

Submicroscopic infections and two malaria vectors are major contributors to residual malaria in an Amazonian village in Peru

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Supplementary Materials

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Supplementary Table S7. Incidence Rate Ratios (IRRs) from a Poisson regression evaluating the association between *Ny. darlingi* counts, geographic clusters, and season (dry vs. wet).

Supplementary Figure S1. Monthly abundance of *Ny. darlingi* and grouping of high, medium and low transmission by Scott-Knott test ($\alpha = 0.05$), Santa Rita, Peru, 2021-2022. The y-axis depicts the number of *Ny. darlingi* per cluster; the x-axis corresponds to the collection months. The black dots represent the outliers in the boxplot. The beige area shows the months of decline of PCR-detected *P. vivax* infections across all transmission intensity groups.

Supplementary Figure S2. Temporal trends of *Ny. darlingi* abundance and malaria prevalence in 2021 with Pearson correlation analysis.

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Supplementary Figure S4. Monthly Nanay River level, precipitation and *Ny. darlingi* abundance, Santa Rita, Peru, 2021-2022.

Supplementary Table S1. Socio-demographic characteristics of study participants, Santa Rita, Peru, 2021-2022. For molecular testing, samples were selected from participants with 10 to 12 months of follow-up.

Characteristic	N = 353*	N = 109**
Gender		
Male	178 (50.4%)	49 (45%)
Female	175 (49.6%)	60 (55%)
Age group (years)		
< 5	38 (10.8%)	6 (5.5%)
5 - 17.9	103 (29.2%)	42 (39%)
18 - 59.9	158 (44.8%)	49 (45%)
> 60	54 (15.3%)	12 (11%)
Education		
Illiterate	41 (11.6%)	12 (11%)
Preschool or Primary school	163 (46.2%)	55 (50%)
Secondary school	129 (36.5%)	38 (35%)
High school	18 (5.1%)	4 (3.7%)
Main occupation		
Indoor related	102 (28.9%)	33 (30%)
Outdoor related	86 (24.4%)	25 (23%)
Other	11 (3.1%)	2 (1.8%)
Not working	154 (43.6%)	49 (45%)
Lifetime malaria history		
Yes	294 (83.3%)	101 (93%)
No	59 (16.7%)	8 (7.3%)
Last year malaria history		
Yes	35 (9.9%)	34 (31%)
No	318 (89.1%)	75 (69%)
Mosquito net possession		
LLIN	184 (52.1%)	48 (44%)
Not LLIN	82 (23.2%)	12 (11%)
Both	86 (24.4%)	49 (45%)
Not mosquito net	1 (0.3%)	0 (0%)
Mosquito net use the previous night		
Always	332 (94.1%)	97 (89 %)
More than half of the times	21 (5.1%)	12 (11%)
No	0 (0%)	0 (0%)
Average positivity rate		0.07 (0, 0.09) ¹

* Number of all participants

** Number of participants with 10 to 12 months of follow-up

¹ mean (Q1, Q2). It measures, across all follow-up visits of the individual, the proportion of positive PCR tests.

Supplementary Table S2. Human census and annual parasite index (API), Santa Rita and contiguous communities, Peru, 2020-2023.

Community	Census 2021*	API 2020	API 2021	API 2022	API 2023
San Jose de Lupuna	352	52.6	39.8	65.3	11.4
San Pedro	235	50.6	8.5	93.6	8.5
Santa Rita	383	33.3	23.5	91.4	13.1

* Census performed as part of the present study by Universidad Peruana Cayetano Heredia (UPCH) personnel.

Supplementary Table S3a. Household characteristics of study participants, Santa Rita, Peru, 2021-2022

Characteristic	N = 83
Wall material	
Brick, cement	5 (6.3%)
Wood	69 (87%)
Other	5 (6.3%)
Lost to follow-up	4
Floor material	
Dirt floor	48 (61%)
Cement	24 (30%)
Wood	6 (8%)
Other	1 (1.2%)
Lost to follow-up	4
Roof material	
Palm	1 (1.3%)
Tin	78 (99%)
Lost to follow-up	4
Source of water	
Open well	10 (13%)
Public tap	5 (6.3%)
River, rain	21 (27%)
Spring	43 (54%)
Lost to follow-up	4
Sanitation facility	
Flush toilet	4 (5.1%)
Ground hole, cesspool	8 (10%)
No facility, field	21 (27%)
Pit latrine	46 (58%)
Lost to follow-up	4
Source of fuel	
Charcoal	1 (1.3%)
Firewood	70 (89%)
Gas	7 (8.9%)
Unknown	1 (1.3%)
Lost to follow-up	4
Cluster	
Household landscapes	33
Forested areas	3
Crops	29
Seasonally flood-prone areas	18
Degraded forest patches	0

Supplementary Table 3b. Bivariate analysis of sociodemographic and behavioral variables of only the participants with 10-12 follow-ups, by PCR infection status, Santa Rita, Peru, 2021-2022.

