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# Cohort profile: The Mâncio Lima cohort study of urban malaria in Amazonian Brazil

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## ABSTRACT

**Purpose** This population-based open cohort study aims to investigate biological and sociodemographic drivers of malaria transmission in the main urban hotspot of Amazonian Brazil.

**Participants** Nearly 20% of the households in the northwestern town of Mâncio Lima were randomly selected and 2,690 participants were enrolled since March 2018. Sociodemographic, occupational, behavioral, and morbidity information was collected during consecutive study visits. Blood samples from participants older than 3 months were used for malaria diagnosis and human genetic studies: those from study participants with laboratory-confirmed malaria have been cryopreserved for genotypic and phenotypic characterization of parasites. Serology was introduced in 2020 to measure the prevalence and longevity of SARS-CoV-2 antibodies.

**Findings to date** Malaria prevalence rates were low (up to 1.0% for *P. vivax* and 0.6% for *P. falciparum*) during 5 consecutive cross-sectional surveys between March-April 2018 and October-November 2020; 63% of infections diagnosed by microscopy were asymptomatic. Malaria risk is heterogeneously distributed, with 20% study participants contributing 86% of the overall burden of *P. vivax* infection. Adult males are at greatest risk of infection and human mobility across the urban-rural interface may contribute to sustained malaria transmission. Local parasites are genetically diverse and fragmented into discrete inbred lineages that remain stable over time and space.

**Future plans** Two follow-up visits, with similar study protocols, are planned in 2021. We aim to identify high-risk individuals that fuel onwards malaria transmission and represent a priority target for more intensive and effective control interventions.

**Registration** This study has been registered at ClinicalTrials.gov (NCT03689036).

Abstract word count: 249

**Key words** malaria; Amazon; epidemiology; risk factors; SARS-CoV-2 antibodies

**Strengths and limitations of this study**

► This is the first population-based cohort study to address the emerging risk of malaria transmission in urbanized spaces in the Amazon.

► Nearly 20% of the households in town of Mâncio Lima, the main urban malaria hotspot in Brazil, were randomly drawn from census listings and 2,690 participants were enrolled and followed-up since March 2018.

► Sociodemographic, occupational, behavioral, and morbidity information and capillary blood samples were collected during five study visits; venous blood from confirmed malaria infections have been cryopreserved for genotypic and phenotypic parasite characterization.

► Main potential limitations include: (a) generalizability of the study results since the cohort population comprises residents in a single malaria hotspot in the Amazon; (b) loss to follow-up due to the high mobility of the target population; and (c) risk of recall bias during interviews.

## INTRODUCTION

Although local malaria transmission has decreased substantially over the past two decades, 120 million people continue to have some risk of infection in Latin America and the Caribbean.[1] The Amazon Basin accounts for approximately 90% of the malaria cases in the Americas; 72% of them are due to *Plasmodium vivax*. Rural communities such as riverine villages, frontier farming settlements, gold mining enclaves, and Amerindian reserves are disproportionally affected.[2]

Despite the low force of infection found across the Amazon, nearly one order of magnitude lower than that in rural Africa,[3] a minority of highly exposed individuals develops clinical immunity to malaria following infection and eventually constitutes a substantial infectious reservoir comprised of asymptomatic parasite carriers that are overlooked by routine surveillance.[3-8] Importantly, blood from asymptomatic *P. vivax* carriers can infect local malaria vectors despite low mean parasite density.[9]

The Amazon has experienced an accelerated urban growth, characterized by massive rural-to-urban migration, unplanned housing, and inadequate infrastructure, that challenges its conventional representation as a densely forested territory interspersed by small and isolated human settlements.[10] Urban residents now account for 72.5% of the population of the Amazon Basin of Brazil and almost 20% of the 24.4 million Amazonian live in cities with >500,000 inhabitants.[11] Importantly, malaria transmission has been increasingly documented within and near densely populated urban centers of the Amazon,[12-14] with occasional large outbreaks in cities.[15] We hypothesize that asymptomatic carriers continuously move malaria parasites across

the urban-rural interface and contribute significantly to outbreaks and sustained malaria transmission in urbanized spaces in the region.[16]

The dynamics, determinants and public health consequences of urban malaria remain largely unexplored as cities and towns grow and proliferate in the Amazon. This population-based open cohort study was set up to investigate a wide range of biological and sociodemographic factors that drive malaria endemicity in the main urban transmission hotspot of Amazonian Brazil. The long-term goal is to provide scientific evidence that can be translated into effective public health interventions for malaria control and elimination. The original study has since expanded to include SARS-CoV-2 antibody measurements during the ongoing COVID-19 pandemic in this hard-hit region.[17]

## COHORT DESCRIPTION

### Study site

The Mâncio Lima cohort study is part of the National Institutes of Health (NIH)-funded Amazonian International Center of Excellence for Malaria Research network, with the overall aim of investigating malaria epidemiology, vector biology and ecology, diagnostics, transmission biology, and clinical pathogenesis across a range of endemic settings in the Amazon Basin of Peru and Brazil (https://www.niaid.nih.gov/research/amazonian-international-center-excellence-malaria-research). The study site, the town of Mâncio Lima (07°36'51"S, 72°53'45"W), is situated in the upper Juruá Valley region of Acre State, westernmost Brazil, close to the border with Peru (Supplemental Fig. 1). Because urban areas in Brazil are defined according to

relatively arbitrary administrative rules that do not necessarily consider population density and other internationally adopted criteria,[18] we delimitated the town of Mâncio Lima essentially as done by the Brazilian Institute of Geography and Statistics (IBGE) but extended the urban area to two urbanized neighborhoods (Iracema and Pé da Terra) situated along the main road that crosses the town, following "urbanicity" criteria developed for use in this setting.[19] At the time of the study onset, the municipality of Mâncio Lima had an annual parasite incidence (API; number of new laboratory-confirmed malaria cases per 1,000 people per year) estimated at 422.8, the highest for a municipality in Brazil.[20] With a typical equatorial humid climate, the area receives most rainfall between November and April, but malaria transmission occurs year-round. *Plasmodium vivax* accounts for 84% of local malaria cases and *P. falciparum* for 14%; <2% are coinfections with both species.[21] *P. vivax* infections are routinely treated with chloroquine (total dose, 25 mg of base/kg over 3 days) and primaquine (0.5 mg of base/kg/day for 7 days) and *P. falciparum* infections are treated with a fixed-dose combination of artemether (2-4 mg/kg/day) and lumefantrine (12-24 mg/kg/day) for 3 days.[22] Both treatment regimens remain highly efficacious in this area.[23,24]

**Study population**

A baseline population census was conducted in Mâncio Lima between November 2015 and April 2016. We enumerated 9,124 permanent residents in the urban area, with ages ranging between <1 month and 105 years, distributed into 2,329 households.[21] The cohort study sample comprises all members of randomly chosen urban households in Mâncio Lima. We used simple probability sampling to draw 534 households from the list of those enumerated during the baseline census survey. We allowed for up to 2.9% non-localized or empty houses and refusals and aimed to

enroll at least 20% of all households in the town. Because the target sample size was not reached during the baseline visit, we used a list of randomly chosen substitute households during the second visit to replace households that declined participation or were not located. Because this cohort study was designed to evaluate a wide range of sociodemographic, clinical, and laboratory exposures in the same population, no formal a-priori sample size and power calculations were made.

**Participant recruitment and follow-up**

Figure 1 presents the study flowchart. During the first study visit, between March and April 2018, we targeted the 534 households drawn from the census listings; 1,391 residents from 354 households were located and agreed to participate. To achieve the desired sample size, 147 "substitute" households were randomly selected and approached during the second visit. The ongoing cohort is dynamic and new residents joining the household (those who moved in or were born between study visits) are enrolled during the follow-up visits. Study participants leaving the sampled households are retained in the cohort as long as they can be located by the field team and their new residences, which are labelled as new households, are situated in the urban area of Mâncio Lima. As a consequence, the total number of households in the sample has increased since the second visit (Fig. 1). Some study participants could not be located during a follow-up visit but are later "rescued" during the next visits, as indicated in Fig. 1; those who die or leave the town are withdrawn. Five house visits were carried out until November 2020 and two follow-up visits are planned for 2021. Subject to funding, follow-up will continue after 2021.

< Figure 1 >

**Data and sample collection**

Structured questionnaires have been applied to study participants during study visits to obtain and update the demographic, socioeconomic, occupational, behavioral, and morbidity information listed in Table 1. GPS coordinates were obtained for all dwellings. Data were entered using tablets programmed with REDCap[25] and subsequently exported to Stata SE 15.0 (StataCorp, College Station, USA) for statistical analysis. Dates of follow-up visits and the number of participants interviewed in each visit are shown in Table 2.

< Table 1 >

< Table 2 >

During each visit, household members older than 3 months are invited to provide a finger-prick blood sample for on-site malaria diagnosis by microscopy and antigen-based rapid diagnostic tests. Giemsa-stained thick blood smears have at least 100 fields examined for malaria parasites under 1000× magnification by experienced local microscopists. Blood aliquots have been stored at -20°C for extraction of human and parasite DNA. Confirmatory molecular diagnosis of malaria has been made with a genus-specific quantitative PCR protocol that targets the mitochondrial genome of human-infecting malaria parasites[26] followed by species-specific tests. Human genetic studies are underway to identify Duffy blood group polymorphisms that modulate the ability of *P. vivax* merozoites to invade human red blood cells[27] and analyses of single-nucleotide polymorphisms and copy number variation in the gene encoding the cytochrome P450 (CYP) enzyme CYP2D6 that affect the metabolization of the antimalarial drug primaquine.[28] SARS-CoV-2 IgG antibodies were measured by ELISA in samples collected between October and November 2020; to estimate the longevity of SARS-CoV-2 antibody responses, seropositive individuals will be retested within 6 and 12 months.

Study participants with laboratory-confirmed malaria during the study visits are invited to contribute a 5 ml venous blood sample for further genotypic and phenotypic characterization of infecting parasites. Additional samples have been collected between the visits from consenting malaria patients from Mâncio Lima and surrounding rural sites to map the circulation of parasite lineages over time across the region. BioR01 Plus leukocyte-depletion filters (Fresenius Kabi, Bad Homburg, Germany) are used to reduce human DNA contamination before parasites′ genome sequencing.[29]

**Additional data sources**

We have routinely retrieved all malaria case notifications in the study population that were entered into the electronic malaria notification system of the Ministry of Health of Brazil (Portuguese acronym, SIVEP-Malaria) since October 2015. Because malaria is a notifiable disease in Brazil and diagnostic testing and treatment are not available outside the network of government-run health care facilities, the electronic database comprises the vast majority of laboratory-confirmed malaria episodes countrywide.[30]

Streams, wetlands rich in moriche palm trees, and natural and human-made fish farming ponds are widespread across the town of Mâncio Lima and serve as breeding habitats for malaria vectors.[31] Potential mosquito breeding sites have been mapped and are continuously monitored for larval density by the local staff of the National Malaria Control Program.

**Patient and public involvement**

Study participants had no role in the design, recruitment and conduct of the study. However, they have been regularly informed on project objectives and main results by WhatsApp messages and short videos. Links to some of these videos are provided in Supplemental Table 1. Local health officers, including National Malaria Control Program personnel, are routinely briefed on study findings during follow-up visits.

## FINDINGS TO DATE

The Mâncio Lima cohort study of urban malaria is ongoing. Table 3 shows the characteristics of participants and results of onsite malaria diagnosis during five study visits. A total of 2,690 residents were interviewed during at least one follow-up visit and 981 (36.5%) participated in all visits (Supplemental Fig. 2). Capillary blood was obtained from 77.6-84.4% % of participants in each visit, with a total of 7,781 samples collected (Table 3). Reasons for not providing blood samples included age below 3 months and inability or refusal to perform a finger puncture. Compared with the total population of Mâncio Lima enumerated during the baseline census, the study sample has a very similar distribution according to sex and age groups (Supplemental Fig. 3) and place of residence (Supplemental Fig. 4). Overall malaria prevalence rates determined by microscopy during the study visits ranged between 0.1% and 1.0% for *P. vivax* and 0.0% and 0.6% for *P. falciparum*; 39 of 62 (62.9%) infections were asymptomatic within one week prior to blood collection. Ongoing analyses indicate that our PCR protocol detects up to 10 times more malaria infections than microscopy in cohort participants.

