Elaphostrongylus spp. from Scandinavian cervidae – a scanning electron microscope study (SEM)

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Abstract: Nematodes of the genus *Elaphostrongylus* collected from moose (*Alces alces* L.), reindeer (*Rangifer tarandus tarandus* L.), and red deer (*Cervus elaphus* L.), respectively, were studied by means of scanning electron microscopy. Morphological differences in the ribs of the genital bursa were demonstrated. The *Elaphostrongylus* species from reindeer and red deer differed from each other in four ribs of the genital bursa. These results agree with the morphological characters of *E.cervi* and *E.rangiferi* described by Cameron (1931) and Mitskevitch (1960). The genital bursa of *Elaphostrongylus* sp. from moose, in accordance with the description of *E.alces* by Steen et al. (1989) showed characteristics differing from those found in *Elaphostrongylus* spp. from reindeer and red deer respectively. These results support the hypothesis that there are three separate species of *Elaphostrongylus* present in Scandinavian Cervidae.

Key words: Morphology, scanning electron microscopy, taxonomy, *Elaphostrongylus alces, Elaphostrongylus rangiferi, Elaphostrongylus cervi.*

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Sammandrag: Rundmaskar inom släktet *Elaphostrongylus* funna hos älg (*Alces alces* L.), ren (*Rangifer tarandus tarandus* L.) och kronhjort (*Cervus elaphus* L.) studerades med hjälp av svepelelektronmikroskop. De hanliga bursorna med sina stödjeribbor uppvisade variationer i utseende, längd och placering mellan dessa rundmaskar. De arter av *Elaphostrongylus* funna hos ren och kronhjort skilde sig åt avseende fyra stödjeribbor på de hanliga bursorna. Dessa resultat stämmer väl överens med de karaktärer som tidigare är beskrivna av Cameron (1931) och av Mitskevich (1960). Den hanliga bursan hos arten *Elaphostrongylus* funnen hos älg, vilken tidigare är beskriven av Steen et al. (1989), visade upp ett utseende som skilde sig från bursorna hos de *Elaphostrongylus*-arter funna hos ren och kronhjort. Dessa resultat stöder hypotesen om tre skilda arter av *Elaphostrongylus* hos Skandinaviska hjortdjur.

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Introduction

The genus *Elaphostrongylus* was erected by Cameron (1931) for a protostrongylid species which he described and named *E.cervi.* Boev and Shul'ts (1950) proposed *Elaphostrongylus* to be the type genus of the subfamily *Elaphostrongylinae.* Four species of the genus *Elaphostrongylus* have been described on the basis of

light microscopy (LM): *E.cervi* Cameron, 1931, *E.panticola* Lyubimov, 1945, *E.rangiferi* Mitskevich, 1960, and *E.alces* Steen et al., 1989. The taxonomy is based mainly on the structure of the male bursa. However the differentiation of species has been questioned by several authors. Kutzer and Prosl (1975) considered *E.rangiferi* to be synonymous with *E.cervi.* Pryadko and Boev (1971) stated that *E.rangiferi* and *E.panticola* are subspecies of *E.cervi.* Kontrimavichus et al. (1976) expressed the opinion that the differences in morphology are due to adaptation to different host species.

The aim of this study was to compare the males of the genus *Elaphostrongylus* spp. found in moose (*Alces alces* L.), reindeer (*Rangifer tarandus tarandus* L.), and red deer (*Cervus elaphus* L.), respectively, by means of scanning electron microscopy (SEM) in order to elucidate the taxonomy of the genus.

Materials and methods

Nematodes were collected from Swedish moose found dead or shot during the hunting season in counties in which reindeer were or were not present. The sampling of nematodes from Swedish reindeer was performed on slaughtered animals from counties also inhabited by moose. Specimens from red deer were collected in Denmark from game animals, both free living and

Table 1. Characteristics of the genital bursae of *Elaphostrongylus* spp. from moose (*Alces alces* L.), reindeer (*Rangifer tarandus* L.) and red deer (*Cervus elaphus* L.)

