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From the Field

Field Capture, Chemical Immobilization, and Morphometrics of a Little-studied South American Carnivore, the Lesser Grison

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ABSTRACT We assessed chemical immobilization and success of trapping devices on a little-known South American mustelid, the lesser grison (*Galictis cuja*). From December 1998 to June 2006, we live-trapped ($n = 23$) and radiomarked ($n = 9$) free-ranging lesser grisons in 2 areas of the Pampas grasslands of Argentina. Both box-traps and rubber-padded leg-hold traps allowed us to capture lesser grisons. However, leg-hold traps had greater capture efficiency. Although the liberation time was longer with tiletamine–zolazepam than ketamine–xylazine, field immobilization of lesser grisons with both drug combinations was effective and safe. The high frequency of thermal regulation problems suggests that body temperatures of chemically restrained lesser grisons should be routinely monitored. Neither collars nor backpack harnesses were effective methods for attachment of radiotransmitters. Trapped individuals showed male-skewed sexual size dimorphism and sex ratio. Capture times suggest that lesser grisons are largely diurnal. Most of the data presented in this study are the first published on the lesser grison and provide baseline information for future research and conservation.

KEY WORDS anesthesia, box-trap, capture rate, grasslands, leg-hold trap, Mustelidae, radiotelemetry.

The lesser grison (*Galictis cuja*) is a small Neotropical mustelid with a distribution in South America ranging from southern Peru and central Chile throughout Paraguay, extending south of Chubut province in Argentina (Redford and Eisenberg 1992). In spite of this relatively wide distribution, few studies have documented the biology of the lesser grison and the basic ecology of this species is poorly understood (Table S1, available online at www.onlinelibrary.wiley.com). Although the lesser grison has been classified as "Least Concern" by the IUCN Red List of Threatened Species (Reid and Helgen 2008), its status in Argentina is "Vulnerable" and the scarcity of knowledge of the biology of the species may prevent adequate determination of conservation status of the species (Ojeda et al. 2012). The production of basic information for the lesser grison (as well as for greater grison, *G. vittata*) is a priority for carnivore studies in the Neotropics (Oliveira 2009).

Live-trapping is often necessary to study mammals (Michalski et al. 2007). In general, mustelids are dangerous to manipulate because of their speed, agility, and aggressive nature (Fowler 1995). Information about the capture, manipulation, and chemical immobilization for lesser grisons as well as for most of the Neotropical mustelids is scarce (Kreeger et al. 2002, West et al. 2007). We captured lesser grisons as part of a larger investigation of the carnivore guild of the Pampas grasslands of Argentina. We assessed chemical restraint of captured individuals to determine an appropriate immobilization dosage range to achieve an adequate field-handling period that minimized recovery time. We also compared the trapping devices used based on their efficiency

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and injuries they caused to lesser grisons. We recorded the difficulties experienced with the attachment of radio-transmitters. Additionally, to provide baseline information on natural history of the lesser grison, we reported morphometric measures and information on activity patterns. Because sexual dimorphism size is prominent in many mustelids, with males being larger than females (Rozhnov and Abramov 2006, Monakhov 2009, Bornholdt et al. 2013), we compared body mass and morphometric features between genders in our sample of trapped individuals.

STUDY AREA

The Pampas grassland (443,000 km^2) is one of the most populated parts of Argentina, South America, and the ecological region with the greatest level of degradation in the country (Bertonatti and Corcuera 2000). Field work occurred in 2 study areas of the Pampas grasslands with different levels of anthropogenic disturbance. The study areas were located approximately 108 km from each other in southern Buenos Aires province. In the protected area (i.e., Ernesto Tornquist Provincial Park), vegetation was dominated by natural short-grass prairie species, including *Stipa* spp., *Piptochaetium* spp., *Briza* spp., and *Festuca* spp., which were intensively grazed by a population of free-ranging horses (*Equus caballus*; Fig. 1a). In our second study area, the land was typically used for intensive agriculture (wheat, barley, soybeans, and sunflowers) and extensive livestock grazing (cattle and sheep) with natural grass patches limited to marginal areas (Fig. 1b). The climate was temperate dry, the annual precipitation ranged from 600 mm to 800 mm, and mean monthly temperatures were 8° C to 23° C.