< Table 3 >

Analyses published to date combined baseline information with routinely collected malaria surveillance data. We found a marked heterogeneity in malaria risk in the study population. Adult males are at greatest risk of infection; poor housing and residence in the less urbanized periphery of the town are additional predictors of elevated malaria risk.[21] Nearly 14% of the study participants, mostly young children and the elderly, comprise a very low-risk fraction of the population who tend to remain uninfected.[21] We used compartmental susceptible-infected-susceptible transmission models to quantify malaria risk heterogeneity at the community level. We estimate that 20% of the residents contribute 86% of the overall burden of *P. vivax* infection in Mâncio Lima.[3] These high-risk individuals eventually develop clinical immunity to malaria and constitute the asymptomatic infectious reservoir that fuels onwards transmission.

To explore the relative contribution of human mobility to malaria in Mâncio Lima, we investigated patterns and determinants of urban-to-rural mobility, which is mostly work-related and places travelers at risk of malaria, and rural-to-urban mobility caused by malaria treatment seeking, which poses an additional risk of infection to urban residents. This information was retrieved from travel histories collected during follow-up visits. We found that the rural localities most frequently visited by urban residents are those with the most intense malaria transmission. These are also the most frequent sources of imported malaria cases diagnosed in the town. The most mobile study participants are unemployed men 16 to 60-years old who maintain both urban and rural residences.[32]

Parasites collected during the study were used to map local and regional transmission pathways of *P. vivax.* We found high genome-level diversity in the *P. vivax* population of Mâncio Lima,

but parasites were fragmented into discrete inbred lineages that co-circulate in the host population over extended periods of time.[33] Microsatellite genotyping of local parasites also revealed the persistence of near-clonal parasite lineages in the town, which have been interpreted as evidence for sustained local *P. vivax* transmission.[34] We also found significant ancestry sharing between parasites collected at a distance >700 km, suggesting that *P. vivax* lineages from the Mâncio Lima hotspot seed regional malaria transmission.[33]

**Strengths and limitations**

This is the first population-based cohort study of urban malaria in the Amazon. We combine longitudinally collected data and samples to identify demographic, socioeconomic, occupational, behavioral, and biological contributors to sustained malaria transmission in urbanized spaces that may represent targets for control interventions. Importantly, the prospective study design enables us to discern temporal associations between exposures and the outcomes of interest and to map the spread of parasite lineages over space and time.

Malaria-related outcomes in the study population have been measured in two ways. First, the point prevalence of infection is determined during each follow-up visit. Conventional and molecular diagnostic tests are carried out on all blood samples, that are collected regardless of any current or recent symptom, allowing us to detect submicroscopic and asymptomatic parasite carriage. Second, the incidence of clinical malaria between study visits is estimated from malaria case notifications in the study population. These are essentially symptomatic infections diagnosed by conventional microscopy. The main limitation of routine surveillance data is that

blood samples are not available for further confirmatory diagnostic tests. Moreover, although surveillance comprises virtually all malaria episodes diagnosed by microscopy, submicroscopic and asymptomatic malaria episodes experienced by the study population between follow-up visits are overlooked.

There has been considerable cohort attrition from initial recruitment to the most recent follow-up visit, 32 months later. The study population is very mobile and many people are involved in seasonal work in surrounding rural areas. Study participants, especially those who have a second residence, often leave the town either temporally or definitively. This may reduce the statistical power to test for associations between less prevalent exposures and outcomes. New residents in the study households are continuously enrolled and returning participants are recued, keeping the total number of participants remarkably stable between visits 2 and 5. However, it remains to be determined whether the remaining and newly arriving participants are representative of the initial study population regarding main exposures.

Another study limitation is the risk of recall bias during interviews. For example, information regarding recent mobility and malaria-related symptoms is entirely dependent on participants′ reports. It is difficult to know with any precision how many antecedent episodes of malaria infection an individual might have had.

Finally, the cohort includes residents in a single malaria hotspot in the Amazon, which may compromise the generalizability of the main results. However, we argue that malaria transmission is increasingly common in similar urbanized spaces in the Amazon.[12-15] Extensive

deforestation and environmental degradation have displaced vectors to more urbanized areas across the region, where suitable larval habitats, such as natural water bodies and human-made fish farming ponds, are now widespread.[13,15,31,35-38]

## COLLABORATION

Datasets from the Mâncio Lima cohort study are not yet openly available, but will be deposited in the ClinEpiDB (https://clinepidb.org/ce/app) repository of population-based epidemiological studies. A repository database has been organized at the University of São Paulo, where future published papers will be made available (https://uspdigital.usp.br/repositorio/). Researchers who are interested in potential collaboration should contact the principal investigator, Marcelo U. Ferreira (muferrei@usp.br), to complete a research plan for evaluation by the Amazonian International Center of Excellence for Malaria Research steering committee.

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**Contributors**. M.U.F., J.M.V. and M.C.C. planned the cohort. M.U.F., P.T.R. and I.C.J. coordinated the fieldwork, with the help of J.T. I.C.J., M.U.F. and M.C.C. supervised data management and analysis. I.C.J. and M.U.F. wrote the first manuscript draft with input from all authors, who have read and approved the final version of the manuscript.

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**Competing interests**. None declared.

**Patient consent for publication.** Not required.

**Ethics approval**. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Study protocols have been approved by the Institutional Review Board of the Institute of Biomedical Sciences, University of São Paulo, and by the National Committee of Ethics in Research, Ministry of Health of Brazil (CAAE numbers 64767416.6.0000.5467 and 30481820.3.0000.5467). Written informed consent was obtained from all study participants or their parents/guardians.

**Provenance and peer review**. Not commissioned; externally peer-reviewed.

**Data availability statement**. Additional data are available on reasonable request.

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**Figure legend.**

Figure 1. Participant flowchart of the Mâncio Lima cohort study of urban malaria.

Table 1. Data collected for the Mâncio Lima cohort study of urban malaria

| Measures | Description |
|---|---|
| **Questionnaire data** | |
| Demographic | Age; sex; pregnancy status; birthplace (municipality, state, and if rural or urban); literacy; second residence outside the urban area (no/yes and where) |
| Socioeconomic | Housing characteristics and ownership of assets; beneficiary of social programs (social security pension, conditional cash transfer program) |
| Occupational | Individual work status; head of the household work status (does not work; formal employee; informal employee; employer) |
| Behavioral | Regular fishing (no/yes); sleeping by the river (no/yes); sleeps with open or closed window (no/yes); time that go to bed and wake up; bathing inside or outside the house |
| Clinical | Laboratory-diagnosed malaria since the last follow-up visit (how many positive diagnosis and parasite species); malaria symptoms in the 7 days prior to the interview |
| Travel history | Overnight trip outside the town since the last follow-up visit (no/yes, place most visited and duration) |
| **Laboratory data** | |
| Malaria microscopy | Giemsa-stained thick smears |
| Malaria RDT | QuickProfile Pf/Pv immunochromatographic test (LumiQuick, Santa Clara, USA). |
| Malaria PCR | Genus-specific quantitative PCR protocol that targets the mitochondrial genome followed by species-specific amplification |
| Duffy/DARC genotyping | TaqMan assays (Applied Biosystems, Foster City, USA) to genotype rs2814778 and rs12075 |
| CYP2D6 genotyping | TaqMan assays (Applied Biosystems) to genotype 12 CYP2D6 single nucleotide polymorphisms and quantify *CYP2D6* gene copy number |
| SARS-CoV-2 antibodies | ELISA for IgG antibodies to the subdomain S1 of the SARS-CoV-2 spike protein (Euroimmun, Lübeck, Germany) |
| Parasite genome sequencing | Whole-genome sequencing on an Illumina NovaSeq next-generation sequencer |
| Parasite phenotypes | Ex-vivo assays of drug sensitivity and red blood cell invasion |

Table 2. Timeline of measurements for the Mâncio Lima cohort study of urban malaria

| Variables | Census survey (Nov 15-Apr 16) | Visit 1 (Mar-Apr 18) | Visit 2 (Oct-Nov 18) | Visit 3 (Apr-May 19) | Visit 4 (Oct-Nov 19) | Visit 5 (Oct-Nov 20) |
|---|---|---|---|---|---|---|
| **Questionnaire data** | | | | | | |
| No. participants interviewed | 9,124 | 1,394 | 2,009 | 2,017 | 2,130 | 2,074 |
| Demographic | √ | √ | √ | √ | √ | √ |
| Socioeconomic | √ | √ | √ | √ | √ | √ |
| Occupational | | √ | √ | √ | √ | √ |
| Behavioral | | √ | √ | √ | √ | √ |
| Clinical | | √ | √ | √ | √ | √ |
| Travel history | | | | √ | √ | √ |
| **Laboratory data** | | | | | | |
| No. blood samples collected[a] | | 1,082 | 1,696 | 1,578 | 1,768 | 1,677 |
| Malaria microscopy | | √ | √ | √ | √ | √ |
| Malaria RDT | | | √ | √ | √ | √ |
| Malaria PCR | | | √ | √ | √ | √ |
| Duffy/DARC genotyping | | √ | √ | √ | √ | √ |

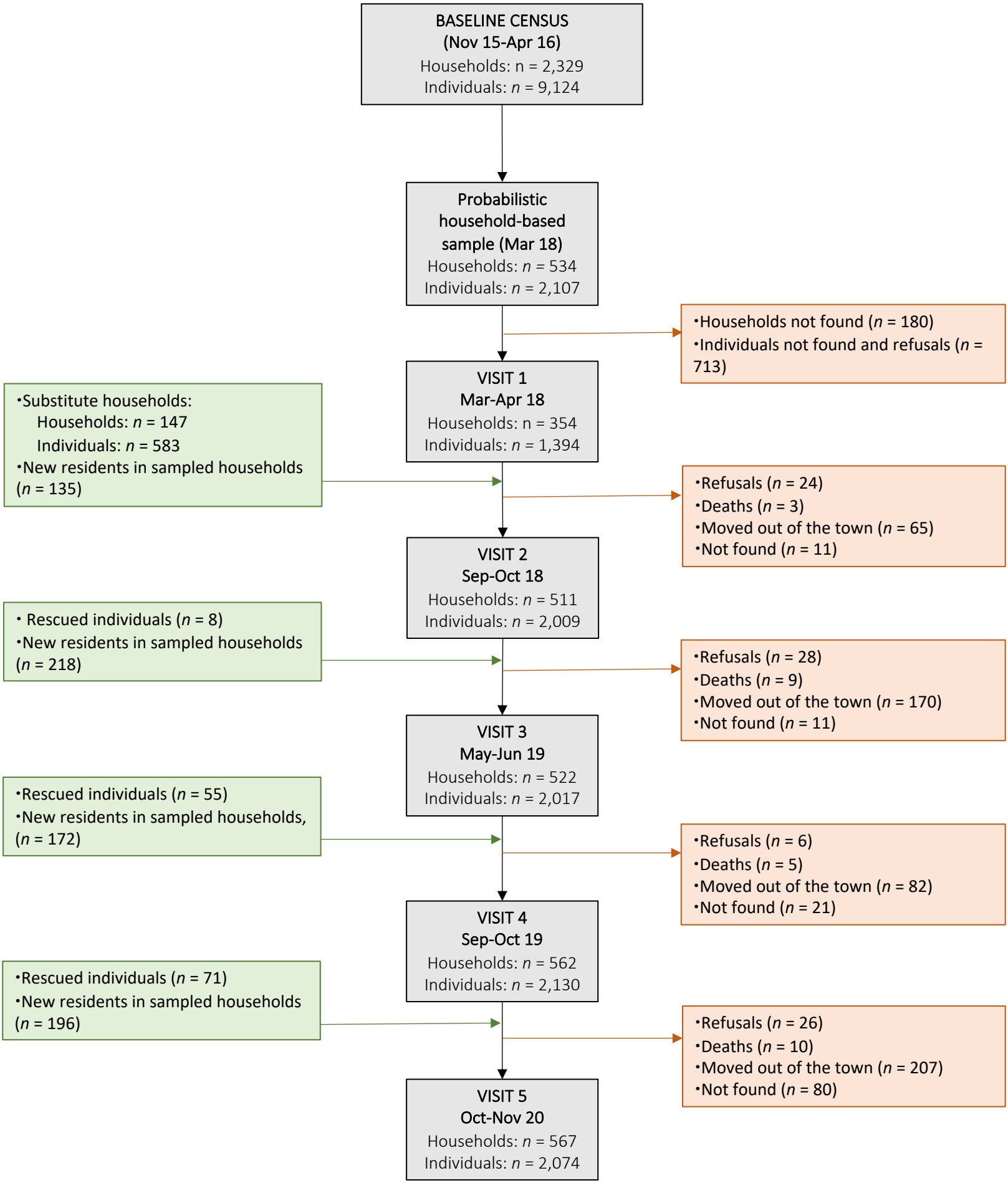
| | | | | | |
|---|---|---|---|---|---|
| CYP2D6 genotyping | √ | √ | √ | √ | √ |
| SARS-CoV-2 antibodies | | | | | √ |
| Malaria parasite sequencing and phenotyping[b] | √ | √ | √ | √ | √ |

√ denotes that the measurement has been made at the noted study visit.
[a]Finger-prick capillary blood samples collected for malaria diagnosis were used for human genetic and SARS-CoV-2 studies
[b]Venous blood samples were collected from consenting study participants with laboratory-confirmed malaria and leukocyte-depleted for whole-genome sequencing and ex-vivo phenotypic assays.

