

Head co-ossification, phragmosis and defence in the casque-headed tree frog *Corythomantis greeningi*

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Abstract

Some anurans have a peculiar casqued head with the skin co-ossified with the underlying bones. This type of skull usually is associated with phragmosis, a protective behaviour in which the animal enters a hole and closes it with the head. Although co-ossification of the head in lissamphibians frequently has been associated with water economy, recent studies of *Corythomantis greeningi*, a casque-headed tree frog from semi-arid areas in north-eastern Brazil, suggest that cranial co-ossification contributes little to conservation of water in the frog. Instead, during phragmotic behaviour, the co-ossified head protects the animal against predators and indirectly enhances water balance. Thus, the primary role of co-ossification is defence, a hypothesis that is the focus of this study, which describes the morphology of the head of *C. greeningi* with an emphasis on the co-ossification and the venom glands. We report on behavioural features and on the toxicity of the cutaneous secretion produced by the abundant venom glands that are associated with large spicules on the skull.

Key words: Amphibia, phragmosis, co-ossification, *Corythomantis greeningi*, defence

INTRODUCTION

The ecological success of anuran species that inhabit arid or semi-arid environments is based on diverse behavioural, physiological and morphological characteristics. The morphology of the skin has been studied in this context because this organ constitutes the interface between the environment and the animal, and represents the organism's ultimate natural barrier to water loss (Duellman & Trueb, 1986; Toledo & Jared, 1993; Stebbins & Cohen, 1995). The sculpturing of anuran skin, in association with dermal morphology, glandular secretions and wiping behaviour (Kobelt & Linsenmair, 1986; Toledo & Jared, 1993; Lillywhite *et al.*, 1997; Jared *et al.*, 1999) apparently reduce water loss in specialized amphibians. In some casque-headed anurans, the dermis is co-ossified with the underlying cranial bones to form a unified structure (Trueb, 1970). It has also been proposed that head co-ossification, together with phragmotic behaviour (blocking of the entrance of a burrow or a hole using part of the body, as defined by Wheeler, 1927) confer protection

against water loss, particularly because most casque-headed anurans live in arid environments (Duellman & Trueb Klaas, 1964; Trueb, 1970; Seibert, Lillywhite & Wassersug, 1974).

Jared *et al.* (1999) and Navas, Jared & Antoniazzi (2002) working with *Corythomantis greeningi*, a monotypic genus of casque-headed hylid from the semi-arid region of north-eastern Brazil (Carvalho, 1941; Sazima & Cardoso, 1980), have demonstrated the ability of captive specimens to enter test tubes backwards and close the entrance with their heads, a behaviour named as 'experimental phragmosis' by Jared *et al.* (1999) and Navas *et al.* (2002). Although co-ossification sometimes has been associated with water economy, studies of *C. greeningi* in phragmosis indicate that there is no significant reduction in water evaporation due to skull impermeability (Navas *et al.*, 2002). In contrast, De Andrade & Abe (1997) have demonstrated the role of co-ossification in water economy in *Aparasphenodon brunoi*. This species, a casque-headed hylid from the Brazilian Atlantic Rainforest (Carvalho, 1941; Sazima & Cardoso, 1980), performs phragmosis inside bromeliad axils (Carvalho, 1939), and was found to be more susceptible to water loss through the skin than through the head. On the other hand, phragmotic behaviour is

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important for preventing desiccation, both in *C. greeningi* and *A. brunoi*, as experimentally concluded by Navas *et al.* (2002) and De Andrade & Abe (1997).

In this study the head morphology of *Corythomantis greeningi*, is described in detail in order to understand better its functional significance. We present behavioural observations and report on the toxicity of the cutaneous secretions. Details of the natural history, morphology of the integument and the calcified dermal layer, as well as the physiology involved in water economy of this species, are available elsewhere (Jared, Toledo & Antoniazzi, 1995; Jared *et al.*, 1999; Katchburian *et al.*, 2001; Navas *et al.*, 2002).

We conclude that casque-headed frogs may differ in their ability to retain water. In the case of *C. greeningi*, reduced skin permeability and phragmosis directly improve water economy. In this species, the co-ossified head might have evolved originally as a protective lid for phragmotic individuals, but not as a structure reducing water permeability through the head. The protective function is reinforced by the development of behavioural traits, and mechanical and chemical systems of defence against predation involving cutaneous bone structures and venom glands (Jared *et al.*, 1995; Toledo & Jared, 1995).

MATERIAL AND METHODS

The animal and its habitat

Specimens of *Corythomantis greeningi* Boulenger, 1896, were collected at Fazenda São Miguel, district of Angicos, State of Rio Grande do Norte (5°39'43"S, 36°36'18"W), Brazil, on 3 expeditions made during January and February, from 1999 to 2001. Sixteen live specimens (body mass: male, 21.10 g, \pm 1.00; female, 41.40 g, \pm 3.20) were captured and maintained in a vivarium at the Laboratory of Cell Biology of Instituto Butantan (São Paulo), where behavioural observations were conducted. The animals were maintained in terraria containing earth as a substrate, with dry branches above and pieces of plastic pipe for shelter. Food was supplied every other day and consisted of beetles *Tenebrio molitor*, crickets *Gryllus* sp. and cockroaches *Pycnocellus surinamensis*. Two specimens of *C. greeningi* were deposited in the amphibian collection of the Zoological Museum of the University of São Paulo (MZUSP 71954, 71955).

