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RECEIVED 29 April 2025

ACCEPTED 13 August 2025

PUBLISHED 16 September 2025

CITATION

Ortega-Monroy R, Tomasini-Ovilla HA,
Santillan-Valenzuela F, Montoya A and
Gonzalez-Ceron L (2025) Molecular
polymorphism of *Plasmodium vivax* Duffy
binding protein domain II from Nicaragua,
and global diversity patterns.
Front. Malar. 3:1620450.
doi: 10.3389/fmala.2025.1620450

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Molecular polymorphism of *Plasmodium vivax* Duffy binding protein domain II from Nicaragua, and global diversity patterns

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Background: *Plasmodium vivax* Duffy binding protein domain II (PvBDP_{II}) is a promising vaccine candidate due to its crucial role in reticulocyte invasion. However, polymorphism is a concern as it may confer evasion of blocking antibodies. In this study, nucleotide and protein polymorphism were analyzed in parasites collected during a decline in malaria cases in Nicaragua.

Methods: Genomic DNA was extracted from *P. vivax* blood samples obtained from symptomatic patients in Nicaragua during 2012–2013. The *Pvdbp_{II}* gene was amplified and sequenced, and genetic structure, genealogical relationships, and amino acid polymorphism were analyzed. For comparison and to elucidate global variation of this gene, homologous sequences from other geographical regions were included.

Results: Sixty-three consensus sequences from Nicaragua were obtained, revealing 12 non-synonymous mutations and 6 haplotypes (Hd=0.704). Nucleotide diversity was lower ($\pi=0.0044$) than in other endemic regions. The Z test of selection (dN/dS) was positive (3.17; $p < 0.001$), similar to parasite populations worldwide (Latin America, Middle East, Asia, Southeast Asia, Africa, Papua New Guinea). Network analysis revealed that Nicaraguan *pvd_{II}* haplotypes differed by 3–12 mutational steps. High-frequency haplotypes from six other Latin American countries were shared, showing signs of diversification and limited population structure. Five Nicaraguan haplotypes corresponded to the 10 most frequent globally. Haplotypes defined solely by amino acid changes at positions 417, 437, and 503 were of the Sal-I type (NWI), NWK, and KRK. These, along with KRI, were shared across most regions at varying frequencies. Based on these variations, two main divergent groups were identified.

Conclusions: The low diversity observed in *pvdbp_{II}* suggests a population contraction aligning with the decline of malaria cases in Nicaragua during the sampling period. The *PvDBP_{II}* haplotypes found may represent those best adapted in Nicaragua and in other endemic regions globally, encompassing both Sal-I-related and divergent types. It would be beneficial to assess the ability of the most frequent and persistent haplotypes to elicit phenotype-transcending immunity, which is critical for the development of a multicomponent vaccine and for monitoring its effectiveness.

KEYWORDS

Plasmodium vivax, Nicaragua, *PvDBP_{II}*, polymorphism, nucleotide diversity

Introduction

Malaria is caused by protozoan parasites of the genus *Plasmodium* and transmitted by female mosquitoes of the genus *Anopheles*. More than 85 countries are at risk of malaria transmission. In 2023, there were 263 million clinical cases, representing an increase of 11 million compared with the previous year (World Health Organization, 2024).

In the Region of the Americas, malaria cases declined by 65.4%, from 1.5 million to 0.6 million between 2000 and 2022, and in 2023 (World Health Organization, 2024). *Plasmodium vivax* accounted for approximately 73% of the 505,642 confirmed malaria cases, while about 27% were caused by *Plasmodium falciparum* (World Health Organization, 2024). The Central and South America have experienced significant changes in malaria transmission. In Nicaragua, malaria cases gradually decreased between 2000 and 2010, reaching more than 90% reduction (from 23,281 cases in 2000 to 896 in 2010 (World Health Organization, 2023). The number of cases fluctuated slightly between 2011 and 2014 (1,171–1,564 per year) and then drastically increased from 2,886 in 2015 to 23,323 and 16,158 in 2021 and 2022, respectively (World Health Organization, 2023). Although Nicaragua reported fewer cases in 2023 (6,716), it remained the country with the highest malaria burden in Central America (World Health Organization, 2024). Malaria elimination requires continuous assessment and improvement of control measures and strategies. Understanding parasite molecular diversity contributes to knowledge of disease epidemiology, delineates transmission patterns, and informs the development of vaccine candidates and antimalarial drug resistance monitoring. Studies of genetic and antigenic diversity can also help predict and monitor the effectiveness of interventions by detecting reductions in parasite diversity or the emergence of biologically important phenotypes (Arnott et al., 2012; Escalante and Pacheco, 2019).

P. vivax merozoites invade reticulocytes through complex interactions between parasite ligands and reticulocyte receptors. The Duffy binding protein (DBP) domain II (DBP_{II}) participates in the formation of a tight, irreversible junction with the erythrocyte

Duffy antigen receptor (DARC), which is essential for successful invasion (Singh et al., 2005). The *pvdbp* gene, located on chromosome six, encodes a 140-kDa type 1 integral membrane protein that is secreted into the microneme. Its structure contains seven domains: a signal peptide, two cysteine-rich domains (II and VI), three hydrophobic domains (III–V), and one transmembrane domain (VII) (Adams et al., 1992; Ranjan and Chitnis, 1999). The cysteine-rich DBP_{II} consists of 170 amino acids spanning residues 198–522. This domain is highly immunogenic, and during infection it induces blocking antibodies that prevent *P. vivax* merozoite invasion of reticulocytes (Michon et al., 1998, 2000). These blocking antibodies are thought to be the mechanism by which a *PvDBP_{II}*-based vaccine could confer protection, making this protein a promising target for vaccine development (Tsuboi et al., 1994). Both linear and conformational epitopes may be important in eliciting a protective immune response. Antibodies reactive to *PvDBP_{II}* linear epitopes have been detected in residents of endemic areas in Papua New Guinea following repeated *P. vivax* exposure (Cole-Tobian et al., 2002; Xainli et al., 2003; Cole-Tobian et al., 2007).