**Table 3.** Number of malarial infections diagnosed by rapid diagnostic test (RDT) and conventional microscopy, according to the presence of malaria-related symptoms, during 5 consecutive cross-sectional surveys in the Mâncio Lima, cohort (2018-2020).

| | | Study visit | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | 4 | | 5 | |
| Symptoms | Species | RDT | Microscopy | RDT | Microscopy | RDT | Microscopy | RDT | Microscopy | RDT | Microscopy |
| Yes | *P. falciparum* | - | 3 | 4 | 0 | 0 | 3 | 1 | 1 | 0 | 0 |
| | *P. vivax* | - | 2 | 4 | 8 | 2 | 4 | 0 | 0 | 0 | 2 |
| | Mixed | - | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | No. tested | - | 218 | 307 | 308 | 182 | 182 | 205 | 205 | 137 | 137 |
| No | *P. falciparum* | - | 5 | 4 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| | *P. vivax* | - | 12 | 5 | 5 | 2 | 5 | 3 | 3 | 0 | 1 |
| | Mixed | - | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| | No. tested | - | 864 | 1,389 | 1,388 | 1,396 | 1,396 | 1,563 | 1,563 | 1,540 | 1,540 |
| Total | *P. falciparum* | - | 8 | 8 | 3 | 3 | 6 | 4 | 2 | 0 | 1 |
| | *P. vivax* | - | 14 | 9 | 13 | 4 | 9 | 3 | 3 | 0 | 3 |
| | Mixed | - | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 |
| | No. tested | - | 1,082 | 1,696 | 1,696 | 1,578 | 1,578 | 1,768 | 1,768 | 1,677 | 1,677 |
| Prevalence (%) | *P. falciparum* | - | 0.6 | 0.4 | 0.1 | 0.1 | 0.3 | 0.2 | 0.1 | 0.0 | 0.0 |
| | *P. vivax* | - | 1.0 | 0.4 | 0.6 | 0.2 | 0.4 | 0.1 | 0.1 | 0.0 | 0.1 |
| | Mixed | - | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Dates of study visits: 1, April-May, 2018; 2, September-October, 2018; 3, May-June, 2019; 4, September-October, 2019; and 5, October-November, 2020. Symptoms (at least one present within the past two weeks): fever, chills, sweating, headache, nausea, vomiting, myalgia, and arthralgia.


BASELINE CENSUS
(Nov 15-Apr 16)
Households: n = 2,329
Individuals: n = 9,124
Probabilistic household-based sample (Mar 18)
Households: n = 534
Individuals: n = 2,107
•Households not found (n = 180)
•Individuals not found and refusals (n = 713)
VISIT 1
Mar-Apr 18
Households: n = 354
Individuals: n = 1,394
•Substitute households:
Households: n = 147
Individuals: n = 583
•New residents in sampled households (n = 135)
•Refusals (n = 24)
•Deaths (n = 3)
•Moved out of the town (n = 65)
•Not found (n = 11)
VISIT 2
Sep-Oct 18
Households: n = 511
Individuals: n = 2,009
• Rescued individuals (n = 8)
•New residents in sampled households (n = 218)
•Refusals (n = 28)
•Deaths (n = 9)
•Moved out of the town (n = 170)
•Not found (n = 11)
VISIT 3
May-Jun 19
Households: n = 522
Individuals: n = 2,017
•Rescued individuals (n = 55)
•New residents in sampled households, (n = 172)
•Refusals (n = 6)
•Deaths (n = 5)
•Moved out of the town (n = 82)
•Not found (n = 21)
VISIT 4
Sep-Oct 19
Households: n = 562
Individuals: n = 2,130
•Rescued individuals (n = 71)
•New residents in sampled households (n = 196)
•Refusals (n = 26)
•Deaths (n = 10)
•Moved out of the town (n = 207)
•Not found (n = 80)
VISIT 5
Oct-Nov 20
Households: n = 567
Individuals: n = 2,074

Supplemental Material for:

# Cohort profile: The Mâncio Lima cohort study of urban malaria in Amazonian Brazil

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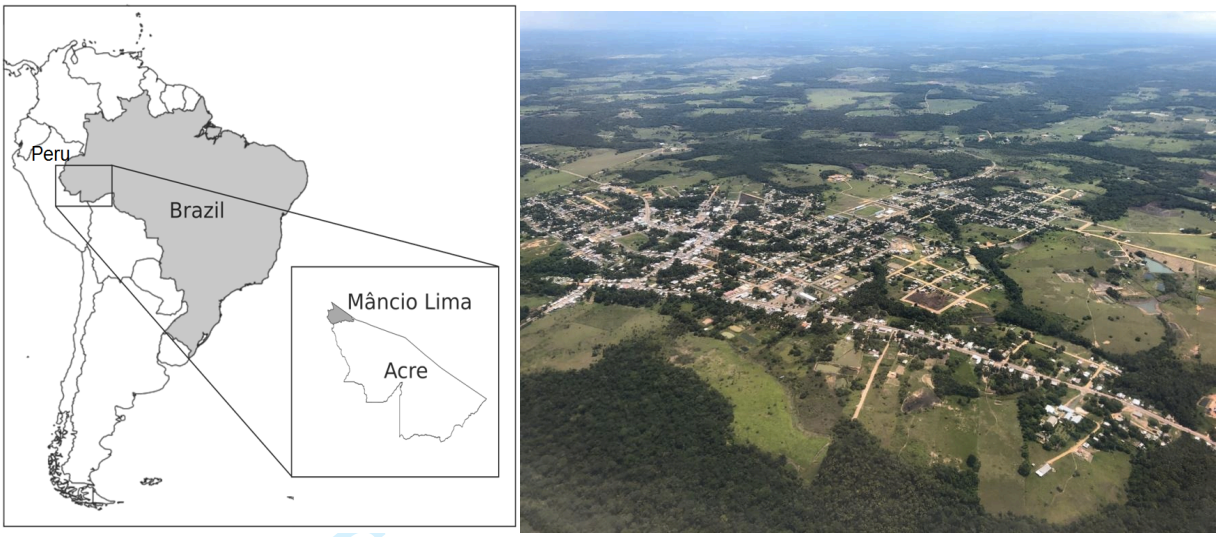
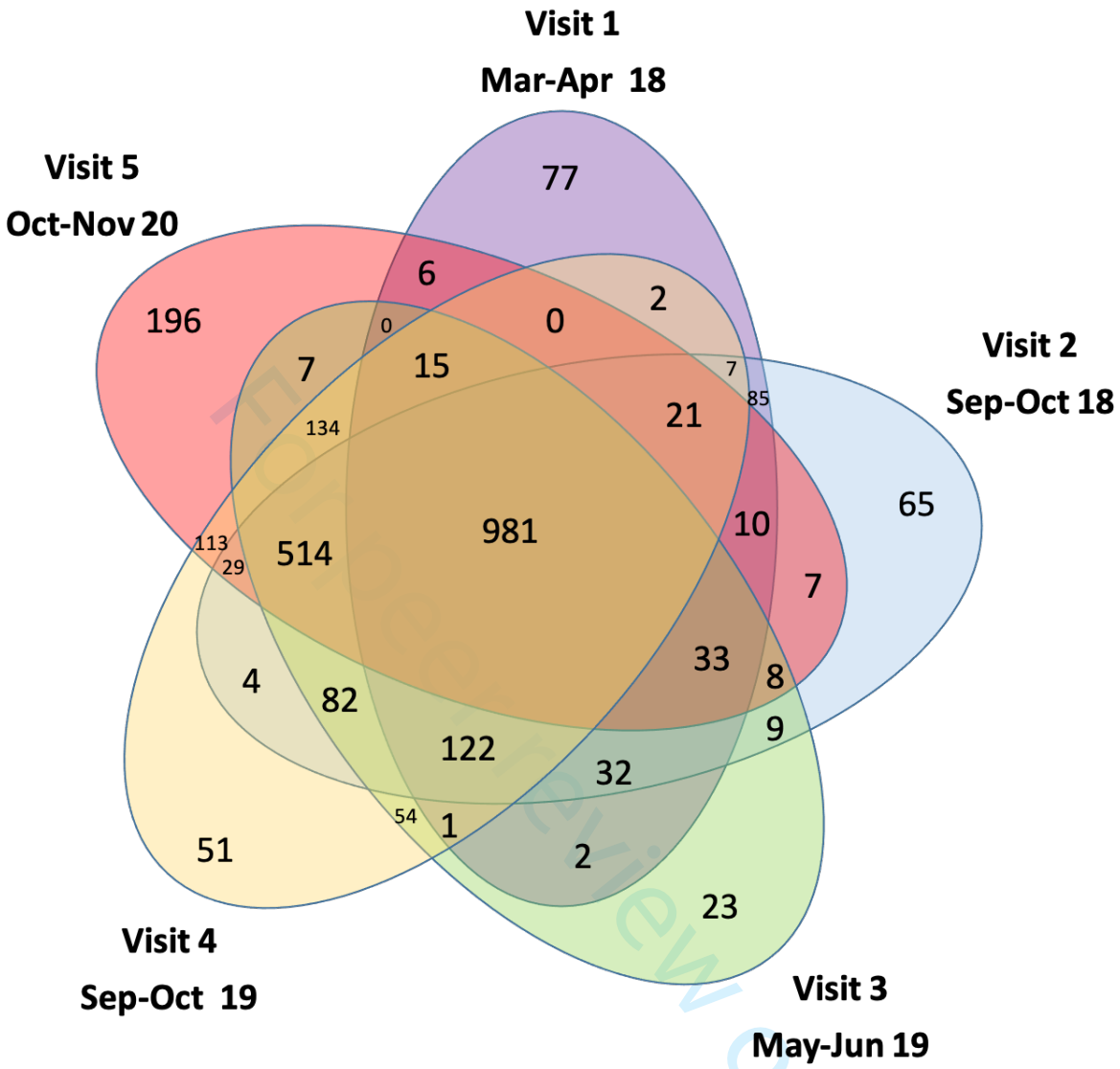
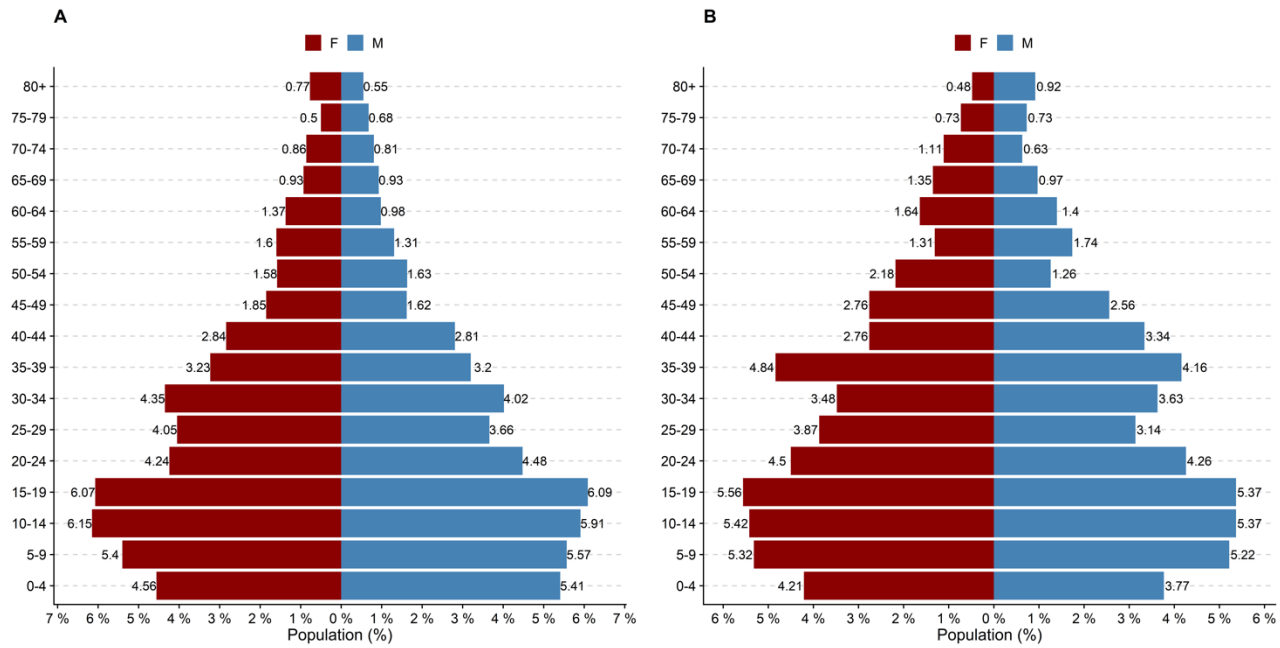


