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Natural history of the pichi (Zaedyus pichiy) in Mendoza Province, Argentina

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Natural history of the pichi (*Zaedyus pichiy*) in Mendoza Province, Argentina

A Dissertation

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in partial fulfillment of the
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Doctor of Philosophy
in
Conservation Biology

by

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ABSTRACT

The pichi *Zaedyus pichiy* (Xenarthra, Dasypodidae) is a poorly known, diurnal armadillo inhabiting arid and semi-arid regions of Argentina and Chile that has endured substantial population declines. My objective was to elucidate different aspects of the natural history of *Z. pichiy* as a first step towards establishing a conservation plan. Wild and captive pichis were studied. Body temperature of wild pichis averaged 35.2±1.2 °C and was highly variable (range 32.2 – 38.3 °C). Temperature measurements of semi-captive males showed that pichis can survive energetically challenging periods by entering hibernation or daily torpor. Stomach contents of poached animals revealed that pichis feed predominantly on insects but also ingest plant material, vertebrates and arachnids. This opportunistic, omnivorous feeding strategy allows them to thrive where food type and availability vary seasonally. The reproductive cycle of pichis was studied by means of histological and fecal hormone analyses. Pichis are seasonal breeders that produce one yearly litter of 1 to 2 offspring, and the initiator of breeding season seems to be an increase in daylength. The absence of regular estrous cycles and corpora lutea in non-pregnant females, and immediate mating attempts after pairing, all suggest that pichis are induced ovulators. Clinical examinations and hematological, serological and coproparasitological analyses of free-ranging pichis, and necropsies and histological examinations of confiscated pichis and roadkills, indicate that the populations are currently in good health. While parasites were often found, no severe pathologies were observed. Infections with potentially zoonotic diseases were rare: only a few pichis were seropositive for *Trypanosoma cruzi,* none had antibodies against *Toxoplasma gondii*, and none of the histologically examined individuals presented lesions attributable to these pathogens. Elevated ambient humidity levels often caused moist dermatitis with epidermal detachment in captive pichis. Poaching is currently considered to have a much higher negative impact on the wild populations than disease epidemics. Mortality due to heavy poaching activity may be difficult, if not impossible, to compensate by the current birth rates. This preliminary database on the natural history and reproduction of pichis will assist efforts to conserve this little-known species of armadillo.

Keywords: Armadillos, conservation, conservation biology, Dasypodidae, diet, diseases, health evaluation, hematology, hibernation, natural history, pathologies, poaching, reference values, reproduction, thermoregulation, torpor, wildlife health, Xenarthra, *Zaedyus pichiy*, zoonoses

INTRODUCTION[1]

Locally known as "piche", "quirquincho" or "blanquito", *Zaedyus pichiy* (Xenarthra, Dasypodidae) occurs farther south than any other armadillo species. Its range extends from the provinces of Mendoza, San Luis and Buenos Aires, south to the Santa Cruz river in Argentina, and south to the Strait of Magellan in Chile (Aguiar and Fonseca in press; Meritt and Benirschke 1973; Wetzel 1985b), at altitudes up to 2500 m. *Z. pichiy* rarely has been kept and studied in captivity and seldom studied in the wild (Superina 2000), which explains the scarcity of scientific information about its life history; most knowledge of this species is based on anecdotal reports from locals.

Materials and Methods

The present report is based on published literature, field studies and observations of captive pichis carried out between 2001 and 2005. To obtain samples from wild pichis, vehicle, walking and horseback transects of varying length were carried out in randomly chosen areas in the entire provincial territory of Mendoza, Argentina (32° 00' S to 37° 30' S and 66° 30' W to 70° 00' W; Figure 1), except in the high Andes in the west where pichis are absent. Transects were performed in all seasons and at all times of day. Pichis were run down, captured by hand, and restrained manually to take morphometric measurements, measure their rectal temperature with a thermocouple, and collect blood and fecal samples. Rectal temperature was measured no later than 5 minutes after capture and blood samples extracted within 15 minutes. All pichis were released at their capture site within 45 minutes.

Observations of captive pichis were obtained from five males and six females kept individually in pens made of wire mesh (2 x 1.5 x 2.5 m each); soil to a depth of 2 m provided a natural substrate for digging. The complex of enclosures was located in Luján de Cuyo, Mendoza province, within the pichis' native range, to ensure their exposure to natural light cycles and

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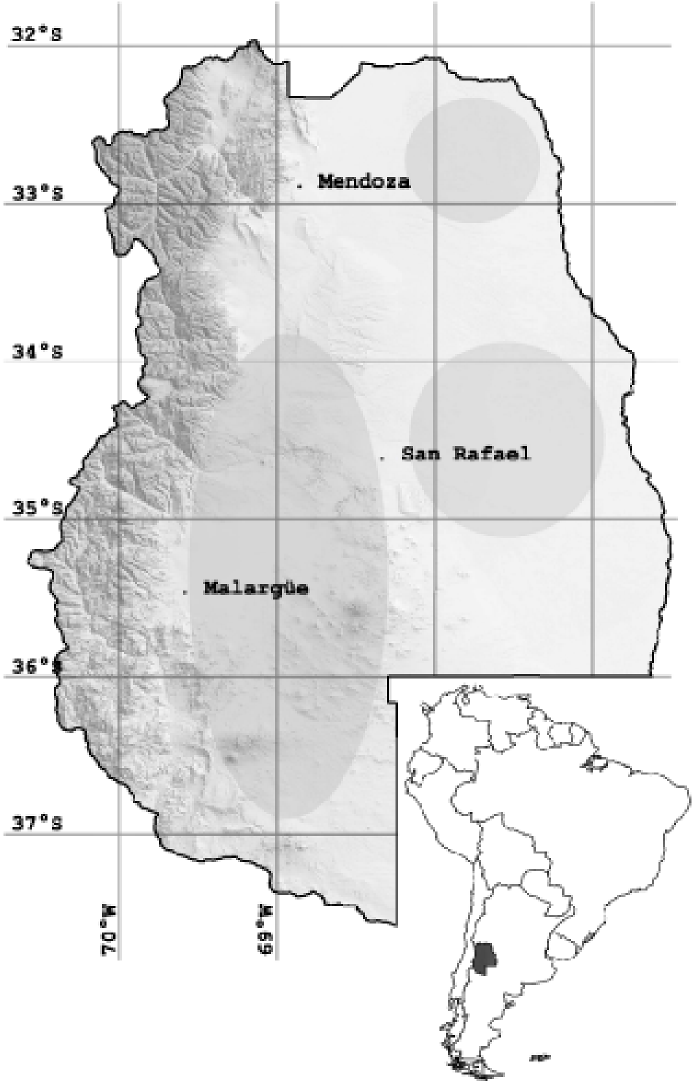
[1] Based on: Superina, M. (in press): The ecology of the pichi *Zaedyus pichiy* in western Argentina. In: The Biology of the Xenarthra (S. F. Vizcaíno and W. J. Loughry, eds.). University Press of Florida.

variations in ambient temperature. Males and females were brought together during the mating season.

A temperature data logger (Thermochron iButton, Maxim Dallas Semiconductors, Sunnyvale, CA) was implanted into the subcutaneous tissue of a captive adult male to record its body temperature at intervals of 90 minutes during the estimated torpor season. During the experiment, the animal was kept in a metal box measuring 80 x 60 x 50 cm filled with wood shavings, and had free access to food and fresh water.

Figure 1: Sampling areas in Mendoza province, Argentina.



Independent-samples t-tests were used to test for significant differences between morphometric data of male and female pichis, and of northern and southern populations, respectively. Quantitative data are reported as means ± SD.

Morphology

Pichis are small, hairy armadillos that can be distinguished from other species by their sharply pointed marginal scutes, short ears, and a light longitudinal dorsal line extending from the posterior end of the scapular shield (or the first band) to the posterior end of the pelvic shield. Their scapular and pelvic shields are separated by an average of seven bands (range 6 to 9), and one complete movable band is present on the anterior edge of the scapular shield (Wetzel 1985a, Superina, unpublished data). Carapace color varies from light yellow to almost black. The tan ventral hair increases in density and length during winter. In contrast to *Euphractus sexcinctus*, *Chaetophractus villosus* and *Chaetophractus vellerosus*, pichis lack pelvic glands (Wetzel 1985b). Also unlike other *Euphractinae*, pichis lack teeth on the premaxillary bone and have eight maxillary and nine mandibular pairs of teeth (Wetzel 1985b).

Table 1: Morphometric measurements of adult wild-caught pichis from all localities in Mendoza province, Argentina.

	Mean (cm)	SD	N
Head length	6.9	0.3	117
Head width	5.3	0.3	117
Ear length	1.5	0.2	80
Carapace length	18.8	1.4	118
Carapace width	21.7	1.2	115
Tail length	10.6	0.9	116
Hind foot length	4.8	0.4	81

Note: *Head length was measured from the tip of the snout to the upper border of the head shield; head width at the broadest part of the head shield; carapace length from the anterior edge of the scapular shield to the posterior edge of the pelvic shield; and carapace width on the fourth band.*

Morphometric data for adult, wild-caught specimens from Mendoza province are shown in Table 1. There is some evidence of sexual dimorphism. Female pichis weigh less than males (mean for 28 females = 906 ± 127 g, mean for 53 males = 983 ± 177 g, $p < 0.05$), and this difference is reflected in a significantly smaller carapace width for females (21.2 ± 1.1 cm, n=41) than males (22.0 ± 1.1 cm, n=71, $p < 0.001$). Geographic variation also occurs. Individuals from southern Mendoza are smaller and have a proportionally more slender head than their conspecifics from northern Mendoza. The head length to head width ratio of northern individuals was 0.09 ± 0.02 (n = 14), but 0.08 ± 0.01 (n = 92) in southern pichis. The small sample of northern animals precludes statistical testing at this time, nonetheless, the difference is visually distinctive (Figure 2) and makes it quite easy to tell if a pichi is from the north or south.

Figure 2: Head morphology of Zaedyus pichiy *from southern (left) and northern (right) Mendoza province, Argentina.*

Habitat

Pichis live in arid habitats with firm sandy soils, such as the Patagonian steppe in southern Mendoza (Figure 3) and the Chacoan *monte* desert in its northern reaches (Figure 4). In Patagonia, the arid climate and poor volcanic soil, covered with sands of variable depth, together determine the scarce vegetation (Candia et al. 1993), which is composed of grasslands with isolated shrubs, areas with open bush communities, and basaltic steps (Candia and Dalmasso 1995). In the Chacoan *monte*, the dry climate and sandy ground favor the growth of characteristic *monte* vegetation, which consists of small trees, shrubs, and scarce pasture (Peralta de Galmarini and Martinez Carretero 1995). In both habitats, pichis are more commonly found near shrubs than on open lands.

Figure 3: Patagonian steppe in southern Mendoza province, Argentina (36.2° S, 68.9° W).

Figure 4: Chacoan monte *in northern Mendoza province, Argentina (32.3° S, 67.9° W).*

Physiology

A major difference between armadillos and most other eutherian mammals is their relatively low body temperature and a reduced ability to maintain it at a constant level. The body temperature of wild pichis averages 35.2 °C, but may fluctuate considerably over the course of a day as well as with changes in ambient temperatures. Compared to other armadillo species, *Z. pichiy* shows the largest daily variation in body temperature, ranging from 22-36 °C under laboratory conditions (Roig 1971b). I recorded body temperatures in 89 wild, non-torpid individuals from Mendoza province and found ranges from 32.2-38.3 °C.

Z. pichiy seems to be very susceptible to sudden changes in environmental temperature. Laboratory studies suggest that pichis display clear signs of inactivity and respiratory arrhythmia during hypothermia. Their behavior returns to normal after prolonged cold exposure. A sudden rise in environmental temperature from 0 °C to 15 °C overexerts the thermoregulatory ability of cold-acclimated animals, leading to an increase in rectal temperature of 16 °C within an hour and eventually to death (Roig 1971b).

The pichi is the only known xenarthran that can enter torpor (Roig 1971b). Torpor usually lasts from mid-May to mid-August. Carcasses of wild pichis confiscated by law enforcement agencies in Mendoza indicate the animals build a subcutaneous fat layer (up to 1.5 cm thick) just prior to the onset of torpor. The rectal temperature of a wild, torpid pichi was 18.8 °C, while an adult male in captivity lowered its body temperature to 10 °C during several torpor bouts. According to several reports from locals, pichis do not leave their burrows at all during winter, which suggests they might hibernate to cope with the harsh climate. Recent research on individuals maintained in semi-captive conditions confirmed that pichis enter deep hibernation, but they are also capable of entering shallow daily torpor (see Chapter 2).

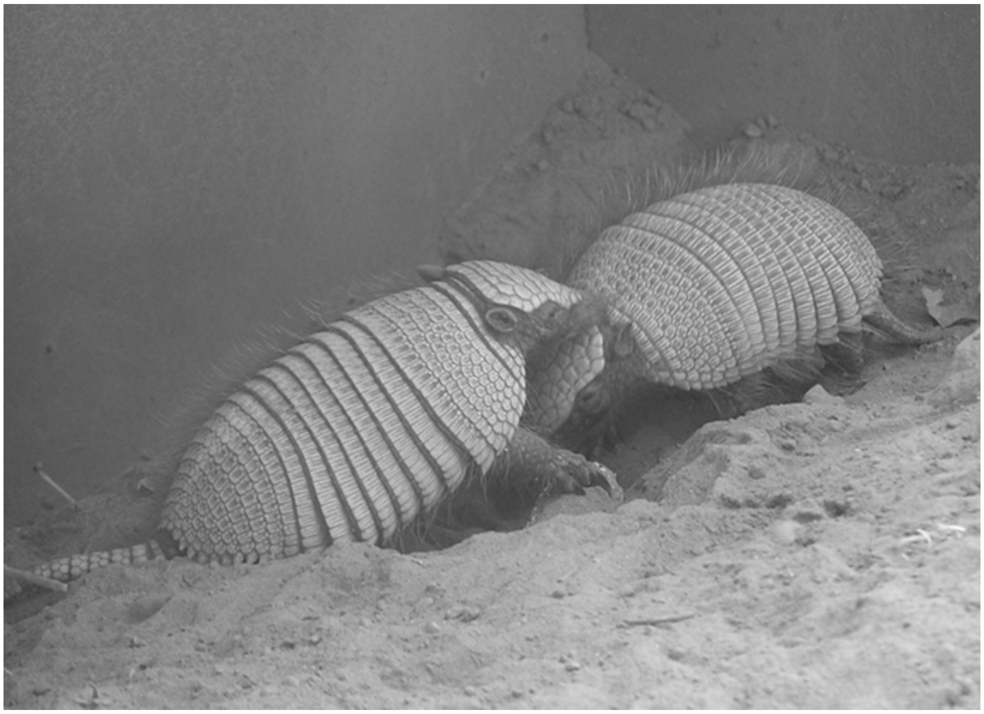
Food habits

Pichis are considered carnivores-omnivores with a preference for carrion. According to Redford (1985), their natural diet also includes insects, especially ants and their larvae, other invertebrates, and plant material. The diet of pichis in the Cerro Nevado area of Mendoza Province, Argentina, will be discussed in Chapter 3.

Reproduction

Field studies indicate that pichis begin their mating season in mid- to late September. Mating has been observed in September and might extend through October. Male armadillos are ascrotal (Anderson and Benirschke 1966); the testes of adult pichis cannot be seen nor palpated in non-reproductive individuals, but are easily recognized during the reproductive season. In seasonal breeders, testis size increases during the reproductive season due to structural modifications of the gonads and enhanced production of spermatozoa (Goeritz et al. 2003). Pichis with prominent testes have been observed only between September and February, which suggests sperm production is limited to spring and summer. Pregnant females have been observed in October and November, and lactating females between November and February. Pichis with small carapaces and a body weight below 600 g, suggesting they were juveniles, have been recorded in January, February and March, but no other months. Similarly, most subadult pichis, i.e., individuals weighing between 600 and 840 g and showing no signs of reproductive activity, have been caught between January and March.

Figure 5: Captive-born pichis Zaedyus pichiy*, aged approximately 40 days, at first emergence.*



Captive pichis reached sexual maturity at 9 to 10 months of age. Mounting was observed between September and December and births of 1 to 2 offspring occurred after a gestation period of approximately 60 days. The offspring did not emerge until they were fully weaned at 40 days of age (Figure 5). These observations, as well as the field data described above, are consistent with those of Redford and Eisenberg (1992) and suggest pichis are seasonal breeders that reproduce in spring and have only one litter per year. The reproduction of pichis is discussed in more detail in Chapter 1.

Behavior

Wild pichis seem to have predominantly diurnal and solitary habits. No wild pichis were caught before 10:00 or after 20:30. Several individuals kept in captivity, however, were more active at night (Altmann and Scheel 1980). Social interactions between wild individuals only were observed during the breeding season. During this same time, captive adult males aggressively defended their territory against other males. Additionally, fights between males and females occurred. Aside from aggression connected with reproduction, fights over food resources have been observed between individuals sharing an enclosure.

Several types of vocalizations have been recorded in captive pichis. A purring sound is emitted when an animal is threatened, which can change into a grunt-like vocalization or a scream (similar to that of *C. vellerosus*) when it is picked up.

In the field, pichis either lie motionless on the ground when threatened, run to hide in an existing burrow, or run under a shrub where they start digging a new shelter. Pichis tend to hide their burrow entrances under vegetation. The roots not only provide support and protection (Greegor 1985): pichis might also feed on the roots or associated insects. Similar to *C. vellerosus* (Greegor 1985), pichis thrust sand backwards while digging and seal the burrow as they excavate. Burrows can be several meters long and of variable depth; maximum depth of burrows excavated during winter was 1.5 m. Only one of over 70 excavated burrows of wild pichis contained nesting material, which suggests that, unlike *Dasypus novemcinctus* (Clark 1951; Taber 1945), this species does not build nests inside its burrows. In captivity, pichis may return to the same burrow for several days, but still dig small, temporary burrows almost every day.

Conservation status

Virtually nothing is known about the size or density of pichi populations in any part of their current range. Although hunting pichis is illegal in Mendoza province, this species is currently one of the main preys of poachers, who use it as a protein source. Intense hunting pressure seems to have depleted populations over the years, as reflected in lower encounter rates and reduced catch rates by poachers. The latter has been confirmed during interviews with locals and hunters, who have reported reduced sightings of pichis in the past years, and from law enforcement agencies, who have noted a considerable decrease in numbers of confiscated pichis. Increasing human population size and expansion of human activities, such as agriculture, into pichi habitat will likely cause further losses (Aguiar and Fonseca in press).

CHAPTER 1: REPRODUCTION OF PICHIS (*ZAEDYUS PICHIY*)

Introduction

Reproductive strategies vary considerably among species, but most studies have focused on only 0.3% of all known mammalian species – in particular humans, domestic livestock and traditional laboratory animals. The reproductive cycle of 97.9% of all mammalian species still remains virtually unstudied (Wildt et al. 2003). Because the survival of a species is inevitably tied to its ability to reproduce, data on its reproductive cycle and behavior are paramount when deciding how best to manage it (Dixson et al. 2003). Among other factors, they are essential pre-requisites for developing and implementing *in situ* and *ex situ* conservation strategies.

The pichi *Zaedyus pichiy* (Mammalia, Xenarthra) is a small armadillo native to Argentina and Chile that is heavily poached. In Mendoza Province, in central-west Argentina, this species is currently the main target of subsistence hunters. Although no past or recent census data are available, a decline in field observations and reduced sightings reported by locals suggest that its wild populations have suffered considerable declines in the past four years. It is difficult to evaluate the chances of survival of *Z. pichiy*, however, because information on its ecology is scarce. *Z. pichiy* has rarely been kept and studied in captivity, and is virtually unknown in the wild. Only anecdotal information is available on its reproductive cycle, and this lack of solid data makes it difficult to evaluate the pichi's chances of surviving heavy poaching and to develop and implement an effective conservation plan.

Most available information on the reproductive cycles of some armadillo species, in particular *Dasypus novemcinctus, Tolypeutes matacus* and *Chaetophractus villosus*, is based on studies of captive individuals. Although *D. novemcinctus* has been studied extensively due to its use in leprosy research (e.g., Enders 1965; Storrs 1971), breeding success for captive nine-banded armadillos is uncommon. However, *T. matacus* and *C. villosus* are often kept in zoos where they reproduce well (Superina 2000). Other species, such as *Z. pichiy* and *Chaetophractus vellerosus*, have only rarely bred in captivity (Superina 2000), while *Priodontes maximus*, *Chlamyphorus truncatus, Calyptophractus retusus*, and *Cabassous spp.* have never reproduced under captive conditions (Chebez 1994; Superina 2000). As a consequence, data on their reproductive cycles are scarce and mainly based on anecdotal reports. While *Dasypus* exhibits delayed implantation and polyembryony, the other genera, including *Zaedyus*, do not seem to share this pattern (Loughry et al. 1998).

Differences among species of armadillos also include variations in seasonality. For example, *Euphractus sexcinctus* is suspected to breed all year long, while other species seem to be seasonal breeders (Superina 2000). Most armadillo species probably have only one litter per year, although it has been suggested that *C. villosus* could give birth to several litters per year (Parera 1996). Up to three yearly litters have been produced by captive *C. villosus* (Ratajszczak and Trzesowska 1997). Published data on the reproductive cycle of *Z. pichiy* are limited to the information presented in Redford and Eisenberg (1992), who suggest that wild pichis reach sexual maturity between 9 and 12 months of age, have a gestation period of approximately 60 days, and give birth to 1 to 3 offspring in January and February, which are fully weaned by six weeks. Clearly, the reproductive strategy of pichis requires further investigation.

Long-term studies of armadillos through direct observation in their natural habitat are difficult and sometimes not feasible due to their semi-fossorial habits. An alternative method to assess their reproductive cycle is to study the metabolites of reproductive steroid hormones in fecal matter. Steroid monitoring in feces was developed almost 30 years ago to measure estrogen metabolites in humans (Monfort 2003). The technique has since been applied to domestic species, and the use of fecal steroid monitoring in captive and free-living wildlife has been growing rapidly since its first use in a non-domesticated species, the macaque, in 1987 (Monfort 2003). Today, fecal hormone metabolite analysis is widely used to obtain basic data on the reproductive cyclicity of wild and captive animals (Brown et al. 1997; Czekala et al. 1994; Ganswindt et al. 2003; Graham et al. 2002; Schwarzenberger et al. 1999). It also has applied uses, such as non-invasive pregnancy diagnosis (Schwarzenberger et al. 1993; Wasser et al. 1991). Due to the considerable interspecific variation in steroid metabolites and metabolite concentrations (Brown et al. 1997), however, a specific protocol needs to be developed for each species. As photoperiod and environmental temperature seem to be major factors influencing gonadal activity in seasonal breeders (Parreira and Cardoso 1993), such studies are ideally carried out with animals maintained in open enclosures in their native range, where they are exposed to natural light cycles and variations in ambient temperature. Furthermore, keeping the study animals in an environment that imitates their natural habitat allows them to express their natural behavior, which helps reducing stress that might alter reproductive hormone levels.

In addition to providing a convenient, non-invasive sampling method, fecal steroid monitoring allows the characterization of reproductive events more precisely than the analysis of blood levels, because (1) blood levels vary according to minor oscillations in steroid secretion, and may be influenced by capture and handling stress, while fecal matter reflects the hormone levels over a period of hours rather than a single point in time; (2) metabolite concentrations are frequently two to four times higher than the concentration of the parent steroid in the blood (Brown et al. 1996; Peter et al. 1996). Because nonmetabolized progesterone is scarce in feces and fecal steroid hormone assays cross-react with several progesterone metabolites, it is more appropriate to use the term progestagens instead of progesterone when referring to fecal metabolites of progesterone (Schwarzenberger et al. 1997).

Another method to determine the reproductive cycle of wild animals is to study their reproductive organs directly. Samples for histological studies can be obtained through (hemi-) castration or extraction of biopsies of live, anaesthetized individuals or after euthanasia, which are the most common techniques in domestic and laboratory animals (e.g., Bansode et al. 2003; França and Godinho 2003). An alternative in endangered species is to sample roadkills and dead, poached individuals confiscated by law enforcement agencies. This sampling method allows avoiding castration and euthanasia of wildlife only to extract reproductive organs. Some potential caveats should, however, be considered. First, the exact time of death and origin of the study animals are often not known. Since the collected tissues may be in varying stages of autolysis, not all samples will be appropriate for histological analysis. Second, the sampled animals may not constitute a representative subsample of a wild population because reproductive status or parasite infestation may have increased their activity or altered their behavior, thus increasing their predation or poaching risk. Third, the total quantity and temporal distribution of samples may depend on poaching activity, as well as frequency and success of anti-poaching patrols. It could therefore be difficult to obtain similar numbers of samples from all seasons or all reproductive stages. Nevertheless, the sampling method is an ethically justifiable way to collect organs from an endangered species and often the only legally acceptable means to obtain samples from protected wildlife. Comparison of the collected materials with data obtained from live or freshly dead animals will allow investigators to evaluate or reduce the influence of the mentioned caveats on the study results.

The purpose of this study was to characterize the reproductive cycle and fecal hormone metabolite levels of male and female pichis. My working hypothesis was that gonadal competence of pichis is limited to austral spring months, indicating seasonal reproduction. I studied the reproductive cycle through direct observation, histological analyses, and by measuring fecal immunoreactive estrogens and progestagens in females, and fecal immunoreactive androgens in males.

Materials and Methods

Sample collection

Fecal samples: Twenty-one study animals (11 males, 10 females) were caught in the wild or confiscated by law enforcement agencies in southern (36° S, 69° W) or northern (32.5° S, 68.5° W) Mendoza Province, Argentina (Table 2, Table 3). They were maintained in individual, open pens made of wire mesh (2 x 1.5 x 2.5 m). Soil to a depth of 2 m provided a natural substrate for digging, and the aboveground border of the pens was covered with galvanized sheet metal, 50 cm high, to prevent the pichis from escaping by climbing. The complex of enclosures was located in Luján de Cuyo, Mendoza, Argentina (33.0° S, 68.9° W), within the pichis' native range, to ensure their exposure to natural light cycles and variations in ambient temperature. Males and females were housed in contiguous pens and paired during the estimated breeding season to allow mating. Collection of deposited feces was not possible because pichis usually defecate in their burrows. However, since pichis usually defecate as soon as they are caught, they were captured by hand when above ground and fecal samples collected into zip-lock plastic bags. Fresh feces were collected 1 to 4 times weekly for periods up to 17 months. Samples were frozen within an hour of collection and stored at –20 °C.

Additional samples were collected from 22 wild males and 18 wild females in Mendoza Province, Argentina. Pichis were run down on foot, captured by hand, and restrained manually to take morphometric measurements and to determine their reproductive status by visual inspection and palpation. Fecal samples were collected into zip-lock plastic bags, and the animals released at the capture site immediately after sampling. Samples were placed in liquid nitrogen within an hour of collection, transferred to the lab, and stored at –20 °C.

Table 2: Identification of captive-kept male Zaedyus pichiy*, duration of fecal sample collection and origin of the animals.*

Males	Sampling period (months)	Origin
ZP94	5	Northern Mendoza
ZP95	5	
ZP146	16	
ZP98	3	Southern Mendoza
ZP101	3	
ZP105	3	
ZP142	11	
ZP143	6	
ZP155	11	
ZP158	10	
ZP160	4	

Table 3: Identification of captive-kept female Zaedyus pichiy*, age at onset of fecal sample collection, and reproductive success during sampling seasons.*

Female	Age at onset of the study	Sampling period (months)	Year	Reproductive success
ZP99	adult	3	2004	None
ZP104	adult	3	2004	Stillbirth?
ZP108	adult	17	2005	Abortion, stress-related
			2006	Birth & lactation 2 females
ZP144	subadult	11	2005	Birth & lactation 1 male, 1 female
			2006	Birth & lactation 2 males
ZP150	subadult	5.5	2005	No pregnancy
			2006	Birth & lactation 1 female
ZP152	subadult	10.5	2005	No pregnancy
		3.5	2006	Birth & lactation 1 male
ZP153	subadult	12	2005	Birth 1 male
			2006	Stillbirth - birth & lactation 2 females
ZP154	subadult	7	2005	Abortion; stung by spider
ZP162	subadult	7	2005	Stillbirth?
		3.5	2006	Birth & lactation 1 male, 1 female
ZP185	Subadult	3.5	2006	Birth & lactation 2 males

Tissue samples: Necropsies and visual inspections for external signs of reproductive status were performed on 144 roadkills and dead pichis that had been confiscated from poachers by law enforcement agencies. Additional samples were collected from captive pichis that died during the study period. The animals were classified as juveniles, yearlings or adults based on morphological signs, such as carapace length and width, and the presence or absence of scars. Animals that could not be assigned to an age class were excluded from the analyses. Ovaries and testes were collected and their maximum length and width were measured with a caliper. Organ volume was calculated with the formula for the volume (V) of an ellipsoid: $V=4/3\cdot\pi ab^2$, where a=1/2 maximum length and b=1/2 maximum width. Testes and ovaries were stored in 10% formalin. They were fixed in Bouin's fluid, desiccated, and embedded in paraffin wax. A section of 5 µm was cut from the equatorial region and stained with hematoxylin-eosin.

Fecal hormone analysis

Fecal samples were lyophilized and pulverized, then 0.20 g was solubilized in 5 ml of 90% ethanol:distilled water, shaken during 30 min., and centrifuged at 2000 rpm for 20 min. The supernatant was recovered and the pellet resuspended in 5 ml of 90% ethanol, shaken during 1 minute, and recentrifuged. Both supernatants were combined, dried completely, and redissolved in 1 ml methanol. Samples were vortexed, diluted in 4 ml PBS buffer, and kept at –20 °C until their analysis. Solid-phase radioimmunoassays (Coat-A-Count Progesterone and Coat-A-Count Total Testosterone, Diagnostic Products Corporation, Los Angeles, CA, USA) were used to quantify fecal progestagens and testosterone, respectively. Fecal estrogen levels were determined by means of a liquid-phase radioimmunoassay (Ultra-sensitive estradiol RIA, Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Samples were thawed, vortexed, centrifuged for 5 minutes at 10000 rpm, and assayed in duplicate. Assays were validated by demonstrating parallelism between standard curves and serial dilutions of fecal extracts. Extraction efficiencies for hormones added to fecal samples were as follows: 95% at 8 ng progesterone; 70% at 10 ng estradiol; and 73% at 40 ng testosterone. The intra-assay coefficients of variation at 40% binding were <10% for all hormones. To reduce errors due to inter-assay variation, samples obtained from an individual during one reproductive season were analyzed in the same assay run. Inter-assay coefficients of variation based on three identical samples included in each assay run were 19% for progestagens, 9% for estradiol, and 16% for testosterone. Cross-reactivities are listed in Table 4 (progesterone), Table 5 (estradiol), and Table 6 (testosterone). Average water content of the fresh feces was 52±14% (n=829).

Table 4: Cross-reactivity of the antibodies to progesterone included in the DPC Progesterone RIA with other steroids, as reported by the manufacturer

Compound	Cross-reactivity
Progesterone	100%
Androstenediol	ND
Corticosterone	0.9%
Cortisol	0.03%
Danazol	0.006%
11-Deoxycorticosterone	2.2%
11-Deoxycortisol	0.01%
DHEA-SO4	0.002%
20α-Dihydroprogesterone	0.2%
Estradiol	ND
17α-Hydroxyprogesterone	3.4%
Medroxyprogesterone	0.3%
Pregnane	ND
5β-Pregnan-3α-ol-20-one	0.05%
5α-Pregnan-3,20-dione	9.0%
5β-Pregnan-3,20-dione	3.2%
Pregnenolone	0.1%
5-Pregnen-3β-ol-20-one-sulfate	0.05%
Testosterone	0.1%

ND: not detectable

Table 5: Cross-reactivity of the estradiol antiserum included in the DSL Ultra-Sensitive Estradiol RIA with other steroids, as reported by the manufacturer.

