Phyllomedusa 18(2):283–292, 2019
© 2019 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v18i2p283-292

SHORT COMMUNICATION

A case of Dipteran parasitism in *Trachycephalus typhonius* (Anura: Hylidae), with a summary of myiasis parasitism in anurans

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Keywords: amphibians, infestation, larvae, Sarcophagidae.

Palavras-chave: anfíbios, infestação, larvas, Sarcophagidae.

Some species of Diptera cause myiasis in anurans, and infestations can occur in organs or other vertebrate tissues for varying lengths of time, depending on the species of parasite (Schell and Burgin 2001, Bolek and Coggins 2002). Larvae feed on tissues as they develop within or on the body of their host, which often results in the death of the host, although some individuals do survive and heal (Eaton *et al.* 2008, Souza-Pinto *et al.* 2015). Some parasitic dipteran species need living hosts to continue their life cycle (Kraus 2007). Dipteran infestations have been well documented in humans and domestic animals because their impacts concern public health and economics (Hall and Wall 1995). Nevertheless, wild animals also host dipteran parasite larvae, but records are scarce and poorly documented (Travers and Townsend 2010, Pinto *et al.* 2017).

Dipteran families such as Calliphoridae, Chloropidae, Phoridae, and Sarcophagidae have been reported parasitizing anuran species around the world, e.g. *Bufo bufo* (Linnaeus, 1758) and *Hyla arborea* (Linnaeus, 1758) (Meisterhans and Heusser 1970, Eaton *et al.* 2008, López *et al.* 2016). In Brazil, documented occurrences are restricted to 10 species, distributed among the families Bufonidae, Hylidae, Leptodactylidae, and Ranidae (Lopes 1981, Souza Jr. *et al.* 1990, Eizemberg *et al.* 2008, Carvalho-Filho *et al.* 2010, Mello-Patiu and Luna-Dias 2010, Oliveira *et al.* 2012, Müller *et al.* 2015, Souza-Pinto *et al.* 2015, Pinto *et al.* 2017). Herein, we present the

Received 30 January 2019
Accepted 03 October 2019
Distributed December 2019

first record of a dipteran larva in *Trachycephalus typhonius* (Linnaeus, 1758) from the Pantanal region of Mato Grosso do Sul state, Brazil. In addition, we provide a summarized appendix of all records of dipteran larvae as myiasis in anurans.

Trachycephalus typhonius is a hylid treefrog that is widely distributed in Argentina, Colombia, Paraguay, Peru, Venezuela, Trinidad Tobago and Brazil (Frost 2018). On 10 November 2017, during field work in the Environmental Protection Area (APA) Baía Negra, (19°01'6.27" S, 57°33'23.06" W; 89 m a.s.l.) in the municipality of Ladário, Mato Grosso do Sul state, Brazil, we captured an adult male *T. typhonius* (ZUFMS-AMP12463, CRC= 76.34 mm). The individual was taken to the laboratory, euthenized with 5% Xylocaine® that was distributed on the abdomen and absorbed into its skin, fixed in 10% formalin, and preserved in 70% alcohol. After fixation, we observed a sarcophagid larva (1.44 mm long, 0.45mm wide) in one of its nasal cavities (Figure 1). This is the first record of a sarcophagid parasite of *T. typhonius.*

Larvae of Sarcophagidae differ from those of other dipteran families by their size and the position of posterior spiracles, which are located inside a concavity (Mello-Patiu *et al.* 2009). Specific identification of the larva we observed was not possible owing to its immature stage; only adults of this dipteran family can be confidently identified (Mello-Patiu and Luna-Dias 2010). Sarcophagids are characterized by their diurnal habits, which contrast with the nocturnal activity of most anuran species (Hagman *et al.* 2005), including *T. typhonius* (Uetanabaro *et al.* 2008). The low occurrence of parasitism by these dipterans in anurans may reflect their discrete activity periods.

