Chapter 5

Phylogeography, Vectors and Transmission in Latin America

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1. Introduction

The overall focus of this chapter is the impact of phylogeographic studies on information pertinent to vector control, and an update on the relative importance and taxonomic status of five malaria vectors, some of which are species complexes, in the subgenus *Nyssorhynchus*: *Anopheles albimanus* Wiedmann, Albitarsis Complex, *Anopheles aquasalis* Curry, *Anopheles darlingi* Root, and *Anopheles nuneztovari* s.l. Gabaldón, considering literature predominantly since 2000. This cut-off date is to avoid repetition or overlap with some of the same subjects that have been covered in other places [1-4].

It is also of interest to vector control and elimination programs that, since 2000 and a more recent compilation that included a list of confirmed or potential Latin American malaria vectors [5], some vector species have been implicated in additional regions or countries by enzyme-linked immunosorbent assay [ELISA; 6], PCR techniques [7], VecTest [8] or more definitive biological and epidemiological evidence has been provided. Examples of these include *An. rangeli* Gabaldón, Cova Garcia and Lopez, initially implicated in Amapá state, Brazil [9] and subsequently in Putumayo, southern Colombia [10]; and *An. triannulatus*, (Neiva and Pinto) incriminated more broadly from Amazonian Brazil [11], then locally from Amapá, Brazil [12]. Furthermore, based on high frequency, biting behavior, seasonality, ELISA and nested-PCR, for the first time, *An. rondoni* (Neiva and Pinto) has been implicated in Matapá, Pará state, Brazil [13]. This is an understudied species, and its potential as a vector in other localities and regions in its distribution (Argentina, Bolivia, Brazil) is worth investigating.

A relatively early summary of information on the five most important malaria vectors in Latin America was published in 1986 [14]. This publication focused on four species in the *Nysso-*

rhynchus subgenus: *Anopheles albimanus*, *Anopheles aquasalis*, *Anopheles darlingi*, *Anopheles nuneztovari* and one in the *Anopheles* subgenus, *Anopheles pseudopunctipennis* Theobald. Naturally, more than 20 years later, this list of five is debatable, although most researchers would still consider *An. darlingi* to be the primary vector overall, and *An. albimanus* to be one of the most important. Nevertheless, some aspects of this publication are still relevant, and it serves as a useful historical introduction.

A review published in 2012 [15] summarized the overall findings of much of the available literature on genetic diversity of malaria vectors, including those in Latin America, and concluded that Pleistocene (0.01-2.6 mya; 16) environmental changes have been the primary drivers of divergence, at least at the species and population levels. These changes and earlier ones during the Miocene (2.6-5.3 mya)/Pliocene (5.3-23.0 mya) were hypothesized to have influenced the phylogeography of some co-distributed neotropical vector species, including *Anopheles darlingi* and selected Albitarsis Complex members [17].

2. Biology and vector status

2.1. *An. albimanus*

Throughout its broad, mostly coastal distribution (Figure 1), *Anopheles albimanus* is an important local vector and is considered to be ecologically adaptable (18). In general this species is crepuscular, zoophilic, exophagic, exophilic and seasonally abundant (19-20). Despite heteogeneity of several attributes, such as host-feeding behaviour, longevity, insecticide resistance and susceptibility to Plasmodium species, thoughout its distribution, it has maintained single species status (20).

2.1.1. Colombia

An. albimanus is distributed in Colombia along the Atlantic and Pacific coasts (Figure 1). It is the main malaria vector on the Pacific coast, but its presence is considered a risk factor in other regions, even where malaria transmission is low [22]. Adult abundance of *An. albimanus* is associated with malaria transmission. The El Niño-Southern Oscillation Event (ENSO), that affects global climatic conditions every 2 to 7 years, has been strongly associated with increases in malaria cases, particularly in areas where *An. albimanus* is the main malaria vector, such as the Pacific coast [23]. *An. albimanus* breeding sites are very diverse, ranging from temporary small ponds to lagoons, and even include artificial containers. Its human biting rates can range between a few specimens per night night up to thousands, depending on the availablity of breeding sites in and around villages. Despite its considerable distribution on both coasts, *An. albimanus* has been found naturally infected with *P. vivax* only along the Pacific [24] (Table 1). In this region, its biting activity shows at least two peaks, one around midnight and a second one of less intensity before dawn, both indoors and outdoors [4]. Consistent use of insecticide-treated nets (ITNs) in this region could potentially reduce malaria transmission risk. In the Buenaventura peri-urban area, around 20% of the bites occur indoors and the main biting activity is outside houses between 18-21h. Then, at midnight, there is a second peak inside houses. Finally, between 05-06h, activity increases again outdoors [4].

Figure 1. Distribution of *Anopheles albimanus* highlighted in green [21].

Taxon	Country	Local transmission	Regional transmission	Evidence	Reference
An. albimanus	Colombia		Pacific region	ELISA, PCR	24
Albitarsis Complex*	Brazil	Marabá, Pará; Matapi River, Amapá; Amazon region; Macapá, Amapá; N. Amazon region, Roraima; Boa Vista, Roraima; Serra do Navio, Amapá	Amazon region	ELISA, PCR, VecTest	9; 11-13; 25-28; Póvoa 2010 (unpub. data)
	Colombia	Puerto Carreño, Vichada		ELISA	29
	Venezuela	Sifontes, Bolivar		ELISA	30-31
An. aquasalis	Brazil	São Luis, Maranhão; Belém, Pará		ELISA	32-33
	Guyana	Mahdia		VecTest	34
	Suriname	Brokobondo; Galibi; Paramaribo		ELISA	35
	Venezuela	coastal areas			1
An. darlingi	Brazil	Marabá, Pará; Belém, Pará; Matapi River, Amapa; Macapá, Amapá; Boa Vista, Roraima; Serra do Navio, Amapá; Pará, N. Brazil; Anajás, Pará; Goianesia do Para, Pará	Amazon region	ELISA, PCR	9; 11-13; 25; 27-28; 33; 36-37; Póvoa 2012 (unpub. data)
	Colombia	Puerto Carreño, Vichada; Quibdó, Chocó; Dibulla, Guajira	Córdoba; Villavicencio, Meta; Putumayo	ELISA	4; 10; 29; 38-41
	French Guiana	Camopi; Saint Georges de l'Oyapock; Maroni River; Upper Maroni Amazonian forest; Village of Loca; Village of Twenke		ELISA	42-45
	Peru		eastern region	ELISA	46
	Suriname	Maroni River		ELISA	43; 47