The district of Angicos where *C. greeningi* were collected is located in a large semi-arid region embracing about 800 000 km² in north-eastern Brazil and characterized by high temperatures and limited rainfall (300–800 mm/year). This huge area is referred to as 'Caatinga' and is characterized by thorny, xeromorphic vegetation and by an abundance of cacti (Souzareis, 1976; Fernandes, 1999). Rain is seasonal, but somewhat unpredictable and occurs mainly in January–March; it is during this time that more than 40 species of native anurans emerge (Rodrigues, 2003).

Behavioural studies

Because previous studies indicated that individual *C. greeningi* adopt phragmotic behaviour inside test tubes (Jared *et al.*, 1999; Navas *et al.*, 2002), we provided pieces of plastic pipe to accommodate the frogs inside the terraria. We observed the behaviour of the animals, especially the nature and extent of the movements of the head, when their heads were grasped in a manner possibly similar to how a predator might seize the frogs.

Morphological analysis

The crania of 3 individuals that died naturally while in captivity were prepared for anatomical observations. The heads were immersed in a solution of sodium hypochloride for removal of the soft tissues. The crania were photographed, divided sagittally into 2 pieces, mounted in metal stubs, coated with gold in a sputtering device, and examined in a Jeol JSM 5300 scanning electron microscope (SEM). In order to examine head co-ossification in detail, 3 additional animals received lethal doses of thionenbutal and the whole heads were fixed in Karnovsky fixative (Karnovsky, 1965), decalcified in 7% EDTA and 0.5% paraformaldehyde in sodium cacodylate buffer 0.1 M, pH 7.2, and embedded in paraffin. Semi-serial sagittal sections were stained in haematoxylin–eosin and examined with a light microscope equipped with Nomarski optics.

In order to study the venom glands, small pieces of the decalcified head skin were prepared for transmission and scanning electron microscopy. After post-fixation in 1% osmium tetroxide, some samples were dehydrated and embedded in epoxy resin and ultra-thin sections were examined in a LEO 906E transmission electron microscope. After post-fixation and dehydration, samples for SEM were frozen in liquid nitrogen and fractured with a razor blade. Subsequently, they were dried by the critical-point method, covered with gold, and examined in the same microscope used for the crania.

Lethality and LD₅₀ calculation of cutaneous venom

Samples of the cutaneous secretions of adult *C. greeningi* were obtained by immersing the tree frogs in distilled water and manually stimulating the skin of the whole body for 5 min. The resulting milky solution was stored at –70 °C and lyophilized; this dried secretion was then used for LD₅₀ determination in male Balb/C isogenic mice donated by the animal facility of Instituto Butantan. The mice used in this test had body weights between 18 g and 22 g and were 7 or 8 weeks old. The animals were treated and maintained under strict ethical conditions according to animal welfare international recommendation (Committee Members, International Society on Toxicology, 1991). The lethal toxicity of *C. greeningi* venom was assessed by intraperitoneal injection of various venom concentrations (25, 50, 75, 115, 150 µg per animal)