Variable	N ¹	Neg, N = 1,113 ¹	Pos, N = 88 ¹	P-value ²
Bathing location	1,199			0.300
In		80 (7.2%)	3 (3.4%)	
Out		307 (28%)	23 (26%)	
River		724 (65%)	62 (70%)	
Main occupation	1,201			0.012
Indoor related		383 (34%)	30 (34%)	
Outdoor related		231 (21%)	31 (35%)	
Other		37 (3.3%)	2 (2.3%)	
Not working		462 (42%)	25 (28%)	
In school	1,201			0.077
Yes		732 (66%)	66 (75%)	
No		381 (34%)	22 (25%)	
Education	1,201			0.500
Illiterate		128 (12%)	6 (6.8%)	
Preschool or Primary school		565 (51%)	45 (51%)	
Secondary school		379 (34%)	35 (40%)	
High school		41 (3.7%)	2 (2.3%)	
Travel in previous month	1,201			
Yes		250 (22%)	16 (18%)	0.400
No		863 (78%)	72 (82%)	
Gender	1,201			0.400
Male		491 (44%)	43 (49%)	
Female		622 (56%)	45 (51%)	
Age group (years)	1,201			0.006
<15		496 (45%)	23 (26%)	
15-30		101 (9.1%)	8 (9.1%)	
30-50		225 (20%)	27 (31%)	
>50		291 (26%)	30 (34%)	
Migration	1,201			0.060
Yes		866 (78%)	76 (86%)	
No		247 (22%)	12 (14%)	
House structure	1,201			0.120
Dwellings with partial or no enclosure		283 (25%)	29 (33%)	
Dwellings with complete enclosure		830 (75%)	59 (67%)	
House fumigated	1,201			
Yes		967 (87%)	72 (82%)	0.200
No		146 (13%)	16 (18%)	
Floor material	1,201			0.004
Cement		370 (33%)	19 (22%)	
Unfinished floor		701 (63%)	60 (68%)	
Walking palm wood		42 (3.8%)	9 (10%)	
Sanitation facility	1,201			0.004
Inadequate sanitation		518 (47%)	55 (63%)	
Basic sanitation		595 (53%)	33 (38%)	
Cluster	1,201			0.120
Household landscapes		453 (41%)	40 (45%)	
Forested areas		55 (4.9%)	1 (1.1%)	
Crops		446 (40%)	40 (45%)	
Seasonally flood-prone areas.		159 (14%)	7 (8%)	
Degraded forest patches		0.000	0.000	
Water source	1,201			0.3
Public water tap		711 (64%)	58 (66%)	
Open well		116 (10%)	5 (5.7%)	
River, rain		286 (26%)	25 (28%)	
Proportion of outdoor time (18:00–21:00)		0.11 (0.100, 0.126)	0.161	0.8361

N: total number of *Plasmodium vivax* PCR tests; Neg, N (%): total number of negative *Plasmodium vivax* PCR tests (percent total); Pos, N (%): total number of positive *Plasmodium vivax* PCR tests (percent total).

¹ number of PCR tests conducted; ² Pearson's Chi-squared test; Fisher's exact test.

Walking palm wood: common name for *Socratea exorrhiza*; Inadequate sanitation: ground hole or no facility; Basic sanitation: indoor/outdoor toilet or at least a latrine.

Numbers in bold in the *p*-value column are statistically significant at 0.05 level.

For this analysis, there were two categories of Sanitation facility: Inadequate - various ground holes, pit latrine; Basic - indoor or outdoor toilet or at least latrine.

Supplementary Table S3c. Factors associated with PCR-detected infection: rate ratios from Poisson regression, Variable selection was performed using a stepwise procedure based on the Akaike Information.

