RESEARCH ARTICLE

# *Mansonella*, including a Potential New Species, as Common Parasites in Children in Gabon

Gaël Mourembou[1,2], Florence Fenollar[1], Jean Bernard Lekana-Douki[3,4], Angelique Ndjoyi Mbiguino[5], Sydney Maghendji Nzondo[3], Pierre Blaise Matsiegui[6], Rella Zoleko Manego[6], Cyrille Herve Bile Ehounoud[1], Fadi Bittar[1], Didier Raoult[1], Oleg Mediannikov[1] *

**1** Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Marseille, France, **2** Ecole Doctorale Régionale d'Afrique Centrale, Franceville, Gabon, **3** Unité de Parasitologie Médicale (UPARAM) CIRMF, Franceville, Gabon, **4** Département de Parasitologie Mycologie et de Médecine Tropicale, Université des Science de la Santé, Libreville, Gabon, **5** Département de Microbiologie, Laboratoire National de Référence IST/sida, Faculté de Médecine, Université des Sciences de la Santé, Libreville, Gabon, **6** Centre de Recherches Médicales de la Ngounié, Fougamou, Gabon

* olegusss1@gmail.com

## Abstract

### Background

Like other tropical African countries, Gabon is afflicted by many parasitic diseases, including filariases such as loiasis and mansonellosis. This study aimed to assess the prevalence of these two filarial diseases in febrile and afebrile children using quantitative real-time PCR and standard PCR assays coupled with sequencing.

### Methodology/Principal Findings

DNA from blood specimens of 1,418 Gabonese children (1,258 febrile and 160 afebrile) were analyzed. Overall, filarial DNA was detected in 95 (6.7%) children, including 67 positive for *M. perstans* (4.7%), which was the most common. *M. perstans* was detected in 61/1,258 febrile children (4.8%) and 6/160 afebrile children (3.8%, $P = 0.6$). Its prevalence increased statistically with age: 3.5%, 7.7% and 10.6% in children aged ≤5, 6–10 and 11–15 years, respectively. *M. perstans* prevalence was significantly higher in Koulamoutou and Lastourville (12% and 10.5%, respectively) than in Franceville and Fougamou (2.6% and 2.4%, respectively). *Loa loa* was detected in seven febrile children including one co-infection with *M. perstans*. Finally, 21 filarial DNA positive were negative for *M. perstans* and *Loa loa*, but ITS sequencing could be performed for 12 and allowed the identification of a potential new species of *Mansonella* provisionally called "DEUX". *Mansonella* sp. "DEUX" was detected only in febrile children.

### Conclusions/Significance

Further study should be performed to characterize *Mansonella* sp. "DEUX" and evaluate the clinical significance of mansonellosis in humans.

## Author Summary

Approximately 114 million people in Africa, mostly located in 33 Sub-Saharan African countries, are infected with *Mansonella perstans*, a filarial nematode. The ability of *M. perstans* to induce severe clinical features has only recently been considered. Unfortunately, no study has evaluated its burden in febrile patients in Gabon, a tropical African country where febrile and parasitic illnesses are common. There, we developed molecular tools to detect *M. perstans* and other *Mansonella* spp., as well as *Loa loa*, another filarial nematode in blood specimens of febrile and afebrile Gabonese children. Our findings suggest that there is no direct link between *M. perstans* and fever among the local population (61/1,258 febrile children [4.8%] versus 6/160 afebrile children [3.8%]), whereas *Loa loa* and another potential new species *Mansonella* sp. "DEUX" were only found in febrile patients (seven and twelve, respectively). Further study should be performed to characterize *Mansonella* sp. DEUX and evaluate the clinical significance of mansonellosis in humans.

## Introduction

Tropical African countries are afflicted by many febrile diseases including HIV, tuberculosis, malaria, and bacteremia; it has been suggested that their emergence can be influenced by infectious factors such as filarial parasites, including *Mansonella perstans* [1,2]. In 2012, Dolo *et al.*, during assessment of the effects of filariasis on anemia and pro-inflammatory responses associated with clinical malaria in Mali, reported that the geometric mean of hemoglobin levels was significantly lower in 31 malaria patients without filariasis than in 31 with filariasis [3]. They added that these filariases, including *M. perstans*, significantly decrease plasma levels of Interleukin-1ra, Inducible Protein-10 and Interleukin-8 at the time of presentation with clinical malaria [3]. Brown *et al.* reported in 2004 that *M. perstans* did not have an adverse effect on HIV infection, as a higher load of CD4 was detected in HIV patients co-infected with *M. perstans* compared to those without this parasite [4]. In 2006, Brown *et al.* also added that the progression to active tuberculosis among HIV-1 infected Ugandans was not associated with *M. perstans* [5].

Until now, no distinct and specific clinical features have been unequivocal associated with *M. perstans* infections, unlike some other human filariasis [1]. For this reason, the potential ability of *M. perstans* to induce pathology has been largely neglected. However, a significant spectrum of clinical features related to this parasite has been described [6,7,8,9]. Some of these manifestations have been reported from endemic areas in Uganda but without statistical links to *M. perstans* infection [10]. Four reasons were proposed to explain the lack of linkage: (a) it is possible that some of the earlier reported manifestations are rare and therefore were not present with high enough frequency to show a significant association, (b) the high prevalence of microfilaremia reduced the ability to detect a significant difference in frequency of manifestations between the microfilaremia positive and microfilaremia negative groups, (c) previously reported manifestations may be more common in expatriates exposed to infection later in life than in individuals who have grown up in the endemic environment and (d) manifestations may be related more to adult worm infections or to exposure to infectious larvae than to microfilaremia [10].

