Entomological profile and new registers of the genera Anopheles (Diptera, Culicidae) in a Brazilian rural community of the District of Coxipó do Ouro, Cuiabá, Mato Grosso

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Abstract. The order Diptera is constituted of insects that possess numerous varieties of habitats, these winged, commonly called mosquitoes or stilt, comprise a monophyletic group. Malaria transmitters in Brazil are represented by mosquitoes of the Anopheles genus, being the principal vector species Anopheles (Nyssorhynchus) darlingi Root. Collectings were accomplished in the rural area of Cuiabá in the region of Coxipó do Ouro/MT, and a total 4,773 adult mosquitoes of the genus Anopheles were obtained. The prevailing species in the collectings where An. (Nys.) darlingi with 3,905 (81.8%), considered the vector of major epidemiological expression in the region, followed by Anopheles (Nyssorhynchus) argyritarsis (Robineau-Desvoidy) 267 (5.6%) and Anopheles (Nyssorhynchus) triannulatus (Neiva & Pinto) 226 (4.7%). This report might be useful to entomological surveillance, demonstrating that the Coxipó do Ouro/MT locality might be elected as an area to be monitored, once the presence of such vector in this type of environment indicates a potential malaria transmission risk for the neighbouring regions in the state of Mato Grosso.

Keywords: Anopheles darlingi; Culicidae; Malaria; Mosquito; Tropical Medicine.

The order Diptera is constituted of insects that possess numerous varieties of habitats, these winged, commonly called mosquitoes or stilt, comprise a monophyletic group (Reidenbach 2009); belong to the order Diptera, suborder Nematocera, suborder Culicomorpha and family Culicidae, which is divided into two subfamilies, Culicinae and Anophelineae. The Culicinae are recognized 11 tribes, with 110 genera and approximately 3,570 species described (Harrbach 2020).

The subfamily Anophelineae has three genera and includes 489 species formally described and constituted by species of the genus Bironella, present only in the Australian region, Chagasia, restricted to the neotropical region, and Anopheles, cosmopolitan, presenting 476 species formally described and named eight subgenera; Anopheles (185 species), Baimaia (1), Myzomia (=Cellia) (225), Christya (2), Kerteszia (12), Lophopodomyia (6), Nyssorhynchus (40) and Stethomyia (5) (Harrbach 2020). Of the three genera, the only one of major importance is the genus Anopheles, cosmopolitan mosquitoes responsible for malaria transmission (Consoli & Oliveira 1994; Colluci & Sallum 2006).

The geographic distribution of Anopheles is wide and can be found in several niches, from deserts to tropical forests (Kiszewski et al. 2004). Some species have considerable plasticity of adaptation to sites and different types of habitats (John 2008).

Mosquitoes of the genus Anopheles are arthropods responsible for the transmission of the etiologic agent of malaria. Five species of protozoa of the genus Plasmodium can cause human malaria: Plasmodium falciparum Welch, Plasmodium vivax Grassi & Feletti, Plasmodium malariae Feletti & Grassi, Plasmodium ovale Stephens, and Plasmodium knowlesi Sinton e Mulligan. In Brazil, there are three species associated with malaria in humans: P. vivax, P. falciparum and P. malariae. Plasmodium ovale is restricted to certain regions of the African continent and to imported cases of malaria in Brazil. Plasmodium knowlesi, a monkey parasite that has been recorded in human cases, occurs only in Southeast Asia. Man is the main reservoir of epidemiological importance for human malaria (Brazil 2017). The vectors of malaria in Brazil are popularly known as “carapãna”, “muriçoça”, “sóvela”, “mosquito-nail” and “bicudo” (Brazil 2017).

In Brazil, the subgenus of the Anophelines of medical importance are Nyssorhynchus and Kerteszia, and in the first subgenus is the species considered the main vector of Anopheles (Nyssorhynchus) darlingi (Root) malaria that is considered the vector of major epidemiological expression because it prefers feeding by human blood, and found in great abundance in environments domesticated by man (Foratini 2002; Colluci & Sallum 2006), this mosquito is highly

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anthropophilic and endophagic, stinging in and around the residences (BRASIL 2017).