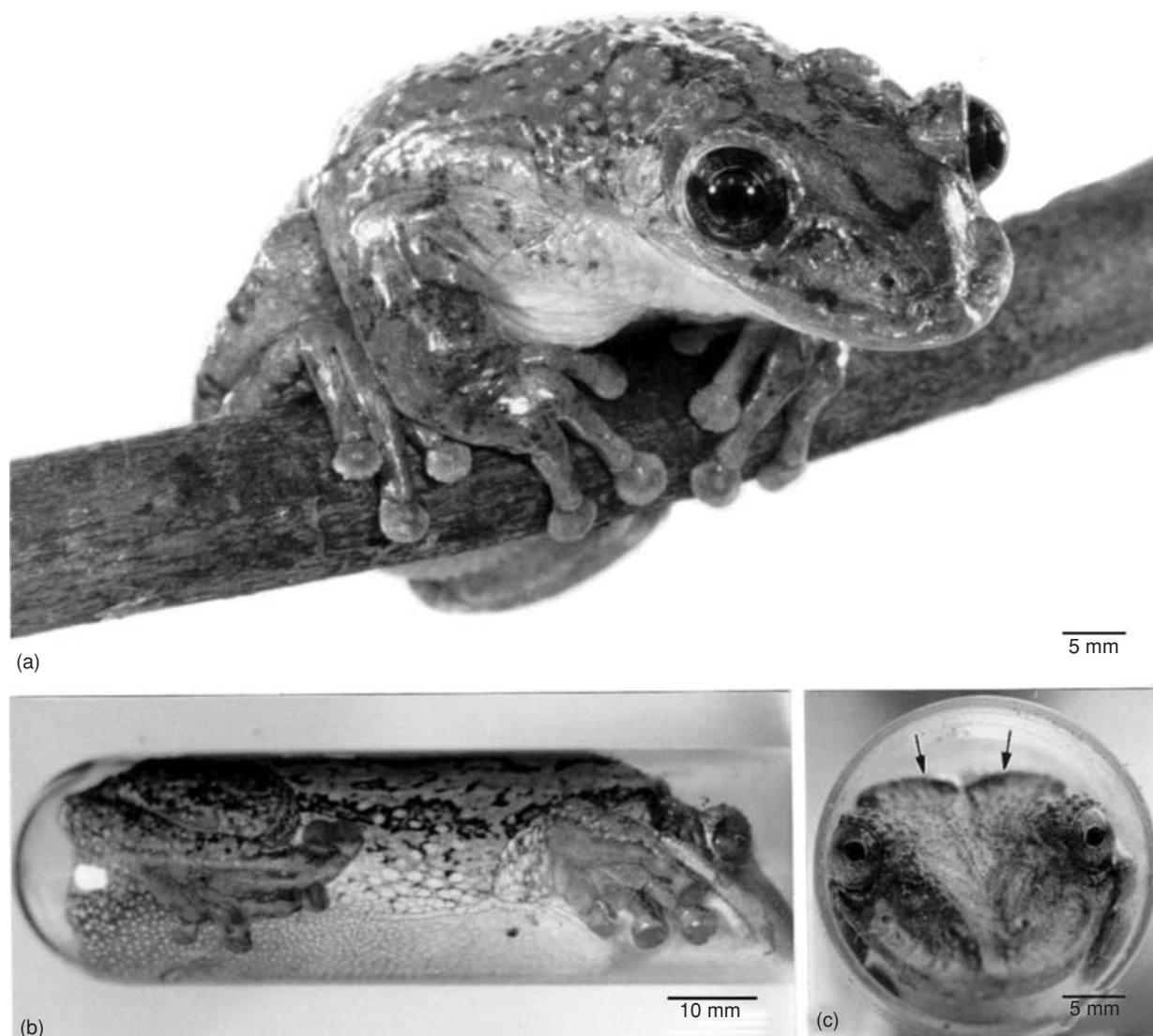


Fig. 1. (a) The casque-headed tree frog *Corythomantis greeningi*. (b) A female *C. greeningi* in phragmotic posture inside a test tube. The head is bent and its top remains exposed to the exterior. (c) Frontal view of *C. greeningi* protected inside a test tube. The area where co-ossification is present is clearly delimited. The arrows point to the well-delimited posterior region of the head.

in 200 μ l of 0.85% NaCl solution. Four animals were used for each venom dose. The LD₅₀ was determined by the Spearman–Karber method (World Health Organization, 1981).

RESULTS

Behavioural observations

Corythomantis greeningi (Fig. 1a) can move its head in various directions without modifying the position of the body. This wide range of movements enabled it to bend the head towards the chest, achieving nearly a 90° angle between the head and body. Previous tests have indicated that captive individual *C. greeningi* adopt phragmotic behaviour inside test tubes when deprived of water (Jared *et al.*, 1999; Navas *et al.*, 2002) (Fig. 1b, c). Daily

observation of the terraria where the animals were maintained indicated their preference for remaining inside the pieces of plastic pipe. Manipulation induced the secretion of large amounts of a viscous and sticky secretion with a characteristic smell. In addition, when the head was firmly immobilized by hand, the animals responded with head movements in many different directions, scraping the rough and spiny head against the palm of the hand, inflicting painful pricks and scratches, much like the abrasion of rough sandpaper against the skin.

Anatomy of the head

The head is dorso ventrally flat and extremely rough (even the eyelids); there are many small spicules along the anterior, lateral and posterior borders (Fig. 2a). The texture and colour of the top of the head resembles bark of the common trees *Prosopis juliflora* and *Mimosa* sp.