Compound	Cross-reactivity
Estrone	2.40%
Estrone-β-D-glucuronide	0.20%
Estrone-3-sulfate	0.01%
Equilin	0.34%
D-Equilenin	3.40%
17α-Estradiol	0.21%
16 Keto Estradiol	0.21%
17β-Estradiol-3-glucuronide	2.56%
Estradiol-3-SO_4	0.17%
Estriol	0.64%
Testosterone	ND
DEA	ND
Diethyl Stilbesterol	ND
17βE2-17-glucuronide	ND

ND: not detectable (<0.01%)

Table 6: Cross-reactivity of the antibodies to testosterone included in the DPC Total Testosterone RIA with other steroids, as reported by the manufacturer

Compound	Cross-reactivity
Aldosterone	0
5β-Androstan-3α,17β- diol	0.4%
Androstenedione	0.5%
5α-Androstan-3β,17β- diol	0.04%
5-Androsten-3β,17β-diol	0.2%
5α-Androstan-3,17- done	0.05%
Androsterone	0.004%
Corticosterone	0.002%
Cortisol	0.005%
Cortisone	0.02%
Danazol	0.09%
11-Deoxycortisol	0
Dexamethasone	0.003%
DHEA	0.002%
DHEA-sulfate	0.006%
5α-Dihydrotestosterone	3.3%
Estradiol	0.02%
5(10)-Estren-17α-ethinyl-17β-ol-3-one	0.2%
4-Estren-7α-methyl-17β- ol-3-one	1.1%
4-Estren-17-ol-3-one	20%
(19-Nortestosterone)	20%
Estrone	0.01%
Ethisterone	0.7%
Fluoxymesterone	0.01%
19-Hydroxyandrostenedione	2.0%
11-Ketotestosterone	16%

Table 6 continued

Compound	Cross-reactivity
Methyltestosterone	1.7%
Norethindrone	0.1%
Norethyrodrel	0
Prednisone	0
Progesterone	0
Spironolactone	0
11β-Hydroxytestosterone	0.8%
Triamicinolone	0.2%

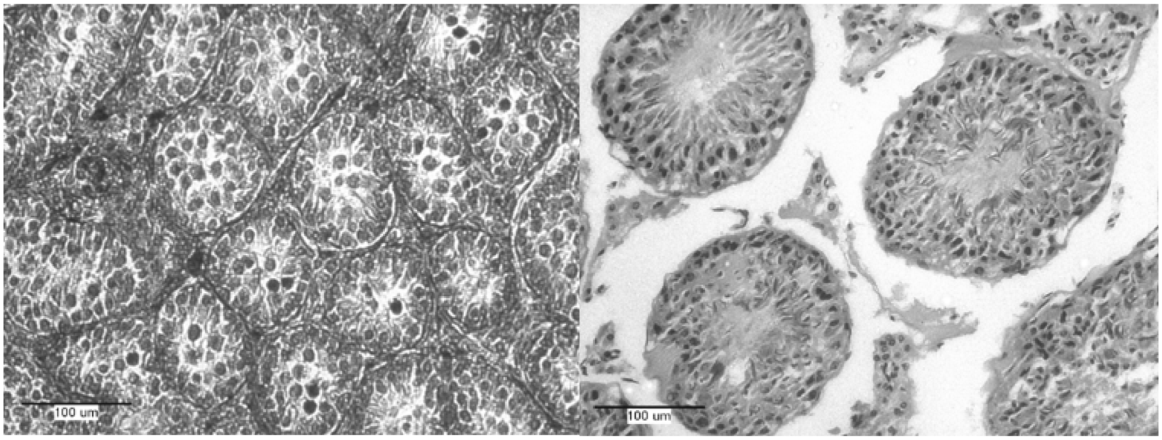
Histological analysis

Males

Spermatogenic activity was evaluated in 10 seminiferous tubules per animal using the spermatogenic index (SI) developed by Grocock and Clarke (1974) as a semi-quantitative measure of reproductive competence. In addition, presence or absence of spermatocytes in the epididymis was recorded. SI scores were assigned from 0–5 according to the following criteria (cf. Grocock and Clarke 1974):

SI 5 = Large seminiferous tubules that display complete spermatogenesis.
SI 4 = Relatively large seminiferous tubules showing complete spermatogenesis, but with less abundant elongated spermatids and spermatozoa (Figure 6, right).
SI 3 = Tubules showing presence of spermatozoa and spermatids, but in lower numbers than in the higher categories.
SI 2 = Seminiferous tubules with Sertoli cells, spermatogonia, primary spermatocytes and round spermatids, but no elongated spermatids.
SI 1 = Small tubules containing only Sertoli cells, spermatogonia, and primary spermatocytes.
SI 0 = Very small tubules containing only Sertoli cells and spermatogonia (Figure 6, left).

Figure 6: Left: *Inactive testis (Spermatogenic Index 0); seminiferous tubules are very small and contain only Sertoli cells and spermatogonia. Few interstitial cells are visible between tubules.* Right: *Testis with high spermatogenic activity (Spermatogenic Index 4); complete spermatogenesis is visible in the relatively large seminiferous tubules.*

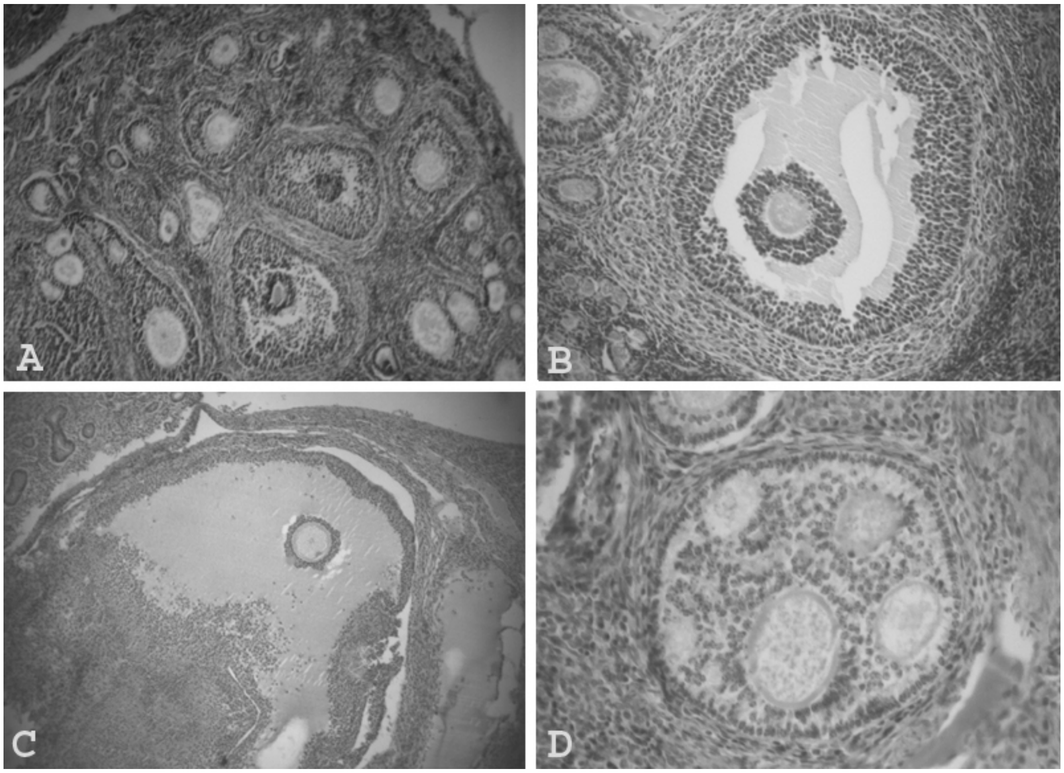


Females

Ovaries were examined by means of light microscopy to determine the presence or absence of the following structures (Figure 7):

- Early secondary follicles: Fully grown oocyte surrounded by a zona pellucida, several layers of granulosa cells, a basal lamina, and a theca interna and externa. Small fluid-filled spaces are visible between granulosa cells.
- Late secondary follicles: Larger than early secondary follicles and with a large, clearly visible follicular antrum.
- Pre-ovulatory follicles: Cumulus oophorus visible, considerably larger fluid-filled antrum than late secondary follicles.
- Corpora lutea: Clearly delimited, large structures consisting of theca lutein and granulosa lutein cells.
- Polyovular follicles: Several oocytes enclosed in the same follicle.

Figure 7: a) Overview of an active ovary with different stages of follicular growth and several polyovular follicles (x40); b) late secondary follicle (x100); c) pre-ovulatory follicle (x40); d) polyovular follicle with 4 oocytes (x100)



Data analysis

Statistical analyses were performed using SPSS (Version 11.0, SPSS Inc, Chicago, Ill., USA) and GraphPad Prism (Version 3.0a for Macintosh, GraphPad Software Inc., San Diego, CA, USA). Unless otherwise stated, results are presented as mean ±SEM. P values below 0.05 were considered statistically significant.

Males

The reproductive season of male pichis from southern and northern Mendoza were calculated separately. For each geographic group, individual values were combined to obtain one mean value per week. The onset of reproductive season was defined as the first increase above the upper limit of the 95% confidence interval (CI) of all fecal samples collected from animals of the same geographic area. The end of the reproductive season was defined as the first value below the upper limit of the 95% CI. Off-season baseline values were calculated for each geographic group as the average of all testosterone values measured outside the reproductive season. Independent-samples t-tests were used to compare hormone levels of captive pichis during reproductive season and off-season, to compare off-season hormone levels of northern and southern pichis, and to test for significant differences between hormone levels of captive and wild pichis both during the reproductive season and off-season. The sample size and distribution over time did not allow statistical analyses on the size or the histological analysis of testes extracted from dead pichis. Photoperiod was calculated from data provided by the Servicio de Hidrografía Naval, Armada Argentina (http://www.hidro.gov.ar). Climate data were obtained from the Servicio Meteorológico Nacional, Fuerza Aérea Argentina, Comando de Regiones Aéreas, Argentina.

Females

Parturition date (day 0) was defined as the day on which progestagen levels were lowest after a hormone peak > 400 ng/g. This approximation was necessary because the exact date of birth could not be determined through direct observation because pichis give birth inside their burrow and offspring do not emerge until they are at least 30 days old. Progestagen levels were log-transformed for statistical analysis. Hormone levels were grouped into intervals of 5 days each from day –40 to +40 and, due to low sample size in some periods, into intervals of 10 days from days –60 to –40, and +40 to +60. Two-way analyses of variance (ANOVA) with Bonferroni post-hoc tests were used to compare hormone levels over time of females that successfully raised their offspring and of females that lost their offspring shortly before or after delivery. Estradiol levels outside pregnancy were screened for regular occurrence of peaks, and the intervals between peaks calculated. Independent-samples *t*-tests were used to compare hormone levels of captive and wild juvenile pichis. Statistical tests were not performed to compare hormone levels of wild and captive adult pichis due to the low number of sampled wild females per reproductive status, and because visual inspection and palpation did not allow identification of wild females in early pregnancy or differentiation between females in early or late lactation. No statistical analyses were performed on the size and histological findings of ovaries.

Results

Most male and female captive pichis reached sexual maturity at 9 to 10 months of age. Mounting attempts were only registered between August and December. In this period, chasing followed by mating attempts usually occurred within minutes of pairing.

Testes from 65 wild-born males and ovaries from 79 wild-born females were collected from dead animals confiscated by law enforcement agencies or from individuals that died in captivity. Eight males (2 yearlings, 6 adults) and 7 females (3 juveniles, 1 yearling, 3 adults) originated from northern Mendoza and the remaining animals were from southern Mendoza. Most samples were collected in February and March. This sampling schedule depended on the confiscations made by inspectors and rangers. Poaching activity is highest between the end of pichi reproductive season and the start of the hibernation season, i.e., between February and April. Testes of 13 males and ovaries of 18 females could not be analyzed histologically due to advanced autolysis.

Males

In males, gonadal competence was limited to a period of three to five months in spring and early summer and was reflected in enlarged testes and significantly elevated fecal testosterone levels ($p<0.001$, Figure 8). These physiological and morphological changes were accompanied by an increased aggressiveness. Males kept in neighboring enclosures grunted and reached through the mesh fence with their forelegs in an attempt to fight with their rival. Aggressive behavior, as well as injuries attributable to fights with conspecifics, was also observed in gonadally competent wild males.

Figure 8: Weekly fecal testosterone levels of captive male Zaedyus pichiy *from a) northern (n=3) and b) southern (n=8) Mendoza Province, Argentina. Dashed lines represent baseline levels outside breeding season. Week 18 = First week of May.*

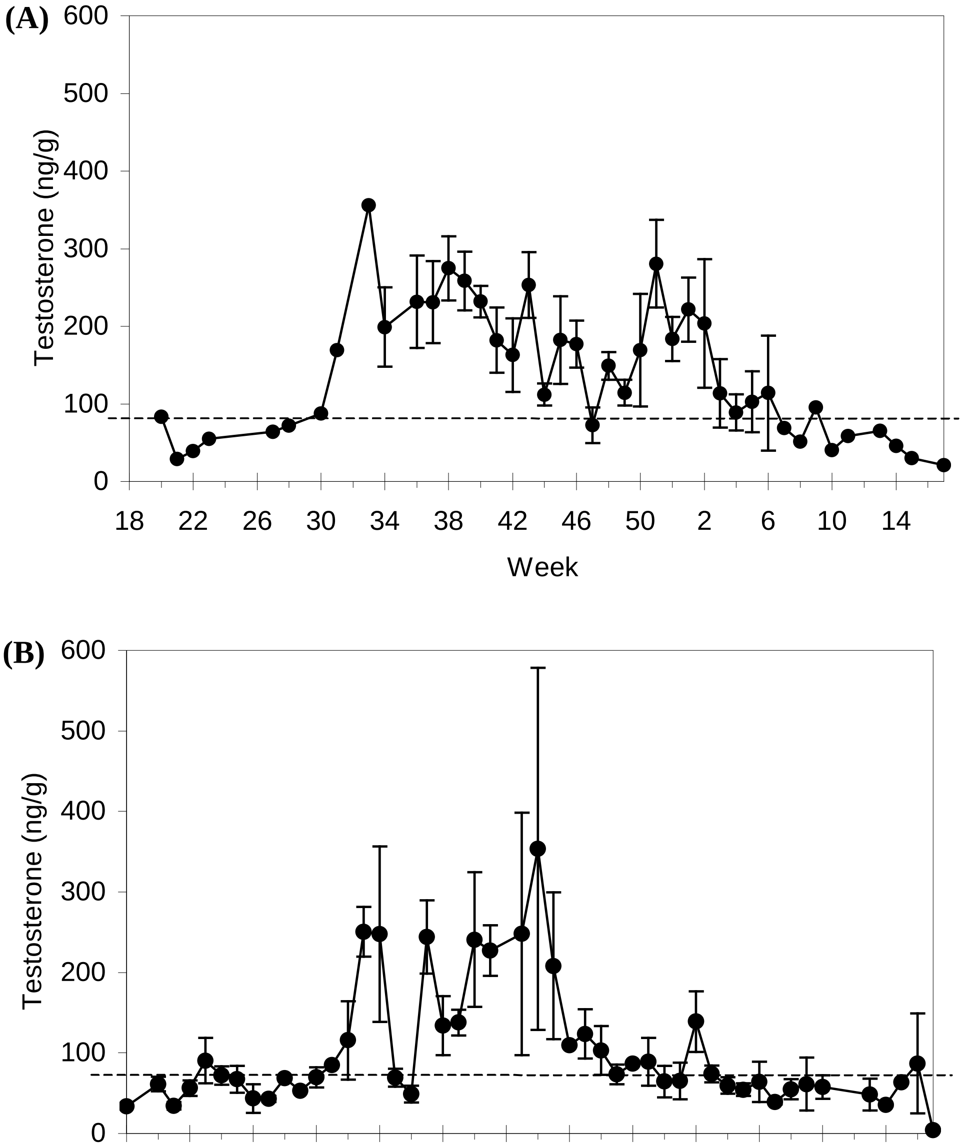
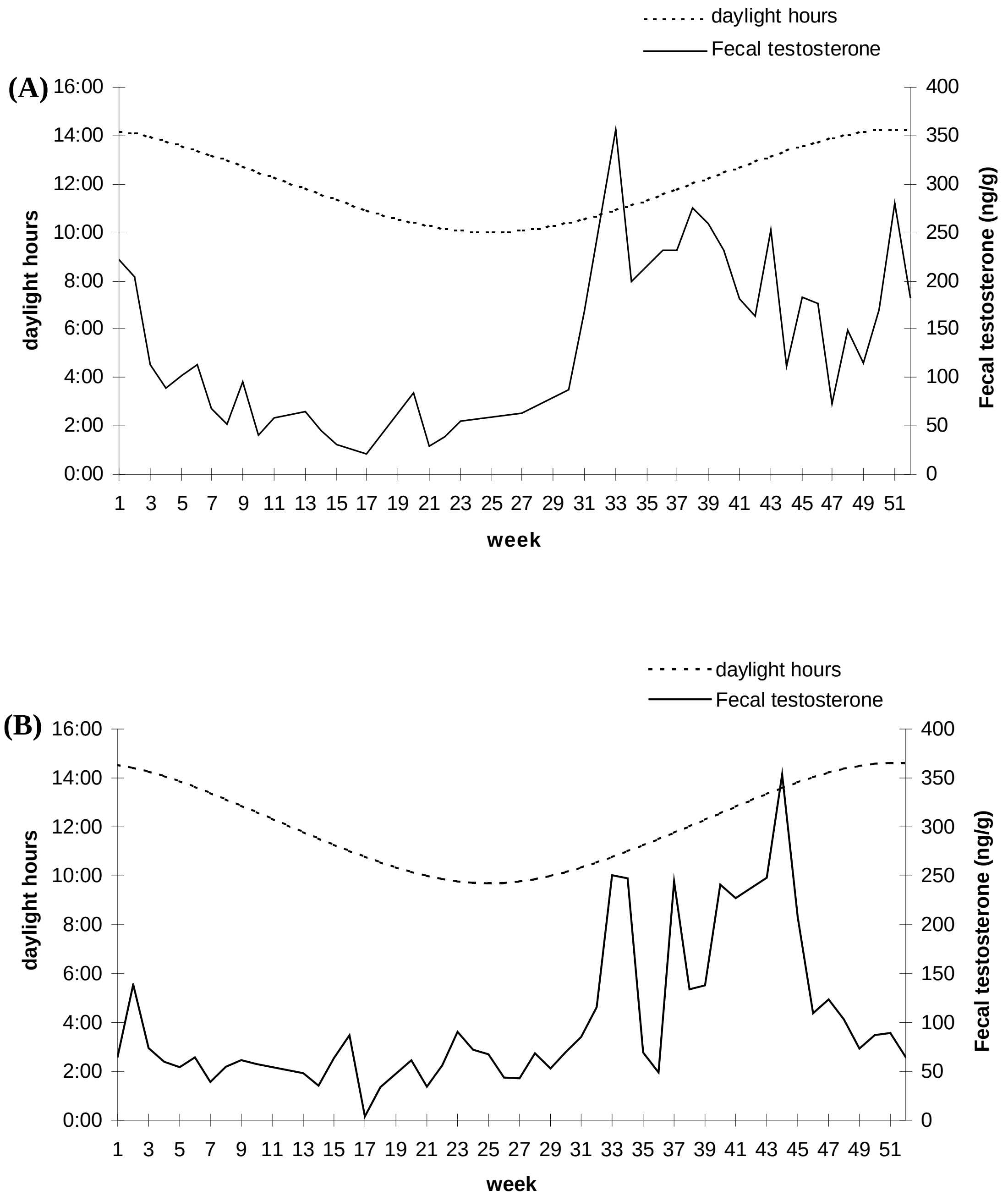


Figure 9: Weekly average fecal testosterone levels of wild-born, captive-kept Zaedyus pichiy *from a) northern (n=3) and b) southern (n=8) Mendoza Province, Argentina, and variation in daylight time at the respective capture sites. In both groups, fecal testosterone levels start increasing 7 to 8 weeks after winter solstice.*



Reproductive season length varied between males of different origins. In animals from northern Mendoza province, fecal testosterone levels remained above the upper limit of the 95% CI from week 32 to week 2 (approx. August 7 to January 10). In individuals from southern Mendoza the levels were elevated from week 33 to week 47 (approx. August 14 to November 25, Figure 8). In pichis from both parts of the province, hormone levels started increasing after winter solstice and reached the upper limit of the 95% CI at a photoperiod of 10 hours and 45 minutes (Figure 9). Pichis from northern Mendoza returned to this limit at a photoperiod of 14 hours and 15 minutes after summer solstice, while their conspecifics from southern Mendoza did so at 14 hours and 15 minutes of daylight before summer solstice. Off-season levels were similar in pichis of different geographic origin ($p>0.05$). Hormone levels of wild males were not significantly different from testosterone levels of captive pichis ($p>0.05$).

Spermatogenic indices (SI) and testis volumes were lowest in juveniles and highest in adults, with yearlings having intermediate levels (Table 7). In adults, SI scores were consistently high in November and showed large variations in animals sampled in February and March. Spermatozoa were only present in the epididymides of 9 adults.

Table 7: Histological analysis of testes of wild confiscated pichis Zaedyus pichiy *of different age classes. SI = Spermatogenic Index*

Age class	Month	n	Average SI (range)	# males with spermatozoa in epididymis	Average Testicular volume in cm^3 (range)
Juvenile	January	1	0	0	0.06
	February	2	0.5 (0-0.9)	0	0.25 (0.14-0.35)
	May	1	0.1	0	0.25
Yearling	February	5	0.5 (0-1.2)	0	0.50 (0.10-1.25)
	March	9	1.5 (0.1-2.9)	0	0.78 (0.16-1.78)
Adult	January	1	0.1	0	0.50
	February	12	1.5 (0.1-4.1)	2	1.43 (0.32-2.88)
	March	16	2.0 (0.1-3.8)	4	1.42 (0.38-2.15)
	April	1	0.9	0	0.51
	October	1	0.9	0	not measured
	November	3	4.2 (3.9-4.8)	3	4.14 (n=1)

Females

Assuming a gestation length of approximately 60 days (Redford and Eisenberg 1992), successful mating occurred on the first day of pairing in two females (ZP144, year 2006; ZP153, year 2006), and 7 to 18 days after pairing in other females (Table 8). Seven captive females included in this study gave birth during the study period; litters consisted of up to two offspring of the same or of different sex. Two females (ZP144 and ZP153) delivered offspring twice in two consecutive reproductive seasons (Table 3, Table 8). Five females aborted or did not raise their offspring. One of them, ZP153, lost her first litter in 2006, became pregnant again immediately thereafter and gave birth to two offspring, which were raised successfully in the presence of the male.

Table 8: Interval between date of pairing and estimated date of parturition, and interval from birth to first emergence of offspring of captive-kept Zaedyus pichiy.

Female (year)	Interval pairing – parturition	Interval parturition – first emergence
ZP144 (2005)	66	41
ZP144 (2006)	58	35
ZP153 (2005)	67	36
ZP153 (2006)	59	33
ZP108 (2006)	78	30
ZP185 (2006)	73	31
ZP162 (2006)	*	37

* Female ZP162 was not separated from the male during the months prior to onset of reproductive season.

Figure 10: Progestagen levels in a) pregnant and lactating female Zaedyus pichiy*, b) pichis that aborted or did not raise their offspring; c) a female pichi that lost its first litter, conceived again, and raised its second litter. Day of parturition (Day 0) was defined as the lowest progestagen level after a peak of >400 ng/g. Note that ZP153 gave birth in 2005 (graph a) and in 2006 (graph c).*

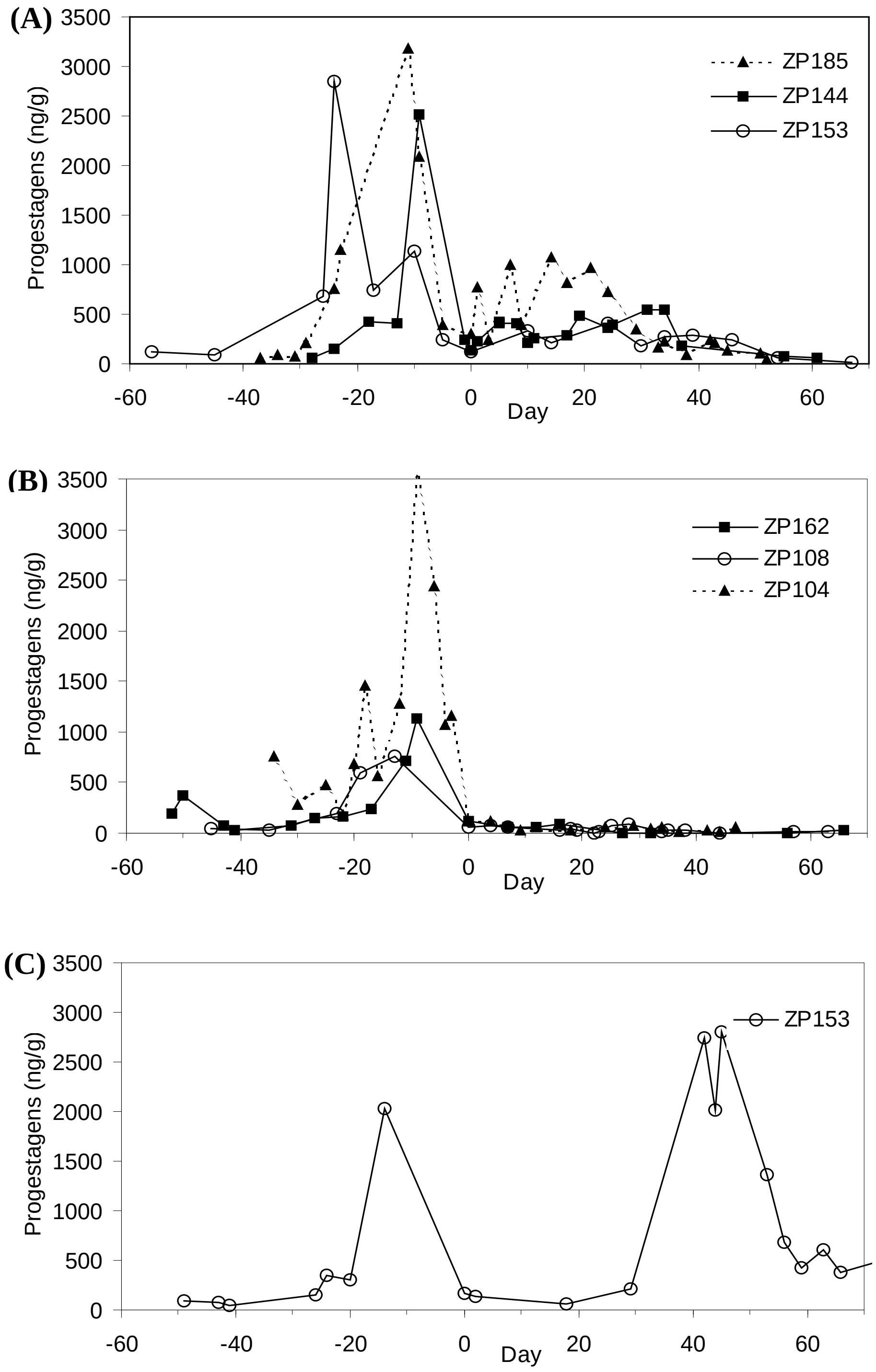
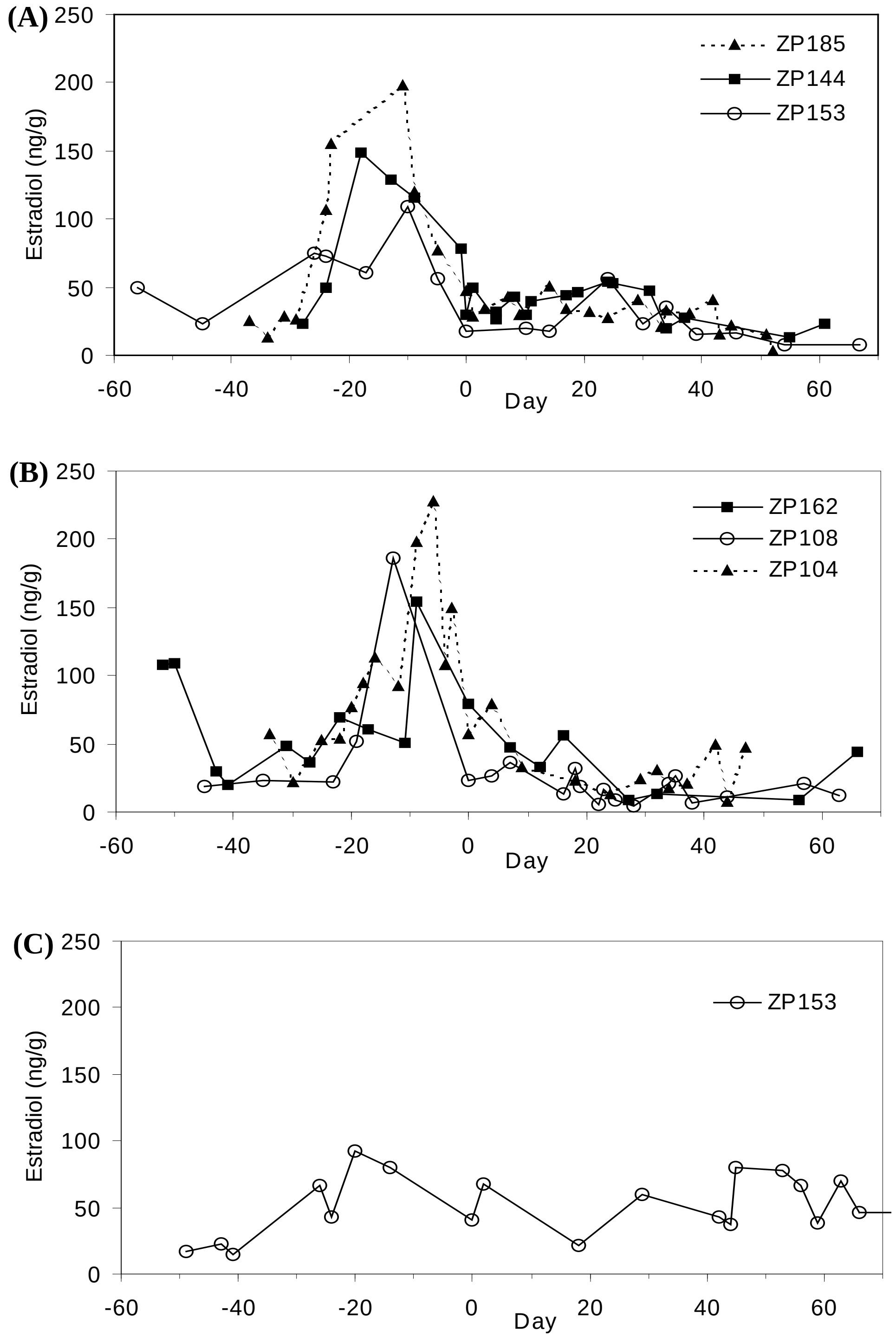


Figure 11: Estradiol levels in a) pregnant and lactating female Zaedyus pichiy, *b) pichis that aborted or did not raise their offspring; c) a female pichi that lost its first litter, conceived again, and raised its second litter. Day of parturition (Day 0) was defined as the lowest progestagen level after a peak of >400 ng/g. Note that ZP153 gave birth in 2005 (graph a) and in 2006 (graph c).*

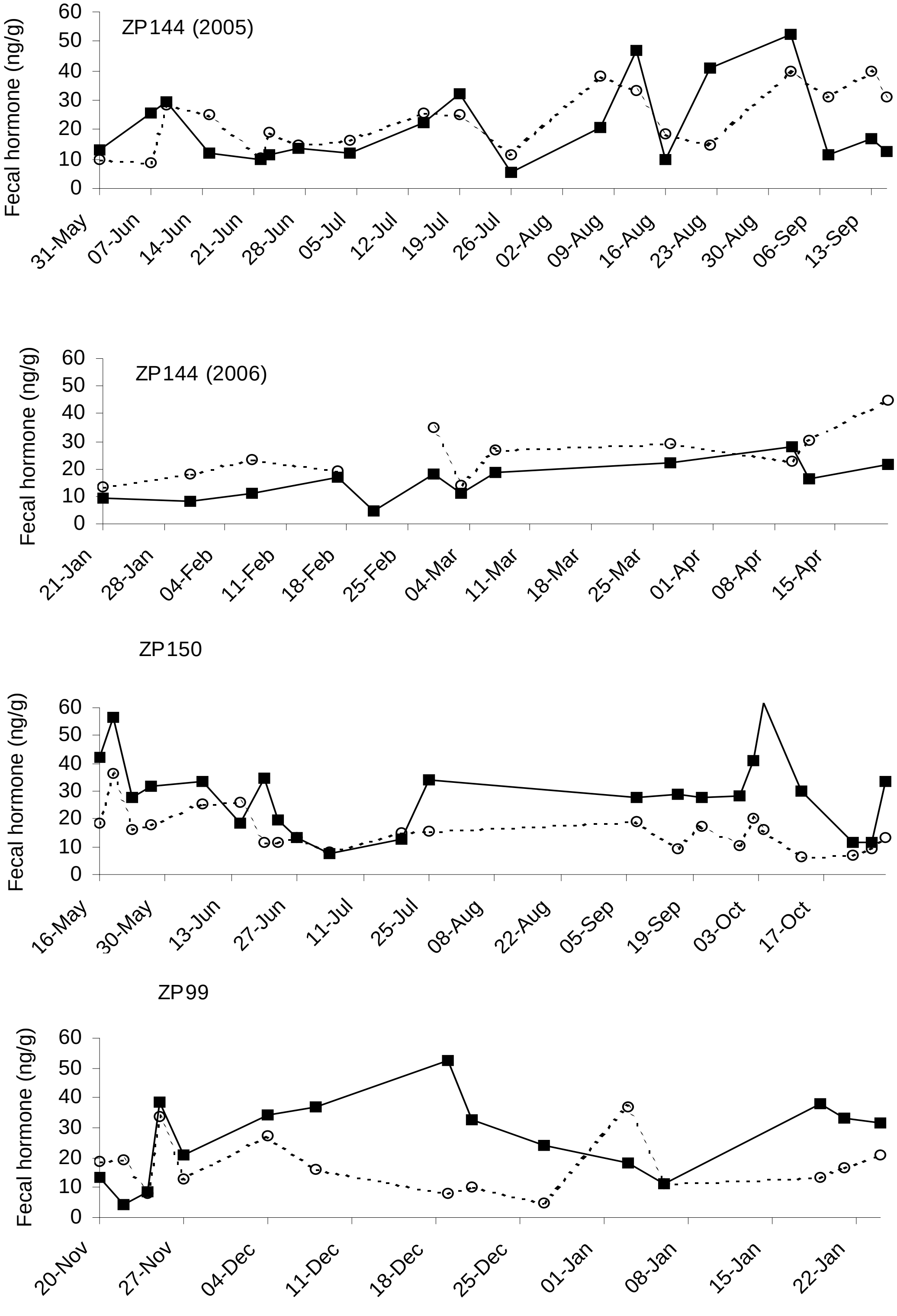


In all females studied, fecal progestagen levels remained low during the first half of pregnancy, increased about tenfold to peak levels of up to 3500 ng/g dry feces, and fell sharply prior to parturition (Figure 10). Fecal estrogen levels followed a similar pattern: they remained low for the first month, then peaked at levels around 200 ng/g before returning to baseline levels around parturition (Figure 11). Progestagen levels of pregnant females that successfully raised their offspring were not significantly different from those of pichis that lost their litters ($p>0.05$), while they varied significantly over time ($p<0.0001$). Estradiol levels varied significantly between groups ($p<0.05$) and over time ($p<0.0001$), with pregnant females that raised their offspring having significantly lower levels on day -10 ($p<0.05$) and a tendency to lower levels on day -5. Postpartum progestagen levels were significantly higher in lactating pichis than in non-lactating females ($p<0.0001$) and varied significantly over time ($p<0.001$); a significant difference was observed from day 5 to 40 ($p<0.05$ on day 5, $p<0.01$ from day 10 to day 40). Postpartum estradiol levels were similar in both groups ($p>0.05$). Offspring remained in their burrows during the first 35±4 days (Table 8). Females were observed carrying food to their burrow several days prior to the first emergence of their offspring. At first emergence, the young foraged for solid food either in proximity of the female or by themselves. The weaning period coincided with the return of progestagen levels to baseline.

