

## SHORT COMMUNICATION

# Putative poison gland in the thorny catfish *Acanthodoras spinosissimus* (Siluriformes: Doradidae)

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## ABSTRACT

*Acanthodoras* is the only genus of catfish known to secrete a conspicuous and abundant milky-looking substance through an axillary pore located just below the base of the posterior cleithral process. Despite this remarkable feature, there is no published information on the anatomical structures that produce the secretion and its possible biological/ecological functions. Dissection and histological analysis of preserved specimens of *A. spinosissimus* revealed the presence of a sacular axillary gland with large, binuclear secretory cells, similar to those found in other poisonous catfish. Secretory cells near the lumen appear to lose nuclei and become filled with secretory products, possibly with proteinaceous elements, as indicated by their eosinophilic appearance. As far as we know, the sacular morphology of the gland appears to constitute a unique characteristic of *Acanthodoras* among Doradidae catfishes. Further studies are necessary to determine the chemical composition of the secretion, as well as its possible uses by the catfish in its natural environment.

**KEYWORDS:** Amazon basin, defense, venom gland, freshwater

## Suposta glândula de veneno no bagre rebeça *Acanthodoras spinosissimus* (Siluriformes: Doradidae)

### RESUMO

*Acanthodoras* é o único gênero de bagre conhecido por secretar uma substância de aparência leitosa conspícua e abundante através de um poro axilar localizado logo abaixo da base do processo cleitral posterior. Apesar dessa característica marcante, não há informações publicadas sobre as estruturas anatômicas que produzem a secreção, nem sobre suas possíveis funções biológicas/ecológicas. A dissecação e análise histológica de espécimes preservados de *A. spinosissimus* revelaram a presença de uma glândula axilar sacular com células secretoras binucleares, semelhantes às encontradas em outros bagres venenosos. As células secretoras próximas ao lúmen parecem perder os núcleos e são preenchidas com produtos secretores, possivelmente com elementos proteicos, conforme indicado por sua aparência eosinofílica. Até onde sabemos, a morfologia sacular da glândula parece constituir uma característica única de *Acanthodoras* entre os bagres Doradidae. Mais estudos são necessários para determinar a composição química da secreção, bem como seus possíveis usos pelo bagre em seu ambiente natural.

**PALAVRAS-CHAVE:** Bacia Amazônica, defesa, glândula de veneno, água doce

Siluriformes comprise the most morphologically diverse and widely distributed Ostariophysi, with 39 described families (Fricke *et al.* 2021). The epidermis of Siluriformes, as in other fish, has become highly specialized, performing multiple functions (Elliot 2011). Groups of cells present in the skin of fish gave rise to epidermal glands that evolved for different functions (Cameron and Endean 1973), such as the

production of mucus to facilitate locomotion in the aquatic environment, and the secretion of proteinaceous compounds (crinotoxins and other substances) as a defense apparatus in association with the spines of pectoral and dorsal fins (Quay 1972; Cameron and Endean 1973). In addition, axillary glands have been described for some families of Siluriformes (Aksysidae, Ariidae, Callichthyidae, Ictaluridae, Mochokidae

**CITE AS:** Sodr , J.G.; Colaço-Fernandes, T.R.; Rapp Py-Daniel, L.H.; Birindelli, J.L.O.; Zuanon, J. 2021. Putative poison gland in the thorny catfish *Acanthodoras spinosissimus* (Siluriformes: Doradidae). *Acta Amazonica* 51: 250-254.

and Plotosidae) (Wright 2015), which were found to secrete bactericidal substances in the callichthyid genus *Corydoras* (Kiehl *et al.* 2006).