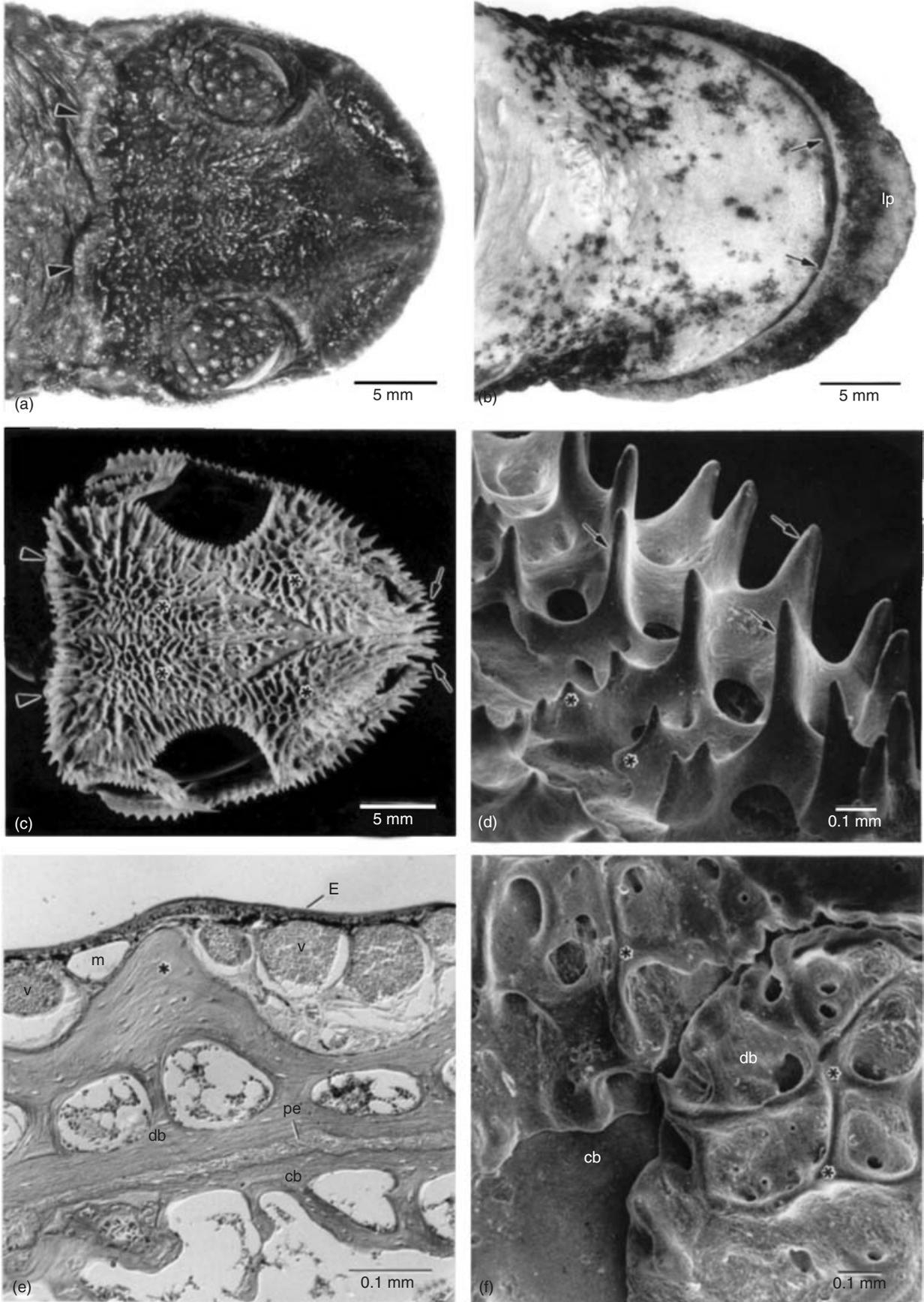


Fig. 2. For legend see facing page.

found in the region where *C. greeningi* inhabits. The head has a distinct occipital crest (Figs 1c & 2a). The mouth is subterminal owing to the presence of a pronounced, bony rostral projection (Fig. 2b).

The macroscopical observation of the skull shows that its dorsal surface is covered by extensive, co-ossified dermal roofing bones that, forming an integumentary cranial co-ossification where the dermis is ossified and joined with the cranial bones, comprises a single bony piece. Dermal roofing bones have bony projections over their entire surfaces. Bony spicules occur across the occiput and around the orbit; dense aggregations configured into a cluster of spines are located on the maxillae, premaxillae and nasals (Fig. 2c). Scanning electron microscopy revealed that the surfaces of the dermal roofing bones are heterogeneous; in some areas, the bone is smooth, whereas in others it is irregular and rugose (Fig. 2f). The more central areas of the dermal bones, on the top of the head have irregular protuberances, but not spicules (Fig. 2f); this contrasts with the margins of the bones, which have spiny projections (Fig. 2d).

Histology of the head

The sagittal sections of the head reveal that the epidermis of the head skin is thinner than that of the body. The dermal stratum spongiosum has many mucous and granular (venom) glands similar to those present in the body skin (Jared *et al.*, 1995 and Fig. 2e). The granular glands of the head skin occupy a large volume of the stratum spongiosum, particularly in the bony flange of the maxillary arcade (Fig. 3a) where large dermal bone spicules are more patent. The stratum compactum of the head of *C. greeningi* is unusual because it is not formed of collagen fibres, but of ossified tissue (the dermal bones) that lies over the cranial bones, forming the co-ossification, according to Trueb (1970). Between the dermal bones and the cranial bones there is a thin periosteal-like layer (Fig. 2e).

Overall, the histological study confirmed the patterns reported by SEM and added interesting new insights, particularly regarding the relationship between dermal bone and skin. In some regions the bony dermal protuberances reach the basal portions of the epidermis (Fig. 2e), as is usual for dermal ossifications. However, at the skull margins, the large spicules seem to force

the epidermis outward, giving the impression that the epidermal tissue is almost at the point of rupturing. Many of the spicules are surrounded by abundant venom glands (Fig. 3b).

Electron microscopy of the venom glands of the head

Like the venom glands on the postcranial part of the body, those of the head are syncytial and enveloped by a myoepithelial layer (Fig. 3d). The syncytium is characterized by the peripheral nuclei and is filled with spherical granules of similar size immersed in a cytoplasmic matrix (Fig. 3c). Examination of the ultrathin sections revealed that these granules are composed of a central region with medium electron density and a more electron-dense peripheral area (Fig. 3d).