Table 9: Intervals between successive estradiol peaks in three non-pregnant, non-lactating captive Zaedyus pichiy *females.*

Female No.	Period included in analysis	Interval between Estradiol peaks (days)					Mean (SEM)
ZP144	June 9 – Sept 13	14	21	24	26	11	
	Feb 7 – Apr 21	21	27	25			21.1 (2.2)
ZP99	Nov 25 – Jan 24	9	17	13	21		15.0 (3.0)
ZP150	May 19 – June 27	19	20				
	Sept 7 – Oct 2	14	11				16.0 (2.5)

Figure 12: Fecal hormone levels of 3 captive non-pregnant, non-lactating female Zaedyus pichiy. *Straight line: Fecal progestagen; dashed line: Fecal estradiol*



Non-pregnant, non-lactating females did not present any clear, high estradiol peaks followed by increases in progestagen indicating ovulation and luteal phase, respectively (Figure 12). Instead, fecal estradiol and progestagens seemed to fluctuate independent of each other. Intervals between successive estradiol peaks were relatively regular for a given individual; however, they varied among females (Table 9).

Table 10: Fecal hormone levels of wild female Zaedyus pichiy. *Reproductive status was determined by means of visual inspection and palpation at time of capture.*

Female No.	Sampling date	Progestagen (ng/g)	Estradiol (ng/g)	Reproductive status
117	15/Jan/05	163.0	5.1	?
119	15/Jan/05	136.5	16.4	Lactation
120	26/Jan/05	248.0	12.8	Lactation
166	16/Oct/05	34.0	6.9	n/s
169	21/Oct/05	73.5	9.3	Lactation?
170	22/Oct/05	43.7	17.4	n/s
174	15/Jan/06	44.6	4.2	Lactation
175	15/Jan/06	74.7	6.2	Lactation
176	15/Jan/06	59.1	15.8	(Juvenile)
177	15/Jan/06	56.3	4.2	(Juvenile)
179	15/Jan/06	81.5	14.4	Lactation
185	23/Jan/06	86.6	29.1	(Juvenile)
308	11/Nov/06	180.6	25.2	Lactation
309	11/Nov/06	150.2	50.5	n/s
311	11/Nov/06	994.6	48.7	Pregnancy
320	17/Dec/06	347.3	7.9	n/s
321	17/Dec/06	49.5	9.0	Lactation?
322	17/Dec/06	202.6	17.3	Lactation

n/s = no signs of pregnancy or lactation

Hormone levels of captive and wild juvenile females were not significantly different ($p>0.05$). Hormones of adult wild females were within range of captive females (Table 10).

Early secondary follicles were found in the ovaries of most pichis of all age classes (Table 11). Late secondary follicles were present in approximately two thirds of juveniles, yearlings, and adults, while pre-ovulatory follicles were present in only two yearlings and four adults. Corpora lutea were rare. They were found in histological preparations from only one pregnant adult female and one adult female that had aborted a few days before death. Both females also had pre-ovulatory follicles. Ovaries were smaller in juveniles than yearlings and adults, and largest in pregnant females.

Table 11: Number of juvenile, yearling and adult Zaedyus pichiy *in which follicles of different stages or corpora lutea were observed during histological analyses.*

Age class	Month	n	Follicles*			Corpora lutea*	Avr. ovary volume in mm^3
			Early secondary	Late secondary	Pre-ovulatory		
Juvenile	February	3	2	1	0	0	25.1
	March	5	5	4	0	0	28.6
Yearling	February	6	5	4	1	0	34.4
	March	10	7	6	1	0	32.9
Adult	February	10	8	8	1	0	36.1
	March	23	20	12	0	0	33.9 (n=21)
	May	1	1	0	0	0	n/m
	October	1	0	1	1	1	n/m
	November	2	1	2	2	1	47.9

n/m = not measured.

* Indicates number of females in which a certain structure was observed

Almost all lactating females and two thirds of all non-lactating subadult or adult females presented early secondary follicles (Table 12). Late secondary follicles were present in approximately two thirds of the lactating females, half of the non-lactating females, and in the two pichis that were pregnant or had recently aborted (Table 12). Pre-ovulatory follicles were found in only a few lactating and non-lactating individuals and in both pregnant females. Polyovular follicles were present in 4 of 8 juveniles, 5 of 16 yearlings, and 7 of 36 adults.

Table 12: Number of lactating, non-lactating and pregnant Zaedyus pichiy *carrying follicles of different stages or corpora lutea. Only yearlings and adults are included.*

Reproductive status	n	Follicles*			Corpora lutea*
		Early secondary	Late secondary	Pre-ovulatory	
Lactating	24	22	16	1	0
Not lactating	29	22	16	3	0
Pregnant **	2	0	2	2	2

* Indicates number of females in which a certain structure was observed

** 1 female aborted days before death

Discussion

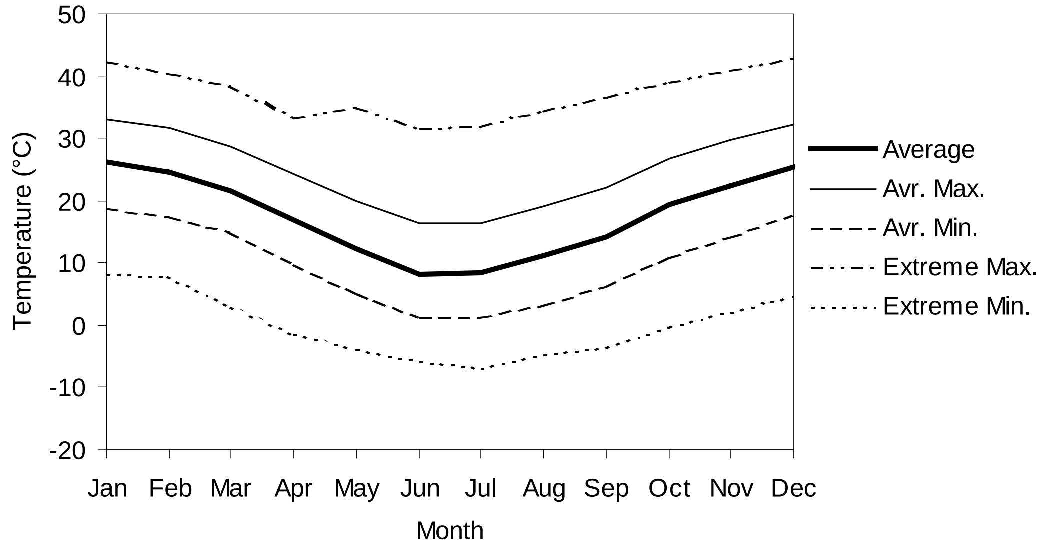
Most captive pichis reached sexual maturity at 9 to 10 months of age, but some females did not reproduce until their second year. Mounting was observed between September and December. Births of 1 to 2 offspring occurred after a gestation period of approximately 60 days; according to locals, litters of three offspring are also possible. These observations are consistent with those of Redford and Eisenberg (1992) and suggest that pichis are seasonal breeders that reproduce in the spring and have only one litter per year.

Seasonal reproduction

The reproductive season for male pichis from southern Mendoza was almost six weeks shorter than that in northern Mendoza. Fecal androgen metabolite levels of southern pichis started increasing around August 19 and returned to baseline around November 25, while testosterone levels of northern pichis were elevated from the beginning of August until mid-January (Figure 8). In animals from either part of the province, the reproductive season started at a photoperiod of 10 hours and 45 minutes after winter solstice and ended when 14 hours and 15 minutes of daylight were attained (Figure 9). The only difference between northern and southern animals was that the former concluded their reproductive season after summer solstice while the latter did so before summer solstice. In accordance with observations in other mammals (Kriegsfeld and Nelson 1999; Trainor et al. 2006), photoperiod seems to be an important cue regulating reproduction in pichis. Photoperiod reliably predicts environmental changes, such as temperature, precipitation, and food availability (Kriegsfeld and Nelson 1999; Trainor et al. 2006) and allows the animals to synchronize the energetically demanding reproductive period with appropriate environmental conditions.

Northern and southern pichis live in different habitat types and are exposed to different environmental conditions. The arid northern and eastern parts of the province belong to the Low Monte ecoregion (Olson et al. 2001), in which the dry climate and sandy soils favor the growth of the characteristic *monte* vegetation, consisting of small trees, shrubs and sparse pasture (Peralta de Galmarini and Martinez Carretero 1995). Although temperature extremes of 43 °C in summer, and -7 °C in winter have been recorded at the weather station closest to the desert of northern Mendoza (San Juan INTA, 31°37’ S, 68°32’ W, Figure 13a), maximum temperatures of up to 48 °C occur in the Mendoza desert. Summer rains constitute about half the average yearly precipitation of 150 mm. Southern Mendoza represents the northernmost tip of the Patagonian steppe (Olson et al. 2001). The combination of an arid climate and the poor volcanic soil, covered with sand of variable depth, is responsible for the sparse vegetation (Candia et al. 1993), characterized by grasslands with isolated shrubs, areas with open bush communities, and basaltic steps (Candia and Dalmasso 1995). The winter is much harsher in this part of the province than in the north, with snowfall and temperatures as low as -15 °C (Figure 13b). The average yearly precipitation of 320 mm consists of snowfall in winter and summer rains. The probability of survival of females and offspring (born late in the reproductive season) that did not gain sufficient weight and fat reserves prior to the onset of the hibernation season is therefore low. In contrast, milder environmental conditions during winter in northern Mendoza are expected to be correlated with higher food availability and reduced metabolic requirements for foraging and thermoregulation, thus increasing the chances of survival of late-born offspring and their mothers. In addition, southern males are already gonadally incompetent when the females wean their first litter after a gestation length of 60 days and 45 days of lactation (Figure 14). Fecal testosterone levels of northern males, however, are still above baseline when the first litter is weaned. As a consequence, the prolonged breeding season and the more favorable environmental conditions in northern Mendoza may allow northern pichis - but not southern ones – to produce two litters per year. This strategy would allow northern pichis to maximize their reproductive output under optimum conditions. It may, however, be detrimental to offspring and females if the second lactation is followed by a harsh winter because the interval between weaning and the initiation of hibernation (less than 6 weeks, Figure 14) would not allow them to accumulate enough energy reserves to survive the cold period.

Figure 13: Twenty-year averages of maximum and minimum ambient temperature, as well as monthly temperature extremes, in a) San Juan (31°37' S / 68°32' W, north of northern Mendoza) and b) Malargüe (35°30' S / 69°35' W, southern Mendoza), Argentina.



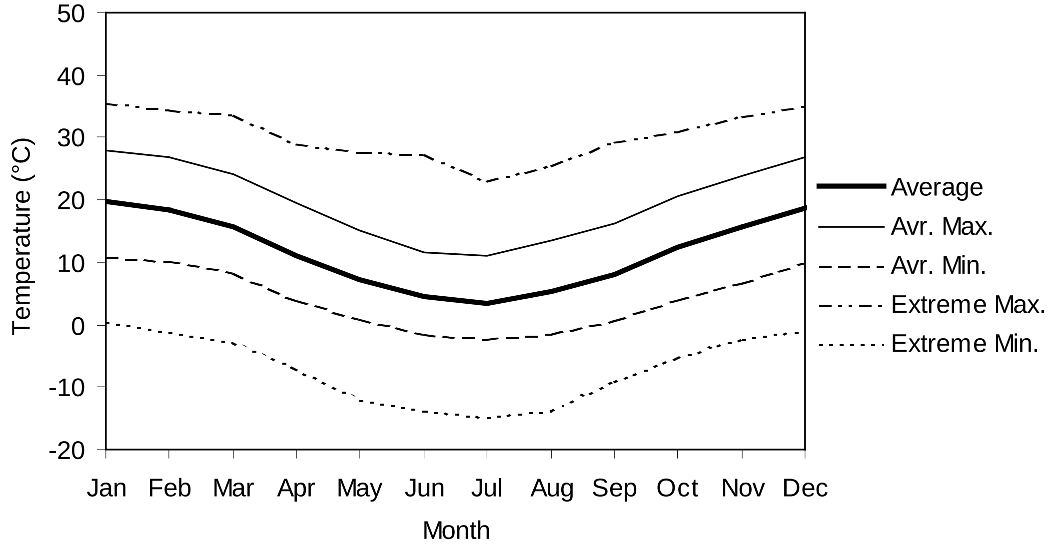
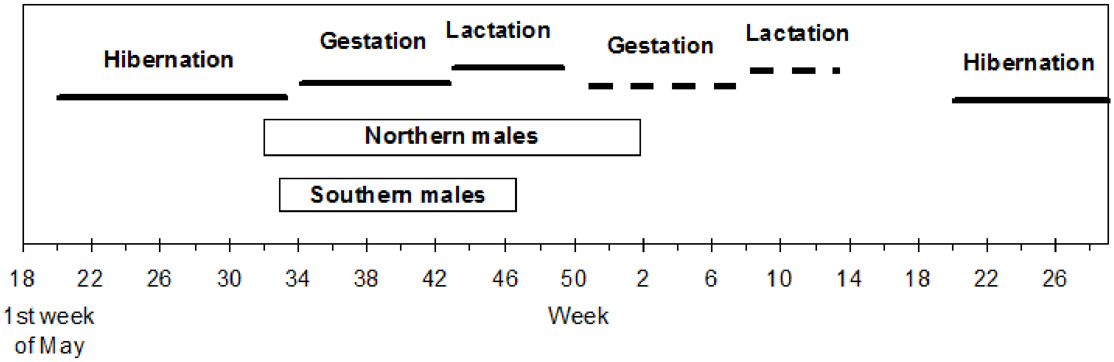


*Figure 14: Reproductive season length in pichis (*Zaedyus pichiy*) from northern and southern Mendoza and its relation to hibernation season, gestation, and lactation.*



Interestingly, the difference in the length of the reproductive season between animals originating from the north or from the south persisted during the two-year study period although they were kept at the same latitude and under similar environmental conditions. It should be noted that only one male (ZP146, year 2005) from the northern population could be sampled from week 22 to 33. The exact start of the reproductive season of northern pichis could therefore vary from the reported date (beginning of August). An increased testis size was registered in male ZP146 on August 17, 2006 (week 33). This finding suggests that the animal had entered reproductive season in a similar period of time as reported above, thus partially confirming the beginning of the reproductive season in northern populations. In any case, an earlier or later initiation of the reproductive season would not change the main conclusion that the reproductive season of northern males extends into the summer months. Variation in reproductive season length along a latitudinal gradient has been observed in mice of the genus *Peromyscus*, in deer of the genus *Odocoileus,* and in lagomorphs (Bronson 1985). In these three groups, as well as in pichis, longer reproductive seasons occur for populations living at lower latitudes. The difference in reproductive season length observed in pichis may therefore represent a genetic divergence caused by selective adaptation to different environmental conditions.

Changes in size and weight can be observed in the testes and ovaries of seasonally breeding mammals, since the reproductive organs are larger and heavier during the reproductive season (Janse van Rensburg et al. 2002). Adult males of many species of seasonally breeding mammals, such as roe deer (*Capreolus capreolus*) or fallow deer (*Dama dama*), show circa-annual cycles of testicular involution and recrudescence, accompanied by temporary arrest or activation of spermatogenesis (Goeritz et al. 2003; Gosch and Fischer 1989). Increased testis size during the reproductive season is caused by structural modifications of the gonads and enhanced production of spermatozoa (Goeritz et al. 2003). As expected, largest testes and highest SI scores were observed in November (Table 7). Although males characteristically achieve gonadal competence earlier and terminate it later than females (Bronson 1985), it was surprising to find active spermatogenesis and mature spermatocytes in the epididymides of males sampled in February and March. A successful fertilization at this time of the year would result in offspring being born in April or May, i.e., when adult pichis usually initiate hibernation (see Chapter 2). A closer look at the data revealed that the male with the largest testes and highest SI scores sampled in February (ZP95) had been caught in the wild in northern Mendoza and died in captivity after intense rainfall had flooded its enclosure. Two weeks prior to death, its fecal testosterone levels were around baseline; they increased slightly and reached 188 ng/g the day before its death. Two potential explanations for the late gonadal activity of ZP95 arise from these findings: First, testosterone levels are elevated over a longer period of time in pichis from northern Mendoza. Consequently, their spermatogenic activity is expected to be prolonged in comparison with southern pichis. Furthermore, viable spermatozoa have been found in the epididymides of androgen-deprived monkeys up to a month after administration of a GnRH antagonist (Yeung et al. 1999), and presence of spermatozoa in the epididymis of ZP95 four weeks after the end of the reproductive season is therefore plausible. Second, its maintenance in captive conditions and in proximity to females could have led to a prolonged gonadal competence. What speaks against the latter, is the fact that five other captive males (two from northern and three from southern Mendoza) that died the same day as ZP95 due to flooding of their enclosures had low testosterone levels and/or low SI scores and no spermatocytes in their epididymides, in contrast to ZP95 which had abundant spermatocytes. Neither captive conditions nor the prolonged reproductive season in northern Mendoza can explain the average SI score of 4.0 and abundant spermatocytes in the epididymis of an adult wild pichi from southern Mendoza that was sampled on March 28. Presumably, this male, as well as the captive ZP95, represent what could be called “statistical outliers”, which help to maintain a degree of variability in reproductive season length in the wild populations and which could result in a selective advantage should environmental conditions change.

Induced ovulation?

In 2006, two females gave birth 58 and 59 days, respectively, after being paired with a male (ZP144) or after having lost the first litter (ZP153; Table 8). If gestation length in pichis is approximately 60 days, as mentioned by Redford and Eisenberg (1992), successful mating must have occurred shortly after pairing in these females. This would be possible if females had been paired close to ovulation or if pichis are induced ovulators. Several reasons suggest that the latter is the more likely possibility. First, males immediately initiated chasing sequences and mounting attempts whenever they were paired with a female during reproductive season. It is unlikely that pairings were, by chance, always carried out around ovulation in all cases. Second, pichis have solitary habits and presumably large home ranges. If ovulation in pichis is tied to mating, this maximizes the chances of a successful reproduction in case of an encounter between male and female individuals during reproductive season. And third, if ovulation is tied to mating, corpora lutea will almost exclusively be present in pregnant females (Boone et al. 2004; Sumar 1999). Indeed, no corpora lutea were observed in any non-pregnant pichi. It cannot be ruled out, however, that the absence of corpora lutea in the analyzed pichis is due to the low number of females that were sampled during breeding season or because pichis have a very short luteal phase. What speaks against this is the fact that fecal hormone analyses revealed no pattern in the variations of progestagen and estradiol levels that would indicate a regular ovarian cycle (Figure 12), in accordance with findings reported in other induced ovulators (Boone et al. 2004; Milligan 1982). The progesterone assay may not have cross-reacted with the (unknown) main fecal progesterone metabolite of pichis, which may have prevented detection of an increase in progestagen levels during the luteal phase of the estrus cycle. Nevertheless, if pichis presented regular, spontaneous ovulations, the pre-ovulatory estrogen peak would have been reflected in the fecal estradiol levels because estradiol-17β is usually excreted in its native form, as free estrone, and/or as 17α-estradiol, all of which are recognized by immunoassays that are specific for estradiol as well as assays that have been developed for determining total estrogens (Brown et al. 1997). A potential caveat is that few samples could be collected from unmated females during reproductive season and estrus cycles therefore may have remained undetected. This would require, however, that all females that later became pregnant were paired exactly before the first ovulation of the reproductive season, which is unlikely because pairings were performed at random dates. No estradiol peaks were observed after pairing that would indicate when ovulation occurred, but this may be the result of the irregular sampling schedule that depended on emergence and capture of the pichis. Confirmation of the hypothesis that pichis are induced ovulators requires a controlled experiment to study the hormone levels and ovulatory rates in two groups of females, one isolated from males and the other paired with males.

Pregnancy

Gestation lengths determined in this study are only an approximation because the exact dates of mating and parturition could not be determined visually and fecal samples were not collected every day. Parturition day was defined as the lowest progestagen measurement after a peak >400 ng/g dry feces based on the observations of one pregnant female (ZP144), which closed the burrow entrance on November 20, 2005 and did not emerge for two days. Based on the date of pairing and the emergence of two offspring 41 days later, it was concluded that female ZP144 had given birth inside the burrow on November 20. The low hormone levels in the fecal sample that had been collected the day of ZP144's immergence prior to delivery was decisive for setting Day 0 in other females as the day of lowest progestagen levels, although in giant anteaters (*Myrmecophaga tridactyla*), for instance, fecal progestagen and estradiol levels are highest on the day of parturition (Schauerte 2005). The same definition for Day 0 was used for females that lost their offspring. Although there is a high probability that ZP108 aborted due to stress (see below), it is not known whether the other females aborted in late pregnancy or gave birth at term and did not raise their offspring. In the former case, Day 0 would represent the day of abortion, while the day of parturition would have occurred an unknown period of time later. Hence, the significantly higher estradiol levels in females that did not raise their offspring probably do not reflect a physiological difference leading to the loss of the litter, but instead are related to the incorrectly defined Day 0.

It is not clear why fecal progestagen levels remained low during the first half of pregnancy. A similar pattern has been reported in giant anteaters (Patzl et al. 1998), but the authors did not give an explanation for their finding. It is possible that the main site of progesterone production shifts from the ovaries to the placenta and/or the adrenal glands around mid-pregnancy, leading to a massive increase in steroid hormone synthesis and elevated progestagen levels. It remains to be studied whether different metabolites may be produced from progesterone of ovarian, placental or adrenal origin. If this is the case, the cross-reactivity of the radioimmunoassay's antibodies to progesterone with the metabolites of ovarian, placental or adrenal progesterone may have differed.

Several factors may be responsible for the longer interval between pairing and parturition in other females (Figure 12): ZP144 and ZP153 were subadults in 2005 and might not have been receptive when first paired; other subadult females, such as ZP152 and ZP150, did not breed at all in their first year (Table 3). Similarly, ZP185 was less than a year old when first paired in 2006, but the interval between pairing and parturition was longer than in ZP144 and ZP153. Because males were not separated from the females during approximately 50 days, it is possible that ZP185, as well as ZP108 in 2006, aborted in the first weeks of pregnancy and successfully mated immediately afterwards. A confirmation of this hypothesis requires further research because progestagen levels remain low in the first half of pregnancy – as occurs, for example, in giant anteaters (Patzl et al. 1998) – and it is therefore not possible to determine whether these females conceived immediately after pairing, aborted, and conceived again, or whether they conceived for the first time two weeks after pairing.

Five females aborted or did not raise their offspring (Table 3). ZP154 presumably aborted after being stung by a venomous spider, while other abortions or stillbirths may have been related to stress. Captive pregnant or nursing armadillo females are highly susceptible to external disturbances (Superina et al. in press). In 2005, advanced pregnancy was diagnosed in ZP108 through ultrasound examination, but the female aborted shortly thereafter, possibly due to stress during transport and examination.

No stress factor or other cause of abortion could be identified in female ZP162, which in 2005 lost her litter in the last third of pregnancy. The hormone profile of female ZP104 suggests that she gave birth or aborted 45 days after being brought into captivity in November 2004. She did not have contact with males in captivity and therefore must have conceived prior to being captured in the wild. Considering the long interval between capture and abortion, it seems reasonable to assume that stress related to the transport and acclimatation to captive conditions can be excluded as the cause for the loss of her litter. It is, however, unknown whether abortion in the last third of pregnancy, stillbirth, or failure to nurse the offspring was responsible for the loss. Males of some armadillo species, such as *Tolypeutes matacus*, can react very aggressively to the presence of offspring. Juvenile mortality can therefore be up to 100% if males are not removed prior to parturition (Superina et al. in press). Infanticide by males or stress-related neglect of the offspring by females can occur, but do not seem to be the rule in captive pichis. ZP153 was not separated from the male prior to delivery in 2006, which may have caused the loss of her first litter. Immediately thereafter, she became pregnant again and gave birth to two offspring, which were raised successfully in presence of the male. Another female that was not sampled for this study gave birth and nursed her offspring in presence of the male. It should be noted, however, that the study animals were maintained in relatively large, open enclosures with abundant substrate for digging that allowed the pregnant or lactating females to retreat from males. It is possible that infanticides are more frequent in pichis kept in smaller enclosures, where females consider the presence of males or humans more of a threat to their offspring (Superina et al. in press).

The case of female ZP153 deserves further attention. She aborted or lost her offspring shortly after birth and gave birth to two healthy offspring 60 days later (Figure 10c, Figure 11c). This inter-birth interval corresponds to the gestation length, i.e., late secondary or pre-ovulatory follicles must have been present when the first litter was lost for immediate fertilization to have occurred. This would be possible if follicular growth were not inhibited in pregnant pichis. Indeed, late secondary and pre-antral follicles were present in the ovaries of two females that were pregnant at time of death (ZP97) or aborted only a few days before death (ZP154). In several species, such as cattle, sheep, horses, and camelids, cohorts of follicles develop synchronously in waves (Adams et al. 1990; Fortune 1994; Nagy et al. 2004; Souza et al. 1996).

Follicular waves and development of dominant follicles occur in early pregnancy of heifers and dairy cows (Ginther et al. 1989; Savio et al. 1990), mares (Allen 2001), camels (Elwishy et al. 1981), and llamas (Adams et al. 1990). Development of large pre-ovulatory follicles also occurs in pregnant guinea pigs, rabbits, and hamsters (Greenwald and Terranova 1988). Sustained development of follicular waves during pregnancy and presence of late secondary or pre-ovulatory follicles at the time of abortion in females ZP108, ZP185, and ZP153 would explain the rapid conception after loss of the first litter.

Lactation

Postpartum progestagen levels were significantly higher in lactating females than in females that aborted or did not raise their offspring (Figure 10). The reason for the elevated progestagen levels in lactating pichi females is not clear. Lactation is maintained through a species-dependent, complex interaction of prolactin, growth hormone, corticosteroids, thyroid hormones, insulin, and parathyroid hormone (Neville et al. 2002; Tucker 1988). Adrenal glands are enlarged in wild lactating pichis, suggesting an increased corticosteroid production during lactation. Presumably, because progesterone and pregnenolone are the precursors of all other steroid hormones (Sanderson 2006), an elevated corticosteroid synthesis to maintain lactation may result in a concomitant increase in progesterone levels due to overspill.

High serum progesterone levels have also been reported in lactating nine-banded armadillos (Peppler and Stone 1980), but the authors did not provide any explanation for their observation. In this species, serum progesterone levels are elevated during delayed implantation (Labhsetwar and Enders 1968), and implantation occurs even if ovariectomy is carried out during the delay period (Buchanan et al. 1956), suggesting an extra-ovarian origin of progesterone. In addition, captive nine-banded armadillos and, to a lesser extent, wild conspecifics submitted to environmental stress present elevated progesterone levels of adrenal origin (Rideout et al. 1985). It is therefore reasonable to assume that an increased release of adrenocorticotropin after a suckling stimulus not only induces the release of glucocorticoids for lactogenesis, but also causes the observed increased progesterone levels in lactating pichis. Corticosteroid analyses are planned to determine the extent of steroid hormone production in lactating versus non-lactating pichis.

Progestagen levels decreased sharply around the first emergence of the offspring. Weaning was initiated several days before first emergence with the females carrying food into the burrows, and juveniles were observed foraging and ingesting solid food when first above ground. The significant decline in progestagen therefore seems to be related to a reduction in suckling stimuli and milk production, supporting the hypothesis that increased progesterone levels during lactation are of adrenal origin and a by-product of corticosteroid production.

Elevated progesterone levels in non-pregnant, lactating Common hamsters have been attributed to matings during lactation (Franceschini et al. 2007). Serum progesterone levels of lactating Djungarian hamsters, however, were low even in the case of concurrent gestation and lactation (Roy and Wynne-Edwards 1995). This explanation can be excluded in pichis because most females in the present study were separated from males during lactation, and no mounting attempt or birth of a second litter was observed in the female that shared an enclosure with the male during lactation.

Some pichi females were not separated from the males prior to giving birth, while others were separated, then paired again after parturition. None of them conceived again, and no postpartum estradiol peak was observed, suggesting that pichis have a lactational anestrus related to the high progesterone levels during lactation. Follicular growth occurs in several species during lactation and follicular waves have been observed in lactating llamas (Sumar 1999), but high prolactin levels and altered LH and FSH pulse frequencies impair follicular development and prevent ovulation (Greenwald and Terranova 1988). It is therefore not surprising that late secondary follicles were found in a high percentage of lactating pichi females (Table 12). Two possible explanations arise for the observation of large pre-ovulatory follicles in only one lactating female. For example, in some cases follicular growth may be sustained until an advanced stage of development and the pre-ovulatory follicles will undergo atresia due to low LH and FSH levels. Alternatively, the female may have lost or weaned her offspring and the absence of suckling stimuli led to the removal of factors that had inhibited maturation of follicles and, potentially, ovulation.

The absence of corpora lutea in the ovaries of lactating females (Table 12) also argues against the occurrence of spontaneous postpartum ovulation and of an ovarian origin of the increased circulating progesterone during this stage.

