Chapter 12

Malaria Transmission in South America—Present Status and Prospects for Elimination

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Additional information is available at the end of the chapter

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Abstract

Four countries (Brazil, Colombia, Peru, and Venezuela) together contributed ~80% of the 875,000 malaria cases reported in the Latin American region (2016). During the 10-year period (2005–2015) when global malaria incidence was dramatically reduced, Brazil and Colombia were an integral part of this trend, on track to meet the mid-term 2020 goal established by the World Health Organization. In Colombia, since 2015 at the cessation of a five-year globally funded malaria program, both incidence and proportion of *Plasmodium falciparum* infections have increased, mainly due to the budget constraints. Similarly, despite a strong record and major recognition for reducing malaria, in 2017, Brazil has seen a resurgence of malaria cases, but no increase in the proportion of *Plasmodium falciparum* to *P. vivax*. A globally funded malaria control program in Peru from 2005 to 2010 resulted in appreciable reduction in the annual parasitic incidence down to 1/1000 by 2011–2012, but soon after, the annual malaria incidence began to rise and by the end of 2017, there were 53,261 reported cases. To add to Venezuela's political and financial woes, malaria continues to increase, such that, 300,189 cases were reported by the end of week 42, 2017. The only rational pathway to malaria elimination is sustained nation-level financial support that does not fall prey to political vicissitudes.

Keywords: malaria, Brazil, Colombia, Peru, Venezuela, epidemiology, transmission landscape, vector biology, interventions

1. Introduction

Malaria transmission control and eventual elimination is one of the greatest worldwide challenges in public health. The World Health Organization (WHO) has established a well-delineated and ambitious plan for control and elimination of the disease by 2030 [1], with a mid-term 2020 global target of reduction of at least 40% in malaria case incidence and malaria mortality rate. Significant advances were made in most of the endemic countries in Latin America, particularly from 2000 to 2015 [2], when the incidence of cases declined by 62% (1,181,095 in 2000 to 451,242 in 2015) and malaria-related deaths by 61.2% (410 to 159). The main strategies used have been rapid diagnosis, treatment with artemisinin-based combination therapy (ACT), indoor residual spraying (IRS), and insecticide-treated bednets (ITNs) or long-lasting insecticide-treated nets (LLINs) [3, 4].

However, malaria is still an important public health concern in the whole Neotropical region, more so during 2016, when a substantial increase in case incidence (875,000) was estimated [1]. Of the 18 endemic countries of Latin America, nine showed an increase in cases of more than 20% compared to 2015 [5], whereas the highest percentage increase (36%) of change in case incidence rate took place in 2014–2016. This was mainly due to the situation in Venezuela. In 2016, Venezuela (34.4%) and Brazil (18%) together accounted for more than 50% of the total reported cases, followed by Colombia (15.3%) and Peru (14.3%). According to the WHO report [1], malaria cases in Colombia nearly doubled in 2016 compared to 2015, despite an earlier reduction; in Peru cases have also been rising steadily since 2011, which has resulted in a loss of the gains achieved since 2000. In Venezuela, there has been a persistent increase in cases since 2000 and even more so since 2015 due to economic and political mismanagement [6] and Guyana recorded an increase in the proportion of *P. falciparum* (42%) to *P. vivax* cases (58%), the highest in South America [1, 7]. In contrast, Suriname observed declining malaria transmission trends to near-elimination levels through a rigorous control and education campaign, together with fortuitous flooding that destroyed populations of the primary malaria vector in the interior [8]. In 2016, Suriname reported only seven cases of *P. falciparum* and 69 of *P. vivax* [1].

The malaria landscape in Latin America consists of low transmission interspersed with diverse hot-spots where transmission is spatially and temporally focused [4, 9–11]. At a regional scale, reported malaria cases where the Annual Parasite Index (API) is >100 are concentrated in the municipalities of Bolívar, Delta Amacuro, and Sucre (Venezuela); Acre, Amapá, and Amazonas (Brazil); Amazonas, Antioquia, Chocó, and Vichada (Colombia); and Loreto (Peru) [1]. Nine countries reported zero local *P. falciparum* cases; Bolivia and Guatemala reported <10 cases. Twelve countries (Argentina, Paraguay, Costa Rica, Belize, Mexico, French Guiana, Suriname, Dominican Republic, Honduras, Bolivia, Haiti, and Brazil) are projected to have attained ≥40% reduction in case incidence by 2020, and five (El Salvador, Ecuador, Guatemala, Guyana, and Colombia) are on target for 20–40% reduction [1].

Currently, an estimated 102 million people are living in areas at risk of malaria transmission in Latin America, of which at least 28 million live in high-risk localities (>10 cases/1000 inhabitants). Most malaria cases in South America result from *P. vivax* (69%) infections, followed by *P. falciparum* (27%), and most occur in the Amazon rain forest. Colombia differs from most of its neighbors in having a large proportion of malaria transmission outside the Amazon, such as the northwest, along the Pacific Coast and in the east, bordering Venezuela [12]. There has been renewed interest in understanding the biology, epidemiology, and the specific challenges of *P. vivax*, particularly since the decline of *P. falciparum* [4, 13–15]. *Plasmodium malariae*, responsible for <1% of cases in this region, is rarely considered in malaria reports, but is likely underestimated because it is difficult to diagnose using microscopy, has a slow growth rate, is generally asymptomatic in humans, and is considered less pathogenic compared with *P. falciparum* and *P. vivax* [2].

To stay on track and advance towards elimination, some of the main challenges in this region, identified by WHO (2017), are a lack of sustainable and predictable international and domestic funding, risks posed by political conflict in malaria endemic zones (e.g., Venezuela), environmental change and anomalous climate patterns [16–19], the emergence of parasite resistance to antimalarials [20–22], and insecticide resistance in mosquito vectors (reviewed in [23, 24]). Additional regional challenges to ongoing efforts to decrease malaria incidence include a significant rise in malaria cases in recent years in Venezuela [6], evidence of submicroscopic and asymptomatic infections [25], increases in peri-urban and gold mining-related malaria [26], and an upsurge in cases of *P. falciparum* in Colombia and Peru [1, 2].

Throughout this chapter, we adopt the new nomenclature proposed for the subfamily Anophelinae by Foster and collaborators [27]. Consequently, *Anopheles* (*Nyssorhynchus*) *darlingi* is herein referred to as *Nyssorhynchus darlingi*. The most important *Nyssorhynchus* vectors involved in this malaria landscape epidemiology are anthropophilic and/or opportunistic and ecologically/behaviorally variable [28]. Patterns of transmission vary regionally, depending on climate, biogeography, ecology, and anthropogenic activities. Transmission is exacerbated by deforestation for timber extraction, agricultural settlements, and mining and development of dams for hydroelectric projects. The creation of breeding sites (such as fish ponds, microdams, forest streams blocked by road construction, and mining pools) [29–31] and spatial mobility of humans, where there is little public health infrastructure (if any), also facilitate transmission in endemic malaria regions and beyond [32–34]. Factors such as infectivity of vectors by *P. vivax* or *P. falciparum* at levels rarely above 1% and heterogeneous entomological inoculation rates (EIRs) combined with low-to-moderate human blood indices (HBI) can result in high-risk for malaria transmission in certain habitats, often associated with anthropogenic change [29, 35, 36]. Inadequate housing protects no one and is a major impediment for reducing and ultimately ending human-mosquito contact [37].

The main objectives of this chapter are: (1) to evaluate the available intervention options that may be generalizable among the main vector species, (2) to determine scenarios where hotspot-specific vector biology and ecological interventions have the best prospects for success, and (3) to propose ways to test and combine current and novel interventions against the diversity of malaria vector species and habitats. This chapter focused on the four countries that together contributed the highest proportion (81.6%) of all reported malaria cases in Latin America in 2016, namely Venezuela, Brazil, Colombia, and Peru [1].

