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FACULDADE DE CIÊNCIAS BIOLÓGICAS

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**ANALISE HISTOLÓGICA DOS FERRÕES DE RAIAS EM SOURE/ ILHA
DO MARAJÓ**

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Trabalho de Conclusão de curso apresentado à
Faculdade de Ciências biológicas da
Universidade Federal do Pará, como requisito
parcial para a obtenção de grau de Licenciado em
Ciências Biológicas.

ORIENTADOR: ADRIANO BIANCALANA

SOURE-PARÁ

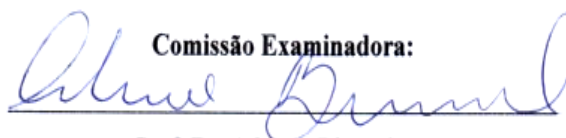
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obtenção de grau de Licenciado em Ciências
Biológicas, aprovado com o conceito Excelente.

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*Dedico este trabalho à minha segunda mãe,
Cecília Maria Borges Gonçalves (in
memoriam), que sempre acreditou em mim e na
educação como ferramenta transformadora.*

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SUMÁRIO

LISTA DE FIGURAS.....	vii
LISTA DE ABREVIATURAS E SIGLAS.....	ix
RESUMO.....	x
1. INTRODUÇÃO.....	1
2. MATERIAL E MÉTODOS.....	2
2.1 OBTENÇÕES DE AMOSTRAS BIOLÓGICAS.....	2
2.2 HISTOLOGIA.....	2
2.3 HISTOQUÍMICA.....	2
2.4 FOTOMICROGRAFIA.....	2
3. RESULTADOS.....	3
3.1 CAPTURA DE ANIMAIS.....	3
3.2 HISTOLOGIA GERAL.....	3
3.3 RESULTADOS HISTOQUÍMICOS.....	9
4. DISCUSSÃO.....	9
5.CONCLUSÃO.....	12
REFERÊNCIAS BIBLIOGRÁFICAS.....	13
ANEXO: NORMAS DA REVISTA TOXICON.....	16

LISTA DE FIGURAS

Fig. 1. Fotomicrografia da estrutura morfológica do ferrão de *Dasyatis guttata*. A) Imagem da espécie *Dasyatis guttata*. B) Corte transversal do ferrão indicando a localização das células secretoras de veneno (seta) no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) Maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as suas vesículas (seta). Células epiteliais (EC). Xylidine Ponceau. D) Maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as suas vesículas (seta). Células epiteliais (EC). Azul de Toluidina. E) Corte evidenciando as células glandulares (GC) e os grânulos (seta) entre as células epiteliais (EC). Hematoxilina e Eosina. F) Corte histológico com enfoque na célula glandular que apresenta uma única vesícula delimitando substâncias presentes no interior da célula (seta). Hematoxilina e Eosina. G) Região mineralizada (MR) com estrutura de medula óssea (BM). Hematoxilina e Eosina. H) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.4

Fig. 2. Fotomicrografia da estrutura morfológica do ferrão de *Potamotrygon orbignyi*. A) Imagem da espécie *Potamotrygon orbignyi*. B) corte transversal do ferrão indicando a localização das células secretoras de veneno no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) maior aumento do epitélio evidenciando as células secretoras de veneno (SC) vesícula das células secretoras de veneno (seta) e as células epiteliais (EC). Azul de Toluidina. D) maior aumento do epitélio evidenciando as células secretoras de veneno (SC), vesícula das células secretoras de veneno (seta) e as células epiteliais (EC). Xylidine Ponceau. E) corte evidenciando as células glandulares (GC). Vesículas das células secretoras de veneno (seta) e Células epiteliais (EC). Hematoxilina e Eosina. F) Imagem em maior aumento da região mineralizada (MR). É possível observar estruturas semelhantes à medula óssea (BM). Hematoxilina e Eosina. G) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.....6

Fig. 3. Fotomicrografia da estrutura morfológica do ferrão de *Potamotrygon scobina*. A) Imagem da espécie *Potamotrygon scobina*. B) corte transversal do ferrão indicando a localização das células secretoras de veneno (seta) no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) maior aumento do epitélio

evidenciando as células secretoras de veneno (SC) e as células epiteliais (EC). Azul de Toluidina. D) maior aumento do epitélio evidenciando as células secretoras de veneno (SC), vesícula das células secretoras de veneno (seta) e as células epiteliais (EC). Xylidine Ponceau. E) corte evidenciando as células glandulares (GC). Vesículas das células secretoras de veneno (seta) e Células epiteliais (EC). Hematoxilina e Eosina. F) maior aumento da região mineralizada (MR). Sendo possível observar estruturas semelhantes à medula óssea (BM). Hematoxilina e Eosina. G) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.....7