METHODS

We captured lesser grisons from December 1998 to June 2006, during 4–5 trapping sessions/year (15–30 days/session). We used 2 trapping devices—iron mesh-wire box-traps (40 × 40 × 120 cm, custom built) and leg-hold traps (Oneida Victor ®Soft Catch Coil Spring Trap 1 1/2; Oneida Victor®, Euclid, OH, USA). Leg-hold traps were modified by adding extra padding of rubber to reduce potential limb injuries; sometimes we deployed >1 leg-hold trap near bait (Luengos Vidal et al. 2003). We checked traps 4 times/day to prevent the risks of thermal stress and injuries related to prolonged capture (Luengos Vidal et al. 2003). We baited traps with chicken meat or live chickens. Captured lesser grisons were restrained with a Y pole or with a restraining box with a sliding wall, depending on trap type.

We chemically immobilized grisons by intramuscular injection with a 4/1.5 combination of ketamine hydrochloride (50 mg/mL Ketalar®, Parke-Davis, Morris Plains, NJ, USA) and xylazine hydrochloride (20 mg/mL Rompun®, Mobay Corp., Animal Health Division, Shawnee, KS, USA) or tiletamine hydrochloride and zolazepam hydrochloride Z; Telazol® or Zoletil®) by hand syringes. Initial amounts were based on visual mass estimation. If necessary to maintain anesthesia, an additional injection of 50% of the initial dose of ketamine was used in both protocols as recommended by Kreeger et al. (2002).

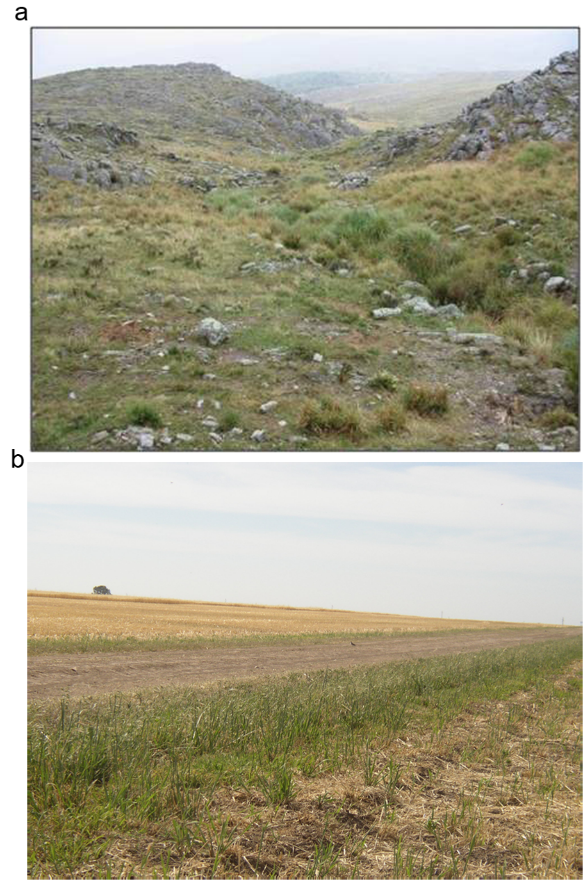

Figure 1. Examples of landscapes of the study areas in the Pampas grassland of Argentina, South America, where we assessed the chemical immobilization and success of trapping devices on the lesser grison (*Galictis cuja*), December 1998 to June 2006. (a) Protected area with vegetation dominated by natural short-grass prairie species; and (b) agricultural area where the land was typically used for intensive agriculture and extensive livestock grazing.

We examined all captured animals for presence of injuries and rated them using the scoring system from Hubert et al. (1996), which has been used for other carnivores (Hubert et al. 1997, Blundell et al. 1999). This scoring system assigned injuries to one category each time a particular injury was observed and each category was assigned a point score related to the gravity of the damage (from 0: apparently normal to 100: dead). We ear-tagged all animals and fitted a sample of captured animals with a radiotransmitter (Table 1). We held each anesthetized animal in a recovery cage (i.e., a trap cage with soft lining in the inner walls) and monitored the animal until it was fully recovered (able to stand and walk without falling or lack of coordination), and we then released the animal at the capture site. Capture, handling, and marking followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011) and the Argentina Society of Mammalogy (Giannoni et al. 2005). Organismo Provincial para el Desarrollo Sostenible from Buenos Aires province authorized all field activities.

