Journal of Helminthology (2002) **76,** 119–124 DOI: 10.1079/JOH2001108

Dictyocaulus capreolus n. sp. (Nematoda: Trichostrongyloidea) from roe deer, *Capreolus capreolus* and moose, *Alces alces* in Sweden

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Abstract

Dictyocaulus capreolus n. sp. recovered from roe deer, *Capreolus capreolus* and moose, *Alces alces* in Sweden is described and figured. Morphological studies revealed the new species to be closest to *D. eckerti* and *D. africanus* on the basis of mouth shape, all three species having an elongate mouth opening. The other species of the genus, including *D. viviparus*, all have a circular to oval mouth opening. *Dictyocaulus capreolus* n. sp. can be distinguished from *D. eckerti* and *D. africanus* on the basis of the morphology of the buccal capsule and the bursa. These morphological studies support earlier evidence of the presence of a new species of *Dictyocaulus* in roe deer and moose that could be distinguished from *D. eckerti* and *D. viviparus* using either a PCR-linked hybridization assay or image analysis software to study the dimensions of the buccal capsule.

Introduction

Bovine parasitic bronchitis is caused by *Dictyocaulus viviparus* which is endemic in temperate regions of the world where cattle are kept on permanent pastures for seasonal grazing (Eysker, 1994). However, lungworms of the genus *Dictyocaulus* occur in both bovid and cervid hosts. According to Gibbons & Khalil (1988), the genus *Dictyocaulus* contains six species of which cattle are the primary host for *D. viviparus* and cervids for *D. eckerti*. Experimental cross infection is possible (Bierioschek *et al.*, 1996), and for many years it has been suggested that wild cervids may serve as important reservoirs of *D. viviparus* (Nilsson, 1971).

In Sweden moose (*Alces alces*) and roe deer (*Capreolus capreolus*) are abundant (approximately 400 000 and 1×10^6 respectively) and both are regularly infected with lungworms. There are also many opportunities for co-grazing in cattle raising areas. Consequently, these cervids have been considered as important reservoir hosts. Field isolates of lungworms from moose and roe deer were recently examined and compared with those from cattle. Sequence data from the second internal ribosomal spacer (ITS-2) indicated the presence of a novel *Dictyocaulus* species in roe deer and moose (Höglund *et al.*, 1999). In a more extensive survey, morphological examination was used in combination with a genetic approach to identify individual worms (Divina *et al.*, 2000). A PCR-linked hybridization assay to probe worm DNA with species-specific oligonucleotide probes was developed and the identity of 273 lungworms from cattle, moose and roe deer were determined. This assay demonstrated that Swedish cattle harboured monospecific *D. viviparus* infection. In contrast, infections composed entirely of the new *Dictyocaulus* were found in roe deer, whereas in the moose a mixed infection between the new *Dictyocaulus* and *D. eckerti* were recorded. The objective of the present study is to describe and illustrate the morphology of the new *Dictyocaulus* in roe deer and moose in Sweden.

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Materials and methods

Lungs from wild cervids were received from local hunters in south and central Sweden. Specimens collected during December 1997 to and including October 1998 from roe deer and moose in Sweden were straightened, the anterior and posterior ends cut with a scalpel and fixed in 10% formalin for morphological studies. The middle body region of the specimens was preserved by freezing −75°C for molecular studies (Divina *et al.*, 2000). Additional specimens were collected in October and November 1999 and preserved in 70% alcohol. Eight males and nine females from roe deer and eight males and six females from moose were examined as temporary mounts in lactophenol or lactoglycerol. *En face* view and cross sections of the body were cut with the aid of a razor blade fragment fixed in a needle-holder and mounted in glycerine jelly. Specimens were also prepared for scanning electron microscopy (SEM) using 3% glutaraldehyde as fixative and OsO_4 postfixative. They were then dehydrated in increasing concentrations of ethanol and critical point dried using liquid CO_2 as the transitional fluid. Dried specimens were mounted on stubs, coated with Ag/Pa and examined in a JEOL JSM-820 electron SEM microscope.