2. Current malaria situation

2.1. Brazil

Brazil had been reporting the highest number of malaria cases in Latin America for many years, but this shifted in 2015. Venezuela, with the growing economic and political crisis, had the dubious distinction of the highest estimated incidence of malaria in the region [38]. Recently, Brazil reported the second highest number of malaria cases (18%), down from 24% of cases in 2015 [1, 38]. Furthermore, Brazil recorded a 76.8% decrease in malaria incidence during 2000–2014 [4], even though transmission was observed to be ongoing in 808 municipalities in 2013 [13]. Nearly all malaria cases (99.5%) in Brazil are reported in the Amazon region, an enormous territory that covers an estimated 60% of Brazil and consists of nine States: Acre, Amazonas, Amapá, Maranhão, Mato Grosso, Pará, Rondônia, Roraima, and Tocantins [4]. The State with the most malaria cases and highest API since 2005 is Acre; the region within Acre with the highest-risk cluster is Vale do Juruá [39] including the municipalities of Cruzeiro do Sul, Mâncio Lima, and Rodrigues Alves that are persistent malaria hot-spots [40]. Other States with API >50 as of 2015 include Amapá, Amazonas, Pará, and Roraima (**Figure 1**).

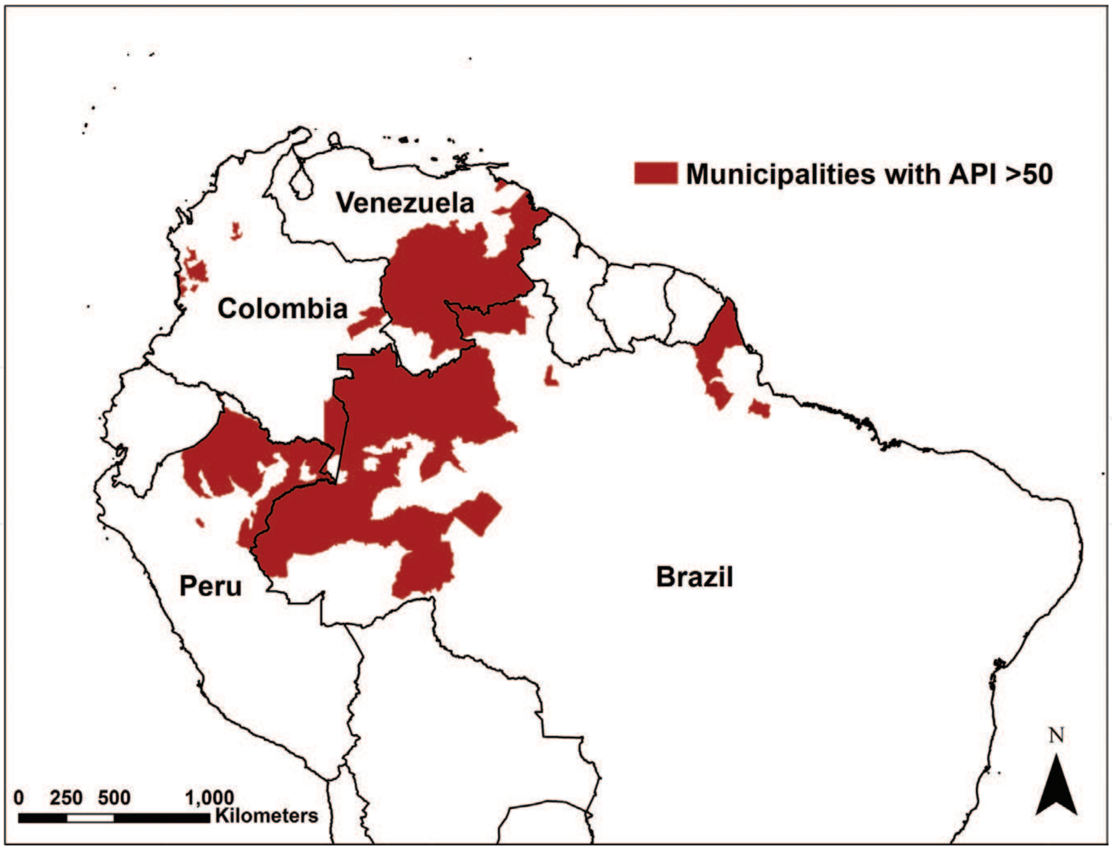

Figure 1. Geographical location of municipalities in Brazil, Colombia, Peru and Venezuela reporting Annual Parasite Index (API) >50 for data based on 2015 [38, 41].

Country	**2014**		**2015**		**2016**		**2017**	
	Number of malaria positive cases		Number of malaria positive cases		Number of malaria positive cases		Number of malaria positive cases	
	P. vivax	***P. falciparum***	***P. vivax***	***P. falciparum***	***P. vivax***	***P. falciparum***	***P. vivax***	***P. falciparum***
Brazil	117,009	22,234	122,743	15,445	110,343	13,829	172,876	21,017
Colombia	20,129	20,634	21,987	26,061	32,635	49,974	22,405	29,404
Peru	54,819	10,416	49,287	12,569	41,287	15,319	40,564	12,697
Venezuela	62,850	27,843	100,880	35,509	179,554	61,034	246,859	53,330

Note: Source of malaria case numbers 2014–2016 is WHO (2017); 2017 data are from individual Ministry of Health websites from each of the four countries.

Table 1. Number of malaria cases of ***Plasmodium vivax*** and ***P. falciparum*** in Brazil, Colombia, Peru, and Venezuela (2014–2017) [1, 42–45].

Across the Brazilian Amazon, the proportion of *P. falciparum* cases has been declining steadily for several years (**Table 1**), and in 2015, this parasite comprised approximately 11% of all cases, with *P. vivax* responsible for the remaining 89% [4]. In 2015, the Brazilian Ministry of Health (MOH) launched The Plan for Elimination of Malaria in Brazil, which focuses on the elimination of *P. falciparum* [46]. It is comprehensive, but substantial challenges remain: behavioral heterogeneity of the primary vector *Ny. darlingi* means that LLINs are only partially effective; most Amazonian housing structures do not meet criteria for routine IRS application; larviciding is most effective for accessible stagnant water bodies, e.g., fish ponds, especially those associated with hot-spots, but not effective for many natural water bodies, which may be difficult to identify and reach, or for streams and rivers with slow-moving water, which are typical *Ny. darlingi* habitats [47, 48]. By the end of 2016, *P. falciparum* still accounted for 11% of all malaria infections reported, and near the end of 2017, this was 10.8% (**Table 1**). In 2016–2017, Brazil was challenged by malaria resurgence, including in municipalities that were in the prevention phase and others with low malaria transmission. Furthermore, the total number of malaria cases in Brazil has increased from 105,057 cases during the period January 1 to December 31, 2016, to 154,343 cases during the period January 1 to October 31, 2017, an increase of 47% [42].

