Malaria Journal

RESEARCH

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First large-scale assessment of pyrethroid resistance in *Anopheles darlingi* (Diptera: Culicidae) in Brazil (2021-2024): a crucial step in informing decision-making in malaria control

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Abstract

Background Malaria continues to pose a significant public health threat in northern Brazil. Current control strategies for *Anopheles darlingi*, the primary malaria vector in the Amazon region, depend on long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) with pyrethroid insecticides. Despite decades of insecticide use, there are very few records of pyrethroid resistance in this mosquito species in Brazil, likely due to a lack of investigations, underscoring the urgent need for further actions.

Objectives To assess the susceptibility of *An. darlingi* from all malaria-endemic regions in Brazil to the pyrethroids used by the Malaria Prevention and Control Programme (NMCP) for vector control.

Methods Adult females *An. darlingi* were collected from 28 locations in the states of Amapá, Acre, Amazonas, Pará, Rondônia, Roraima, Mato Grosso, Maranhão, and Tocantins. These locations were chosen because of their high malaria incidence in recent years. The collected mosquitoes were sent to the Laboratory of Biology, Control, and Surveillance of Insect Vectors to produce F1 progeny. Discriminating concentration (DC) WHO tube bioassays were performed on deltamethrin (0.05%), etofenprox (0.5%), and permethrin (0.75%). The intensity of resistance was evaluated by comparing the mortality rates of mosquitoes exposed to papers treated with 1 × and 5 × the DC of these insecticides.

Results Of the 19 *An. darlingi* populations evaluated, only four were susceptible to deltamethrin (Tapauá, Jacareacanga, Cantá, and Caracaraí). For etofenprox, 13 populations were resistant, whereas five were susceptible (Tapauá, Porto Velho, Porto Grande, Cantá, and Caracaraí). With respect to permethrin, 18 populations were evaluated, of which 12 were classified as susceptible and 6 as resistant (Coari, Manaus, Barcelos, Guajará, Rodrigues Alves, and Cruzeiro do Sul). Resistance intensity tests indicated that all populations, except Barcelos, presented low resistance to pyrethroids according to the WHO classification.

Conclusions The first large scale detection of pyrethroid resistance in *An. darlingi* in Brazil is concerning and calls for urgent action to prevent its spread in the Amazon region. This study represents a critical step toward establishing comprehensive resistance monitoring and management plans for malaria vectors in Brazil.

Keywords Malaria, *Anopheles darlingi*, Pyrethroid resistance, Amazon, Deltamethrin, Etofenprox, Permethrin

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Background

Malaria remains a significant public health challenge in the northern region of Brazil, where almost all national cases (99.9%) are concentrated. In 2023, 140,267 cases were reported in the country, representing a 9% increase compared with 2022, when 128,969 cases were recorded [1, 2].

Mosquitoes of the genus *Anopheles*, which include over 470 species [3], are the primary vectors of *Plasmodium* parasites that cause malaria in humans [4]. *Anopheles* (*Nyssorhynchus*) *darlingi* plays a preponderant role in transmission of malaria parasites in the Americas, particularly in the Amazon regions of Bolivia, Venezuela, Colombia, and Peru [5–7]. In Brazil, in addition to *An. darlingi*, which has been recorded in all Amazonian states, other species also play important roles in disease transmission across the country's diverse ecosystems [6, 8]. *Anopheles aquasalis*, for instance, acts as a significant vector in coastal areas, whereas species such as *Anopheles cruzii*, *Anopheles bellatrix*, and *Anopheles homunculus* are recognized as vectors within the Atlantic Forest biome [6, 8]. Moreover, several other species have been reported in the country as naturally infected with *Plasmodium vivax* and/or *Plasmodium falciparum*, suggesting their potential role as local vectors. Notably, these include *Anopheles peryassui, Anopheles benarrochi, Anopheles tadei, Anopheles oswaldoi, Anopheles rangeli,* and *Anopheles triannulatus*, among other species whose vectorial capacities are still under investigation [8].

Owing to improvements in diagnostic methods and access to malaria treatment coupled with the large-scale deployment of vector control measures (e.g., long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) with pyrethroid insecticides), Brazil experienced a significant reduction in malaria cases between 2010 and 2016 [1]. Unfortunately, pyrethroid resistance has been documented in *Anopheles* mosquitoes in various countries, raising concerns about the sustainability of these interventions [7–15].

For many years, there have been no significant records of insecticide resistance in malaria vectors in Amazonian countries, except in *Anopheles albimanus,* the species with the highest number of reports of insecticide resistance in Latin America [16–21]. However, resistance has recently been detected in *An. darlingi* in Bolivia, Colombia, Peru, and French Guiana, likely because of increased selective pressure [17, 22–24]. In Brazil, however, despite the epidemiological significance of malaria, data on insecticide susceptibility or resistance in *Anopheles* mosquitoes remain scarce, with only four studies conducted to date [25–28]. This gap can be attributed to the absence of a structured resistance monitoring program, unlike the well-established monitoring programme for *Aedes aegypti* in the country [26].

