

RESUMO

Tabanídeos são insetos onde somente a fêmea é hematófaga, e além do incômodo provocado por sua picada, em animais de produção, companhia e humanos, podem também transmitir agentes causadores de enfermidades. Devido ao hábito alimentar, esses animais possuem em sua saliva componentes com poder anti-hemostático, vasodilatador, inibidor de imunoglobulinas, entre outros. Além disso, são também vetores mecânicos de uma série de agentes patogênicos importantes, como os tripanossomatídeos. Tendo em vista estas características, foi realizado um estudo de bioprospecção de componentes da glândula salivar de tabanídeos da floresta ombrófila mista (Sul do Brasil) e pesquisa da presença de *Trypanosoma evansi* e *Trypanosoma vivax* no aparelho bucal destes insetos, além do levantamento populacional e estudo de preferência anatômica para repasto. Foram capturados, semanalmente, entre as 15h e 18h, em quatro sítios na região de Lages, tabanídeos (Diptera, Tabanidae) no período de fevereiro de 2018 a fevereiro de 2019. As capturas com iscas usando um equino de coloração escura ocorreram durante três horas. Quatro observadores munidos de copos de vidro coletaram as moscas que pousaram no cavalo, este permanecia em estação para os procedimentos de coleta, e a cada 30 minutos era colocado em marcha, durante 5 minutos. Os exemplares capturados foram armazenados conforme localização do repasto, no dispositivo SECTAB e foram escolhidas as espécies mais abundantes e de maior tamanho, para facilitar a dissecação e fornecer material em quantidade suficiente para análise. Os insetos coletados foram mortos em frascos mortíferos contendo clorofórmio. Após, foi procedida a identificação taxonômica, sendo as glândulas salivares e os aparelhos bucais extraídos logo após a coleta. As glândulas salivares foram dissecadas sob um estereomicroscópio e transferidas para microtubo contendo tampão fosfato - salina (PBS pH 7.2), foram armazenados em TNE. O DNA do aparelho bucal e proteínas das glândulas salivares foram extraídos e analisados especificamente para a prospecção dos tripanossomatídeos e para a análise proteômica. O número de espécies de tabanídeos variou de zero no outono/inverno a sete no verão (dezembro a fevereiro). *Dichelacera (D.) alcicornis* Wied. (276) foi a espécie mais abundante seguida de *Chrysops fuscipex* Lutz (94) e *C. patricia* Pechuman (56). As capturas de pico ocorreram durante o verão (dezembro a fevereiro). O comportamento de pouso para repasto sanguíneo variou de acordo com os gêneros e espécies: *Chrysops* sp. (98,93%) preferiram a cabeça, *Fidena nigripes* (100%) tórax e abdomen, já *Dichelacera* sp. (73,91%), *Poeciloderas* sp. (95,65%) e *Acanthocera kroeberi* (100%) preferiram os membros. No material do aparelho bucal do gênero *Dichelacera* spp. e da espécie *Dichelacera (D.) alcicornis* foi encontrado DNA de *T. evansi*. Nas glândulas salivares das espécies *D. (D.) alcicornis*, *D. (D.) januarii* e *Fidena nigripes* foram identificadas 70 proteínas com funções nos processos metabólicos dos ácidos carboxílicos, proteínas, carboidratos, bem como funções estruturais, oxirredutoras, de transporte.

Palavras chave: Tabanídeos, saliva, peptídeos, proteínas, *Trypanosoma evansi*, vetores.

