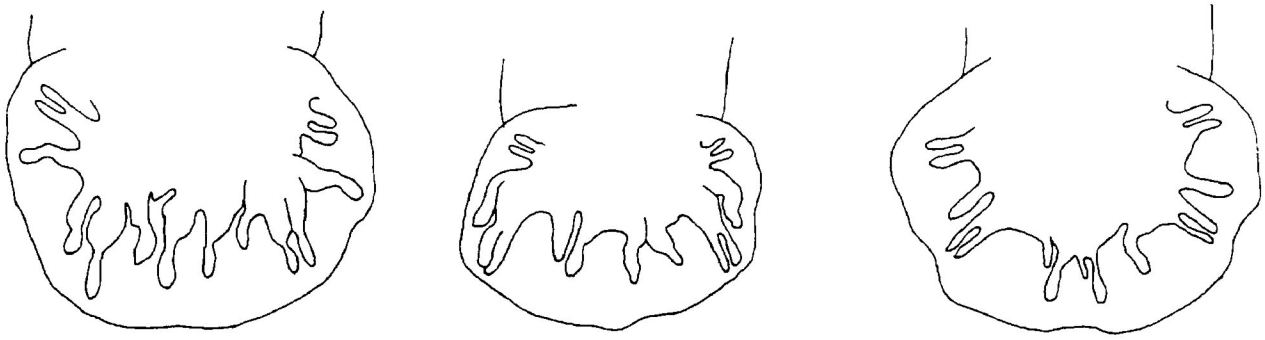


P. odocoilei



E. cervi rangiferi

100 μm

Table 1: Dimensions (in μm) of North American elaphostongyline nematodes*

	N	*Parelaphostrongylus tenuis*	N	*P. andersoni*	N	*P. odocoilei*	N	*Elaphostrongylus cervi*
Male								
length (mm)	8	55(31-62)*	3	21(20-22)	4	22(21-25)	7	34(31-39)
width	8	162(92-200)	3	81(72-91)	4	110(100-121)	7	179(156-191)
esophagus	5	640(562-770)	3	685(660-714)	4	620(546-662)	7	634(614-662)
gubernaculum	17	109(89-137)	24	58(49-68)	19	80(70-91)	9	74(56-88)
crura	17	27(22-32)	24	13(8-18)	19	20(15-27)	-	-
spicules	15	223(202-249)	24	121(106-138)	18	159(145-170)	9	218(205-225)
spicule branch	13	82(72-92)	21	37(30-57)	14	43(37-48)	-	-
Female								
length (mm)	7	79(66-90)	1	35			16	47(38-55)
width	7	209(120-250)	1	126	-	-	16	200(133-237)
esophagus	7	694(623-796)	1	695	1	724	16	653(573-741)
nerve ring from anterior	6	104(90-126)	1	52	1	53	14	134(102-158)
excretory pore from anterior	5	139(109-164)	1	119	1	92	14	169(140-205)
vulva from posterior	23	181(138-233)	15	155(113-178)	5	157(142-188)	23	233(181-302)
anus from posterior	23	53(35-62)	15	56(36-70)	5	43(35-59)	23	63(49-79)

* Mean values are given, followed by the range in parentheses.

* Not all characters could be measured in every specimen.

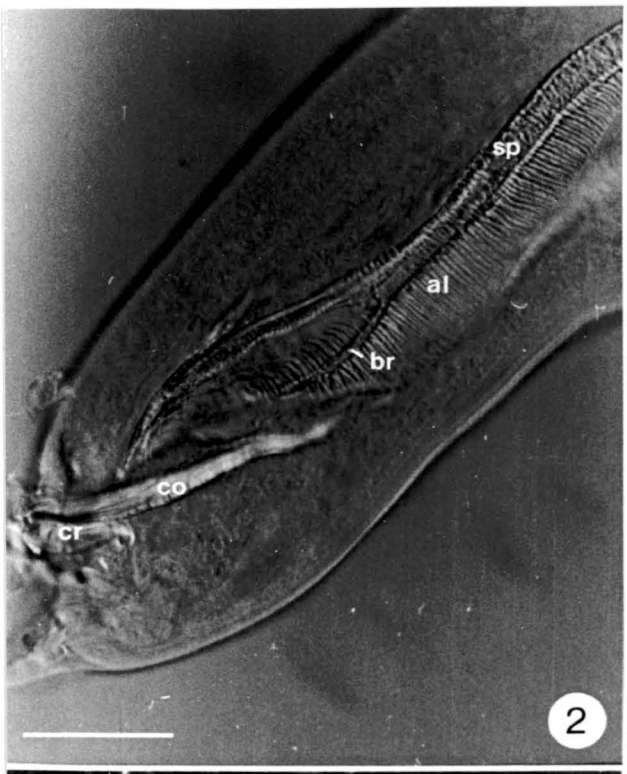
usually present (Fig. 1).

A pair of spicules and a gubernaculum are present in males (Figs. 2-5). The gubernaculum consists of a slender corpus that extends dorsally over the distal portion of the spicules. Crurae are present distally on the dorsal surface of the gubernaculum of *Parelaphostrongylus* species. They are small and inconspicuous in *P. andersoni* (Table 1), but are more prominent in *P. tenuis* and *P. odocoilei* (Figs. 2 and 4, respectively).

The spicules are sigmoid-shaped, curving medially and ventrally. Each has two medially directed, membranous alae arising from the spicule shaft. The alae are supported by sclerotized thickenings or ribs arising perpendicularly from the spicule shaft and extending close to the margin of the alae (Fig. 2). The dorsal ala extends along the entire length of the spicule from the capitulum to the tip. The ventral ala arises about one third the way along the spicule and also extends to the tip.

Although the morphology of the distal region of the spicule shaft was difficult to resolve at the light microscopical level, it is similar in the three *Parelaphostrongylus* species (Figs. 2, 4, 5). The shaft branches dorsally in the distal region of the spicule. The branch arises more proximally in *P. tenuis* (Fig. 2) than in *P. odocoilei* (Fig. 4) and *P. andersoni* (Fig. 5) and thus is more obvious (see also Table 1). The distal portion of the spicule shaft of *E. c. rangiferi* appears to be unbranched when viewed using light microscopy (Fig. 3).

Figs. 2 - 5. Light micrographs of the posterior ends of male elaphostrongyline nematodes, lateral view, showing spicules (sp), alae (al), dorsal branch (br), corpus of gubernaculum (co), and crurae (cr). Note that the spicules of *E. cervi* are unbranched and that gubernacular crurae are absent in this species. Fig. 2. *P. tenuis*. Fig. 3. *E. cervi*. Fig. 4. *P. odocoilei*. Fig. 5. *P. andersoni*. Scale bar = 40um.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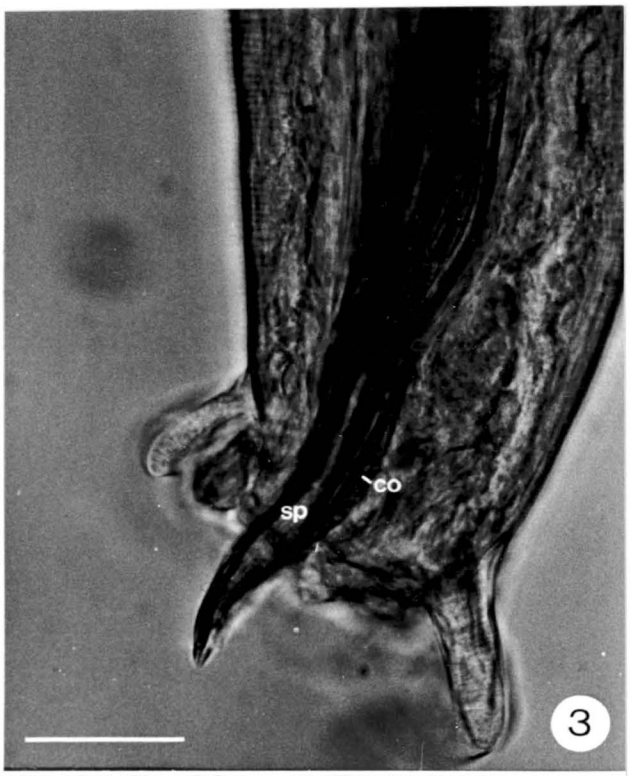
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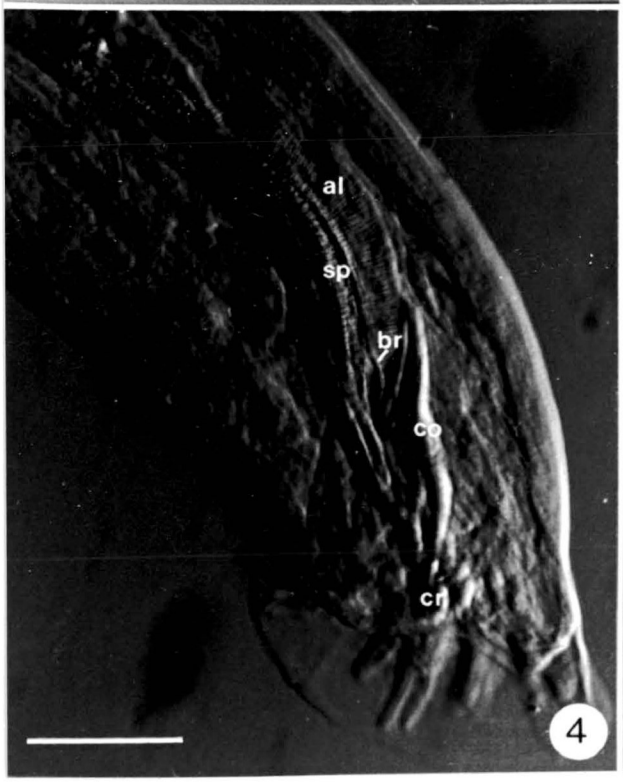
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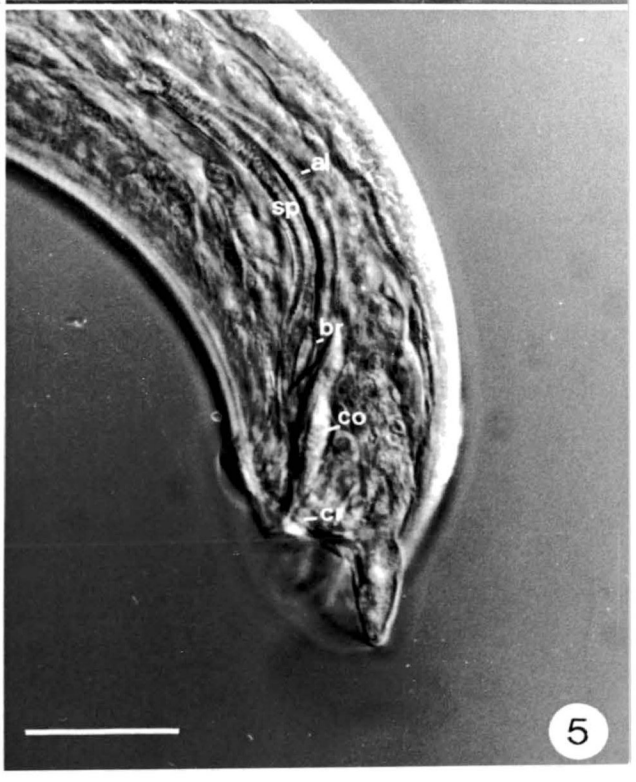
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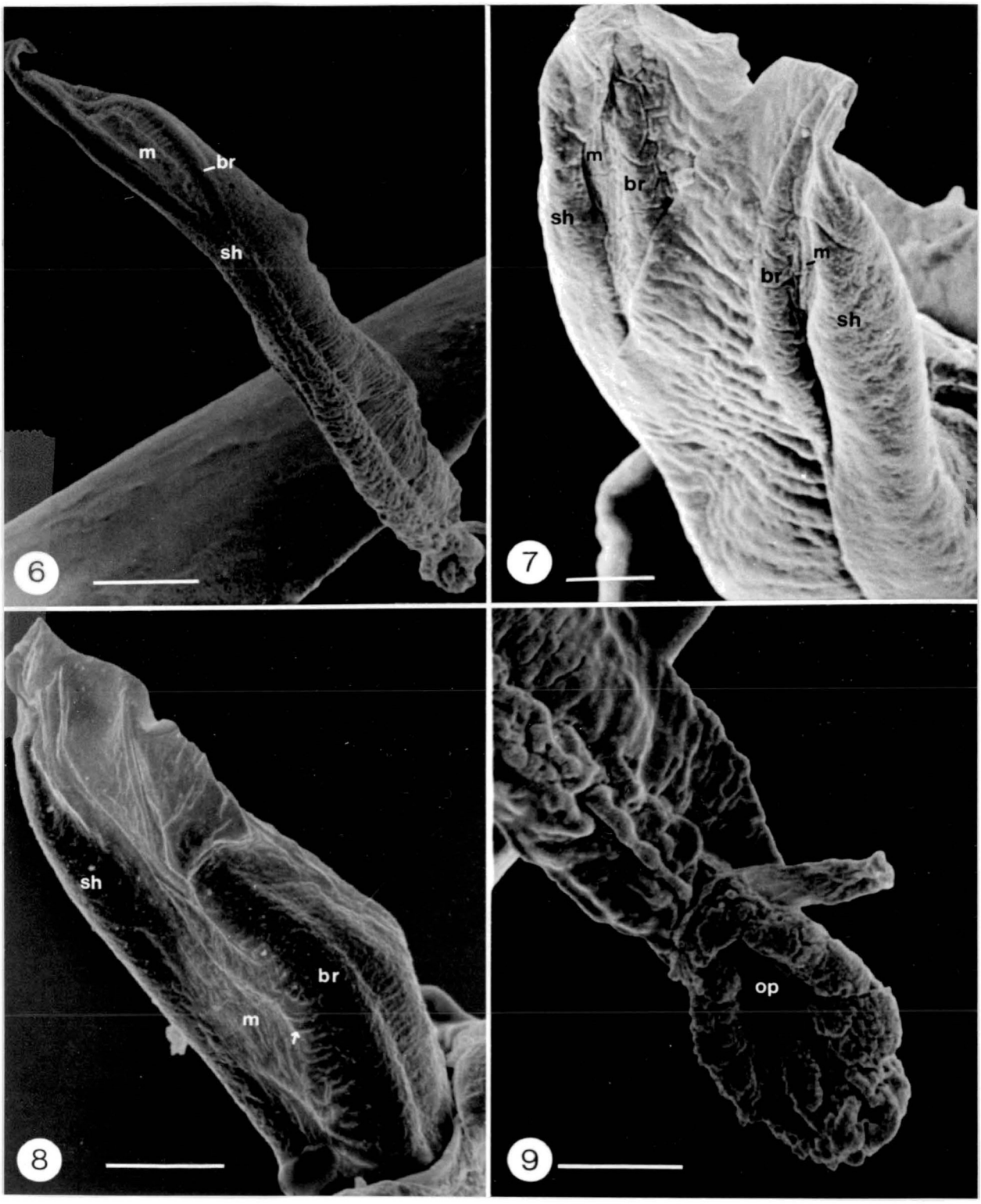
Scanning electron microscopy

Spicules

In some specimens, the spicules and gubernaculum protruded from the cloaca and could be prepared directly for SEM study while other specimens had to be digested in pepsin or bleach. Pepsin digest released the spicules and gubernaculum from fresh specimens with little damage. However, bleach solution was required to free these structures from alcohol fixed specimens and some surface damage resulted.