Of the four existing studies, two were conducted in Mazagão, Amapá [25, 28], both evaluating the susceptibility of *An. darlingi* and *Anopheles marajoara* to pyrethroids. No resistance was detected in *An. darlingi*, but *An. marajoara* showed signs of possible resistance to deltamethrin, which requires attention [25, 28]. The other two studies were conducted in Cruzeiro do Sul, Acre [26, 27]. Resistance was detected in *An. darlingi* to the pyrethroids etofenprox [26, 27], deltamethrin [27], cypermethrin [27], alpha-cypermethrin [27] and lambda-cyhalothrin [27]. In addition to the *An. darlingi* populations from Cruzeiro do Sul, Sucupira (2017) [27] also evaluated *Anopheles* from Vitória do Xingu-PA, where all mosquitoes tested were susceptible to these insecticides [27]. Despite the relevance of these studies, insecticide resistance in *Anopheles* in Brazil needs to be mapped more comprehensively, covering more locations to provide a complete overview of resistance in the country.

In 2022, the Ministry of Health launched the National Malaria Elimination Plan, aiming to eradicate the disease in Brazil by 2035 [1]. This plan sets ambitious goals, including the elimination of malaria caused by *P. falciparum* by 2025 and *P. vivax* by 2030. In terms of vector control, one of the key actions of this plan is the implementation of a monitoring and management programme for *Anopheles* resistance to insecticides, highlighting the importance of entomological surveillance to achieve malaria elimination and understanding vector resistance status to guide appropriate insecticide use [1].

Resistance monitoring is crucial for carefully selecting insecticides and is an essential component of resistance management strategies [29]. A structured program allows for both an initial diagnosis of resistance and, over time, a more detailed understanding of the resistance profiles of local mosquito populations, which is essential for implementing more locally effective control strategies. Considering that resistance can be reversed, strategic management is cost-effective [29].

This study aims to fill an important knowledge gap regarding the resistance of *An. darlingi* to pyrethroid insecticides in Brazil by investigating 19 locations across six states with high malaria incidence rates. By mapping this vector's susceptibility to insecticides, it is expected that the data obtained will contribute to improving vector control strategies, guiding more effective and sustainable interventions. Additionally, this study provides support for the establishment of a continuous insecticide resistance monitoring system for *Anopheles* mosquitoes in the country.

Methods

Mosquito collection

Between 2021 and 2024, adult females of *An. darlingi* were collected from 28 high malaria incidence sites across nine states in the Amazon region of Brazil: Amazonas—Coari, Guajará, Lábrea, Santa Isabel do Rio Negro, Manaus, São Gabriel da Cachoeira, Barcelos, Tapauá, and Tefé; Acre—Cruzeiro do Sul, Mâncio Lima, and Rodrigues Alves; Amapá—, Porto Grande and Calçoene; Pará—Anajás, Bagre, and Jacareacanga; Roraima– Alto Alegre, Pacaraima, Caracaraí and Cantá; and Rondônia—Porto Velho and Candeias do Jamari; Tocantins—Araguatins; Mato Grosso—Aripuanã and Colniza; and Maranhão– Jenipapo dos Vieiras (Fig. 1).

The collections were conducted in collaboration with the Municipal Health Departments through their respective Entomological Surveillance Coordinations or equivalent agencies. Mosquito collections were conducted at night, between 6 and 10 PM, focusing on peridomestic areas close to potential larval habitats. Peridomestic areas were chosen due to the greater abundance of *An. darlingi* in these areas compared to indoor environments, as observed in previous studies and corroborated by data in the literature [25, 30]. The peridomestic areas were defined as the spaces around the houses, from 5 to 10 m away from the residential structures, where there is greater interaction between the inhabitants and the external environment [25].

The human landing catch (HLC) technique was employed with adequate protection measured according to the Ministry of Health protocols [25], hence adhering to the approved research ethics committee (CAAE: 45,663,232.2.1001.0001) to ensure the safety of field technicians and minimize the risk of malaria transmission [31].

Identification and maintenance of mosquitoes in the laboratory

The mosquitoes were morphologically identified in the field using the dichotomous key of Consoli and Oliveira [32], this method was applied by experienced professionals trained in the identification of *Anopheles* mosquitoes, ensuring the accuracy and reliability of the results. Females identified as *An. darlingi* were feed with a sugar solution of 10% (p/v) and subsequently transported to the Laboratory of Biology, Control, and Surveillance of Insect Vectors (LBCVIV-FIOCRUZ) in Rio de Janeiro, RJ. Once in the laboratory, they were artificially fed (Hemotek®) with citrated rabbit blood, following an ethical license (CEUA LW-27/21), to establish the F1 generation.

Bioassays for resistance evaluation

Insecticide resistance assessments were conducted using the F1 generation through discriminating concentration (DC) bioassays following the World Health Organization (WHO) tube assay method with insecticide-impregnated papers [33]. The insecticides tested included deltamethrin (type II pyrethroid), etofenprox (non-ester pyrethroid), and permethrin (type I pyrethroid).

