Metacercariae of *Eumegacetes medioximus* (Digenea: Eumegacetidae) in larvae of Odonata from Brazil

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Abstract: During studies on the participation of larval Odonata in the life cycle of trematodes carried out at the Pampulha reservoir, Belo Horizonte, State of Minas Gerais, Brazil, between May and September 2011, dragonfly larvae, *Orthemis discolor* (Burmeister, 1839) and *Perithemis mooma* Kirby, 1889, were found harboring metacercariae identified as *Eumegacetes medioximus* Braun, 1901. This is the first report and morphological description of metacercariae of *E. medioximus* in the Neotropical region.
Keywords: *trematodes, insects, dragonfly, parasites.*

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Resumo: Durante estudos sobre a participação de larvas de Odonata no ciclo biológico de trematódeos realizados na represa da Pampulha, Belo Horizonte, Minas Gerais, Brasil, entre maio e setembro de 2011, larvas de libélulas, *Orthemis discolor* (Burmeister, 1839) e *Perithemis mooma* Kirby, 1889, foram encontradas apresentando metacercárias identificadas como *Eumegacetes medioximus* Braun, 1901. Este é o primeiro relato e caracterização morfológica de metacercárias de *E. medioximus* para a região neotropical.
Palavras-chave: *trematódeos, insetos, libélula, parasitos.*

Introduction

The involvement of insects belonging to the order Odonata Fabricius, 1793 in the life cycle of digeneans has been known since the beginning of the twentieth century (Sinitsin 1905). Several digenean that parasitize birds and amphibians, such as the species of *Eumegacetes* Looss, 1900 (Eumegacetidae), *Gorgoderina* Looss, 1902 (Gorgoderidae), *Haematoloechus* Looss, 1899 (Plagiorchiidae), *Halipegus* Looss, 1899 (Derogenidae), and *Prosthogonimus* Lühe, 1899 (Prosthogonimidae), have been reported parasitizing dragonfly larvae as second intermediate or paratenic hosts in North America, Europe and Asia (Stafford 1931, Boddeke 1960, Hall 1960, Snyder & Janovy Jr 1994, Kumari & Madhavi 1994, Zelmer & Esch 1998, Bolek & Janovy Jr 2007, Bolek et al. 2010). Although several species of these helminths have been recorded as adult parasites in vertebrate hosts from Brazil (Travassos et al. 1969), there are no reports of natural infection of larval dragonflies by digeneans in this country.

In the present study the natural infection of larval dragonflies with metacercariae identified as *Eumegacetes medioximus* Braun, 1901 is reported for the first time in the Neotropical region.

Material and Methods

During malacological surveys conducted at the Pampulha reservoir (43° 59' 35" W and 19° 50' 50" S), Belo Horizonte, Minas Gerais, Brazil, between May and September 2011, naiads of Odonata belonging to the suborder Anisoptera were collected using a hand net and transported alive to the laboratory.

After morphological identification in accordance with Santos (1973), Costa et al. (2004) and Costa & Santos (2009), the odonate naiads were teased apart using dissecting needles and examined for parasites under a stereomicroscope. The cysts recovered were initially studied in temporary mounts using light microscope. The metacercariae were mechanically excysted using metal needles, slightly pressed between glass slides, fixed in formalin at 70 °C, stained with alum acetocarmine, dehydrated in an ascending series of alcohol, cleared in beechwood creosote and mounted in Canada balsam.

Photographs were obtained using a Leica ICC50 HD digital camera attached to a light microscope and measurements were obtained by analyzing the captured images using the Leica Application Suite (LAZ EZ) software, version 2.0. A drawing was made with aid of a camera lucida. The parasite was identified based on taxonomic keys and descriptions according Travassos et al. (1969) and Lotz & Font (2008). Measurements are presented in micrometers. Specimens studied were deposited in the collection of the Invertebrate Taxonomy and Biology Laboratory (DPIC), Instituto de Ciências Biológicas, UFMG, Brazil.

Results and Discussion

Odonate naiads, identified as *Orthemis discolor* (Burmeister) and *Perithemis mooma* Kirby (Anisoptera: Libellulidae), had metacercariae in the abdominal cavity. Six similar cysts were found in the content of the proventriculus of a specimen of Striated Heron, *Butorides striata* (Linnaeus), found dead in the same locality. After morphological analysis and morphometry, the parasite was identified as belonging to the family Eumegacetidae Travassos, 1922 and the species characterized below.