SEM studies confirmed the gross spicule morphology seen in light microscopical preparations and elucidated the form of the spicules near the tips. Examination of the distal half of the spicules of *P. tenuis* provided the following morphological details that were consistent in all specimens examined. A thin branch of the spicule shaft arises dorsally at a point approximately two thirds the distance along its length and extends to its distal tip (Figs. 6-8). A thin membrane spans between the spicule shaft and the dorsal branch (Figs. 6-8). In some specimens the membrane is taut (Figs. 6 and 8). In others, it appears to be folded, and the branch is closer to the shaft (Fig. 7). The dorsal branch has numerous short, sclerotized projections which extend into the membrane (Fig. 8). The branch appears to converge with the main shaft at the spicule tip but actually terminates in the dorsal ala (Fig. 8). When the spicule is viewed ventrally, the dorsal branch is obscured by the ventral ala. The spicule has a prominent enlargement or capitulum

Figs. 6-9. Spicules of *P. tenuis*. Fig. 6. Lateral view of a spicule mounted on a minuten pin. A branch (br) arising from the shaft (sh) extends to the tip of the spicule. A membrane (m) is located between the branch and the shaft. Scale bar = 20 um. Fig. 7. Tips of both spicules protruding through the cloaca, medioventral view. The distal branch (br) is smaller in diameter than the remainder of the shaft (sh). Scale bar = 5um. Fig. 8. A single spicule protruding through the cloaca. Note the membrane (m) between the branch (br) and the shaft (sh), and sclerotized projections (arrow) into the membrane. Scale bar = 10um. Fig. 9. Spicule capitulum with lateral opening (op). Scale bar = 10um.


m
br
sh
6
m
br
sh
m
br
sh
7
sh
br
m
8
op
9

with a lateral opening at the proximal end (Fig. 9). The capitulum is of similar form in the other three species.

The morphology of the spicules of *P. odocoilei* (Fig. 10) and *P. andersoni* (Fig. 11) closely resembles that of *P. tenuis*. A dorsal branch of the shaft extends to the spicule tip and a membrane spans the region between the shaft and its branch. In *P. odocoilei* a v-shaped thickening is situated ventrolaterally on the spicule shaft anterior to the dorsal branch (Fig. 10). This formation is not present in the other species. The spicule shaft of *E. c. rangiferi* is unbranched along its entire surface (Figs. 12 and 13). The alae are wider in *E. c. rangiferi* than in *Parelaphostrongylus* species; this is especially evident in the distal region of the spicule (Fig. 13).

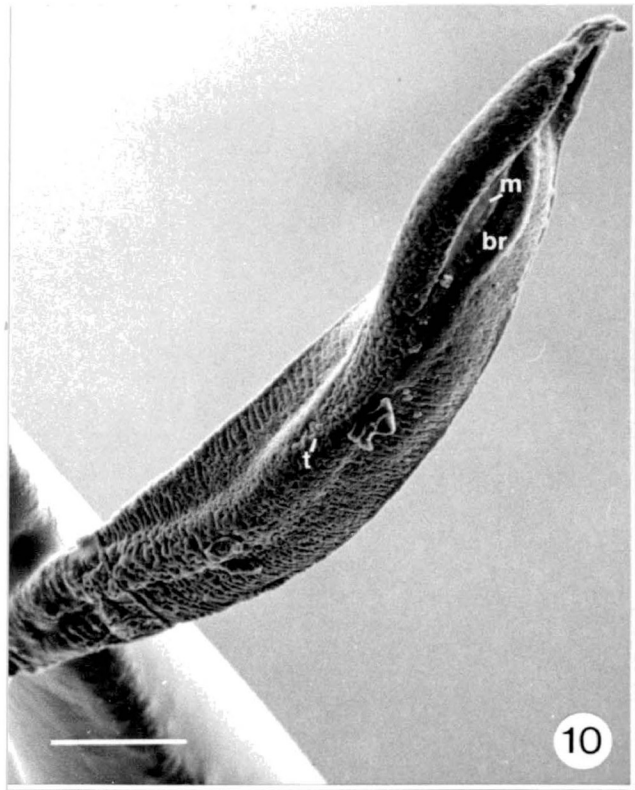
Gubernaculum

The gubernaculum of *P. tenuis* is undivided, but has a dorsal, longitudinal groove which becomes deeper and wider toward the distal end (Fig. 14). The ventral surface of the corpus is flat (Fig. 15). It is thin proximally, but becomes progressively wider toward the distal end.

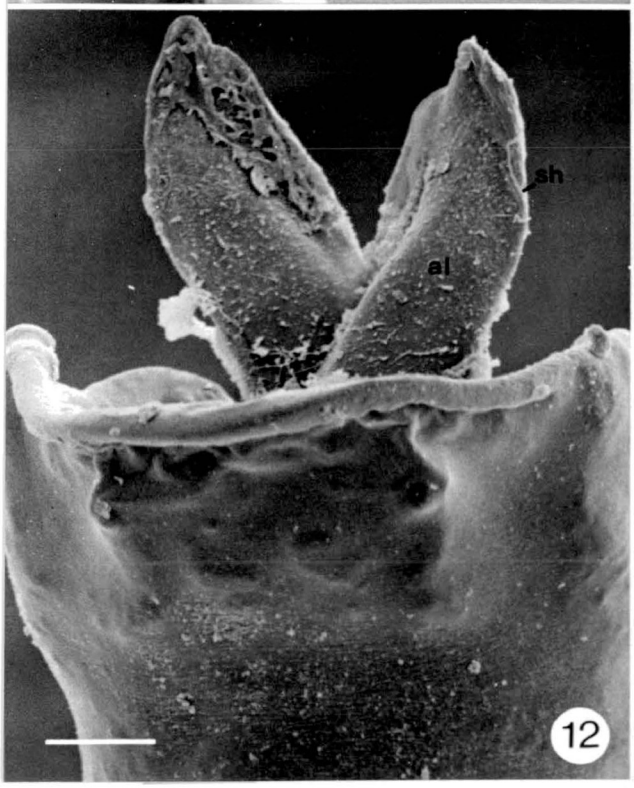
The gubernacular crurae of *P. tenuis* extend dorsolaterally from the corpus, becoming more expansive distally (Fig. 16). From four to six prominent lobes are present on the dorsal surface and curve laterally outward (Figs. 16 and 17). Two small, ventrally directed protuberances, one on each side of the crurae, are present (Fig. 18). It is not clear whether they are part of the crurae or extensions of the edge of the cloaca.

The corpus of the gubernaculum of *P. odocoilei* is similar in form to that of

Fig. 10. *P. odocoilei* spicule mounted on a minuten pin, dorsolateral view. A v-shaped thickening (t) is proximal to the branch (br) of the shaft. A membrane (m) is located between the branch and the shaft. Scale bar = 15um. Fig. 11. Distal tip of spicule of *P. andersoni*, showing branch (br) and membrane (m); dorsolateral view. Scale bar = 4um. Figs. 12 and 13. Spicules of *E. c. rangiferi* protruding through the cloaca, dorsal view, showing unbranched shaft (sh) and alae (al). Scale bars; Fig. 12 = 15um; Fig. 13 = 10um.


m
br
t
10


m
br
11


sh
al
12

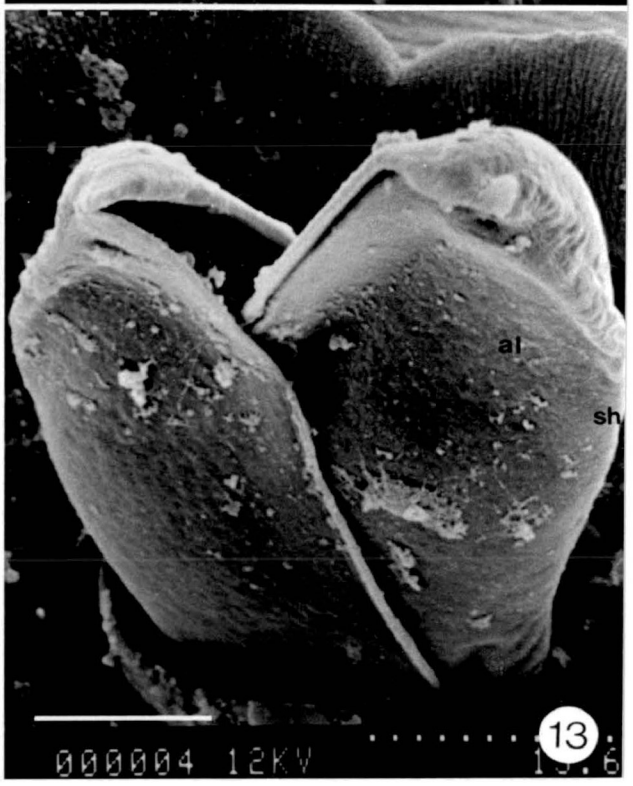
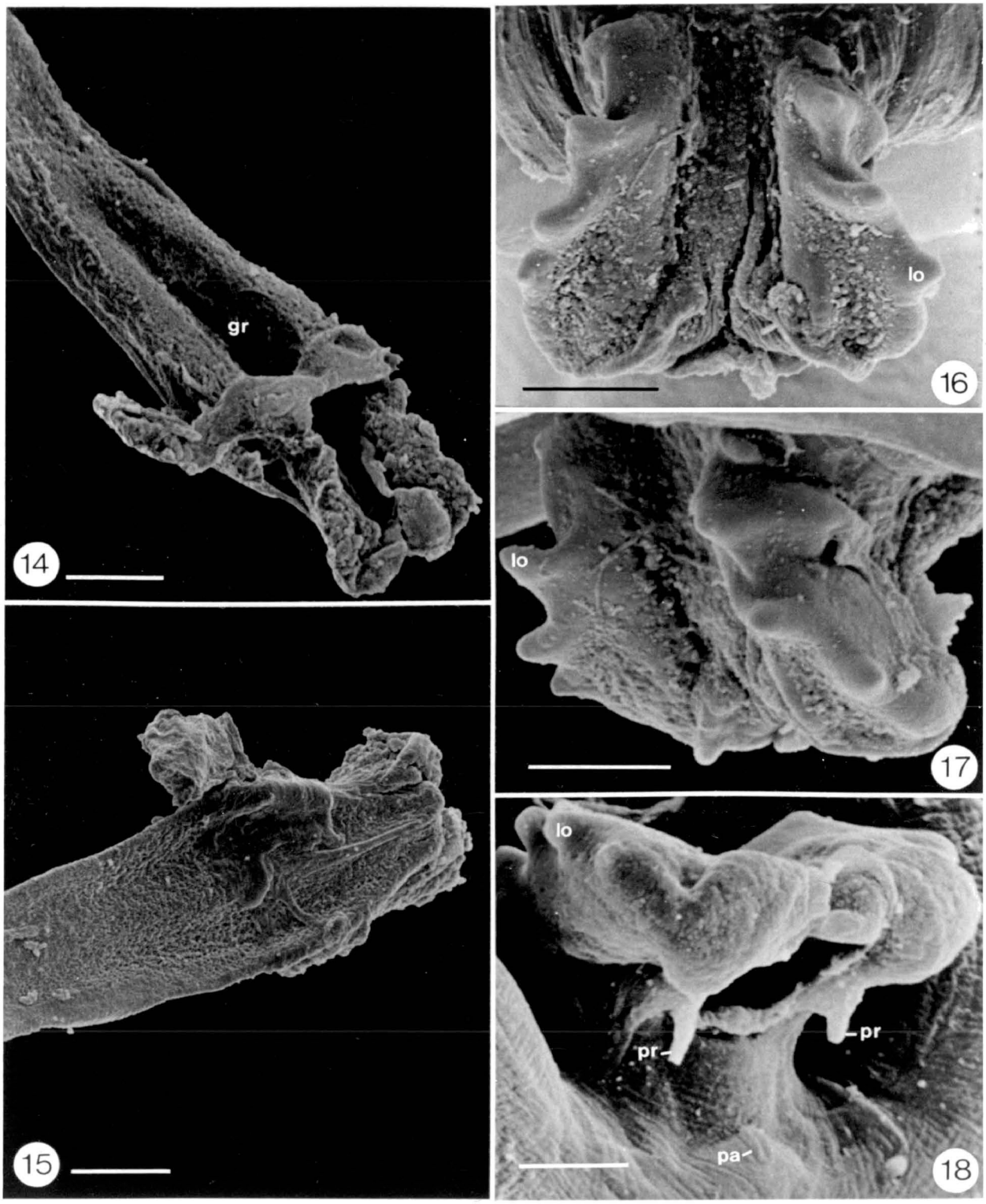
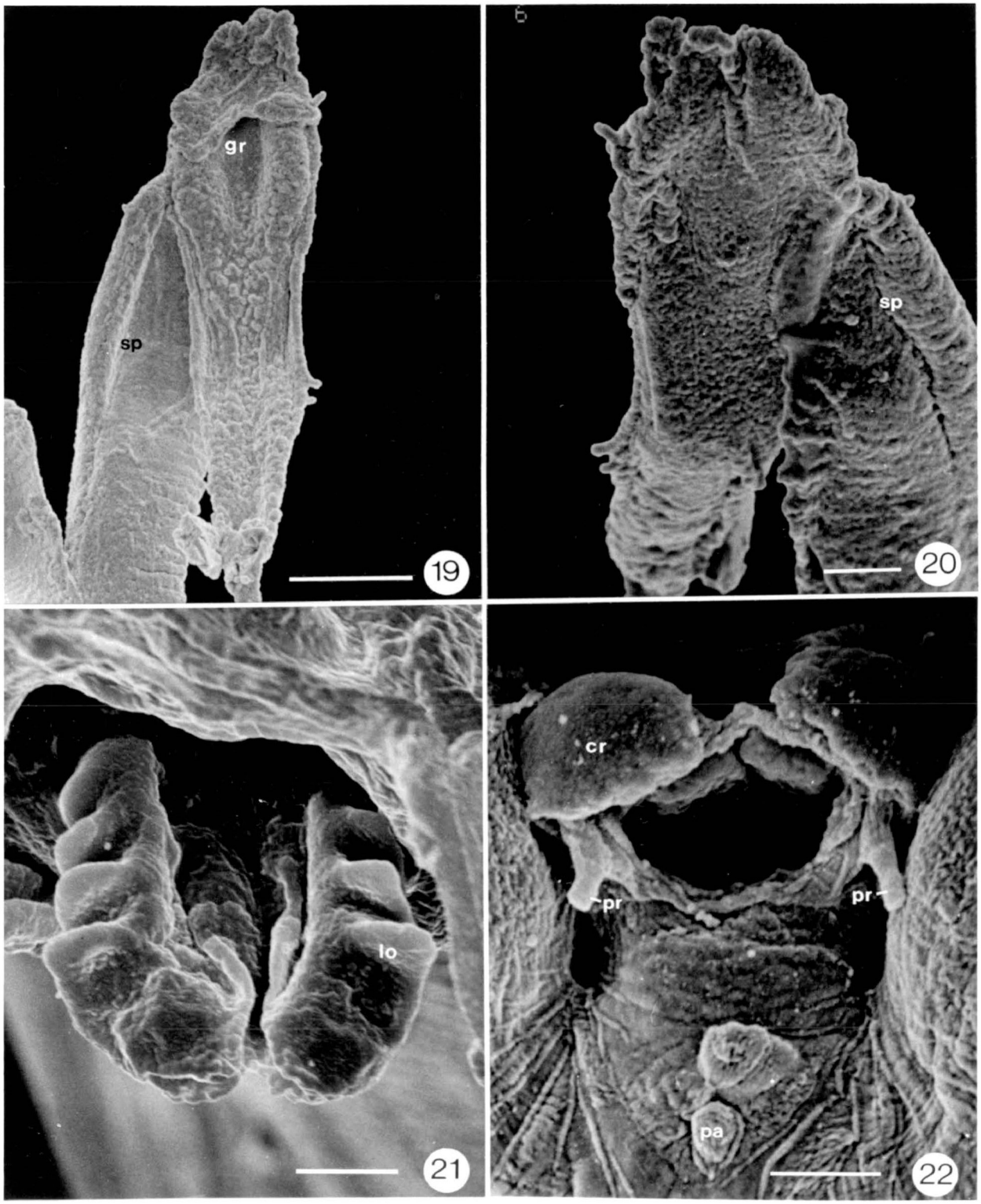