As there was no specific DC established for *An. darlingi*, we adopted the concentrations recommended for anophelines by the WHO in their 2022 guidelines: 0.05% for deltamethrin, 0.5% for etofenprox, and 0.75% for permethrin [33]. For each insecticide, 4 exposure tubes and 2 control tubes were used, with each tube containing 20 to 25 females, totaling between 80 and 100 females exposed to the insecticide per assay. Knockdown was recorded 1 h after exposure, and mortality was assessed 24 h after the start of the experiment [33]. To ensure the reproducibility of the results, at least two tests were conducted on different days for each insecticide. More detailed information on the number of females used for each insecticide and population can be found in the Table 1.

To serve as a reference for susceptibility, bioassays were conducted using the laboratory-maintained strain of *An. darlingi* from the LBCVIV colony, which has been maintained for three years under controlled conditions. This strain does not exhibit a known resistance mechanism and was used as a negative control (expected 100% mortality in bioassays).

(See figure on next page.)

Fig. 1 Geographic distribution of *Anopheles darlingi* collection sites in Brazil. **A** The map highlights the nine Brazilian states that compose the Amazon region (Amazonas, Acre, Rondônia, Mato Grosso, Maranhão, Pará, Tocantins, Amapá, Roraima), represented by distinct colors as detailed in the legend. Specific collection sites located in these states are indicated by abbreviations corresponding to the municipalities where *Anopheles darlingi* females were collected between 2021 and 2024: São Gabriel da Cachoeira (SGC), Santa Isabel do Rio Negro (SIR), Barcelos (BAR), Manaus (MN), Coari (CO), Tefé (TF), Tapauá (TP), Lábrea (LB), Guajará (GJ), Mâncio Lima (ML), Rodrigues Alves (RA), Cruzeiro do Sul (CZS), Porto Velho (PV), Candeias do Jamari (CJ), Colniza (COL), Aripuanã (ARI), Jacareacanga (JAC), Bagre (BG), Anajás (AN), Porto Grande (PG), Calçoene (CAL), Oiapoque (OIA), Araguatins (ARG), Jenipapo dos Vieiras (JPV), Cantá (CT), Caracaraí (CAR), Alto alegre (AA) and Pacaraima (PAC). **B** The second map provides additional context by illustrating the geographic distribution of the Amazon region within South America