	Elaphostrongylus sp. from moose.	*Elaphostrongylus* sp. from reindeer.	*Elaphostrongylus* sp. from red deer.
Dorsal rib	Five. The dorsal rib is divided into two dorso-dorsal, two medio-dorsal and one externo-dorsal rib.	One. The dorsal rib is divided into two buckles with two branches each.	One. The dorsal rib is split into two branches, with complementary branches.
Externo-dorsal rib	Two. One rib dx. ending at 8/10 (n=7) of the bursa. One rib sin., slightly longer.	Two. One rib dx. and one rib sin. with two branches, ending at 5/10 (n=12) of the bursa.	Two. One rib dx. and one rib sin., ending at 5/10 (n=5) of the bursa.
Lateral rib	Four. Two ribs dx. and two ribs sin. One rib sin. is short, while one rib sin. and the two ribs dx. are slightly longer than the externo-dorsal rib dx.	Four. Two ribs dx. and two ribs sin. well developed, ending close to the margin of the bursa.	Four. Two ribs dx. and two ribs sin. They are thin, ending at the margin of the bursa.
Externo-lateral rib	One. Rib dx. ending at 7/10 (n=8) of the brusa.	Two. One rib dx. and one rib sin. ending at 7/10 (n=12) of the bursa	Two. One rib dx. and one rib sin. ending at 7/10 (n=6) of the bursa.
Ventral rib	Four. Two ribs dx. and two ribs sin., ending close to the margin of the bursa.	Four. Two ribs dx. and two ribs sin., ending close to the margin of the bursa.	Four. Two ribs dx. and two ribs sin., ending close to the margin of the bursa.
Shape of the bursa.	Oval.	Circular.	Circular.

dx. = ad dextram
sin. = ad sinistram

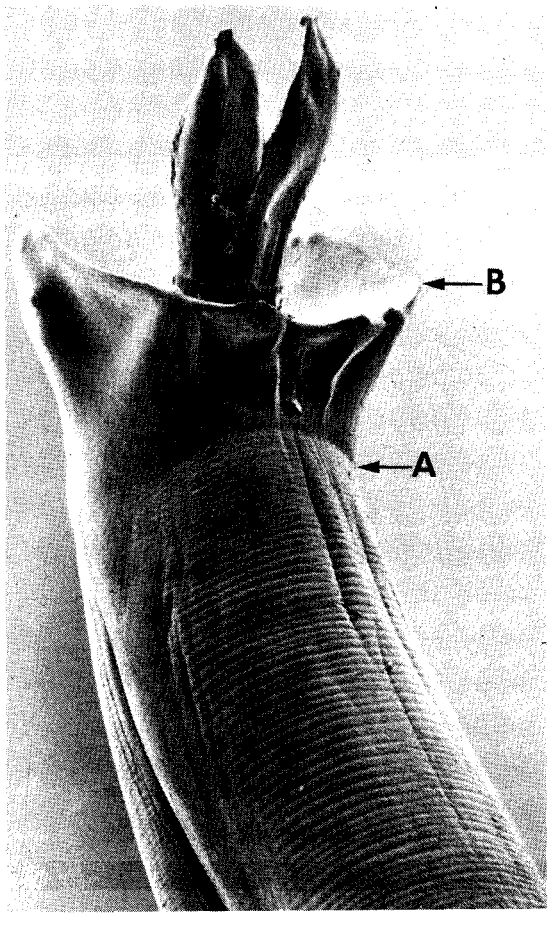


Fig. I: *Elaphostrongylus* sp. from reindeer. Male genital bursa. Ventral view. Base of the bursa (arrow A) margin of the bursa (arrow B).

farmed where neither moose nor reindeer excist.

Fourteen *Elaphostrongylus* males from each of moose and reindeer, and eight from red deer were examined. Seven males of *Elaphostrongylus* from moose originated from counties also inhabited by reindeer whereas the remaining seven did not.

The nematodes were immersion fixed for at least 24 h in a mixture of 3 % glutaraldehyde and 3 % paraformaldehyde in 0.1 M phosphate buffert (pH 7.4). The specimens were then postfixed in phosphate buffered 1 % OsO_4 at 4°C for 1 h, rinsed, dehydrated in an ethanol series ending in absolute ethanol. The ethanol was then replaced stepwise by Freon TF, which served as an intermediate fluid. The specimens were critical-point dried using liquid CO_2, mounted on stubs with double adhesive tape, and sputter coated with palladium. The specimens were examined and photographed in a JEOL JSM 820 at 5–15 kv.