Trachycephalus typhonius is arboreal and active at night, but it perches on leaves and branches during the day and is easily spotted by human observers (Duré and Kehr 2006). Thus, the frogs are easily available for infestation by sarcophagids because infestation would occur by day, a period associated with low host activity during time the frogs are less likely to eat the adult flies (Souza-Pinto *et al.* 2015).

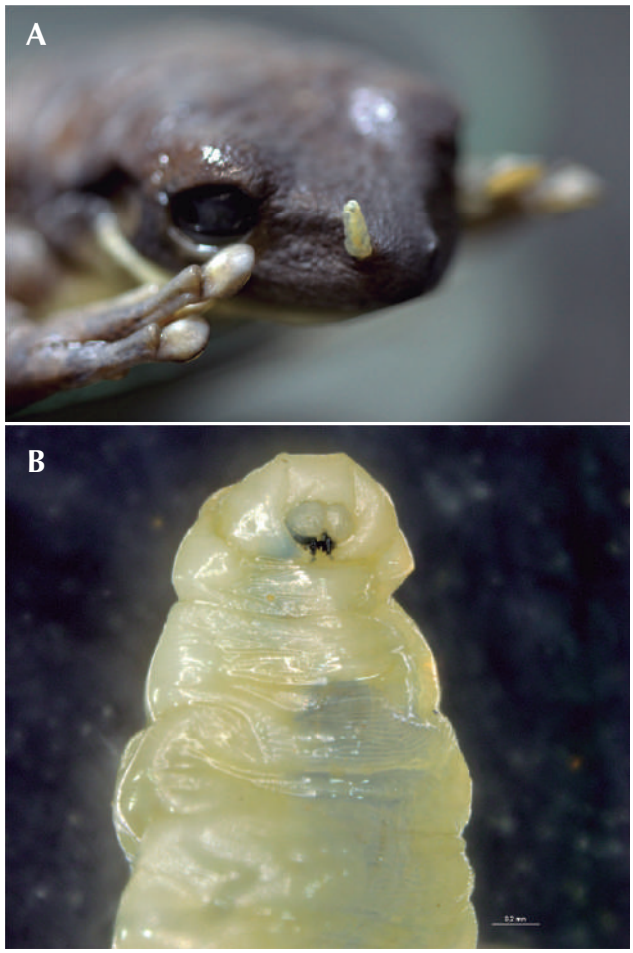

Figure 1. (**A**) Individual of *Trachycephalus typhonius* showing a Sarcophagidae larva in the nasal cavity. (**B**) Head details of a Sarcophagidae larva. Scale bar = 0.2 mm.

Species of Sarcophagidae are known to cause myiasis in anurans (e.g., Crump and Pounds 1985, Travers and Townsend 2010). The general pattern of infestation begins when adult flies deposit their eggs on the skin of hosts, after which the eggs hatch and first instar larvae penetrate the skin, migrating to the final infestation site in the body of the host, where they develop (Crump and Pounds 1985, Kraus 2007). Egg deposition by dipterans on anurans usually occurs on healthy hosts (Medina *et al.* 2009), as we observed parasitism on an apparently healthy frog lacking skin lesions.

Worldwide, 63 anuran species, distributed in 15 families, were recorded as dipteran hosts, with the highest number of occurrences in Australia and Brazil (10 records each) (Appendix I). Among the four dipteran families that parasitize anurans, Sarcophagidae is most prevalent ($N = 24$), followed by Calliphoridae ($N = 19$), Chloropidae ($N = 18$), and Phoridae ($N = 1$). Three records included in Appendix I have not been identified.