Analysis of nucleotide diversity in *pvdbp_{II}* provides information on circulating antigenic variants and the feasibility of a universal vaccine (Nobrega de Sousa et al., 2011). Domain II accounts for 93% of the polymorphism in the *PvDBP* molecule and may play a role in parasite immune evasion (Cole-Tobian et al., 2007; Chootong et al., 2014; Sousa et al., 2010; VanBuskirk et al., 2004). The 10 most frequent global amino acid variations in *PvDBP_{II}* include R308S, K371Q, D384G, Q385K, K386N/E, R390H, N417K, L424I, W437R, and I503K (Sousa et al., 2011; Almeida-de-Oliveira et al., 2020). Residues N417K, W437R, and I503K have been implicated in immune evasion (VanBuskirk et al., 2004). More recently, residues D309–A326, Q401–N417, L315–K334, and Q401–W420 have been identified as participating in conformational epitopes (Urusova et al., 2019).

Central America appears to be the last region colonized by *P. vivax*, with parasite populations exhibiting low diversity and similarity to the Sal-I strain (Taylor et al., 2013). Previous genetic studies using markers such as *pvama1* and *pvmdr1*

suggest that *P. vivax* in Nicaragua (Gonzalez-Ceron et al., 2017; 2023) may represent a unique population, possibly not exposed to pyrimethamine and distinct from South American populations (Gonzalez-Ceron et al., 2020). To characterize *PvDBP* diversity in Central America, this study analyzed nucleotide diversity and molecular polymorphism of *P. vivax* DBP domain II using isolates from Nicaragua collected in 2012–2013. Homologous sequences from Latin America and other geographical regions were included for comparative analysis and to assess global variation.

Materials and methods

The study was approved by the Ethics Committee of the National Institute of Public Health (CI1042) and the National Centre for Diagnosis and Reference, Ministry of Health, Nicaragua. The study followed bioethics guidelines (CITI program). All participants older than 18 years provided written informed consent, and minors aged 7–17 years gave assent accompanied by the written informed consent of one parent or guardian, in accordance with the Declaration of Helsinki. Patient personal information was encrypted, and only the municipality of residence was recorded.

P. vivax samples

P. vivax diagnosis was conducted by parasitological analysis of thick blood smears at the national sentinel laboratory network of the Ministry of Health. Blood samples were obtained from symptomatic patients living in affected areas of Nicaragua between 2012 and 2013 (Gonzalez Ceron et al., 2023; Gutierrez et al., 2016). *P. vivax* infected blood samples were spotted onto Whatman #2 filter paper, dried, and stored in the dark for later use. Sixty-five previously obtained samples from different municipalities were selected for this study.

Gene amplification and sequencing

DNA extraction was performed using the QIAamp Blood Minikit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Three circles of 5 mm each were cut from the dried blood spots. A final elution volume of 50 μ L containing extracted DNA was stored at -20°C for subsequent analysis. The *Pvdbp* domain II was amplified using nested PCR. The first PCR was performed in a 20 μ L reaction mixture containing: 9.2 μ L H₂O, 4 μ L 5x Buffer, 1.6 μ L 25mM MgCl₂, 1 μ L 1.2mM dNTP, 2 μ L 10 μ M DBPF1 (5' -GATAAACTGGGGAGGAAAAAGAT- 3') and DBPR1 (5' -CTTATCGGATTTGAATTGGTGGC- 3') primers, 0.2 μ L GoTaq[®] DNA polymerase (Promega, Madison, WI, USA), and 2 μ L extracted DNA. The nested PCR was

performed in a 50 μ L reaction containing: 25.5 μ L H₂O, 10 μ L 5x Buffer, 4 μ L 25mM MgCl₂, 2.5 μ L 1.2mM dNTP, 5 μ L 10 μ M DBPF3 (5' -CCTCGAATGGTGGCAATCCT- 3') and DBPR3 (5' -CCTC TTCAACGGAACAAACGCA- 3') primers, 0.5 μ L of GoTaq[®] Polymerase and 2.5 μ L of first PCR product to obtain a fragment of \sim 984 bp.

Both PCR reactions were carried out under the following conditions: 95°C for 3 min; 35 cycles of 94°C for 40 s, 58°C for 40 s, and 72°C for 90 s; and a final extension at 72°C for 5 min. Reactions were performed in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA).

Amplified fragments were resolved on 1% agarose gels stained with ethidium bromide and visualized under ultraviolet (UV) light using a digital photo documentation system (UVP Inc., Upland, CA, USA). PCR products were purified using the MiniElute PCR Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions and quantified using a NanoDrop ND2000 spectrophotometer (Thermo Scientific, USA). Samples with a concentration of at least 30ng/ μ L were sequenced by the Sanger method with DBPF3 and DBPR3 primers at the High-Throughput Genomics Unit, University of Washington, Seattle, USA. Electropherograms were reviewed in BioEdit 7.2.5 (Hall, 1999) using the Sal-I reference sequence (XM_001608337.1). All sequences showed single peaks, suggesting a single genotype per amplified product. Consensus sequences were deposited in GenBank (accession numbers PV581685–PV581747).

Genetic analysis

Parameters of diversity and neutral evolution estimates were run in dnaSP v6.12 (Rozas et al., 2017), including the number of mutations (M), segregating sites (S), haplotypes (H), haplotype diversity (Hd), and synonymous and non-synonymous nucleotide changes (S, NS). Nucleotide diversity (π), defined as the average number of nucleotide differences per site between two or more sequences in a population, and genetic diversity (θ -w), defined as the variety in the DNA segment composition within a population, were also estimated.

Tajima's D is based on the expectation that the estimates of S/a1 and k are unbiased estimates of θ -w under a neutral model (Tajima, 1989). Here, S is the number of segregating sites, n is the number of nucleotide sequences, and k is the average number of nucleotide differences between pairs of sequences. A negative Tajima's D value indicates an excess of low-frequency polymorphism, which may suggest directional selection or population expansion, whereas a positive value suggests balancing selection or population contraction. D* and F* of Fu and Li evaluate whether the pattern of polymorphism is consistent with a neutral model. D* is based on the difference between η s, the number of singletons (sites segregating at a frequency of 1/n or (n – 1)/n), and η , the total number of mutations, whereas F* is based on the difference between η s and k (Fu and Li, 1993). Tests were run in DnaSP (Rozas et al., 2017).

