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## Brief Report

# Warble infestations by *Hypoderma tarandi* (Diptera; Oestridae) recorded for the first time in West Greenland muskoxen <sup>☆</sup>



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## ABSTRACT

In the northern hemisphere, Caribou (*Rangifer* spp.) populations are known to be infested with the skin-penetrating ectoparasite, *Hypoderma tarandi* (Diptera; Oestridae). Although regarded as host specific, *H. tarandi* has been reported from other species, and has become of increasing concern as a zoonosis infecting humans. In February 2012, concurrent with the hunting of muskoxen, we examined carcasses for muscle and tissue parasites, and recorded warble larvae infestations. DNA extracted from samples of larvae was amplified targeting 579 bp of the COI gene, and subsequently sequenced, to be confirmed as *H. tarandi*. Infestation by oestrid flies has not previously been reported in muskoxen in West Greenland.

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## 1. Introduction

Only two ungulate species occur in West Greenland, the indigenous caribou (*Rangifer tarandus groenlandicus*) and muskoxen (*Ovibos moschatus*). The latter were introduced in the mid 1960s and have expanded from 27 animals to an estimated population of 24,000 individuals (Cuyler et al., 2009). As elsewhere in the northern hemisphere, the caribou population of West Greenland is known to be infested with the skin-penetrating ectoparasite *Hypoderma tarandi* (Diptera; Oestridae). For example, prevalences of up to 100% have been reported in the Kangerlussuaq-Sisimut population (Cuyler et al., 2010). Although caribou (*Rangifer* spp.) are primary hosts for *H. tarandi* (Kutz et al., 2012), first-instar larval infestations have been reported from other species: muskoxen in Northwest Territories, Canada (Gunn et al., 1991), and red deer (*Cervus elaphus*) in western Norway (Nilssen and Gjersteg, 1988), and have become of increasing concern as a zoonosis infecting humans (Faber and Hendriks, 2006; Lagace-Wiens et al., 2008).

In this paper we describe a post-mortem survey of muskoxen in Greenland in which *H. tarandi* was found.

## 2. Material and methods

In February 2012, a total of 424 muskoxen were examined post-mortem for muscle and tissue parasites at the Kangerlussuaq muskox abattoir. All muskoxen were shot in an area situated south of Kangerlussuaq (66°15'N–67°N) (Fig. 1) where hunting of muskoxen occurs twice yearly: January–March and August–October.

During slaughter, the hides were removed quickly without the possibility of inspection of the subcutaneous tissues, but the carcasses were subsequently examined for warble lesions. The warble larvae recovered were stored in 70% ethanol and sent to the University of Copenhagen. After morphological examination, DNA extraction was performed using a commercial kit (Epicentre Biotechnologies Complete DNA and RNA Purification Kit) following the Total Nucleic Acids Purification Protocols for tissue samples.

A part of the mitochondrial gene encoding for subunit I of cytochrome oxidase (COI) was amplified by PCR using the primers UEA7 5'-TAC AGT TGG AAT AGA CGT TGA TAC-3' and UEA10 5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3' (Simons et al., 1994). This region has been shown to contain sufficient variability among *Hypoderma* spp. for species identification (Otranto et al., 2003). After enzymatic purification, the PCR products were sequenced in both directions by MacroGen (Seoul, Korea).

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The genetic relationship between the unknown *Hypoderma* species were compared to *H. bovis* (AF497761), *H. lineatum* (AF497762), *H. diana* (AF497763), *H. tarandi* (AF497764), *H. actaeon* (AF497765), and *H. sinense* (AY350769) using *Dermatobia hominis* (AY463155) as an out group. Sequences were analyzed by Neighbour-Joining (NJ) and Minimum Evolution (ME) tree methods using Kimura-2-parameter distance and by Maximum Parsimony where the tree was obtained using the Close-Neighbor-Interchange algorithm. The robustness of the topology was assessed with 1000 bootstraps. The analysis was done using MEGA version 5 (Tamura et al., 2011).

### 3. Results and discussion

Of the total of 424 muskoxen examined, warble larvae were found on carcasses of 16 animals (3.8% of the examined individuals). Since hides were not examined, larvae could have been overlooked, and the prevalence and intensity of the infestation were, therefore, probably higher. Seven of the animals found infected were calves, each with 5–15 larvae, 5 were adult males, each with 5–10 larvae,

and 4 were adult females (one lactating) with 1–2 larvae each. Eight larvae were examined morphologically. Three larvae were 5 mm in length and white in color, 4 larvae were 10 mm in length and creamy white in color, and 1 larva were 15 mm in length and light brown in color. Based on the descriptions in Colwell et al. (2006), the larvae recovered were early to late second instars.