Envenomation symptoms and venom lethality

The symptoms of envenomation were recorded during a 72-h period of observation after venom injection. Death usually was preceded by signs of prostration, cachexia and neurological disorder evidenced by disrupted movements such as circling behaviour. The estimated LD₅₀ for Balb/C mice was 69.75 µg ± 1.33.

DISCUSSION

Corythomantis, as well as *Hyla*, *Osteocephalus*, *Trachycephalus*, *Aparasphenodon*, *Pterohyla* and *Tripurion* are all genera of hylids that include species possessing casqued heads (Trueb, 1970) that are involved in integumentary co-ossification, characterized by the deposition of bone in the dense connective tissue of the dermal layer of the skin (Trueb, 1966). Boulenger (1896), when first describing *C. greeningi*, observed 'the curious shape of the head', using the Greek term *corytho*, which means casque or helmet. Most of these hylids, including *C. greeningi* (Jared *et al.*, 1999), inhabit dry environments. Although the presence of cranial co-ossification in this species was reported by Trueb (1970), details about the morphology and function of this structure have not been well elaborated.

Wheeler (1927), dealing with insects, coined the term phragmosis and extended it to other groups of

Fig. 2. (a) Top of the head of *Corythomantis greeningi* showing the extremely rough integument, including the eyelids. The arrowheads point to the posterior border of the head. (b) General view of the head in ventral position, showing the labial flanges (lp) and the mouth (arrows) in ventral position. (c) Skull in dorsal view. The dermal roofing bones (asterisks), in the form of many protuberances and spicules, especially at the borders, cover most of the skull. The arrowheads designate the posterior region of the skull and the arrows point out the clustering of the spicules at the anterior. (d) Scanning electron microscopy of the border of the skull showing the major spicules (arrows) and the minor spicules (asterisks) covering the region. (e) Histological sagittal section of head, showing the cranial co-ossification, which is formed by the dermal bone (db) superposed over the cranial bones (cb). Between them, there is a periosteal-like structure (pe). The asterisk indicates a dermal bone protuberance. m, mucous gland; v, venom glands; E, epidermis. Haematoxylin–eosin staining. Nomarski contrast. (f) Scanning electron micrograph of part of the skull showing the smooth cranial bone (cb), covered by dermal bone (db). The asterisks indicate dermal bone protuberances.