Dictyocaulus capreolus n. sp.

(figs 1–17)

Description. Oral opening elongated oval, dorso-ventrally flattened. Single ring of six cephalic papillae observed, two lateral amphids. Buccal capsule oval, flattened dorso-ventrally, wall thick, kidney-shaped in optical section, poorly sclerotized tooth present. Cuticle with numerous longitudinal cuticular ridges of arête type (ridge type as defined by Durette-Desset, 1971), low in height, perpendicular to body surface.

Male. Body 24–62 mm long; 0.136–0.312 mm just anterior to bursa. Head 0.060–0.100 mm wide. Cephalic vesicle generally present 0.104–0.280 mm wide, length variable. Oesophagus 0.832–1.464 mm long. Buccal capsule 0.032–0.056 mm wide, 0.008–0.016 mm long, wall 0.007–0.012 mm wide. Buccal capsule wall measured by image analysis software (Divina *et al.*, 2000) 0.0089–0.0238 mm long, 0.0053–0.0126 mm wide. Anterior to nerve ring, excretory pore 0.276–0.396 mm, 0.352–0.600 mm respectively. Cervical papillae not observed. Spicules 0.224–0.280 mm long, porous texture, two sclerotized alae, one extends from proximal third ending in branch, second ala starts in distal half ending near tip, small transparent membrane on outer margin of spicule present, single slender branch clearly separate from broad main stem. Gubernaculum present, 0.022–0.072 mm long, porous texture, irregularly oval in dorso-ventral view, uneven in width, variable in shape in lateral view. Bursa bell-shaped, lobes not separated, heart-shaped in dorso-ventral view. Ventrals with short common stem, parallel, antero-ventral (ray 2) markedly shorter than postero-ventral ray (ray 3), does not reach bursal margin, postero-ventral almost reaches bursal margin; antero-lateral ray (ray 4) short, does not reach bursal margin; medio- and postero-lateral rays (rays 5, 6) completely fused, separate from antero-lateral, reach bursal margin, longer in lateral and dorso-ventral view than externo-dorsal ray (ray 8); externo-dorsal ray separate from dorsal ray, shorter than dorsal ray; dorsal ray (ray 9, 10) divided to base, each branch with three small divisions at distal tip. Genital cone simple, a pair of short dorsal raylets (papillae 7), single median ventral papilla (papilla 0), associated membranes absent.

Female. Body 34–81 mm long; 0.340–0.592 mm wide in vulvar region. Head 0.072–0.176 mm wide. Cephalic vesicle generally present, 0.116–0.320 mm wide, length variable. Oesophagus 0.880–1.616 mm long. Buccal capsule 0.032–0.068 mm wide, 0.008–0.020 mm long, wall 0.006–0.014 mm wide. Buccal capsule wall measured by image analysis software (Divina *et al.*, 2000) 0.0091–0.0234 mm long, 0.0049–0.0149 mm wide. Anterior to nerve ring, excretory pore 0.296–0.400 mm, 0.200–0.576 mm respectively. Cervical papillae not observed. Vulva opens 12.92–31.56 mm from tail tip, lips slightly swollen. Reproductive system didelphic, amphidelphic. Combined length of vestibules 0.992–1.872 mm. Anterior sphincter 0.076–0.100 mm long; posterior sphincter 0.068–0.088 mm long. Anterior infundibulum 0.032–0.064 mm long; posterior infundibulum 0.040–0.056 mm long. Immature eggs in uterus 0.088–0.104 mm × 0.056–0.064 mm; mature embryonated eggs 0.076–0.092 mm × 0.040–0.056 mm. Tail 0.336–0.632 mm long; phasmids 0.120–0.228 mm anterior to tail tip.

Type host. *Capreolus capreolus*, roe deer.

Other hosts. *Alces alces*, moose.

Type locality. Fjälkinge, Skåne, Sweden.

