## Further Evidence of Cougars (Puma concolor) in Ontario, Canada

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Previous studies have indicated that Cougars (*Puma concolor*) were present on the Ontario landscape from 1935 to 2010. During 2012 and 2014, six pieces of evidence were collected that verified that Cougars were present in Ontario at that time. (1) A scat found near Collingwood, Ontario, was confirmed as containing Cougar DNA. (2) A Cougar was photographed by a member of the public near Pefferlaw, Ontario, and the photograph was proven to be authentic. (3) A Cougar was photographed near Kenora, Ontario. (4) A Cougar was observed near Kenora, Ontario, and tracks confirmed the sighting. (5) A Cougar attacked a dog near Bracebridge, Ontario; the animal was subsequently shot by police and DNA evidence indicated that it had at one time been in captivity. (6) A cougar was photographed and later captured near Grafton, Ontario.

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By the early 20th century, Cougars (Puma concolor) had apparently been extirpated from much of their historical range in eastern North America including Ontario, Canada (Bolgiano and Roberts 2005). However, Gerson (1988) reported documented sightings of Cougars in Ontario during 1935-1983. Rosatte (2011a) provided further evidence that Cougars existed on the Ontario landscape between 1991 and 2010. DNA analysis has confirmed the existence of free-ranging Cougars in Ontario and Quebec and most of eastern Canada (Cumberland and Dempsey 1994; Bertrand et al. 2006; Mallory et al. 2012; Le Duing et al. 2013). In Ontario, Cougars are classified as an endangered species (OMNR 2010). In this paper, we provide additional evidence, collected during 2012–2014, that supports the view that free-ranging Cougars occur in Ontario.

On 7 June 2012, a scat was found by a member of the public near Collingwood, Ontario (44.475°N, 80.275°W) (Figure 1). Swabs of the scat were submitted to the Natural Resources DNA Profiling & Forensic Centre (NRDPFC) at Trent University, Peterborough Ontario, for DNA analysis. To determine the species, a region of the cytochrome B gene within the mitochondrial DNA was amplified (30 cycles at annealing temperature 54°C) using Cougar-specific primers, PumaCB-F (5'-CCG AGA AGR TAT GGA GCC ATA A-3') and PumaCB-R (5'-CTA TAC ATC AGA CAC AAT GAC TGC C-3'). This amplification is sensitive down to 10 pg of DNA. The fragment size generated



FIGURE 1. Scat found by a resident near Collingwood, Ontario, in June 2012, contained Cougar (*Puma concolor*) DNA. Photo: Jeanette Varga-Papp.

using primers PumaCB-F and PumaCB-R was 121 base pairs (without the primer sequences) and 170 base pairs (with the primer sequences). The amplified DNA was subsequently sequenced on an Applied Biosystems 3730 DNA Analyzer (Life Technologies, Burlington, Ontario, Canada) and the resulting sequences were analyzed using the phylogenetic software package MEGA5.2 (Tamura *et al.* 2011). The sequences were compared to several cytochrome B sequences in the National Centre for Biotechnology Information database by conducting a Basic Local Alignment Search Tool (BLAST) search. DNA from the scat aligned with and was most closely related to *Puma concolor* (Cougar) (GenBank accessions KC567624 and JN999997).

In mid-January 2013, a member of the public reported that he had taken a photograph of a Cougar near Pefferlaw, Ontario, (44.315°N, 79.203°W) at about 0100 (Figure 2). A site visit by Ontario Ministry of Natural Resources (OMNR) staff revealed a few inconsistencies with vegetation in the original photo but these differences may have been due to the fact the photo was taken at night. There was no evidence to indicate that the photo had not been taken on the property, and an interview with the photographer suggested that the photo was valid. Using the tree in the photo as a source of scale, we estimated that the animal was about 110 cm head and body length and 66 cm in height along its back. These dimensions were in the range for North American Cougar populations (Logan and Sweanor 2001). The large size ruled out smaller cats, such as Bobcat (Lynx rufus) (Anderson and Lovalo 2003), which also occur in southern Ontario (Rosatte 2011b; Naughton 2012). Six Cougar experts from across North America confirmed that the animal in the photo was a subadult Cougar.