The Z test of selection (dN/dS) was estimated using the Modified Nei–Gojobori model with Jukes–Cantor correction in MEGA v11 (Tamura et al., 2021). This test calculates the ratio of non-synonymous (dN) to synonymous (dS) substitutions per site across all sequence pairs, and the variance of the difference was computed using the bootstrap method with 1,000 replicates. To delineate genealogical relationships among *pvdvp_{II}* haplotypes from Nicaragua and other regions, median-joining networks were generated using Population Analysis with Reticulate Trees in PopART v1.7 (Bandelt et al., 1999). Genetic structure differences in the distribution of variants were also analyzed to identify groups of sequences sharing similar patterns of variation, using a Bayesian clustering approach with Markov chain Monte Carlo (MCMC) estimation under an admixture model (Porrás-Hurtado et al., 2013). Tests were run 20 times with a burn-in of 50,000 and 100,000 iterations for K values ranging from 2 to 5 in STRUCTURE v2.3.4 (Porrás-Hurtado et al., 2013). The most probable number of clusters or subpopulations was estimated using the online tool Structure Harvester <https://taylor0.biology.ucla.edu/structureHarvester/> (accessed 30 June 2023) (Earl and vonHoldt, 2012). Genetic differentiation between two or more populations was calculated using the F_{ST} index (Wright, 1951) in dnaSP v6.12.03 (Rozas et al., 2017). F_{ST} values range from 0 to 1, where 0 indicates genetically identical populations and 1 indicates completely unrelated populations.

To compare and gain a broader understanding of *PvDBP_{II}* diversity, homologous sequences from various geographical sites were retrieved from NCBI. *Pvdvp_{II}* homologous sequences, accessible via NCBI (<https://pubmed.ncbi.nlm.nih.gov>) and PlasmoDB (<http://plasmodb.org/plasmo/>), were acquired and are listed as follows: Brazil (BRA, $n = 122$) EU812839.1 - EU812960.1 (Sousa et al., 2010); Mexico (MEX, $n = 35$) KP759780.1 - KP759814.1 (Gonzalez-Ceron et al., 2015), Ecuador (ECU, $n = 51$) Pv001-Pv091 (Nunez et al., 2023); Peru (PER, $n = 11$) Peru06-Peru10, Peru1008, Peru1021, Peru1022, Peru2025, Peru257-Peru262 (Hupalo et al., 2016); Colombia (COL, $n = 38$) U50590.1 - U50575.1 (Ampudia et al., 1996), and 30102100448 - 30102100440 (Hupalo et al., 2016); Iran (IRN; $n = 12$) KF791926.1 - KF791921.1 (Valizadeh et al., 2014a), KF318359.1 - KF318358.1 (Valizadeh et al., 2014b), and KF751810.1 - KF751807.1 (Nateghpour et al., 2017); Pakistan (PKN; $n = 118$) (Nguyễn et al., 2024); India (IND; $n = 61$) MN549534.1 - MN549594.1 (unpublished), Sri Lanka (SLK; $n = 100$) GU143914.1 - GU144013.1 (Premaratne et al., 2011), China (CHN; $n = 124$) MZ765947.1 - MZ766070.1 (Shi et al., 2021); South Korea (SK; $n = 13$) JN989484.1 - JN989472.1 (Ju et al., 2013); Thailand (THL; $n = 22$) EF368159.1 - EF368180.1 (Gosi et al., 2008); Myanmar (MYN; $n = 167$) MN233573.1 - MN233489.1, MN233488.1 - MN233407.1 (Hu et al., 2019); Papua Nueva Guinea (PNG, $n = 88$) AF469515.1 - AF469602.1 (Cole-Tobian et al., 2002); Ethiopia (ETH; $n = 75$) MZ062409.1 - MZ062224.1 (Lo et al., 2021); Sudan (SUD; $n = 97$) MG805657.1 - MG805616.1 (Hoque et al., 2018), MZ062409.1 - MZ062224.3 (Lo et al., 2021); Uganda (UGA; $n = 31$) KX009560.1 - KX009537.1 (unpublished), JX174528.1 - JX174522.1 (unpublished); Botswana (BTW, $n = 8$) MZ062409.1 - MZ062224.2 (Lo et al., 2021).

Amino acid and haplotype polymorphism.

DNA sequences were translated into amino acid sequences, and the frequency of the most common amino acid substitutions and haplotypes found in Nicaragua was compared with those from other geographical regions. Additionally, the frequency of the eight amino acid combinations derived from polymorphic residues 417, 437, and 503 was compared regardless of other substitutions. These polymorphic residues are implicated in immune evasion (VanBuskirk et al., 2004).

P. vivax circulation in Nicaragua using *pvdvp_{II}* versus *pvdvp_{II}* - *pvama_{I-II}*

To test the sensitivity of *pvdvp_{II}* haplotypes in tracking *P. vivax* circulation in Nicaragua, these haplotypes were mapped to the municipality level using ArcGIS v10.8.1. Furthermore, since *pvama_{I-II}* sequences were available for most isolates, their geographical distribution was compared with that of a concatenated sequence comprised of *pvdvp_{II}* and *pvama_{I-II}*. Sixty-one isolates covering both *pvdvp_{II}* (from this study) and *pvama_{I-II}* sequences (GenBank: ON730710.1–ON730730.1, ON730732.1–ON730736.1, ON730738.1–ON730746.1, ON730749.1–ON730774.1) (Gonzalez Ceron et al., 2023) were used. *Pvdvp_{II}* (1,806 bp) and *pvama_{I-II}* (915 bp) sequences were concatenated in MEGA v11, and haplotypes were resolved in DnaSP. Genetic relationships were investigated using a maximum likelihood phylogenetic tree in MEGA.