Most of the anuran hosts documented in Appendix I are terrestrial ($N = 11$ families), and the most frequently parasitized families are Bufonidae ($N = 11$ records) and Myobatrachidae ($N = 9$ records). The terrestrial habits of these anurans may provide greater exposure to parasitic dipterans searching for potential hosts (Hagman *et al.* 2005). Contact with water, even briefly, can kill dipteran larvae (Ziser and Nettles 1979, Eaton *et al.* 2008); thus, if frogs frequent bodies of water, it would be difficult for parasitic larvae to survive. Among hylids, dipteran larvae have been documented as parasites in seven species, with five records in Brazilian species (Meisterhans and Heusser 1970, Eaton *et al.* 2008, Eizemberg *et al.* 2008, Mello-Patiu and Luna-Dias 2010, Oliveira *et al.* 2012, Souza-Pinto *et al.* 2015, Pinto *et al.* 2017). Most hylids are arboreal and, while some species have both terrestrial and aquatic habits, most are associated with terrestrial vegetation, perching on leaves and branches (Vitt and Caldwell 2013); this would allow adult dipterans to access these anuran hosts. Thus, habitat type of anuran hosts can influence their risk of being parasitized by dipterans, with arboreal and terrestrial species being the most likely to be affected. Conversely, species with aquatic or semi-aquatic habits may avoid being parasitized by dipteran larvae as water submersion can lead to mortality of parasitic larvae.

Acknowledgments.—We thank Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for authorization to capture anurans (number 56729-3). ICOS and SSC thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001. KC thanks Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for her scholarship. DJS thanks CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for his research fellowship (311492/2017-7).

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Editor: Jaime Bertoluci

Appendix I. *Records of Dipteran larvae as myiasis parasites of anurans worldwide. Diptera families:[1] Calliphoridae, [2]Sarcophagidae, [3]Chloropidae, and [4]Phoridae.*

Host species	Diptera species	References
Alytidae		
Alytes obstetricans Laurenti, 1768	*Lucilia bufonivora*[1]	Brumpt 1934a
Bufonidae		
Anaxyrus americanus Holbrook, 1836	*Lucilia elongata*[1]	Briggs 1975
	Lucilia silvarum[1]	Anderson and Bennett 1963, Bleakney 1963, Bolek and Coggins 2002
Anaxyrus boreas Baird and Girard, 1852	*Lucilia elongata*[1]	James and Maslin 1947
	Lucilia silvarum[1]	Eaton *et al.* 2008
Atelopus varius Lichtenstein and Martens, 1856	*Lepidodexia bufonivora*[2]	Crump and Pounds 1985, Pounds and Crump 1987
Bufo sp.	*Lucilia bufonivora*[1]	Spence 1954
	Lucilia sericata[1]	Stewart and Foote 1974
Bufo bufo Linnaeus, 1758	*Lucilia ampullacea*[1]	Glaw *et al.* 2014
	Lucilia bufonivora[1]	Brumpt 1934a,b, Hendriks 1974, Garanin and Shaldybin 1976, Strijbosch 1980, Nijs 1984, Spieler 1990, Albrecht *et al.* 1996, Zavadil 1997, Gosá *et al.* 2009, Salazar *et al.* 2012, Arias-Robledo *et al.* 2019
	Lucilia silvarum[1]	Duncker 1891, Stadler 1930, Heim de Balsac 1933, Sandner 1955, Eaton *et al.* 2008
	Lucilia sp.[1]	Gerber 1950, Janzen 1994
	Wohlfahrtia vigil[2]	Cepelák 1952, Povolný and Verves 1997
Bufotes viridis Laurenti, 1768	*Lucilia bufonivora*[1]	Garanin and Shaldybin 1976
Duttaphrynus melanostictus Schneider, 1799	*Lucilia porphyrina*[1]	Dasgupta 1962
	Sarcophaga ruficornis[2]	Roy and Dasgupta 1977
Epidalea calamita Laurenti, 1768	*Lucilia bufonivora*[1]	Vestjens 1958, Zavadil 1997, Kordges 2000
Rhinella diptycha Cope, 1862	Undetermined[2]	Souza-Pinto *et al.* 2015
Rhinella nattereri Bokermann, 1987	*Lepidodexia bufonivora*[2]	Lopes and Vogelsang 1953

