**Materials and Methods**

*Samples and morphological identification of the filaroid nematodes*

In March 2007, four adults red-handed tamarins were found road killed on the edge of the national road 1, near the coast of northern French Guiana. Two males (TMDl and TMD2) were near Iracoubo: 5°28'27.5"N 53°11'12.7"W and 5°29'16.4"N 53°13111.S"W. Two others, TMD3 (male) and TMD4 (female) were near Saut Sabbat: 5° 24' 24.S" N 53°42'20. "W and 52.2" N 53°36'30 4"W.

Adult filarioid worms were preserved in 70% ethanol for DNA-based studies and in Amman’s for light microscopic observation. Morphological identification at species level was performed according to the morphology of the anterior and the posterior ends of the worm, position of some organs (i.e., vulva, nerve ring. etc.) along with the morphometric parameters as described in (Bain *et al.* 1986). Measurement and microscopic examination were performed using a DM-LB2 microscope and Leica Las version 4.5.0 software (Leica Microsystems, Wetzlar, Germany).

*Molecular identification* and *phylogenetic analysis*

Genomic DNA (gDNA) was extracted from a piece of tissue (~ 1 mm from the middle of body) of each adult worm using QIAGEN DNA tissues kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Two lysis steps were performed prior to the extraction procedure: (i) mechanical lysis performed on the FastPrep-24™ 5G homogenizer under high-speed agitation for 3 cycles of 40 seconds each in the presence of glass powder, (ii) enzymatic digestion of proteins with buffer G2 supplemented with 25% proteinase K for 24 hours at 56°C. The extracted DNA was eluted in a total volume of 100 µL and stored at – 20°C.

The gDNA was amplified using the pan-Nematoda-18S primers (Laidoudi *et al.*, 2019) and pan-filaroid *cox*1 based PCR [Pan-fil *cox*1] (Laidoudi *et al.*, 2020a) (Table S2) and later sequenced to generate a 1127–1155 and 509 bp product, respectively. A third PCR [Hspec-28S] (Medkour *et al.*, 2020) was used to amplify 493–523 bp from the third part of the helminthic 28 rRNA gene (Table S2). PCR ampliﬁcations were performed in a thermal cycler (Applied Biosystem, Paris, France) as described elsewhere (Laidoudi *et al.*, 2019, 2020c). DNA amplicons were purified using NucleoFast 96 PCR plates (Macherey Nagel EURL, Hoerdt, France) prior to sequencing reaction with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Big-Dye PCR products were purified on Sephadex G-50 Superfine gel filtration resin and sequenced using ABI Prism 3130XL sequencer.

DNA sequences were assembled using ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia). The absence of co-amplifications of nuclear mitochondrial genes (numts) was verified for the *cox*1 DNA sequences, by alignment against the complete mitochondrial genome of *Acanthocheilonema viteae* (HQ186249) using the ClustalW application in Bioedit v.7.2.5. (Hall *et al.*, 2011). In addition, visual inspection of the sequence chromatograms for ambiguities, indels and stop codons of the translated *cox*1 sequences was performed using Chromas Pro 2.0.0 software as recommended (Song *et al.*, 2008). The DNA sequences of the nuclear 18S and 28S rRNA genes and mitochondrial *cox*1 as well as the *cox*1 amino acid sequence were separately subjected to preliminary analysis using Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990).

Both DNA and amino acid sequences of the filarial *cox*1 gene were aligned against the representative members of all Onchocercidae clades (ONC1 to ONC5) (Lefoulon *et al.*, 2015). Alignment was performed using the ClustalW (Hall *et al.*, 2011). DNA sequences of *Filaria* *latala* (GenBank accession number: KP760186) and its protein sequence (Protein Id: ALR73845) (Lefoulon *et al.*, 2015) and that of the Spirurida *Abbreviata* *caucasica* (GenBank accession number: MT231294; Protein Id: QIP66136) (Laidoudi *et al.*, 2020b) were used as outgroups to root the trees. The Akaike Information Criterion (AIC) option in MEGA6 (Tamura *et al.*, 2013) was used to determine the best nucleotide substitution model fitted to each sequence alignment. The General Time Reversible (GTR, *+G, +I*) (Waddell and Steel, 1997) and Le Gascuel (2008) (LG, +G) models were selected for the DNA and protein alignments respectively. The maximum likelihood method (ML) based on 1000 bootstraps was inferred for each alignment to generate the phylogenetic trees in MEGA6 (Tamura *et al.*, 2013).

Multi-loci sequence typing (MLST) phylogeny was performed on the basis of the *cox*1 and the 18S genes. Briefly, *cox*1 and 18S gene datasets representing all Onchocercidae clades (ONC1 to ONC5) were constructed from the previously published sequences (Lefoulon *et al.*, 2015). Aligned using MAFFT (Katoh *et al.*, 2002), concatenated using Seaview (Gouy *et al.*, 2010). Sequences from *F. latala* (GenBank accession number: *cox*1: KP760186; 18S: KP760135) and *A. caucasica* (GenBank accession number: *cox*1: MT231294; 18S: MN956825) were used as out groups to root the tree. The ML phylogram was generated using IQTREE (Nguyen *et al.*, 2015) and the ultra-fast bootstrap (UFBoot) with 1000 replicates (Minh *et al.*, 2013). The best fitting evolutionary model (GTR, *+G, +I*) was selected using Modelfinder (implemented as functionality of IQ-TREE). The analysis was performed on Galaxy Australia server ([https://usegalaxy.org.au/](about:blank)).

The 28S sequence was aligned against 13 sequences representing three Onchocercidae clades (ONC2, ONC3 and ONC5) retrieved from GenBank and Worm-Parasite databases (<https://parasite.wormbase.org/index.html>). Sequence alignment was performed using the ClustalW application within Bioedit software v.7.2.5. (Hall *et al.*, 2011). DNA sequences from *Gongylonema pulchrum* (GenBank accession number: LR215834), *Parascaris* spp. (GenBank accession number: DQ145716 and PRJNA386823) and *Ascaris* spp. (GenBank accession number: PRJNA62057 and AY210806) were used as out groups. The best nucleotide substitution model was fitted using MEGA6 (Tamura *et al.*, 2013). The Tamura 3-parameter model (T92, *+G, +I*) (Tamura, 1992) was selected. The ML method based on 1000 bootstraps was inferred using MEGA6 (Tamura *et al.*, 2013).

Finally, all phylograms were processed using iTOL v4 software (Letunic and Bork, 2019). The interspecific pairwise distance (IPD) was used to estimate the evolutionary divergence between the *cox*1 gene and its translated amino acid sequence as well as the concatenated sequences (*cox*1 and 18S) and the 28S rRNA gene among the Onchocercidae clades. Standard errors were determined by a bootstrap procedure with 1000 replicates. Analyses were inferred using MEGA6 software (Tamura *et al.*, 2013), based on the Maximum Composite Likelihood model (Tamura *et al.*, 2004).

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