

September 15, 2022

To: Commissioner Basil Seggos

New York Department of Environmental Conservation

625 Broadway

Albany, New York 12233-1010

Via: Basil.Seggos@dec.ny.gov

CC: DEC Wildlife Bureau (wildlife@dec.ny.gov)
Assemblymember Steve Englebright, (EngleS@nyassembly.gov)
Governor Kathy Hochul

RE: December 2021 hunting incident of gray wolf underscores need for DEC to increase protections

Dear Commissioner Seggos,

In December 2021, a hunter in New York shot and killed a large canid subsequently identified by genetic analysis at Trent University as 98% wolf. This is not the only such incident. Rather, it is the most recent of multiple instances of large canids, which turned out to be wolves, being shot and killed by coyote hunters in New York and elsewhere in the Northeast over the past few decades.

In light of these events, we are writing to urge the New York Department of Environmental Conservation (DEC) to take immediate action to increase protections for wolves potentially dispersing through and recolonizing the Northeast, including the state of New York (Glowa et al. 2009).¹ DEC's mission is to "conserve, improve, and protect New York's natural resources." Apex predator populations are crucial to healthy ecosystems. The absence of highly interactive species that are key to maintaining habitat and other natural functions, such as wolves and cougars, has left a functional void in our ecosystems that has degraded overall environmental quality. Conservation efforts to conserve and improve New York's natural resources must include protecting and restoring a crucial missing apex predator that once roamed across the state. A future in which recolonizing wolves in our region are able to successfully establish packs and an eventual population is only possible with an active and immediate response by DEC. Yet DEC has failed to take any actions to educate the public on potential wolf presence or the fully protected status of wolves in New York under both state and federal law, despite DEC's own awareness of wolves legal status in New York and that several wolves have been shot and

¹ The proper taxonomic treatment of wolves in Northeastern North America has yet to be fully resolved by the scientific community as the area is a zone of historic admixture between putative wolf species, subspecies and/or their hybrids (Rutledge et al. 2012, Rutledge et al. 2015, Wilson et al. 2000, Wilson et al. 2003). Nevertheless, for legal purposes they are generally all considered gray wolves (*Canis lupus*). Regardless of whether such wolves are ultimately scientifically classified as gray wolves (*Canis lupus*), Great Lakes wolves (*Canis lupus x lycaon*), eastern wolves (*Canis lycaon* or *Canis lupus lycaon*), or something else, there can be no dispute that they are currently protected under state and federal law as wolves.

killed in New York by hunters over the past few decades (Glowa et al. 2009, Maine Wolf Coalition).

We make the following four requests to the DEC to ensure proper protection of federally and state-protected wolves throughout the region and to restore vital ecological health to New York's ecosystems.

1. *We urge the DEC to confirm the recent killing of a wolf in central New York and publicly acknowledge the potential of wolves to disperse through and recolonize the region.*

As you are aware, in December 2021, a hunter in Central New York shot and killed a large canid species weighing approximately 85 pounds. The hunter reported the incident to the DEC, which subsequently sent staff to take a DNA sample of the animal for analysis. The hunter, upon request, also provided the Northeast Ecological Recovery Society with tissue samples for an independent analysis.

This independent DNA analysis was conducted by Trent University's Natural Resources DNA Profiling & Forensic Centre, a leading institution with expertise in wild canid ancestry located in Ontario, Canada (e.g., Rutledge et al. 2012, 2015; Wilson et al. 2000, 2003). Trent University's analysis confirmed the sample as an admixed nature composed of 98% wolf genetics predominated by Great Lakes wolf ancestry. Trent University ran this sample with an in-house database consisting of 350 reference genotypes from samples assigned to Ontario (ON) *Canis* groups (60 Great Lakes wolf, 60 eastern wolf, 60 eastern coyote), 60 ON domestic dog, 60 Saskatchewan (SK) coyote (unhybridized *C. latrans*), and 50 Northwest Territories (NT) gray wolf (unhybridized *C. lupus*) (Appendix A).