Characteristic		IRR ¹	95% CI ¹	p-value
Gender				
	Male	—	—	
	Female	0.62	0.39, 1.00	0.049
Age group (years)				
	<15	—	—	
	>50	2.47	1.38, 4.44	0.002
	15-30	2.84	1.05, 7.12	0.031
	30-50	3.19	1.66, 6.19	<0.001
Main occupation				
	Indoor related	—	—	
	Outdoor related	2.28	1.33, 3.98	0.003
	Other	3.13	0.48, 11.7	0.14
	Not working	0.61	0.33, 1.12	0.11
Sanitation facility				
	Inadequate sanitation	—	—	
	Basic sanitation	0.48	0.28, 0.80	0.006
Migration				
	Yes	—	—	
	No	0.31	0.13, 0.64	0.003
Education				
	Illiterate	—	—	
	Preschool or Primary school	5.65	1.69, 35.1	0.018
	Secondary school	4.46	1.21, 28.9	0.052
	High school	1.76	0.20, 15.4	0.6
Home type				
	Dwellings with partial or no enclosure	—	—	
	Dwellings with complete enclosure	0.02	0.00, 0.15	<0.001
Floor material				
	Cement	—	—	
	Unfinished floor	0.86	0.43, 1.76	0.7
	Wood-Pona	2.15	0.86, 5.14	0.091
Source of water				
	Treated water	—	—	
	Open well	0.66	0.22, 1.58	0.4
	River, rainwater	0.03	0.00, 0.20	<0.001

¹ IRR = Incidence Rate Ratio, CI = Confidence Interval

Supplementary Table S4. Composition of anopheline mosquito species per month for 12 months, Santa Rita, Peru, 2021-2022.

Month	Season	Mosquito species	N = 14,825	Composition per month (%)
Feb - 2021	Wet	<i>Ny. darlingi</i>	212	39.92
		<i>Ny. dunhami</i>	34	6.40
		<i>Ny. benarrochi</i> B	43	8.10
		<i>Ny. oswaldoi</i> s.l.	16	3.01
		<i>Ny. tadei</i>	2	0.38
		<i>Ny. triannulatus</i>	62	11.68
		<i>An. forattinii/An. near costai</i>	2	0.38
		<i>An. nimbus</i>	1	0.19
		<i>An. near costai</i> G1	147	27.68
		<i>An. peryassui</i>	12	2.26
Mar - 2021	Wet	<i>Ny. darlingi</i>	897	60.57
		<i>Ny. dunhami</i>	36	2.43
		<i>Ny. benarrochi</i> B	16	1.08
		<i>Ny. oswaldoi</i> s.l.	14	0.95
		<i>Ny. triannulatus</i>	51	3.44
		<i>An. near costai</i> G1	464	31.33
		<i>An. forattinii/An. near costai</i>	1	0.07
		<i>An. mattogrossensis</i>	1	0.07
		<i>An. peryassui</i>	1	0.07
Apr - 2021	Wet	<i>Ny. darlingi</i>	2,599	81.63
		<i>Ny. dunhami</i>	19	0.60
		<i>Ny. benarrochi</i> B	6	0.19
		<i>Ny. oswaldoi</i> s.l.	5	0.16
		<i>Ny. triannulatus</i>	21	0.66
		<i>Ny. rangeli</i>	1	0.03
		<i>An. forattinii/An. near costai</i>	2	0.06
		<i>An. near costai</i> G1/G2	528	16.58
		<i>An. peryassui</i>	3	0.09
May - 2021	Wet	<i>Ny. darlingi</i>	2,696	80.45
		<i>Ny. dunhami</i>	13	0.39
		<i>Ny. benarrochi</i> B	2	0.06
		<i>Ny. oswaldoi</i> s.l.	33	0.98
		<i>Ny. triannulatus</i>	24	0.72
		<i>An. near costai</i> G1	571	17.04
		<i>An. forattinii/An. near costai</i>	1	0.03
		<i>An. mattogrossensis</i>	2	0.06
		<i>An. peryassui</i>	8	0.24
		<i>An. spp.</i>	1	0.03
Jun - 2021	Wet	<i>Ny. darlingi</i>	1,500	77.36
		<i>Ny. dunhami</i>	17	0.88
		<i>Ny. benarrochi</i> B	2	0.10
		<i>Ny. oswaldoi</i> s.l.	16	0.83
		<i>Ny. tadei</i>	2	0.10
		<i>Ny. triannulatus</i>	27	1.39
		<i>An. forattinii/An. near costai</i>	3	0.15
		<i>An. near costai</i> G1	370	19.08
		<i>An. peryassui</i>	2	0.10
Dec - 2021	Wet	<i>Ny. darlingi</i>	2,104	80.37
		<i>Ny. dunhami</i>	290	11.08
		<i>Ny. benarrochi</i> B	4	0.15
		<i>Ny. oswaldoi</i> s.l.	24	0.92
		<i>Ny. tadei</i>	1	0.04
		<i>Ny. rangeli</i>	4	0.15
		<i>Ny. triannulatus</i>	22	0.84
		<i>Ny. sp.</i>	9	0.34
		<i>An. near costai</i> G1	157	6.00
		<i>An. peryassui</i>	3	0.11
Jan - 2022	Wet	<i>Ny. darlingi</i>	518	66.58
		<i>Ny. dunhami</i>	97	12.47
		<i>Ny. benarrochi</i> B	1	0.13
		<i>Ny. triannulatus</i>	54	6.94
		<i>An. forattinii/An. near costai</i>	1	0.13
		<i>An. near costai</i> G1	49	6.30
		<i>An. peryassui</i>	3	0.39
		<i>An. squamifemur</i>	1	0.13
		<i>An. spp.</i>	54	6.94