We defined induction time as the time from initial injection until complete immobilization (i.e., no response to tactile or auditory stimuli). We defined manipulation time as

Table 1. Manufacturer, attachment type, and performance of very-high-frequency radiotransmitters attached to 9 lesser grisons (M = male, F = female) monitored in the Pampas grassland, Argentina, South America, from 1998 to 2006.

ID	Company[a]	Attachment type[b]	Hours of tracking	Cause of failure	Transmitter recovery
M1	AVM	Collar	<12	Radiocollar drop-off	Yes
M2	AVM	Collar	<24	Radiocollar drop-off	Yes
M3	AVM	Collar	<12	Radiocollar drop-off	Yes
F1	TS	Collar	<12	Radiocollar drop-off	Yes
M4	TS	Backpack	<24	Loss of signal gain[c]	No
M5	AVM	Collar	480	Radiocollar drop-off	Yes
F2	TS	Collar	<12	Loss of signal gain[c]	Yes
M6	TS	Backpack	<12	Loss of signal gain[c]	No
M7	TS	Backpack	40	Backpack drop-off	Yes

[a] AVM = AVM Instrument Company Ltd., Colfax, California, USA; and TS = Telemetry Solutions, Inc., Concord, California, USA.
[b] Collar; made of strong textile belt that only encircled the neck; backpack: made of strong textile belt encircling the neck and with a loop that surrounded the torso, with connecting straps between them for reinforcement.
[c] Loss of signal gain could have been due to transmitter failure (manufacturing defect), or potential damage to collar from the animals.

the time that elapsed from injection until first nonstimulated head movement, recovery time as the interval between injection and when an animal recovered its ability to maintain an upright posture, and liberation time as the interval from injection to complete recovery (normal gait, unimpaired and coordinated locomotion). Criteria for an "effective dose" combination were as follows: 1) mean induction time <11 min because ketamine is a short-acting drug and intramuscular injections take effect within 3–5 min with complete immobilization within 5–10 min (McKenzie 1993); 2) sedation of sufficient depth to allow safe handling for ≤15 min; and 3) maintenance of rhythmic cardiac and breathing rate. We used nonparametric Kruskal–Wallis tests to compare manipulation time, recovery time, and liberation time between effective drug protocols (i.e., protocols that allowed us to work with animals such that they experienced little or no mobility, no pain, and no awareness of their state of capture and physical restriction). We considered hypothermia and hyperthermia to occur when rectal temperatures were below 35° C or above 39° C, respectively (West et al. 2007).

We determined respiration rate visually by observing chest movements (breaths/min), determined resting heart rate with a stethoscope (beats/min), and measured rectal temperature using a digital thermometer. These parameters were taken at 10-min intervals as soon as possible after immobilization and until handling procedures were completed. Additionally, we checked capillary refill time to assess blood pressure. We applied a commercial ointment (Lacryvisc® gel; Alcon Laboratorios S.A., Mexico) to the eyes to lubricate the cornea and conjunctiva, and placed a cloth over the eyes to minimize the risk of injuries produced by light.

We recorded data on body mass, sex, and morphometric features (external alveolar distance between upper canines; total body length; tail length; leg length; head length; rostral width; ear length; neck circumference; chest circumference). We weighed grisons to the nearest 0.1 kg with a 10-kg spring scale (Pesola®; PESOLA AG, Schindellegi, Switzerland). We took tooth, ear, and foot measurements to the nearest 0.1 cm with a vernier caliper. We took all other measurements to the nearest 1.0 cm with a 200-cm measuring tape. We used t-tests ($\alpha = 0.05$) to compare body mass and morphometric features between male and female lesser grisons. We also calculated a sexual dimorphism index, as $100 \times ([\text{mean males}/\text{mean females}] - 1)$ (see Krüger 2005, Rodríguez-Refojos and Zuberogoitia 2009) for each variable to further evaluate sexual dimorphism.