Some of the roadblocks in reducing and eliminating *P. vivax* include the high frequency of low-density *P. vivax* infections and the difficulty of their diagnosis by microscopy, particularly in areas approaching elimination and the persistence of liver stage hypnozoites that may be responsible for relapses [2, 4, 49]. Peri-urban and urban malaria transmission has been difficult to eliminate in cities such as Manaus (Amazonas State) and Cruzeiro do Sul (Acre State). In 2015, Manaus reported 7300 cases, most of which were acquired during work or other activities in neighboring municipalities, suggesting that interventions need to be focused on the mobile proportion of the human population [13]. Better transmission control is thought to lead to a lower *P. falciparum:P. vivax* ratio, reflecting the rapid and stable reduction of cases in urban settings compared with a lower and more heterogeneous reduction in rural and indigenous areas [13]. In a study based in and around the small cities of Mâncio Lima and Rodrigues Alves, Acre State; three development gradients, i.e., urban-rural, rural-riverine, and housing location were analyzed for multiple households. The lowest risk (OR = 0.55, 1.23–1.12) of having a household with malaria was along the rural-riverine gradient, the most forested of the three; in contrast, the highest risk (OR = 1.92, 1.03–3.92) was along the urban-rural gradient, where urbanization was associated with roads, basic services, water treatment, electricity from a power grid, and less forest access [40]. This is an interesting and important finding, because malaria is so often assumed to be rural, associated with nearby water bodies and often linked to the forest environment. However, malaria risk is clearly linked with poverty, as another important finding of this study was that malaria risk is higher for poor individuals living in rural areas than those living in urban areas [40]. The poor in urban areas generally are exposed less frequently to biting, infected *Nyssorhynchus* and *Anopheles* mosquitoes, and have better access to health services than the poor in rural areas [40].

A valuable epidemiological tool was developed in 2010 to identify malaria outbreaks via an automated algorithm [50]. Use of the algorithm aimed to mobilize local control managers to act as rapidly as possible and they identified *P. vivax* as the primary causative pathogen for nearly all outbreaks, most of which occur in low or interrupted transmission areas where the likelihood of reintroduction is high. In 2014 and 2015, as many 112 and 111 outbreaks were identified, respectively [13]. The effectiveness of this tool has not been validated but it demonstrated usefulness in transmission reduction, which could lead to widespread adoption in Brazil.

2.2. Colombia

In 2016, Colombia recorded 83,227 cases, the third highest number in Latin America, which comprised 15.3% [1, 41]. Thus, malaria continues to be a serious public health problem and transmission is heterogeneous, presenting zones of low unstable transmission with endemic-epidemic patterns including various hot-spots [12]. From 2000 to 2014, Colombia made solid gains against malaria (50–75% reduction in cases), mainly due to interventions such as diagnostic health posts and vector control. However, these gains have been undermined since the Colombia Malaria Project ended in 2015; case numbers doubled between 2015 and 2016 [41].

For the past decade, *P. vivax* accounted for approximately 70% of reported cases, with the remainder exclusively *P. falciparum* [12]. However, in 2016, this proportion shifted alarmingly in favor of *P. falciparum* constituting 60% of reported cases [1, 41, 43, 51, 52]. This parasite species predominates along the Pacific Coast, one of the endemic hot-spots, where there is a high occurrence of Colombian Afro-descendant individuals who are Duffy-negative [53].

Taken together, eight Colombia Departments accounted for 90.8% of all the 2016 noncomplicated malaria cases. These are Chocó, Nariño, and Cauca (western Colombia), Antioquia and Córdoba (northwestern), Guainía and Vichada (central-eastern along the border with Venezuela), and Amazonas (southeastern). Among various Departments, Chocó was worst affected and contributed 53% of all reported cases during 2014–2015 [38]. Nevertheless, up to the 49th epidemiologic week of 2017, Chocó registered a lower proportion of cases (30.7% [43]) compared with the same period in 2015, because several health posts ceased reporting due to national, State, and municipal budgetary constraints with the closure of the Colombia Malaria Project (2015). In the Departments of Arauca and Guajira in eastern Colombia, bordering Venezuela, there was an increase in cases compared to the average number registered during 2012–2016. Of the 860 non-autochthonous cases reported overall, most (76.7%) were *P. vivax* and nearly all (93.1%) were from Venezuelan patients [43].

Malaria transmission in Colombia has mainly been rural, but a recent study indicated that between 2008 and 2012, urban and peri-urban malaria transmission described as endemic, unstable and of low intensity, occurred in many municipalities in the Pacific Coast and a few in eastern Colombia [53]. However, the authors indicated that a serious limitation was not having a clear consensus on the definition of urban and peri-urban. Nevertheless, there appears to be a trend of decreasing rural and a concurrent progressive increase of urban malaria. Possible explanations of this phenomenon are human migration resulting from ongoing-armed conflict, illegal mining, or illicit crop activities, and the movement of asymptomatic carriers.

In western and northwestern Colombia, with the existing healthcare and disease prevention programs, gold-mining (mostly illegal) has played an important role in the maintenance of malaria as shown by public health surveillance data based on 2010–2013 [26]. This study showed that gold-mining was predominant in seven Colombian Departments that contributed 89.3% (270,753 cases) of the national malaria cases during this period; of which, 31.6% of the cases were from mining areas. The worst of these were located in Antioquia, Córdoba, and Buenaventura municipalities in Valle del Cauca.

Vector control interventions in Colombia rely on the use of insecticides, larvicides, and ITNs [54] to reduce human-vector contact. Some research groups focused on mosquito vector biology aiming to provide baseline information for the development and implementation of appropriate vector control interventions by the evaluation of ecology and biology of vector species, improved species identification, spatio-temporal distribution, biting behavior and preferences, and natural infection by *Plasmodium* [55–60].

A comprehensive early warning system, as part of the Integrated National Adaptation Pilot project and the Integrated Surveillance and Control System at the municipality level, has been implemented in four pilot sites in Colombia, where it showed promise, providing new data on malaria incidence and seasonality, vector species presence and abundance, entomological indices and feeding frequencies, climate variables, human population information, and some data on vector control activities [61]. Limitations that remained included the scarcity and difficulty of accessing cultural qualitative and quantitative factors and the limited preparedness of State and municipal health authorities to implement malaria dynamic models [61].

2.3. Peru

The most recent WHO data showed that Peru reported an estimated 14.3% of all malaria cases in the region for 2016; this amounted to 56,606 cases, of which 73% were *P. vivax* [1]. This estimate has been rising fairly steadily since 2010–2011, ever since cessation of the international financial support provided by the Global Fund Malaria Project "PAMFRO" that had successfully reduced the annual incidence to <1 case/1000 inhabitants for 2010 and 2011 [62]. After 2011, there was a surprisingly rapid malaria resurgence, hypothesized to be due to: (1) budgetary constraints; (2) the perception that malaria was under control; and (3) a concurrent regional dengue epidemic in Loreto [63]. Transmission may have been worsened due to the historic Loreto flood of 2011–2012 that inundated and damaged many riverine communities [62]. During the period between 2002 and 2013, 79% of cases were *P. vivax* and 21% *P. falciparum* [11]. A worrisome trend has been the recent increase in the proportion of *P. falciparum* in 2016 (27%) and 2017 (24%) (**Table 1**).

Numerous malaria endemic riverine and highway villages exist near the Iquitos-Nauta highway and along the Itaya and Nanay Rivers to the south and west of Iquitos. Inhabitants of two of these villages, Lupuna and Cahuide, took part in a cross-sectional survey in January 2013 (off-peak malaria season), with census data taken in mid-2012. One substantial determination was that prevalence of *P. vivax* and *P. falciparum* was many times higher by packed red blood cell (PRBC)-PCR compared with microscopy (25 vs. 3.6% and 5 vs. 0.2%, respectively) [33]. Routine surveillance, using the more sensitive PCR detection method and treatment that includes individuals with very low parasitemia who maintain local transmission even during the off-peak malaria season, acting as potential parasite reservoirs, could be an effective addition to prompt diagnosis and treatment to further reduce malaria regionally. In addition, the overall heterogeneous distribution patterns of *P. vivax* and *P. falciparum* differ sharply in Lupuna and Cahuide, i.e., *P. vivax* is transmitted more locally within villages and *P. falciparum* is more often acquired at a distance, related to occupation, and transported on a regional basis [33].

