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Short Communication

Experimental evidence for embryo retention in the high-elevation Chilean lizard *Liolaemus leopardinus* (Squamata: Liolaemidae)

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Liolaemus leopardinus Müller and Hellmich, 1932 is a medium-to-large, high-elevation lizard endemic to central Chile (Pincheira-Donoso and Núñez 2005) and considered Endangered by IUCN (Díaz *et al.* 2016). The species is active approximately six months during austral late spring, summer, and fall and inactive during the cool and snowy austral winter. It is limited to a narrow altitudinal band (1800–3000 m a.s.l.), and lives in colonies on rocky outcrops (Fox and Shipman 2003, Pincheira-Donoso *et al.* 2008). It is viviparous, breeds during austral spring and summer, and embryo gestation is about 60 days long (Leyton and Valencia 1992); females give birth to two to four youngs in late fall. Results from Fox and Shipman (2003) and a pilot study by Fox and Núñez in 2005 showed that *L. leopardinus* forms social groups. This behavior was observed again by Santoyo-Brito and Fox during two field seasons in the late austral spring and summer of 2011–2012 and 2012–2013 at their field site (35 km northeast of Santiago at 2760 m a.s.l.; 33°14' S, 70°16' W), in the Andean cordillera of central Chile. In all field seasons it was common to observe groups of lizards on top of rocks, along rocky ledges, or in crevices. The number of individuals in each group was variable, ranging from small groups formed by a male and a female (sometimes pregnant), at times with one to three juveniles, to larger groups up to 6–10 (or more) adults and juveniles within rock crevices.

During our 2011–2013 thorough behavioral study of sociality of *L. leopardinus,* we made some observations on reproductive abnormalities in adult females. In the austral late-fall seasons of 2012 and 2013, we collected pregnant adult females at our field site. All individuals were caught via noosing, mass was determined with a 60-g spring scale (PESOLA No. 20060) and snout–vent length (SVL) and tail length measured with a ruler. Reproductive status of each female was determined by palpation. In the field, pregnant females give birth to up to four neonates in late March and early April. Mean SVL and

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mass of six free ranging neonates were 41.0 mm (SE = 0.44) and 1.9 g (SE = 0.15), respectively. Parturition occurs typically in a single event, while the female is solitary underneath flat rocks, away from the social groups, on rocky cliffs and piles (ESB, pers. obs.).

In early February 2012 we caught 13 pregnant adult females; mean SVL and mass of these lizards was 92.3 mm (SE = 0.94) and 24.6 g (SE = 0.81), respectively. Each lizard was housed individually in a shoebox (20.2 cm wide × 34.4 cm long × 11.8 cm high) with a mesh lid held with rubber bands or the central part of the plastic lid was removed and replaced with nylon mesh glued to the edges of the lid. After 23 days of the maintenance of these females, none of them had given birth; thus, we switched to group maintenance (for 23 days) in a large, indoor laboratory pen (1.0 × 1.5 m floor space) because we had previously had success in the 2005 pilot study with females giving birth in large pens in groups in the laboratory. The floor of the pen was covered with natural substrate from the field site (a mixture of dirt and gravel). We also placed a few flat rocks inside the pool for the lizards to use as refuges and basking spots.

In late February and early March 2013, we caught 13 pregnant adult females; mean SVL and mean mass = 93.5 mm (SE = 0.73) and 36.7 g (SE = 1.31), respectively. This time all females were kept in two groups for 51 days, each in an inflatable circular wading pool (1.5 m diameter × 30.5 cm deep). The substrate was similar to the one used in 2012. In both years, lizards housed in groups were usually found next to each other in intimate contact underneath the flat rocks early in the morning and at night. We also frequently observed groups of lizards basking on top of rocks during the day. In both seasons, lizards were fed daily *ad libitum* with larval *Tenebrio* sp. and larval *Chilecomadia moorei* Silva Figero, 1915. Lighting was from above using 40-watt incandescent bulbs in lamps with reflectors set 16 cm above favorite basking sites. Lamps were placed inside the enclosures, and lights were set to a photoperiod of 12:12 h (light: dark).

