First report of *Anopheles benarrochi* B in a remote malaria-endemic area in southern Amazonian Ecuador

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INTRODUCTION

- Despite extensive vector control efforts to stop malaria transmission in Ecuador[1], this disease persists in residual transmission sites especially in northern coastal Ecuador and the Amazon basin close to the Ecuador-Peru border[2]. In recent years, malaria burden has increased from 1,315 cases in 2017; to 1,718 and 2,081 cases in 2018 and 2019, respectively. In 2019, more than half of the malaria cases in Ecuador were reported in Morona Santiago (790, 38%) and Pastaza (512, 25%) provinces located in the southern Amazon basin[3].

- The Achuar Indigenous territory, a remote area in the southern Amazonian Ecuador, is a hotspot for malaria transmission. It covers over 12,000 km^2 and is composed of 64 communities (7,000 inhabitants) along the Pastaza river. Constant exchange and travel across the Ecuador-Peru border facilitates malaria introduction from endemic areas of Peru such as Loreto[4].

- The Anopheline fauna of Ecuador has been studied mainly in the coastal region with limited information for the Amazon region[5] where *Anopheles benarrochi* B[6] has been reported in a malaria endemic area in the northeastern Amazon. *Anopheles benarrochi* s.l. has been found naturally infected with *Plasmodium falciparum* and *P. vivax* in the northeastern Peruvian Amazon suggesting that members of this species complex may play a role in malaria transmission in endemic areas throughout the Amazon where *An. darlingi* is not dominant[7]. In this study, we conducted anopheline mosquito collections in two Achuar communities, identified specimens morphologically and performed species molecular confirmation by COI barcoding[8,9]. DNA barcoding is useful for discovering and confirming new species and is essential for future systematics and biodiversity studies[8].

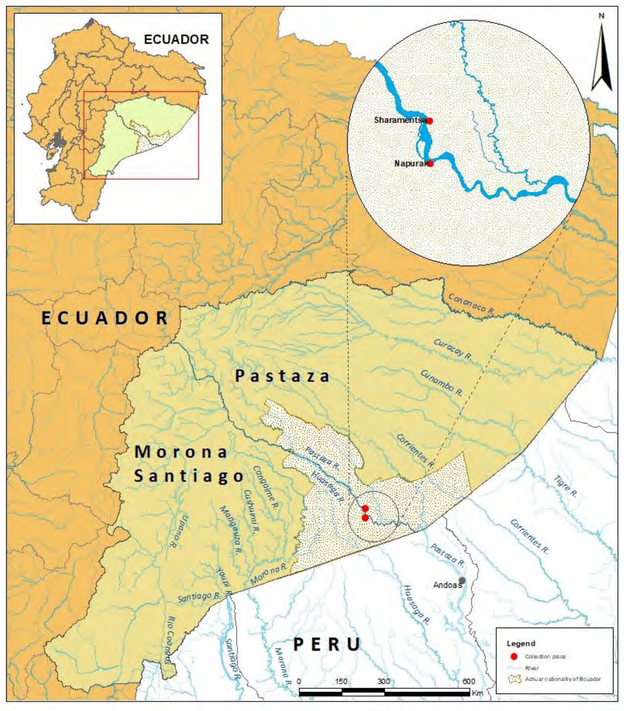

Figure 1. Study sites: Sharamentsa and Napurak villages, Achuar Indigenous territory in southern Amazonian Ecuador.

MATERIAL & METHODS

- **Study site**: Adult mosquitoes were collected between June and December, 2019 from two communities Napurak (2°31'13.53"S; 76°59'32.32"W) and Sharamentsa (2°27'50.22"S; 76°59'36.74"W), both located along the Pastaza river in the Achuar indigenous territory, a remote area of the Ecuadorian Amazon (Figure 1).

- **Mosquito collection**: Collections were performed using CDC light traps (1800-0600 h) (Figure 2A, 2B) and hourly protected human landing catches (HLC) (2000-2400 h) (Figure 2C). Mosquitoes were stored in silica gel in the field laboratory (Figure 2D), located at Sharamentsa community (2E).

- **Morphological identification**: Samples were taken to the Entomological laboratory of Universidad San Francisco de Quito, where morphological identification was carried out by the project team with the support of the colleagues of Instituto Nacional de Investigación en Salud Pública (INSPI), Quito[10,11].