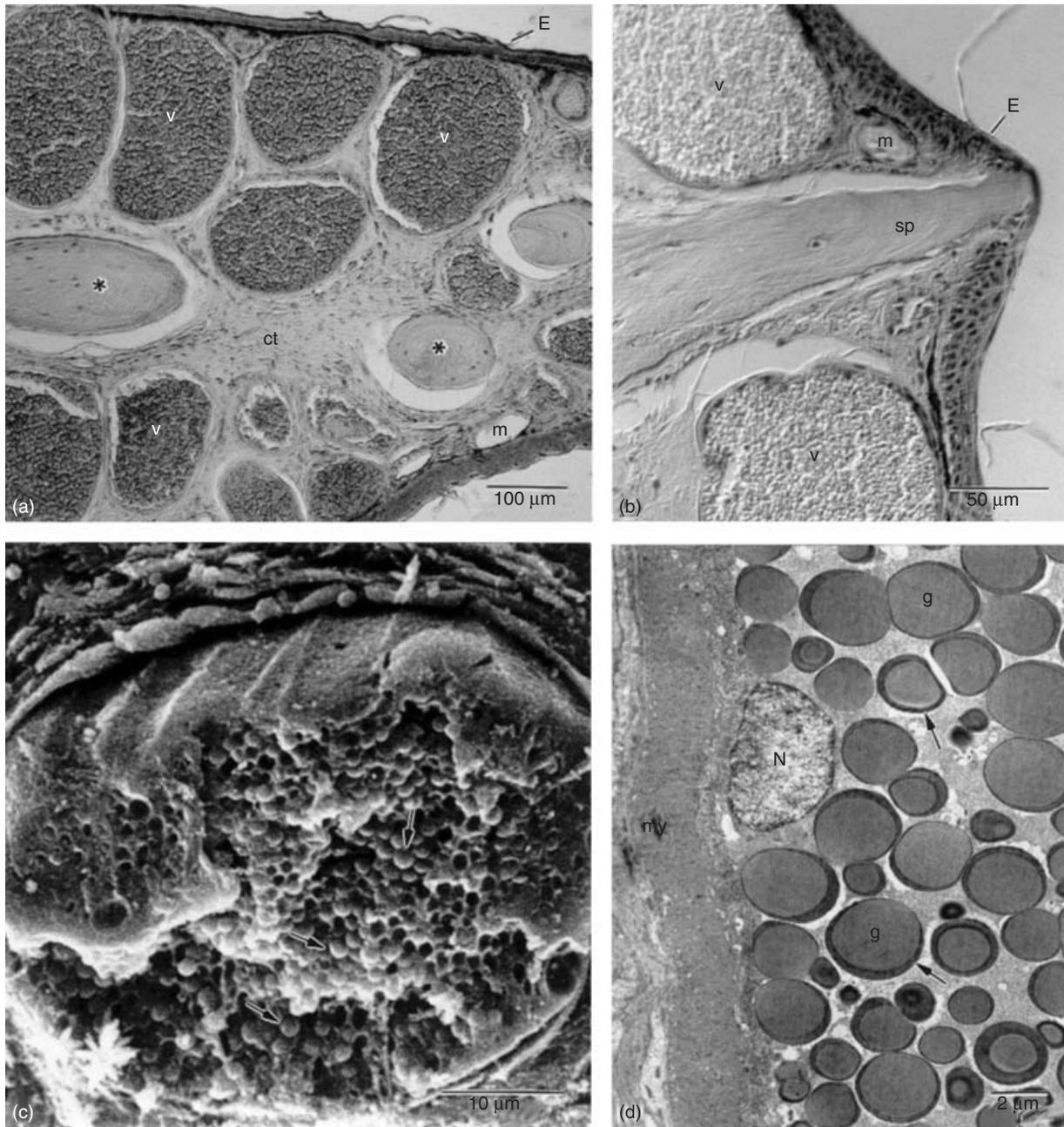


Fig. 3. (a) Histological section of the labial flanges of *Corythomantis greeningi* where a large number of venom glands (v) is present. E, epidermis; ct, connective tissue of the epidermis. Asterisks indicate dermal bones. Haematoxylin–eosin staining. (b) Histological section of a head bone spicule (sp) projecting itself up to the epidermis (E). Around the spicule venom granular glands (v) are observed. m, mucous gland. Haematoxylin–eosin staining. Nomarski contrast. (c) Scanning electron micrograph of a fractured syncytial venom gland, filled with spherical secretion granules (arrows), immersed in a matrix. (d) Transmission electron microscopy of part of the syncytial venom gland. The granules (g) are not homogeneous, but formed of an inner region and a more electron-dense external region (arrows). my, myoepithelial layer; N, syncytial nucleus.

animals, reporting that Barbour (1926) was the first to observe this behaviour among amphibians. In 1912, on a scientific expedition to Cuba, Barbour (1914) observed *Bufo empus* (shell toad or *sapo de concha* in Spanish) ‘that lives in a burrow for which its hard, shelly head forms a perfect stopper’. Similarly, hylids perform phragmosis by closing tree holes. As Stuart (1935) observed in the Guatemalan hylid *Triprrion petasatus*: ‘once in a small hole

the species plugs the cavity with its casque-head, and it is almost impossible to remove a specimen thus protected’. Thus, the casque-head has long been linked to phragmosis. Barbour (1926), indeed, hypothesized that *C. greeningi* would be phragmotic based only on the morphological features of the head. Nevertheless, natural history field studies of *C. greeningi* conducted by Jared *et al.* (1999) did not register such a dramatic case of phragmosis as

that previously described for *Tripriion petasatus*, although some specimens were found inside rock crevices, tree holes and dry water pipes with diameters corresponding to that of their heads. Experimental phragmosis with *C. greeningi* has demonstrated that the head with the broad maxillary flange, functions as a lid (Jared *et al.*, 1999; Navas *et al.*, 2002). The cryptic effect is reinforced by the texture and colour pattern of the top of the head, which resembles tree bark, probably making phragmosis in nature effective because the head would be difficult for a potential predator to identify. Moreover, phragmotic behaviour may help to maintain humidity inside the frog's shelter, which would be important in semi-arid regions where rain is unpredictable (Souzaris, 1976; Fernandes, 1999). In fact, Navas *et al.* (2002) demonstrated that *C. greeningi* can distinguish between humid holes and dry holes when it searches for an ideal location to perform phragmosis.

Although cranial co-ossification usually is associated with water economy, Navas *et al.* (2002) suggested that this association is not valid for *C. greeningi*. On the other hand, phragmosis *per se* in this species and in *A. brunoi* may reduce water evaporation by more than 90% (De Andrade & Abe, 1997; Navas *et al.*, 2002). De Andrade & Abe (1997), making a comparison in evaporative water loss between *C. greeningi* and *A. brunoi*, showed that in *C. greeningi* no significant difference was found between the evaporative water loss in the head and in the body skin. In contrast, *A. brunoi* was found to be more susceptible to water loss through the skin, although the rate of evaporative water loss through the head surface is smaller than that through its body skin. Therefore, even living in the rainforest, *A. brunoi*, when compared with *C. greeningi*, seems to be more dependent on cranial co-ossification during phragmosis.

Due to the characteristics of the morphology of the skull and body integument, as well as of the behaviour of *C. greeningi*, we propose that cranial co-ossification, at least in this species, plays an important role in defence against predation. Investment in osseous enlargement of the head through increment of the dermal bones, with protuberances and spines, may serve as a mechanical defence. This peculiar structure of the head is probably efficient in keeping the animal safe inside holes and making it very difficult for a potential predator to remove the animal from its hole. The extent of head movements may favour a perfect closure of the hole, maintaining the animal inside. A nearly 90° forward flexion of the head, as observed in *C. greeningi*, seems to be common in phragmotic amphibians and, according to Trueb (1970), is associated with an enlargement of the rhomboideus anterior muscle. In fact, Firschein (1951) noticed that preserved specimens of *Pternohyala fodiens* are commonly found with the head forming a 90° angle with the body. We have observed the same fact in preserved specimens of *C. greeningi* and *Tripriion petasatus* from the MZUSP collection.

