Occurrence of *Anopheles (Anopheles) fluminensis* Root in the City of São Paulo, Brazil

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Malaria is considered one of the most important tropical diseases, affecting millions of people in the world¹. In Brazil, approximately 97% of the malaria cases are focused in only six states of the Amazon region: Acre, Amapá, Amazonas, Pará, Rondônia and Roraima. The majority of the cases occur in rural areas, having reports of disease also in urban areas². The transmission of autochthonous malaria outside of the Amazon region occurs in several Brazilian states, mainly those associated with the ecosystem of the Serra do Mar³. In the State of São Paulo, the disease is characterized by sporadic outbreaks in the west and persistent transmission in the east of the state where only mildly symptomatic or asymptomatic cases, caused by Plasmodium vivax, occur with low parasitemia. Anopheles (Kertezsia) cruzii and An. (Ker) bellator (Diptera: Culicidae) have been incriminated as the main local vectors of the pathogen^{2, 3}. However, fluctuations in weather conditions can promote the emergence of other potential vectors^{1, 4}.

According to the Superintendencia de Controle de Endemias (SUCEN), the epidemiological situation of malaria in the State of São Paulo is under control, with no systematic entomological surveillance as there was in the past. Currently, scientific research is now the main source of information about the distribution of the anopheline species in the State of São Paulo⁵.

In a study that aimed to understand the biodiversity of Culicidae in municipal parks of the City of São Paulo, the discovery of *An. (Anopheles) fluminensis* Root in the city was an important result since this species had never been recorded before, but there is a record about *An. fluminensis* in some areas of the State of São Paulo⁶. *Anopheles fluminensis* is also present in South American countries (Argentina, Bolivia, Brazil and Peru)⁷. This species was described from male specimens collected in the city of Itaperuna (State of Rio de Janeiro, Brazil), and from larval stages that were collected along the edge of a small river in the "Baixada Fluminense"⁸. Regarding the ecology of *An. fluminensis*, a study in northern Paraná state suggested that the species is poorly adapted to anthropogenic environments. This species is an organism of forested area, characterized as a species of nocturnal habits in a study in the Serra do Mar region^{9,10}.

In the City of São Paulo— the largest city in South America, the *An. fluminensis* was found in the Anhanguera Municipal Park, where vegetation consists of eucalyptus (*Eucalyptus saligna*) and *Pinus* sp, with native species of flora in remnant riparian vegetation¹¹, being shelter to several birds and mammals, including howler monkeys (*Alouatta alouatta*) and other primates that are reservoirs of *Plasmodium* species. Anhanguera Park is close to two of the core areas of Green Belt biosphere reserve in the State of São Paulo, the Serra da Cantareira, and the Serra do Japi, taking the Cities of Cajamar and Caieiras as neighboring municipalities¹¹. The climate is classified as humid subtropical, high altitude, with a well-defined dry season, in which the average annual temperature is 24.4°C with rainfall ranging from 1400–1590 mm/yr.

During the monthly survey of Culicidae from October 2010 to December 2011, 27 immature An. fluminensis were collected (found in 8 out of 14 months), of which 11 specimens were raised to the adult stage (9 females and 2 males). Adult specimens were collected at dusk, with CO₂-baited CDC illuminated traps installed near ground level (1 m), and with vacuum aspirators, where the forest was denser. Specimens were identified according to Forattini⁴ and confirmed molecularly by sequence analysis of the second internal transcribed spacer of ribosomal DNA (ITS2). The immature forms were collected using dippers in two distinct environments; in a pool of ground water and along a lake margin, both shaded. All the specimens were deposited as adult vouchers in the Entomologic Reference Collection of Faculdade de Saúde Pública, São Paulo University, Brazil. This is the first record of the species in the City of São Paulo (S 23° 25'068, W 46° 47'320).

According to Wilkerson and Peyton¹², species of An. (*Anopheles*) Series *Arribalzagia* exhibits three unique



Fig. 1: Anopheles (Anopheles) fluminensis— (a) Wing with sector pale (I), subcostal area (II), main pale (III); (b) Light scales on upper mesanepimeron; (c) Absence of light scale rows in the sternum I; (d) Hind tarsomere-4 (Ta-4) with three light spots; and (e) Aedeagus with about five pairs of apical leaflets.

areas along the wing costal region classified as: sector pale (I); subcostal area (II); and pre-apical pale (III) (Fig. 1a) and has no accessory pale spot sector as in *Cellia*, *Lophopodomyia*, *Kerteszia* and *Nyssorhynchus* subgenera. These patches are of importance for the taxonomy of the genus *Anopheles*. In accordance with the identification keys of Forattini⁴, *An. fluminensis* can be distinguished from other morphologically similar species by having light scales on upper mesanepimeron (Fig. 1b), absence of light scale rows in the sternum I (Fig. 1c), hind tarsomere-4 (Ta-4) with three light spots (Fig. 1d), and aedeagus with about five pairs of apical leaflets (with thin margins) (Fig. 1e). The specimens collected in Anhanguera Park have these characters which allowed for its identification.

Regarding vector competence, Hayes *et al*¹³ found *An. fluminensis* salivary glands infected by sporozoite forms, being the third highest infection rate of species in the region of Junin, Peru. Forattini⁴ provides that *An. fluminensis* can act as a vector in some cases, depending on local conditions, and the coexistence of other species which are incriminated as main vectors. Neves *et al*⁶, in a study performed near the municipality of Itanhaém, São Paulo, Brazil, showed *An. fluminensis* specimens infected naturally by *P. malariae*.

The discovery of *An. fluminensis* in the City of São Paulo is important because it demonstrates some adaptation to the most urbanized and anthropogenic environments, which can influence changes in the epidemiological profile of malaria in the region, but the role of this species as a secondary vector remains unknown. The transmission of malaria in the region extra-amazonica is also a public health problem. The fauna of anophelines in the municipality of São Paulo includes species that can secondarily participate in the transmission of the disease in the city. It should be noted that the results of these studies are important to help those responsible for epidemiological surveillance.

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