Additional localities. Roe deer (Bro, Vallentuna, Täby and Östuna in Uppland; Haninge, Sörmland; Svedala, Skåne). Moose (Glanshammar, Närke; Öregrund and Alunda in Uppland; Mörkö, Stockholm).

Deposition of type specimens. The Natural History Museum, London, UK. Holotype number 2001.5.16.1; paratypes from *Capreolus capreolus* numbers 2001.5.16.2–7, from *Alces alces* numbers 2001.5.16.8–11.

Discussion

Railliet & Henry (1907) established the genus *Dictyocaulus* for nematodes recovered from the bronchi and bronchioles of artiodactylids. Skrjabin *et al.* (1954) reviewed the history and taxonomy of the genus. Gibbons & Khalil (1988) revised the genus and following the same classification the specimens described are assigned to the genus *Dictyocaulus* in the superfamily Trichostrongyloidea on the basis of the morphology of the cephalic region, female reproductive system, spicules and bursa. Gibbons & Khalil (1988) accepted the validity of six species, namely, *D. filaria* (Rudolphi, 1809) Railliet & Henry, 1907,

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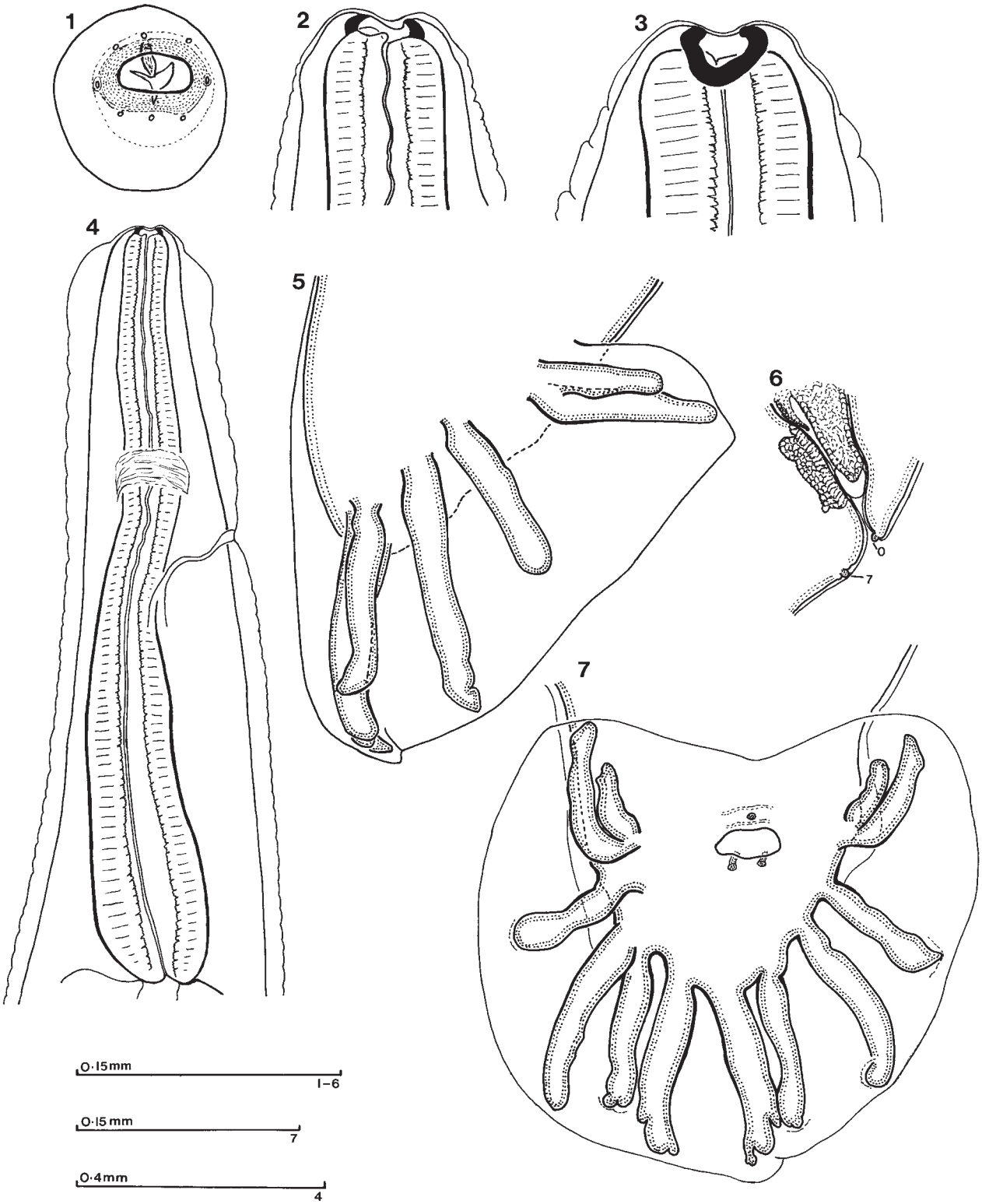