The lengths of the ribs were measured in proportion to the distance from the base (Fig. I B) to the margin (Fig. I A) of the genital bursa (Fig. I).

Results

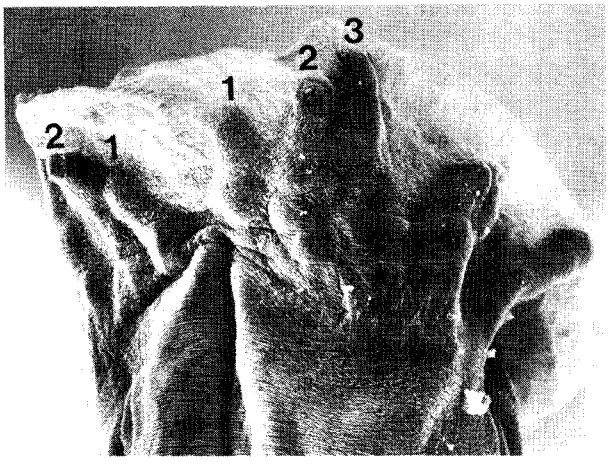
Common morphological traits

The bursas lack distinct divisions into lobes. The bursal ribs are well developed. The genital cones are pronounced.

Specific morphological traits

Elaphostrongylus sp. from moose (Fig. II–IV), (Table 1).

The dorsal rib is divided into two dorso-dorsal (Fig. II;1,1), two medio-dorsal (Fig. II;2,2), and one externo-dorsal rib *ad dextram* (Fig. II;3), all split to the base of the dorsal rib. The dorso-dorsal and the medio-dorsal ribs end as knobs on the inner and the outer surface (Fig. II;2,2) of the bursa. The externo-dorsal rib *ad dextram* ends at eight – tenths (Fig. II;3) (n=7) of the bursa on the outer surface as a pronounced knob (Fig. IV;3). The slightly longer externo-dorsal rib *ad sinistram* (Fig. III;3) is situated close to the lateral ribs (Fig. III;4,5), ending as a knob on the inner surface of the bursa. The two lateral ribs *ad dextram* (Fig. IV;4,5) are as long as the left externo-dorsal rib (Fig. III;3) and end as two knobs on the inner surface of the bursa. The posterio-lateral rib *ad sinistram* (Fig. III;4) was the same length as the two right lateral ribs and like them ends as a knob on the inner surface of the bursa (Fig. IV;arrow 4). The medio-lateral rib *ad sinistram* (Fig. III;5) is shorter than the posterio-lateral rib *ad sinistram* ending as a knob (Fig. III;5) on the outer surface of the bursa. The externo-lateral rib (Fig. IV;6) is visible only at the right lateral view, well separated from the two lateral ribs, ending at seven-tenths (n=8) of the bursa as a pronounced knob (Fig. IV;6) on its outer surface. The two ventral ribs (Fig. IV;7,8) end together close to the margin of the bursa as knobs (Fig. IV;7*,8*) on its inner surface, on both the right and left sides. The shape of the bursa is oval (Fig. IV,V). The genital cone has a papilla on the ventral side (Fig. V arrow A).



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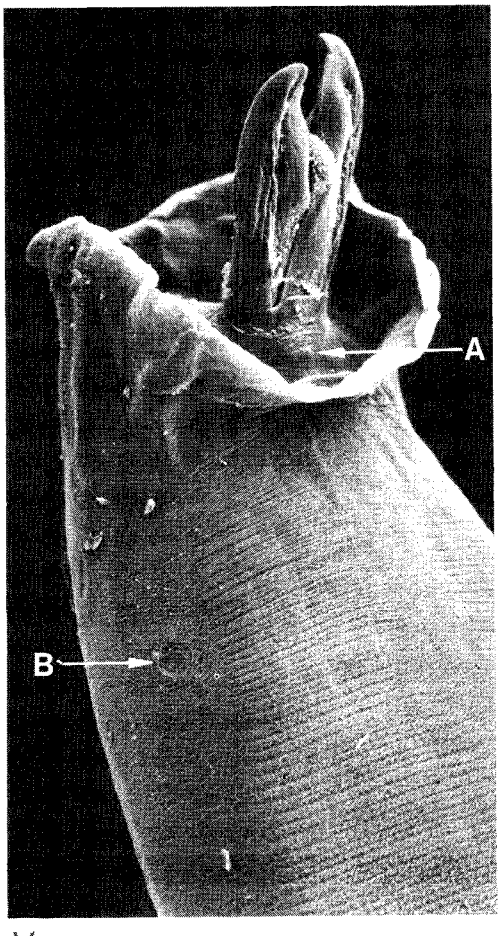
III



