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Parasite composition of a raccoon transported to Iceland confirm its American origin

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ABSTRACT
In 2018, a live raccoon was detected by a mink hunting dog in a burrow on the coast close to Keflavík International Airport in Iceland and subsequently shot. Dissection confirmed this non-native vagrant to be a subadult, immature female. Presence of some subcutan and kidney fat reserves suggested transportation to Iceland as a stowaway hiding in goods in an aeroplane - rather than having been locked up starving for weeks in a container on a freight ship. Raccoons are native in the Nearctic but were released in Europe in the last century. Parasitological examinations of the raccoon in Iceland revealed the presence of at least 13 parasite species. Five of them are common in American raccoon populations (*Eimeria nuttally, Capillaria procyonis, C. putori, Molineus barbatus, Placoconus lotoris*) but do not occur in European conspecifics. This confirms the American origin of the animal.

Keywords: Iceland · Nearctic origin · parasite fauna · *Procyon lotor* · raccoon · translocation

YFIRLIT
Sníkjudýr sem fundust í þvottabirni á Íslandi staðfesta ameríska uppruna dýrsins
Árið 2018 fann minkahundur lifandi þvottabjörn *Procyon lotor* í holu við sjávarsíðuna skammt frá Keflavíkurflugvelli og var hann felldur. Krufning staðfesti að þarna var á ferðinni stálpuð, ókynþroska birna. Fitubirgðir undir húð og við nýru gáfu til kynna að birnan hafi tekið sér far til landsins falin í vörum flugvélar en ekki komið hingað sveltandi vikum saman í gámi flutningaskips. Náttúruleg heimkynni þvottabjarna eru Vestanhafs en á síðustu öld voru þeir fluttir til Evrópu þar sem þeir eru í dag víða algengir. Rannsóknir á sníkjudýrum birnunnar leiddu í ljós að minnsta kosti 13 tegundir. Fimm þeirra eru algengar í þvottabjörnum í Ameríku (*Eimeria nuttally, Capillaria procyonis, C. putori, Molineus barbatus, Placoconus lotoris*) en finnast ekki í þvottabjörnum í Evrópu. Því staðfestir sníkjudýrafána dýrsins það að birnan kom hingað frá Ameríku.

INTRODUCTION
The terrestrial mammalian fauna of Iceland is limited. The Arctic fox (*Vulpes lagopus*) was the only indigenous mammal when Norse settlers arrived with their livestock and pets in the late 9th century. Polar bears (*Ursus maritimus*), occasionally visiting Iceland through the centuries, have either been killed or swum or walked back to the pack ice (Skirnisson 2009). Humans unintentionally imported four rodent species and, in the late 18th century, reindeer (*Rangifer tarandus*) was introduced. In 1931, the American mink (*Neovison vison*) was imported

for fur farming but soon escaped and, within few decades, a feral population had colonized all suitable habitat on the island (Skírnisson 1993) and, in recent decades, domestic rabbits (*Oryctolagus cuniculus*) have established stable populations locally in Iceland.

The importation of live animals is strictly controlled by the Icelandic Food and Veterinary Authority (MAST) to hinder the introduction of parasites and other disease agents to indigenous livestock and pets. Imported dogs and cats are quarantined, examined for contagious diseases and parasites, and systematically treated before being handed over to the owners free of any infections (Skirnisson et al. 2018).

Feral mink is usually considered a pest in Iceland and is frequently hunted with specially trained dogs that track and locate the animals in the wild. In 2018, a trained mink dog detected a raccoon (*Procyon lotor*) in a small hideaway close to the sea-shore in Hafnir, SW Iceland. This finding was unexpected as raccoons are neither kept as pets nor occur in the wild in Iceland. The raccoon was shot with a small shotgun used for mink hunting and afterwards handed over to MAST and taken to the Institute for Experimental Pathology (IEP) at Keldur. They examined the carcass for the presence of certain viral infections and non-indigenous parasites, for example *Trichinella* spp.

Raccoons are native and widespread wild mammals in North and Central America . have been introduced to other areas in the world, including Europe. Two pairs were brought to Germany in 1934 and deliberately released into the wilderness in northern Hesse (Fischer et. al 2015). A decade later, several raccoons escaped from a fur farm further to the east in Germany, in Brandenburg. In both areas feral populations became established and gradually spread to adjacent areas (Gey 1998). At present, they are widely distributed in several European countries, with the highest population density in Germany (Hohmann & Bartussek 2002).

In the 1990s, Gey (1998) studied the parasite fauna of two raccoon populations in Germany and detected five endoparasite species. However, reviewing the literature on the parasite fauna of native raccoons in America, he reported 110 species altogether, 60 endoparasites and 50 ectoparasites. Thus, the different parasite composition of raccoons living in Europe and America is well known; raccoons from the Nearctic frequently host several parasite species that are absent in Europe.