Fig. 4. Fotomicrografia da estrutura morfológica do ferrão de *Plesiotrygon iwamae*. A) Imagem da espécie *Plesiotrygon iwamae*. B) corte transversal do ferrão indicando a localização das células secretoras de veneno (seta) no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as células epiteliais (EC). Azul de Toluidina. D) maior aumento do epitélio evidenciando as células secretoras de veneno (SC), células glandulares (GC) e as células epiteliais (EC). Xylidine Ponceau. E) corte evidenciando as células glandulares (GC) e as Células epiteliais (EC). Hematoxilina e Eosina. F) maior ampliação da região mineralizada (MR). Sendo possível observar estruturas semelhantes à medula óssea (BM). Hematoxilina e Eosina. G) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.....8

LISTA DE ABREVIATURAS E SIGLAS

AT: Azul de toluidina

BM: Bone marrow

C: Connection

CT: Connective tissue

EC: Epithelial cells

EDTA: Ácido Etilenodiamino tetra- acético

ET: Epithelial tissue

GC: Glandular cells

HE: Hematoxilina e eosina

ICMBIO: Instituto Chico Mendes de Conservação da Biodiversidade

MR: Mineralized region

SC: Secretory cells

SISBIO: Sistema de autorização para coleta de material biológico e para a realização de pesquisa em unidades de conservação federais e cavernas.

XP: Xylidine ponceau

ANALISE HISTOLÓGICA DOS FERRÕES DE RAIAS EM SOURE/ ILHA DO MARAJÓ

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Resumo

As raias são animais que quando pisoteados possuem um movimento reflexo de cauda, que ocasiona grave acidente com seu ferrão. Como representantes podemos citar a família Dasyatidae e a Potamotrygonidae, sendo essa última pertencente ao ambiente dulcícola. No município de Soure, na ilha do Marajó no estado do Pará- Brasil, todo ano apresenta registros de acidentes com esses animais. Devido à gravidade das lesões e a importância médica relacionada, é relevante entender o funcionamento celular nesse processo, estudando a morfologia dos ferrões das raias e analisar a diferença entre elas. Para isso os agulhões foram removidos das espécies estudadas e fixados em formalina 10%. Posteriormente, foram descalcificados em solução contendo EDTA 5% para realização dos procedimentos histológicos e depois analisados no microscópio de luz. Através das análises microscópicas as espécies *Dasyatis guttata*, *Potamotrygon orbignyi*, *Potamotrygon scobina* e *Plesiotrygon iwamae* apresentaram ferrão com organização celular semelhante, no entanto as células secretoras de veneno estavam presentes em regiões diferentes do epitélio de animais dulcícolas e marinhos, além disso, esse tecido apresentou constituição frágil, podendo maximizar a gravidade dos acidentes. Foi possível visualizar uma conexão entre a região óssea e o tecido conjuntivo e ver estruturas semelhantes à medula óssea na região mineralizada. Dessa forma

estudos de caracterização dos ferrões é um passo importante para entender sobre o mecanismo de defesa desses animais.

Palavras-chave: Água doce, Marinha, Raias, Veneno; Morfologia.

Abstract

The Stingray are animals that when trampled underfoot have a reflex movement of tail, which causes serious accident with its stinger. As representatives we can mention the family Dasyatidae and Potamotrygonidae, the latter belonging to the freshwater environment. In municipality of Soure, on the island of Marajó, in the state of Pará in Brazil, every year presents records of accidents with these animals. Due to the severity of the injuries and the related medical importance, it is relevant to understand the cellular functioning in this process, studying the morphology of the stinger of the stingrays and to analyze the difference between them. For this, the stinger were removed from the studied species and fixed in 10% formalin. Subsequently, they were decalcified in a solution containing 5% EDTA for histological procedures and then analyzed by light microscope. Through microscopic analysis the species *Dasyatis guttata*, *Potamotrygon orbignyi*, *Potamotrygon scobina* and *Plesiotrygon iwamae* presented stinger with similar cellular organization, however, venom secreting cells were present in different regions of epithelium of freshwater animals and marine animals. In addition, this tissue has a fragile constitution, what can maximize the severity of accidents. It was possible to visualize a connection between the bone region and connective tissue and to see structures similar to the bone marrow in the mineralized region. Thus studies of stinger characterization is an important step to understand about the defense mechanism of these animals.

Keywords: Freshwater, Marine, Stingray, Poison, Morphology.

1. INTRODUÇÃO

As raias são animais venenosos que estão relacionados a muitos acidentes com seres humanos (Garrone Neto & Haddad, 2010; Sampaio, 2014). Esses animais possuem grande distribuição. As marinhas, por exemplo, da família Dasyatidae podem ocorrer em toda a costa Brasileira, já a família Potamotrygonidae pertence exclusivamente ao ambiente dulcícola e pode ser encontrada na maior parte dos sistemas fluviais da América do Sul tropical (Rosa, 1985).

A família Potamotrygonidae a principio pertencia ao ambiente marinho e ao gênero Himantura (família Dasyatidae) a aproximadamente 20 milhões de anos atrás, no Mioceno (Lovejoy, 1996). Contudo em águas da América do sul, a família Potamotrygonidae, desenvolveu adaptações osmóticas e hoje vivem em ambientes dulcícolas (Thorson, 1967).