Mato Grosso state, which is part of the endemic area, currently responsible for 1.0% of malaria cases (BRASIL 2011). The distribution of the cases of the disease presents a not homogeneous behavior and is especially concentrated the municipality of Colniza, considered medium risk and Nova Bandeirantes, considered low risk (Mato Grosso 2016). Malaria control is based on early diagnosis, case management and vector control, and for the latter method it is necessary to know the behavior of the vector in the target locality (BRASIL 2017).

Even malaria suffering declining in number year by year still poses problems for the authorities by their difficult control. In the state of Mato Grosso, according to the state health department's survey, 216 cases were reported in the year 2016. In that same year, the five municipalities of Mato Grosso with the highest number of cases were: Colniza, Aripuanã, Juína, Brasnorte and Rondolândia (PARENTE et al. 2012).

Data from the World Health Organization (WHO 2013) indicate that malaria is the tropical infectious-contagious disease that causes most social and economic problems in the world, only outnumbered by AIDS deaths (PARENTE et al. 2012).

One of the major concerns regarding this vector is reproductive success in the face of anthropic environmental impact, human changes, such as human settlements, agriculture and indigenous peoples, miners and hydroelectric enterprise construction (Hiwat & Bretas 2011).

It is essential to develop entomological studies that subsidize options in the adoption of control mechanisms that effectively reduce the rate of transmission and mortality caused by malaria.

The present study reports the occurrence of the anophelines fauna and behavior of the species that occur in the community of Coxipó do Ouro, Cuiabá district, seeking to understand the entomological link in the transmission of malaria in the municipality of Mato Grosso.

**MATERIAL AND METHODS**

**Area of study.** Sampling of specimens was performed, between January/2014 and November/2016, from 17:00 to 23:00 h, totaling six h of catch for three consecutive nights in each of the five months of each year (Table 1). A total of 4,773 winged adults female and 62 larvae were collected in rural areas of Cuiabá-MT in the community of Coxipó do Ouro 15°27’20.98” S; 55°58’35.22” W (Figure 1).

**Mosquito collection.** For the collections of adult females Anophelines specimens, two methods were used: Shannon trap and Method of capture by human attraction (Human Landing Catch method/HLC) as recommended by WHO (2013) and MARCONDES et al. (2007), where captors are fully protected and using black stockings of thick tissue covering the legs of the catches so that the insect has difficulty performing blood repast, but is still attracted. Adult female mosquitoes were captured between 17:00 and 23:00 h.

The human bait collections were performed on three consecutive nights by four individuals working in teams of two in three-hour shifts, capturing Anophelines mosquitoes using the technique with “Castro’s catcher” suction tube (MARCONDES et al. 2007; RIBEIRO et al. 2013).

After capture, the insects were stored in entomological pots of plastic containers of volume 500 mL, inserted with a maximum of 40 to 50 insects. The collected specimens were conditioned and fed with cotton soaked in sugar water (10%) and stored in polystyrene boxes, covered with a wet towel, thus avoiding the presence of predators and helping the survival of the captured insects.

For sampling of the immature Anophelines was used the method of shellfuls in the borders of the River Coxipó of the gold being 50 shellfuls per month of sampling sampled totaling 750 shellfuls. The collected larvae were counted and identified with a Coleman optical microscope with a 100x magnification at the Entomology Laboratory of the Universidade Federal do Mato Grosso (Federal University of Mato Grosso). Identification was performed using the keys for classification of CONSOLI & OLIVEIRA (1994) and FORATTINI (2002).

**Morphological identification of Diptera.** The collected adult individuals were stored in appropriate containers, sorted, identified, assembled and preserved in the Entomology laboratory of the Universidade Federal do Mato Grosso (UFMT). The collected specimens were identified in the Medical Entomology Laboratory of the Faculty of Medicine of the Federal University of Mato Grosso in small

**Figure 1.** Location of the collection point in the region of the Coxipó do Ouro river, Mato Grosso, Brazil, from 2014 to 2016 (left). Risk map of Brazil by municipality of infection. Source: Sivep-Malaria and Sinan /SVS/MS/2017 (right).
groups (30 specimens), the mosquitoes adult females were killed in the freezer at –20 °C for 5 min. Using microscope-40X stereoscope, for identification at the specific level using the taxonomic keys: Zavortink (1927); Deane et al. (1947); Lane (1953); Farani & Linthicum (1981); Consoli & Oliveira (1994); Forattini (2002).