*M. perstans*, whose vectors are tiny blood-sucking flies called midges belonging to *Culicoides* genus, infects approximately 114 million people in Africa, mostly located in 33 Sub-Saharan African countries [1,2,11]. Moreover, *M. perstans* currently remains unaffected by the most common anti-filarial drugs [1,2]. In Gabon, a central African country, most information on

*M. perstans* has been retrieved when studying other filariases. Its prevalence was recently reported to be 10.2%, including a 3.2% co-infection with *Loa loa* in 270 villages of Gabon [12]. *M. perstans* is widespread in Sub-Saharan Africa but no study has reported its prevalence among febrile children in Gabon. This study aimed mainly to assess the prevalence of mansonellosis and *Loa loa* in febrile and afebrile children from Gabon using molecular methods.

## Materials and Methods

### Ethics statement

After approval from the Central Ethics Committee of Gabon (N°00370/MSP/CABMD and N° 0023/2013/SG/CNE), 1,258 febrile and 160 afebrile children were recruited from 2011 to 2014. Samples from the Republics of Côte d'Ivoire and Senegal were obtained under Ethics numbers N°86/MSLS/CNERN-dkn and N°00.87 MSP/DS/CNERS, respectively. Written Informed Consent from parents or legal guardians of each child was systematically received before inclusion in the study.

### Study areas and included population

This study took place in four areas of Gabon located in three provinces of the country: Franceville (Haut-Ogooue province), Koulamoutou and Lastourville (Ogooue Lolo province), and Fougamou (Ngounie province). Among children, 997 (873 febrile and 124 afebrile) were included in Franceville, 171 in Lastourville (155 febrile and 16 afebrile), 83 in Fougamou (63 febrile and 20 afebrile), and 167 febrile children in Koulamoutou. All the children were recruited from pediatric outpatient wards of Reginal hospital centers (Franceville and Koulamoutou), Medical Center (Lastourville), and Medical Research Unit (Fougamou).

Finally, seven blood samples from two other areas of West Africa that had previously been found positive for *M. perstans* were included in the analysis in order to compare sequences and construct phylogenetic trees. Two samples were from the Republic of Côte d'Ivoire and five samples were from Senegal.

For each child, 100µl of DNA were extracted from 200µl of blood samples (collected in EDTA tubes) using a DNA Blood Kit E.Z.N.A (Omega Bio-Tek, Norcross, U.S.A) according the manufacturer's guide. The DNA was extracted in Franceville, stored at -20°C and sent in ice packs to URMITE, Marseille, France for molecular analysis.

### Molecular analyses

**Quality controls.** Assessment of the quality of DNA extracts (internal control) was performed for each sample using a quantitative real-time PCR (qPCR) targeting the human ß-actin gene [13]. All primers and probes used in this study are summarized (Table 1). For all PCR assays performed, a mix of PCR alone was used as negative control for every ten samples. Positive controls (DNA of the targeted parasite), two per assay, were also included to validate each reaction.

**Primers and probes.** All samples were first screened with qPCR using the set of primers and probe targeting the Inter Transgenic Spacer (ITS) that was able to amplify all species of *Mansonella* genus as well as *Loa loa* [11]. All samples positive for ITS qPCR were systematically tested with a qPCR targeting *M. perstans* [11] as well as a qPCR targeting *Loa loa*. In addition, they were also all amplified with standard PCR assays targeting ribosomal DNA ITS1 and 5S rRNA regions coupled with sequencing [14,15]. Finally, a new set of primers and probe were specifically designed to amplify a portion of the ITS1 sequence of a potential new species of *Mansonella* identified in this study.

**Table 1. Summary of all the PCR systems used in this study.**

| Filaria | Targeted sequences | Type of PCR | Primers | Size of amplicons (pb) | Taqman probes | References |
|---|---|---|---|---|---|---|
| *Mansonella* species and *Loa loa* | ITS | qPCR | F: 5'- CAATTACTAGGAAGGCGTCC-3' <br> R: 5'- AATAGCGGATTTGGCAGCTA -3' | 237 | 6-FAM-CGGTGATATTCGTTGGTGTCT-TAMRA | [11] |
| *Mansonella perstans* | ITS | qPCR | F: 5- CCTTCGAGCAATTACTAGGA-3' <br> R: 5'- TGACTTAATTGCCACTATAAGC-3' | 187 | 6-FAM-TTCACTTTTATTTAGCAACATGCA-TAMRA | [11] |
| *Mansonella* sp « DEUX » | ITS1 | qPCR | F: 5'- CGAATATCACCGTTAATTCAGTTGT-3' <br> R: 5'-AACGAGCTTCAAAACAAACACA-3' | 179 | 6- FAM-TTCACTTTTATTAGCAACATGTATGAA-TAMRA | This study |
| *Loa loa* | 15r3, 5–6 | qPCR | F: 5'-CGAAAAATTATAGGGGGAAAC-3' <br> R: 5'-TCGTAGACCAAACTGCGAAC-3' | 148 | 6- FAM- TCAAGAGCCGATATACTGAAAG CTATC-TAMRA | [32,33] |
| *M. perstans, Loa loa* and *W. branchrofti* | ITS1 | Standard | F: 5'-GGTGAACCTGCGGAAGGATC-3' <br> R: 5'-CTCAATGCGTCTGCAATTCGC-3' | 485 | NA | [14,15] |
| | | PCR | | | | |
| *Filarioidea* | 5S | Standard PCR | S2: 5'-GTTAAGCAACGTTGGGCCTGG-3' <br> S16: 5'-TTGACAGATCGGACGAGATG-3' | 421 | NA | [14,15] |

NA = Not applicable

**PCR assays.**   The CFX96 Touch detection system test (Bio-Rad, Marnes-la-Coquette, France) was used to perform qPCR. FAST qPCR MasterMix (Eurogentec, Liege, Belgium) was prepared according the manufacturer's protocol and used during this step as previously reported [13].