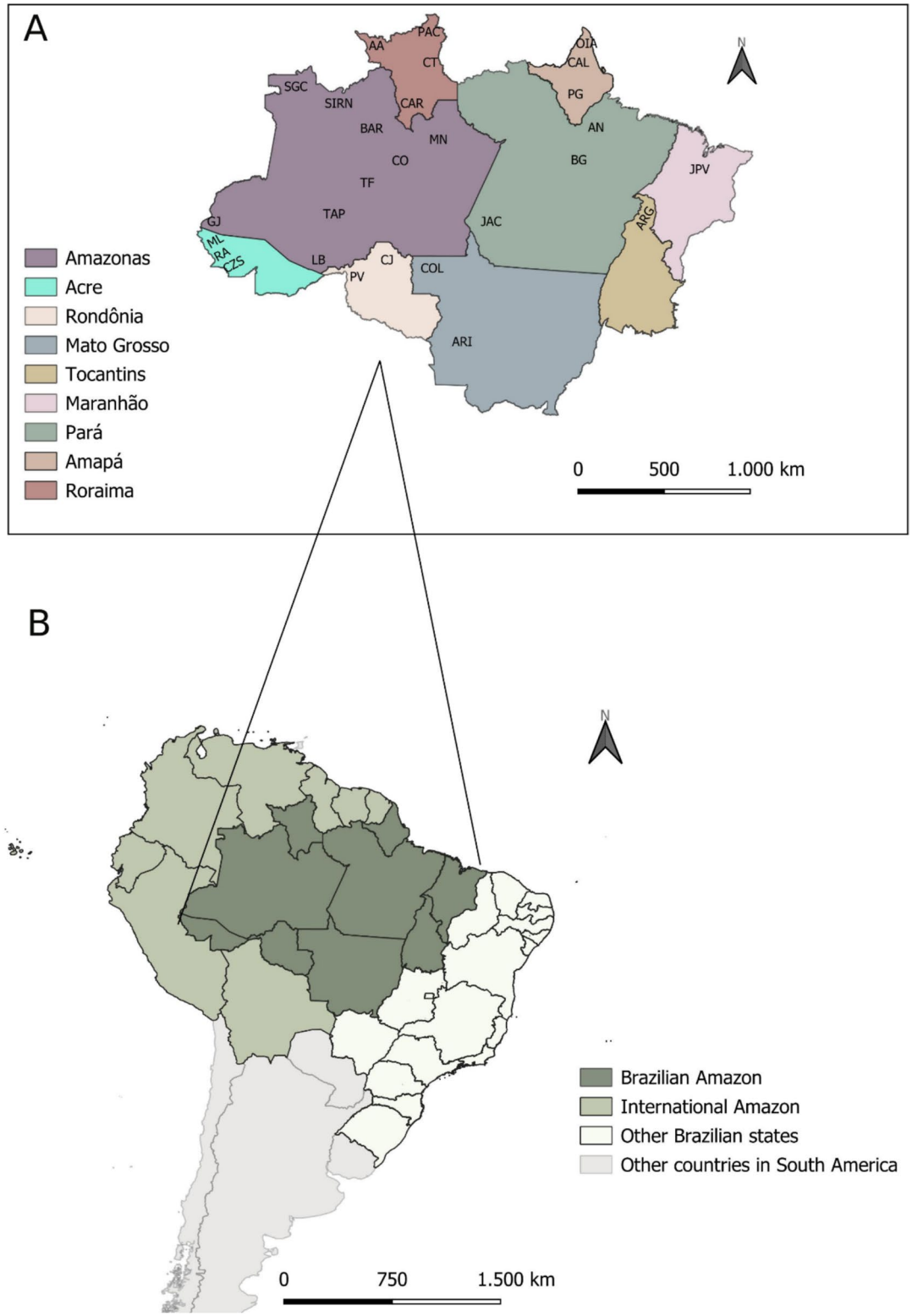

Fig. 1 (See legend on previous page.)

Table 1 General data of the populations used in the study

State	Population	Date of collection	N° of females collected	N° of adults tested from F1	Bioassay (N° of females used)
Acre	CZS	Apr/2021	393	2585	Deltamethrin (468)
	CZS	May/2021	211	2161	Etofenprox (450) and Permethrin (450)
	CZS	Aug/2022	300	2000	5 × CD all insecticides (300 females by insecticide)
	RA	May/2021	26	333	Etofenprox (150)
	RA	Aug/2022	150	401	Deltamethrin (240)
	RA	Jul/2024	135	2485	Permethrin (390), Etofenprox (450) and 5 × CD deltamethrin (240)
	ML	May/2021	26	670	Etofenprox (370)
	ML	Aug/2024	120	1600	Deltamethrin (300) and Permethrin (450)
Amapá	OIA*	Dec/2021	0	0	–
	PG	Jul/2021	300	2671	Deltamethrin (450), Etofenprox (480), Permethrin (390)
	CAL*	Oct/2023	53	100	–
Amazonas	SGC	Aug/2022	100	549	Permethrin (286)
	SGC	May/2023	145	1337	Deltamethrin (360), Etofenprox (360)
	CO	Aug/2021	200	2500	Deltamethrin (361), Etofenprox (281), Permethrin (365)
	CO	Apr/2023	100	1191	5 × CD Deltamethrin (240), Etofenprox (150), Permethrin (120)
	GJ	Apr/2022	172	192	Etofenprox (100)
	GJ	Jul/2024	133	2000	Deltamethrin (360), Etofenprox (320), Permethrin (365)
	LB	Jun/2022	160	2385	Deltamethrin (480), Etofenprox (538), Permethrin (297)
	SIRN*	Mar/2023	8	39	–
	TAP	May/2024	600	5800	Deltamethrin (450), Etofenprox (458), Permethrin (452)
	MN	Jun/2023	159	2500	Deltamethrin (450), Etofenprox (450), Permethrin (459)
	BAR	Aug/2023	280	3000	Deltamethrin (480), Etofenprox (450), Permethrin (540)
	TF	Aug/2023	317	4000	Deltamethrin (541), Etofenprox (539), Permethrin (450)
Rondônia	PV	Apr/2022	360	3300	Deltamethrin (871), Etofenprox (356), Permethrin (425)
	CJ	Apr/2022	300	5711	Deltamethrin (731), Etofenprox (725), Permethrin (421)
Pará	AN	Aug/2022	400	6036	Deltamethrin (703), Etofenprox (735), Permethrin (416)
	BG	Sep/2022	390	3074	Deltamethrin (576), Etofenprox (581), Permethrin (300)
	JAC	May/2022	157	887	Deltamethrin (418)
	PAC*	Mar/2022	20	153	–
Roraima	AA *	Mar/2022	0	0	–
	CAR	Jun/2024	220	1260	Deltamethrin (240), Etofenprox (240), Permethrin (240)
	CT	Jun/2024	146	1227	Deltamethrin (240), Etofenprox (241), Permethrin (240)
Tocantins	ARG*	May/2022	0	0	–
Mato Grosso	ARI*	Apr/2022	8	0	–
	COL*	Apr/2022	8	0	–
Maranhão	JPV *	Jun/2022	8	0	–

* No bioassays conducted due to the insufficient number of F1 females available

Assessment of resistance intensity

To evaluate the resistance intensity, additional bioassays were conducted using concentrations at five times the standard discriminating concentrations (5XDC) of the respective insecticide: 0.25% for deltamethrin, 2.5% for etofenprox, and 3.75% for permethrin.