One female (ZP185) was sampled daily after the second pairing. Several mounting attempts were observed; however, the absence of an estradiol peak (Figure 11a) and the fact that no signs of pregnancy or birth could be detected, suggest that ZP185 did not ovulate. It may seem unusual that mounting attempts occurred although the female was not receptive; nevertheless, they have also been observed in captive pregnant females and may be related to captive maintenance. Due to their solitary habits, encounters between wild male and female pichis are expected to be rare in absence of hormonal and pheromonal cues that would indicate female receptivity. Under captive conditions, however, males are constantly in close proximity to females and may therefore react even to low levels of pheromones.

Polyovular follicles

Polyovular development seems to be a natural polymorphism. Polyovular follicles were found in half of the juveniles, one third of the yearlings, and one fifth of all adults. Follicles containing more than one oocyte have been identified in many species; among others, they are relatively common in the domestic bitch (Telfer and Gosden 1987) and have been observed in the armadillos *C. vellerosus* and *Z. pichiy* (Codón and Casanave 2000). Consistent with earlier reports (Bodemer and Warnick 1961; Telfer and Gosden 1987), they were more frequent in immature ovaries than in the ovaries of adult pichis. Due to their low incidence, their contribution to the litter size is expected to be very low (Telfer and Gosden 1987), although Greenwood and Sargeant (1994) suggested that in striped skunks polyovular follicles may be responsible for the disparity between corpus luteum and embryo counts.

Follicular waves

Confirmation of the occurrence of follicular waves in pichis would require monitoring the growth of follicles over time by daily ultrasonography. The present study provides additional indirect evidence for the development of follicular waves in pichis, but it cannot be excluded that the emergence of ovulatory-sized follicles occurs randomly. Early and late secondary follicles were found in a large proportion of the studied females, independent of their age or the time of year in which they were sampled (Table 11). Similarly, pre-ovulatory follicles occurred during and outside the breeding season and independently of the female's reproductive status. The incidence of pre-ovulatory follicles is probably higher than is reflected in this study. Emergence of successive dominant follicles can occur on the ipsilateral or contralateral ovary (Vaughan et al. 2004), but the origin of the sampled animals (mainly poached, partially eviscerated pichis) did not allow collecting and analyzing both ovaries from all pichis and dominant follicles could have been present on the ovary that had not been sampled.

Could there be an ecological advantage related to the production of pre-ovulatory follicles during pregnancy and lactation? A high risk of abortion or neonatal mortality would increase the probability that females need to mate and ovulate again. Stress-induced abortions, as well as abandonment or injuries of neonates are common in captive armadillos (Superina et al. in press). In the wild, environmental stress, such as intense summer rains or stress related to predator attacks, may lead to loss of the offspring and require new matings. The offspring must be born as early as possible to maximize their chances of growing and putting on sufficient fat reserves before the hibernation season. Having a "backup follicle" in an advanced stage of development, i.e., a late secondary or pre-ovulatory follicle, is beneficial for the female because it allows her to conceive soon after losing her offspring. Studies in the mouse, rat, and hamster have shown that follicular growth from initial recruitment into the growing pool of follicles to ovulation take around 20 to 30 days, and up to 6 months in ewes, the first stages of growth being the slowest (Greenwald and Terranova 1988). Although follicle growth rates of pichis are unknown, even an interval of 15 days from abortion or loss of the newborn to the next ovulation could be a critical delay that impairs the chances of survival of the offspring and the mother. Alternatively, the formation of pre-ovulatory follicles during pregnancy may be necessary for the development of accessory corpora lutea, as occurs in mares (Allen 2001). The low number of pregnant females that could be evaluated histologically makes it difficult to confirm or refute this last possibility.

Anestrus

The histological findings suggest that in spite of being seasonal breeders, pichis present follicular growth during most, if not all of the year (Table 11). Because no samples could be obtained from the hibernation season, it cannot be confirmed whether follicular growth is sustained during that period. Growth and regression of follicular waves during anestrus are not uncommon in mammals showing this pattern of follicle development; their presence has been confirmed in ewes (Souza et al. 1996) and in mares in the second half of the anovulatory period (Donadeu and Ginther 2002). Watson et al. (2002) reported a small increase in estrogen levels related to the growth of large follicles during follicular waves in anestrous mares, but Donadeu and Ginther (2002) failed to detect an association between estradiol levels and growth of follicles and suggested that the fluctuations in estradiol levels may not have been of ovarian origin. The small estradiol fluctuations around baseline that were analyzed in three non-pregnant, non-lactating captive pichis showed a relatively regular pattern of 15, 16 or 21 days, and may be related to wavelike follicular growth (Figure 12). When the intervals between estradiol peaks of 7 females were pooled and classified according to the date at which they occurred, the difference between in-season (14.4±2.3 days, n=7) and off-season (20.7±1.4 days, n=15) intervals was significantly different (independent-samples t-test, $p<0.05$). Because samples were not collected every day, it is possible that not all estradiol peaks were detected and, for instance, an interval of 26 days actually represents two follicular waves of 13 days each. To minimize this error, periods with long sampling intervals were excluded from this analysis. Nevertheless, the difference in in-season versus off-season estradiol peak intervals is striking and may represent a delayed follicular growth outside breeding season.

The hormone fluctuations around baseline that were observed in fecal hormones of female pichis outside breeding season may be influenced by two potentially confounding factors, the sampling schedule and the daily variation of the diet fed to the study animals. The elusive character of pichis and their habit of defecating inside their burrows impeded the establishment of a regular sampling schedule. The samples were therefore obtained whenever the animals could be caught, i.e., sometimes at first emergence in the morning but in other occasions at noon or at dusk. Sousa and Ziegler (1998) found significant diurnal fluctuations in fecal progesterone levels of captive marmosets (*Callithrix jacchus*). The fluctuations observed in pichi fecal hormone levels could thus reflect circadian rhythms of fecal hormone metabolite excretion. The lack of reliable scientific information on the diet of wild pichis made it difficult, if not impossible, to develop a standardized, balanced artificial diet. Food composition therefore varied daily and seasonally. Because the dietary fiber content has been reported to influence hormone excretion (Wasser et al. 1993), it is possible that the fecal hormone levels in pichis also showed some level of variation due to the changing diet.

Conclusions

The present findings indicate that pichis are seasonal breeders that regulate reproduction through photoperiodic cues. Reproductive season is prolonged at lower latitudes, suggesting that the production of two yearly litters of 1 to 2 (exceptionally 3) offspring is possible in part of the area of distribution of *Z. pichiy*.

Several unexpected features were found in female pichis. Follicular growth seems to occur in a wave-like manner in all reproductive stages. The absence of any signs of a regular estrus cycle and the observation that during reproductive season, mounting attempts – and, in some cases, fertilization – occur immediately after pairing suggest that pichis are induced ovulators. Fecal progestagen levels were elevated only in the second half of pregnancy and, surprisingly, also during lactation. The latter may be of adrenal origin and related to high corticosteroid production for the maintenance of lactation.

Hormonal analyses indicate that fecal hormone level analysis is not a useful method to determine the proportion of pregnant females in wild pichi populations non-invasively because estradiol and progestagen levels are low both in non-pregnant, non-lactating females and in females in their first month of pregnancy and a large proportion of pregnant females could be overlooked. High progestagen and estradiol levels are indicative of females in their second half of pregnancy, while a combination of high progesterone and low estradiol levels represents lactating females.

CHAPTER 2: HIBERNATION AND DAILY TORPOR IN PICHIS (*ZAEDYUS PICHIY*)

Introduction

The pichi (*Zaedyus pichiy*) is an armadillo (Xenarthra, Dasypodidae) of a relatively small body size (ca. 1 kg) that inhabits arid and semi-arid habitats with firm sandy soils in central and southern Argentina and Chile (Wetzel 1985b), at altitudes up to 2500 m (Superina in press). Temperatures typically encountered in the area of distribution of pichis vary considerably along a latitudinal gradient. Average maximum and minimum temperatures in winter (July) range from 16 °C and 2 °C, respectively, in the northernmost part of their distribution (Mendoza; 32°50' S, 68°47' W) to 5 °C and -4 °C, respectively, in the southernmost part (Gobernador Gregores; 48°47' S, 70°10' W). Average maximum and minimum temperatures in summer (January) range from 32 °C and 18 °C, respectively, in the north to 21 °C and 8 °C, respectively, in the south (Servicio Meteorológico Nacional, Fuerza Aérea Argentina). Little is known about the biology of this solitary, diurnal species that has recently been classified as Near Threatened by the IUCN Red List of Threatened Species (Superina in press; Superina et al. 2006). Like other armadillos, pichis appear to be poorly equipped to cope with cold ambient temperatures (T_a), having low rates of heat production, a high thermal conductance resulting from a relatively thin fur limited mostly to their ventral surface, and body temperatures that are low, variable and highly sensitive to changes in ambient temperature (McNab 1980, 1985; Roig 1971b). Pichis have been described as carnivores-omnivores with a preference for carrion (Redford 1985), but recent observations suggest that they feed predominantly on invertebrates, grubs and plant material (see Chapter 3); these food resources are either absent or very scarce during winter. Therefore, the combination of low T_a and decreased food availability during winter potentially subjects these small, poorly insulated mammals with a low heat production capacity to a significant energetic challenge.

A common strategy in mammals to deal with energetic challenges is to enter a state of hypometabolism, either in the form of shallow daily torpor or prolonged and deep seasonal hibernation (Körtner and Geiser 2000). Hibernation and daily torpor occur in a wide variety of mammalian and avian taxa (Geiser 1998; Heldmaier et al. 2004), but other than a brief description by Roig (1971b) of daily torpor of captive armadillos presumed to be *Z. pichiy* (see discussion), there is no information about torpor or hibernation in any Xenarthra species available (Geiser 1998).

The objective of this study was therefore to describe the patterns of body temperature changes in captive pichis kept in outdoor enclosures and subjected to natural light cycles and variations in T_a during the winter to determine whether pichis enter shallow daily torpor or prolonged deep hibernation.

Materials and Methods

The body temperature of four wild-born, captive-kept male pichis (*Zaedyus pichiy*) was monitored from April 2006 until August to October 2006 depending on the individual. All animals were adults at the onset of the study. Two animals (ZP155 and ZP160) were captured in southern Mendoza Province, Argentina (36° S, 69° W), 9 and 6 months prior to the onset of the study; two individuals (ZP142 and ZP158) were confiscated from poachers in southern Mendoza 14 and 10 months prior to the onset of the study (Table 13).

Table 13: Origin of the study animals, their capture or confiscation date, and dates of implantation and removal of the data loggers

Male	Origin	Capture date	Implantation date	Removal date
ZP142	Confiscated	27-Feb-05	11-Apr-06	(found dead 25-Aug-06)
ZP155	Wild-caught	1-May-05	21-Apr-06	26-Sept-06
ZP158	Confiscated	18-Jun-05	18-Apr-06	1-Dec-06
ZP160	Wild-caught	28-Aug-05	21-Apr-06	15-Aug-06

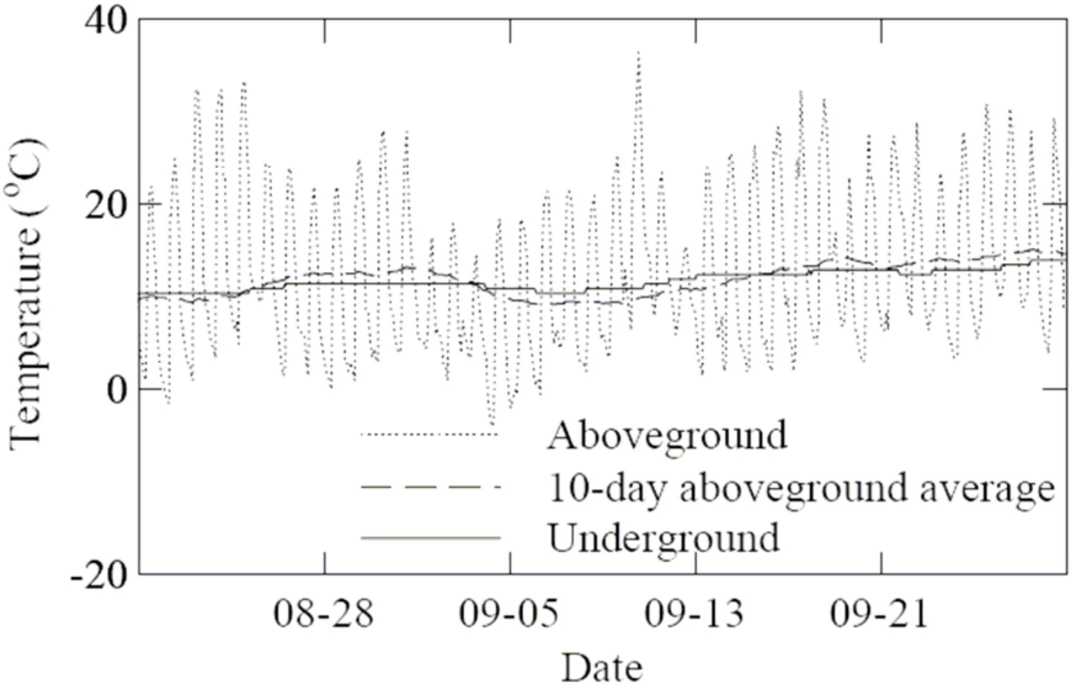
Pichis were kept in individual pens made of wire mesh and sheet metal, of 2 m x 1.5 m x 2.5 m each; soil to a depth of 2 m provided a natural substrate for digging burrows. For the duration of the experimental period, food, consisting of a varying mixture of fruits, vegetables, meat, dry cat food, rice, and a vitamin-mineral supplement, was offered once daily; water was provided *ad libitum*.

Subcutaneous temperature (T_{sc}) was measured using temperature data loggers (Thermochron iButton, Maxim Integrated Products Inc., Sunnyvale, CA; resolution 0.5 °C, accuracy 1 °C) implanted into subcutaneous tissue under the carapace border posterior to the right front leg. Data loggers were programmed to register temperature every two hours. At the time of data logger implantation (between April 11 and April 21, 2006), the body mass of the individuals was 1128±101 g (range 1050 to 1270 g). Animals were pre-medicated with 0.02 $mg \cdot kg^{-1}$ atropine; anesthesia was induced with 8% sevoflurane and maintained at 3-4%. The implantation site was infiltrated with lidocaine prior to incision, the sterilized data logger inserted, and the skin sutured. Enclosures were checked daily for loose soil in burrow entrances as a sign of emergence. One animal (ZP142) was found dead in its burrow at 80 cm depth on August 25. The other data loggers were extracted surgically between August 15 and December 1, 2006. At the time of data logger removal, the body mass of the individuals was 967±289 g (range 800 to 1300 g). Prior to the surgical removal of the implants, rectal temperature was measured with a digital thermometer (TES Electrical Electronic Corp., Taipei, Taiwan) at a depth of two to three centimeters, at exactly the same moment as a scheduled data logging event to compare rectal temperature and T_{sc}. On average, rectal temperature measurements were 1.0±0.3 °C higher than T_{sc} measured with the implants. The T_{sc} reported here is therefore a biased estimate of body temperature, and we cannot exclude the possibility that measurements were affected by surface contact with substrate or sun exposure.

Burrow temperature was not measured for the whole duration of the study. However, a data logger was placed 1 m above ground near the enclosures to register aboveground temperature every two hours. From mid-August to late September 2006, an additional data logger was buried at the typical depth of burrows (80 cm). The temperature measured by this data logger was significantly correlated ($r=0.88$, $P<0.001$, Pearson correlation) to the average of aboveground temperatures measured during the previous 10 days; this 10-day average of aboveground temperature was used as an estimate of burrow temperature (Figure 15).

Statistical analyses were performed using Systat 8.0 (Systat Software Inc., San Jose, Calif., USA). Values are presented as mean ± SD.

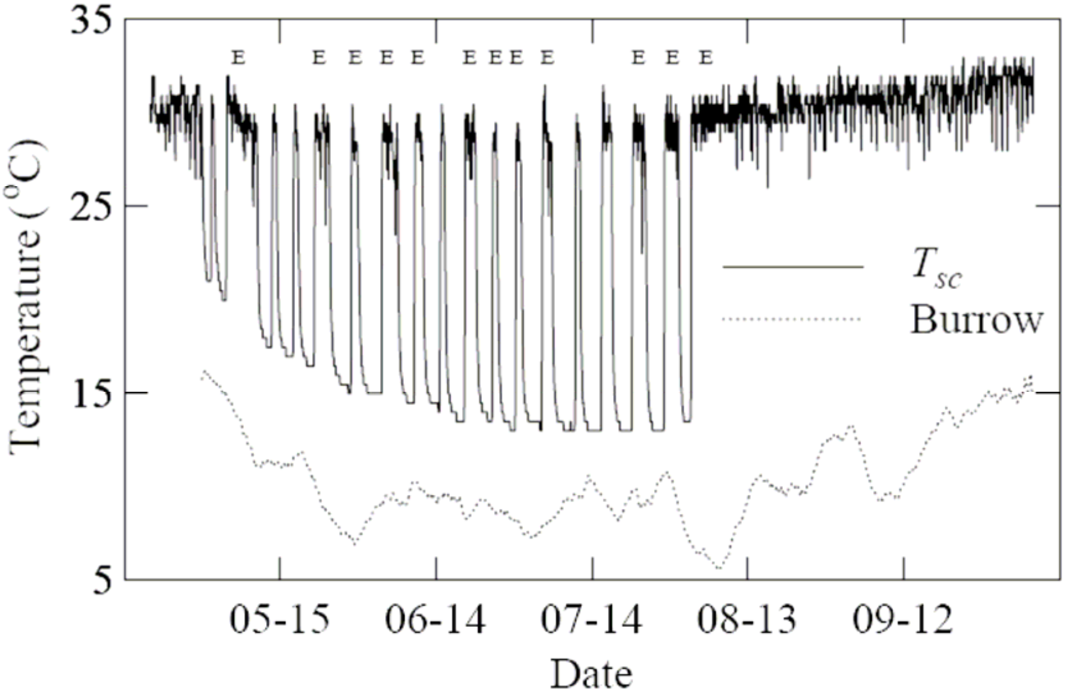
Figure 15: Temperature measured with data loggers placed 1 m above ground and 0.8 m below ground. The average temperature measured above ground for the previous 10 days was correlated (r=0.88, P<0.001) to temperature measured below ground and was used as an estimate of burrow temperature.



Results

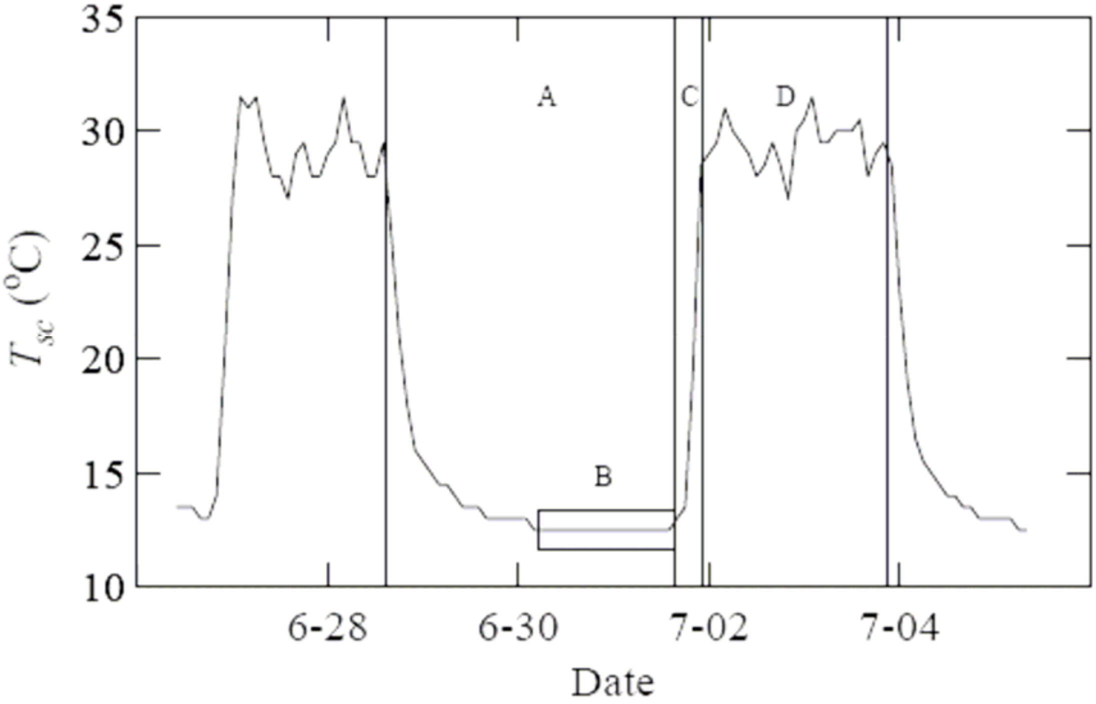
During the winter months (April to August 2006), all individuals exhibited changes in T_{sc} that are typical of body temperature changes that occur in hibernating mammals, consisting of extended periods of torpor interrupted by periods of euthermia (Figure 16). The behavior of the animals during periods of euthermia was variable: They emerged from their burrows 58% of the time, and animals that emerged usually ate some of the provided food. Entry into hibernation, defined as the start of the first torpor event when T_{sc} dropped below 25°C and lasted for at least 24 hours, occurred between April 24 and 29 of 2006. Hibernation lasted 73-95 days, with the last arousal from such torpor events occurring between July 06 and August 01, 2006. One individual (ZP142) never emerged after July 14 and was found dead in its burrow on August 25. Based on T_{sc} measured by its data logger, it died during hibernation on July 14.

Figure 16: Pattern of changes in subcutaneous temperature (T_{sc}*) of* Zaedyus pichiy *(ZP158) during five months of 2006. Burrow temperature is an estimate calculated as the average temperature measured 1 m aboveground for the previous 10 days. E = Inter-torpor periods during which the animal emerged*



Torpor events (Figure 17) lasted an average of 75±20 hr (range 20-112 hr), during which T_{sc} stabilized to an average of 14.6±2.1°C (range 12.5-21 °C). The duration of arousal events, calculated as the time between when T_{sc} first started to rise until mean euthermic temperature was reached, lasted 10.2±1.9 hr (range 8-14 hr). Maximal rewarming rates, calculated as the fastest rate of increase in T_{sc} between three observations (4 hr) during arousal events, were 0.055±0.0069 $°C \cdot min^{-1}$ (range 0.042 to 0.067 $°C \cdot min^{-1}$). Periods of euthermia that interrupted periods of torpor lasted 44±38 hr (range 6 to 192 hr) during which the average T_{sc} was 29.1±0.7 °C (range 27.2 to 30.6 °C).

Figure 17: Typical subcutaneous temperature (T_{sc}*) changes of a* Zaedyus pichiy *(ZP160) over 10 days during hibernation. Area A = duration of the torpor event; Area B = time during which torpid subcutaneous temperature was measured; Area C = arousal period; Area D = inter-torpor period of euthermia.*



Torpid T_{sc} changed during the course of the hibernation season (Figure 16, Figure 18), decreasing as estimated burrow temperature decreased, with torpid T_{sc} being on average 4.8 °C above estimated burrow temperature. Closer examination of the relationship between torpid T_{sc} and estimated burrow temperature (Figure 19) revealed that at an estimated burrow temperature of 10 °C and below, torpid T_{sc} stopped decreasing and stabilized to an average of 13.5±0.9 °C (range 12-15.5 °C).

Figure 18: Changes in minimal subcutaneous temperature (T_{sc}*) during torpor of four pichis, and in estimated burrow temperature, during the hibernation season. Burrow temperature is an estimate calculated as the average temperature measured 1 m aboveground for the previous 10 days.*

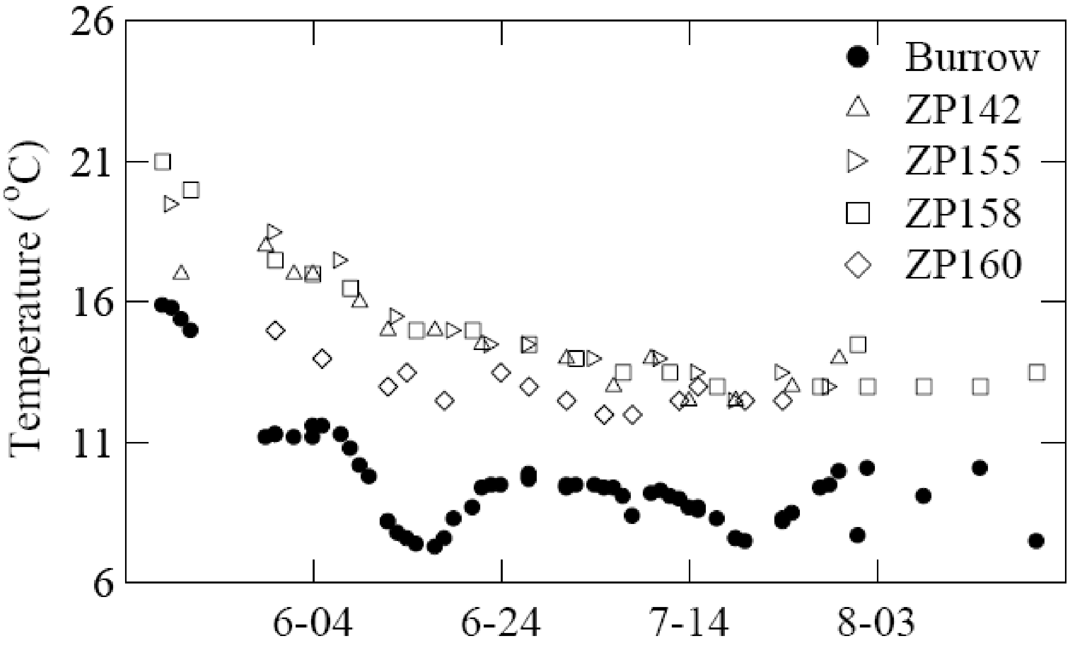
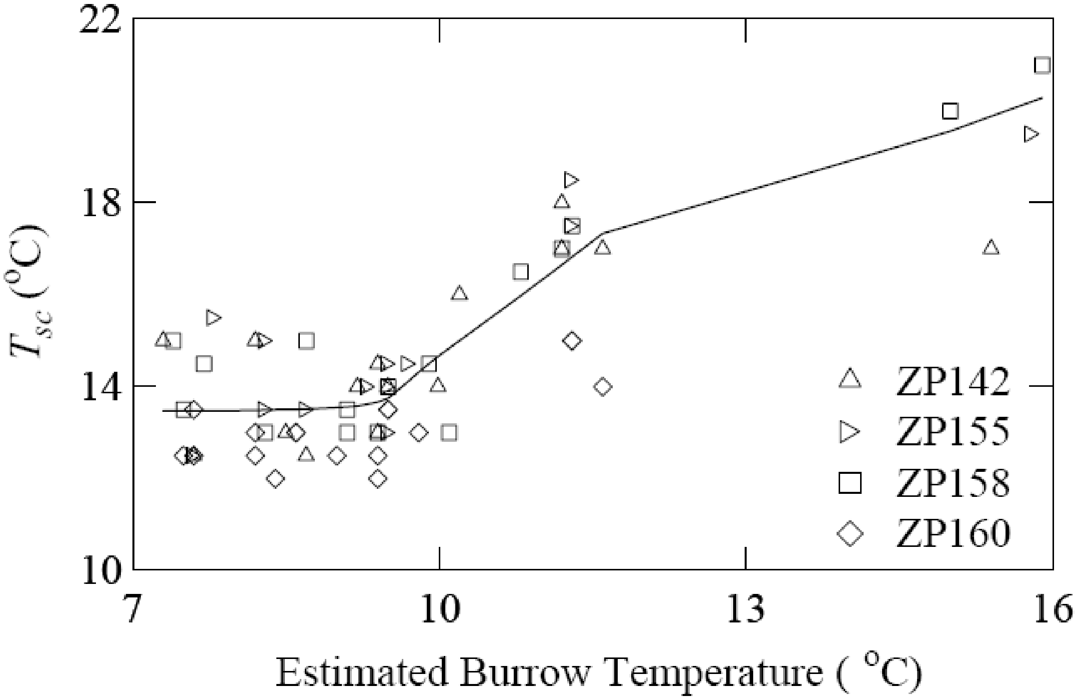


Figure 19: Relationship between torpid subcutaneous temperature (T_{sc}*) in four pichis and burrow temperature, estimated as the average temperature measured 1 m aboveground for the previous 10 days. Line smoothed using the LOWESS function (tension set at 0.85) of Systat.*



During the weeks that followed the hibernation season, T_{sc} was highly variable but increased linearly over time for all three individuals at an average rate of 0.03 °C per day (Figure 20). During the same time period, T_{sc} was also correlated (Pearson correlation) to estimated burrow temperature (ZP155: r=0.39, p<0.001; ZP158: r=0.33, p<0.001; ZP160: r=0.11, p=0.02). Part of the post-hibernation variability in T_{sc} could be attributed to daily torpor, when T_{sc} typically dropped from 30 to 31 °C to below 30 °C (sometimes as low as 24.5 °C) for 4 to 6 hrs. The timing of torpor events was highly variable (Figure 21), occurring consistently at night in one individual (ZP158) but at variable times for two other males. For each individual, date-adjusted T_{sc} were calculated (Packard and Boardman 1987) by adding the individual's mean post-hibernation T_{sc} to the residual of the individual's regression of post-hibernation T_{sc} as a function of date (in Julian days). Similar to the timing in the occurrence of torpor, date-adjusted T_{sc} varied inconsistently according to time of day (Figure 21), with only one individual exhibiting a distinct pattern of highest and least variable temperatures during midday (the higher variability at night was related to the daily torpor).