The DEC has yet to release an official statement on this incident, which occurred over 8 months ago, but a public spokesperson for the agency refuted the analysis to the media, stating the canid was most closely related to an eastern coyote and referenced a competing genetic analysis conducted by Wildlife Genetics Institute at East Stroudsburg University in Pennsylvania. We urge the DEC to either provide sufficient and proper refuting evidence or confirm the independent analysis from Trent University that this canid species was in fact a wolf.

This is not the first incident of wolf presence in New York. Of particular note are two other recent confirmed documented cases of wolves within the state (plus one killed in 1968²):

- On December 19, 2001, a coyote hunter shot and killed an 85 pound male wolf in Day, New York. The DEC did not investigate or report the animal to the U.S. Fish and Wildlife Service (USFWS). A USFWS agent investigated the report and subsequently confiscated the remains of the animal, which DNA analysis later confirmed was a wolf.
- On April 12, 2005, a 99 pound wolf was shot and killed in Sterling, New York. DNA analysis confirmed the animal to be a gray wolf according to a July 14, 2005, letter from

² On January 10, 1968, an adult male gray wolf was killed by an automobile on Tote 10 in the Royal Mountain Ski Area near Rockwood, New York. The USFWS is aware of the skull of the 1968 wolf, which is currently housed in the Museum of Natural History, Smithsonian Institution, Washington, D.C.

Sree Kanthaswamy, Ph.D., of the Veterinary Genetics Laboratory at the University of California, Davis.

These confirmed killings are in New York only, but multiple other documented killings from across the northeast have occurred (Glowa et al. 2009) as well as numerous other unconfirmed sightings and observations of large canids within the state.

DEC has been aware for quite some time of the potential for wolves to disperse through or recolonize the region (NYDEC *Canis lupus* Species Assessment 2015). Wolves are currently present and breeding south of Algonquin Provincial Park in Ontario, roughly 100 miles from the New York border, a dispersal distance that has been recorded consistently in other wolf populations across the country (COSEWIC. 2015, Morales-González et al. 2022). Mammal species other than wolves have been recorded crossing the Saint Lawrence, including lynx and fisher (Koen et al. 2015, Carr et al. 2007). Further, wolves have been documented traveling hundreds of miles during dispersal, which means that wolves of the Great Lakes region are also within potential dispersal distance (Weiss et al. 2014).

The DEC's 2015 species assessment for the gray wolf confirmed the potential for successful recolonization of wolves to suitable habitats across the state. Analyses have confirmed suitable habitat for wolves, particularly in the Adirondack/Tug Hill plateau region of upstate New York (Caroll, 2003). Notably, the DEC also acknowledged that recolonization of wolves across the state would require protection from intentional killing (NYDEC *Canis lupus* Species Assessment 2015).

In addition, the DEC's 2015 species assessment recognized the need to change the common name from gray wolf to just "wolf," expanding protections to all wolf species/subspecies that were once native to the area. Furthermore, the potential for eastern wolves to colonize New York is also high. This is significant because the United States does not have any known populations of this North American evolved wolf.

New York is located within dispersal distance for wolves currently residing in Canada and contains some of the best remaining habitat for wolves in the Northeast. As has been shown with this recent incident and other past documented wolves killed in the Northeast, they have the potential to recolonize and establish populations in the region (Glowa et al. 2009). However, the potential for wolf recolonization in the area is reliant upon the DEC providing proper protections for the endangered species.

- 2. DEC is legally required to keep gray wolves on the state endangered species list and must ensure compliance with 6 NYCRR 182.8 by prohibiting their killing and "take" by hunters.*

In 2019, the DEC proposed to take the gray wolf off the state endangered species list, despite objections from New York lawmakers and multiple conservation organizations. We are disappointed by the DEC's lack of compliance with state statute mandating wolf protection in New York, oppose all efforts to delist the species in the state, and strongly support policies and

measures to protect future recolonization of the imperiled species across the state's remaining suitable habitat. Additionally, we support the DEC's change to the common name "wolf" to recognize and expand protection to all wolves native to this region.