Month	Season	Mosquito species	N = 14,825	Composition per month (%)
Jul - 2021	Dry	<i>Ny. darlingi</i>	203	41.86
		<i>Ny. dunhami</i>	10	2.06
		<i>Ny. benarrochi</i> B	1	0.21
		<i>Ny. oswaldoi</i> s.l.	6	1.24
		<i>Ny. rangeli</i>	1	0.21
		<i>Ny. triannulatus</i>	3	0.62
		<i>An. near costai</i> G1	255	52.58
		<i>An. peryassui</i>	5	1.03
		<i>An. spp.</i>	1	0.21
Aug - 2021	Dry	<i>Ny. darlingi</i>	45	57.69
		<i>Ny. dunhami</i>	5	6.41
		<i>Ny. oswaldoi</i> s.l.	4	5.13
		<i>Ny. triannulatus</i>	13	16.67
		<i>An. near costai</i> G1	8	10.26
		<i>An. forattinii/An. near costai</i>	2	2.56
		<i>An. peryassui</i>	1	1.28
Sep - 2021	Dry	<i>Ny. darlingi</i>	34	69.39
		<i>Ny. dunhami</i>	1	2.04
		<i>Ny. benarrochi</i> B	3	6.12
		<i>Ny. oswaldoi</i> s.l.	3	6.12
		<i>Ny. triannulatus</i>	2	4.08
		<i>An. near costai</i> G1	5	10.20
		<i>An. peryassui</i>	1	2.04
Oct - 2021	Dry	<i>Ny. darlingi</i>	70	56.45
		<i>Ny. dunhami</i>	7	5.65
		<i>Ny. benarrochi</i> B	2	1.61
		<i>Ny. oswaldoi</i> s.l.	3	2.42
		<i>Ny. triannulatus</i>	7	5.65
		<i>An. near costai</i> G1	32	25.81
		<i>An. peryassui</i>	2	1.61
		<i>Ny. sp</i>	1	0.81
Nov - 2021	Dry	<i>Ny. darlingi</i>	116	56.04
		<i>Ny. dunhami</i>	34	16.43
		<i>Ny. oswaldoi</i> s.l.	7	3.38
		<i>Ny. triannulatus</i>	5	2.42
		<i>An. forattinii</i>	41	19.81
		<i>An. forattinii/An. near costai</i>	1	0.48
		<i>An. near costai</i> G1	1	0.48
		<i>An. peryassui</i>	2	0.97

Supplementary Table S5. Total anopheline seasonal composition, Santa Rita, Peru, 2021-2022.

Season	Mosquito species	N = 14,825	Composition per species (%)
Wet	<i>Ny. darlingi</i>	10,526	75.8
	<i>Ny. dunhami</i>	506	3.6
	<i>Ny. triannulatus</i>	261	1.9
	<i>Ny. oswaldoi s.l.</i>	108	0.8
	<i>Ny. benarrochi B</i>	74	0.5
	<i>Ny. rangeli</i>	5	0.0
	<i>Ny. tadei</i>	5	0.0
	<i>Ny. spp.</i>	9	0.1
	<i>An. peryassui</i>	32	0.2
	<i>An. near costai G1</i>	2,286	16.5
	<i>An. mattogrossensis</i>	3	0.02
	<i>An. forattinii/An. near costai</i>	10	0.1
	<i>An. squamifemur</i>	1	0.01
	<i>An. nimbus</i>	1	0.01
	<i>An. spp.</i>	55	0.4
Dry	<i>Ny. darlingi</i>	468	49.7
	<i>Ny. dunhami</i>	57	6.1
	<i>Ny. triannulatus</i>	30	3.2
	<i>Ny. oswaldoi s.l.</i>	23	2.4
	<i>Ny. benarrochi B</i>	6	0.6
	<i>Ny. rangeli</i>	1	0.1
	<i>An. peryassui</i>	11	1.2
	<i>An. near costai G1</i>	342	36.3
	<i>An. forattinii/An. near costai</i>	3	0.3
	<i>An. spp.</i>	2	0.2