Figure 20: Changes in subcutaneous temperature (T_{sc}*) of three pichis in the weeks following hibernation. The range of dates differs between individuals, reflecting the difference in the timing of the end of the hibernation season and of the removal of implanted data loggers. Solid lines fitted by linear regression analysis (ZP155:* $R^2=0.30$*,* $p<0.001$*, slope=0.038; ZP158:* $R^2=0.22$*,* $p<0.001$*, slope=0.028; ZP160:* $R^2=0.02$*,* $p=0.021$*, slope=0.015).*

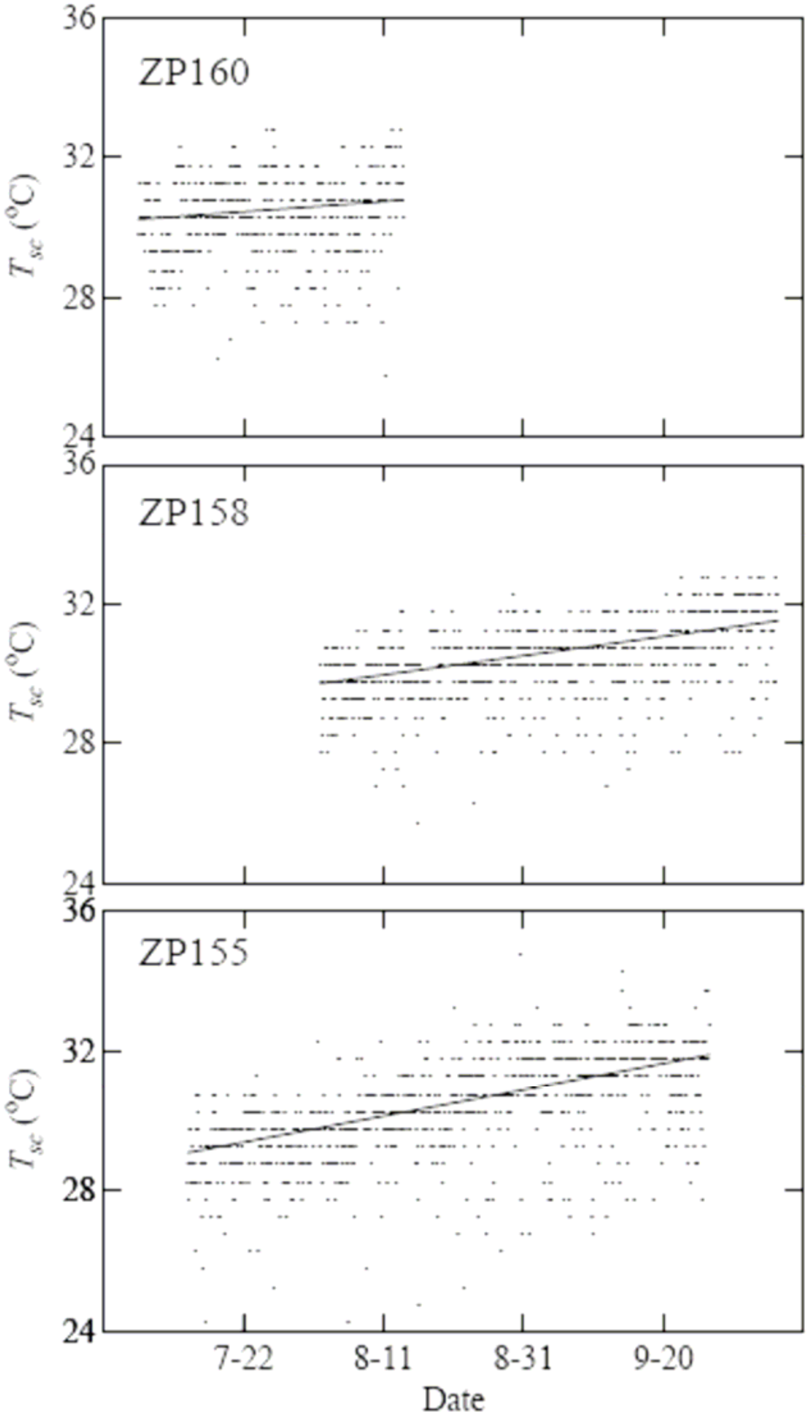
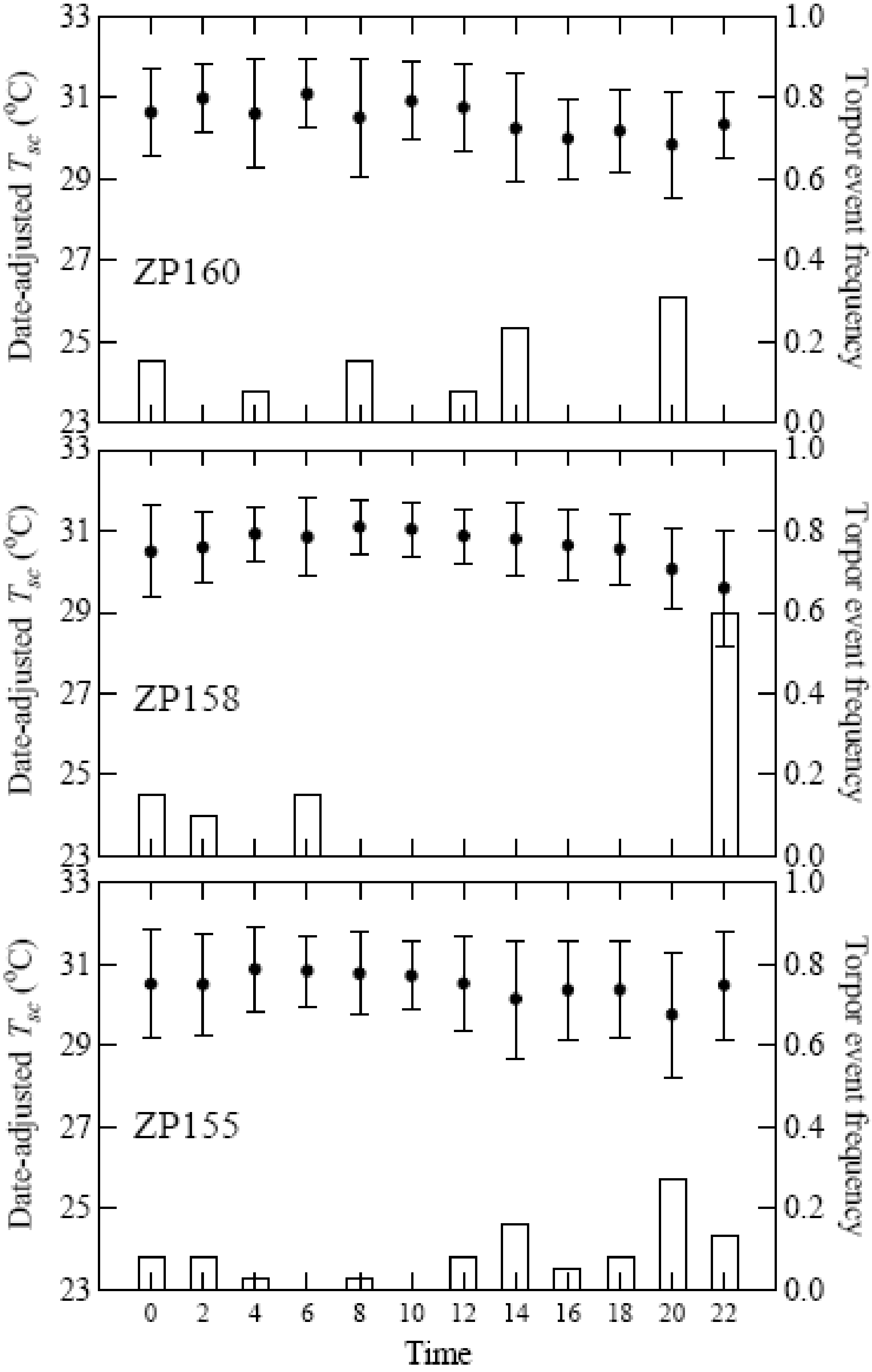


Figure 21: Circadian changes in date-adjusted subcutaneous temperature (T_{sc}; solid dots, mean ± SD) and in the relative frequency of torpor events (bars).



Discussion

Our results show that *Zaedyus pichiy* is a true hibernator, exhibiting extended periods of torpor interrupted by periods of euthermia that are typical of all mammalian hibernators. The present report is the first documentation of hibernation and the first detailed description of torpor in a xenarthran. Roig (1971b) reported that *Z. pichiy* can reduce its body temperature to 22 °C during resting periods, but his temperature measurements did not exceed 24 hours per individual and may have involved at least some individuals of another armadillo species (see below). Hatcher (1903), on the other hand, mentions that a little armadillo inhabiting Patagonia enters hibernation, but this report seems to be anecdotal and is rather confusing. The study area and the fact that the carapace of the observed armadillo had a serrated edge suggest that the author was indeed referring to *Z. pichiy*. Nevertheless, he classifies the animal as *Tatusia hybrida*, a scientific name that was previously used for *Dasypus hybridus* but not for *Z. pichiy*, and mentions that *Tatusia* rolls up into a complete ball, a characteristic that is exclusive for armadillos of the genus *Tolypeutes.*

Hibernation in mammals typically results in energy savings of about 90% energy as compared to normothermia (Heldmaier et al. 2004). For pichis, entering hibernation is probably critical to the winter survival of these small, poorly insulated mammals with a low thermogenic capacity, by reducing the energetic challenge imposed by periods of low T_a combined with reduced availability of their main food item (soil invertebrates). Our observation of hibernation in captive individuals is consistent with the observations that sightings of wild individuals in the field are rare during winter and limited to juveniles in poor body condition.

All our captive pichis entered hibernation despite being provided with food and water *ad libitum*, but their periods of euthermia were more variable and torpor events were shorter than in other mammals of similar mass (French 1985). The pichis did not always emerge during periodic arousals and often ate when aboveground during these euthermic intervals. Periodic emergence and foraging is common in various insectivorous hibernators, such as hedgehogs, echidnas, and bats (Körtner and Geiser 2000), whereas woodchucks (*Marmota monax*) do not feed during euthermia and do not return to torpor if provided with food during arousal times (Davis 1967).

Food hoard-supplemented eastern chipmunks (*Tamias striatus*) spent almost twice as much time in euthermia than controls and had higher minimum body temperatures (Humphries et al. 2003). Considering that sightings of wild pichis during hibernation season are rare and their food availability is low during winter, it is possible that the captive animals spent more time in euthermia than their wild conspecifics because food was available during arousals.

Dates of entry into hibernation were relatively consistent and occurred approximately two weeks after the autumnal equinox, but hibernation season length varied considerably among individuals. The first animal concluded its last torpor event about two weeks after winter solstice, while the last individual emerged almost a month later. It is possible that the date of entry into hibernation of free-ranging individuals differs from the initiation of hibernation determined in our study animals because the latter were provided with food *ad libitum*. Emergence of the last individual coincided with the initiation of reproductive season. Mating soon after emergence is essential to allow the offspring to grow to subadult size and deposit enough fat prior to immergence into hibernation, and to ensure that females recover from the metabolically challenging lactation period and increase their fat deposits before the next hibernation season.

Overall, the changes in T_{sc} during torpor events in pichis followed a pattern that is typical for hibernators, consisting of a gradual decrease in body temperature, the maintenance of a relatively stable minimum temperature for a variable length of time, and an arousal during which body temperature rapidly increases to euthermia (Heldmaier et al. 2004). Torpor depth increased gradually and followed the reduction in daily average T_a, but only to a minimal T_{sc} of, on average, 13.5 °C. Further reduction in T_a probably led to a stimulation of thermoregulatory heat production to prevent body temperature from falling below the threshold, as it has been described in other species (Heldmaier et al. 2004). Estimates of maximal rewarming rates are only approximate due to the relatively low accuracy of the data logger and the long time interval between measurements. Rewarming rates in mammals are inversely related to body mass (Geiser and Baudinette 1990), but the low number of hibernators of similar body mass (Heldmaier et al. 2004) makes a comparison of rewarming rates in pichis and other species difficult. In addition, xenarthrans have lower basal metabolic rates and lower body temperatures during euthermia than other mammals (McNab 1985). Nevertheless, pichis appear to be similar to large insectivores in their rates of rewarming (Geiser and Baudinette 1990), which is a reasonable comparison as both have similarly low metabolic rates compared to other eutherian mammals (McNab 1986).

All xenarthrans have relatively low and variable body temperatures during normothermia (McNab 1985). The present study revealed substantial diurnal variation in T_{sc} during the post-hibernation period. Daily reductions in T_{sc} to below 30 °C, sometimes to 24.5 °C, suggest that pichis not only hibernate but also enter daily torpor. The timing of maximum daily temperature was highly variable. In one male (ZP158), T_{sc} was usually, but not always, highest around noon, and this animal entered torpor consistently at night, as expected from the pichi's diurnal habits. Other males, however, sometimes presented highest T_{sc} at night and sometimes during the day. Similarly, they were torpid at variable times of the day. Because the data logger was implanted in the subcutaneous tissue, it is possible that some of the variability in the temperature measurements was caused by contact of the implantation site with the soil or by exposure to sunlight. Also, the fact that the study animals were maintained in semi-captive conditions may have influenced their activity patterns. For instance, some pichis regularly emerged soon after food was placed in their enclosures, but this timing may be different to the period of day during which they would forage in the wild.

Roig (1971b) described a pattern of change in body temperature in captive pichis kept at a constant T_a of 15 °C with highest body temperature occurring around midnight and the lowest around noon. The body mass of the animals studied by Roig (1971b) varied between 1.25 and 2.35 kg, whereas the largest body mass we observed in > 200 captive and free-ranging pichis was 1.5 kg (Superina, unpublished data). Perhaps, some of the animals used by Roig were not *Z. pichiy*, but another species occurring in the same area, such as *Chaetophractus villosus*, a species that is most active at dusk and presents its highest body temperature in the evening (Casanave and Affanni 1994). If this is the case, then torpor may be present in other Xenarthra species than the pichi.

The variability in their daily temperature patterns leads us to think that T_{sc} may vary depending on changing activity levels related to the reproductive season or increased foraging activity. Because testosterone levels start increasing around the end of hibernation season, it is also possible that the increase in T_{sc} is related to hormonal changes related to the reproductive season. Nevertheless, the observed gradual increase in T_{sc} after hibernation may also reflect, to a certain degree, the steady increase in T_a.

In conclusion, our observations are consistent with the standard response of hibernating mammals and suggest that *Z. pichiy* enters hibernation during winter months and is capable of entering daily torpor outside hibernation season. The present study represents the second description of a South American hibernator; although presumed to occur in a wide variety of South American mammals, hibernation has only been described in one South American mammal, the marsupial *Dromiciops gliroides* (Bozinovic et al. 2004). Daily torpor has been reported from four South American rodent species (*Phyllotus darwini rupestris*, Bozinovic and Marquet 1991; *Eligmodontia typus*, Bozinovic and Rosenmann 1988a; *Calomys musculinus*, Bozinovic and Rosenmann 1988b; *Calomys venustus*, Caviedes-Vidal et al. 1990), the mouse-opposum *Thylamys elegans* (Bozinovic et al. 2005), the marsupial *Marmosa elegans* (Roig 1971a), and from the insectivorous bat *Myotis chiloensis* (Bozinovic et al. 1985). Therefore, as suggested by Heldmaier et al. (2004), hibernation and daily torpor may be more common than previously thought.

CHAPTER 3: DIET OF THE PICHI *ZAEDYUS PICHIY* (XENARTHRA: DASYPODIDAE) IN MENDOZA PROVINCE, ARGENTINA

Introduction

The family *Dasypodidae* includes 21 extant armadillo species that show a remarkable range in size, area of distribution, and food habits (Fonseca and Aguiar 2004; Redford 1985). In his review on the diet of wild armadillos, Redford (1985) broadly classified the nine genera into three main groups: Generalized carnivores-omnivores (*Euphractus*, *Chaetophractus*, *Zaedyus*), generalist insectivores (*Chlamyphorus*, *Calyptophractus*, *Dasypus*), and specialist insectivores (*Priodontes*, *Cabassous*, *Tolypeutes*). Nevertheless, Redford (1985) emphasized that his classification only considered the “modal” food habits of armadillos and that the diet of most species could vary temporally and geographically. Later studies indicated that *Tolypeutes* fed on a broader variety of food items than initially thought and would best be described as an opportunistic insectivore (Bolkovic et al. 1995). It is possible that further research will lead to additional changes in the classification of armadillos.

The pichi (*Zaedyus pichiy*) is a small (approx. 1 kg) armadillo that lives in arid and semi-arid habitats. It occurs farther south than any other armadillo species, inhabiting firm sandy grounds on open lands from the provinces of Mendoza, San Luis and Buenos Aires, south to the Santa Cruz River in Argentina, and south to the Strait of Magellan in Chile, at altitudes up to 2500 m (Meritt and Benirschke 1973; Superina in press; Wetzel 1985b). Scientific information about this diurnal, semi-fossorial species is scarce, but recent studies have started to shed some light on its ecology (Superina in press). Pichis are the only known armadillos that hibernate during winter and can enter daily torpor outside hibernation season (see Chapter 2). The size and density of pichi populations, as well as the home range of these solitary armadillos, is not known in any part of their current range. Pichis are heavily poached and used as a protein source in large parts of their range (Fonseca and Aguiar 2004; Superina in press). This depletion of wild populations has recently led to the inclusion of *Z. pichiy* in the IUCN Red List of Threatened Species, where it is listed as Near Threatened (Superina et al. 2006).

Pichis have been classified by Redford (1985) as carnivores-omnivores that "probably eat whatever they can whenever they can" and show a seasonal variation in their diet composition. Although their diet also includes plant roots and tubers, it has been suggested that they forage on the insects parasitizing the roots and not the plant itself (Redford 1985). In 1833, Darwin documented beetle larvae, roots and a reptile in the stomach of a pichi (Keynes 2000). Krieg (1961) described the pichi as an omnivore with a preference for a carnivore diet and mentioned that it likes carrion, but prefers ant larvae and pupae as well as subterranean termite nests. This predilection for insects has also been observed by Meritt (1973, cited in Redford 1985), who documented a pichi that consumed tarantula spiders as well as soft-bodied grubs, but also vegetable matter, and Krumbiegel (1940), who identified several ant species, a spider, larvae of the genus *Euceticus* (Lepidoptera: Psychidae), the stomach of a small unidentified mammal, and a large amount of sand in the stomach of a wild pichi. Surprisingly, the latter also contained larvae of the genus *Macronema* (Trichoptera: Hydropsychidae), suggesting that the animal had been foraging near a stream or a pond although pichis are usually restricted to dry areas and avoid humid environments. Because these studies were based on occasional observations of very few individuals (usually one), we considered it necessary to evaluate the diet of a larger number of pichis to test the hypothesis that pichis are carnivores-omnivores. We analyzed stomach contents of pichis because they provide a more comprehensive perspective of an animal's diet than the analysis of fecal matter (Putman 1984). Armadillos usually do not chew their food to ingest a large biomass of insects in a short period of time, which facilitates the identification of food items in the stomachs because they are almost intact. The collection of stomach contents from roadkills or from dead armadillos that have been confiscated by law enforcement agencies is an ethically acceptable means to study the diet of threatened or endangered species without having to sacrifice any individuals.

Materials and Methods

The Nevado area (35°40' S, 68°36' W) is an extra-Andean mountainous range in the Patagonian Steppe ecoregion (Olson et al. 2001), located 200 km east of the Andes in Mendoza Province, Argentina. Its maximum altitude is 3833 m. The arid climate is harsh, with snowfall and temperatures as low as –15 °C in winter and up to 35 °C in summer at lower altitudes. The average yearly precipitation of 320 mm consists of snowfall in winter and summer rains. In this area, pichis have been observed at altitudes up to 2500 m.

Twenty-nine dead pichis were confiscated during two anti-poaching patrols on February 12, 2006 (6 males, 13 females; Group 1) and March 7, 2006 (2 males, 8 females; Group 2) near Cerro Nevado. The first confiscation occurred in an area locally known as Cerro Las Tetas, where pichis live at an altitude of approximately 1700 m. The vegetation is a shrub steppe on sandy and basaltic soils consisting of *Neosparton aphyllum* (Verbenaceae); *Ephedra ochreata* (Ephedraceae); *Atriplex sp.* (Chenopodiacea); *Panicum urvilleanum*, *Stipa spp.*, *Poa spp.* (Gramineae); *Grindelia chiloensis*, and *Senecio spp.* (Compositae). The second confiscation site, locally known as Cerro Las Balas, is situated approximately 19 km south of the first one. The main poaching area around Cerro Las Balas lies between 1950 and 2200 m above sea level. The vegetation is less diverse and consists mainly of *Panicum urvilleanum*, *Stipa spp.*, and isolated shrubs of *Adesmia pinifolia* (Leguminosae).

Stomach contents were collected during necropsies and fixed in 5% formaldehyde for subsequent analyses. The samples were sifted under running water through a sieve with 1 mm mesh size to separate soil from food items. The soil - water mixture was then sifted through tulle to recover sand and other sediments, which were placed in small polyethylene bags. Total volume of each sample devoid of soil was measured in a beaker, and food items over 35 mm length were separated prior to extraction of several aliquots of 11.5 ml in which all items were identified and separated into 16 categories (Table 14). Aliquots were taken from each sample until the number of categories reached a stable maximum, such as to reach the asymptote of a species accumulation curve. The maximum number of items was reached after the second and fourth aliquots. The food items were identified under a stereoscopic microscope (Nikon model C-PS, Nikon Corporation, Japan) and placed in previously weighed polyethylene bags. All samples were then placed in an oven at 40 °C, dried to constant weight within 48 to 72 hours, and weighed with a digital scale (Sartorius AG, Göttingen, Germany) to the nearest milligram. Average dry weight was calculated per type of food item and per animal, and percent aggregate weight was calculated for each item and each group (Korschgen 1980). Data analyses were performed with a statistical software program (SPSS, version 11.0, SPSS Inc., Chicago, Illinois, USA). The proportions of sand and food items ingested by pichis confiscated on different dates, and of items ingested by males and females, were compared with Mann-Whitney U tests.

Results

Twenty-nine stomach samples were evaluated for this study. The stomach contents of three pichis of Group 1 could not be identified because they were too digested; these samples were excluded from further analyses. Sand accounted for an aggregate dry weight of 79.19% of the stomach contents in Group 1 (n=16, confiscated on February 12) and 48.17% of the stomach contents in Group 2 (n=10, confiscated on March 7; Mann-Whitney U test, $p<0.001$). The ingested food items are presented in Table 14 as percent aggregate dry weight and relative frequency after removal of sand. The predominant food item, estimated as percent dry weight of the stomach content, was highly variable in the pichis of Group 1, while 8 out of 10 animals of Group 2 had primarily foraged on Coleoptera larvae (Table 15). The two groups ingested a similar number of different food categories (Fisher's exact test, $p>0.05$), but the proportions of some food items differed significantly. Coleoptera, ants, and plant material were found in all examined stomach samples. Adult coleoptera were present in 25 out of 26 samples and represented 4.57% of the aggregate dry weight in Group 1 and 8.02% in Group 2 (Mann-Whitney U test, $p>0.05$). Only one adult could be identified; it belonged to the tenebrionid species *Nyctelia laevis*. Larval stages of different beetle families were present, with Scarabeidae representing the bulk of all larvae ingested by both groups (Table 14). Group 2 had ingested significantly more larvae than Group 1 ($p=0.001$). Carabid and curculionid larvae were only found in stomachs of Group 2, and tenebrionid larvae had been ingested by all pichis of Group 2 but only 2 out of 16 animals of Group 1. Plant material included grass seeds, leaves and fine roots and accounted for a larger proportion of the stomach contents in Group 1 than in Group 2 ($p<0.001$). One pichi had ingested a flower of *Grindelia chiloensis* (Asteraceae). Workers were the most common caste of ants, but winged stages, soldiers (mainly of *Pheidole aberrans*), pupae, and eggs were also observed. All pichis had foraged on more than one ant species (range 2 to 7 species). *Solenopsis patagonica* was the most common ant, followed by *Pheidole aberrans* (Table 16). The majority of pichis had ingested eggs or pupae of at least one ant species.

Table 14: Food items found in stomachs of Zaedyus pichiy *collected near Cerro Nevado, Mendoza, Argentina. Group 1 was confiscated on February 12, 2006, and Group 2 on March 7, 2006.*

Food item	Group 1 (n=16) Aggregate percent weight[1]	No. of stomachs[2]	Group 2 (n=10) Aggregate percent weight[1]	No. of stomachs[2]
Coleoptera:				
Adults	4.57	15	8.02	10
Carabidae larvae	0.00	0	0.09	4
Scarabeidae larvae	13.89	9	51.77	9
Tenebrionidae larvae	0.22	2	8.11	9
Curculionidae larvae	0.00	0	0.01	2
Unidentified larvae	0.08	1	0.38	3
Arachnida	2.25	8	1.55	8
Plant material	17.24	16	1.85	10
Ants	15.30	16	15.33	10
Fungi	0.45	1	0.00	0
Diptera	28.41	4	2.22	2
Vertebrata	0.32	1	3.18	1
Nematoda	Trace	1	0.00	0
Phasmatodea	0.00	0	0.73	2
Unidentified insects	0.03	2	Trace	1
Unidentified material	17.24	16	6.77	9

[1] Estimated after removal of sand

[2] Number of stomachs that contained the food item

Arachnids were more common in Group 2 and included scorpions and spiders, but represented only a low aggregate percent weight in both groups. Scorpions of the genus *Brachistosternus sp.* were identified in 4 stomachs and *Zabius fuscus* in one, while the species ingested by the remaining pichis could not be identified. One pichi had eaten a tarantula

(*Mygalomorpha*). Fly larvae (*Diptera*) accounted for 28.41% aggregate weight of Group 1, but only 2.22% aggregate weight of Group 2. Basidiomycetes (fungi) were found in one pichi belonging to Group 1. Vertebrates were rarely observed. One pichi had ingested a lizard of the species *Liolaemus josei*, while the stomach of another one contained hair of an unidentified mammal. Nematodes were present in one, and *Paradoxomorpha* sp. (Phasmatodea: Pseudophasmatidae) in two stomachs. The category of unidentified insects included chitin fragments that could not be assigned to any taxon. The category "unidentified material" included small organic particles. The proportion of food items ingested by males did not differ significantly from the proportion ingested by females.

Table 15: Predominant food item ingested by Zaedyus pichiy *based on percent weight after removal of sand.*

Food item	Group 1 * (n=16)	Group 2 * (n=10)
Coleoptera adults	3	0
Scarabeidae larvae	3	7
Tenebrionidae larvae	0	1
Arachnida	1	0
Plant material	5	0
Ants	3	2
Diptera	1	0

* Number of stomachs in which the food item represented the largest proportion of all ingested items

Table 16: Ant species found in stomachs of Zaedyus pichiy *collected near Cerro Nevado, Mendoza, Argentina*

Ant species
Solenopsis patagonica
Pheidole aberrans
Dorymyrmex jorgenseni
Dorymyrmex tener
Dorymyrmex richteri
Dorymyrmex sp.
Camponotus sp.
Pogonomyrmex sp.
Subfamily Formicinae
Subfamily Myrmycinae

Discussion

The present study suggests that in Mendoza Province, pichis are opportunistic feeders. The evaluated animals had mainly fed on invertebrates, but had also ingested varying amounts of plant material. Individual stomachs often contained a large quantity of the same food item ranging from beetles to plants, suggesting that they fed on whatever food source was available. In the absence of similar studies on the diet of pichis, we will compare our results with the diet of two other carnivore-omnivore armadillos, the screaming hairy armadillo *Chaetophractus vellerosus* (Greegor 1980) and the yellow armadillo *Euphractus sexcinctus* (Dalponte 2004), although the methods used by Greegor and Dalponte differ from ours.

The large amount of sand found in all stomachs was striking and accounted for up to 96% of the stomach content's dry weight. The average soil content was higher than in *Chaetophractus vellerosus* (Greegor 1980). Presumably, this large quantity of soil is ingested accidentally because armadillos cannot efficiently separate insects and other food items from the inorganic material surrounding them when capturing their prey with their tongue covered with sticky saliva. Soil could, however, be ingested deliberately as a source of minerals and trace elements (Bolkovic et al. 1995) or to help triturating the food items. Taber (1945) suggested that soil ingestion may be necessary for a solid consistency of feces, and Redford (1987) postulated that armadillos ingest soil deliberately to neutralize the effects of the chemical defense mechanisms of the ingested insects.

Insects were the predominant food item observed in the stomachs of pichis belonging to both evaluated groups (Table 14). They were present in all studied stomachs and accounted for 62.5% aggregate weight of the stomach contents after removal of sand in Group 1, and 86.7% in Group 2. Insects were also the major food item ingested by *C. vellerosus* (summer: 46% volume, winter: 25.7%, Greegor 1980) and *E. sexcinctus* (61.3% volume, Dalponte 2004). Although pichis also ate beetles that live aboveground, subterranean larvae of different families constituted the bulk of the identified Coleoptera. Pichis have a well-developed sense of smell, which helps them locate prey items that are below ground. The predominant larvae belonged to the families Scarabeidae and Tenebrionidae. Group 2 had fed on a wider variety of beetle larvae than Group 1; no carabids or curculionids were observed in the stomachs of Group 1, while they were present in 4 and 2 stomachs of Group 2, respectively. Larvae ingested by pichis of Group 2 were considerably larger than those eaten by animals of Group 1, which may partially explain why beetle larvae constituted a significantly larger proportion of the stomach contents of Group 2.

All pichis had ingested ants of different species, stages, and castes. They may have ingested workers when finding a trail while foraging aboveground, but the presence of eggs and pupae in most stomachs indicates that they had also attacked anthills. Indeed, we have often observed wild pichis foraging on anthills and tracks of pichis near anthills with signs of predation. We are not aware of any ant species that exists in the areas of confiscation but was not present in the stomach samples. This observation, added to the fact that all stomachs contained several (up to 7) ant species, suggests that pichis ingest any ant they can find. Because *S. patagonica* and *P. aberrans* are the most common ants in the Nevado area, it was not surprising that they were also most abundant in our samples. The observation of eggs and larvae of different species in a single stomach indicates that pichis consecutively predate on several nests. Similar observations have been reported from anteaters, which usually fed from one ant colony for less than a minute before moving to the next and foraged on up to 80 colonies per day (Montgomery 1985). Presumably, armadillos and anteaters remain on one anthill as long as they can withstand the defense of soldiers. Although the pichi's carapace provides an excellent protection against the attack of soldiers, we often found ants attached to the legs and the abdomen of pichis we captured near anthills.

It was surprising to find specimens of *Paradoxomorpha* sp. (Phasmatodea), locally known as "chinche molle", in two pichi stomachs. These insects are seasonally abundant and relatively large (the ingested specimens were 7 cm long) and therefore appear to be an ideal prey item. Nevertheless, one of the defense mechanisms of Phasmids is to secrete repugnant or caustic chemicals. In some *Paradoxomorpha*, the secretion is produced by thoracic glands and contains ethyl ether or orthoformic acid (Hogue 2003). Although we could not determine the species of *Paradoxomorpha* that had been ingested by pichis, the strong smell of "chinche molle" suggests that they are capable of producing similar chemicals that may deter potential predators. It is unlikely that pichis ingested them by accident due to their large size, and the fact that both pichis had also fed on large quantities of other food items (they accounted for less than 4% dry weight in both cases) suggests that they did not prey upon Phasmids as a consequence of the unavailability of alternative food sources.

Vertebrate remains were rarely observed in the stomachs. The lizard species ingested by one pichi is commonly found in the Nevado area, at altitudes between 1000 and 2500 m (V. Corbalán, personal communication). Although this study suggests that lizards are rarely eaten by pichis, their ingestion probably depends on many factors, such as lizard density and environmental conditions. Reptiles are less active at cold temperatures, which makes them an easier prey for pichis at night or on cool days. For instance, lizards were found in the stomachs of 4 out of 10 dead pichis that had been confiscated near Cerro Diamante, Mendoza Province, in January 2007 (Superina, unpublished data). Carrion was not present in any of the studied samples, but the hair identified in one stomach suggests that at least one pichi had been eating parts of a mammal. However, it is possible that the ingestion of carrion is more common than reported in this study because it is digested faster than other food items and may therefore have been overlooked. Captive pichis eagerly eat raw meat, and the stomach of one roadkill sampled in southern Mendoza contained a bone fragment that seemed to be a part of the jaw of *Ctenomys sp*., suggesting that pichis may sometimes forage on animal carcasses. Nevertheless, in 6 years of fieldwork and during observations of over 150 wild pichis, only one wild individual was found scavenging on a dead guanaco although the presence of dead wildlife was relatively common. Talmage and Buchanan (1954) suggested that armadillos are attracted by the maggots parasitizing on animal carcasses and not the carrion itself. Indeed, 6 of the evaluated pichis had ingested fly larvae that are commonly found on animal remains. Diptera larvae were ingested by only 4 out of 16 animals of Group 1, but accounted for more than one fourth of the aggregate percent weight of this group (Table 14).

Vertebrates seem to be a less important part of the diet of pichis than of other omnivore armadillos. Dalponte (2004) estimated a 23.3% occurrence of vertebrate remains in the stomachs of 12 *E. sexcinctus*. Although vertebrates only accounted for 4.0% of the volume of stomachs analyzed, it is noteworthy that three out of 12 yellow armadillos had ingested small rodents (Sigmodontinae), while another individual had fed on a small snake and a bird and other stomachs contained armadillo scutes and skin fragments of a domesticated mammal (Dalponte 2004). *C. vellerosus* seems to be the most carnivorous of all armadillos studied to date. Mammals, lizards, birds, and anurans added up to 27.7 percent volume of the stomach content of screaming hairy armadillos in summer, while the proportion of vertebrates was considerably lower (13.9%) in winter (Greegor 1980). The lack of reliable information on the diet of *C. villosus* and *C. nationi* precludes a comparison with these species. Nevertheless, it is probable that the former relies heavily on vertebrates, especially carrion, because *C. villosus* is commonly found near animal carcasses (Superina 2000).

Scorpions and spiders were found in over 60% of the analyzed stomachs – mainly in animals of Group 2 – but only accounted for a low aggregate percent weight (Table 14). A similar proportion of *C. vellerosus* had ingested arachnids in the winter months, while this food item was less common in screaming hairy armadillos captured during summer (Greegor 1980). *E. sexcinctus* seems to rely less on arachnids; fragments of large spiders were found in only 25% of the stomachs (Dalponte 2004). Interestingly, no Solifugae were found in any pichi stomach although this order includes one of the most abundant arachnids in the Nevado area. Most solifuges have nocturnal habits and hide under rocks during the day. However, there are some diurnal species that are active only during warm sunny days (Maury 1998). It is possible that pichis do not feed on them because they are not able to catch these fast-moving arachnids or because of the inverted activity cycles of the diurnal armadillos and the solifuges.