Standard PCRs were performed using GeneAmp PCR System 2720 thermal cyclers (Applied Biosystems, Bedford, MA, USA) while sequencing was performed with an ABI Prism 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems). For sequencing, obtained amplicons were directly purified using the PCR filter plate Millipore NucleoFast 96 PCR kit following the manufacturer recommendations (Macherey–Nagel, Düren, Germany). Sequencing reactions were carried out using the Big-Dye Terminator, version 1.1, cycle sequencing kit DNA according to the manufacturer's instructions (Applied Biosystems, Foster City, USA) [16]. All PCR products were sequenced using the primers summarized in Table 1 with an initial denaturation step at 96°C (1 minute), followed by 25 cycles of denaturation at 96°C (10 seconds), annealing at 50°C (5 seconds), and extension at 60°C (3 minutes). Sequencing products were purified using the Millipore MultiScreen 96-well plates (Merck, Molsheim, France), containing 5% Sephadex G-50 (Sigma-Aldrich, L'Isle d'Abeau Chesnes, France). Finally, ChromasPro 1.34 software (Technelysium Pty. Ltd., Tewantin, Australia) was used to correct sequences and BLASTn searches were performed in the NCBI (http://blast.ncbi.nlm.nih.gov.gate1.inist.fr/Blast.cgi).

**Phylogenetic trees.**   Consensus phylogenetic trees showing the relationships between the species of filarial parasites under study were made based on comparisons of the ITS1 and 5S sequences. The sequences compared were from the gene database GenBank (www.ncbi.nlm.nih.gov) and from previous studies (those which were unavailable in the Genbank database) [14,15]. The sequences were aligned using ClustalW, and phylogenetic inferences were obtained using Bayesian phylogenetic analysis with TOPALi 2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK) within the integrated MrBayes application and using the HKY85 substitution model. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree [17].

## Statistical analysis

Epi Info software (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used to perform data analysis: Mantel-Haenszel $\chi 2$ and Fisher exact tests. Statistical significance was considered for a two tailed *P* value lower than 0.05.

## Results

DNA extracts from blood specimens of 1,418 children (1,258 febrile and 160 afebrile) were included in the analysis since all of them were positive for human ß-actin qPCR. In summary, 703 males and 673 females were included; no data on sex was available for 42 children. Overall, 864 out of 1,418 children (61%) were included during the rainy season and 554 during the dry season (39%). Data on age were available for 1,374 children and are summarized (Fig 1).

## Detected filaria

Overall, 95 out of 1,418 samples (6.7%) were positive based on ITS qPCR. Among them, 67 (4.7%) were positive for *M. perstans* and 7 for *Loa loa* (including 1 co-infection with *M. perstans*), whereas 21 were negative using these two qPCR tests. Identification of *M. perstans* and *Loa loa* was confirmed with sequencing and the BLAST search tool. All children with *Loa loa* were febrile; two were from Franceville, two from Koulamoutou, one from Lastourville, and two from Fougamou.
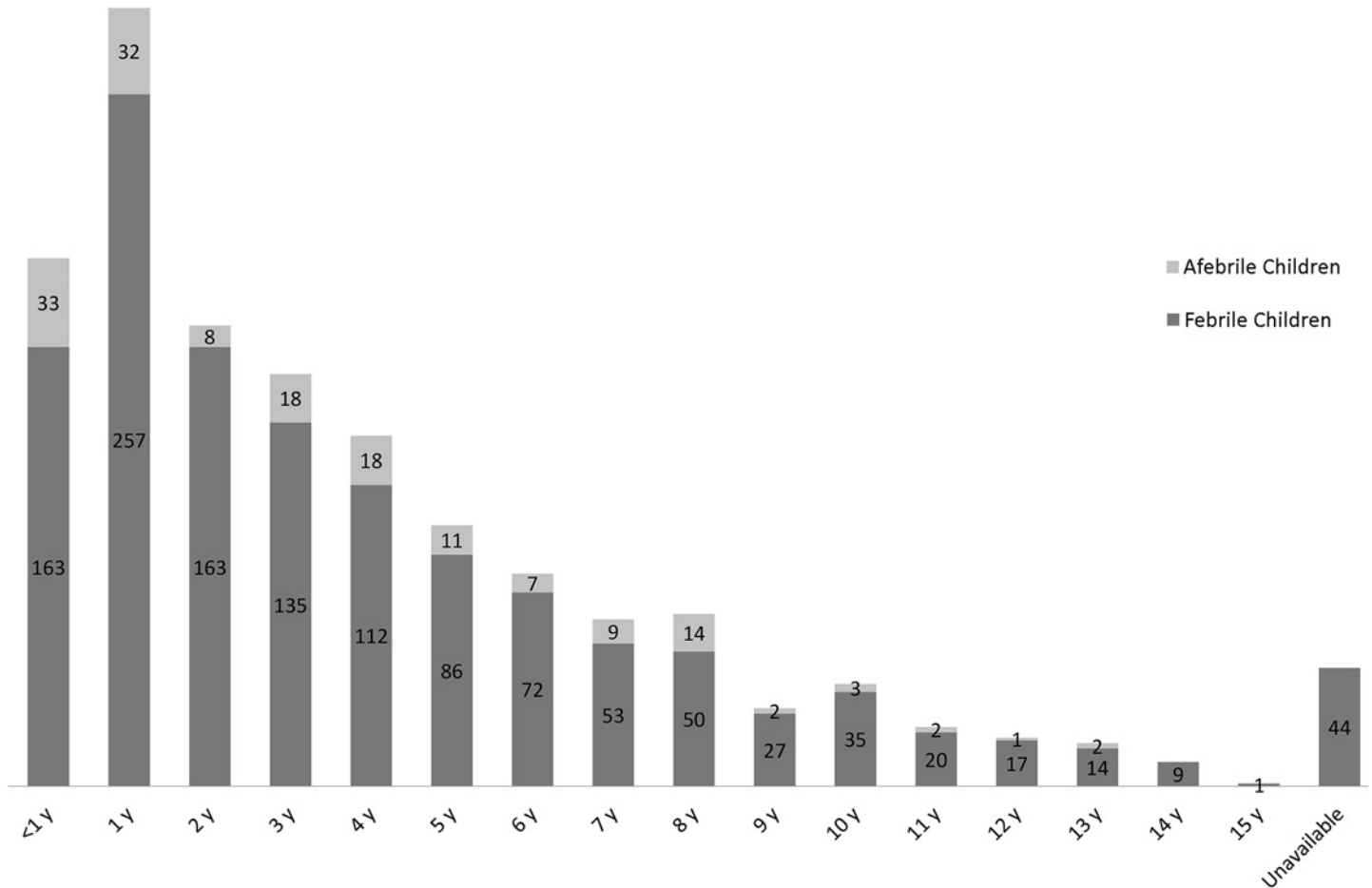
**Fig 1. Age distribution of all children included in this study.** (y = year-old).