É frequente a ocorrência de acidentes envolvendo esses animais nos rios da bacia amazônica e esse é um dos principais motivos pelo qual esses animais são temidos (Charvet-Almeida et al., 2002; Carvalho et al., 2003). Por possuírem o habito bentônico, se enterra no substrato e ao serem pisadas na região dorsal giram a cauda que contem o ferrão em direção ao agressor introduzindo o aguilhão (Sampaio, 2014).

Os acidentes provocados por peixes recebem o nome de Ictismo que pode ser sarcotóxico, isto é, através da ingestão de toxinas presentes em órgãos e no próprio tegumento dos animais, ou acantotóxico quando é através da mordida ou ferroadada, sendo esse último mecanismo utilizado por raias (Funasa, 2001).

A ferrada provoca intensa ação inflamatória (Antoniazzi et al., 2011). Isto provavelmente por que as raias possuem ferrões que são pequenas estruturas dérmicas que contem serrilhas nas laterais e estes estão localizados na região dorsal da cauda. O aguilhão ainda possui um sulco glandular contendo células de veneno, presente na região interna das laterais (Carvalho et al., 2003).

Existem poucos trabalhos de descrição do aguilhão de raias apesar dos inúmeros acidentes ocasionados por esses animais, diante disso, o objetivo desta pesquisa é analisar a morfologia do ferrão e comparar entre as espécies marinhas e dulcícolas capturadas no município de Soure localizado no estado do Pará em Brasil.

2. MATERIAL E MÉTODOS

2.1 OBTENÇÕES DE AMOSTRAS BIOLÓGICAS

Os ferrões foram coletados de três amostras diferentes das espécies: *Potamotrygon scobina*, *Potamotrygon orbignyi*, *Plesiotrygon iwamae* e *Dasyatis guttata*, em Soure, (S00°44'32,4";W048°30'24,9") PA. (Sob licença fornecida pelo sistema sisbio/icmbio nº 60966-1) Através de armadilha fixa chamada “Curral”.

2.2 HISTOLOGIA

Após a coleta o material foi fixado em solução contendo formol 10% e levados ao laboratório de biologia celular e molecular da Universidade federal do Pará. Depois de 24h no fixador as amostras foram submetidas ao processo de descalcificação em EDTA 5%. O ferrão foi fragmentado e a região apical foi desidratada e embebida em parafina histológica na orientação transversal (Pedroso et al., 2007), para posteriormente serem analisados ao microscópio óptico.

2.3 HISTOQUÍMICA

Os cortes foram submetidos às colorações Hematoxilina e Eosina para uma visão geral do tecido, Xylidine Ponceau para verificar a presença de proteínas totais e azul de Toluidina para análise de açúcares ácidos e ácidos nucleicos presentes nas células.

2.4 FOTOMICROGRAFIA

A micrografia foi obtida a partir de um microscópio óptico Nikon (eclipse E200) equipado com uma câmera digital e software de análise S-EYE (v. 1.3.2.297).

3. RESULTADOS

3.1 CAPTURA DE ANIMAIS

Foram capturados 34 animais no período de 20 de Abril de 2017 a 20 de Outubro de 2018 no município Soure na ilha de Marajó no estado do Pará- Brasil. E todos foram identificados a nível de espécie. A identificação dos animais foi baseada nos trabalhos de Lasso et al. (2013) e Gomes et al. (2010) onde foi possível identificar quatro espécies, três pertencentes a raias fluviais e uma única espécie marinha. Sendo 14 animais coletados da espécie *Potamotrygon orbignyi*, 10 de *Potamotrygon scobina*, 8 de *Plesiotrygon iwamae* e apenas 3 de *Dasyatis guttata*.

3.2 HISTOLOGIA GERAL

Os resultados demonstraram que a estrutura do ferrão da espécie marinha *Dasyatis guttata* (Fig. 1 A) é composta por uma região óssea localizada no centro do agulhão, ao seu redor se encontra o tecido conjuntivo e mais externamente uma cobertura de tecido epitelial (Fig. 1B).

No epitélio estão presentes as células secretoras de veneno (Fig. 1C-D) que geralmente tendem a se localizar próximas as serras laterais do ferrão, essas células possuem vesículas de formato Fusiforme em seu citoplasma e são compostas de material proteico (Fig. 1C).

As demais células epiteliais apresentam formato arredondado e no espaço entre as células é possível observar grânulos de secreção. As células glandulares são outro tipo celular presente no epitélio (Fig. 1E).

Ainda foi possível observar um tipo celular que morfológicamente é muito semelhante com as células glandulares, porém é menor, possui um núcleo lateralizado e as moléculas do interior dessas células parecem estar contidas em uma única vesícula (Fig. 1F).

Foi possível observar que tanto o epitélio de animais marinhos quanto o de animais dulcícolas é frágil, pois na maioria das coletas em campo os ferrões não se apresentavam com o tecido totalmente preservado, e ao manusear se soltavam com facilidade. Nos cortes histológicos dessas regiões observou-se que em áreas onde não havia epitélio, o tecido conjuntivo associado ao osso estava preservado.