al
sh
13
000004 12KV

Fig. 14. Gubernaculum of *P. tenuis*, dorsal view of corpus. Most of the crurae have been removed. Note the deep longitudinal groove (gr) in the corpus. Scale bar = 10um. Fig. 15. Gubernaculum of *P. tenuis*, ventral view. Scale bar = 10um. Figs. 16-18. Crurae of *P. tenuis*. Note the prominent dorsal lobes (lo); Fig. 16. Dorsal view. Scale bar = 10um. Fig. 17. Ventral view. Scale bar = 10um. Fig. 18. Lateral view. Note protuberances (pr) ventral to the crurae. Note also a bilobed papilla (pa) on the cuticular surface ventral to the cloaca. Scale bar = 5um.


gr
14
lo
16
lo
17
lo
pr
pr
pa
18
15

Figs. 19 and 20. Gubernaculum of *P. odocoilei*. Most of the crurae have been removed. The corpus is adhering to one of the spicules (sp). Fig. 19. Dorsal view. Note the longitudinal groove (gr) in the distal region of the corpus. Scale bar = 5um. Fig. 20. Ventral view. Scale bar = 5um. Fig. 21. Crurae of *P. odocoilei*, dorsal view, showing dorsal lobes (lo). Scale bar = 5um. Fig. 22. Cloaca of *P. odocoilei*, showing distal distal edges of crurae (cr), papilla (pa) ventral to the cloacal opening, and protuberances (pr) associated with the crurae and the edge of the cloaca. Scale bar = 3um.


gr
sp
19
sp
20
lo
21
cr
pr
pr
pa
22

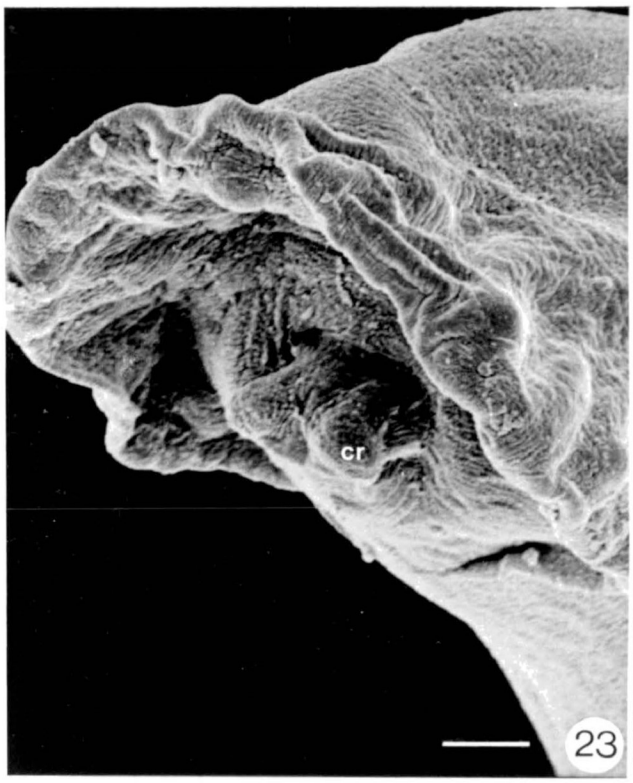
P. tenuis. A dorsal groove is present in the distal region (Fig. 19). Although the groove is deep, it does not split the corpus (Figs. 19 and 20). As in *P. tenuis*, a series of dorsal lobes is present on the crurae (Fig. 21). Each of the lobes is similar in size in *P. odocoilei*, giving an overall rectangular appearance to the crurae (Fig. 21). Two ventrally directed protuberances, similar to those observed in *P. tenuis* (Fig. 18), are present in *P. odocoilei* (Fig. 22).

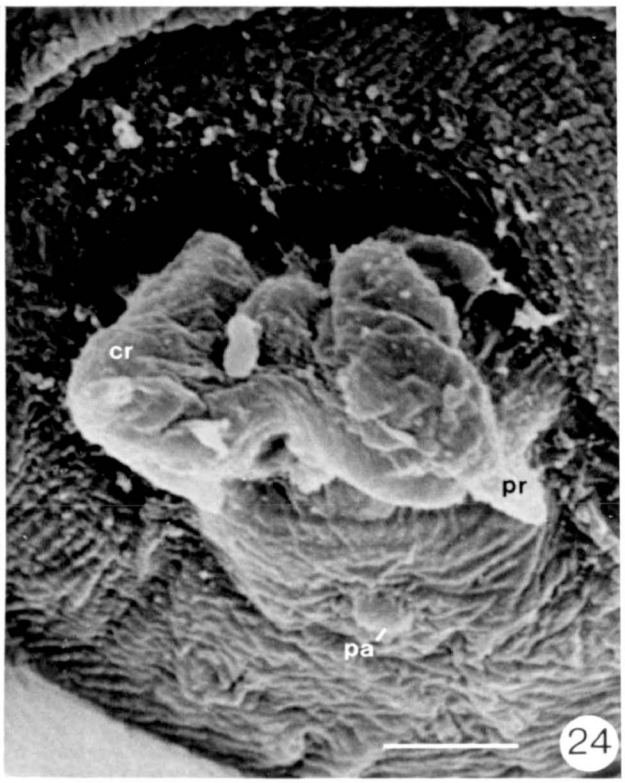
Short crurae are present in *P. andersoni* (Figs. 23 and 24). No distinct lobes were observed on their upper surface. Two ventrally directed protuberances were observed in the distal region of the crurae (Figs. 24 and 30) as in the other two *Parelaphostrongylus* spp. It is not clear whether these protuberances are part of the crurae or whether they arise from the cuticle at the edge of the cloaca (Figs. 18, 22, 24, 30). They could not be seen using light microscopy in any of the species. No such protuberances were observed in *E. c. rangiferi*.

Bursae

The structure of the base of the dorsal ray varied among the species. In *P. tenuis* and *E. c. rangiferi*, the edge of the base is smooth (Figs. 25 and 26, respectively), whereas in *P. odocoilei* (Fig. 27) and *P. andersoni* (Fig. 28) it is more heavily cuticularized forming a ridge. In the latter two species, the branches of the dorsal ray appear to be situated ventral to its base, whereas in *P. tenuis* and *E. c. rangiferi*, they are smooth extensions of the base of the ray (Figs. 25 and 26). In several specimens of *E. c. rangiferi*, thin cuticular ridges extend from the externo-lateral rays to the branches of the dorsal ray (Fig. 26). Similar ridges extend along the bursal rays in *Parelaphostrongylus* species (eg. Fig. 27).

Figs. 23 and 24. Crurae of *P. andersoni* protruding through the cloaca. Fig. 23. Dorsolateral view showing crurae (cr). Fig. 24. Dorsal view showing crurae (cr), ventral protuberance (pr), and papilla (pa) ventral to the cloaca. Scale bar = 3um.


cr
23


cr
pr
pa
24

Figs. 25-28. Dorsal views of bursae showing the dorsal rays (dr). Fig. 25. *P. tenuis*. Fig. 26. *E. c. rangiferi*. Scale bar = 20um. Fig. 27. *P. odocoilei*. Note the thickened cuticle around the base of the ray in this species and in *P. andersoni*. Fig. 28. *P. andersoni*.

