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## **Histopathology of large epidermal cysts on the invasive Puerto Rican Crested Anole (*Anolis cristatellus*) in Miami, Florida, USA.**

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### **Abstract**

Large masses were observed on the head and bodies of non-native Puerto Rican crested anoles (*Anolis cristatellus*) in Miami, Florida USA. Following examination, the masses were found to be epidermal inclusion cysts. The cysts did not appear to interfere with body condition or behavior. This is the first record of epidermal inclusion cysts in *A. cristatellus* in either the native (Puerto Rico) or non-native (Florida) range.

**Keywords:** invasive species, Florida, *Anolis*, cyst.

### **Introduction**

Emerging human activities are having detrimental consequences in wildlife ecosystems, increasing the frequency and effects of biological invasions and introduction of novel diseases. Globalization and increased connectedness are causal factors for the prevalence of invasive species, which represent the second leading cause of extinction in the US (Crowl *et al.*, 2008). Recognizing and recording any alteration in basic behavioral and morphological conditions of any wildlife organism is important if we are to accurately document how novel stressors are influencing biodiversity (McNamara, 2015). To understand dispersion of invasive species and diseases, observational and experimental approaches are required at local, regional, continental and global scales, with which biotic and abiotic effects and impacts can be evaluated (Crowl *et al.*, 2008).

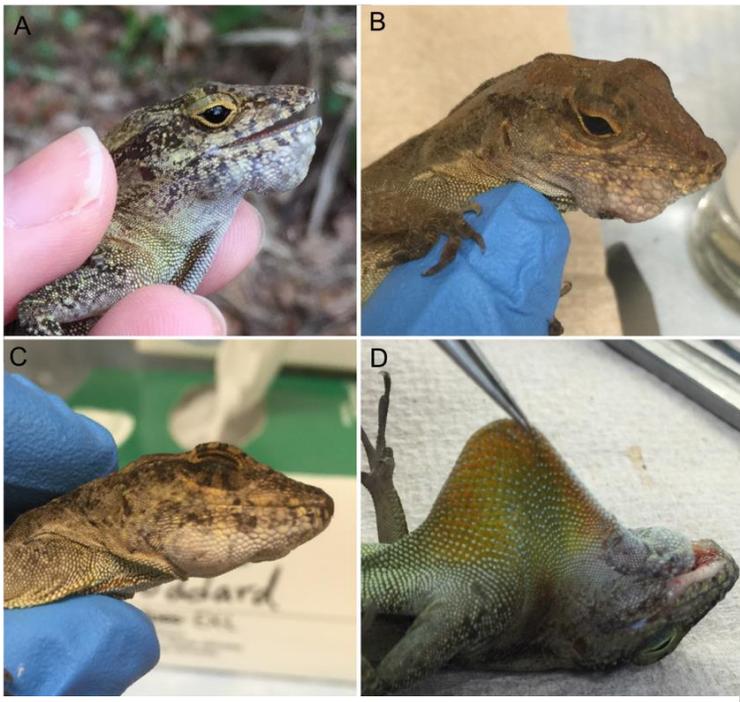
Here, we report the first histopathological examination of large, external cyst-like masses observed on a non-native exotic lizard – Puerto Rican crested anoles (*Anolis cristatellus*) – in Miami, Florida USA. Epidermal inclusion cysts refer to an epidermoid cyst resulting from the implantation of epidermal cells in the dermis. They are benign lesions that can be of sebaceous or follicular origin, commonly reported in mammals, specifically in humans, dogs, sheeps, cats and horses. Lesions can be caused by several mechanisms such as sequestration of epidermal rests in embryonic life, occlusion of the pilosebaceous unit, or trauma/injuries (Parker, 1995; Fomm, 2018). In reptiles, cases of epidermal inclusion cysts have been reported in Painted

Turtles (*Chysemys picta*), which were located around the tympanum and seem to have arisen from epidermal pockets similar to sebaceous cysts reported in mammals (Christiansen *et al.* 2004).

### Case Study

Puerto Rican crested anoles (*A. cristatellus*) were originally introduced to two independent locations in Miami FL in the 1970s (Kolbe *et al.* 2016). Genetic analyses confirmed that the two populations – Key Biscayne and South Miami/Pinecrest – were the result of independent introductions (Kolbe *et al.* 2007). The Key Biscayne population was first detected in 1975 and originates from the capital of Puerto Rico, San Juan (Schwartz & Thomas 1975, Bartlett & Bartlett 1999), while the South Miami population was discovered in 1976 on the Red Road canal (Snapper Creek) and originates from northeast Puerto Rico (Wilson & Porras 1983). While the population on Key Biscayne has remained relatively constrained, dispersal of the South Miami population is ongoing and *A. cristatellus* may now be found throughout the South Miami/Pinecrest/Coral Gables/Coconut Grove neighborhoods (Kolbe *et al.* 2016).

Tumor-like external masses were first observed on adult male individuals of *A. cristatellus* in 2013 in the South Miami population. The masses are soft to touch, ovoid, and appear as a swollen protrusion from the skin (see Fig 1A-D). In some populations in the South Miami region, almost all individuals will possess at least one facial mass (and anecdotally this is heavily skewed towards males).



