

Short communication

Molecular detection of *Mansonella mariae* incriminates *Simulium oyapockense* as a potentially important bridge vector for Amazon-region zoonoses

Túllio Romão Ribeiro da Silva^{a,b}, Luiz Henrique de Aquino Narzetti^{a,c}, James Lee Crainey^{a,*}, Carlos Henrique Costa^{a,d}, Yago Vinícius Serra dos Santos^{a,e}, Lorena Ferreira de Oliveira Leles^{a,e}, Felipe Arley Costa Pessoa^a, Ana Carolina Paulo Vicente^f, Sérgio Luiz Bessa Luz^a

^a Instituto Leônidas e Maria Deane/ILMD/Fiocruz Amazônia, Laboratório de Ecologia de Doenças Transmissíveis na Amazônia, Manaus, AM, Brazil

^b Programa de Pós-graduação Stricto Sensu em Biologia Parasitária, Instituto Oswaldo Cruz/IOC/FIOCRUZ, Rio de Janeiro, RJ, Brazil

^c Programa Institucional de Bolsas de Iniciação Científica (PIBIC), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil

^d Programa de Pós-graduação Stricto Sensu em Biologia Computacional e Sistemas do Instituto Oswaldo Cruz (PGBCS/IOC/Fiocruz), Brazil

^e Programa de Pós-Graduação Stricto Sensu em Biologia da Interação Patógeno Hospedeiro (PPGBIO-Interação), Instituto Leônidas e Maria Deane/ILMD/Fiocruz Amazônia, Manaus, AM, Brazil

^f Instituto Oswaldo Cruz/IOC/FIOCRUZ, Laboratório de Genética Molecular de Microrganismos, Rio de Janeiro, RJ, Brazil



ARTICLE INFO

Keywords:

Arthropod borne disease
Urban expansion
Brazilian Amazon
Periurban zones
Emerging infections disease
Zoonotic disease
Zoonoses
S. oyapockense
Mansonella mariae

ABSTRACT

Objective: To assess the emergent zoonotic disease risk posed by the voracious human-biting blackfly species *Simulium oyapockense* in the peripheral regions of an expanding urban centre situated deep in the Brazilian Amazon rainforest.

Methods: We performed nine human landing catches at three periurban sites surrounding the Brazilian Amazon town of São Gabriel da Cachoeira. Using the detection of non-human primate filarial parasites as an indicator of the zoonotic disease threat posed by a biting insect, we screened 3328 *S. oyapockense* blackflies for the presence of zoonotic filarial DNA with an ITS-1 PCR assay and Sanger sequencing.

Results: Between 98 and 100% of the biting insects captured during our nine collections were identified as *S. oyapockense*; at our three collection sites and during our three seasonally-distinct collections this species was captured at rates between 28 and 294 blackflies per hour. PCR screening of the march-collected *S. oyapockense* detected infectious-stage (L3) *Mansonella mariae* parasites (which are only known to infect non-human primates) in >0.15% of the tested head samples.

Conclusions: Our results show that residents of the periurban regions of São Gabriel da Cachoeira are routinely exposed to the bites of *S. oyapockense* blackflies which have previously fed on non-human primates.

Urban expansion into the Brazilian Amazon brings increasing numbers of sylvatic pathogens into contact with a growing regional population (Lowe et al., 2020). Although it is well recognised that this expansion has emergent and re-emergent disease risks associated with it, it is still far from clear which pathogen reservoirs and disease vectors pose the greatest threat to human health (Lowe et al., 2020).

In the forested regions surrounding the northern tributaries of the Amazon River, blackflies from the Amazonicum species-group

(including *S. oyapockense*) have been recorded biting humans >6000 times a day and are known to play a key role in the regional transmission of *M. ozzardi* and *O. volvulus* (Shelley et al., 1997). Despite this, however, simuliids are not typically discussed as an emerging disease risk, with most disease-vector concern focused on anthropophilic mosquitoes (Lowe et al., 2020).