*Acanthodoras* are small Doradidae catfishes known to secrete a conspicuous milky-looking substance through an axillary pore just below the posterior cleithral process, which is considered to be poisonous by aquarium keepers (Burgess 1989). The few published studies that included species of *Acanthodoras* (e.g., Sabaj and Ferraris 2003; Birindelli 2014) deal with taxonomic and/or phylogenetic analyses, and do not refer this secretion. The genus is considered to include three valid species, *Acanthodoras spinosissimus* (Eigenmann & Eigenmann, 1888), *Acanthodoras depressus* (Steindachner, 1881), and *Acanthodoras cataphractus* (Linnaeus, 1758), distributed in the Amazon and Orinoco basins and coastal drainages of the Guianas (Sabaj and Ferraris 2003). These thorny catfishes have sedentary habits, a conspicuous color pattern consisting of a boldly contrasting lightly-colored longitudinal band (varying from white to orange) on a dark olivaceous to nearly black background. Both *A. cataphractus* and *A. spinosissimus* are commonly sold as ornamental fishes (Anjos *et al.* 2009), sometimes mixed with specimens of the similar-looking doradid *Agamyxis pectinifrons*. Despite being common among aquarium-fish keepers and in scientific fish collections, there is no published information on the anatomical structures that produce the secretion produced by these fish, and its possible biological/ecological functions. This study aimed to fill this knowledge gap by means of the anatomical and histological analysis of the axillary secretory structure of *A. spinosissimus* and its comparison with the apparently similar structures found in other thorny catfishes.

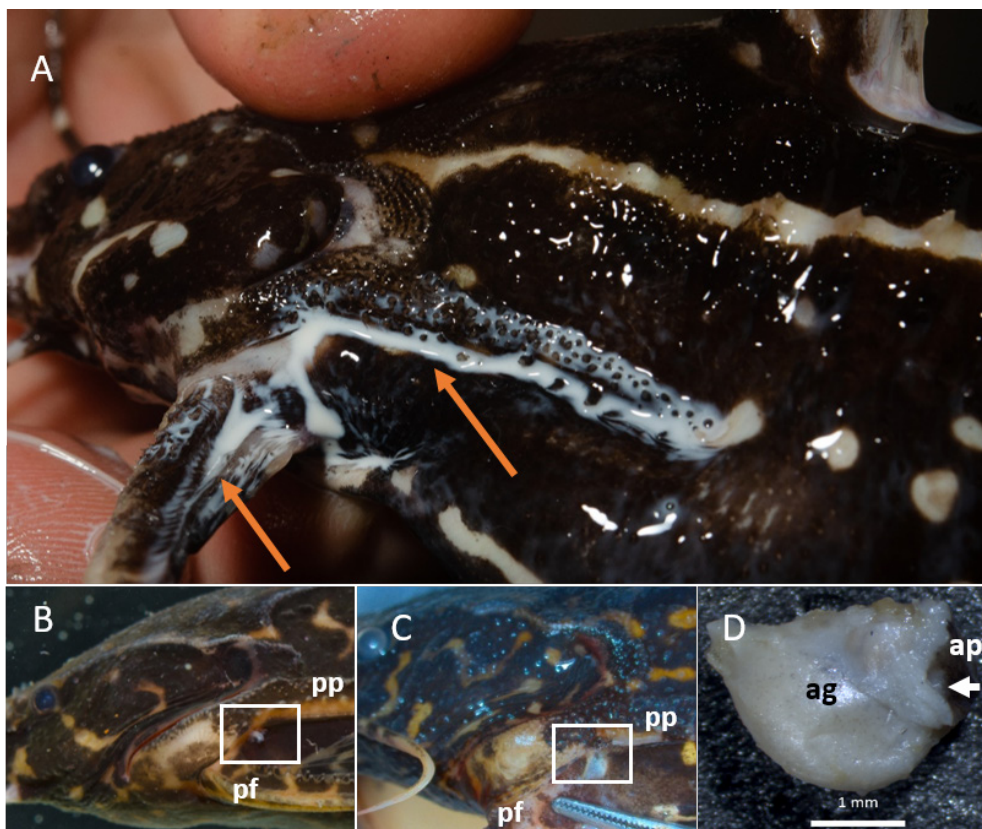
Four specimens of *A. spinosissimus* were caught in the Santa Maria Stream (03°06'23"S, 60°19'07"W), an affluent of the Negro River, near the city of Manaus, Amazonas state, Brazil. The fishes were transported to the laboratory of the Instituto Nacional de Pesquisas da Amazônia (INPA), euthanized with a lethal dose of eugenol, and examined before preservation in 10% formalin. After a thorough examination of the external characteristics of the axillary pore, the freshly dead specimens were dissected for an overall anatomical analysis of the gland. The gland was examined and carefully extracted with surgical equipment under a stereomicroscope. Anatomical details of the gland were also recorded with digital images using a stereomicroscope Zeiss Stemi 2000 with an attached camera. Later, the gland was fixed in 10% buffered formaldehyde for 48 hours, dehydrated in an increasing alcohol series (70-100%), clarified in xylol and impregnated in Paraplast resin for histological analysis. Glands were sectioned in a rotating microtome Leica RM2245 at a thickness of 5 µm and slides were stained in Hematoxylin-Eosin (H&E) for further microscopic description.