Taxon	Country	Local transmission	Regional transmission	Evidence	Reference
	Venezuela	Sifontes, Bolivar; Upper Orinoco River (southern)		ELISA	30-31; 48
An. nuneztovari s.l.	Brazil	Matapi River, Amapa; Serra do Navio, Amapá; Anajás, Pará	Amazon region	ELISA	9; 11-12; 37
	Colombia	Tierralta, Córdoba		ELISA	38
	French Guiana	Saint Georges de l'Oyapock		ELISA	42
	Venezuela	Ocidente de Venezuela		ELISA	49

*, Reference 27 refers to *Anopheles albitarsis* E (now *An. janconnae*) and Póvoa 2010 (unpublished data) refers to the first evidence of *An. oryzalimnetes* as a potential vector. References 12 and 25 refer to *An. marajoara* as a vector, while the remaining references refer to *An. albitarsis* s.l. as vectors.

Table 1. Regional South American vectors subgenus *Nyssorhynchus*: evidence for malaria vector status.

2.1.2. Peru

An. albimanus in Peru is considered the main malaria vector along the Pacific coast, particularly in the north, where it is seasonal, and linked to agriculture [50]. Due to high insecticide application, mainly in rice fields, *An. albimanus* is resistant to all insecticides used in public health in this area [51]. Flooded rice fields provide ideal mosquito breeding habitat and *An. albimanus* density is associated with rice crops. Since 2005, the Peruvian Minister of Health, together with the Agricultural sector, implemented a modified irrigation system, so that the fields are dry for a week, and then intermittently irrigated, resulting in a decrease in mosquito larvae by 87% [52]. An important follow-up question would be whether this *An. albimanus* larval control has actually resulted in a decrease in malaria incidence rate (MIR), or in local *An. albimanus* adult female abundance, as measured by human biting rate (HBR) and entomological inoculation rate (EIR).

In several other South American countries (Ecuador, Panama, Venezuela) where *An. albimanus* is a malaria vector, as determined by sporozoite detection or other comparable information in earlier studies, data based on newer techniques are not available. However, some recent investigations have drawn attention to new distributions or larval habitat characterizations that pinpoint areas of fruitful potential research and possible targets for control measures [53-56].

2.2. Albitarsis Complex

Presently, there are eight recognized species (*An. albitarsis* s.s., *An. albitarsis* F, *An. albitarsis* G, *An. albitarsis* I, *An. deaneorum*, *An. janconnae*, *An. marajoara*, *An. oryzalimnetes)* and one lineage (*An. albitarsis* H) in the Albitarsis Complex [57]. The species described as near *An. janconnae* from Colombia [58] is now considered to be *An. albitarsis* I [57]. The overall distribution of members of this complex is wide-ranging, including both Central and South America, as well as some Caribbean Islands [59] (Figure 2).