The diet of all evaluated pichis included plant material, such as grass seeds, leaves, roots, and flowers. The ingested quantities, however, varied considerably between the two groups (Table 14). Plants were the predominant food item of 5 pichis of Group 1, but of none of the pichis belonging to Group 2 (Table 15). Although we did not study the abundance of dietary items in the two confiscation sites, we suspect that pichis of Group 2 ingested less plant matter because of a higher availability of their preferred food item (insects). This assumption is supported by observations in yellow armadillos and screaming hairy armadillos. Plant material was the main food of screaming hairy armadillos in winter but not in summer, possibly because the reduced availability of ground-dwelling insects during the cold period obliged them to rely on another food source (Greegor 1980). It was also an important food item of wild *E. sexcinctus*, in which it accounted for almost one third of the stomach contents (Dalponte 2004). Stomachs of yellow armadillos inhabiting intensively cultivated areas contained significantly less plant matter than stomachs of individuals in the Pantanal, which seemed to have been related to the higher abundance of insects in the agricultural region. It is therefore probable that all omnivore armadillos ingest insects whenever they are available and rely on alternative food items when insects are less abundant.

Basidiomycetes were identified in one stomach based on the presence of spores. Mycelia-like structures were also found in the stomachs of 10 other pichis of Group 1, but we classified them as unidentified material because the absence of reproductive structures did not allow a conclusive identification. It is therefore possible that other pichis had ingested small amounts of fungi. We can only speculate on the origin of these fungi. None of the identified ant species are known to cultivate fungi as a food source, suggesting that the pichis did not ingest them while foraging on an anthill. The fact that many basidiomycetes are mycorrhizal symbionts (Hibbett et al. 2000) leads us to think that pichis may ingest fungi accidentally while foraging on the roots of shrubs. However, the absence of studies on mycorrhizal symbionts in Mendoza Province does not allow us to confirm or reject this hypothesis.

The interval between the two confiscations (less than a month) is too short to reflect a temporal variation in the diet of pichis. The shifts in diet we observed between the two groups are therefore probably related to the different geographic locations. Although we do not know the exact capture sites of the evaluated pichis, the locations where they have been confiscated from poachers are distant enough from each other to state that they were not extracted from the same area or from a single population. The lack of studies on the abundance of invertebrates in these sites does not allow us to evaluate whether pichis of Group 2 ingested more beetle larvae simply because they were more common in their area than in the first confiscation site or because pichis have a preference for this food item and actively search for them. It is, however, interesting to note that pichis of Group 1 inhabited an area with higher plant diversity and ingested a higher proportion of plant material. Possibly, the amount of plant material that pichis ingest depends, among others, on the available plant species. Pichis often dig their burrows at the base of *Neosparton aphyllum* or *Ephedra ochreata*. The most obvious reasons for this behavior are that pichis hide the burrow entrance under shrubs as a protection against predators, and that the roots provide structural support for the burrow and prevent collapses. An additional benefit of making a burrow under these shrubs could be that pichis forage on insect larvae that are associated with the roots, such as ground beetle (Scarabeidae) larvae, or on the roots themselves, either to ingest plant material or to use the water stored in them.

It should be noted that the present results only reflect the diet of pichis in a certain time of the year, and that seasonal variation in food availability may lead to differences in the type or proportion of ingested food items. For instance, reduced insect activity in winter may result in an increased ingestion of plant material, as it has been observed in *C. vellerosus* (Greegor 1980). Furthermore, this study only determined the diet of individuals having their origin in the Cerro Nevado area of Mendoza Province, Argentina, while the diet of pichis inhabiting other areas or different altitudes may consist of other food items. In the Low Monte ecoregion of northern Mendoza, for instance, pichis frequently forage on *Prosopis* pods (Superina, unpublished data), a food item that is not available in our study area. Pichis in southern Mendoza have been observed to eagerly ingest fruits of *Ephedra ochreata*, a shrub that is present in parts of the Cerro Nevado area but did not carry fruits at the time of confiscation of the animals studied here.

In conclusion, our observations are consistent with the original classification of *Zaedyus pichiy* as a carnivore-omnivore, but it seems to be the least carnivorous armadillo of this group. *Z. pichiy* is best described as an opportunistic omnivorous armadillo that mainly relies on invertebrates but also ingests plant material, vertebrates, and arachnids whenever these items are available. This omnivorous, opportunistic feeding strategy allows pichis to cope with the large seasonal variation in food availability caused by the varying environmental conditions. Further studies on the diet of pichis inhabiting different areas, and of samples collected in different seasons, are needed to understand the extent of omnivory of this little armadillo.

CHAPTER 4: HEALTH EVALUATION OF WILD AND CAPTIVE PICHIS (*ZAEDYUS PICHIY*)

Introduction

Disease epidemics are an integral part of ecosystems that may play a regulatory role when densities of sub-populations are high, but can cause local extinctions as host ranges contract and population sizes diminish (May 1988; Munson and Karesh 2002; Woodroffe 1999). Even apparently harmless pathogens may become important when animal populations are stressed through human activities, such as agriculture, industry, tourism, or hunting activity (Scott 1988). Monitoring the prevalence of pathogens in wild animal populations is therefore necessary to evaluate the impact of human activities on wild animal health (Karesh et al. 1995). Furthermore, both public and government expect an efficient intervention from conservation scientists in case of sudden population declines of wild populations, which, among others, might be caused by disease outbreaks (Karesh et al. 1995). Disease recognition and control in wild populations, however, are usually hindered by a lack of knowledge about the pathogens that are present (Woodroffe 1999). In addition, it is difficult to determine whether an animal is healthy or affected by a disease if the parameters describing a healthy individual are not known. Baseline data, such as knowledge of present and historical occurrence of pathogens and hematological reference values of healthy individuals, are key to identifying a wildlife health problem and detect its source. They are crucial to investigate the causality between human encroachment into wildlife habitat and disease occurrence. Furthermore, such information is essential to implement adequate management strategies (Karesh et al. 1995).

Veterinarians working with confiscated wildlife are often confronted with an additional problem: Reintroduced animals may introduce new infectious agents or parasites into an area to which the native fauna is immunologically naïve, causing disease outbreaks in the local populations that can have deleterious or even devastating effects (Cunningham 1996; Woodford 1994). In contrast to this, the reintroduced individual may be exposed to new diseases that could affect or even kill it. Prior to releasing a seized animal into its natural habitat, it is therefore essential to evaluate the health of both the reintroduction candidate and its conspecifics at the reintroduction site to minimize the risk of disease transmission. It is, however, very difficult to carry out a complete health check of reintroduction candidates if it is not known for which diseases the animals should be screened, i.e., if no information is available about the pathogens that commonly affect the species.

Pathogens affecting wild animals may also pose a risk to the health of human beings. As many zoonotic pathogens seem to have a wildlife reservoir (Kruse et al. 2004), humans that come into close contact with wild animals are exposed to pathogens that might not cause disease in their natural hosts, but may become a threat to humans once they cross the species barrier. The association between human and animal health problems has recently led to increased efforts to merge these two fields (Enserink 2007). Hunters are especially prone to acquire a zoonotic pathogen while killing or consuming wildlife (Monath 1999).

The pichi *Zaedyus pichiy* is a small armadillo species endemic to Argentina and Chile. Although neither historical nor current census data are available, lower encounter rates in Mendoza Province, in central west Argentina, suggest that its wild populations have decreased considerably in the past years. These apparent population declines may have been caused – at least partially – by poaching and by disease epidemics. Pichis are the main prey of poachers in Mendoza Province who use them as a protein source, and locals have reported that disease outbreaks of unknown etiology severely affected the wild populations in some areas of Mendoza. These two factors are not mutually exclusive and could have acted in conjunction, either simultaneously or consecutively. The lack of knowledge on endemic pathogens of wild pichis makes it difficult to evaluate the role of diseases in these population declines. Although wild specimens of other armadillo species have been screened for specific pathogens, such as *Leptospira* (Carillo et al. 1972), *Salmonella* (Quevedo et al. 1978) or *Sarcocystis* (Lindsay et al. 1996), only one study (Mazza et al. 1936) included pichis. Similarly, hematological reference values exist for *Dasypus novemcinctus* (D'Addamio et al. 1978; Purtilo et al. 1975), *D. hybridus* (Cuba-Caparó 1976), *D. septemcinctus* (Coppo et al. 1979) and *Chaetophractus villosus* (Casanave and Polini 1999; Polini and Casanave 1999), but not for pichis. Humans are in close contact with pichis during illegal hunting and consumption. Therefore, the question arises whether these animals pose a risk to human health through the transmission of diseases. It has long been known that armadillos can carry potentially zoonotic diseases such as Chagas and toxoplasmosis (Barr et al. 1991; Barretto and Ribeiro 1979; Forrester 1992; Ramírez et al. 1984; Sogorb et al. 1977). These diseases could be transmitted to hunters and consumers of pichis during killing, evisceration, or consumption of pichis and lead to serious health problems or even death. Consequently, learning more about pathogens of pichis has implications not only for wild pichi populations but also for humans who interact closely and frequently with these animals.

The objectives of this study were therefore as follows: (1) Establish hematological reference values for healthy pichis; (2) Describe the pathologies affecting wild and captive populations; (3) Determine the prevalence of potentially zoonotic pathogens in pichi populations.

Materials and Methods

Project location, sample and data collection

Live wild pichis

Samples were collected from wild pichis during vehicle, walking, and horseback transects of varying length in randomly chosen areas of Mendoza Province, central west Argentina (66°30’ to 70°00’ W and 32°00’ to 37°30’ S), except in the high Andes in the west where pichis are absent. The southern part of Mendoza Province belongs to the Patagonian steppe ecoregion (Olson et al. 2001). The arid climate and the poor volcanic soil, covered with sands of variable depth, together determine the scarce vegetation (Candia et al. 1993), which is composed of grasslands with isolated shrubs, areas with open bush communities, and basaltic steps (Candia and Dalmasso 1995). Trees are absent from this region. The arid northeastern part of Mendoza is part of the Low Monte ecoregion (Olson et al. 2001). The dry climate and sandy grounds favor the growth of the characteristic *monte* vegetation, which consists of small trees, shrubs and scarce pasture (Peralta de Galmarini and Martinez Carretero 1995).

Transects were performed in all seasons and at all times of day between November 2001 and December 2006. One hundred and thirty nine wild-caught pichis (87 males, 52 females) were run down and captured by hand. Capture sites were registered with a hand-held GPS and environmental temperature was measured with a digital thermometer. Thirty-three animals were considered juveniles, 22 were classified as yearlings, and 84 were adults based on their body mass, carapace length and width, the presence and absence of scars, and capture date. Physical examinations and sample collections were carried out under manual restraint. Clinical examinations focused on body condition, visible external lesions and scars, symptoms of pathological processes, ectoparasite load, and reproductive status. The animals were measured, weighed with a spring scale (Pesola AG, Baar, Switzerland), and their rectal temperature registered with a digital thermometer (TES Electrical Electronic Corp., Taipei, Taiwan). Ticks and fleas were collected and stored in 96% ethanol for identification. Fecal samples were collected for coproparasitological analyses and kept refrigerated until shipment to the lab. Blood samples were obtained by venipuncture of the medial coccygeal vein. Due to the small size of this vein and rapid coagulation, blood was collected directly from the needle into heparin-coated and uncoated microcapillaries (Biocap S.A., Buenos Aires, Argentina), which where subsequently sealed with plasticine (Critoseal, Oxford Labware, St.Louis, MO). Thin smears of fresh blood were done *in situ* and air-dried. Coated microcapillaries were stored in an insulated container and transported to the lab, while uncoated microcapillaries were first kept at environmental temperature for two hours to allow coagulation, then transferred to the insulated container. Rectal temperature was measured no later than 5 minutes after capture and blood samples extracted within 15 minutes. All pichis were released at their capture site within 45 minutes.

Live captive pichis

Wild-caught and captive-born pichis were maintained during 1 to 26 months in a private facility in Luján de Cuyo, Mendoza, Argentina (33° S, 69° W). The enclosure consisted of individual pens made of wire mesh and sheet metal, of 2 x 1.5 x 2.5 m each; soil to a depth of 2 m provided a natural substrate for digging. Food, consisting of a varying mixture of fruits, vegetables, meat, dry cat food, rice, and a vitamin-mineral supplement, was offered once daily; water was provided *ad libitum*. Pichis were visually inspected at feeding time; individuals presenting lesions or symptoms of disease were removed from the enclosure and maintained separately until their recovery. Blood samples from twenty-five animals (11 wild-caught, kept in captivity > 16 months; 14 captive-born) were obtained under manual restraint between January 8 and January 30, 2007.

Dead captive and wild pichis

Collaborating inspectors and rangers of the Mendoza Department of Natural Renewable Resources refrigerated dead pichis they confiscated until they could be turned over to the author. Necropsies were performed on roadkills (12 males, 4 females) and confiscated pichis (58 males, 82 females, 5 of unknown sex), as well as on captive pichis that died during the study period (7 males, 5 females). The animals were classified as juveniles, yearlings or adults based on morphological signs, such as carapace length and width, and the presence or absence of scars. They were examined for macroscopic lesions, and representative tissue samples of reproductive organs, heart, lungs, liver, kidney, diaphragm, skeletal muscle, skin, intestine, lymph nodes, adrenal glands, and any macroscopic lesion were collected where available. Not all organs could be sampled from all individuals because many pichis were eviscerated at the time of confiscation. Tissues were stored in 10% formalin.

Sample analysis

Bacteriological cultures, blood smears, and hematology, serum chemistry, and serology samples of captive and wild animals were analyzed in collaboration with Laboratorio Mera, Mendoza, Argentina. Blood smears were fixed with methanol, stained with Giemsa solution (Merck, Buenos Aires, Argentina), and examined by light microscopy for the differential leukocyte count. Packed cell volume (PCV) was determined using the microhematocrit method. The hemocytometer method was used for total erythrocyte and total leukocyte count determination. Serum biochemical assays were performed by spectrophotometric methods using commercial kits (Wiener Lab, Rosario, Argentina) and included the determination of the following parameters: Blood urea nitrogen (BUN), enzymatic method (540 nm); alanine aminotransferase (ALT) and aspartate aminotransferase (AST), colorimetric method (505 nm); Total Proteins, Biuret method (540 nm); Albumin, colorimetric method (625 nm); Globulins, Total protein concentration minus albumin concentration; Calcium, Cresolphtalein complexone method (570 nm); Alkaline Phosphatase (AP), optimized kinetic method (405 nm).

Microbiological samples were inoculated in sheep blood agar, MacConkey Agar and Sabouraud Agar and cultured aerobically at 37 °C. Identification of isolates was done with routine microbiological techniques (Finegold and Baron 1986). Commercially available test kits based on indirect hemagglutination (Toxotest HAI, Wiener Lab, Rosario, Argentina; and Chagas HAI, Polychaco S.A.I.C., Buenos Aires, Argentina, respectively) were used to test serum samples for antibodies against *Toxoplasma gondii* and *Trypanosoma cruzi.* Samples were considered positive if agglutination was present at dilutions of at least 1/16. Ectoparasites and fecal samples were submitted to the Centro de Parasitología y Vectores, La Plata, Argentina for analysis. Ectoparasites were identified based on morphological characteristics. Fecal samples were analyzed by means of the modified McMaster method and Willis flotation technique with Sheather solution (Hawken 1983; Hendrix 1998). Tissues for histopathological analysis were fixed in Bouin's fluid, embedded in paraffin, sliced at 5 µm, and stained with hematoxylin-eosin. Histological slides were screened by Dr. Michael Garner (Northwest ZooPath, Monroe, WA, USA) under light microscopy for signs of pathological alterations and presence of pathogens.

Data analysis

Data analyses were performed with a statistical software program (SPSS, version 11.0, SPSS Inc., Chicago, Illinois, USA). For the determination of reference values, hematology and serum chemistry data were explored by means of basic statistics, stem and leaf plots, boxplots, and histograms. Outliers were deleted and excluded from further analyses, and mean, standard deviation, median, and range were calculated for each blood parameter. Values for packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), blood urea nitrogen (BUN), protein, albumin, calcium, ALT, AST, AP, percentage neutrophils, and percentage lymphocytes, were normally distributed; Student's t tests were therefore used to evaluate significant differences between gender, age, and reproductive status for these parameters (Sokal and Rohlf 2001). Nonparametric statistical analyses were utilized to evaluate these differences in white blood cell count (WBC) and differential WBC (except percentage neutrophils and lymphocytes) because they were not normally distributed. Fisher's Exact and Chi-square tests were used to compare the prevalence of histopathological lesions per affected organs in juveniles, yearlings and adults, and in males and females, respectively. Calculated *p* values ≤ 0.05 were considered statistically significant.

Results

Hematology, serum chemistry, and serology

The results of the hematological and serum chemistry analyses of wild and captive pichis are summarized in Table 17 and Table 19, respectively. Adult wild pichis presented a significantly higher PCV and RBC than juveniles (independent-samples t test, p=0.028 and 0.004, respectively; Table 18), but more samples from juveniles are needed to confirm this difference. The PCV was significantly higher in males than females (independent-samples t test, p = 0.002), while females had higher eosinophil percentages and counts (Mann-Whitney U test, p = 0.008 and 0.013, respectively; Table 18). No other significant differences between genders or age classes were found. The blood values of non-lactating adult females were within the range of values for lactating females, and reproductively active adult males, as determined by enlarged testes, had similar blood values as males sampled outside the breeding season (p>0.05 in all cases).

Blood values for captive pichis were similar to the values for their wild conspecifics, except for significantly lower PCV, neutrophil percentages, and absolute and relative band neutrophil values, and higher absolute and relative lymphocyte counts in captive animals (Table 19).

All of the 24 tested wild pichis, and all of the 11 evaluated captive pichis, were seronegative for *Toxoplasma gondii*. Two out of 25 tested animals were seropositive for *Trypanosoma cruzi*, presenting titers of 1/16 and 1/64, respectively.

Table 17: Hematological and blood chemistry values of wild Zaedyus pichiy. *Note that not all parameters were determined for each individual because of the low quantity of blood that could be extracted per animal.*

Parameter	***n***	**Mean ± SD**	**Median**	**Range**
PCV (%)	75	49.2 ± 6.0	49.0	36 – 60
RBC (10^6/µl)	25	4.30 ± 1.05	4.14	2.60 – 6.50
Hemoglobin (g%)	1	16.0		
MCV (fl)	25	120.2 ± 30.0	115.6	72 - 185
WBC (10^3/µl)	63	4.3 ± 2.2	3.7	1.0 – 10.3
Neutrophils (%)	73	58.4 ± 17.1	58.0	18 – 94
Band neutrophils (%)	69	0.3 ± 0.5	0.0	0 – 2
Lymphocytes (%)	72	27.1 ± 12.8	27.0	3 – 58
Eosinophils (%)	72	7.7 ± 7.7	4.0	0 – 29
Monocytes (%)	72	3.8 ± 2.4	3.0	0 – 11
Basophils (%)	72	1.1 ± 1.5	0.0	0 – 6
Neutrophils (10^3/µl)	50	2.34 ± 1.31	2.04	0.50 – 5.38
Band neutrophils (10^3/µl)	64	0.008 ± 0.021	0.00	0.00 – 0.10
Lymphocytes (10^3/µl)	53	1.17 ± 0.82	1.04	0.14 – 3.40
Eosinophils (10^3/µl)	53	0.29 ± 0.26	0.22	0.00 – 0.92
Monocytes (10^3/µl)	53	0.16 ± 0.14	0.12	0.00 – 0.53
Basophils (10^3/µl)	57	0.023 ± 0.038	0.00	0.00 – 0.12
Blood Urea Nitrogen (mg/dl)	71	30.9 ± 10.1	31.2	13.1 – 57.3
Protein (g/dl)	14	6.1 ± 1.0	6.3	4.1 – 7.9
Albumin (g/dl)	13	3.3 ± 0.4	3.5	2.5 – 4.0
Calcium (mg/dl)	20	9.8 ± 1.8	9.9	7.2 – 13.5
ALT (U/l)	11	8.8 ± 4.3	8.0	3 – 16
AST (U/l)	4	13.0 ± 7.2	13.5	5 – 20
AP (U/l)	3	144.0 ± 93.5	179	38 – 215

Table 18: Hematological and blood chemistry values of wild Zaedyus pichiy *for which a significant difference was observed between males and females, or between adults and juveniles. PCV and RBC values were compared with independent-samples t tests, while absolute and relative eosinophil counts were compared with Mann-Whitney U tests. Symbols * and $^{\#}$ indicate significant differences ($p \leq 0.05$); symbols **, $^{\#\#}$ and $^{\wedge\wedge}$ indicate highly significant differences ($p \leq 0.01$)*

Parameter		***n***	**Mean ± SD**	**Median**	**Range**
PCV (%)	Males	39	51.3 ± 5.4**	50.0	39 – 60
	Females	35	47.2 ± 5.6**	48.0	37 – 58
	Juveniles	17	47.1 ± 5.6*	48.0	37 – 57
	Adults	45	50.7 ± 5.5*	50.0	39 – 60
RBC (10^6/µl)	Juveniles	4	3.48 ± 3.10$^{\#\#}$	3.40	3.20 – 3.90
	Adults	6	4.52 ± 1.08$^{\#\#}$	4.41	2.60 – 6.50
Eosinophils (%)	Males	41	5.54 ± 5.65$^{\wedge\wedge}$	3.00	0.0 – 19.0
	Females	30	10.67 ± 9.26$^{\wedge\wedge}$	6.50	1.0 – 29.0
Eosinophils (10^3/µl)	Males	27	0.21 ± 0.22$^{\#}$	0.11	0.0 – 0.78
	Females	25	0.37 ± 0.27$^{\#}$	0.31	0.02 – 0.92

Table 19: Hematological and blood chemistry values of captive Zaedyus pichiy. *The symbol* * *indicates a significant difference* ($p \leq 0.05$)*, and the symbol* ** *indicates a highly significant difference* ($p \leq 0.01$) *to values of wild* Zaedyus pichiy *listed in Table 17.*

Parameter	***n***	**Mean ± SD**	**Median**	**Range**
PCV (%)	23	46.0 ± 7.0*	4.0	29 – 57
RBC ($10^6/\mu l$)	9	3.50 ± 1.37	4.05	1.68 – 5.35
MCV (fl)	9	136 ± 44.3	115.0	91 – 223
WBC ($10^3/\mu l$)	23	5.2 ± 2.2	4.9	2.1 – 10.3
Neutrophils (%)	22	49.3 ± 16.2*	48.0	24 – 85
Band neutrophils (%)	22	0.6 ± 0.9*	0.0	0 – 3
Lymphocytes (%)	22	37.7 ± 15.6**	37.0	8 – 68
Eosinophils (%)	22	7.9 ± 5.7	7.0	0 – 19
Monocytes (%)	22	3.6 ± 3.3	2.5	0 – 15
Basophils (%)	22	0.9 ± 1.3	0.0	0 – 4
Neutrophils ($10^3/\mu l$)	22	2.48 ± 1.20	2.54	0.91 – 5.14
Band neutrophils ($10^3/\mu l$)	22	0.004 ± 0.006**	0.00	0.00 – 0.25
Lymphocytes ($10^3/\mu l$)	22	1.92 ± 1.04**	1.82	0.22 – 4.23
Eosinophils ($10^3/\mu l$)	22	0.44 ± 0.46	0.29	0.00 – 1.95
Monocytes ($10^3/\mu l$)	22	0.19 ± 0.18	0.10	0.00 – 6.15
Basophils ($10^3/\mu l$)	22	0.005 ± 0.009	0.00	0.00 – 3.09
Blood Urea Nitrogen (mg/dl)	17	28.3 ± 10.8	28.4	13.5 – 49.9
ALT (U/l)	6	12.3 ± 10.7	9.0	2 – 33
AST (U/l)	6	23.5 ± 34.5	5.0	4 – 90
AP (U/l)	5	196.0 ± 62.7	200	108 – 258

Live wild-caught pichis

Signs of pathological processes were observed in 25 of 139 animals and included emaciation, apathy, dehydration, decalcified carapace border, pale mucosa, inflammation and abscesses on the penis, and infected wounds. Thirty-one pichis presented fresh lesions, such as skin lesions, missing or worn out scutes, or fractured scutes. Males and females were equally affected (23% vs. 21%; Mann-Whitney U test, $p>0.05$). The prevalence of fresh lesions was not significantly different in adults, yearlings, and juveniles (24%, 36%, and 9%, respectively; Kruskal-Wallis Test, $p>0.05$). Scars were observed in 64 animals and affected mainly the bands, head shield, carapace border, and tail. Forty-six percent of all males and an identical percentage of females presented one or more scars. No significant difference in the prevalence of scars was observed among age classes (51% adults; 50% yearlings; 30% juveniles; Kruskal-Wallis Test, $p>0.05$).

The average rectal temperature outside hibernation season was 35.2±1.2 °C. It was highly variable (range 32.2 – 38.3 °C, n=89), but this variability was not related to environmental temperature or age. The temperature of one adult male sampled during hibernation was 18.9 °C, similar to the temperature measured within its burrow (18 °C).

Fecal analyses were performed on 54 animals. *Aspidodera* spp. were the most commonly found endoparasites (83% of studied pichis), followed by *Eimeria* oocysts (59%) and trichostrongylids (28%, Table 20). Five animals (9% of studied pichis) were free of parasites, and the same quantity was infested with 4 or 5 different parasite types (Table 21). Fleas of the species *Malacopsylla grossiventris* or *Phthiropsylla agenoris* were found in 84 of 139 armadillos. One pichi from northern Mendoza carried ticks of the species *Amblyomma pseudoconcolor*. Males did not carry significantly more ticks or fleas than females, but significantly more yearlings than juveniles were infested with ectoparasites (analysis of variance, $p=0.045$). No blood parasites were found in any of the examined animals.

Table 20: Endoparasites identified during coproparasitological analyses of 54 wild Zaedyus pichiy.

	N infested pichis	Percent infested pichis
Aspidodera eggs, larvae, and/or adults	45	83.3
Eimeria oocysts	32	59.3
Trichostrongylidae eggs	15	27.8
Unidentified coccidia	10	18.5
Unidentified nematode eggs	4	7.4
Mathevotaenia eggs	3	5.6
Isospora oocysts	3	5.6
Cyclobulura eggs	2	3.7
Trichuris eggs	2	3.7
Cestode eggs or fragments	2	3.7

Table 21: Endoparasite diversity determined in 54 wild Zaedyus pichiy *by means of coproparasitological analyses. Only a few animals were free of endoparasites or were infested with four or five different parasite types, while two thirds of the studied pichis were infested with two or three parasite types.*

Endoparasite types	N	Percent pichis
0	5	9.3
1	8	14.8
2	20	37.0
3	16	29.6
4	3	5.6
5	2	3.7

Captive pichis

Health problems were observed in only a few captive pichis. The lack of information about appropriate doses of antibiotics, anesthetics and other drugs for pichis required an extrapolation based on published doses for other armadillo species (Superina 2000) or domestic cats.

Six animals that had been temporarily maintained in 200 liter barrels filled with sand developed humid cutaneous lesions and skin detachment on paws, abdomen, and face. The affected animals were kept in a dry environment during treatment. Lesions were cleaned twice daily with povidone iodine, and 25 mg/kg flunixin-meglumine (Apronal, Laboratorios König S.A., Avellaneda, Argentina) applied for analgesia once daily (SID) during three days. Cefalexin (Cefalexin Plus, Proagro S.A., Rosario, Argentina) was used SID at a dose of 60 mg/kg via intramuscular (IM) injections for antibiotic treatment until the results of the microbiological study of skin lesions were obtained. Bacteriological analysis revealed infection with *Citrobacter* and *E. coli* susceptible to enrofloxacin. Antibiotic therapy was therefore changed to IM injections of 1.25 mg/kg enrofloxacin (Floxagen, Vetanco S.A., Buenos Aires, Argentina) twice daily (BID) during 5 days. Treatment had to be suspended in one male after it developed anasarca, probably as an allergic reaction to enrofloxacin. Another bacterial culture with antibiogram was performed a week later because the lesions persisted in spite of antibiotic treatment. Some bacterial colonies had acquired resistance to enrofloxacin, which required a change to 20 mg/kg trimethoprim-sulfa (Raxidal, Intervet International GmbH, Unterschleissheim, Germany), administered IM SID during 5 days. Supportive treatment included IM injections of 2 mg/kg menadione (Mestil-Ka, Fada Pharma, Buenos Aires, Argentina) during 5 days. The animals were released in their enclosures after complete healing of the lesions.

Two males required isolation and antibiotic treatment of infected lesions during the reproductive season. One affected adult male was apathetic and emaciated, with soil adhered to extensive facial lesions. Necrotic tissue was removed, the wounds cleaned twice daily with hydrogen peroxide and povidone iodine, and 0.5 mg/kg dexamethasone (Decadron, Sidus S.A., Pilar, Argentina) and 20 mg/kg trimethoprim-sulfa applied IM once daily during 5 days.

Artificial feeding by means of a type K-35 gastric tube of 1.4 mm diameter (Koler S-14, Deplamed S.R.L., San Martín, BsAs, Argentina) with Ensure (Abbott Laboratories, Columbus, OH, USA) was necessary during the first two days of treatment. The second case involved a subadult male that presented extensive facial and carapace lesions with inflammation and swelling. Initial therapy consisted of wound cleaning with hydrogen peroxide and povidone iodine, and IM administration of 0.5 mg/kg dexamethasone and 15000 IU penicillin with 20 mg dihydrostreptomycin (Fatromicina S, Laboratorio Fatro S.p.A., Bologna, Italy). Trimethoprim-sulfa was then injected once daily IM at a dose of 20 mg/kg until the results of the laboratory analyses were obtained. IM application of 10 mg/kg ketamine (Ketamina 50, Holliday-Scott S.A., Buenos Aires, Argentina) and 0.1 mg/kg diazepam (Diazepan Lamar, Laboratorios Lamar, Buenos Aires, Argentina) was ineffective to induce anesthesia for extraction of a skin biopsy. Inhalatory anesthesia was therefore induced with 8% sevoflurane (Sevorane, Abbott Laboratories Argentina, S.A., Buenos Aires, Argentina) and maintained at 3% until biopsies could be taken from the facial lesions under local anesthesia with lidocaine 2% (Lidocaína Enco, Laboratorios Enco, Mendoza, Argentina). The hematological analyses revealed that the animal was anemic and had a leukocytosis with an elevated percentage of band neutrophils. Cytology and histopathology suggested a chronic ulcerative dermatitis with bacterial colonization and granulation tissue formation. Bacteriological culture revealed that the carapace lesions were infected with *Pseudomonas*, while *E. coli* was isolated from the facial lesions. Antibiotics were changed to 40 mg/kg ciprofloxacin (Ciprofloxacina Lazar, Dr. Lazar y Cía S.A.Q.e I., Carapachay, Argentina) *per os* SID during 10 days according to the antibiogram. Due to prolonged bleeding after IM injections, 2 mg/kg menadione was administered IM during 5 days. The wounds were completely healed 2 weeks after the first intervention and the animal was released in its enclosure.

On August 22, 2005, an adult male was found lying aboveground close to its burrow entrance. It was apathetic, anorectic and icteric with a body temperature of 19 °C. Hematological and serum chemistry analyses revealed a PCV of 29%, presence of nucleated RBC, yellow plasma, 19100 WBC/µl, 41% neutrophils, 9% band neutrophils, 28% lymphocytes, 4% monocytes, 18% eosinophils; elevated hepatic enzymes (ALT 90 U/l, AP 54 U/l), and high BUN levels (110 mg/dl). The animal was quarantined, and therapy was initiated with 1.25 mg/kg enrofloxacin IM BID. Three days later, the animal was less apathetic, but it remained icteric and still refused to eat; leukocytosis had increased to 24000 WBC/µl, percentage of neutrophils was now 77%, and the hepatic enzymes remained elevated (ALT 150 U/l, AP 70 U/l). Antibiotic therapy was changed to 50 mg/kg amoxicillin (Clamoxyl L.A., Pfizer S.R.L., Buenos Aires, Argentina). Subcutaneous hemorrhages were observed on the icteric abdominal skin 6 days after the first symptoms; supportive treatment was therefore initiated with 2 mg/kg menadione IM during 5 days, and IM injections of 30 mg/kg iron (Suiferro Fuerte, Chienfield S.A., Munro, Argentina) and 0.2 ml/kg Vitamin B complex (Pura B, Holliday-Scott S.A., Buenos Aires, Argentina). Blood values on September 1st revealed a PCV of 20%, regenerative neutrophilia with 34850 WBC/µl, abundant nucleated RBC, and hepatic enzymes and BUN within normal levels (AST 12 U/l, ALT 8 U/l, AP 22 U/l, BUN 14 mg/dl). A marked hematuria and yellowish diarrhea were present in addition to the persisting jaundice, but the animal was more active than in the previous days and, for the first time since it had been quarantined, eagerly ate the offered food. The liver parenchyma did not present visible alterations during ultrasound examination, but the gall bladder could not be identified. Coproparasitological analysis revealed a low infestation with coccidia and *Aspidodera*. Doxicyclin (Vibramicina, Pfizer SRL, Buenos Aires, Argentina) was administered *per os* SID during the next 10 days at a dose of 10 mg/kg. On September 5, the PCV had returned to normal levels (38%), and the jaundice regressed gradually. The patient was released in its enclosure on September 15.