The study was approved by the ethics committee for the use of animals in scientific research of INPA, and registered with the protocol # 051/2015 CEUA/INPA. The license to collect fishes was provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis to JZ (IBAMA, permanent permit #101932).

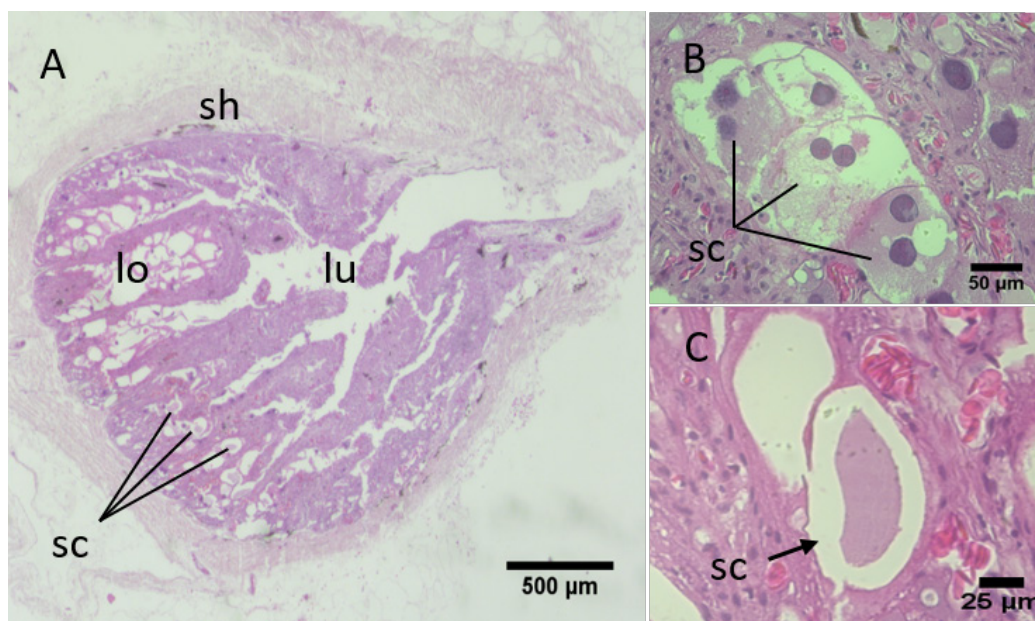
The axillary gland of *A. spinosissimus* releases a milky secretion through a conspicuous axillary pore located just below the posterior cleithral process (Figure 1a-c). The gland has a sac-like shape (Figure 1d) and is highly vascularized, being encapsulated by a connective tissue sheath that invaginates and separates lobes endowed with large, binucleated secretory cells, which are located on the periphery of the gland (Figure 2a). Secretory cells near the lumen appear to lose nuclei and the cytoplasm becomes filled with secretion, possibly containing proteinaceous elements, as suggested by its eosinophilic appearance (Figure 2b). Proteinaceous products had already been found in the secretions of axillary glands of other catfishes (see Wright 2015). Although cells filled with secretory substances were frequent in the histological preparations, the empty lumen of the gland also showed secretory cells with the presence of vacuoles (Figure 2c), possibly indicating cell depletion, as also observed in the Plotosidae catfish *Cnidoglanis macrocephalus* (Valenciennes 1840) (Cameron and Endean 1971).