In 2019, when the DEC proposed removing state protections for gray wolves, the U.S. Fish and Wildlife Service was preparing to federally delist them. The gray wolf was officially delisted across most of the Lower 48 United States in November 2020, effective January 4, 2021. That federal delisting was immediately challenged in court by multiple conservation organizations. In February 2022, the delisting rule was overturned and federal protections were restored, including those for wolves within New York state. Due to their federal status, the DEC must adhere to 6 NYCRR § 182.3, which legally requires coordinated state listing of all federally listed threatened or endangered species that are "native species present or formerly present in New York."

This legally-required state/federal coordinated listing mandates that wolves remain a state-protected species. Any wolves present within the state are currently protected under both federal and state law as an endangered species. (6 NYCRR Part 182.5, 50 C.F.R. part 17). As a state-listed endangered species, the DEC is required to prohibit any activity that is likely to result in "take" of the species. (6 NYCRR 182.8).

3. *We urge the DEC to establish best management practices to protect fragile, newly recolonizing wolf populations within the state.*

This recent incident of the wolf shot in New York in December 2021 must be taken into consideration in DEC's future management actions to protect wolves and actions to prohibit future take of a state and federally protected species. The wolf shot in December was misidentified as a coyote, which is a common issue for protecting wolf populations across North America (Newsome et al. 2015). Misidentification is especially a challenge in the Northeast where 'coyotes' are larger due to hybridization with wolves (Way 2021) and wolves are often smaller due to the eastern wolf and their hybrids being a smaller bodied canid (Benson et al. 2012). We urge the DEC to follow best management practices for protecting fragile wolf populations, which include banning or restricting coyote hunting (e.g., night hunting, hunting with dogs, limited times of the year, limited regions, etc.) in order to avoid intentional and unintentional take of wolves in the region.

Regulating coyote hunting through strictly-enforced bans or restrictions to protect fragile or newly colonizing wolf populations is not a new management strategy and has proven effective in protecting wolves in other regions of North America, even where the size difference between the two canids is more pronounced. This management measure was taken in Wisconsin when wolves began to recolonize the state. Beginning in 1980, the "[c]oyote season [was] closed in northern management units to protect the nascent wolf population" (Wisconsin Department of Natural Resources 2020). The prohibition on shooting coyotes applied during the "gun" deer season for 33 years from 1980 to 2013. A ban on coyote hunting was also implemented in North Carolina in an effort to protect critically endangered red wolf populations, a canid species

morphologically indistinguishable from coyotes under most field conditions (North Carolina Wildlife Resources Commission 2017).

Way and Hirten (2019) noted that body size and genetics are often the only way to tell one *Canis* type apart from another. It is possible that small to medium sized wolves (e.g., 60-65 pounds) are killed and assumed to be large 'coyotes'. The DEC should consider more genetic testing to fully understand the extent and presence of wolves across the state.

Furthermore, the Ontario Ministry of Natural Resources and Forestry has a current ban on coyote hunting and trapping within 40 Ontario townships surrounding Algonquin, Killarney, Queen Elizabeth II Wildlands and Kawartha Highlands Provincial Parks to help protect wolves of the region. Therefore, there is sufficient precedent to support a ban or strict regulation of coyote hunting to mitigate the accidental illegal killing of wolves.

In addition, the DEC should conduct immediate hunter education and outreach to inform hunters within the state of (1) the potential for wolves moving through the region; (2) the state and federally protected status of wolves in the state; (3) prohibitions on shooting large canids that could potentially be a wolf; and (4) the legal repercussions of killing a federally and state protected species.