RESULTS AND DISCUSSION

Mato Grosso is located in the Central West region of Brazil, and the biome is closed as a characteristic. The Cerrado is the second largest Brazilian and South American plant formation, and is considered the most biodiverse savanna formation in the world, surpassed only by the Amazon Forest. In Mato Grosso the area occupied by this biome is approximately 300,000 km², equivalent to 34% of the state territory. Due to its location, it shares specimens with most Brazilian biomes (Amazon Forest, Caatinga and Atlantic Forest) (Brasil 2018). Characteristic of tropical regions, the Cerrado presents two well-defined seasons: rainy summer and dry winter (Dantas et al. 2016).

Fifteen campaigns were carried out for collecting adults and larvae, during the period studied (2014, 2015, 2016) there were a total of 750 shellfuls to obtain immature specimens. From this technique, 62 were positive for Anophelines larvae, 20 larvae of the species *An. argyritarsis* were identified, and the others only at the generic level.

A total of 4,773 adult females of the genus *Anopheles* were collected from January 2014 to November 2016, representing seven morphospecies: *Anopheles* (Nyssorhynchus) *darlingi* (Root) 3,905 (81,8%), *Anopheles* (Nyssorhynchus) *argyritarsis* (Robineau-Desvoidy) 267 (5,6%), *Anopheles* (Nyssorhynchus) *triannulatus* (Neiva & Pinto) *Anopheles* (Nyssorhynchus) *albitarsis* (Lynch-Arribálzaga) 144 (3,0%), *Anopheles* (Nyssorhynchus) *minor* (Lima) 111 (2,3%), *Anopheles* (Nyssorhynchus) *benarrochi* (Galbadon) 67 (1,4%) e *Anopheles* (Nyssorhynchus) *oswaldoi* (Periassu) 53 (1,1%) (Table 1).

The species with the highest number of individuals collected was the *An. darlingi* species with 3,905 individuals, totaling 81.8% of the specimens collected in 2014, 2015 and 2016. *An. darlingi* is the main vector of malaria in Brazil probably throughout the continent South American (Forattini 2002; Collucci & Sallum 2006, Brasil 2019).

Considering its role as the main transmitter of *Plasmodium falciparum* (Welch), it is responsible for the transmission of other species of *Plasmodium vivax* (Grassi & Feletti) and *Plasmodium malariae* (Feletti & Grassi) (Brasil 2017). Other authors in their research, concluded that *Anopheles* species may be involved in the transmission dynamics, in addition to the *Plasmodium* protozoan, may be involved in microfilariae transmission (Amuzi et al. 2010) and arboviruses (Medlock et al. 2005).

Specimens of the genus *Plasmodium* may also parasitize non-human primates and constitute a natural reservoir for human malaria. The only two parasite species described in South American primates are *Plasmodium brasilianum* (Gonder & Von Berenberg-Gossler) having this species, a wide geographic distribution, being found in the Amazon Rainforest from Panama to Brazil (Perelman et al. 2011) and *Plasmodium simium* (Grassi & Feletti) species found in Atlantic Forest areas of the South and Southeast regions (De Almeida et al. 2015) of may under special conditions infect humans (LalRemruata et al. 2015).

Reports of anthroponoontic were described in Brazil and Venezuela concerning these two species. Venezuelan researchers LalRemruata et al. (2015) argued in their studies that human infections occurring in that country denote that *P. brasilianum* in Venezuela were genetically identical to *P. brasilianum* in host monkeys and *P. malariae* in humans. The records reported by Brasil et al. (2017) showed the evidence found in the Atlantic Forest in Rio de Janeiro/Brazil on *P. simium*, indicating that this species may correspond to the description of a sixth type of human malaria.