Data analysis

Resistance levels were determined according to WHO guidelines [30]. Confirmed resistance occurs when mortality (corrected, if necessary) is less than 90%; possible resistance is indicated by mortality (corrected, if necessary) between 90 and 98%; and susceptibility is indicated by mortality (corrected, if necessary) equal to or greater than 98%, classifying the vector population as susceptible to the insecticide [25]. For each bioassay,

the percentage of mortality was calculated, and the average mortality rate, along with standard deviations, was derived from the replicates conducted on different days. Microsoft Excel for Microsoft 365 MSO (version 2310 Build 16.0.16924.20054) 64-bit was used for data analysis, while graphics were plotted using GraphPad Prism (version 6.01).

Results

Mosquito collection

Of the collections conducted in 28 municipalities, only 19 yielded enough *An. darlingi* females to generate a new generation and conduct subsequent bioassays. No bioassays were conducted with the populations from the states of Mato Grosso, Maranhão, and Tocantins, as shown in Table 1.

Of the 19 populations assessed, only four were susceptible to deltamethrin: Tapauá (Amazonas), Jacareacanga (Pará), and Cantá and Caracaraí (Roraima). With respect to etofenprox, five populations were susceptible: Tapauá (Amazonas), Porto Velho (Rondônia), Porto Grande (Amapá), and Cantá and Caracaraí (Roraima). A larger number of populations were susceptible to permethrin: 11 out of the 18 populations tested. The seven populations resistant to this insecticide were Coari, Manaus, Barcelos, Guajará (Amazonas), Cruzeiro do Sul, Mâncio Lima, and Rodrigues Alves (Acre). The remaining populations were susceptible to permethrin (Fig. 2). The reference laboratory strain of *An. darlingi* (LBCVIV) also shows full susceptibility (100%) to all 3 insecticides, hence validating the tests using WHO DCs.

Figure 3 provides an overview of the status of pyrethroid resistance in *An. darlingi* populations across the Brazilian Amazon. Notably, populations from the states of Amazonas and Acre are prominent, as the majority of those assessed in these states exhibited resistance to all three insecticides.

We evaluated the intensity of resistance in 13 populations classified as resistant to deltamethrin, 12 populations resistant to etofenprox, and 7 populations resistant to permethrin. It was not possible to assess the intensity of resistance to the three pyrethroids in the populations from Manaus and Mâncio Lima due to the limited number of F1 females available. In total, 5640 mosquitoes were exposed to 5xDC. The results of the bioassays revealed that resistance to the three pyrethroids was classified as low in the populations from São Gabriel da Cachoeira, Tefé, Coari, Lábrea, Guajará, Rodrigues Alves, Cruzeiro do Sul, Porto Velho, Candeias do Jamari, Porto Grande, Anajás, and Bagre, as their mortality rates were $\geq$98% at 5xDC. Conversely, the mortality rates of mosquitoes from Barcelos were <98% for deltamethrin (86%) and etofenprox (91%). Since 10xDC was not evaluated due to the insufficient number of insects for this additional test, the resistance level of *An. darlingi* population from Barcelos could only be classified as moderate (Table 2).

Discussion

The first large-scale survey of pyrethroid resistance in *An. darlingi* was conducted in 9 states of the Brazilian Amazon region. The 28 municipalities selected for this study were among the areas with the highest malaria endemicity in Brazil in recent Years [1], where two main strategies were employed for vector control: long-lasting insecticidal (LLINs) and indoor residual spraying (IRS). Both methods use pyrethroids due to their low toxicity to mammals and prolonged residual efficacy [34].

The effectiveness of these strategies has been proven in other endemic regions, such as Africa, where the distribution of insecticide-treated nets resulted in a significant decrease in malaria incidence between 2000 and 2015 [35]. Following this success, LLINs were introduced in Brazil in 2007, with intensified distribution between 2010 and 2015, prioritizing vulnerable communities. Since then, nets treated mainly with permethrin, alpha-cypermethrin, and deltamethrin, all pyrethroids, have been used in the country [36].

In parallel, IRS has been conducted quarterly. Until the 1990 s, IRS in Brazil was conducted using DDT [37, 38]. With the ban on DDT due to environmental and public health concerns, the National Malaria Control Programme (NMCP) adopted the use of pyrethroids as substitutes [38, 39]. Initially, a wettable powder formulation of cypermethrin was used for this purpose. From 2003 to 2014, alpha-cypermethrin was the main insecticide employed in IRS [34, 37]. However, in response to the need for insecticide diversification, the NMCP replaced alpha-cypermethrin with Etofenprox, a non-ester pyrethroid [34].