The objectives of the present study were to determine the sex, age and condition of the raccoon found in the coastal area in Iceland and to identify the parasite composition and food remains detected in the gastrointestinal tract. This paper will compare the findings to the continent-specific parasite fauna of raccoons in order to find out if the animal arrived in Iceland from America or Europe and consider the likely way of transportation to Iceland.

MATERIALS AND METHODS

The raccoon was shot on March 20, 2018 in a small burrow on the sea coast (63°56′54′′N, 22°38′32′′W) south of the village Hafnir, SW Iceland. The site is approximately 4 km southwest of Keflavík International Airport, and 8 km southwest of the harbour area in Helgavík. The carcass was brought to IEP Keldur the following day. To evaluate possible virological infections, samples were taken from the fresh carcass which was then put in a freezer at -18°C. Two weeks later, the carcass was thawed out overnight at room temperature. After photographing, weighing, sexing and taking standard body measurements the raccoon was examined for ectoparasites. Hairs were collected with a comb from different parts of the body and ears and examined directly under a stereo microscope. After digestion in 10% KOH (Foreyt 2001), hair samples were also examined under the microscope for the presence of ectoparasites.

During autopsy, inner organs were macroscopically examined for abnormalities. Tissue and muscle samples were systematically taken and fixed in 10% buffered formalin. Later, histological sections were prepared, stained with haematoxylin–eosin (H&E) stain (Suvarna et al. 2008) and examined microscopically for the presence of parasites. The gallbladder was

cut up and examined for helminths under a stereoscope. The liver was cut into slices and visually checked for anomalies. Scrapings from the urinary bladder were examined for the presence of worms and eggs under the microscope.

The digestion method was applied to search for *Trichinella* sp. (Mayer-Scholl et. al 2017) with 39 grams of muscle used (tongue, diaphragm and masseter; 15, 16 and 8 grams, respectively).

The contents of the stomach, intestine and rectum were separately isolated and washed under tap water into a sieve with a 125 µm mesh size. All helminths were separated from indigestible prey remains under a stereoscope, identified to species or genus levels, and individuals of each prey species counted or their number roughly estimated (Gay 1998, Skirnisson 2016).

A faecal sample was taken from the rectum and examined for helminth eggs and protozoan cysts or oocysts by using the formalin-ethyl acetate sedimentation technique (FEAST) (Allen & Riedly 1970, Skírnisson et al. 2018).

Undigested food remains were compared to intact specimens in order to determine recently consumed prey.

RESULTS AND DISCUSSION

Sex, age and condition

The raccoon was a subadult female, weighing 3.0 kg. Head and body length was 44 cm, tail 24 cm, ear length 60 mm and hind foot 106 mm. Teeth were sharp. Undeveloped uterus and ovaries confirmed that the female was not sexually mature. According to Zeveloff (2002), approximately half of female raccoon yearlings do not mature and breed until their second year of life. The body mass and measurements were in accordance with data presented for subadult females in America (Chapman & Feldhamer 1982, Mech et al. 1968).

A few grams of subcutaneous fat were noted under the skin on the inside of the front legs and laterally on the belly. Approximately two grams of kidney fat were also present, while mesenteric fat was absent. The condition of the raccoon was estimated to be normal and the animal was not believed to have recently been starving.

Parasites and food composition

At least 13 distinct parasites were identified in the intestinal tract and in tissues of the raccoon, nine could be identified to species level, four to generic level and at least two distinct representatives of genus *Microsomacanthus* were found (Table 1). However, no ectoparasites were found, and no *Trichinella* sp. was detected. No parasites were found in the gallbladder and the liver.

No food remains were found in the stomach. However, remains of four distinct prey species were found in the small intestine and the rectum, remains that represent the prey selection of the animal earlier in the day or maybe on the day before its death. The most voluminous parts of these remains were formed by covert feathers, finely crushed leg bones and toes of a Purple sandpiper (*Calidris maritima*), a common shore bird on the shore where the raccoon was shot. Moreover, small-cut fragments of Great spider crab (*Hyas araneus*), shell fragments of a periwinkle (*Littorina* sp.), and few setae of an unidentified rainworm (Oligochaeta) were detected.