Dead pichis

Tissue samples were collected from 173 dead pichis. The tissues of 18 pichis were not analyzed histologically due to advanced autolysis. The gender of five individuals could not be determined because their reproductive organs had been extracted by poachers; these animals were excluded from further analyses. Consequently, results are reported for a total of 150 pichis (87% of sampled dead animals). Of these, 120 had been confiscated from poachers, 10 were hit by cars, 10 drowned in captive conditions after intense rainfall that flooded their enclosures, and 10 died for other reasons, including lack of adaptation to captive conditions (1 juvenile male), hypothermia (1 adult female), and unknown causes (8 individuals; Table 22).

Table 22: Cause of death of wild and captive Zaedyus pichiy *included in the histopathological study.*

	Males			Females			
	Juvenile	Yearling	Adult	Juvenile	Yearling	Adult	Total
Poached	4	12	30	9	23	42	120
Hit by car	1	0	6	1	0	2	10
Drowned	0	1	5	1	1	2	10
Other	0	1	2	4	0	3	10
TOTAL	5	14	43	15	24	49	150

The study animals were not examined for macroscopic external lesions because most pichis presented extensive destruction of the carapace either due to dog bites or superficial burning (poached individuals) or because they were hit by a car. Macroscopic lesions on internal organs were rarely observed. Several lactating females and one adult male presented enlarged adrenal glands. The liver was yellowish or ochre in 5 adults and 1 yearling. An enlarged, sometimes granulated spleen was observed in 7 animals. Three adult males and 1 juvenile female had macroscopic lesions on the heart consisting of a whitish apex or a whitish spot on the ventricle. The heart was dilated in 3 adults. One juvenile male presented a circular lesion of 0.9 x 1.3 cm with myiasis on the right leg, one juvenile female had a subcutaneous abscess caudal to the right elbow, and one adult female had a round lesion on the cecum and enlarged intestinal lymph nodes.

Histopathological analysis revealed microscopic lesions in almost all studied pichi lungs (98% of studied animals) and over 75% of all examined skeletal muscles, hearts, and gastrointestinal tracts, while female reproductive organs and spleens rarely presented microscopic lesions (Table 23). In general, the prevalence of affected organs was similar in male and female pichis, as well as in individuals of different age classes. Lesions of the adipose tissue were more common in adult males than in females of the same age class ($p<0.0001$, Fisher's exact test), while the latter had significantly more hepatic lesions than the former ($p<0.05$).

Table 23: Organs affected by microscopic lesions in juvenile, yearling and adult male and female Zaedyus pichiy. *The first number indicates the quantity of individuals presenting microscopic alterations, while the second number indicates the total of analyzed samples. Note that not all organs were available for sampling in all 150 examined individuals. Superscripts mark significant differences. Symbols* * *and* $^{+}$*: p<0.05 (Fisher's exact test); symbol* $^{\#}$ *: p<0.05 (Chi-square test); symbol* ****: p<0.0001 (Fisher's exact test).*

	Males			Females			
	Juvenile	Yearling	Adult	Juvenile	Yearling	Adult	Total
Skin	2/4	6/8	13/26	6/11	6/12	12/28	45/89
Skeletal muscle	3/5	13/14	29/41	10/15	19/24	37/46	111/145
Adipose tissue	0/4	3/13	$16/40^{***}$	3/15	1/24	$2/45^{***}$	25/141
Heart	1/4	9/13	28/37	8/12	17/20	34/43	97/129
Lungs	4/4	14/14	39/40	11/12	20/20	41/42	129/132
Lymph node	0/3	0/5	2/16	0/6	2/8	5/21	9/59
Liver	1/4	$4/9^{*}$	$18/30^{+}$	$6/12^{\#}$	$11/12^{*\#}$	$33/39^{+\#}$	73/106
Kidney	0/5	2/14	14/35	2/14	7/22	14/40	39/130
Ureter	0/1	3/4	8/14	3/7	7/9	15/18	36/53
Spleen	0/2	0/5	1/17	0/7	1/8	0/17	2/56
Tongue	0/1	1/6	3/17	2/6	1/11	4/14	11/55
Stomach	0/1	0/2	1/5	1/2	0/2	1/3	3/15
Intestine	1/1	1/1	7/11	3/3	5/5	10/12	27/33
Colon	1/1	2/2	4/6	1/3	7/7	5/8	20/27
Adrenal glands	0/4	0/8	3/21	0/7	0/13	5/31	8/84
Testes	0/3	2/7	3/20				5/30
Ovary				0/3	0/17	0/22	0/42
Uterus				2/10	0/12	1/25	3/47

Hepatic lesions were also more common in yearling females than in males of the same age class ($p<0.05$), and their prevalence was significantly different among age classes of female pichis ($p<0.05$, Chi-Square), with adults being more affected than juveniles. The most common microscopic lesions were inflammations of the gastrointestinal tract, varying degrees of pulmonary inflammation, signs of pulmonary congestion, edema, hemorrhage, and atelectasis attributed to acute pulmonary shock, rhabdomyolysis of the skeletal muscle, and cardiac necrosis (Table 24).

Table 24: Prevalence of the predominant lesions per organ system in juvenile, yearling and adult male and female Zaedyus pichiy *observed during histopathological analysis.* [1] *indicates histopathological findings associated with parasitism or parasite migration.* [2] *indicates histopathological findings possibly associated with parasitism or parasite migration. Note that one individual may have presented several lesions in one organ, and that not all organs were available for sampling in all individuals. See Table 23 for numbers of analyzed tissue samples per organ and sex/age group.*

	MALES			FEMALES			ALL
	Juvenile	Yearling	Adult	Juvenile	Yearling	Adult	PICHIS
SKIN							
Dermatitis [1]	25%	62.5%	46.2%	36.4%	41.7%	42.9%	43.8%
Abscess	25%	0	0	0	0	0	1.1%
Burn artifacts	0	25%	0	27.3%	8.3%	3.6%	7.9%
Intrafollicular mite [1]	0	12.5%	0	0	0	0	1.1%
SKELETAL MUSCLE							
Rhabdomyolysis	80%	7.1%	58.5%	66.7%	62.5%	73.9%	66.9%
Inflammation	0	0	7.3%	0	8.3%	8.7%	6.2%
Sarcocystis cysts [1]	0	14.3%	9.8%	0	20.8%	8.7%	10.3%
Microgranuloma [1]	0	0	2.4%	0	41.7%	2.2%	2.1%
Necrosis	0	0	4.9%	0	12.5%	0	3.4%

Table 24 continued

	MALES			FEMALES			ALL
	Juvenile	Yearling	Adult	Juvenile	Yearling	Adult	PICHIS
HEART							
Myocarditis [2]	0	15.4%	5.4%	0	10%	4.7%	8.5%
Microgranuloma [1]	0	0	0	0	5%	2.3%	1.6%
Sarcocystis cysts [1]	0	0	2.7%	0	0	0	0.8%
Necrosis	25%	61.5%	67.6%	58.3%	75%	72.1%	67.4%
ADIPOSE TISSUE							
Inflammation	0	7.7%	0	0	4.2%	2.2%	2.1%
Microgranuloma [1]	0	0	2.5%	0	0	2.2%	1.4%
LUNGS							
Inflammation [2]	75%	92.9%	87.5%	58.3%	85%	83.3%	83.3%
Besnoitia cysts [1]	0	7.1%	22.5%	25%	25%	14.3%	18.2%
Microgranuloma [1]	25%	143%	15%	16.7%	20%	28.6%	20.5%
Bacterial emboli / abscess	0	0	2.5%	8.3%	0	0	1.5%
Acute pulmonary shock	75%	92.9%	82.5%	41.7%	90%	78.6%	79.5%
LIVER							
Periportal lymphocytic inflammation [2]	25%	44.4%	46.7%	33.3%	91.7%	79.4%	61.3%
Other types of hepatitis [2]	0	0	3.3%	16.7%	0	2.6%	3.8%
Hemosiderosis	0	0	13.3%	0	0	5.1%	5.7%
Microgranuloma [1]	25%	0	3.3%	0	0	2.6%	2.8%
KIDNEY							
Pyelonephritis [2]	0	21.4%	11.4%	7.1%	18.2%	15%	13.8%
Chron. interstitial nephritis	0	0	0	7.1%	4.5%	7.5%	3.8%
Microgranuloma [1]	0	0	2.9%	0	0	0	0.8%
Tubular mineralization	0	0	8.6%	0	0	20%	8.5%
Tubular necrosis	0	0	5.7%	0	0	2.5%	2.3%

Table 24 continued

	MALES			FEMALES			ALL
	Juvenile	Yearling	Adult	Juvenile	Yearling	Adult	PICHIS
URETER							
Inflammation [2]	0	75%	57.1%	42.9%	66.7%	83.3%	66%
SPLEEN							
Besnoitia cysts [1]	0	0	5.9%	0	0	0	1.8%
TONGUE							
Inflammation [2]	0	16.7%	11.8%	33.3%	0	28.6%	16.3%
Sarcocystis cysts [1]	0	0	0	0	0	7.1%	1.8%
Microgranuloma [1]	0	0	5.9%	0	0	0	1.8%
INTESTINE							
Enteritis/Gastritis [2]	100%	100%	63.6%	100%	100%	83.3%	93.9%
Protozoan cysts [1]	0	0	0	0	0	8.3%	3%
ADRENAL GLANDS							
Focal inflammation	0	0	0	0	0	3.2%	1.2%
Shock	0	0	9.5%	0	0	3.2%	3.6%
Nodular hyperplasia	0	0	4.8%	0	0	3.2%	2.4%
REPRODUCTIVE ORGANS							
Endometritis / orchitis [2]	0	14.3%	15%	20%	0	0	7.3%
Microgranuloma [1]	0	14.3%	0	0	0	0	1.2%
OTHER TISSUES							
Peritonitis						3	3
Mediastinitis						2	2
Inflammation around large nerve			1			1	2

Discussion

Hematology and serum chemistry

The present study provides the first hematological and serum chemistry reference values for wild-caught and captive-kept *Zaedyus pichiy* of different genders and age classes. The number of biochemical parameters that could be determined per animal was limited due to several difficulties related to blood collection. The small diameter of the coccygeal vein and the rapid clotting prevented the extraction of large quantities of blood. In addition, capture stress and low environmental temperature often seemed to reduce blood flow in this vein, thus impeding blood extraction. Nevertheless, puncture of the coccygeal vein was considered the method of choice for sample collection because it did not require chemical restraint of the animals. Although the saphenous vein can be used for cannulation in anesthetized armadillos (Moore 1983), it is difficult to puncture in unsedated pichis because it often rolls away from the hypodermic needle and cannot be seen or palpated in obese individuals. The cephalic vein is not easy to puncture in armadillos because the carapace, the short forearm, and the long elbow make it difficult to immobilize the leg and apply a tourniquet or digital pressure. The jugular vein has been described as a less convenient venipuncture site in armadillos because it cannot be located visually or by palpation and often collapses during aspiration (Moore 1983), while cardiac puncture is unacceptable in live animals because it damages the myocardium and can lead to death.

A comparison of blood values of pichis and other species is difficult because different studies use varying sampling and analytic techniques. The RBC count of pichis was much lower than in other mammals, while the mean cell volume (MCV) was almost twice the average MCV of 288 mammalian species, but lower than in giant anteaters (Gascoyne and Hawkey 1992). These differences were not unexpected because the size and number of red blood cells vary considerably across taxa (Gascoyne and Hawkey 1992). When compared to other armadillos, the RBC count was slightly higher than in *C. villosus* (Casanave and Polini 1999) and *D. septemcinctus* (Coppo et al. 1979), but lower than in *D. hybridus* (Cuba-Caparó 1976).

The PCV was significantly higher in males than females, in adults than in juveniles, and in wild pichis than in captive individuals (Table 18). The reason for the former is not known, but may be related to behavioral differences, such as males having larger home ranges than females and thus suffering higher degrees of dehydration and hemoconcentration due to the low water availability in their environment. Age differences in the PCV are normal in domestic mammals, with newborn usually having high RBC and PCV values that fall rapidly during the first weeks and gradually increase starting around the second month of life until adult levels are reached at about one year of age (Jain 1993). In contrast to their wild conspecifics, captive animals had free access to water, which may have prevented dehydration and hemoconcentration, leading to a lower PCV.

The low WBC count in wild and captive pichis was striking. Total WBC count varies greatly among species and is influenced by stress, diseases, and allergic reactions. Pichis had a considerably lower WBC count than any other studied armadillo species (*C. villosus*, Casanave and Polini 1999; *D. septemcinctus*, Coppo et al. 1979; *D. hybridus*, Cuba-Caparó 1976; D'Addamio et al. 1978; *D. novemcinctus*, Purtilo et al. 1975). The WBC count was also lower than in most other mammalian species, except for a few non-domestic ruminants (Hawkey 1977; Jain 1993). Neutrophils may be proportionally higher during acute infections, while lymphocytes typically increase proportionally during chronic infections (Kraft and Dürr 2000). The neutrophil : lymphocyte ratio is usually not calculated during routine evaluations of individual animals, but it is a good indicator to compare the relationship between these white blood cells among species. It was similar in *Z. pichiy* (2.0) and *D. septemcinctus* (1.9, Coppo et al. 1979), higher in pichis than in *C. villosus* (1.42, Casanave and Polini 1999) and *D. hybridus* (1.71, Cuba-Caparó 1976), while *D. novemcinctus* had a neutrophil : lymphocyte ratio of 2.26 to 2.54 (D'Addamio et al. 1978; Purtilo et al. 1975). It is possible that the neutrophil : lymphocyte ratio of captive pichis was lower (1.29) because they were used to human presence and handling. Presumably, capture and sampling were stressful events for wild pichis that led to an increased release of catecholamines and a consequent increase in neutrophils, thus leading to a shift of the neutrophil : lymphocyte ratio. Eosinophil counts were higher in females than in males, but the reason for this difference is unknown.

Mean BUN levels of pichis were almost three times higher than in *D. septemcinctus* (Coppo et al. 1979), but half the levels found in wild *D. novemcinctus* (Ramsey et al. 1981). Urea is mainly excreted through the kidney and can be used as an indicator of renal function. It may be elevated if blood sampling is performed after food intake, due to a high protein diet, or because of a reduced excretion via urine (Kraft and Dürr 2000). Elevated BUN levels in pichis may have been related to a combination of these factors. Samples were extracted from wild-caught animals that were probably foraging at the time of capture and thus may have had elevated postprandial BUN levels. Furthermore, pichis have been classified as carnivores-omnivores (Redford 1985), but seem to feed predominantly on invertebrates, grubs and plant material in Mendoza Province (see Chapter 3). Regardless of their exact diet composition, the BUN levels are expected to be high because both insectivore and carnivore diets are rich in protein and a high protein diet leads to elevated BUN levels (Dunbar et al. 1997; Fuller et al. 1985; Oyarzun et al. 1996). Finally, the low water availability in their natural habitat may require the pichis to minimize urine excretion to conserve body water, and a concomitant reduced urea excretion would lead to high BUN levels.

Pichis presented higher calcium and albumin levels than seven-banded armadillos (Coppo et al. 1979), but calcium levels were comparable to those reported for wild *D. novemcinctus* by Ramsey et al. (1981). Protein levels of *Z. pichiy* were within the range reported for wild *D. novemcinctus* (Ramsey et al. 1981) and for wild *D. septemcinctus* (Coppo et al. 1979). Depending on the species, the alanine aminotransferase (ALT) or aspartate aminotransferase (AST) is more appropriate to evaluate hepatic function (Kraft and Dürr 2000), but further studies need to be performed in pichis to determine the relevance of each hepatic enzyme for clinical evaluations. Wild pichis had lower ALT levels than captive nine-banded armadillos (Giacometti et al. 1972) and wild seven-banded armadillos (Coppo et al. 1979). The values obtained for AST were lower than levels given for wild nine-banded armadillos by Ramsey et al. (1981), and for *D. septemcinctus* by Coppo et al. (1979). Alkaline Phosphatase is usually elevated during growth phases, i.e., in juvenile animals, and in cases of cholestasis (Kraft and Dürr 2000). Alkaline phosphatase (AP) was higher in pichis than in *D. septemcinctus* (Coppo et al. 1979).

Health status of wild pichi populations

In general, the studied wild populations were considered to be in good health; they were mainly affected by the consequences of parasite infestation. The health problems observed in captive pichis will be discussed in the same chapter as the pathologies of their wild conspecifics because several symptoms occurred in both groups.

The exact time of death and origin of the confiscated pichis were often not known. The collected tissues of some individuals were therefore in varying stages of autolysis, and not all samples were appropriate for histological analysis. Nevertheless, the sampling method is considered to be an ethically justifiable means to perform population studies on endangered species that require extraction of tissue samples. Furthermore, it is often the only legally acceptable means to obtain samples from protected wildlife. Two caveats should be considered, however, when using poached animals for population studies. First, the sampled animals may not constitute a representative subsample of a wild population because reproductive status or parasite infestation may have changed their activity or altered their behavior, thus increasing or decreasing their predation or poaching risk. Second, the total quantity and temporal distribution of samples may depend on poaching activity, as well as the frequency and success of anti-poaching patrols. For instance, poachers are rarely active during the reproductive season of wild pichis because of the common belief that eating reproductively active pichis causes indigestion, while poaching during hibernation season is restricted to the warmer northern parts of Mendoza Province, where chances are higher that a pichi emerges during the day than in southern Mendoza. It may therefore be difficult to obtain similar numbers of samples from all seasons or all reproductive stages.

Body temperature

Body temperature was low and relatively variable, as expected for xenarthrans (McNab 1985). In this study, female pichis had similar rectal temperatures to males, while Burns and Waldrip (1971) reported that male *D. novemcinctus* had higher body temperatures than females. Johansen (1961), on the other hand, did not find any difference related to gender in *D. novemcinctus*. No correlation was found between ambient and body temperature. Although rectal temperature was measured as soon as possible after the animals were caught in the wild, it is possible that it was elevated because of stress related to capture and handling. The rectal temperature of a wild hibernating male was higher than temperatures measured in other torpid pichis, which lowered their body temperature to an average of 14.6 °C (see Chapter 2). Because torpid body temperature seems to depend on burrow temperature (Chapter 2), it is possible that this male's torpid temperature setpoint was higher than in the other animals because of a higher soil temperature.

Trauma

Fresh lesions and scars were observed in a large proportion of wild-caught pichis. In males, skin and carapace lesions, and lesions and abscesses on the penis may have been caused by intraspecific fights during the reproductive season. Territorial fights have been observed both in wild and captive male armadillos and have led to severe injuries of captive pichis. Both affected captive males were maintained in individual pens, with other males living in contiguous enclosures. While interactions between captive males were rare outside the breeding season, gonadally competent males often reached through the mesh fence with their forelegs in an attempt to fight with a potential rival. Due to their semi-fossorial habits, soil adhered to the moist lesions of injured pichis that retreated into their burrows, thus providing an ideal environment for bacterial growth. Nevertheless, because males did not present more fresh lesions or scars than females, territorial fights are probably not the main cause for the high prevalence of these affections. Some lesions clearly had been inflicted by foxes and birds of prey, and others may have been related to the pichi's flight behavior and its semi-fossorial habits. While their osseous carapace is not an efficient protection against predators – both the teeth of mammalian predators and the claws of birds of prey can easily fracture it –, it allows them to seek shelter and start digging a new burrow under dense, sometimes thorny shrubs where their predators cannot reach them. Although thorns do not penetrate the carapace, they can cause lesions between the carapace bands. Indeed, 20% of the fresh lesions and over 25% of scars affected the carapace bands or the skin folds between bands. A large proportion of scars consisted of missing or worn-out scutes on the head shield and its superior border (18%) or on the carapace border (15%). Unlike other armadillo species, the carapace border of pichis consists of sharply pointed, sometimes hook-like scutes. When foraging or digging a burrow, pichis usually start moving the soil with their head. Sustained contact of the head shield with sand and stones may lead to abrasion and the worn-out aspect of scutes observed in many animals. In addition, pichis often dig their burrows under shrubs. Presumably, this allows them to hide the burrow entrance, while the roots provide support and reduce the risk of the burrows collapsing. It is possible that the loose upper border of the head shield and the scutes of the carapace border sometimes get caught in a root or another obstacle while the animal is backing up, leading to injuries or fracture of scutes.

Infected wounds were rare although fresh lesions were relatively common in wild pichis. Other than the abscess noted on the base of the penis of an adult male, only one adult male had infected lesions, but this individual had not been caught in the wild for this study. Prior to its confiscation from poachers, the pichi had been severely injured by a hunting dog and was agonizing in a bag for several days. Its recovery required extended surgical intervention and over a month of daily treatment. Most of the other observed lesions were superficial and probably healed rapidly. Only four individuals presented leukocytosis with neutrophilia, indicating an infection. No external lesions were observed in three of these animals, while one pichi with increased numbers of band neutrophils only presented a superficial wound on the left front leg; the infections thus probably affected one or more internal organs. It is possible, however, that infections are more frequent in wild pichis than observed in this study but remained undetected. If infection induces behavioral changes, such as reduced activity or pichis remaining in their burrows until they recover, their capture and sampling rate would be reduced in comparison with healthy individuals. Alternatively, pichis with infected lesions or generalized infections may be an easy prey for predators and therefore be removed from the population at an early stage of disease.

Lesions related to the cause of death

Several organs presented lesions that were attributed to the stress and shock caused by the capture of pichis by poachers and their dogs. In the skeletal muscle and tongue, the acute stress response was manifested as rhabdomyolysis, while mild myocardial necrosis related to stress or shock was the most common finding in the cardiac muscles. Capture stress or trauma, such as hunting dog bites, caused an acute pulmonary shock with varying degrees of congestion, edema, hemorrhage, and atelectasis in the lungs of several animals. A few pichis presented renal tubular necrosis, which was attributed to hypoxia associated with shock, although it cannot be excluded that these lesions were caused by endotoxemia. The acute shock also led to mild congestion or hemorrhage in the adrenal glands of three adult pichis, while two adults presented a mild nodular hyperplasia that may have been the result of previous episodes of stress or aging.

Many skin samples had burn artifacts, such as edema and clots, associated with poaching. Poachers often hold the killed and eviscerated pichis over an open flame for half a minute or so to avoid decomposition of their prey until the end of their poaching expedition, which can last several days. Nevertheless, these burn artifacts did not significantly impede the microscopic interpretation of skin samples.

Pathologies related to water availability

Both excessive humidity and a reduced availability of water in the pichi's environment have been found to cause negative effects in wild pichis. The state of dehydration observed in one adult male, the tubular mineralization of the kidneys of 11 adult animals that were evaluated histologically, and the elevated PCV and BUN levels mentioned above may be explained by the low precipitation rates and absence of open water sources in the natural habitat of pichis. Elevated ambient humidity, on the other hand, has been reported to cause disease in wild populations and has caused cutaneous lesions in captive pichis. Several captive individuals developed a moist dermatitis with epidermal detachment while being maintained in a humid environment. The lesions were infected with bacteria that are commonly found in soil. In humans, prolonged water contact increases the permeability of the skin's stratum corneum and leads to formation of intercellular pools of water within the stratum corneum (Warner et al. 2003), which, among other effects, facilitates bacterial colonization. Furthermore, moderate lateral friction is sufficient to cause skin abrasion after prolonged exposure to water in humans (Willis 1973). Considering that pichis live in arid environments where extended skin contact with water is rare, it is possible that their epidermis is more susceptible to prolonged exposure to humidity than humans. Presumably, the temporary maintenance of some pichis in 200 liter barrels, in which the substrate may have retained humidity due to a reduced exposure to sunlight, led to an increased permeability of the skin. Epidermal detachment may then have occurred during intense contact of the macerated stratum corneum with the abrasive substrate. These symptoms have not been observed in wild pichis during this study, but several inhabitants of rural areas in Mendoza Province reported that a disease locally known as “pichi plague”, characterized by red spots on the skin covering large abscesses, has led to local extinctions. The locals consistently associated this disease with extended periods of rainfall. Consequently, it is possible that the “pichi plague” is caused by bacterial colonization of the macerated subcutaneous tissue with an opportunistic pathogen.

Parasitism

Over 90% of all evaluated pichis were infested with intestinal parasites (Table 20, Table 21). The large majority were nematodes – especially trichostrongylids – followed by protozoans and cestodes. The most common species were *Aspidodera fasciata* and *A. scoleciformis* (Heterakoidea). Adult specimens of this genus were also regularly observed in fecal matter of *Z. pichiy* collected for other studies. *Aspidodera* are common parasites of armadillos and seem to have a direct life cycle (Cavalcanti Proença 1937; Chandler 1946; Fujita et al. 1995; Navone 1986). Other *Trichostrongylidae* that could not be further identified were present in 15 examined pichis. A wide variety of trichostrongylids have been described in armadillos, many of which seem to be specific parasites of armadillos (Durette-Desset 1970; Navone 1987; Talmage and Buchanan 1954). Nematode eggs of the genus *Trichuris* were found in two pichis. Reports of infestation of wild armadillos with *Trichuris* are rare and limited to Talmage and Buchanan (1954) who mention that *Trichuris subspiralis* has been recorded in *Dasypus hybridus*, *Tolypeutes tricinctus* and *Cabassous unicinctus*. *Trichuris* eggs are sometimes observed in armadillos kept in captive conditions (Superina 2000). *Cyclobulura* eggs were not common, but adults of this parasite were observed in the cecum or large intestine of three evaluated pichis. *Cyclobulura lainsoni* is the only species of the subfamily Labiobuluridae that has been described for a xenarthran. It has been isolated from silky anteaters (*Cyclopes didactylus*) from Belem, Brasil (Quentin 1977) and differs morphologically from the species observed in pichis. *Cyclobulura* of *Z. pichiy* therefore constitutes a new parasite species.

Eimeria oocysts were identified in 32 of 54 analyzed fecal samples. The presence of oocysts of varying size suggests that pichis are hosts of several *Eimeria* species. *Isospora* oocysts were less common and require further investigation because no other armadillo species has been found to carry parasites of this genus. The category of unidentified coccidia oocysts mentioned in Table 20 includes oocysts of different diameters. Some of them are probably parasites that do not infest pichis but are present in their gastrointestinal tract due to ingestion of the parasite's invertebrate host.

Tapeworm eggs or fragments were present in 5 fecal samples. In three cases, the eggs could be identified as *Mathevotaenia* sp., a cestode genus that parasitizes rodents, insectivores, xenarthrans, marsupials, and primates (Navone 1988). Further identification of the cestode eggs was not possible in one case, and one fecal sample contained proglottids of a tapeworm of the subfamily Hymenolepidinae, but the absence of a scolex precluded a more precise identification of the parasite.

The fleas collected from wild *Z. pichiy* were identified as *Malacopsylla grossiventris* and *Phthiropsylla agenoris.* Both species are commonly found on armadillos (Mauri and Navone 1993). The presence of *Amblyomma pseudoconcolor* in a pichi represents a new host record and extends the area of distribution of this hard tick (Superina et al. 2004).

The large majority of histopathological findings were associated with parasitism or parasite larva migration (Table 24). The most prominent skin lesions involved perivascular inflammation consistent with hypersensitivity, which sometimes extended to the adipose tissue. In wild mammals, this lesion is most often associated with parasitism or arthropod bites. In the pichis, it was most likely due to parasitism by fleas, ticks, and mites. Only a single intrafollicular mite was found in one of the armadillos, but these ectoparasites can be difficult to demonstrate histologically, especially when they are present in low numbers.

Parasitism was the most probable cause of gastritis, as well as of the mild to moderate, chronic enteritis and colitis observed in several pichis. Occasionally, this process was associated with protozoa – mainly coccidia – or nematodes. Microgranulomas associated with migrating nematode larvae were noted in sections of skeletal muscle, adipose tissue, heart, tongue, lungs, liver, kidney, and reproductive organs. It is not clear which nematode species caused these lesions during larval migration because no larvae were observed in any tissue sample. *Aspidodera* are the most common intestinal parasites of pichis (Table 20), but larval migration has not been reported in this genus, and none of the other Heterakoidea species studied to date seems to have a larval phase outside the gut (Read and Skorping 1995). Larval migration occurs in some, but not all trichostrongylid nematodes (Read and Skorping 1995), but further studies are needed to determine whether the development of the *Trichostrongylidae* that parasitize armadillos includes a tissue phase.

Several other organ lesions may have been related to parasite migration. The most common finding in the liver was mild periportal lymphocytic inflammation consistent with a low grade, ascending inflammatory process of the biliary tree. This lesion is often caused by biliary tract parasitism, but ascarid ova were only observed in one examined animal with a portal inflammatory lesion. Other types of hepatic inflammations were rare and affected only 4 animals; they were probably associated with low-grade sepsis or parasite migrations. Two thirds of all evaluated ureters were affected by inflammation involving the adventitial and muscular tunics of the ureter and the surrounding connective tissue or adipose tissue. The eosinophil component of the inflammatory response suggests parasitism, and migrating nematode larvae were observed directly adjacent to the inflamed ureter of one individual. No luminal parasites were seen in any of the examined pichi ureters, but it is possible that parasites ascend through the lumen to the kidney and cause a lymphocytic pyelonephritis, as observed in 18 pichis. The mild eosinophilic inflammation observed in the testicular and spermatic cord tunics of some males were consistent with parasite migration.

Several animals had *Sarcocystis* cysts in the skeletal muscle, which were sometimes associated with mild lymphocytic inflammation in the muscle, presumably due to rupture of the cysts. It is probable that the mild lymphocytic inflammation observed in the tongue and the myocardium of several animals was caused by rupture of protozoan cysts, although the same were only noted in the tongue of an adult female and in the heart muscle of an adult male. Several *Sarcocystis* species have been identified in the skeletal muscle and tongue of North and South American armadillos (Howells et al. 1975; Lindsay et al. 1996; Tanhauser et al. 2001), and the prevalence can be as high as 100% (Lindsay et al. 1996). The nine-banded armadillo has been identified as a naturally infected intermediate host of *Sarcocystis neurona* (Cheadle et al. 2001; Tanhauser et al. 2001). Opossums are definitive hosts of *S. neurona* (Fenger et al. 1995), but the definitive hosts of the other *Sarcocystis* species infecting armadillos, including pichis, are not known. Humans may serve as intermediate or definitive hosts for a variety of species of this coccidian parasite (Greve 1985). Nevertheless, the zoonotic potential of the species affecting wild pichis is considered to be low because hunters and consumers usually only eat well-cooked pichi meat, while a transmission would require ingestion of raw or undercooked musculature. Furthermore, the intermediate, as well as the definitive hosts of each *Sarcocystis* species are considered to be specific or limited to closely related species (Fayer 2004). The evolution of a two-host life cycle involving pichis and humans would require that humans often ingest armadillo meat and that pichis are in frequent contact with sporocysts shed in human feces, but the latter is unlikely considering the low density of human settlements within pichi habitat.

Besnoitia cysts were present in the lungs of 24 wild pichis. No other report on *Besnoitia* infection of any armadillo species or of a wild mammal species of Argentina has been found in the literature; the only previous report of *Besnoitia* from Argentina involved domestic rabbits (Venturini et al. 2002). This study therefore documents for the first time besnoitiosis in an armadillo and in a wild mammal native to Argentina. Pulmonary besnoitiosis was sometimes associated with acute inflammatory processes, and this protozoan or other parasites may have been the cause of the relatively high prevalence of chronic inflammation in the lungs. *Besnoitia* cysts were also present in visceral organs, although this finding was rare and limited to the spleen of one adult pichi. The indirect life cycle of *Besnoitia* includes a definitive host and an intermediate host that varies depending on the involved parasite species (Ayroud et al. 1995; Elsheikha et al. 2004; Glover et al. 1990). The *Besnoitia* species infecting wild pichis, as well as its definitive host, are unknown. Although carnivores have been described as definitive hosts of several *Besnoitia* species, it is not clear whether they are involved in the life cycle of all species (Ayroud et al. 1995).