While resistance to pyrethroids in anopheline is well documented in other countries with malaria case reports [8–24], data from Brazil remain scarce. Most existing records come from isolated research projects [25–28], highlighting the absence of a national resistance monitoring program. This study fills this gap, revealing resistance to deltamethrin in 15 of the 19 *An. darlingi* populations evaluated. These results are not surprising, considering the widespread use of deltamethrin not only against *Anopheles* but also in national campaigns to control *Ae. aegypti,* where resistance has been well documented [40–50]. Resistance to deltamethrin in anophelines has already been documented in other countries in Central and South America, Africa, and Asia [16, 22–24], including recent reports of reduced susceptibility in *An.*

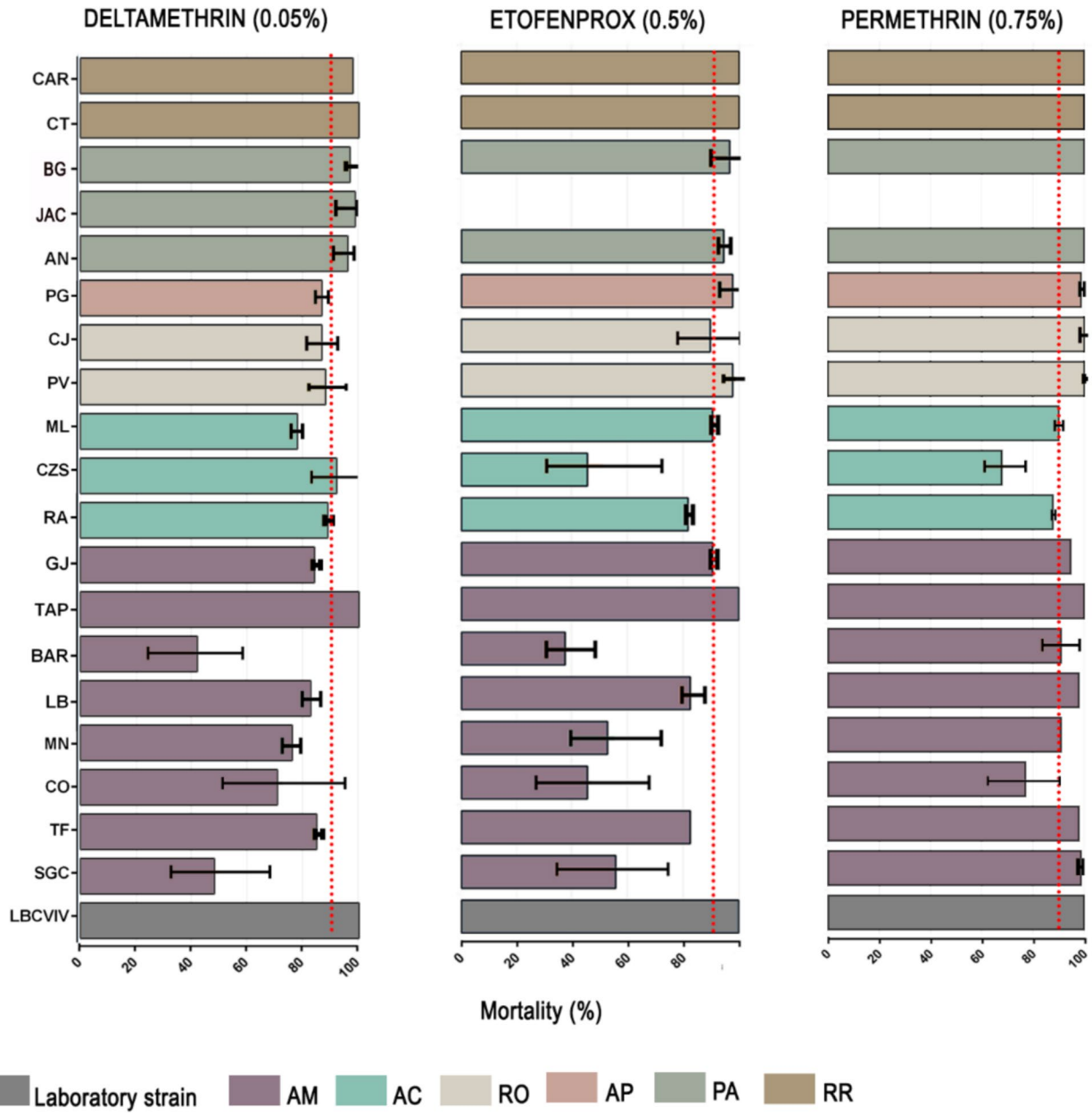

Fig. 2 Bioassays for insecticide resistance to pyrethroids with *Anopheles darlingi* from the Brazilian Amazonian (2021–2024). Mortality percentage of female mosquitoes 24 h after exposure to the discriminating concentrations of the insecticides deltamethrin, etofenprox, and permethrin in WHO bioassays with impregnated paper. Mortality below 90% indicates resistance (dotted line). Populations are identified with the following abbreviations: SGC (São Gabriel da Cachoeira), BAR (Barcelos), MN (Manaus), TF (Tefé), CO (Coari), TAP (Tapauá), LB (Lábrea), ML (Mâncio Lima), RA (Rodrigues Alves), CZS (Cruzeiro do Sul), PV (Porto Velho), CJ (Candeias do Jamari), JAC (Jacareacanga), BG (Bagre), AN (Anajás), and PG (Porto Grande). Populations sharing the same color belong to the same state

darlingi on the French Guiana-Brazil border [51]. In addition to deltamethrin, our study also evaluated resistance to etofenprox and permethrin. Bioassays revealed resistance to etofenprox in 13 of the 18 populations tested, aligning with limited studies conducted in Brazil and other Latin American countries [26, 27].