Most malaria cases are concentrated in the Amazon region (Acre, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima and Tocantins). However, there are reports of cases of the disease being carried out in endemic areas in other Brazilian states (Brasil 2019) (Figure 1).

These records of human movement between regions with few or many endemic diseases confirm the risk of malaria due to the presence of the vector in both environments as recorded in these collections. Recreation with bars and clubs of associations and economic activities involved are among the main activities that put people at risk of contracting *Plasmodium* and disseminating them in other regions (Tadei et al. 2007).

In a study conducted in localities near the Manso hydroelectric station in the State of Mato Grosso (Forattini 1993; Ribeiro et al. 2007); these researchers collected specimens of Anophelines in the area of implantation of this plant, being also identified a high number of individuals of the species *An. darlingi*, corroborating with the records found in this study.

Epidemiologically, the presence of specimens of the genus *Anopheles* can be considered an important genus Culicidae family that includes many species vectors of *Plasmodium* causing human malaria. In Brazil, 11 species of them have epidemiological importance and great impact in the transmission of the disease: *An. (Nys.) darlingi*; *Anopheles*
(Nyssorhynchus) australis Curry; An. (Nys.) albitarsis; Anopheles (Nyssorhynchus) marajoara Galvão & Damasceno; Anopheles (Nyssorhynchus) janconnae Wilkerson & Sallum; Anopheles (Nyssorhynchus) deaneorum Rosa-Freitas; An. (Nys.) oswaldoi; Anopheles (Kerteszia) cruzii Dyar & Knab; Anopheles (Kerteszia) bellator Dyar & Knab e Anopheles (Kerteszia) homunculus Komp (Brasil 2017).

We can observe that three of these species were identified in this study: An. triannulatus (140, 4.2%), third largest species in a sample of specimens identified in this study, followed by An. albitarsis (96; 2.9%); An. oswaldoi (28; 0.8%), showing the amplitude, adaptability and dispersion of the genus Anopheles in the regions of Mato Grosso.

Three species of captured Anopheles are important in the epidemiology of malaria transmission: An. darlingi is considered one of the most efficient malaria vectors in the Americas region (FORATTINI et al. 1993; SINKA et al. 2011); An. triannulatus was considered to be one of the important vectors in Mato Grosso (MISSAWA et al. 2011), and was found naturally infected with Plasmodium (SINKA et al. 2011), and this species is incriminated in the transmission of malaria in Brazil (TASEI & DUTTON-TATTERS 2000), Peru and Venezuela (BENARROCH 1931; ARAMBURU et al. 1999), a fact that MORENO et al. (2013) tracked the distribution of this transmitter in several countries of the globe.

Still, Anopheles argyritarsis Robineau-desvoidy, where we cite the captures of the field insertions of its immature forms, recorded in this study, showing the importance of this vector in Mato Grosso. In the municipality of Várzea Grande/Mato Grosso, reports on this species were recorded by SILVA et al. (2008) who found immature forms of this species in water boxes in association with larvae of Aedes albopictus (Skuse). CARRERA-ALVES (2001) in the city of Rio de Janeiro/Brazil; found larvae of An. argyritarsis and pupa of An. australis, in deactivated artificial containers found in open sky.

FORATTINI et al. (1998), in a research carried out in São Paulo/ Brazil, found this type of synanthropy, where two larvae of An. argyritarsis were identified, together with immatures of Ae. albopictus and Culex quinquefasciatus (Say). This information corroborates and contributes to show the high plasticity of this arthropod in adapting to new environments that are not ideal for development and growth mainly sites devoid of emergent vegetation, which are the first choice sites for these anophelines.

Previous studies have confirmed that An. argyritarsis, a vector specimen of Plasmodium vivax transmission (FARAN & LINHICUM 1981); although in relation to this species. FORATTINI (2002) reports in his studies that the vector capacity of An. argyritarsis has been the subject of questioning, fact attributed to possible misidentifications. This species, although it can be found in houses, is not truly domiciled and shows great indifference to human blood (FORATTINI 2002).