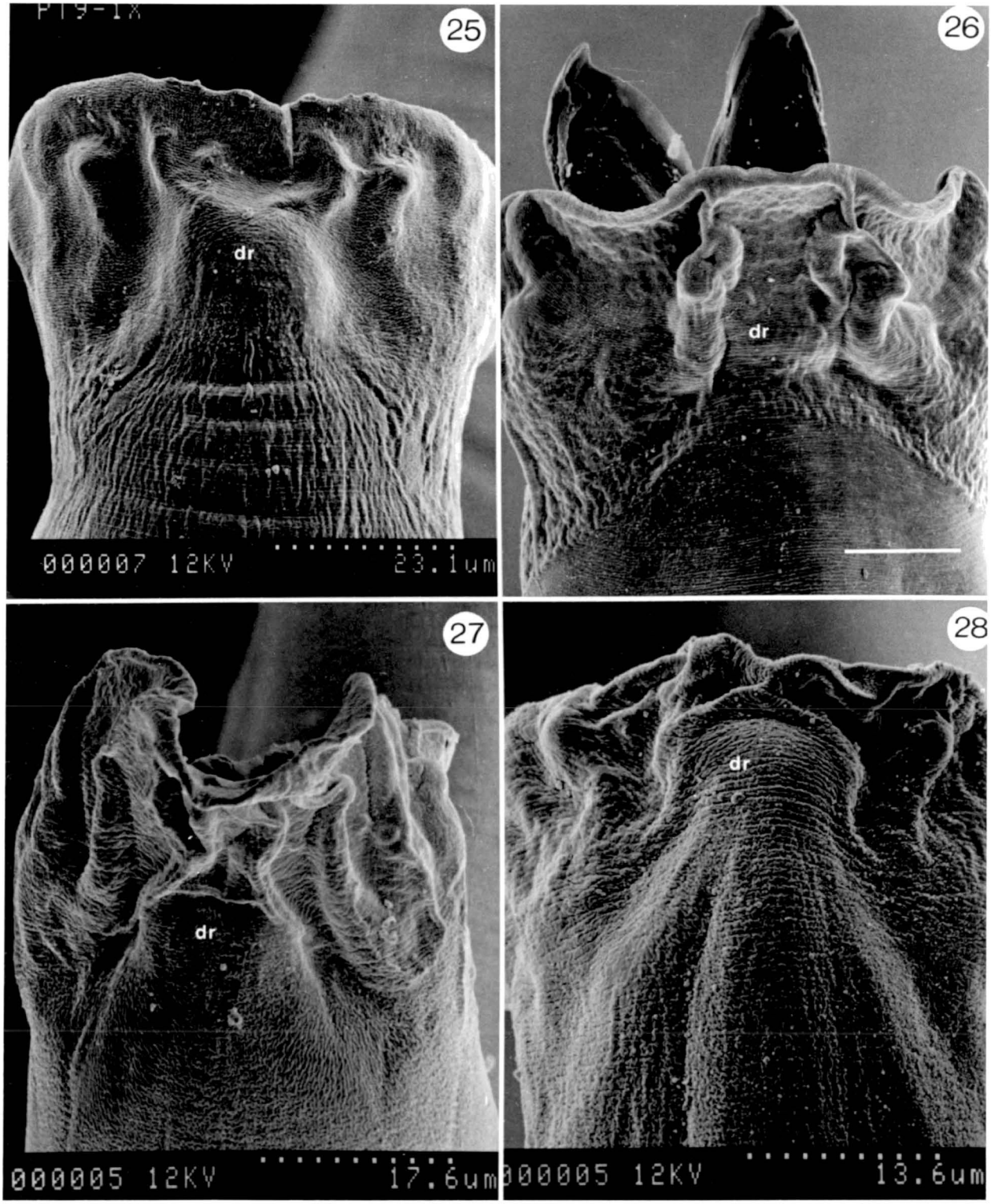
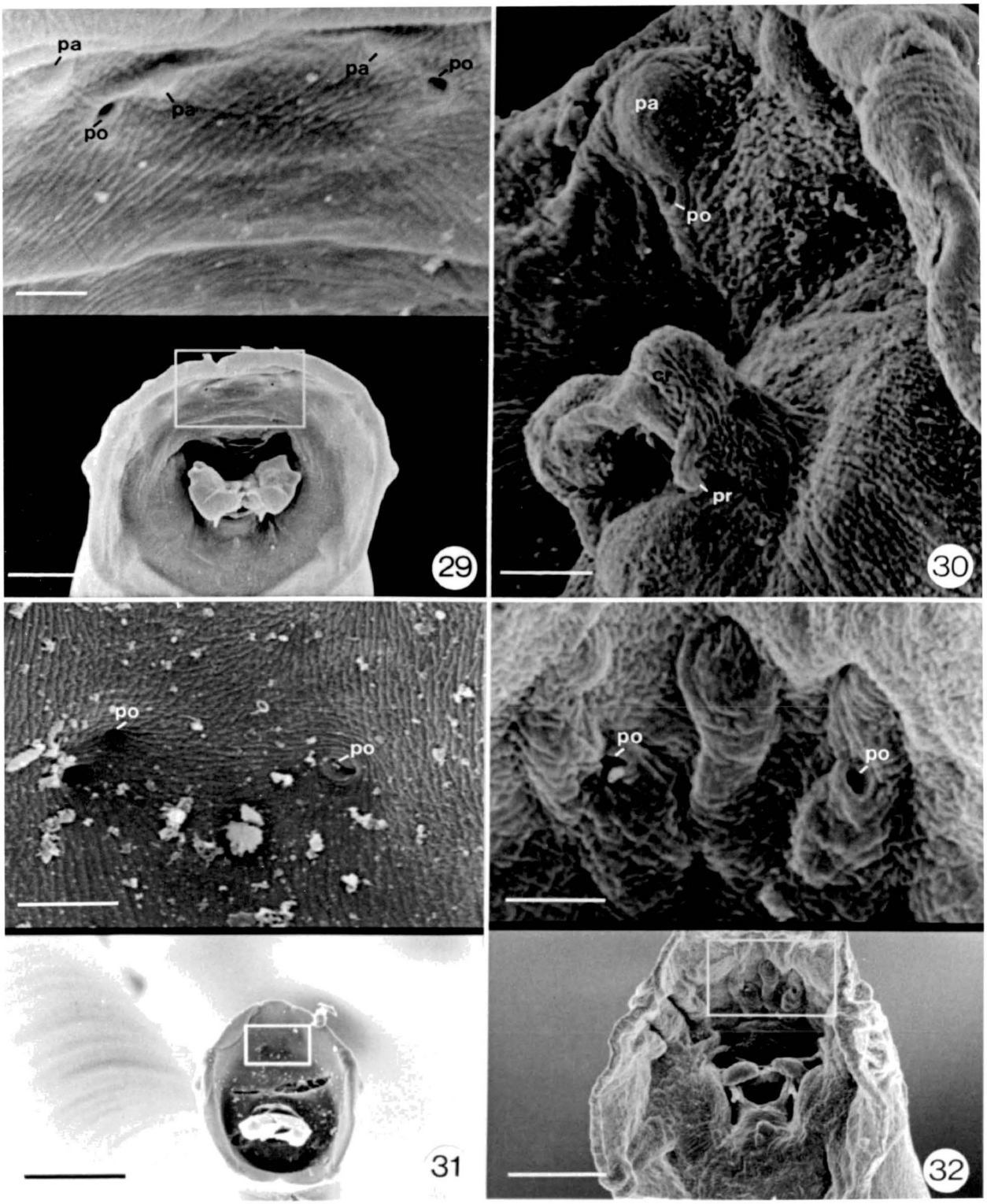

PT9-1X
25
dr
000007 12KV 23.1um
26
dr
27
dr
000005 12KV 17.6um
28
dr
000005 12KV 13.6um

Fig. 29. Bursa of *P. tenuis*, ventral view, showing pores (po) and associated papillae (pa) (enlarged). Lower scale bar = 15um; upper scale bar = 5um. Fig. 30. Cloaca of *P. andersoni*, ventrolateral view showing pore (po) and associated papilla (pa). Note also the protruding gubernacular crurae (cr) and one of two ventral protuberances (pr). Scale bar = 10um. Fig. 31. Bursa of *E. c. rangiferi*, ventral view showing pores (po) associated with the dorsal rays (enlarged). Lower scale bar = 50um; upper scale bar = 5um. Fig. 32. Bursa of *P. odocoilei*, ventral view, showing pores (po) associated with the dorsal rays (enlarged). Lower scale bar = 15um; upper scale bar = 3um.


pa
pa
pa
po
po
29
pa
po
cr
pr
30
po
po
po
po
31
32

Several pores are visible ventrally in the bursa of *Parelaphostrongylus* spp. and *E. c. rangiferi* (Figs. 29-32). In ventral view in which the bursal rays were not visible (Figs. 29, 30, 31), the location of the pores corresponds roughly to the expected positions of the tips of the bursal rays. In those specimens in which the rays were visible in ventral view (eg. Fig. 32), pores were located at or near their tips. Several papilla-like structures protruding from the cuticle of the bursa are visible near the pores (Figs. 29, 30). A small papilla is located ventral to the cloaca in the three *Parelaphostrongylus* species (Figs. 18, 22, and 24). This papilla was not seen in *E. c. rangiferi*.

Morphology of the cephalic region

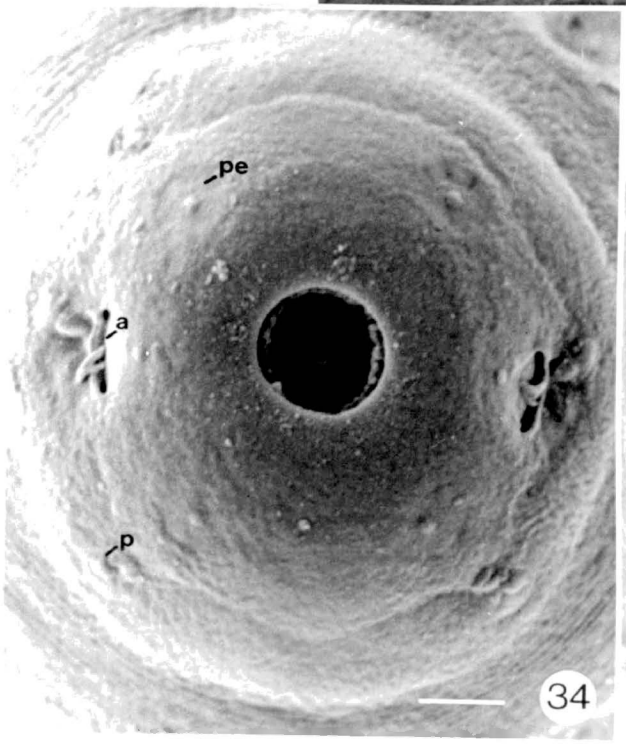
The cephalic region in each species is narrower and set off somewhat from the rest of the body (Fig. 33). It is characterized by having four outer, paired papillae and a circle of six inner papillae (Figs. 34 and 35). The six inner papillae are each centered within a circular depression, or perityl, that is somewhat lower than the surrounding cephalic surface (Figs. 34 and 35). The lateral amphids are elliptical pores. A shallow, sloping surface is present within each pore. In some specimens, elongate structures resembling cilia protruded through these slits (Fig. 34).

Female morphology

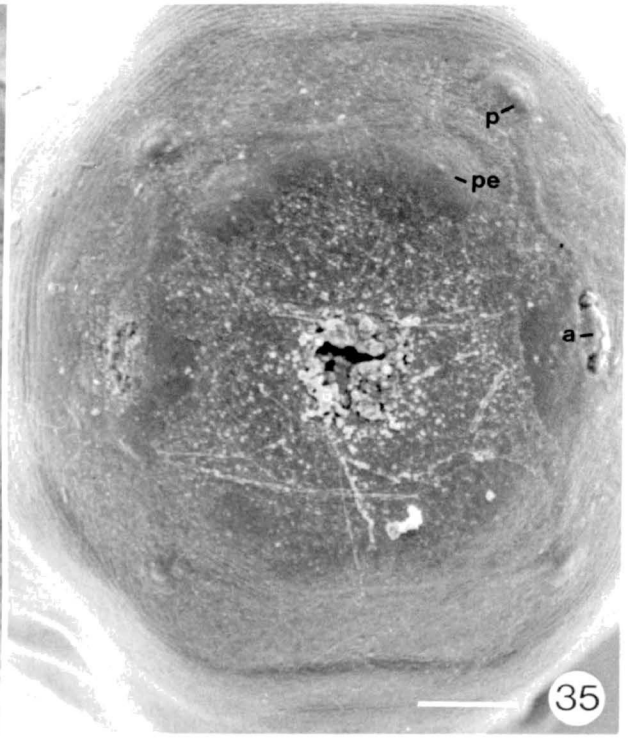
The tail of female *E. c. rangiferi* (Fig. 36) is more bluntly rounded than the more pointed tails of *Parelaphostrongylus* species. The caudal regions in *P. odocoilei*

Fig. 33. Cephalic region of *P. odocoilei*, anterolateral view. Scale bar = 10um. Fig. 34. Cephalic region of *P. odocoilei*, en face. Scale bar = 4um. Fig. 35. Cephalic region of *E. c. rangiferi*, en face. Note perityls (pe), amphids (a), and paired cephalic papillae (p). Scale bar = 10um.