Regarding permethrin, resistance was observed in six of the 18 populations evaluated, providing the first documentation of this phenomenon in *An. darlingi* in Brazil.

The complexity of the pyrethroid resistance landscape in the Brazilian Amazon region is evident, highlighting

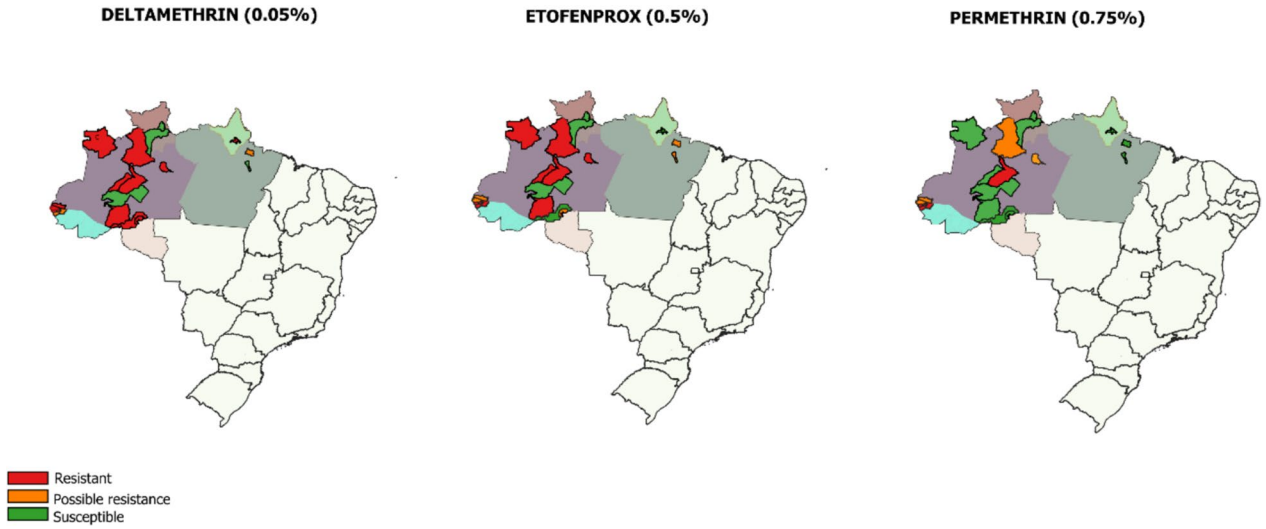

Fig. 3 Overview of pyrethroid resistance in *Anopheles darlingi* from the Brazilian Amazonian region. The map of Brazil illustrates the results of insecticide resistance bioassays for deltamethrin, etofenprox, and permethrin in *Anopheles darlingi* collected from various localities in the Amazon region between 2021 and 2024. Red indicates resistant populations, orange indicates populations with possible resistance, and green denotes susceptible populations

the urgent need for a national monitoring programme and new vector control strategies. The low intensity of resistance observed in most populations suggests that alternating different classes of insecticides may be an effective strategy, except in places like Barcelos, where

Table 2 Mortality rates of *Anopheles darlingi* exposed to the discriminating concentration and five times the DC

	Mortality (%)					
	Deltamethrin		Etofenprox		Permethrin	
	DC	5XDC	DC	5XDC	DC	5XDC
Municipality	**(0.05%)**	**(0.25%)**	**(0.5%)**	**(2.5%)**	**(0.75%)**	**(3.75%)**
SGC—AM	48	99	56	99	**99**	–
TF—AM	85	100	83	100	**98**	–
CO—AM	71	98	46	100	77	100
MN—AM	76	–	53	–	91	–
LB—AM	83	99	83	98	**98**	–
BAR—AM	42	86	38	91	91	99
TAP—AM	**100**	–	**100**	–	**100**	–
GJ—AM	84	100	91	100	95	100
RA—AC	89	98	82	100	88	100
CZS—AC	92	100	46	100	68	100
ML—AC	78	–	91	100	90	100
PV—RO	88	100	**98**	–	**99.6**	–
CJ—RO	87	99	90	100	**100**	–
PG—AP	87	100	**98**	–	**99**	–
ANJ—PA	96	100	95	100	**100**	–
JAC—PA	**99**	–	–	–	–	–
BG—PA	97	100	97	100	**100**	–
CT—RR	**100**	–	**100**	–	**100**	–
CAR—RR	**98**	–	**100**	–	**100**	–

Bold values indicate susceptible populations (i.e. mortality ≥ 98% at DC). Rates of ≤ 98% at 5XDC indicate at least a moderate level of resistance

resistance is more pronounced. However, it is necessary to understand the resistance mechanisms involved.