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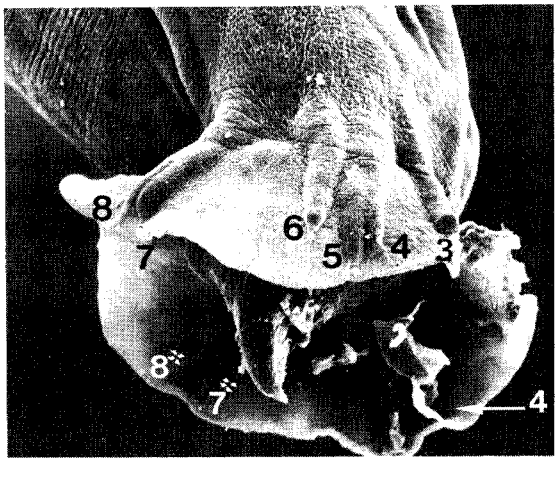


IV

Fig. II–V: *Elaphostrongylus* sp. from moose. Male genital bursa.

Fig. II: Dorsal view. Dorsal rib, split to the base, divided into two dorso-dorsal (1,1), two medio-dorsal (2,2), and one externo-dorsal (3) rib dx. The medio-dorsal ribs end as knobs (2,2) on the outer surface of the bursa.

Fig. III: Lateral view sin. The externo-dorsal rib sin. (3) is situated close to the posterio-lateral rib sin. (4) and the medio-lateral rib sin. (5). The medio-lateral rib sin. ends as a knob (5) on the outer surface of the bursa.

Fig. IV: Lateral view dx. The externo-lateral rib (6) is visible only at the right side ending as a pronounced knob (6) on the outer surface of the bursa, separated from the two lateral ribs dx. (4,5). The lateral ribs dx. and the posterio-lateral rib sin. (arrow 4) end as knobs on the inner surface (arrow 4) of the bursa. The two ventral ribs (7,8) end close together, as knobs (7*,8*) close to the inner margin. Note the oval shape of the bursa.

Fig. V: Ventro-lateral view. The genital cone has a papilla (arrow A) on the ventral side. Note the phasmid-like structure (arrow B), and the oval shape of the bursa.

Fig. VI–VIII: *Elaphostrongylus* sp. from reindeer. Male genital bursa.

A phasmidlike structure is seen in lateral view (Fig. V arrow B). *Elaphostrongylus* sp. in moose from different localities, irrespectively if reindeer was present, showed the same morphological structure of the bursa.

Elaphostrongylus sp. from reindeer (Fig. VI–VIII) (Table 1)

The dorsal rib has a broad base divided into two buckles (Fig. VI;1,1) with two branches each. One branch is short (Fig. VI arrows) ending on the inner surface of the bursa, the other is thinn (Fig. VI;2,2) and visible as an indentation on the inner surface of the bursa. The externo-dorsal ribs *ad sinistram* (Fig. VI;3, arrow a) *et ad dextram* are separated from both the dorsal rib and the lateral ribs (Fig. VI;4,5). The externo-dorsal ribs are split into two branches: one short (Fig. VI;3) ending at fifth-tenths (n=12) of the bursa with a pronounced knob (Fig. VII;3) on its outer surface: one thin, ending diffusely on the bursa (Fig. VI,VII;arrow a).

There are two lateral ribs on each side (Fig. VI, VIII; 4,4*,5,5*). These are well developed, longer than the other ribs, ending together as knobs (Fig. VIII;4*,5*) close to the inner margin of the bursa. The externo-lateral rib (Fig. VI,VII,VIII;6) and the lateral ribs on both sides seem to be comected at the base. The externo-lateral ribs are half the length of the lateral ribs ending at seven-tenths (n=12) of the bursa in pronounced knobs (Fig. VI,VII,VIII;6) on its outer surface. The two ventral ribs (Fig. VIII;7,8) on each side are longer than the externo-lateral ribs but shorter than the two lateral ribs. One of the ventral ribs ends as a knob (Fig. VIII;7) and the other as a pit (Fig. VIII;8) together on the inner surface, close to the margin of the bursa. The shape of the bursa is circular. The genital cone is well developed with a papilla located medially on the dorsal side (Fig. VIII arrow).

