

## Anopheline Species Complexes in Brazil. Current Knowledge of Those Related to Malaria Transmission

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*A summary of the problems related to the systematics of primary and secondary Brazilian anophelines vectors of malaria is presented.*

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Many neotropical anopheline species are either candidates or formed by complex of cryptic species. The taxonomic elucidation of these complexes reflects on the epidemiology of malaria transmission and ultimately to the control.

In Brazil, there are 54 species belonging to five subgenera of *Anopheles* Meigen (*Nyssorhynchus*, *Kerteszia*, *Stethomyia*, *Lophopodomys*, *Anopheles*). Anopheline species reported as human malaria vectors in the country belong to the subgenera *Nyssorhynchus* and *Kerteszia* (Deane 1986, Consoli & Lourenço-de-Oliveira 1994).

In the subgenus *Nyssorhynchus*, the species found harboring human plasmodia include *Anopheles darlingi* Root 1926, *An. aquasalis* Curry 1932, *An. albitarsis sensu lato* Lynch-Arribáizaga 1878 (including *An. deaneorum* Rosa-Freitas 1989), *An. oswaldoi* Peryassú 1922, *An. nuneztovari* Gabaldon 1940 and *An. triannulatus* (Neiva & Pinto 1922). In the subgenus *Kerteszia* natural infections were reported for *An. cruzii* Dyar & Knab 1908, *An. bellator* Dyar & Knab 1906 and *An. homunculus* Komp 1937. It is our opinion that other species reported naturally infected do not play a role in malaria maintenance as they are exophilic, zoophilic, of low density and their distribution and frequency do not coincide with that of malaria. Except for *An. darlingi*, the natural history of the species listed above points out for zoophilic and/or exophilic behavior in some areas, in such a fashion

that their role in malaria transmission is doubted (Deane 1986). Are these characteristics an indication that these species are indeed complexes?

To decide whether a given species is high polymorphic or a complex of closely related species, integrated approach studies on distinct populations, including on that of the type-localities and where morphological/behavioral/molecular differences have been reported, are mandatory. Most of the Brazilian anopheline species has been taxonomically investigated by morphology, behavior and molecular tools such as isoenzymes and DNA analyses (mitochondrial and ribosomal DNA restriction analysis, random amplification and sequencing of specific regions) as summarized on Table.

More than 99% of the malaria cases reported in Brazil occur in the Amazon in which transmission is due to *Nyssorhynchus* species only.

*An. darlingi* is the most important Brazilian malaria vector (Shannon 1933, Rachou 1958). The species is the most anthropophilic and endophilic among the Amazonian anophelines. It is frequently found infected and its distribution and density are clearly related to malaria transmission. Even though many populations of the species have been lately reported as biting outdoors, *An. darlingi* continues successfully transmitting malaria both indoors and at the close vicinity of the houses (Lourenço-de-Oliveira 1995). Isoenzymatic, behavioral and mitochondrial DNA studies on either Brazilian (Rosa-Freitas et al. 1992, Freitas-Sibajev et al. 1995) or other Latin-American (Manguin et al. 1998) populations, showed that *An. darlingi* is a monotypic species.

*An. aquasalis* is the lowland coastal vector in Brazil. Chromosomal banding pattern and mtDNA

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TABLE  
Summary of differences in behavior, morphology, isoenzyme, mtDNA, rDNA, RAPD and cytogenetic data reported in the literature for populations of neotropical anopheline species related to malaria transmission

Species	Behavior	Morphology	Isoenzyme	mtDNA	rDNA (ITS2)	RAPD	Cytogenetics	References	Conclusion
<i>Anopheles darlingi</i>	<sup>1</sup> (Peak and place of biting)	@ (Except from Belize)	@	@	ND	ND	<sup>1</sup>	Consoli & Lourenço-de-Oliveira 1994 Freitas-Sibajev et al. 1995 Harbach et al. 1993 Kreutzer et al. 1972 Manguin et al. 1998 Rosa-Freitas et al. 1992	Monotypic
<i>An. aquasalis</i>	<sup>1</sup> (Host and place of biting)	<sup>1</sup> (Egg)	@	<sup>1</sup>	@	ND	@	Conn et al. 1993a Cova-Garcia et al. 1977 Flores-Mendoza 1994 Monaca-Perez & Conn 1991	High polymorphic (UI)
<i>An. albitarsis</i>	<sup>1</sup> (Host and place of biting)	@ (Except <i>An. deaneorum</i> )	<sup>1</sup>	<sup>1</sup>	ND	<sup>1</sup>	<sup>1</sup>	Kreutzer et al. 1976 Narang et al. 1993 Rosa-Freitas et al. 1990 Wilkerson et al. 1995	Complex (4 species)
<i>An. oswaldoi</i>	<sup>1</sup> (Host and place of biting)	<sup>1</sup> (Male genitalia)	UI	ND	<sup>1</sup>	ND	ND	Causey et al. 1946 Consoli & Lourenço-de-Oliveira 1994 Flores-Mendoza pers. comm. Klein & Lima 1990 Marrelli et al. 1998	Complex: (at least 2 forms - UI)
<i>An. nuneztovari</i>	<sup>1</sup> (Host and place of biting)	<sup>1</sup> (Egg, male genitalia and female)	<sup>1</sup>	<sup>1</sup>	<sup>1</sup>	ND	<sup>1</sup>	Delgado & Rubio-Palis 1992 Fritz et al. 1994 Hribar 1994, 1995 Linley et al. 1996	Possibly a complex
<i>An. triannulatus</i>	@	<sup>1</sup> (Male genitalia, larva and egg)	<sup>1</sup>	<sup>1</sup>	ND	<sup>1</sup>	ND	Silva-do-Nascimento 1995 Silva-do-Nascimento pers. com.	Complex: (at least 2 species - UI)
<i>An. cruzii</i>	<sup>1</sup> (Acrodendrophily)	<sup>1</sup> (Larva)	ND	ND	<sup>1</sup>	ND	<sup>1</sup>	Deane et al. 1971 Malafrente et al. 1997 Ramirez et al. 1989 Zavortink 1973	High polymorphic (UI)

