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ANTIPREDATORY BEHAVIORS OF THE COLOMBIAN ENDEMIC GLASSFROG *CENTROLENE SAVAGEI* (ANURA: CENTROLENIDAE)

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Abstract

Predation has been an important agent of natural selection which has led the evolution of several characteristics of amphibians, such as toxic and distasteful skin secretions, cryptic and aposematic coloration, and a variety of defensive postures and behaviors. This work describes for the first time the behaviors displayed by the glass frog *Centrolene savagei*, to avoid predation. These behaviors consisted in assume defensive postures, release cloacal fluids and production of odoriferous secretions, while individuals were visual and tactile estimulated. The defense postures and production of odoriferous secretions is established as the first record of such behaviors for the family Centrolenidae, demonstrating that many aspects of the natural history of glass frogs are still unknown.

Key words: Centrolenidae, *Centrolene savagei*, defensive behavior, defensive posture, odoriferous secretions.

COMPORTAMIENTO ANTIPREDATORIO DE LA RANA DE CRISTA ENDÉMICA DE COLOMBIA *CENTROLENE SAVAGEI* (ANURA: CENTROLENIDAE)

Resumen

La depredación ha sido un importante agente de selección natural que ha dirigido la evolución de varias características de los anfibios, tales como las secreciones de sustancias toxicas y desagradables, la coloración críptica y aposemática, y una variedad de posturas y comportamientos defensivos. En este trabajo se describe por primera vez los comportamientos realizados por la rana de cristal *Centrolene savagei*, para evitar la depredación. Dichos comportamientos consistieron en asumir pasturas de defensa, liberar fluidos cloacales y producir secreciones olorosas al momento de ser estimulados visual y táctimente los individuos. La adopción de posturas de defensa y la secreción de sustancias olorosas, se constituye como el primer registro de dichos comportamientos para la familia Centrolenidae, demostrando así que muchos aspectos de la historia natural de las ranas de cristal siguen siendo desconocidos.

Palabras clave: Centrolenidae, *Centrolene savagei*, comportamiento defensivo, postura defensiva, secreciones olorosas.

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Predation has been important in the evolution of many amphibian characteristics, such as toxic and distasteful skin secretions, cryptic and aposematic coloration, and a variety of defensive postures and behaviors (WELLS, 2007; VITT & CALDWELL, 2009). Once an amphibian actually encounters a predator, its behavioral responses depend on the morphology and ecology of the animal and the identity and proximity of the predator (WELLS, 2007). TOLEDO *et al.* (2011) make an important collection of defensive behaviors in anurans, registering a total of 30 different types of behaviors (some with a few sub-categories). Despite the notable advances in the understanding of phylogenetic relationships and taxonomy in Centrolenidae (CISNEROS-HEREDIA & MCDIARMID, 2007; GUAYASAMIN *et al.*, 2009), little is known about its defensive behaviors.

Centrolene savagei (GUAYASAMIN *et al.*, 2009) formerly *Cochranella savagei* (RUIZ-CARRANZA & LYNCH, 1991) is an endemic glass frog to the Colombian Andes, inhabiting montane and sub montane forests on both flanks (East and Eest) of the Central and Western Cordilleras at elevations between 1400 and 2410 m (RUIZ-CARRANZA & LYNCH, 1991, 1997; ROJAS-MORALES *et al.*, 2011). This glass frog has been listed by the IUCN as Vulnerable under criteria B1ab (iii) (BOLÍVAR *et al.*, 2004). This contribution describes for the first time the defensive behaviors of *C. savagei*, based on observations of different individuals at the municipality of Manizales, department of Caldas, Colombia. During our observations none of the individuals was collected, however, voucher specimens of *C. savagei* from this location, , are housed in the Museo de Historia Natural de la Universidad de Caldas (MHN-UC 0253-4).