A estrutura óssea do ferrão possui organização com medula óssea (Fig. 1G) e apresenta uma região de conexão entre a área mineralizada e o tecido conjuntivo (Fig. 1H).

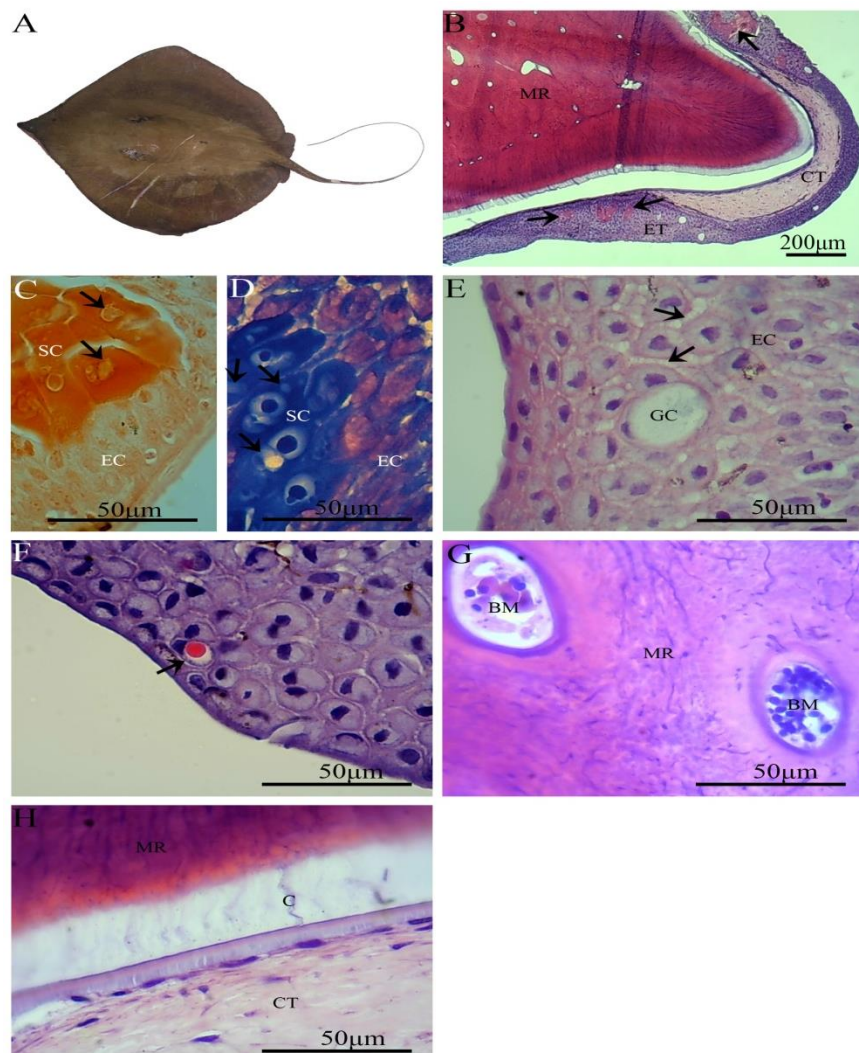


Fig. 1. Fotomicrografia da estrutura morfológica do ferrão de *Dasyatis guttata*. A) Imagem da espécie *Dasyatis guttata*. B) Corte transversal do ferrão indicando a localização das células secretoras de veneno (seta) no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) Maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as suas vesículas (seta). Células epiteliais (EC). Xylidine Ponceau. D) Maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as suas vesículas (seta). Células epiteliais (EC). Azul de Toluidina. E) Corte evidenciando as células glandulares (GC) e os grânulos (seta) entre as células epiteliais (EC). Hematoxilina e Eosina. F) Corte histológico com enfoque na célula glandular que apresenta uma única vesícula delimitando substâncias presentes no interior da célula (seta). Hematoxilina e Eosina. G) Região mineralizada (MR) com estrutura de medula óssea (BM). Hematoxilina e Eosina. H) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.

Os resultados demonstraram que o ferrão da espécie dulcícola *Potamotrygon orbignyi* (Fig. 2A) é composto de uma região mineralizada localizada no centro do aguilhão, ao redor dessa região se encontra o tecido conjuntivo e mais externamente uma cobertura feita pelo tecido epitelial (Fig. 2B), o mesmo ocorre para as espécies *Potamotrygon scobina* e *Plesiotrygon iwamae* (Fig. 3B, Fig. 4B).

Nessas espécies dulcícolas as células secretoras de veneno são alongadas, possuem vesículas fusiformes em seu citoplasma e armazenam material proteico (Figs. 2C-D, 3C-D, 4C-D). Já as demais células do tecido epitelial secretoras de muco possuem um formato mais arredondado e não armazenam conteúdo proteico (Figs. 2C-D, 3C-D, 4C-D), outro tipo celular frequente nesses animais são as células glandulares, que são descritas na literatura como células garrafais devido ao formato alongado e uma possível conexão para fora do epitélio (Figs. 2E, 3E, 4E). As células especializadas na produção de veneno em

animais de água doce se distribuem por todo o epitélio que recobre o ferrão e não tem uma região de concentração preferencial específica.