In 2012, not a single pregnant female gave birth, either in solitary or group housing, although all of them ate well and maintained their health. In early April, the mean weight of all 13 lizards was 21.6 g (SE = 0.48); significantly less than the mass recorded upon capture in early February (Paired t-test: $t_{(12)} = 4.052$, $p = 0.002$). None of the lizards showed a sudden loss of weight, and all looked healthy. That same year, two of those females were sacrificed after 51 days in captivity (13 February to 4 April) and we found no embryos, but instead abundant yellow fat bodies. Another female from the field and inadvertently killed on 09 March was dissected and she contained two late-term embryos inside, each 40 mm SVL (Figure 1).

In 2013, nine of the 13 pregnant females kept in the pools gave birth to 25 live, healthy, fully developed neonates. A few of the newborns were still covered with a clear embryonic membrane when first observed: none presented a chewed appearance, and we did not find any undeveloped embryos. Unfortunately, we did not keep track of the post-partem weight of all females kept in the pools. However, the mean weight of two of those pregnant females when first captured was 32.0 g and the post-partem mean weight immediately after giving birth was 16.0 g. During both years we did not observe any agonistic or cannibalistic behavior by adults against newborns or young individuals in the field or in captivity.

Social behavior refers to the broad array of possible interactions between two or more members of the same population (Allaby 2009, Gardner *et al.* 2016). However, due to the apparent lack of sociality generally accepted in the past (Doody *et al.* 2013), squamate reptiles have been typically labeled as non-social (Wilkinson *et al.* 2010, Wilkinson and Huber 2012), a situation that has led researchers to overlook the diversity and often complex social behavior in this large taxon (Doody *et al.* 2013, Gardner *et al.* 2016). Recently, the assumption of absence of sociality in squamate species has been questioned and literature reporting

squamate species found in social aggregations has skyrocketed (Graves and Duvall 1995, Mouton *et al.* 1999, O'Connor and Shine 2003, Davis *et al.* 2011, Gardner *et al.* 2016). Close proximity with conspecifics may offer benefits (e.g., thermoregulation, osmoregulation, protection against predation among others) during embryonic development (Graves and Duvall 1995).

The difference between the mean mass of pregnant females ($N = 26$) in the two years—24.6 g (SE = 0.81) in 2012 and 36.7 g (SE = 1.31) in 2013—could be due to the advanced pregnancy of the females caught in very late February and early March 2013. Blackburn *et al.* (2003) indicate that squamates' inviable eggs can (1) be retained in the oviducts for a very long period of time, (2) be extruded *en masse* during parturition, (3) pass down the oviduct and out of the cloaca as single events, (4) undergo dissolution and ooze out of the cloaca, and (5) be aborted and ingested by the mother. From the 26 lizards kept in captivity, we never observed any signs of abortion (i.e., amniotic fluid, egg masses, ooze in females' cloaca, remains of egg yolk, or material of aborted embryos or eggs attached to feces). However, as it is subtle, it is possible that we failed to observe gradual expulsion of liquefied embryonic material from pregnant mothers. Cannibalism, mostly on young individuals, has been reported in nine different *Liolaemus* species, of which two instances have occurred in captivity (Pincheira-Donoso 2000, Robles and De la Riva 2017). During our various behavioral studies of *L. leopardinus* (2003, 2005, 2001–12, and 2012–13) we never observed cannibalistic behavior—on either sex—by free-ranging adult males and females or by adult females kept under laboratory conditions.

Thus, based on the lack of evidence over the assumptions that females could have aborted their products (Blackburn 1998, Blackburn *et al.* 2003) or that adult females could have consumed an entire neonate (Halloy and Halloy 1997, Pincheira-Donoso 2012, Robles and De la Riva 2017), we conclude that the set of pregnant females maintained in captivity retained their embryos possibly due to the stress of the artificial conditions (Blackburn *et al.* 2013) and the lack of beneficial social interactions among conspecifics during embryonic development (Graves and Duvall 1995). As far as we know, this is the first report of embryo retention in the genus *Liolaemus.*

Figure 1. Adult female *Liolaemus leopardinus* with two late-term embryos. Female was collected at El Colorado, Chile, and inadvertently killed in mid austral fall in 2012. Implanted radio (09 March 2012) was also recovered.

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References

Allaby, M. 2009. *A Dictionary of Zoology*. 3rd edition. New York. Oxford University Press. 689 pp.

Blackburn, D. G. 1998. Resorption of oviductal eggs and embryos in squamate reptiles. *Herpetological Journal 8:* 65–71.