Elaphostrongylus sp. in reindeer from localities with or without moose showed similar morphological structures of the genital bursa, which differed markedly from those found in *Elaphostrongylus* sp. from moose.

Elaphostrongylus sp. from red deer (Fig. IX-XI) (Table 1)

The dorsal rib is split into two branches visible as indentations on the inner surface of the bursa. Each branch is split into complementary branches (Fig. IX;arrows), visible as convexities on the inner surface of the bursa.

The externo-dorsal ribs *ad dextram et ad sinistram* (Fig. IX;3,3) are separated from the dorsal rib and slightly shorter than the dorsal rib's complementary branches. Both end at the middle (n=5) of the bursa as pronounced knobs (Fig. IX;3,3) on its outer surface.

There are two lateral ribs on each side (Fig. X;4,5,5). These are thin, ending close together as knobs (Fig. X;4,5,5) at the inner margin of the bursa. The externo-lateral ribs *ad sinistram* (Fig. X;6) *et ad dextram* are shorter than the lateral ribs and end as pronounced knobs (Fig. X;6) at seven-tenths (n=6) on the outer surface of the bursa. The two ventral ribs on each side are (Fig. X;7,7;8,8) thinner and longer than the externo-lateral ribs and end close together on the inner surface near the margin of the bursa. One is shaped as a knob (Fig. X;7,7) and the other as a pit (Fig. X;8,8). The shape of the bursa is circular (Fig. X). The genital cone is well developed, supported by a pair of lateral bulges on the dorsal side (Fig. XI arrows).

Discussion

The *Elaphostrongylus* spp. investigated from three different Scandinavian Cervidae species reveal pronounced and constant differences in the shape of the genital bursa, the bursal ribs, branches, and the genital cone (Table 1).

The bursae of *Elaphostrongylus* spp. from reindeer and red deer are circular, and the dorsal ribs are seen as buckles, indentations and convexities, often split into branches.

Elaphostrongylus sp. from moose differs strongly from those of the other two *Elaphostrongylus* spp. The bursa and the ribs have characteristic shapes i.e the bursa is oval and has well developed long and slender ribs diverging from the base.

It is noticeable that the differences in the appearance of the bursae in *Elaphostrongylus* sp. in moose and reindeer are constant even if the hosts share the same habitat.

Kontrimavichus et al. (1976), however, have suggested that the differences between the species in the genus *Elaphostrongylus* are results of their parasitism in different hosts. They demand a cross-infection study of hosts by the different elaphostrongyles to be essential for cla-

Fig. VI:
Dorsal view. The dorsal rib is divided into two buckles (1,1) with two branches each. One branch is short (arrows), ending on the inner surface, the other (2,2) is thinner and visible as an indentation of the inner surface of the bursa.
The externo-dorsal ribs are split into two branches, one short (3) and one thin (arrow a). The externo-lateral ribs (6) are half the length of the lateral ribs (4,5).

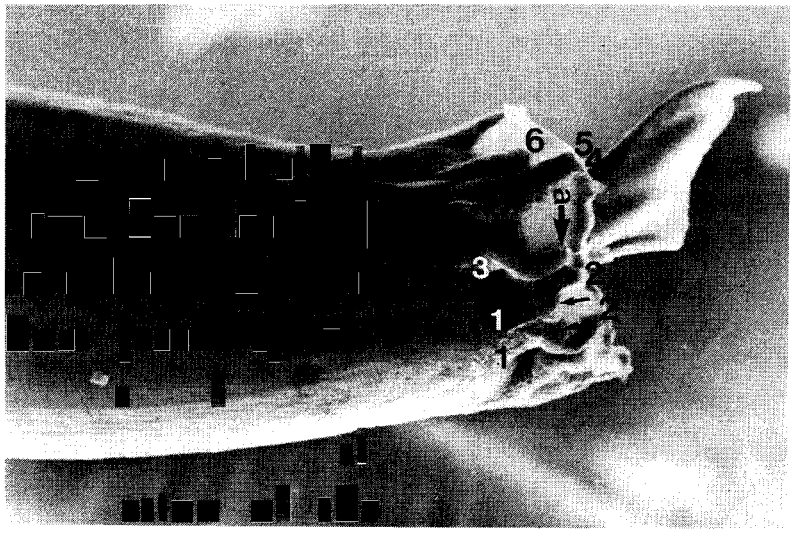


Fig. VII:
Lateral view sin. The short branch of the externo-dorsal rib ends a a pronounced knob (3) on the outer surface, while the thin branch (arrow a) ends diffusely of the bursa. The externo-lateral rib (6) ends in a knob (6) on the outer surface of the bursa.