- **Molecular identification**: DNA from whole mosquito was extracted using the QIAgen DNeasy® Tissue kit at USFQ. COI barcodes were amplified using LCO1490 and HCO2198 primers targeting the universal "barcoding" region of the mitochondrial *COI* I gene[12] following Mosquito Barcoding Initiative (MBI) protocols[8]. Purified PCR products were sequenced using a BigDye terminator cycle-sequencing kit (Applied BioSystems®) and a SeqStudio Genetic Analyzer (Applied BioSystems®) at NAMRU-6. Sequences were compared with expertly identified specimens in the MBI database available on the Barcode of Life Database (BOLD; *www.boldsystems.org*).

Figure 2. A) and B) CDC Light trap collection; C) protected human landing collection; D) processing specimens in the field laboratory; E) Sharamentsa community.

RESULTS

- Forty-eight anopheline mosquitoes were collected; thirty specimens (11 from Napurak, 19 from Sharamentza) were morphologically identified and DNA barcoded.

- Morphological identification indicated the presence of *Anopheles oswaldoi* (6), *Anopheles strodei* (5), and *Anopheles (Nys.)* sp. (8), in Sharamentsa; *Anopheles benarrochi* s.l. (3), *An. oswaldoi* (1), *An. rangeli* (1), and *An.* (*Nys.*) sp. (6) in Napurak (Table 1).

- Molecular species confirmation indicates that all thirty specimens were *An. benarrochi* B. This clearly showed a misidentification based on the morphology of adult females (Figure 3), which is common in *Anopheles* mosquitoes from the Amazon region.

Locality	Date of collection	Morphological ID	Collection Method		No. Total sequenced
			HLC	CDC	
Sharamentsa	Jul - 2019	*Anopheles oswaldoi*	5		5
	Dec - 2019	*Anopheles oswaldoi*	1		1
	Jul - 2019	*Anopheles strodei*	4	1	5
	Jun - 2019	*Anopheles (Nys.) spp.**	4	2	6
	Dec - 2019	*Anopheles (Nys.) spp.**	2		2
Napurak	Dec - 2019	*Anopheles benarrochi*	3		3
	Dec - 2019	*Anopheles oswaldoi*	1		1
	Dec - 2019	*Anopheles rangeli*	1		1
	Dec - 2019	*Anopheles (Nys.) spp.**	6		6
TOTAL					**30**

HLC = Human landing collection; CDC = CDC light trap; *Unidentified mosquitoes

Table 1. *Anopheles* species and total numbers collected by locality, date and collection method in Sharamentsa and Napurak, Achuar communities, Ecuador.

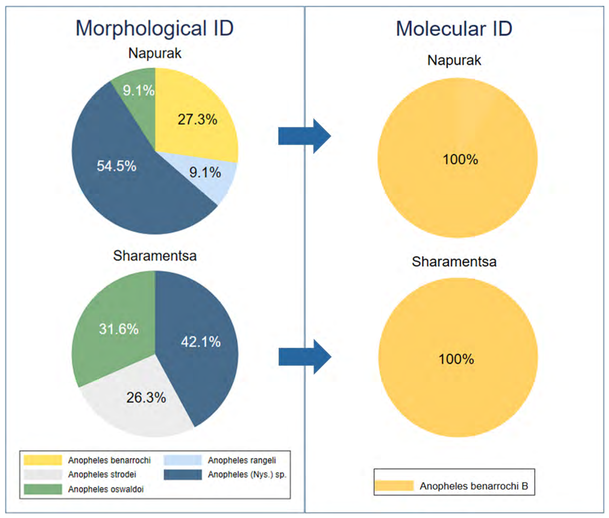

Figure 3. Anopheline species composition (%) based on morphological and molecular identification (COI barcodes) (n=30)

CONCLUSIONS

✓ This is the first report of *Anopheles benarrochi* B in the southern Amazonian Ecuador. Detection of this species in malaria endemic communities along the Pastaza river suggests a potential role in malaria transmission. Future studies will investigate species abundance and natural infection with malaria parasites.

✓ Species identification based exclusively on morphological traits could be misleading for Amazonian anophelines. The application of DNA barcoding techniques proved highly useful in confirming true identities of the morphologically complex Amazonian Anopheles. This approach allowed the discovery of previously unreported taxa in these remote malaria endemic communities.

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Source of support: Funding was provided by the Armed Forces Health Surveillance Branch (AFHSB) and its Global Emerging Infections Surveillance and Response Section (GEIS), 2019 ProMIS ID P0143_19_N6_04 & 2020 ProMIS ID P0144_20_N6_04.