The parasite composition of the raccoon also suggested its earlier prey selection, as some of the parasites found were acquired by eating prey species on the shore that serve as intermediate hosts in the parasites' life cycle (Table 1). Thus, the presence of a fourth stage *Anisakis simplex* larva in the stomach of the raccoon confirmed consumption of a fish hosting the third stage parasite larvae some days earlier. The same fish, or maybe some other marine fish species on the shore, infected with metacercariae of *Cryptocotyle lingua* (Figure 1b) in its skin (black spot disease), had also been consumed. Thirdly, the presence of approximately 10,000 mature *Microphallus piriformis* (Figure 1a) in the small intestine confirmed that the raccoon had actively been eating periwinkles *Littorina* spp., infected with metacercaria of this small digenean. Fourthly, the presence of at least two distinct *Microsomacanthus* cestode species (one shown on Figure 1c) confirmed the consumption

Table 1. Parasites of a subadult female raccoon *Procyon lotor,* unintentionally transported to Iceland in late winter 2018 with information on the infection site of the parasite in the host, and if the parasite occurs in Iceland (Ic), America (Am) or Europe (Eu), according to Gey (1998) and Skirnisson (2016). Unknown intensity of infection is noted with "Present".

Parasite	Intensity	Infection site	Occurence
Protozoans			
Sarcocystis sp.	Present	Tongue and musculature*	Am, Eu,
Eimeria nuttally	Present	Faecal sample (rectum)	Am
Cestodes			
Microsomacanthus spp.	~100	Small intestine	Ic
Digeneans			
Microphallus piriformis	~10.000	Small intestine	Ic
Cryptocotyle lingua	~200	Small intestine	Ic
Nematodes			
Capillaria procyonis	Present	Tongue*	Am
Capillaria putori	51	Stomach	Am
Capillaria plica	Present	Urinary bladder	Am, Eu
Molineus barbatus	1	Small intestine	Am
Placoconus lotoris	63	Small intestine and colon	Am
Anisakis simplex	1	Stomach	Ic, Am, Eu
Acanthocephalans			
Polymorphus phippsi	2	Entangled in feathers in colon	Ic

*Confirmed with microscopical examination of histological samples

of some infected amphipods on the shore; amphipods are reported to host cysticercoids of several *Microsomacanthus* species in Iceland (Skírnisson 2016). Finally, two mature *Polymorphus phippsi* acanthocephalans were also identified. However, as they were detected within a bundle of feathers, rolled up in the lumen of the intestine, not free or attached to the intestinal wall of the raccoon, they are believed to have been parasites of the aforementioned Purple sandpiper.

In general, the above findings are consistent with reports on the general feeding habits and wide adaptations of raccoons and indicate that the animal did not have problems in finding appropriate food in the area. The raccoon had most likely survived for several days, even for some weeks, in Iceland when it was shot.

The seven remaining parasites found include two protozoans and five nematodes (Table 1). Tissue cysts of *Sarcocystis* sp. were detected in histological sections from the tongue and the pelvic musculature. In America, raccoons are reported to be an intermediate host for five, and final host for a further three, *Sarcocystis* species (Gey 1998). Which species occurred in the vagrant raccoon in Iceland remains unknown, however.

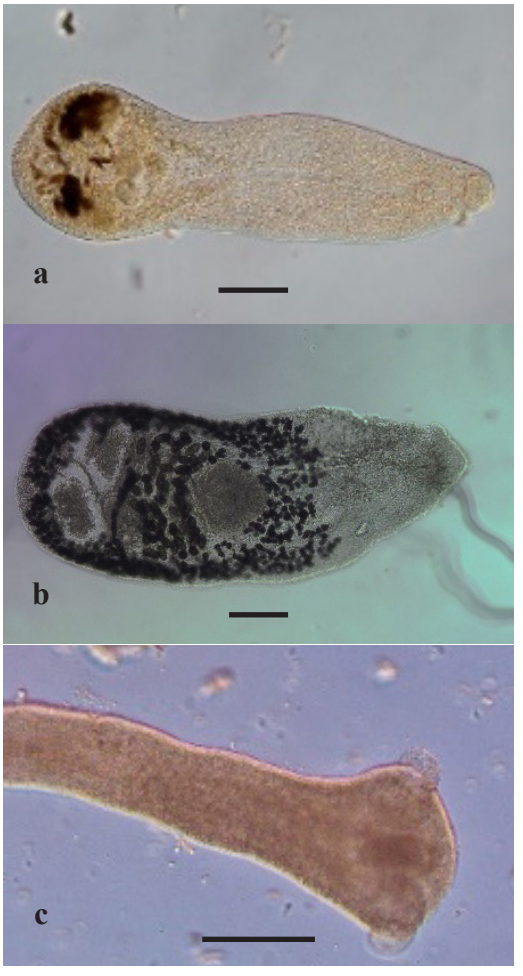

Figure 1. Photomicrographs of three indigenous parasite species detected in the intestine of a vagrant raccoon, *Procyon lotor*, that acquired helminth infections by consuming the respective intermediate hosts on the coast in Iceland (see text): **a** *Microphallus piriformis*, **b** *Cryptocotyle lingua,* **c** *Microsomacanthus* sp. Scale bars **a,b** 250 µm, **c** 100 µm.