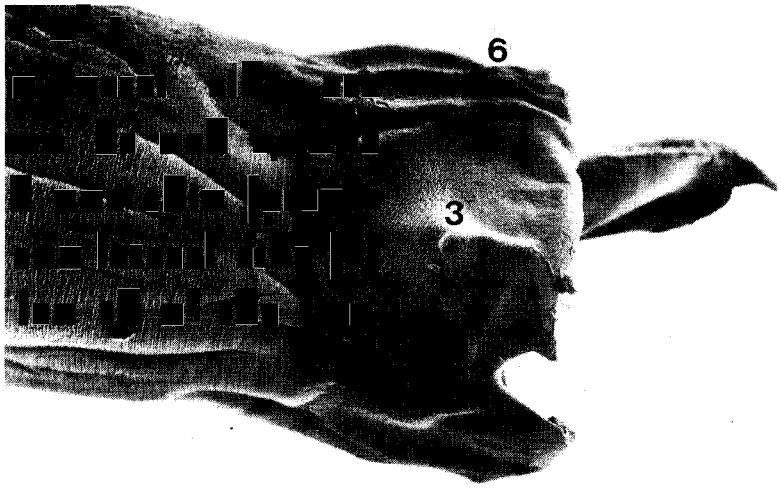


Fig. VIII:
Dorso-lateral view. The lateral ribs (4,5) end close to the inner margin of the bursa with two knobs (4*,5*). The ventral ribs (7,8) are shorter than the lateral one and end as a knob (7) and a pit (8), respectively. The genital cone has a papilla (arrow) located medially on the dorsal side.

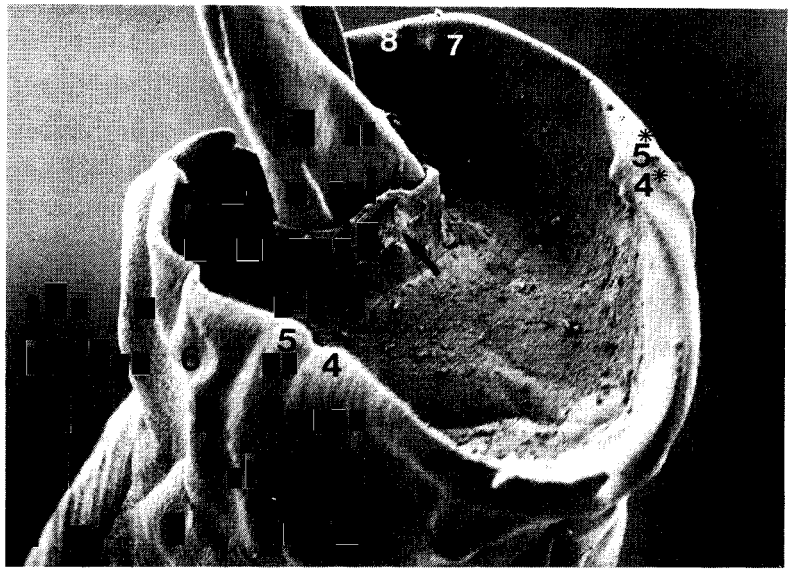


Fig. IX–XI:
Elaphostrongylus sp. from red deer. Male genital bursa.

Fig. IX:
Dorsal view. The dorsal rib is split into two branches. Each branch is split into complementary branches (arrows). The externo-dorsal ribs (3,3) are shorter than the complementary branches of the dorsal rib. They end in a pronounced knob (3,3) on the outer surface of the bursa.