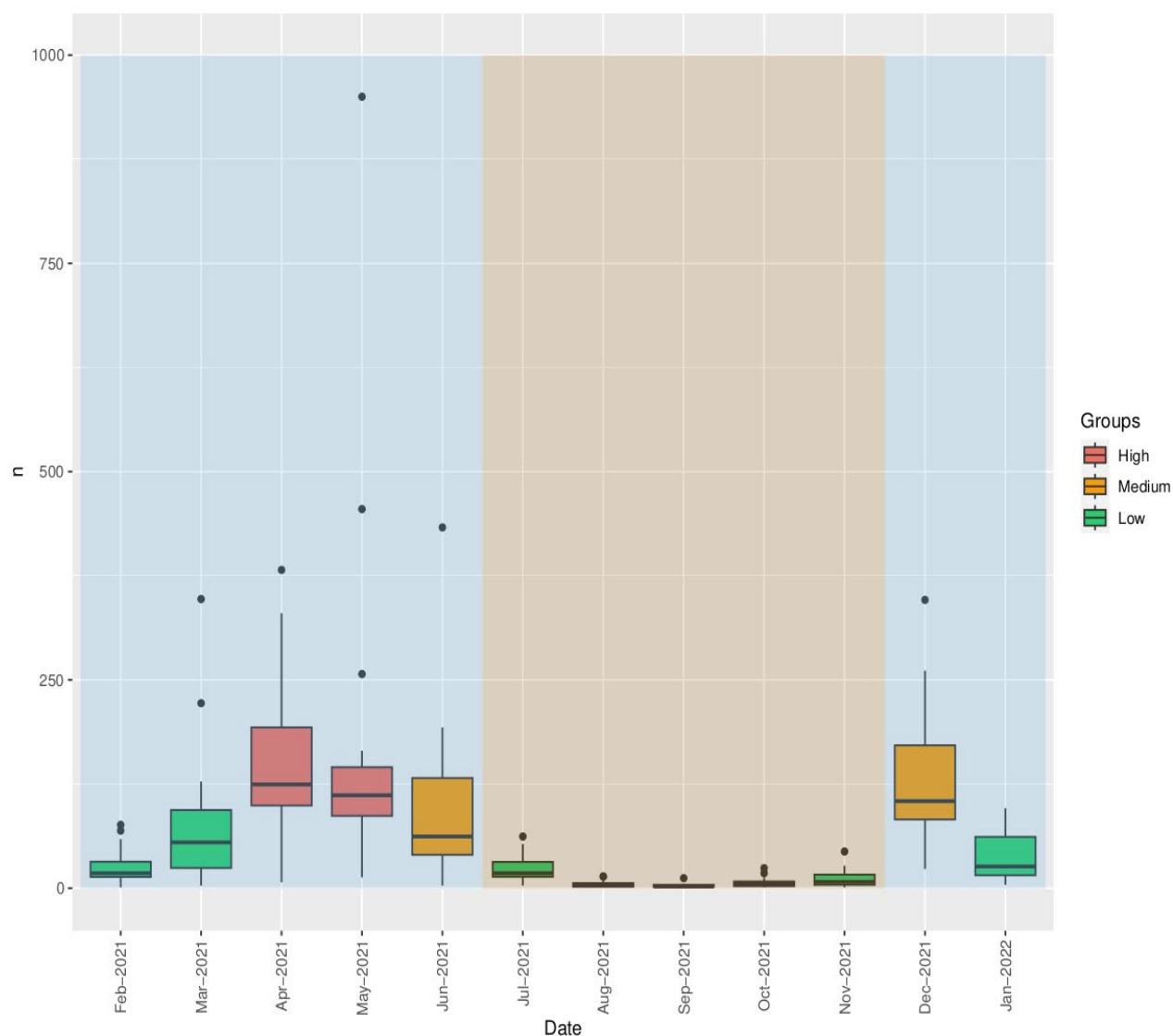
Supplementary Table S6. Infection Rate (IR), Human Biting Rate (HBR) and Entomological Inoculation Rate (EIR) of *An. near costai* G1 by month and cluster in Santa Rita, Peru, 2021-2022.

