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Biological aspects of immature stage of *Nyssomyia whitmani* (Antunes and Coutinho) (Diptera, Psychodidae, Phlebotominae) in laboratory conditions



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ABSTRACT

Nyssomyia whitmani (Antunes and Coutinho, 1939) has been considered as a complex of cryptic species, and some of the populations of this complex plays an important role in the transmission of *Leishmania* spp. in Brazil. The present study reports the biological aspects concerning the productivity out of eggs and the development time of the descendants of females obtained in Dourados municipality, Mato Grosso do Sul state. The females were captured with modified electric aspirators, fed in hamsters and further individualized in containers for breeding. At the insectary, temperature and relative humidity were maintained on average of 24.5 °C and 67.3%, respectively. From 944 females 3737 eggs were obtained, 748 (20.0%) evolved to the stage of larvae, and 93 (12.4%) of these reached adult stage. The life cycle lasted 80.6 days and the last larval instar was the longest. The use of a higher protein diet revealed a significant improvement in larval development.

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Introduction

Nyssomyia whitmani is recorded in 720 Brazilian municipalities and it is distributed over 25 states, including the Federal District (Queiroz et al., 1994; Membrive et al., 2004; Teodoro et al., 2006; Galati, 2014). In Mato Grosso do Sul this species has been recorded in many municipalities (Galati et al., 1996, 2006; Oliveira et al., 2003, 2006; Braga-Miranda et al., 2006; Nascimento et al., 2007; Nunes et al., 2008; Almeida et al., 2010).

The species was found by visualization of flagellate forms or detection of *Leishmania* (V.) *braziliensis* DNA in some states as Ceará, Maranhão, São Paulo, Paraná and Mato Grosso do Sul (Pessoa and Coutinho, 1941; Azevedo et al., 1990; Queiroz et al., 1994; Luz et al., 2000; Oliveira-Pereira et al., 2006; Paiva et al., 2010) and therefore, it was implicated as a probable vector of the parasite at the studied sites. The first information about the species life cycle *Nyssomyia whitmani* was obtained by Barretto (1942) from specimens collected from the southeast region of Brazil. However, more research about population dynamics of this sandfly species and its immature

stages of biology are still needed to understand the insect's pattern of abundance and its relationship with the environment.

In spite of the few published studies about their immature forms (larvae and pupae) and the duration of these stages, this study aimed to obtain information on oviposition and the life cycle of *Ny. whitmani*, which comprises the stages of egg, larvae and pupae, in order to assist the understanding of this species ecology.

Materials and methods

The captures were held between 2012 and 2013, from 18:00 h to 21:00 h, using an electric aspirator attached to a 6 V battery in a chicken coop located at the Fazenda Coqueiro farm, in the municipality of Dourados, Mato Grosso do Sul (MS), Highway MS162, Km 10 Dourados-Ithaum (22° 12' S and 54° 54' W). The specimens were transported to the Laboratório de Parasitologia Humana da Universidade Federal do Mato Grosso do Sul (UFMS), located at Campo Grande/MS, according to the methodology of Rangel et al. (1985, 1986).

Apple slices were placed inside the cage to feed the males and females until their arrival at the laboratory. In the evening, a hamster (*Mesocricetus auratus*) previously anesthetized with an intramuscular injection (ketamin 75 mg/kg and xylazine 10 mg/kg

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Table 1

Analysis of collected females, total of females that laid eggs, total eggs laid, total hatched larvae, total emerged adults and average temperature (°C) and relative humidity (RH%) during the eight collection periods between 2012 and 2013.

Collection month/year (2012–2013)	Females	Females that laid eggs (%)	Number of eggs (eggs/females)	Total of larvae (%)	Total of pupae	Adults (%)	Temperature (°C) and humidity (UR%)
Collection 1	215	13 (6.0)	390 (30.0)	52 (13.3)	–	–	24.7°/63.9%
Collection 2	151	8 (5.3)	324 (40.5)	104 (32.1)	–	–	25.6°/65.7%
Collection 3	187	18 (9.6)	448 (25.0)	102 (22.7)	–	–	25.4°/64.0%
Collection 4	10	4 (40.0)	84 (21.0)	6 (7.1)	–	–	24.2°/63.0%
Collection 5	90	6 (6.7)	86 (14.3)	–	–	–	23.9°/68.3%
Collection 6 ^a	5	5 (100.0)	88 (17.6)	41 (46.6)	13 (31.7)	11 (84.6)	23.7°/71.7%
Collection 7	148	78 (52.7)	1640 (21.0)	431 (26.3)	149 (34.5)	136 (91.2)	24.1°/69.3%
Collection 8	138	33 (23.9)	677 (20.5)	18 (2.7)	3 (16.6)	2 (66.6)	24.6°/72.7%
Total	944	165 (17.48)	3737 (22.6)	748 (20.02)	165 (22.0)	149 (90.3)	24.5°/67.3%

^a Beginning of a new larval diet.