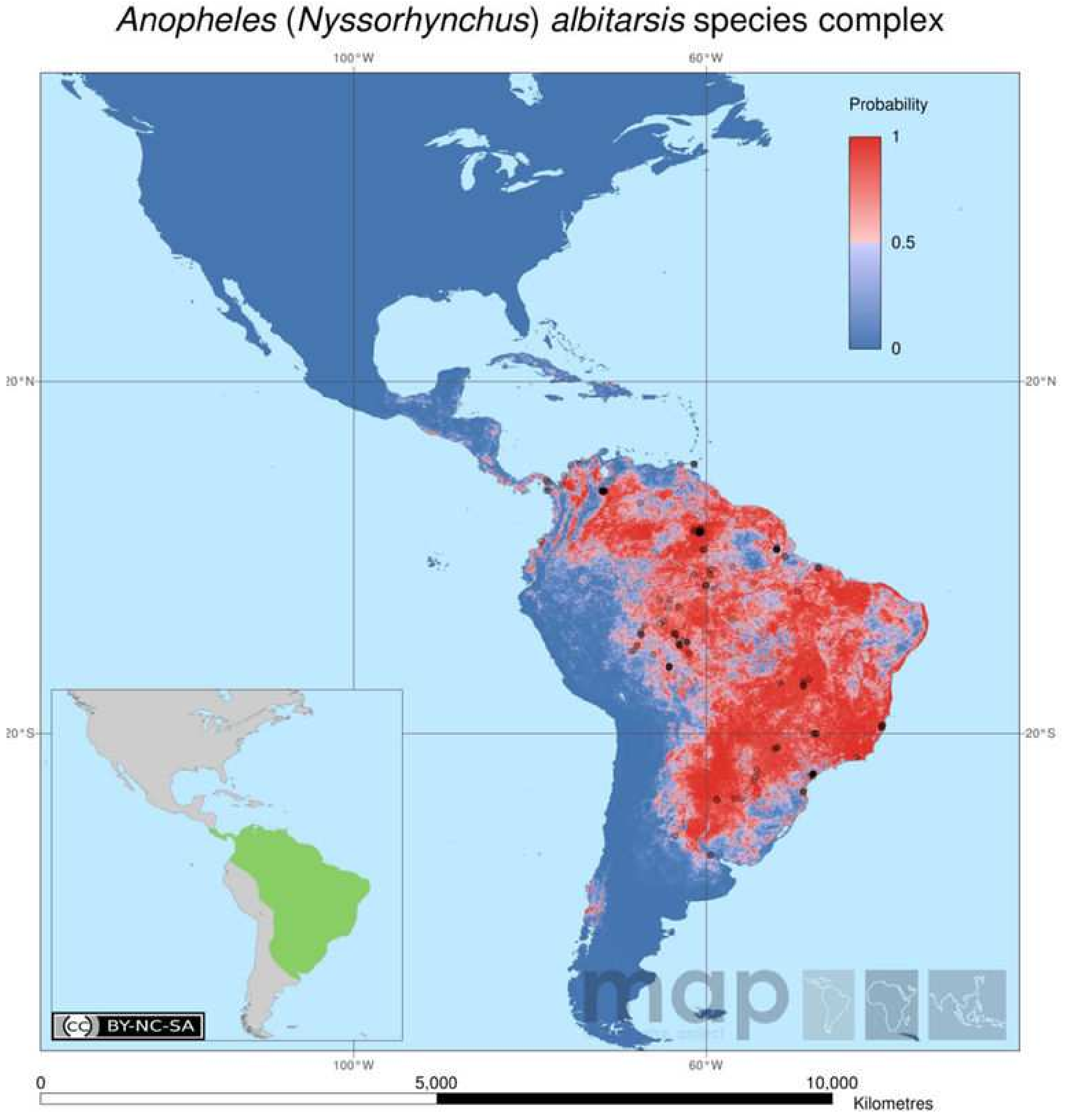

Figure 2. This map shows the predicted probability of occurrence of *An. albitarsis* in the Americas [2]

2.2.1. Brazil

Six species of the Albitarsis Complex are known from Brazil to date: *An. albitarsis* s.s., *An. albitarsis* G, *An. deaneorum*, *An. janconnae*, *An. marajoara*, and *An. oryzalimnetes*, [57,60] (Figure 3). The most broadly distributed member of this complex is *An. marajoara* Galvão and Damasceno [57,59]. It can be very abundant locally [12], and its breeding site types vary from swampy shores of lakes and ponds to small road puddles; it is generally associated with sunlight and often with aquatic or semi-aquatic vegetation [59]. It has been found infected by *Plasmodium falciparum* (Welsh), *Plasmodium vivax* (Grassi & Feletti) 210 and *P. vivax* 247, and *Plasmodium malariae* (Grassi & Feletti), and is a peri-urban as well as a rural vector, depending on locality, availability of breeding sites and hosts [12, 25]. It is also associated with deforested areas of the Vale do Ribeira in the southeastern Atlantic Forest of Brazil [61]. Although the EIR in Amapá state, Brazil was found to be lower than that of *An. darlingi*, it is an important local vector, at least in lowland rainforest in parts of the eastern Amazon [12, 62].

The distribution of *An. janconnae* Wilkerson and Sallum [classified previously as *An. albitarsis* E; [27, 60] appears to be limited to northern Amazonian Brazil, including along the Amazon River [57, 60]. Larval habitat types in several localities in Roraima state ranged from marsh to seepages to stream margins, and, based on analyses of several environmental variables, *An. janconnae* could be classified as a habitat specialist [McKeon, Conn & Povoa, unpublished data, 2012]. *An. janconnae* was incriminated as a local malaria vector around Boa Vista, the capital of Roraima state [27]. It is likely that the infected specimens identified as *An. albitarsis* s.l. from this region [26, 28] (Table 1), are *An. janconnae*, at least according to the geographic distribution [57].

An.oryzalimnetes (Wilkerson and Motoki), previously *An. albitarsis* B [60] has a broad distribution in Brazil that includes the Amazon region and southern Brazil [57]. It is frequently associated with rice fields, and is anthropophilic [60]. It was determined to be positive by ELISA for *Plasmodium* in Pará state [M.M. Povoa 2010, unpublished data], and may play a role in local transmission.

2.2.2. Colombia

At least three members of *An. albitarsis* s.l. are present in Colombia. *Anopheles marajoara* (some collections of which, according to the map [57], may be *An. albitarsis* I) is widely distributed [22], and its biology is similar to that described above under *An. marajoara* in Brazil. It is conisidered to be a regional vector in Colombia [22, 39, 63]. In the municipality of Puerto Carreno in eastern Colombia near the Venezuelan border, it was detected infected with *P. falciparum* [29] at a surprisingly high rate (1.92%; 3/152 specimens infected). Here, its peak biting time was 18-19h, with a minor peak from 20-21h, and it was collected both indoors and outdoors. It is suggested that together with *An. darlingi*, the dominant vector in the area, it is responsible for maintaining local malaria transmission in this municipality [29].

The second member of the complex is sympatric with *An. darlingi* in the east, and probably involved in malaria transmission. This species has been identified by various names, including *An. allopha*, *An. marajoara*, near *An. janconnae* [58], and most recently as *An. albitarsis* I [57]. Its known distribution thus far is restricted to Colombia. Relatively little is known about its biology, because of species identification issues, and there is no direct evidence yet for its involvement in malaria transmission. It appears that the specimens from Vichada, Colombia, identified as *An. marajoara* [64], are *An. albitarsis* I [57], so the distribution of *An. marajoara* in Colombia, and its involvement in malaria transmission, need re-evaluation.

The third species, *An. albitarsis* F, was first described from Puerto Carreño, near the Venezuelan border, [65]. In this locality it was found in sympatry with *An. darlingi* and a species in the Albitaris Complex now defined as *An. albitarsis* I. Its distribution is hypothesized to include Colombia, Venezuela and Trinidad [57]. Because it can easily be confused morphologically with *An. marajoara*, and it is found in regions of malaria endemnicity, this species is of some epidemiological importance.

2.2.3. Venezuela

It now appears that at least *An. albitarsis* I and possibly also *An. albitarsis* F are present is Venezuela [57]. Furthermore, *An. albitarsis* I could be sympatric in some regions with *An. marajoara* (the identification of which needs to be confirmed in Venezuela using molecular techniques), a local vector of *P. vivax* in western Venezuela [66]. *An. marajoara* also plays a significant role, together with *An. darlingi*, in malaria transmission in five localities in southern Venezuela [30-31]. In this gold mining region, where transmission is yearlong, the peak biting time, (19-21h, mostly before midnight), was comparable with most other reports of *An. marajoara*. Although *An. marajoara* was feeding both indoors and outdoors, it was significantly exophagic.