ABSTRACT

Tabanids are insects, in which only the female is hematophagous. In addition to the irritation caused by its bite in farm animals, as well as in humans, tabanids can also transmit disease-causing agents. Due to tabanids feeding habits, their saliva contains anti-hemostatic agents, vasodilators, immunoglobulin inhibitors, among other components. Besides that, they also act as mechanical vectors of several important pathogens, such as trypanosomatids. Due to these facts, a bioprospecting study on the salivary gland components from the mixed ombrophilous forest (Southern Brazil) tabanids was performed. It was also investigated the presence of *Trypanosoma evansi* and *Trypanosoma vivax* in the oral tract of these insects, in addition to a population survey as well as assessment of anatomical preference for repast. Tabanids (Diptera, Tabanidae) were captured, weekly, between February 2018 to February 2019 at 3 to 6 pm in four different farms in Lages. The capture occurred for three hours and used a dark-colored horse as a bait. We collected the horse landed flies using a glass cup. The horses exercised every 30 minutes, for 5 minutes, and remained in station for collection procedures. The captured specimens were stored according to repast location, in a SECTAB device. The most abundant and larger specimens were chosen to facilitate dissection and provide sufficient material for analysis. The collected insects were killed in chloroform containing flasks. After that, a taxonomic identification was performed, with salivary glands and oral appliances extracted soon after the collection. The salivary glands were dissected under a stereomicroscope and transferred to a microtube containing phosphate - saline buffer (PBS pH 7.2), and stored in TNE. The oral DNA and salivary gland proteins were extracted and analyzed specifically for trypanosomal prospection and proteomic analysis. The number of tabanid species fluctuated from zero in the fall/winter to seven in the summer (December to February). *Dichelacera (D.) alcicornis* Wied. (276) was the most abundant species followed by *Chrysops fuscipex* Lutz (94) and *C. patricia* Pechman (56). Peak catches occurred during the summer (December to February). The landing behavior for blood repast varied according to genera and species: *Chrysops* spp. (98.93%) preferred the head, *Fidena nigripes* (100%) thorax and abdomen, while *Dichelacera* spp. (73.91%), *Poeciloderas* sp. (95.65%) and *Acanthocera kroeberi* (100%) preferred the members. In the mouthpiece material of the genus *Dichelacera* spp. and from the species *Dichelacera (D.) alcicornis* was found DNA of *T. evansi*. In the salivary glands of the species *D. (D.) alcicornis*, *D. (D.) januarii* and *Fidena nigripes*, 70 proteins with functions in the metabolic processes of carboxylic acids, proteins, carbohydrates, as well as structural, oxidoreductive, transport functions were identified.

Keywords: Tabanids, saliva, peptides, *Trypanosoma evansi*.

6.5 REFERÊNCIAS

ALVES-SILVA J.; *et al.* An insight into the sialome of *Glossina morsitans morsitans*. **BMC genomics**, v. 11, p. 213, 2010.

AMERI, M.; *et al.* An immunoglobulin binding protein (antigen 5) of the stable fly (Diptera: Muscidae) salivary gland stimulates bovine immune responses. **J. Med. Entomol.**, v. 45, p. 94–101, 2008.

AN, S.; *et al.* A novel allergen Tab y 1 with inhibitory activity of platelet aggregation from salivary glands of horseflies. **Allergy**, Copenhagen, v. 66, n. 11, p. 1420-1427, 2011.

AN, S.; *et al.* Purification and Characterization of Two New Allergens from the Venom of *Vespa magnifica*. **PLoS ONE**, v. 7, n. 2, e31920, 2012.

BASSI, R. M. A.; CUNHA, M. C. I.; COSCARON, S. A study of behavior of tabanids (Diptera, Tabanidae) from Brazil. **Acta Biol. Paran.**, v. 29, p. 101–115, 2000.

BARROS, A. T. M.; GORAYEB, I. D. S. A checklist and identification key to the Tabanids (Diptera: Tabanidae) from Nhecolandia subregion of the Pantanal, State of Mato Grosso do Sul, Brazil. **Rev. Bras. de Bio.**, v. 56, p. 547-551, 1996.

BARROS, A. T. M. Seasonality and relative abundance of Tabanidae (Díptera) captured on horses in the Pantanal, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 96, p. 917-923, 2001.