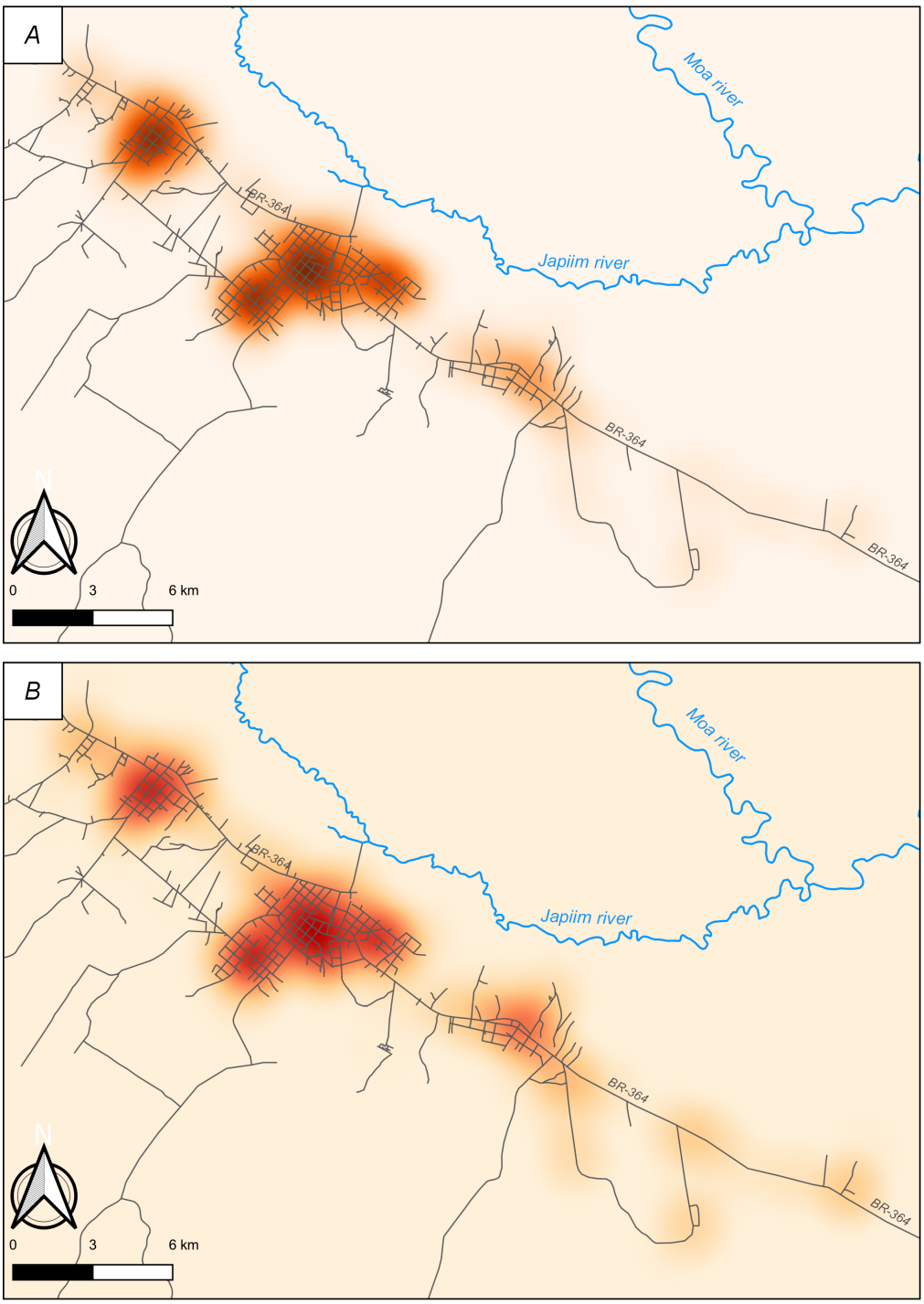

**Supplemental Fig. 1. Study Site.** The left panel shows the location of the Municipality of Mâncio Lima, in northwestern Brazil, next to the border with Peru. The right panel shows an aerial photography of the town of Mâncio Lima. (Photography by Rodrigo M. Corder.)




**Supplemental Fig. 2. Participants in consecutive study visits.** The Venn diagram shows the number of individuals interviewed in each study visit and in different combinations of them. Note that 981 individuals participated in all study visits.




**Supplemental Fig. 3. Representativeness of the study population according to sex and age groups.** Compared with the total population of Mâncio Lima enumerated during the baseline census carried out between November 2015 and April 2016 (A), the study sample has a very similar distribution according to sex and age groups (B).




**Supplemental Fig. 4. Representativeness of the study population according to place of residence.** Compared with the total population of Mâncio Lima enumerated during the baseline census carried out between November 2015 and April 2016 (A), the study sample has a nearly equal distribution according to place of residence (B).

**Supplemental Table 1. Short videos about the Mâncio Lima cohort study**

| Access | Topic | Producer |
|---|---|---|
| https://youtu.be/oEwJPHiBNmc | Field study set-up, March 2018 | TV USP |
| https://www.youtube.com/watch?v=CBx7z0ApznI | House-to-house visits, March 2018 | TV USP |
| https://youtu.be/wE3gJfdRpVo | Impact of COVID-19 on malaria, August 2020 | Agência FAPESP[a] |

[a]Five short videos on COVID-19 research in the field site were produced; a teaser can be found at: https://www.youtube.com/watch?v=lg1Ug-T_whQ&list=PLPdNbZy8nStgk8tZiXn7M7E9EeWXQ2zl9&index=1.

BMJ Open

# Cohort profile: The Mâncio Lima cohort study of urban malaria in Amazonian Brazil



| | |
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| | |

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# Cohort profile: The Mâncio Lima cohort study of urban malaria in Amazonian Brazil

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## ABSTRACT

**Purpose** This population-based open cohort study aims to investigate biological and sociodemographic drivers of malaria transmission in the main urban hotspot of Amazonian Brazil.

**Participants** Nearly 20% of the households in the northwestern town of Mâncio Lima were randomly selected and 2,690 participants were enrolled since April 2018. Sociodemographic, housing quality, occupational, behavioral, and morbidity information and travel histories were collected during consecutive study visits. Blood samples from participants >3 months old were used for malaria diagnosis and human genetic studies; samples from participants with laboratory-confirmed malaria have been cryopreserved for genetic and phenotypic characterization of parasites. Serology was introduced in 2020 to measure the prevalence and longevity of SARS-CoV-2 IgG antibodies.

**Findings to date** Malaria prevalence rates were low (up to 1.0% for *P. vivax* and 0.6% for *P. falciparum*) during 5 consecutive cross-sectional surveys between April-May 2018 and October-November 2020; 63% of infections diagnosed by microscopy were asymptomatic. Malaria risk is heterogeneously distributed, with 20% study participants contributing 86% of the overall burden of *P. vivax* infection. Adult males are at greatest risk of infection and human mobility across the urban-rural interface may contribute to sustained malaria transmission. Local *P. vivax* parasites are genetically diverse and fragmented into discrete inbred lineages that remain stable across space and time.

**Future plans** Two follow-up visits, with similar study protocols, are planned in 2021. We aim to identify high-risk individuals that fuel onwards malaria transmission and represent a priority target for more intensive and effective control interventions.

**Registration** ClinicalTrials.gov (NCT03689036).

Abstract word count: 249

**Key words** malaria; Amazon; epidemiology; risk factors; SARS-CoV-2 antibodies

**Strengths and limitations of this study**

► This is the first population-based cohort study to address the emerging risk of malaria transmission in urbanized spaces in the Amazon.

► Nearly 20% of the households in town of Mâncio Lima, the main urban malaria hotspot in Brazil, were randomly drawn from census listings and 2,690 participants were enrolled and followed-up since April 2018.

► Sociodemographic, occupational, behavioral, and morbidity information, travel histories, and capillary blood samples were collected during five study visits; venous blood from confirmed malaria infections have been cryopreserved for genetic/genomic and phenotypic parasite characterization.

► Main potential limitations include: (a) generalizability of the study results since the cohort population comprises residents in a single malaria hotspot in the Amazon; (b) loss to follow-up due to the high mobility of the target population; and (c) risk of recall bias during interviews.