Sequence analysis identified three haplotypes among the 5 *Hypoderma* spp. larvae included and these showed 98–99% identity to *H. tarandi* (AF497764) whereas the identity to *H. lineatum* (AF497762) was 86–87% (BLAST search, Genbank). Irrespective of the tree building method, identical tree topology was obtained and the five *Hypoderma* spp. from muskoxen clustered together with *H. tarandi* (Fig. 2). The p-distances within this group were between 0% and 1.7%. While the variation in the COI gene may be up to 6% for members of the same species, p-distances of more than 10% normally implies different species (Blouin, 2002). Based on the cluster analysis and the p-distances, we therefore suggest that the warbles obtained from the muskox in Greenland are *H. tarandi*.

Infestation by oestrid flies has not previously been reported in muskoxen in West Greenland. *H. tarandi* is normally regarded as host specific (Kutz et al., 2012) and infestations found in muskoxen seem to be the result of a cross-contamination from caribou, in which infestations are highly prevalent. Cross transmission studies on *H. lineatum* indicate the possibility of multiple-species infections but with limited development beyond first-instar larvae (Colwell and Otranto, 2006). This study shows development beyond this larval stage in muskoxen. Whether development to subsequent larval instars or into adult flies is possible regarding *H. tarandi* with muskoxen as hosts is not known. There are no records of complete development of *H. tarandi* in humans. However, the reported cases of ophthalmomyiasis caused by *H. tarandi*, which often lead to loss of sight, are of concern. Such cases are uncommon, but have been reported from the Canadian Arctic, Scandinavia and the Netherlands (Faber and Hendriks, 2006; Lagace-Wiens et al., 2008; Kan et al., 2013). These publications have led to an increased awareness of *H. tarandi* as a zoonosis, and any increase in the number of cases is thought to be due to this increased awareness rather than a true increase in incidence (Kan et al., 2013). However, an increasing human population in the northern latitudes has impact on the epizootiology in the Arctic. Resource exploration, encroachment of domestic animals on wildlife habitats, and movement and relocation of wildlife alters the landscape and prerequisites for parasitic infections (Kutz et al., 2004).



Fig. 1. The location of the muskox population studied in Kangerlussuaq, Greenland (circle).

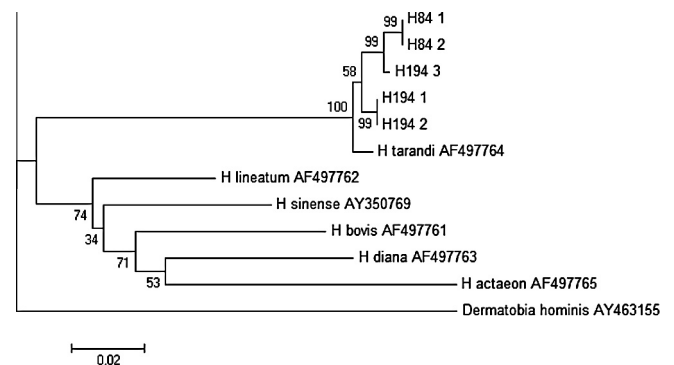


Fig. 2. Neighbour-Joining dendrogram showing the genetic relationship between *Hypoderma* spp. based on 579 bp of the Cox-1 gene using Kimura-2-parameter distance with *Dermatobia hominis* included as an out group. Warbles H84 and H194 are obtained from two muskoxen on Greenland whereas the other sequences were obtained from GenBank with accession number given after each species. Bootstrap values are indicated at the nodes (1000 replicates). Scale bar: number of base substitutions per site.

In comparison with other parts of the Arctic, the density of the muskox population in West Greenland is high, with an observed summer density of 1.3 animals per km<sup>2</sup> in the Kangerlussuaq region (Cuyler et al., 2009). As parasite abundance typically increases with host density, the warble infestations described here, and the recent discovery of *Taenia ovis krabbei* (Raundrup et al., 2012) in muskoxen indicate that there may be an overall increase of parasitic infections in the West Greenland ungulates.

The arctic climate forces organisms to evolve capabilities to withstand extreme weather conditions, high seasonality, as well as long periods without sunlight or with midnight sun. In arctic regions low biological diversity is evident, and are thereby highly vulnerable to invasions, and even minor environmental perturbations could alter the life history patterns of already established pathogens (Kutz et al., 2009). Thus, as the annual mean temperature has increased at almost twice the rate in the Arctic compared to the rest of the world (Anisimov et al., 2007), vegetation changes and invasion of new species have already been observed (Davidson et al., 2011). In addition, warmer and extended summer seasons, present a prolonged grazing season for the muskox and a longer transmission period for parasites. Further, as the morphological development of invertebrate species progresses faster at increased temperatures, pupae on the tundra mature earlier, and an extended infestation season as well as flight range of warble flies are expected.

How the future epidemiology of *H. tarandi* develops in West Greenland is however difficult to assess as insufficient parasitological data in the Arctic makes predictions about impacts from new species establishments a challenging task (Kutz et al., 2004).

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