IM in 2:1 rate) was placed inside the cage, in order that the females could blood feed on it during an average time of 40 min. After 48 h, the females were individualized in a 0.5 cm plaster-lined plastic container with 2.5 cm diameter and 3.5 cm height, which were capped with voile fabric. A piece of cotton soaked in undiluted honey was placed on top of the voile fabric, as a source of carbohydrate for the females (Brazil and Brazil, 2003).

The containers were kept in covered plastic boxes (34.0 cm × 24.5 cm × 9.0 cm) with filter paper in the base, or in foam boxes (31.0 cm × 20.0 cm × 22.0 cm) with plaster on the walls and the base, in order to conserve moisture and temperature of the microenvironment. These parameters were controlled with a digital thermo-hygrometer, which maintained an average temperature of 24.5 °C and air relative humidity of 67.3%. After death, females were dissected for species identification, through the observation of spermathecae morphology and identified according Galati (2003). Once the females of *Ny. whitmani* were identified, their eggs were grouped, counted and transferred with fine-tipped brushes to a 0.5 cm plaster-lined Petri dishes (8.5 diameter and 1.5 cm height) according Rangel et al. (1985). After transferring the eggs, soil collected from the hen house where the insects were once collected was added. After the larvae hatched, lyophilized liver (OXOID – dehydrated liver) was introduced in their diet. Daily observations were made until the emergence of the winged adults.

The larvae at all instars (L1, L2, L3 and L4) and the pupae were counted. Larvae hatching and ecdysis were recorded to verify the species biological cycle. To estimate the duration of *Ny. whitmani* life cycle, including incubation period, duration of larval instars and pupal period until adult emergence, the minimum duration (minimum time, in consecutive days, between the observation of the first individual of larval period until the observation of the first individual of the second instar and so on); and the maximum duration (maximum time, in consecutive days, between the observation of the first individual of the instar until the observation of the last individual of the same instar), and median were calculated.

Results

From the 944 individualized females, 3737 eggs were obtained from the oviposition of 165 females. The minimum and maximum times of development of the immature forms were 54.3 and 106.9 days, respectively.

From the total of females fed, 17.48% laid eggs, with the lowest percentage in the second collection (5.3%) and the highest in the 60 (100%), followed by seventh collection (52.7%). The average number of eggs per female was 6.1 (Table 1). The eggs were elliptical, presented dark tonality and were laid singly or in small clusters. The mean incubation period of the eggs was 9.5 days and its median was 12 days. At hatching, first instar larvae (L1) presented a single pair of caudal setae and whitish bodies and heads, with a cephalic

portion that became darkened after a few hours. This phase lasted a minimum of 7.0 and the maximum of 11.2 days, with a median of 5 days (Table 2).

The L2 larvae presented two pairs of caudal setae and fed on the hen house soil set in the containers with more avidity when compared to L1 larvae. The maximum and minimum times were 4.9 and 9.4 days, respectively (median of 7 days) (Table 2).

It was possible to check with the naked eye that the third instar larvae was a few times larger than the second and maintained two pairs of caudal setae. This instar lasted a minimum of 6.1 days and maximum of 18.0 days, with a median of 10 days (Table 2).

The fourth and last larval instar (L4) was characterized by the presence of a spot in the last tergite. It was the longest period among the immature forms with a median of 21 days (range 16.1–29.8), and after this time, the larvae had their last ecdysis. It was observed that in this period the larvae reduced its feeding and locomotion. At the beginning of the pupal stage, the pupae presented yellowish and light brown eyes, which changed to a blackened shade as the end of the phase approached and remained until they reach adult form. This period showed a range between a minimum of 10.7 days and a maximum of 22.7 days, with a median of 16.7 days. The biological cycle presented a median of 80.6 days (Table 2). There was no difference between emerged males and females.

Discussion

Barretto (1941, 1942) reported lower values for the life cycle when studying populations of *Ny. whitmani* from Southeast of Brazil, with averages of 50.5 days. When studying the biology of *Nyssomyia intermedia* and *Ny. whitmani*, Barretto (1941, 1942) verified the rapid and gradual development of eggs when temperature was set between 25° and 27 °C. Rangel et al. (1985), reported a range of 26–56 days (average 41 days) when monitoring the life cycle of *Ny. intermedia* at 25 °C and 86% humidity. Andrade-Filho et al. (2004) noted a life cycle of 34.9 days at temperatures ranging between 25 °C and 26 °C and 80% humidity, when studying this last species.

Table 2

Duration (days) of egg, larval and pupal development of *Ny. whitmani* under laboratory conditions at temperature of 24.5 °C (±1 °C) and RH ~67% over the eight collection periods between 2012 and 2013.

Instar	Minimum (days)	Maximum (days)	Median (days)
Egg	9.5	15.9	12.0
Larva 1	7.0	11.2	5.0
Larva 2	4.9	9.4	7.0
Larva 3	6.1	18.0	10.0
Larva 4	16.1	29.8	21.0
Pupa	10.7	22.7	16.7
Total	54.3	106.9	80.6

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