As part of a study investigating mansonellosis transmission, we carried out three sets of standard human landing catches to capture

* Corresponding author at: Laboratório de Ecologia de Doenças Transmissíveis na Amazônia (EDTA), Instituto Leônidas e Maria Deane (Fiocruz), Rua Teresina, 476, Adrianópolis, Manaus, Amazonas, CEP 69027-070, Brazil.

E-mail address: james.lee@fiocruz.br (J.L. Crainey).

<https://doi.org/10.1016/j.meegid.2021.105200>

Received 11 September 2021; Received in revised form 27 December 2021; Accepted 28 December 2021

Available online 3 January 2022

1567-1348/© 2022 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1
Biting insects captured at three sites around the periphery of São Gabriel da Cachoeira.

Collection date	Collection site	Total number of biting insects captured	<i>S. oyapockense</i> as a percentage of the total capture	Total Biting insect capture per hour	Total <i>S. oyapockense</i> capture per hour
March Collection 02 to 08/03/2016	Comunidade Areal	375	100%	46.8	46.8
	Porto de Camanaus	896	100%	224	224
	Casa de Camanaus	2057	100%	257.1	257.1
September Collection 25 to 30/09/2017	Comunidade Areal	238	100%	59.5	59.5
	Porto de Camanaus	2366	100%	283.2	283.2
	Casa de Camanaus	1176	100%	294	294
October collection 26 to 28/10/2017	Comunidade Areal	308	98.7%	38.5	38
	Porto de Camanaus	523	99.6%	65.3	65.1
	Casa de Camanaus	112	100%	28	28

Table 1 Collections were all performed between 8 am and mid-day; using between two and four vector collectors with approval from the FIOCRUZ/IOC ethics committee (Reference number: CAAE: 41678515.1.0000.5248) and following a protocol detailed in Romão Ribeiro da Silva et al., 2019. The coordinates of the collection sites were as follows. Comunidade Areal: 0°8'60"S/66°57'7.2"W; "Porto de Camanaus": 0°8'56"S/66°56'8.79"W, and "Casa de Camanaus": 0°8'51"S/66°56'23.23"W."

