Fox Rothschild LLP
ATTORNEYS AT LAW

Mail: P. O. Box 5231, Princeton, NJ 08543-5231
Princeton Pike Corporate Center
997 Lenox Drive
Lawrenceville, NJ 08648-2311
Tel 609.896.3600 Fax 609.896.1469
www.foxrothschild.com

Vincent A. Vietti
Office Managing Partner

Filed via ePermits

Date: June 29, 2023

RE: 3-200-37a: Import / Export / Re-export of Live Animals under CITES/ESA
Application Number CS0582384

POWER OF ATTORNEY
OF
Judith Berens

I, Judith Berens, with an address of 2143 D Road, Loxahatchee, Florida 33470 make, constitute, and appoint my Attorney, Dr. Nany E Halpern with an address of 997 Lenox Drive, Lawrenceville, NJ 98648 and Sheri Sublet, with an address of 1158 Pellum Road, Waltersboro, SC 29488 as my agent.

I am the applicant of application number CS0582384, submitted on January 23, 2023 on behalf of Panther Ridge Conservation Center, Inc., and I hereby appoint my/our attorney, Nancy E. Halpern, DVM, Esquire, partner at Fox Rothschild LLP to transact all business in the U.S. Fish and Wildlife Service connected therewith for the referenced application identified above.

Signed on July 5, 2023

Judith Berens

Executive Director/President, Panther Ridge Conservation Center, Inc.

Fox Rothschild LLP
ATTORNEYS AT LAW

WITNESSES (both of whom are

18 years of age or older):

[redacted]

Octavio Herrera., Witness

[redacted]

Sherika Allen, Witness

(NAME OF WITNESS)

STATE OF FLORIDA

COUNTY OF [COUNTY])

) ss. Palm Beach.

)

The foregoing instrument was acknowledged before me by means of ☒ physical presence or ☐ online notarization this [DATE] 10th day of [MONTH] July, [YEAR] 2023 by [PRINCIPAL NAME] Sherika Allen, who is personally known to me or has produced [TYPE OF IDENTIFICATION] FLDL as identification.

Given under my hand and seal of office, on [MONTH] [DATE], [YEAR].

July 10th 2023.

[NOTARY NAME TYPED/PRINTED/STAMPED] Octavio Herrera.

[NOTARY TITLE/RANK] Notary Public.

[NOTARY SERIAL NUMBER, IF ANY]

HH 350713

OCTAVIO HERRERA
Notary Public - State of Florida
Commission # HH 350713
My Comm. Expires Jan 18, 2027

January 15, 2023

U.S. Fish and Wildlife Service, International Affairs
Division of Management Authority, Branch of Permits
5275 Leesburg Pike, MS: IA
Falls Church, Virginia 22041

To USFWS:

The purpose of importation of the Ocelots in this application is for both zoological display and scientific research.

I am the Executive Director of TPFECF (Twin Pine Farm & Exotics Conservation Foundation) located in South Carolina. I have assisted Panther Ridge in the importation of these two animals; we actively work with Texas A&M on a few scientific research projects with other species and we are delighted to start a new project including Ocelots.
We have imported various species, native to other countries, that are rare to the United States. Each animal we bring in is quarantined, observed, vetted, documented, microchipped & dna samples are obtained to be entered into a studbook. We assure that the animals brought in by TPFECF go through this process to protect their future in the United States; ensuring their species is properly managed and the husbandry remains strong in captivity.

We look forward to being able to contribute to this scientific research and study of the Ocelot feline species being done by Texas A&M and Brian Davis.

Sincerely,

Sheri Sublett
Executive Director
TPFECF
Twin Pine Farm & Exotics Conservation Foundation
info@TPFECF.org
843-310-4441

FWS Form 3-200-37a (Rev. 01/2020)
U.S. Department of the Interior

OMB Control No. 1018-0093
Expires 08/31/2023

IMPORT/EXPORT/RE-EXPORT OF LIVE ANIMALS (CITES/ESA)

☒New ☐Reissue/Renew ☐Amendment

Complete Sections **A or B**, and C, D, and E of this application. U.S. address may be required in Section C.**

A. Complete if applying as an individual

1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2 Date of birth (mm/dd/yyyy)	5.a. Telephone number	5.b. Alternate telephone number	6. E-mail address

B. Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution	1.b. Doing business as (dba)	
PANTHER RIDGE CONSERVATION CENTER INC		

2. Tax identification no.	3.a. Description of business, agency, Tribe, or institution	3.b. Website URL (if applicable)
57-115211	Wildlife & Wild Felid Endangered Conservatory, Accredited Zoo, Nonprofit 501(c)(3) open to the public promoting education through conservation	Https://www.PANTHERridge.org

4.a. Principal officer (P.O.) last name	4.b. P.O. first name	4.c. P.O. middle initial	4.b. P.O. Title
Berens	Judith	M	Executive Director/President

5. Primary contact name	6. Primary e-mail address
Sheri L. Sublett	Zooery@lowcountry.com

7.a. Business telephone number	7.b. Alternate phone no.	8.a. Primary contact telephone no.
561-795-8914	561-797-9544	843-217-9188

C. All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)
2143 D road

1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country
Loxahetchee	Florida	33470	Palm Beach	USA

2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)
1158 Pellum Road

2.b. City	2.c. State	2.d. Zip code/Postal code	2.e. County/Province	2.f. Country
Walterboro	SC	29488	Colleton	USA

D. All applicants MUST complete

1. Include a check or money order, payable to the U.S. FISH AND WILDLIFE SERVICE, **a nonrefundable processing fee** [50 CFR 13.11(d)(4)]. Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))

2. If you are requesting a reissue/renew/amendment, what is your permit/file number?

3. Certification: I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13 of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

Judith M. Berens — Judith M Berens — 4/2/22

The individual/principal officer of the business must print and sign the application. (No photocopied or stamped signatures) — Date (mm/dd/yyyy)

** Further instructions for the above application may be found on our ePermits website. See the last page for information on the Privacy Act, Paperwork Reduction Act, Estimated Burden, and Freedom of Information Act aspects of this application form.

Mail your application(s) to Division of Management Authority, Branch of Permits, MS:IA 5275 Leesburg Pike, Falls Church, VA 22041-3803.

E. IMPORT/EXPORT/RE-EXPORT OF LIVE ANIMALS (CITES/ESA)

General Information

This application covers activities involving LIVE CITES and ESA listed animals.

Review this application carefully and **provide complete answers to all of the questions**. If you are applying for multiple species, be sure to indicate which species you are addressing in each response. **If more space is needed, attach a separate sheet with your responses, numbered according to the questions.**

Please allow at least 90 days for the application to be processed.

How do I determine whether the species is protected under CITES and/or the ESA?

CITES	ESA
To determine whether an animal species is protected under CITES, when the species was listed, or whether exemptions apply to your requested activity, see the list of CITES species	To determine whether an animal species is protected under the ESA, please review the list of ESA-listed species in the Code of Federal Regulations. Please be aware that any permit request involving an **ESA endangered species** must be published in the Federal Register for a required 30-day public comment period.

Questions
If you have any questions regarding an action you are requesting authorization for please contact the Division of Management Authority at managementauthority@fws.gov.

Please note: for renewal or amendment of a multi-use permit being requested **within the 5-year** Federal Register public notice period, use application 3-200-52

This form should NOT be used for:

- Pre-Convention, Pre-Act, or antique (antique exemption criteria) specimens (use application 3-200-23)
- Captive Bred Wildlife Registration (use application form 3-200-41)
- ESA Plants (use application form 3-200-36)
- Import of LIVE African Elephants from Botswana, Namibia, South Africa, and Zimbabwe and Southern White Rhinoceros from eSwatini and South Africa (use application form 3-200-37f)

Electronic Information Submission

Electronic submission of inventories, photographs, and receipts: For hard copy applications, if you wish to provide information electronically, please include a flash drive containing this information with your physical application.

1. Name and address where you wish the permit to be mailed, **if different from physical address**. If you would like expedited shipping, please enclose a self-addressed, pre-paid, computer-generated, courier service airway bill. If unspecified, all documents will be mailed via regular mail through the U.S. Postal Service.

   Sheri Lynn Sublett
   Twin Pine Farm & Exotics Conservation
   1158 Pellum Road
   Walterboro SC 29488
2. Point of contact if we have questions about the application (name, phone number, and email).

   Sheri L. Sublett; (843) 217-9188 or (843) 310-4441; info@tpfecf.org

3. Have you or any of the owners of the business (if applying as a business, corporation, or institution), been assessed a civil penalty or convicted of any criminal provision of any statute or regulation relating to the activity for which the application is filed; been convicted, or entered a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act; forfeited collateral; OR are currently under charges for any violation of the laws mentioned above?

X No __ Yes

If you answered "Yes" to Question 3, provide: a) the individual's name; b) date of charge; c) charge(s); d) location of incident; e) court, and f) action taken for each violation. Please be aware that a "Yes" response does not automatically disqualify you from getting a permit.

4. Type of Activity: ☒ Import
☐ Export
☐ Re-export (e.g. exporting a specimen that was previously imported into the United States)

5. The current location of the **animal(s) (if different from the physical address)**:

Name: Mystic Monkeys & Feathers Wildlife Park
Address: Portion 41 of the farm Buffelsdrift
City:
State/Province: 179 JR, Rust de Winter, LIMPOPO
Postal Code:
Country: South Africa

6. Recipient/Sender:

- If **export/re-export**, provide name and **physical address** of the recipient in the foreign country.
- If **import**, provide name and **physical address** of the exporter/re-exporter in the foreign country.

Name: Mystic Monkeys & Feathers Wildlife Park
Address: Portion 41 of the farm Buffelsdrift
City:
State/Province: 179 JR, Rust de Winter, LIMPOPO
Postal Code:
Country: South Africa

7. For **each animal** involved in the import/export/re-export, provide (you may use the table below):

a. Scientific name (genus, species, and *if applicable*, subspecies) Leopardus Pardalis
b. Common name Ocelot
c. Approximate or actual birth/hatch date (mm/dd/yyyy) Female: 1/11/2022
Male: 2/28/2021
d. Wild or captive-born Captive born

e. Quantity

f. Sex (males.females.unknown sex, 10.2.3)

g. Permanent markings and/or identification information (microchip #, leg band #, tattoos, studbook #).

a. Scientific name (genus, species, and *if applicable*, subspecies)	b. Common Name	c. Approximate or Actual Birth/Hatch Date (mm/dd/yyyy)	d. Wild (W) or Captive-born (C)	e. Quantity	f. Sex (male. female. unknown sex, ex: 1.0.0)	g. Permanent markings/ID information (e.g., microchip #, leg band #, tattoo, studbook #, etc.)
EXAMPLE: *Pan troglodytes*	Chimpanzee	08/01/2006	C	1	1.0.0 OR male	Studbook# 152; Microchip# 00056321-00
Leopardus Pardalis	Ocelot	12/28/2021	C	1	Male	933071000204510
Leopardus Pardalis	Ocelot	1/11/2022	C	1	Female	933071000204430

Source of Specimen

8. For **each captive-born/captive-hatched animal(s),** provide a signed and dated statement from the breeder or other appropriate documentation (e.g. Species 360 report) that includes the following:

   a. Scientific name (genus, species, and *if applicable*, subspecies), Attached

   b. Common name,

   c. Name and address of the facility where the animal was bred and born,

   d. Birth/hatch date (mm/dd/yyyy),

   e. Identification information (studbook, microchip, leg band, etc.),

   f. Name and address of the facility where the parental stock is located,

   g. A statement from the breeder that the animal was bred and born at the breeder's facility (including the facility's name and address), and

   h. If you are not the breeder, provide documentation demonstrating the history of transactions (e.g., chain of custody or ownership of the animal).

9. For **each animal(s) taken from the wild**, provide:

   a. Scientific name (genus, species, and *if applicable*, subspecies),

   b. Common name,

   c. Specific location (e.g. county, state, province, country) where the animal was removed from the wild;

   d. The name of the individual(s) who collected the animal(s) and their authorization to do so, including copies of foreign and domestic (Federal, State, and/or Tribal) government collecting permits, licenses, contracts and/or agreements;

e. Method of collection, including capture protocol and any injury and mortality experienced during collection, transport, and holding;

f. Information related to any remuneration, either financial or in-kind, provided for acquiring the animal(s);

g. Efforts to use captive specimens (e.g., captive-born, captive-held) in lieu of taking animals from the wild.

10. For **each animal being re-exported** (e.g., exporting animal(s) previously imported into the United States), provide:

a. A copy of the CITES export or re-export document issued by the appropriate CITES office in the country from which the wildlife was imported (this document is **stamped cancelled** by USFWS Office of Law Enforcement upon import inspection); and

b. A copy of your Declaration for Importation or Exportation of Fish or Wildlife (Form 3-177) **cleared** by USFWS Office of Law Enforcement.

c. A copy of the ESA permit that authorized the original import.

d. If you did not make the original import, provide documentation outlining chain-of-ownership since import, including:

1. A copy of the importer's clearance documents (a, b & c above) and,
2. Subsequent invoices (or other documentation) showing the history of transactions leading to your ownership of the animals after import (chain of custody).

Description and Justification For Requested Activity

Describe the purpose of your proposed activity.

Attached

11. If **scientific research**, provide:

a. A copy of the research proposal (outlining the purpose, objectives, and methods),
b. Detailed information on capture methods including:
   i. who will be capturing the animals
   ii. equipment used
   iii. measures taken to prevent injury and mortality
c. An explanation of whether other similar work has already been conducted or is currently being conducted,
d. A copy of the study's Institutional Animal Care and Use Committee (IACUC) form (*if applicable*),
e. Peer-reviewed scientific papers published from this research (*if applicable*).

12. If **conservation education and/or zoological display**, provide:

Attached

a. Objectives of proposed activity in support of an education program,

b. Copies of educational materials (e.g., handouts, text of signage or public presentations), incorporating the following information:

i. Status in the wild

ii. Current threats

iii. Conservation efforts

13. If **captive propagation for the conservation and survival of the species**, provide:

a. A description of how the species will be propagated (e.g. artificial insemination, breeding pairs/groups),

b. Documentation showing your participation in an established breeding program (example: current breeding plan outlining your role in the program AND letter from the breeding coordinator confirming your participation in this breeding program.)

c. How your breeding stock is managed to maintain genetic vitality, including:

i. avoidance of inbreeding,

ii. considerations of average kinship,

iii. differences in paternal and maternal average blood relationships/relatedness,

iv. carrying capacity of your facility,

v. disposition of progeny.

d. Plans and agreements for future re-introduction (*if applicable*).

14. Please provide a detailed description on how the proposed activities will **enhance or benefit the wild population within its native range** (e.g., direct or indirect conservation efforts) and provide documentation (e.g., **signed** memorandums of understanding) demonstrating your commitment to supporting the program and how the program contributes directly to the species identified in your application.

Technical Expertise & Facilities

- For **export/re-export**, provide information for the **receiving institution.**
- For **import**, provide information for **your institution**.
- For **import to multiple facilities**, provide information for **all receiving institutions.**

15. CV or resume outlining the technical experience of each caretaker working with, maintaining, and/or propagating **each** species, as it relates to the proposed activities, including experience with similar species. Attached resume

16. Current inventory of the species at the facility (males.females.unknown sex, e.g., 10.2.3), See attachments

17. Number of years the species has been maintained at the facility, 24 years

18. Number of births per species per year over the last 5 years, 0 of this soecies

19. Number of mortalities per species (or similar species) per year over the last 5 years and steps taken to avoid or decrease such mortalities, See attachment

20. A detailed description, diagrams, and photos clearly depicting the existing facilities **where the wildlife will be maintained** including: dimensions, construction materials, and protection from the elements. Do not provide blueprints; See attachment

21. Approximate carrying capacity for the species at the facility. Can house up to ten of this species

Transport/Shipment of Live Animals

22. Transport conditions for live animals must comply with the CITES Guidelines for Transport of Live Animals. All air transport must also comply with the International Air Transport Association (IATA) live animal regulations (contact airline for information). As such, provide:

a. The type, size, and construction of any shipping container and,

b. The arrangements for watering or otherwise caring for the wildlife during transport.

Wooden custom crate, 1200lX500wX500w, one divider within, openings on each side, with wire mesh for protection to administer zupreem feline canned diet during travel and a PVC construct with an opening on the outside of crate on both sides (for both animals) that allows water to be poured in from ohtside down the pvc and into water dish.

All international shipment(s) must be through a designated port. A list of designated ports (where an inspector is posted) is available. If you wish to use a port not listed, please contact the Office of Law Enforcement for a Designated Port Exemption Permit (form 3-200-2).

CITES Appendix I & Marine Mammal Species

- For **export** of a **CITES Appendix I-listed species**, provide a copy of the CITES import permit, or evidence one will be issued by the Management Authority of the country to which you plan to export the specimen(s). In accordance with Article III of the CITES treaty, it is required that import permits are issued before the

corresponding export permit.

- For **import** of **CITES Appendix-I listed species**, provide information to show the import is not primarily for commercial purposes as outlined in Resolution Conf. 5.10 (Rev. CoP 15).
- For **import** of **live CITES Appendix-I marine mammals**, provide a copy of your FWS or NMFS Marine Mammal Protection Act (MMPA) permit or authorization.

NOTICES

PRIVACY ACT STATEMENT

Authority: The information requested is authorized by the following: the Bald and Golden Eagle Protection Act (16 U.S.C. 668), 50 CFR 22; the Endangered Species Act (16 U.S.C. 1531-1544), 50 CFR 17; the Migratory Bird Treaty Act (16 U.S.C. 703-712), 50 CFR 21; the Marine Mammal Protection Act (16 U.S.C. 1361, et seq.), 50 CFR 18; the Wild Bird Conservation Act (16 U.S.C. 4901-4916), 50 CFR 15; the Lacey Act: Injurious Wildlife (18 U.S.C. 42), 50 CFR 16; Convention on International Trade in Endangered Species of Wild Fauna and Flora (TIAS 8249), 50 CFR 23; General Provisions. 50 CFR 10; General Permit Procedures, 50 CFR 13; and Wildlife Provisions (Import/export/transport), 50 CFR 14.

Purpose: The collection of contact information is to verify the individual has an eligible permit to conduct activities which affect protected species. This helps FWS monitor and report on protected species and assess the impact of permitted activities on the conservation and management of species and their habitats.

Routine Uses: The collected information may be used to verify an applicant's eligibility for a permit to conduct activities with protected wildlife; to provide the public and the permittees with permit related information; to monitor activities under a permit; to analyze data and produce reports to monitor the use of protected wildlife; to assess the impact of permitted activities on the conservation and management of protected species and their habitats: and to evaluate the effectiveness of the permit programs. More information about routine uses can be found in the System of Records Notice, Permits System, FWS-21.

Disclosure: The information requested in this form is voluntary. However, submission of requested information is required to process applications for permits authorized under the listed authorities. Failure to provide the requested information may be sufficient cause for the U.S. Fish & Wildlife Service to deny the request.

PAPERWORK REDUCTION ACT STATEMENT

We are collecting this information subject to the Paperwork Reduction Act (44 U.S.C. 3501) in order provide the U.S. Fish and Wildlife Service the information necessary, under the applicable laws governing the requested activity, for which a permit is requested. Information requested in this form is purely voluntary. However, submission of requested information is required in order to process applications for permits authorized under the applicable laws. Failure to provide all requested information may be sufficient cause for the U.S. Fish and Wildlife Service to deny the request. According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. OMB has approved this collection of information and assigned Control No. 1018-0093.

ESTIMATED BURDEN STATEMENT

We estimate public reporting for this collection of information to average 2 hours, including time for reviewing instructions, gathering and maintaining data and completing and reviewing the form. Direct comments regarding the burden estimate or any other aspect of the form to the Service Information Clearance Officer, Fish and Wildlife Service, U.S. Department of the Interior, 5275 Leesburg Pike, MS: BPHC, Falls Church, VA 22041-3803, or via email at info_Coll@fws.gov.

Please do not send your completed form to this address.

e-mail: blouduiker@mweb.co.za
website: www.mysticmonkeys.co.za
Tel: +27 82 566 4929
Fax: +27 0866774020
Vat: 4310157740

Postnet Suite 27
Private Bag X1604
Bela-Bela
Limpopo

Portion 41 of the Farm Buffelsdrift
179JR, Rust de Winter, LIMPOPO

29 March 2022

Ocelot information:

I Christa Saayman, ID number 6308130073084, owner of Mystic Monkeys & Feathers Wildlife Park hereby confirm the following:

1) Scientific name:
2) Common name: Ocelot
3) Name and address of the facility where the animal was bred and born:
   Mystic Monkeys & Feathers Wildlife Park,
   Portion 41 of the Farm Buffelsdrift 179JR, Rust de Winter,
   Limpopo, South Africa
4) Birth date (mm/dd/yyyy)
   - Ocelot female = 11/ 1/2022
   - Ocelot male = 28/12/2021
5) Identification information (microchip)
   Ocelot female = 11/ 1/2022 microchip number: (933071000204430)
   Ocelot male = 28/12/2021 microchip number: (933071000204510)
6) Name and address of the facility where the parental stock is located:
   Mystic Monkeys & Feathers Wildlife Park,
   Portion 41 of the Farm Buffelsdrift 179JR, Rust de Winter,
   Limpopo, South Africa
7) Dimentions of the crates (2 in 1):
   1200 Lenght X 500 Width X 500 Height

Hope that you find above mentioned in order!

MYSTIC MONKEYS &
FEATHERS WILDLIFE PARK
CHRISTA SAAYMAN - OWNER
CELL: (+27)82 566 4929

Kind regards

Christa Saayman
Owner of Mystic Monkeys & Feathers Wildlife Park

LIMPOPO PROVINCIALGOVERNMENT
Dept. of Economic Development,
Environment & Tourism

Permit No: CPM-005-00001
(to Establish and Operate a Wild Animal Park)

CERTIFICATE OF ORIGIN

(1) EXPORTER	NO: 019745	ORIGINAL
MYSTIC MONKEYS AND FEATHERS WILDLIFE PARK PORTION 41 OF THE FARM BUFFELSDRIFT 179JR RUST DE WINTER, LIMPOPO PROVINCE REP. OF SOUTH AFRICA	ISSUED BY: THE GREATER BOKSBURG CHAMBER OF COMMERCE AND INDUSTRY **ASSOCIATION NOT FOR GAIN** Cnr 11th Avenue & 4th Street Boksburg North 1459 Gauteng R.S.A. Tel: 083 417 3585 Fax: 086 696 8048 P.O. Box 133 Boksburg 1460 Republic of S.A.	
(2) CONSIGNEE PANTHER RIDGE CONSERVATION CENTER INC. 2143 D ROAD LOXAHATCHEE FL 33470		
(3) COUNTRY OF ORIGIN REP. OF SOUTH AFRICA		
(4) MODE OF TRANSPORT AIR	(5) REMARKS	

(6) ITEM NO	(7) MARK AND NUMBERS	(8) DESCRIPTION OF GOODS	(9) QUANTITY	(10) INVOICE PRICE
01	AS ADD.	1:1 X LIVE OCELOTS (LEOPARDUS PARDALIS) ANIMAL CHIP NUMBERS: 1. 933071000204430 (FEMALE) 2. 933071000204510 (MALE) COUNTRY OF ORIGIN: SOUTH AFRICA ZOO BORN AT MYSTIC MONKEYS AND FEATHERS WILDLIFE PARK	2 / TWO	AS PER ATT. INV.

I, the undersigned, hereby certify on behalf of the **GREATER BOKSBURG CHAMBER OF COMMERCE AND INDUSTRY** that there has been lodged with the Chamber a sworn statement by a duly authorised signatory, on behalf of the exporter, to the effect that the information relating to the merchandise described above is true and correct.

CERTIFICATE ONLY VALID WHEN ENDORSED WITH THE STAMP OF THE GREATER BOKSBURG CHAMBER OF COMMERCE AND INDUSTRY

Boksburg Chamber of Commerce & Industry
Address: 367 Commissioner Street
Boksburg 1459
Tel No. 010 285 0313 Cell No: 083 417 3585
E-mail: admin@gbccci.co.za

19/04/2022

for **The Greater Boksburg Chamber of Commerce & Industry**

Alterations only approved when validated

SCHOOL OF VETERINARY MEDICINE
& BIOMEDICAL SCIENCES
Department of Veterinary Integrative Biosciences
Department of Small Animal Clinical Sciences

Brian W. Davis, Ph.D.
Assistant Professor, Research

January 1, 2023

U.S. Department of the Interior
5275 Leesburg Pike MS: BPHC
Falls Church, VA 22041-3803

To whom it may concern,

My name is Dr. Brian W. Davis. I am an evolution nary and medical geneticist at Texas A&M University School of Veterinary Medicine and Biomedical Sciences. I am also the founder and Executive Director of Exotic Genome, a biobank that acts as a repository for DNA and tissues for veterinary research. Please see my faculty information and publication record located at http://scholars.tamu.edu.

I have agreed to work and collaborate with Twin Pine Farm Exotics Conservation Foundation and Panther Ridge Conservation Center regarding ocelots (*Leopardus pardalis*) being imported from South Africa. As part of my work at Texas A&M and with Exotic Genome, I will sample their DNA and retain it in my biobank. Genomic information will be generated for these animals and compared to data available from mine and my collaborators' previous work on North, Central, and South American felids, as well as the remainder of family Felidae. Our previous work in ocelots has focused on the Y chromosome, but larger population information is being generated. Please see attached articles for details.

If any further information is required, please feel free to contact me.

Sincerely,

Brian W Davis, Ph.D.
Research Assistant Professor, Texas A&M University
Department of Veterinary Integrative Biosciences
Department of Small Animal Clinical Sciences
College of Veterinary Medicine and Biomedical Sciences
Texas A&M University bdavis@cvm.tamu.edu

Veterinary Medical Research Building, Room 318
4458 TAMU
College Station, TX 77843

Tel. 979.458.7818
bdavis@cvm.tamu.edu

Downloaded from genome.cshlp.org on January 4, 2023 - Published by Cold Spring Harbor Laboratory Press

Phylogenomic evidence for ancient hybridization in the genomes of living cats (Felidae)

Gang Li,[1] Brian W. Davis,[1,2,4] Eduardo Eizirik,[3] and William J. Murphy[1,2]

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Inter-species hybridization has been recently recognized as potentially common in wild animals, but the extent to which it shapes modern genomes is still poorly understood. Distinguishing historical hybridization events from other processes leading to phylogenetic discordance among different markers requires a well-resolved species tree that considers all modes of inheritance and overcomes systematic problems due to rapid lineage diversification by sampling large genomic character sets. Here, we assessed genome-wide phylogenetic variation across a diverse mammalian family, Felidae (cats). We combined genotypes from a genome-wide SNP array with additional autosomal, X- and Y-linked variants to sample ~150 kb of nuclear sequence, in addition to complete mitochondrial genomes generated using light-coverage Illumina sequencing. We present the first robust felid time tree that accounts for unique maternal, paternal, and biparental evolutionary histories. Signatures of phylogenetic discordance were abundant in the genomes of modern cats, in many cases indicating hybridization as the most likely cause. Comparison of big cat whole-genome sequences revealed a substantial reduction of X-linked divergence times across several large recombination cold spots, which were highly enriched for signatures of selection-driven post-divergence hybridization between the ancestors of the snow leopard and lion lineages. These results highlight the mosaic origin of modern felid genomes and the influence of sex chromosomes and sex-biased dispersal in post-speciation gene flow. A complete resolution of the tree of life will require comprehensive genomic sampling of biparental and sex-limited genetic variation to identify and control for phylogenetic conflict caused by ancient admixture and sex-biased differences in genomic transmission.

[Supplemental material is available for this article.]

There is an emerging consensus that gene flow frequently occurs following speciation despite the establishment of reproductive barriers that otherwise maintain species-level distinctiveness (Roca et al. 2005; Good et al. 2008; Ellegren et al. 2012; Garrigan et al. 2012; Toews and Brelsford 2012; Cahill et al. 2013, 2014; Cui et al. 2013; Martin et al. 2013; Kutschera et al. 2014; Sullivan et al. 2014). However, incomplete lineage sorting (ILS) is assumed by default to underpin most cases of phylogenetic discordance. Few studies in the literature account for hybridization by analyzing all inheritance patterns (uniparental, sex-biased, biparental) with high-resolution data, or instead have focused on only a few species (Roca et al. 2005; Cahill et al. 2013, 2014; Trigo et al. 2013; Khan et al. 2014). The cat family Felidae contains 38 recognized species within eight lineages (designated henceforth by a capitalized name, e.g., Puma lineage) that vary in the breadth of their geographic occurrence (Buckley-Beason et al. 2006; Johnson et al. 2006). While the relationships within many felid clades are robust to variation in subgenomic sampling, several inter-generic and inter-specific relationships remain unresolved and have not been assessed genome-wide to determine the specific drivers of discordance observed in previous studies (Johnson et al. 2006; Davis et al. 2010).

Although hybrid zones between related cat species have been reported (Schwartz et al. 2004; Homyack et al. 2008; Trigo et al. 2008, 2013), the extent to which ancient and contemporary introgression has occurred is poorly understood on broad geographic and genomic scales. Recent genetic evidence suggests complex patterns of admixture in felids of the Neotropical genus *Leopardus*, including the presence of cryptic species (Trigo et al. 2008, 2013). These observations are matched by the prevalence of felid hybridization in captivity (Gray 1972), which has generated numerous hybrids of both large cats and medium to small cats. These include the gigantic liger, a hybrid between a male lion and female tiger, as well as domestic cat inter-specific hybrid breeds, including the Bengal and Savannah, which are common household pets worldwide. This proclivity for hybridization is facilitated by the strong colinearity among felid genomes coupled with recent genetic divergence (Wurster-Hill and Centerwall 1982; Davis et al. 2009; Cho et al. 2013). The genomes of modern felids thus present a unique resource to study the dynamics of introgression and the genetic basis of reproductive isolation in both controlled crosses and natural populations (Davis et al. 2015).

Previous studies have demonstrated that robust phylogenetic signal can be obtained by querying domestic animal SNP arrays with DNA from related species of the same genus, family, or order, despite having diverged tens of millions of years from the array reference genome (Decker et al. 2009; McCue et al. 2012). Here, we generated genome-wide SNP data from 38 cat species and analyzed

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these separately and together with Y-linked variation and whole mitogenomes, allowing us to disentangle different maternal, paternal, and biparental histories within a diverse family of mammals. We assessed genome-wide patterns of intra-lineage phylogenetic discordance and identified signals of ancient hybridization throughout the genomes of many cat species. Many of these nuclear signatures were accompanied by patterns of mitonuclear discordance. Our results allow further insight into the evolutionary processes leading to the diversification of extant cats of the world and provide a roadmap for future in-depth population genomics in this group as a model system for better understanding the speciation process.

Results

We generated Illumina whole-genome genotyping data (~63,000 SNPs) for 100 felid DNA samples covering virtually all recognized species and validated the consistency of genotyping quality and utility for phylogenomic inference (see Supplemental Table S1; Supplemental Fig. S1; Methods). SNP call rates ranged from 92% to 99% across felids and were generally correlated with previous estimates of phylogenetic divergence (Johnson et al. 2006). Within each felid lineage, SNP call rates for each species were very similar (Supplemental Table S1), indicating that comparisons between members of the same felid lineage (i.e., admixture tests) should not be compromised by array ascertainment bias.

After excluding low-quality SNPs and heterozygous sites, we generated maximum likelihood (ML) phylogenies for the combined SNP supermatrix (Fig. 1) and for each chromosome (Supplemental Fig. S2). No individual chromosome was strongly discordant with the biparental SNP-based phylogeny, except for the Y Chromosome data set from Johnson et al. (2006), which strongly supported a closer association of the Bay cat lineage with the *Panthera* lineage (also observed by Luo et al. 2014). To confirm that SNP-based characters were robust for phylogenetic inference, we compared our results to trees derived from an independent 50-kb supermatrix of complete felid vomeronasal receptor (*V1R*) gene repertoires for 27 felids (Montague et al. 2014). The *V1R* ML topology showed strong congruence with the SNP-based phylogeny and confirmed the nested position of the Bay

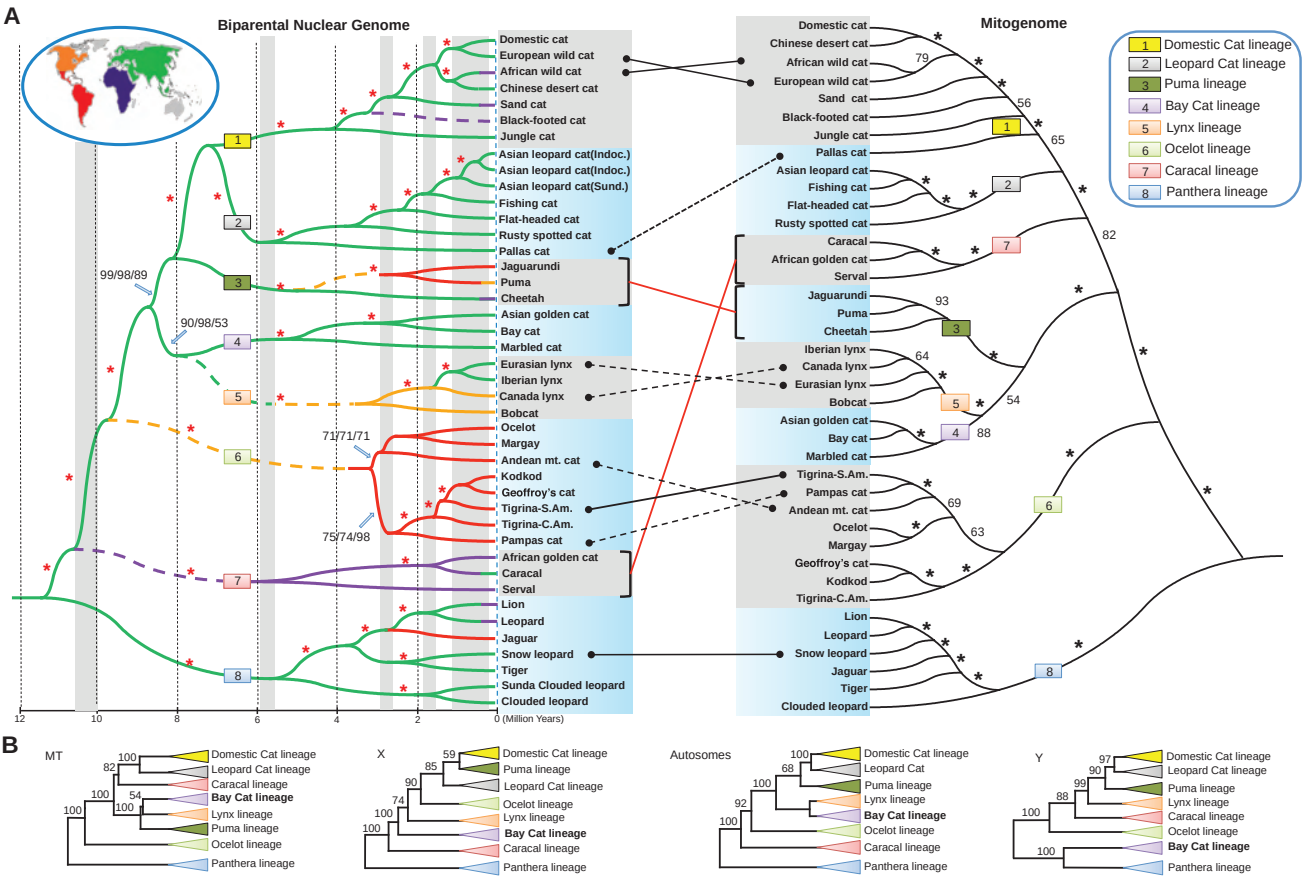

Figure 1. Discordant phylogenetic patterns across maternal, paternal, and biparentally inherited subgenomic partitions. (*A*) Comparison of nuclear genome and mitogenome phylogenies. The nuclear phylogeny/time tree excludes the Y Chromosome partition. Lines *between* the two trees indicate alternative placement of particular species (black) or clades (red). Dashed lines represent poorly supported alternative placements. The time tree is based on the average divergence times across eight individual MCMCtree analyses (Supplemental Table S3). Lineages are color-coded based on their current/historical distributions (see *inset* map). Dashed lines indicate hypothesized dispersal events out of Eurasia. Gray vertical bars indicate periods of extended low sea level (Haq et al. 1987) that may have facilitated dispersal between continents/islands. Asterisks indicate ML bootstrap support values of 100 in all analyses (all SNPs/binary-coded SNPs/SNP + gene supermatrix). (*B*) Phylogenies showing relationships and bootstrap support values for eight felid lineages based on the mitogenome, X Chr (5761-bp), Y Chr (5352-bp), and autosomal (123,906-bp) SNP + gene supermatrix partitions. Bootstrap support for monophyly of each lineage is 100% (data not shown). The Bay cat clade is displayed in boldface to highlight the different topological positions based on different modes of inheritance.