## Mansonella perstans

*M. perstans* is the most prevalent filarial parasite detected in 67 children, including 61 febrile (4.8%, 61/1,258) and 6 afebrile children (3.8%, 6/160; $P$ = 0.6). Overall, *M. perstans* was detected in 3.5% of children ≤ 5 years of age (36/1,036), 7.7% of six to ten year-olds (21/272), and 10.6% of 11 to 15 year-olds (7/66), ([Fig 2]). The prevalence was statistically significantly
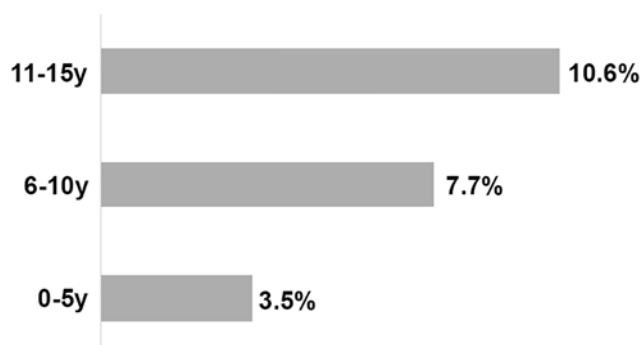


**Fig 2. Prevalence of *M. perstans* according to age for all positive children.** (y = year-old).
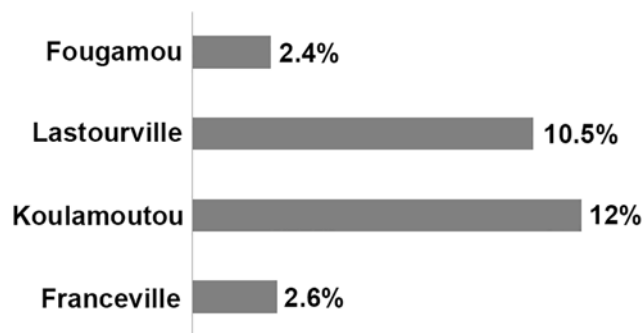
**Fig 3. Prevalence of *M. perstans* according to the studied areas.**

doi:10.1371/journal.pntd.0004155.g003

lower in children $\leq 5$ years of age than in those between six and ten year-olds ($P = 0.004$) and in those between 11 and 15 year-olds ($P = 0.01$). No statistical difference of prevalence was observed in children between ages six and ten and those between 11 and 15 year-olds ($P = 0.4$). Furthermore, no difference in *M. perstans* prevalence was observed between males (4.3%, 30/703) and females (4.9%, 33/673 $P = 0.6$). The prevalence of *M. perstans* was lower in the dry season (3.9%, 34/864) than in the rainy season (6%, 33/554), but without statistical significance ($P = 0.09$).

The lowest prevalence of *M. perstans* was observed in Haut-Ogooue and Ngounie areas, Franceville (2.6%, 26/997), and Fougamou (2.4%, 2/83), respectively. The prevalence was higher in the 2 sites of Ogooue Lolo province: Koulamoutou (12%, 20/107) and Lastourville (10.5%, 18/171), (Fig 3). Besides, these differences of prevalence of *M. perstans* were statistically significant.

The ITS1 and 5S sequences that were obtained were of high quality. Sequences from all Gabonese samples were identical for both of the two sequences. However, while the ITS1 sequences of *M. perstans* from Republic of Côte d'Ivoire and Senegal were completely identical to each other, they differed from all sequences from Gabon by only one base pair (position 383 from the beginning of the amplicon). Comparison of the 5S sequences from the different analyzed areas permitted identification of five genetic variants of *M. perstans*: three from Senegal, one from Côte d'Ivoire and one from Gabon (Fig 4A).