## INTRODUCTION

Although local malaria transmission has decreased substantially over the past two decades, 120 million people continue to have some risk of infection in Latin America and the Caribbean.[1] The Amazon Basin accounts for approximately 90% of the malaria cases in the Americas; 72% of them are due to *Plasmodium vivax*. Rural communities such as riverine villages, frontier farming settlements, gold mining enclaves, and Amerindian reserves are disproportionally affected.[2]

Despite the low force of infection found across the Amazon, nearly one order of magnitude lower than that in rural Africa,[3] a minority of highly exposed individuals develops clinical immunity to malaria following infection and eventually constitutes a substantial infectious reservoir comprised of asymptomatic parasite carriers that are overlooked by routine surveillance.[3-8] Importantly, blood from asymptomatic *P. vivax* carriers can infect local malaria vectors despite low mean parasite density.[9]

The Amazon has experienced an accelerated urban growth, characterized by massive rural-to-urban migration, unplanned housing, and inadequate infrastructure, that challenges its conventional representation as a densely forested territory interspersed by small and isolated human settlements.[10] Urban residents now account for 72.5% of the population of the Amazon Basin of Brazil and almost 20% of the 24.4 million Amazonian live in cities with >500,000 inhabitants.[11] Importantly, malaria transmission has been increasingly documented within and near densely populated urban centers of the Amazon,[12-14] with occasional large outbreaks in cities.[15] We hypothesize that asymptomatic carriers continuously move malaria parasites across

the urban-rural interface and contribute significantly to outbreaks and sustained malaria transmission in urbanized spaces in the region.[16]

The dynamics, determinants, and public health consequences of urban malaria remain largely unexplored as cities and towns grow and proliferate in the Amazon. This population-based open cohort study was set up to investigate a wide range of biological and sociodemographic factors that drive malaria endemicity in the main urban transmission hotspot of Amazonian Brazil. The long-term goal is to provide scientific evidence that can be translated into effective public health interventions for malaria control and elimination. The original study has since expanded to include SARS-CoV-2 IgG antibody measurements during the ongoing COVID-19 pandemic in this hard-hit region and investigate possible interactions between dengue and COVID-19.[17]

## COHORT DESCRIPTION

### Study site

The Mâncio Lima cohort study aims to investigate malaria epidemiology, diagnostics, transmission dynamics, and clinical pathogenesis in Amazonian Brazil. The study site, the town of Mâncio Lima (07°36'51"S, 72°53'45"W), is situated in the upper Juruá Valley region of Acre State, westernmost Brazil, close to the border with Peru (Supplemental Fig. 1). Because urban areas in Brazil are defined according to relatively arbitrary administrative rules that do not necessarily consider population density and other internationally adopted criteria,[18] we delimitated the town of Mâncio Lima essentially as done by the Brazilian Institute of Geography and Statistics (IBGE) but extended the urban area to two urbanized neighborhoods (Iracema and

Pé da Terra) situated along the main road that crosses the town, following "urbanicity" criteria developed for use in this setting.[19] At the time of the study onset, the municipality of Mâncio Lima had an annual parasite incidence (API; number of new laboratory-confirmed malaria cases per 1,000 people per year) estimated at 422.8, the highest for a municipality in Brazil.[20] With a typical equatorial humid climate, the area receives most rainfall between November and April, but malaria transmission occurs year-round. *Plasmodium vivax* accounts for 84% of local malaria cases and *P. falciparum* for 14%; <2% are coinfections with both species.[21] *P. vivax* infections are routinely treated with chloroquine (total dose, 25 mg of base/kg over 3 days) and primaquine (0.5 mg of base/kg/day for 7 days) and *P. falciparum* infections are treated with a fixed-dose combination of artemether (2-4 mg/kg/day) and lumefantrine (12-24 mg/kg/day) for 3 days.[22] Both treatment regimens remain highly efficacious in this area.[23,24]

**Study population**

A baseline population census was conducted in Mâncio Lima between November 2015 and April 2016. We enumerated 9,124 permanent residents in the urban area, with ages ranging between <1 month and 105 years, distributed into 2,329 households.[21] The cohort study sample comprises all members of randomly chosen urban households in Mâncio Lima. We used simple probability sampling to draw 534 households from the list of those enumerated during the baseline census survey. We allowed for up to 2.9% non-localized or empty houses and refusals and aimed to enroll at least 20% of all households in the town. Because the target sample size was not reached during the baseline visit, we used a list of randomly chosen substitute households during the second visit to replace households that declined participation or were not located. Because this cohort study was designed to evaluate a wide range of sociodemographic, clinical, and laboratory

exposures in the same population, no formal a-priori sample size and power calculations were made.

**Participant recruitment and follow-up**

Figure 1 presents the study flowchart. During the first study visit, between April and May 2018, we targeted the 534 households drawn from the census listings; 1,391 residents from 354 households were located and agreed to participate. To achieve the desired sample size, 147 "substitute" households were randomly selected and approached during the second visit, in October-November 2018. The ongoing cohort is dynamic and new residents joining the household (those who moved in or were born between study visits) are enrolled during the follow-up visits. Study participants leaving the sampled households are retained in the cohort as long as they can be located by the field team and their new residences, which are labelled as new households, are situated in the urban area of Mâncio Lima. As a consequence, the total number of households in the sample has increased since the second visit (Fig. 1). Some study participants could not be located during a follow-up visit but are later "rescued" during the next visits, as indicated in Fig. 1; those who die or leave the town are withdrawn. Participants who died, moved away from the study site, and those who withdrew their consent to participate were considered lost for follow-up. Five house visits were carried out until November 2020 and two follow-up visits are planned for 2021. Subject to funding, follow-up will continue after 2021.

< Figure 1 >

**Data and sample collection**

Structured questionnaires have been applied to study participants during study visits to obtain and update the demographic, socioeconomic, occupational, behavioral, and morbidity information listed in Table 1, which indicates which key variables are individual (one value for each participant) or household-level (the same value attributed to all household members). Dates of follow-up visits and the number of participants interviewed in each visit are shown in Table 2. Both individual and household-level information was collected during study visits. For the vast majority of variables, information is missing for <5% of participants. GPS coordinates were obtained for all dwellings. Data were entered using tablets programmed with REDCap[25] and subsequently exported to Stata SE 15.0 (StataCorp, College Station, USA) for statistical analysis. Because study participants are nested into households, which introduces dependency among observations, we have been using mixed-effects logistic or Poisson regression models with random effects at the household level and robust variance for data analysis.

< Table 1 >

< Table 2 >

During each visit, household members older than 3 months are invited to provide a finger-prick blood sample for on-site malaria diagnosis by microscopy and antigen-based rapid diagnostic tests. Giemsa-stained thick blood smears have at least 100 fields examined for malaria parasites under 1000× magnification by experienced local microscopists. Blood aliquots have been stored at -20°C for extraction of human and parasite DNA. Confirmatory molecular diagnosis of malaria has been made with a genus-specific quantitative PCR protocol that targets the mitochondrial genome of human-infecting malaria parasites[26] followed by species-specific tests. Human genetic studies are underway to identify Duffy blood group polymorphisms that modulate the ability of *P. vivax* merozoites to invade human red blood cells[27] and analyses of

single-nucleotide polymorphisms and copy number variation in the gene encoding the cytochrome P450 (CYP) enzyme CYP2D6 that affect the metabolization of the antimalarial drug primaquine.[28] SARS-CoV-2 IgG antibodies were measured by ELISA in samples collected between October and November 2020; to estimate the longevity of SARS-CoV-2 antibody responses, seropositive individuals will be retested within 6 and 12 months.

Study participants with laboratory-confirmed malaria during the study visits are invited to contribute a 5 ml venous blood sample for further genetic/genomic and phenotypic characterization of infecting parasites. Additional samples have been collected between the visits from consenting malaria patients from Mâncio Lima and surrounding rural sites to map the circulation of parasite lineages over time across the region. BioR01 Plus leukocyte-depletion filters (Fresenius Kabi, Bad Homburg, Germany) are used to reduce human DNA contamination before parasites′ genome sequencing.[29]

**Additional data sources**

We have routinely retrieved all malaria case notifications in the study population that were entered into the electronic malaria notification system of the Ministry of Health of Brazil (Portuguese acronym, SIVEP-Malaria) since October 2015. Because malaria is a notifiable disease in Brazil and diagnostic testing and treatment are not available outside the network of government-run health care facilities, the electronic database comprises the vast majority of laboratory-confirmed malaria episodes countrywide.[30]

Streams, wetlands rich in moriche palm trees, and natural and human-made fish farming ponds are widespread across the town of Mâncio Lima and serve as breeding habitats for malaria vectors.[31] Potential mosquito breeding sites have been mapped and are continuously monitored for larval density by the local staff of the National Malaria Control Program.

**Patient and public involvement**

Study participants had no role in the design, recruitment and conduct of the study. However, they have been regularly informed on project objectives and main results by WhatsApp messages, podcasts, and short videos. Links to some of these videos are provided in Supplemental Table 1. Local health officers, including National Malaria Control Program personnel, are routinely briefed on study findings during follow-up visits.

**FINDINGS TO DATE**

The Mâncio Lima cohort study of urban malaria is ongoing. Supplemental Table 2 shows the sociodemographic characteristics of participants at enrollment. A total of 2,690 residents were interviewed during at least one follow-up visit and 981 (36.5%) participated in all visits (Supplemental Fig. 2). Capillary blood was obtained from 77.6-84.4% % of participants in each visit, with a total of 7,781 samples collected (Table 3). Reasons for not providing blood samples included age below 3 months and inability or refusal to perform a finger puncture. Compared with the total population of Mâncio Lima enumerated during the baseline census, the study sample has a very similar distribution according to sex and age groups (Supplemental Fig. 3) and place of residence (Supplemental Fig. 4). Overall malaria prevalence rates determined by

microscopy during the study visits ranged between 0.1% and 1.0% for *P. vivax* and 0.0% and 0.6% for *P. falciparum*; 39 of 62 (62.9%) infections were asymptomatic within one week prior to blood collection (Table 3). Ongoing analyses indicate that our PCR protocol detects up to 10 times more malaria infections than microscopy in cohort participants, but further standardization and validation are in progress.

< Table 3 >

Analyses published to date combined baseline information with routinely collected malaria surveillance data. We found a marked heterogeneity in malaria risk in the study population. Adult males are at greatest risk of infection; poor housing and residence in the less urbanized periphery of the town are additional predictors of elevated malaria risk.[21] Nearly 14% of the study participants, mostly young children and the elderly, comprise a very low-risk fraction of the population who tend to remain uninfected.[21] We used compartmental susceptible-infected-susceptible transmission models to quantify malaria risk heterogeneity at the community level. We estimate that 20% of the residents contribute 86% of the overall burden of *P. vivax* infection in Mâncio Lima.[3] These high-risk individuals eventually develop clinical immunity to malaria and constitute the asymptomatic infectious reservoir that fuels onwards transmission.

To explore the relative contribution of human mobility to malaria in Mâncio Lima, we investigated patterns and determinants of urban-to-rural mobility, which is mostly work-related and places travelers at risk of malaria, and rural-to-urban mobility caused by malaria treatment seeking, which poses an additional risk of infection to urban residents. This information was retrieved from travel histories collected during follow-up visits. We found that the rural localities most frequently visited by urban residents are those with the most intense malaria transmission.

These are also the most frequent sources of imported malaria cases diagnosed in the town. The most mobile study participants are unemployed men 16 to 60-years old who maintain both urban and rural residences.[32]

Parasites collected during the study were used to map local and regional transmission pathways of *P. vivax.* We found high genome-level diversity in the *P. vivax* population of Mâncio Lima, but parasites were fragmented into discrete inbred lineages that co-circulate in the host population over extended periods of time.[33] Microsatellite genotyping of local parasites also revealed the persistence of near-clonal parasite lineages in the town, which have been interpreted as evidence for sustained local *P. vivax* transmission.[34] We also found significant ancestry sharing between parasites collected at a distance >700 km, suggesting that *P. vivax* lineages from the Mâncio Lima hotspot seed regional malaria transmission.[33]

We have recently shown that serologically proven prior dengue infection is associated with increased subsequent risk of clinically apparent COVID-19 in this cohort.[17] Dengue IgG antibodies were detected in 37.0% of the 1,285 cohort participants tested in October-November, 2019, with 10.4 seroconversion events per 100 person-years over the following 12 months. In October-November, 2020, 35.2% of the participants tested had anti-SARS-CoV-2 IgG and 57.1% of the 448 SARS-CoV-2 seropositives reported clinical manifestations of COVID-19 at the time of infection. Participants aged >60 years were twice more likely to have symptomatic COVID-19 than under-five children. Importantly, prior dengue infection was associated with twice the risk of clinically apparent COVID-19 upon SARS-CoV-2 infection after adjustment for identified confounders.[17]

**Strengths and limitations**

This is the first population-based cohort study of urban malaria in the Amazon. We combine longitudinally collected data and samples to identify demographic, socioeconomic, occupational, behavioral, and biological contributors to sustained malaria transmission in urbanized spaces that may represent targets for control interventions. Importantly, the prospective study design enables us to discern temporal associations between exposures and the outcomes of interest and to map the spread of parasite lineages over space and time.