33

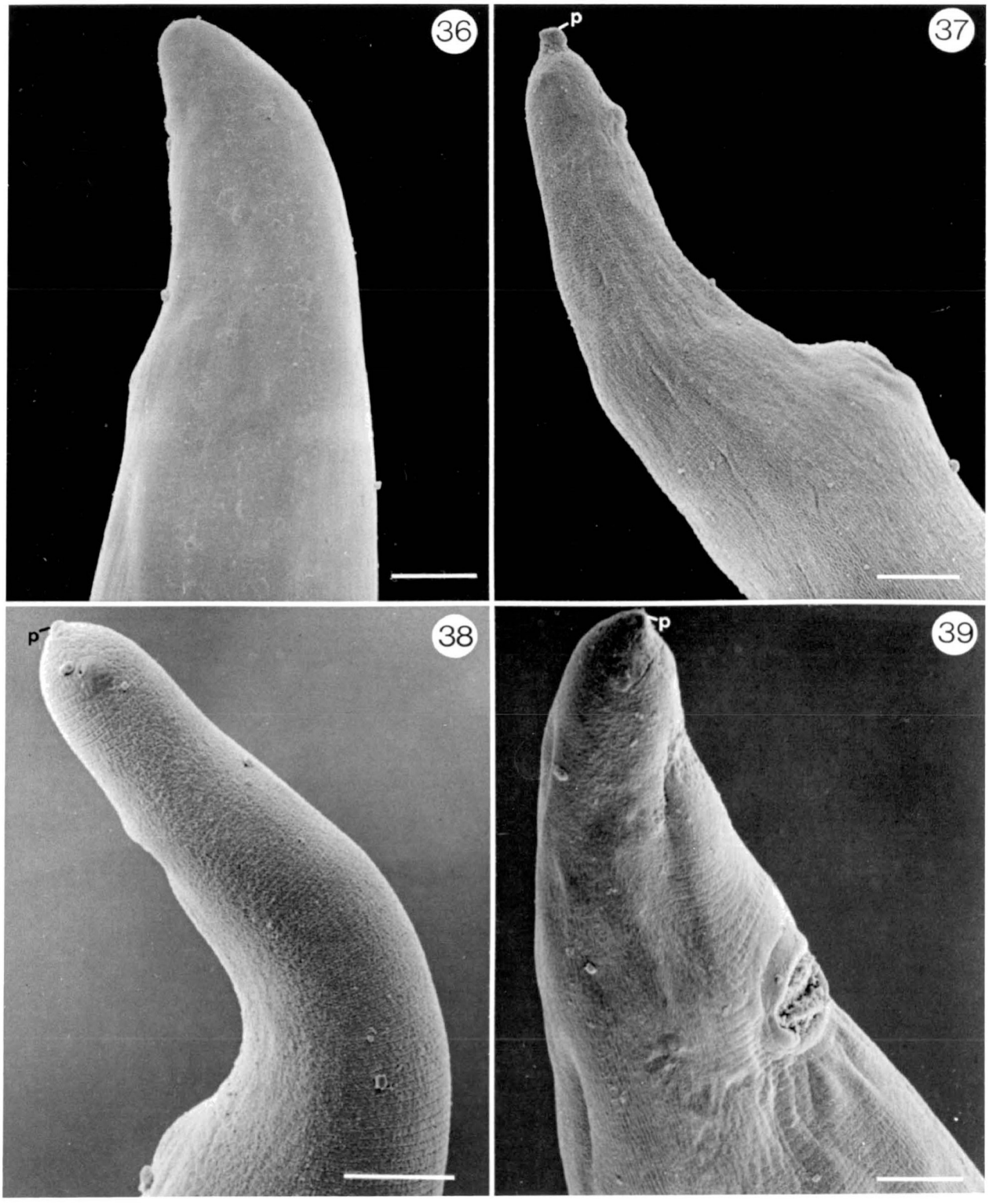


34

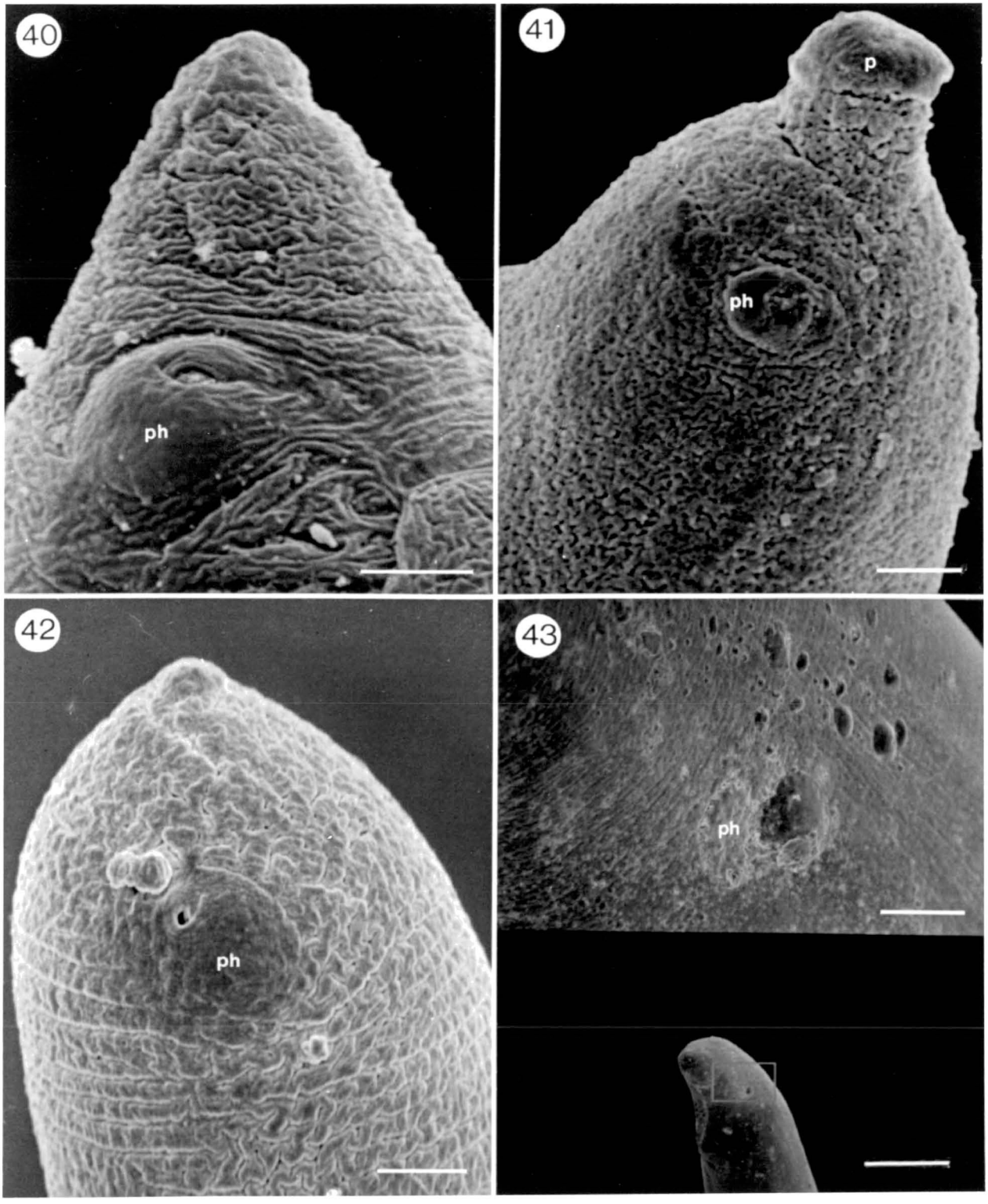


35

Figs. 36-39. Tail region of females, lateral view. Fig. 36. *E. c. rangiferi*. Scale bar = 30um. Fig. 37. *P. odocoilei*. Scale bar = 30um. Fig. 38. *P. andersoni*. Scale bar = 15um. Fig. 39. *P. tenuis*. Scale bar = 15um. Note the papilla-like projection (p) at the distal tip in *Parelaphostrongylus* spp.


36
37
p
38
p
39
p

Figs. 40-43. Tips of female tails showing phasmids (ph) with acentral pore, lateral view. Fig. 40. *P. tenuis*. Scale bar = 3um. Fig. 41. *P. odocoilei*. Note prominent papilla-like projection (p) at the tip of the tail. Scale bar = 3um. Fig. 42. *P. andersoni*. Scale bar = 3um. Fig. 43. *E. c. rangiferi* (region showing phasmid enlarged). Lower scale bar = 75um; upper scale bar = 7.5um.


40
ph
41
p
ph
42
ph
43
ph

and *P. andersoni* (Figs. 37 and 38, respectively), constrict immediately posterior the vulva. In *P. tenuis,* it is not constricted but tapers gradually toward the tip of the tail (Fig. 39).

In most specimens of the three *Parelaphostrongylus* spp., a small, rounded papilla-like projection was observed at the distal tip of the tail (Figs. 37-42). This projection is not clearly delineated from the remainder of the tail in *P. tenuis* (Figs. 39 and 40). It is most prominent in *P. odocoilei* (Figs. 37 and 41). In one specimen of *P. odocoilei* (out of five examined), the papilla-like projection was absent. It was also absent in three of the 15 specimens of *P. andersoni* that were examined. No papilla-like projection was observed in *E. c. rangiferi.*

The phasmids of each of the four species take the form of a pore that is acentrally located upon a slightly elevated, smoother region of cuticle (Figs. 40-43).

Phylogenetic analysis

Within the family Protostrongylidae, it is assumed that the genera *Elaphostrongylus* and *Parelaphpstrongylus* are each other's closest relatives based on the absence of a telamon (a supporting apparatus in the posterior region) and a gubernacular capitulum in males and on the similar overall bursa morphology and similar location in hosts of the same family. Therefore, to determine the phylogenetic relationships within the genus *Parelaphostrongylus*, *Elaphostrongylus cervi rangiferi* was chosen as the outgroup.

A total of nine binary characters was employed in the analysis (Table 2). The resulting data matrix (Table 3) was used to construct the most parsimonious cladogram depicting the phylogeny of *Parelaphostrongylus* spp. (Fig. 44).

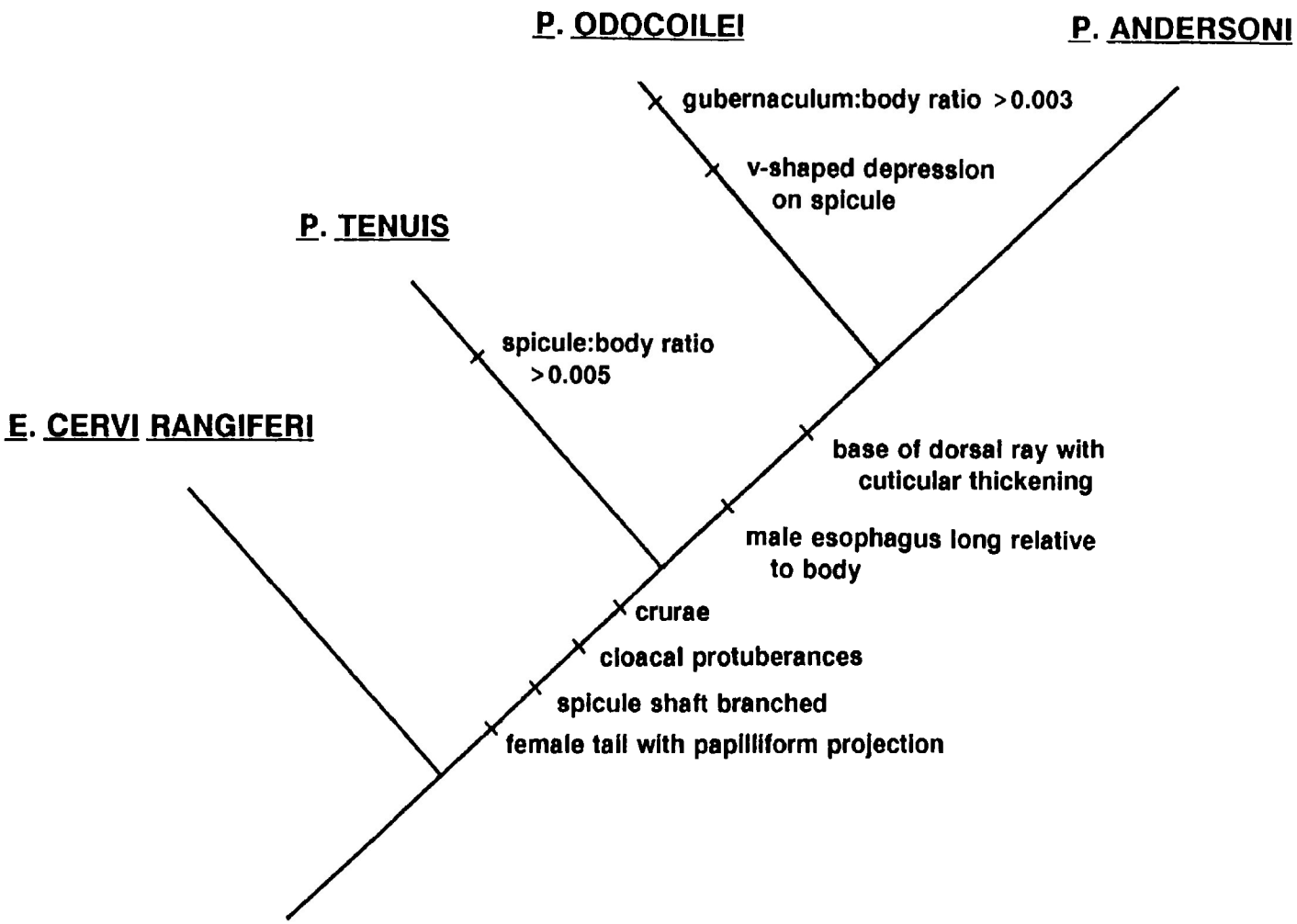
Table 2. List of characters used to reconstruct the phylogeny of the genus *Parelaphostrongylus*, using *Elaphostrongylus cervi rangiferi* as the outgroup. Plesiomorphic character states are coded 0; apomorphic character states are coded 1.

1. gubernaculum	crurae absent (0) crurae present (1)
2. spicules	shaft unbranched (0) shaft distally branched (1)
3. female tail	tail without caudal papilla-like projection (0) tail with caudal papilla-like projection (1)
4. cloacal protuberances	ventrally directed protuberances at rim of cloaca absent (0) protuberances present (1)
5. dorsal ray	base without cuticular thickening (0) base with cuticular thickening (1)
6. ratio of male esophageal length to total body length	less than 0.022 (0) greater than 0.022 (1)
7. ratio of gubernaculum length to total body length	less than 0.0028 (0) greater than 0.0028 (1)
8. ratio of spicule length to total body length	greater than 0.005 (0) less than 0.005 (1)
9. spicule texture	spicule shaft anterior to distal third of uniform texture (0) v-shaped depression on spicule shaft anterior to the distal third (1)

Table 3. Data matrix used for the reconstruction of the phylogeny of *Parelaphostrongylus* spp. based on the characters outlined in Table 2

	Character
	1 2 3 4 5 6 7 8 9
E. c. rangiferi	0 0 0 0 0 0 0 0 0
P. tenuis	1 1 1 1 0 0 0 1 0
P. odocoilei	1 1 1 1 1 1 1 0 1
P. andersoni	1 1 1 1 1 1 0 0 0

Fig. 44. Cladogram depicting the phylogenetic relationships of *Parelaphostrongylus* spp.


P. ODOCOILEI
P. ANDERSONI
gubernaculum:body ratio >0.003
v-shaped depression on spicule
P. TENUIS
spicule:body ratio >0.005
E. CERVI RANGIFERI
base of dorsal ray with cuticular thickening
male esophagus long relative to body
crurae
cloacal protuberances
spicule shaft branched
female tail with papilliform projection

Strong evidence for monophyly of the genus *Parelaphostrongylus* is shown by the similar spicule morphology, presence of crurae on the gubernaculum, the presence of a caudal papilla-like structure in females, and the presence of ventral protuberances at the outer rim of the cloaca. The genus is divided into two clades, one consisting of *P. tenuis*, the other consisting of *P. odocoilei* and *P. andersoni*. The muscleworm clade is characterized by the two synapomorphies of the cuticular thickening around the base of the dorsal ray and the greater esophageal length relative to the overall body length.