Fig. X:
Caudal view. Ventral side (arrow). The lateral ribs (4;5,5) end as knobs (4;5,5) at the inner margin of the bursa. The externo-lateral ribs (6) are shorter than the lateral ribs (4;5,5) and end as knobs (6) on the outer surface of the bursa. The ventral ribs (7,7, 8,8) end close together at the inner margin of the bursa, one as a knob (7,7) and one as a pit (8,8). The shape of the bursa is circular.

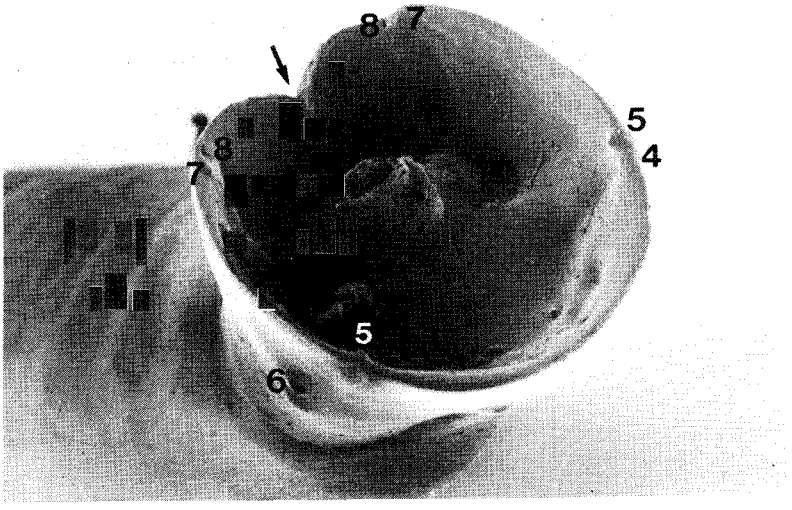
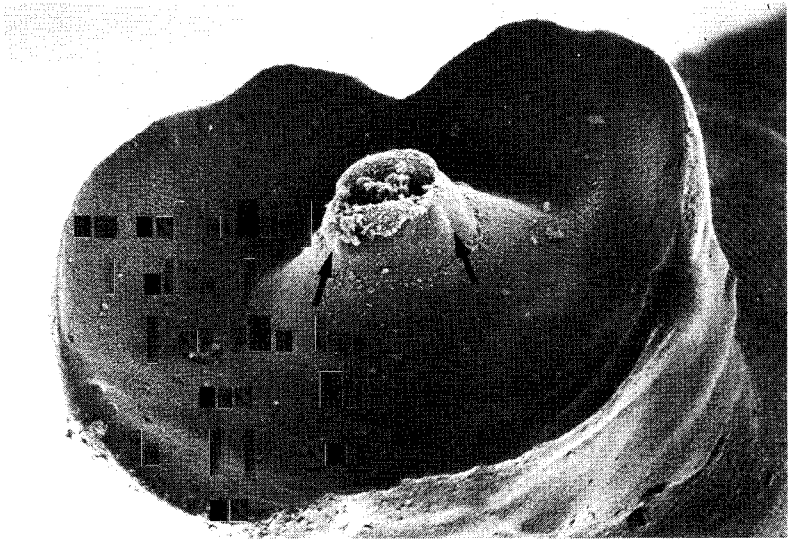


Fig. XI:
Caudal view. The genital cone has two pairs of lateral bulges on the dorsal side (arrows). Note the circular shape of the bursa.



rifying the differentatiation of the species of the genus.

In 1964, Enigk and Hildebrandt performed a cross-infection study with the genus *Dictyocaulus* in order to investigate the species of *D.filaria, D.viviparus* and *D.eckerti. D.filaria* turned out to be a specific species with other hosts than *D.viviparus. D.eckerti* was estimated to be synonymous of *D.viviparus.* Later *D.viviparus* has been considered to have different strains with different patogenicity for Cervidae and Bovidae (Corrigal et al 1988).

These circumstances should as well be considered when evaluating the taxonomy of the genus *Elaphostrongylus* .

However, the detailed morphology of the genital bursae as elucidated by SEM in *Elaphostrongylus* spp. from red deer, reindeer, and moose strengthens earlier descriptions based on light microscopy of *E.cervi* by Cameron (1931), *E.rangiferi* by Mitskevich (1960) and *E.alces* by Steen et al. (1989).

The results therefore support the current taxonomic designation of these parasites as being three distinct species.

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