The DEC has repeatedly and wrongly informed hunters that any large canids observed can be identified as eastern coyotes, which has misinformed hunters about the potential return of wolves in the region. This has effectively removed any degree of hunter obligation to properly identify animals before killing them. The eastern coyote or coywolf (Way 2021) is about 60-65% coyote, 25-30% wolf, and 10% dog. They average 30-40 pounds, with exceptional individuals weighing 50-55 pounds (Way 2013, 2021), yet can appear larger due to their longer body and lighter frame compared to a similar sized dog. Actual wolves are generally much bigger and heavier but can be difficult to distinguish from other canids due to individual variability and field conditions (e.g., low light, distance of sighting). This variability makes it very difficult to properly discern these closely related canids (Way and Hirten 2019). New York's coyote hunting season opens on October 1st and trapping season on October 25th. Therefore, the DEC must act swiftly to curtail the potential for more violations of federal and state law—and, even more tragically, potentially additional dead wolves.

4. *We urge the DEC to protect critical habitat linkages to facilitate wolf dispersal into the region.*

As an additional measure to facilitate wolf dispersal into the region we also urge the DEC to assist conservation groups and outdoor recreationists currently working on restoring and protecting critical habitat linkages -- wildlife corridors, or wildways -- by keeping New York's wildlands interconnected and connected to natural habitats in Ontario and New England. These vital links include the Algonquin to Adirondack axis (A2A), the Adirondack to Tug Hill nexus across the Black River Valley, the Mohawk Link between Adirondack Park and Catskill Park, the Emerald Necklace of the Finger Lakes region, the Adirondack to Green Mountain link across the

Southern Lake Champlain Valley, Split Rock Wildway linking the Champlain Valley with the High Peaks, and riparian corridors throughout the region. (Reining et al. 2006)

Conclusion

The DEC has a legal duty to take proactive measures to protect any wolf that exists within the state. The actions we request DEC to take are initial steps to ensure protection for any newly recolonizing wolves in the region. A more thorough analysis of best management practices for protecting fragile wolf populations will be required by the DEC's species assessment currently underway in order to ensure full and robust protection for the species.

With direct action by DEC to protect newly recolonizing wolf populations in the region, New York ecosystems have the potential to flourish and be restored to ecological health. We request a timely response from the DEC, including a confirmation of the wolf shot in 2021 and how the DEC plans to increase protections for wolves within the state.

Thank you for your time and attention.

Sincerely,

Renee Seacor, JD
Carnivore Conservation Advocate
Project Coyote & The Rewilding Institute

Jonathan Way, Ph.D.
Founder, Wildlife Biologist
Eastern Coyote/Coywolf Research

Amaroq Weiss, MS, JD
Senior Wolf Advocate
Center for Biological Diversity

John M. Glowa, Sr.
President
The Maine Wolf Coalition, Inc.

Joe Butera
President & Co-founder
Northeast Ecological Recovery Society

David Gibson
Managing Partner
Adirondack Wild: Friends of the Forest Preserve

Peter Bauer
Executive Director
Protect the Adirondacks

Dave R. Parsons, MS
Carnivore Conservation Biologist & Science Advisor
The Rewilding Institute & Project Coyote

Willie Janeway
Executive Director
Adirondack Council

Francisco J. Santiago-Ávila, PhD
Science and Conservation Manager
Project Coyote & The Rewilding Institute

Michael Kellet
Executive Director
RESTORE: The North Woods

Tara Thornton
Deputy Director
Endangered Species Coalition

Jennifer Sherry
Wildlife Science and Policy Specialist
Natural Resources Defense Council

Christine Schadler, MS, MA
Project Coyote Representative, Vermont & New Hampshire
New Hampshire Wildlife Coalition

Ron Sutherland, PhD
Chief Scientist
Wildlands Network

William S. Lynn, PhD
Research Scientist
Marsh Institute, Clark University

Frank Vincenti
Director
Wild Dog Foundation

Cornelia Norton Hutt
Board of Directors, Chair
Red Wolf Coalition, Inc.