DISCUSSION

Morphology

The results of this study provide a more detailed description of the morphology of the Elaphostrongylinae. It is evident from these results that difficulties have been encountered in previous light microscopical study of structures such as the spicules. Varying transparency of the spicules and obstruction by other tissues have confounded attempts to determine their precise morphology. Most published illustrations of the spicules of *P. tenuis* (Dougherty 1945; Anderson 1956; Whitlock 1959) are generally similar to the morphological features described in this SEM study, but the descriptions provided are inconsistent or inaccurate in several respects. Dougherty (1945), in the initial description of *P. tenuis*, described "a longitudinal slit in the distal half of the lamina" of the spicules. His illustration of the lateral view of the spicule shows an elliptical aperture in the shaft approximately 2/3 the distance along its length. It does not extend to the distal tip of the shaft. Furthermore, both sides of the shaft bordering the aperture are of equal width, whereas SEM shows that the dorsal branch is narrower than the main shaft. Dougherty (1945) did not describe or illustrate a membrane between the branch and the shaft. The membrane is virtually invisible when viewed *in situ* under the light microscope.

Whitlock (1959) stated that the spicules of *P. tenuis* are not split. He describes, however, "an accessory, tapering, bending point" which originates "from

the area of the ventral ala." This bending point forms one edge of "a complex, spoon-shaped structure". The rest of the shaft constitutes the other edge of the "spoon" (Whitlock 1959). This description, although somewhat imprecise, seems to be more consistent with SEM depictions of the spicules, but no mention is made of a ramification of the main shaft. Anderson (1956) made no mention in his description that the spicules of *P. tenuis* were split but instead depicted broader alae near the tips of the spicules.

In the initial description of *P. odocoilei* (Hobmaier and Hobmaier 1934), the distal region of the spicules was not described in detail. Most subsequent descriptions of this muscleworm, although illustrating the distal branching pattern, do not provide details of this region of the spicules. Brunetti (1969) described the distal spicule region as a "spoon-shaped structure" formed by an extension of the dorsal ala. In a light micrograph provided, the dorsal ala appears to be separated from the spicule shaft near the distal tip. However, the branch of the spicule shaft that is attached to the dorsal ala was not included in Brunetti's description of the spicules.

Platt and Samuel (1978a) stated that the spicules of *P. odocoilei* lack a "foramen" in the shaft as they believed was present in specimens of *P. tenuis* studied for comparison. Although their drawings show one of the alae separated from the spicule shaft, no mention of this feature was made in the text. The branch of the distal region of *P. odocoilei* spicules is more difficult to observe under the light microscope than that of *P. tenuis*.

There has been considerable variability in light microscopical descriptions of the spicules of *P. andersoni* despite original drawings by Prestwood (1972) that are consistent with SEM findings of this study. A dorsal branching of the shaft is illustrated, and the region in between the branch and the shaft is drawn in a manner that suggests a clear, membranous area extending to the spicule tip (Prestwood, 1972). Pybus and Samuel (1981) described "a distal bifurcation" in the spicules of *P. andersoni* and their drawing shows a longitudinal split in the distal region of the spicule. These authors amended Prestwood's spicule description by stating that the "distal bifurcation" is actually longer than originally depicted, "and may extend up to 1/4 the length of the spicule". Although no measurements of this region were provided by Prestwood (1972), her illustrations indicate that the branch arises distally, approximately 6/7 the distance along the spicule. Spicules of *P. andersoni* measured in this study branched at a similar point.

More recently, Lankester and Hauta (1989), in the first report of *P. andersoni* in caribou, stated that there is no foramen in the distal region of the spicules. Instead, it was described as being "a lightly sclerotized region of the spicule shaft." This lightly sclerotized zone probably corresponds to the membrane in between the branch and the shaft. No branching of the shaft was illustrated by Lankester and Hauta (1989).

The membrane between the branch and the shaft of *Parelaphostrongylus* spp. is flexible. In some specimens it is relatively smooth whereas folds were seen in others. This feature may allow the spicule tips to be compressed when inserted into

the female. The functional role of the spicules presumably is to dilate the vagina of the female during copulation and to aid in the transfer of sperm (McLaren 1976; Clark *et al.* 1973; Lee 1973). Ultrastructural studies of other nematodes have shown that the spicules are innervated (Dick and Wright 1974; Lee 1973; Wharton 1986; Wright 1978), suggesting that they may also serve as sensory structures that help in locating the vulva. Similar ultrastructural studies probably would reveal nerves inside the spicules of *Parelaphostrongylus* spp. It would be interesting to determine if both the branch and the shaft were innervated because it might indicate that both parts of the spicule tip are active in copulation. Possibly the branch of the spicule is compressed toward the shaft when inserted and separates once the vulva has been penetrated. This would aid in anchoring the worms during copulation. The morphology of the spicule tips may also function, as suggested by Khalil (1978), as "pass-words" by the males to identify themselves to females.

SEM examination of the *E. c. rangiferi* spicules shows that the spicule shaft is unbranched. These findings are consistent with published light microscopical descriptions (Cameron 1931; Lankester and Northcott 1979). The unbranched shaft in this species has no transparent regions as in *Parelaphostrongylus* spp. SEM views of *Elaphostrongylus* spp. have also been published showing spicules without a branching shaft (Steen and Johansson 1990).

The corpus of the gubernaculum of *P. odocoilei* was previously thought to be distally split (Hobmaier and Hobmaier 1934; Brunetti 1969; Platt 1978; Platt and Samuel 1978a). The first description of *P. odocoilei* by Hobmaier and Hobmaier

(1934) characterized the gubernaculum as "2 small chitinous ribbons". Brunetti (1969) described the posterior third of the corpus as being split, and from the photomicrograph of the gubernaculum that he provided, it does, indeed, appear to be split. Platt and Samuel (1978a) also stated that the corpus is split along one third of its length. It is likely that thinner or more lightly sclerotized cuticle make the solid groove in the corpus difficult to observe. A deep groove in this region, however, is visible using SEM. A similar groove in *P. tenuis* has not been perceived as a split by previous workers, possibly because of the larger size and heavier sclerotization of the gubernaculum.

The gubernacular crurae of *P. andersoni* are much smaller than in the other *Parelaphostrongylus* species. Short crurae were described and illustrated by Prestwood (1972). Lankester and Hauta (1989) could not observe crurae on specimens of *P. andersoni* from caribou, but in the present study using SEM, crurae could be observed protruding through the cloaca in specimens from both caribou and white-tailed deer.

The crurae protruded through the cloaca in many of the *Parelaphostrongylus* specimens examined, and it is therefore reasonable to speculate that they may function directly in copulation and be inserted into the vulva of the female. Chitwood and Chitwood (1950) stated that the gubernaculum ordinarily is not everted during copulation other than in forms with denticulate or dentate crurae. These may be adapted for gripping the vulvar lips and holding the vulva open. The outward bending of the accessory piece of *Syphacia obvelata* (which is situated in a

similar location within the worm to the crurae of *Parelaphostrongylus* species) upon eversion of the spicules, may enable it to aid in positioning of the spicule (Dick and Wright 1974). The crurae of *Parelaphostrongylus* spp. may similarly be protruded during copulation. Their dorsal lobes provide a rugged surface that may aid in aligning and anchoring the male cloaca with the vulva of the female for successful sperm transfer.

The nature of the ventrally directed protuberances associated with the crurae in *Parelaphostrongylus* spp. could not be determined. They are not visible using the light microscope, suggesting that they are not sclerotized and may be concealed within the cloaca when the crurae are retracted. Similar sensory protuberances in *Aphelenchoides blastophthorous* (Tylenchida: Aphelenchina) lie hidden within the cloacal sac when the spicules are retracted but are exposed when the spicules are protuded (Clark and Shepherd 1977). In *Parelaphostrongylus* spp., it appears that they arise at the juncture of the ventral part of the crurae and the outer edge of the cloaca. Possibly they are sensory papillae involved in copulation. Perianal papillae have been described in many nematodes. Papillae similar to those seen here occur in the ventral region of *S. obvelata* (Dick and Wright 1974), and two similar papillae, one on each side of the cloaca, are present in *Trichuris trichiura* (Gibbons 1986). The protuberances described here in *Parelaphostrongylus* spp. bear a resemblance to the ventral raylets or papillae found in some trichostrongylids (Gibbons 1986; Gibbons and Khalil 1983). Further investigations, including the use of transmission electron microscopy, are required to determine the precise location of the protuberances

relative to surrounding structures and to elucidate their function.

The greater degree of cuticularization around the base of the dorsal ray in *P. odocoilei* and *P. andersoni* gives the impression that it is elevated more dorsally than that of *P. tenuis* and *E. c. rangiferi*. Platt (1978) stated that the "mound-like" dorsal ray of *P. odocoilei* "is situated in a more dorsal position than in *P. tenuis* and *P. andersoni*." Platt (1984) also described the shape of the dorsal ray of *P. andersoni* and *P. odocoilei* as a "compact bulb." Actually, the dorsal ray of *P. tenuis* can also be interpreted as being a compact bulb, although the relative thickness of the dorsal ray is difficult to assess accurately using light microscopy. Observations on the bursae of each species in lateral view using SEM did not reveal any differences in the degree of elevation or shape of the dorsal ray in *Parelaphostrongylus* spp.

Pores associated with the bursal rays were observed in several specimens; papillae were often located close to the pores. No intraspecific consistencies were apparent, probably because the ray morphology also varied. Sense organs at the tips of the bursal rays are known to occur in other nematodes (Bird and Bird 1991), and the presence of one or more papillae surrounding the pores at the ray tips suggests that they may be developed for a role in copulation. The movements of the bursa are complex, as shown by Croll and Wright (1976), who observed copulation in *Nematospiroides dubius*. Presumably the sensory structures at the tips of the rays of the elaphostrongyline nematodes aid in locating the female tail and in aligning the vulva with the male cloaca.

The single papilla located immediately ventral to the cloaca in

Parelaphostrongylus spp. is in a similar position to the anal papillae of *S. obvelata* (Dick and Wright 1974). In the latter species, they are considered to be mechanoreceptors sensitive to pressure or lateral deflections (Dick and Wright 1974). A similar role could be hypothesized for the single papilla found in *Parelaphostrongylus* spp. Although an anal papilla could not be seen in specimens of *E. c. rangiferi* in this study, it has been described in an *Elaphostrongylus* sp. from moose (Steen and Johansson 1990). These authors also describe additional papillae and "lateral bulges" surrounding the dorsal region of the cloaca. Such structures were not observed here in either *Elaphostrongylus* spp. or *Parelaphostrongylus* spp.

The general arrangement of the cephalic sensory structures was similar in each of the four species. Wright (1975) described the cephalic and labial papillae of *Nippostrongylus brasiliensis*. Both types were believed to function as mechanoreceptors in this species (Wright 1975), and may serve a similar purpose in elaphostrongyline nematodes. Other anterior sensory structures include amphids which are said to function as chemoreceptors (Wright 1975; Bird and Bird 1991; Wharton 1986). The amphids of *N. brasiliensis* contain sensory dendrites within the amphidial canal (Wright 1975). Possibly the cilium-like structures seen protruding through the amphidial pores of several elaphostrongyline specimens are the tips of these dendrites.

The six shallow circular depressions within which the labial papillae are located were termed perityls by Platt (1978; 1984). Perityls were described as being homologous to the six lips found in *Heterostrongylus* spp. by Anderson (1978), and

were further defined by Platt (1984) as being nonmovable lips. In addition, Platt (1984) states that perityls are absent in *E. c. rangiferi* but present in *Parelaphostrongylus* spp. Specimens of *E. c. rangiferi* were not available to Platt in his cladistic study, and he relied on a description of the species from Lankester and Northcott (1979) in which six labial papillae were illustrated without a surrounding shallower base. The results of the present study, however, have shown that areas that might be termed perityls are present in each of the four elaphostrongyline species including *E. c. rangiferi*. They have also been depicted in a study of the genus *Elaphostrongylus* by Gibbons *et al.* (1991).

Light microscopical studies have revealed morphological differences in the tails of female *Parelaphostrongylus* spp. Anderson (1956) described a trilobed, caudal protuberance at the caudal extremity of *P. tenuis* females, and suggested that it is comprised of cuticle and has no sensory function. Whitlock (1959) described two lateral and one terminal papilla on the tip of the tail. The two lateral papillae are probably the phasmids seen in this study. Brunetti (1969) described the *P. odocoilei* female tail as ending in a small papilliform projection. Other studies of this worm (Hobmaier and Hobmaier 1934; Platt 1978; Platt and Samuel 1978a) do not illustrate or describe this structure. A caudal, papilliform projection on females of *P. andersoni* has not been described. Its small size makes it difficult to observe, and it is absent in some specimens. Nevertheless, it is consistently smaller than that of *P. odocoilei*.

The two lateral pores on slightly elevated areas on the female tails are likely

the phasmids. The phasmids of only a small number of nematode species have been examined using electron microscopy (Gibbons 1986). Future descriptions of the phasmids of other members of the Metastrongyloidea may provide useful comparative information.