BOUVIER, G. Notes sur le Tabanides de la region de Campinas (Estado de São Paulo). **Mem. Inst. Oswaldo Cruz**, v.50, p. 581-595, 1952.

BRADFORD, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal. Biochem.**, v. 72, p. 248-256, 1976.

CHRISTEN, S. E.; *et al.* SECTAB - A New Device for Tabanid Storage in Field Collections. **Neotrop. Entomol.**, v. 38, n. 6, p. 883-884, 2009.

CIDADE-BRASIL. **Município de Lages. 2019.** Página inicial. Disponível em: <<https://www.cidade-brasil.com.br/municipio-lages.html#desc>>. Acesso em: 20 de jun. de 2019.

CIPRANDI, A.; HORN, F.; TERMIGNONI, C. Saliva de animais hematófagos: fonte de novos anticoagulantes. **Rev. Bras. Hematol. Hemoter.**, v. 25, p. 250-262, 2003.

CORDERO, M. E.; *et al.* Proteomic analysis of detergent-solubilized membrane proteins from insect-developmental forms of *Trypanosoma cruzi*. **J. Proteome Res.**, v. 8, n. 7, p. 3642-3652, 2009.

CONESA, A.; *et al.* Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. **Bioinformatics**, v. 21, p. 3674-3676, 2005.

FAIRCHILD, G. B. Notes on Neotropical Tabanidae. XII. Classification and distribution, with keys to genera and subgenera. **Arq. Zoo.**, São Paulo, v. 17, p. 199-255, 1969.

FERREIRA, R. L. M.; RAFAEL, J. A. Criação de imaturos de mutuca (Tabanidae: Diptera) utilizando briófitas e areia com substrato. **Neotrop. Entomol.**, v. 35, p. 141-144, 2006.

FORTES, E. **Parasitologia Veterinária**. 3. ed. São Paulo: Ícone, 1997.

FREYE, H. B.; LITWIN, C. Coexistent anaphylaxis to Diptera and Hymenoptera. **Annals of Allerg., Asth. & Immunol.**, Jackson, v. 76, n. 3, p. 270-272, 1996.

FUKUMOTO, S.; *et al.* Tick troponin I-like molecule is a potent inhibitor for angiogenesis. **Microv. Research**, v. 71, p. 218-221, 2006.

GREVELINK, S. A.; YOUSSEF, D. E.; LOSCALZO, J. Salivary gland extracts from the deerfly contain a potent inhibitor of platelet aggregation. **Proc. Natl. Acad. Sci. USA**, v. 90, p. 9155-9158, 1993.

HEMPOLCHOM, C.; *et al.* Proteomes of the female salivary glands of *Simulium nigrogilvum* and *Simulium nodosum*, the main human-biting black flies in Thailand. **Acta Tropica**, v. 194, p. 82-88, 2019.

JIANG, W.G.; *et al.* Angiomotin and angiomotin like proteins, their expression and correlation with angiogenesis and clinical outcome in human breast cancer. **BMC Cancer**, p. 6-16, 2006.

MA, D.; *et al.* Anti-thrombosis repertoire of bloodfeeding horsefly salivary glands. **Mol. & Cell. Proteomics**, v. 8, p. 2071-2079, 2009.

KAZIMÍROVÁ, M.; SULANOVÁ, M.; TRIMNELL, A. R. Anticoagulant activities in salivary glands of tabanid flies. **Med. Vet. Entomol.**, v. 16, p. 301-309, 2002.

KAZIMÍVORÁ, M.; *et al.* Identification of Anticoagulant Activities in Salivary Gland Extracts of four Horsefly Species (Diptera, Tabanidae). Anticoagulant Proteins and Diagnostic Agents. **Haemostasis**, v. 31, p. 294-305, 2001.

KLEIN, R. M. **Mapa fitogeográfico do Estado de Santa Catarina**. Itajaí: Herbário Barbosa Rodrigues. 24p., 1978.