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cat lineage and more basal positions for the Caracal and Ocelot lineages (Supplemental Fig. S3). We then generated a robust felid phylogeny (Fig. 1A) based on biparentally inherited data (~130-kb from SNPs + *V1R* genes + published genes) that removed the confounding effects of paternal evolutionary history (Fig. 1B).

Mitonuclear discordance

Next, we contrasted the maternal and biparental evolutionary history of felids by assembling complete mitochondrial genomes of nearly all recognized species (except *Neofelis diardi* and *Leopardus guttulus*) using light coverage whole-genome sequencing (see Methods; Supplemental Table S1). Remapping the raw reads to each assembly revealed extremely high and even depth of coverage for each mitochondrial genome (avg. 135-fold) compared to the expected nuclear genome coverage (avg. 0.3-fold coverage). This process supported the true cytoplasmic origin of each mitogenome assembly. When we compared the mitogenome-based phylogeny to our nuclear genome species tree, we identified nine topological conflicts, including strongly supported differences within *Panthera* (i.e., the position of the snow leopard) as well as the relative position of the Puma and Caracal lineages within Felidae (Fig. 1B). The mitogenomic phylogeny supported a sister-group relationship between the Bay cat and Lynx lineages that was also observed in the biparental phylogeny but not in the Y Chromosome phylogeny (Fig. 1B), indicating the Bay cat lineage Y Chromosome tracks a unique history relative to the other genomic partitions.

One potential and underappreciated source of mitonuclear discordance is the inadvertent amplification and sequencing of nuclear mitochondrial pseudogenes (numts) (Antunes et al. 2007). When we compared our reference mitogenomes to GenBank entries, we found that half of the published big cat (i.e., genus *Panthera* and *Puma concolor*) mitogenomes contained long stretches of high sequence divergence that were consistent with numt contamination (Supplemental Fig. S4; Supplemental Table S2). These results underscore the confounding impact that numts may have on phylogenetic relationships (Antunes et al. 2007; Davis et al. 2010) and caution against indiscriminate use of published mitochondrial DNA (mtDNA) sequences.

Felid diversification and evidence for hybridization

We then applied Bayesian divergence time and ancestral area reconstruction approaches to explore spatial and temporal patterns of diversification throughout modern felid evolution (Supplemental Figs. S5, S6; Supplemental Tables S3, S4). Our time tree indicated that the ancestors of each felid lineage originated within and dispersed out of Asia in the late Miocene, obviating the need to invoke hypotheses requiring multiple dispersal events back to Asia from North America (Fig. 1; Johnson et al. 2006). Rather, the ancestors of the Puma and Lynx lineages probably dispersed simultaneously to the Americas via the reopening of the Bering Land Bridge ~5.9 million years ago (MYA) (Koufos et al. 2005) as documented by the first occurrence of fossil Felinae in North America shortly thereafter (Qiu 2003). Older divergence times (≥10 MYA) for the progenitors of the Caracal and Ocelot lineages leave open the possibility that the ancestors of these two lineages dispersed out of Asia into Africa and the Americas, respectively, via land bridges established earlier rather than later in the Miocene (Haq et al. 1987; Koufos et al. 2005).

Given our observation of substantial phylogenetic discordance across the felid phylogeny, along with biogeographic reconstructions that indicate ample spatial overlap in the history of cat lineages, we scanned the genome-wide SNP matrix for phylogenomic patterns suggestive of inter-specific hybridization. Our first approach used sliding window-based likelihood ratio tests to identify broad (>1 Mb) chromosomal regions that harbored significantly different phylogenetic signal from the species tree (Fig. 2A,B), which would not be expected when serial species divergences were separated by millions of years (Hobolth et al. 2007, 2011). In order to distinguish discordant regions that are a result of hybridization from ILS, we estimated the *D*-statistic from the ABBA/BABA test (Table 1), which assesses phylogenetic asymmetry of nonspecies trees and the proportion of the genome that is shared between two taxa due to admixture (Durand et al. 2011). Taken together, these approaches provide genome-wide evidence for historical gene flow within the majority of the eight felid lineages (Table 1; Fig. 2).

On one end of this continuum, the Puma, Caracal, and Bay cat lineages showed fewer discordant windows and possessed *D*-statistics and *Z*-scores lower than most other clades (Table 1; Fig. 2). These lineages each contain three ecologically distinct species that diverged over long evolutionary time frames relative to other felid groups, thus ILS-based discordance is less expected. In contrast, the remaining five felid lineages possessed numerous signatures of phylogenetic discordance, some of which may be attributable to historical hybridization (Table 1; Fig. 2). One of the best documented felid hybrid zones is between the bobcat and Canada lynx, which share a broad trans-continental range overlap in North America that has likely persisted to varying degrees as climate fluctuated across time. Genetic studies have identified several populations where hybridization is common along the US/Canada border (Schwartz et al. 2004; Homyack et al. 2008; Koen et al. 2014). ABBA/BABA tests indicate ancient signatures of bobcat-Canada lynx gene flow (Table 1), consistent with an extended period of gene flow that continues to the present day. Our results also indicate introgression between the Canada lynx and Eurasian lynx (Table 1), which likely occurred during recurrent emergence of the Bering Land Bridge during the Pleistocene.

The greatest amount of phylogenetic discordance was observed within the Ocelot lineage, which diversified within the past 2–3 million years (MY) in the Neotropics, where many species coexist in sympatry or parapatry. Recent genetic analyses demonstrated complex speciation/hybridization affecting at least four species within this group: two tigrina species, one from northeastern Brazil (*Leopardus tigrinus*) that possesses pampas cat (*L. colocolo*) mitochondria within a tigrina-like nuclear background, and a recently proposed sister species from southeastern Brazil (*L. guttulus*, not sampled here) which hybridizes with the Geoffroy's cat (*L. geoffroyi*) in a narrow hybrid zone in southern Brazil (Trigo et al. 2008, 2013, 2014). As predicted, the northeastern tigrina we genotyped possessed pampas cat mtDNA within a tigrina nuclear DNA background (Supplemental Fig. S8) but also contained nuclear signatures of ancient hybridization with the Geoffroy's cat (Table 1; Fig. 3A), suggesting an extended history of admixture between the three species lineages. A Central American tigrina (Fig. 1; Supplemental Fig. S8) showed large mitochondrial and nuclear divergence (11.0%–15.3% and 0.5%–0.6%, respectively) from Brazilian tigrinas, as well as from the Geoffroy's cat and kodkod (*L. guigna*). Tigrinas have not been extensively sampled for genetic variation across the northern part of their range; however, our data support previous observations based solely on mtDNA (Johnson et al. 1999; Trigo et al. 2008) of the potential existence of an additional, presently unrecognized Central American cat species.

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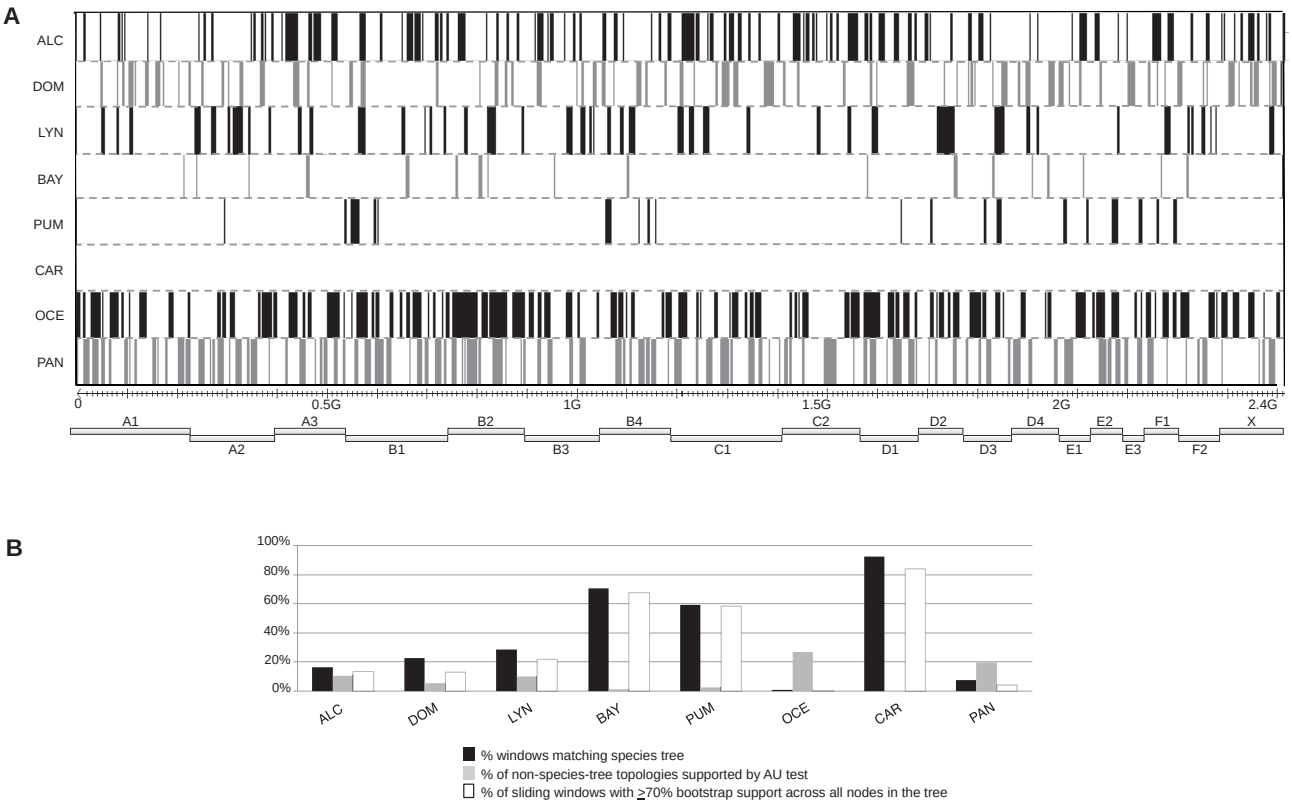

Figure 2. Signatures of genome-wide phylogenetic discordance. (*A*) Results of sliding window-based approximately unbiased (AU) tests for each of the eight felid lineages (see Methods for details). Horizontal panels indicate genomic regions (vertical bars) that produced significantly better topologies than the species tree topology. Felid chromosomes are displayed in bars *below* the scale spanning the length of the feline genome. Abbreviations for lineages are as follows: (ALC) Asian leopard cat, (DOM) Domestic cat, (LYN) Lynx, (BAY) Bay cat, (PUM) Puma, (CAR) Caracal, (OCE) Ocelot, (PAN) *Panthera*. (*B*) Summary statistics for results in Figure 1A. The white bars represent the percentage of sliding windows supporting the species tree topology (black bars) with bootstrap support ≥ 70% (Hillis and Bull 1993). The gray bars indicate the proportion of nonspecies tree topologies identified by the AU test that were significantly better than the species tree (Fig. 1A).

The Asian Leopard cat lineage (genus *Prionailurus*) includes a similar radiation of small-bodied cats distributed throughout Southeast Asia. The leopard cat is broadly sympatric with other members of the genus, and recent molecular evidence indicates that its Indochinese and Sundaic populations display species-level measures of Y Chromosome and mitochondrial divergence (Luo et al. 2014). Whole-genome SNP data from 13 Asian leopard cats sampled across these regions confirmed a species-level separation of more than one million years, which was further supported by deep mtDNA divergence (Fig. 3B). Interestingly, mitogenome phylogenies support a closer relationship between the fishing cat and Indochinese leopard cat populations than between the latter and supposed conspecifics, a pattern consistent with ancient hybridization when coupled with evidence of inter-species gene flow from SNP-based admixture tests (Table 1). This unique pattern of mitonuclear discordance suggests capture of an unsampled or extinct *Prionailurus* sp. mitochondrion in the ancestral lineage of the fishing cat. As with the Ocelot lineage, further phylogeographic sampling of Asian leopard cats throughout their broad distribution, along with their closely related congeners, will further aid in delimiting new species boundaries more conclusively.

Within the Domestic cat lineage, species/subspecies of the closely related *Felis silvestris* complex are known to hybridize in nature (e.g., Nussberger et al. 2013; Le Roux et al. 2015) and hybrids of the domestic cat and Jungle cat are the progenitors of an exotic cat breed known as the Chausie. Similarly, we find evidence for two episodes of ancient admixture within the Domestic cat lineage derived from *D*-statistics inferred from the SNP matrix (Table 1). To demonstrate that our SNP-based *D*-statistics were not artifacts due to ascertainment bias driven by factors such as genotyping error or recurrent mutation, we analyzed ~20–30× Illumina whole-genome sequence coverage for three of the non-*silvestris* members of the Domestic cat lineage: Black-footed cat, Sand cat, and Jungle cat, and a close outgroup species, the Asian leopard cat. We aligned these reads to the domestic cat v6.2 assembly, generated reference assemblies, and calculated *D*-statistics after stringent filtering of the SNV data set (mean = 26.6 million SNVs per species) (see Methods). Our results (Supplemental Table S5; Fig. 3C) provide highly significant *D*- and *Z*-scores between the same pairs of taxa observed with the SNP array-based statistics (Table 1).

As an additional validation of the SNP-based *D*-statistics, we remapped the domestic cat SNP probes to the new *Felis* genome sequences and recalculated the statistics from a reduced set of highest confidence orthologous SNPs, for which we required 99% pairwise identity between the domestic cat probe sequence and 100% identity to the SNP call in the reference assemblies of the other felids. The results (Supplemental Table S6) demonstrated that even with a smaller, high confidence set of orthologous SNPs that match the sequence probe (and the SNP call) precisely,

Table 1. Measures of phylogenetic discordance and admixture within felid lineages

Lineage	% of windows matching species tree	% of nonspecies-tree topologies supported by likelihood ratio tests	Species tree (P1,P2) P3	Outgroup	ABBA/BABA results				
					D	*Z*	*P*	ABBA	BABA
Ocelot	1.5	26.0	(Kodkod, Tigrina_S.Am.), Pampas cat [a]	Serval	0.102 ± 0.057	1.782	0.0748	173	141
			(Kodkod, Geoffroy's cat), Tigrina (S.Am.) [a]	**Serval**	**0.146 ± 0.048**	**3.064**	**0.0022**	**232**	**173**
			(Ocelot, Margay), Pampas cat	Serval	0.063 ± 0.063	1.466	0.1427	271	239
			(Ocelot, Margay), Tigrina (S.Am.)	Serval	0.029 ± 0.045	0.079	0.9368	293	295
Panthera	14	14.3	(Leopard, Lion), Jaguar	Sunda Clouded leopard	0.013 ± 0.043	0.310	0.7565	264	257
			(Lion, Leopard), Tiger	Sunda Clouded leopard	0.031 ± 0.048	0.652	0.5144	215	202
			(Snow leopard, Tiger), Leopard	Sunda Clouded leopard	0.056 ± 0.042	1.345	0.1788	291	260
			(Tiger, Snow leopard), Lion[a,b]	Domestic cat	0.041 ± 0.001	33.39	0	357,875	329,510
Lynx	37.3	8.7	**(Iberian lynx, Canada lynx), Bobcat** [a]	**Asian Leopard cat**	**0.176 ± 0.056**	**3.182**	**0.0015**	**204**	**143**
			(Iberian lynx, Eurasian lynx), Canada lynx	**Asian Leopard cat**	**0.199 ± 0.047**	**6.112**	**1.9×10^{-5}**	**274**	**183**
Leopard cat	26.6	7.6	**(Sunda ALC, Indochinese ALC), Fishing cat** [a]	**Pallas cat**	**0.179 ± 0.047**	**3.847**	**0.0001**	**277**	**193**
Domestic cat	33.1	4.4	**(Sand cat, Domestic cat), Black-footed cat**	**Jungle cat**	**0.152 ± 0.023**	**6.630**	**3.4×10^{-11}**	**1033**	**761**
			(Black-footed cat, Sand cat), Jungle cat	**Bobcat**	**0.090 ± 0.028**	**3.223**	**0.0013**	**690**	**577**
			(Domestic cat, Sand cat), Jungle cat	**Bobcat**	**0.186 ± 0.024**	**7.626**	**2.4×10^{-14}**	**926**	**636**
Puma	65.7	1.5	(Jaguarundi, Puma), Cheetah	Serval	0.024 ± 0.044	0.546	0.5850	257	245
Bay cat	80.4	1.0	(Bay cat, Asian golden cat), Marbled cat	Tiger	0.139 ± 0.042	2.976	0.0029	312	243
Caracal	98.0	0.0	(African golden cat, Caracal), Serval	Asian golden cat	0.058 ± 0.069	0.842	0.3997	109	97

Boldface indicates significant *D*-statistics ($Z \geq 3.0$) with SNP and/or SNV data.
[a]Species comparisons with documented mitochondrial introgression or hybridization.
[b]Whole-genome SNV alignment results.

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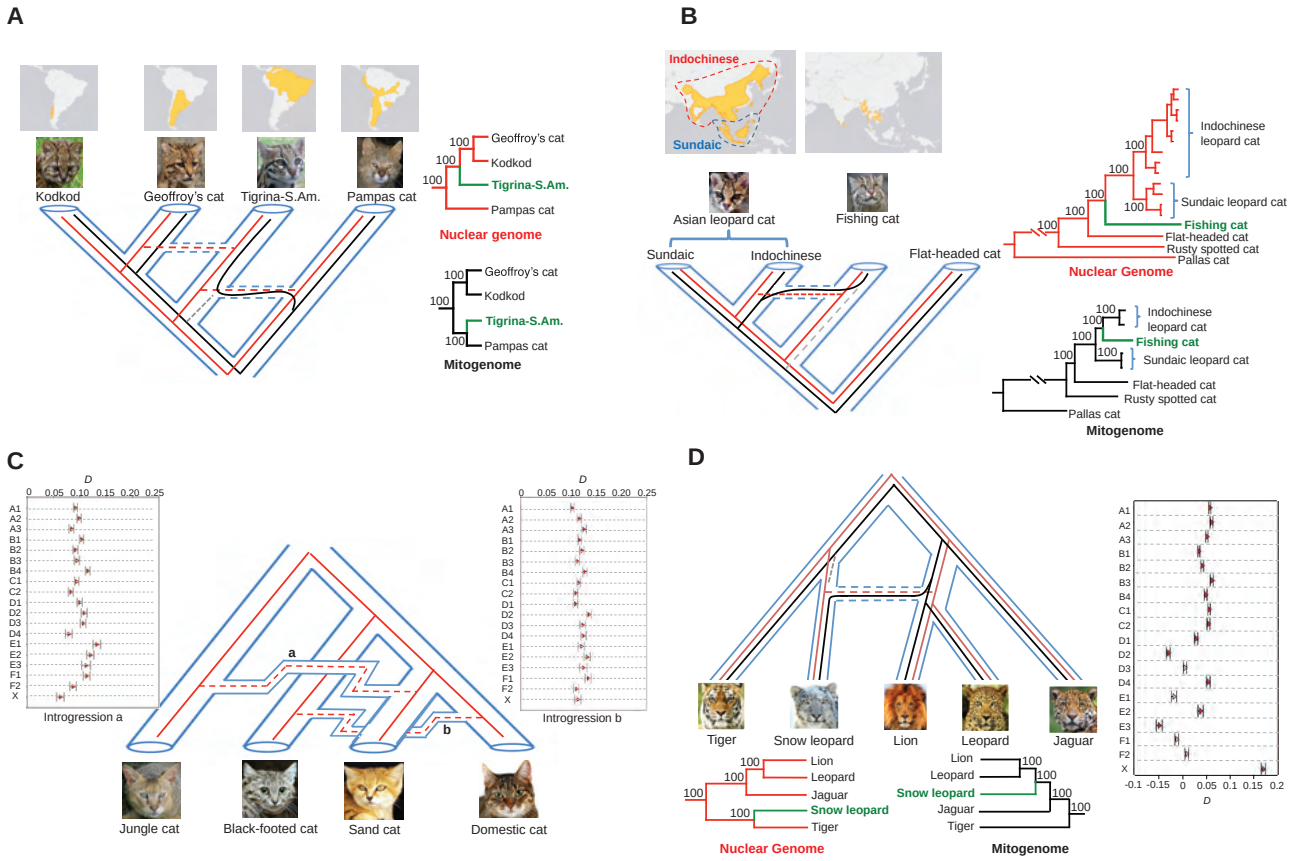

Figure 3. Patterns of hybridization within felid lineages. (*A*) Ocelot lineage, showing the discordant position of the northern tigrina (green) between nuclear (red) and mitogenome (black) phylogenies. *Within* the species tree is shown nuclear (red) and mitogenome (black) genealogies, with dashed red lines indicating genomic evidence for ancient hybridization (not scaled to time). (*B*) Asian leopard cat lineage, showing the discordant position of the fishing cat (green) relative to the Asian leopard cat populations in nuclear and mitogenome trees. (*C*) Domestic cat lineage, showing the results of admixture tests based on whole-genome SNV alignments which reveal two strong introgression signals (a and b) across the whole genome of *Felis* species. (*D*) *Panthera* lineage, showing the discordant position of the snow leopard (green) between nuclear and mitogenome phylogenies. Admixture tests from whole-genome SNV alignments support gene flow from the lion/leopard lineage to the snow leopard. Note the significantly skewed *D*-statistic for the X Chromosome (*right*).

the *D*-statistics remain significant. These results confirm that phylogenetic noise or genotyping errors were not biasing the results and further validated the use of the array-based *D*-statistics. As a third measure to assess the potential impact of phylogenetic noise in the SNP array data generated for the non-*Felis* lineages, we jackknifed the outgroup taxon for the significant comparisons shown in Table 1 of the manuscript, and in nearly all cases the *Z*-scores remained significant (Supplemental Table S7).

Big cat hybridization and remarkable patterns of X Chromosome divergence

Big cats of the genus *Panthera* readily reproduce in captivity (Gray 1972), yielding many possible hybrids among the parent species, including the liger (male lion × female tiger). The recent sequencing of several big cat genomes (Cho et al. 2013) provided an opportunity to perform a high-resolution test of the hypothesis that ancient introgression may have led to the mitonuclear discordance in the phylogenetic position of the snow leopard (Figs. 1, 3D). Genome-wide analysis of tiger, lion, and snow leopard single nucleotide variants (mean = 20.8 million SNVs/species), using the domestic cat as the outgroup, identified significant signatures of admixture between the lion and snow leopard genomes (Supplemental Table S8; Fig. 3). The most striking signal was observed for the X Chromosome, where sliding window-based divergence time estimates were significantly younger than autosomes and notably enriched for topologies supporting a sister relationship between snow leopard and lion (Supplemental Fig. S9; Fig. 4A), similar to the mitogenome phylogeny where the snow leopard is sister to lion and leopard. Furthermore, our estimates for lion/snow leopard X Chromosome divergence were very similar to the mean mitogenomic divergence (~2.1 MYA) (Supplemental Fig. S5B).

The distribution of the alternative topologies on *Panthera* X Chromosomes was nonrandom, with more than half occupied by massive blocks of reduced divergence time. These blocks correspond to one ~45-Mb recombination cold spot and at least two smaller 5- to 10-Mb cold spots on the domestic cat X Chromosome (Schmidt-Kuntzel et al. 2009), a genomic feature virtually absent on autosomes (Fig. 4B). Intriguingly, a large recombination cold spot, also flanked by high recombination rate regions, is found on the domestic pig X Chromosome in nearly the same syntenic region (Ma et al. 2010; Ai et al. 2015). Both species share colinearity with the human chromosome over the vast majority of the

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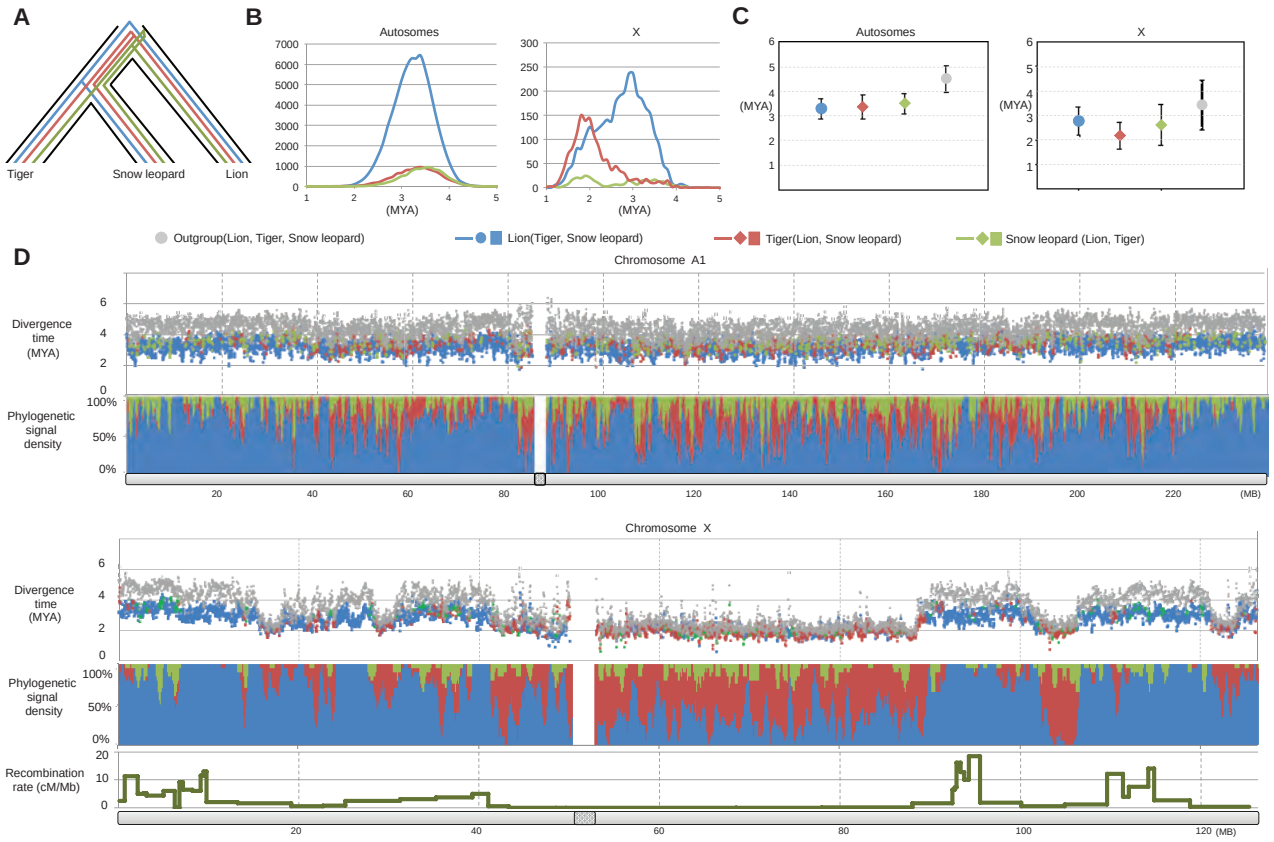

Figure 4. Evidence for admixture within *Panthera* genome sequences. (*A*) Species tree topology of the three *Panthera* species defined by black outline. Blue lines represent coalescent patterns of tiger + snow leopard shared alleles, green indicates lion + tiger shared alleles, and red represents lion + snow leopard shared alleles. (*B*) Distribution of trees supporting each topology from the genome-wide sliding window analysis (*y*-axis) plotted against divergence time (*x*-axis), shown for autosomes and the X Chromosome (see Supplemental Fig. S9 for individual chromosome plots). (*C*) Mean and standard error for divergence times for each category of sister-species relationship within the three taxa. (*D*) Phylogeny and relative divergence time (*y*-axis, in millions of years ago) for each window plotted along the largest autosome (Chromosome A1, *above*) and the X Chromosome (*below*). Dots are color-coded for each of the three sister-species relationships (see legend). The gray dots indicate the age of the base of *Panthera*. Note the drop in divergence times for both internal and basal nodes in regions corresponding to extremely low recombination rates (*bottom*). Recombination data are from the domestic cat linkage map (Schmidt-Kuntzel et al. 2009).

chromosome (Murphy et al. 1999; Davis et al. 2009; Ma et al. 2010), which would preclude an inversion-based mechanism to explain this extremely large nonrecombining region that is shared across divergent mammalian orders.

Recently, Ai et al. (2015) found similar patterns of introgression between high- and low-altitude populations of pigs within the large X Chromosome recombination cold spot and suggested that these events were driven by an adaptive sweep. Natural selection is most effective in low-recombination regions (Smith and Haigh 1974), particularly on the X Chromosome, where both beneficial and deleterious recessive alleles are exposed in hemizygous males (Charlesworth et al. 1987). However, distinguishing positive from background selection can be difficult, as both types of selection will shorten the time to the most recent common ancestor (O'Fallon 2013). We suggest that the marked inter-node depth and time reduction of (lion + snow leopard) phylogenies within but not outside of low-recombination regions is also consistent with a similar selective sweep across this region (Fig. 4B).

Moreover, we propose that the reduced lion–snow leopard X Chromosome divergence, together with the retention of a lion/leopard-related mitochondrion within the snow leopard genome (Fig. 1) can be best explained under a scenario of hybridization, similar to that invoked to explain recent X Chromosome divergence between chimpanzee and human (Patterson et al. 2006). If fertile F1 female hybrids, derived from snow leopard-ancestor males and lion/leopard-ancestor females, backcrossed to males from the snow leopard ancestor, a strong selective sweep favoring a newly beneficial X Chromosome allele(s), coupled with severely limited recombination across a large portion of the X Chromosome (Fig. 4), could account for the presence of this large introgressed region within the genome of modern snow leopards. The persistence of phylogenetically discordant X Chromosome haplotypes is expected as they maintain a mitochondrion-like signature longer than other nuclear markers, being carried twice as often by females than males. This trend would be exacerbated in X Chromosome regions with extremely reduced recombination.

Discussion

Like most mammals, felid males disperse farther than females (Sunquist and Sunquist 2002), hence the maternally transmitted mitochondrial genome is not predicted to reflect species boundaries as accurately as Y Chromosome and autosomal markers (Currat et al. 2008; Petit and Excoffier 2009). This prediction is supported

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by the greater discordance between the felid mitochondrial phylogeny and different nuclear partitions, the latter of which are more similar (Fig. 1). In addition, felid inter-species hybrids follow Haldane's Rule (Haldane 1922) specifically as it relates to hybrid sterility: Females are generally fertile, while males are overwhelmingly sterile (Gray 1972; Davis et al. 2015). Unisexual sterility coupled with male-biased dispersal promotes situations where the mitochondrion of one species or lineage may persist within the genomic background of a different species following introgression into one of the parental populations (e.g., Roca et al. 2005). This pattern is exemplified by the northeastern tigrina and the fishing cat genomes, which possess mitochondrial DNA more similar to that of a different species.

Such sex-biased asymmetries in fertility and dispersal may also explain the observed discordancies at deeper branches of the felid phylogeny when interpreted jointly with Bayesian ancestral geographic reconstructions (Supplemental Fig. S6). In one example, the mitogenomic phylogeny strongly supports the Puma lineage as sister to the Lynx+Bay cat clade, while the nuclear genome places the Puma lineage as sister to the Asian leopard cat+Domestic cat clade. Although ILS could explain this pattern, we interpret the strongly supported alternate topologies and the reduced relative divergence times between genomic partitions (Supplemental Fig. S5A,B) as evidence for historical admixture and mitochondrial capture between ancestors of the Puma lineage and Lynx+Bay cat lineage within Asia, preceding migration to North America in the Late Miocene (Fig. 1). Similarly, the Caracal clade origin is ~2 MY and multiple inter-nodes younger in the mitogenome tree than in the biparental tree (Fig. 1A,B; Supplemental Fig. S5A,B), a pattern that is also consistent with a scenario involving mitochondrial capture.

Sex-biased asymmetry can also produce rare instances of Y Chromosome discordance, the nuclear marker that is least expected to show discordance in species with male-biased dispersal (Petit and Excoffier 2009). Such an example exists within the felid phylogeny, where the 9.5 MYA coalescence of the Bay cat and *Panthera* lineage Y Chromosomes (Luo et al. 2014) post-dates the estimated biparental lineage divergence by >2 MY, spanning several internal nodes that are discordant between the two topologies. We interpret this pattern as indication of an episode of ancient admixture within Eurasia where these two lineages originated and diversified (Supplemental Fig. S6). Introgression of a *Panthera*-like Y Chromosome into the Bay cat lineage ancestor early during post-speciation divergence could have been derived from the generation of fertile male backcross hybrids, and their transient increased fitness over nonhybrid males. This could effectively result in a selective sweep by a *Panthera*-like Y Chromosome within the nuclear genome background of a Bay cat lineage ancestor. In this particular scenario, it is worth noting that the ancestors of each of these cat lineages were much less divergent at the putative time of hybridization than many living felids that currently produce viable and fertile inter-specific hybrids (Gray 1972; Schwartz et al. 2004).

Although phylogenetic discordance is often attributed to ILS, the low effective population size of mitochondrial and Y Chromosome DNA, and that of felid species in general, coupled with the influence of male-mediated dispersal and Haldane's Rule on introgression, suggests that the conflicting phylogenetic signals between genomic partitions are best explained by hybridization (Currat et al. 2008; Petit and Excoffier 2009; Cahill et al. 2013). Our observations of historical admixture between many pairs of cat species are consistent with evidence for contemporary inter-specific hybridization in nature and captivity spanning large phylogenetic distances. Ongoing whole-genome sequencing efforts in different felid species should provide much greater resolution and insight into the genomic landscape of admixture and provide further tests of these SNP array-based inferences.

Hybridization is a natural component of the evolutionary process, yet anthropogenic influences may further promote inter-specific hybridization in low-density carnivore populations (Allendorf et al. 2001). This is especially critical for the majority of cat species worldwide, where habitat encroachment, poaching, deforestation, and climate change are drastically reducing felid numbers. We have highlighted groups of species where further population genomic sampling will better define the architecture of admixture and genes underlying adaptive introgression and divergence. Differentiating between natural and human-mediated hybridization will be critical to develop effective conservation efforts on behalf of these threatened carnivores.

