

## FIRST RECORD OF *Anopheles (Anopheles) costai* FONSECA & RAMOS, 1939 IN ESPÍRITO SANTO STATE, BRAZIL

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### SUMMARY

Field collections of immatures and adults of Culicidae were carried out in the Rio Doce Valley, Espírito Santo State, aiming to increase knowledge on the anopheline species of that region. Considering all Culicidae species collected, among other anopheline it is noteworthy the presence of *Anopheles darlingi*, *An. oswaldoi* and *An. costai*. Regarding to *An. darlingi*, responsible for malaria outbreak in the low lands in that state, it was expected to confirm its presence in the area. The register of specimens of *An. costai* in the field collections is relevant, increasing the geographical distribution of the species and representing better knowledge of the Series Arribalzagia of the subgenus *Anopheles*.

**KEYWORDS:** *Anopheles costai*; Distribution; First register; Espírito Santo; Brazil.

### INTRODUCTION

Human malaria remains one of the major public health problems in Brazil. In the Amazon area, where 95% of all Latin America malaria cases are reported, the primary vector is *An. darlingi* Root, an endophilic and anthropophilic species highly susceptible to infection with *Plasmodium* parasites<sup>8,9</sup>. In localities situated outside the Amazon region, every year cases of malaria originated either from areas situated in the Amazon or other countries, i.e. African and Latin American countries are notified. However, in the dynamics of malaria transmission within the southern Atlantic Forest ecosystem, where *Anopheles (Kerteszia)* mosquitoes are frequent, malaria transmission is associated with two species, i.e. *An. cruzii* and *An. bellator*, both considered primary vectors.

Out of more than 500 species of *Anopheles* in the world approximately 100 occur in the Neotropical Region<sup>12</sup>. Of those found in Latin America, 29 have been indicated as potential human malaria vectors<sup>10</sup>. While in the Amazon area the primary vector is *An. darlingi*<sup>5,16</sup> in Mexico and Central America *An. (Anopheles) pseudopunctipennis* and *An. albimanus*<sup>14</sup> are major vectors. However, these are not the only malaria vectors and research based on radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) has identified other anopheline species playing potentially important roles in malaria transmission in Latin America<sup>1-4,8,9,11,16,18</sup>.

In the published literature data, there are fifteen *Nyssorhynchus* species that have been registered as potential vectors of human

*Plasmodium*<sup>10</sup>. However, these data should be analyzed with caution because the literature may include false-positive data for mosquitoes that have just or recently fed on human blood infected with malaria parasites, mainly because infections have been detected by direct microscopic observation, immunological and molecular approaches. Epidemiological studies designed to incriminate malaria vector species are rare or incomplete. In Brazil, where several species of the genus *Anopheles* are registered, it is plausible to hypothesize that in any area, where both a competent vector species in high density and infected humans are present, it will be possible to have active *Plasmodium* transmission and consequently, autochthonous cases of malaria.

The subgenera *Nyssorhynchus* and *Kerteszia* include several species that have been demonstrated to be competent to transmit *Plasmodium*. Consequently, species of these two subgenera have been studied in several biological aspects, including taxonomy, systematic, ecology and control. The subgenus *Anopheles* contains several species that occur in Brazil, however it is poorly known and species identification is usually problematic. Consequently, a proper knowledge of the genus *Anopheles* in ecological and systematic aspects, including phylogenetic relationships among the species is relevant. Within these circumstances, to design an effective malaria control program, it is fundamental to know potential vector species that occur in the area where the program will be implemented.

It is well known that a fundamental step towards the recognition of the target species is the accurate identification of field captured specimens. As a natural consequence of epidemiological studies on

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malaria conducted in the hilly area of the Espírito Santo State, where *An. cruzii* of the subgenus *Kerteszia* has been registered and autochthonous cases of malaria<sup>13</sup>, field collection of immatures have been carried out aiming to obtain mosquito samples with all stages associated. Collection of immatures were carried out in both hilly areas and in low lands in the Rio Doce Valley. As a result, we are reporting for the first time the occurrence of *An. costai* in Espírito Santo State. This report enlarge the geographical distribution range of the taxon and contributes for a better knowledge of the subgenus *Anopheles* in Brazil.

## MATERIALS AND METHODS

Immatures and adults of Culicidae were collected in January 2006 in localities situated in the Rio Doce Valley, Espírito Santo State, in the following municipalities and respective localities: i) Linhares, Lagoa Juparanã (19° 18.311' S, 40° 05.84' W) and Chapadão do XV (19° 20.917' S 40° 07.103' W); ii) Sooretama (19° 02.674' S 40° 03.098' W), Joerama-A, Córrego Cupido (19° 02.753' S 40° 00.682' W) and Pequi Farm (19° 04.984' S 40° 06.880' W); iii) Jaguaré, Barra Seca (18° 59.693' S 40° 00.129' W) and Lagoa do Macuco, Marianelli Farm (19° 02.089' S 39° 56.909' W).