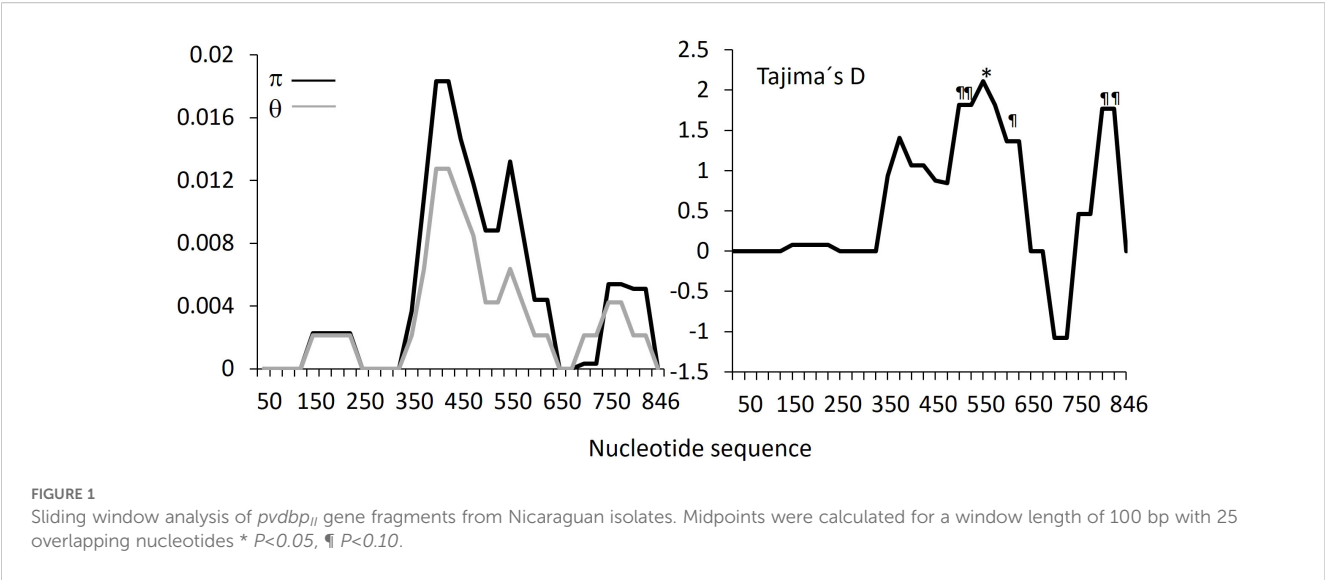
Results

Diversity parameters for *pvdvp_{II}*, Nicaragua.

Sixty-three consensus *pvdvp_{II}* sequences of 891 bp were obtained, corresponding to nucleotides 595–1485 of the Sal-I strain. Only nonsynonymous mutations were detected, and the mutation at codon 441 was a singleton (Supplementary Table 1). No mixed-genotype infections were detected. Nucleotide and genetic diversity were low ($\pi = 0.006$ and $\theta-w = 0.004$, respectively). A sliding window analysis of nucleotide diversity showed higher values across nucleotides 900–1500 (Figure 1). Six haplotypes were detected, with haplotype diversity (H_d) of 0.704. Tajima's D for *pvdvp_{II}* was 1.531, and Fu and Li's D* and F* statistics were 1.411 and 1.326, respectively; none were significant. The R^2 coefficient of linkage disequilibrium for the Nicaraguan parasites was 0.356.

Haplotype networks

A median-joining network using 63 sequences from Nicaraguan parasites showed six haplotypes separated by 1–12 mutational steps.



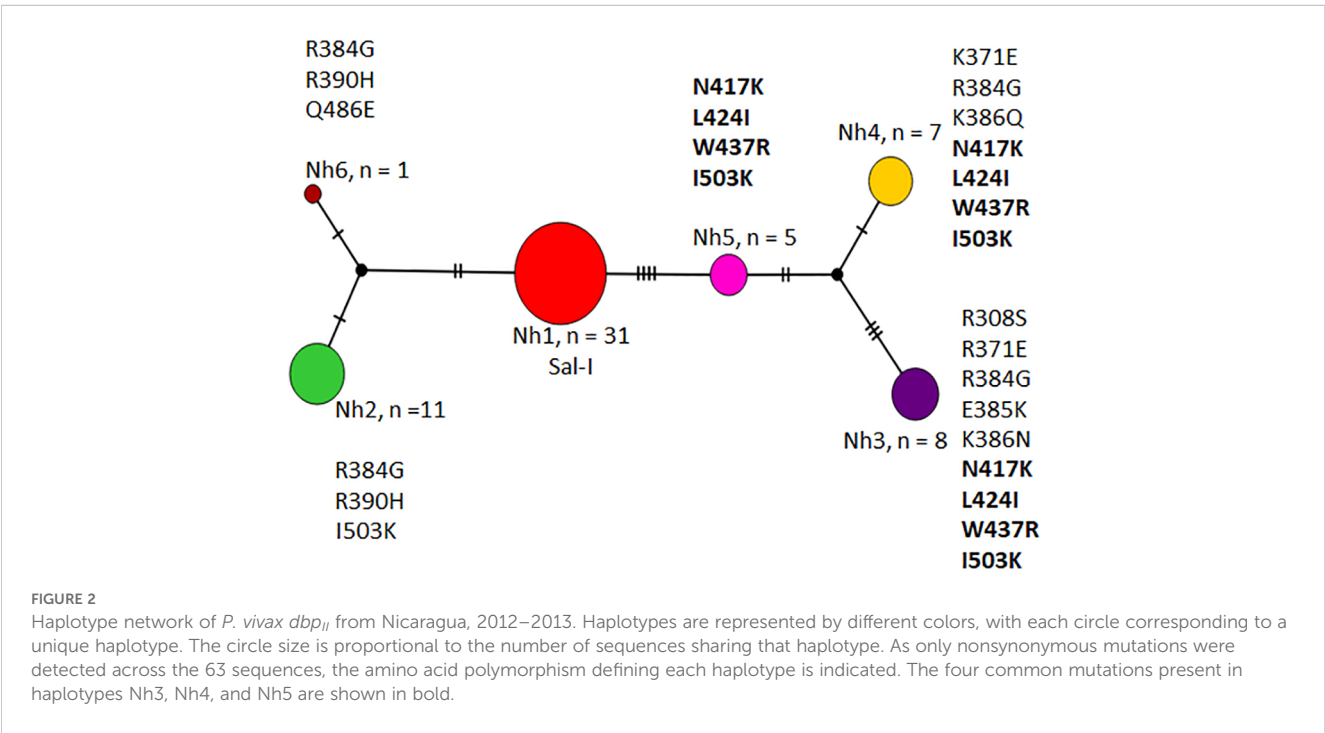
Haplotype Nh1 was the most frequent and was separated by three mutational steps from Nh2 and Nh6, and by four steps from Nh5. Nh4 and Nh3 were separated by one and three mutational steps from Nh5, respectively. Nh2 and Nh6 shared two nonsynonymous mutations (R384G and R390H), while Nh3, Nh4, and Nh5 (divergent haplotypes) shared four nonsynonymous mutations (N417K, L424I, W437R, and I503K) (Figure 2).