## *Mansonella* sp. "DEUX"

Among the 21 positive samples with ITS qPCR, but negative with *M. perstans* and *Loa loa* qPCR assays, sequencing of ITS1 was carried out for 12 samples; not enough DNA was available for 9 samples from Franceville. The sequences were almost identical to each other (two samples had the same single nucleotide polymorphism) and differed significantly from sequences of *M. perstans*: nine nucleotide polymorphisms and five deletions/insertions for the sequenced portion (94% of identity). The BLAST search did not yield any identification. Unfortunately, DNA sequences of *Mansonella streptocerca*, another possible human pathogen [15], were unavailable in the Genbank. The only available gene sequence (5S ribosomal RNA) of *M. streptocerca* was printed in the manuscript of Fischer *et al.* [15]. So, in order to identify if the *Mansonella* from 12 samples was *M. streptocerca*, we sequenced the 5S rRNA of these samples. The comparison of both sequences (the one printed in the manuscript and the one obtained from our samples) showed a difference (Fig 4B). This *Mansonella* sp. (provisionally called "DEUX") was detected only in febrile patients. The 12 patients were from Koulamoutou (four patients), Lastourville (four patients), and Fougamou (four patients).
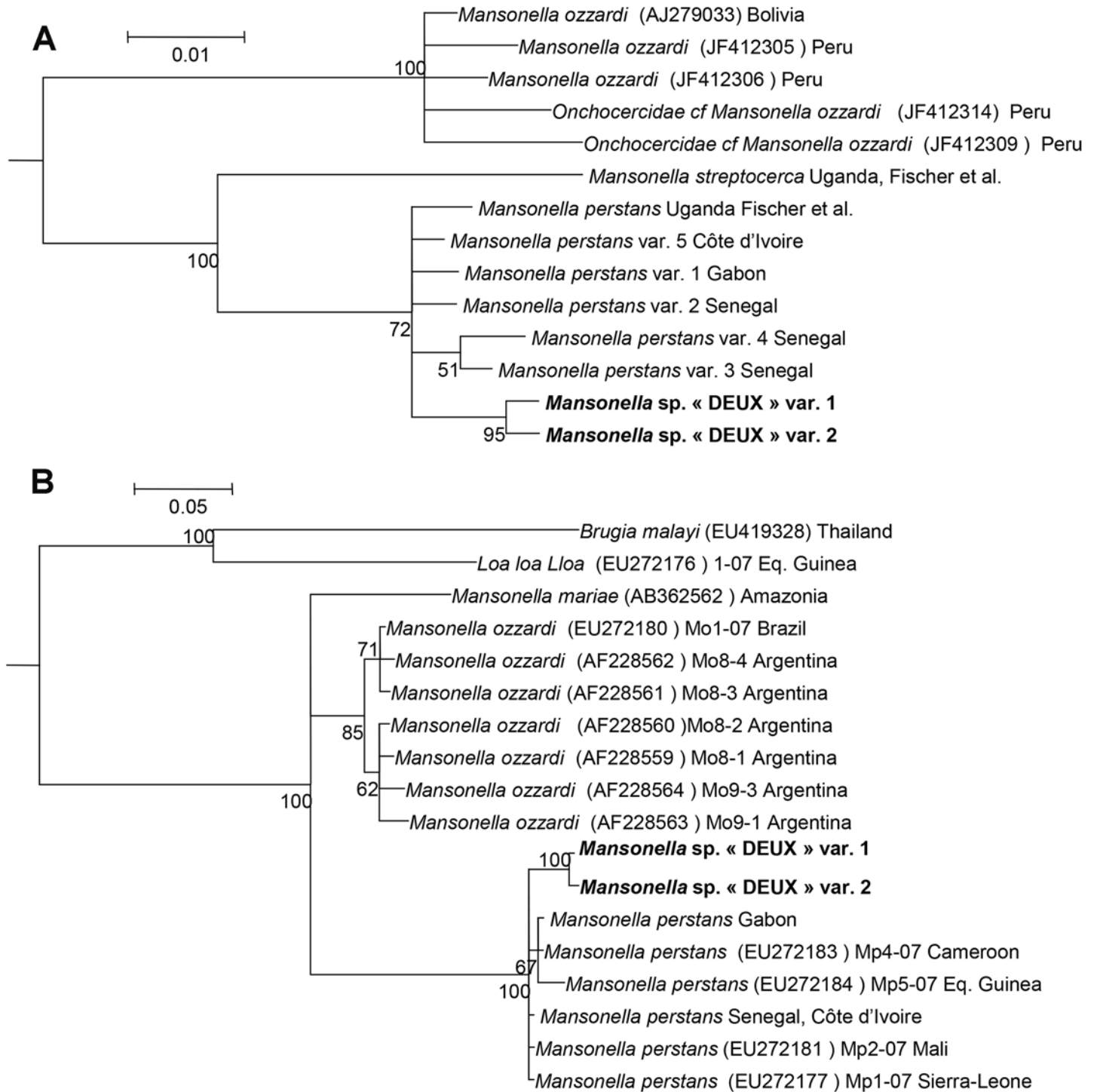
**Fig 4. Consensus phylogenetic tree showing the relationships between species of *Onchocercidae* in the study.** A. Consensus phylogenetic tree showing the relationships between species of *Onchocercidae* in the study based on 5S sequence comparisons. The scale bar represents a 1% nucleotide sequence divergence. GenBank accession numbers are indicated when the sequences originated from Genbank, and the geographic origin of the filariae is indicated at the end when possible. B. Consensus phylogenetic tree showing the relationships between species of *Onchocercidae* in the study based on ITS1 sequence comparisons. The scale bar represents a 5% nucleotide sequence divergence. GenBank accession numbers are indicated when the sequences originated from Genbank, and the geographic origin of the filariae is indicated at the end when possible.

doi:10.1371/journal.pntd.0004155.g004

## Nucleotide sequences

All nucleotide sequences obtained during this study were stored in the Genbank under the following numbers: KR080185-KR080190 for the ITS1 sequences from the two genetic variants of *Mansonella* sp. "DEUX" from Gabon, from *M. perstans* from Gabon, Senegal, and Republic of Côte d'Ivoire and for *Loa loa* from Gabon, respectively; KR080177-KR080184 for the 5S sequences from the two genetic variants of *Mansonella* sp. "DEUX" from Gabon, for *Loa loa* from Gabon, and for *M. perstans* from Côte d'Ivoire, Gabon and three genetic variants from Senegal, respectively.