2.3. ***Anopheles aquasalis***

This brackish-water breeder is found along the Pacific as far south as Ecuador and along the Atlantic to southern Brazil [1,14] (Figures 3,4). It is rarely found far from the ocean, but it can tolerate quite low salt ion concentrations, and has been detected in freshwater springs. It can be present in enormous numbers in marshy coastal areas, so that even if it is not extremely susceptible to *Plasmodium*, it can maintain malaria transmission when its abundance is high, especially during the rainy season [67]. It persists as an important local vector in Sucre state, eastern Venezuela, where a series of pioneering studies have identified hotspots of local transmission that are very useful for prevention and control efforts [68-69]. It has also been incriminated as a vector of *P. falciparum* and [or] *P. vivax* in Maranhão [32] and Pará states in Brazil [33, 70]. In Linden and Madia, Guyana [34], *An. aquasalis* was detected infected with *P. vivax* using VecTest, but the total sample size of anophelines collected was very small (*n*=45). In three towns in Suriname, Paramaribo, Brokopondo and Galibi, *An. aquasalis* was also detected infected with *Plasmodium* by ELISA [35], and is likely responsible for local, coastal transmission in this region.

2.4. ***Anopheles darlingi***

The species considered to be the most important vector in the Amazon basin is *Anopheles darlingi* [2,3] (Figure 5). It is anthropophilic, adaptive and it has been incriminated in many localities in many countries, where it is often labeled a national vector (Table 1). As such, it has been the focus of a very wide range of research, monitoring and control efforts, and the publication for the first time of its complete genome is an exciting new development (GenBank accession number ADMH00000000).

2.4.1. Bolivia

An. darlingi is distributed in the northeastern Bolivian Amazon, in the departments of Pando, Beni and Santacruz de la Sierra, along the border with Brazil [71]. It shows a biting peak between 19-21h, with 83% of the bites occurring before 22h, when most local people go to bed. After this time, numbers decline, with little or no activity between 02:30-05h [72]. There have been relatively few studies on this species in Bolivia, and data are very scarce.

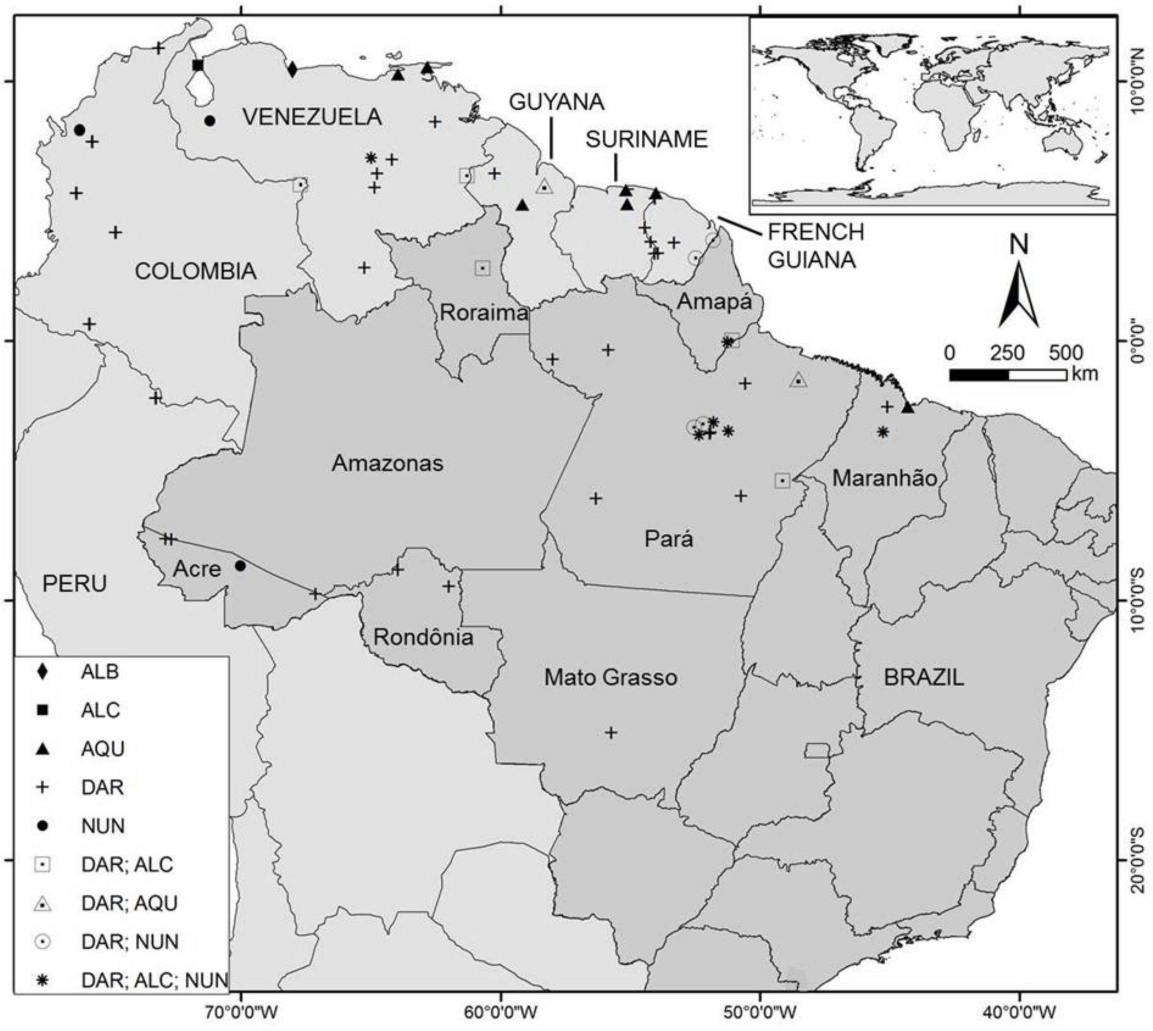

Figure 3. South American localities where malaria vectors have been incriminated by various methods since the year 2000. Species codes: ALB, *Anopheles albimanus*; ALC, Albitarsis Complex; AQU, *An. aquasalis*; DAR, *An. darlingi*; NUN, *An. nuneztovari*. The darker grey area is Brazil.