Methods

SNP genotyping and analysis

We used 150–300 ng of DNA from each felid species to genotype ~63,000 SNPs on the Illumina feline array. The 63K array was designed using the domestic genome sequence as a reference with 62,897 SNPs ascertained from six domestic cats and an African wild cat (*Felis lybica*) (Mullikin et al. 2010), distributed across all 18 autosomes and the X Chromosome. The vast majority of the SNPs on the array were ascertained in a pool of domestic cat breeds from low-coverage whole-genome sequencing (Mullikin et al. 2010). Illumina array genotypes for all individuals were filtered to include only SNP call rates >95%. We excluded all SNPs included in the array design that assay felid lineage-specific apomorphies and phenotype-associated mutations (Eizirik et al. 2003; Johnson et al. 2006). Heterozygous sites (predominantly found in the domestic cat and its closest relatives, the European wild cat, African wild cat, and Chinese desert cat) (Supplemental Table S1) were scored as ambiguities in phylogenetic analyses only.

Since the creation of the 63K chip, revisions to the feline genome assembly required reassessment of the physical marker location of the SNP probe sequences. We compared each probe sequence to the *Felis catus* v6.2 (felCat5) assembly using BLAST (Altschul et al. 1997). After removal of SNPs with negative and/or highly duplicated probe mapping results, 59,628 SNPs remained in our analyses. The highest nondomestic cat SNP calling rate was observed between the three wildcat subspecies/species of the *Felis silvestris* complex (i.e., European wild cat, African wild cat, and Chinese desert cat; average call rate > 99.4%). The lowest SNP call rate (86%) was observed for the Andean mountain cat DNA sample, extracted from a museum hide, which may have contributed to its poor phylogenetic placement within the Ocelot lineage.

To demonstrate that a potential bias of nucleotide identification caused by the two-dye Illumina genotyping system on nondomestic cat species did not influence phylogenetic accuracy, we also performed a second phylogenetic analysis in which the nuclear SNP matrix was translated into a binary matrix ("0" representing G or C; "1" representing A or T), following Decker et al. (2009).

Mitochondrial genome sequencing and assembly

To obtain high-quality complete mitochondrial genome sequences and to avoid sequencing nuclear mitochondrial pseudogenes, we isolated DNA from mitochondria-enriched preparations of

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fibroblast cells for 35 felid species where cell lines or tissues were available. Mitochondrial enrichment was performed with a two-step sucrose gradient procedure (Jones et al. 1988) that yields both mitochondrial and nuclear-enriched pellets. Each pellet was extracted using the Qiagen DNeasy kit. Standard Illumina fragment libraries (~300-bp average insert size) were prepared for each mitochondria-enriched DNA isolate and were sequenced to ~0.3× genome-wide depth of coverage on the Illumina HiSeq 2000 platform. We generated de novo mitochondrial genome assemblies with SOAPdenovo2 (Luo et al. 2012) by evaluating a series of *k*-mer sizes. Following assembly, all raw Illumina reads were mapped to assembled contigs using default settings in BWA-MEM to assess coverage depth (Li and Durbin 2009). Mapping results were analyzed using SAMtools, including removal of PCR duplicates (Li et al. 2009). Mitochondrial contigs were identified with BLAST (Altschul et al. 1997) comparisons to the domestic cat mitochondrial genome and confirmed by read-depth statistics.

Sequence alignment, phylogenetic reconstruction, and tree topology comparison

The SNP matrix was combined with several published genes and gene supermatrices, including a 5.6-kb *CES7* gene matrix (Li et al. 2011), the 23-kb multigene supermatrix of Johnson et al. (2006), and a 49.7-kb vomeronasal (*V1R*) gene supermatrix (Montague et al. 2014). Sequence alignments were performed with MAFFT (Katoh and Toh 2010). We applied MODELTEST (Posada and Crandall 1998) to select the best-fitting substitution model for each data set. RAxML 7.2.8 (Stamatakis 2006) was used for maximum likelihood tree searching and bootstrapping. We also analyzed a more conservative subset of the SNP matrix (40,225 SNPs) that excluded all SNP markers whose flanking probe sequence failed to produce a single unique BLAST hit with 100% nucleotide identity to the felCat5 (v6.2) genome reference sequence. This precaution was taken because SNPs on the array were identified from previous low coverage (1.9×) assemblies of the domestic cat genome, i.e., versions felCat3/4 (Pontius et al. 2007; Mullikin et al. 2010).

Divergence time estimation and ancestral area reconstruction

We used the MCMCtree v4.8a software in the PAML-4 package (Yang 2007) to estimate divergence times using previously published fossil constraints for Felidae (Johnson et al. 2006). Analyses were run for 100,000 generations with a burn-in of 10,000 generations. Analyses were run twice to check for convergence. The final time trees in Supplemental Figure S5, A and B are based on the mean of analyses that varied fossil constraint (hard- versus soft-bounded), rate model (independent versus autocorrelated), and character sampling (only for the nuclear gene supermatrix, both with and without the SNP matrix) (Supplemental Tables S3, S4). Ancestral area reconstructions were performed with the Bayesian Binary MCMC (BBM) approach implemented in the software RASP (Ali et al. 2012).

Detection of historical gene flow

We used Consel (Shimodaira and Hasegawa 2001) to perform approximately unbiased (AU) tests to compare different tree topologies across the whole-genome SNP matrix. We divided the whole-genome matrix into 10-Mb sliding windows (step = 100-kb), chosen to contain adequate sequence variation within each felid lineage (Supplemental Table S9) to allow for examination of variation in phylogenetic signal across each chromosome. Maximum likelihood trees for each window were built using RAxML (GTR + Γ substitution model) and compared to the null hypothesis (i.e., the species tree topology).

To detect admixture over longer evolutionary time scales, we applied the ABBA/BABA approach of Green et al. (2010) to the SNP matrix as well as whole-genome SNV data sets (see below). This method identifies imbalances in alternative topology frequencies under a four-taxon scenario (three ingroup taxa and one outgroup). For analyses based on the Illumina SNP matrix, we tested alternative phylogenetic scenarios only within members of the eight felid lineages, to eliminate any ascertainment bias that would result from the SNPs being discovered in the domestic cat (*Felis silvestris catus*). Although each SNP character state that is observed within any of the non-*Felis* cat lineages may be due to recurrent mutation, relative to the domestic cat from which they were discovered, these mutations are unique within each of the felid lineages and will track lineage-specific changes within that clade and in its immediate ancestors. Whether the mutation occurred within the immediate ancestor of the domestic cat and the ancestors of another felid lineage is irrelevant because we did not include members of the Domestic cat lineage as outgroup taxa when calculating SNP array-based *D*-statistics for the other seven felid lineages. All *D*-statistics calculated within the Domestic cat lineage were validated using whole-genome sequencing SNV data, to rule out ascertainment bias effects within the members of the genus *Felis*.

D-statistics and *Z*-scores calculations were performed with the R package evobiR (http://www.uta.edu/karyodb/evobiR/index.html), which measures signatures of alternative phylogenetic asymmetry and the proportion of the genome that is shared between two taxa due to admixture, respectively (Durand et al. 2011). ABBA/BABA tests performed on the SNP data set included heterozygous sites by generating pseudohaploid sequences, following Green et al. (2010) and Cahill et al. (2013, 2014). Statistical significance of the *Z*-score was assessed for each replicate by converting the *Z*-score into a two-tailed *P*-value (Eaton and Ree 2013). One thousand bootstrap iterations were used to measure the standard deviation of the *D*-statistic derived from the SNP-array data, because the ABBA/BABA SNP sites are largely unlinked (~1 SNP per 4 Mb) (Eaton and Ree 2013, Streicher et al. 2014). We defined significant *D*-statistics as those having *Z*-scores ≥ 3 (following Green et al. 2010; Cahill et al. 2013).

Whole-genome sequencing and alignment of *Felis* species

We constructed standard 300-bp insert Illumina fragment libraries using high molecular weight DNA extracted from fibroblast cell cultures of sand cat (*Felis margarita*), jungle cat (*Felis chaus*), and Asian leopard cat (*Prionailurus bengalensis*). For each sequencing library, we generated ~25× genome coverage with 2 × 125-bp reads on the Illumina HiSeq 2500 platform. In addition, we analyzed ~713 million raw Illumina sequence reads (2 × 100-bp, Illumina HiSeq 2000) from a black-footed cat, *Felis nigripes*. Reads were trimmed (Trim Galore, http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and mapped to the v6.2 domestic cat genome assembly using BWA-MEM (Li and Durbin 2009) with default settings. SAMtools (Li et al. 2009) was used to call raw single nucleotide variants (SNV). We filtered the SNV data as follows: (1) We identified high-quality SNVs (quality > 100); (2) we restricted our analysis to variants with mapping quality ≥ 30 and excluded variants from regions with read-depth variation greater or less than 50% of the genome-wide average; (3) we evaluated the likelihood score for each genotype (i.e., likelihood of double-strand alternative genotype equal to 0); and (4) we merged the filtered SNV data to whole-genome alignments conforming to the structure of the domestic cat v6.2 reference genome assembly. *D*-statistics were

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calculated in a similar fashion as for the SNP data set; however, statistical significance of the *Z*-score was assessed with a block jackknife test (5-Mb block size) with 100 replicates resampled with replacement.

Whole-genome analysis of *Panthera* species

To detect signals of phylogenetic discordance and introgression within the whole genomes of tiger, lion, and snow leopard, we generated *D*-statistics and performed a sliding window-based divergence time scan across all chromosomes. Illumina short reads from the three *Panthera* species (Cho et al. 2013) were aligned to the repeat-masked domestic cat genome assembly (serving as the outgroup) using BWA (Li and Durbin 2009) with the following settings: bwa aln –n 0.08. SNV data for three *Panthera* species was obtained and filtered applying the same strategy used for the *Felis* whole-genome data. Whole-genome four-way alignment blocks were created and analyzed in 100-kb windows (with 25-kb moving steps) across the whole-genome alignment. *D*-statistics were calculated in the same manner as the *Felis* data set (described above). Maximum likelihood trees were constructed for each window with RAxML (Stamatakis 2006) (GTR+GAMMA substitution model). We used MCMCtree in PAML to calculate the relative divergence time for each node, assuming independent rates and employing three soft-bounded constraints established from the 95% credibility intervals of our biparental matrix time tree (Supplemental Table S3): a minimum of 8 MY and maximum of 16 MY for the split between the domestic cat and *Panthera*, and a maximum of 7 MY for the basal *Panthera* node. These secondary constraints were consistent with the fossil constraints of Johnson et al. (2006).

Data access

Mitochondrial sequence data generated for this study have been submitted to GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) under accession numbers KP202255–KP202295. The Illumina genotype matrix is deposited in DRYAD (doi:10.5061/dryad.751cv). Whole genome Illumina sequencing reads from *Felis chaus*, *Felis margarita*, *Felis nigripes*, and *Prionailurus bengalensis* are deposited in the NCBI Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra/) under accession numbers SRX1058146, SRX1058385, SRS1087700, and SRX1058464, respectively. Scripts used for sliding-window analysis can be found in the Supplemental Material.

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Author contributions: G.L. and W.J.M. conceived the study, designed the experiments, and wrote the manuscript, with input from all authors. G.L. and W.J.M. analyzed the data. B.W.D. prepared mitochondria and nuclear-enriched DNAs. E.E. contributed reagents and sequences.

References

Ai H, Fang X, Yang B, Huang Z, Chen H, Mao L, Zhang F, Zhang L, Cui L, He W, et al. 2015. Adaptation and possible ancient interspecies introgression in pigs identified by whole-genome sequencing. *Nat Genet* **47:** 217–225.

Ali SS, Yu Y, Pfosser M, Wetschnig W. 2012. Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). *Ann Bot* **109:** 95–107.

Allendorf FW, Leary RF, Spruell P, Wenburg JK. 2001. The problems with hybrids: setting conservation guidelines. *Trends Ecol Evol* **16:** 613–622.

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25:** 3389–3402.

Antunes A, Pontius J, Ramos MJ, O'Brien SJ, Johnson WE. 2007. Mitochondrial introgressions into the nuclear genome of the domestic cat. *J Hered* **98:** 414–420.

Buckley-Beason VA, Johnson WE, Nash WG, Stanyon R, Menninger JC, Driscoll CA, Howard J, Bush M, Page JE, Roelke ME, et al. 2006. Molecular evidence for species-level distinctions in clouded leopards. *Curr Biol* **16:** 2371–2376.

Cahill JA, Green RE, Fulton TL, Stiller M, Jay F, Ovsyanikov N, Salamzade R, St John J, Stirling I, Slatkin M, et al. 2013. Genomic evidence for island population conversion resolves conflicting theories of polar bear evolution. *PLoS Genet* **9:** e1003345.

Cahill JA, Stirling I, Kistler L, Salamzade R, Ersmark E, Fulton TL, Stiller M, Green RE, Shapiro B. 2014. Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Mol Ecol* **24:** 1205–1217.

Charlesworth B, Coyne JA, Barton NH. 1987. The relative rates of evolution of sex-chromosomes and autosomes. *Am Nat* **130:** 113–146.

Cho YS, Hu L, Hou HL, Lee H, Xu JH, Kwon S, Oh S, Kim HM, Jho S, Kim S, et al. 2013. The tiger genome and comparative analysis with lion and snow leopard genomes. *Nat Commun* **4:** 2433.

Cui R, Schumer M, Kruesi K, Walter R, Andolfatto P, Rosenthal GG. 2013. Phylogenomics reveals extensive reticulate evolution in *Xiphophorus* fishes. *Evolution* **67:** 2166–2179.

Currat M, Ruedi M, Petit RJ, Excoffier L. 2008. The hidden side of invasions: massive introgression by local genes. *Evolution* **62:** 1908–1920.

Davis BW, Raudsepp T, Pearks Wilkerson AJ, Agarwala R, Schäffer AA, Houck M, Chowdhary BP, Murphy WJ. 2009. A high-resolution cat radiation hybrid and integrated FISH mapping resource for phylogenomic studies across Felidae. *Genomics* **93:** 299–304.

Davis BW, Li G, Murphy WJ. 2010. Supermatrix and species tree methods resolve phylogenetic relationships within the big cats, *Panthera* (Carnivora: Felidae). *Mol Phylogen Evol* **56:** 64–76.

Davis BW, Seabury CM, Brashear W, Li G, Roelke-Parker M, Murphy WJ. 2015. Mechanisms underlying mammalian hybrid sterility in two feline interspecies models. *Mol Biol Evol* **32:** 2534–2546.

Decker JE, Pires JC, Conant GC, Mckay SD, Heaton MP, Chen KF, Cooper A, Vilkki J, Seabury CM, Caetano AR, et al. 2009. Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics. *Proc Natl Acad Sci* **106:** 18644–18649.

Durand EY, Patterson N, Reich D, Slatkin M. 2011. Testing for ancient admixture between closely related populations. *Mol Biol Evol* **28:** 2239–2252.

Eaton DA, Ree RH. 2013. Inferring phylogeny and introgression using RADseq data: an example from flowering plants (*Pedicularis*: Orobanchaceae). *Syst Biol* **62:** 689–706.

Eizirik E, Yuhki N, Johnson WE, Menotti-Raymond M, Hannah SS, O'Brien SJ. 2003. Molecular genetics and evolution of melanism in the cat family. *Curr Biol* **13:** 448–453.

Ellegren H, Smeds L, Burri R, Olason PI, Backström N, Kawakami T, Künstner A, Mäkinen H, Nadachowska-Brzyska K, Qvarnström A, et al. 2012. The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature* **491:** 756–760.

Garrigan D, Kingan SB, Geneva AJ, Andolfatto P, Clark AG, Thornton KR, Presgraves DC. 2012. Genome sequencing reveals complex speciation in the *Drosophila simulans* clade. *Genome Res* **22:** 1499–1511.

Good JM, Hird S, Reid N, Demboski JR, Steppan SJ, Martin-Nims TR, Sullivan J. 2008. Ancient hybridization and mitochondrial capture between two species of chipmunks. *Mol Ecol* **17:** 1313–1327.

Gray AP. 1972. *Mammalian hybrids, a check-list and bibliography*, rev. ed. Commonwealth Agricultural Bureaux, Bucks, England.

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai W, Fritz MH, et al. 2010. A draft sequence of the Neandertal genome. *Science* **328:** 710–722.

Haldane JBS. 1922. Sex ratio and unisexual sterility in hybrid animals. *J Genet* **12:** 101–109.

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Haq BU, Hardenbol J, Vail PR. 1987. Chronology of fluctuating sea levels since the Triassic. *Science* **235:** 1156–1167.
Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol* **42:** 182–192.
Hobolth A, Christensen OF, Mailund T, Schierup MH. 2007. Genomic relationships and speciation times of human, chimpanzee, and gorilla inferred from a coalescent hidden Markov model. *PLoS Genet* **3:** e7.
Hobolth A, Dutheil JY, Hawks J, Schierup MH, Mailund T. 2011. Incomplete lineage sorting patterns among human, chimpanzee, and orangutan suggest recent orangutan speciation and widespread selection. *Genome Res* **21:** 349–356.
Homyack JA, Vashon JH, Libby C, Lindquist EL, Loch S, McAlpine DF, Pilgrim KL, Schwartz MK. 2008. Canada lynx-bobcat (*Lynx canadensis* × *L. rufus*) hybrids at the southern periphery of lynx range in Maine, Minnesota and New Brunswick. *Am Mid Nat* **159:** 504–508.
Johnson WE, Slattery JP, Eizirik E, Kim JH, Raymond MM, Bonacic C, Cambre R, Crawshaw P, Nunes A, Seuanez HN, et al. 1999. Disparate phylogeographic patterns of molecular genetic variation in four closely related South American small cat species. *Mol Ecol* **8:** S79–S94.
Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O'Brien SJ. 2006. The late Miocene radiation of modern Felidae: a genetic assessment. *Science* **311:** 73–77.
Jones CS, Tegelstrom H, Latchman DS, Berry RJ. 1988. An improved rapid method for mitochondrial DNA isolation suitable for use in the study of closely related populations. *Biochem Genet* **26:** 83–88.
Katoh K, Toh H. 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **26:** 1899–1900.
Khan FAA, Phillips CD, Baker RJ. 2014. Timeframes of speciation, reticulation, and hybridization in the bulldog bat explained through phylogenetic analyses of all genetic transmission elements. *Syst Biol* **63:** 96–110.
Koen EL, Bowman J, Lalor JL, Wilson DE. 2014. Continental-scale assessment of the hybrid zone between bobcat and Canada lynx. *Biol Conserv* **178:** 107–115.
Koufos GD, Kostopoulos DS, Vlachou TD. 2005. Neogene/Quaternary mammalian migrations in Eastern Mediterranean. *Belgian J Zool* **135:** 181–190.
Kutschera VE, Bidon T, Hailer F, Rodi JL, Fain SR, Janke A. 2014. Bears in a forest of gene trees: Phylogenetic inference is complicated by incomplete lineage sorting and gene flow. *Mol Biol Evol* **31:** 2004–2017.
Le Roux JJ, Foxcroft LC, Herbst M, MacFadyen S. 2015. Genetic analysis shows low levels of hybridization between African wildcats (*Felis silvestris lybica*) and domestic cats (*F. s.catus*) in South Africa. *Ecol Evol* **5:** 288–299.
Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25:** 1754–1760.
Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Proc GPD. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25:** 2078–2079.
Li G, Janecka JE, Murphy WJ. 2011. Accelerated evolution of *CES7*, a gene encoding a novel major urinary protein in the cat family. *Mol Biol Evol* **28:** 911–920.
Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, et al. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. *Gigascience* **1:** 18.
Luo SJ, Zhang Y, Johnson WE, Miao L, Martelli P, Antunes A, Smith JL, O'Brien SJ. 2014. Sympatric Asian felid phylogeography reveals a major Indochinese-Sundaic divergence. *Mol Ecol* **23:** 2072–2092.
Ma J, Iannuccelli N, Duan Y, Huang W, Guo B, Riquet J, Huang L, Milan D. 2010. Recombinational landscape of porcine X chromosome and individual variation in female meiotic recombination associated with haplotypes of Chinese pigs. *BMC Genomics* **11:** 159.
Martin SH, Dasmahapatra KK, Nadeau NJ, Salazar C, Walters JR, Simpson F, Blaxter M, Manica A, Mallet J, Jiggins CD. 2013. Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Res* **23:** 1817–1828.
McCue ME, Bannasch DL, Petersen JL, Gurr J, Bailey E, Binns MM, Distl O, Guerin G, Hasegawa T, Hill EW, et al. 2012. A high density SNP array for the domestic horse and extant Perissodactyla: utility for association mapping, genetic diversity, and phylogeny studies. *PLoS Genet* **8:** e1002451.
Montague MJ, Li G, Gandolfi B, Khan R, Aken BL, Searle SM, Minx P, Hillier LW, Koboldt DC, Davis BW, et al. 2014. Comparative analysis of the domestic cat genome reveals genetic signatures underlying feline biology and domestication. *Proc Natl Acad Sci* **111:** 17230–17235.
Mullikin JC, Hansen NF, Shen L, Ebling H, Donahue WF, Tao W, Saranga DJ, Brand A, Rubenfield MJ, Young AC, et al. 2010. Light whole genome sequence for SNP discovery across domestic cat breeds. *BMC Genomics* **11:** 406.
Murphy WJ, Sun S, Pecon-Slattery J, Chen ZQ, O'Brien SJ. 1999. Extensive conservation of sex chromosome organization between cat and human revealed by parallel radiation hybrid mapping. *Genome Res* **9:** 1223–1230.
Nussberger B, Greminger MP, Grossen C, Keller LF, Wandeler P. 2013. Development of SNP markers identifying European wildcats, domestic cats, and their admixed progeny. *Mol Ecol Resour* **13:** 447–460.
O'Fallon B. 2013. Purifying selection causes widespread distortions of genealogical structure on the human X chromosome. *Genetics* **194:** 485–492.
Patterson N, Richter DJ, Gnerre S, Lander ES, Reich D. 2006. Genetic evidence for complex speciation of humans and chimpanzees. *Nature* **441:** 1103–1108.
Petit RJ, Excoffier L. 2009. Gene flow and species delimitation. *Trends Ecol Evol* **24:** 386–393.
Pontius JU, Mullikin JC, Smith DR, Lindblad-Toh K, Gnerre S, Clamp M, Chang J, Stephens R, Neelam B, Volfovsky N, et al. 2007. Initial sequence and comparative analysis of the cat genome. *Genome Res* **17:** 1675–1689.
Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.
Qiu ZX. 2003. Dispersals of Neogene carnivorans between Asia and North America. *Bull Am Mus Nat Hist* **279:** 18–31.
Roca AL, Georgiadis N, O'Brien SJ. 2005. Cytonuclear genomic dissociation in African elephant species. *Nat Genet* **37:** 96–100.
Schmidt-Kuntzel A, Nelson G, David VA, Schaffer AA, Eizirik E, Roelke ME, Kehler JS, Hannah SS, O'Brien SJ, Menotti-Raymond M. 2009. A domestic cat X chromosome linkage map and the sex-linked *orange* locus: mapping of *orange*, multiple origins and epistasis over *nonagouti*. *Genetics* **181:** 1411–1425.
Schwartz MK, Pilgrim KL, McKelvey KS, Lindquist EL, Claar JJ, Loch S, Ruggiero LF. 2004. Hybridization between Canada lynx and bobcats: genetic results and management implications. *Conserv Genet* **5:** 349–355.
Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* **17:** 1246–1247.
Smith JM, Haigh J. 1974. The hitch-hiking effect of a favourable gene. *Genet Res* **89:** 391–403.
Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.
Streicher JW, Devitt TJ, Goldberg CS, Malone JH, Blackmon H, Fujita MK. 2014. Diversification and asymmetrical gene flow across time and space: lineage sorting and hybridization in polytypic barking frogs. *Mol Ecol* **23:** 3273–3291.
Sullivan J, Demboski JR, Bell KC, Hird S, Sarver B, Reid N, Good JM. 2014. Divergence with gene flow within the recent chipmunk radiation (*Tamias*). *Heredity* **113:** 185–194.
Sunquist M, Sunquist F. 2002. *Wild cats of the world*. Univ. of Chicago Press, Chicago.
Toews DPL, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Mol Ecol* **21:** 3907–3930.
Trigo TC, Freitas TRO, Kunzler G, Cardoso L, Silva JCR, Johnson WE, O'Brien SJ, Bonatto SL, Eizirik E. 2008. Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L. geoffroyi* and *L. tigrinus* in southern Brazil. *Mol Ecol* **17:** 4317–4333.
Trigo TC, Schneider A, de Oliveira TG, Lehugeur LM, Silveira L, Freitas TRO, Eizirik E. 2013. Molecular data reveal complex hybridization and a cryptic species of Neotropical wild cat. *Curr Biol* **23:** 2528–2533.
Trigo TC, Tirelli FP, Freitas TRO, Eizirik E. 2014. Comparative assessment of genetic and morphological variation at an extensive hybrid zone between two wild cats in southern Brazil. *PLoS One* **9:** e108469.
Wurster-Hill DH, Centerwall WR. 1982. The interrelationships of chromosome-banding patterns in canids, mustelids, hyena, and felids. *Cytogenet Cell Genet* **34:** 178–192.
Yang ZH. 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol Biol Evol* **24:** 1586–1591.

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Reduced genetic diversity and isolation of remnant ocelot populations occupying a severely fragmented landscape in southern Texas

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conservation; microsatellites; mtDNA; *Leopardus albescens*; population structure; Felidae.

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Abstract

The ocelot *Leopardus pardalis* has become a conservation priority in the US as a result of severe population decline and loss of habitat during the 20th century. Only two small populations remain in this country. Their short-term viability is threatened by the disappearance of dense thornshrub communities, human-caused mortality and demographic stochasticity. The influence these factors have on ocelot persistence must be considered to develop effective conservation initiatives. We therefore examined neutral genetic diversity and connectivity among ocelots in the US and northeastern Mexico using 25 autosomal microsatellites and a 395-bp segment of the mitochondrial control region. Genetic variation was lowest in the population occurring on Laguna Atascosa National Wildlife Refuge, Texas (autosomal microsatellite $H_E = 0.399$ and mtDNA-haplotype diversity = 0) and highest in northeastern Mexico (0.637 and 0.73, respectively), while intermediate on private lands in Willacy County, Texas (0.553 and 0.252, respectively). Significant genetic differentiation between the two Texas populations was observed, despite their close proximity (~30 km). Both populations were also significantly divergent from northeastern Mexico. The absence of any detectable gene flow implies that the human modified landscape of the Lower Rio Grande Valley in southern Texas acts as a strong barrier to ocelot movement, disrupting metapopulation dynamics and contributing to loss of diversity. As a consequence, continued genetic erosion among the Texas populations is expected. The lack of movement through the fragmented landscape also suggests it is unlikely ocelots will recolonize unoccupied habitat patches along the Lower Rio Grande and the delta interior where agriculture and urban land uses predominate. The continued rapid development will exacerbate this problem. These factors threaten the persistence of the Texas populations and limit their recovery. Translocations are necessary to link ocelot populations in the US.

Introduction

Many species exhibit metapopulation dynamics, particularly in ecosystems with discontinuously distributed habitat. A metapopulation consists of smaller, local populations that periodically undergo extinction and recolonization events, often with an influx of individuals from a larger, more stable core population (Levins, 1969; Hanski, 1999). In this system, the persistence of local populations is dependent on the relative rates of extinction and recolonization; these in turn are largely dependent on the levels of connectivity (Levins, 1969; Hanski, 1999). In areas dominated by small, fragmented habitat patches where extinctions occur frequently, only high levels of dispersal can maintain local populations.

The connectivity of populations maintained by dispersal can be reduced in a landscape fragmented by unsuitable habitat resulting from anthropogenic perturbations. In areas where dispersal is compromised, and the remaining

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habitat patches are small, extirpations of local populations occur more frequently than recolonizations. Fixation of deleterious alleles and loss of adaptive alleles as a result of genetic drift and inbreeding compromise fitness and synergistically interact with demographic stochasticity to further reduce viability of populations (Hedrick & Kalinowski, 2000; Reed, 2005; Reed, Nicholas & Stratton, 2007). Genetic diversity and population connectivity are therefore of major concern when developing management strategies for threatened species occupying a fragmented landscape (Reed & Frankham, 2003; Reed, 2004; O'Grady *et al.*, 2006). Spatial and temporal patterns in variation can provide insight on movement between populations (Avise, 2004), potential for inbreeding depression (Hedrick & Kalinowski, 2000) and the likelihood that extant populations will recolonize unoccupied habitat patches.

Most felids require large habitat tracks and occur at relatively low densities; therefore, they have been particularly impacted by anthropogenic pressures including habitat loss, predator control and the fur trade (Nowell & Jackson, 1996). In North America, species such as the mountain lion *Puma concolor* have begun to recover, and others including the bobcat *Lynx rufus* remain abundant even in areas with high human densities (Nielsen & Wolf, 2001; Riley & Malecki, 2001; Pimm, Dollar & Bass, 2006; Ruell *et al.*, 2009). In contrast to these success stories, the ocelot *Leopardus pardalis albescens* is yet to make a recovery in the US and is listed as endangered by US Fish & Wildlife Service and Texas Parks & Wildlife; although, across large parts of Central and South America it is common in many Neotropical lowland ecosystems (Caso *et al.*, 2008). Historically, its northern distribution extended into Arizona, Arkansas, Louisiana and Texas within the US (Murray & Gardner, 1997). Removal of dense cover and other anthropogenic factors caused major population reductions during the 20th century (Tewes and Everett, 1986). Currently, within the US the ocelot persists in only two, small populations inhabiting a severely fragmented landscape in southern Texas (Haines *et al.*, 2005; 2006*b*; Janečka *et al.*, 2008).

Ocelots in Texas are closely associated with remnant dense thornshrub communities (Shindle & Tewes, 1998; Harveson *et al.*, 2004; Jackson & Zimmerman, 2005; Horne *et al.*, 2009). One of the populations consists of 20–40 individuals occupying the Laguna Atascosa National Wildlife Refuge (LANWR) in Cameron Country (Haines *et al.*, 2005; Janečka *et al.*, 2008). The other one occurs on private lands in Willacy Country ~30 km northwest of LANWR (Haines *et al.*, 2006*a,b*). Janečka *et al.* (2008) estimated the effective size (N_E) of this population to be smaller than in LANWR ($N_E = 3$ compared with $N_E = 14$–16, respectively).

The two areas appear isolated, and previous studies have shown low diversity in both mitochondrial and microsatellite markers, particularly in LANWR (Walker, 1997; Janečka *et al.*, 2007; 2008). The nearest known ocelot populations are ~150 km to the south in the State of Tamaulipas, Mexico and are believed to be larger and less fragmented (Caso, 1994; Janečka *et al.*, 2007). Currently, they are separated by high human densities, widespread development and extensive croplands, particularly in the Rio Grande delta. Previous phylogenetic analysis has shown a very close relationship between Tamaulipas and Texas populations, both of which are classified within the subspecies *L. p. albescens* (Murray & Gardner, 1997; Janečka *et al.*, 2007).

Population viability analysis suggests high probability of extinction in LANWR ($P = 0.65$ within 100 years; Haines *et al.*, 2005). However, the risk was lower when the two US populations were considered in a metapopulation model ($P = 0.15$ within 50 years; Haines *et al.*, 2006*c*). The positive relationship between viability and dispersal is mediated through both demographic rescue and genetic exchange (Hanski, 1999). Therefore, the landscape connectivity has a large influence on the persistence of this species within the US, and must be incorporated with other ecological and demographic information during conservation planning.

We therefore used 25 autosomal microsatellites and a portion of the mtDNA control region to characterize genetic diversity and population structure in ocelots. Our goals were to (1) compare genetic diversity among the Texas and northeastern Mexico populations; (2) determine the degree to which fragmented populations in Texas are isolated from each other and from Mexico. These issues must be considered in order to develop effective recovery plans that ensure the persistence of the last wild Neotropical felid breeding within the US.

Methods

Study area and sample collection

All sampling sites (Fig. 1) were located in the Tamaulipas Biotic Province, which stretches from southern Texas to Tamaulipas, Mexico. This region is a transition zone between Nearctic and Neotropical fauna characterized by thornshrub, scrub forest and mixed grassland–forest habitats (Blair, 1950). Blood samples were collected from wild-caught ocelots during previous radio-telemetry studies conducted between 1986 and 2005 (Tewes, 1986; Laack, 1991; Caso, 1994; Beltran & Tewes, 1995; Shindle & Tewes, 2000; Haines, Tewes & Laack, 2005; Laack *et al.*, 2005; Haines *et al.*, 2006*a,b,c*; Horne *et al.*, 2009).

The three primary study localities included: (1) LANWR, Cameron County (i.e. Cameron, $n = 52$); (2) Yturria Ranch and other private ranches in northern Willacy County (i.e. Willacy, $n = 34$); (3) Los Ebanos Ranch, Tamaulipas (i.e. Mexico, $n = 17$; supporting information Table S1). A few additional individuals were captured in four other areas: (1) Santa Ana National Wildlife Refuge, Texas (SANWR, $n = 1$); (2) Port of Brownsville, ($n = 1$); (3) Los Zoyates Ranch, Tamaulipas ($n = 3$); (4) Miradores Ranch, Tamaulipas ($n = 3$). Ocelots that died as a result of vehicle collisions in Texas were also sampled near the following localities: Port Mansfield ($n = 6$), Raymondville ($n = 2$), Lyford ($n = 1$), Highway 186 in Willacy County ($n = 1$), Rio Hondo ($n = 1$) and Sarita ($n = 2$).

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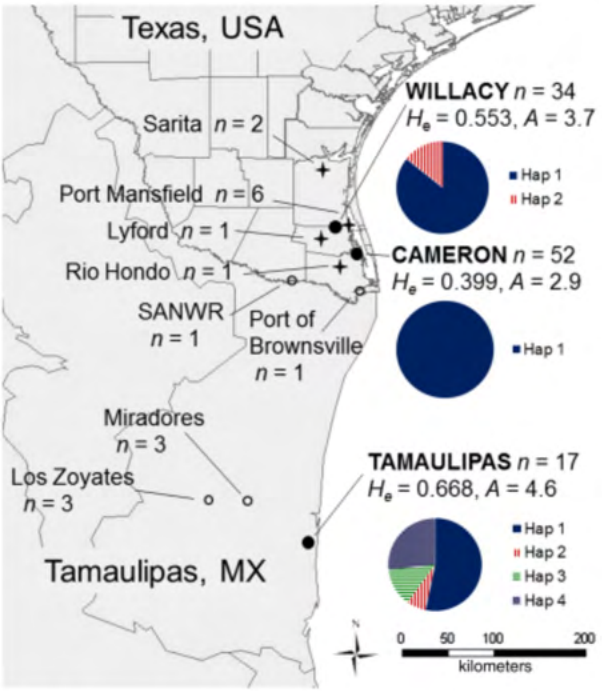

Figure 1 Map showing sampling localities in US and Mexico. Solid points represent primary populations, open circles localities outside these areas where ocelots were radio-collared, and crosses represent locations where road-killed ocelots where found. H_E = expected heterozygosity, A = mean allele number. Pie charts show mtDNA haplotype frequencies in the three populations.