A região mineralizada possui estruturas semelhantes à medula óssea (Figs. 2F, 3F, 4F) que contem diversas células e ainda apresenta uma forte conexão com o tecido conjuntivo (Figs. 2G, 3G, 4G). Observou-se também que o tecido conjuntivo é bastante vascularizado e possui hemácias nucleadas.

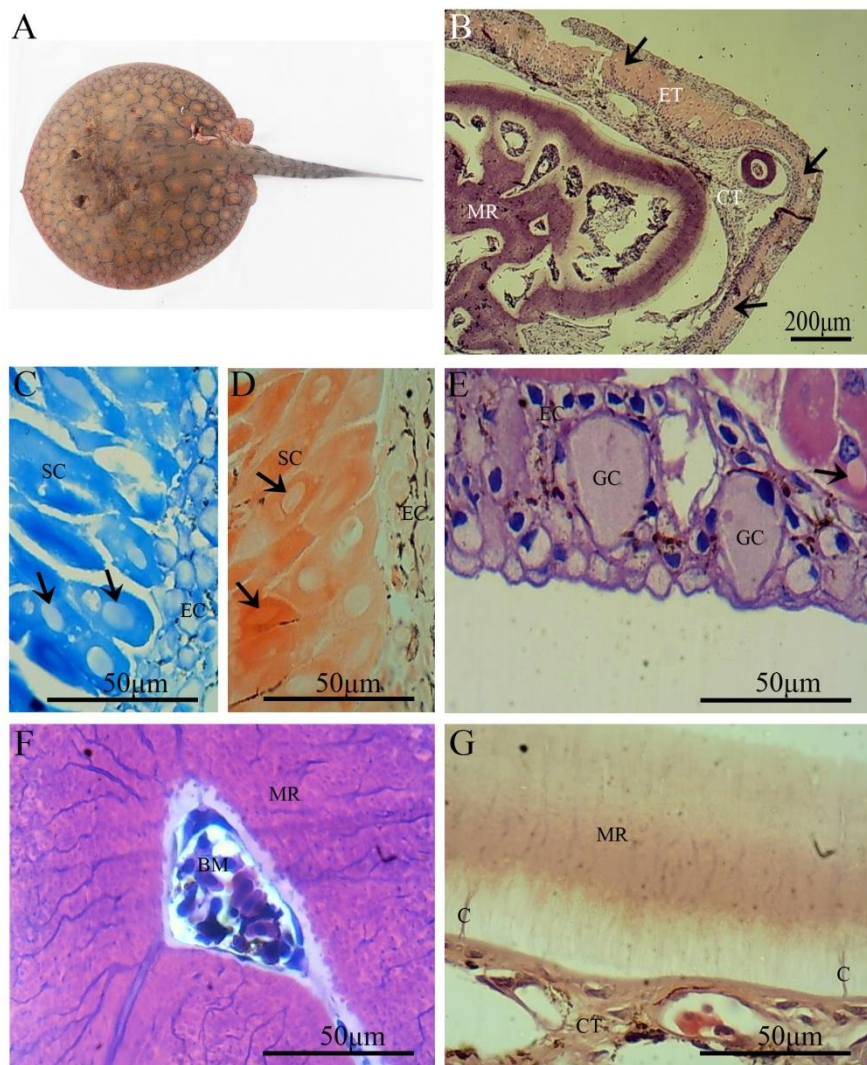


Fig. 2. Fotomicrografia da estrutura morfológica do ferrão de *Potamotrygon orbignyi*. A) Imagem da espécie *Potamotrygon orbignyi*. B) corte transversal do ferrão indicando a localização das células secretoras de veneno no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) maior aumento do epitélio