Most years, between 90 and 95% of all malaria cases and 99.4% of *P. falciparum* are reported from Loreto Department, in northern Amazonian Peru [64]. In 2017, this amounted to 50,702 cases (96.2% of those across Peru); there were also small foci in Amazonas State (822 cases in 2017), west of Loreto, and in San Martin (415 cases in 2017), south of Loreto [64]. There was a serious *P. vivax* outbreak in the gold-mining region of the southern Amazon, in Madre de Dios and neighboring Ucayali until about 2011 [65], but only 6 cases were reported in Madre de Dios and 79 in Ucayali in 2017 [64]. In Tumbes and Piura, along the northwestern coast, malaria has greatly diminished and what remains is epidemic, sporadic, and peri-urban, likely the result of reintroduction [64, 66–68].

Loreto Department comprises an estimated 30% of Peruvian territory and there are about one million inhabitants [69]. Malaria transmission is highly seasonal, coinciding mainly with the heavy rainy season (January to June) and Andean snowmelt, that together increase river levels up to 10 m, causing major fluctuations in the abundance of the main regional malaria vector *Ny. darlingi* [70, 71]. Most malaria infections are found in rural and remote villages whose inhabitants live along the Amazon River, and its many tributaries, in enclosed or partially enclosed wooden houses [62, 72]. There has been increasing recognition, beginning with a ground-breaking study [73], of hyperendemic foci linked to occupational activities (such as timber extraction, farming, and charcoal production) and human mobility [33].

2.4. Venezuela

Whereas the continent achieved a significant decline in malaria-related morbidity (62%) and mortality (61%) between 2000 and 2015 as part of the implementation of the Global Malaria Action Plan 2008–2015 [41], Venezuela, in contrast, was the alarming exception in the region, displaying an unprecedented 365% increase in malaria cases between 2000 and 2015 [6]. In 2016 alone, 240,588 malaria cases were officially reported [1], whereas by the end of 2017, this number had increased to 300,189 total cases [45]. Astonishingly, the number of cases reported in 2017 in Venezuela is higher than that reported in the last 29 years (1988–2016) [74].

Economic and political mismanagement have precipitated a general collapse of Venezuela's health system creating an ongoing humanitarian crisis with severe social consequences [75, 76]. Consequently, a malaria epidemic has been fueled by financial constraints that prevented the procurement of malaria commodities (insecticides, drugs, diagnostic supplies, mosquito nets, etc.), epidemiological surveillance, reporting activities, vector-control and disease-treatment efforts, high internal human migration associated with illegal gold mining, and underlying malnutrition due to a general lack of provision and implementation of services. In 2016, *P. vivax* malaria accounted for 76% of all cases, followed by *P. falciparum* (18%), *P. malariae* (<1%), and *P. vivax*/*P. falciparum* mixed (6%) infections [1].

Although *P. falciparum* malaria occurs mostly in the lowland rain forests of the Venezuelan Guayana region, *P. vivax* malaria is endemic in the coastal plains and savannas, as well as the lowland Guayana forests [17]. Currently, an estimated 80% of malaria in Venezuela is associated with gold mining areas in the forest ecosystem of the southeastern region, where local transmission is maintained in few but persistent disease hot-spots by *Ny. darlingi* and *Ny. albitarsis* s.l. ([77–79]; Grillet unpublished). Infection Rates (IR) of *Ny. albitarsis* s.l. and *Ny. darlingi* collected during 2009–2012 in Sifontes, Bolivar State, were very high: 5.4 and 4.0%, respectively [80]. Gold mining extraction activities substantially reduce forest vegetation cover, which seems to favor aquatic vector habitat production, especially for *Ny. albitarsis* s.l. ([79]; Grillet unpublished). Mining activities in turn result in highly mobile human populations that migrate in search of jobs, working, and sleeping outdoors, exposed to continuous mosquito biting for long periods of time. Many of these economic migrants are previously unexposed to *Plasmodium* and some of them return to nonendemic malaria regions, e.g., near the capital Caracas, with circulating gametocytes, reintroducing *Plasmodium* to areas where malaria had been eliminated previously [81]. Although, most disease transmission in Venezuela has been rural, recent observations suggest a significant change in the landscape epidemiology of malaria since 2013—urban and peri-urban malaria transmission are now associated with some cities close to Caracas [Grillet unpublished]. Finally, case spillover has overloaded frontier health care infrastructure in Brazil and Colombia where in 2016, 78 and 81%, respectively, of imported malaria cases originated from Venezuela [2]. The continued upsurge of malaria in Venezuela threatens to become uncontrollable, jeopardizing the hard-won gains in the Americas' elimination agenda and global malaria targets.

For decades, Venezuela was a leader in vector control and public health policies in Latin America, especially after being the first WHO-certified country to eliminate malaria in much of its territory in 1961 as a result of a very aggressive, vertical malaria control campaign [82]. This campaign consisted of the interruption of malaria transmission through systematic and integrative infection and vector control. Additionally, the program included the detailed knowledge of malaria microepidemiology (at local level, case management, consisting of diagnosis, patient treatment, and mass drug administration), mapping malaria cases, malaria health information system updated weekly, community participation through volunteer community health workers, application of larvicides, and sanitary engineering such as housing improvement and water management. This public health success helped to galvanize interest in global elimination [82]. The Venezuelan approach for malaria elimination in the past differs little from current prevention, control and elimination, except that it was implemented in an epidemiological landscape where insecticide and parasite resistance were absent, political will was significant, and government support was very strong. Vector control and case prevention require long-term investment and sustainability without which it is difficult to envision elimination as a viable outcome.

3. The main malaria vectors

3.1. *Nyssorhynchus darlingi*

The most widespread and dominant malaria vector in the Amazon region is *Nyssorhynchus darlingi* (**Figure 2**) [27, 28, 84, 86]. Localities where *Ny. darlingi* has been formally incriminated by ELISA or other molecular techniques are shown in **Figure 3**, although the full distribution of *Ny. darlingi* extends from southern Mexico through northern Argentina [84]. This species shares several characteristics with invasive species (e.g., *Aedes albopictus*) and other primary malaria vectors such as *An. gambiae* s.s., including fast growth, phenotypic plasticity, rapid reproduction, moderate-high dispersal ability, ecological competence, and association with humans [28, 104–106]. In Loreto Department, Peru, since *Ny. darlingi* reinvaded, or re-expanded its range into the peri-Iquitos area about 1998 [107], it has spread along numerous Peruvian river drainages to the north and west [70, 108]. In Brazil and Peru, it is ranked the number one vector [4, 29, 109]; in Colombia, it is one of three main vectors, the other two being *Ny. albimanus* and *Ny. nuneztovari* [87, 110, 111]; and in Venezuela, it shares top billing with *Ny. albitarsis* s.l. [77, 78, 80]. A recent review highlights the very low insecticide resistance in *Ny. darlingi* detected in the Neotropics, i.e., one population in Choco, western Colombia is resistant to DDT, permethrin, lambda-cyhalothrin, and deltamethrin [23].