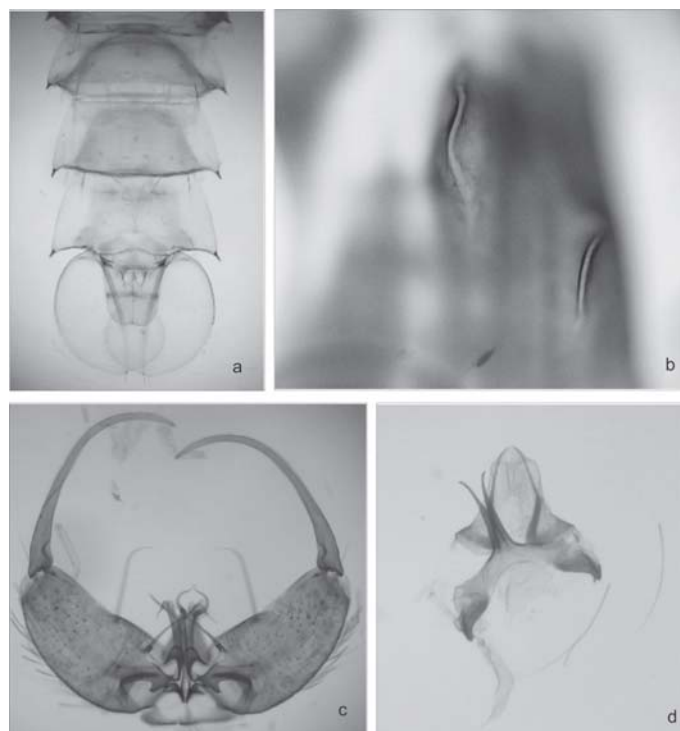
Immatures were taken from breeding habitats such as ground ponds and swamps by using regular entomological collecting pans. Fourth-instar larvae were individually separated in small plastic vials using the same water of the breeding habitats. Larvae were kept separate until the emergence of the correspondent pupae and adults. After the emergence of the adults, they were kept alive for 24 hours before being killed with ethyl acetate. Species identifications were based on all stages, including male genitalia.

Adults were captured using Shannon trap. Adult collections were carried out in the extra domiciliary environment, in both open areas and inside remaining forest located nearby the edges of the Sooretama Forest Reserve. Adults were kept alive in small glass vials, where they were blood fed. They were transported to the laboratory inside boxes and kept alive for about 48 hours, when they were induced to oviposition by cutting one of the wings.

## RESULTS

Culicidae species which were collected during the field work carried out in areas of the Rio Doce Valley are listed in Table 1. It is worth to

note the presence of *An. darlingi*, the major vector of *Plasmodium* in Brazil. Samples of this species were captured in both a community farm area designated Assentamento Joerama A and in the Pequi Farm, both in Sooretama municipality. Additionally, topotypes of *An. oswaldoi* were collected inside a forest fragment situated in Marianelli Farm situated in the area of Lagoa do Macuco, in Jaguaré municipality. The Rio Doce Valley area represents the type-locality of *An. oswaldoi*. Two adult females of *An. oswaldoi* were captured in Shannon trap using both human and light attraction. Among the anopheline specimens collected in Lagoa do Macuco, Jaguaré and in Joerama A, Sooretama municipality, a few samples of *An. (Anopheles) costai* were found. Figure 1 shows the major morphological characters employed in the recognition of *An. costai*.



**Fig. 1** - Morphological characters that are important for identification of *Anopheles costai*. The microphotos were taken from an adult male specimen collected in Marianelli Farm, Jaguaré municipality, Espírito Santo State, Brazil. a: Terminal segments (apex of V-IX, paddle) of the abdomen of a male specimen; b: Wing case of a pupa, showing two protuberances; c: Male genitalia, general aspect; d: Ninth tergal lobe of the male genitalia and prototiger.

**Table 1**

Presence of species of the genus *Anopheles* collected and their respective collection localities in Espírito Santo State, Brazil, in January 2006

Species	Localities					
	Lagoa do Macuco	Joerama A	Lagoa Juparanã	Chapadão do XV	Fazenda Pequi	Fazenda Marianelli
<i>An. oswaldoi</i>	X					
<i>An. triannulatus</i>	X	X			X	
<i>An. albitarsis</i> s.l.	X	X	X	X	X	
<i>An. minor</i>	X	X				X
<i>An. darlingi</i>		X			X	X
<i>An. kimpilcarorii</i>						X
<i>An. costai</i>	X	X				

In addition to the Anophelinae species listed in Table 1, the following Culicidae were collected: *Aedeomyia (Aedeomyia) squamipennis*; *Culex (Aedinus) accelerans*; *Culex (Culex) coronator*; *Culex (Melanoconion) ensiformis*; *Culex (Melanoconion) idottus*; *Culex (Melanoconion) spp.*; *Uranotaenia (Uranotaenia) geometrica* and *Uranotaenia (Uranotaenia) natalie*.