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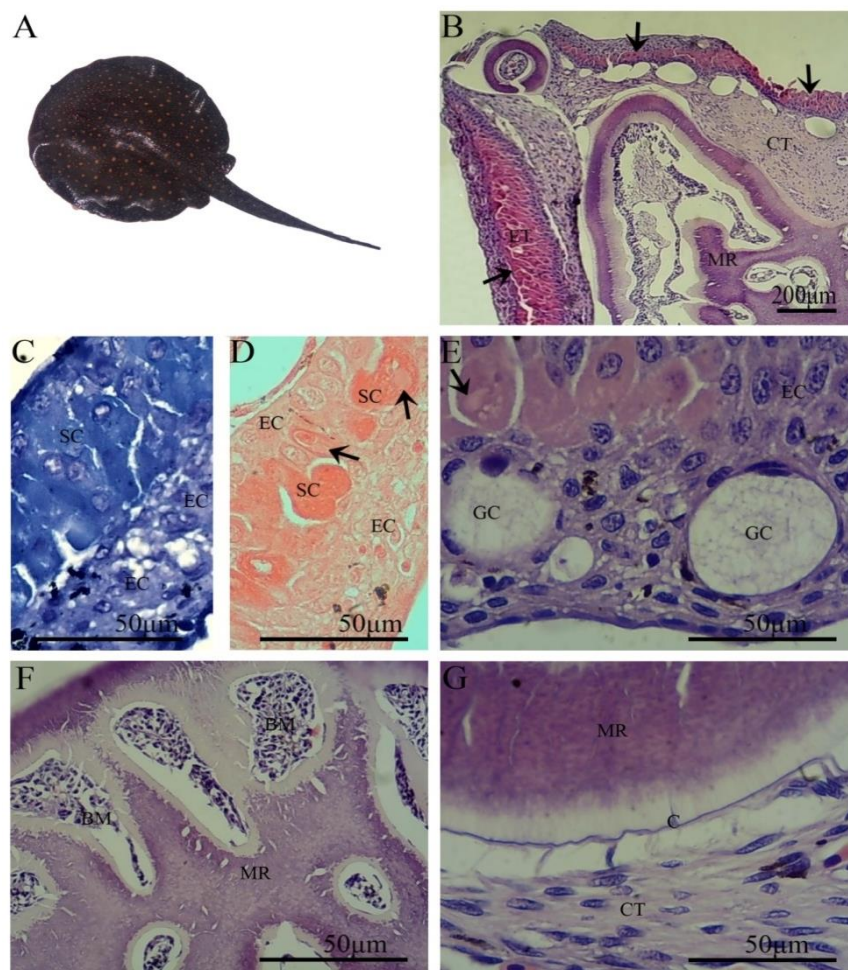


Fig. 3. Fotomicrografia da estrutura morfológica do ferrão de *Potamotrygon scobina*. A) Imagem da espécie *Potamotrygon scobina*. B) corte transversal do ferrão indicando a localização das células secretoras de veneno (seta) no tecido epitelial (ET). Tecido conjuntivo

(CT) e região mineralizada (MR). Hematoxilina e Eosina. C) maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as células epiteliais (EC). Azul de Toluidina. D) maior aumento do epitélio evidenciando as células secretoras de veneno (SC), vesícula das células secretoras de veneno (seta) e as células epiteliais (EC). Xylidine Ponceau. E) corte evidenciando as células glandulares (GC). Vesículas das células secretoras de veneno (seta) e Células epiteliais (EC). Hematoxilina e Eosina. F) maior aumento da região mineralizada (MR). Sendo possível observar estruturas semelhantes à medula óssea (BM). Hematoxilina e Eosina. G) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.

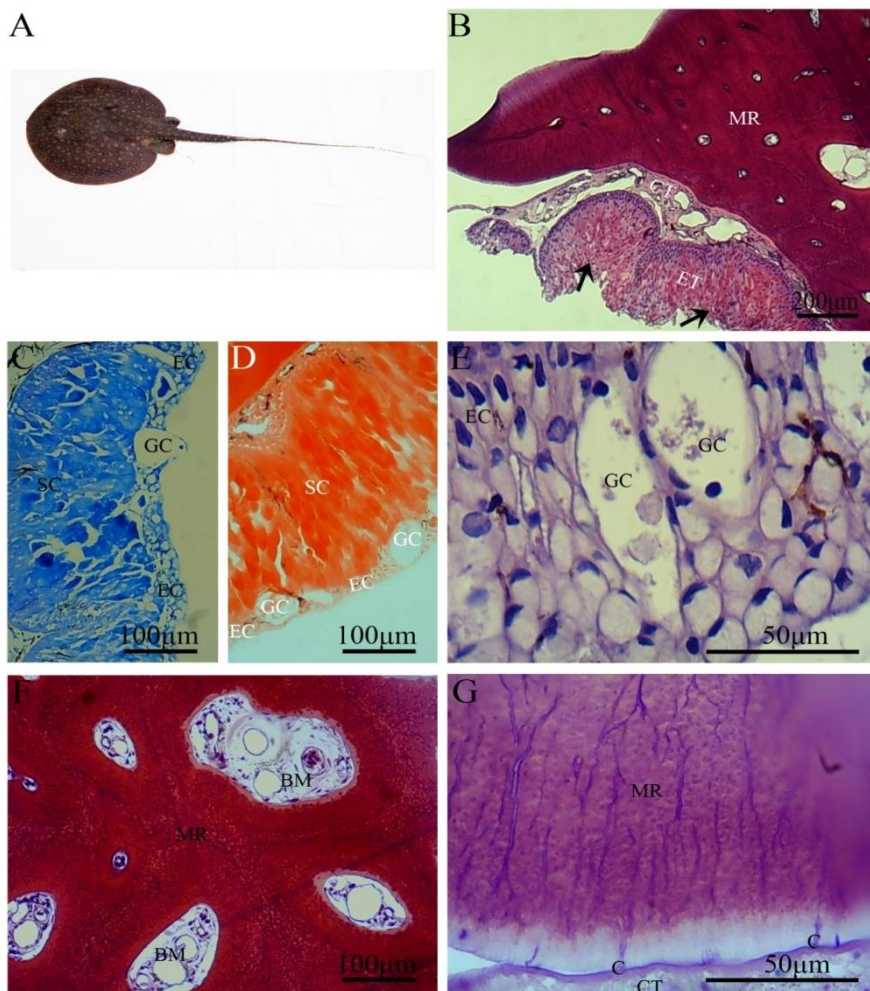


Fig. 4. Fotomicrografia da estrutura morfológica do ferrão de *Plesiotrygon iwamae*. A) Imagem da espécie *Plesiotrygon iwamae*. B) corte transversal do ferrão indicando a