Blackburn, D. G., K. K. Weaber, J. R. Stewart, and M. B. Thompson. 2003. Do pregnant lizards resorb or abort inviable eggs and embryos? Morphological evidence from an Australian skink, *Pseudemoia pagenstecheri. Journal of Morphology 256:* 219–234.

Davis, A. R., A. Corl, Y. Surget-Groba, and B. Sinervo. 2011. Convergent evolution of kin-based sociality in a lizard. *Proceedings of the Royal Society B 278:* 1507–1514.

Díaz, S., P. Espejo, G. Lobos, J. Mella, H. Núñez, and M. Ruiz de Gamboa. 2016. *Liolaemus leopardinus*. The IUCN Red List of Threatened Species 2016: e. T12004A69940961. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T12004A69940961.en. Captured on 30 March 2017.

Doody, J. S., G. M. Burghardt, and V. Dinets. 2013. Breaking the social-non-social dichotomy: a role for reptiles in vertebrate social behavior research? *Ethology 119:* 95–103.

Fox, S. F. and P. A. Shipman. 2003. Social behavior at high and low elevations: environmental release and phylogenetic effects in *Liolaemus*. Pp 310–355 *in* S. F. Fox, J. K. McCoy, and T. A. Baird (eds.), *Lizard Social Behavior.* Baltimore. Johns Hopkins University Press.

Gardner, M. G., S. K. Pearson, G. R. Johnston, and M. P. Schwarz. 2016. Group living in squamate reptiles: a review of evidence for stable aggregations. *Biological Reviews 91:* 925–936.

Graves, B. M. and D. Duvall. 1995. Aggregation of squamate reptiles associated with gestation, oviposition, and parturition. *Herpetological Monographs 9:* 102–119.

Halloy, M. and S. Halloy. 1997. An indirect form of parental care in a high altitude viviparous lizard, *Liolaemus huacahuasicus* (Tropiduridae). *Bulletin of the Maryland Herpetological Society 33:* 139–155.

Leyton, V. and J. Valencia. 1992. Follicular population dynamics: its relation to clutch and litter size in Chilean *Liolaemus* lizards. Pp 123–134 *in* W. C. Hamlet (ed.), *Reproductive Biology of South American Vertebrates*. New York. Springer-Verlag, Inc.

Mouton, P. L. F. N., A. F. Flemming, and E. M. Kanga. 1999. Grouping behaviour, tail-biting behaviour and sexual dimorphism in the armadillo lizard (*Cordylus cataphractus*) from South Africa. *Journal of Zoology 249:* 1–10.

O'Connor, D. and R. Shine. 2003. Lizards in 'nuclear families': a novel reptilian social system in *Egernia saxatilis* (Scincidae). *Molecular Ecology 12:* 743–752.

Pincheira-Donoso, D. 2000. Hallazgo de predación intraespecífica en *Liolaemus chilensis* (Lesson, 1831) (Sauria, Tropiduridae). *Revista de Integración Biológica de Concepción 1:* 33–35.

Pincheira-Donoso, D. 2012. Intraspecific predation in the *Liolaemus* lizard radiation: a primer. *Animal Biology 62:* 277–287.

Pincheira-Donoso, D. and H. Núñez. 2005. Las especies chilenas del género *Liolaemus* Weigmann, 1834 (Iguania: Tropiduridae: Liolaeminae): taxonomía, sistemática y evolución. *Publicación Ocasional del Museo Nacional de Historia Natural, Chile 59:* 7–486.

Pincheira-Donoso, D., J. A. Scolaro, and P. Sura. 2008. A monographic catalogue on the systematics and phylogeny of South American iguanian family Liolaemidae (Squamata, Iguania). *Zootaxa 1800:* 1–85.

Robles, O. J. and I. De la Riva. 2017. Cannibalism in the Andean lizard *Liolaemus orientalis*. *Studies on Neotropical Fauna and Environment 52:* 244–247.

Wilkinson, A., K. Kuenstner, J. Mueller, and L. Huber. 2010. Social learning in a non-social reptile (*Geochelone carbonaria*). *Biology Letters 6:* 614–616.

Wilkinson, A. and L. Huber. 2012. Cold-blooded cognition: reptilian cognitive abilities. Pp. 129–143 *in* J. Vonk and T. K. Shackelford (eds.), *The Oxford Handbook of Comparative Evolutionary Psychology*. Oxford. Oxford University Press.

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