The distribution in Brazil includes the lowlands of the Amazonian biome, the Cerrado, and the southern Atlantic forest [84, 112, 113]. *Nyssorhynchus darlingi* is adaptable and flexible in its behavior: exophagic and endophagic; anthropophilic and opportunistic; though generally exophilic [28, 71, 97, 114]. The standard entomological indices range widely across its distribution [71, 80, 96, 97, 103, 114]. One frequently recognized characteristic of *Ny. darlingi* is the speed with which it colonizes deforested Amazonian patches and a variety of anthropogenic water bodies such as gold mining pools, brick-making depressions, wells, cisterns, and fishponds, as well as natural breeding site types linked to rivers or flooded forest [29, 60, 111, 115, 116]. Its adaptation to novel environments may lead to increased vectorial capacity and survival, as well as greater risk of malaria transmission [117, 118]. The most likely drivers of *Ny. darlingi* divergence at a macro-geographic scale, across its broad distribution, are biogeographic or geographic boundaries and Pleistocene environmental changes [113, 119]. At a regional scale, isolation-by-distance has been shown to influence population structure [120], whereas at a micro-geographic scale, current local environmental conditions have a marked effect [113, 119–122].

In Colombia, *Ny. darlingi* is distributed on either side of the Andes mountain range in lowland regions characterized by biogeographical and ecological heterogeneity [111]. West of the Andes, in the Urabá-Bajo Cauca and Alto Sinú (UCS) region, *Ny. darlingi* is the most common *Nyssorhynchus* species, exhibits endo and exophagy, is infected with *P. vivax*, and maintains transmission even at low abundance [60, 87, 111]. In most localities included in this study, the peak biting activity of *Ny. darlingi* was after 20:00 or 21:00 h when people conduct indoor and/or outdoor activities increasing the risk of vector-human contact. East of the Andes [111] and in southern Colombia, peak biting activity is at sunset [92] when no one is protected under ITNs. The dominance of *Ny. darlingi* in most of northwestern Colombian localities seems to be favored by ecological perturbations resulting from various human activities, such as alluvial mining, livestock, small-scale rice

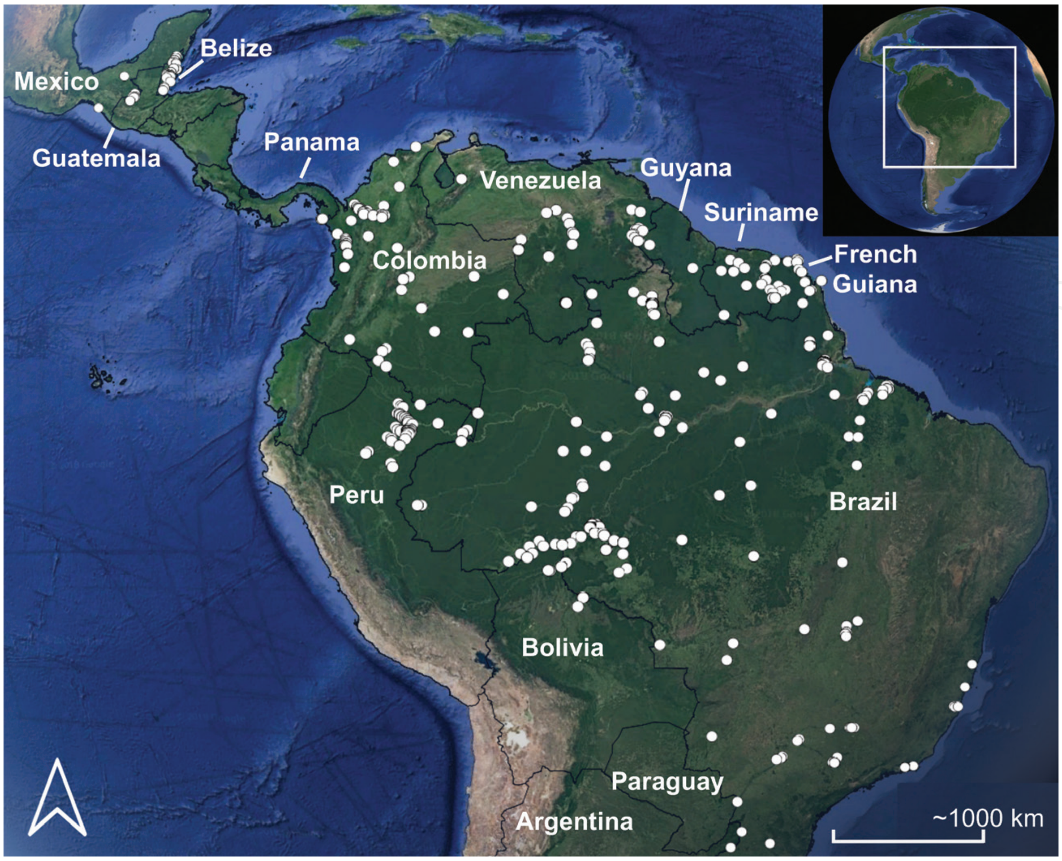

Figure 2. Distribution of *Nyssorhynchus darlingi* (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

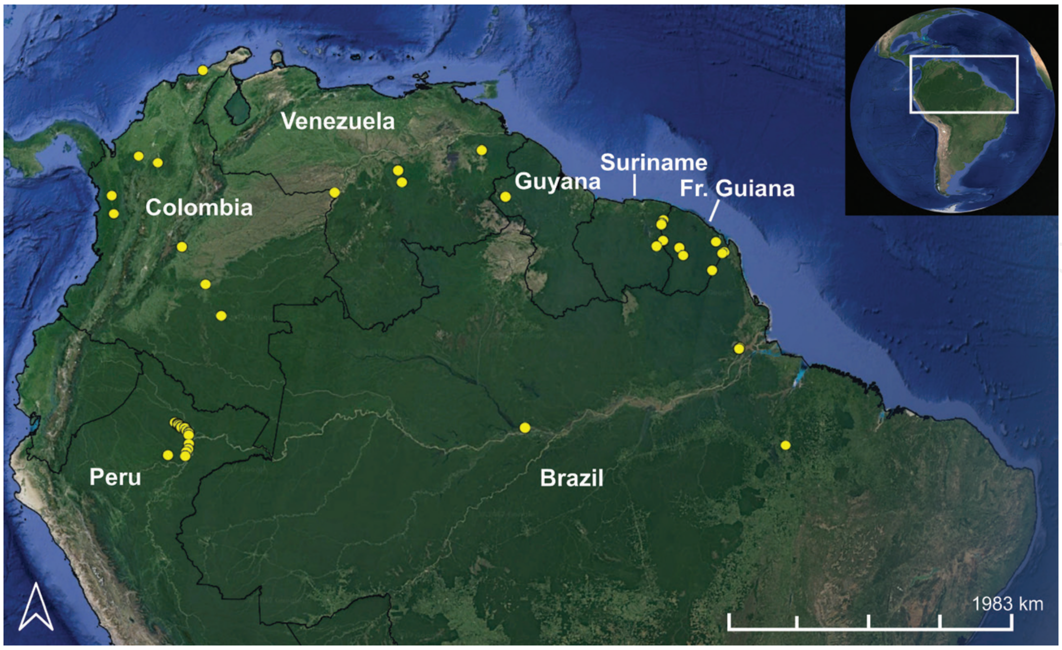

Figure 3. Localities (denoted by yellow dots) where the primary malaria vector *Nyssorhynchus darlingi* has been reported infected with *Plasmodium vivax* or *Plasmodium falciparum* incriminated by molecular methods during 2005 to 2017 [8, 35, 59, 60, 73, 80, 87–103]. Map made in Google Earth Pro [83, 84].

production, and forest fragment landscapes [60]. Vector control strategies that include ITNs are recommended for containment of *Ny. darlingi* populations [60, 87, 111, 123].