localização das células secretoras de veneno (seta) no tecido epitelial (ET). Tecido conjuntivo (CT) e região mineralizada (MR). Hematoxilina e Eosina. C) maior aumento do epitélio evidenciando as células secretoras de veneno (SC) e as células epiteliais (EC). Azul de Toluidina. D) maior aumento do epitélio evidenciando as células secretoras de veneno (SC), células glandulares (GC) e as células epiteliais (EC). Xylidine Ponceau. E) corte evidenciando as células glandulares (GC) e as Células epiteliais (EC). Hematoxilina e Eosina. F) maior ampliação da região mineralizada (MR). Sendo possível observar estruturas semelhantes à medula óssea (BM). Hematoxilina e Eosina. G) corte histológico que mostra a conexão (C) entre a região mineralizada (MR) e o tecido conjuntivo (CT). Hematoxilina e Eosina.

3.3 RESULTADOS HISTOQUÍMICOS

Reações histoquímicas indicaram que as células secretoras de veneno, presente no epitélio dos ferrões, respondem positivamente ao corante XP evidenciando a presença de proteínas totais (Figs. 1C, 2D, 3D, 4D). Além disso, o citoplasma das células apresentou positividade ao corante AT (Figs. 1C, 2D, 3D, 4D) indicando a presença no citoplasma dessas células de carboidratos ácidos e ou ainda grande atividade de síntese proteica, evidenciada pela presença do mRNA citoplasmático.

4. DISCUSSÃO

As espécies *Dasyatis guttata*, *Potamotrygon orbignyi*, *Potamotrygon scobina* e *Plesiotrygon iwamae* apresentam ferrão com organização celular semelhante, isto é, todas elas possuem uma região mineralizada no centro ligado ao tecido conjuntivo que externamente está associado a um epitélio.

Em animais de água salgada as células especializadas na produção de veneno se localizam próximo às serrilhas laterais e formam agregados (Dehghani et al., 2010). Já nas espécies dulcícolas há uma distribuição das células secretoras de veneno por toda a extensão do epitélio. Ambas as espécies apresentam vesículas no interior das células de formato

279 fusiforme e também possuem substâncias proteicas evidenciadas pela reação positiva quando
280 submetidas ao corante Azul de Bromofenol (Dehghani et al., 2010; Pedroso et al., 2007).

281 De fato, essas células apresentam essa morfologia e são ricas em conteúdo
282 proteico como foi evidenciado pelo corante XP na presente pesquisa. Segundo Russell (1965)
283 componentes presentes no veneno da raia *Urolophus halleri* seriam a Serotonina, 5
284 nucleotidase e Fosfodiesterase identificados a partir de estudos com eletroforese em disco.
285 Além das proteínas podemos dizer que outro constituinte das células especializadas são os
286 carboidratos ácidos, visto que quando submetidas á coloração com o AT reagiram
287 positivamente. No entanto não foi possível caracterizar essas moléculas, nem mesmo
288 estabelecer a atividade metabólica de proteínas e açúcares, um passo importante para a
289 compressão dos mecanismos de funcionamento do veneno nos processos inflamatórios.
290 Estudo que deve ser realizado em pesquisas futuras.

291 As raias fluviais e marinhas possuem células epiteliais não especializadas na
292 produção de veneno, que possuem um formato arredondado e são caracterizadas como células
293 de muco que contem muitos grânulos de secreção em seu citoplasma (Dehghani et al., 2010 ;
294 Pedroso et al., 2007). Nas análises microscópicas foi possível observar que esses grânulos se
295 encontram também no espaço extracelular.

296 Acredita-se que as células produtoras de muco presentes no epitélio dos peixes
297 possuem diversas funções, como a locomoção do animal, pois diminui o atrito com a água o
298 que pode gerar redução dos gastos de energia (Shephard, 1994). Estudos realizados com muco
299 da raia *Hypanus americanus* demonstraram atividade antimicrobiana contra o fungo *Candida*
300 *albicans*. Além disso, ao ser injetado na pata de camundongos foi possível observar uma forte
301 ação inflamatória e o aparecimento de edema, que estão relacionados à ação de peptidases e
302 peptídeos derivados de proteínas (Coelho et al., 2019).