The haplotype network of global isolates was highly complex, with multiple genealogical groups and no discernible geographic structure, as reported previously (Nguyễn et al., 2024). Figure 3A shows a network of Latin American *pvdBP11* haplotypes present in at least two individuals. Intra-haplotype mutational steps ranged from 1 to 30. Four of the six haplotypes from Nicaragua represented the

most frequent haplotypes (Nh1, Nh2, Nh3, and Nh5), albeit at different frequencies (Figure 3A). A higher number of unique haplotypes were detected in Ecuador > Brazil > Colombia compared with other Latin American sites.

Structure analysis

The Evanno test for structure analysis based on *pvdBP11* suggested five distinct subpopulations in Latin America; however, the Delta K value was low (0.549). Population structure at K = 5 and K = 4 did not differ significantly (Figure 3B). Most admixture was observed in Brazil and Colombia. Nicaraguan subpopulations were



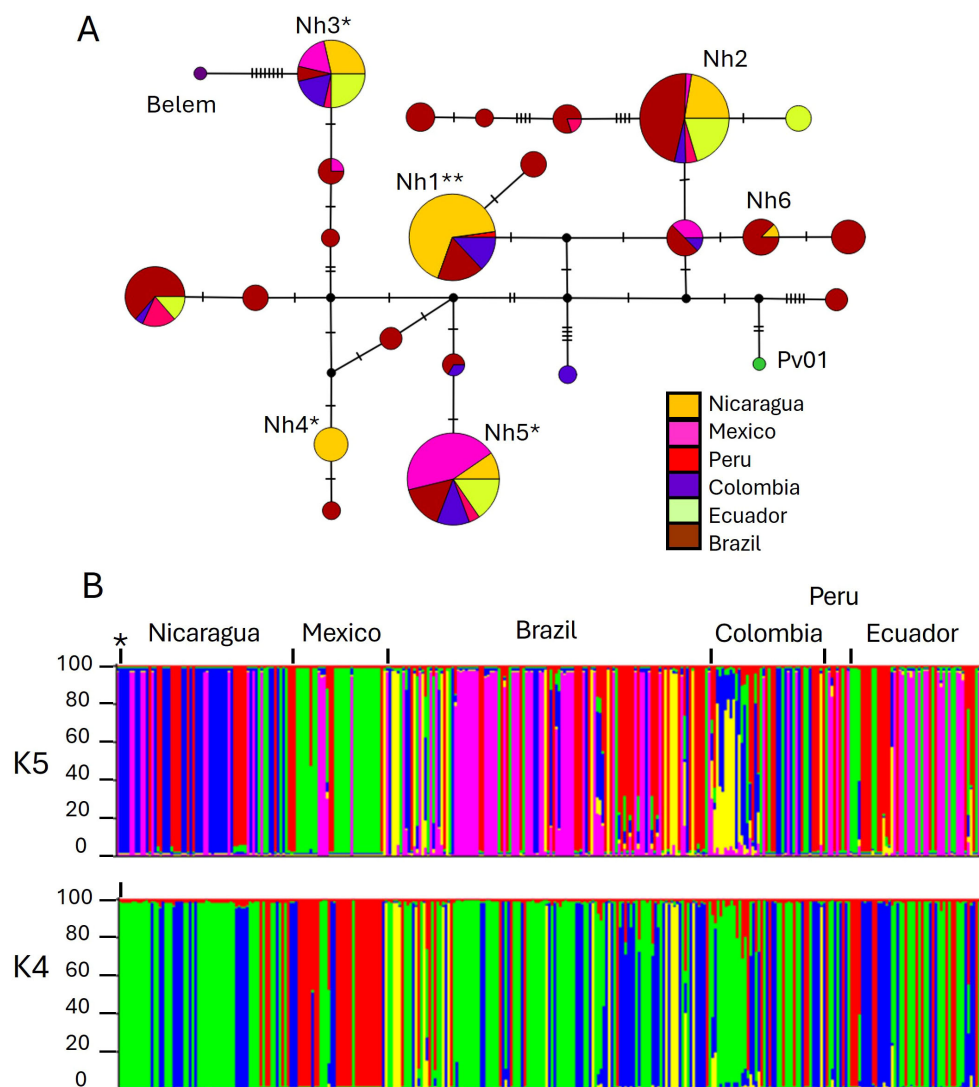


FIGURE 3

Haplotype network and genetic structure of *P. vivax dbp_{II}* from Latin America. (A) Median-joining haplotype network of *pvd_{bpII}* haplotypes. Each circle represents a unique haplotype, and its size is proportional to haplotype frequency (based on 269 sequences, including only haplotypes with two or more sequences). Haplotypes are connected by lines, with short vertical stripes indicating the number of mutational steps between them. Haplotypes Nh1–Nh6 denotes Nicaraguan haplotypes. Reference strains such as Sal-I (**), Pv01, and Belem were included. *Haplotypes showing four simultaneous amino acid changes at residues N417K, L424I, W437R, and I503K are marked. (B) Genetic structure of 319 *P. vivax* isolates. Each color represents one subpopulation. The Y-axis indicates the percentage of shared ancestry among individuals. The blue (K = 5) or green (K = 4) subpopulation includes the *Sal-I strain. Individuals displaying more than one color indicate mixed ancestry.

shared with South American parasites, with the greatest difference observed against Mexico.

Genetic parameters of *pvd_{bpII}* in Nicaragua, Latin America and globally

This analysis included 1,234 sequences covering 224 codons (739–1410 nucleotides) from 19 affected countries: 318 sequences from six regions in Latin America (LA; 25.8%), including Nicaragua; 137 from the Middle East (11.1%); 155 from South Asia (12.6%); 189 from Southeast Asia (15.3%); 135 from East Asia (10.9%); 210 from Africa (17%); and 88 from Papua New Guinea

(PNG; 7.1%). A total of 133 polymorphic sites and 144 mutations were identified, of which 60 were singletons. Of the 244 haplotypes detected, 70 were present in two or more individuals, representing 85.9% of all sequences.

Nicaraguan parasites showed the second-lowest nucleotide diversity (π) in LA and the third-lowest globally. *Pvd_{bpII}* haplotype diversity in Nicaragua was lower than in Peru, Brazil, and Colombia but higher than in Mexico. Overall, genetic diversity in South America was higher than that in Mesoamerica. Globally, the highest nucleotide and haplotype diversity were observed in Thailand and South Korea, respectively (Supplementary Table 2).

