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Molecular identification of *Taenia* spp. in wolves (*Canis lupus*), brown bears (*Ursus arctos*) and cervids from North Europe and Alaska

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ABSTRACT

Taenia tapeworms of Finnish and Swedish wolves (*Canis lupus*) and Finnish brown bears (*Ursus arctos*), and muscle cysticerci of Svalbard reindeer (*Rangifer tarandus platyrhynchus*), Alaskan Grant's caribou (*Rangifer tarandus granti*) and Alaskan moose (*Alces americanus*) were identified on the basis of the nucleotide sequence of a 396 bp region of the mitochondrial cytochrome *c* oxidase subunit 1 gene. Two species were found from wolves: *Taenia hydatigena* and *Taenia krabbei*. The cysticerci of reindeer, caribou and one moose also represented *T. krabbei*. Most of the cysticercal specimens from Alaskan moose, however, belonged to an unknown *T. krabbei*-like species, which had been reported previously from Eurasian elks (*Alces alces*) from Finland. Strobilate stages from two bears belonged to this species as well. The present results suggest that this novel *Taenia* sp. has a Holarctic distribution and uses *Alces* spp. as intermediate and ursids as final hosts.

1. Introduction

Cestodes of the genus *Taenia* (Eucestoda: Cyclophyllidea: Taeniidae) are parasites of terrestrial mammals, characteristically occurring as adult tapeworms in carnivorous definitive hosts, and developing as cystic metacestode stages, called cysticerci or coenuri, in their prey. In the Holarctic, cervids serve as principal intermediate hosts for several species of *Taenia*[1–3]. In general, small cysticerci in the cardiac and skeletal muscles in cervids are supposed to be caused by a single Holarctic species, *Taenia krabbei*, which typically uses canids as definitive hosts (see e.g. [2]).

Recently, muscle cysticerci from Eurasian elks (*Alces alces*)[1] in Finland were characterized using morphological criteria and mitochondrial DNA sequences [4]. The host, location, size and morphology of the cysticerci indicated that they might belong to *T. krabbei*. In the molecular phylogenetic analysis, however, the specimens were placed distant from the previously characterized *T. krabbei* isolates from Svalbard (Norway) [5] suggesting the presence of two distinct species. Because of insufficient materials for morphological comparison, it was not possible to solve whether the Finnish or Svalbard species should hold the original name, and which one of them should be regarded as a new species. The Finnish species was represented only by cysticerci from Eurasian elks, and the specimens from Svalbard were adult stages from Arctic foxes (*Vulpes lagopus*). The latter were not, however, in adequate condition to allow detailed morphological examination [4].

On the barren Svalbard archipelago, the Arctic fox serves as the sole definitive host of the taeniid species identified as *T. krabbei*, and the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) acts as the intermediate host [6]. The aim of this study was to find out the definitive host of the Finnish *T. krabbei*-like species by identifying *Taenia* tapeworms of the wolf (*Canis lupus*) and the brown bear (*Ursus arctos*) since they are obviously the only carnivores preying on the Eurasian elk in Fennoscandia. In addition, the role of the semi-domesticated reindeer (*Rangifer tarandus tarandus*) as an intermediate host was examined. Furthermore, muscle cysticerci of the Svalbard reindeer were identified molecularly to confirm the life cycle of the *T. krabbei*-like species on Svalbard, and the geographical distribution of these two *Taenia* species was further investigated by identifying muscle cysticerci of cervids from Alaska (USA).

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[1] Also known as moose in English. The nomenclature used for mammals in this article follows mainly Wilson DE, Reeder DM, editors. Mammal species of the world. A taxonomic and geographic reference. 3rd ed. Baltimore: Johns Hopkins University Press; 2005. Available from: http://www.bucknell.edu/msw3/.

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2. Materials and methods

Parasite specimens analyzed in this study were acquired mostly by post mortem examinations of animals killed by legal hunting. Hunting was independent from the present work; no animals were killed on the authors' request. The specimens were collected from several areas in North Europe and North America. For details of sampling locations, see Fig. 1.

2.1. Tapeworms from bears

Intestines of 53 brown bears were collected in 1996–2002 and stored frozen at the Finnish Game and Fisheries Research Institute. The bears were from eastern Finland, about half of them were from the North Karelia region (Fig. 1, area 9). The intestines were later examined to find out the diet of the bears [7]. In the course of that study the intestinal helminths were collected and preserved in ethanol at +4 °C.

Another set of bears, 51 animals, was studied for intestinal helminths in 2010. The bears had been shot in August–October; 47 in North Karelia, three in the northeastern Northern Ostrobothnia region (or Koillismaa; Fig. 1, area 7) and one in the Kainuu region (area 8). The animals were legally killed adults or yearlings (two individuals) with the exception of one accidentally shot cub. There were equal numbers of females and males; the gender of one bear was not documented. The body weights of the adults varied from 53 to 280 kg. The intestines of 41 animals were dissected fresh on the field during the first 4 days of the bear-hunting season, whereas the rest of the samples (10) were frozen and dissected later. The tapeworms were preserved in 70% ethanol at −20 °C.

The species of all the *Taenia* tapeworms from both bear sample sets were identified by molecular methods (see Section 2.5. below).

