

Chemical recognition of prey in *Liolaemus ceii* (Donoso-Barros, 1971)

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The order Squamata, has traditionally been divided into two major clades: Scleroglossa and Iguania (Estes, et al., 1988), based on the main sensory modality (visual or chemical) they use to explore and interpret the environment (Mason, 1992; Ord and Martins, 2006). The first clade is characterised by the chemical detection and recognition of prey; Scleroglossids are active predators that frequently protrude and retract their tongue (tongue flicks) when prey is detected. On the other hand, iguanids are mainly ambush predators, which always or regularly rely on the visual detection of the prey.

Liolaemus is the second most diverse genus in Iguania, with approximately 250 species (Abdala, et al., 2015). The chemical sensory modality is very important in this genus and it is known that numerous species exhibit self-recognition (Troncoso-Palacios and Labra, 2012; Labra and Hoare, 2015), recognition of conspecifics (Labra, 2006; 2008; Vicente and Halloy, 2016) and chemical discrimination of conspecifics and closely related congeners (Labra, 2011). However, the chemical recognition of prey is less known. It is curious that in the diverse genus *Liolaemus* chemical recognition of prey has so far only been studied in three species. In *Liolaemus zapallarensis*, chemical recognition of prey could not be shown, supporting what is established for Iguania (Deperno and Cooper, 1993). However, *L. lemniscatus* and *L. pictus* do present chemical recognition of prey and both species are able to detect scents of insects in soil and volcanic ash (Labra, 2007; Mora and Labra, 2017).

It is clear that chemical recognition of prey in *Liolaemus* is vastly understudied. Hence, we hope that

the present study will be an important contribution by presenting a video report on chemical recognition of prey in *Liolaemus ceii* (Donoso Barros, 1971). We collected thirteen lizards (6 females, 7 males) near Alumine, Neuquen (Route 13 between Kilka and Primeros Pinos: 38° 54' 14.70''S; 70° 43' 59.50''W; datum WGS84), in February 2014, during the pre-hibernation season. The lizards were captured by hand and individually placed in cloth bags with their identification labels. In the lab, they were placed in plastic boxes (36 × 27 × 19 cm) covered with a lid of plastic mesh. Enclosures had 3 cm of sandy substrate, a rock to be used as shelter and basking place, and a small bowl with water *ad libitum*. We kept lizards in an isolated room with a summer photoperiod (13:11, L: D), using halogen lamps, which also allowed maintaining a mean ambient temperature of 30 °C during the day. Every two days we fed each lizard with two *Tenebrio molitor* larvae, dusted with vitamins for lizards. Prior to the experiments, lizards remained in their enclosures for one week, in order to habituate them to the experimental conditions. Before each trial, the focal lizard was removed from its enclosure and held in a cloth bag for 10 min, as this procedure minimises the stress associated with handling the lizards (Labra, 2011). The bag was then opened to allow the animal to move freely into a treatment enclosure. We then videotaped the behaviour of the lizard for 8 min using a digital video camera (Sony DCR-SR67) installed at 50 cm above the enclosure and connected to a monitor. We stored the digital videos for further analyses, which we performed with VLC Media Player 2.2.1. The individuals were collected for systematic studies so that at the end of each experimental trial, animals were sacrificed and deposited in Museo de Ciencias Naturales de Salta (MCN).

The example video (see supplementary files) lasts for 6 min 22 s. It shows a young female of *L. ceii* with a snout-vent length (SVL) of 52.35 mm. We determined the onset of chemical exploration as the moment when the female made the first tongue-flicks (latency time

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57.03 s). This is the time when the animals become habituated and explore the environment (Labra and Niemeyer, 1999). During the first 5 min of the video, the lizard moved for a total of 2 min 40 s (total time motion). The number of tongue flicks was 47 (that is a measure of chemical exploration; Schwenk, 1995). Later, it exhibited escape behaviour for 35 s at the wall of the plastic box. Finally, the lizard moved and made another tongue-flick on the substrate, waited for a moment and then started to dig under the sand where it found one larva (*Tenebrio molitor*). The lizard consumed the prey right after its discovery. During the focal period, the prey never moved, which leads us to believe that the lizard could not have detected the prey by visual or audible cues. This report is important because it shows that *L. ceii*, an Iguanian lizard, localized its prey without the need of vision. Moreover, the lizard made numerous tongue-flicks before it found the prey, which further points to the fact that there is chemical exploration and recognition of the prey.

Iguania lizards are active foragers which principally detect their prey using vision, however, for *L. lemniscatus* and *L. pictus* chemical prey recognition was shown (Labra, 2007; Mora and Labra, 2017). This work describes lab observations which could indicate that *L. ceii* presents a similar mechanism for prey recognition. Although despite the high number of tongue flicks (47) during almost seven min, only the in the last were these performed near the place where the prey was hidden. Thus, we asked if *L. ceii* detects chemosignals via the vomeronasal organ or if there is also an implication of the olfactory system. Although the mechanism is still unknown, *L. ceii* shows clear evidence of chemical exploration and detection of its prey. The use of visual cues similar to *L. lemniscatus* and *L. pictus* does not seem very likely. The genus *Liolaemus* comprises herbivores, omnivores and a majority of insectivores (Espinoza, et al., 2004). The fact that iguanian lizards are considered mainly as visual predators might point towards the hypothesis that chemical detection of prey in *Liolaemus* is a derivate condition in the genus. Future studies will help to clarify and redefine concepts and ideas about prey detection in Iguania.

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