Kimberly Baker
Public Land Advocate
Environmental Protection Information Center

Betsy Klein
Founder and President
Plan B to Save Wolves

Timothy Coleman
Executive Director
Kettle Range Conservation Group

Beatrice Friedlander
Board President
Attorneys for Animals, Inc.

Jennifer Rosado
Citizen Scientist
The Maine Wolf Coalition, Inc.

Carter C. Niemeyer, MS
Consultant
US Fish and Wildlife Service (retired)

Brooks Fahy
Executive Director
Predator Defense

Adam DeParolesa
Founder/ President
Northeast wolf refuge

Zee Soffron
Director
North American Wolf Foundation

Karol Miller
President
The 06 Legacy

Robert Crabtree
Chief Scientist
Yellowstone Ecological Research Center

Marilyn Jasper
Chair
Public Interest Coalition

Adrian Treves, PhD
Professor, Founder, Director
Carnivore Coexistence Lab

Nancy Warren
Executive Director
National Wolfwatcher Coalition

Brenna Galdenzi
President
Protect Our Wildlife, Vermont

Courtney Vail
Campaign Director
Oceanic Preservation Society

David Johns
Professor (retired), School of Government
Portland State University

Lynn Okita
Board Chair
Western Wildlife Outreach

Suzanne Fouty, PhD
Hydrologist/Soils Specialist
USDA Forest Service (retired)

Bradley J. Bergstrom, Ph.D.
Member, Science Advisory Board
Project Coyote

Appendices:

- Genetic Characterization of NY Canis Samples from 2021 hunting incident in central New York analyzed by Trent University Natural Resources DNA Profiling & Forensic Centre

Literature Cited:

Benson, J.F., B.R. Patterson, and T.J. Wheeldon. 2012. Spatial genetic and morphologic structure of wolves and coyotes in relation to environmental heterogeneity in a *Canis* hybrid zone. *Molecular ecology* 21: 5934–5954. <https://doi.org/10.1111/mec.12045>

Carr, D., Bowman, J., Kyle, C.J., Tully, S.M., Koen, E.L., Robitaille, J-F, and Wilson, P.J.. 2007. "Rapid Homogenization of Multiple Sources: Genetic Structure of a Recolonizing Population of Fishers," *Journal of Wildlife Management* 71(6), 1853-1861, (1 August 2007). <https://doi.org/10.2193/2006-274>

Carroll, Carlos. 2003. Impacts of Landscape Change on Wolf Viability in the Northeastern U.S. and Southeastern Canada: Implications for Wolf Recovery. *Wildlands Project Special Paper No. 5*. Richmond, VT: Wildlands Project. 31 pp.

COSEWIC. 2015. COSEWIC assessment and status report on the Eastern Wolf *Canis sp. cf. lycaon* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xii + 67 pp. Available: www.registrelep-sararegistry.gc.ca/default_e.cfm

Glowa, J., W. L. Pepperman, C. L. Schadler, J. Butera, and J. G. Way. 2009. Petition submitted to protect wolves and allow wolf recovery in the northeast. Submitted to U.S. Department of Interior on 31 January 2009. 17 pages. Available: <http://www.easterncoyoteresearch.com/downloads/ESApetition2009final.pdf>

Koen, E.L., Bowman, J., and Wilson, P.J.. Isolation of peripheral populations of Canada lynx (*Lynx canadensis*). *Canadian Journal of Zoology*. 93(7): 521-530. <https://doi.org/10.1139/cjz-2014-0227>

Maine Wolf Coalition. 2022. "Northeast Wolf Recovery: Wolves of the Northeast." <http://mainewolfcoalition.org/wolves-in-the-northeast/>

Morales-González, A., Fernández-Gil, A., Quevedo, M. and Revilla, E. (2022), Patterns and determinants of dispersal in gray wolves (*Canis lupus*). *Biol Rev*, 97: 466-480. <https://doi.org/10.1111/brv.12807>