In relation to the collection periods from 2014 to 2016, there was a higher index in the first year (2014), where 1,735 (36.3%) were recorded, followed by the year (2015), where there were 1,589 (33, 3%) and finally the year 2016, where 1,449 (30.4%) were registered (Table 1). In the region of gold mining in Nova Gurita, researchers from Mato Grosso (MACHÉL & OLIVEIRA 2014) collected 481 specimens of the genus. In 2008, in the municipality of Colniza/Mato Grosso (MACHÉL & MISSAWA 2012) collected 3,160 high-density specimens in May of that year.

We can still check the records of RIBEIRO et al. (2013) who obtained 2,762 specimens from seven different species of Anopheles, evaluating the nictemeral distribution in the area of influence of the Manso hydroelectric station. In 2015, MARTINS et al. found the first records of An. darlingi in a hydroelectric station in Minas Gerais/Brazil, and the impacts generated by these reservoirs. These researchers found An. darlingi species as the most prevalent species, and these results are in accordance with the results found in this study, showing that this species of Anophelines, the representative with the highest prevalence index in the Midwest of Brazil.

In Mato Grosso State, the records of the species captured through different collection techniques (human bait, mosquito trap and Shannon trap) performed by MISSAWA et al. (2011) demonstrate the importance of this genus in the center-west region of the country, citing: An. darlingi, An. benarrochi, Anopheles (Anopheles) mediopunctatus (Theobald), Anopheles (Nyssorhynchus) nigritarsis (Chagas), An. oswaldoi, Anopheles (Anopheles) peryassui (Dyar et Knab), Anopheles (Nyssorhynchus) rangeli (Gabald, Covo-gar et Lopez), and An. triannulatus, as captured species.

In Goiás/Brazil, MANGEL et al. (2010) found a variety of Anophelines species that presented the highest diversity in the studied municipalities were An. argyritarsis and An. darlingi, however these researchers found a higher density in Anopheles (Nyssorhynchus) evansae (Brethes). We can observe the rich of species that may be present in the different niches of the Central West region of Brazil. In contrast to the techniques used in the study by these researchers, it can be observed in this study that the technique of human bait was superior in the capture of the winged specimens, 4,161 (87.2%) in relation to the Shannon technique 612 (12.8%), (Table 2), showing opposite values to those found by MISSAWA et al. (2011).

The collection period occurred from 17:00 to 23:00 h An. darlingi was the most prevalent species in Shannon trap and human bait, present at all collection times and in all years of capture. In human bait An. darlingi and An. argyritarsis, 87.4% of the captured insects were added together. TEGODORO et al. (1995) evaluating Culicidae diversity in the Paraná river/Brazil, detected the presence of An. triannulatus and An. albitarsis...

| Species Captured     | Human isca | Shannon | Total |
|----------------------|------------|---------|-------|
|                      | (N)        | %       | (N)   | %     | (N)   | %     |
| An. (Nys.) albitarsis| 82         | 2,8     | 62    | 16,2  | 144   | 3,0   |
| An. (Nys.) argyritarsis| 192       | 6,5    | 75    | 19,6  | 267   | 5,6   |
| An. (Nys.) benarrochi| 42         | 1,4    | 25    | 6,5   | 67    | 1,4   |
| An. (Nys.) darlingi| 3555       | 120,9  | 350   | 91,6  | 3905  | 81,8  |
| An. (Nys.) minor | 91         | 3,1    | 20    | 5,2   | 111   | 2,3   |
| An. (Nys.) oswaldoi| 35         | 1,2    | 18    | 4,7   | 53    | 1,1   |
| An. (Nys.) triannulatus| 164       | 5,6    | 62    | 16,2  | 226   | 4,7   |
| Total                | 4161       | 142    | 612   | 160   | 4773  | 100   |

Table 2. Number and percentage of anophelines species captured according to harvesting techniques in the years 2014, 2015 and 2016, in Coxipó do Ouro district, Cuiabá/MT – Brazil.

in Shannon trap. An. Triannulatus in Shannon trap (2,741) followed by human bait (788).