Tajima's D values varied widely across regions, ranging from −1.562 in PNG to 1.92 in India. Similarly, Fu and Li's D* and F* values

ranged from −5.57 to 1.74 and from −4.74 to 1.91 in PNG and Brazil, respectively. The Z test of selection also varied among parasite populations and was positive and significant in parasites from Nicaragua, Mexico, and Colombia, as well as in populations from the Middle East, Asia, Africa, and PNG (Supplementary Table 2).

The fixation index (F_{ST}) was estimated between parasite populations from different geographical regions. F_{ST} values between Nicaraguan and other LA parasites were low (0.032–0.114), except with Mexican parasites (0.247). Low F_{ST} values were also observed between parasites from Nicaragua and those from India (0.043) and Iran (0.064) (Supplementary Table 3).

Frequency of PvDBPII amino acid substitutions and haplotypes in Nicaragua, Latin America and globally

All mutations detected in Nicaraguan parasites were shared with other geographical regions (Table 1). The most frequent substitution in Nicaraguan parasites was I503K (n = 63; 49.2%), followed by D384G (42.9%). The simultaneous occurrence of four changes at N417K, L424I, W437R, and I503K was observed in 31.8% of isolates from Nicaragua. These were present in all Latin American (LA) regions and at frequencies ranging from 26% to

100% globally. In Nicaragua, mutations at K386N and K386Q occurred at similar frequencies (12.7% and 11.1%, respectively), but K386Q was infrequent globally (Table 1).

No exclusive PvDBPII haplotypes defined by 14 residues (R308S, L333F, K371E, N375D, D384G, E385K, K386N/Q, R390H, N417K, I419M, L424I, W437R, S447K, I503K) were detected in Nicaraguan parasites. Five of the six haplotypes corresponded to the 10 most frequent haplotypes estimated in our study (gh1–gh10), four of which occurred at >5% frequency (Table 2). Gh2 (Sal-I/Nh1), gh3 (Nh5), and gh5 (Nh3) were present in all LA regions, whereas gh7, gh8, gh9, and gh10 had the least distribution. Gh10 (Nh4) from Nicaragua was also observed in the Middle East and Africa. In LA, both Sal-I-related and divergent haplotypes were widely distributed (Table 2). Supplementary Table 4 shows the frequency of the most frequent haplotypes by country.

The frequencies of PvDBPII phenotypes defined by combinations at residues N417K, W437R, and I503K—known to participate in antibody evasion (VanBuskirk et al., 2004)—were also estimated, regardless of other substitutions. Phenotypes corresponding to the Sal-I sequence (NWI) and NWK were present in all regions at varying frequencies. The divergent phenotype KRK was detected in 31.7% of Nicaraguan parasites and showed wide variation in LA and outside the continent

TABLE 1 Frequency (%) of polymorphisms in PvDBPII from Nicaragua compared with other geographical sites.

Origin	Polymorphic residues									
	R308S	K371E	D384G	E385K	K386N	R390H	N417K	L424I	W437R	I503K
Nicaragua	12.7	23.8	42.9	12.7	12.7	19.1	31.8	31.8	31.8	49.2
Mexico	17.1	20	31.4	17.1	17.1	11.4	88.6	88.6	85.7	91.4
Colombia	13.6	24.3	48.7	21.6	21.6	8.1	51.4	51.4	37.8	37.8
Ecuador	19.6	31.4	66.7	19.6	19.6	37.3	60.8	60.8	56.9	86.3
Peru	18.2	45.5	81.8	18.2	18.2	36.4	63.6	63.6	72.7	54.6
Brazil	6.6	26.2	81.2	19.7	22.1	49.2	40.2	47.5	49.2	43.4
Pakistan	16.9	34.7	87.3	29.7	28.8	61.9	36.4	50.8	37.3	53.4
Iran	5.3	42.1	68.4	10.5	10.5	26.3	52.6	63.2	63.2	73.7
India	11.5	34.4	75.4	22.9	22.9	47.5	36.1	47.5	36.1	52.5
Sri Lanka	13	34	94	20	20	66	36	49	37	55
China	14.52	26.6	83.1	46.8	47.6	54	60.5	71.8	68.6	49.2
South Korea	0	53.9	100	0	0	100	53.9	100	46.2	46.2
Thailand	22.7	22.7	81.8	50	45.5	59.1	45.5	86.4	68.2	50
Myanmar	6.6	11.4	96.4	43.1	38.9	85	75.5	83.8	73.7	47.9
PNG	55.7	1.1	54.6	10.2	10.2	40.9	26.1	52.3	26.14	28.4
Ethiopia	30.7	56	92	40	37.3	52	66.7	72	68	53.3
Sudan	29.2	40.6	77.1	16.7	16.7	36.5	65.6	90.6	65.6	72.9
Uganda	0	29	90.3	0	0	90.3	45.2	100	45.2	32.3
Botswana	100	0	100	0	0	100	0	100	0	100

TABLE 2 *P. vivax* DBPII: most frequent global haplotypes.

Haplotype	Frequency (%) LA/Global	Codon number														
		308	333	371	375	384	385	386	390	417	419	424	437	447	503	
		Amino acid (Sal-I strain)														
		R	L	K	N	D	E	K	R	N	I	L	W	S	I	
gh1 (Nh2)	14.1/10.4	G	.	.	H	K	
gh2 (Nh1)	15.4/8.2	
gh3 (Nh5)	14.7/7.2	K	.	I	R	.	K	
gh4	5.6/6.9	.	.	E	.	G	.	.	.	K	M	I	R	.	.	
gh5 (Nh3)	9.1/4.5	S	.	E	.	G	K	N	.	K	.	I	R	.	K	
gh6	2.5/4.4	K	
gh7	0/3.8	S	.	.	.	G	.	.	H	.	.	I	.	K	.	
gh8	0/3.7	.	F	.	D	G	K	N	H	K	.	I	R	.	K	
gh9	0/2.9	.	F	E	.	G	K	Q	.	K	.	I	R	.	.	
gh10 (Nh4)	2.2*/2.6	.	.	E	.	G	.	Q	.	K	.	I	R	.	K	

Total sequences: Latin America (LA, n = 318) and Global (n = 1235). Nh1-Nh5 were haplotypes named for Nicaraguan. PvDBPII sequences. Sal-I strain sequence XM_001608337. *only Nicaragua.

(Table 3). Haplotypes with the NWI and KRK phenotypes were found in most regions, followed by NWK and KRI. While Nicaragua had the top three phenotypes globally, parasite populations from Myanmar and Sudan exhibited seven of the eight phenotypes (Table 3).