Figure 4. Distribution of *Anopheles aquasalis* highlighted in orange [21]

2.4.2. Brazil

Most of the newest incriminations of the continued involvement of *An. darlingi* in malaria transmission originate in Amazonian Brazil (Table 1, Figure 3). Rather than summarizing each new investigation, this section focuses on the findings on *An. darlingi* in a longitudinal study that investigated bloodmeal hosts, transmission, and seasonal abundance in three riverine villages along the Matapí River in Amapa state, northern Amazonian Brazil [12, 62, 73]. An ELISA analysis for IgG of common vertebrates found that the highest human blood indices (HBI) were in *An. darlingi* and *An. marajoara*. What was unexpected was that the HBIs of *An. darlingi* varied significantly among the three villages, which are only 1.5-7.0 km apart, likely because of host availability. It was found to be important to conduct a census of animals in each locality to be able to interpret the HBI results correctly. Even though *An. darlingi* was the most abundant species collected at human landing catches in each village, the HBI of *An. darlingi* resting collections, from under houses or in vegetation, ranged from 0.017-0.405, demonstrating how opportunistic this species can be, despite its anthropophily [12]. From the same study sites 113,117 mosquitoes collected from 2003-2005 were analyzed by ELISA. For this part of the study, *An. darlingi* and *An. marajoara* had the highest proportion of positives and also the highest EIRs, and thus the highest human-vector contact. Nevertheless, *An. darlingi* is still considered to be more important in this study area than *An. marajoara* because of its higher EIR [12]. Seasonal abundance was measured for 32 consecutive months of collection and showed that *An. darlingi* was most abundant during the wet-dry transition period between June and August, and that a strong positive correlation of *An. darlingi* abundance with rainfall lagged by several months. The latter finding may indicate that rainfall could be an important factor in predicting vector abundance, at least locally.

2.4.3. Colombia

The distribution of *An. darlingi* in Colombia is widespread but heterogeneous, and hypothesized to be interrupted by the Andes. It is found mainly south and east of the Andes, including the Amazon region, bordering Brazil and Peru, but also north and west of the Andes, along

Figure 5. Distribution of *Anopheles darlingi* highlighted in pink [21].

the main Colombian rivers (such as Magdalena, Cauca and Atrato) [22, 74]. *An. darlingi* is the main malaria vector throughout its distribution. Although associated with forest environments, it was also detected in the peri-urban area of the cities of Quibdó (Chocó) and Villavicencio (Meta), where malaria transmission occurs [40]. Breeding sites are the typical streams with slow water movement, but stationary water bodies such as natural and constructed fish ponds also provide good habitats. Similar to Peru (see below), the biting behaviour in Colombia is mostly before midnight, from 18-24h, with a smaller peak at sunrise (05-06h), but there is also persistant biting activity throughout the night [39].

2.4.4. French Guiana

In French Guiana, there has been renewed activity on malaria vectors, with most findings incriminating *An. darlingi* as the primary vector (Table 1). Between 2000-2002 in three Amerindian villages in the Upper-Maroni region of the Amazon forest, *An. darlingi* bit throughout the night, with peaks at 21:30-03:30h and again after 05:30h [44]. The biting rate was very high (255.5 bites/person/night) and specimens were infected with *P. falciparum*, *P. vivax* and *P. malariae*. Behavior was characterized as endo-exophagic and exophilic. The malaria transmission risk exists all year but probably it is greater during the rainy season when vectorial capacity was estimated to be higher [44]. A combination of ITNs and repellent is recommended; IRS is not efficient because of the housing materials and relative inaccessibility of this region. A second study in the Maroni area compared villages of Amerindian Wayanas and the Aloukous [45]. Significant findings include: the peak local malaria case reporting is the same timeframe (August to October) as the highest IMT (numbers of infected mosquitoes surviving long enough to transmit) of *An. darlingi*; the possibility that the persistent yearlong transmission is focused or perhaps limited to the Amerindian villages; and different bionomics of *An. darlingi* in the two villages which lead investigators to conclude that in this region there may exist two distinctive subspecies of *An. darlingi*. An analysis of collections from 2006-2011, in several regions of French Guiana, detected *An. darlingi* infected by *P. vivax* from Camopi and Saint Georges de l'Oyapock, both near the Oyapock River along the eastern border with Brazil [42].

2.4.5. Peru

An. darlingi is the main malaria vector species in eastern Amazonian Peru, the area with the highest malaria transmission in the country. It invaded this region in the 1990s [75], and its distribution now includes peri-urban settlements around the city of Iquitos, Loreto province. This change has been attributed to logging, agriculture and urban expansion, associated with deforestation [76-77]. To date, its greatest abundance is associated with areas of at least partial deforestation [78]. The main breeding sites in the Peruvian Amazon are streams and river margins in forested areas [77], however, the density of fish ponds has shown a positive association with malaria cases along roads in Loreto, suggesting that such ponds could be important local sources of this species [79]. *An. darlingi* was found naturally infected with *P. vivax* and *P. falciparum* in Loreto [46]. The human biting activity, which is similar indoors and outdoors, peaks two hours after sunset [77]. Because of the early evening biting peak, personal protection may be necessary to supplement bed-net use.

2.4.6. Panama

An. darlingi was recently detected in Panamá for the first time, in the eastern Darien region, near the border with Colombia, associated with the highest prevalence of drug-resistant *P. falciparum* [80]. This species was only collected by human landing catch, and not recorded in light traps, confirming its high anthropophily. Despite the extension and increased frequency of deforestation in Panama, *An. darlingi* has been detected only in the east. Its discovery suggests that unplanned deforestation should be avoided to prevent further expansion of this very anthropophilic species, and hence potential *P. falciparum* transmission, to other regions in the country.

2.4.7. Suriname

A timely and important new development in Suriname is the apparent collapse of populations of *An. darlingi* in the sparsely inhabited interior, in and around three study communities, correlated with two main factors: the introduction of ITNs and climatic events, *i.e.*, unusual flooding which coincided with the beginning of the control activities in 2006 [47]. However, it should be noted that indoor residual spraying (IRS), active case detection (ACD), and a public awareness campaign also were implemented throughout the interior in 2006. As the authors point out, for Suriname, the next challenge is to try to find ways to use these methods to reduce or eliminate transmission among the gold-mining communities, where people are very mobile, and often active (not using ITNs) during potential biting times of *An. darlingi*. The latter are notoriously plastic, and vary locally and regionally. An important determination will be whether these results [47] can serve as a model for some communities where *An. darlingi* and malaria transmission are endemic in other countries.