MA, D.; *et al.* Purification and characterization of two new allergens from the salivary glands of the horsefly, *Tabanus yao*. **Allergy**, v. 66, p. 101-109, 2011.

MILETTI, L. C.; *et al.* Prevalence, seasonality and behaviour of Tabanidae (Diptera) captured on a horse in the Planalto Serrano of Santa Catarina State, Brazil. **Int. J. Trop. Insect. Sci.** v. 31, n. 1-2, p. 122-126, 2011.

OLIVEIRA, M. T.; BARAU, J. G.; JUNQUEIRA, A. C. M., Structure and evolution of the mitochondrial genomes of *Haematobia irritans* and *Stomoxys calcitrans*. The Musc. (Diptera: Calyptratae) perspe. **Mol. Phylogenet. Evol.**, v. 48, p. 850-857, 2007.

RAJSKÁ, P.; *et al.* Effects of horsefly (Tabanidae) salivary gland extracts on isolated perfused rat heart. **Med. Vet. Entomol.**, v. 21, p. 384-389, 2007.

RAJSKÁ, P.; *et al.* Vasodilatory activity in horsefly and deerfly salivary glands. **Med. Vet. Entomol.**, v. 17, p. 395-402, 2003.

RAMOS, C. J. R.; *et al.* **Trypanosoma evansi e T. vivax em tabanídeos, da floresta ombrófila mista na região de Lages, SC.** Lages, 2019. No prelo.

REDDY, V. B.; *et al.* Chrysoptin is a potent glycoprotein IIb/IIIa fibrinogen receptor antagonist present in salivary gland extracts of the deerfly. **J. Biol. Chem.**, v. 275, n. 21, p. 15861-15867, 2000.

RIBEIRO, J. M.; VALENZUELA, J. G. Purification and cloning of the salivary peroxidase/catechol oxidase of the mosquito *Anopheles albimanus*. **J. Exp. Biol.**, v. 202, p. 809–816, 1999.

RIBEIRO, J. M. C.; ARCA, B. From sialomes to the sialoverse: An insight into the salivary potion of blood feeding insects. **Adv. Insect. Physiol.**, v. 37, p. 59–118, 2009.

RIBEIRO, J. M. C.; *et al.* An insight into the sialome of the horse fly, *Tabanus bromius*. **Insect. Biochem. Mol. Biol.**, v. 65, p. 83–90, 2015.

SANTA CATARINA. Fundação do Meio Ambiente (FATMA). **Lista comentada de espécies exóticas invasoras no estado de Santa Catarina: espécies que ameaçam a diversidade biológica.** Silvia R. Ziller (consultora). Floarianópolis: FATMA, 2016. 88p.

TURCATEL, M.; CARVALHO, C. J. B.; RAFAEL, J. A. Horse flies (Diptera: Tabanidae) of Paraná State, Brazil: pictorial identification key for subfamilies, tribes and genera. **Biota Neotrop.**, v. 7, p. 265-278, 2007.

VIBRANS, A. C.; SEVEGNANI, L.; GASPER, A. L.; *et al.* **Floresta ombrófila mista.** Blumenau: Edifurb, 2013. 440p.

VOLFOVA, V; TOTHOVA, V; VOLF, P. Hyaluronidase activity in the salivary glands of tabanid flies. **Insect. Biochem. Mol. Biol.**, v. 73, p. 38-46, 2016.

WAGNER, G.; *et al.* The *Trypanosoma rangeli* trypomastigote surfaceome reveals novel proteins and targets for specific diagnosis. **J. Proteom.**, v. 82, p. 52-63, 2013.

WANG, X.; *et al.* An insight into the transcriptome and proteome of the salivary gland of the stable fly, *Stomoxys calcitrans*. **Insect. Biochem. Mol. Biol.**, v. 39, p. 607–614, 2009.