2.2. Tapeworms from wolves

Seventy-five wolves, which died in Finland between November 2007 and December 2009, were routinely studied for intestinal helminths during the post mortem examinations at the Finnish Game and Fisheries Research Institute or the Finnish Food Safety Authority Evira. About 80% of the wolves were killed by legal hunting, and the rest were killed in traffic accidents, shot illegally or died because of other causes. The wolves were mainly from eastern Finland; only six were from the western or southwestern part of the country. Weights of the wolves varied from 5.5 to 52 kg; 49 were males and 26 females. The whole carcasses or intestinal tracts were stored frozen until dissected. Tapeworms were collected and preserved in ethanol at +4 °C or −20 °C. They were identified to the generic level based on the macroscopic appearance. All *Taenia* tapeworms of 26 randomly selected *Taenia* positive wolves were counted, and the species of each worm specimen was determined molecularly. The selected wolves were from the Kainuu or Koillismaa regions except for one animal that was from the Southern Ostrobothnia region (Fig. 1, area 10). The fragmented tapeworm individuals were differentiated from each other either based on the presence of scoleces or, if scoleces were not found, by identifying the anterior parts of distinct strobilae.

In addition, 28 wolves, shot in Sweden during licensed hunting in January 2010, were examined for intestinal helminths in necropsies at the National Veterinary Institute of Sweden. The wolves were from the central, western and southwestern parts of the country. To protect the laboratory staff against the threat of echinococcosis, the intestines were frozen and stored at −80 °C before examination. *Taenia* tapeworms were preserved in 70% ethanol at −20 °C. As the Finnish material, they were identified to the generic level based on the macroscopic appearance. In the course of another parasitological study [8], some of these *Taenia* specimens were further identified by the hook dimensions and using a multiplex PCR assay. In the present study, 49 *Taenia* tapeworms from nine Swedish wolves were identified with molecular methods. These wolves were from the counties of Västra Götaland, Värmland and Dalarna (Fig. 1, areas 2, 3 and 4, respectively).

2.3. Meat inspection of semi-domesticated reindeer

In the autumn 2010, 11,457 reindeer from all over the Finnish Lapland region (Fig. 1, area 6), 1501 from the Koillismaa region and 806

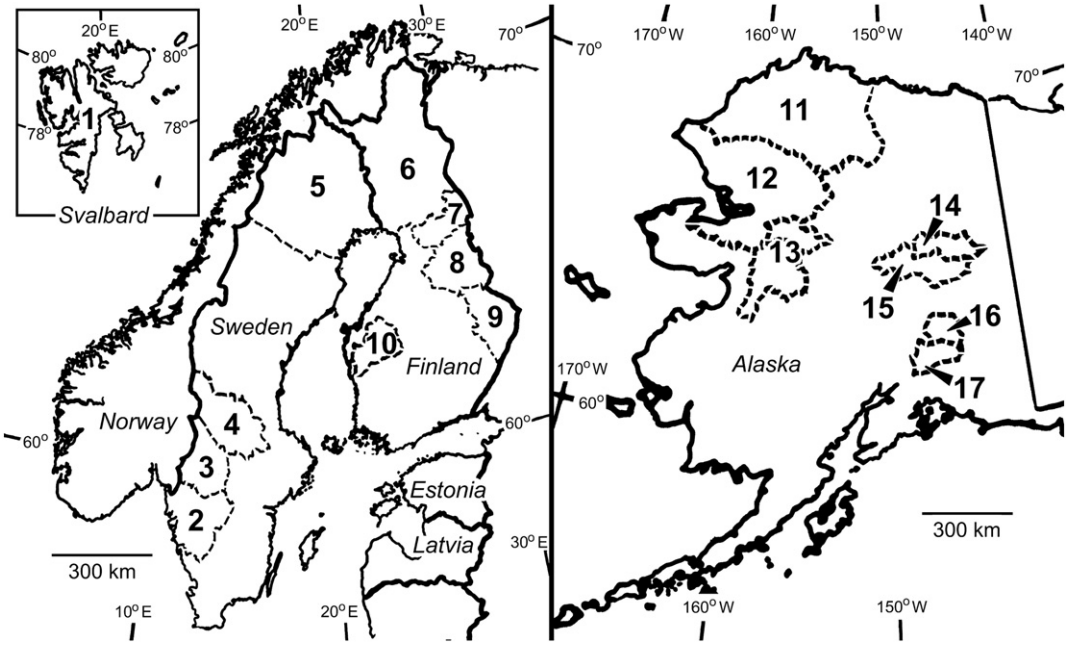

Fig. 1. Sampling locations of *Taenia* specimens of this study: 1, Spitsbergen Island; 2, Västra Götaland; 3, Värmland; 4, Dalarna; 7, Koillismaa; 8, Kainuu; 9, North Karelia; 10, Southern Ostrobothnia; 11, Northwest Alaska; 12, Northwest Arctic coast; 13, Koyukuk River; 14, White Mountains; 15, Minto Flats and Tanana River valley; 16, Gulkana River; 17, Matanuska River valley. Carcasses of semidomesticated reindeer studied for muscle cysticerci originated from Koillismaa (7), Finnish Lapland (6) and Norrbotten (5). Dash lines indicate boundaries of Swedish counties, Finnish regions and Alaskan game management units or subunits.