2.4.8. Venezuela

Investigations along the Upper Orinoco River, southern Venezuela from 1994-1995 confirmed that *An. darlingi* was responsible for most, if not all of the local transmission of *P. falciparum*, *P. vivax* and *P. malariae* [48], that children under the age of 10 were at greatest risk, and that the EIR of *An. darlingi* was 129 positive bites/person/year. In a gold-mining region in southern Venezuela, studies from 1999-2000 [30-31] also determined that *An. darlingi* was one of two main vectors (the other was *An. marajoara* but see above under Albitarsis Complex). Surprisingly, many of the bionomic aspects of the two species in the five localities studied were quite similar (both more abundant during the rainy season, both biting indoors and outdoors with pronounced endophagic behavior), although *An. marajoara* was more abundant overall. The most striking bionomics difference between the two species was the peak biting time: *An. darlingi* bit throughout the night with two minor peaks (23-0h and 03-04h), whereas *An. marajoara* had a peak from 19-21h [30-31].

2.5. *Anopheles nuneztovari* s.l.

Anopheles nuneztovari s.l. is restricted to northern and Amazonian South America (Figure 6) and has been considered to be two genetically, ecologically and epidemiologically distinctive geographic populations, with the perception that the Colombian/western Venezuelan population was a regional vector (anthrophophilic and endo-exophagic) and the Amazonian population, mostly zoophilic and exophagic, was not [1, 5, 11]. Initially, evidence for malaria transmission by *An. nuneztovari* s.l. was found predominantly in Colombia and western Venezuela [22, 49]. However, a series of positive ELISA results and incriminations of malaria transmission involvement from localities in the Brazilian Amazon (Table 1; Figure 3) since 2000 soon undermined this relatively simple view. *An. goeldii*, which had been synonymized with *An. nuneztovari*, was resurrected as a valid species [81]. This work proposed different geographical distributions for each species, with *An. goeldii* in the Amazon region and *An. nuneztovari* more restricted to Colombia and Venezuela. The report of the discovery of *An. nuneztovari* infected with *Plasmodium* from Saint Georges de l'Oyapock, French Guiana, using results from a longitudinal study (2006-2011) is of at least local relevance, but it will be taxonomically important to determine whether this species is actually *An. nuneztovari*, or might possibly be *An. goeldii*, since susceptibility of *An. goeldii* to *Plasmodium* has not yet been tested [42].

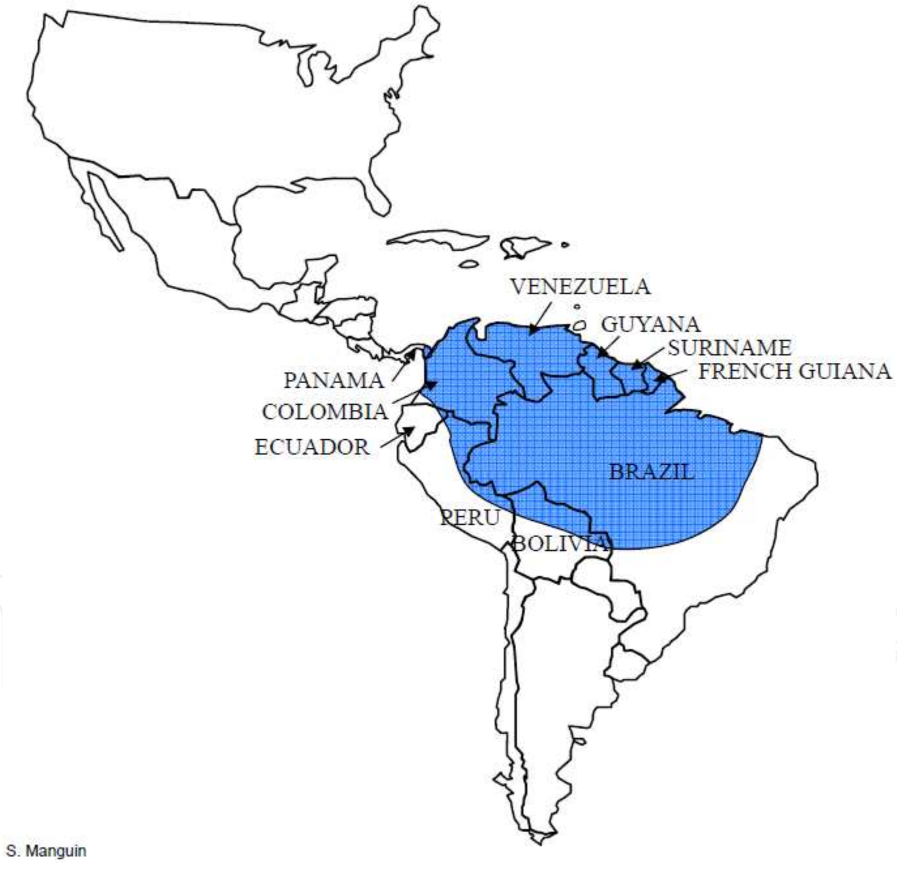

Figure 6. Distribution of *Anopheles nuneztovari* s.l. highlighted in blue [21].

An. nuneztovari s.l. is widely distributed in Colombia [22], particularly in the east, along the Venezuelan frontier, in the northwest region (Departments of Córdoba and Antioquia), where approximately 50% of the malaria cases occur, and in some areas along the Pacific

Coast in the west, notably along the San Juan river (Chocó), and in the Buenaventura area (Valle). Specimens from Tierralta, Córdoba, a region of crop and livestock production, where *An. nuneztovari* s.l. was the most abundant species collected by human landing catches, were infected by *P. vivax* [38]. The breeding sites include small, permanent ponds, sunlit flooded pastures, and it has been determined that aquaculture ponds are one of this species' most frequent breeding places. In the west, in Cimitarra (Santander), such ponds, characterized as permanent, completely exposed to sun and containing emerging vegetation, particularly grasses, represent approximately 81% of the breeding sites [63]. In Colombia, *An. nuneztovari* s.l. shows differing biting behavior by region. An exophagic tendency has been described in the northwest (Córdoba) [82], whereas in the east (Santander), a more endophagic behavior has been described [63]. The endophagic-exophilic variability makes control by residual insecticides very difficult.