Autosomal microsatellite genotyping

Blood samples were stored in lysis buffer (Longmire, Maltbie & Baker, 1997). The DNA extractions were performed with a PureGene® DNA Purification Kit (Gentra Systems, Minneapolis, MN, USA). We initially screened 41 autosomal microsatellites isolated in the domestic cat *Felis catus* by Menotti-Raymond *et al.* (1999; 2003*a*,*b*; 2009) to select loci that would be informative in ocelots (supporting information Table S2). These were broadly distributed throughout the genome based on their position in the domestic cat radiation hybrid and linkage maps (NCBI MAP VIEWER BUILD 0.1; Menotti-Raymond *et al.*, 2003*a*; 2009; Davis *et al.*, 2009). Felids have a high level of chromosomal conservation with >95% of locus locations and order preserved across their family (Davis *et al.*, 2009). Therefore, we assumed that the genomic positions for majority of loci are shared between the ocelot and the domestic cat. Of the loci screened, the 31 that exhibited robust amplification and unambiguous alleles were used to genotype the sampled ocelots (supporting information Table S2).

We used two methods for labeling PCR amplicons. The first 15 microsatellites were genotyped using forward primers 5′-labeled with a fluorescent dye (FAM, HEX, or TET; supporting information Table S2). Reaction mixes contained 0.2 mM of each dNTP, 1 × PCR HotMaster™ *Taq* buffer (Eppendorf, Hamburg, Germany) with 2.5 mM $MgCl_2$, 0.25 units of HotMaster™ *Taq*, 0.24 mM of each primer and 20 ng of DNA template. The PCR conditions included an initial denaturing step of 94 °C for 60 s, 30 cycles of 94 °C for 30 s, 53 °C for 30 s, 72 °C for 60 s, and a final extension step of 72 °C for 2 min. The m13 primer dye-labeling approach (Guo & Milewicz, 2003) was used for the remaining 16 loci (supporting information Table S2). Reactions contained 0.8 mM of each dNTP, 1 × PCR Platinum *Taq* buffer (Invitrogen, Carlsbad, CA, USA), 2.5 mM $MgCl_2$, 0.2 units of Platinum *Taq*, 0.027 mM forward primer with a m13 sequence tag on the 5′-end, 0.4 mM of the 5′-dye-labeled m13 primer (FAM, NED, VIC, or PET), 0.4 mM of the reverse primer, and 20 ng of DNA template. The PCR reaction conditions were 94 °C for 2 m, 40 cycles of 94 °C for 15 s, 55 °C for 30 s, 72 °C for 60 s, and a final extension step of 72 °C for 5 min.

Direct labeled amplicons were genotyped on the ABI 3100 automated sequencer (Applied Biosystems, Forest City, CA, USA) in the Laboratory of Plant Genomics and Technology, Texas A&M University and m13-labeled amplicons on an ABI 3730 sequencer in the Equine Molecular and Cytogenetics Laboratory, Department of Veterinary Integrative Biosciences, Texas A&M University. Allele calls were made using GENOTYPER 2.0 (Applied Biosystems). Amplicons from two previously genotyped ocelot samples were included with every genotyping run to ensure alleles were consistently sized.

Y-chromosome microsatellite genotyping

There is a lack of available microsatellite loci on the Y chromosome for population genetic analysis of felids. We therefore tested 28 microsatellites discovered within introns of seven single copy genes located in the non-recombinant region of the domestic cat Y chromosome (Davis *et al.*, 2009). Primers were designed using Primer3 and PCR tested to ensure they did not amplify an X-linked paralog (Rozen & Skaletsky, 2000). The m13 primer dye-labeling PCR conditions described above were used with 1.5 mM $MgCl_2$ and 58 °C annealing temperature. Alleles were genotyped as described above. Sixteen loci were excluded because of inconsistent PCR amplification. The remaining 12 microsatellites were genotyped in 53 male ocelots (supporting information Table S1).

Mitochondrial control region sequencing

A 395-bp segment of the control region was sequenced and aligned using primers from Jae-Heup *et al.* (2001) modified to match the ocelot mitochondrial DNA sequence (PAN-OCELOT-F primer, 5′-CTCAACTATCCGAAAGAGCTT-3′, PAN-OCELOT-R primer, 5′-CCTGTGGAACATTAGGAATT-3′; Janečka *et al.*, 2007) This fragment aligns with positions 16,833 to 218 of the domestic cat mitochondrial genome, which is located in the central conserved region between repetitive sequences I and II (Lopez, Cevario & O'Brien, 1996; Jae-Heup *et al.*, 2001). The PCR

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amplification was performed in 25 μL volumes containing 0.2 mM of each dNTP, 1 × JumpStart PCR buffer (Sigma Aldrich, St Louis, MO, USA), 1.25 units of JumpStart *Taq*, 0.25 mM forward primer, 0.25 mM reverse primer and 20 ng DNA template. Cycle conditions included an initial denaturing step of 94 °C for 1 min, 30 cycles of 94 °C for 15 sec, 58 °C for 30 s, 72 °C for 2 s, and a final extension step of 72 °C for 2 min. The PCR products were sequenced using an ABI BigDye v. 1.1 Terminator Kit on the ABI 3100 or ABI3730 automated sequencer following the recommendations of the manufacturer. Sequences were obtained in both directions, and contiguous sequences were constructed using SEQUENCHER 3.0.

Genetic diversity – autosomal microsatellite analysis

Measures of genetic variability, including expected heterozygosity (H_E), mean number of alleles (A) and number of private alleles (A_P), were estimated using GENALEX 6.4 (Peakall and Smouse, 2006). Allelic richness (A_R), observed heterozygosity (H_O) and gene diversity (H_S) was estimated and tested for significant differences between populations with 10 000 permutations in FSTAT 2.3.9 (Goudet, 2001). Tests for linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP 3.1 (Guo & Thompson, 1992; Raymond & Rousset, 1995). Populations were tested for deviations from equilibrium at each locus and across all loci. The Bonferroni method was used to correct P-values for multiple comparisons in the HWE and LD tests (Rice, 1989). The three populations were tested for recent reductions in effective population sizes based on excess gene diversity across loci following the methods of Cornuet & Luikart (1996) as implemented in BOTTLENECK 1.2.02 (two-phase model and standardized differences test).

Genetic structure among ocelot populations

Samples were divided into three populations: Cameron ($n = 42$), Willacy ($n = 28$) and Mexico ($n = 12$). Pair-wise F_{st} estimates were derived and tested for significance with 10 000 permutations using the AMOVA framework in GENALEX. Effective numbers of migrants per generation (N_em) were estimated from the mean frequencies of private alleles by GENEPOP (Barton & Slatkin, 1986). One ocelot generation is *c.* 7 years based on life-history data (Janečka *et al.*, 2008).

Assignment tests were conducted by estimating the probability of individuals originating from each of the populations using GeneClass 2 (Piry *et al.*, 2004). The number of mis-assignments is positively related to dispersal between populations (Rannala & Mountain, 1997; Paetkau *et al.*, 2004). Assignment tests were implemented using both frequency-based (Paetkau *et al.*, 2004) and Bayesian methods (Rannala & Mountain, 1997). The proportion of individuals mis-assigned was compared between populations. We also tested for the presence of first generation migrants using GeneClass and STRUCTURE 2.2 (Pritchard, Stephens & Donnely, 2000). Finally, individuals sampled outside of three primary study sites were assigned to their most likely source using the three populations as reference samples in both GeneClass and STRUCTURE.

Bayesian model-based clustering in STRUCTURE was used to explore population structure without regard to geographic origin (Pritchard, Stephens & Donnely, 2000). This approach applies a Bayesian algorithm to estimate the likelihood of K clusters (synonymous with 'populations') and the portion of an individual's genetic variation (Q) attributed to each of the clusters, based on LD and HWE. We estimated the likelihood for $K = 1$–10 using the admixture model for 10 independent runs with 500 000 MCMC generations and burn-in of 100 000. The most likely number of clusters was determined using two approaches; by estimating the posterior probability for each K as recommended by Pritchard, Stephens & Donnely (2000) and by using the method of Evanno, Regnaut & Goudet (2005). The model-defined clusters were compared with the three geographically defined populations.

MtDNA data analysis

Sequence alignment was performed in SEQUENCHER and the number of variable sites, haplotype diversity (D_{hap}), nucleotide diversity (π) and departure of haplotype frequencies from neutrality (Tajima's D test and Fu and Li's D test) were estimated in DNASP 4.10.8 (Tajima, 1989; Fu & Li, 1993; Rozas and Roses, 2006). A minimum spanning network of haplotypes was constructed in ARLEQUIN 3.0 (Excoffier, Laval & Schneider, 2005). Population differentiation of mtDNA variation was examined by estimating pair-wise F_{st} and testing for significance with 10 000 permutations in ARLEQUIN.

Results

Patterns of genetic variation at autosomal microsatellite loci

Five pairs of loci consisted of microsatellites located within 30 centimorgans of each other. From each of these pairs, we excluded the less informative locus (i.e. lower A and H_E), along with FCA262 because it was monomorphic. Autosomal genetic variation was examined among ocelots using the remaining 25 independent, variable loci (Cameron $n = 42$, Willacy $n = 28$, Mexico $n = 12$). Only one (FCA208) was out of HWE in all of the populations. FCA023 and FCA132 were out of HWE in Cameron, along with FCA035 in Mexico. Only 88% of the loci were polymorphic in Cameron, while all of the loci were variable in the other two populations (supporting information Table S3). The three populations had an excess of genetic diversity consistent with a recent bottleneck (Cameron $P = 0.01954$, Willacy $P = 0.00099$, Mexico $P = 0.02240$).

The highest levels of genetic diversity were observed in Mexico and intermediate levels in Willacy (Table 1 and supporting information Table S3). The A_R, H_O and H_S was

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Table 1 Genetic diversity among 25 autosomal microsatellites and 395 bp portion of the mtDNA control region in three ocelot populations

	Autosomal microsatellites					MtDNA control region		
	n	*A*	A_E	A_P	H_E	*n*	π	D_{Hap}
Texas								
Cameron	42	2.88	2.00	6	0.399	39	0	0
1991–1998	29	2.84	2.00	6[a]	0.399	27	0	0
1999–2005	13	2.64	1.89	1[a]	0.379	12	0	0
Willacy	28	3.72	2.51	11	0.553	35	0.00064	0.252
1991–1998	18	3.66	2.52	22[b]	0.536	25	0.00082	0.324
2005	10	2.84	2.11	3[b]	0.480	10	0	0
Mexico								
Ebanos	12	4.64	3.30	42	0.637	15	0.00289	0.667

[a]With respect to the other Cameron temporal group

[b]With respect to the other Willacy temporal group

A, mean number of alleles; A_E, effective number of alleles; A_P, private alleles; H_E, expected heterozygosity; π, nucleotide diversity, D_{Hap}, haplotype diversity.

significantly lower in Cameron ($A_R = 1.841$, $H_O = 0.402$, $H_S = 0.407$) compared with Mexico ($A_R = 2.516$, $H_O = 0.605$, $H_S = 0.669$; $P = 0.0009$, 0.0018, 0.0009, respectively), while only H_O was significantly lower ($P = 0.0275$) compared with Willacy ($A_R = 2.131$, $H_O = 0.561$, $H_S = 0.528$). The differences between Willacy and Mexico were not significant. Mexico had roughly 5.5-fold greater number of private alleles than the two Texas populations despite a much smaller sample size.

In the AMOVA, the overall $F_{st} = 0.180$ was significant ($P = 0.001$), as were all pair-wise values. The highest was between Cameron and Mexico ($F_{st} = 0.272$, $P = 0.001$), and the lowest between Willacy and Mexico ($F_{st} = 0.113$, $P = 0.001$). The F_{st} between the two Texas populations was 0.163 ($P = 0.001$). All three populations also showed significant levels of differentiation in the genic and genotypic tests (X^2 = infinity, $P < 0.0001$ for all pair-wise comparisons in both tests). The N_em estimate was 0.444 per generation between all pairs of populations. There were no mis-assignments and no migrants detected among the three populations.

Model-based clustering without regard for geographic origin consistently found $K = 4$ clusters using Pritchard, Stephens & Donnely (2000) criteria and $K = 3$ clusters using the Evanno, Regnaut & Goudet (2005) method (Fig. 2a). In both cases, all cluster assignments were consistent with the population of origin. There were no clusters composed of individuals from more than one population. When $K = 4$, Willacy was split into two temporal groups, with one consisting of individuals captured in 1991–1998, and the other of those captured 2005. In analyses that tested $K = 2$, one cluster was comprised exclusively of ocelots from Cameron and the second cluster consisted of individuals from both Willacy and Mexico.

Assignment of individuals outside of main populations

Among the two individuals captured and radio-collared outside of the main populations, the one near Port of

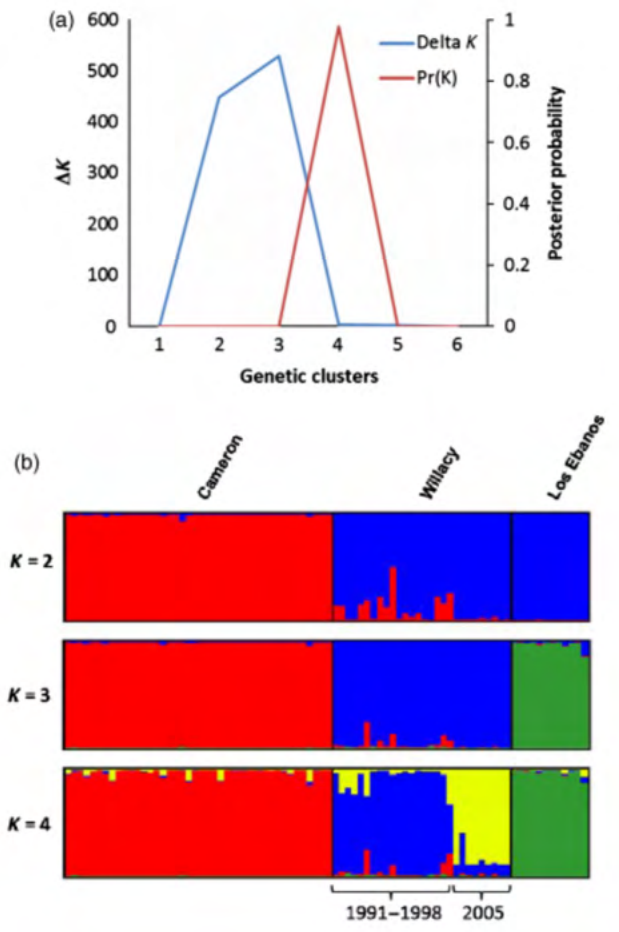

Figure 2 (a) Graph of the posterior probability of each cluster estimated in STRUCTURE and the change in likelihood derived using Evanno, Regnaut & Goudet (2005). The peaks represent the most likely number of clusters using the respective approach. (b) The proportion of individual variation for $K=2$, $K=3$ and $K=4$ assigned to a given genetic cluster in STRUCTURE. Each cluster is represented by a different color.

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Table 2 Population assignments using the Bayesian method in STRUCTURE and GeneClass for ocelots that did not originate in the three primary study areas

					P (STRUCTURE)			*P* (GeneClass)		
Date Collected	County	Location	mtDNA hap	Loci	Ca	Wi	Eb	Ca	Wi	Eb
Road-killed										
11/16/1989	Willacy	Port Mansfield	1	16	0.06	0.92	0.02	0.00	0.46	0.10
1989	Willacy	Port Mansfield	1	16	0.58	0.34	0.08	0.00	0.02	0.00
1989	Willacy	Port Mansfield	1	15	0.30	0.65	0.05	0.00	0.20	0.01
7/29/1991	Willacy	Port Mansfield	1	16	0.03	0.96	0.02	0.00	0.34	0.00
Oct 1993	Willacy	Port Mansfield	1	15	0.04	0.95	0.02	0.00	0.59	0.00
1/12/2004	Willacy	Port Mansfield	3	15	0.15	0.82	0.02	0.00	0.22	0.00
10/27/1997	Willacy	Lyford	1	25	0.02	0.97	0.01	0.00	0.27	0.00
6/17/1999	Willacy	Highway 186	1	15	0.25	0.74	0.01	0.00	0.03	0.00
4/7/1997	Cameron	Rio Hondo	1	15	0.67	0.20	0.13	0.00	0.00	0.00
10/15/1997	Kenedy	Sarita	1	16	0.33	0.63	0.03	0.00	0.09	0.00
8/31/1990	Kenedy	Sarita	1	15	0.28	0.70	0.03	0.00	0.16	0.00
Radio-collared										
5/8/1992	Hidalgo	SANWR	1	30	0.01	0.01	0.98	0.00	0.00	0.00
4/27/1998	Cameron	Port of Brownsville	1	15	0.97	0.02	0.01	0.99	0.00	0.00

Individuals from Texas were road-kills; except for ocelots from Santa Anna National Wildlife Refuge (SANWR) and Port of Brownsville which were live-trapped.
Ca, Cameron; Wi, Willacy; Mx, Mexico; *P*, probability.

Brownsville was assigned to Cameron, and the one in Santa Anna NWR most likely originated from Mexico. Eight of the 11 road-killed ocelots were assigned to the Willacy population (Table 2), including individuals found in Port Mansfield, Sarita and Lyford. Only one of the individuals from Port Mansfield could not be assigned, but had the highest probability of coming from Willacy. There was one road-kill found close to LANWR (i.e. Rio Hondo) that likely came from that population, although it could not be assigned with confidence.

Y-microsatellite diversity

Variation for Y-linked microsatellite loci was determined for 53 male ocelots (Cameron $n = 22$, Willacy $n = 18$, Mexico $n = 3$). Only two of the 12 loci were variable among the individuals examined and both were located in the SMCY gene, although in different introns (SMCY2 in intron 2 and SMCY7 in intron 7). Four alleles were present SMCY2 (168, 169, 172 and 180) and six in SMCY7 (167, 169, 171, 173, 175 and 177). However both loci exhibited a large number of what appeared to be heterozygous genotypes with the shorter allele having a higher peak than the longer allele (nine heterozygotes in SMCY2 and 12 in SMCY7). As the Y-chromosome is haploid, this suggests that the locus may have been duplicated in ocelots. Because of this inconsistency we did not use the Y microsatellites in our analysis of genetic diversity.

Mitochondrial diversity and differentiation

A 395-bp fragment of the control region was sequenced for 77 ocelots (Cameron, $n = 39$; Willacy, $n = 35$; Mexico, $n = 15$). There were four haplotypes (Hap) with three variable sites (Fig. 3), all of which were present in Mexico [frequencies = 0.533 (Hap 1), 0.067 (Hap 2), 0.133 (Hap 3) and 0.267 (Hap 4); GenBank accessions JF930139–JF930142]. Hap 1 also occurred in Cameron (1.00) and Willacy (0.857), however, in Texas Hap 2 was only found in Willacy (0.143). Haps 3 and 4 were not detected in any individuals from Texas. Overall, Mexico had the highest levels of haplotype and nucleotide diversity, followed by Willacy, with no diversity detected in Cameron (Table 1, Fig. 1).

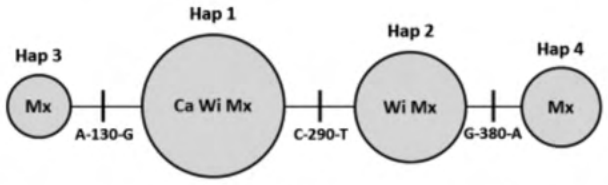

Figure 3 Haplotype (Hap) map of 395-bp portion of the MtDNA control region. Each hatch mark represents a single nucleotide substitution with the specific nucleotide states given. The size of each haplotype circle is based on its frequency among all ocelots, although sizes are not directly proportional [frequencies = 0.877 (Hap 1), 0.066 (Hap 2), 0.019 (Hap 3) and 0.038 (Hap 4)]. Ca, Cameron; Wi, Willacy; Mx, Mexico.

The greatest level of mtDNA differentiation was between the Cameron and Mexico populations with an F_{st} value of 0.451 ($P < 0.001$), and significantly different haplotype frequencies. The lowest level of differentiation was between Willacy and Cameron ($F_{st} = 0.126$, $P = 0.018$). The F_{st} between Willacy and Mexico was 0.180 ($P = 0.005$). A rapid

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loss of diversity was observed in Willacy over a span of only 7 years. In this population, D_H was 0.324 among samples collected 1991–1998, yet in 2005 there was no diversity detected in the mitochondrial control region.

Discussion

Genetic variation within and between ocelot populations

Lowest diversity was observed in ocelots from Cameron, which is the only breeding population occurring on a US federal or state refuge. The level of microsatellite diversity at LANWR was comparable to variation in felid populations that have recently undergone severe bottlenecks, including the critically endangered Amur leopards *Panthera pardus oreintalis* in the Russian Far East and North Korea (Uphyrkina *et al.*, 2002), and those that have been recently isolated by anthropogenic factors, such as mountain lions in the central coast and southern regions of California (Ernest *et al.*, 2003). In addition, no variation was observed for the control region segment in the Cameron population. This is consistent with the small N_e estimates for the time period spanning 1989–1996, ranging from 8.0 to 13.9 (Janečka *et al.*, 2008). The small effective population size coupled with its isolation has increased the effects of drift and inbreeding leading to low genetic diversity compared with Willacy and Tamaulipas.

The Willacy population retained more ancestral variation and was less divergent from Mexico based on estimates of genetic differentiation and the grouping of Willacy and Mexico into one cluster for $K = 2$ (Fig. 2b). Yet, the N_e of Willacy between 1998 and 2005 was estimated to be smaller than in Cameron (maximum estimate of 3.1) despite the intermediate levels of genetic diversity (Janečka *et al.*, 2008). In the cluster analysis of autosomal variation for $K = 4$ there was a division of Willacy into two distinct genetic groups corresponding to ocelots captured in the 1990s and those in 2005. Over that period, there was also a loss of 22 autosomal microsatellite alleles and the mtDNA data shows a reduction from moderate haplotype diversity during mid-1990s, to no diversity in 2005. This suggests that until the 1990s Willacy was likely larger and more widely distributed, but over the last decade has been subject to extreme genetic drift as a result of reductions that led to a very small population size and demographic instability. This scenario is also consistent with the observed patterns in heterozygosity that were indicative of a recent bottleneck. Although diversity was highest in northeastern Mexico, a recent reduction in effective size was also detected in that area, suggesting the Tamaulipas region may likewise be currently undergoing population reductions.

Genetic divergence

Both microsatellite and mtDNA analyses revealed significant genetic subdivision among the three populations. The F_{st} between Cameron and Willacy, which are separated by *c.* 30 km, was higher than between Willacy and Tamaulipas, which are more than 300 km apart. Historical gene flow can influence estimates of F_{st}; therefore, the lower value between the two geographically distant populations is likely the result of greater levels of ancestral variation retained by Willacy compared with the Cameron population, not contemporary gene flow. Estimates of N_em are below one individual per generation between all pairs of ocelot populations, thus indicating an overall lack of connectivity. A value of $N_em = 1$ is roughly considered the minimum for maintaining population connectivity sufficient to prevent genetic divergence (Mills & Allendorf, 1996).

Assignment tests reflect contemporaneous dispersal more precisely than F_{st} estimates because they allow for the identification of recent migrants (Rannala & Mountain, 1997; Paetkau *et al.*, 2004; Manel, Gaggiotti & Waples, 2005). There were no mis-assignments or migrants observed among the three ocelot populations. Furthermore, in the Bayesian analysis using STRUCTURE, all clusters were composed of individuals from only one population. The absence of mis-assignments and migrants, and complete resolution of populations into distinct clusters, illustrates the high levels of isolation and subsequent differentiation.

The extreme genetic divergence observed between proximate areas in Texas <30 km apart is unusual for a medium-sized carnivore. Felids are physically capable of long-distance dispersal (>100 km), and exhibit reasonable amounts of connectivity over large geographic areas (Hellborg *et al.*, 2002; Sunquist & Sunquist, 2002; Ernest *et al.*, 2003). The high amount of differentiation between the two ocelot populations is likely a result of habitat modifications in the Lower Rio Grande Valley, combined with small population sizes, and is analogous to the effects of anthropogenic changes on Iberian lynx *Lynx pardinus* in Spain and black bears *Ursus americanus* in Florida, where significant genetic structure in highly vagile species was linked to anthropogenic dispersal barriers (Johnson *et al.*, 2004; Dixon *et al.*, 2007).

Human activities in southern Texas and northeastern Mexico have eliminated large tracts of dense thornshrub communities preferred by ocelots (Tewes & Everett, 1986; Jahrsdoerfer & Leslie, 1988). The area between the Cameron and Willacy population is characterized by agricultural regions with greatly reduced native plant communities and extensive sorghum and cotton fields many kilometers wide (Fig. 4). The strong habitat selection of ocelots for dense thornshrub, and avoidance of areas with $<75\%$ canopy cover, likely reduces movement between relatively close habitat patches (Horne *et al.*, 2009). Our study suggests that this has resulted in complete isolation of the two remaining Texas ocelot populations. Absence of dispersal between the Cameron and Willacy populations strongly suggested from the genetic data is also supported by ecological research; no dispersal events have been observed between these two areas in nearly 30 years of radio-telemetry work (Tewes, 1986; Laack, 1991; Caso, 1994; Beltran & Tewes, 1995; Shindle & Tewes, 2000; Haines, Tewes & Laack, 2005; Laack *et al.*, 2005; Haines *et al.*, 2006*a*,*c*; Horne *et al.*, 2009).

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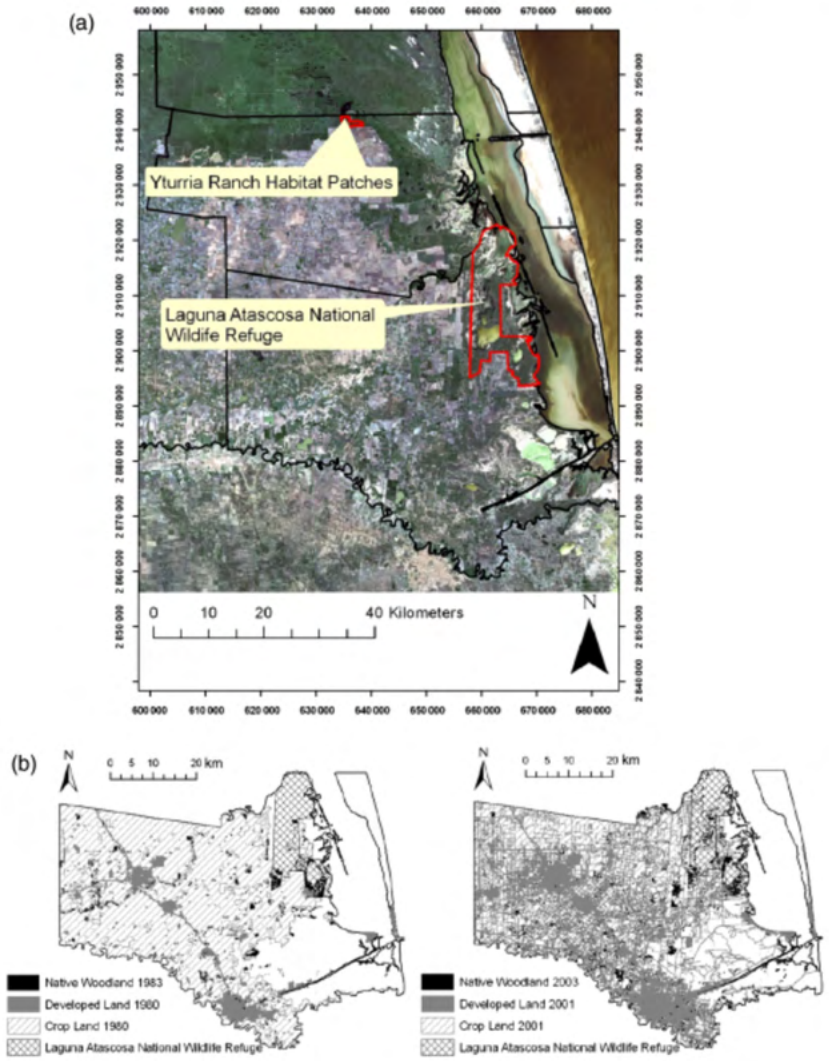

Figure 4 (a) Satellite image showing the distribution of agriculture in the Lower Rio Grande Valley. (b) Map illustrating the amount of development from 1980 to 2001 in Cameron County, Texas.

Road mortality a hindrance to ocelot dispersal

Vehicle collisions are the highest source of anthropogenic mortality for ocelots (Haines, Tewes & Laack, 2005), and further limit dispersal through the agricultural lands surrounding the remaining habitat patches. For instance, two road-killed ocelots found near LANWR were assigned to Cameron and seven road-kills to Willacy. Presumably these cats were moving out of their natal area, yet died in a vehicular accident before reaching the other US breeding population.

Ocelots from Port Mansfield, Sarita and Raymondville belonged to the Willacy population, suggesting that it may extend outside of its known distribution. Interestingly, one of the Port Mansfield road-kills collected in 2004 had mtDNA Hap 2, which was not detected in the 2005 Willacy sample. Native rangeland is present to the north, west and east of Willacy, which is likely more porous to ocelot movement compared with the agricultural landscape that borders the Cameron population (Fig. 4), thereby facilitating dispersal to the north and east. Willacy therefore is a critical source for natural recolonization of areas close to the Yturria habitat patches.

The only evidence for movement between Texas and Tamaulipas was the individual captured in SANWR in 1992, which was assigned to Mexico and also possessed a high number of private alleles from that population. Therefore, this ocelot likely came from habitat south of the Rio Grande River, suggesting the possibility that there is an unknown ocelot population closer to the US border than the one sampled in southern Tamaulipas. This individual is one of the few ocelots documented in SANWR since 1990, and potentially represents rare dispersal events that thus

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far failed in reaching either of the US breeding populations. The ocelots sampled in Miradores and Los Zoyates were assigned to the Mexico population.

Implications for conservation

Ocelot reached the northern extent of their historical range in parts of Texas, Louisiana and Arkansas (Lowery, 1974). This region, along with Tamaulipas, together constitute a closely related phylogenetic clade suggesting former connectivity likely maintained via thornshrub habitat that was previously more widely distributed (Janečka *et al.*, 2007; Jahrsdoerfer & Leslie, 1988). In the past, Mexico may have served as a source for populations in the US. Any potential metapopulation dynamics have now been disrupted by brush removal, row crop agriculture, road development and urbanization that have greatly altered the landscape (Fig. 4). These barriers impede ocelot movement between the few remnant habitat patches. Our genetic data strongly indicate that habitat fragmentation has resulted in virtually complete isolation of the two extant populations in Texas, in addition to an overall loss of genetic variation.

Therefore, the extinction rate for both Texas populations likely exceeds the rate of colonization, and the fates of each population in Texas are independent from each other as well as from existing populations in Mexico. Both populations are well below the minimum population viability size recommended for long-term survival (Franklin, 1980; Shaffer, 1981; Reed *et al.*, 2003). As shown across diverse taxa, including several species of felids, a continued loss of genetic diversity through increased drift and inbreeding as the number of potential breeders declines contributes to reductions in fitness and greater extinction risks (Roelke, Martenson & O'Brien, 1993; Frankham & Ralls, 1998; Hedrick & Kalinowski, 2000; Keller & Waller, 2002; Reed & Frankham, 2003; Reed, Nicholas, & Stratton, 2007, Johnson et al., 2010). Research is needed to determine if inbreeding depression is affecting ocelots in Texas.

Conservation actions can be implemented to offset the continued decline of ocelot populations in the US. Development of habitat corridors in conjunction with safe passages across highways associated with mortality of dispersing ocelots would facilitate exchange between populations, improving the overall stability of the Texas populations (Haines *et al.*, 2006*c*). However, the Lower Rio Grande Valley is a rapidly growing area (Fig. 4b), and the creation of such corridors is not logistically feasible. Translocations represent the only practical method for reconnecting these populations, although the potential effects must be first carefully evaluated (Hedrick, 2010).

In addition to the demographic benefits of translocations, genetic variation in Texas could be partially restored and inbreeding reduced, minimizing the potential for inbreeding depression (Johnson et al., 2010; Hedrick, 2010). Populations of *L. p. albescens* from Tamaulipas are a genetically appropriate source based on their phylogenetic relationship, and would yield the highest increase in variation (Janečka *et al.*, 2007). However, it also would be beneficial to move individuals between Cameron and Willacy. There is significant divergence and high number of private alleles when these populations are compared with each other, suggesting each has preserved a different portion of ancestral variation. A partial reconstruction of historical levels of diversity could be achieved by mixing the Texas populations. Finally, expansion of ocelots into isolated vacant habitat patches will likely also require translocations. These management strategies should enhance the long-term viability of the remaining ocelot populations in Texas.

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References

Avise, J.C. (2004). *Molecular markers, natural history, and evolution*. Sunderland: Sinauer Associates.

Barton, N.H. & Slatkin, M. (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* **56**, 409–415.

Beltran, J.F. & Tewes, M.E. (1995). Immobilization of ocelots and bobcats with ketamine hydrochloride and xylazine hydrochloride. *J. Wildl. Dis.* **31**, 43–48.

Blair, F.W. (1950). Biotic Provinces of Texas. *Tex. J. Science.* **2**, 93–117.

Caso, A. (1994). *Home Range and Habitat Use of Three Neotropical Carnivores in Northeast Mexico*. Master's thesis, Texas A&M University-Kingsville, Kingsville.

Caso, A., Lopez-Gonzalez, C., Payan, E., Eizirik, E., de Oliveira, T., Leite-Pitman, R., Kelly, M. & Valderrama, C. (2008). *Leopardus pardalis. IUCN Red List of threatened species. Ver. 2010.4*. Available at http://www.iucnredlist.org (accessed December 1, 2010).

Cornuet, J.M. & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001–2014.

Davis, B.W., Raudsepp, T., Pearks-Wilkerson, A.J., Agarwala, R., Schaffer, A.A., Houck, M., Chowdhary, B.P. & Murphy, W.J. (2009). A high-resolution cat radiation hybrid and integrated FISH mapping resources for phylogenomic studies across Felidae. *Genomics* **93**, 299–304.

Dixon, J.D., Oli, M.K., Wooten, M.C., Eason, T.H., McCown, J.W. & Cunningham, M.W. (2007). Genetic consequences of habitat fragmentation and loss: the case of the Florida black bear (*Ursus americanus floridanus*). *Conserv. Genet.* **8**, 455–464.

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Ernest, H.B., Boyce, W.M., Bleich, V.C., May, B., Stiver, S.J. & Torres, S.G. (2003). Genetic structure of mountain lion (*Puma concolor*) populations in California. *Conserv. Genet.* **4**, 353–366.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol. Ecol.* **14**, 2611–2620.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**, 47–50.

Frankham, R. & Ralls, K. (1998). Conservation biology: inbreeding leads to extinction risk. *Nature* **392**, 441–442.

Franklin, I.R. (1980). Evolutionary change in small populations. In *Conservation biology: An evolutionary-ecological perspective*: 135–150. Soule, M.E. & Wilcox., B.A. (Eds). Sunderland: Sinauer.

Fu, Y.X. & Li, W.H. (1993). Statistical tests of neutrality of mutations. *Genetics* **33**, 693–709.

Goudet, J. (2001). *FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)*. Available at http://www2.unil.ch/popgen/softwares/fstat.htm (accessed November 14, 2010).

Guo, S.W. & Thompson, E.A. (1992). Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics* **48**, 361–372.