***Eumegacetes medioximus* Braun, 1901 (Figure 1 and 2)**

Synonyms: Eumegacetes perodiosus Travassos, 1922.

Stage: Metacercaria.

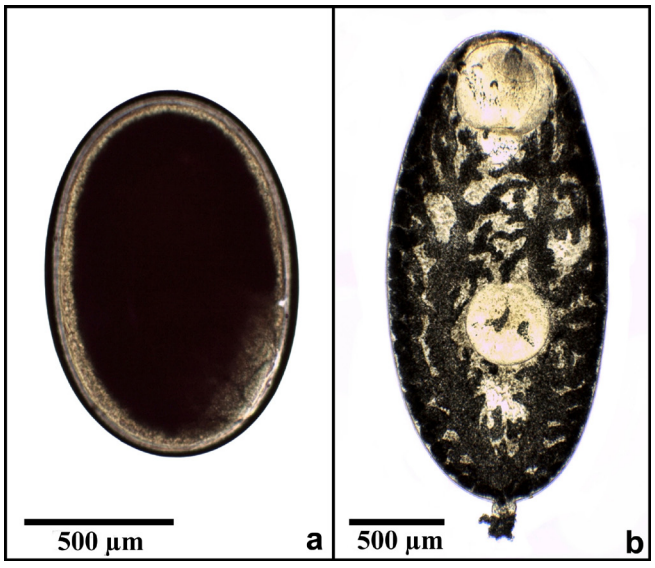

Figure 1. Metacercaria of *Eumegacetes medioximus* found in naiads of Odonata collected at the Pampulha reservoir, Belo Horizonte, Minas Gerais, Brazil. (a) Encysted. (b) Excysted. Unstained wet mount.

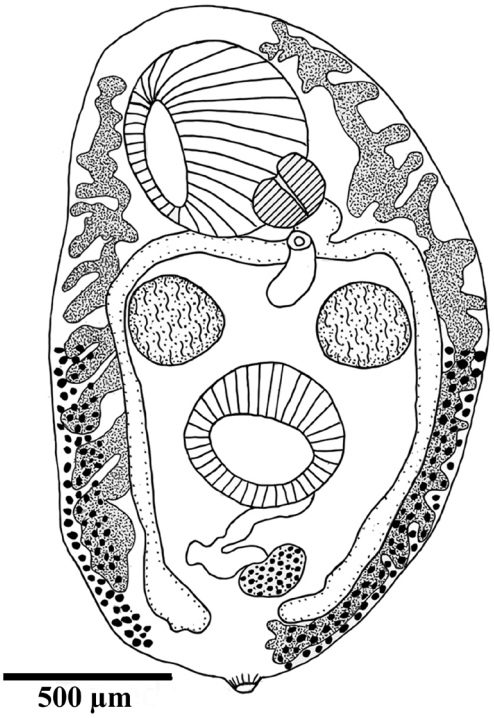

Figure 2. Line drawing of excysted metacercaria of *Eumegacetes medioximus* found in naiads of Odonata collected at the Pampulha reservoir, Belo Horizonte, Minas Gerais, Brazil.

Hosts: Orthemis discolor (Burmeister, 1839) and *Perithemis mooma* Kirby, 1889.

Site of infection: Hemocoel.

Locality: Pampulha reservoir, Belo Horizonte, State of Minas Gerais, Brazil.

Description: Cysts oval to spherical 1254 (1117-1375) in length by 1080 (840-1375) in width. Cystic membrane transparent measuring 45 (34-60) in thickness. Excysted metacercariae oval 1953 (1812-2137) in length by 1224 (937-1428) in width. Oral sucker

subterminal, 529 (363-643) in length by 530 (362-643) in width. Ventral sucker equatorial, 353 (286-432) in length by 407 (296-590) in width. Oral sucker width to ventral sucker width ratio 1.52 (1.27-2.11). Prepharynx short. Pharynx muscular, 203 (142-226) in length by 242 (157-302) in width. Esophagus short. Intestinal caeca extending to the posterior portion of the body. Two testes spherical, opposite, pre-acetabular, intercaecal. Right testis 263 (154-326) in length by 237 (142-314) in width. Left testis 234 (142-307) in length by 233 (135-289) in width. Ovary round, post-acetabular, median, 176 (111-227) in length by 172 (110-204) in width. Vitellaria follicular, extracaecal, in two fields lateral, extending from posterior margin of testes to posterior extremity of body. Uterine primordium inconspicuous, seen only in the anterior region of ovary. Cirrus pouch oval to elongated, median, located in the anterior portion of body, 152 (144-177) in length by 84 (75-95) in width. Genital pore median, bifurcal. Excretory vesicle V- shaped, with arms reaching the level of the anterior region of body, presenting lateral diverticula and filled with dark granules. Excretory pore terminal.