Phylogenetic analysis

No attempts have been made to reconstruct the phylogeny of the Protostrongylidae using modern phylogenetic techniques. Cladistic study would require availability of specimens from all presumed genera in order to uniformly describe detailed morphological characters. An identification key for the Protostrongylidae has been compiled by Anderson (1978). However, holomorphological descriptions of the worms are not provided since several characters useful in keying out some taxa are not important for others, and thus, are not described. Other works on the Protostrongylidae (Skrjabin *et al.* 1952; Yamaguti 1961) similarly, do not provide uniform morphometric data for each genus. Furthermore, original drawings from the primary literature often are simply reproduced and do not provide new illustrations and modern descriptions. For example, the initial illustrations of the genus *Skrjabinocaulus* Boev and Sulimov, 1963 are reproduced in Anderson (1978), but profiles of the worms do not show morphological features as clearly as in illustrations of other taxa. Finally, the currently accepted classification of the Protostrongylidae was not based on phylogenetic analysis, and the family may comprise a polyphyletic assemblage.

The application of phylogenetic systematics to biological classifications was

emphasized by Hennig (1966), who wrote, "Species are gathered into groups according to the degree of their phylogenetic kinship, and these groups are coordinated or subordinated to one another." Recently, more organized rules and conventions for such classifications have been provided (Wiley 1981). Generally, these conventions involve the classification of taxa as monophyletic groups. A full cladistic analysis of the Protostrongylidae and closely related taxa, may thus provide a sound classification for these nematodes and help to detect paraphyletic groups.

Protostrongylid genera other than *Elaphostrongylus* spp. and *Parelaphostrongylus* spp. have not been studied on a comparative basis using SEM. Surface characters are often distorted when viewed under the light microscope, and are not easily seen or interpreted because of the low resolution and limited magnification of the light microscope. SEM studies can reveal more characters for use by taxonomists for identification and classification (Gibbons and Khalil 1990). Therefore a full SEM reexamination of all protostrongylid nematodes would be useful.

Phylogeny of elaphostrongyline nematodes

Results of the present study indicate that some of the characters used by Platt (1984) in reconstructing the phylogeny of North American elaphostrongyline nematodes were incorrectly interpreted. The distally split corpus in *P. odocoilei* is not split, but forms a distal groove. The spicules of *P. andersoni* are not split and those of *P. tenuis* do not have a foramen; the spicule shaft of *P. odocoilei* is branched. Perityls are found in both *Parelaphostrongylus* spp. and in *E. c. rangiferi*, not just the

former. The dorsal ray, described as a compact bulb and used by Platt to separate the muscleworms from *P. tenuis,* appears to be similar in all three species and is therefore a synapomorphy. Also, the branches of the dorsal ray of *P. odocoilei* were said to be terminally located (Platt 1984), but in some specimens, the branches are ventral.

Although the characters used here were different than those of Platt (1984), the most parsimonious cladogram has an identical topology to that of Platt (1984). In this study, males of the most recent ancestor of the *Parelaphostrongylus* clade had gubernacular crurae, a distally branched spicule shaft, and ventrally directed protuberances at the edge of the cloaca; females had a caudal papilliform projection at the tip of the tail. The ancestor of the muscleworm clade had a cuticular thickening around the base of the dorsal ray and a long esophagus relative to overall body length.

The *P. odocoilei* - *P. andersoni* clade is also distinct from the *P. tenuis* clade in that the prepatent period of the muscleworms is generally shorter than that of *P. tenuis*. In white-tailed deer, the prepatent period of *P. tenuis* is 91 days (Anderson 1963); similarly, a 92-day prepatent period was reported for *P. tenuis* in experimentally infected wapiti (Anderson *et al.*, 1966). In *P. andersoni*, however, the shortest reported prepatent period in white-tailed deer is 51 days (Pybus and Samuel 1984), and the highest is 67 days (Nettles and Prestwood 1976). Experimentally infected caribou calves began to pass *P. andersoni* 1st stage larvae 66 days after initial exposure to L3 larvae (Lankester and Hauta 1989). Similarly, the shortest prepatent

period reported for *P. odocoilei* in mule deer is 45 days (Pybus and Samuel 1984), and the longest is 62 days (Platt and Samuel 1978b). In black-tailed deer (*O. h. columbianus*) the longest reported prepatent period is 72 days, and it is similar for *P. odocoilei* in moose (Platt and Samuel 1978b). Thus, the two muscleworms have a shorter prepatent period than *P. tenuis*, a feature that may correspond to the phylogenetic divergence of the muscleworm clade.

Reported prepatent periods for *E. c. rangiferi*, the outgroup used in this study, are extremely variable, making it difficult to postulate whether or not a long prepatent period is the plesiomorphic condition. A caribou calf that was experimentally infected with *E. c. rangiferi* from Newfoundland began to pass 1st-stage larvae 74 days after infection, and a moose calf became patent 64 days after infection with larvae from the same source (Lankester 1977). Watson (1983) infected red deer calves with *E. cervi cervi* and reported a prepatent period of 107-125 days. In a later study, Watson (1986) infected red deer calves which passed 1st stage larvae 86-98 days after exposure. A prepatent period as short as 20 days has been reported for *E. cervi* var. infections in moose (Steen 1991). Therefore, because of the variability of prepatent periods of *E. cervi*, it is difficult to infer the transformation series of this feature with respect to the elaphostrongyline phylogeny.

The *P. odocoilei* - *P. andersoni* clade contains the two species whose adults inhabit the skeletal muscles of their hosts; *P. tenuis* occupies the central nervous system. It is difficult to speculate on the ancestral location within the host for *Parelaphostrongylus* spp. given that members of the outgroup (*E. c. rangiferi*) inhabit

both the central nervous system and the skeletal muscles (Lankester and Northcott 1979; Mason 1989). There is some evidence to suggest that more adult *E. cervi* occur in the skeletal muscles than in the central nervous system. Mitskevich (1964) reported 7.5% of adult *E. cervi* recovered from experimentally infected reindeer in the brain, and 2% under the peritoneum. The majority of the adults, however, were found in the muscles of the neck, forelimbs, and hindlimbs. Lankester and Northcott (1979) recovered 26 *E. c. rangiferi* from the muscles of a naturally infected caribou calf that had displayed neurologic signs, and no worms were found in the cranium or in the vertebral canal. It has been suggested that *E. cervi* develops in the neural parenchyma and later migrates to the skeletal muscles (Anderson 1968; Lankester 1977). In caribou and moose calves, neurologic signs appeared 29 to 33 days after infection, suggesting that upon infection, larvae move directly to the central nervous system, where they apparently develop in the meningeal spaces (Lankester 1977). Subsequent migration to the skeletal muscles may occur via the lateral nerves. Four months after experimental infection of a caribou calf, Lankester (1977) found four *E. c. rangiferi* in the central nervous system and one was in the skeletal muscles. In a moose calf killed three months after infection, 18 worms were in the central nervous system and 18 were in the skeletal muscles (Lankester 1977). The experimental evidence for a migration route is, however, incomplete, and further studies are necessary.

The life cycle of *P. tenuis*, including the sites of maturation, is better known. In white-tailed deer, *P. tenuis* develops from the third to the fifth stage in the neural

parenchyma. Approximately 40 days after infection, immature worms leave the nerve tissue and mature between the dura mater and the leptomeninges (Anderson 1963). Little damage is done to the nerve parenchyma during the development and movement of the worms (Anderson 1965a). No records exist of *P. tenuis* occurring in the skeletal muscles of cervids, although the type specimen, an adult male, was found in a small bronchiole from the lung of a white-tailed deer (Dougherty 1945).

The majority of adult *P. andersoni* and *P. odocoilei* are located in the skeletal muscles. Maturation likely occurs after the worms enter muscle tissue, although gravid females have also been found within the spinal epidural space, suggesting that maturation may occur in locations other than muscle (Pybus and Samuel 1984). Interestingly, it has been suggested by Pybus (1983) that the two muscleworm species may move to the lumbar region of the central nervous system early in their migration before dispersing to the muscles. Being in or near nerve tissue may thus be a prerequisite of maturation in the life cycles of all of the elaphostrongyline nematodes. This hypothesis may be tested by periodically examining the central nervous systems of experimentally infected cervids for maturing nematodes.

Despite the fact that all of these worms may share a common developmental site in the central nervous system, the skeletal muscle habitat of adults of *P. odocoilei* and *P. andersoni* corresponds to the phylogenetic lineage that separates them from *P. tenuis*, which is limited primarily to the subdural space of white-tailed deer. Although the developmental biology of *E. c. rangiferi* is insufficiently known, it might be concluded that the ancestor of the elaphostrongyline clade was associated with the

central nervous system at some point in its life cycle. Specialization of habitats occurred in the two *Parelaphostrongylus* clades, with adult *P. tenuis* being limited to the central nervous system and adults of *P. odocoilei* and *P. andersoni* to the skeletal muscles. Oviposition in all of the species involves the host circulatory system.

The definitive host of a parasite is one in which sexual reproduction occurs (Noble *et al.* 1989). A list of definitive hosts of elaphostrongyline nematodes was compiled by Platt (1978). However, it included several hosts of *P. tenuis* which may be killed by neurologic disease caused by immature worms. Although there is evidence, for example, that *P. tenuis* can reproduce within moose (Anderson 1965b), this and other hosts in which sexual reproduction is retarded or prevented probably are not functional hosts in nature.

The guinea pig is also listed as a definitive host of *P. tenuis* (Platt 1978), but adult *P. tenuis* or their first stage larvae have not been recovered from this experimental animal (Anderson and Strelive 1966a; Spratt and Anderson 1968). Similarly, *P. tenuis* will not mature in sheep (Anderson and Strelive 1966b). Thus, the white-tailed deer is the normal definitive host of *P. tenuis*, but worms may occasionally reach sexual maturity in wapiti (Carpenter *et al.* 1973; Anderson *et al.* 1966; Pybus *et al.* 1989), reindeer (Anderson 1971), possibly mule deer, since gravid females were found in this host (Anderson *et al.* 1966) and possibly fallow deer (Davidson *et al.* 1985; Pybus *et al.* 1992).

In recent years knowledge regarding the host specificity of other elaphostrongyline nematodes has also grown considerably. For example, *P. andersoni*,

which was originally thought to occur only in white-tailed deer (Platt 1978), is now known to be widespread in North American caribou (Lankester and Hauta 1989). It becomes patent experimentally in mule deer (Pybus 1983) and fallow deer (Lankester *et al.* 1990). Infection of *P. andersoni* in moose has, to date, not been reported (Lankester 1987). *Parelaphostrongylus odocoilei* matures naturally in *O. hemionus* subspp., *A. alces*, and woodland caribou (Gray and Samuel 1986). Natural and most likely patent infections have been reported from mountain goats (*Oreamnos americanus*) in the western United States (Pybus *et al.* 1984), and experimental infections have shown that *P. odocoilei* cannot become established in white-tailed deer (Pybus 1983).

The degree of host specificity of *E. cervi* cannot be determined until the taxonomy of the three possible forms is resolved. There may be a form specific to *Rangifer*, one specific to *Cervus*, and one specific to *Alces*. Alternatively, one somewhat variable form may infect all of these hosts and, therefore, be considered to have little specificity.

The Fahrenholz Rule states that host relationships can be inferred from the relationships of their parasites, since the evolution of the parasites parallels that of their hosts (Hennig 1966). The validity of the Fahrenholz Rule can be examined using modern cladistic techniques. A phylogenetic hypothesis depicting the relationships between parasites can be superimposed on one depicting the reconstructed host phylogeny. Thus, hypothesized host and parasite phylogenies derived using the same technique can be studied to infer instances of cospeciation

and coaccomodation (Brooks 1979).

Phylogenetic relationships among North American cervids are not well understood, and attempts to reconstruct the phylogeny of cervids have produced conflicting hypotheses. Giffin (1974) used cladistic techniques to reconstruct the phylogeny of the Artiodactyla, including the Cervidae. An outgroup was not defined, although criteria of phylogenetic relationships as described by Hennig (1966) were used. It was proposed that the sister group to the genus *Odocoileus* was the genus *Rangifer*, and the two groups were united by the synapomorphy of a higher vomer. The genus *Rangifer* was characterized by the autapomorphy of females with antlers. The elaphostrongyline phylogeny, as proposed by Platt (1984), corresponds to this host sister group relationship such that *E. cervi* originated in *Rangifer* spp. and *Parelaphostrongylus* spp. originated with the genus *Odocoileus*.

A similar phylogenetic hypothesis, showing the close relationship between *Odocoileus* and *Rangifer*, has been presented by Groves and Grubb (1987). The synapomorphy linking the genera *Pudu, Mazama, Hippocamelus, Blastocerus, Ozotocerus, Odocoileus,* and *Rangifer* was the presence of a vomerine septum in these genera. Unfortunately, important details including a full character list, choice of an outgroup, and character argumentation were not presented in this study. Nevertheless, the proposed cervid phylogeny corresponds roughly with that of Giffin (1974).

In contrast, a preliminary phylogenetic hypothesis presented by Gustafson (1985) argues that the genera *Blastocerus, Ozotocerus, Hippocamelus, Pudu, Mazama,*

and *Capreolus* are more closely related to *Odocoileus* than is the genus *Rangifer*. This study was based primarily on antler morphology, and it was postulated that the antler pattern shown by *Capreolus* spp. (roe deer) is close to the primitive pattern for all of the remaining Odocoileinae (Gustafson 1985). Detailed antler characters were not used in the study by Giffin (1974).

In addition to morphological data, genetic and molecular biological information is also accumulating with respect to cervid relationships. Baccus *et al.* (1983) showed that considerable variability exists between different taxa. On the basis of a cluster analysis depicting genetic similarity of various proteins from 10 species of ungulates, it was shown that roe deer and moose were more similar to white-tailed deer and mule deer than were caribou and reindeer. In this study, however, the systematic relationships among the Odocoileinae were not clarified.