During surveys conducted in several patches of Low Montane Wet Forest (*sensu* HOLDRIDGE, 1982; HARTSHORM, 2002) in Manizales, Caldas, Colombia, between November 2009 and January 2010, we observed three examples of the defensive behaviors of *C. savagei*, with some intraspecific variation. These observations are as follow:

First individual

On 18 November 2009 at 21:48 h in the creek "Chisperos" (Vereda "*Alto Bonito*" 5º06'36'' N, 75º29'57'' W, 1950 m), Manizales, Caldas, Colombia. A male (snout vent length SVL = 21.2 mm) heard on a leaf of *Oreopanax pallidum* (Araliaceae) at 1.4 m above the stream, next to a clutch with 23 eggs. When we move to 1.5 m near the individual, it responded to our presence by assuming a defensive posture during about 18 sec.., which includes raising his body, extending all the limbs and slightly inflates the thorax (Figure 1A, B). When we approach to about 0.2 m, it released a sharp stream of its cloaca and when captured and handled, individual gave a slight unpleasant odor through their skin, perceptible only a short distance (10 cm approx.). This odor is difficult to define and compare because their aromatic attributes are unique, although is slightly similar to that produced by macerating the leaves of plants of the genus *Piper*.

Second individual

On 11 January 2010 at 22:18 h in the creek "Aguasclaras" (Vereda "*El Águila*" 5º06'27.5'' N, 75º29'30'' W, 2050 m), Manizales, Caldas, Colombia.

A male (SVL = 20.8 mm) perched on a leaf of *Heliconia latispatha* (Heliconiaceae) at 1.64 m above the stream. When we come to about 0.15 m of the individual, it responded to our presence assuming a defensive posture (during about 70 sec.), which consisted of flatten their body on the leaf blade and retracting their limbs (Figure 1C). During this observation the individual did not release a sharp stream as observed in the first individual. At the time of his capture it expelled an odor through the skin, similar to the previous individual.

Third individual

On 20 December 2009 to 22:18 h in the creek "Aguasclaras" (Vereda "*El Águila*" 5º06'27.5'' N, 75º29'30'' W, 2050 m), Manizales, Caldas, Colombia. A male (SVL = 21.8 mm) perched on a leaf of *Xanthosoma saggitifolium* (Araceae) at 1.64 m above the stream. When we come to about 50 cm of the individual, it responded to our presence assuming a posture (about 40 sec.), which was to raise his body slightly inflating thorax. The individual released several sharps streams of their cloaca, similar to that observed in the first individual. At the time of capture, the frog gave an unpleasant odor like perceived in previous individuals.

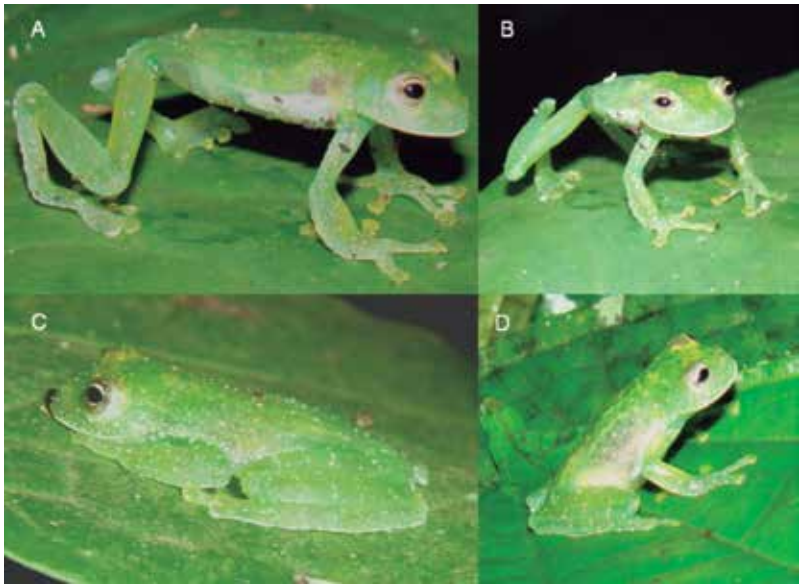

Figure 1. Defensive and typical postures of the glass frog *Centrolene savagei*. (A and B) lateral and frontal view of the defensive posture, which involves raising the rear of the body, extending the limbs (front and rear) and inflate slightly the thorax. (C) Defensive posture consisting of dorsoventral flattening of the body, on the leaf blade retracting the limbs. (D) Typical posture de *C. savagei*. Note the difference in the position of the trunk and extremities with regard to defense postures. Photos: Julián Andrés Rojas-M.