Guo, G. & Milewicz, D.M. (2003). Methodology for using a universal primer to label amplied DNA segments for molecular analysis. *Biotechnol. Lett.* **25**, 2079–2083.

Haines, A.M., Tewes, M.E. & Laack, L.L. (2005). Survival and cause-specific mortality of ocelots in southern Texas. *J. Wildl. Mgmt.* **69**, 255–263.

Haines, A.M., Tewes, M.E., Laack, L.L., Grant, W.E. & Young, J. (2005). Evaluating recovery strategies for an ocelot (*Leopardus pardalis*) population in the United States. *Biol. Conserv.* **126**, 512–522.

Haines, A.M., Grassman, L.I., Tewes, M.E. & Janečka, J.E. (2006*a*). The first ocelot (*Leopardus pardalis*) monitored via GPS telemetry. *Eur. J. Wildl. Res.* **52**, 216–218.

Haines, A.M., Janečka, J.E., Tewes, M.E., Grassman, L.I. & Morton, P. (2006*b*). The importance of private lands for ocelot *Leopardus pardalis* conservation in the United States. *Oryx* **40**, 90–94.

Haines, A.M., Tewes, M.E., Laack, L.L., Horne, J.S. & Young, J.H. (2006*c*). A habitat-based population viability analysis for ocelots (*Leopardus pardalis*) in the United States. *Biol. Conserv.* **132**, 424–436.

Hanski, I. (1999). *Metapopulation ecology*. Oxford: Oxford University Press.

Harveson, P.M., Tewes, P.M., Tewes, M.E., Anderson, G.L. & Laack, L.L. (2004). Habitat use by ocelots in South Texas: implications for restoration. *Wildl. Soc. Bull.* **32**, 948–954.

Hedrick, P.W. (2010). Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conserv. Genet.* **11**, 615–626.

Hedrick, P.W. & Kalinowski, S.T. (2000). Inbreeding depression in conservation biology. *Ann. Rev. Ecol. Syst.* **31**, 139–162.

Hellborg, L., Walker, C.W., Rueness, E.K., Stacy, J.E., Kojola, I., Valdmann, H., Vila, C., Zimmermann, B., Jakobsen, K.S. & Ellegren, H. (2002). Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. *Conserv. Genet.* **3**, 97–111.

Horne, J.S., Haines, A.M., Tewes, M.E. & Laack, L.L. (2009). Habitat partitioning by sympatric ocelots and bobcats: implications for recovery of ocelots in southern Texas. *Southwest. Nat.* **54**, 119–126.

Jae-Heup, K., Eizirik, E., O'Brien, S.J. & Johnson, W.E. (2001). Structure and patterns of sequence variation in the mitochondrial DNA control region of the great cats. *Mitochondrion* **14**, 279–292.

Jackson, V.L. & Zimmerman, E.G. (2005). Landscape metrics associated with habitat use by ocelots in south Texas. *J. Wildl. Mgmt.* **69**, 733–738.

Jahrsdoerfer, S.E. & Leslie, D.M. (1988). Tamaulipan brushland of the Lower Rio Grande Valley of South Texas: descriptions, human impacts, and management options. *USFWS Biol. Report.* **88**, 36–63.

Janečka, J.E., Walker, C.W., Tewes, M.E., Caso, A., Laack, L.L. & Honeycutt, R.L. (2007). Phylogenetic relationships of ocelot (*Leopardus pardalis albescens*) from the Tamaulipan Biotic Province and implications for ocelot recovery. *Southwest. Nat.* **52**, 89–96.

Janečka, J.E., Tewes, M.E., Laack, L.L., Grassman, L.I. Jr, Haines, A.M. & Honeycutt, R.L. (2008). Small effective population sizes of two remnant ocelot populations (*Leopardus pardalis albescens*) in the United States. *Conserv. Genet.* **9**, 869–878.

Johnson, W.E., Godoy, J.A., Palomares, F., Delibes, M., Fernandes, M., Revilla, E. & O'Brien, S.J. (2004). Phylogenetic and phylogeographic analysis of Iberian lynx populations. *J. Hered.* **95**, 19–28.

Johnson, W.E., Onorato, D.P., Roelke, M.E., Land, E.D., Cunningham, M., Belden, R.C., McBride, R., Jansen, D., Lotz, M., Shindle, D., Howard, J., Wildt, D.E., Penfold, L.M., Hostetler, J.A., Oli, M.K. & O'Brien, S.J. (2010). Genetic restoration of the Florida panther. *Science* **329**, 1641–1645.

Keller, L.F. & Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241.

Laack, L.L. (1991) *Ecology of the ocelot (Felis pardalis) in South Texas*. Master's thesis, Texas A&I University, Kingsville.

Laack, L.L., Tewes, M.E., Haines, A.H. & Rappole, J. (2005). Reproductive life history of ocelots *Leopardus pardalis* in southern Texas. *Acta Theriol.* **50**, 505–514.

Levins, R. (1969). Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bull. Entomol. Soc. Am.* **15**, 237–240.

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Lopez, J.V., Cevario, S. & O'Brien, S.J. (1996). Complete nucleotide sequences of the domestic cat (*Felis catus*) mitochondrial genome and a transposed mtDNA tandem repeat (*Numt*) in the nuclear genome. *Genomics* **33**, 229–246.

Longmire, J.L., Maltbie, M. & Baker, R.J. (1997). Use of "lysis buffer" in isolation and its implications for museum collections. Occasional Papers, The Museum of Texas Tech University, Lubbock, 163, 1–3.

Lowery, G.H. Jr (1974). *The mammals of Louisiana and its adjacent waters*. Baton Rouge: Louisiana University Press.

Manel, S., Gaggiotti, O.E. & Waples, R.S. (2005). Assignment: matching biological questions with appropriate techniques. *Trends Ecol. Evol.* **120**, 136–142.

Menotti-Raymond, M., David, V.A., Agarwala, R., Schäffer, A.A., Stephens, R., O'Brien, S.J. & Murphy, W.J. (2003*a*). Radiation hybrid mapping of 304 novel microsatellites in the domestic cat genome. *Cytogen. Genome Res.* **102**, 272–276.

Menotti-Raymond, M., David, V.A., Lyons, L.A., Schäffer, A.A., Tomlin, J.F., Hutton, M.K. & O'Brien, S.J. (1999). A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics* **57**, 9–23.

Menotti-Raymond, M., David, V.A., Roelke, M.E., Chen, Z.Q., Menotti, K.A., Sun, S., Schäffer, A.A., Tomlin, J.F., Agarwala, R., O'Brien, S.J. & Murphy, W.J. (2003*b*). Second-generation integrated genetic linkage/radiation hybrid maps of the domestic cat (*Felis catus*). *J. Hered.* **94**, 95–106.

Menotti-Raymond, M., David, V.A., Schäffer, A.A., Tomlin, J.F., Eizirik, E., Phillip, C., Well, D., Pontius, J.U., Hannah, S.S. & O'Brien, S.J. (2009). An autosomal genetic linkage map of the domestic cat, *Felis silvestris catus*. *Genomics* **93**, 305–313.

Mills, L.S. & Allendorf, F.W. (1996). The one-migrant-per-generation in conservation and management. *Conserv. Biol.* **10**, 1509–1518.

Murray, J.L. & Gardner, G.L. (1997). *Leopardus pardalis* mammal. *Species* **548**, 10–17.

Nielsen, C.K. & Wolf, A. (2001). Spatial organization of bobcats *Lynx rufus* in southern Illinois. *Am. Midl. Nat.* **146**, 45–52.

Nowell, K. & Jackson, P. (1996). *Wild cats: status survey and conservation action plan*. Gland: IUCN.

O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D., Tonkyn, D.W. & Frankham, . (2006). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biol. Conserv.* **133**, 42–51.

Paetkau, D., Slade, R., Burden, M. & Estoup, A. (2004). Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol. Ecol.* **13**, 55–65.

Peakall, R. & Smouse, P.E. (2006). GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295.

Pimm, S.L., Dollar, L. & Bass, O.L. Jr (2006). The genetic rescue of the Florida panther. *Anim. Conserv.* **9**, 115–122.

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L. & Estoupa, A. (2004). GeneClass 2: a software for genetic assignment and first-generation migrant detection. *The J. Hered.* **95**, 536–539.

Pritchard, J.K., Stephens, M. & Donnely, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Rannala, B. & Mountain, J.L. (1997). Detecting immigration by using multilocus genotypes. *Proc. Natl. Acad. Sci. USA* **94**, 9197–9201.

Raymond, M. & Rousset, F. (1995). Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Reed, D.H. (2004). Extinction risk in fragmented populations. *Anim. Conserv.* **7**, 181–191.

Reed, D.H. (2005). Relationship between population size and fitness. *Conserv. Biol.* **19**, 563–568.

Reed, D.H. & Frankham, R. (2003). The correlation between population fitness and genetic diversity. *Conserv. Biol.* **17**, 230–237.

Reed, D.H., O'Grady, J.J., Brook, B.W., Ballou, J.D. & Frankham, R. (2003). Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biol. Conserv.* **113**, 23–34.

Reed, D.H., Nicholas, A.C. & Stratton, G.E. (2007). Genetic quality of individuals impacts population dynamics. *Anim. Conserv.* **10**, 275–283.

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Riley, S.J. & Malecki, R.A. (2001). A landscape analysis of rougar distribution and abundance in Montana, USA. *Environ. Mgmt.* **28**, 317–323.

Roelke, M.E., Martenson, J.S. & O'Brien, S.J. (1993). The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr. Biol.* **3**, 340–350.

Rozas, J. & Rozas, R. (2006). DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* **15**, 174–175.

Rozen, S. & Skaletsky, H.J. (2000). Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics methods and protocols: methods in molecular biology*: 365–386. Krawetz, S. & Misener, S. (Eds). Totowa: Humana Press.

Ruell, E.W., Riley, S.P., Douglas, M.R., Pollinger, J.P. & Crooks, K.R. (2009). Estimating bobcat population sizes and densities in a fragmented urban landscape using noninvasive capture-recapture sampling. *J. Mammal.* **90**, 129–135.

Shaffer, M.L. (1981). Minimum population sizes for species conservation. *BioScience* **31**, 131–134.

Shindle, D.B. & Tewes, M.E. (1998). Woody species composition of habitats used by ocelots (*Leopardus pardalis*) in the Tamaulipan Biotic Province. *Southwest. Nat.* **43**, 273–279.

Shindle, D.B. & Tewes, M.E. (2000). Immobilization of wild ocelots with tiletamine and zolazepam in southern Texas. *J. Wildl. Dis.* **36**, 546–550.

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Sunquist, M.E. & Sunquist, F. (2002). *Wild cats of the world.* Chicago: University of Chicago Press.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.

Tewes, M.E. (1986) *Ecological and behavioral correlates of ocelot spatial patterns*. Doctoral thesis, University of Idaho, Moscow.

Tewes, M.E. & Everett, D.D. (1986). Status and distribution of the endangered ocelot and jaguarundi in Texas. In *Cats of the world: biology, conservation, and management*: 147–158. Miller, S.D. & Everett, D.D. (Eds). Washington: National Wildlife Federation.

Uphyrkina, O., Miquelle, D., Quigley, H., Driscoll, C. & O'Brien, S.J. (2002). Conservation genetics of the Far Eastern leopard (*Panthera pardus oreintalis*). *J. Hered.* **93**, 303–311.

Walker, C.W. (1997) *Patterns of genetic variation in ocelot (Leopardus pardalis) populations for South Texas and Northern Mexico*. Doctoral thesis, Texas A&M University-Kingsville and Texas A&M University, Kingsville.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Ocelots sampled in the US and Mexico that were used for population structure analysis in this study. Ten additional ocelots were genotyped for Y microsatellites but were excluded from this table because they were not used to asses autosomal and mtDNA diversity.

Table S2. The chromosomal position of 41 autosomal microsatellites in the domestic cat genetic linkage and radiation hybrid maps that were screened in ocelots (NCBI Map Viewer Build 0.1, Menotti-Raymond *et al.*, 2003*a*,*b*; 2009; Davis *et al.*, 2009). Microsatellites in bold were selected for population structure analysis. PCR results (res.) are coded as; + = robust amplification, – = no amplification, and M.A. = multiple amplicons of different size. PCR conditions (cond.) refer to whether the primers were fluorescently labeled directly on the 5′ end (1) or using a dye-labeled m13 tag (2).

Table S3. Genetic diversity among 25 autosomal microsatellites in three ocelot populations sampled from 1991–2005. Abbreviations: Ca = Cameron County, Texas; Wi = Willacy County, Texas; Mx = Mexico; H_O = observed heterozygosity, H_E = expected heterozygosity; A_N = effective alleles.

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Future

We are eager to share our message of conservation, especially with young people who will grow up to be tomorrow's stewards of the environment. We welcome:

- Schools (all levels)
- Scouts (all types)
- Summer Camp Excursions
- Senior Care Centers
- Civic Groups

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to schedule your visit.

PANTHER RIDGE
CONSERVATION CENTER

2143 D Road
Loxahatchee, FL 33470

20 minutes west of the Florida Turnpike
Okeechobee exit

Phone: 561-795-8914

Appointments are required.

Email: pantheridge@aol.com

Panther Ridge Conservation Center is a 501(c)3 not-for-profit corporation, and is registered with the state of Florida under the Solicitation of Contributions Act. A copy of the official registration (# CH15293) and financial information may be obtained from the Florida Division of Consumer Services by calling 1-800-425-7353 (toll free from within Florida). Registration does not imply endorsement, approval or recommendation by the state of Florida.

PANTHER RIDGE
CONSERVATION CENTER

PantherRidgeCC.org

Mission

We are dedicated to conservation through education. Our Center is home to over 20 big cats representing nine different species – five are classified as endangered.

We strive to engage the public with our feline ambassadors so that they may become aware of the plight of big cats in the wild.

Once a cat is accepted by Panther Ridge, it will never again be in need of a home, or at risk of neglect or mishandling.

Our animals are treated with kindness, respect and gratitude for their part in conservation.

Commitment

Panther Ridge is an Accredited Facility with the Zoological Association of America and the Feline Conservation Federation. We are one of the few private facilities to be accepted by The Association of Zoos and Aquariums' Species Survival Plan (SSP) for clouded leopards. The SSP oversees the population management of select species and enhances conservation of this species in the wild. We are proud to be part of this important program. It is a safety net against extinction.

Care

The housing, feeding and care of exotic felines is challenging – and costly.

Each cat's diet is specially formulated to meet its own specific requirements. We serve more than 100 pounds of beef, chicken, game and supplements every day.
Annual cost $50,000+

Each resident receives the highest quality of veterinary care. Some cats require daily medication for chronic problems.
Monthly cost $2,000+

Each enclosure exceeds Florida Fish and Game Commission standards. Every habitat provides a safe and stimulating environment.
Cost $25,000-75,000+

Maintenance of existing structures is an ongoing process.
Cost $20,000-25,000+

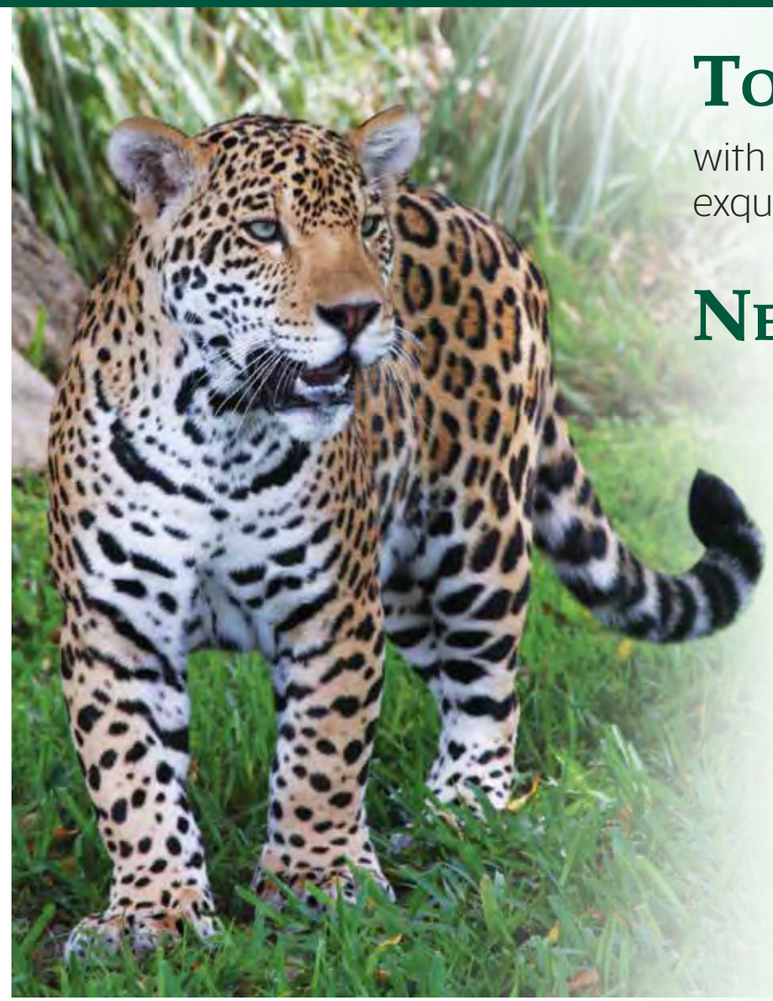
You can help

All but two of the species that resides at Panther Ridge will be extinct in the wild within 20 years. Degradation and loss of natural habitat along with human encroachment are by far the major factors of the loss of multiple species.

Panther Ridge depends on the support of caring and concerned individuals and organizations like you to maintain the health of our resident felines and enables us to be involved in conservation outreach projects.

Please consider a donation to our Center as a very special gift for birthdays and holidays. The "adoption" of one of our magnificent cats is truly a gift of conservation.

Visit PantherRidgeCC.org to learn more about our adoption benefits and other donation options.



Tours

Get up close and personal with our world's most exquisite big cats.

New!

Twilight Tours and Feeding Tours.

Tours are hosted by qualified exotic feline experts. Enjoy behind-the-scenes stories of each cat along with Q and A sessions.

Amazing photo opportunities are everywhere at our beautiful five-acre retreat.

Tour proceeds go toward the feeding and care of the Panther Ridge felines.

Appointments are required for all tours.

Certificate of Excellence

tripadvisor

Panther Ridge is available for private parties.

Our park-like setting is conducive to both casual and elegant corporate affairs and holiday gatherings.

Call 561-795-8914 for details.

kidskonnect

Ocelot
Worksheets

CARD 48

OCELOT

MAMMALS — GROUP 1

ORDER *Carnivora* | FAMILY *Felidae* | GENUS & SPECIES *Felis pardalis*

The ocelot is a small nocturnal cat from North and South America. Camouflaged head-to-tail to blend with its dusky forest habitat it has, arguably, the most beautiful coat of any cat.

KEY FACTS

SIZES
Body length: 65-97cm.
Tail length: 27-40cm.
Weight: 11-16kg.

BREEDING
Sexual maturity: 6-8 months.
Mating: Once or twice a year, depending on location.
Gestation: 70 days.
Litter size: 2-4 kittens.

LIFESTYLE
Habit: Solitary or may live in pairs.
Coat colour: Varies from rich yellow to grey, depending on habitat. Underside speckled white. Ringed tail.
Lifespan: 17 years in captivity. Unknown in wild.

RELATED SPECIES
There are 28 species of small wild cat found worldwide, with the exception of Australasia and the polar regions.

Range of the ocelot

DISTRIBUTION
Ocelots range from Arizona in North America to Argentina in South America.

CONSERVATION
Ocelots have long sufffered from extensive hunting for their valuable pelt. More recently the ocelot received full protection from hunters, under international law, and commercial trade is now banned.

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THE OCELOT'S COAT COMPARED WITH OTHER CATS

Camouflage: Dark blotches on a rich golden brown or silver-grey base colour. Perfect for the equatorial forests that form its habitat. Shown below right are pelt comparisons with the leopard and cheetah. Each cat is suited to its own environment; the leopard to the dry tropical forest, the cheetah to the arid savannah.

The ocelot is about a third of the length of the Bengal tiger. By comparison, the domestic cat is dwarved by the other two.

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The ocelot is classified as a small cat, belonging to the genus *Felis*, but is one of the largest cats in this genus. Like other small cats, it has a bare nose and long, sensitive whiskers but, unlike the domestic cat, it will run swiftly from danger rather than bolt up a tree.

HABITAT

The ocelot is a creature of the forests and scrubland. In the forest its coat is a rich golden colour and is brightly whorled, giving it an amazing camouflage against the golden-brown hue of the trees. When it inhabits scrubland, this cat's coat colour has a duller, greyish tinge, again giving it the best possible camouflage.

An agile climber, the ocelot shares most of its range with the smaller margay. Both species spend time resting in trees but unlike the margay, which can run down trees headfirst, the ocelot climbs down backwards.

Most cats are territorial and the ocelot is no exception, marking its boundaries by spraying trees with urine. Most are solitary but some ocelots form pairs.

Above: In unfamiliar territory, the ocelot will rest up during the day and hunt only at night.

FOOD & HUNTING

Although its sense of smell is highly developed, the ocelot relies more on its hearing and eyesight to detect prey on its nightly hunting expeditions. The hearing of small cats is more acute than big cats and they are better able to pick up the high-pitched squeaks of some of their prey. Whiskers are also important to the ocelot when hunting. These are so sensitive to touch that they help the cat to manoeuvre correctly in tight spaces.

The ocelot's diet consists of agoutis, hares, mice, monkeys and birds. In fact, it will eat anything it can overpower. Although it spends a lot of time in the trees, all hunting is done on the ground, where it pads lightly along on its toes.

The ocelot will lie flat on its belly once a meal is spotted. Biding its time, it will slowly slink forward in this position and then rush at the prey over a short distance before pouncing and catching it with its claws. The ocelot keeps its claws razor-sharp for this very purpose by scratching them against tree trunks.

The prey is then quickly dispatched with a bite to the neck. Small prey is devoured headfirst but with larger prey the ocelot will begin its meal at a soft part of the victim's body.

When hunting in pairs, the oselots will call out so each knows the other's location.

Below: With keen senses and powerful incisors, the ocelot is well equipped to hunt and kill.

BREEDING

It is uncertain when and how often the ocelot breeds but it is possible that if conditions are right it will breed twice a year, in summer and winter. The ocelot's location will affect breeding, too: those in Arizona and Argentina may only breed seasonally.

When the breeding season arrives the ocelot will venture out of its territory in order to find a mate. The first courtship move is actually made by the female who calls loudly to attract a male when she is in season. During mating the male holds the female by the back of the neck until mating is complete. After mating she lashes out at the male but then invites him to mate with her again.

Before the kittens are born just over two months later, the female makes a secure hidden nest. This is sometimes lined with soft down plucked from her underside.

Two to four kittens are born. As each one arrives the mother breaks the birth sac to free the baby. She bites off the umbilical cord and licks the new-born clean and dry, then eats the afterbirth.

The kittens are fully furred when born but are blind and helpless for a few days. The mother will leave them only when necessary. For the first few weeks the ocelot kittens feed only on their mother's milk, and during this time she will eat all their droppings to keep the nest area scrupulously clean.

When the kittens are a little older the mother will bring them small live prey and teach them how to kill. Later they follow her on hunting trips. Once they become competent hunters the mother will drive them from the nest or they will leave of their own accord.

Right: ***The disarming beauty of an ocelot kitten makes it, sadly, much sought after for a pet.***

DID YOU KNOW?

- In the dark a cat's sight is about six times better than that of a man.
- The puma is the largest of the small cats — bigger even than the leopard, a big cat (*Panthera* species) from Africa and Asia.
- Cats are the most carnivorous of all the meat-eating animals, with an almost exclusive diet of vertebrate creatures. They are, therefore, at the top of the food chain with few enemies except man.
- Small cats eat by crouching over their prey — not lying down, in the manner of most big cats.
- When lying down the ocelot sleeps with its forepaws stretched out in front and its head resting on them like a dog. It is the only small cat to do this.
- In 1968 North American fur traders imported a total of 129,000 ocelot pelts.

There are currently 800,000 to 1.5 million ocelots in the world today.

SAVE THE TEXAS OCELOT

MEET THE OCELOT

SLIGHTLY ROUNDED **EARS**

DISTINCT **MARKINGS** WITH DARK BORDERS

LONG, RINGED **TAIL**

MEDIUM-SIZED CAT **15-30LBS**

UNLIKE MOST OTHER CATS, OCELOTS CAN TURN THEIR ANKLE JOINTS AROUND, ALLOWING THEM TO CLIMB DOWN A TREE **HEADFIRST**

TOP PREDATORS IN THE THORNY BRUSH FORESTS OF TEXAS, OCELOTS MOSTLY PREY ON **RABBITS, MICE, RATS AND BIRDS.**

IN TEXAS, OCELOTS RELY ON THORNY BRUSH FOR SHELTER AND PROTECTION AND REQUIRE A LOT OF SPACE.

1 male needs **25** square miles of territory.

OCELOTS IN TROUBLE

OCELOTS ARE FEDERALLY LISTED AS **ENDANGERED** IN THE UNITED STATES.

<80 OCELOTS REMAIN IN THE COUNTRY.

OCELOTS **ONCE RANGED** FROM SOUTH TEXAS UP INTO ARIZONA, ARKANSAS AND LOUISIANA.

THEY ARE **NOW** FOUND ONLY IN SOUTH TEXAS RANCHLANDS AND THE LAGUNA ATASCOSA NATIONAL WILDLIFE REFUGE.

95% OF THE OCELOT'S THORNY BRUSH HABITAT HAS BEEN LOST.

40% of the ocelots studied over the past 30 years have been **KILLED** by vehicles.

SAVING THE ENDANGERED OCELOT

THE U.S. FISH AND WILDLIFE SERVICE AND PARTNERS WORK TO PROTECT OCELOTS IN SOUTH TEXAS BY:

MONITORING OCELOTS LIVING ON THE REFUGE AND PRIVATE LANDS.

PROTECTING, RESTORING AND CONNECTING THORN FOREST HABITAT.

WORKING WITH THE TEXAS DEPARTMENT OF TRANSPORTATION TO INSTALL UNDER-ROAD WILDLIFE CROSSINGS THAT HELP KEEP OCELOTS OFF THE ROAD.

PLANNING TO TRANSLOCATE (MOVE) OCELOTS WITHIN TEXAS AND FROM MEXICO TO BRING MUCH NEEDED GENETIC DIVERSITY TO THE SMALL REMAINING POPULATION.

YOU CAN HELP OCELOTS!

ADOPT AN OCELOT
to support conservation through the Friends of Laguna Atascosa.

LIVE IN TEXAS?
Report ocelot sightings to (956) 748-3608, x111.

LEARN MORE
GO TO
www.fws.gov/refuge/laguna_atascosa
& www.savetexasocelots.org

FOLLOW
"Vivo the Ocelot" on Facebook

Ocelot

ORDER *Carnivora* ▶ FAMILY *Felidae*
▶ GENUS *Felis* ▶ SPECIES *Felis pardalis*

THE OCELOT is found in parts of North, Central and South America, from Arizona and Texas southwards as far as northern Argentina.

LOCATION

STATISTICS

STATUS	Locally common; listed on CITES Appendix I
SOCIAL UNIT	Solitary
LENGTH	55–100cm (21½–39½in)
TAIL	30–45cm (11¾–17½in)
WEIGHT	11.3–15.8kg (24lb 14oz–34lb 12oz)
HABIT	Terrestrial
ACTIVITY	Nocturnal
SEXUAL MATURITY	Female: 18–22 months; male: 30 months
BREEDING SEASON	Varies according to location
GESTATION PERIOD	75–85 days
NUMBER OF YOUNG	1–3
BREEDING INTERVAL	1 year
DIET	Mainly rodents such as mice; rabbits; young deer and peccaries; birds; snakes; fish
LIFESPAN	Up to 10 years in wild; up to 21 years in captivity

Feline Beauty
The coat has a whitish, yellowish or red-grey base, marked with a stunning pattern of dark streaks, rings and spots. The ocelot has two black stripes on each cheek and bars on the inner legs.

Rounded face with short muzzle has jaws that can open wide

Large eyes give good night vision

Short fur is thick and velvety

Body is strong and powerful

Markings act as camouflage, enabling ocelot to blend in with surroundings and stalk prey

Long tail may be ringed or marked with dark bars on upper surface

MYTH OR FACT?
With its beautifully patterned, soft fur and relatively small size, the ocelot was at one time in great demand as a pet. Before traders sold it on, they treated this small cat cruelly, removing its fangs and claws to make it less dangerous and easier to keep in captivity.

Armed Paws
The ocelot has five digits on the front feet and four on the back feet. Each has a curved, retractable claw, perfect for seizing and holding prey, as well as for climbing trees.

RELATED SPECIES
The ***ocelot*** is one of 31 species of ***cat*** in the genus ***Felis*** of small cats. It is closely related to the ***margay*** (*F. weidii*), which is also found in forest and scrubland from northern Mexico to northern Argentina. Another relative is the ***little spotted cat*** (*F. tigrina*), a forest-dweller found from Costa Rica to northern Argentina.


Margay

FACTFILE

Going Undercover with a
Spotted Feline

WELL KNOWN FOR ITS VIVIDLY PATTERNED COAT, the ocelot was at one time highly valued for its pelt and was widely hunted to supply the fur trade. Now that it is legally protected, this solitary feline can be appreciated for its beauty from afar, and is able to live its secretive life under the cover of thick vegetation in the forests and grasslands of North and South America.

Ample Cover
This secretive cat can live in a wide range of habitats, from grasslands to swamps and forests, but it always requires dense vegetation for cover. By day the ocelot rests in a tree hollow, under a thicket or stretched out on a broad branch. Although it is an efficient swimmer and climber, it stays mainly on the ground, travelling up to 7.6km (4¾ miles) each day in search of food. The ocelot is territorial, and the home ranges of males do not overlap. However, a male's territory may overlap with that of several females. Although it spends most of its time alone, this beautiful feline may occasionally meet and socialize with other individuals, communicating with mewing calls and by scent-marking.

Protected Den
In tropical parts of its range, this cat has no set breeding season, but in Mexico and Texas females give birth in autumn and winter. An adult male mates briefly with any female whose territory overlaps his, then moves on to find another partner. When it is time to give birth, the female retires to a den in a dense thicket or under the roots of a fallen tree. The kittens weigh 200–340g (7–12oz) at birth. They stay in or near the den for the first few weeks of life, suckling regularly. They are weaned at six weeks and at about two months they start to hunt with their mother.


A kitten starts to explore after a few weeks, but stays near its mother for up to a year.

Main picture: Conaction Bradford


This cat needs to drink regularly, and in the dry season it has to take what moisture it can from dried-up ponds.

Ambush Attack
At night the ocelot emerges from its shelter to hunt for prey on the ground. Its diet changes with the seasons, but consists mainly of small, ground-dwelling rodents such as mice and rats. It also eats other small animals such as rabbits, snakes, lizards and birds. In the wet season, it may also eat fish and land crabs, which become abundant at that time. With its excellent night vision, the ocelot is well adapted for hunting in dim light. Its spotted and striped fur also allows it to blend into its dappled background. As soon as this agile cat spots passing prey, such as a mouse, it pounces swiftly and pins down the victim in its needle-sharp claws.

A sharp-eyed ocelot can spot even tiny prey as it moves through thick vegetation

In Demand
Once widely hunted for its decorative pelt and captured for the pet trade, the ocelot is now legally protected in most parts of its range. As a result, the species has partially recovered its numbers, with a total population of about 1.5–3 million. Today, the main threat to this cat comes from deforestation. The ocelot has a slow reproductive rate and it cannot survive without cover and abundant small prey.

October 6, 2022

U.S. Fish and Wildlife Service, International Affairs
Division of Management Authority, Branch of Permits
5275 Leesburg Pike, MS: IA
Falls Church, Virginia 22041

To USFWS:

Friends of Laguna Atascosa National Wildlife Refuge, is a 501(c)3 non-profit organization; dedicated to Ocelot Conservation. The group researches and monitors wild Ocelots in their natural Texas habitat. This endangered feline can be found in South America, Mexico, including Arizona & Texas.

Panther Ridge is a non-profit, 501(c)3 feline conservatory located in South Florida; focused on the preservation of wild endangered felines, including the Ocelot. Our facility showcases a number of feline species, exhibiting each in a habitat designed to engage the public. Our board of directors approach conservation through education & awareness, our intimate environment allows a direct one-on-one tourist experience a large zoo cannot achieve. Promoting education is the anchor for conservation.

Supporting and contributing financially to organizations such as LANWR, can change the future survival of threatened species. We donate annually and to this group, commitment includes additional disbursements over a five year span. Funds donated to LANWR in 2021/2022 totaled $1000.00 thus far, future payments are donated with a minimum of $500.00 yearly. We plan to support this group for many years to come; our board stays current on LANWR's news, posts and updates; in our collaboration the public is educated with the most current Ocelot information, facts, and efforts for future species survival, in honor of them.

LANWR's information (included) was sourced directly from their webpage (https://flanwr.org). I certainly have made it a priority to attend Ocelot Conservation Day, which will be on March 5, 2023, held at the Gladys Porter Zoo annually.

Sincerely yours,

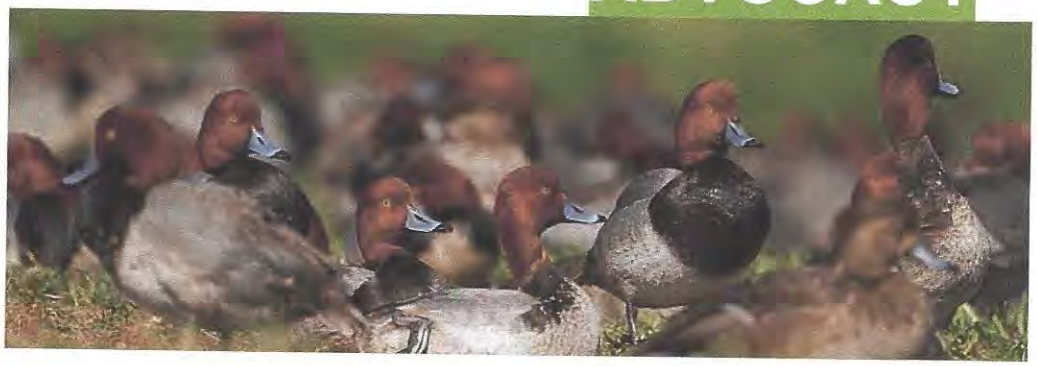
Judy Berens

Judy Berens

561-795-8914
2143 D Road, loxahatchee, Florida 33470
www.pantherridge.org

friends of LAGUNA ATASCOSA national wildlife refuge

ADVOCACY



From the iconic Tamaulipan thornscrub where the elusive ocelot creeps, to coastal prairie where Aplomado Falcons soar, to the shores of the Laguna Madre Bay where thousands of migrating Redhead Ducks gorge on seagrasses during the winter, the diverse landscapes of Laguna Atascosa National Wildlife Refuge (Refuge) provide a home for numerous species and is one of the most biologically diverse areas in North America. The Refuge is home to an incredible biodiversity with more than 450 species of plants, 415 documented species of birds, 130 butterfly, 45 mammals, 44 reptiles and amphibians and approximately 40 fish species. The Refuge also provides a place for the public to enjoy wildlife and open space, through wildlife watching, biking, hiking, photography, environmental education, and hunting. No wonder so many concerned people like you (nature lovers, conservationists, wildlife watchers, volunteers, birders, photographers, outdoor enthusiasts, ranchers, educators, anglers, hunters, businesses and more) have come together to support Friends of Laguna Atascosa National Wildlife Refuge.