RESULTS

We captured 23 individuals on 26 occasions (Fig. 2). We also trapped other nontarget species, such as Pampas fox (*Pseudalopex gymnocercus*), Molina's hog-nosed skunk (*Conepatus chinga*), and Geoffroy's cat (*Leopardus geoffroyi*). All captured lesser grisons were adults with permanent teeth.

Four and 22 captures were in box-traps and leg-hold traps, respectively. On average, 187 trap-days were necessary to capture a lesser grison in our study areas. The capture rate with box-traps was 632 trap-days/capture, whereas the capture rate with leg-holds was 105.8 trap-days/capture (~6× lower). Most captures (64.2%) occurred in native grasses taller than 20 cm, 27% occurred in sites along a railway border with both native and introduced tall grasses,

Figure 2. Two lesser grisons waiting for liberation in a recovery cage after being captured in an agricultural area of the Pampas grassland of Argentina, South America, during study conducted from December 1998 to June 2006. On the right side is a radiocollared male and on the left side is a female, too small (1 kg) to carry a radiotransmitter.

Table 2. Descriptive statistics for 10 morphometric measurements (mass in kg and the remaining variables in cm) and sexual dimorphism index (SDi) of male and female lesser grisons captured in the Pampas grassland, Argentina, South America, during 1998–2006.

	Female			Male					
Variable[a]	Sample size	Mean ± SD	Range	Sample size	Mean ± SD	Range	*t*-value	*P*	SDi (%)
M	$n=6$	1.03 ± 0.15	0.8–1.3	$n=15$	2.12 ± 0.46	1.3–2.8	−8.03	<0.01	105.82
C–C	$n=6$	1.50 ± 0.20	1.2–1.8	$n=13$	1.88 ± 0.10	1.7–2.0	−4.31	<0.01	25.33
TL	$n=6$	66.33 ± 1.21	65.0–68.0	$n=15$	73.03 ± 2.82	68.0–78.0	−5.55	<0.01	10.10
TLb	$n=6$	17.42 ± 2.65	14.0–22.0	$n=15$	18.66 ± 2.15	15.0–23.0	−1.13	0.27	7.11
LL	$n=4$	5.75 ± 0.29	5.5–6.0	$n=8$	7.24 ± 1.64	4.9–10.0	−2.49	0.04	25.91
HL	$n=6$	9.08 ± 0.80	8.0–10.5	$n=16$	9.18 ± 1.58	7.0–12.0	−0.13	0.89	1.10
RW	$n=6$	5.65 ± 0.67	4.5–6.5	$n=11$	8.50 ± 1.30	6.0–10.0	−4.95	<0.01	50.44
NC	$n=6$	15.58 ± 0.92	14.5–17.0	$n=10$	19.25 ± 1.77	17.0–23.0	−4.67	<0.01	23.55
EL	$n=6$	1.54 ± 0.49	1.1–2.1	$n=13$	1.73 ± 0.36	1.2–2.3	−0.98	0.343	12.33
CC	$n=5$	17.00 ± 1.41	15.0–18.0	$n=4$	23.00 ± 2.12	20.5–25.0	−5.10	0.001	35.29

[a] M, mass; C–C, external alveolar distance between upper canines; TL, total length; TLb, tail length (bone); LL, leg length; HL, head length; RW, rostral width; EL, ear length; NC, neck circumference; CC, chest circumference.

15.3% occurred in cropland, and 11.5% occurred in grazed vegetation shorter than 20 cm.

On 3 occasions, we captured 2 animals (26% of the trapped animals; 1 M and 1 F in all cases) simultaneously in the same group of leg-hold traps. Captures were most frequent in winter (38.4%) and autumn (26.9%); 69% of captures were during daylight hours. Finally, lesser grisons were captured by their front limbs in 60.8% of the cases. Of the 4 individuals captured in box-traps, 1 had cutaneous abrasions on the face. Of the animals captured with leg traps, 65.2% did not show any evidence of injuries and the remaining individuals presented minor injuries with scores of ≤5 (1 case of edematous swelling or hemorrhage, 1 case of avulsed nail, and cutaneous laceration of <2 cm in 3 cases).