These are the first reports of defensive behaviors in *C. savagei*. According to the terminology proposed by TOLEDO *et al.* (2011), *C. savagei* displayed five types of defensive behaviors, which are: (1) body-raising, (2) puffing up the body, (3) crouching down, (4) cloacal discharge and (5) secretion of odor. The first four behaviors were displayed when individuals were visually stimulated, and the last when captured.

Many anurans often respond to predators by elevating the body (body-raising) and inflating themselves with air (puffing up the body) (HANSON & VIAL, 1956; MARCHISIN & ANDERSON, 1978; WELLS, 2007; DAS *et al.*, 2010; TOLEDO *et al.*, 2011), which has the function to exaggerate the body size to persuade predators (WELLS, 2007). Body-raising behavior (*sensu* TOLEDO *et al.* (2011), has two sub-categories, depending on the position of the legs to extend, (A) body-raising with legs vertically stretched and (B) body-raising with legs laterally stretched. Body-raising of both types is common to perform synergistically with puffing up the body. The body raising behavior observed in the first and third individual of *C. savagei*, corresponds to type (B) *sensu* TOLEDO *et al.* (2011). This behavior and puffing up the body, are the first case known for the Centrolenidae family (Table 1).

Anurans perform cloacal discharge as a defense behavior and the material expelled can be liquid or solid (TOLEDO *et al.*, 2011). Liquid cloacal discharge (extrusion of bladder contents) is the commonest behavior, occurring in many anurans species (see Appendix 1 of TOLEDO *et al.*, 2011). Solid discharge or defecation (faeces expelled with force from the intestine) has been reported for the bufonid *Anaxyrus terrestris* only, when seized by a snake (MARCHISIN & ANDERSON, 1978). The behavior displayed by the first and third individuals of *C. savagei*, which was to expel a sharp stream of their cloaca (liquid cloacal discharge), has been observed in the glass frog *Hyalinobathrachium valerioi* (VOCKENHUBER, 2008) (Table 1). The significance of this anti-predatory behavior is not understood, but we suggest it might work to deter small arthropod predators, due any chemical property of the fluid aversive to predators, or by mechanical shock on them.

Excrete substances through the skin, is a behavior observed in several species of anurans (see Appendix 1 of TOLEDO *et al.* 2011). These secretions may be of four types: (A) odoriferous, (B) noxious, (C) adhesive and (D) slippery. For example, frogs of the genus *Phyllomedusa* are noted for their noxious secretions and strong odors when handled (CEI & ERSPAMER, 1966; LUTZ, 1966; SAZIMA, 1974; CEI, 1980). Also, the nocturnal aromobatid *Aromobates nocturnus* is characterized by ejecting a foul-smelling discharge at the time it is threatened by a predator (VITT & CALDWELL, 2009).

The behavior observed in *C. savagei*, to release an odor similar to smashed plants, is a behavior observed only in nocturnal frogs species (SMITH *et al.*, 2004). TOLEDO *et al.* (2011) suggests that odors similar to plants can function as chemical camouflage and/or for mimicry. Some anurans produce odors that resemble smashed plants, which may mislead a predator, especially if the frog remains motionless and the predator cannot rely on visual or chemical cues to find its prey (SMITH *et al.*, 2004; TOLEDO *et al.*, 2011). The conduct displayed by individuals of *C. savagei*, which is to release an odor similar to smashed plants, is established as the only known for Centrolenidae (Table 1), although such behavior has also been observed in species such *Centrolene quindianum* and *Nymphargus grandisonae* (SEL and JAR pers. obs).

Table 1. Defensive behaviors in frogs of the family Centrolenidae.