Specimens deposited: DPIC 6220-6222

Remarks: Eumegacetes medioximus was described by Braun (1901) from material collected by Johann Natterer from the Great Jacamar, *Galbula grandis* Latham (Galbulidae), in Brazil in the mid-nineteenth century. It was subsequently recorded in several species of birds in the States of Mato Grosso do Sul and Rio de Janeiro in Brazil (Travassos 1928, Travassos & Freitas 1940, Travassos et al. 1969, Brasil & Amato 1992), and in Venezuela (Lutz 1928, Caballero & Díaz-Ungría 1958). Except for the absence of eggs in the uterus, other morphological traits of the metacercariae studied in the present study are in accordance with reported by these authors. Recently, *Eumegacetes* sp. was found in sparrows, *Passer domesticus* (Linnaeus) (Passeridae), in the State of Rio Grande do Sul (Calegaro-Marques & Amato 2010). Other developmental stages of *E. medioximus* were not previously known.

Members of Eumegacetidae are intestinal parasites of birds with worldwide distribution (Lotz & Font 2008). The life cycle is known for *Eumegacetes artammi* Mehra, 1935, and *Orthetrotrema monostomum* Macy and Basch, 1972, and includes a prosobranch mollusc, *Melanoides tuberculata* (Müller) (Thiaridae), as intermediate host; xiphidiocercariae belonging to the microcotylous group emerge from infected molluscs and penetrate odonate larvae, to form metacercariae, in the case of *E artammi*, or progenetic adults in *O. monostomum* (Kumari & Madhavi 1994, Madhavi & Swarnakumari 1995). Different species of dragonfly larvae have been reported harboring metacercariae of *Eumegacetes* in the United States of America (Stafford 1931, Hall 1960) and India (Rao & Madhavi 1961, Singh & Pande 1968, Prakash & Pande 1970, Kumari & Madhavi 1994). The present study is the first report of metacercariae of *Eumegacetes* in larvae of Odonata in the Neotropics.

The first intermediate hosts of *E. medioximus* remain unknown. Recently, a small xiphidiocercariae with morphological characteristics consistent with cercariae of Eumegacetidae known have been found in *Pomacea lineata* (Spix) (Ampullariidae) at the Pampulha reservoir (HAP, unpublished data), however experimental or molecular evidences are still necessary to confirm that these cercariae are conspecific with *E. medioximus*. Although *M. tuberculata* is the only intermediate host of *Eumegacetes* known so far, larvae of these parasites have not yet been reported in this thiarids in the Neotropical region (Pinto & Melo 2011). Experimental infection studies are needed in order to elucidate the participation of these prosobranch molluscs in the transmission of *E. medioximus*.

Few studies have evaluated the involvement of aquatic insects in the biological cycle of digeneans in South America. Larvae of Odonata and Coleoptera have been found harboring metacercariae of *Gorgoderina* sp. and *Rauschiella repandum* (Rudolphi, 1819), respectively, in Venezuela (Lutz 1926, 1928). Natural infection with metacercariae of *Stomylotrema vicarium* Braun, 1901 (Stomylotrematidae), in aquatic Coleoptera, *Megadytes glauca* Brullé (Dityscidae), and Heteroptera, *Belostoma* spp. (Belostomatidae), have been reported in Argentina and Brazil (Ostrowski de Núñez 1978, Digiani 2002, Amato & Amato 2006).

Over the last decade, studying the biological interactions between insects and trematodes has become important, since metacercariae found in aquatic insects, including Odonata, have been found infected by *Neorickettsia risticii* (Holland et al. 1985) (Anaplasmataceae), the etiological agent for Potomac Horse Fever (Chae et al. 2000, Madigan et al. 2000, Mott et al. 2002, Gibson & Rikihisa 2008).

Despite the diversity of species of trematodes found in Brazil, studies involving the possible role of insects in the transmission of these parasites are still needed.

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