Figs 1–7. *Dictyocaulus capreolus* n. sp. light microscopy. 1, Female, *en face* view; 2, female, anterior end in optical section, lateral view; 3, female, anterior end, lateral view; 4, male, anterior end, right lateral view; 5, bursa, right lateral view; 6, genital cone with papilla 0 and right papilla 7, right lateral view; 7, bursa, ventral view.

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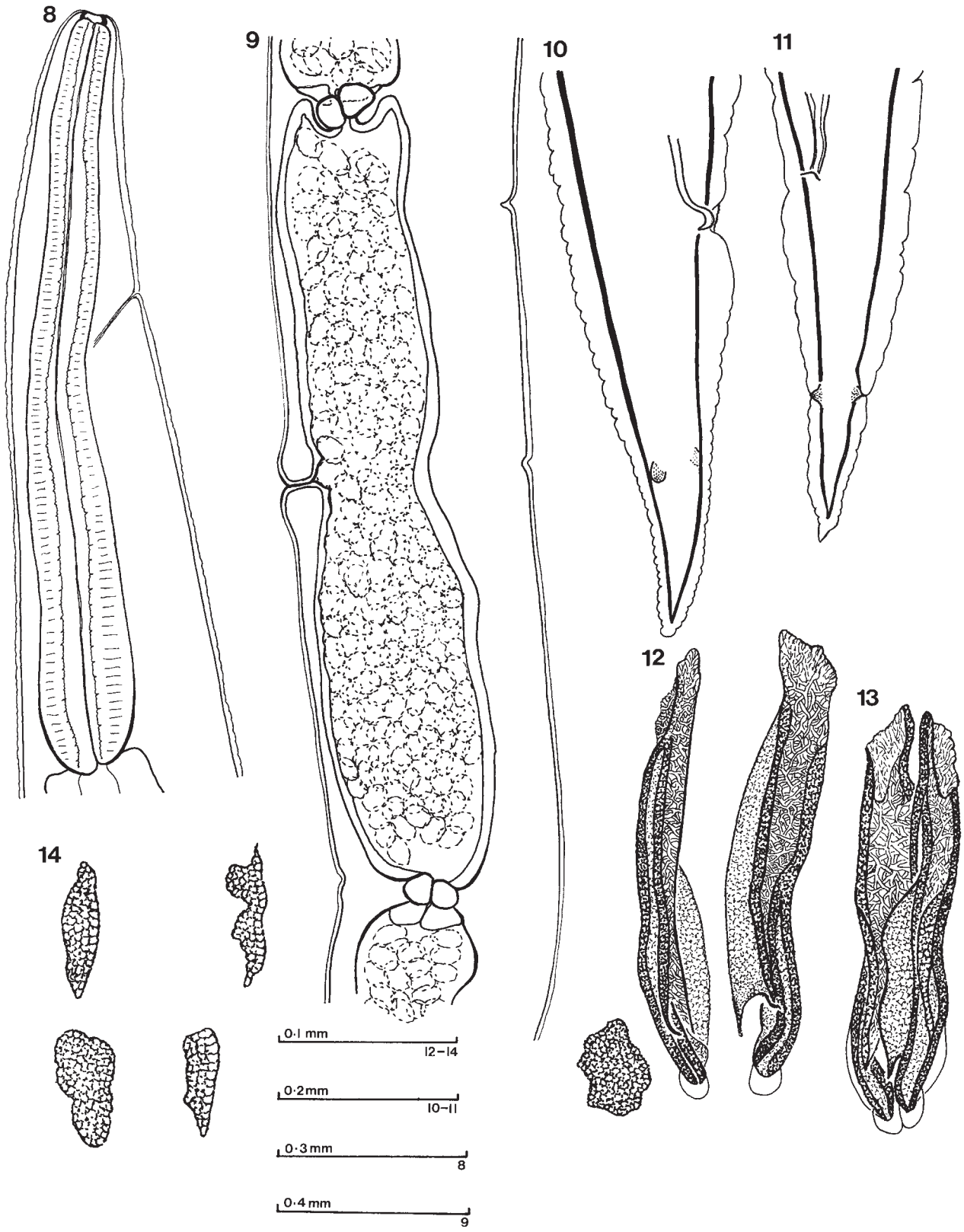