To better understand the molecular mechanisms underlying resistance, samples of surviving and dead mosquitoes were cryopreserved for future analyses. This step is crucial, as there is a significant knowledge gap regarding these mechanisms in *An. darlingi* populations, especially when compared to extensive studies conducted on anophelines in Africa and Asia [13, 52–66].

The mechanisms of pyrethroid resistance in anophelines include modifications in voltage-gated sodium channels (*kdr* mutations) and increased metabolism by specific enzymes [53–58, 60–64]. Although several *kdr* mutations have been identified in *Anopheles* species worldwide, there is currently no evidence of these mutations in *An. darlingi* [60]. Studies in Colombia and French Guiana suggest that metabolic resistance may be the predominant mechanism in the region [51, 65–67].

Although biochemical assays to determine the metabolic mechanisms involved in resistance were not conducted in this study, they represent a crucial step for a comprehensive understanding of resistance dynamics [68]. These analyses are essential for identifying the enzymatic detoxification pathways associated with insecticide resistance and will be fundamental for refining resistance management strategies in the future [69]. There is a significant knowledge gap regarding these mechanisms in *An. darlingi* populations.

This pioneering study establishes a solid foundation for understanding insecticide resistance in *An. darlingi* in Brazil. Despite logistical and structural challenges, it was demonstrated that implementing a monitoring programme is feasible and necessary. Training health professionals across all states in the Amazon region and developing an efficient logistical system for the collection and transport of anophelines are important steps to ensure the continuity of monitoring.

The evidence of widely distributed pyrethroid resistance in *An. darlingi* in the Amazon region demands urgent action. Establishing periodic monitoring of insecticide resistance at predefined locations, using appropriate biological tools, could help adjust surveillance and vector control actions. A model to consider is the integrated insecticide resistance surveillance plan for mosquito vectors developed in France [70], which could provide a coordinated approach to address the growing problem of mosquito resistance in the Amazon region. Furthermore, the establishment of the South American Research Network for the Surveillance and Control of Insecticide Resistance in Arthropod Vectors (WINSA), created by IRD and FIOCRUZ with support from the US-CDC VecNet initiative and WHO-TDR, presents an excellent opportunity to coordinate research on insecticide resistance in mosquitoes across the region and to serves as a platform for regional collaboration and the development of effective mitigation strategies (Corbel et al., pers. commun.).

In summary, this comprehensive study not only reveals the current state of pyrethroid resistance in *An. darlingi* in the Brazilian Amazon but also lays the groundwork for future research and control actions. The implementation of a national resistance monitoring program, along with the development of new vector control strategies, will be crucial for the continued success of malaria control efforts in the region. It is also important to note that other *Anopheles* species in the region, which are involved in malaria transmission, should be monitored for insecticide resistance as well. Monitoring these species is essential to ensure comprehensive vector control and to address potential resistance issues across all malaria vectors in the Brazilian Amazon.

Conclusion

This study provides the first large dataset on the susceptibility of *An. darlingi* populations in the Brazilian Amazon to the pyrethroids deltamethrin, etofenprox, and permethrin. Resistance to these insecticides has been identified in several locations, raising concerns about the efficacy of current vector control strategies. However, the bioassays revealed that the observed resistance was predominantly of low intensity. This finding suggests that alternating distinct insecticides may still be an effective strategy to prolong their effectiveness and optimize control outcomes. Furthermore, this research significantly contributes to the implementation of a national resistance monitoring system for anophelines, laying a solid foundation for ongoing studies and future control measures. While *An. darlingi* is the main vector of *Plasmodium* in the region, other *Anopheles* species also contribute to transmission and should be systematically monitored for insecticide resistance to ensure comprehensive and sustainable vector management strategies.

Abbreviations

DC	Discriminating concentration
FIOCRUZ	Oswaldo Cruz Foundation
F1	First generation of laboratory-bred mosquitoes
IRD	Institut de Recherche pour le Développement
IRS	Indoor residual spraying
LBCVIV	Biology, Control and Monitoring of Insect Vectors Laboratory
LLINs	Long-lasting insecticidal nets
NMCP	National Malaria Prevention and Control Program
WHO	World Health Organization

Acknowledgements
We gratefully acknowledge the support of the Ministry of Health, the National Council for Scientific and Technological Development (CNPq), the Bill and Melinda Gates Foundation, and the health departments of various states and municipalities throughout the Amazon region, whose contributions were essential to the successful conduct of this study.

Author contributions
Study design: JBPL and AJM; Mosquito collection and bioassay execution: QSA and PS; Laboratory mosquito rearing: QSA, PS, and PG; Manuscript writing and revision: QSA, CMR, ACL, DFB, AJM, VC and JBPL.

Funding
This work was supported by the Bill & Melinda Gates Foundation [INV-003970] and the Brazilian Ministry of Health/DECIT/CNPq [443114/2019–6]. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The Ethics Committee of the Institute of Scientific and Technological Research of Amapá, Brazil (CAAE: 45663232.2.1001.0001) approved the conduct of this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 7 January 2025 Accepted: 24 April 2025
Published online: 17 May 2025

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