Appendix I - *Continued.*

Host species	Diptera species	References
Rhinella margaritifera Laurenti, 1768	Undetermined	Carvalho-Filho *et al.* 2010
Centrolenidae		
Hyalinobatrachium fleischmanni Boettger, 1893	Undetermined[2]	Medina *et al.* 2009
Craugastoridae		
Pristimantis thectopternus Lynch, 1975	Undetermined[2]	Gómez-Hoyos *et al.* 2012
Craugastor rhodopis Cope, 1867	*Lepidodexia* (*Notochaeta*) *bufonivora*[2]	Vázquez-Corzas *et al.* 2018
Dendrobatidae		
Ameerega bassleri Melin, 1941	Undetermined[2]	Hagman *et al.* 2005
Ameerega cainarachi Schulte, 1989	Undetermined[2]	Hagman *et al.* 2005
Ameerega peruviridis Bauer, 1986	*Sarcodexia lambens*[2]	Hagman et al. 2005
Eleutherodactylidae		
Eleutherodactylus sp.	*Lepidodexia* sp.[2]	Dodge 1968
Hylidae		
Aplastodiscus arildae Cruz and Peixoto, 1987	*Lepidodexia bufonivora*[2]	Eizemberg *et al.* 2008
Boana atlantica Caramaschi and Velosa, 1996	Undetermined[2]	Oliveira *et al.* 2012
Boana beckeri Caramaschi and Cruz, 2004	*Lepidodexia centenaria*[2]	Mello-Patiu and Luna-Dias 2010
Boana caingua Carrizo, 1991	*Megaselia scalaris*[4]	López *et al.* 2016
Boana polytaenia Cope, 1870	Undetermined[1]	Gomes *et al.* 2018
Boana stenocephala Caramaschi and Cruz, 1999	Undetermined[1]	Gomes *et al.* 2018
Dryaderces inframaculata Boulenger, 1882	Undetermined[2]	Pinto *et al.* 2017
Hyla arborea Linnaeus, 1758	*Lucilia silvarum*[1]	Anderson and Bennett 1963
Pseudacris maculata Agassiz, 1850	*Lucilia silvarum*[1]	Eaton *et al.* 2008
Pseudacris triseriata Wied-Neuwied, 1838	*Lucilia silvarum*[1]	Roberts 1998
Scinax fuscovarius Lutz, 1925	Undetermined[2]	Souza-Pinto *et al.* 2015

Appendix I - *Continued.*

Host species	Diptera species	References
Rheohyla miotympanum Cope, 1863	*Lepidodexia* (*Notochaeta*) *bufonivora*[2]	Vázquez-Corzas *et al.* 2018
Leptodactylidae		
Adenomera diptyx Boettger, 1885	*Lepidodexia* (*Notochaeta*) *adelina*[2]	Mulieri *et al.* 2018
Leptodactylus elenae Heyer, 1978	*Lepidodexia* (*Notochaeta*) *adelina*[2]	Mulieri *et al.* 2018
Leptodactylus latrans Steffen, 1815	Undetermined[2]	Müller *et al.* 2015
Physalaemus albonotatus Steindachner, 1864	*Lepidodexia* (*Notochaeta*) *adelina*[2]	Mulieri *et al.* 2018
Limnodynastidae		
Heleioporus albopunctatus Gray 1841	*Batrachomyia* sp.[3]	McAlpine 1955, Elkan 1965
Myobatrachidae		
Crinia signifera Girard, 1853	*Batrachomyia* sp.[3]	Krefft 1864, Elkan 1965, Lemckert 2000
Geocrinia laevis Günther, 1864	*Batrachomyia* sp.[3]	McAlpine 1955, Elkan 1965
Geocrinia victoriana Boulenger, 1888	*Batrachomyia* sp.[3]	McAlpine 1955
Pseudophryne bibronii Günther, 1859	*Batrachomyia quadrilineata*[3]	McAlpine 1955, Elkan 1965
	Batrachomyia sp.[3]	Krefft 1864, Pengilley 1992
Pseudophryne corroboree Moore, 1953	*Batrachomyia* sp.[3]	Pengilley 1992
Pseudophryne dendyi Lucas, 1892	*Batrachomyia* sp.[3]	McAlpine 1955, Elkan 1965, Pengilley 1992
Pseudophryne pengilleyi Wells and Wellington, 1985	*Batrachomyia* sp.[3]	Lemckert 2000
Uperoleia laevigata Keferstein, 1867	*Batrachomyia* sp.[3]	Lemckert 2000
	Batrachomyia strigipes[3]	McAlpine 1955, Schell and Burgin 2001
Uperoleia marmorata Gray, 1841	*Batrachomyia* sp.[3]	Krefft 1864
Odontophrynidae		
Proceratophrys sp.	*Lepidodexia* sp.[2]	Gómez-Hoyos *et al.* 2012 *apud* Lopes 1981