In contrast, if the head of *C. greeningi*, when the animal is outside the shelter, is grasped by the sides, the voluminous venom glands, located mainly in the

labial flanges, are probably pressed against the spines and protuberances, facilitating venom introduction into the oral mucosa of the predator. When *C. greeningi* is attacked, the head most probably plays an important role in defence, since the animal may use it as a weapon. In fact, it is very difficult to grasp its head for more than a few seconds. Deglutition of an animal such as this would be very difficult because, in addition to mechanical injuries, glands over the entire body release highly lethal toxins, rapidly envenoming the potential predator. An LD₅₀ of around 70 µg is high when compared with, for example, the viperid snake genus *Bothrops*, responsible for the largest number of snakebites in Brazil (Ministério da Saúde, Fundação Nacional da Saúde, 1998). Bothropic venom, in the species *B. jararaca*, presents an LD₅₀ of around 45 µg (Sanchez *et al.*, 1992).

A defence process similar to that which may occur in the head of *C. greeningi* has already been identified in Asiatic salamanders of the genus *Tylostotriton*. When *T. verrucosus* is harassed, the animal stiffens its body and perforates glandular accumulations in the skin with the free extremities of its pointed ribs. In the case of an attack, the ribs, impregnated with the glandular secretions, may serve as potent weapons, introducing venom into the oral mucosa of the predator (Brodie, Nussbaum & DiGiovanni, 1984).

When analysing the illustrations of Trueb (1970) showing casque-headed hylid skulls, it is evident that those of *C. greeningi* and *Tripriion spatulatus* (formerly named *Diaglena spatulatus*) resemble one another. Both have pointed protuberances roofing the cranial bones and many spiny spicules in the skull borders, although those of *C. greeningi* are more spiny. Thus, if *T. spatulatus* had venom glands in the spiny labial borders, such as *C. greeningi* has, these two species, among all hylids studied by Trueb (1970), would be the most well adapted to use the head as a weapon. Coincidentally, both species colonize similar semi-arid environments, which may suggest a convergent adaptation.

In this context, the term 'helmet' (*corytho*) used by Boulenger (1896), seems to be appropriate in relation to its mechanical function in defence. Lutz (1954) in her trails to give an explanation to this bizarre type of skull in *Corythomantis*, as well as in *Aparasphenodon*, suggested that co-ossification in these species would protect them against predators and haematophagous animals (Lutz & Lutz, 1939).

The association of phragmosis with protection against predators had already being raised for *Tripriion petasatus* (Duellman & Trueb Klaas, 1964). Trueb (1970) commented that many structural modifications in the skin of co-ossified heads, such as reducing of gland number, poor vascularization, and diminishing of sensory and protective functions would occur. Contradicting Trueb (1970), our results show that, in *C. greeningi*, the head presents a number of glands similar to that of the body skin. Moreover, in some regions, such as the labial flanges, there is a considerable increase in the number of venom glands. Our observations demonstrate that venom release in the head is important for defence. In fact, when the

animal is manipulated, abundant secretion exudes from the entire body, including the head.

Cranial co-ossification of casque-headed anurans, together with phragmosis, have been traditionally associated as a mechanism against desiccation. In this paper, we have shown that, at least for *C. greeningi*, co-ossification has also evolved additional functions as a mechanical and chemical weapon in the defence against predation.