Incidental findings

The macroscopic alterations were not always reflected in microscopic lesions. A focal lymphocytic inflammation was observed in one enlarged adrenal gland, but the remaining adrenals did not present any microscopic lesions. Presumably, the adrenals are enlarged in lactating pichi females because of an increase in corticosteroid production to maintain lactation (see Chapter 1). Alternatively, the adrenals may have been enlarged because of an increased corticosteroid production caused by chronic stress. The altered color of the liver corresponded to a periportal lymphocytic inflammation in three animals and hemosiderosis in two individuals, while no microscopic lesions were observed in an adult female with yellowish liver. Hemosiderosis is a condition in which hemosiderin, a pigment containing iron oxide, is deposited in tissues. In the studied animals, hemosiderosis was probably caused by a chronic inflammation associated with parasitism. No microscopic lesions were associated with macroscopically enlarged spleens. In the larger hairy armadillo *Chaetophractus villosus*, splenic hematopoiesis persists in adult animals (Galíndez et al. 1997). If the same is true for pichis, the enlarged spleens may be the result of an increased extramedullary hematopoiesis. The white granules noted on some spleens were interpreted as lymphoid tissue of the white pulp, which may have been activated due to a viral or bacterial insult. Although myocardial necrosis related to stress or shock was one of the most commonly observed microscopic alterations (Table 24), only three animals presented corresponding macroscopically recognizable whitish areas, while no microscopic finding was associated with the whitish heart base noted in an adult male. Similarly, no microscopic lesions were found in the dilated hearts, but dilated cardiomyopathy is sometimes not reflected in histopathologically identifiable alterations. The affected animals did not present any evidence of left- or right-sided cardiac insufficiency in lung or liver, respectively, and thus probably did not have clinical symptoms related to the observed cardiac dilation.

A bacterial abscess with sulfur granules suggestive of *Actinomyces* was observed in the lungs of an adult male that had been confiscated from poachers. Many *Actinomyces* species are commensal organisms of the oropharyngeal mucosa, and pulmonary infection may have occurred after aspiration of oropharyngeal secretions or via hematogenous dissemination (Smego and Foglia 1998).

Female reproductive organs were rarely affected by pathologic alterations. A mild endometritis was noted in two females, but the cause for this process could not be identified although parasitism or low grade bacterial infection are considered most likely candidates.

One captive adult male was affected by a severe hepatic problem with subcutaneous hemorrhages and anemia. The presumptive diagnosis of acute hepatitis was based on the massive leukocytosis, substantially elevated hepatic enzymes, and the lack of visualization of the gall bladder during ultrasound examination. Blood clotting abnormalities are common during hepatic diseases because of a reduced production of coagulation factors by the liver. In addition, spontaneous hemorrhages related to hypovitaminosis K are relatively common in captive armadillos having predominantly insectivore food preferences because the reduced ingestion of ants and termites in captivity leads to an insufficient vitamin K synthesis by intestinal bacteria (Superina 2000). Although this problem has not been reported for captive individuals of the genera *Chaetophractus* and *Zaedyus* (Superina 2000), the antibiotics used for treatment of the acute hepatitis may have negatively affected the intestinal bacteria, leading to a hypovitaminosis K that aggravated the coagulation problems and led to subcutaneous hemorrhages and anemia.

A juvenile male that had been rescued by rangers from a cattleguard presented a lesion of 0.9 x 1.3 cm on the right leg that was infested with screwworms. The animal was emaciated, refused to eat in captivity and died a few days later of inanition. Histological analysis of the wound revealed an abscess in the deeper dermis related to a penetrating wound. The origin of the lesion could not be established, but is probably related to the animal falling into the relatively deep cattleguard. Similarly, the cause of the subcutaneous abscess observed in a confiscated juvenile female could not be determined, and the animal died of inanition in spite of attempts to feed it artificially with a gastric tube. Although bacteria of the genus *Klebsiella* were identified in bacteriological culture, histopathology revealed that the necroulcerative lesion was infected with filamentous bacteria. Finally, an adult female that had been confiscated dead from poachers presented a round lesion on the cecum. Histopathological analysis suggests that the animal had suffered a cecal perforation that lead to peritonitis, lymphadenitis of the mesenteric lymph node, and sepsis. The cecum of pichis is relatively large and presumably hosts a large number of bacteria that aid in the enzymatic breakdown of ingested plant material. Although no foreign body was detected in the cecum content, it is possible that perforation occurred by an accidentally ingested sharp fragment, such as a thorn or a piece of wire.

Potentially zoonotic diseases

Trypanosoma cruzi

Trypanosoma cruzi infections were rarely observed although different techniques were used to screen wild pichis for this pathogen. The occurrence of this parasite in pichis was confirmed in Mendoza over 70 years ago (Mazza et al. 1936). In spite of this, the fact that pichis may carry the causative agent of Chagas disease is widely unknown among hunters. The life cycle of *T. cruzi* includes a mammalian host and an insect vector, usually a triatomine bug. Transmission to humans usually occurs through the inoculation of the bug's feces – which contain trypomastigotes, the infective form of *T. cruzi* – through wounds in the skin (mainly due to the bite of the triatomine bug) or through the mucosal surfaces of the face (Tyler and Engman 2001). Experimental studies in mice indicate that oral transmission of *T. cruzi* to humans through the ingestion of infected mammals might be possible (Camandaroba et al. 2002). Additionally, the hunter is inevitably in contact with the pichi's blood while killing it. Because parasites can persist in the blood at low levels even during chronic infection (Espinola Carvalho et al. 2003), direct transmission of trypanosomes from an infected animal to a hunter might also occur through contamination of the mucous membranes or small skin lesions with blood.

No lesions attributable to *T. cruzi* were observed in the histopathological analyses, and no trypanosomes were found in Giemsa-stained blood smears. Only two animals were found to be seropositive. Because the parasite may be periodically absent from the bloodstream during the chronic phase, the screening of blood smears would only have allowed identification of pichis in the acute stage of trypanosomiasis. Circulating antibodies, however, persist throughout the chronic phase of infection and in macaques they can be detected more than 16 years after infection (Espinola Carvalho et al. 2003). A positive serologic result only indicates that the animal has been exposed to the pathogen at some point, but not necessarily that it was infected at the time of sampling or that it had clinical symptoms. A comparison with the antibody titer of a second blood sample, extracted two weeks after the first one, would allow determining whether the pichis were seropositive due to previous exposure to *T. cruzi*, developing immunity after a recent infection, or in remission from an infection. One of the seropositive animals had been confiscated alive from a poacher. It was moribund at the time of confiscation and died a short time later; both blood and tissue samples were therefore available for analysis. The serological result could not be confirmed by histopathology, suggesting that the low titer may have been a false positive result. Alternatively, it is possible that armadillos infected with *T. cruzi* do not develop any lesions. This hypothesis is supported by Barr et al. (1991) who did not observe any macroscopic or microscopic lesions in an infected nine-banded armadillo. However, both this study and Barr et al. (1991) evaluated only one seropositive individual histologically. It is therefore difficult to determine whether the absence of lesions is normal in armadillos infected with *T. cruzi* or if these two infected individuals did not present any alterations due to a low pathogenicity of the agent, a recent infection, or another unknown factor. If *T. cruzi* does not cause lesions or amastigote nests in armadillo tissues, prevalence in wild pichi populations may be higher than determined in this study. The transmission risk from pichis to humans is difficult to evaluate until this last point is elucidated.

The probability of pichis dying rapidly after infection with *T. cruzi*, which would be reflected in low infection rates in wild populations, is expected to be very low. *Dasypus* was the first sylvatic host of *T. cruzi* to be identified; its description dates back to 1912 (Chagas 1912). A high prevalence of *T. cruzi* in wild armadillos has been reported in several studies and from different countries. Seropositive armadillos have been found, among other locations, in Florida (Irons 1971), the New Orleans area (Yeager 1982), Colombia (Barreto et al. 1985), Brazil (Chagas 1912), Argentina (Mazza et al. 1936), and Paraguay (Yeo et al. 2005). Four *Euphractus sexcinctus*, 3 *Dasypus hybridus* and 1 *Dasypus novemcinctus* from northeastern Argentina were seronegative for *T. cruzi* (Superina, unpublished data). The armadillo is considered a natural host and an important reservoir host for *T. cruzi* (Barretto and Ribeiro 1979; Fujita et al. 1994; Lainson et al. 1979; Yaeger 1988; Yeo et al. 2005), and armadillos may have played an important role in the evolution of the *T. cruzi* II lineage that predominates in the southern cone countries of South America and is associated with terrestrial sylvatic ecotopes (Yeo et al. 2005). Presumably, this lineage initially entered the domestic transmission cycle through the maintenance of (infected) armadillos in a domestic environment prior to using them as protein source, thus allowing peridomestic triatomine bugs to transmit the parasite from armadillos to humans (Yeo et al. 2005).

Toxoplasma gondii

It was surprising that none of the serologically or histologically examined pichis were positive for *Toxoplasma gondii*. A wide range of birds and mammals, including humans and felids, serve as intermediate hosts for *T. gondii*, in which the parasite reproduces asexually and forms cysts in different tissues (Kozubsky and Alfano 1996). The intermediate hosts may ingest sporulated (mature) oocysts when consuming plants or soil contaminated with the feces of felids, which are definitive hosts of this parasite (Kozubsky and Alfano 1996), or when feeding on soil invertebrates that can act as mechanical vectors of infective oocysts (Bettiol et al. 2000). Alternatively, pseudocysts and cysts might be ingested through the consumption of infected flesh or entrails (Gállego 1998; Kozubsky and Alfano 1996). All three ways of infection may be possible in pichis because their diet includes invertebrates and – to a lesser degree – carrion, they ingest a substantial amount of soil while foraging, and wild felids are common in their area of distribution.

Although the indirect hemagglutination test used here has a lower sensitivity than other serological tests, it is a widely used technique for epidemiological screenings (Kozubsky and Alfano 1996; Tenter et al. 2000). It cannot be excluded that some false negative results were obtained with this serological test, but it is noteworthy that the histopathological study of 150 pichis did not provide a single positive animal. Considering that *T. gondii* infestation has been determined in several other armadillo species (Forrester 1992; Ramírez et al. 1984; Sogorb et al. 1977), it is unlikely that pichis show the same vulnerability to this parasite as Australian marsupials, which present a very low seroprevalence due to their high mortality rates during primary infection (Obendorf et al. 1996). Although more pichis need to be studied to confirm the findings of the present study, the prevalence of *T. gondii* in wild pichi populations in Mendoza province, and the risk of zoonotic transmission to humans during evisceration and handling or ingestion of undercooked pichi tissues, seem to be low.

Conclusions

This study constitutes the first report of a health evaluation in free-ranging populations of *Zaedyus pichiy*. Necropsies and histological examinations of confiscated pichis and roadkills did not reveal any severe pathologies and suggest that the examined populations are in good health. Poaching is therefore considered to have a much higher negative impact on the wild populations than disease epidemics. Although the zoonotic risk related to poaching and consumption of pichis appears to be low, it should be considered that pichis were only tested for a few potentially zoonotic diseases. Additional studies are needed to evaluate the prevalence of other zoonotic pathogens, such as *Salmonella*, *Leptospira* or *Brucella.* The results reported here lay the foundation for future health assessments of wild pichi populations. They will be essential when disease epidemics are suspected to affect wild pichis, and should be taken into account when evaluating the cause of sudden population declines.

DISCUSSION

Introduction

The pichi *Zaedyus pichiy* is a small, solitary armadillo species of which not much was known prior to the initiation of this project. Charles Darwin collected several specimens during the voyage of H.M.S. Beagle and noted that pichis were “excessively numerous” on sandy plains in Patagonia (Keynes 2000). Neither historical nor current census data are available, but the sustained hunting pressure and low encounter rates during the present study in Mendoza Province, in central west Argentina, suggest that its wild populations have suffered significant declines since Darwin considered them to be excessively abundant.

After Darwin’s brief description of pichis, the knowledge on the natural history of this armadillo species remained virtually the same for almost 150 years. Research on pichis is difficult because their semi-fossorial habits hinder a direct observation of wild individuals, which may explain the scarcity of scientific data on this species. Capture and sampling is further complicated by their solitary habits and an apparent low density. The present study aimed at elucidating some aspects of the natural history of *Z. pichiy* as a first step towards a conservation plan for this species. The research involved wild pichis and individuals kept in semi-captive conditions and combined direct observations with laboratory techniques.

Natural history of pichis

Z. pichiy is endemic to central and southern Argentina and Chile (Wetzel 1985b). This solitary, diurnal mammal inhabits uneven, sandy terrain and sand dunes, but is usually absent from clayey grounds. Several captive pichis that were housed temporarily in a humid environment developed skin diseases, which suggests that the precipitation rate may be an important factor limiting their distribution. Pichis have low rates of heat production, a high thermal conductance, and body temperatures that are low, variable and highly sensitive to changes in ambient temperature (McNab 1980, 1985; Roig 1971b). Their semi-fossorial habits help them coping with daily temperature fluctuations, but this study documents for the first time that they are also capable of entering daily torpor and hibernation. Presumably, they close the burrow entrance as a protection against predators and to prevent excessive cooling (in winter) or heating (in summer) of the burrow. The depth of their den may vary according to climatic conditions. For instance, pichi burrows in southern Mendoza (36° S, 69° W) were as deep as 1.5 m during winter, while captive pichis kept in open enclosures in Luján de Cuyo (33.0° S, 68.9° W) hibernated at 80 cm depth and burrows excavated during winter in the desert of northern Mendoza (32.5° S, 68.5° W) were less than 50 cm deep. Winter temperatures are considerably lower in southern Mendoza than in the north, and it is probable that the maximum burrow depth reflects the level at which soil temperature is not influenced by fluctuations in ambient temperature, as shown by Greegor (1974) for *Chaetophractus vellerosus*. Some captive pichis have been observed using the same burrow for several weeks, while others often dug new burrows almost every day. In the wild, pichis usually hide their burrow entrances under shrubs. The vegetation provides structural support for the burrow, but it is possible that pichis also use the plants as a food source, either by predating on insect larvae that are associated with the roots or by ingesting the roots themselves.

The present study is the first evaluation of the diet of wild pichis that is based on a relatively large number of samples instead of occasional observations. Pichis seem to feed predominantly on insects, but they also ingest plant material, vertebrates, and arachnids whenever these items are available. This opportunistic, omnivorous feeding strategy allows them to thrive in arid environments where food and, especially, insect abundance varies seasonally. The period of lowest food availability is expected to coincide with the metabolically challenging cold period, and pichis reduce their energetic requirements during this time of the year by entering hibernation. It is possible that their omnivorous food habits and the ability to hibernate allowed them to extend their area of distribution to southern Patagonia with its harsh climate, i.e., farther south than any other armadillo species.

The low food availability during winter not only induces hibernation, but also regulates other aspects of the life history of pichis. With a gestation length of 60 days and approx. 45 days of lactation, pichis must reproduce shortly after emerging from torpor to maximize the chances of survival of their young. Offspring must grow to subadult size and put on enough fat reserves to survive their first winter, which is not possible if they are born in late summer or fall. Similarly, females must wean their offspring beginning several months before hibernation to be able to recover from the energetically challenging lactation period. As a consequence, pichis only breed during a limited period of time. This study reveals that the initiation of breeding season is probably triggered by an increase in daylength. Irrespective of the animal's geographic origin, fecal testosterone levels of male pichis started increasing after winter solstice and reached levels above baseline at a photoperiod of 10 hours and 45 minutes. Reproductive season length was two months longer in pichis originating from northern Mendoza, where environmental conditions are more favorable and may allow late-born offspring to survive the cold period. It would be interesting to study the reproductive season length of pichis living at other latitudes, such as in southern Patagonia, to evaluate whether it is initiated and ends at the same daylength as in Mendoza. If this is the case, testosterone levels of male pichis near Gobernador Gregores, Santa Cruz Province (48°47' S, 70°10' W) will increase above baseline at the end of August and return to baseline at the end of October, i.e., the breeding season will be no more than 2 months long.

This study provides new information on the reproduction of pichis, but many aspects still remain unknown because a large proportion of their reproductive behavior cannot be observed due to their semi-fossorial habits. For instance, aboveground chasing sequences and mounting attempts were commonly seen after pairing of male and female captive pichis but mating has never been observed during the study period. It is possible that pichis mate in the burrow. Parturition occurs inside the burrow where the females and their newborn are protected from potential predators, and the offspring do not leave the den until they are approximately 40 days old. As a consequence, it is difficult to determine the exact day of parturition and the number and size of newborn, estimate the rate of perinatal mortality, or observe the female's behavior during parturition and lactation. The offspring already ingest solid food and are relatively independent at first emergence, which in captive pichis occurs at the age of about 40 days. The food competition observed between captive females and their weaned offspring suggests that juveniles soon leave their mother's territory. I was able to observe wild offspring foraging near adult females only once in five years of fieldwork, when three offspring weighing between 490 and 640g were caught near two females that presented signs of lactation. It remains to be studied whether such observations are not more frequent because wild offspring remain in their burrows for longer periods than in captive conditions or because the females expel them from their territories as soon as they are weaned. It is, however, possible that the higher food availability in captive conditions leads to an earlier weaning and more intense food competition than in the wild.

Histological and hormonal analyses, as well as observations of the behavior of captive pichis, suggest that pichis are induced ovulators and that follicular growth is sustained during pregnancy and lactation, but further studies are needed to confirm the presence of these strategies and to evaluate what induces ovulation. Reflex ovulation maximizes the chances of successful breeding of this solitary species in case of an encounter between males and females. Similarly, the combination of induced ovulation and continued follicular growth during pregnancy and lactation allows the females to conceive soon after aborting or losing their suckling young, which increases the chances of survival of the litter resulting from the second conception. This continuous production of pre-ovulatory follicles during gestation and lactation, however, raises the question whether pichis have a high risk of abortion and whether survival rates of newborn are low. If this were the case, it would be important to determine the reasons for these losses.

Conservation of wild pichi populations

Z. pichiy has recently been classified as Near Threatened by the IUCN Red List of Threatened Species (Superina et al. 2006). This new categorization – pichis were previously listed as Data Deficient – was mainly based on preliminary results of my fieldwork. Nevertheless, the exact reasons for the apparent population declines were unknown at the time of evaluation of its conservation status. My findings suggest that at present, poaching is a far more important reason for concern than disease outbreaks. Although hunting wildlife is prohibited by law in Mendoza Province, pichis are intensely sought after by sports hunters who use them as a protein source and sell them on the black market. The impact of such illegal hunting activities can be extreme. Eighty-nine dead pichis were confiscated from three groups of poachers during anti-poaching patrols in the Cerro Nevado area of Mendoza (35°40’ S, 68°36’ W) between February 12 and March 8, 2006. At least one third were lactating females. If we consider that pichis have a litter size of 1 to 2 offspring and many of the orphans probably did not survive by themselves, the real loss to the wild populations may have been between 117 and 145 animals. Female pichis need to ingest larger quantities of food during the metabolically challenging lactation period. As a consequence, they are more active in summer and spend more time aboveground than males or non-breeding females and have a higher probability of being caught by poachers and their dogs. This aggravates the impact of poaching in this time of the year beyond the fact that hunting a lactating female probably leads to the death of its offspring. The sheer size of Mendoza Province, which covers an area of 150,000 km^2, and limited financial resources make it difficult for the few rangers and inspectors to carry out an effective anti-poaching control in the entire provincial territory. To efficiently use the limited time and resources and maximize the protection of wild pichi populations, anti-poaching patrols should therefore be intensified when the females are in lactation, i.e., between the beginning of November and the beginning of March. Ideally, additional patrols should aim at protecting the females during pregnancy, i.e., between September and November. However, the widespread belief that eating pichis during their mating season causes indigestion currently keeps poaching activity low from August to the beginning of November and indirectly protects reproductively active pichis and pregnant females. Nevertheless, this impression may have to be revised soon because the poaching community is steadily shifting from rural subsistence hunters to urban sports hunters who may not be aware of such local beliefs or do not care about them. Patrols will have the least impact during the winter months because pichis are hibernating from mid-May to mid-August and the probability that poachers find active pichis is low.

Although it cannot be ignored that the wild pichi populations are subjected to an intense hunting pressure, a realistic assessment of the impact of poaching requires additional information. For instance, demographic data and information on the size and density of wild populations are essential to estimate population growth rates and to determine whether this species is naturally rare or its populations have been decimated by man or other external factors. Similarly, extraction rates must be known to assess the scale of the poaching problem. Unfortunately, such data are not available for pichi populations, and several factors make it difficult to determine these parameters. No method has been found to determine the age of armadillos, thus making it virtually impossible to evaluate survival rates of different age classes without monitoring a large number of individuals from birth to death. In addition, no information is available about birth and mortality rates, net reproductive rates, the life expectancy, the age at reproductive senescence or population growth rates of pichis or any other armadillo species. Exact litter sizes and juvenile survival rates of pichis are difficult to evaluate because, as shown in this study, the offspring remain in their burrows during the first 40 days of life. Future studies are planned to evaluate whether these data could be obtained through the observation of semi-captive animals by offering them artificial burrows or placing cameras inside their natural burrows. The determination of other demographic parameters requires the long-term monitoring of a wild population inhabiting a delimited area. The latter would also provide invaluable information on population size, structure, and density.

Abba et al. (2007) and Wilkie and Carpenter (1999) evaluated rates of off-take through interviews with hunters and consumers of wildlife. In Mendoza Province, a survey would provide unreliable results and underestimate the real extent of poaching because hunting pichis is illegal and the large majority of interviewees would play down or deny their hunting activity. Due to the same reason, poached pichis that are not consumed by their hunters are not offered for sale in public but rather sold to friends or relatives. As a consequence, extraction rates cannot be estimated by monitoring the availability and abundance of animal carcasses at wild meat markets, as performed by Fa et al. (2000) in Africa. The comparison of species density or biomass in protected and hunted sites has been used as an indirect measure of poaching activity (Bodmer et al. 1997; Carrillo et al. 2000; Cullen Jr. et al. 2001). This technique may be useful to estimate the impact of poaching on pichis of Mendoza Province, but it would require a very careful selection of survey sites because pichis are poached in virtually all of its range, including in protected areas. Alternatively, extraction rates may be estimated by analyzing the quantity of wildlife that is confiscated from poachers by law enforcement agencies (Wright et al. 2000), especially if the patrols are carried out regularly in a determined area. Two caveats should, however, be considered: First, anti-poaching patrols during which no animals have been confiscated are often not registered although they would provide essential information. Furthermore, the general area of poaching can be inferred from the confiscation site, but it is not known whether the poachers extracted their prey from an area of, for instance, 200 or 2000 hectares.

Mortality due to heavy poaching activity may be difficult, if not impossible, to compensate if pichis produce one yearly litter of one or two (possibly three) offspring. Pichis in northern Mendoza could potentially increase their reproductive output by producing two litters per year if the population density drops below a certain level. The risks associated with this strategy are relatively high because females may not have enough time to recover from the metabolically challenging lactation period and increase their fat deposits before the hibernation season and because a second set of offspring would have reduced probabilities of winter survival.

No disease epidemics were reported from the wild pichi populations of Mendoza Province during the study period 2002 - 2007, but according to several locals, a disease of unknown etiology locally known as “pichi plague” had decimated pichi populations in several parts of the province prior to 2002. Although care should be taken when interpreting anecdotal reports from the local population, it is striking that all locals associated the disease with periods of intense rainfall and high environmental humidity levels. In addition, the most common health problems I observed in captive pichis were related to elevated ambient humidity levels. Changes in the patterns of precipitation in the habitat of pichis may lead to an increased incidence of “pichi plague” outbreaks or skin problems in the form of skin detachment and colonization of the lesions with opportunistic pathogens.

The present study is the first systematic, organized research into the pathogens affecting an armadillo species inhabiting an arid environment. The evaluated wild populations were considered to be in good health. It is, however, important to note that pichis were only screened for a few diseases and many more pathogens may exist in the populations but remained undetected. For instance, pichis were not screened for any viral disease. Although some nine-banded armadillos have been found to carry antibodies against the St. Louis Encephalitis virus (Day et al. 1995), the lack of reports on viral diseases in armadillos, as well as the absence of similar studies in any other wild animal species of Mendoza Province, made it virtually impossible to determine for which of the wealth of existing viruses the pichis should be screened. The baseline data on the pathogens present in wild pichi populations, as well as the parameters describing a healthy pichi presented in this study should be interpreted as a starting point for future health studies. The growing human encroachment into pichi habitats and the accompanying increased presence of domestic animals near pichi populations should be closely monitored. Domestic species may carry diseases or act as reservoirs of pathogens against which the wild populations are immunologically naïve, and a transmission of these pathogens to pichi populations could have devastating effects. Epidemiological studies involving domestic animals and other wild species will be key to an integrative conservation management of the pichi populations and their habitat.

The large majority of lesions observed in histopathological examinations of pichis that had been confiscated from poachers were related to tissue migrations of parasite larvae, and a moderate parasite load was found in most individuals. It is important to realize that this equilibrium between hosts and parasites can be influenced by stressors related to environmental changes or increased human activity in their natural habitat, which could affect the health of wild pichis. Similarly, chronic stress could impair the immune system of pichis, increase their disease susceptibility, and lead to the emergence of new diseases in wild populations. The pichi's omnivorous food habits and the fact that it ingests large amounts of soil while foraging, position this species on a high level in the food chain and predispose it to accumulate pollutants. Monitoring the health of pichis will therefore not only provide information on the impact of human activities on wild pichi populations, but may also serve as an indirect measure of environmental health in arid environments.

Environmental stress, such as increased precipitation rates or drought and a concomitant reduction in food availability may not only impair the health of wild pichis, but also affect other aspects of their life history. For instance, encounter rates were extremely low during a 9-month drought period that affected Mendoza Province in 2003. Only a very few juveniles were observed in the summer and fall of 2003/2004. It is probable that some pichis died during the drought period and that most of the surviving animals did not reproduce in the breeding season of 2003 due to a lack of energy resources. Captive armadillos have low reproductive rates and a high neonatal mortality, which has been attributed to elevated stress levels (Superina 2000). *Dasypus novemcinctus*, for instance, is very difficult to breed in captivity, which is believed to be a consequence of elevated progesterone levels of adrenal origin (Rideout et al. 1985). The latter were also elevated in wild nine-banded armadillos subjected to environmental stress (Rideout et al. 1985). It is therefore possible that an increase in environmental stress will impair the reproduction of wild pichis and lead to a further reduction of the populations. Pichi sightings returned to normal levels in the second half of 2004, suggesting that the reduced observations in the previous year had not been caused by a massive die-off. Presumably, pichis were torpid during large parts of the drought period to reduce their energetic requirements. Further studies are planned to test whether food-deprived pichis enter torpor.

In conclusion, this project shed some light onto the natural history of pichis, but also raised some important questions that need to be addressed in future studies. An evaluation of the population density and home range of this solitary species is urgently needed to determine its real conservation status, and further work on its reproductive strategy and survival rates will help understanding the potential of wild populations to recover from the impact of poaching. A long-term monitoring of the health status of wild pichis will be key to evaluating the impact of human activities on pichi populations and their environment.

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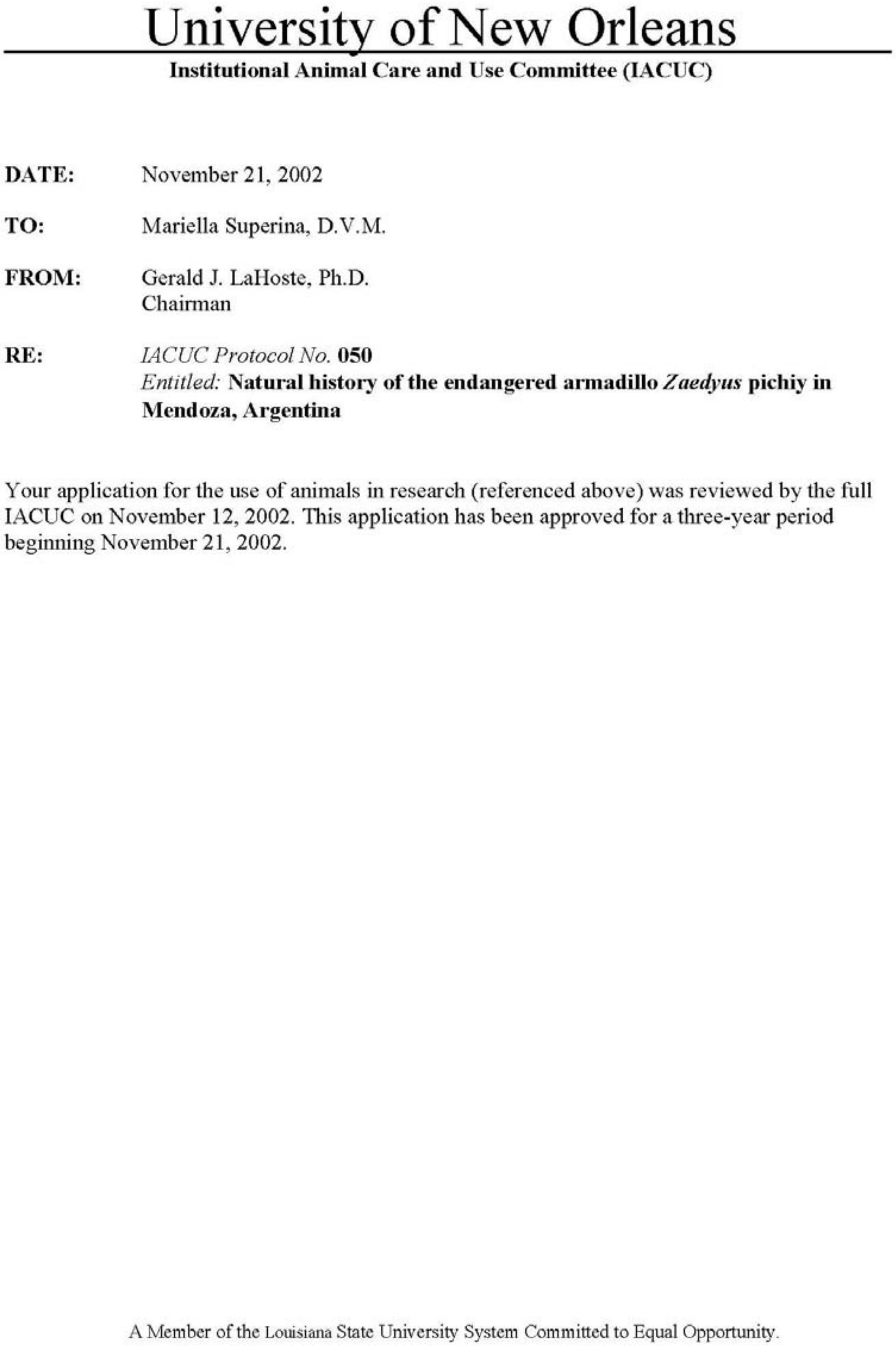
APPENDIX



University of New Orleans
Institutional Animal Care and Use Committee (IACUC)

DATE: November 21, 2002

TO: Mariella Superina, D.V.M.

FROM: Gerald J. LaHoste, Ph.D.
Chairman

RE: *IACUC Protocol No.* **050**
Entitled: **Natural history of the endangered armadillo *Zaedyus* pichiy in Mendoza, Argentina**

Your application for the use of animals in research (referenced above) was reviewed by the full IACUC on November 12, 2002. This application has been approved for a three-year period beginning November 21, 2002.

A Member of the Louisiana State University System Committed to Equal Opportunity.

University of New Orleans

Institutional Animal Care and Use Committee (IACUC)

DATE: June 26, 2007

TO: Mariella Superina, D.V.M.

FROM: Gerald J. LaHoste, Ph.D.
Chairman

RE: *IACUC Protocol No.* **079**
Entitled: **Reproductive cycle of the pichi *(Zaedyus pichiy)* in Mendoza, Argentina**

Your revised application for the use of animals in research (referenced above) has been received and approved. The start date for this protocol is June 26, 2007 and the expiration date is June 26, 2009.

VITA

Mariella Superina was born in Wettingen, Aargau, Switzerland. She received her degree in veterinary medicine (med. vet.) from University of Zurich, Switzerland, in 1998, and a doctoral degree in veterinary medicine (Dr. med. vet.) from University of Zurich, Switzerland, in 2000.