## Discussion

Febrile illnesses are common in Sub-Saharan Africa while filarial parasites, including *M. perstans*, are known to influence their emergence. However, no study evaluated the prevalence of filarial parasites, especially *M. perstans*, among febrile children in Gabon. The data reported here were validated following rigorous criteria. Each PCR assay included a positive control (DNA of targeted filarial parasite) and a negative control (PCR mix alone). All positive samples were sequenced to confirm our results and the sequences obtained were carefully verified.

This study confirmed that *M. perstans* and *Loa loa* are widespread in Gabon [12,18]. However, no significant difference was observed between the prevalence of *M. perstans* in febrile (4.8%) and afebrile (3.8%) children. Similar lack of difference has also been recently reported in Senegal where the prevalence of *M. perstans* was 14.4% (29/201) among febrile and 15% (14/96) in afebrile children [11]. The lack of difference in the prevalence of *M. perstans* between febrile and afebrile subjects seems to support the previously reported observation that in general *M. perstans* is not a major cause of febrile illness [12].

Mansonelloses are most often considered to present few symptoms or even asymptomatic. Only eosinophilia, pruritus, and ocular involvement are typically reported as caused by *M. perstans*. However, severe complications have been observed in *M. perstans* infections, such as in the case of a missionary family returning from an African area endemic to *M. perstans* in which multiorgan failure was reported [9]. Ndibazza *et al.* in 2013 reported that parasites including, *M. perstans* infections were associated with an increase of the rate of clinical malaria unlike to other types of infection such as *Schistosoma mansoni*, which had no consistent association with childhood malaria [19]. Several other clinical features have been also reported and linked to *M. perstans* in studies, including hormonal disturbances, subcutaneous swellings, skin rashes, acute swelling in the forearms, itching, pain or ache organs, extreme exhaustion, fever, neurological, and psychological symptoms [6,7,8,9,20]. The diversified reports about the pathogenicity of *M. perstans* suggest that some factors such as the degree of microfilaremia, coinfection, sex, age, and the geographical origin of the person should greatly influence its pathogenicity [9,21,22]. In addition, the pathogenicity of *M. perstans* could be related to the age of first exposure. That could explain the severity of this infection among expatriates whereas few symptoms have been reported in native populations where asymptomatic carriers have also been observed for other parasites, including *Plasmodium falciparum* [13]. Overall, very few studies have screened rural endemic populations for symptoms [1]. *M. perstans* remains unresponsive to most antifilarial drugs [1]. Antibiotics for *Wolbachia*, its endosymbiont, had been reported as a strategy to combat this parasite [23].

The prevalence of *M. perstans* increased significantly with age. This finding supports those previously reported in Africa [2,11,10]. In fact, the higher prevalence in older children and adults should be related to their common exposure to the *Culicoides* bites during their activities

[24]. For example, fishermen, farmers, and cattle breeders were the most affected occupational categories in a study on filariasis in Bauchi State, Nigeria [25]. Besides, a significant frequency of *M. perstans* was observed according to the studied areas. Indeed, the prevalence was higher in the Ogooue Lolo province (Koulamoutou and Lastourville) than in Ngounie (Fougamou) and Haut Ogooue (Franceville). At Franceville, the vegetation consists mainly of savanna, while Koulamoutou and Lastourville are covered of tropical rainforest, and Fougamou is a grassland forest area. These finding correlate with those recently published by Akue *et al.* in 2011 [12]. Among the three major Gabonese ecosystems, the forest had a higher prevalence of parasites than the savannah and wetlands [12]. Studies summarized by Simonsen *et al.* supports this observation [1]. They reported that the high prevalence of *M. pertans* occurred in many areas where tropical forests alternate with large swamps and open ground [1]. They added that *M. perstans* was common in the rain forest, less common in the forest peripheries, higher in or near densely forested areas and low in the mountain grassland zones of the British Cameroons, and related to the species and biting density of the vectors [1]. Variation among other filarial parasites such as loasis, according to contrasting bioecological zones, was observed in central Africa [26]. Some vector species prefer to breed in decomposing plant material; extensive cultivation of bananas has been identified as a risk factor for transmission [1], this should explain why farmers are one of the most infected occupational categories[25]. Studies in other areas have shown that low-pH soil, low organic soil content, salty soil, and wet soil contributed to *Culicoides* fly breeding [27,28], while temperature may affect vector competence [29].

A novel potential species of *Mansonella* was detected on the basis of molecular analyses. Interestingly, a *Mansonella* species that was morphologically different from *M. ozzardi* was recently identified in blood samples from Peru [30], but no genetic difference was found. In this case, unfortunately, we failed to obtain blood smears, thus, no morphological analyses and comparisons of *Mansonella* sp. "DEUX" were possible. It is important to emphasize that *Mansonella* sp. "DEUX" was only detected in febrile children. We strongly suspect that *Mansonella* sp. "DEUX" may be a new species, because based on ITS1 comparison, *M. perstans* is very homogenous throughout Africa (from Senegal to Gabon) and *Mansonella* sp. "DEUX" differs significantly from *M. perstans* with this spacer (94%). Based on comparison by 5S rRNA sequences, *Mansonella* sp. "DEUX" is different from *M. streptocerca* [14,15]. Other possible variants may include *Mansonella rodhainii*, *Mansonella gorillae*, *Mansonella vanhoofi*, *Mansonella leopoldi*, and *Mansonella lopeensis*, reported from humans and great apes in Africa [31]. Unfortunately, genes from these species are unavailable in the Genbank. The slides containing microfilariae should enable us to describe morphological features of *Mansonella* sp. "DEUX" and to compare them with the aforementioned *Mansonella* spp. Until now, no other valid species belonging to the genus *Mansonella* were reported in Africa [31]. Thus, *Mansonella* sp. "DEUX" may represent a potential new species of *Mansonella* with a possible pathogenic role in humans. An additional morphologic study is necessary in order to identify whether *Mansonella* sp. "DEUX" represents one of the *Mansonella* species not yet molecularly characterized, a genetic variant of *M. perstans* or really a new species.