Month	Cluster	<i>An. near costai</i> G1 (n)	HBR	# Analysed	<i>An. near costai</i> G1 infected	IR	EIR	<i>Plasmodium</i>
Feb - 2021	K1	0	0.00	0	0.00	-	-	<i>P. vivax</i>
	K2	50	11.33	49	0.00	0.00	0.00	
	K3	19	4.75	19	0.00	0.00	0.00	
	K4	78	19.50	74	1.00	0.01	0.26	
	K5	0	0.00	0	0.00	-	-	
Mar - 2021	K1	21	10.50	21	0.00	0.00	0.00	<i>P. spp.*</i>
	K2	151	30.71	148	0.00	0.00	0.00	
	K3	181	45.25	179	0.00	0.00	0.00	
	K4	111	27.75	110	0.00	0.00	0.00	
	K5	0	0.00	0	0.00	-	-	
Apr - 2021	K1	132	66.00	131	0.00	0.00	0.00	<i>P. spp.*</i>
	K2	192	33.00	190	0.00	0.00	0.00	
	K3	105	26.25	104	0.00	0.00	0.00	
	K4	97	24.25	97	1.00	0.01	0.25	
	K5	2	2.00	2	0.00	0.00	0.00	
May - 2021	K1	10	5.00	10	0.00	0.00	0.00	<i>P. malariae/P. brasilianum</i>
	K2	334	45.67	332	1.00	0.00	0.14	
	K3	41	13.67	41	0.00	0.00	0.00	
	K4	176	44.00	176	0.00	0.00	0.00	
	K5	10	5.00	10	0.00	0.00	0.00	
Jun - 2021	K1	4	4.00	3	0.00	0.00	0.00	<i>P. malariae/P. brasilianum</i>
	K2	214	23.42	193	1.00	0.01	0.12	
	K3	56	18.67	54	0.00	0.00	0.00	
	K4	62	31.00	62	0.00	0.00	0.00	
	K5	34	34.00	34	0.00	0.00	0.00	
Jul - 2021	K1	22	7.33	22	0.00	0.00	0.00	<i>P. vivax</i>
	K2	143	21.29	143	0.00	0.00	0.00	
	K3	73	24.33	73	0.00	0.00	0.00	
	K4	14	7.00	14	0.00	0.00	0.00	
	K5	3	3.00	3	0.00	0.00	0.00	
Aug - 2021	K1	1	1.00	1	0.00	0.00	0.00	<i>P. vivax</i>
	K2	0	0.00	0	0.00	-	-	
	K3	0	0.00	0	0.00	-	-	
	K4	7	3.50	7	1.00	0.14	0.50	
	K5	0	0.00	0	0.00	-	-	
Sep - 2021	K1	0	0.00	0	0.00	-	-	<i>P. malariae/P. brasilianum</i>
	K2	2	0.42	2	0.00	0.00	0.00	
	K3	2	2.00	2	0.00	0.00	0.00	
	K4	1	1.00	1	1.00	1.00	1.00	
	K5	0	0.00	0	0.00	-	-	
Oct - 2021	K1	2	2.00	2	0.00	0.00	0.00	<i>P. vivax</i>
	K2	14	2.11	14	0.00	0.00	0.00	
	K3	14	14.00	14	0.00	0.00	0.00	
	K4	2	1.00	2	0.00	0.00	0.00	
	K5	0	0.00	0	0.00	-	-	
Nov - 2021	K1	5	2.50	5	0.00	0.00	0.00	<i>P. vivax</i>
	K2	16	3.00	16	0.00	0.00	0.00	
	K3	4	2.00	4	0.00	0.00	0.00	
	K4	13	4.33	12	0.00	0.00	0.00	
	K5	4	4.00	4	1.00	0.25	1.00	
Dec - 2021	K1	12	6.00	12	1.00	0.08	0.50	<i>P. spp.*</i>
	K2	58	13.83	58	1.00	0.02	0.24	
	K3	34	17.00	34	1.00	0.03	0.50	
	K4	32	8.00	32	0.00	0.00	0.00	
	K5	21	10.50	21	0.00	0.00	0.00	
Jan - 2022	K1	0	0.00	0	0.00	-	-	<i>P. malariae/P. brasilianum</i>
	K2	38	5.06	37	0.00	0.00	0.00	
	K3	6	3.00	4	0.00	0.00	0.00	
	K4	0	0.00	0	0.00	-	-	
	K5	5	5.00	5	0.00	0.00	0.00	

K1, household landscapes; K2, forested areas; K3, crops; K4, degraded patches; K5, flood-prone areas. Total Number of *An. near costai* s.l. specimens captured was 2,628. Although there was one *An. near costai* G2 identified from a subset of *An. near costai* s.l. with *COI* sequencing (n=25), for the purpose of this data summary, we assume the predominant *An. near costai* s.l. species to be *An. near costai* G1. *P. spp.* - we could not identify these samples to species.