Figs 8–14. *Dictyocaulus capreolus* n. sp. light microscopy. 8, Female, anterior end right lateral view; 9, vulvar region, left lateral view; 10, female tail, right lateral view; 11, female tail, latero-ventral view; 12, spicules and gubernaculum dorso-ventral view; 13, spicules *in situ*; 14, gubernaculum from four specimens showing variation in shape in right lateral view.

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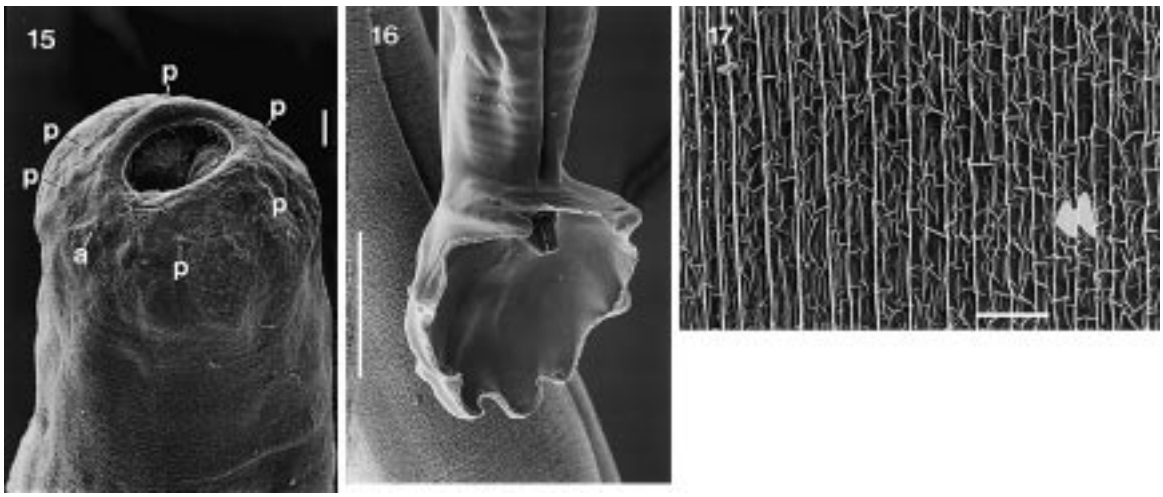

Figs 15–17. *Dictyocaulus capreolus* n. sp. scanning electron microscopy. 15, Cephalic region showing elongated oval mouth opening, cephalic sensory papillae (p) and amphid (a), scale bar = 10 μm; 16, bursa, ventral view, scale bar = 100 μm; 17, cuticle showing numerous longitudinal cuticular ridges, arrowed, scale bar = 10 μm.