Geographic distribution of *P. vivax* haplotypes in Nicaragua municipalities

Most *pvdbpII* haplotypes were widely distributed in Nicaragua. Haplotype Nh1 (Sal-I) was common in RACCN and found in one isolate from the Pacific coast (Figure 4A). Haplotype Nh2 was the second most frequent (17.4%) in RACCN. Haplotypes Nh3–Nh5 were restricted to RACCN municipalities, while Nh4 predominated on the Pacific coast (Figure 4A). Of the six *pvdbpII* haplotypes, only two were exclusive to a single municipality.

The concatenated sequence (*pvdbpII*–*pvama1I-II*) significantly increased resolution, yielding 16 haplotypes and identifying exclusive haplotypes for each region or municipality (Figure 4B). Notably, 30 of 31 parasites with *pvdbpII* Sal-I also exhibited *pvama1I-II* Sal-I. The Mining region harbored two frequent and exclusive haplotypes, while Bonanza had one and Rosita had two. Waspam and Puerto Cabezas displayed six and one exclusive haplotypes, respectively. All three haplotypes detected on the Pacific coast were exclusive (Figure 4B).

A phylogenetic tree revealed several genetic groups that exhibited a degree of homogeneity when analyzed with either *pvdbpII* alone or the concatenated sequence (Figure 5).

Discussion

The low *P. vivax dbpII* diversity found in Nicaragua aligns with a continuous decline in the number of *P. vivax* cases since 2001, including a significant drop between 2007 and 2012. This decline was likely intensified operational activities for malaria control in the country (Pan American Health Organization, 2020; World Health Organization, 2021). Low genetic diversity was also observed in *pvama1I-II* (Gonzalez Ceron et al., 2023), which is consistent with mitochondrial DNA analysis of Central American isolates compared with those from South America or outside the continent (Taylor et al., 2013).

The invasion process of *P. vivax* requires the interaction of the Duffy binding protein domain II (DBPII) and the DARC receptor on reticulocytes (Singh et al., 2005). This molecule shows polymorphism that might hinder its effectiveness as a vaccine candidate (VanBuskirk et al., 2004). *PvdbpII* from Nicaragua exhibited mutation and haplotype patterns similar to those found in Latin America and elsewhere (Sousa et al., 2011; Almeida-de-Oliveira et al., 2020). Haplotype network analysis, STRUCTURE analysis, and the F_{ST} index based on *pvdbpII* suggest limited genetic differentiation between Nicaraguan and other Latin American parasites, a finding echoed in recent studies of global parasites (Nguyễn et al., 2024). In contrast, analysis of *pvmsp142*, *pvama1I-II*, and *pvm-dr1* gene markers has shown a differentiated population in Nicaragua (Gutierrez et al., 2016; Gonzalez-Ceron et al., 2017; 2020). This is supported by the finding that mutations associated with pyrimethamine resistance in *PvDHFR*, which are common in South America, were not present in parasites from Nicaragua or

TABLE 3 Comparison of the frequencies (%) of *P. vivax* BDP_{II} phenotypes in parasites from Nicaragua (2012–2013) and other geographical sites.

Origin	PvDBP _{II} haplotypes by residues: 417–437–503							
	N-W-I*	N-W-K	K-W-I	K-W-K	K-R-I	N-R-I	N-R-K	K-R-K
Nicaragua	50.8	17.5	0	0	0	0	0	31.7
Mexico	8.6	2.7	0	2.7	0	0	0	85.7
Colombia	43.2	5.4	10.1	2.7	8.1	0	0	29.7
Ecuador	1.9	37.3	2	2	9.8	0	0	47.1
Peru	9.1	18.2	0	0	36.4	0	9.1	27.3
Brazil	27.1	23.8	0	0	27.1	2.5	6.7	13.1
Pakistan	29.7	33.1	0	0	17.8	0	0.8	18.6
Iran	10.5	26.3	0	0	15.8	0	10.5	36.8
India	32.8	31.1	0	0	14.8	0	0	21.3
Sri Lanka	18.8	44.6	0	0	25.7	0	1	9.9
China	20.2	11.3	0	0	30.7	0	8.1	29.8
South Korea	46.2	0	0	7.7	7.7	0	0	38.5
Thailand	22.7	9.1	0	0	18.2	9.1	13.6	27
Myanmar	22.8	1.8	1.2	0.6	28.1	0.6	0	44.9
PNG	70.5	3.4	0	0	1.1	0	0	25
Ethiopia	25.3	6.7	0	0	20	0	1.3	46.7
Sudan	28.3	6.3	0	0	0	0	0	65.6
Uganda	54.8	0	0	0	12.9	0	0	32.3

* Sal-I phenotype. Amino acid changes are indicated in italics. *P. vivax* from Botswana had only one phenotype (Asn-Trp-Lys n = 8).

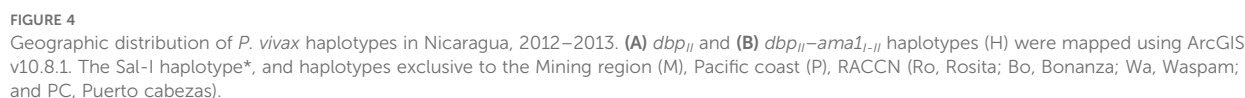
Mexico collected the same period (Gonzalez-Ceron et al., 2020). The presence of similar mutations in *pvdBP_{II}* globally might indicate convergent evolution, possibly similar to genes involved in drug resistance or other genes under selective pressure (Abera et al., 2021; Flannery et al., 2015).

The exclusive presence of nonsynonymous mutations and a positive Z test of selection in Nicaraguan parasites may indicate that this protein is under antibody pressure, consistent with findings from other geographic regions (Almeida-de-Oliveira et al., 2020; Cole-Tobian and King, 2003; Chen et al., 2016; Michon et al., 2000). Varying intensities of antibody selective pressure and/or genetic drift (VanBuskirk et al., 2004), might explain the variation in haplotype frequencies observed between Nicaragua and other geographical regions. Although *PvDBP_{II}* is less diverse compared with other molecules more exposed to the immune system (Gonzalez-Ceron et al., 2015), a proportion of infected individuals develop variable antibody titers, which appear to increase with exposure (Zakeri et al., 2011; Nicolette et al., 2016). These antibodies inhibited the binding of *P. vivax* to human erythrocytes (Grimberg et al., 2007) and may be phenotype-transcending and long-lasting (King et al., 2008; Nicolette et al., 2016; Thawornpan et al., 2022).