Supplementary Table S7. Incidence Rate Ratios (IRRs) from a Poisson regression evaluating the association between *Ny. darlingi* counts, geographic clusters, and season (dry vs. wet).

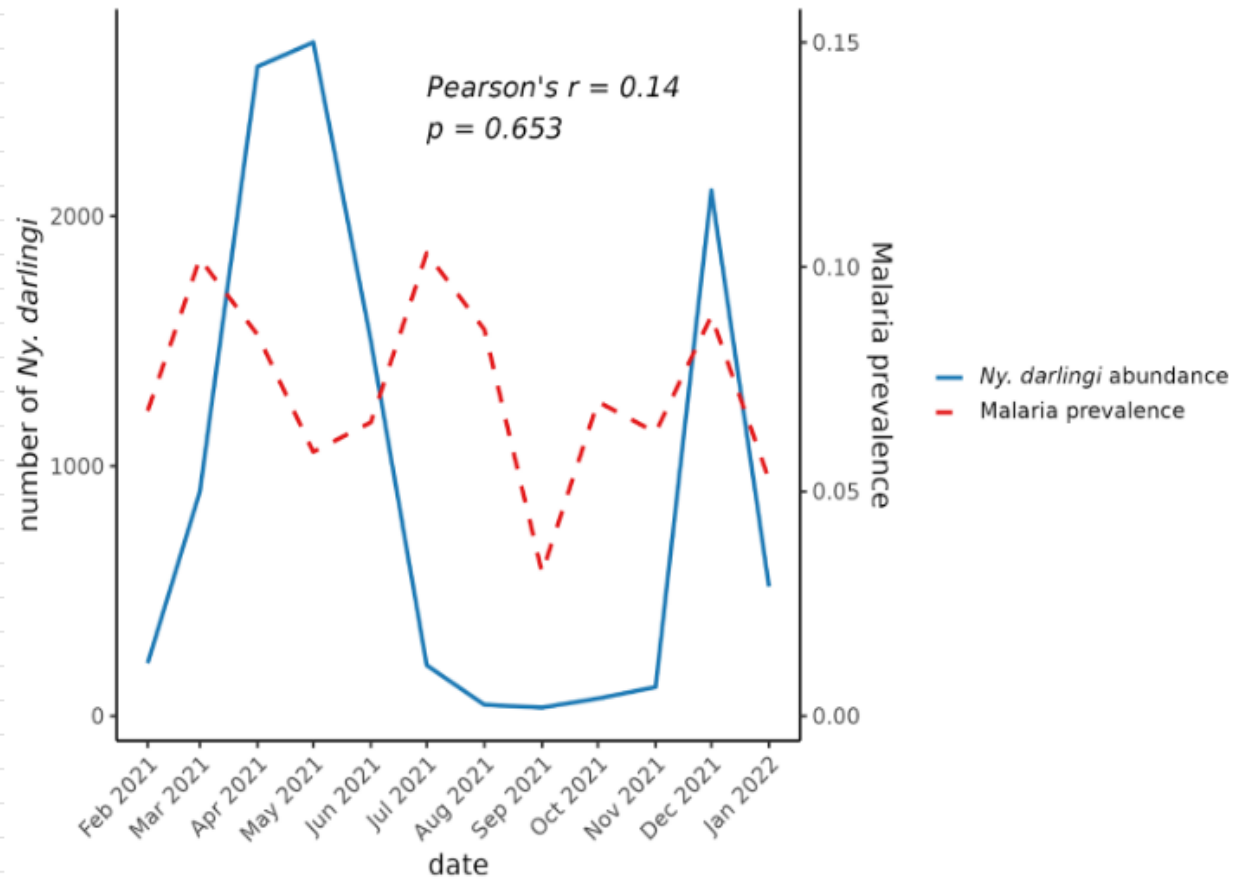
Characteristic		IRR	95% CI	<i>p</i> -value
Cluster				
	Household landscapes	—	—	
	Forested areas	1.51	1.42, 1.60	<0.001
	Crops	1.30	1.22, 1.38	<0.001
	Degraded patches	1.72	1.63, 1.83	<0.001
	Seasonally flood-prone areas	0.52	0.48, 0.57	<0.001
Season				
	Dry	—	—	
	Wet	4.94	4.70, 5.19	<0.001



Supplementary Figure S1. Monthly abundance of *Ny. darlingi* and grouping of high, medium and low transmission by Scott-Knott test ($\alpha = 0.05$), Santa Rita, Peru, 2021-2022.

The y-axis depicts the number of *Ny. darlingi* per cluster; the x-axis corresponds to the collection months. The black dots represent the outliers in the boxplot.