Studies on the genetic structure of *Ny. darlingi* in Colombia have shown that at the micro-geographic scale, in northwestern Colombia, *Ny. darlingi* is characterized by low genetic differentiation and high gene flow [123, 124]. The environmental heterogeneity that is a hallmark of this malaria endemic region does not reach a threshold to impact the population structure of *Ny. darlingi* [124]. A comprehensive genetic study that evaluated *Ny. darlingi* throughout its distribution in Colombia found that at a macro-geographic scale, differentiation into two main groups, west and east of the Andes, was most likely influenced by the Andes; at a micro-geographic scale, differentiation was partly the result of isolation by resistance, probably due to ecological differences, with significant impact on its population structure. In the current malaria scenario in Colombia and considering that Anophelinae mosquitoes adapt to climate and environmental changes, population studies should contribute to the development and implementation of vector control interventions and monitor their effectiveness in important malaria endemic regions of Colombia where *Ny. darlingi* maintains transmission.

Within Peru, only in the peri-Iquitos region of Loreto Department has the genetic structure of *Ny. darlingi* been evaluated, initially using Random Amplified Polymorphic DNA-PCR, that detected substantial homogeneity [125]. When populations from highway and riverine habitats were compared over a decade later using microsatellite markers, two highly admixed subpopulations were detected in each of nine villages [35]. The second major finding was that the 2012–2014 population of *Ny. darlingi* [35] had replaced that of the 2006 [126] and both of these subpopulations had the signature of a recent expansion. The source of the replacement population is unknown, although a broad analysis of microsatellite data across South America suggests that it most likely comes from western Brazil [35].

In Venezuela, *Ny. darlingi* is found in the lowland tropical rainforest, in the southern part of the country (Amazonas and Bolivar States), the piedmont ecoregion characterized by high rainfall and tropical forests in Trujillo State, western Venezuela, and in the llanos in central-western Venezuela, a subregion of the savanna ecoregion [127]. There is very little population structure in Venezuelan *Ny. darlingi* based on isozymes, RAPDs, ITS2 sequences [86], but more sensitive molecular markers, or whole genomes, might detect micro-geographic differences among the diverse ecoregions.

3.2. *Nyssorhynchus albimanus*

Nyssorhynchus albimanus is a malaria vector [27] characterized by ecological adaptability and a widespread, mostly coastal lowland, Neotropical distribution (**Figure 4**) [128]. Its presence usually coincides with areas that experience two annual rainy seasons, precipitation greater than 1000 mm, high relative humidity and a monthly variation in temperature between 22° and 29°C [127, 129, 130]. Despite its absence in Brazil, in Colombia, *Ny. albimanus* constitutes one of the main vectors in rural and peri-urban areas below 400–500 m, predominating along the Colombian Caribbean and Pacific Coasts and on the Island of San Andres [130–133]. These regions have different levels of *Plasmodium* transmission and the importance of *Ny. albimanus* also differs [133]. The Pacific is a humid tropical forest and one of the rainiest regions globally; in contrast, the Caribbean tropical forest is drier and hotter [134]. Malaria cases increase in

Figure 4. Distribution of *Nyssorhynchus albimanus* (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

relation to ENSO patterns and cycles, particularly those transmitted by *Ny. albimanus* along the Pacific Coast of Colombia [61].

The availability of suitable breeding sites determines distribution and abundance of *Ny. albimanus* [130], a species that can thrive in fresh and brackish water, natural habitats (animal tracks, lakes, streams, and wells), and anthropogenic ones (rice fields, lagoons, and mining excavations, among others) [130, 135]. Behaviorally, *Ny. albimanus* is mainly zoophilic, exophagic, and exophilic; yet it can be anthropophilic, depending on local circumstances and abundance [130]. It is also known to be endophagic in local malaria hot-spots along the Pacific Coast, i.e., the urban sector of Buenaventura. The main outdoor biting time is 19:00–23:00 h, when many inhabitants are outside, and therefore exposed to biting and *Plasmodium* transmission [130]. As a vector of *P. falciparum* and *P. vivax*, *Ny. albimanus* has been incriminated in the Pacific region [133] and a new species from the southern Pacific Coast, *Ny. albimanus* B, detected by mitochondrial *COI* sequences, was infected with *P. falciparum* [57]. Despite the high abundance of *Ny. albimanus* in the Caribbean region, no infected specimens were detected [136].

Population genetic studies of *Ny. albimanus* in Colombia confirm its status of a single taxon throughout its distribution, with low population structuring and little genetic differentiation [137]. Two broader studies that included samples from Nicaragua to Ecuador, both nuclear and mitochondrial markers, found evidence for geographic structuring [138] and population contraction across Panama followed by an east-west expansion [139]. Under the hypothesis that malaria vectors are exposed to control pressures and environmental alterations that may lead to genotypic and phenotypic variation, genetic (microsatellite) and phenotypic (wing trait) data in populations of *Ny. albimanus* from the Pacific and Caribbean, despite a significant effect of environmental factors on wing traits, support a regional metapopulation of *Ny. albimanus* [132].

In Peru, *Ny. albimanus* is restricted to the Tumbes region of the northern coast, where it transmits *P. vivax* at the end of the hot rainy season. Local insecticide application, mostly in rice fields, lead to extreme levels of insecticide resistance [23]. A series of meetings and decisions between southern Ecuador and northern Peru health personnel resulted in a highly successful control program that employed a wide array of interventions such that autochthonous malaria was eliminated in El Oro, Ecuador in 2011 and in Tumbes, Peru in 2012 [135].

In Venezuela, *Ny. albimanus* is distributed along the coast and the margins of Valencia Lake, south of Maracay, although it does not appear to contribute to malaria transmission locally [127, 140]. It was found to be as abundant as the known coastal vector *Ny. aquasalis* in Aragua State, northcentral Venezuela, where both species had similar peak biting times during the early evening and were collected biting outdoors [141].

3.3. *Nyssorhynchus albitarsis* s.l.

The *Albitarsis* Complex comprises at least eight species [142] that extend across Central and South America and some Caribbean islands (**Figure 5**). The difficulty of their morphological differentiation complicates recognition of their role(s) in malaria transmission, an important aspect for the implementation of targeted and effective vector control strategies [143]. Three species are known vectors: *Ny. deaneorum*, *Ny. janconnae*, and *Ny. marajoara.* The latter is important regionally in *Plasmodium* transmission in central and eastern Brazil, where its distribution includes Amapá, Mato Grosso, Pará, and Rôndonia [84, 142]. Its role in transmission rivals that of *Ny. darlingi* in some habitat types such as peri-urban Macapá City, Amapá [144] and along the Rio Matapi, Amapá [88]. An entomological survey during an outbreak in western French Guiana, in an illegal gold mining area, detected a high *P. vivax* infectivity rate (6.4%) in specimens of *Ny. marajoara* [99]. An ecological niche model, based on current and future (2070), distributions of *P. falciparum*, *Ny. darlingi*, all species of the *Albitarsis* Complex, climate, biome and topography, projected that, whereas climate change would reduce suitable habitat for *Ny. darlingi,* both *Ny. marajoara* and *Ny. deaneorum* are expected to expand southward, thereby increasing their likely role in *P. falciparum* transmission by the projected date of 2070 [19].

In Colombia, only a few species, in particular *Ny. marajoara,* have been identified morphologically in this complex [90, 145–147] and implicated in urban transmission [145]. This species is thought to be widespread in this country [110]. However, a detailed analysis of many Colombian specimens, identified molecularly, did not detect any individual *Ny. marajoara* [147], in agreement with Ruiz-Lopez et al. [142], whose study indicated that *Ny. marajoara* is restricted to the central-eastern and western regions of Brazil and is most likely absent in Colombia. Further studies need to be done on this vector to better frame its geographic distribution.