New York Department of Environmental Conservation. Gray Wolf (*Canis Lupus*) 2015 Species assessment. https://www.dec.ny.gov/docs/wildlife_pdf/sgcnwolf.pdf

Newsome, T.M., J.T. Bruskotter, and W.J. Ripple. 2015. When shooting a coyote kills a wolf: Mistaken identity or misguided management? *Biodiversity and Conservation* 24: 3145-3149. <https://doi.org/10.1007/s10531-015-0999-0>

North Carolina Wildlife Resources Commission. Hunting Regulations. North Carolina Wildlife Resources Commission; 2017. Available:
<http://www.ncwildlife.org/Portals/0/Regs/Documents/Hunting-Regulations.pdf>

Reining, C., K. Beazley, P. Doran and C. Bettigole. 2006. From the Adirondacks to Acadia: A Wildlands Network Design for the Greater Northern Appalachians. Wildlands Project Special Paper No. 7. Richmond, VT: Wildlands Project. 58 pp

Rutledge, L.Y., P.J. Wilson, C.F.C. Klutsch, B.R. Patterson, and B.N. White. 2012. Conservation genomics in perspective: a holistic approach to understanding *Canis* evolution in North America. *Biological Conservation* 155: 186–192. <https://doi.org/10.1016/j.biocon.2012.05.017>

Rutledge, L.Y., S. Devillard, J.Q. Boone, P.A. Hohenlohe, and B.N. White. 2015. RAD sequencing and genomic simulations resolve hybrid origins within North American *Canis*. *Biology Letters* 11: 20150303. <https://doi.org/10.1098/rsbl.2015.0303>

Way, J.G. 2013. Taxonomic Implications of Morphological and Genetic Differences in Northeastern Coyotes (Coywolves) (*Canis latrans* × *C. lycaon*), Western Coyotes (*C. latrans*), and Eastern Wolves (*C. lycaon* or *C. lupus lycaon*). *Canadian Field-Naturalist* 127(1): 1–16.

Way, J.G. 2021. Coywolf: Eastern Coyote Genetics, Ecology, Management, and Politics. Eastern Coyote/Coywolf Research, Barnstable, MA. 277 pages. E-book. Open Access URL: <http://www.easterncoyoteresearch.com/CoywolfBook/>.

Way, J.G., and J.L. Hirten. 2019. Wild *Canis* spp. of North America: a pictorial representation. *Canadian Field-Naturalist* 133: 295-296.

Weiss, A., Greenwald, N. and Bradley, C. 2014. Making Room for Wolf Recovery. The Case for Maintaining Endangered Species Protections for America's Wolves. https://www.biologicaldiversity.org/campaigns/gray_wolves/pdfs/Making_Room_for_Recovery_web.pdf

Wilson, P.J., S. Grewal, I.D. Lawford, J.N.M. Heal, A.G. Granacki, D. Pennock, J.B. Theberge, M.T. Theberge, D.R. Voigt, W. Waddell, R.E. Chambers, P.C. Paquet, G. Goulet, D. Cluff, and B.N. White. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Canadian Journal of Zoology* 78: 2156–2166. <https://doi.org/10.1139/z00-158>.

Wilson, P. J., S. Grewal, T. McFadden, R.C. Chambers, and B.N. White. 2003. Mitochondrial DNA extracted from eastern North American wolves killed in the 1800s is not of gray wolf origin. *Canadian Journal of Zoology* 81: 936-940.

Wisconsin Department of Natural Resources. A Chronology of Wisconsin Deer Hunting: From Closed Seasons to Record Harvests <https://dnr.wi.gov/news/Weekly/article/?id=3786>

Genetic Characterization of *Canis* Samples

Submitted by Joseph Butera

Report Prepared for Joseph Butera
12 July 2022

By: Audrey Wilson and Matthew Harnden

Reviewed by: Michael Donaldson and Christopher Kyle

Trent University
Natural Resources DNA Profiling & Forensic Centre
2140 East Bank Drive
Peterborough ON Canada
K9L 1Z8

Purpose:

Provide a genetic characterization of a single *Canis* sample submitted by Joseph Butera.