**Figure 1.** Epidermal inclusion cysts on male individuals of *A. cristatellus* in Miami, FL.

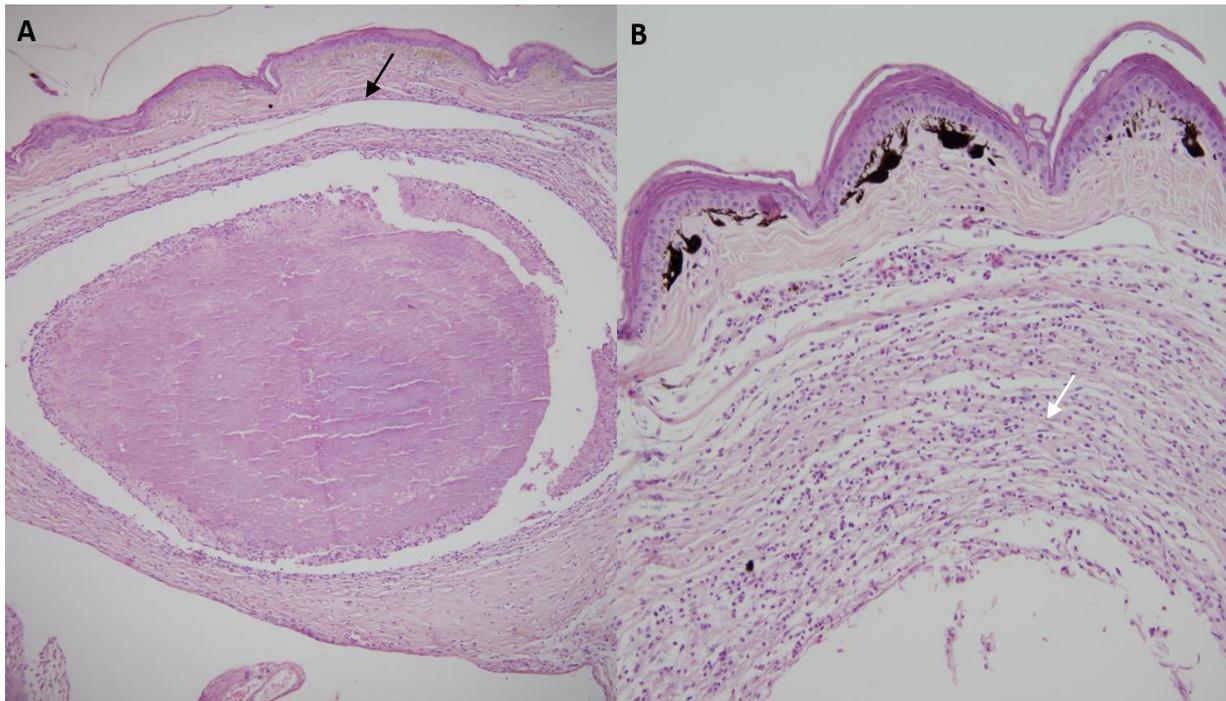
Individuals appeared healthy, with no discernable effects on body condition or behavior. Some individuals have been observed foraging successfully and in entering agonistic interactions with conspecifics. A review of the literature revealed no similar cases to this in other *Anolis* lizards, although some anecdotal reports exist from other anole biologists<sup>3</sup> and in other anole species (*A. carolinensis*, *A. smaragdinus*) from the herpeto-cultural industry (e.g. *Reptile Boards*, *A Reptile and Amphibian Community* blog).

We collected six male individuals with obvious external masses from Fairchild Tropical Botanic Garden, Coral Gables FL

<sup>3</sup> For example, Brian Magnier writes on *Anole Annals*, “Parasitic Fly Larva in *Anolis cristatellus*?” <http://www.anoleannals.org/2016/09/28/parasitic-fly-larva-in-anolis-cristatellus/>

(25.677°N, 80.276°W). All individuals were anesthetized via intracoelomic injections of 0.2-0.4ml of liquid lidocaine diluted to 0.5 g in 10ml of distilled water. Once individuals were sedated, an injection of 0.5 ml of lidocaine was administered intracardially to euthanize the lizard. Procedures were performed following the American Veterinary Medical Association (AVMA) guidelines for the euthanasia of animals (2013 edition).

Following euthanasia, all masses on all individuals were dissected and preserved in 10% neutral buffered formalin. Samples were fixed using 10% neutral buffered formalin for 24 hours and preserved in 70% ethanol and then sent for histopathological analyses in Histopat Laboratory (Bogota, Colombia).



**Figure 2.** Cyst lined (black arrow) by keratinized epithelium with a distinct granular layer without nuclear atypia. In addition, we observed inflammation due to cyst rupture with presence of histiocytes, lymphocytes and scattered eosinophils (white arrow). **A.** (4x). **B.** (10x). H&E (Hematoxylin and Eosin staining).

### *Histopathological Description*

We processed skin biopsies from six *A. cristatellus* specimens with obvious epidermal lesions. Lamellar and ellipsoid fragments were generally ca. 1-1.5 cm long, ca. 0.8 cm wide, and ca. 0.5-0.7 cm thick. The skin surface was corrugated and tan grey. Following a tangential cut, diameter cystic lesions varying in diameter were found with white and oily content. For the histopathology, formalin-fixed tissues were de-paraffinized in xylene and alcohol, embedded in paraffin, sectioned at 2  $\mu$ m and stained with hematoxylin and eosin (H&E).

We observed that all cysts were lined by a keratinized epithelium with distinct granular layers without nuclear atypia. In addition, we observed inflammation due to cyst rupture with presence of histiocytes, lymphocytes, and scattered eosinophils (see Fig 2). No presence or remains of microorganisms or parasites associated with the cysts were observed in any of the samples.

## Conclusions

Here, following our investigations, we report of epidermal inclusion cysts on populations of Puerto Rican crested anoles (*A. cristatellus*) in Miami FL are not associated to microorganisms. We are still unsure of the causal factors of the masses and that avenue represents ongoing research. Further questions include ascertaining whether masses on the Key Biscayne population are the same as the masses assessed in the South Miami population in this study.

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