D. africanus Gibbons & Khalil, 1988, *D. arnfieldi* (Cobbold, 1884) Railliet & Henry, 1907, *D. cameli* Boev, 1951, *D. eckerti* Skrjabin, 1931 and *D. viviparus* (Bloch, 1782) Railliet & Henry, 1907. Durette-Desset *et al.* (1988) reinstated and redescribed *D. noerneri* Railliet & Henry, 1907 from *C. capreolus* in France and proposed *D. eckerti* as a synonym if they should be considered morphologically identical or *D. noerneri* to be maintained for lungworm from European cervids if they are morphologically different. Skrjabin *et al.* (1954) have previously regarded *D. noerneri* as a *species inquirenda* on the basis of the absence of a full description. Jansen & Borgsteede (1990) again proposed *D. noerneri* as a *species inquirenda* as they considered the species is not based on sufficient information to confirm its identity. They therefore confirm that the large lungworm from cervids is *D. eckerti*. Railliet & Henry (1907) erected *Dictyocaulus* for four species from the bronchi of herbivores including '*D. noerneri* n. sp. from roe deer' and listed it as '*D. noerneri*: spicules of 281μm (after Nörner)'. No description or location of type specimens was given. *Dictyocaulus noerneri* is therefore considered *incertae sedis*. The morphological differences between the species of *Dictyocaulus* are small and Durette-Desset *et al.* (1988) and Jansen & Borgsteede (1990) demonstrated in their studies how the thickness of the buccal capsule could be used to differentiate species. Divina *et al.* (2000) reviewed the work of other authors who have used the characters of the buccal capsule and applied it to their study of large lungworms from cattle, moose and roe deer. Divina *et al.* (2000) showed that the buccal capsule wall width and shape together with molecular studies (ITS-2) support the differences of the specimens described from *D. viviparus* and *D. eckerti* and suggest they belong to a new species. Morphological studies revealed an elongate mouth opening similar to *D. eckerti* and *D. africanus*. All the other species of the genus, including *D. viviparus*, have a circular or oval mouth opening. The specimens described also differ from *D. eckerti* in the length of the buccal capsule (0.012–0.014 mm long for *D. eckerti* (= *D. noerneri*) from *C. capreolus* in France as reported by Durette-Desset *et al.* (1988) compared to a mean length of 0.0172 mm in males and 0.0188 mm in females for the specimens described from *C. capreolus* in Sweden as reported by Divina *et al.* (2000) with a range of 0.008–0.0238 mm in males and 0.008–0.0234 mm in females as reported by Divina *et al.* (2000) and the present study), the shape of the bursa (oval in *D. eckerti*, heart-shaped in the specimens described) and in a PCR-linked hybridization assay (*Dictyocaulus* sp. probe OP110 hybridized with all the worm ITS-2 samples from roe deer and 78.2% in moose but did not hybridize with the probe for *D. eckerti* as reported in Divina *et al.* (2000)). The specimens described differ from *D. africanus* in the antero-ventral ray or ray 2 being markedly shorter than the postero-ventral ray or ray 3 (ventral rays equal or slightly subequal in *D. africanus*) and the fused medio-/postero-lateral ray (rays 5 and 6) being slightly longer than the externo-dorsal ray or ray 8 (shorter or equal in *D. africanus*). Morphological and molecular studies on the specimens from *C. capreolus* and *A. alces* in Sweden combine to differentiate them from the nearest described species, namely, *D. eckerti* and *D. africanus*. The specimens are considered new and named *Dictyocaulus capreolus* after the type host. Specimens from *C. capreolus* in other geographical locations require further study using molecular and morphological techniques to establish their specific identity.

Acknowledgements

Professor D.E. Jacobs, The Royal Veterinary College, University of London and Professor A. Uggla, head of SWEPAR for constructive criticism of the draft manuscript. Mrs Elisabeth Wilhelmsson, SWEPAR for technical assistance. Dr T. Mörner, National Veterinary Institute, Uppsala and all Swedish hunters that provided wild cervids.

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(Accepted 21 August 2001)