All the specimens obtained in the field collections were deposited in Reference Collections of Faculdade de Saúde Pública da Universidade de São Paulo.

## DISCUSSION

According to data collected by the Vigilância Ambiental do Sistema Único de Saúde, during the period from 1985 until 2006 4,870 autochthonous and imported cases of malaria were registered in Espírito Santo State. The majority of cases were reported in municipalities situated in the hilly area of that state, where *An. cruzii* of the subgenus *Kerteszia* is a potential vector<sup>13</sup> and in the low lands, where species of the subgenus *Nyssorhynchus* and *Anopheles* are more prominent than *Kerteszia* species. Presence of *An. darlingi* in the Rio Doce Valley was expected, because the local vector control personnel had already registered the species and attributed its presence to malaria epidemics in low land areas.

Regarding to specimens of *An. oswaldoi* collected in Marianelli Farm, they represent topotypes specimens that were obtained from eggs oviposited by field collected females. These specimens of *An. oswaldoi* are deposited in the Entomological collection of Faculdade de Saúde Pública/USP (FSP-USP). These topotypes will be important for studies that objective both morphological and molecular characterization of the species.

Additionally, *An. costai* is morphologically similar to other species of the Series Arribalzagia, especially *An. forattinii*<sup>17</sup> and *An. mediopunctatus*<sup>15</sup>. These three species were misidentified as *An. mediopunctatus* for several years, as a consequence of identifications based only on adult female characters. However, field collected specimens associated with immatures and adult females and males carried out in the Ribeira Valley, southern of Atlantic Forest and in Brazilian Amazon areas made possible to achieve an accurate separation of the three species. *Anopheles costai* was in the synonymy of *An. mediopunctatus* for several years. It was described by FONSECA & RAMOS, in 1939<sup>6</sup> based on specimens collected in São Vicente municipality, São Paulo State. Recently, SALLUM *et al.*<sup>15</sup> resurrected *An. costai* from synonymy. The geographical distribution range of *An. costai* is poorly known in Brazil. The taxon has been registered in localities of São Paulo, Rondônia, Bahia, Minas Gerais and Pará States, as well as in other South American countries<sup>15</sup>. The first report of *An. costai* in the Rio Doce Valley increases the distribution range of the taxon in Atlantic Forest and suggest that specimens identified as *An. mediopunctatus* in that state may include samples of *An. costai* that have been misidentified as *An. mediopunctatus*. Consequently, one should be cautious when identifying specimens as *An. mediopunctatus* because similarly to what occur in the Ribeira Valley, São Paulo, *An. costai* and *An. mediopunctatus* may be sympatric in the Rio Doce Valley. This hypothesis should be tested with additional field collections in the Rio Doce Valley and other poorly sampled areas of Espírito Santo State.

Epidemiological importance of *An. costai* is poorly known, however there are evidences that show a possible involvement of the species in the dynamics of malaria transmission in some areas in Brazil. TADEI & DUTARY-THATCHER<sup>16</sup> hypothesized that *An. mediopunctatus* may be participating of malaria transmission in areas of the Amazon Region. Considering that *An. mediopunctatus* seems to occur only in areas of the Atlantic Forest, southern Brazil, it is possible to hypothesize that the specimens identified by TADEI & DUTARY-THATCHER<sup>16</sup> may be of either *An. costai* or *An. forattinii*, which occur in Amazon areas. Furthermore, KLEIN *et al.*<sup>8,9</sup> showed that specimens of *An. costai* that were misidentified as *An. mediopunctatus*, are susceptible to be infected by *Plasmodium falciparum* and *P. vivax*.

In conclusion, although morphological characters are available to identify most of the *Anopheles* species, distinction of closely-related species is problematic, and additional differential traits should be employed, for example those of male genitalia and external morphology of the eggs. Additionally, while research in malaria vectors is based on data provided by single or few field captured mosquitoes, the design of adequate control measures will not be achieved. Consequently, ways to achieve an accurate species identification should be considered by researchers working with Latin American anophelines in order to produce reliable and high-quality results that will provide basis for effective surveillance and control strategies in the countries where malaria occurs.

## RESUMO

### Sobre o encontro de *Anopheles (Anopheles) costai* Fonseca & Ramos, 1939 no Estado do Espírito Santo, Brasil

Empreenderam-se coletas de imaturos e adultos de Culicidae no Vale do Rio Doce, Estado do Espírito Santo, Brasil, com o objetivo de ampliar o conhecimento sobre os anofelíneos dessa região. Do material obtido, além de outros anofelíneos e demais mosquitos, destaca-se a presença de *Anopheles darlingi*, *An. oswaldoi* e *An. costai*. Quanto ao *An. darlingi*, responsável por surtos de malária nas regiões baixas daquele Estado, já se esperava a confirmação de sua presença. Assinala-se como relevante a captura de espécimes de *An. costai*, ampliando-se a distribuição geográfica da espécie, bem como, trazendo contribuição para o conhecimento da Serie Arribalzagia do subgênero *Anopheles*.

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