WHAT WE ADVOCATE FOR

We meet our mission by focusing on three main issues:

- Acquisition of south Texas lands for transfer to the Refuge
- Restoration of Tamaulipan thornscrub habitat for ocelots and coastal prairie for Aplomado Falcons
- Raising awareness of the Refuge through outreach and education, including the annual Ocelot Conservation Day held since 1997.

FUTURE GOALS

Friends of
Laguna Atascosa
National Wildlife Refuge

- Friends will continue to play an active role in helping Laguna Atascosa NWR to grow in land conservation, habitat restoration and research.
- Develop programs that reflect the refuge importance with connections to the community and that promote a greater sense of environmental responsibility. Educating the next generation who will carry the legacy forward.
- Become a champion for growth, both in membership and diversity, and in resource capacity.
- Conduct outreach through special events, the web site, brochures and other promotional materials.
- Advocate on a local and national level for funding and other issues that affect wildlife conservation in South Texas.
- Continue to operate and improve the Visitor Center's nature store, which offers educational, interpretive and promotional materials benefiting the refuge.
- Grow our Board capacity, skills, and diversity to meet the challenges ahead.

Friends of
Laguna Atascosa
National Wildlife Refuge

ACCOMPLISHMENTS

- Organized and hosted the annual Ocelot Conservation Festival in South Texas since 1997. In 2019 and 2020 , we also brought ocelot awareness to the Longhorn Cattle Company at our 'Afternoon with an Ocelot.' In the past we've hosted an ocelot Soiree, a fundraising event for Ocelot Conservation. The annual 5K run through the Gladys Porter Zoo is a very popular event.

- Funded the purchase of live traps, trail cameras, and radio & GPS tracking collars for ocelot research.

- Partnered with USFWS to restore 153 acres of coastal prairie along Holly Beach, removed 8,500 lbs. of trash from the area, and and installed nearly 5,000 feet of fencing to prevent illegal dumping and offroading!

- Have donated approximately $7,000 since 2004 to help monitor and protect Kemp's ridley sea turtles.

- Sponsor Save Texas Ocelots.org specialty license plate. Friends of Laguna Atascosa receives $22.00 from the sale or renewal of each plate from the TxDMV, 100% of which goes to ocelot conservation.

- Successfully write and manage grants, including funding for the Ocelot Translocation Project, Bahia Grande Wetland Restoration, boardwalk overlooks along Laguna Madre at Plover Point and Redhead Ridge, and Aplomado Falcon Habitat Restoration at Bahia Grande.

Friends of
Laguna Atascosa
National Wildlife Refuge

Ongoing Commitments of the Friends Using Self-Generated Funds

- Support for Ocelot monitoring at Laguna Atascosa NWR
- Support for Student Interns working with the Ocelot Program and Visitor Service Program
- Support for Student of the University of Texas at Brownsville engaged in environmental research at Laguna Atascosa NWR
- Support for public hunting program at Laguna Atascosa NWR

Point of Contact:

Nicole Ekstrom
Executive Director
Friends of Laguna Atascosa National Wildlife Refuge
Mailing: P.O. Box 13519, Port Isabel, TX 78578
Shipping: 22817 Ocelot Rd., Los Fresnos, TX 78566
Physical: 22688 Buena Vista Blvd. Los Fresnos, TX 78566
Mobile: 956-832-3905
Website: https://flanwr.org
Online Store: https://flanwrnaturestore.square.site/
Facebook: https://www.facebook.com/VivatheOcelot/
http://www.flanwr.org/

From: Sheri Sublett info@tpfecf.org
Subject: Re: Friends of Laguna Atascosa National Wildlife Refuge Donation receipt
Date: Oct 15, 2022 at 5:47:03 PM
To: JUDY BERENS pantheridge@aol.com

Thank you Judy

Sincerely,

Sheri Sublett
Twin Pine Farm & Exotics Conservation Foundation
WWW.TPFECF.ORG
(843)310-4441 (office)
(843)217-9188 (mobile)
Facebook.com/tpfecf

On Oct 14, 2022, at 1:20 PM, JUDY BERENS <pantheridge@aol.com> wrote:

Hi Sherri,

FYI

Sent from my iPhone

Begin forwarded message:

From: Friends of Laguna Atascosa National Wildlife Refuge <admin@flanwr.org>
Date: October 14, 2022 at 7:41:38 AM EDT
To: pantheridge@aol.com
Subject: Friends of Laguna Atascosa National Wildlife Refuge Donation receipt
Reply-To: Nicole Ekstrom <director@flanwr.org>

Judy,

Thanks so much for the generous donation! We will put this in our ocelot fund for use in our ocelot programs, including land acquisition and habitat restoration of thornscrub habitat for ocelots. We have our annual Ocelot Conservation Day on March 5, 2023. If you could join us that would be great. We can set you up with a booth to let people know

about your organization. Thanks and keep in touch! - Nicole Ekstrom, Executive Director, director@flanwr.org, (956) 299-0907

DONATION RECEIPT

Friends of Laguna Atascosa National Wildlife Refuge

Receipt number: 00003
Date: Thu, October 06, 2022
Amount: $1,000.00
Tender: Wild Apricot Payment

Donation received from:
Judy Berens
pantheridge@aol.com
Panther Ridge Conservation Center, Inc

Thank you for your donation!

As a 501(c)(3) organization, gifts and donations to Friends of Laguna

Atascosa National Wildlife Refuge are tax-deductible.

September 2022 NEWSLETTER

friends of
LAGUNA ATASCOSA
national wildlife refuge

IN THIS ISSUE

Friends has a brand new website! Check out flanwr.org to find out about upcoming events, volunteer needs, and how you can help support us!

OPERATIONAL HOURS

Visitor's Center
Wednesday - Friday
7:00am-2:00pm

Nature Store
Thursday-Saturday
8:00am-4:00pm

Refuge Access
Sunday - Saturday
Sunrise to Sunset

New Youth Programs

Friends is kicking off the fall season with new programs and events. We will have two new youth programs, events, and membership-only clubs.

New youth programs will include a monthly Wild Friends Storytime and Homeschool Science Series.

The Wild Friends Storytime, geared for six and under, will include feeding the birds, a story featuring a native animal or habitat from the Refuge, and a craft. Our first Storytime on September 3 will feature the book: "Just Like Us! Birds" by Bridget Heos. Students will learn all about bird nests and make their own bird nest craft.

The Homeschool Science Series, geared for elementary and middle school students, will include hands-on activities that will provide a more in-depth understanding of Refuge habitats and wildlife. Our first event will be a Family Fish Camp at Bahia Grande on September 23. At this event, students will learn about fish identification, habitat, rules and regulations, fishing equipment, and get to fish at the Bahia Grande!

EVENTS

9/3 - 9am (VC)
Wild Friends Storytime and Feed the Birds

9/8 - 6pm
Bahia Grande Documentary
at Quinta Mazatlan

9/10 - 9am (VC)
Feed the Birds and Docent Walk

9/17 - 9am (VC)
Feed the Birds and Docent Walk

9/23 - 9am
Family Fish Camp
at Bahia Grande

9/24 - 9am
National Public Lands Day Cleanup
at Steve Thompson Wildlife Dr

WHAT TO WATCH FOR

Kiskadee Cruisers Run Club

Garden Club

Oct 9-15 National Wildlife Refuge Week

for more information and registration flanwr.org/events

Creature Feature: Rio Grande Chirping Frog

You might wonder what kind of animal makes a sound like squeaky shoes in the middle of the night. This secretive little creature is actually a tiny frog which most people have never heard of let alone seen. The Rio Grande Chirping Frog can only be found in the lower Rio Grande Valley and south into a small portion of the Mexico border. These spotted, mottled, olive-green and brown amphibians are barely a half inch long. Although they have been introduced into various locations in Texas, this is likely due to inadvertent transportation from the plant nursery business. They prefer heavy leaf litter and vegetation, while also having a preference for seeking shelter under large rocks and flower pots. The warmth received from the rocks is likely the reason why these frogs are able to survive outside of their usual habitat and have developed populations in cities such as San Antonio and Houston, to name a few. The most interesting feature about the Rio Grande Chirping Frog is their breeding habits. During spring and early summer, females lay 5-13 tiny eggs barely underneath the surface of moist soil. This is highly unlike other species of frogs, which deposit their eggs in the water. After about 15 days, the eggs hatch into tiny froglets, undergoing metamorphosis inside the egg. This unique process is known as direct development. Due to their small size, their main diet consists of tiny insects such as flies, spiders, and springtails. Next time you are sitting outside on a warm summer night, listen for the squeaky calls of the chirping frogs, and maybe, just maybe, you will have the privilege of observing these amazing creatures in their natural habitat.

SUPPORT US

Save Texas Ocelots License Plates

The Nature Store @ Laguna Atascosa

GO SHOPPING!

Adopt an Ocelot

Members-Only Events on the Horizon

Looking for another reason to become a member of the Friends? In October, Friends will be starting members-only clubs! We will be starting with a run club, the Kiskadee Cruisers Run Club, and a Garden Club.

The Kiskadee Cruisers Run Club will have its kickoff event with a 5k run for the Walk for the Wild on October 15 during National Wildlife Refuge Week. During this event, participants will run a guided 5k (3.1 mile) route through a Refuge trail. This trail will begin adjacent to Laguna Atascosa Lake and feature three ponds for wildlife viewing. A water station and selfie station will also be present. The event will start at 6 am at Osprey Overlook on Saturday, October 15.

Friends is starting a members-only Garden Club that will meet monthly to revitalize the garden in front of the Refuge Visitor's Center. Activities will include weeding, planting native pollinator plants, and watering. Our first event is Monday, October 3 at 9 am.

In addition to being invited to members-only clubs, members are entitled to 1 vote per member bundle and can attend the membership Annual meeting, are eligible for a position on a committee & on the board of directors, receive a 15% discount on merchandise purchased at the store (must show card), and receive early-bird announcements for major events.

To find out more about how to become a member, go to flanwr.org/members

Board of Directors 2021 - 2022

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Robert Severson

Vice President
Pete Moore

Secretary
Joyce Hamilton

Treasurer
Dr. Thomas deMaar

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Dr. Greg Garcia

Dr. Christopher Gabler

Dr. Sharon Wilcox

David Lohse

Shane Wilson

Cathy Harrington

STAFF

Executive Director
Nicole Ekstrom

Bookstore Manager
Michaela Wiersema

Ocelot

Scientific Name: *Leopardus pardalis*

Federal Status: Endangered, 3/30/72• State Status: Endangered

Description

The Ocelot is a beautiful medium-sized spotted cat with body dimensions similar to the bobcat (30-41 inches long and 15-30 lbs). Its body coloration is variable; with the upper parts gray or buff with dark brown or black spots, small rings, blotches, and short bars. A key feature is the parallel stripes running down the nape of the neck. The under parts are white spotted with black. The Ocelot's long tail is ringed or marked with dark bars on the upper surface. The backs of the rounded ears are black with a white central spot.

Ocelot
© USFWS Tom Smylie

Ocelot kittens
© USFWS Linda Laack

Habitat

In Texas, Ocelots occur in the dense thorny shrub lands of the Lower Rio Grande Valley and Rio Grande Plains. Deep, fertile clay or loamy soils are generally needed to produce suitable habitat. Typical habitat consists of mixed brush species such as spiny hackberry, brasil, desert yaupon, wolfberry, lotebush, amargosa, whitebrush, catclaw, blackbrush, lantana, guayacan, cenizo, elbowbush, and Texas persimmon. Interspersed trees such as mesquite, live oak, ebony, and hackberry may also occur.

Canopy cover and density of shrubs are important considerations in identifying suitable habitat. Optimal habitat has at least 95% canopy cover of shrubs, whereas marginal habitat has 75-95% canopy cover. Shrub density below the six foot level is the most important component of Ocelot habitat. Shrub density should be such that the depth of vision from outside the brush line is restricted to about five feet. Because of the density of brush below the six foot level, human movement within the brush stand would often be restricted to crawling.

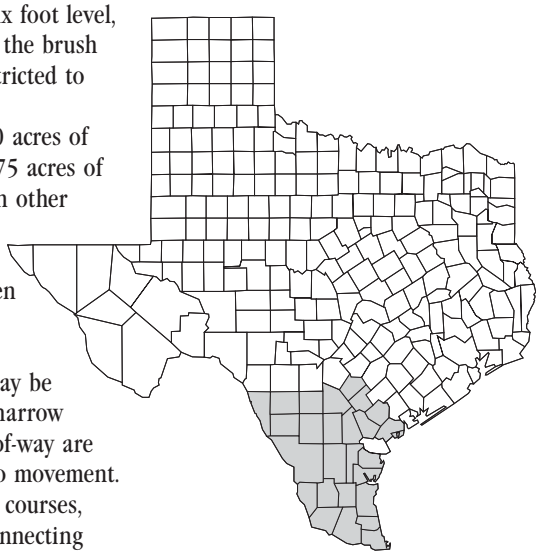
Tracts of at least 100 acres of isolated dense brush, or 75 acres of brush interconnected with other habitat tracts by brush corridors, are considered very important. Even brush tracts as small as 5 acres, when adjacent to larger areas of habitat, may be used by Ocelots. Roads, narrow water bodies, and rights-of-way are not considered barriers to movement. Brushy fence lines, water courses, and other brush strips connecting areas of habitat are very important.

Historical records indicate that the Ocelot once occurred throughout south Texas, the southern Edwards Plateau Region, and along the Coastal Plain. Over the years, the Ocelot population declined primarily due to loss of habitat and predator control activities. Today, Texas counties that contain areas identified as occupied habitat are: Cameron, Duval, Hidalgo, Jim Wells, Kenedy, Kleberg, Live Oak, McMullen, Nueces, San Patricio, Starr, Willacy, and Zapata.

Life History

Ocelots normally begin their activities at dusk, when they set out on nightly hunts for rabbits, small rodents, and birds. They move around during the night, usually within a well-established home range (area of activity) of one to two square miles for females and three to four square miles for males. Most mornings they bed down in a different spot within the territory. Male Ocelots tend to travel more than females. Males generally cover an extensive area in a short time, whereas females cover less area but use the home range more intensively.

Female Ocelots occupy a den for their kittens in thick brush or dense bunchgrass areas surrounded by brush. The den is often a slight depression with the dead leaves and mulch scraped away. The usual litter size is one or two kittens. The



mother goes off to hunt at night, but spends each day at the den site. The kittens begin to accompany their mother on hunts at about 3 months of age. They stay with her until they are about a year old. Studies have shown that kittens are born from late spring through December.

Threats and Reasons for Decline

Historically, the South Texas Plains supported grassland or savanna-type climax vegetation with dense mixed brush along dry washes and flood plains of the Rio Grande. The extensive shrub lands of the Lower Rio Grande Valley have been converted to agriculture and urban development

over the past 60 years. Much of this land, particularly the more fertile soils, has been cleared for production of vegetables, citrus, sugarcane, cotton, and other crops. Unfortunately for the Ocelot, the best soil types also grow the thickest brush and thus produce the best habitat. Less than 5% of the original vegetation remains in the Rio Grande Valley.

Only about 1% of the South Texas area supports what is currently defined as optimal habitat. Most of this habitat occurs in scattered patches probably too small to support Ocelots for extended periods. As a result, young cats dispersing from areas of suitable habitat have no place to go and most are probably hit by cars or die of disease or starvation. Road mortality is a more recent reason for decline. As Ocelot habitat in South Texas becomes fragmented by bigger highways with faster traffic, Ocelots have become increasingly vulnerable to being struck by vehicles while crossing roads. About half of the Ocelot mortality documented in the past 20 years has been from road mortality.

The Ocelot population in Texas is very small, probably no more than 80 to 120 individuals. Approximately 30 to 35 live in the chaparral remaining at or near the Laguna Atascosa National Wildlife Refuge. Unless vigorous conservation measures are taken soon, this beautiful cat may join the list of species extirpated from the United States.

Recovery Efforts

Much information has been obtained recently concerning Ocelot biology in south Texas. However, there is still much to be learned regarding reproduction, rearing of young, dispersal, home range, and movements. Efforts to inform landowners and the public about the habitat needs, land management options, and biology of the Ocelot are critical to recovery.

Conservation of remaining habitat, and maintenance or creation of brush corridors connecting these habitats, is necessary for survival of the Ocelot population in Texas. The U.S. Fish and Wildlife Service, Texas Parks and Wildlife Department, The Nature Conservancy, and many local landowners have been working to protect, acquire and restore Ocelot habitat in the Rio Grande Valley. Restoration generally involves revegetating previously cleared areas with native trees and shrubs.

The U.S. Fish and Wildlife Service and the Texas Department of Transportation are also working together to try and reduce Ocelot road mortality by installing Ocelot underpasses under roads where Ocelots are known to frequently cross.

Where To Learn More About Ocelots

The best places to visit to learn more about the Ocelot are the Laguna Atascosa National Wildlife Refuge near Rio Hondo (956) 748-3607, Santa Ana National Wildlife Refuge near Alamo (956) 787-3079, Bentsen-Rio Grande Valley State Park near Mission (956) 585-1107, Las Palomas Wildlife Management Area near Edinburg (956) 447-2704, and Audubon's Sabal Palm Grove Sanctuary near Brownsville (956) 541-8034.

How You Can Help

You can be involved with the conservation of Texas' nongame wildlife resources by supporting the Special Nongame and Endangered Species Conservation Fund. Special nongame stamps and decals are available at Texas Parks and Wildlife Department (TPWD) field offices, most state parks, and the License Branch of TPWD headquarters in Austin.
The Feline Research Program at the Caesar Kleberg Wildlife Research Institute (Texas A&M University-Kingsville) also accepts contributions to its Cat Conservation Fund. These funds are dedicated to the research and recovery of free-ranging wild cats of Texas. For more information, contact the Feline Research Program at (361) 593-3922.

The non-profit group, Friends of Laguna Atascosa Refuge, has an Adopt-an-Ocelot program in which 100% of the donated funds go towards ocelot conservation. For a small donation, participants receive an adoption packet that includes life histories and pictures of ocelots living at Laguna Atascosa National Wildlife Refuge, ocelot facts, and an adoption certificate. To learn more, contact Linda Laack at (956) 748-3607 or write Adopt-an-Ocelot, P.O. Box 942, Rio Hondo, Texas 78583.

The public is asked to report sightings of Ocelots to the Feline Research Program, Texas Parks and Wildlife Department, or U.S. Fish and Wildlife Service. Be sure to note tail length, size, color, habitat, behavior, location, date, and time of day seen.

Sub-tropical forest habitat
© TPWD

Habitat loss in the Lower Rio Grande Valley
© TPWD Bill Reaves

For More Information Contact

Texas Parks and Wildlife Department
Wildlife Diversity Branch
4200 Smith School Road
Austin, Texas 78744
(512) 912-7011 or (800) 792-1112
or
U.S. Fish and Wildlife Service
Laguna Atascosa National Wildlife Refuge
P.O. Box 450
Rio Hondo, Texas 78583
(956) 748-3607
or
U.S. Fish and Wildlife Service
Ecological Services – LRGV Office
Route 2, Box 202-A
Alamo, Texas 78516
(956) 784-7560

Management guidelines are available from the Texas Parks and Wildlife Department or U.S. Fish and Wildlife Service for landowners and managers wishing to conserve and improve habitat for the Ocelot.

References

Burt, W.H. and R.P. Grossenheider. 1964. *A field guide to the mammals.* Houghton Mifflin Company, Boston, Mass. 284pp.

Davis, W.B. and D.J. Schmidly. 1994. *The mammals of Texas.* Texas Parks and Wildlife Press. Austin, Texas. 338pp.

Tewes, M.E. and D.J. Schmidly. 1987. "The neotropical felids: jaguar, ocelot, margay, and jaguarundi" *in* M. Novak, J. Baker, M.E. Obbard and B. Malloch (eds.) Wild Furbearer Management and Conservation in North America. Ministry of Natural Resources, Ontario. 697-711.

U.S. Fish and Wildlife Service. 1990. *Listed cats of Texas and Arizona recovery plan (with emphasis on the ocelot)*. Endangered Species Office, Albuquerque, N.M.

Walker, E.P., F. Warnick, K.I. Lange, H.E. Uible, and P.F. Wright. 1975. *Mammals of the world. Vol. 2.* John Hopkins Univ. Press, Baltimore. 1500pp.

Funds for the production of this leaflet were provided by the U.S. Fish and Wildlife Service, under Section 6 of the Endangered Species Act.

Resume and Experience of Judy Berens

I obtained my first Personal Pet License from the Florida FWC in 1994, and my first cat was an ocelot. In 1998, I added another ocelot that was scheduled for confiscation, and a sickly 3 month old puma cub, who recovered from a bad case of metabolic bone disease.

In 1999, I purchased a 10 acre farm, incorporated Panther Ridge Sanctuary and began building enclosures. Over the next several years, I took in 3 more pumas and a serval. By 2003, it was time to become a 501c3. I added another puma and a tiger owned by another party, who helped with the care of the cats. And the cats kept coming - - a black leopard and a puma with a 2 day old cub that came from a facility that was being closed down by the USDA. Soon thereafter, I bought 2 clouded leopards and took in a caracal that needed to be re – homed.

From 2004 – 2009 I attended all of the Felid Tag conferences and became aquainted with many AZA people. During that time period, I hosted an FCF husbandry course, became a professional member and an accredited facility of the FCF, added 2 ocelots, 2 jaguars and 2 cheetahs and attended my first ZAA meeting. In 2007, Panther Ridge Sanctuary changed it's name to Panther Ridge Conservation Center, to more accurately reflect our support for in situ projects for various species, especially cheetahs and clouded leopards, and our mission for the future. The center has supported Cheetah Conservation Botswana, Cheetah Outreach (plus having sourced and exported two female Anatolians to them), Jim Sanderson's Andean Mountain cat project and the Clouded Leopard Project spearheaded by Ric Schwartz of the Nashville Zoo and the late Jo Gayle Howard.

During the summers of 2004 and 2005 Panther Ridge endured 3 major hurricanes. Fortunately, our caging was very well built and the emergency plans that I had developed early on worked perfectly. All of the cats were back out in their enclosures after the storms passed and we had checked the integrity of the caging materials. 2005 also brought us 4 clouded leopards from the Audubon Zoo.

They were aged 12 – 14, and had been used in the artificial insemination program there when hurricane Katrina devastated the zoo.

Our dietary plan has been thoroughly researched with the help of my vet and experts on various species. I have conferred with nutritionists from White Oak, Busch Gardens and the Palm Beach Zoo. Food items, including whole prey, are used as one form of enrichment. Our volunteers have a broad range of experience in enrichment from previous endeavors. One of them has spent months in South America working at various conservation projects, another is an excellent dog trainer and another is quite advanced in operant training, which we are using now to condition as many cats as possible for veterinary procedures without the need of tranquilization.

I am a firm believer in the support of in situ projects and the importation of genetically significant cats for breeding in this country…..it may be the only way many of these species will avoid extinction.

In 2014 Tropical Storm Isaac dumped 20" of rain on us over just a few days. We enacted our hurricane plan, as all but 2 of our enclosures were under a foot of water. USDA is now requiring emergency plans as the FWC in Florida has done for years. I cannot stress how important this is, and all staff and volunteers at Panther Ridge must commit the plans to memory. In November of 2018, Panther Ridge moved to a 5 acre parcel in Loxahatchee Florida. We have built a totally new facility from top to bottom.

My animal experience includes:

Tiger (pantera tigris), Leopard (pantera pardus), Clouded Leopard (neofelis nebulosa), Ocelot (leopardus pardalis), Cougar (puma concolor), Jaguar (pantera onca),Cheetah (acinonyx jubatus), Caracal (caracal caracal), Bobcat (lynx rufus), Serval (leptailurus serval) and Fishing cat (prionailurus viverrinus)

Panther Ridge currently holds 20 cats…PRCC and I are currently licensed by the Florida Fresh Water Fish and Game Commission, for felidae, and by the USDA.

Sadie Ryan

[redacted] Royal Palm Beach, FL 33411

Education

University of Wisconsin – La Crosse
Bachelor of Science in Biology, August 2014

Related Experience

Head Keeper — March 2016-present
Panther Ridge Conservation Center — Wellington, FL – Loxahatchee Groves, FL

- Primary caretaker and trainer of over 22 Class I & II carnivores including cheetahs, jaguars, cougars, leopards, servals, caracals, ocelots, fishing cats and clouded leopards
- Implemented and manage new volunteer & intern programs, redesigned and rebranded website
- Responsible for daily posting on all social media outlets and quarterly newsletters
- Care includes but not limited to shifting, diet prep, exhibit maintenance, cleaning enclosures, detailed animal observations and documentation, grounds maintenance, daily behavioral training sessions, assist in medical procedures, implementing enrichment items and activities
- Responsible for giving educational guided tours to guests and scheduling tours
- Critical care on neonate species including caracal, jaguar, fishing cat, cougar, cheetah, and clouded leopard
- Helped in entire facility relocation, animal transportation and layout to new property

Cheetah/Carnivore Intern — September 2015-March 2016
Wildlife Safari — Winston, OR

- Responsible for the care of Class 1 carnivores, including African lions, Sumatran tigers, cheetahs, black bears and brown bears. Care includes, but not limited to, shifting animals, cleaning enclosures, diet prep, daily behavioral training, enrichment, and all animal observations detailed in daily logs and ZIMS
- Worked free contact with 18 cheetahs
- Presented daily encounter programs for public, including keeper talks and public training sessions
- Participated in both on- and off-site educational programs with ambassador cheetahs
- Weekly cross training in ungulate department - caring for 25 different exotic and native species including but not limited to giraffes, rhinos, zebra, elk, bison, ostrich, hippos, eland, etc.

Carnivore/Primate Intern — December 2014-March 2015
Tiger World — Rockwell, NC

- Provided daily care and enrichment for over 40 different species
- Monitored and documented daily animal health, food logs and observations
- Assisted in the maintenance of the grounds and exhibits
- Conducted educational tours, keeper talks and acted as a source of information to guests

Seasonal Zookeeper — May 2014-October 2014
Santa's Village Azoosment Park & A Zoo To You — East Dundee, IL

- Performed basic husbandry care for exotic and domestic animals including preparing and distributing diets
- Educated guests about animals and correct animal feeding/interaction protocols
- Assisted in exotic animal shows, led children's pony rides, and supervised guest interactions in petting zoo
- Observed animal behaviors and participated in capture and restraint practice

Other Experience

West Nile Virus Field/Lab Tech Internship — April 2015-August 2015
Clarke Environmental — Roselle, IL

- Identified genus and species of samples brought into lab and performed virus testing on adult samples
- Passed the Illinois Department of Agriculture General Standard Exam in order to participate in ongoing local and remote field evaluations of new pesticide formulations

Undergraduate Research — October 2013-May 2014
Biology Department, Mississippi River Center Studies — La Crosse, WI

- Measurement of carbon quality and quantity in sediments from various regions of the Upper Mississippi River Valley
- Presented findings at the Mississippi River Research Conference

USDA

Expiration Date: 01-03-2023

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Animal Care

This is to certify that
PANTHER RIDGE CONSERVATION CENTER INC

is a licensed Class C - Exhibitor
under the

Animal Welfare Act

(7 U.S.C. 2131 et seq.)

Certificate No. 58-C-1003

Customer No. 12392

Elisabeth Goldstein

Deputy Administrator

Special Authorization Permit

FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION
DIVISION OF LAW ENFORCEMENT, CAPTIVE WILDLIFE OFFICE
620 SOUTH MERIDIAN STREET
TALLAHASSEE, FLORIDA 32399-1600

Permittee Name:	Judith Berens	Permit #:	SA-22-0005
Permittee Address:	2143 D Road	Effective Date:	2/16/2022
City, State, Zip Code:	Loxahatchee, Florida 33470	Expiration Date:	12/12/2022

AUTHORIZED PERSONS:	AUTHORIZATIONS:
Elizabeth Felton	Class II: Felidae
Sadie Ryan	Class I: Felidae (including cougars and cheetahs) Class II: Felidae

ARE AUTHORIZED TO:
Possess the listed wildlife on behalf of the permittee in a traveling exhibition capacity (itinerary shall be submitted to Florida Fish and Wildlife Conservation Commission prior to travel) and/or in a delivery situation to a properly licensed dealer, veterinarian, or licensed facility.

AUTHORIZED LOCATION:
Panther Ridge Conservation Center, Inc.- 2143 D Road, Loxahatchee, Florida 33470

Permittee Signature J Berens Date 2/16/22

Not valid unless signed. Your signature confirms that all information provided to issue the permit is accurate and complete, and indicates acceptance and understanding of the provisions and conditions listed below. **Any false statements or misrepresentations when applying for this permit may result in felony charges and will result in revocation of this permit.**

Authorized by: Lieutenant John ~~Conlin~~, Division of Law Enforcement

Authorizing Signature JTCONLIN N400 Date 2/16/2022

PERMIT CONDITIONS AND PROVISIONS:

1. Each authorized person must demonstrate the experience requirements necessary to temporarily possess the listed wildlife, pursuant to Rule 68A-6.004, Florida Administrative Code.
2. This Special Authorization Permit is only valid when it is accompanied by a License to Possess Class I and/or Class II Wildlife for Exhibition or Public Sale (ESA), Applicant ID 75719, License ID 157231.
3. The ESA licensee must provide financial responsibility guarantee for Class I wildlife possessed by the persons authorized on this permit to cover the animals when in travel status.

LICENSE TO POSSESS CLASS III WILDLIFE FOR EXHIBITION OR PUBLIC SALE

Florida Fish and Wildlife Conservation Commission
PO Box 6150, Tallahassee, FL 32314-6150
(850) 488-6253

ESC 28240
Applicant ID: 75719 License ID: 403-157218

Cost: $50.00

Issued: 02/16/2022
Expires: 03/17/2023

PANTHER RIDGE CONSERVATON CENTER, INC.
2143 "D" ROAD
LOXAHATCHEE, FL 33470

Location Address:
BERENS, JUDITH
2143 "D" ROAD
LOXAHATCHEE. FL 33470

LICENSEE AUTHORIZATIONS

This license DOES NOT authorize the sale of federally-designated Endangered and Threatened species, state-designated Threatened species or state-designated Species of Special Concern, per Rule 68A-27.003 and 68A-27.005 F.A.C., respectively, or the possession of more than the limit, per Rule 68A-25.002 F.A.C.

CLASS 3

ALL CLASS III MAMMALS EXCEPT CAPUCHIN, SPIDER AND WOOLLY MONKEYS

A Person whose substantial interests are affected by FWC's action may petition for an administrative proceeding (hearing) under section 120.569 and 120.57 of Florida Statutes. A person seeking a hearing on FWC's action shall file a petition for hearing with the agency within 21 days of receipt of written notice of the decision. The petition must contain the contain the information and otherwise comply with section 120.569, Florida Statutes, and the uniform rules of the Florida Division of Administration, 28-106, Florida Administrative Code. If the FWC receives a petition, FWC will notify the permittee. The enclosed Explanation of Rights statement provides additional information as to the rights of parties whose substantial interests are or may be affected by this action.

Certificate of Accreditation

Promoting the responsible ownership, management, and propagation of animals in both private and public domains

ZAA

ZOOLOGICAL ASSOCIATION
OF AMERICA

Panther Ridge Conservation Center

has been awarded this certificate for meeting the qualifications of

ZAA Accreditation

Dr. Barbara Baker

Dr. Barbara Baker
ZAA Board Chair

Accredited Since 2015

SEAL of ACHIEVEMENT

Holly Hunt

Holly Hunt
ZAA Accreditation Chair

Accredited Through 2026

ZAA

ZOOLOGICAL ASSOCIATION OF AMERICA

May 21, 2021

Judy Barrens
Panther Ridge Conservation Center
2143 D Rd
Loxahatchee
Fl 33470

Dear Judy

On behalf of the Zoological Association of America (ZAA), I would like to congratulate you on the successful completion of Panther Ridge Conservation Center's, ZAA Facility Accreditation assessment and audit! Accreditation is an important part of ZAA's facility membership program and exemplifies our organization's commitment to excellence in animal husbandry, and facility operations.

Your facility, Panther Ridge Conservation Center, continues to meet all ZAA's objectives:

- Professional standards for husbandry and animal care practices.
- Accurate animal and medical records.
- Appropriate, safe, and quality existence for animals kept in a captive environment.
- Safe environment for humans; both staff and visitors.
- Enhanced survival of species using appropriate methods.

As an accredited facility, you will have access to the Surplus and Want Lists available through the ZAA website at www.zaa.org . Your facility's accreditation number is, AC-197.

Please note your facility must get reaccredited every five years or upon relocation. Your next reaccreditation assessment and audit is May 2026.

We hope you proudly display your accreditation certificate to show everyone that your facility meets all ZAA's accreditation standards.

Congratulations again and thank you for your continued support in promoting the responsible ownership, management, and propagation of animals in your accredited facility.

Sincerely,
John Seyjagat

Executive Director
john@zaa.org
4433925897

Objective:

-Restore & Enhance the survival of the Ocelot Species in our nation.

-Educating the public to inspire and promote the conservation and future of the Ocelots & their wild and natural habitats.
-Displaying the Ocelots with signage that will teach the tour groups about this species.
-Donating a portion of the tour profits to Ocelot Conservation.
-Working and Assisting Fish & Wildlife with their efforts in building back the wild population of this
species through propagation.
-Contributing to the scientific research conducted at Texas A&M.
-

Note* currently applying with Fish & Wildlife Federal grants for ESA species survival and conservation plan.

Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Thu 2/15/2024 2:09 PM

To:Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>;NHalpern@foxrothschild.com <NHalpern@foxrothschild.com>

Good afternoon,

Thank you for your application on behalf of Panther Ridge Conservation Center Inc for the import of two live ocelots (*Leopardus pardalis*). I apologize for the delay. I have reviewed this application and found that it is incomplete and information is required to proceed with the processing the request. Please provide the following:

1. Information regarding the dam and sire of the two ocelots requested for import, including where they were born and birth dates.

2. Question # 13. Justification for the requested activity under captive propagation for the conservation and survival of the species. The current justification is inadequate for the following reasons:
    a. In the application the stated purpose of import is for scientific research and conservation education/zoological display. However, *Leopardus pardalis* is listed as Endangered under the Endangered Species Act. As such, under 50 CFR 17.22 the issuance criteria for an ESA permit considers scientific purposes, enhancing the propagation or survival, or incidental take for endangered wildlife.
        i. It is unlikely that the current application will meet enhancement for education/zoological display as this justification is not among of the criteria that is considered for Endangered species; this purpose is considered for Threatened listed species [50 CFR 17.32(a)(1)].
        ii. It is also unlikely that the current application will meet enhancement for scientific purposes given that the facility requesting the import is not proposing to conduct the research themselves and is therefore not the primary researcher. In addition, to meet enhancement under scientific research it would need to be adequately demonstrated that import of live ocelots are necessary for the proposed research rather than importing samples collected from those animals. Such a justification was not provided in the proposal.
    b. Based on the information provided in the application, captive propagation for conservation and survival of the species appears to be more applicable to the requested import rather than scientific research, which is not the predominate purpose for the import, and education/zoological display, which is not among the purposes in the federal regulations that are considered for exemption from prohibited activities concerning endangered listed species. All items under Question # 13 must be responded to.