Gabon remains a significant target of filariasis including *Loa loa*, *M. perstans*, and a potential new species of *Mansonella*, *Mansonella* sp. "DEUX". *M. perstans* was commonly observed in both febrile and afebrile children whereas *Loa loa* and *Mansonella* sp. "DEUX" were observed only in febrile children. Even if *M. perstans* does not appear to be directly linked to febrile episodes among the local population, its real clinical impact has not yet been determined. Moreover, further study should be performed to characterize *Mansonella* sp. "DEUX" morphologically and evaluate its clinical significance in humans.

## Supporting Information

**S1 Checklist. STROBE checklist.**
(DOC)

## Acknowledgments

## Author Contributions

Conceived and designed the experiments: FF JBLD ANM DR OM. Performed the experiments: GM CHBE FB. Analyzed the data: GM FF JBLD ANM PBM RZM OM. Contributed reagents/materials/analysis tools: JBLD SMN PBM CHBE FB OM. Wrote the paper: GM FF JBLD DR OM.

## References

1. Simonsen PE, Onapa AW, Asio SM. *Mansonella perstans* filariasis in Africa. Acta Trop. 2011; 120 Suppl 1:S109–S120. doi: 10.1016/j.actatropica.2010.01.014 PMID: 20152790

2. Downes BL, Jacobsen KH. A Systematic Review of the Epidemiology of Mansonelliasis. Afr J Infect Dis. 2010; 4(1):7–14. PMID: 23878696

3. Dolo H, Coulibaly YI, Dembele B, Konate S, Coulibaly SY, Doumbia SS, et al. Filariasis attenuates anemia and proinflammatory responses associated with clinical malaria: a matched prospective study in children and young adults. PLoS Negl Trop Dis. 2012; 6(11):e1890. doi: 10.1371/journal.pntd.0001890 PMID: 23133692

4. Brown M, Kizza M, Watera C, Quigley MA, Rowland S, Hughes P, et al. Helminth infection is not associated with faster progression of HIV disease in coinfected adults in Uganda. J Infect Dis. 2004; 190 (10):1869–79. PMID: 15499545

5. Brown M, Miiro G, Nkurunziza P, Watera C, Quigley MA, Dunne DW, et al. *Schistosoma mansoni*, nematode infections, and progression to active tuberculosis among HIV-1-infected Ugandans. Am J Trop Med Hyg. 2006; 74(5):819–25. PMID: 16687687

6. Bregani ER, Ceraldi T, Rovellini A, Ghiringhelli C. Case report: intraocular localization of *Mansonella perstans* in a patient from south Chad. Trans R Soc Trop Med Hyg. 2002; 96(6):654. PMID: 12625144

7. Lansoud-Soukate J, Dupont A, De Reggi ML, Roelants GE, Capron A. Hypogonadism and ecdysteroid production in *Loa loa* and *Mansonella perstans* filariasis. Acta Trop. 1989; 46(4):249–56. PMID: 2571252

8. Scott G. Pathogenicity of *Acanthocheilonema perstans*. J Trop Med Hyg. 1962; 65:230–2. PMID: 13992525

9. Fux CA, Chappuis B, Holzer B, Aebi C, Bordmann G, Marti H, et al. *Mansonella perstans* causing symptomatic hypereosinophilia in a missionary family. Travel Med Infect Dis. 2006; 4(5):275–80. PMID: 16905458

10. Asio SM, Simonsen PE, Onapa AW. *Mansonella perstans* filariasis in Uganda: patterns of microfilaraemia and clinical manifestations in two endemic communities. Trans R Soc Trop Med Hyg. 2009; 103 (3):266–73. doi: 10.1016/j.trstmh.2008.08.007 PMID: 18809192

11. Bassene H, Sambou M, Fenollar F, Clarke S, Djiba S, Mourembou G, et al. High prevalence of *Mansonella perstans* filariasis in rural Senegal. Am J Trop Med Hyg. 2015; 93(3):601–6. doi: 10.4269/ajtmh.15-0051 PMID: 26078318

12. Akue JP, Nkoghe D, Padilla C, Moussavou G, Moukana H, Mbou RA, et al. Epidemiology of concomitant infection due to *Loa loa* and *Mansonella perstans* in Gabon. PLoS Negl Trop Dis. 2011; 5(10): e1329. doi: 10.1371/journal.pntd.0001329 PMID: 22022623

13. Mourembou G, Fenollar F, Socolovschi C, Lemamy GJ, Nzoughe H, Kouna LC, et al. Molecular Detection of Fastidious and Common Bacteria as Well as *Plasmodium* spp. in Febrile and Afebrile Children in

Franceville, Gabon. Am J Trop Med Hyg. 2015; 92(5):926–32. doi: 10.4269/ajtmh.14-0699 PMID: 25802432

14. Jimenez M, Gonzalez LM, Carranza C, Bailo B, Pérez-Ayala A, Muro A, et al. Detection and discrimination of Loa loa, *Mansonella perstans* and *Wuchereria bancrofti* by PCR-RFLP and nested-PCR of ribosomal DNA ITS1 region. Exp Parasitol. 2011; 127(1):282–6. doi: 10.1016/j.exppara.2010.06.019 PMID: 20599994