On 31 October 2013, a Cougar was photographed by a member of the public, as it snarled and growled at four dogs at a private residence about 5 km northeast of Kenora, Ontario (49.775°N, 94.415°W). OMNR staff (L. Anderson) viewed the photo and confirmed that the animal was a Cougar. However, the photographer would not provide OMNR with an electronic copy of the photo at that time. Coincidently, on 15 November 2013, a Cougar was observed by a trapper (from about 65 m distance) about 17 km northeast of Kenora, Ontario, (49.817°N, 94.254°W). The animal was described as "deer-coloured" with a long tail, and about the size of an adult shepherd dog.

OMNR staff visited the location of the sighting and photographed tracks left by the animal in the snow. The tracks were about 9.0 cm in diameter and had characteristics that were consistent with a Cougar (Rosatte 2011a) (Figure 3). The distance from the heel of the hind foot to the toe of the front foot when the Cougar was walking on a snow-covered fallen tree trunk was 158 cm. When walking on flat ground across a roadway, the stride distance from the front of the toe on the right hind foot to the front of the toe on right front foot was about 85-90 cm. This stride distance was consistent with Cougar as opposed to Canada Lynx (Lynx canadensis) or Bobcat (Montana Fish, Wildlife & Parks 2015: 14). The location of the tracks was about 12 km east of the site where the photo of a Cougar was taken on 31 October. Given the tremendous mobility of Cougars, it is possible that these sightings and tracks were from the same animal.

Additional evidence involves a Cougar that had once been in captivity. During early July 2012, a number of Cougar sightings in the greater Bracebridge, Ontario, area were reported to the Ontario Provincial Police, Bracebridge detachment. On 7 July 2012, a Cougar attacked a dog (a German shepherd mix) in a residential yard about 15 km north of Bracebridge, near Utterson (45.211°N, 79.329°W). The Cougar was shot by the police because of the potential danger to the public (Figure 4) and the dog was euthanized as a result of its injuries. The Cougar was examined at the scene by OMNR staff and police, and it appeared that the front claws were missing. The rear claws were present, but



FIGURE 2. Photo of a Cougar (*Puma concolor*) taken near Pefferlaw, Ontario, in January 2013. Photo: Mike Wahrer and Al Dixon.



FIGURE 3. Photo of tracks where a Cougar (*Puma concolor*) was observed near Kenora, Ontario, on 15 November 2013. Photo: Lil Anderson.

were well worn suggesting the animal may have been a captive animal that had escaped or was released.

The carcass was frozen at the Bracebridge OMNR office and transported on 10 July 2012 to the Canadian Cooperative Wildlife Health Centre (CCWHC), Guelph, Ontario, for post-mortem analysis. On 11 July 2012, hair and muscle samples were submitted to the NRDPFC for genetic analysis. During 2013, samples were also shipped to the United States Forest Service, Rocky Mountain Research Station, Wildlife Genetics Laboratory, in Missoula, Montana, in an attempt to determine the geographic origin of the Cougar by comparing its DNA profile with profiles in a database of Cougars from the western United States.