The male:female ratio of captured animals was 1:0.5. Males were larger for all morphometric measures, with 70% of them statistically significant (Table 2). Body mass, rostral width, and chest circumference showed the greatest values of sexual dimorphism (105.82; 50.44; 35.29, respectively; Table 2).

We used the ketamine–xylazine combination in 20 captures, tiletamine–zolazepam in 4 captures, and 2 animals were not sedated (Table 3). Immobilization with ketamine–xylazine was effective in 80% of the cases. In the other 4 cases (20%), we had problems injecting the first dose because the animals were agitated and moving and thus, it was likely that they received partial doses. Manipulation time ($H=1.7$, $n_1=16$, $n_2=4$, $P=0.21$), recovery time ($H=3.63$, $n_1=16$, $n_2=4$, $P=0.06$), and liberation time ($H=6.35$, $n_1=16$, $n_2=4$, $P=0.005$) were longer with tiletaine–zolazepam than ketamine–xylazine, although only the difference in liberation time was statistically significant between the drug combinations (Table 3).

The mean (±SD) heart rate with effective ketamine–xylazine doses was 169.27 ± 69.79 beats/min and 234 ± 16.81 beats/min with tiletamine–zolazepam. The respiration rates of animals injected with ketamine–xylazine were very irregular. Most individuals (14/24) presented thermal problems, with prevalence of hypothermia (Table 3) over hyperthermia with both drug combinations, but we could not find any relationship with season or period of day of the capture.

We equipped 9 animals with radiotransmitters, using traditional collars ($n=6$) and backpacks ($n=3$; Fig. 2; Table 1). Seven of 9 transmitters dropped off or ceased transmitting in <24 hr. We found most radiotransmitters inside or very near underground burrows. In only one case were we able to record location data during 20 days for a male; the positions recorded ($n=6$ locations) for this animal covered an area of 473.8 ha (100% Minimum Convex Polygon) with a minimum daily travel distance of 2 km.

DISCUSSION

In addition to evaluations of chemical restraint and radiotransmitter attachment, we report natural history information for a widespread but poorly studied small carnivore, including morphometry, sexual dimorphism, and sex ratio of trapped individuals. This information will

Table 3. Dosage, reaction times, and percent of induced animals among thermal categories among drug combinations used to anesthetize lesser grisons trapped in the Pampas grassland, Argentina, South America, during 1998–2006. Reaction times were defined as: Mt—manipulation time, the time that elapsed from injection until first nonstimulated head movement; Rt—recovery time, the interval between injection and when an animal recovered its ability to maintain an upright posture; and Lt—liberation time, the interval from injection to complete recovery; Normothermic—normal state of body temperature.

		mg/kg	Mt (min)	Rt (min)	Lt (min)			
Drug combination	*n*	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	<35° C (%)	Normothermic (%)	>39° C (%)
Effective ketamine	16	25.9 ± 12.8	58.9 ± 27.6	80.3 ± 32.3	142.4 ±64.5	37.5	37.5	25
Effective xylazine	16	2.2 ± 1.1	58.9 ± 27.6	80.3 ±32.3	142.4 ± 64.5	37.5	37.5	25
Effective tiletamine–zolazepam	4	22.7 ± 9.3	16 0 ± 176.6	270 ±212.1	600 ± 360.6	50	25	25
Ineffective ketamine	4	23.2 ± 7.7	41.7[a] ± 27.5[a]	87.5[a] ±46[a]	165[a] ± 21.2[a]	25	75	0
Ineffective xylazine	4	2.28 ± 2	41.7[a] ± 27.5[a]	87.5[a] ± 46[a]	165[a] ± 21.2[a]	25	75	0

[a] Indicates the result of the total doses injected (sum of several doses injected subsequently in some individual).

support future research and conservation planning for a species that tends to be particularly susceptible to anthropogenic threats (Miller et al. 1999, Valenzuela-Galván et al. 2008). Lesser grison captures occurred mostly during daylight hours in accordance with the observations recently reported for the high Andes (Tellaeche et al. 2014) and this suggests that lesser grisons would be largely diurnal. Several of our captures were of 2 animals in the same group of traps. This behavioral trait was previously described for the same species in the Patagonia region by Redford and Eisenberg (1992), and is in agreement with other anecdotal observations from Argentina (Jayat et al. 1999, Tellaeche et al. 2014) and the hypothesis that *G. cuja* tends to form monogamous pairs (Nowak 2005).