Executive Summary:

The sample submitted yielded sufficient DNA to allow for genetic assignment among Canadian *Canis* categories in context of an in-house reference database. The sample was of an admixed nature predominated by Great Lakes wolf ancestry.

Items Receipt:

One 15 mL tube was received by the Natural Resources DNA Profiling and Forensic Centre on March 22nd, 2022. The tube contained a tongue sample cut into two pieces, suspended in ethanol. The tube was labelled as follows:

Sample: 85-pound canid's tongue for DNA testing
Collected: 1/15/2022, New York State
By: Joseph Butera
83-37 267 St. Floral Park, NY 11004 USA
LorJoewolf@J420.com

The sample was given the laboratory ID “CAN_T” and stored in a refrigerator upon arrival.

Results Summary:

A CAN_T mtDNA sequence was assigned a *C. lycaon* haplotype and inferred ancestral origins according to Wilson et al. 2003 (**Table 1**). We identified the sample as male based on Zfx/Zfy amplification for sex identification (**Table 1**). We obtained a genotype for the sample at 12 microsatellite loci (**Table 2**) and conducted an ancestry proportion test in *STRUCTURE* (**Table 1**).

Conclusion:

Laboratory ID	Genetic assignment based on microsatellite data and ancestry Q-value > 80% in context of current <i>Canis</i> database
CAN_T	admixed; predominated by Great Lakes wolf ancestry

Table 1. Summary of sexing, mtDNA haplotype, and nuDNA microsatellite results

Laboratory ID	Type	Sex (Zfx/Zfy)	mtDNA Haplotype and Inferred Ancestral Lineage		Microsatellite Loci (of 12)	Microsatellite Inferred Ancestry					
						Dog	SK Coyote	Eastern Coyote	NT Grey Wolf	Great Lakes Wolf	Eastern Wolf
CAN_T	Tissue	Male	CRL5_C3	<i>C. lycaon</i>	12	0.007	0.009	0.003	0.345	0.526	0.109

Table 2. Microsatellite genotyping data

Laboratory ID	Microsatellites locus (cxx#)																							
	225		200		123		377		250		204		172		109		253		442		410		147	
CAN_T	163	167	211	217	147	153	154	156	131	131	200	200	156	156	149	149	109	109	166	166	114	118	172	174

Methods:

DNA Extraction

A small portion of tissue was taken using a scalpel and digested in 0.5 mL of tissue lysis buffer plus 40 µL of Proteinase K. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following manufacturer protocols. The sample was processed in duplicate.

DNA Amplification

The mitochondrial DNA (mtDNA) control region was amplified and sequenced with primers AB13279 and AB13280. PCR and sequencing conditions used as described in Wheeldon et al. (2010) with the following adjustments in amplification conditions: an initial denaturing at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 2 minutes. Amplified products were separated on an agarose gel stained with ethidium bromide and visualized under UV light. Amplified products were cleaned with ExoSAPIT (ThermoFisher) and sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher).

For sex amplification, DNA was amplified at a region of the Zfx/Zfy genes on the X and Y-chromosomes and a region of the Sry gene on the Y-chromosome (P1-5EZ and P2-3EZ: Aasen and Medrano 1991; Y53-3C and Y53-3D: Fain and LeMay 1995). Sex-specific amplified products were visualized under UV light.