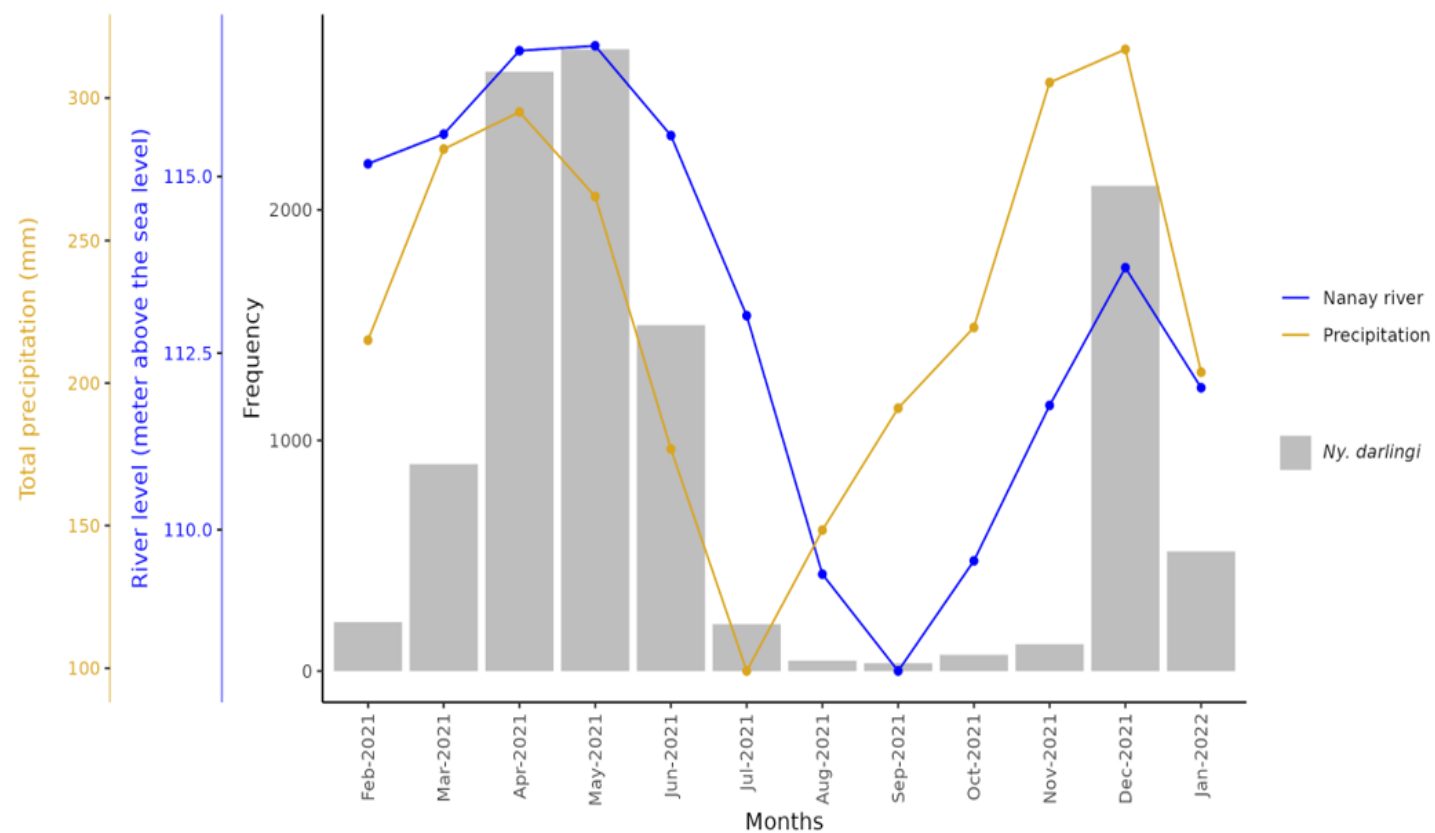
The beige area shows the months of decline of PCR-detected *P. vivax* infections across all transmission intensity groups.



Supplementary Figure S2. Temporal trends of *Ny. darlingi* abundance and malaria prevalence in 2021 with Pearson correlation analysis.



Supplementary Figure S3. Spatial distribution of malaria infections and *An. near costai* G1 abundance by season in Santa Rita, Peru, 2021-2022. Triangles represent the number of *An. near costai* G1 mosquitoes captured, with the darkest blue being the highest number, and circles represent the number of human *P. vivax* infections identified by PCR.



Supplementary Figure S4. Monthly Nanay River level, precipitation and *Ny. darlingi* abundance, Santa Rita, Peru, 2021-2022.