A single eimeriid species was found. Given other evidence suggesting the origin of the raccoon (see below), it was assumed to be one of two eimerids, both with similar-sized oocysts, which infect raccoons in America. As no micropyle was detected on the oocysts examined in the present study, the species involved was identified as *Eimeria nuttally*. The other eimerid reported from raccoons, *Eimeria procyonis*, usually has an micropyle. *E. procyonis* was carried by raccoons over to Europe and is at present commonly identified from raccoons on both continents (Gey 1998).

Three *Capillaria* species are known to infect raccoons (Gey 1998). In America, *C. procyonis* occurs in the mouth epithelia, tongue and oesophagus and *C. putorii* parasitizes in the stomach and the intestine. Both species were found in the raccoon in Iceland (Table 1). However, these species are not reported in Europe. The third species, *C. plica,* was detected in scrapings from the urinary bladder of the raccoon in Iceland (Figure 2). This species occurs on both continents and is known to infect other species in Europe, e.g. red fox *Vulpes vulpes* (Gey 1998).

The two remaining nematode species found were *Molineus barbatus* and *Placoconus lotoris* (Figure 2, Table 1). Both are well known parasites of American raccoons and are not found in Europe (Gey 1998).

Based on the parasite composition of the vagrant raccoon transferred to Iceland and the geographical distribution of the distinct species (Table 1), it seems clear that the raccoon arrived from America.

Way of transportation

Raccoons can be transported to Iceland either in ships or by airplane. The harbour of Helgavík is located approximately 8 km away from the seashore where the raccoon was captured. Enquiries at the harbour office revealed that freight ships transporting containers from America have not been brought to the harbour for more than a decade. Therefore, importation by ship to Helgavík should be excluded. However, importation of a raccoon to Iceland in a container has already been documented (Anonymous 1998). In late October 1998, a raccoon, creeping on a wooden pallet close to the door of a container filled with hot tubs was observed when the container was opened. Almost four weeks earlier, the container had been loaded in Toronto, Canada, locked up, brought to New York harbour, taken aboard on a container ship and transported to Iceland.

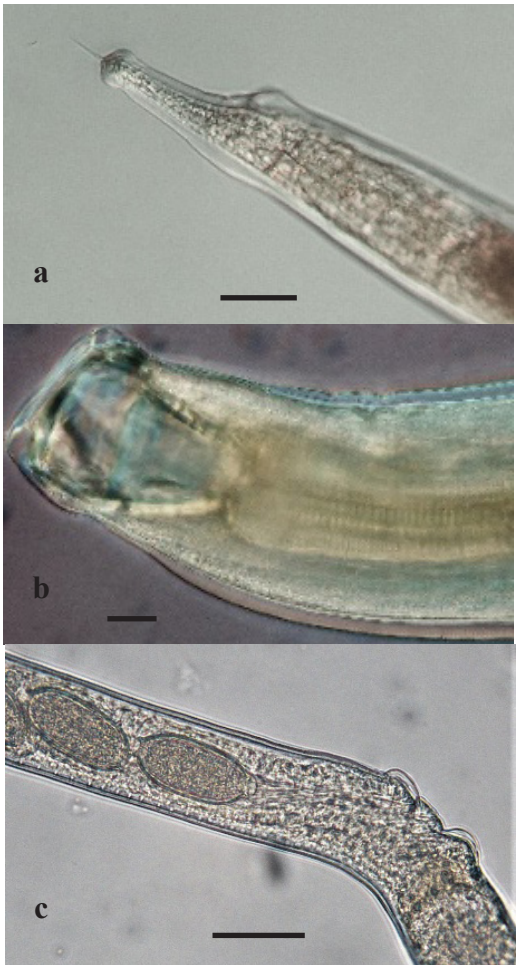

Figure 2. Photomicrographs of three intestinal nematodes detected in a vagrant raccoon, *Procyon lotor,* found on the coast close to Keflavík International airport in Iceland. Note that these parasites occur in raccoons in America but not in Europe (Gay 1998): **a** Posterior end of a female *Molineus barbatus*, **b** Buccal capsule of *Placoconus lotoris,* **c** Vulva region of *Capillaria putori. S*cale bar 50 µm.

When detected, the raccoon was still alive but very weak, unable to move and about to die after being locked up in the container for almost a month. In the present case, the good physical condition of the raccoon did not suggest recent starving in a closed container without access to food and water. On the contrary, the raccoon is considered to have come to Iceland by airplane by hiding in goods that were loaded onto the plane. Every day, several cargo and passenger planes leave from different airports in the USA, and are unloaded 6-8 hours later at the Keflavík International Airport, about 4 kilometres away from the site in Hafnir where the raccoon was detected.

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