12 autosomal microsatellite loci (cxx225, cxx200, cxx123, cxx377, cxx250, cxx204, cxx172, cxx109, cxx253, cxx442, cxx410, and cxx147) were amplified as three multiplex reactions for each sample in a total reaction volume of 15 µL with 1X Qiagen Multiplex Mastermix (Qiagen), 0.12 µM of primers, and 2 µL of DNA. Products were amplified under the following conditions: 94°C for 15 minutes, 32 cycles of 94°C for 30 seconds, 56-58°C for 90 seconds, 72°C for 1 minute, with a final extension at 60°C for 45 minutes.

Sequenced Analysis

The mtDNA control region was sequenced using an ABI 3730 genetic analyzer (Applied Biosystems). Sequences were edited and aligned in the software package MEGA v11 and consensus sequences were aligned with sequences of known haplotypes from the NRDPFC *Canis* database consisting of published haplotypes from Wilson et al 2003, Rutledge et al 2010, and Wheeldon et al. 2010. Additionally, sequences were compared to the National Center for Biotechnology Information (NCBI) database with the Basic Local Alignment Search Tool (BLAST). Haplotypes were assigned based on the NRDPFC database, and in reference to any identical sequences in the NCBI database. Putative species of origin were assigned to each sample based on the mtDNA sequences in context of published haplotypes and putative ancestry origin according to Wilson et al. (2003).

Microsatellite Genotyping

All autosomal microsatellite amplifications were analyzed on an ABI 3730 (Applied Biosystems) genetic analyzer.

Inferring Ancestry Proportion using STRUCTURE

To infer *Canis* ancestry proportions of CAN_T, we used an in-house database consisting of 350 reference genotypes from samples assigned to Ontario (ON) *Canis* groups (60 Great Lakes wolf, 60 eastern wolf, 60 eastern coyote), 60 ON domestic dog, 60 Saskatchewan (SK) coyote (unhybridized *C. latrans*), and 50 Northwest Territories (NT) grey wolf (unhybridized *C. lupus*). We estimated population clustering and ancestry proportions using *STRUCTURE* v2.3.4 (Pritchard et al. 2000, Falush et al. 2003, 2007) with default settings. We implemented the F-model (i.e., the correlated allele frequencies model) and ran the admixture model 50 times at $K = 1$ through $K = 10$ for $5.0E+5$ iterations following an initial burn-in of $2.5E+5$ iterations. Results were evaluated using the online tool CLUMPAK vbeta (Kopelman et al. 2015), where we retrieved *STRUCTURE* plots and admixture proportions (Q-values) across the runs. We assigned the individual to populations based on a Q-value threshold of 0.800 (note, this value is a commonly used threshold in the literature, but other values could be applied); otherwise, we considered the individual to be admixed.

References:

- Aasen E, and Medrano J. 1991. Amplification of the Zfy and Zfx Genes for Sex Identification in Humans, Cattle, Sheep and Goats. *Biotechnology*. 8: 1279-81.
- Fain SR, and LeMay JP. 1995. Gender identification of humans and mammalian wildlife species from PCR amplified sex linked genes. *Proc. Am. Acad. Forensic Sci.* 1:34.
- Falush D, Stephens M, and Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*. 164: 1567-1587.
- Falush D, Stephens M, and Pritchard JK. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*. 7:574-578.
- Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, and Mayros I. 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*. 15: 1179-1191.
- Pritchard JK, Stephens M, and Donnelly P. 2000. Inference of population structure from multilocus genotype data. *Genetics*. 155:945–959.
- Rutledge L, Garroway C, Loveless K, and Patterson BR. 2010. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. *Heredity*. 105:520-531.
- Wheeldon TJ, Patterson BR, and White BN. 2010. Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. *Molecular Ecology*. 19:4428-4440.
- Wilberg MJ, and Dreher BP. 2004. GeneCap: a program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. *Molecular Ecology Notes*. 4: 783-785.
- Wilson PJ, Grewal S, McFadden T, Chambers RC, and White BN. 2003. Mitochondrial DNA extracted from eastern North American wolves killed in the 1800s is not of gray wolf origin. *Canadian Journal of Zoology*. 81(5): 936-940.