Both models of traps allowed us to capture lesser grisons. However, the use of rubber-padded foot-hold traps greatly increased the capture rate. The greater efficiency of foot-hold traps has also been reported by other studies on co-occurring carnivores, including the Molina's hog-nosed skunk (Castillo 2011), Pampas fox (Luengos Vidal et al. 2003), and small carnivores in other areas (Michalski et al. 2007). The trapping rate for the lesser grison was greater than for sympatric Pampas foxes, but less than for Geoffroy's cats (Castillo et al. 2009), in agreement with the hypothesis that lesser grisons would not be particularly affected by the modification of the natural grasslands of the Argentine Pampas caused by agriculture (Ojeda et al. 2012).

The anesthesia period for both drug protocols was sufficient to take morphometric measurements, collect biologic samples, and attach radiotransmitters. We suggest that field immobilization of lesser grisons with both drug combinations is effective and safe. Although we found differences in reaction time between both protocols, the variation in the sample sizes does not guarantee definitive conclusions. The longer recuperation time with tiletamine–zolazepam should also be considered when selecting a drug combination in lesser grisons. The use of antagonists or reversal agents might facilitate a shorter time to release these animals. However, the prevalence of thermal problems (also reported for other carnivores; Castillo et al. 2012, Luengos Vidal et al. 2014) suggests that body temperatures of chemically restrained lesser grisons should be routinely monitored to ensure that efforts to restore the animal to normal body temperature can be initiated promptly.

In agreement with Bornholdt et al. (2013), we observed a clear pattern of sexual size dimorphism, which is typical of most mustelids (Moors 1980, Shine 1989). Sexual dimorphism may arise for several reasons such as sexual selection, intrinsic differences in the reproductive roles, and niche separation between the sexes (Thom et al. 2004); more information would be necessary to hypothesize what factor may be determining its occurrence in the lesser grisons. Our measurements taken from live specimens were not directly comparable with those taken on dried skins from museum collections by Bornholdt et al. (2013). However, the mean body lengths we recorded for both sexes were outside the ranges of those reported by for lesser grison specimens collected from most of its range (Bornholdt et al. 2013); this is intriguing and deserves further investigation. It is worth mentioning that our morphometric data also extend the known range of body mass for this species, from 1.25–2.5 kg to 0.8–2.8 kg (Yensen and Tarifa 2003).

Sex ratios in trapped samples of mustelids are consistently skewed toward males (Buskirk and Lindstedt 1989). Buskirk and Lindstedt (1989) believed that male bias in trapping mustelids is due to trap spacing in relation to size of mustelid home range; but in our trapping grid the distance between traps was small, so we do not think that the spacing of the traps influenced the sex of animals. To our knowledge, this is the first work to report that this trend is likely to apply to a South American mustelid, by showing that male lesser grisons represent 67% of all captured individuals.

The home range size we reported for a male was based on a single sample and should be considered a minimum estimate. Nevertheless, to our knowledge, it is the first estimate of this parameter for the lesser grison. Although we radiotagged 9 individuals, we were unable to obtain enough locations to properly study their spatial ecology. Neither collars nor backpacks were effective methods of attaching radiotransmitters. The similarity between the circumference of the neck and head, and the ability of these mustelids to rotate their head, appear to be potential reasons that they managed to easily remove or damage the radiotransmitters.

Given the distances traveled, the speed, and the home range of the single individual that we were able to radiotrack for almost a month, we discourage the use of implanted transmitters in the lesser grison because of their lack of significant gain (Eagle et al. 1984, Green et al. 1985, Koehler et al. 2001). The reduced signal may be further attenuated by the animal's position in burrows and nearby terrain features (Philo et al. 1981, Zschille et al. 2008). Implanted transmitters may be adequate to monitor animals with small home ranges but may not be appropriate to monitor wide-ranging animals (Koehler et al. 2001, Proulx and MacKenzie 2012), as appears to be the case of the lesser grison.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1. Review of information published on the lesser grison (*Galictis cuja*) classified by primary research topic.