Outro tipo celular bastante frequente no epitélio que recobre o ferrão desses animais são as células glandulares, que foram descritas como células garrafais por apresentarem um formato alongado e uma conexão para fora do epitélio, mas em geral são células grandes que contem proteína hialina e só foram descritas por Pedroso et al. (2007) em animais dulcícolas. No entanto, essas células podem conter um formato arredondado e conter açúcares em sua composição. Esse mesmo tipo celular também foi encontrado na espécie *Dasyatis guttata*.

Durante a pesquisa outro tipo de célula glandular foi identificado, que é menor, em relação ao outro tipo de célula glandular típica. Apresenta um núcleo na lateral da célula e uma única vesícula ocupando o citoplasma armazenando algum tipo de secreção, com constituição provável de proteínas e de carboidratos.

Poucos autores descrevem a região óssea do ferrão, Pedroso et al. (2007) apenas cita ser uma região mineralizada. No entanto, foi possível identificar que tanto animais marinhos quanto animais dulcícolas possuem estruturas semelhantes à medula óssea. Ademais, essa estrutura pode ter como função a produção de células sanguíneas (Junqueira & Carneiro, 2013). Além de que, a região óssea possui conexão com o tecido conjuntivo, evidenciando que apesar do tecido epitelial ser um tecido relativamente frágil, existe uma região que conecta o tecido conjuntivo à região óssea.

Os diferentes graus de gravidade dos acidentes envolvendo animais de água doce e marinha pode ser explicada pela ampla distribuição do veneno no epitélio (nas de água doce), enquanto que em animais marinhos se encontra restrito a regiões específicas (Pedroso et al., 2007).

No caso das raias de água doce as células secretoras de veneno se distribuem estrategicamente por todo o epitélio, o que demonstra uma estratégia de defesa diferente das raias marinhas que possuem células de veneno somente próximo às estruturas em forma de

serra do ferrão. Isso pode estar relacionado aos ambientes onde esses animais habitam. Pois antes eles vivam no mesmo ambiente a aproximadamente 20 milhões de anos atrás no Mioceno (Lovejoy, 1996), contudo família Potamotrygonidae se diversificou em águas da América do sul e para isso teve que desenvolver adaptações para o ambiente dulcícola (Thorson, 1967).

Contudo, outros fatores devem ser levados em consideração para determinação da gravidade do acidente, já que pode ocorrer diferença na toxicidade entre as espécies de raias, que podem ainda estar relacionadas à maturidade sexual e o sexo do animal (Monteiro dos santos et al., 2019).

5. CONCLUSÕES

Dessa forma podemos dizer que é difícil avaliar a gravidade dos acidentes com raias de água doce ou salgada somente considerando a quantidade e a posição das células de veneno. Pois como foi dito, outros fatores podem influenciar. No entanto, é notório que todos os animais estudados têm um padrão morfológico semelhante de ferrão, apesar de suas peculiaridades, e que o tecido que recobre o ferrão apresenta constituição frágil, podendo maximizar a gravidade dos acidentes. Entretanto, o tecido conjuntivo é fortemente conectado a região óssea.

Na região de Soure a maioria dos acidentes está, provavelmente, relacionada a animais de água doce. Uma vez que a maioria das espécies capturadas foi dulcícola, o que pode justificar a gravidade dos acidentes.

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ANEXO

NORMAS DA REVISTA TOXICON



TOXICON

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TABLE OF CONTENTS

•	Description	p.1
•	Audience	p.2
•	Impact Factor	p.2
•	Abstracting and Indexing	p.2
•	Editorial Board	p.3
•	Guide for Authors	p.5



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Toxicon has an open access mirror [Toxicon: X](#), sharing the same aims and scope, editorial team, submission system and rigorous peer review. **An introductory offer Toxicon: X - full waiver of the Open Access fee.**

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- papers on novel findings related to the chemical, pharmacological, toxicological, and immunological properties of natural toxins
- molecular biological studies of toxin and other genes from poisonous and venomous organisms that advance understanding of the role or function of toxins
- clinical observations on poisoning and envenoming where a new therapeutic principle has been proposed or a decidedly superior clinical result has been obtained. *Toxicon* will not accept single-case reports unless they describe new, previously unreported, clinical features; envenomings or poisonings by rare animals, plants, fungi or microorganisms for which there is little or no clinical information in the literature; or treatment that employs a new therapeutic principle for which effectiveness is convincingly demonstrated. Such case reports must include: (1) expert species identification; (2) meticulous clinical documentation of symptoms, signs, laboratory data, treatment and clinical outcomes; (3) originality (adding to knowledge of the clinical phenotype); (4) where feasible, photographic documentation of clinical signs.
- material on the use of toxins as tools in studying biological processes and material on subjects related to venom-antivenom problems
- articles on the translational application of toxins, for example as drugs and insecticides
- epidemiological studies on envenoming or poisoning, so long as they highlight a previously unrecognised medical problem or provide insight into the prevention or medical treatment of envenoming or poisoning. Retrospective surveys of hospital records, especially those lacking species identification, will not be considered for publication. Properly designed prospective community-based surveys are strongly encouraged.
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