The post mortem at the CCWHC revealed that the Cougar was a young (determined by tooth wear) adult female weighing 48 kg that had died due to gunshot trauma. Extremely large amounts of subcutaneous and internal fat were found, the muscle mass was normal, and there was slight wear on the teeth. The Cougar was a healthy animal, in excellent body condition, with no evidence of significant pre-existing disease. The brain tested negative for rabies by the fluorescent antibody technique. The liver was olive-green with a slightly fatty texture. The gallbladder was full, and the stomach contained grass, hair, and two pieces of fabric. Most of the intestinal tract was empty, and lesions were not apparent in the liver, thyroid, tonsil, esophagus, small



FIGURE 4. Photo of a dead adult female Cougar (*Puma concolor*) that attacked a dog near Bracebridge, Ontario, on 7 July 2012. Photo: C. MacDonald.

intestine, stomach, heart, or brain (as determined by histologic examination). A single large *Sarcocystis* cyst was observed within the tongue muscle and a few small foci of interstitial inflammation were found in the kidneys. A thick band of acellular eosinophilic material (possibly amyloid) occurred at the corticomedullary junction of the adrenal glands, and the mammary gland was inactive. The Cougar was pregnant, with an approximately 14-cm (crown to rump) fetus present. No tattoos, microchips, or other identifying markings were found; however, the lack of claws on the forefeet indicated probable surgical removal. The body condition and absence of claws on the front feet suggested that the animal had recently been captive and cared for.

The geographic origin of the adult female Cougar is unknown. However, there was a large exotic cat facility 300 m from where the Cougar had been shot. Blood samples collected from two captive male Cougars at that facility in October 2012 revealed that they could be related to the adult female Cougar at the sibling level. In addition, one of the male Cougars could not be excluded as father of the fetus based on examination of six microsatellite loci at the NRDPFC. Regarding the strength of the parentage assignment, in this case, we were only concerned with distinguishing between two possible fathers of the fetus. One of the male Cougars was excluded at one of the six loci, whereas the other could not be excluded at any of the six loci. This evidence showed that the adult female had been in captivity at some time, during which she became impregnated. The owner of the exotic cat facility subsequently pled guilty to charges relating to the case.

Analysis of the samples sent to the Wildlife Genetics Laboratory in Montana was inconclusive with respect to the geographic population from which the adult female and the two male Cougars originated. This result may have been due to the fact that these Cougars originated from a mixture of populations or from a population the laboratory had yet to sample, e.g., South American genotype Cougars. The Wildlife Genetics Laboratory database contained genotypes from 1770 animals collected from South Dakota, North Dakota, Nebraska, Montana, Wyoming, Colorado, Idaho, New Mexico, Arizona, Texas, Florida, and Oregon.

About two weeks before the shooting of the Cougar in Utterson, a Cougar was observed during the evening by several people about 5 km south of Utterson near Port Sydney, Ontario. Given the movement capabilities of Cougars (up to 50 km/night; Rosatte 2011a), it is possible that these sightings involved the same Cougar. Regardless, the implications of a formerly captive pregnant adult female Cougar roaming central Ontario is significant from a genetic perspective (although its chances of surviving in the wild are unknown).

Last, a Cougar was observed and photographed near Grafton, Ontario (43.993°N, -78.023°W) by a member of the public on 10 July 2014 (Figure 5). On 11 July, OMNR staff captured the animal using a baited bear trap, and it was transported to a zoo in Peterborough, Ontario, for assessment. It was determined to be a healthy young male Cougar, about three years of age, with its claws intact. The origin of the Cougar is unknown (as of 21 July 2014), but its disposition (fairly tame) led to the suspicion that it was in captivity at some time.

The new data presented here provide further support of the view that Cougars currently exist on the Ontario landscape. As in previous studies, it is unknown



FIGURE 5. Photo of a Cougar (*Puma concolor*) near Grafton, Ontario, on 10 July 2014. Photo: Jean Wilson.

whether the animals were escaped or released captives, immigrants from western North America, native Cougars, or a genetic mixture of several sources, as was found in Quebec and New Brunswick (Rosatte 2011a; Le Duing *et al.* 2013). The Cougar that was shot near Bracebridge, Ontario, provides evidence to support the theory of Rosatte (2011a) that escaped or released Cougars and their progeny are one of the sources of Cougars in Ontario. It also provides evidence that escaped or released animals are among the Cougars that are currently on the Ontario landscape.

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