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REFERENCES

- Barbour, T. (1914). A contribution to the zoogeography of the West Indies, with especial reference to amphibians and reptiles. *Mem. Mus. Comp. Zool.* **44**: 242–243.
- Barbour, T. (1926). *Reptiles and amphibians. Their habits and adaptations*. Cambridge, MA: The Riverside Press.
- Boulenger, G. A. (1896). Descriptions of new Batrachians in British Museum. *Ann. Mag. Nat. Hist. (Lond.)* **6**: 401–406.
- Brodie, E. D., Nussbaum, R. A. & DiGiovanni, M. (1984). Antipredator adaptations of Asian salamanders (Salamandridae). *Herpetologica* **40**: 56–68.
- Carvalho, A. L. (1939). Nota previa sobre os habitos de uma interessante 'perereca' bromelicola do litoral SE brasileiro (*Aparasphenodon brunoi* Mir. Rib. 1920), *O Campo*, Rio de Janeiro, Agosto 25–26.
- Carvalho, A. L. (1941). Notas sobre o gênero *Corythomantis* Boulenger e *Aparasphenodon* Miranda Ribeiro. *Papéis Dep. Zool.*, São Paulo **1**: 101–110.
- De Andrade, D. V. & Abe S. A. (1997). Evaporative water loss and oxygen uptake in two casque-headed tree frogs, *Aparasphenodon brunoi* and *Corythomantis greeningi* (Anura, Hylidae). *Comp. Biochem. Physiol.* **118**: 685–689.
- Duellman, W. E. & Trueb Klaas, L. (1964). The biology of the hylid frog *Triprion petasatus*. *Copeia* **1964**: 308–321.
- Duellman, W. E. & Trueb, L. (1986). *Biology of amphibians*. New York: McGraw-Hill Book Company.
- Fernandes, A. (1999). Provincia das caatingas ou nordestina. *An. Acad. Bras. Ci.* **71**: 299–310.
- Firrschein I. L. (1951). Phragmosis and the 'unken reflex' in a Mexican hylid frog, *Pternohyla fodiens*. *Copeia* **1951**: 71.
- Jared, C., Antoniazzi, M. M., Katchburian, E., Toledo, R. C. & Freymüller, E. (1999). Some aspects of the natural history of the casque-headed tree frog *Corythomantis greeningi* Boulenger (Hylidae). *Ann. Sci. Nat.* **3**: 105–115.
- Jared, C., Toledo, R. C. & Antoniazzi, M. M. (1995). A histological study of the integument of *Corythomantis greeningi* (Amphibia, Anura, Hylidae). *Rev. Bras. Biol.* **55**: 509–515.
- Karnovsky, M. J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Morphol.* **27**: 137–138A.
- Katchburian, E., Antoniazzi, M. M., Jared, C., Faria, F. P., Souza Santos, H. & Freymüller, E. (2001). Mineralized dermal layer of the Brazilian tree frog *Corythomantis greeningi*. *J. Morphol.* **248**: 56–63.
- Kobelt, F. & Linsenmair, K. E. (1986). Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. I: the skin of *Hyperolius viridiflavus nitidulus* in wet and dry season conditions. *Oecologia* **68**: 533–541.
- Lillywhite, H. B., Mittal, A. K., Garg, T. K. & Agrawal, N. (1997). Integumentary structure and its relationship to wiping behaviour in the common Indian tree frog, *Polypedates maculatus*. *J. Zool. (Lond.)* **243**: 675–687.
- Lutz, B. (1954). Anfíbios anuros do Distrito Federal. *Mem. Inst. Oswaldo Cruz* **52**: 155–238.
- Lutz, A. & Lutz, B. (1939). Notes on the genus *Phyllomedusa* Wagler. Observations on small Phyllomedusinae without vomerine teeth or conspicuous parotids found in the region of Rio de Janeiro. *Ann. Acad. Bras. Sci.* **11**: 219–263.
- Ministério da Saúde, Fundação Nacional de Saúde (1998). *Manual de diagnóstico e tratamento de acidentes por animais peçonhentos*. Brasília: Governo Federal Brasileiro.
- Navas, C. A., Jared, C. & Antoniazzi, M. M. (2002). Water economy in the casque-headed tree frog *Corythomantis greeningi* (Hylidae): role of behaviour, skin, and skull skin co-ossification. *J. Zool. (Lond.)* **257**: 525–532.
- Rodrigues, M. T. (2003). Herpetofauna da Caatinga. In *Ecologia e Conservação da Caatinga*: 183–238. Leal, I. R., Tabarelli, M. & Silva, J. M. C. (Eds). Recife: Editora Universitária da UFPE.
- Sanchez, E. F., Freitas, T. V., Ferreira-Alves, D. L., Velarde, D. T., Diniz M. R., Cordeiro, M. N., Agostini-Cotta, G. & Diniz, C. R. (1992). Biological activities of venoms from South American snakes. *Toxicon*, **30**: 95–103.
- Sazima, I. & Cardoso, A. J. (1980). Notas sobre a distribuição de *Corythomantis greeningi* Boulenger 1896 e *Aparasphenodon brunoi* Miranda-Ribeiro, 1920 (Amphibia, Hylidae). *Iheringia, Sér. Zool. Porto Alegre* **55**: 3–7.
- Seibert, E. A., Lillywhite, H. B. & Wassersug, R. J. (1974). Cranial co-ossification in frogs: relationship to rate of evaporative water loss. *Physiol. Zool.* **47**: 261–265.
- Stebbins, R. C. & Cohen, N. W. (1995). *A natural history of amphibians*. Princeton, NJ: Princeton University Press.
- Souzaeins, A. C. D. (1976). Climate of Caatinga. *An. Acad. Bras. Ci.* **48**: 325–335.
- Stuart, L. C. (1935). A contribution to a knowledge of the herpetology of a portion of the savannah region of central Petén, Guatemala. *Misc. Publ., Mus. Zool. Univ. Michigan* **29**: 1–56.
- Toledo, R. C. & Jared, C. (1993). Cutaneous adaptations to water balance in amphibians. *Comp. Biochem. Physiol.* **105**: 593–608.
- Toledo, R. C. & Jared, C. (1995). Cutaneous granular glands and amphibian venoms. *Comp. Biochem. Physiol.* **111**: 1–29.
- Trueb, L. (1966). Morphology and development of the skull in the frog *Hyla septentrionalis*. *Copeia* **1966**: 562–573.
- Trueb, L. (1970). Evolutionary relationships of casque-headed tree frogs with co-ossified skulls (Family Hylidae). *Univ. Kansas Publ., Mus. Nat. Hist.* **18**: 547–716.
- Wheeler, W. M. (1927). Physiognomy of insects. *Quar. Rev. Biol.* **2**: 1–36.
- World Health Organization (1981). *Progress in the characterisation of venoms and standardisation of antivenoms*. Geneva: World Health Organization.