Albitarsis Complex species appear to be uncommon in Peru but this could reflect a general lack of *Nyssorhynchus* taxon sampling and molecular identification, particularly outside the Amazon region of Loreto.

Although there are several published reports of *Ny. marajoara* as an important regional malaria vector in Bolivar State, Venezuela, along with *Ny. darlingi* [77, 78, 148], a different species,

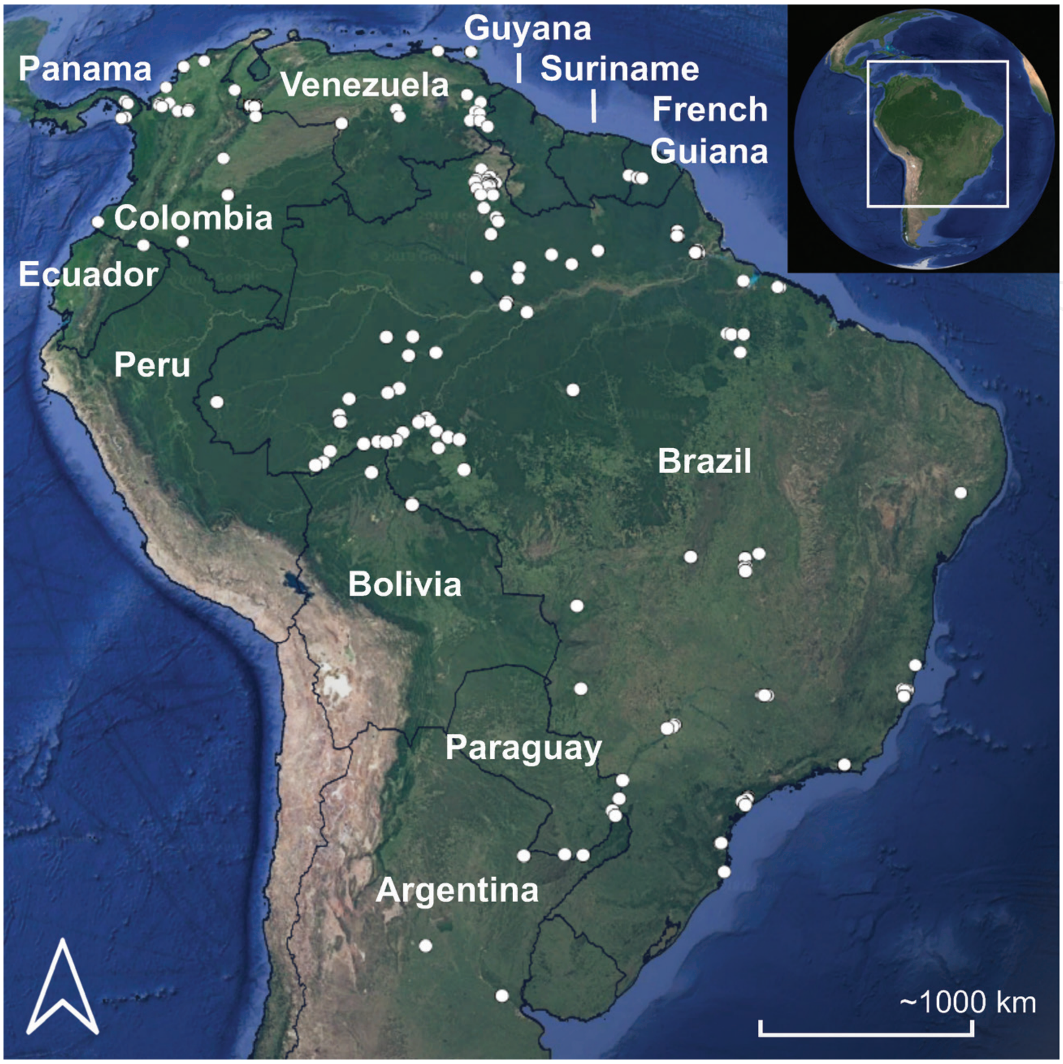

Figure 5. Distribution of the *Albitarsis* Complex (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

Ny. albitarsis F in the *Albitarsis* Complex [142], was identified from the Caura Basin, Bolivar State [96]. In the most recent publication from the malaria hot-spot Sifontes, the specimens infected by *Plasmodium* are referred to only as *Ny. albitarsis* s.l. [80]. Hopefully, the correct species identities and distribution will soon be determined in this very crucial Venezuelan hot-spot.

3.4. The *Nuneztovari* Complex

The *Nuneztovari* Complex, extending through much of northern South America, includes *Ny. nuneztovari* (**Figure 6**), *Nyssorhynchus dunhami*, and *Nyssorhynchus goeldii* [149]. Like the *Albitarsis* Complex, species in the *Nuneztovari* Complex are similar morphologically and difficult to identify accurately. Scarpassa and collaborators [150] presented strong molecular evidence that additional species exist in Brazil and briefly reviewed the role of *Ny. nuneztovari* as a malaria vector in five Amazonian States. *Nyssorhynchus nuneztovari* is restricted to Colombia and western Venezuela, *Ny. goeldii* to Amazonian Brazil, and *Ny. dunhami* to central-western Brazil, Colombia and Amazonian Peru [71, 150]. It is difficult to evaluate the identification of

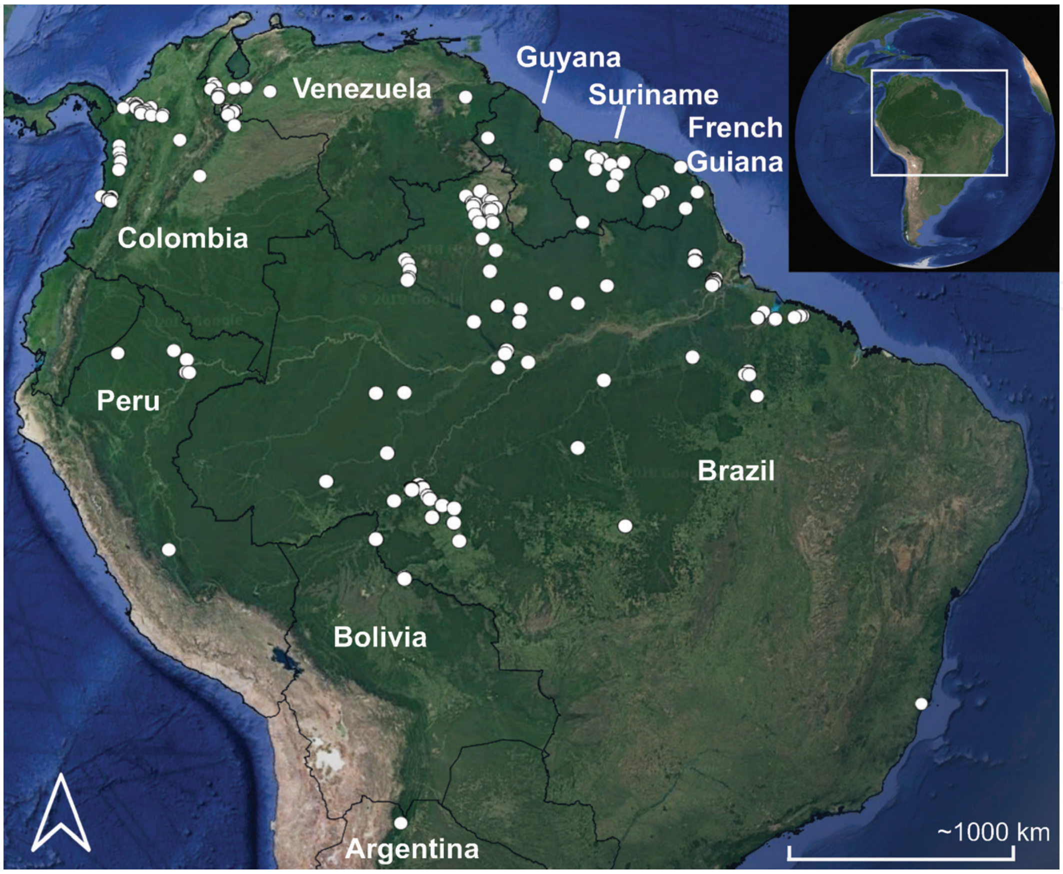

Figure 6. Distribution of the Nuneztovari Complex (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

these species in earlier publications, because distributions of *Ny. nuneztovari* and *Ny. goeldii* overlap, as do those of *Ny. goeldii* and *Ny. dunhami* [149, 150].