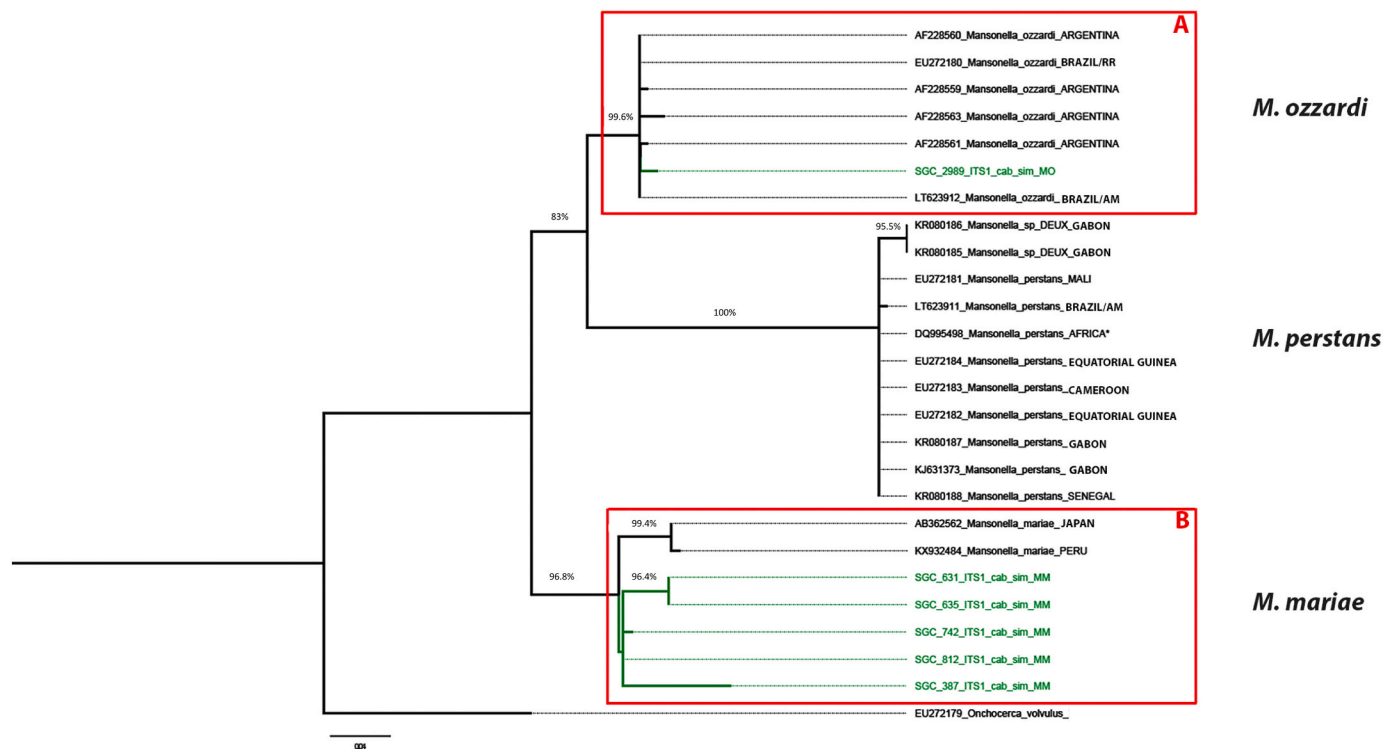


Fig. 1. An ITS-1-based maximum likelihood tree showing the molecular classification of the filarial ITS-1 sequences obtained from this study. Box A highlights the *M. ozzardi* ITS-1 sequence (MZ726991) obtained from this study within a bootstrap supported grouping containing other *M. ozzardi* reference sequences. Box B shows *Mansonella mariae* ITS-1 sequences (MZ726992–6) obtained from this study clustering in a bootstrap supported cluster with *M. mariae* reference sequences.

biting insects from three periurban collections sites around the periphery of the Brazilian Amazonian town of São Gabriel da Cachoeira (SGC) (Romão Ribeiro da Silva et al., 2019). As can be seen in Table 1, the blackfly species *S. oyapockense* represented >98% of the biting insects captured at all three of our periurban collection sites and during all three seasonally distinct collections. Although biting rates were not as high as has been observed in other parts of the upper tributary regions of the Amazon basin (Shelley et al., 1997), they were still found to be

persistently high during all of our collections, ranging from 38 to 294 *S. oyapockense* bites per hour.

However, as it is only blackflies that have previously taken a blood meal (parous blackflies) that can cause zoonosis, this persistent *S. oyapockense* biting does not directly translate to a persistent emerging zoonotic disease risk. In the region of the Amazon basin where our study was conducted, *S. oyapockense* parous biting rates are highly seasonal and therefore so is the zoonotic disease risk these bites pose (Shelley

et al., 1997). From past parous *S. oyapockense* biting rate estimates, we would expect the *S. oyapockense* we captured in March to present a high zoonotic disease risk and that those captured in September and October to present only a minor risk (Shelley et al., 1997). As our study aimed to use the presence of zoonotic filarial parasite DNA in human-biting *S. oyapockense* as an indicator of the zoonotic disease risk their bites pose, we thus chose to focus our PCR-screening on our 3328 march-collected blackflies.

After individual-insect dissections, DNA from *S. oyapockense* thoraxes was extracted in pools. A total of 65 pools were prepared: seven from material collected from “Comunidade Areal” site, 40 from material collected at the Porto de Camanaus collection site and 18 pools from the blackflies captured at the “Casa de Camanaus” site. Using a ribosomal ITS-1 PCR assay capable of identifying the presence of a variety of filarial parasite species (Ta-Tang et al., 2010), filarial DNA was detected in 14 of these pools. Individual PCR screening of the 700 *S. oyapockense* dissected head samples, pertaining to the PCR positive thorax samples, identified six infectious stage (L3) filarial larvae. As can be seen in Fig. 1, one of these ITS-1 sequences derived from *M. ozzardi*; whereas the other five ITS-1 sequences derived from *M. mariae* parasites, which, so far, have only been found infecting non-human primates (Bain et al., 2015).