In a cladistic study of the mitochondrial-DNA phylogeny of the Cervidae, Cronin (1991) suggested that *Rangifer* is not the sister group to the genus *Odocoileus*. The mitochondrial-DNA information suggests that caribou are a monophyletic group separate from the Cervinae and Odocoileinae. These results agree with the genetic study of Baccus *et al.* (1983), as well as with the morphological analysis of Gustafson (1985). It is clear, then, that phylogenetic reconstructions of the Cervidae have produced somewhat opposing results. A cladistic analysis including all cervid taxa and as many morphological characters as possible is necessary in order to provide a robust phylogenetic hypothesis regarding cervid relationships. The studies described above are, for the most part, only partially complete since full character analysis and

outgroup comparison have not been applied. Therefore the phylogenetic evidence for a sister group relationship between *Odocoileus* and *Rangifer* is, at this time, incomplete. Nonetheless, if *Rangifer* were indeed the sister group to *Odocoileus*, this would imply a cospeciation event involving *E. cervi* speciating with *Rangifer*, as has been proposed by Platt (1984). The speciation of the genus *Parelaphostrongylus* could also have occurred with the speciation of *Odocoileus* spp.

Platt (1984) also hypothesized that this cospeciation of the Elaphostrongylinae with *Rangifer* and *Odocoileus* was a Nearctic event. There are conflicting views regarding the biogeographical history of New World cervids, and this is due to the scant paleontological record of North American Pliocene and Pleistocene deer (Fry and Gustafson 1974). The Cervidae are believed to have originated in Eurasia in the Late Oligocene and Early Miocene (Pilgrim 1941; Baker 1984; Eisenberg 1987). There are no records of antlered forms in North America until the Early Pliocene when they are believed to have entered from Eurasia (Eisenberg 1987). From Early Pliocene ancestors that appeared first in Eurasia and later in North America, the "modern" cervids, including *Odocoileus, Alces,* and *Rangifer*, were derived (Baker 1984).

Giffin (1974) stated that *Rangifer* evolved in North America on the basis of the synapomorphies that document the common ancestry of *Rangifer* and the New World deer *Mazama* and *Odocoileus*. This relationship presumably implies a Nearctic origin and a later emigration by *Rangifer* to the Palearctic. This hypothesis is supported by other authors (eg Brokx 1972; Baker 1984); emigration to the

Palearctic is said to have occurred during the Pleistocene. Unfortunately the paleontological evidence does not indicate a point of origin for *Rangifer* nor does it reveal an accurate time period for such an emigration.

The opposite view was presented by Kurtén (1971), who stated that *Rangifer* originated in the Old World and entered North America in the late Pleistocene. Pleistocene records of *Rangifer* in Eurasia are widespread. The oldest known Eurasian fossil reindeer was found at Suessenborn, Germany, and is most likely over 400,000 years old (Kurtén 1968). Late Pleistocene records are also widespread in Spain, Italy, southern Russia, Germany, and France (Kurtén 1968; Stuart 1991). Pleistocene records of *Rangifer* in North America extend as far south as Alabama (Churcher *et al.* 1989). Therefore, *Rangifer tarandus* had an extensive Holarctic distribution by the late Pleistocene, and the precise evolutionary origin of the genus, whether in the Nearctic or in the Palearctic, is not clear.

A more compromising hypothesis on the origin of *Rangifer* was proposed by Guthrie and Matthews (1971), who suggested that numerous mammals, including *Rangifer*, evolved in the Beringian faunal zone which united the Palearctic and Nearctic regions during the Pleistocene. These Beringian mammals emigrated first to the Palearctic in the mid-Pleistocene, and later dispersed to the Nearctic during the late Pleistocene. The earliest known *Rangifer* fossils were discovered in the Cape Deceit fauna of Alaska, and are said to predate the Gunz 2 stage in Europe and the late Kansan stage of the North American mid-continent (Guthrie and Matthews 1971). The Cape Deceit record is also the earliest recorded *Rangifer* specimen for

North America (Churcher *et al.* 1989). A Beringian origin for *Rangifer* is compatible with the Holarctic Pleistocene distribution of this species described by other authors. Also, its presumed status as the sister group to *Odocoileus* (Giffin 1974) could still be possible if speciation occurred during the Pliocene when the ancestral cervid emigrated from the Palearctic. Divergence of the *Rangifer* clade presumably would have occurred in the eastern Palearctic and Alaska.

The cospeciation of *E. cervi* in *Rangifer*, coupled with the subsequent emigration of *Rangifer* to the Palearctic from the Nearctic, accounts, according to Platt (1978; 1984), for the current Holarctic distribution of *E. cervi*. Platt also concluded that the presence of *E. cervi* in Newfoundland as reported by Lankester and Northcott (1979) provided evidence for a Holarctic distribution of this species. However, there currently are no known records of *E. cervi* in continental North America and it is believed that *E. cervi* may have been introduced to Newfoundland by the introduction of reindeer from Norway in 1908 (Lankester and Fong 1989). If *E. cervi* did, indeed, speciate in *Rangifer*, this must have occurred after populations of the host had emigrated to the Palearctic from Beringia. Possibly *E. cervi* became extinct in North American caribou but there is no evidence to support this suggestion. Thus, although the speciation of the host and the parasite may correspond, there is little biogeographical evidence to suggest that *E. cervi* originated in Nearctic *Rangifer*.

It is most likely that *E. cervi* evolved in a Palearctic cervid, and although *Rangifer* cannot be excluded as the possible original host, other cervids may also be

considered. *Elaphostrongylus cervi* var. is known to occur in several present day Eurasian cervids, including red deer, reindeer, and moose (see discussion above). The ranges of these hosts may have overlapped during the Pleistocene (Guthrie 1966; Stuart 1991), as they do today in parts of Scandinavia, for example. Therefore, because of the possibility of a host-switching event involving *E. cervi* and any of these hosts, the parasite may have originated in a host other than *Rangifer.*

Interestingly, *Alces alces* originated in the Palearctic and later emigrated to the Nearctic (Kelsall and Telfer 1974; Geist 1987; Peterson 1955; Gustafson 1985), as did *Cervus elaphus* (Baker 1984; Geist 1985; Guthrie 1966). Neither of these species has been found to be infected with *E. cervi* within North America. Possibly, they dispersed into the Nearctic before *E. cervi* became widespread in its various Palearctic hosts. A similar explanation accounts for the absence of *Nematodirus* spp. (Trichostrongyloidea) in North American moose and wapiti while they are widespread in Palearctic cervids (Hoberg *et al.* 1989). Undoubtedly, future paleontological discoveries and information regarding the present distribution of *E. cervi* will provide more accurate insights regarding the original host of this species. The corresponding host and parasite phylogenies, however, indicate that *E. cervi* is very closely associated with *Rangifer.* Its origin in this host, however, can be questioned.

The current distribution of *Parelaphostrongylus* species, corresponding host and parasite phylogenies, and the information on host specificity discussed above, provide evidence that this genus originated within the host genus *Odocoileus.* Platt (1984)

provided a detailed hypothesis describing the speciation of *P. tenuis* prior to the evolution of extant *Odocoileus* species and the subsequent cospeciation of *P. odocoilei* and *P. andersoni* with *O. hemionus* and *O. virginianus*, respectively. The speciation of *P. tenuis* was thought not to be a response to speciation in the host lineage (Platt 1984), but instead, originated prior to the formation of *O. virginianus*.

Alternatively, it may be possible that both *P. tenuis* and *P. andersoni* cospeciated with *O. virginianus*. This speciation of the two parasites within one host is possible with specialization of habitats in the central nervous system and in muscles. Thus, populations of an ancestral *Parelaphostrongylus* species may have undergone one of the forms of allopatric speciation (*sensu* Wiley 1981;Brooks and McLennan 1991) arising from the isolation of the two populations within the host. Competition between the two parasites would be minimal if adults occupied different habitats. Indeed, concomitant infections of *P. tenuis* and *P. andersoni* within white-tailed deer have been identified (Pybus *et al.* 1990). Based on the suspected migration route of adult *E. cervi*, it may be possible that the ancestral *Parelaphostrongylus* species may also have favoured both the skeletal muscles and the central nervous system.

Conversely, a cospeciation event involving *P. andersoni* in *O. virginianus* and *P. odocoilei* in *O. hemionus* from a muscleworm ancestor (Platt 1978; 1984) is perhaps more likely when host and parasite phylogenies are compared. The two *Odocoileus* species presumably differentiated allopatrically in two centres of distribution, east and west of the prairies, with *O. hemionus* having more specific

habitat requirements than *O. virginianus* (Brokx 1972). This differentiation presumably allowed for the cospeciation of *P. odocoilei* with mule deer and *P. andersoni* with white-tailed deer (Platt 1978). Based on the lower pathogenicity of *P. odocoilei* in black-tailed deer (*O. h. columbianus*) compared to mule deer (*O. h. hemionus*), Pybus (1983) suggested that the former may have had a longer association with *P. odocoilei* than the latter host, although mule deer were said to be suitable definitive hosts, based on natural populations of *P. odocoilei* in mule deer from Alberta (Pybus 1983).

Evidently the white-tailed deer serves as a suitable definitive host for *P. andersoni* as infections have only moderate pathogenic effects in this host (Nettles and Prestwood 1976; Pybus 1983). In this study and in those of Platt (1978; 1984), it is concluded that *P. andersoni* cospeciated with white-tailed deer. It has been suggested that *P. andersoni* may have originated in *Rangifer* instead of in *O. virginianus*, since the parasite has been found to be widespread in North American caribou (Lankester and Hauta 1989). The origin of *P. andersoni* in *Rangifer* would not be congruent with the phylogenetic hypothesis of the Elaphostrongylinae since it would imply the speciation of *P. tenuis* in an ancestral *Odocoileus* species followed by cospeciation of the muscleworm species, *P. andersoni* in *Rangifer* and *P. odocoilei* in *O. hemionus*. The more parsimonious explanation is that cospeciation of the two muscleworms with two hosts of the same genus occurred, and if *Rangifer* is the sister group of *Odocoileus*, the host phylogeny (Giffin 1974) and the parasite phylogeny would correspond more closely.

The widespread distribution of *P. andersoni* in North American caribou and its possible origin in this host might suggest that the parasite would also occur in Eurasian *Rangifer tarandus* (Lankester and Hauta 1989). No *Parelaphostrongylus* spp., including *P. andersoni*, have so far been found in Eurasian cervids. Their absence in Eurasia provides further evidence for a Nearctic origin in *Odocoileus* spp. A host-switching event most likely occurred in which *P. andersoni* from white-tailed deer were transferred to caribou. Caribou were found south of the Pleistocene ice sheets throughout the Wisconsian, as were *Odocoileus* spp. (Churcher *et al.* 1989; Kurtén and Anderson 1980, as in Stuart 1991; Pielou 1991). During the Wisconsian, the ranges of *Odocoileus* and *Rangifer* may have overlapped in several regions in North America. A site in Alabama contained *Rangifer* fragments dating to approximately 11,820 years B.P. as well as bone fragments of *Odocoileus virginianus* (Churcher *et al.* 1989). It has been suggested that the mixture of typical boreal forest inhabiting species with grassland taxa in this site may reflect seasonal visitations by southern forms during warm months and southern migrations by the boreal taxa during sustained cold periods (Churcher *et al.* 1989). If similar, prolonged overlap of this nature occurred throughout the rest of North America, it is possible that *P. andersoni* may have become established in caribou through contact with white-tailed deer.

The morphological data base on other cervid parasites, particularly the trichostrongyloids, is growing (Fruetel 1987; Lichtenfels and Pillit 1983; Hoberg and Rickard 1988; Rickard and Hoberg 1990; Durette-Desset 1985; Hoberg *et al.* 1989), and an improved understanding of such characters as spicule morphology, synlophe,

and the position of the vulva, will allow more detailed cladistic analysis. Several *Nematodirella* species live in cervid hosts including *R. tarandus, A. alces, C. elaphus,* and *O. hemionus*. Other artiodactyls such as mountain goats (*Oreamnos americanus*), gazelles (*Gazella* spp.), and muskox (*Ovibus moschatus*) are also parasitized (Lichtenfels and Pilitt 1983). Comparing phylogenies of *Nematodirella* species with a cervid phylogeny may support or contradict the coevolutionary history of *Parelaphostrongylus* species in cervids. Also, the broader host specificity of *Nematodirella* species in artiodactyls may eventually be useful in coevolutionary studies of this group.

Hoberg *et al.* (1989) postulated a coevolutionary relationship of *Nematodirus tarandi* in *Rangifer* species and *N. odocoilei* in *Odocoileus* species based on the sister group relationship between *Odocoileus* and *Rangifer* that was described by Giffin (1974). Although there is morphological similarity of the larvae and adults of the two nematodes (Fruetel 1987), a more robust phylogenetic hypothesis of *Nematodirus* species would strengthen the coevolutionary postulate.

The presence of particular *Nematodirus* species in Palearctic *Alces* species and *Cervus* species, and their absence in these hosts in North America indicates that the two hosts dispersed into the Nearctic prior to a host - switching event that occurred in the Palearctic (Hoberg *et al.* 1989). Conversely, Durette-Desset (1985) suggested that some Caprinae and Odocoileinae passed from North America into Eurasia via Beringia, since Palearctic *Nematodirus* species were supposedly "morphologically more evolved than those in North America". The absence of *Parelaphostrongylus*

species in the Palearctic, however, would contradict this latter hypothesis, assuming that *Parelaphostrongylus* species would have dispersed along with their cervid hosts. Furthermore, the biogeographical evidence that cervids and bovids migrated from the Nearctic to the Palearctic during the Pleistocene is sparse (Geist 1985; Hoberg *et al.* 1989). In most discussions of trichostrongyloid coevolution with cervid hosts, no hypothesized phylogenies of either the hosts or the parasites have been presented. Cladistic studies of both may provide a stronger coevolutionary hypothesis regarding trichostrongyloid nematodes and their mammalian hosts.

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