3. Question # 15. Provide the CV or resume for Judith Berens. While a summary of her experience is helpful, it lacks some of the details we look for from a CV or resume.

4. Question # 16. What is the current inventory of ocelots maintained at Panther Ridge Conservation Center Inc. (by sex)?

5. Question # 19. The number of mortalities for the species at Panther Ridge Conservation Center Inc. and cause of death over the past 5 years.

6. Question # 20. A detailed description, diagrams, and photos clearly depicting the existing facilities where the wildlife will be maintained including dimensions, materials, and protection from the elements. Only three photos of the facility were provided which is inadequate. Response to this question should include a thorough written technical description along with photographs.

7. Question # 21. How was the carrying capacity determined?

8. Question # 22. What are the units of the dimensions of the crate? What is the material of the divider between the two animals? Will anyone being accompanying the animals during transport?

9. Provide a copy of the invoice specified on the Certificate of Origin between Mystic Monkeys and Feathers Wildlife Park and Panther Ridge Conservation Center Inc.

If you have questions or need clarification on the above, please let me know. Thank you.

In accordance with 50 CFR 13.ll(e), if the requested information is not received by this office within 45 calendar days of the date of this email, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application, and all required fees, for the Service to consider your proposed activity.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

Lamberson, Amanda M

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Thursday, March 28, 2024 12:53 PM
To: Lamberson, Amanda M
Cc: JUDY BERENS
Subject: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center
Attachments: Question 2 #13 .pdf; AZA ocelot SSP sustainability partner policy and application document .pdf; Mystic Monkey Invoice.pdf; Mystic statement and responses .pdf; Question 6 #20.pdf

Follow Up Flag: Flag for follow up
Flag Status: Flagged

This email has been received from outside of DOI - Use caution before clicking on links, opening attachments, or responding.

Good Afternoon,

Thank you for taking the time to review the application CS0582384 for Panther Ridge.
We have answered your questions below; there is a total of six (6) attachments that correlate with our responses.

Sincerely,

Sheri Sublett
Executive Director
Twin Pine Farm & Exotics Conservation Foundation
(843)310-4441
(843)217-9188 (mobile)
TPFECF@Lowcountry.com
www.TPFECF.org
Facebook.com/TPFECF

On Feb 15, 2024, at 2:09 PM, Lamberson, Amanda M <amanda_lamberson@fws.gov> wrote:

Good afternoon,

Thank you for your application on behalf of Panther Ridge Conservation Center Inc for the import of two live ocelots (*Leopardus pardalis*). I apologize for the delay. I have reviewed this application and found that it is

incomplete and information is required to proceed with the processing the request. Please provide the following:

1. Information regarding the dam and sire of the two ocelots requested for import, including where they were born and birth dates. ***Ocelot female: 11/01/2022 Parents: Mother: Twix Birth date: 09/22/2016 (On premises) Father: Thunder Birth date: 09/10/2017 (On premises) Ocelot male: 12/28/2021 Parents: Mother: Mica Birth date: 07/26/2017 (On Premises) Father: Mike Birth date: 06/01/2017 (On premises)***

2. Question # 13. Justification for the requested activity under captive propagation for the conservation and survival of the species. The current justification is inadequate for the following reasons:
    a. In the application the stated purpose of import is for scientific research and conservation education/zoological display. However, *Leopardus pardalis* is listed as Endangered under the Endangered Species Act. As such, under 50 CFR 17.22 the issuance criteria for an ESA permit considers scientific purposes, enhancing the propagation or survival, or incidental take for endangered wildlife.
        i. It is unlikely that the current application will meet enhancement for education/zoological display as this justification is not among of the criteria that is considered for Endangered species; this purpose is considered for Threatened listed species [50 CFR 17.32(a)(1)].
        ii. It is also unlikely that the current application will meet enhancement for scientific purposes given that the facility requesting the import is not proposing to conduct the research themselves and is therefore not the primary researcher. In addition, to meet enhancement under scientific research it would need to be adequately demonstrated that import of live ocelots are necessary for the proposed research rather than importing samples collected from those animals. Such a justification was not provided in the proposal.
    b. Based on the information provided in the application, captive propagation for conservation and survival of the species appears to be more applicable to the requested import rather than scientific research, which is not the predominate purpose for the import, and education/zoological display, which is not among the purposes in the federal regulations that are considered for exemption from prohibited activities concerning endangered listed species. All items under Question # 13 must be responded to.

    See attachments:

    1. " Question 2 #13"

    2. " AZA AZA ocelot SSP sustainability partner policy and application"

3. Question # 15. Provide the CV or resume for Judith Berens. While a summary of her experience is helpful, it lacks some of the details we look for from a CV or resume.

   See attachment: "Judy Berens 2024 Professional Resume"

4. Question # 16. What is the current inventory of ocelots maintained at Panther Ridge Conservation Center Inc. (by sex)?

   Total current inventory: 1

   Details: "Delilah F Age 21

5. Question # 19. The number of mortalities for the species at Panther Ridge Conservation Center Inc. and cause of death over the past 5 years.

   Total number of Ocelot mortalities: 2

   Details: 1. "Toltec" M age 16 (Neurological)

   2. "Cody" M age 25 (Old Age)

6. Question # 20. A detailed description, diagrams, and photos clearly depicting the existing facilities where the wildlife will be maintained including dimensions, materials, and protection from the elements. Only three photos of the facility were provided which is inadequate. Response to this question should include a thorough written technical description along with photographs.

   See attachment: "Question 6 #20"

7. Question # 21. How was the carrying capacity determined?

   See attachment: "Mystic statement and responses"

8. Question # 22. What are the units of the dimensions of the crate? What is the material of the divider between the two animals? Will anyone being accompanying the animals during transport?

   ****See attachment: "Mystic statement and responses"***

9. Provide a copy of the invoice specified on the Certificate of Origin between Mystic Monkeys and Feathers Wildlife Park and Panther Ridge Conservation Center Inc.

See attachment: "Mystic Invoice"

Total number of attachment files: 6

13. Captive propagation for the conservation & survival of the species

a. The species will be propagated by using artificial insemination and natural breeding techniques. Upon arrival the cats will have genetic testing performed which will be added to the Ocelot Genome. Panther Ridge will work in collaboration with the Ocelot SSP and coordinate propagation together.

b. Please see attached "*AZA ocelot SSP sustainability partner policy and application document file*".

c. Breeding stock will be genetically tested for relatedness.

1. Male and female will have no contact with any other animals.

2. With the guidance of the Ocelot SSP and the Ocelot studbook keeper, the offspring of this pair will be matched appropriately with the best genetically viable partner for future conservation.

3. Based upon the Ocelot genetic genome bank & the results from the Ocelot Studbook matches, the offspring will be paired properly to the best matched mate.

4.The carrying capacity at Panther Ridge is currently three (3) pairs with possibility of future expansion

5. Based on conservation priorities from the SSP and studbook coordinator the offspring will be managed appropriately to enhance the species survival. Offspring will be used for educational programs, outreach initiatives, or public exhibits to raise awareness about conservation issues, species diversity, and the importance of ocelot conservation.

d. N/A (reintroductions of future possibility coordinated with Laguna Atascosa, ocelot SSP and Texas A&M)

ASSOCIATION OF ZOOS & AQUARIUMS

Sustainability Partner Policy & Application

Information & Application to Become an Approved Sustainability Partner in an AZA Species Survival Plan® Program

The digitized Full Application to become an Approved Sustainability Partner can be obtained by contacting conservation@aza.org.

Approved by the AZA Board of Directors 1 December 2009, Revised March 2014

The Association of Zoos and Aquariums (AZA) is a professional organization representing accredited zoological parks and aquariums and certified related facilities. Among its objectives, AZA strives to raise professional standards that foster the continued development of superior zoos and aquariums and best practices in animal population management.

AZA-accredited zoos and aquariums serve as centers of excellence in wildlife conservation, and public education and create animal exhibits that provide society the opportunity to develop personal connections with the animals and nature. As such, AZA-accredited zoos and aquariums are concerned about ecosystem health, take responsibility for species survival, contribute to research, and promote the highest standards of animal care and welfare in the management of small populations of earth's precious wildlife species.

Expectations for Approved Sustainability Partners in an AZA Species Survival Plan® (SSP) Program

AZA SSP Programs focus on the conservation of select and typically threatened or endangered species through the cooperative management of small populations at AZA-Accredited Zoos and Aquariums and Certified Related Facilities. These institutions undergo a thorough accreditation review process that includes the submission of an extensive application as well as an intensive, on-site inspection by a team of experts to ensure the highest standards of animal care and management are met.

AZA believes that the highest standards of animal care, welfare, and population management are of paramount importance, and Approved Sustainability Partners are expected to agree and abide by AZA's Code of Professional Ethics, SSP Full Participation Policy, Animal Acquisition, Transfer and Transition Policy, and relevant Accreditation Standards, especially those related to animal care and welfare.

Sustainability Partners are *not considered accredited or certified*. They are referred to as Sustainability Partners in SSPs and may not display the AZA SSP logo. Like AZA accreditations and certifications, approvals for a Sustainability Partner's continued participation in an SSP Program must be renewed every five years.

Who is Eligible to Apply for Approval as a Sustainability Partner?

If an AZA SSP Program determines that a potential Sustainability Partner cares for animals that can provide genetic, demographic, conservation, husbandry, population management, and/or animal welfare benefits to the SSP Program, the following are eligible to apply for approval as Sustainability Partners:

1. **Zoos/aquariums located outside the U.S. that are members of the World Association of Zoos and Aquariums (WAZA) or one of WAZA's Super-Regional Zoological Associations**

   Note: Super-Regional Zoological Associations of WAZA include: Asociación Latinoamericana de Parques Zoológicos y Acuarios (ALPZA), Asociación Mesoamericana y del Caribe de Zoológicos y Acuarios (AMACZOOA), Zoo and Aquarium Association - Australasia (ZAA), Asociación de Zoológicos Criaderos y Acuarios de México (AZCARM), Canadian Association of Zoos and Aquariums (CAZA), Eurasian Regional Association of Zoological Parks and Aquariums (EARAZA), European Association of Zoos and Aquaria (EAZA), Japanese Association of Zoos and Aquariums (JAZA), African Association of Zoos and Aquaria (PAAZAB), South East Asian Zoos Association (SEAZA), South Asian Zoo Association for Regional Cooperation (SAZARC). Note: these are called super-regional associations because all other WAZA-member zoological associations fall under these larger umbrella organizations.

   Submission Requirements
   A. A Letter of Invitation & Justification from an AZA SSP Program for the applicant to participate in their SSP Program;
   B. A completed and signed Sustainability Partner Application; and
   C. A Letter affirming their membership in WAZA or their Super-Regional Zoological Association.

2. **Wildlife facilities located within or outside the U.S. that are not open to the public on a regularly scheduled and predictable basis.**

   Note: Wildlife facilities include, but are not limited to ranches, refuges, rehabilitation centers, research facilities, sanctuaries, survival centers, breeding facilities, private individuals, and educational outreach organizations.

   Submission Requirements
   A. A Letter of Invitation & Justification from an AZA SSP Program for the applicant to participate in their SSP Program;
   B. A completed and signed Sustainability Partner Application; and
   C. Letters of Sponsorship from the Directors of two different AZA-accredited institutions.

3. **Wildlife facilities located within or outside the U.S. that are open to the public on a regularly scheduled and predictable basis.**

   Note: Wildlife facilities include, but are not limited to ranches, refuges, rehabilitation centers, research facilities, sanctuaries, survival centers, breeding facilities, private individuals, and educational outreach organizations.

   Submission Requirements
   A. A Letter of Invitation & Justification from an AZA SSP Program for the applicant to participate in their Program;
   B. A completed and signed Sustainability Partner Application;
   C. Letters of Sponsorship from the Directors of two different AZA-accredited institutions;
   D. Verification of participation in the SSP Program in the SSP Program's most recent two SSP Breeding and Transfer Plans, or of participation for at least the last 5 years; if there have not been two Plans; and
   E. Submission of the most recent USDA report(s), if applicable.

Application Processes

Submission Process

Applications are reviewed by AZA's Wildlife Conservation Management Committee (WCMC) throughout the year via committee conference calls, as well as twice each year – during the AZA

Annual Conference and the AZA Mid-Year Meeting. No fees are charged for the Sustainability Partner application.

The SSP Coordinator must work with the applicant to ensure all application materials are complete. The SSP Coordinator must submit the completed application and required letters to the AZA Conservation & Science Department (conservation@aza.org), and ensure that the applicant does not participate in their SSP Program until they are formally approved as a Sustainability Partner.

The AZA Conservation & Science Department will review the application materials, and forward applications deemed to be complete and appropriate for review to the WCMC Vice Chair for Partnerships who will act as the coordinator of the Sustainability Partner's application (Appendix G). The Vice Chair of Partnerships will review application materials to identify potential needs for clarification. The Vice Chair of Partnerships will notify the AZA Conservation & Science Department when the application is ready for WCMC review, and distribute the application to the committee for review.

Review Process

WCMC will evaluate the benefits of the applicant being approved as an SSP Program Participant in the context of the entire AZA *ex-situ* population which includes all SSP animals at AZA-Accredited Institutions, Certified Related Facilities, and Approved Sustainability Partners.

Reviews are held either by conference call or in closed sessions, and are attended by WCMC members and advisors and AZA staff; in addition, members of the AZA Board of Directors, the SSP Coordinator, and/or the corresponding Taxon Advisory Group (TAG) Chair may attend as well. Approval decisions are based on the information that exists at the time of the application review, not on future plans. Crucial elements in WCMC's consideration include:

- Completion of application
- Application support letters
- Whether there is sufficient evidence that the applicant follows the tenets of AZA's Code of Professional Ethics, SSP Full Participation Policy, Acquisition, Transfer and Transition Policy, and relevant Accreditation Standards, especially those related to animal care and welfare.

WCMC may take one of the following actions:

- **Approval:** WCMC will grant approval when it determines that the applicant facility meets the requirements of an approved Sustainability Partner.
- **Table Approval:** WCMC may table a facility's materials if it determines that certain conditions must be met or additional information submitted before the facility can be considered as meeting Sustainability Partner requirements. In addition, WCMC must believe that the facility can meet those requirements within one year.
- **Deny Approval:** WCMC will deny approval when a facility does not meet the minimum requirements (see "expectations") to be recognized as an approved Sustainability Partner at the present time and, in its opinion, would require in excess of one year to successfully do so.

Once a decision is reached, the WCMC Vice Chair for Partnerships will inform the SSP Coordinator and the facility's Owner/CEO/Director of the outcome. An official letter noting the decision and points of discussion will be sent from the AZA Conservation & Science Department to the SSP Coordinator and the facility Director within 30 days of the application review.

Approved Sustainability Partners Responsibilities

Once approval has been granted the Approved Non-Member SSP Program Participant will:

- Agree to adhere to AZA's Code of Professional Ethics, SSP Full Participation Policy, Animal Acquisition, Transfer and Transition Policy, and relevant Accreditation Standards, especially those related to animal care and welfare.

- Appoint an Institutional Liaison (IL) to serve as the primary point of contact for SSP communications.
- Not display the SSP logo.
- On an as-needed basis, agree to allow the sponsoring AZA-accredited institution, staff, Board, WCMC, TAG, and/or SSP representatives to visit the applicant institution/zoo and view their facility, upon request, to ensure adherence to AZA policies and animal care and welfare practices. Such visitors will provide feedback to the SSP, TAG, and the WCMC.
- Submit a new, complete application for Sustainability Partner before the end of the five-year approval period in order to continue participation in the SSP Program.

Loss of Approval Status

A Sustainability Partner may have its approved status revoked if it fails to meet any of the Sustainability Partner responsibilities identified above and in the application.

If AZA/WCMC deems it appropriate, the SSP Program may work with a Sustainability Partner that loses its Approved status for up to two years to help manage the population, facilitate disposition of animals owned by AZA institutions and, when possible, mentor re-approval of Sustainability Partner status. In such instances, the Sustainability Partner will not be an active participant in the SSP but still may be considered during the planning processes. During this time the SSP will not move SSP animals to the former Sustainability Partner facility.

For reinstatement as a Sustainability Partner, the potential Sustainability Partner's benefit to the SSP population and adherence to AZA's Code of Professional Ethics, Animal Acquisition, Transfer and Transition Policy, and Animal Care and Welfare Standards must be reassessed.

Sustainability Partner Application

General Information

Organization Information:

1. Applicant Organization Name:
2. Type of Organization: (check all that apply)
   - ☐ Zoo Aquarium Sanctuary / Refuge / Rehabilitation or Survival Center
   - ☐ Educational Organization Breeding facility Private individual / breeder
   - ☐ Research Facility Privately owned / Financed Non-profit U.S. – 501(c) 3
   - ☐ Type of organizational governance (e.g. Board of Directors etc)._____
3. Street Address:

   City: State: Country
4. If located outside of the United States, is the Organization a member of:

   WAZA: Yes No

   WAZA Super-Regional Zoological Association: Yes No

   Name:
5. Is this Organization open to the public on a regularly scheduled and predictable basis?

   Yes Hours: No
6. Telephone Number: FAX number:
7. Website Address:

SSP Program Information

8. Name of SSP Program being applied for:
9. SSP Coordinator Name:
10. SSP Coordinator Telephone Number: SSP Coordinator E-mail:
11. Name of the corresponding TAG:
12. TAG Chair Name:
13. TAG Chair Telephone Number: TAG Chair E-mail:
14. Number of SSP Animals that are to be included in the SSP Program:

Representative Information

15. Name of the Applicant Organization's Director/CEO:
16. Applicant Director/CEO Telephone Number:
17. Applicant Director/CEO E-mail:
18. Name of the Applicant Organization's Institutional Liaison (IL):
19. Applicant IL Telephone Number:
20. Applicant IL's E-mail Address:

Supporting Materials

1. *Zoos/aquariums located outside the U.S. that are members of the World Association of Zoos and Aquariums (WAZA) or one of WAZA's Super-Regional Zoological Associations*

   ☐ Does this application include a completed Letter of Invitation and Justification from the SSP Program Coordinator?

☐ Does this application include a completed Letter Affirming the applicant's membership from WAZA or a Super Regional Zoological Association?

- Name of Association:
- Association Director Name:
- Association Director's Telephone Number:
- Association Director's E-mail Address:

2. *Wildlife facilities located within or outside the U.S. that <u>are not open</u> to the public on a regularly scheduled and predictable basis.*

☐ Does this application include a completed Letter of Invitation and Justification from the SSP Program Coordinator?

☐ Does this application include completed letters of sponsorship from two directors of two different AZA-accredited institutions?

Name of AZA-Accredited Sponsoring Institution # 1:
AZA Institution #1 Director Name:
Institution #1 Director's Telephone Number:
Institution #1 Director E-mail Address:

Name of AZA-Accredited Sponsoring Institution # 2:
AZA Institution #2 Director's Name:
Institution #2 Director's Telephone Number:
Institution #2 Director's E-mail Address:

3. *Wildlife facilities located within or outside the U.S. that <u>are open</u> to the public on a regularly scheduled and predictable basis.*

☐ Does this application include a completed Letter of Invitation and Justification from the SSP Program Coordinator?

☐ Does this application include completed letters of sponsorship from two directors of two different AZA-accredited institutions?

Name of AZA-Accredited Sponsoring Institution # 1:
AZA Institution #1 Director Name:
Institution #1 Director's Telephone Number:
Institution #1 Director E-mail Address:

Name of AZA-Accredited Sponsoring Institution # 2:
AZA Institution #2 Director's Name:
Institution #2 Director's Telephone Number:
Institution #2 Director's E-mail Address:

☐ Does this application include verification of participation in the most recent, two final SSP Breeding and Transfer Plans, or verification of participation for at least the last five years if two final Plans have not been completed?

☐ Does this application include the most recent USDA report(s), if applicable?

Applicant Agreement to Accept All Sustainability Partner Responsibilities

By signing below, I, (Name_____), (Title-this must be the Applicant Director or CEO) of the (Applicant Organization Name), fully agree to meet the responsibilities listed below if approved as a Sustainability Partner in the SSP Program and further acknowledge that failure to do so may result in a revocation of this approved status:

- ☐ I and my Staff will adhere to AZA's Code of Professional Ethics, SSP Full Participation Policy, Animal Acquisition, Transfer and Transition Policy, and Animal Care and Welfare Standards.
- ☐ I will provide the AZA SSP Program with complete studbook histories for all animals proposed for inclusion in the SSP managed population within 30 days of approval.
- ☐ I will not display the SSP logo.
- ☐ I will complete the full application process before the end of the five-year approval period ends in order to continue my Organization's participation in the SSP Program.
- ☐ I will ensure that the sponsoring AZA-accredited institution, staff, Board, WCMC, TAG, and/or SSP representatives can schedule a visit to the applicant institution/zoo upon request to view the facilities and ensure adherence to AZA policies and animal care and welfare practices.

Applicant Director/CEO Signature: _____ Date: _____

Letter of Invitation and Justification from an AZA SSP Program for a Sustainability Partner to Participate in their Program

The letter from an SSP Coordinator should:

1. Clearly identify the SSP for which the potential Sustainability Partner participant's involvement is being requested, as well as the SSP Coordinator's identity, telephone number and e-mail address;
2. Make a clear formal request for approval of the potential Sustainability Partner;
3. Identify very specifically why the potential Sustainability Partner's participation would benefit the SSP program and how approval of the potential Sustainability Partner for participation in the SSP will provide genetic, demographic, population management, welfare, conservation or other benefits to the SSP Program;
4. Provide confirmation that the SSP Coordinator has discussed in detail with the potential Sustainability Partner the responsibilities that the Non-Member will assume upon approval as an SSP Participant including assurance that the Sustainability Partner applicant has fully read and understood:
    - AZA's Code of Professional Ethics,
    - the Full Participation in the SSP Policy,
    - the Animal Acquisition, Transfer and Transition Policy, and the
    - relevant Accreditation Standards, especially those related to animal care and welfare.
5. Provide a clearly stated description of how the Sustainability Partner applicant provides a level of animal care and welfare in keeping with AZA's standards and identify how the SSP Coordinator has developed this understanding. This section should include any forms of information that corroborate the applicant's adherence to AZA standards of animal care and welfare (e.g. photographs, veterinary statements, USDA inspection reports, etc.).
6. Identify any additional factors that substantiate how approval of the potential Sustainability Partner's participation will benefit the SSP Program and conservation of the species.
7. End with the signature of the SSP Coordinator and the date.

Letter Affirming Membership in a Recognized Zoological Association for Organizations Outside the United States that are not Members of AZA, which are Applying for Approval in an AZA SSP Program

The letter from the appropriate association should:

1. Clearly identify the name of the organization /entity applying for approval as a participant in an AZA SSP Program;
2. Clearly identify the name of Association: *(WAZA or a WAZA Super-Regional Zoological Association);*

   Association Director Name:

   Association Director's Telephone Number:

   Association Director's E-mail Address:
3. Affirm that the applicant organization / entity is a current member in good standing of the Association providing this letter;
4. Provide clearly stated assurance that the applicant organization / entity provides a level of animal care and welfare in keeping with the standards of the Association providing this letter and identify how this information is known;
5. End with the signature of the Association Director and the date.

Letters From the Directors of Two AZA-accredited Institutions Sponsoring the Approval of a Non-Member Organization as an SSP Sustainability Partner

The letters from two, separate AZA-accredited Institution Directors should:

1. Identify the SSP for which the potential Sustainability Partner participant's involvement is being sponsored;
2. Provide a description articulating why you believe that the potential Sustainability Partner applicant will provide a benefit to the SSP Program;
3. Provide a statement that you understand that your signature on the letter of sponsorship serves as your assurance that the applicant organization provides a level of animal care and welfare equivalent to or above that of the relevant AZA Accreditation Standards. In this section please describe when you (or identify who on your senior staff) have visited the applicant's facility and outline the observations / experiences that substantiate why you believe the applicant adheres, or will adhere, to AZA's Code of Professional Ethics, SSP Full Participation Policy, Animal Acquisition, Transfer and Transition Policy, and relevant Accreditation Standards, especially those related to animal care and welfare.
4. Identify any additional factors that substantiate how approval of the potential Sustainability Partner applicant's participation will benefit the SSP Program and conservation of the species
5. End with your signature and the date.

JUDY BERENS

Phone
561-797-9544

Email
pantheridge@aol.com

Location
2143 D Road, Loxahatchee, FL 33470

EDUCATION

VASSAR COLLEGE
BA cum laude Biology/ Economics
1971

UNIVERSITY OF MIAMI, CORAL GABLES, FL
MBA
1973

RELATED EXPERIENCE

INDEPENDENT MORTGAGE BROKER
Miami, Fl / 1974 – 1976

ASSISTANT TO CHAIRMAN OF CARESSA SHOES
1981 – 1983

COMPANY ACQUIRED IN LEVERAGED BUYOUT IN 1983
Miami, Fl

RELEVANT EXPERIENCE

Intern/ assistant to Jean Hatfield, a breeder of ocelots and other exotics
1991 – 1992

Acquired FWC Florida license to possess Class 2 Felidae and acquired my first ocelot 1993

Founded Panther Ridge Sanctuary –1999 taking in and caring for unwanted and or injured class 2 cats.

Apprentice to Catherine Hilker, Animal Ambassador Director, Cincinnati Zoo
1986-1987

Acquired FWC Florida license to possess Class 1 Felidae 2001. Wellington, Fl
Acquired my first leopard.

Founded Panther Ridge Conservation Center, Inc , an IRS 501(c) 3 nonprofit organization.
2003

Serving as President and Executive Director 2003 to present, 21 years.
Wellington, Fl.

Became an Accredited Facility and Professional Member of Zoological Association of America.
2004 to present

Clouded Leopard Species Survival Plan member
2013 to present

ZAA Animal Management Plan member for cheetah and fishing cats.

AZA jaguar studbook manager, Stacy Johnson, has all relevant information on our jaguars.

Panther Ridge is applying to become American Humane Conservation Certified.

Panther Ridge Conservation Center has housed ocelots continuously since 1993, 5 ocelots in total. I live on premises and have interacted with ocelots every day during that time. At present there is one 20 – year - old ocelot here. Our oldest ocelot lived to be 25 years old and was humanely euthanized in 2018.

USDA and FWC records are impeccable. Please contact them if you wish. AZA recommendation from Rick Schwartz, Director of the Nashville Zoo in Tennessee is available upon request.

MATERIALS LIST FOR CONSTRUCTION FOR OCELOTS:

Everything is black vinyl coated or color infused galvanized wire to look black.

Uprights:
Corner posts 3" sunk in the ground 3' in cement

Line posts 2 1/2" same installation

Bottom rails 1 7/8" mid height horizontal rails same as bottom rails

Chain link for FWC class 2 cats is 11 1/2 gauge that looks like 9 gage - used on walls and roofs (These are FWC, ZAA and AZA requirements.)
All fittings, clamps, tension bars and latches are heavy duty commercial.

Doors are double latched on outer door of lock outs for Class 2
 - additional latches, locks and chains for Class 1

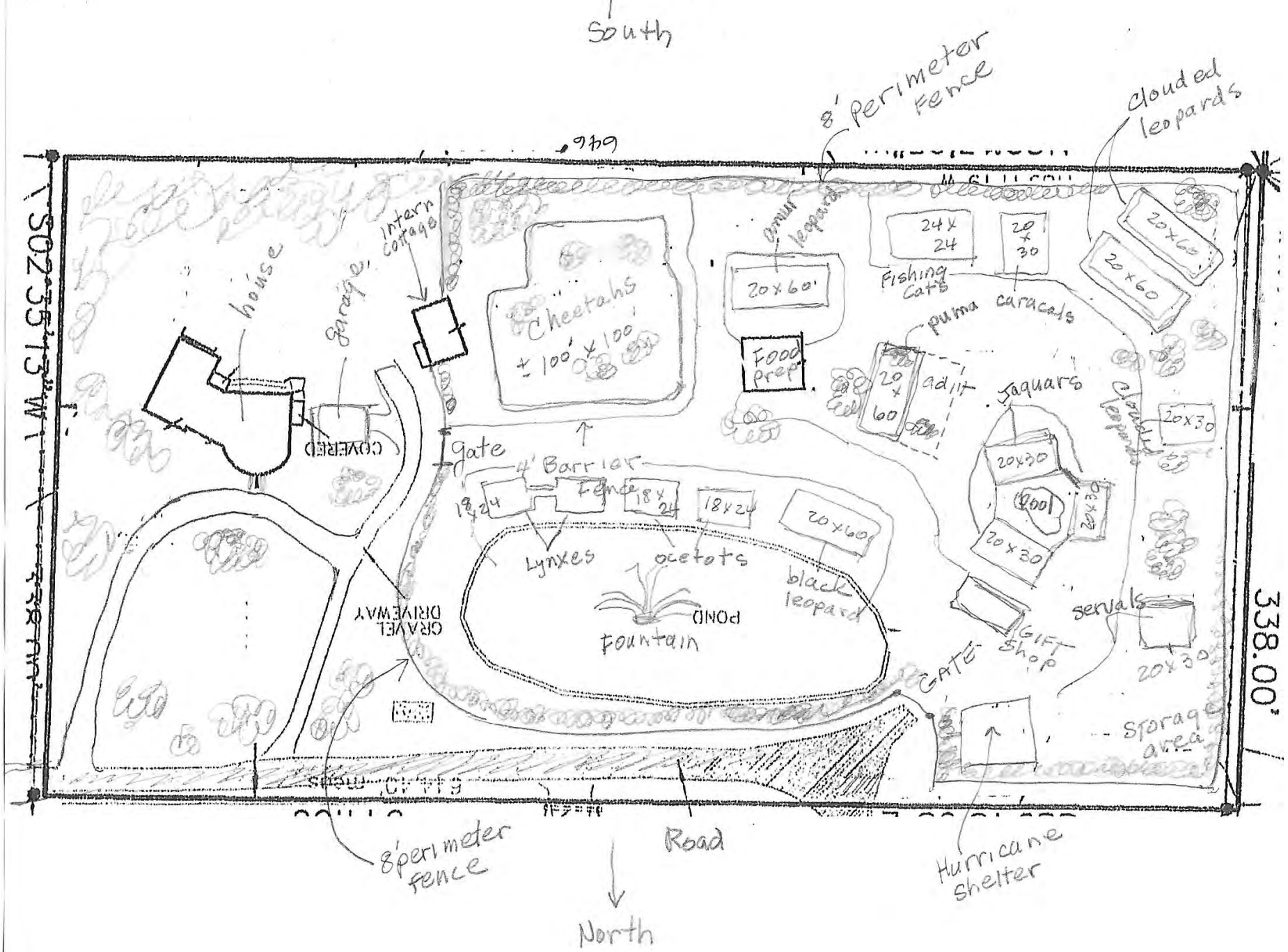
Existing enclosure that can be used for ocelots is a 20' x 60' and closes off to make 3 20'x20' enclosures.
Wide doors between them open the entire area if desired.
Lock out on each end is 4'x4' and has either a guillotine door made of 3/4" Star board heavy plastic material or a sliding chain link open/close set up.

All den boxes, tables, ramps made our of 2"x 6" pressure treated wood. Non toxic plants throughout. Larger poles and logs are suspended and large scratching logs are scattered in the habitat.

Commercial thickness custom tarps 12' x 20' are attached over each section for sun and rain protection.

Elevated den boxes 3' x 5' or 4' x 4' are in each section and a tree house is in one of the sections.

Substrate is either grass or sanitized playground mulch of uniform size.


South
8' perimeter fence
clouded leopards
house
garage
intern cottage
Cheetahs
± 100' x 100'
20x60'
Food Prep
Fishing Cats
24 x 24
20 x 30
puma
caracals
20x60
20x60
20 x 60
addit
Jaguars
20x30
20x30
Pool
20x30
20x30
gate
4' Barrier Fence
18x24
18x24
18x24
20x60
Lynxes
ocelots
black leopard
pond
fountain
GRAVEL DRIVEWAY
GIFT Shop
servals
20x30
GATE
storage area
338.00'
S02°35'13"W
8' perimeter fence
Road
Hurricane shelter
North

e-mail: blouduiker@mweb.co.za
website: www.mysticmonkeys.co.za
Tel: +27 82 566 4929
Fax: +27 0866774020
Vat: 4310157740

Postnet Suite 27
Private Bag X1604
Bela-Bela
Limpopo

Portion 41 of the Farm Buffelsdrift
179JR, Rust de Winter, LIMPOPO

28 March 2024

TO WHOM IT MAY CONCERN:

I, Christa Saayman, owner of Mystic Monkeys & Feathers Wildlife Park in South Africa hereby state and confirm the following:

Information about the Ocelots:
1) **Scientific name:** Leopardus pardalis
2) **Common name:** Ocelot
3) **Name and address of facility of origin:**
Mystic Monkeys & Feathers Wildlife Park
Portion 41 of the Farm, Buffelsdrift 179JR,
Rust de Winter, Limpopo, South Africa
4) **Birth date of Ocelot:** (mm/dd/yyyy)
Ocelot female= 11/01/2022
Ocelot male= 28/12/2021
5) **Identification information (microchip number):**
Ocelot female= 933071000204430
Ocelot male= 933071000204510
6) **Name and address of the facility where the parental stock is located:**
Mystic Monkeys & Feathers Wildlife Park
Portion 41 of the Farm, Buffelsdrift 179JR,
Rust de Winter, Limpopo, South Africa
7) **Dimensions of the crates:**
1200 Lenght X 500 Width X 500 Height (X2 boxes)
As these cats have grown now, we will put them in their own crate with no divider in between. The crates will be build according to IATA standards.
8) Accompanying the animals during shipment isn't necessary as they are adults and need no special care. Predators don't eat every day, they can go a week without food, so they should be fine during transportation.
9) The carrying capacity was determined by our breeding stock as we get a lot of babies and cannot keep all of them. The Ocelots are in a Zoo, where they get fresh water, sanitation and food.

I hope you find above mentioned in order.

Please let me know should you require any further information!

Christa Saayman
Owner of Mystic Monkeys & Feathers Wildlife Park

MYSTIC MONKEYS &
FEATHERS WILDLIFE PARK
CHRISTA SAAYMAN - OWNER
CELL: (+27)82 566 4929

LIMPOPO PROVINCIALGOVERNMENT
Dept. of Economic Development,
Environment & Tourism

Permit No: CPM-005-00001
(to Establish and Operate a Wild Animal Park)

e-mail: blouduiker@mweb.co.za
website: www.mysticmonkeys.co.za
Tel: +27 (0)82 566 4929

POSTNET SUITE 27
PRIVATE BAG X1604
BELA-BELA
0480

VAT NR / BTW NO: 4310157740

INVOICE

TO / AAN:	PANTHER RIDGE CONSERVATION CENTER INC 2143 D ROAD LOXAHATCHEE FL 33470	**DATE / DATUM:**	28 March 2024
		VAT NO: /	
		BTW NO:	
		INVOICE NO. /	
		FAKTUURNR:	MM2222

QUANTITY / HOEVEELHEID	DESCRIPTION / BESKRYWING	UNIT PRICE / EENHEIDSPRYS	VALUE / WAARDE
1 :1	Ocelot/ *Leopardus pardalis*	n/a	$0
	Crates		$500
	Freight cost		$865
	FOB		

RUST DE WINTER PROPERTY HOLDINGS CC
T/A RUST TWO
ABSA BANK
MONTANA (CODE :632 005)
REK/ACC: 407-782-5586

SUB TOTAL / SUBTOTAAL $1365

RUST DE WINTER PROPERTY HOLDINGS CC / Trade as RUST 2
Bank: ABSA BANK,
Bank address: Corner of Potgieter and Marx street, Pick a Pay Mall, Bela-Bela, South Africa
Swift code: ABSAZAJJ, Sorting code: ZA632005 / Account number: 407-782-5586

Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Wed 4/3/2024 1:28 PM

To:Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Cc:JUDY BERENS <pantheridge@aol.com>

Good afternoon Ms. Sublett,

Thank you for the additional information on behalf of Panther Ridge Conservation Center. Based on the information provided with regards to justifying import for captive propagation for the conservation and survival of the species, it seems Panther Ridge Conservation Center is seeking approval to become a Sustainability Partner in the AZA SSP Program. This would certainly assist in facilitating meeting enhancement. However, for the purposes of application CS0582384, if Panther Ridge Conservation Center has not already been accepted into the AZA program or other cooperative breeding program, then the application remains incomplete. Unless you can provide documentation showing Panther Ridge Conservation Center's participation in an established breeding program, such as through a current breeding plan outlining Panther Ridge Conservation Center's role in the program and letter from the breeding coordinator confirming participation, we will be required to administratively close this application. Once such information is acquired it can be provided upon reapplication. If you do have such documentation, however, while the 45-day deadline was March 31, 2024, as I did not respond until today I can give you until April 9, 2024 to provide any documents to complete the application.