Malaria-related outcomes in the study population have been measured in two ways. First, the point prevalence of infection is determined during each follow-up visit. Conventional and molecular diagnostic tests are carried out on all blood samples, that are collected regardless of any current or recent symptom, allowing us to detect submicroscopic and asymptomatic parasite carriage. Second, the incidence of clinical malaria between study visits is estimated from malaria case notifications in the study population. These are essentially symptomatic infections diagnosed by conventional microscopy. The main limitation of routine surveillance data is that blood samples are not available for further confirmatory diagnostic tests. Moreover, although surveillance comprises virtually all malaria episodes diagnosed by microscopy, submicroscopic and asymptomatic malaria episodes experienced by the study population between follow-up visits are overlooked.

There has been considerable cohort attrition from initial recruitment to the most recent follow-up visit, 32 months later. The study population is very mobile and many people are involved in seasonal work in surrounding rural areas. Study participants, especially those who have a second residence, often leave the town either temporally or definitively. This may reduce the statistical power to test for associations between less prevalent exposures and outcomes. New residents in the study households are continuously enrolled and returning participants are recued, keeping the total number of participants remarkably stable between visits 2 and 5. However, it remains to be determined whether, regarding main study exposures, newly arriving and remaining participants are similarly representative of the target population.

Another study limitation is the risk of recall bias during interviews. For example, information regarding travel, recent mobility and malaria-related symptoms is entirely dependent on participants′ reports. It is difficult to know with any precision how many antecedent episodes of malaria infection an individual might have had.

Finally, the cohort includes residents in a single malaria hotspot in the Amazon, which may compromise the generalizability of the main results. However, we argue that malaria transmission is increasingly common in similar urbanized spaces in the Amazon.[12-15] Extensive deforestation and environmental degradation have displaced vectors to more urbanized areas across the region, where suitable larval habitats, such as natural water bodies and human-made fish farming ponds, are now widespread.[13,15,31,35-38]

## COLLABORATION

Datasets from the Mâncio Lima cohort study are not yet openly available, but will be deposited in the ClinEpiDB (https://clinepidb.org/ce/app) repository of population-based epidemiological studies. A repository database has been organized at the University of São Paulo, where future published papers will be made available (https://uspdigital.usp.br/repositorio/). Researchers who are interested in potential collaboration should contact the principal investigator, Marcelo U. Ferreira (muferrei@usp.br), to complete a research plan for evaluation by the Amazonian International Center of Excellence for Malaria Research steering committee.

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**Contributors**. M.U.F., J.M.V. and M.C.C. planned the cohort. M.U.F., P.T.R. and I.C.J. coordinated the fieldwork, with the help of J.T. I.C.J., M.U.F. and M.C.C. supervised data management and analysis. I.C.J. and M.U.F. wrote the first manuscript draft with input from all authors, who have read and approved the final version of the manuscript.

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**Competing interests**. None declared.

**Patient consent for publication.** Not required.

**Ethics approval**. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Study protocols have been approved by the Institutional Review Board of the Institute of Biomedical Sciences, University of São Paulo, and by the National Committee of Ethics in Research, Ministry of Health of Brazil (CAAE numbers 64767416.6.0000.5467 and 30481820.3.0000.5467). Written informed consent was obtained from all study participants or their parents/guardians.

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**Data availability statement**. Additional data are available on reasonable request.

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**Figure legend.**

Figure 1. Participant flowchart of the Mâncio Lima cohort study of urban malaria.

Table 1. Data collected for the Mâncio Lima cohort study of urban malaria

| Measures | Description |
|---|---|
| **Questionnaire data** | |
| Demographic | Age; gender; pregnancy status; birthplace; literacy; second residence outside the urban area (no/yes)[a] |
| Socioeconomic | Housing characteristics and ownership of assets; beneficiary of social programs (social security pension, conditional cash transfer program) |
| Occupational | Individual work status (does not work; formal employee; informal employee; employer) |
| Behavioral | Regular fishing (no/yes); sleeping by the river (no/yes); sleeps with open or closed window (no/yes); time that go to bed and wake up; bathing inside or outside the house; use of bed nets during the night before interview. |
| Travel history | Overnight trip outside the town since the last follow-up visit (no/yes, place most frequently visited and duration of stay) |
| Morbidity | Laboratory-diagnosed malaria since the last follow-up visit (how many positive diagnosis and parasite species); malaria symptoms in the 7 days prior to the interview |
| **Laboratory data** | |
| Malaria microscopy | Giemsa-stained thick smears |
| Malaria RDT | QuickProfile Pf/Pv immunochromatographic test (LumiQuick, Santa Clara, USA). |
| Malaria PCR | Genus-specific quantitative PCR protocol that targets the mitochondrial genome followed by species-specific amplification |
| Duffy/DARC genotyping | TaqMan assays (Applied Biosystems, Foster City, USA) to genotype rs2814778 and rs12075 |
| CYP2D6 genotyping | TaqMan assays (Applied Biosystems) to genotype 12 CYP2D6 single nucleotide polymorphisms and quantify *CYP2D6* gene copy number |
| SARS-CoV-2 antibodies | ELISA for IgG antibodies to the recombinant subdomain S1 of the SARS-CoV-2 spike protein (EI 2606-9601 G; Euroimmun, Lübeck, Germany) |
| Dengue antibodies | ELISA for IgG antibodies to dengue virus serotype 2 viral particles (EI 266b-9601 G; Euroimmun, Lübeck, Germany) |
| Parasite phenotypes | Ex-vivo assays of drug sensitivity and red blood cell invasion |
| Parasite genome sequencing | Whole-genome sequencing on an Illumina NovaSeq next-generation sequencer |

[a]Household-level variables are underlined.

Table 2. Timeline of measurements for the Mâncio Lima cohort study of urban malaria

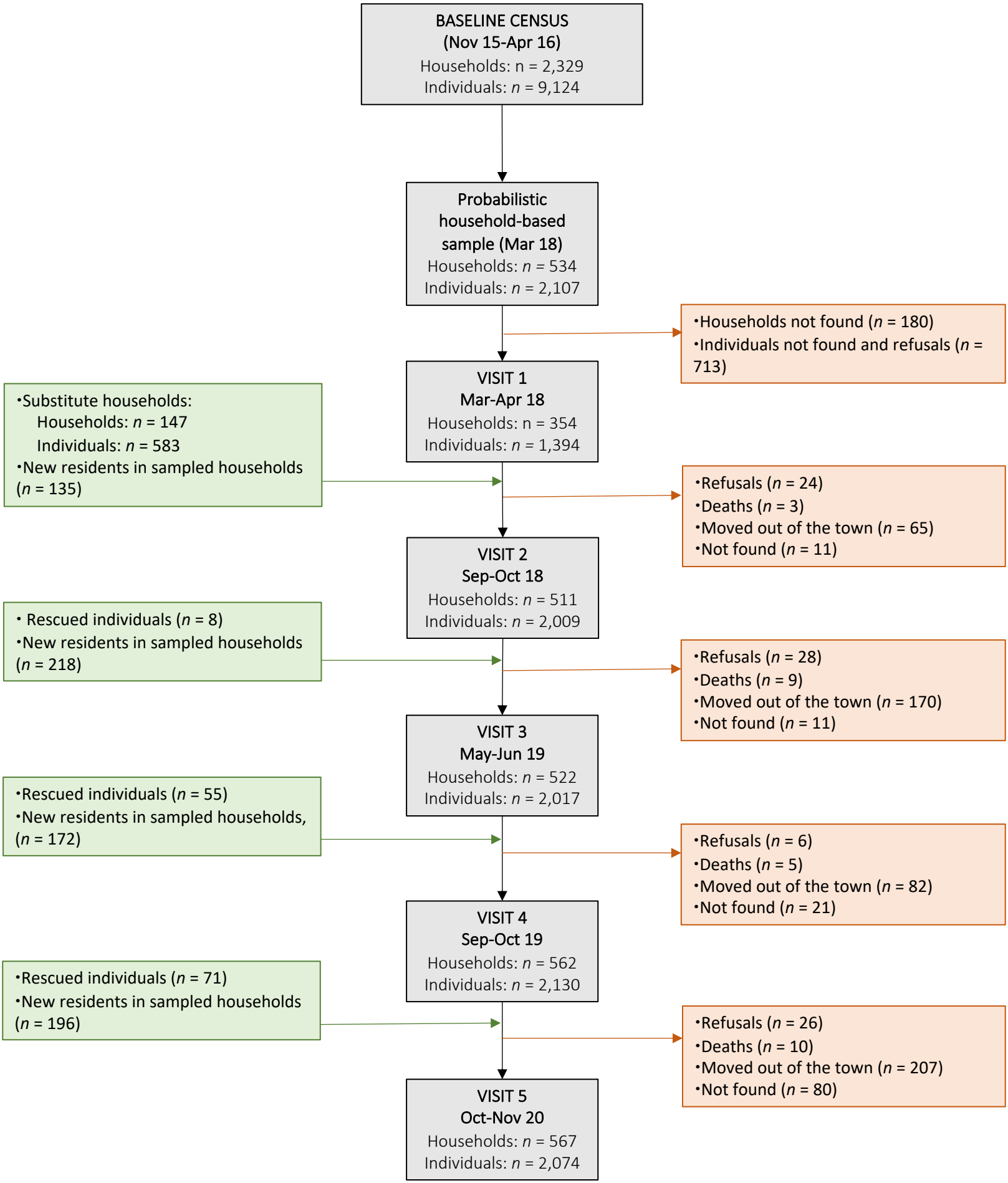
| Variables | Census survey (Nov 15-Apr 16) | Visit 1 (Apr-May 18) | Visit 2 (Sep-Oct 18) | Visit 3 (Apr-May 19) | Visit 4 (Oct-Nov 19) | Visit 5 (Oct-Nov 20) |
|---|---|---|---|---|---|---|
| **Questionnaire data** | | | | | | |
| No. participants interviewed | 9,124 | 1,394 | 2,009 | 2,017 | 2,130 | 2,074 |
| Demographic | √ | √ | √ | √ | √ | √ |
| Socioeconomic | √ | √ | √ | √ | √ | √ |
| Occupational | | √ | √ | √ | √ | √ |
| Behavioral | | √ | √ | √ | √ | √ |
| Clinical | | √ | √ | √ | √ | √ |
| Travel history | | | | √ | √ | √ |
| **Laboratory data** | | | | | | |
| No. blood samples collected[a] | | 1,082 | 1,696 | 1,578 | 1,768 | 1,677 |
| Malaria microscopy | | √ | √ | √ | √ | √ |
| Malaria RDT | | | √ | √ | √ | √ |
| Malaria PCR | | | √ | √ | √ | √ |
| Duffy/DARC genotyping | | √ | √ | √ | √ | √ |
| CYP2D6 genotyping | | √ | √ | √ | √ | √ |
| SARS-CoV-2 antibodies | | | | | | √ |
| Dengue antibodies | | | | | √ | √ |
| Malaria parasite sequencing and phenotyping[b] | | √ | √ | √ | √ | √ |

√ denotes that the measurement has been made at the noted study visit.
[a]Finger-prick capillary blood samples collected for malaria diagnosis were used for human genetic and SARS-CoV-2 studies
[b]Venous blood samples were collected from consenting study participants with laboratory-confirmed malaria and leukocyte-depleted for whole-genome sequencing and ex-vivo phenotypic assays.