Although blood surveys from the SGC region have detected *M. ozzardi* and *M. perstans* in the blood of residents and indeed the existence of co-infections with both parasites (Crainey et al., 2020), there are hitherto no records of the *M. mariae* causing human infections in this region or indeed in any other region (Tavares da Silva et al., 2017; Ta-Tang et al., 2018). Our results thus strongly suggest that a high proportion of the biting *S. oyapockense* captured in March (between 0.15% [5/3328] and 0.71% [5/700]), previous took blood meals from non-human primates and thus could have roles as zoonotic bridge vectors, putting the residents living in the periphery of SGC in contact with non-human primate blood pathogens. Extrapolating from our data one would expect that a periurban resident of SGC who spent around 12 h a day outside during the first week of March 2018 to have been bitten by *S. oyapockense* blackflies which had previously fed on a non-human primate between 5 and 32 times.

Beyond demonstrating the emerging zoonotic disease threat that *S. oyapockense* poses in the expanding periurban regions of SGC in the upper Amazon tributaries, this study’s incrimination of *S. oyapockense* as a vector of *M. mariae* also contributes to understanding of mansonellosis host-parasite interaction dynamics. For example, our results show that bites from the region’s *S. oyapockense* blackflies routinely expose residents to L3 *M. mariae* parasites and suggest that residents may be exposed to more L3 *M. mariae* parasites than they are to L3 *M. ozzardi* parasites. Given that *M. mariae* has not yet been reported in local blood survey, this suggests that *M. mariae* may not be able to develop in a human host (Ta-Tang et al., 2021). Similarly, as both *M. ozzardi* and *M. perstans* are prevalent in the area it also seems unlikely that contact with L3 *M. mariae* provides protection against mansonellosis infections in the way contact with *Onchocerca ochengi* appears to provide against *O. volvulus* infections (Eisenbarth et al., 2016). Interestingly too, our results suggest that Yanomamis in the nearby onchocerciasis focus could have antibodies against L3 *M. mariae* parasites, which have not been tested for cross-reactivity with the Ov-16 assay currently being used by the WHO for its regional onchocerciasis epidemiological monitoring (Botto et al., 2016; Luz et al., 2014).

Declaration of Competing Interest

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge Dr. Jeane Marcelle Cavalcante do Nascimento for her assistance with morphological identification of biting insects. JLC, SLBL and FACP all gratefully acknowledge support from the Fundação de Amparo à Pesquisa do Estado do Amazonas for project grants: FAPEAM; 062.01282/2018; 002.00200/2019, and PROEP-FIOCRUZ 001/2014. JLC, ACPV and FACP also all gratefully acknowledge their CNPq productivity grants.