In *Plasmodium*, amino acid substitutions in molecules involved in parasite development and in vaccine candidates frequently modify the protein's antigenic profile, potentially allowing parasites to escape

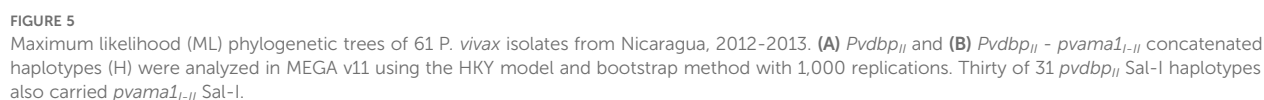
heterologous antibody responses. Experimental studies have shown that antibodies targeting *PvDBP_{II}* Sal-I (N417-W437-I503) inhibit erythrocyte adhesion to COS cells expressing the homologous phenotype. However, naturally occurring mutants showed varying levels of inhibition, with NRK and KRK variants showing the strongest inhibition (VanBuskirk et al., 2004). Similar results were observed in *P. vivax* *ex vivo* short-term assays using heterologous monoclonal antibodies (Rawlinson et al., 2019). Remarkably, heterologous haplotypes carrying NWI/NWK versus KRK, NRK, and KRI were the most prevalent globally, including in Nicaragua.

Further experiments using a mouse model demonstrated the induction of cross-reactive inhibitory antibodies by different *PvDBP_{II}* phenotypes prevalent in Thailand and the humoral responses were either phenotype-transcending or phenotype-specific (Thawornpan et al., 2022). Haplotypes with variations similar to global haplotypes gh8 and gh9 showed >50% inhibition of adhesion *in vitro* against all polymorphic haplotypes except the Sal-I phenotype. In contrast, one haplotype similar to the highly frequent gh1 induced strain-specific antibodies. Sera from *P. vivax*-infected individuals showed variable inhibition, blocking adhesion of between two and five *PvDBP_{II}* phenotypes (Thawornpan et al., 2022). Conserved continuous or conformational B-cell epitopes may contribute to phenotype-transcending immunity (VanBuskirk et al., 2004; Sousa et al., 2011; Ntumngia et al., 2012; Chootong et al., 2014; Valizadeh et al., 2014a;



Previous findings suggest that the overall pattern of polymorphism is more relevant than individual amino acid substitutions in generating phenotype-transcending humoral responses. However, the quality and quantity of antibody responses vary among individuals. In Nicaragua, two main *Pv*DBPII phenotype groups were detected, representing highly frequent and widespread haplotypes globally: gh1, gh2, and gh6,

In Nicaragua, over half a million people are at risk of malaria, with *P. vivax* being the predominant species. In RACCN, where malaria transmission is most persistent, indigenous groups such as the Miskito population (Ministerio de salud, 2014), are particularly vulnerable due to natural disasters, climate change, economic



instability, and limited access to health services (Ministerio de salud, 2014; Larrañaga et al., 2013; Soto et al., 2022). Notably, the RACCN accounted for 67.6% of all malaria cases in Nicaragua between 2013 and 2018. During this period, reported *P. vivax* cases in the region increased from 950 in 2013 to 14,501 in 2018, alongside a high rate of presumed relapse episodes (Soto et al., 2022). Puerto Cabezas was the most affected municipality. Significant population movement from areas such as Waspam to Puerto Cabezas may have contributed to the dispersal of *P. vivax* haplotypes, a factor that warrants further evaluation. The *pvdvpII* marker alone is insufficient for parasite surveillance, but it could be informative when used in combination with other markers.

Because sampling in Nicaragua was conducted after transmission had declined, the prevalent *pvdvpII* haplotypes in Nicaragua might correspond to those that were best adapted. These haplotypes would persist, but their frequencies could fluctuate—a phenomenon previously observed using temporal networks for *pvamaII-II* (Flores-Alanis et al., 2017) and *pvmsp142* (Flores-Alanis et al., 2022) in southern Mexico.

More integrated and temporal studies could help explain how haplotypes (both Sal-I-related and divergent) interact and fluctuate in a population over time, and explore evidence of immune evasion in natural infections. In addition, studying histocompatibility leukocyte antigen (HLA) associations with homologous and heterologous antibody responses would be beneficial. For instance, in the Brazilian Amazon, antibody responses against the Sal-I haplotype were associated with specific HLA antigens (Kano et al., 2016). Moreover, some Nicaraguan haplotypes were among the most frequent globally, providing potential candidates for mono- or polyvalent vaccines beyond just the Sal-I haplotype.

Conclusions

P. vivax dbpII in Nicaragua showed low genetic diversity, and while its mutations and haplotypes were shared with other geographic areas both within Latin America and globally, their frequencies varied. Non-synonymous mutations predominated and were located at sites involved in B-cell epitopes under immunological selective pressure, consistent with findings in other regions. This information contributes to the development of PvBDPII-based vaccines, suggesting that the extent and type of polymorphism relevant for vaccine design may already have been identified. However, ongoing parasitological surveillance remains crucial to detect new variants that could potentially lead to immune evasion.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/genbank/>, accession numbers: ON730710-ON730774.

Ethics statement

The studies involving humans were approved by Ethic Committees of the National Center for Diagnosis and Reference (CNDR) of the Health Ministry in Nicaragua, and the National Institute of Public Health, Mexico (CI1042). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

RO-M: Methodology, Formal Analysis, Writing – original draft. HT-O: Methodology, Writing – original draft. FS-V: Methodology, Data curation, Formal Analysis, Writing – original draft. AM: Conceptualization, Writing – original draft, Funding acquisition, Resources, Methodology. LG-C: Writing – review & editing, Supervision, Writing – original draft, Formal Analysis, Investigation, Funding acquisition, Project administration, Conceptualization, Validation.

Funding

The author(s) declare financial support was received for the research and/or publication of this article. This work was supported by AMI/RAVEDRA-PAHO/WHO-Nicaragua and Global Fund-Malaria Component-Nicaragua, and CONACyT-Mexico: project CB-2009-01-131247.

Acknowledgments

To the personnel of the sentinel sites in Nicaragua for their commitment in sample collection, identification and storage.

Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmala.2025.1620450/full#supplementary-material>

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