Species	A	B	C	D	E	F	G	References
Centrolene geckoideum						X		RUEDA-ALMONACID (1994)
Centrolene quindianum					X			Personal Observation
Centrolene savagei	X	X	X	X	X			In this work
Hyalinobathrachium valerioi	X							VOCKENHUBER (2008)
Nymphargus grandisonae					X			Personal Observation
Vitreorana uranoscopa							X	TOLEDO *et al.* (2010)

Defensive behaviors. A. cloacal discharge, B. crouching down, C. body-raising, D. puffing up the body, E. secretion (odour), F. noxious secretion and G. thanatosis or death feigning.

Predatory pressure on glass frogs certainly determined and shaped the evolution of various features and anti-predatory behaviors, which are not yet well understood or known. Most species of centrolenids call and breed in the riparian vegetation so that individuals are exposed to predators that use streams and creeks as foraging sites (HAYES, 1983). Glass frogs at different stages (embryos, larvae and adults) are preyed mainly by arthropods, such as crabs (HAYES, 1983), amblipigids (HERTZ & LOTZKAT, 2010), spiders (HAYES, 1983), opilionids (HAYES, 1983; VOCKENHUBER, 2008), orthopterans (HAYES, 1983), wasps (DRAKE & RANVESTEL, 2005; DELIA *et al.*, 2010), ants (HAWLEY & CHASTAIN, 2007; VOCKENHUBER *et al.*, 2009), fly larvae (VILLA, 1977) and dermapterans (VOCKENHUBER, 2008).

A glass frog's characteristic that evolved in response to predatory pressure is its mimetic aspect (SCHWALM *et al.*, 1977). Most of this frogs are a shade of green, ranging from light lime green to dark green; some species have green bones as a result of green bile salts and also contain a unique pigment in their skin that reflects the same wavelength infrared radiation that plants do, possibly another adaptation for concealment (SCHWALM *et al.*, 1977). In the glass frog *Hyalinobatrachium valerioi*, males perform parental care of the clutches in both day and night (24 h) (MCDIARMID, 1978; VOCKENHUBER *et al.*, 2009). That centrolenid have a reticulate green pattern on a yellowish to pale-gold background, and an attending male is strikingly similar in appearance to their egg clutches , especially during the day (MCDIARMID, 1978). MCDIARMID (1978) suggests that the dorsal coloration of *H. valerioi* has evolved for prevent predation in response to its diurnal parental care If the predator searching for an egg clutch is small, enough to be repulsed by the male frog, selection should favor a strong resemblance between the guarding male and his clutch. This will increase the probability of successful defense when the predator mistakes the male with the clutch. If the predator is a frog-eating species, it may mistake the frog with another egg clutch, which is not a suitable prey, and continue its search (MCDIARMID, 1978).

The predation pressure determined the evolution of different anti-predatory behaviors in glass frogs, such as defense posturing, the release of odors (both in this work) and eggs parental care (MCDIARMID, 1975). In the last, for example, some species as *Hyalinobathrachium colymbiphyllum*, *H. valerioi* and *H. fleishmanni*, present a complex behavior of defense to its clutches, which can include hit with

the fore and hind legs the arthropods that trying feeding on embryos (DRAKE & RANVESTEL, 2005; VOCKENHUBER *et al.*, 2008; DELIA *et al.*, 2010). Our observations are the first published on defense posturing and the release of odors in glass frogs (Table 1), showing that many aspects are still unknown about the behavior and ecology of these frogs. Unfortunately, little is known about the anti-predatory behaviors in centrolenids, which hinders the understanding of the evolution of their behavioral characters We suggest that systematic and ecological studies on centrolenids should include the behavioral repertoire of the species to better determine their evolution in a phylogenetic framework.

Our observations are the first published on defense posturing and the release of odors in glass frogs (Table 1), showing that many aspects are still unknown about the behavior and ecology of these frogs. Unfortunately, little is known about the antipredatory behaviors in centrolenids, which hinders the understanding of the evolution of this character within these taxa. We suggest that systematic and ecological studies on centrolenids should pay more attention to the behavioral repertoire of the species to better determine their evolution in a phylogenetic framework.

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