Most of my other questions were adequately responded to. However, for "Question # 21. How was the carrying capacity determined?", this question was intended for Panther Ridge Conservation Center to answer, not Mystic Monkeys and Feathers Wildlife Park. In addition, the other information on the enclosures is still somewhat unclear. On the map of the facility it is indicated a 20x60 enclosure is designated for black leopard, and two 18x24 enclosures are designated for ocelot, yet the letter specifies a 20'x60' enclosure would be used for ocelots. If this is not accurate then either the diagram or letter needs to be updated. Second, while additional photos are helpful, it is not enough for us to see the full the enclosure. More photographs would be needed to clearly see and evaluate the full enclosure. Please let me know if you have any questions.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Thursday, March 28, 2024 12:52 PM
To: Lamberson, Amanda M <amanda_lamberson@fws.gov>
Cc: JUDY BERENS <pantheridge@aol.com>
Subject: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

MATERIALS LIST FOR CONSTRUCTION FOR OCELOTS:

Everything is black vinyl coated or color infused galvanized wire to look black.

Uprights: Corner posts 3" sunk in the ground 3' in cement

Line posts 2 1/2" same installation

Bottom rails 1 7/8" mid height horizontal rails same as bottom rails

Chain link for FWC class 2 cats is 11 1/2 gauge that looks like 9 gage - used on walls and roofs (These are FWC, ZAA and AZA requirements.)

All fittings, clamps, tension bars and latches are heavy duty commercial.

Doors are double latched on outer door of lock outs for Class 2 - additional latches, locks and chains for Class 1

Existing enclosure that can be used for ocelots is a 20' x 60' and closes off to make 3 20'x20' enclosures.

Wide doors between them open the entire area if desired.
Lock out on each end is 4'x4' and has either a guillotine door made of 3/4" Star board heavy plastic material or a sliding chain link open/close set up.

All den boxes, tables, ramps made our of 2"x 6" pressure treated wood. Non toxic plants throughout. Larger poles and logs are suspended and large scratching logs are scattered in the habitat.

Commercial thickness custom tarps 12' x 20' are attached over each section for sun and rain protection.

Elevated den boxes 3' x 5' or 4' x 4' are in each section and a tree house is in one of the sections. Substrate is either grass or sanitized playground mulch of uniform size.

DRONE PHOTOGRAPHS OF PANTHER RIDGE FELINE CONSERVATION CENTER:

ZAA

ZOOLOGICAL ASSOCIATION OF AMERICA

May 21, 2021

Judy Barrens
Panther Ridge Conservation Center
2143 D Rd
Loxahatchee
Fl 33470

Dear Judy

On behalf of the Zoological Association of America (ZAA), I would like to congratulate you on the successful completion of Panther Ridge Conservation Center's, ZAA Facility Accreditation assessment and audit! Accreditation is an important part of ZAA's facility membership program and exemplifies our organization's commitment to excellence in animal husbandry, and facility operations.

Your facility, Panther Ridge Conservation Center, continues to meet all ZAA's objectives:

- Professional standards for husbandry and animal care practices.
- Accurate animal and medical records.
- Appropriate, safe, and quality existence for animals kept in a captive environment.
- Safe environment for humans; both staff and visitors.
- Enhanced survival of species using appropriate methods.

As an accredited facility, you will have access to the Surplus and Want Lists available through the ZAA website at www.zaa.org . Your facility's accreditation number is, AC-197.

Please note your facility must get reaccredited every five years or upon relocation. Your next reaccreditation assessment and audit is May 2026.

We hope you proudly display your accreditation certificate to show everyone that your facility meets all ZAA's accreditation standards.

Congratulations again and thank you for your continued support in promoting the responsible ownership, management, and propagation of animals in your accredited facility.

Sincerely,
John Seyjagat

Executive Director
john@zaa.org
4433925897

Certificate of Accreditation

Promoting the responsible ownership, management, and propagation of animals in both private and public domains

ZAA

ZOOLOGICAL ASSOCIATION OF AMERICA

Panther Ridge Conservation Center

has been awarded this certificate for meeting the qualifications of

ZAA Accreditation

Dr. Barbara Baker

Dr. Barbara Baker
ZAA Board Chair

Accredited Since 2015

Holly Hunt

Holly Hunt
ZAA Accreditation Chair

Accredited Through 2026

April 4, 2024

To Whom It May Concern,

The Cheetah Animal Management Program (AMP) for the Zoological Association of America (ZAA) is a cooperatively managed *ex situ* program for cheetahs. This program was the flagship AMP for ZAA and is coordinated across ZAA accredited members to create a sustainable cheetah population. The Cheetah AMP maintains a regional studbook and utilizes a population biologist and a program manager to make recommendations for breeding and transfers to ensure the greatest genetic variability.

The Cheetah AMP and its members educate and spread awareness about cheetah conservation to the public. In addition, participating facilities support cheetah conservation through annual donations to in situ field partners, including Cheetah Conservation Fund, Cheetah Conservation Botswana, and Cheetah Outreach Trust. This conservation linkage is an important component to the AMP structure.

Panther Ridge has been an active member of the ZAA Cheetah AMP since the program's inception in 2017. Panther Ridge has cared for and managed multiple cheetahs for the AMP with a focus on educating the public and supporting cheetah conservation annually. If you have any questions about Panther Ridge's participation in the ZAA Cheetah AMP, please feel free to contact me.

Sincerely,

Jason Ahistus
ZAA Cheetah AMP Species Manager
Carnivore Programs Advisor
Fossil Rim Wildlife Center
Phone: 218-341-5830
Email: jasona@fossilrim.org

Laura Carpenter
Cincinnati Zoo and Botanical Garden
2300 Vine St.
Cincinnati, OH 45220

April 24, 2024

To Whom It May Concern:

As SSP coordinator for the ocelot SSP I would like to write this letter of support for Judy Berens, owner of Panther Ridge, to help facilitate the approval of her application for the importation of two ocelots from South Africa.

Judy has kept both large and small cats, including ocelots, at her facility for over 30 years. Her facility is ZAA accredited, I am in support of her application for Sustainability Partnership within the AZA for the ocelot SSP.

Since this pair of ocelots would provide new genetics and reproductive potential for the SSP population, it would be of value to us both for the application to be approved.

Thank you for considering her application.

Sincerely,

Laura Carpenter
Ocelot SSP Coordinator and Studbook Keeper
Laura.carpenter@cincinnatizoo.org
(513) 364-7678

April 12, 2024

USFW Division of Management Authority
Branch of Permits
MS: IA 5275 Leesburg Pike
Falls Church, VA 22041-3803

THE
DALLAS
WORLD
AQUARIUM

Dear Division of Management Authority,

I am writing this letter to support the proposed importation of (2) unrelated young ocelots, Leopardis pardalis, from South Africa to the Panther Ridge Conservation Center, Inc. in Loxahatchee, Florida.

I have known Judy Berens, the Center's Founder and Director for many years. In May of 2018, The Dallas World Aquarium received a male ocelot from Panther Ridge on loan when we were in need of an exhibit animal.

I know Judy to be extremely knowledgeable in the care and husbandry of numerous species of felids, including ocelots.

The Panther Ridge Conservation Center provides important services not only in the care, rescue, and rehabilitation of endangered and threatened cat species, but also in terms of conservation education to the South Florida community in which they are located. In those roles, they are highly regarded as a top non-profit charity.

Please feel free to contact me if needed and thank you for the opportunity to support these activities.

Sincerely,

Daryl L. Richardson
Director
The Dallas World Aquarium

1801 NORTH GRIFFIN
DALLAS, TEXAS USA 75202
PHONE: 1.214.720.2224
FAX: 1.214.720.2242
WWW.DWAZOO.COM

April 28, 2024

To Whom It May Concern,

Panther Ridge Conservation Center, Inc has been working with the Clouded Leopard Species Survival Plan (CL SSP) since 2012 to assist in maintaining a sustainable zoo-based population of genetically valuable clouded leopards. Although they are not an AZA Sustainability Partner, Panther Ridge has expressed interest in joining the SSP in that capacity and the application has recently been shared in order to move that partnership forward. Panther Ridge communicates information about births, deaths and transfers in order to assist in the maintenance of the International Studbook and has provided genetically valuable offspring to the SSP in the to maintain genetic diversity in the population.

Please contact me if you should have questions or need further clarification.

Sincerely,

Jilian Fazio, Ph.D.
Director
Turtle Back Zoo
Clouded Leopard SSP Coordinator
International Studbook Keeper
jfazioi@parks.essexcountynj.org
908-447-6921

April 29th, 2024

To Whom It May Concern:

Please accept this correspondence as a letter of recommendation and confidence for Judy Berens of Panther Ridge. I have worked personally with Judy for over thirty years, and I have always had fair and professional dealings with her. I have been to her facility and have always found it in immaculate condition and all her animals have been healthy, well cared for with appropriate space, shelter, and enrichment.

I am particularly impressed with her success breeding clouded leopards. This species is arguably the most difficult cat to breed in captivity and she has produced numerous litters in cooperation with the Clouded Leopard SSP. Additionally cubs can be difficult to raise and there has historically been a high infant mortality rate with clouded leopards, but Judy has had great success and she is an expert in juvenile socialization to ensure the cubs can go into future breeding programs which is essential for this species. She also has long time experience with ocelots which is another difficult species to breed in captivity and she has had similar success with this species. As an AZA Accredited Zoo, I would not have any reservations sending felines to her facility as I know they will always be well cared for, and Judy always will do right by any cat under her care.

Please feel free to reach out to me if you need additional information.

Sincerely,

Rick Schwartz
President and CEO
615-833-1534
Rschwartz@nashvillezoo.org
3777 Nolensville Pike, Nashville, TN 37211

Committed to global and local conservation through research, habitat protection, breeding programs and education initiatives.

Judy Berens/Panther Ridge Conservation Center
2143 D Road
Loxahatchee, Florida 33470
Pantheridge@aol.com
(561) 797-9544
May 1, 2024

Memorandum of Understanding

To:
United States Fish and Wildlife Service
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

Subject: Agreement for Participation in the AZA Animal Management Program - Sustainable Partnership Ocelot SSP

Dear To Whom It May Concern,

This memorandum of understanding (MOU) confirms the agreement between Judy Berens/Panther Ridge and the United States Fish and Wildlife Service regarding the participation of two ocelots, to be imported from South Africa, in the Association of Zoos and Aquariums (AZA) Animal Management Program.

1. **Purpose**:
The purpose of this MOU is to outline the terms under which the two ocelots will be incorporated into the AZA Animal Management Program, ensuring their care, conservation, propagation and future sustainability within controlled environments.

2. **Commitments**:
a. **Judy Berens/Panther Ridge** agrees to comply with all standards and guidelines set forth by the AZA for animal welfare and management.
b. **Judy Berens/Panther Ridge** commits to maintaining appropriate documentation and records concerning the health, breeding, and welfare of the ocelots and to share this information with the AZA as required.
c. **AZA Ocelot SSP** agrees to provide oversight and support, ensuring that the importation and subsequent care of the ocelots align with federal regulations and conservation goals.

3. **Duration**:
This MOU will remain in effect for the lifespan of the ocelots, their offspring and all future ocelots held at Panther Ridge Conservation Center.

5. **Signatory Authority**:
This document will be executed by Judy Berens of Panther Ridge Conservation Center and the appointed liaison officer for Panther Ridge, Sheri Sublett of Twin Pine Farm & Exotics Conservation Foundation (TPFECF).

By signing below, both parties agree to the terms outlined in this Memorandum of Understanding.

Sincerely,

Judy Berens
Founder
Panther Ridge Conservation Center
May 1, 2024

Acknowledged and Agreed:

Sheri L. Sublett
Liaison Officer
Panther Ridge Conservation Center
(Executive Director of TPFECF)
May 1, 2024

This MOU serves as a testament to our shared commitment to the preservation and sustainable management of wildlife species, reflecting our dedication to conservation and responsible stewardship.

[End of Memorandum]

e-mail: blouduiker@mweb.co.za
website: www.mysticmonkeys.co.za
Tel: +27 82 566 4929
Fax: +27 0866774020
Vat: 4310157740

Postnet Suite 27
Private Bag X1604
Bela-Bela
Limpopo

Portion 41 of the Farm Buffelsdrift
179JR, Rust de Winter, LIMPOPO

28 March 2024

TO WHOM IT MAY CONCERN:

I, Christa Saayman, owner of Mystic Monkeys & Feathers Wildlife Park in South Africa hereby state and confirm the following:

Information about the Ocelots:
1) **Scientific name:** Leopardus pardalis
2) **Common name:** Ocelot
3) **Name and address of facility of origin:**
Mystic Monkeys & Feathers Wildlife Park
Portion 41 of the Farm, Buffelsdrift 179JR,
Rust de Winter, Limpopo, South Africa
4) **Birth date of Ocelot:** (mm/dd/yyyy)
Ocelot female= 11/01/2022
Ocelot male= 28/12/2021
5) **Identification information (microchip number):**
Ocelot female= 933071000204430
Ocelot male= 933071000204510
6) **Name and address of the facility where the parental stock is located:**
Mystic Monkeys & Feathers Wildlife Park
Portion 41 of the Farm, Buffelsdrift 179JR,
Rust de Winter, Limpopo, South Africa
7) **Dimensions of the crates:**
1200 Lenght X 500 Width X 500 Height (X2 boxes)
As these cats have grown now, we will put them in their own crate with no divider in between. The crates will be build according to IATA standards.
8) Accompanying the animals during shipment isn't necessary as they are adults and need no special care. Predators don't eat every day, they can go a week without food, so they should be fine during transportation.

I hope you find above mentioned in order.

Please let me know should you require any further information!

Christa Saayman
Owner of Mystic Monkeys & Feathers Wildlife Park

MYSTIC MONKEYS &
FEATHERS WILDLIFE PARK
CHRISTA SAAYMAN - OWNER
CELL: (+27)82 566 4929

LIMPOPO PROVINCIALGOVERNMENT
Dept. of Economic Development,
Environment & Tourism

Permit No: CPM-005-00001
(to Establish and Operate a Wild Animal Park)

Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Fri 5/24/2024 12:07 PM

To:Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>

Good afternoon Ms. Sublett,

I reviewed the additional information. Some of the information provided was adequate and some clarification is still required. I consulted with a supervisor and the letters of support should be adequate to show intent to participate in a breeding program. The following two items still require clarification.

- The photographs and updated letter still does not answer the discrepancy I noted between the hand drawn diagram and information provided with the photographs. Ms. Berens provided the diagram, if it is not accurate then she needs to explain that in writing. The drone photographs can be used to indicate which enclosures among them are designated for ocelots in addition.
- Ms. Berens must answer the question of how the carrying capacity was determined. This could be based on ZAA or AZA guidance, recommendation from another facility, past experience, etc. for example.

The Division of Scientific Authority also has further questions in order to make their finding under CITES:

- Please describe the diet fed to the ocelots and how diets are prepared.
- Approximately how many guests visit Panther Ridge every year?
- Are there any off-exhibit areas that are closed to guest viewing? If so, are there any areas capable of housing ocelots?
- Please describe volunteer training protocols for those who care for ocelots.
- Please provide updated licenses/certificates. Those previously provided have expired.
- Please provide the CV for the veterinarian on staff.
- Please describe the veterinary care given to the ocelots, as well as avenues for emergency care to be provided if needed.
- Please describe security to prevent theft of specimens and measures taken to rectify any previous theft or security problem.
- Please confirm that no births have taken place in the last five years.
- Please describe the funding sources available on a long-term basis to cover the cost of maintaining the facility and the specimens imported. Tax Form 990 for the year 2022 shows a large net income loss (130,848 USD) in comparison to recent years. Please explain.

I will provide 45 days to respond to the new questions.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

In accordance with 50 CFR 13.ll(e), if the requested information is not received by this office within 45 calendar days of the date of this email, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application, and all required fees, for the Service to consider your proposed activity.

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Monday, May 20, 2024 11:50 AM
To: Lamberson, Amanda M <amanda_lamberson@fws.gov>
Subject: Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Good Morning!

Sorry about the file attachments in the last email. Two of them were small files but one of them contained many pictures so it was very hard to attach it to the email; I even tried to compress it into a ZIP and it didn't make much of a difference.
I just logged into my account and uploaded the three files to the application. Please let me know if it worked! One file is a revised mystic monkey statement (eliminating the carrying capacity), the second file contains reference letters, ZAA accreditation and a memorandum, the third is the largest file and contains habitat photos and drone photos.

Thank you!

Sincerely,

Sheri Sublett
Executive Director
Twin Pine Farm & Exotics Conservation Foundation
(843)310-4441
(843)217-9188 (mobile)
TPFECF@Lowcountry.com
www.TPFECF.org
Facebook.com/TPFECF

On May 20, 2024, at 8:48 AM, Lamberson, Amanda M <amanda_lamberson@fws.gov> wrote:

Good morning Sheri,

Thank you for your email. Could I request that you please upload these documents directly to the application file? Our email security system will not allow me to access links to external downloads. Please let me know if you have any trouble uploading them.

Kind regards,

Amanda Lamberson

Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Sunday, May 19, 2024 10:28 PM
To: Lamberson, Amanda M <amanda_lamberson@fws.gov>
Subject: Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

---

Download Attachment
Available until Jun 18, 2024

Dear Ms. Lamberson,

We very much appreciate the forty five day extension granted to us.
Please see the attached three (3) files which include the documentation and correspondence to complete the application: CS0582384.

Thank you, we look forward to hearing from you.

Click to Download
Panther Ridge Habitat Design Construction Photographs Drone.pdf
0 bytes

Click to Download
5.20.2024 USFWS Panther Ridge Oxcelot Application.pdf.pdf
0 bytes

Click to Download
Mystic Monkey Ocelot Q&A.pdf
0 bytes

Sincerely,

Sheri Sublett
Executive Director

Twin Pine Farm & Exotics Conservation Foundation

(843)310-4441
(843)217-9188 (mobile)
TPFECF@Lowcountry.com
www.TPFECF.org
Facebook.com/TPFECF

> Good afternoon Ms. Sublett,
>
> Thank you for the additional information on behalf of Panther Ridge Conservation Center. Based on the information provided with regards to justifying import for captive propagation for the conservation and survival of the species, it seems Panther Ridge Conservation Center is seeking approval to become a Sustainability Partner in the AZA SSP Program. This would certainly assist in facilitating meeting enhancement. However, for the purposes of application CS0582384, if Panther Ridge Conservation Center has not already been accepted into the AZA program or other cooperative breeding program, then the application remains incomplete. Unless you can provide documentation showing Panther Ridge Conservation Center's participation in an established breeding program, such as through a current breeding plan outlining Panther Ridge Conservation Center's role in the program and letter from the breeding coordinator confirming participation, we will be required to administratively close this application. Once such information is acquired it can be provided upon reapplication. If you do have such documentation, however, while the 45-day deadline was March 31, 2024, as I did not respond until today I can give you until April 9, 2024 to provide any documents to complete the application.
>
> Most of my other questions were adequately responded to. However, for "Question # 21. How was the carrying capacity determined?", this question was intended for Panther Ridge Conservation Center to answer, not Mystic Monkeys and Feathers Wildlife Park. In addition, the other information on the enclosures is still somewhat unclear. On the map of the facility it is indicated a 20x60 enclosure is designated for black leopard, and two 18x24 enclosures are designated for ocelot, yet the letter specifies a 20'x60' enclosure would be used for ocelots. If this is not accurate then either the diagram

or letter needs to be updated. Second, while additional photos are helpful, it is not enough for us to see the full the enclosure. More photographs would be needed to clearly see and evaluate the full enclosure. Please let me know if you have any questions.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

- *Carrying Capacity:*

  Currently, Panther Ridge has the capacity to carry four Ocelots. Our institution has the land to expand and build additional enclosures as needed for our Sustainable Partnerships with AZA (American Zoological Association).

- *Please describe the diet fed to the ocelots and how diets are prepared.*

  All cats at Panther Ridge eat a daily diet at least once a day, some older cats will receive two meals which consists of chicken, beef, horse, and fish depending on the individual. Due to the carnivorous nature of Ocelots, their diet consists of lean muscle meat from beef and poultry, as well as fish. This meat serves as the primary protein source and provides essential amino acids necessary for muscle maintenance and overall health. Overall vitamin used daily is “Oasis Feline T” with additional supplementation with bone meal. For dietary enrichment, other varieties of food can be offered as well as “snacks” during the day. Enrichment foods can include organ meats, whole prey, fish, cantaloupe, goat’s milk, other types of meat (deer, goat, lamb, etc.), bone broth and other approved foods. Once a week a “whole prey day” will be fed out providing a full diet consisting of types of birds, bunnies, rodents, and hoof-stock to the cats that enjoy it.

Methods of feeding diets can be used for enrichment as well as daily training with diets, hiding whole prey in enclosures and tour snacks are a few methods. All whole prey fed out is pre-frozen. If from Panther Ridge's breeding program of rats and bunnies, then they must be frozen for two weeks minimum prior to feeding out. Live prey is to never be provided with the exception of some types of fish. These whole prey items provide essential nutrients like protein, fat, vitamins, and minerals in a natural and balanced form. Whole prey items typically include bones, which are important for dental health and provide calcium and other minerals essential for bone strength and overall skeletal health. Supplementation is also added before distribution to ensure they receive all the essential nutrients they need.

Cats are weighed on a monthly basis. Diets are formulated based off ideal body condition and their current weight.

Diets are made up daily by measuring the pre-determined amount of meat and cutting them into appropriate sizes for the individual cat. Fat is also trimmed off the meat.

- *Approximately how many guests visit Panther Ridge every year?*

  Approximately 4,000-5,000 people visit Panther Ridge per year. Guests are permitted onto the property by scheduling a guided tour; by appointment only.

- *Are there any off-exhibit areas that are closed to guest viewing? If so, are there any areas capable of housing ocelots?*

  There is an off-exhibit enclosure for the ocelots, if necessary. We do guided tours by appointment only once a day, which last an hour or two. Guests are required to leave the property after the tour has ended. At no point in time do guests wander at their own leisure throughout the day.

- *Please describe volunteer training protocols for those who care for ocelots.*

  All volunteers go through our docent program. Docents may participate in animal husbandry alongside keepers with approved species. Docents learn the same precautionary protocols as a keeper would for all class two felines Panther Ridge houses. At no point in time, do docents work with the cats without a keeper staff present.

- *Please provide updated licenses/certificates. Those previously provided have expired.*

  See Attachments

- *Please provide the CV for the veterinarian on staff.*
  See Attachment

- *Please describe the veterinary care given to the ocelots, as well as avenues for emergency care to be provided if needed.*
  Scheduled health assessments are conducted as needed by our veterinarian to monitor our ocelots' overall health. This includes physical examinations at least once a year, checking for any signs of illness or injury, and assessing their body condition. Bloodwork is done on a yearly basis or as requested by our vet. Our ocelots are vaccinated against common diseases to prevent outbreaks within the facility's population. They are also treated regularly with preventives for parasites like ticks, fleas, and intestinal worms.
  A major role in our everyday care for ocelots is monitoring their behavior. Observing their behavior helps detect any changes that might indicate underlying health issues or stress. Environmental enrichment is provided to ensure their psychological well-being. Panther Ridge has a well-defined emergency response plan in place, outlining procedures to follow in case of an emergency involving the ocelots. We are equipped with essential medical supplies and equipment to provide immediate care to injured or ill ocelots. This includes items like first aid kits, medications, and equipment for minor procedures. If an ocelot requires urgent veterinary care beyond what can be provided on-site,

arrangements are made for transportation to a veterinary clinic or hospital equipped to handle exotic species. Effective communication and coordination among staff members are essential during emergencies. Clear lines of communication ensure that everyone is aware of the situation and can act promptly to address it. By implementing these measures, the conservation center ensures that its ocelots receive the necessary veterinary care and attention, both as part of their routine health maintenance and in case of emergencies.

Our veterinary staff is as follows:

- Stephanie Johnston, DVM
  Animal Clinic of West Lake Worth
  (The clinic is located fourteen miles away from Panther Ridge; we have Dr. Johnston's cellular phone number for emergencies and on call).
- Kim Golden, DVM
  ACCESS Emergency Animal Hospital
  (The clinic is located six miles from Panther Ridge).
- Jena Dicharry, DVM and Susan Clubb, DVM
  The Rainforest Clinic for Birds and Exotics
  (The clinic is located two miles away from Panther Ridge).
- Lucia Alvarez, DVM
  Boca Veterinary Clinic
  (The clinic is located approximately thirty three miles away).

- Other specialists are used from time to time, but ACCESS has most specialties “in house”.

  All of our ocelots have lived well into their senior years, with our oldest living to the astounding age of 25.

- *Please describe security to prevent theft of specimens and measures taken to rectify any previous theft or security problem.*

  Panther Ridge has a clear history of nothing ever been stolen. All enclosures containing animals are locked at all times unless keepers are entering/exiting the enclosures. Our eight foot perimeter fence, entrances and gates are locked at all times unless staff and guests are entering and/or leaving the facility. There is staff on site 24/7 with the executive director’s living quarters situated on the property as well as a house for staff members.

- *Please confirm that no births have taken place in the last five years.*

  There has not been any Ocelot’s born at Panther Ridge within the last five years. Our female is post reproductive.

- *Please describe the funding sources available on a long term basis to cover the cost of maintaining the facility and the specimens imported. Tax Form 990 for the year 2022 shows a large net income loss (130,848 USD) in comparison to recent years. Please explain:*

  Panther Ridge Conservation Center lost money in 2022 because of major capital improvements that were done that year. Several new habitats were built and landscaped that year. Shortfalls can be covered by funds from the founder's trust, which can comfortably handle the expenses. Our finances are secured by the trust document.

Stephanie Johnston DVM

3000 Carl Bolter Dr
Delray FL 33444
petdoctorj@msn.com

I was drawn to this profession to be an advocate for the voiceless. It has ever been my mission to straddle the philosophical midline between the animal activist and the utilitarian. This to provide an educated voice of reason for animal welfare.

Education

1994
Bachelor of Science in animal science
NC State University
*Focus on animal husbandry, nutrition, and farm science

1998
Doctor of Veterinary Medicine
Atlantic Veterinary College
*Focus on small animal, wildlife, and exotic animal medicine and surgery

Employment

1998-2002
Associate Veterinarian
Wiles Road Animal Hospital
*Provided medicine and surgery for dogs, cats, exotic pets, and wildlife

2002-2004
Associate Veterinarian
Cypress Creek Animal Hospital
*Provided medicine and surgery for dogs, cats, exotic pets, and wildlife

2004-present
Self Employed
Integrated Veterinary Medicine
*Provided medicine and surgery for dogs, cats, exotic pets, and wildlife via house call services
*Provided per diem medicine and surgery for more than 30 practices in Broward, Dade, and Palm Beach counties
*Provided USDA inspection services for multiple zoo collections

2021-present
Associate Veterinarian
American Veterinary Group
*Providing medicine and surgery for dogs, cats, exotic pets, and wildlife

Non-employment activities

2002-2004
served as medical director of Sawgrass Nature Center
2004-present
serving as medical director for Panther Ridge Conservation Center

Permit to Possess 26 or More Class I and/or Class II Animals for Commercial Use (ESB)

Florida Fish Wildlife Conservation Commission
PO BOX 6150, Tallahassee, FL 32314-6150
850-488-6253

For LE Use Only

CustomerID:	1006237926	Permit #	Effective Date:	Expiration Date:
		890-000013	05/17/2024	05/17/2025

Authorized Persons / Locations

Permittee: JUDY BERENS

Responsible Party: Judy Berens

Facility Address: 2143 D Road
Loxahatchee FL, 33470

Sponsor Name: N/A

Authorized Wildlife

Class I - Felidae (including cougars and cheetahs)

Class II - Felidae

Permit Conditions

ESA/B #1 Wildlife maintained under this permit at this facility shall be subject to inspection by Commission personnel, pursuant to Section 379.304, F.S., and Rule 68A-4.006, F.A.C.
ESA/B #2 This permit does not authorize the importation of nonnative wildlife. A separate importation permit must be acquired for importation of any nonnative wildlife into Florida.
ESA/B #3 This permit does not authorize the possession of species listed in Chapter 68-5, F.A.C., as Conditional or Prohibited.
ESA/B #4 Permittee shall comply with the standard caging requirements for all wildlife authorized and housed under this permit, pursuant to Chapter 68A-6, F.A.C., unless otherwise authorized or regulated by other rules of the Commission.
ESA/B #5 Permittee shall comply with facility requirements for all wildlife authorized and housed under this permit, pursuant to Chapter 68A-6, F.A.C.
ESA/B #6 Permittee shall comply with record keeping requirements as specified in Chapter 68A-6, F.A.C., for wildlife authorized or possessed under this permit.
ESA/B #7 Permittees in possession of Class I wildlife shall demonstrate consistent and sustained commercial activity, in accordance with Rule 68A-6.005, F.A.C.
ESA/B #8 Permittees shall report required escapes of wildlife, in accordance with Rule 68A-6.018, F.A.C.
ESA/B #9 Permittees shall report required injuries from wildlife, in accordance with Rule 68A-6.018, F.A.C.
ESA/B #10 Permittee shall comply with transportation requirements for all wildlife authorized under this permit when away from permitted facility, in accordance with Rule 68A-6.014, F.A.C.
ESA/B #11 Wildlife used for public contact shall be exhibited in a manner that prevents injury to the public and the wildlife, pursuant to Rule 68A-6.016, F.A.C.
ESA/B #12 Any species requiring a permit for possession shall only be transported in the state of Florida by an individual permitted for possession of the wildlife being transported. Such permit shall be in possession of the individual(s) engaging in activity authorized by this permit and shall be available upon request by Commission personnel.
ESA/B #13 Any employee in possession of wildlife away from permitted facility shall comply with experience requirements for such wildlife, in accordance with Rule 68A-6.004, F.A.C.

USDA

Expiration Date: 01-03-2027

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Animal Care

This is to certify that
Panther Ridge Conservation Center Inc

is a licensed Class C - Exhibitor
under the

Animal Welfare Act

(7 U.S.C. 2131 et seq.)

Certificate No. 58-C-1003

Customer No. 12392

Maximum Number Of Animals Authorized: **50**

Authorized Dangerous Animal Group(s): **Exotic/Wild Felines and Hybrids;**

Deputy Administrator

APHIS FORM 7007 (JUL 2019)

Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Lamberson, Amanda M <amanda_lamberson@fws.gov>
Thu 7/18/2024 4:09 PM
To:Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>

Good afternoon Sheri,

I have reviewed everything and only have two follow up requests at this time.

1. Thank you for the information on what the ocelot capacity amount is, but I am still needing information on *how* it was determined from Ms. Berens.

2. The "Vector Map Panther Ridge CC" attachment did not go through on my end. Please re-send.

Thank you.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

In accordance with 50 CFR 13.ll(e), if the requested information is not received by this office within 45 calendar days of the date of this email, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application, and all required fees, for the Service to consider your proposed activity.

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Tuesday, July 9, 2024 4:42 PM
To: Lamberson, Amanda M <amanda_lamberson@fws.gov>
Subject: Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Hi Amanda:

Sounds terrific thank you for the reply.

Sincerely,

Sheri Sublett
Executive Director

Twin Pine Farm & Exotics Conservation Foundation

(843)310-4441
(843)217-9188 (mobile)
TPFECF@Lowcountry.com

www.TPFECF.org
Facebook.com/TPFECF

On Jul 9, 2024, at 3:37 PM, Lamberson, Amanda M <amanda_lamberson@fws.gov> wrote:

Hi Sheri,

No problem, I will work on reviewing everything this week and let you know if we have any follow-up questions. If not, we'll continue processing the application.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Monday, July 8, 2024 1:12 PM
To: Lamberson, Amanda M <amanda_lamberson@fws.gov>
Subject: Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Dear Ms. Amanda Lamberson,

I hope you had a wonderful holiday, I do apologize for the time it has taken for this reply.
Attached, you will find a total of five (5) files.

1. Is an updated map of Panther Ridge; the hand-drawn map that was originally turned in is aged and outdated by a number of years. Please disregard the first file with the hand drawn map and replace with the recent file submitted that included the drone photos, written descriptions, enclosure pictures, along with this updated, attached vector map.

2. Florida Wildlife Permit

3. Federal (USDA Class C Permit)

4. Resume of the Attending Veterinarian

5. Answers to the Questions from the Division of Scientific Authority

Please let me know if you have any further questions. Thank you for your time and we hope to hear back from you soon. Take care!
Vector Map Panther Ridge CC.jpg
<Stephanie Johnston DVM CV.docx.pdf>

New USDA 2027.pdf.pdf
Panther ridge Florida permit .pdf
<Panther Ridge Scientific Authority Q&A.pdf>

Sincerely,

Sheri Sublett
Executive Director

Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Lamberson, Amanda M <amanda_lamberson@fws.gov>
Tue 8/6/2024 9:53 AM
To:Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>

Good morning Sheri,

Thank you for this information and apologies for the delayed response. I will continue processing the application and will follow up if we need anything further.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

From: Twin Pine Farm & Exotics <twinpinefarmexotics@lowcountry.com>
Sent: Tuesday, July 23, 2024 11:11 AM
To: Lamberson, Amanda M <amanda_lamberson@fws.gov>
Subject: Re: [EXTERNAL] Re: Application Incomplete: CS0582384 - Panther Ridge Conservation Center

Dear Ms. Lamberson,

1. Recommended minimum space **per cat** is as follows:
    - **Weight 10–20 kg = 4 x 2 x 2.5 m (13 x 6.5 x 8 ft.) per cat (l x w x h)**

The carrying capacity was determined using a formula and guideline produced from AZA (American Zoological Association) for small cats. The capacity was also determined by Panther Ridge staff and Executive Director.

2. I have attached the vector map to this email.

Sincerely,

Sheri Sublett
Executive Director

Twin Pine Farm & Exotics Conservation Foundation

(843)310-4441
(843)217-9188 (mobile)
TPFECF@Lowcountry.com
www.TPFECF.org
Facebook.com/TPFECF

On Jul 18, 2024, at 4:09 PM, Lamberson, Amanda M <amanda_lamberson@fws.gov> wrote:

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