**Table 3.** Number of malarial infections diagnosed by rapid diagnostic test (RDT) and conventional microscopy, according to the presence of malaria-related symptoms, during 5 consecutive cross-sectional surveys in the Mâncio Lima, cohort (2018-2020).

| | | Study visit | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | 4 | | 5 | |
| Symptoms | Species | RDT | Microscopy | RDT | Microscopy | RDT | Microscopy | RDT | Microscopy | RDT | Microscopy |
| Yes | *P. falciparum* | - | 3 | 4 | 0 | 0 | 3 | 1 | 1 | 0 | 0 |
| | *P. vivax* | - | 2 | 4 | 8 | 2 | 4 | 0 | 0 | 0 | 2 |
| | Mixed | - | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | No. tested | - | 218 | 307 | 308 | 182 | 182 | 205 | 205 | 137 | 137 |
| No | *P. falciparum* | - | 5 | 4 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| | *P. vivax* | - | 12 | 5 | 5 | 2 | 5 | 3 | 3 | 0 | 1 |
| | Mixed | - | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| | No. tested | - | 864 | 1,389 | 1,388 | 1,396 | 1,396 | 1,563 | 1,563 | 1,540 | 1,540 |
| Total | *P. falciparum* | - | 8 | 8 | 3 | 3 | 6 | 4 | 2 | 0 | 1 |
| | *P. vivax* | - | 14 | 9 | 13 | 4 | 9 | 3 | 3 | 0 | 3 |
| | Mixed | - | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 |
| | No. tested | - | 1,082 | 1,696 | 1,696 | 1,578 | 1,578 | 1,768 | 1,768 | 1,677 | 1,677 |
| Prevalence (%) | *P. falciparum* | - | 0.6 | 0.4 | 0.1 | 0.1 | 0.3 | 0.2 | 0.1 | 0.0 | 0.0 |
| | *P. vivax* | - | 1.0 | 0.4 | 0.6 | 0.2 | 0.4 | 0.1 | 0.1 | 0.0 | 0.1 |
| | Mixed | - | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Dates of study visits: 1, April-May, 2018; 2, September-October, 2018; 3, May-June, 2019; 4, September-October, 2019; and 5, October-November, 2020. Symptoms (at least one present within the past two weeks): fever, chills, sweating, headache, nausea, vomiting, myalgia, and arthralgia.


BASELINE CENSUS
(Nov 15-Apr 16)
Households: n = 2,329
Individuals: n = 9,124
Probabilistic household-based sample (Mar 18)
Households: n = 534
Individuals: n = 2,107
•Households not found (n = 180)
•Individuals not found and refusals (n = 713)
VISIT 1
Mar-Apr 18
Households: n = 354
Individuals: n = 1,394
•Substitute households:
Households: n = 147
Individuals: n = 583
•New residents in sampled households (n = 135)
•Refusals (n = 24)
•Deaths (n = 3)
•Moved out of the town (n = 65)
•Not found (n = 11)
VISIT 2
Sep-Oct 18
Households: n = 511
Individuals: n = 2,009
• Rescued individuals (n = 8)
•New residents in sampled households (n = 218)
•Refusals (n = 28)
•Deaths (n = 9)
•Moved out of the town (n = 170)
•Not found (n = 11)
VISIT 3
May-Jun 19
Households: n = 522
Individuals: n = 2,017
•Rescued individuals (n = 55)
•New residents in sampled households, (n = 172)
•Refusals (n = 6)
•Deaths (n = 5)
•Moved out of the town (n = 82)
•Not found (n = 21)
VISIT 4
Sep-Oct 19
Households: n = 562
Individuals: n = 2,130
•Rescued individuals (n = 71)
•New residents in sampled households (n = 196)
•Refusals (n = 26)
•Deaths (n = 10)
•Moved out of the town (n = 207)
•Not found (n = 80)
VISIT 5
Oct-Nov 20
Households: n = 567
Individuals: n = 2,074

Supplemental Material for:

# Cohort profile: The Mâncio Lima cohort study of urban malaria in Amazonian Brazil

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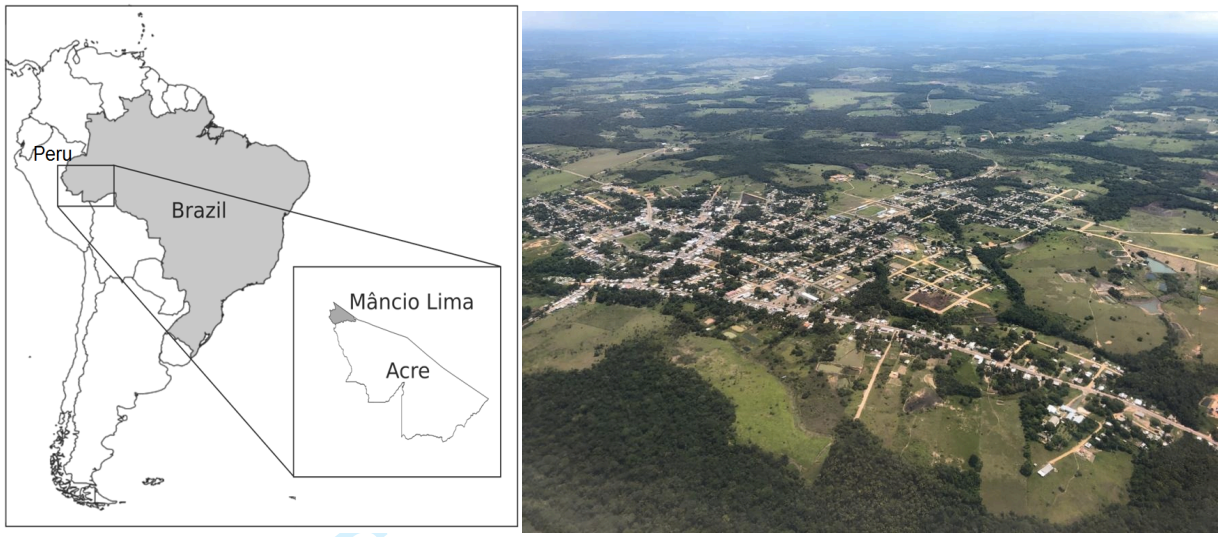
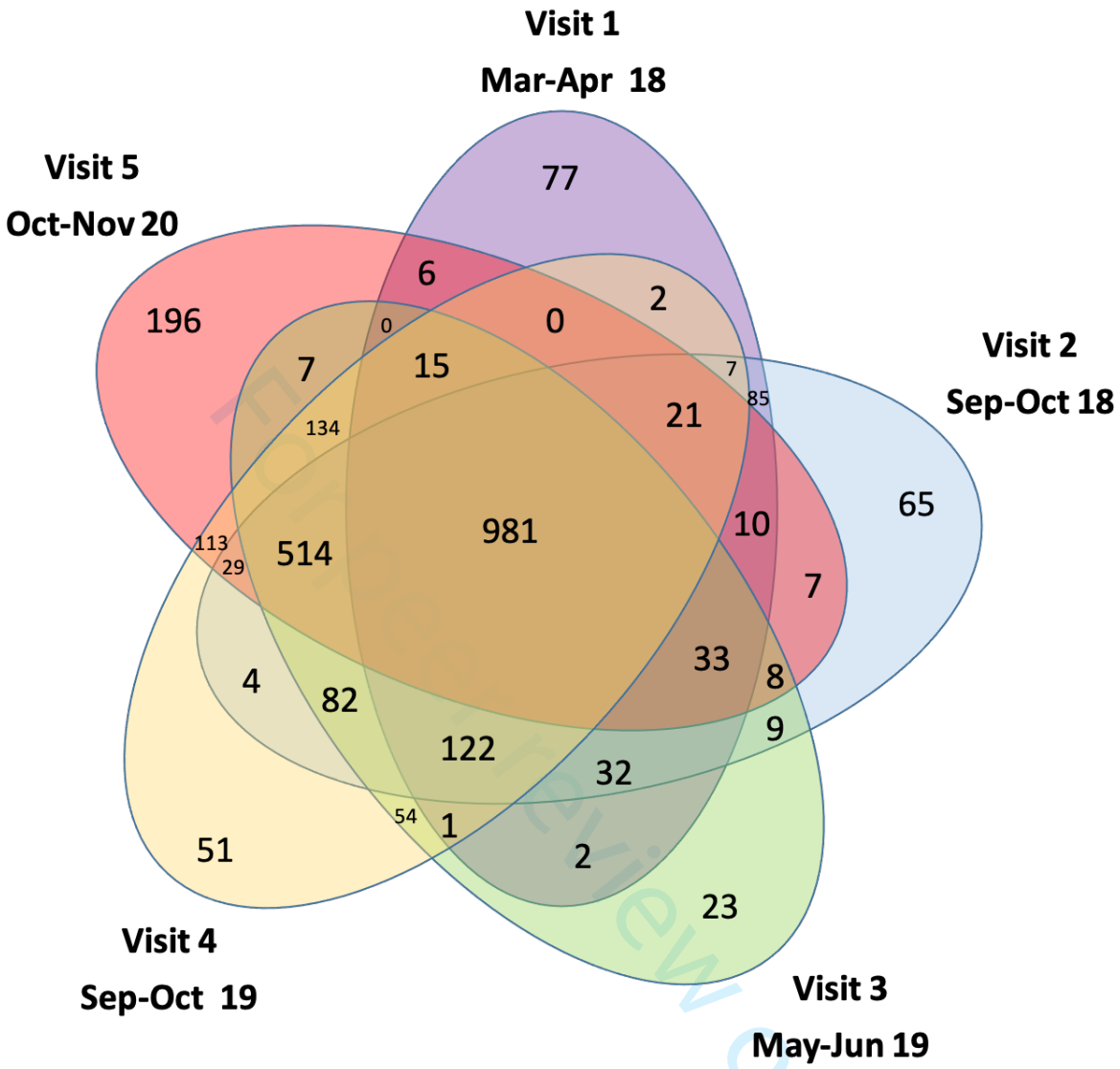
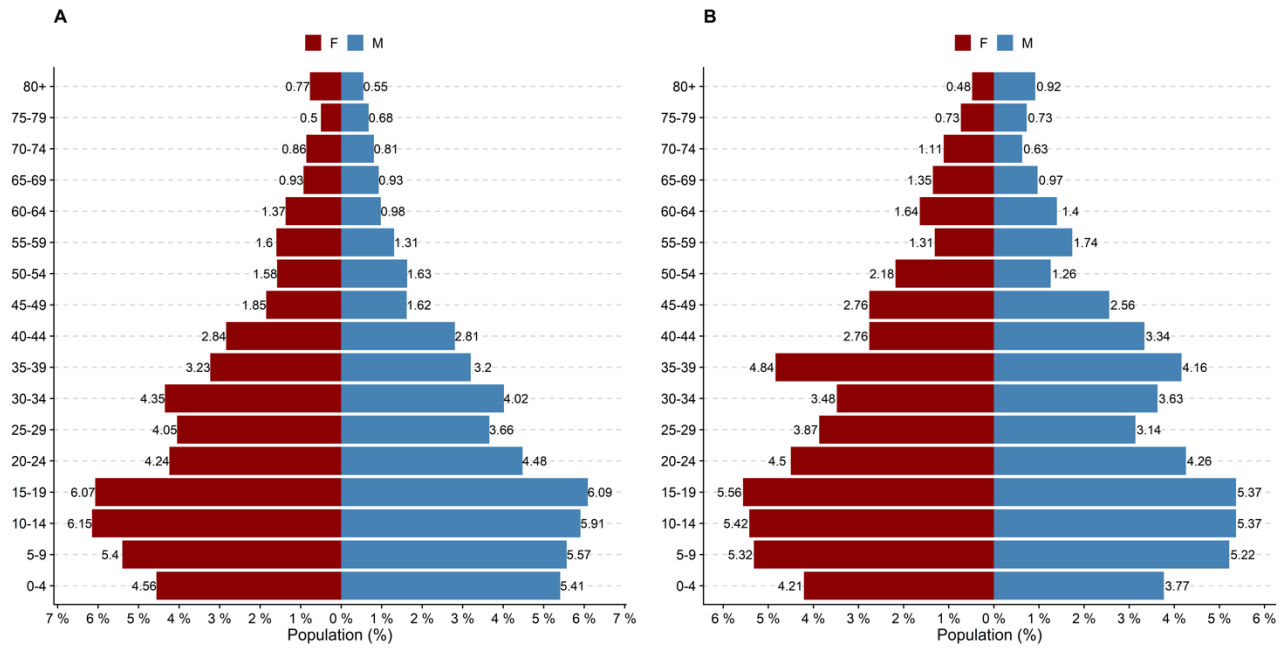