ND: non determined; UI: under investigation.

restriction profiles of specimens from Venezuela and Brazil were identical (Moncada-Pérez & Conn 1991, Conn et al. 1993a). Isoenzymes from three populations of Venezuela and Surinam (Steiner et al. 1981) and two from Brazil (Flores-Mendoza 1994) with behavioral differences also revealed only intraspecific variation. Egg morphology of *An. aquasalis* varies intraspecifically (Maldonado et al. 1997). In fact, variation was seen in a single female oviposition (Flores-Mendoza 1994). Results of mitochondrial DNA and egg morphology analyses however, suggest that there might be an interspecific division in *An. aquasalis* populations north and south of the Amazon River delta (Conn et al. 1993a, Linley et al. 1993).

*An. albitarsis* is a complex formed by, at least, four sibling species: *An. marajoara* Galvão & Damasceno 1942, *An. albitarsis sensu stricto* Lynch-Arribáizaga 1878, *An. deaneorum* and a fourth form to be formally described (Rosa-Freitas et al. 1990, Wilkerson et al. 1995). Since *An. deaneorum* is the only morphologically distinguishable member of the albitarsis complex, the role of each member in malaria transmission has not been determined yet. The incrimination of other members of the complex, except *An. deaneorum* (Klein et al. 1991a, b), were based solely on their presumed geographical distribution.

*An. oswaldoi* has been regarded as a potential malaria vector in some localities of the Amazon (Arruda et al. 1986, Oliveira-Ferreira et al. 1990, Branquinho et al. 1996) although some authors believe that most populations of this species are much more related to the natural environment and prefer to feed on animals than on man indoors (Deane et al. 1948, Consoli & Lourenço-de-Oliveira 1994, Lourenço-de-Oliveira & Luz 1996). The taxonomic status of the species is under investigation (PhD work of CFM). Preliminary results from morphological analyses demonstrate that at least two forms are present under *An. oswaldoi*: *An. oswaldoi sensu stricto* and *An. konderi* Galvão & Damasceno 1942, distinguished practically only by the shape of the apex of aedeagus (Causey et al. 1946, Lounibos et al. 1997).

Morphology, behavior, cytogenetics, isoenzymes and mtDNA studies favor the existence of at least two cryptic species in *An. nuneztovari*: one in Venezuela and Colombia northwest of Orinoco and another in the Amazon (Conn et al. 1993b, Fritz et al. 1994, Linley et al. 1996). The species is considered a primary malaria vector in Venezuela and Colombia (Gabaldon 1969, Gabaldon et al. 1975). In Brazil however, the species is not related to malaria transmission, although natural infection by *Plasmodium vivax* has been detected in areas where *darlingi* was the primary

vector (Arruda et al. 1986).

*An. triannulatus* is constituted by at least three sibling forms. These forms can be differentiated morphologically (egg, larva and male genitalia) and isoenzymatically (Silva-do-Nascimento 1995). The typical *triannulatus* is the most known and largely distributed form. The other two forms seem to be restricted mostly to central Brazil and are not related to malaria transmission.

The mosquitoes of the subgenus *Kerteszia* share the common characteristic of using bromeliads as breeding places. An exception is *An. (Ker.) bambusicolus* Komp 1937 that also breeds in bamboo.

*An. (Kerteszia) cruzii* and *An. bellator* were primary vectors of the malaria once endemic in southeastern/southern Brazil (Rachou 1958). *An. cruzii* is currently involved in the maintenance of the oligosymptomatic malaria occurring in the valleys of the Atlantic Coastal Rain Forest in both Rio de Janeiro and São Paulo states (Carvalho et al. 1988, Azevedo 1997, Branquinho et al. 1997). Larval differences were observed in *An. cruzii* populations from Rio de Janeiro and Santa Catarina (Zavortink 1973). Besides, chromosomal banding pattern differences were also found among several *An. cruzii* populations (Ramirez 1989, Dessen pers. comm.). *An. homunculus* is a morphologically close related species and there is the possibility of being a sibling species in the *cruzii* complex (PhD work of CJCP). The remaining *Kerteszia* species do not seem to be important in malaria transmission in Brazil.

In summary, *An. darlingi* is a monotypic species. *An. aquasalis* and *An. nuneztovari* are possibly complexes. *An. albitarsis*, *An. triannulatus* and *An. oswaldoi* are complexes of species. Anopheline species of the subgenus *Kerteszia* are still under investigation (Table).

The refinement of the taxonomic tools and the addition of other populations are likely to lead to new insights into the knowledge and understanding of the neotropical species complexes.

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