Appendix I - *Continued.*

Host species	Diptera species	References
Pelobatidae		
Pelobates fuscus Laurenti, 1768	*Lucilia* sp.[1]	Gerber 1950
	Lucilia bufonivora[1]	Garanin and Shaldybin 1976
Pelodryadidae		
Ranoidea caerulea White, 1790	*Batrachomyia mertensi*[3]	Lindner 1958, Elkan 1965, Vogelnest 1994
Ranoidea citropa Péron, 1807	*Batrachomyia* sp.[3]	Krefft 1864, Elkan 1965
Ranoidea genimaculata Horst, 1883	*Batrachomyia* sp.[3]	Hoskin and McCallum 2007
Ranoidea jungguy Donnellan and Mahony, 2004	*Batrachomyia* sp.[3]	Hoskin and McCallum 2007
Ranoidea myola Hoskin, 2007	*Batrachomyia* sp.[3]	Hoskin and McCallum 2007
Ranoidea phyllochroa Günther ,1863	*Batrachomyia nigritarsis*[3]	Skuse 1889, McAlpine 1955, Elkan 1965
Ranoidea wilcoxii Günther, 1864	*Batrachomyia* sp.[3]	Hoskin and McCallum 2007
Phyllomedusidae		
Agalychnis saltator Taylor, 1955	Undetermined	Travers and Townsend 2010
Ranidae		
Lithobates berlandieri Baird, 1859	*Lepidodexia* (*Notochaeta*) *bufonivora*[2]	Vázquez-Corzas *et al.* 2018
Lithobates catesbeianus Shaw, 1802	*Lepidodexia* sp.[2]	Souza Jr. *et al.* 1990
	Lucilia bufonivora[1]	Hall 1948
Lithobates sylvaticus LeConte, 1825	*Lucilia elongata*[1]	Bolek and Janovy Jr. 2004
	Lucilia silvarum[1]	Roberts 1998, Bolek and Janovy Jr. 2004, Eaton *et al.* 2008
Papurana supragrisea Menzies, 1987	*Batrachomyia krausi*[3]	Evenhuis 2006, Kraus 2007
Pelophylax perezi López-Seoane, 1885	*Lucilia bufonivora*[1]	Gosá *et al.* 2009
Rana arvalis Nilsson, 1842	*Lucilia bufonivora*[1]	Brumpt 1934a, Zumpt 1965, Garanin and Shaldybin 1976
Rana dybowskii Günther, 1876	Undetermined[2]	Povolný and Verves 1997
Rana temporaria Linnaeus, 1758	*Lucilia bufonivora*[1]	Brumpt 1934a,b, Zumpt 1965, Koskela *et al.* 1974, Albrecht *et al.* 1996, Kordges 2000
Not reported	*Lucilia bufonivora*[1]	Tantawi and Whitworth 2014