15. Fischer P, Buttner DW, Bamuhiiga J, Williams SA. Detection of the filarial parasite *Mansonella streptocerca* in skin biopsies by a nested polymerase chain reaction-based assay. Am J Trop Med Hyg. 1998; 58(6):816–20. PMID: 9660471

16. Nkamga VD, Huynh HT, Aboudharam G, Ruimy R, Drancourt M. Diversity of Human-Associated *Methanobrevibacter smithii* Isolates Revealed by Multispacer Sequence Typing. Curr Microbiol. 2015; 70(6):810–5. doi: 10.1007/s00284-015-0787-9 PMID: 25708582

17. Mediannikov O, Diatta G, Kasongo K, Raoult D. Identification of *Bartonellae* in the soft tick species *Ornithodoros sonrai* in Senegal. Vector Borne Zoonotic Dis. 2014; 14(1):26–32. doi: 10.1089/vbz. 2013.1326 PMID: 24359424

18. Van HM, Chabaud B, Akue JP, Ivanoff B. Filariasis due to Loa loa and *Mansonella perstans*: distribution in the region of Okondja, Haut-Ogooue Province, Gabon, with parasitological and serological follow-up over one year. Trans R Soc Trop Med Hyg. 1987; 81(3):441–6. PMID: 3479854

19. Ndibazza J, Webb EL, Lule S, Mpairwe H, Akello M, Oduru G, et al. Associations between maternal helminth and malaria infections in pregnancy and clinical malaria in the offspring: a birth cohort in entebbe, Uganda. J Infect Dis. 2013; 208(12):2007–16. doi: 10.1093/infdis/jit397 PMID: 23904293

20. Anosike JC, Dozie IN, Onwuliri CO, Nwoke BE, Onwuliri VA. Prevalence of *Mansonella perstans* infections among the nomadic Fulanis of northern Nigeria. Ann Agric Environ Med. 2005; 12(1):35–8. PMID: 16028864

21. Keiser PB, Coulibaly YI, Keita F, Traore D, Diallo A, Diallo DA, et al. Clinical characteristics of post-treatment reactions to ivermectin/albendazole for *Wuchereria bancrofti* in a region co-endemic for Mansonella perstans. Am J Trop Med Hyg. 2003; 69(3):331–5. PMID: 14628953

22. Bouyou-Akotet MK, Moussavou Boussougou MN, Ovono-Abessolo F, Owono-Medang M, Kombila M. Influence of *Mansonella perstans* microfilaraemia on total IgE levels in Gabonese patients co-infected with *Loa loa*. Acta Trop. 2014; 131:11–5. doi: 10.1016/j.actatropica.2013.11.012 PMID: 24280145

23. Gehringer C, Kreidenweiss A, Flamen A, Antony JS, Grobusch MP, Bélard S. Molecular evidence of *Wolbachia* endosymbiosis in *Mansonella perstans* in Gabon, Central Africa. J Infect Dis. 2014; 210 (10):1633–8. doi: 10.1093/infdis/jiu320 PMID: 24903665

24. Agbolade OM, Akinboye DO, Olateju TM, Ayanbiyi OA, Kuloyo OO, Fenuga OO. Biting of anthropophilic *Culicoides fulvithorax* (Diptera: Ceratopogonidae), a vector of *Mansonella perstans* in Nigeria. Korean J Parasitol. 2006; 44(1):67–72. PMID: 16514285

25. Anosike JC, Onwuliri CO. Studies on filariasis in Bauchi State, Nigeria. II. The prevalence of human filariasis in Darazo Local Government area. Appl Parasitol. 1994; 35(4):242–50. PMID: 7812311

26. Wanji S, Tendongfor N, Esum M, Atanga SN, Enyong P. Heterogeneity in the prevalence and intensity of loiasis in five contrasting bioecological zones in Cameroon. Trans R Soc Trop Med Hyg. 2003; 97 (2):183–7. PMID: 14584374

27. Lubega R, Khamala CPM. Larval habitats of common Culicoides *Latreille* (Diptera, Ceratopogonidae) in Kenya. Bull Entomol Res. 1976; 66(3):421–5.

28. Narladkar BW, Deshpande PD, Shivpuje PR. Bionomics and life cycle studies on *culicoides* sp. (Diptera: Ceratopogonidae). J Vet Parasitol. 2006; 20(1):7–12.

29. Mullens BA, Gerry AC, Lysyk TJ, Schmidtmann ET. Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpreting laboratory data in a field context. Vet Ital. 2004; 40(3):160–6. PMID: 20419655

30. Marcos LA, Arrospide N, Recuenco S, Cabezas C, Weil GJ, Fischer PU. Genetic characterization of atypical *Mansonella* (Mansonella) ozzardi microfilariae in human blood samples from northeastern Peru. Am J Trop Med Hyg. 2012; 87(3):491–4. doi: 10.4269/ajtmh.2012.11-0379 PMID: 22826497

31. Bain O, Mutafchiev Y, Junker K, Guerrero R, Martin C, Lefoulon E, et al. Review of the genus *Mansonella Faust*, 1929 sensu lato (Nematoda: Onchocercidae), with descriptions of a new subgenus and a new subspecies. Zootaxa. 2015; 3918(1):151–93. doi: 10.11646/zootaxa.3918.2.1 PMID: 25781087

32. Touré FS, Bain O, Nerrienet E, Millet P, Wahl G, Toure Y, et al. Detection of *Loa loa*-specific DNA in blood from occult-infected individuals. Exp Parasitol. 1997; 86(3):163–70. PMID: 9225766

33. Ajuh PM, Akue JP, Boutin P, Everaere S, Egwang TG. *Loa loa*: structural diversity of a 15-kDa repetitive antigen. Exp Parasitol. 1995; 81(2):145–53. PMID: 7556556