3. Phylogeography

3.1. *Anopheles albimanus*

An exemplary study, based on large sample sizes using microsatellite markers and a mtDNA *ND5* gene fragment, laid the groundwork for several *Anopheles albimanus* phylogeographic ideas [83]. These researchers detected restricted gene flow that they hypothesized to be the result of the physical barrier of the Central American Cordillera. Recent work, more geographically focused on one country or a region, with additional local sampling, provides additional insights into phylogeography in Central America [20, 85] and Colombia [84].

A mitochondrial DNA *COI* gene fragment and microsatellites were used to test for congruence with biogeographical provinces [86] in Colombia. In this case [84], one population, Turbo, was from Magdalena (Caribbean), three were from Maracaibo (Caribbean), and the four were from Choco (Pacific). The eight populations tested were clearly differentiated into two coastal regions, Caribbean and Pacific, with evidence for a late Pleistocene expansion (estimated to 21,994 years ago) or a selective sweep. Even though there was evidence for historical restrictions to gene flow (*COI* data), the microsatellites detected contemporary gene flow between the regions. Interestingly, a SAMOVA analysis found an unusual division. Only the three most easterly populations along the Caribbean coast grouped together. The fourth and most western Caribbean population, Turbo, was consistently more closely related to the four Pacific populations. Taken together, these data suggest possible semi-permeable boundaries among the three biogeographical provinces tested. Most relevant to malaria is the fact that the evidence for contemporary gene flow indicates that insecticide resistance genes, for example, could spread readily in these Colombian regions [84].

An. albimanus from Central America was examined using a fragment of the mtDNA *COI* gene to test the original hypothesis [83]. Physical barriers to gene flow were not detected (i.e., the Central America Cordillera was porous for *An. albimanus*) and contemporary isolation by distance was not supported [20]. Three divergent, co-occuring haplotype groups were detected using a statistical parsimony network, and these were not evenly distributed across Costa Rica and Panama. A new hypothesis suggested that they could be the result of multiple introductions into the region, probably caused by historical fragmentation and subsequent secondary contact. A more wide-ranging study incorporated the samples from Colombia [84], Ecuador, and Nicaragua with those from Costa Rica and Panama [20] and added two molecular fragments: the nuclear *white* gene and the ITS2. A SAMOVA analysis defined three large population demes, one from Nicaragua, Costa Rica and the Atlantic coast of western Panama; a second one incorporating the Pacific coast of western Panama, central-eastern Panama and the Caribbean Colombian coast; and a third one restricted to the Pacific coast of Colombia and Ecuador [85]. There were also four haplogroups, based on the *COI* fragment, which differed little from those found in the earlier Panamanian study [20] except for the addition of a fourth, restricted to the Pacific coast of Colombia and Ecuador, and separated by 18 mutation steps from its nearest haplogroup. Interestingly, because it tracks an earlier history, the *white* gene network showed much less divergence, supporting the overall conclusion that the primary time-frame for anopheline divergence at the species level is Pleistocene [15]. In summary, the combined *An. albimanus* data set strongly supported the presence of a single species in this region, which was expected, but also found very robust evidence for Pleistocene geographic fragmentation followed by range expansion across southern Central America [85].

3.2. The Albitarsis Complex

Following the newest revelations about the number of species (eight) plus a novel lineage (*An. albitarsis* H) in *An. albitarsis* s.l. [57], parts of an earlier study on the biogeography and population genetics of this complex [17] need to be reconsidered and modified. This is particularly the case for *An. janconnae*, which is more restricted than thought (under the taxonomic name of *An. albitarsis* E), the expanded distribution of *An. albitarsis* F (which now includes Venezuela and Trinidad as well as Colombia), the complexities of the distribution of *An. marajoara*, which really may have a very broad range, newly described *An. albitarsis* G, distributed along the Brazilian Amazon, and *An. albitarsis* I, restricted to northwestern Colombia [57]. Despite these problems, one recent study can be used to illustrate the phylogeography of at least *An. albitarsis* G [as far as it is known; 57] and part of the range of *An. marajoara* [87]. *An. albitarsis* G [lineage 2 in reference 87] may be restricted to localities near the Amazon River or its tributaries. It has little population structure and the small subdivisions that were detected in haplotype networks were unrelated to geographic locality. The evidence from the mtDNA *COI* fragment used in this study indicates that this lineage is older than *An. marajoara* [lineage 1 in reference 87]. On the other hand, the *white* gene and ITS2 data detected a single network between *An. albitarsis* G and *An. marajoara*, indicating that the divergence is recent. The most compelling result in this study concerning *An. marajoara* is that SAMOVA defined two population demes along the Amazon River, splitting this species into western and eastern entities with differing genetic characteristics. The boundary is located near Rio Jari in Amapá state, not far from one detected in *An. darlingi* [88] and an earlier one seen in a study of *An. nuneztovari* s.l. using restriction fragment length analysis of the mtDNA genome [89]. For *An. marajoara*, this boundary is permeable, since there were shared haplotypes on either side [87]. A denser sampling of all three species could more rigorously test whether this is the result of underlying geological boundaries or perhaps more recent climatic events.