Reed (1924) documented the presence of epidermal glands and axillary pores in a number of siluriform species, including the presence of tubular axillary glands in some Doradidae catfish, such as *Lithodoras dorsalis* (Valenciennes 1840) and *Nemadoras humeralis* (Kner 1855). As far as we know, *Acanthodoras* seems to be the only genus of Doradidae with a saccular axillary gland that secretes an abundant milky-looking substance, which occurs when the fish is taken out of the water or when grabbed underwater. The freshwater callichthyid *Corydoras aeneus* (Gill 1858) was described to possess a tubular axillary gland (Greven *et al.* 2006), and *Corydoras sterbai* Knaack, 1962 is known to release a clear glandular secretion with bactericidal properties when stressed (Kiehl *et al.* 2006). *Cnidoglanis macrocephalus*, a species previously known to release venom through the fin spines, also has a saccular gland at the posterior cleithral process (Cameron and Endean 1971), which is anatomically similar to that described here for *A. spinosissimus*. The development of the secretory cells is remarkably similar in both species, with immature cells found on the periphery of the gland and losing the nuclei during their migration to the lumen. The secretion is liberated to the exterior when mature cells filled by secretory products break off and release the content in the lumen of the gland, as observed in the tadpole madtom, *Noturus gyrinus* (Mitchill, 1817) (Ictaluridae) Wright (2015).

Whereas most species of Doradidae have a single-pored axillary gland, others possess a glandular tissue associated with



**Figure 1.** A – *Acanthodoras spinosissimus* secreting the milky putative poison (arrows); B – Axillary pore (rectangle) of *A. spinosissimus*, located below the postcleithral process (pp), near the base of the pectoral fin (pf). C – Opened pore (rectangle); D – Axillary gland of a specimen of *A. spinosissimus* of 90 mm standard length, indicating the position of the axillary pore. pp = postcleithral process; pf = pectoral fin; ag = axillary gland; ap = axillary pore. This figure is in color in the electronic version.



**Figure 2.** Histological aspects of the axillary gland of *Acanthodoras spinosissimus*. A – Longitudinal section of the gland; B – Binucleated secretory cells; C – Secretory cell filled with secretory product. sh = gland sheath; lo = lobule; lu = lumen; sc = secretory cell. Staining with Hematoxylin-Eosin. This figure is in color in the electronic version.



several pores in the axillary region, including *Rhynchodoras* (Birindelli *et al.* 2007), *Doras* (Sabaj Pérez and Birindelli 2008), *Anduzedoras*, *Hassar* (Birindelli *et al.* 2011), *Nemadoras* (Sabaj Pérez *et al.* 2014), *Tenellus*, *Trachydoras*, and the basal species of *Leptodoras* (Sabaj 2005). Nothing is known about the function and anatomical characteristics of the epidermal glands present in these species, how their structure is compared to that described here for *Acanthodoras*, or to that described for other singled-pored doradids (e.g., *Lithodoras dorsalis*).

The axillary gland of the *Acanthodoras* species probably has a defensive function. These catfishes release the secretion abundantly when stressed, and this is associated with loud stridulatory sounds produced by rotating movements of the pectoral spine and also by vibrating the swim bladder with fast contracting drumming muscles (Boyle *et al.* 2015). The bold color pattern of species of *Acanthodoras* may be aposematic and also contribute to warn out potential predators, a condition already hypothesized by Carvalho *et al.* (2003) for juvenile raphael catfish (*Platydoras*, Doradidae) engaged in cleaning interactions with dangerous clients. Finally, the axillary-gland secretion has a strongly bitter taste (JZ, pers. obs.) and may help repelling predators or forcing them to release a seized individual of *Acanthodoras*. Ongoing studies by our research group may help clarify these points.

We described, for the first time, a saccular axillary gland that secretes a milky substance in Doradidae. Comparisons to other thorny catfishes are still very limited, as almost no information is available for other doradids in the literature. Therefore, more detailed anatomical descriptions of these glands could provide insights into the habits and evolution of the thorny catfishes, as well as possibly interesting convergences found in catfishes of other families.

## ACKNOWLEDGMENTS

We thank the staff of the Laboratório Temático de Microscopia Óptica e Eletrônica - LTMOE at Instituto Nacional de Pesquisas da Amazônia (INPA) for the involvement in histological preparation of samples. We also wish to thank Dr. Wallace Duncan for a critical review that greatly improved the manuscript and Dr. Douglas Aviz Bastos for generously providing the photos shown in Figure 1. The Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq supported this study through a PCI grant and a MSc scholarship to JGS, and a productivity grant to JZ (#313183/2014-7), and JLB (#302872/2018-3).

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**RECEIVED:** 16/05/2021

**ACCEPTED:** 29/06/2021

**ASSOCIATE EDITOR:** David Santana



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