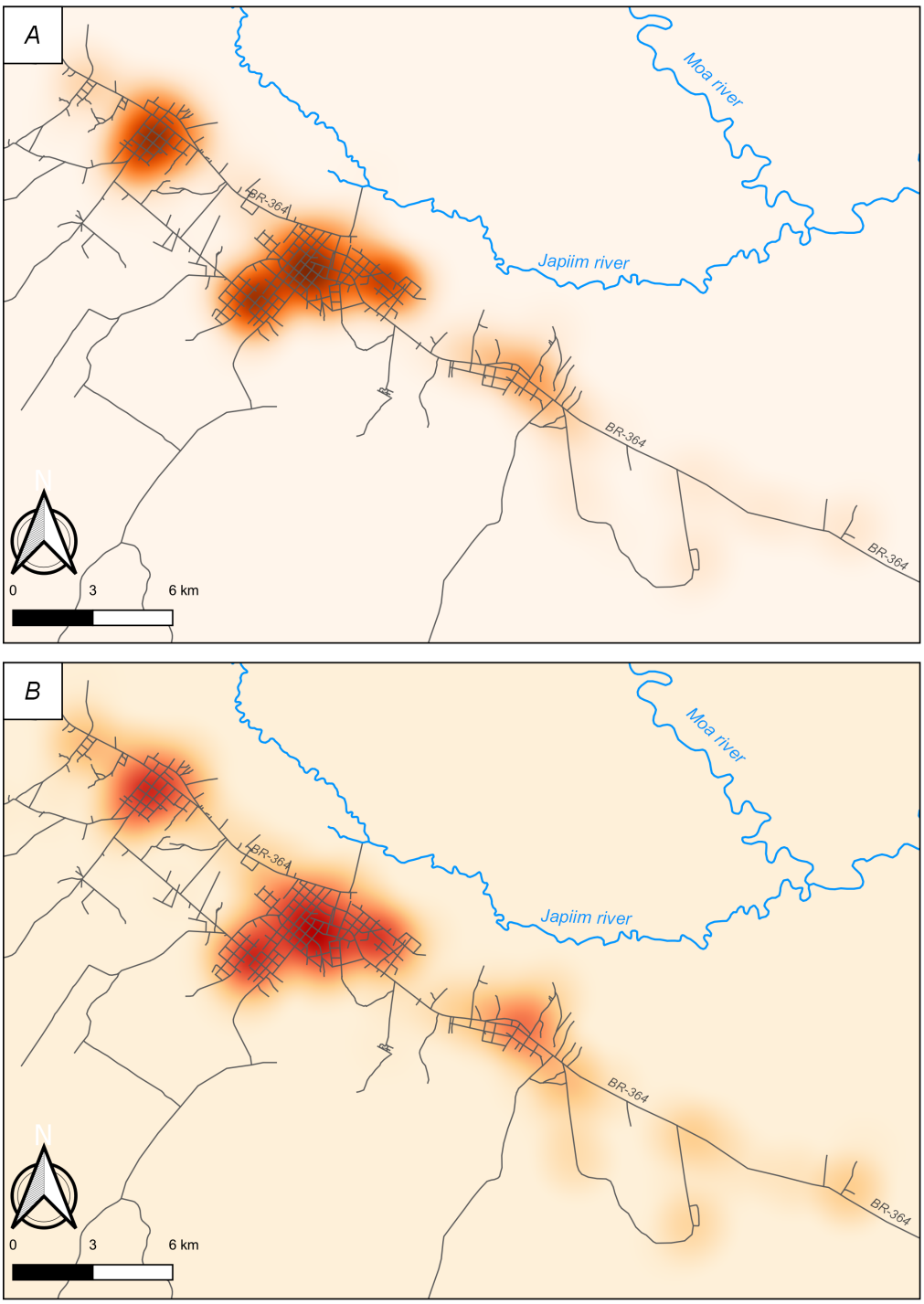

**Supplemental Fig. 1. Study Site.** The left panel shows the location of the Municipality of Mâncio Lima, in northwestern Brazil, next to the border with Peru. The right panel shows an aerial photography of the town of Mâncio Lima. (Photography by Rodrigo M. Corder.)




**Supplemental Fig. 2. Participants in consecutive study visits.** The Venn diagram shows the number of individuals interviewed in each study visit and in different combinations of them. Note that 981 individuals participated in all study visits.




**Supplemental Fig. 3. Representativeness of the study population according to sex and age groups.** Compared with the total population of Mâncio Lima enumerated during the baseline census carried out between November 2015 and April 2016 (A), the study sample has a very similar distribution according to sex and age groups (B).




**Supplemental Fig. 4. Representativeness of the study population according to place of residence.** Compared with the total population of Mâncio Lima enumerated during the baseline census carried out between November 2015 and April 2016 (A), the study sample has a nearly equal distribution according to place of residence (B).

**Supplemental Table 1. Short videos about the Mâncio Lima cohort study**

| Access | Topic | Producer |
|---|---|---|
| https://youtu.be/oEwJPHiBNmc | Field study set-up, March 2018 | TV USP |
| https://www.youtube.com/watch?v=CBx7z0ApznI | House-to-house visits, March 2018 | TV USP |
| https://youtu.be/wE3gJfdRpVo | Impact of COVID-19 on malaria, August 2020 | Agência FAPESP[a] |

[a]Five short videos on COVID-19 research in the field site were produced; a teaser can be found at: https://www.youtube.com/watch?v=lg1Ug-T_whQ&list=PLPdNbZy8nStgk8tZiXn7M7E9EeWXQ2zl9&index=1.

**Supplemental Table 2. Sociodemographic, occupational, and behavioral characteristics, housing quality, and travel history at the enrollment of the Mâncio Lima cohort study population (individuals who participated in at least one of five consecutive study visits; n= 2,690)**

| Type of measure | Variable | Description | Value |
|---|---|---|---|
| **Demographic** | Age (years) | Range | 0-103 |
| | | Mean | 26.4 |
| | | Median | 22 |
| | | Standard deviation (SD) | 19.8 |
| | Gender | Male | 1,311 (48.7%) |
| | | Female | 1,379 (51.3%) |
| | | Total | 2,690 (100) |
| | Pregnant? (only females) | No | 1,325 (96.1%) |
| | | Yes | 43 (3.1%) |
| | | Does not know/did not answer | 11 (0.8%) |
| | | Total | 1,379 (100%) |
| | Birthplace | Mâncio Lima | 1394 (51.8%) |
| | | Other, Juruá Valley | 985 (36.6%) |
| | | Other, Amazon | 255 (9.5%) |
| | | Other, elsewhere in Brazil | 8 (0.3%) |
| | | Does not know/did not answer | 48 (1.8%) |
| | | Total | 2,690 (100%) |
| | Literacy (only ≥10 years old) | Illiterate | 159 (7.6%) |
| | | Literate | 1,911 (91.8%) |
| | | Does not know/did not answer | 12 (0.6%) |
| | | Total | 2,082 (100%) |
| | Highest level of schooling (only ≥25 years old) | Never studied | 190 (15.5%) |
| | | Incomplete elementary school | 493 (40.3%) |
| | | Complete elementary school | 32 (2.6%) |
| | | Incomplete high school | 279 (22.8%) |

| | | | |
|---|---|---|---|
| | | Complete high school | 69 (5.6%) |
| | | Incomplete college | 119 (9.7%) |
| | | Complete college | 43 (3.5%) |
| | | Total | 1,225 (100%) |
| | Second residence outside the urban area? | No | 2,017 (75.0%) |
| | | Yes | 446 (16.6%) |
| | | Does not know/did not answer | 227 (8.4%) |
| | | Total | 2,690 (100%) |
| **Household** | Wall material | Masonry | 926 (34.4%) |
| | | Wood | 1,762 (65.6%) |
| | | Rammed earth | 1 (0.0%) |
| | | Other | 1 (0.0%) |
| | | Total | 2,690 (100%) |
| | Floor material | Masonry | 247 (9.2%) |
| | | Wood | 850 (31.6%) |
| | | Ceramics | 1,386 (51.5%) |
| | | Cement | 202 (7.5%) |
| | | Other | 5 (0.2%) |
| | | Total | 2,690 (100%) |
| | Roofing material | Asbestos | 2,176 (80.9%) |
| | | Aluminium | 460 (17.1%) |
| | | Wood | 15 (0.6%) |
| | | Clay tile | 17 (0.6%) |
| | | Other | 22 (0.8%) |
| | | Total | 2,690 (100%) |
| | Presence of a suspended ceiling beneath the roof? | No | 2,046 (76.1%) |
| | | Yes | 644 (23.9%) |
| | | Total | 2,690 (100%) |

| | | |
|---|---|---|
| How many rooms are there in this house? | Range | 1-16 |
| | Mean | 5.6 |
| | Median | 5 |
| | SD | 1.7 |
| How many rooms are utilized as bedrooms? | Range | 1-8 |
| | Mean | 2.5 |
| | Median | 2 |
| | SD | 0.9 |
| How many beds are there in your house? | Range | 1-7 |
| | Mean | 3 |
| | Median | 3 |
| | SD | 1.1 |
| How many insecticide-treated bednets are there in your house? | Range | 0-7 |
| | Mean | 1.5 |
| | Median | 1 |
| | SD | 1.5 |
| How many toilets are there in your house? | Range | 0-5 |
| | Mean | 1.2 |
| | Median | 1 |
| | SD | 0.6 |
| Number of people in the household | Range | 1-13 |
| | Mean | 4.9 |
| | Median | 4 |

| | | | |
|---|---|---|---|
| | | SD | 2.2 |
| | Any household member is beneficiary of social programs (e.g. social security pension, conditional cash transfer)? | No | 539 (20.0%) |
| | | Yes | 2,151 (80.0%) |
| | | Total | 2,690 (100%) |
| **Occupational** | Individual work status (only ≥10 years old) | Does not work | 1,259 (60.5%) |
| | | Formal employee | 207 (9.9%) |
| | | Informal employee | 567 (27.2%) |
| | | Employer | 14 (0.7%) |
| | | Does not know/did not answer | 35 (1.7%) |
| | | Total | 23,082 (100%) |
| **Behavioral** | Regular fishing | No | 2,350 (87.4%) |
| | | Yes | 296 (11.0%) |
| | | Does not know/did not answer | 44 (1.6%) |
| | | Total | 2,690 (100) |
| | Sleep by the river | No | 2,465 (91.6%) |
| | | Yes | 180 (66.9%) |
| | | Does not know/did not answer | 45 (1.7%) |
| | | Total | 2,690 (100%) |
| | Sleep with open or closed window | Closed | 2,639 (98.1%) |
| | | Open | 11 (0.4%) |
| | | Varies | 8 (0.3%) |
| | | Does not know/did not answer | 32 (1.2%) |
| | | Total | 2,690 (100%) |
| | Sleep time | Mean (hours:minutes) | 07:52 pm |
| | | Median | 21:00 pm |

| | | | |
|---|---|---|---|
| | Wake-up time | Mean hours:minutes) | 06:24 am |
| | | Median | 06:00 am |
| | Bathing inside or outside the house? | Inside | 1,256 (46.7%) |
| | | Outside | 1,322 (49.1%) |
| | | Both | 67 (2.5%) |
| | | Does not know/did not answer | 45 (1.7%) |
| | | Total | 2,690 (100%) |
| | Slept under bednet past night? | No | 957 (35.6%) |
| | | Yes, not insecticide-treated | 526 (19.6%) |
| | | Yes, insecticide-treated | 1,141 (42.4%) |
| | | Does not know/did not answer | 66 (2.4%) |
| | | Total | 2,690 (100%) |
| **Travel history** | Overnight trip outside the town within the past 6 months? | No | 1,825 (67.8%) |
| | | Yes | 857 (31.9%) |
| | | Does not know/did not answer | 8 (0.3%) |
| | | Total | 2,690 (100%) |
| | Most common travel destinations and total duration of stay | 1st | City of Cruzeiro do Sul: 4,334 days |
| | | 2nd | Timbaúba: 4,318 days |
| | | 3rd | Ramal do Feijão Insosso, 2,300 days |
| | | 4th | Puyanawa reserve: 2,097 days |
| | | 5th | Ramal do Batoque, 2,037 days |