3.3. *An. darlingi*

Several studies on the phylogeography of *An. darlingi* have been undertaken. The earliest one [90] used the mtDNA *COI* fragment and detected a significant genetic division between Central America/northwestern Colombia, and the rest of South America. According to the statistical parsimony network, the more widespread and ancestral haplotypes were in Amazonian and southern South America, suggesting that the Central American/Colombian haplotypes may have originated there. This division was also supported by sequences of the *white* gene, which found two genotypes, genotype I, restricted to the Amazon, and genotype II, in northwestern Colombia and Venezuela, and Central America [91]. A microsatellite analysis of 1,376 samples also strongly supported the initial *COI* genetic division, and found substantial structure within the Amazon Basin [91]. The conclusion was that there were two main drivers for this division: differences in effective population size among the divisions, and physical distances between the populations. A more sophisticated analysis of the mtDNA *COI* fragment included additional Brazilian samples and excluded the Central American samples [88]. These researchers detected six main population groups in South America, and found ancestral distribution to be central Amazonia. They proposed that populations became isolated by three barriers: the Amazon River, the Andes and the southeastern Brazilian coastal ranges. They also found that limited dispersal across some landscape types has promoted differentiation between other proximate populations. A local study of *An. darlingi* in Córdoba and Antioquia, Colombia, using mtDNA *COI*, microsatellites and the *white* gene [74] supported the earlier geographic hypothesis [90], discovering that the five populations tested were more closely related to the Central American populations of *An. darlngi* that they were to South American *An. darlingi*. Because of local high gene flow among the five populations, similar control strategies could be implemented in these two contiguous Colombian states. Similarly, newly detected *An. darlingi* from Panama were most closely related to Colombian and Central American *An. darlingi* [80]. Concordant phylogeographies were determined for the two neotropical vectors *An. darlingi* and *An. triannulatus* [92]. With the mtDNA *COI* fragment, SAMOVA detected four similar population subdivisions: one in southern coastal Brazil, two in central Brazil and one northeast of the Amazon. Both species originated south of the Amazon River and seem to have followed a similar expansion pathway to their present-day distributions. Other neotropical anophelines with similar distributions may share a common spatial and demographic history with these species, and remain to be evaluated.

3.4. *An. aquasalis*

The only study that attempted to analyse *An. aquasalis* within a phylogeographic framework was conducted using a fragment of the mtDNA *COI* gene with specimens from five localities on either side of the Amazon, in Amapá and Pará states, Brazil [93]. The most important findings from this study inferred that despite the width of the mouth of the Amazon, this freshwater delta was not a barrier for the salt-water tolerant *An. aquasalis*, likely because of so much tidal mixing, and the numerous islands and channels in the region. However, gene flow was restricted, based on isolation by distance that was detected using a Nested Clade Analysis [94]. The relative regional importance of *An. aquasalis* as a malaria vector has waned since the earlier publications [1, 14], so there may be fewer opportunities to pursue phylogeographic questions, especially because the distribution is relatively limited. However, no one has compared specimens from the Atlantic and Pacific coasts, and it is possible that population structure similar to that found for *An. albimanus* [85], could be detected in *An. aquasalis*, considering that both species share a relatively narrow coastal distribution in South America, and were subjected to the same kinds of Pleistocene environmental changes.

3.5. *An. nuneztovari* s.l.

The revision of the taxonomic status of *An. nuneztovari* s.l., that now includes *An. nuneztovari* s.s. and *An. goeldii* [81] has implications for the interpretation of the first study of *An. nuneztovari* phylogeography, which focused on the nuclear *white* gene [95]. Five lineages were detected [95], 2 and 3 in Colombia/Venezuela and 1, 4 and 5 in Amazonian Brazil. The earliest divergence, during the Pliocene (5.3-23.0 mya), is between Colombia and Venezuela west of the Andes (lineage 3) and Amazonian lineage 4. The most likely hypothesis to explain this divergence is an early uplift of the East Andean Cordillera [96]. Curiously, the levels of genetic divergences among the five lineages were high, although the minimum spanning network of the haplotypes connected all of them. There were five localities where two lineages were sympatric: in Brazil - Boa Vista, Roraima state; Altamira, Pará state and near Pôrto Velho, Rondonia state; Guayaramerín, Beni, Bolivia; and Rio Socuavó, Zulia, Venezuela. These localities are of special interest, since they may be admixture zones or hotspots of divergence. The simplest hypothesis to explain the five lineages taxonomically is that the two in Colombia/Venezuela are *An. nuneztovari* s.s. and the three in Brazil are *An. goeldii*. The sharing of haplotypes across the Andes, between eastern and western Venezuela [95], is congruent with and supports findings for *An. albimanus* [83], The Albitarsis Complex [57] and *An. darlingi* (Conn, unpublished data) that have hypothesized that the eastern Andean Cordillera is only a partial barrier for anopheline mosquitoes.

A second phylogeographic study was undertaken with some of the same samples, plus new ones from Amazonian Brazil, using a mtDNA *COI* fragment [97], which charts a more recent history of divergence, all within the Pleistocene, compared with the *white* gene fragment. In this work, there were two major monophyletic clades, I and II. Specimens from Bolivia/Colombia/Venezuela represent the most basal subclade, IIC; whereas the Amazonian specimens were found in clades I and II-A and II-B. There were also several localities of sympatry among the clades: five in Amapá, Amazonas and Pará states, Amazonian Brazil, and one in Suriname. None of these are the same as the ones detected by the *white* gene study, perhaps suggesting that these were later areas of sympatry. There was an intriguing connection detected between the specimens from Colombia/Venezuela and those from Amazonian Bolivia, which had previously been seen when sequences of the rDNA ITS2 were used [98]. This may be the signature of the marine incursion hypothesis [95]. One of clades I, II-A or II-B likely represents *An. goeldii*, but additional analyses are needed to determine which one, and also to test the hypothesis of multiple species in the Amazon.

4. Conclusions

There have been many changes in the incrimination, identification and several new insights into the phylogeography of the species discussed in this chapter. The most important taxonomic changes are those in the Albitarsis Complex, with the discovery of two new species, *An. albitarsis* G and I, and a new lineage, *An. albitarsis* H. Nothing is known about their involvement in malaria transmission, although their ranges all include malaria endemic areas, or their local contribution to diversity or to a better understanding of the complex patterns of Amazonian biogeography and phylogeography. The relative paucity of new work on *An. aquasalis* is a reminder that its relative importance appears to be lessening, although it is still likely to be important locally, particularly when in high abundance. Obviously, the importance of *An. darlingi* in still on the rise in several localities in many countries, attributable mainly to its remarkable adaptability and association with landscape changes. The resurrection of *An. goeldii* from synonymy is also a milestone, because it provides a first step toward resolving a longstanding discussion about the possible importance of *An. nuneztovari* s.l. in local transmission in Amazonian Brazil. It may also clarify some aspects of the recent phylogeographic inferences based on *white* and the mtDNA *COI* genes. Lastly, the detection of concordant phylogeographies, one of which is *An. darlingi* in Brazil, depict a clear path towards future research which will have important epidemiological consequences.

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