References

- Bain, O., Mutafchiev, Y., Junker, K., Guerrero, R., Martin, C., Lefoulon, E., Uni, S., 2015. Review of the genus *Mansonella* Faust, 1929 sensu lato (Nematoda:Onchocercidae), with descriptions of a new subgenus and a new subspecies. *Zootaxa* 3918 (2), 151–193. <https://doi.org/10.11646/zootaxa.3918.2.1>.
- Botto, C., Basañez, M.G., Escalona, M., Villamizar, N.J., Noya-Alarcón, O., Cortez, J., Vivas-Martínez, S., Coronel, P., Frontado, H., Flores, J., Graterol, B., Camacho, O., Tovar, Y., Borges, D., Morales, A.L., Ríos, D., Guerra, F., Margeli, H., Rodríguez, M. A., Unnasch, T.R., Grillet, M.E., 2016. Evidence of suppression of onchocerciasis transmission in the Venezuelan Amazonian focus. *Parasit. Vectors* 9 (4). <https://doi.org/10.1186/s13071-016-1313-z>.
- Crainey, J.L., Costa, C.H.A., de Oliveira Leles, L.F., Ribeiro da Silva, T.R., de Aquino Narzetti, L.H., Serra Dos Santos, Y.V., Costa Conteville, L., Costa Pessoa, F.A., Carvajal Cortés, J.J., Vicente, A.C.P., Rubio Muñoz, J.M., Bessa Luz, S.L., 2020. Deep sequencing reveals occult *Mansonella* infections in residents from the Brazilian Amazon Village of São Gabriel da Cachoeira. *Clin. Infect. Dis.* 71 (8) <https://doi.org/10.1093/cid/ciaa082>.
- Eisenbarth, A., Achukwi, D.M., Renz, A., 2016. Ongoing transmission of *Onchocerca volvulus* after 25 years of annual Ivermectin mass treatments in the Vina du Nord River valley, in North Cameroon. *PLoS Negl. Trop. Dis.* 10 (2) <https://doi.org/10.1371/journal.pntd.0004392>.
- Lowe, R., Lee, S., Lana, R.M., Codeço, C.T., Castro, M.C., Pascual, M., 2020. Emerging arboviruses in the urbanized Amazon rainforest. *B.M.J.* 371 <https://doi.org/10.1136/bmj.m4385>.
- Luz, S.L., Crainey, J.L., Shelley, A.J., Rubio, M., 2014. Outstanding insecurities concerning the use of an Ov16-based ELISA in the Amazonia onchocerciasis focus. *Mem. Inst. Oswaldo Cruz* 109 (4), 506–508. <https://doi.org/10.1590/0074-0276140079>.
- Romão Ribeiro da Silva, T., Crainey, J.L., Costa Pessoa, F.A., Vinicius Serra Dos Santos, Y., Pereira-Silva, J.W., Ferreira de Oliveira Leles, L., Vicente, A.C., Bessa Luz, S.L., 2019. Blackflies in the ointment: *O. volvulus* vector biting can be significantly reduced by the skin-application of mineral oil during human landing catches. *PLoS Negl. Trop. Dis.* 13 (4) <https://doi.org/10.1371/journal.pntd.0007234>.
- Shelley, A.J., Lowry, C.A., Maia-Herzog, M., Dias, A.P.A.L., Moraes, M.A.P., 1997. Biosystematic studies on the Simuliidae (Diptera) of the Amazonia onchocerciasis focus. *Bul. Nat. Hist. Mus. Ent.* 66, 1–120.
- Ta-Tang, T.H., López-Vélez, R., Lanza, M., Shelley, A.J., Rubio, J.M., Luz, S.L.B., 2010. Nested PCR to detect and distinguish the sympatric filarial species *Onchocerca volvulus*, *Mansonella ozzardi* and *Mansonella perstans* in the Amazon region. *Mem. Inst. Oswaldo Cruz* 105 (6). <https://doi.org/10.1590/s0074-02762010000600016>.
- Ta-Tang, T.H., Crainey, J.L., Post, R.J., Luz, S.L., Rubio, J.M., 2018. Mansonellosis: current perspectives. *Res. Rep. Trop. Med.* 9 (doi: 0.2147/RRTM.S125750. eCollection 2018).
- Ta-Tang, T.H., Luz, S.L.B., Crainey, J.L., Rubio, J.M., 2021. An overview of the management of mansonellosis. *Res. Rep. Trop. Med.* 12, 93–105. <https://doi.org/10.2147/RRTM.S274684>.
- Tavares da Silva, L.B., Crainey, J.L., Ribeiro da Silva, T.R., Suwa, U.F., Vicente, A.C., Fernandes de Medeiros, J., Pessoa, F.A., Luz, S.L., 2017. Molecular verification of new world *Mansonella perstans* parasitemias. *Emerg. Infect. Dis.* 23 (2) <https://doi.org/10.3201/eid2303.161159>.