In Colombia, *Ny. nuneztovari* is an important malaria vector on both sides of the Andes presenting morphological, behavioral, and genetic heterogeneity throughout the country [151, 152]. In northwestern Colombia, it was found to be the most prevalent species, confirming an earlier study [58], and showed endo and exophagic behavior [152]. It was naturally infected by *P. vivax* VK247 [60, 87], positive for *P. vivax* VK210, and VK247 in the Pacific Coast [103]. In eastern Colombia, there are no recent reports of *Ny. nuneztovari* infected with *Plasmodium*, but more importantly, there is a lack of investigation of malaria outbreaks along the frontier with Venezuela with no record of species identification and vector incrimination. Because of the humanitarian crisis in Venezuela, the numbers of malaria cases have increased dramatically since 2010 [1, 153]. In the most recent study of Colombian *Ny. nuneztovari*, it was reported to be abundant and dominant in localities where anthropogenic activities such as livestock, fish-farming, and small to medium-scale agriculture were common, attributed to its adaptability to environmentally impacted habitats [152]. Common larval habitats were artificial fishponds and wetlands, particularly in the west and northwest [58, 152].

Regionally, this species shows the highest biting activity after 20:00 h, which suggests high transmission risk when people are at home, but not necessarily under nets. ITNs could be one component of an effective vector control intervention. In a locality in the northeast, Tibú, contiguous with Venezuela, peak biting of *Ny. nuneztovari* was after 21:00 h. This population differed genetically from other Colombian populations and its behavior was similar to *Ny. nuneztovari* from Venezuela. The populations exhibited endo and exophagic behavior in all localities and the results of the study indicated that region-specific interventions on both sides of the Andes would be most effective [152]. EIR values detected for Colombian *Ny. nuneztovari* were 3.5–3.6 in the northwest and 7.2 in the west. The highest value was in Buenaventura, on the Pacific Coast, where *Ny. albimanus* is considered the primary vector [133], but, according to the new study, *Ny. nuneztovari* also has a role in transmission in peri-urban Buenaventura [152].

In Peru, *Ny. nuneztovari* has been detected in five Departments: Pasco, Junín, Loreto, Ucayali, and Madre de Dios [154] and its presence confirmed in Loreto [155]. It may have a role in local malaria transmission, but remains unexplored. *Nyssorhynchus nuneztovari* is known as an important regional vector in western Venezuela where it occurs in seven States [156]. It was first identified morphologically in Bolivar State by Moreno et al. [157], from the malaria hot-spot of Sifontes municipality and was found infected by *P. vivax* (0.52%) [80]. It has also been found to be as abundant as *Ny. darlingi* in the Lower Caura River Basin, Bolivar State, where it was mostly active at sunset, although biting also throughout the night. Nevertheless, it was not detected infected by *Plasmodium* (although *Ny. darlingi* was), so the latter is more important in relation to malaria risk in the Caura River area [96].

4. Conclusions and recommendations

As discussed by Packard [37], for sustainable malaria control, focusing on decreasing incidence towards elimination, effective measures need to be considered, including those related to human ecology. Examples include a significant improvement in living and housing conditions, redesigning of anthropogenic landscapes from those that favor mosquito vectors to a remodeled landscape that is both adequate for humans and inadequate for vector mosquitoes. The sustainability and success of a malaria control program depends on a combination of diagnosis of human infection, treatment with anti-malarial drugs, and vector control. Moreover, proposed changes will need to be maintained such that the malaria baseline will not be affected by either interruption or disruption of a control program [1]. It would be sensible to include malaria control in the One Health Program, to align it with the elimination of extreme poverty, a goal of the global sustainable development program.

The recent elimination of malaria on the Peru-Ecuador border was a successful strategy and included strengthening surveillance and treatment, resource sharing, the use of operational research to inform policy, and novel interventions [135]. The current program depends on prompt, effective diagnosis and treatment with no charge, community personnel trained to collect blood smears from febrile persons within their communities, case reporting to a national surveillance system that includes a five-category case definition (indigenous, imported, introduced, induced, and cryptic), active foci and case investigations, mapping and elimination of larval habitats, and the use of ITNs and LLINs. This could serve as a model for the current situation along the Venezuelan border with its neighbors, Colombia and Brazil. One very important aspect of this program is that it took 20 years to achieve its goals [135].

Worldwide, some of the innovations adopted for prevention, control, and eventual elimination of malaria transmission during the past ~10 years have included the development and deployment of LLINs [158, 159], the completion and exploration of many mosquito and parasite genomes [160–163], major progress on genome editing in vector mosquitoes [164–166], new interventions such as house eaves [167] and push-pull systems [168], and better evaluation of larval source management (LSM) as a potential component of integrated control management systems [169]. Global policies and recommendations provide a useful framework and roadmap guided by the Global Technical Strategy of Malaria Control and Elimination (2016–2025), a reconsideration of the vectorial capacity formula for elimination [170] and the Plan for Elimination of Malaria in Brazil (UN/OMS 2015; [4]).

During the same 10-year timeframe, several novel tools and strategies have been envisaged that focus on the Neotropical malaria control and eradication landscape: (1) successful colonization of the main malaria vector *Ny. darlingi* [171, 172]; (2) development of predictive models on climate change scenarios for Neotropical malaria vectors and *Plasmodium* [18, 19]; and (3) collection of baseline larval habitat characteristics in malaria endemic regions that can guide larval source reduction [29, 48, 58, 173] and may prove effective as part of a broader array of vector interventions in certain landscape types such as abandoned gold mining pools [174] and possibly commercial fish ponds [31].

The most serious challenge to malaria eradication in South America from the viewpoint of vector control is that most vector species are primarily exophilic, often exophagic, and frequently bite early in the evening. Therefore, it is essential to determine and monitor the local biting behavior of a mosquito vector species.

Identified gaps in vector interventions throughout South American endemic areas are:

1. Sustained funding for vector surveillance and intervention;
2. Ongoing training programs for vector biologists and promoting community participation;
3. Use of species distribution models to map potential distribution and epidemiology to focus interventions and planning;
4. New efforts to control exophagic vectors and targeting aquatic stages should be part of integrated control and elimination programs that prioritize hot-spots;
5. More accurate and timely identification of transmission in hot-spots;
6. Routine evaluation of application strategies and insecticide resistance.

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Conflict of interest

The authors declare no conflict of interest.

List of abbreviations

ACT	artemisinin-based combination therapy
API	annual parasite index
COI	cytochrome c oxidase I
DDT	dichlorodiphenyltrichloroethane
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
ENSO	El Niño-Southern oscillation
HBI	human blood index
IR	infection rate
IRS	indoor residual spraying
ITN	insecticide-treated net
ITS2	internal transcribed spacer 2
LLIN	long-lasting insecticide-treated net
LSM	larval source management
MOH	Ministry of Health
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
WHO	World Health Organization

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