In the Shannon trap, the most active h of the prevailing species (An. darlingi) in this study were between 18:00 at 22:00 h, in human bait An. argyritarsis had a higher density of 22:00 at 23:00 h, while A. triannulatus Neiva & Pinto and An. minor Lima prevailed from 17:00 at 19:00 h, while An. benarrochi, An. oswaldoi, and An. albifasciatus were detected at 17:00 at 18:00 h. The performance of An. albifasciatus and An. argyritarsis was recorded from 22:00 to 23:00 h (Figure 2).

When analyzing the activity period of the dominant species An. darlingi in the research area of the Coxipó do Ouro/Mato Grosso district, we can observe that this species was active mainly in the initial collection times from 17:00 at 18:00 h with number of specimens collected and again a second peak of hematophageal activity at 21:00 at 22:00 h. When we compare this information with the results obtained by MISSAWA et al. (2011), these researchers showed in their casuistic proximity of the hematophageal activity of this species that had its initial peak from 18:00 at 19:00 h and then resuming its frequency at a second peak from 22:00 to 23:00 h.

Other researchers in their series reported values close to those found in this study. RIBERIO et al. (2013) found a high prevalence in the period from 18:00 at 19:00 h of An. darlingi, using human bait technique in the period 2005-2006. MACIEL & OLIVEIRA (2014) collected 481 specimens of the genus with peak of greater activity from 18:00 at 21:00 h.

When we compare the results obtained in studies in Mato Grosso by MACIEL & MISSAWA (2012) we can observe a similarity in the schedules obtained in this study and conclude that this vector species showed to be very active first from 18:00 at 19:00 h resuming the secondary peak of their food activities at 21:00 at 22:00 h. TADEI & DUTARY-TATCHER (2000) report that the highest activity period of An. darlingi occurs during the first three h of the escotophase, varying from 17:00 at 21:00 h. This report is in accordance with this work. However, these arguments can be compared with the records of RUND et al. (2013) that observed the rhythmic patterns of Anopheles gambiae Giles and found that the olfactory organs of these arthropods feel the odors of humans in the pre-twilight and twilight periods being more attracted hematophagy at night.

The vector species An. darlingi, has a preferential feeding activity at twilight times, being this variation to be differentiated by determining factors, such as temperature and season of the year. FORATTINI et al. (1993) and FORATTINI 2002 argue in their results that these variations of the rhythm in the nyctemeral cycle of An. darlingi are indicative of the existence of diverse populations and, consequently, of different behavior. However, in a similar cycle observed in Aripuanã/Mato Grosso/Brazil, CHARLOONG & WILKES (1979) verified that the twilight peaks are predominantly composed of nulliparous females. As different researchers have registered different preferential activity schedules in their cases (TADER & DUTARY- TATCHER 2000; VOORHAM 2000; SANTOS et al. 2005).

The natural history of this subfamily of mosquitoes has received the attention of researchers from various parts of the world (SINKA et al., 2011; SINKA et al., 2012). These studies have led to a process of knowledge of their biological characteristics in order to discover their vulnerabilities to more easily monitor and combat them (FORATTINI 2002). Currently, vectors serve as a tool for growing studies. The study of Anopheles species in a region where malaria is endemic is important for the targeting of control measures, especially since the same species in the same place can change their habits over time, mainly due to environmental changes.

The data obtained in the present study indicate that the ecological changes followed by the human occupation observed in the research should be considered. This study showed that changes in the environment, whether they are in urban areas, prospectors or rural areas. This finding raises the discussion about the need to understand the importance of social and population characteristics, such as mobility, that affect the population dynamics of species transmitting pathogens, possibly contributing to the increase of disease transmission, among them malaria.

The populations of Anopheles, which are located in the study area, are due to the locations of water from these environments, some species demonstrate the ability to
undergo variations in their biology and to adapt to different environmental conditions.

The high degree of synanthropism of species of the genus *Anopheles*, prevalent *An. darlingi*, is a species considered important in the epidemiology of malaria in the region, point to the need to monitor these species in environments preserved, anthropized and modified by man.

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**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

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