Aus der

Medizinischen Universitätsklinik und Poliklinik Tübingen

Abteilung VII, Tropenmedizin

(Schwerpunkt: Institut für Tropenmedizin, Reisemedizin, Humanparasitologie)

Genetic differences between M. perstans and Mansonella sp „DEUX“ in three polymorphic marker regions

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von
Fischer, Mara

2025

Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Privatdozentin Dr. J. Held
2. Berichterstatter: Privatdozentin Dr. M. Schütz

Tag der Disputation: 27.09.2024

Widmung

Für Cosima

Table of contents

Table of tables VI

Table of figures IX

List of abbreviations X

1 Introduction 1

1.1 *Mansonella* species 1
1.2 Phylogenetic relationships within the genus *Onchocerca* 1
1.3 *Mansonella* sp. “DEUX” 4
1.4 Genotyping assays for *Mansonella* spp. 5
1.5 Life cycle, vectors and transmission 8
1.6 *Mansonella* diagnostics 9
1.7 Clinical presentation 10
1.8 Endosymbiosis with *Wolbachia* and its implications for treatment 11
1.9 Aim of this thesis 12

2 Materials14

2.1 Chemicals 14
2.2 Kits 14
2.3 Laboratory equipment 14
2.4 Software 15

3 Methods 16

3.1 Study area and acquisition of *Mansonella* DNA 16
3.1.1 Study cohort A 16
3.1.2 Study cohort B 17
3.1.3 Additional DNA extraction 17
3.2 PCR setup 18
3.3 Electrophoresis 18
3.4 Amplicon purification for sequencing 19
3.4.1 Amplicon purification via exoSAP 19
3.4.2 Amplicon purification via gel 19
3.5 Sequencing 19

3.6 Phylogenetic analysis 20
3.6.1 Reference sequences 20
3.6.2 Phylogenetic analysis of sequences 20
3.7 PCR optimization 22
3.7.1 Automated capillary gel electrophoresis 22
3.7.2 Primer design 23
3.7.3 *12S* rDNA PCR optimization 24
3.7.4 *cox1* PCR optimization 28
3.7.5 *28S* rDNA optimization 31
4 Results 34
4.1 Sample collection of study cohort A and clinical presentation 34
4.2 PCR optimizations 34
4.2.1 *12S* rDNA 34
4.2.2 *cox1* 36
4.2.3 *28S* rDNA 37
4.2.4 PCR amplification and sequencing 40
4.3 Phylogenetic analyses 41
4.3.1 Primary objective: Single nucleotide polymorphisms between *M. perstans* and *Mansonella* sp. “DEUX” 42
4.3.2 Secondary objective 1: Investigation of the intraspecific differences in *M. perstans* and *Mansonella* sp. “DEUX” in *12S* rDNA, *cox1* and *28S* rDNA 46
4.3.3 Secondary objective 2: Comparison with previously deposited sequences 49
4.3.4 Secondary objective 3: Visualization of the phylogenetic relationship 51
4.3.5 Clustering ouf the previously excluded sequences 56
4.3.6 Overview of the phylogenetic analysis 56
5 Discussion 59
5.1 Sample collection 59
5.2 Polymerase chain reaction 60
5.2.1 PCR optimization 60
5.2.2 Primer design 60

5.2.3 PCR amplification 61
5.2.4 Sequencing 61
5.3 Phylogenetic analysis 62
5.3.1 Species differentiation based on *12S* rDNA, *cox1* and *28S* rDNA 63
5.3.2 Intraspecific differences and the potential for genotyping 63
5.3.3 Association of genotypes with country of origin or host species 64
5.3.4 Sequences excluded from analysis 65
5.3.5 Interpretation of the incongruences between Bayesian trees and maximum likelihood trees and the clustering of the outgroups 65
5.3.6 The status of *Mansonella* sp. "DEUX" as a distinct species within the onchocercid family 67
5.4 Outlook 68
5.5 Conclusion from this thesis 69
6 Summary 71
7 Zusammenfassung 72
8 Bibliography 74
9 Erklärung zum Eigenanteil 83
10 Acknowledgements 84
11 Supplementary files 85
11.1 Clincal presentation 85
11.1.1 Questionnaire for study participants 85
11.1.2 Clinical symptoms as reported by participants 86
11.1.3 Distributions of symptoms as reported by participants 86
11.2 Phylogenetic analysis 86
11.2.1 Sequences generated in this thesis 87
11.2.2 Additionally analyzed sequences, accessed 20.04.2023 111
11.2.3 Outgroups, accessed 20.04.2023 113

Table of tables

Table 1: Overview of samples analyzed in this study 16
Table 2 Sample and marker preparation for gel electrophoresis 18
Table 3: Reaction mix composition and cycling conditions for exoSAP 19
Table 4: Sequences and further characteristics of sequencing primers 20
Table 5: Overview of utilized reference sequences. 20
Table 6: QIAxcel size markers and alignment markers 23
Table 7: *12S* rDNA primer sequences and further characteristics 24
Table 8: *12S* rDNA preamplification final reaction mix composition and cycling conditions 27
Table 9: *12S* rDNA nested PCR final reaction mix composition and cycling conditions 27
Table 10: *Cox1* primer sequences and further characteristics 28
Table 11: *Cox1* preamplification final reaction mix composition and cycling conditions 30
Table 12: *Cox1* preamplification final reaction mix composition and cycling conditions 30
Table 13: *28S* rDNA primer sequences and further characteristics 31
Table 14: *28S* rDNA preamplification final protocol reaction mix composition and cycling conditions 33
Table 15: *28S* rDNA nested PCR final protocol reaction mix composition and cycling conditions 33
Table 16: Overview of amplified and sequenced samples 41
Table 17: Overview of analyzed samples 42
Table 18: *12S* rDNA SNPs in *Mansonella sp*. "DEUX" sequences generated in this thesis and their placement on the *M. perstans* reference sequence 43
Table 19: Additional *12S* rDNA SNPs present in a majority of *Mansonella sp*. "DEUX" sequences generated in this thesis and their placement on the *M. perstans* reference sequence 43
Table 20: *Cox1* SNPs in *Mansonella sp*. “DEUX” sequences generated in this thesis and their placement on the *M. perstans* reference sequence 44

Table 21: *28S* rDNA SNPs in *Mansonella sp*. “DEUX” sequences generated in this thesis and their placement on the *M. perstans* reference sequence...........45
Table 22: Overview of SNPs between *Mansonella* species in sequences generated in this thesis and comparison with interspecific nucleotide divergences between other onchocercid species. 46
Table 23: Overview of the number of sequences and genotypes from samples investigated in this thesis 49
Table 24: Overview of analyzed sequences including previously published sequences 50
Table 25: Overview of host species, source material and country of origin of analyzed sequences including previously published sequences.......................50
Table 26: Overview of the number of sequences and genotypes with the inclusion of additional sequences 51
Table 27: Intraspecific differences within *M. perstans* and *Mansonella* sp. “DEUX“. 57
Table 28: Comparison of intraspecific nucleotide divergences to previous results 63
Table 29: Distribution of clinical symptoms in participants without infection, with infection with mixed filarial species and Mansonella mono- or coinfections 86
Table 30: *12S* rDNA sequences generated in this thesis 89
Table 31: *cox1* sequences generated in this thesis..100
Table 32: *28S* rDNA sequences generated in this thesis 111
Table 33: Characteristics of additionally analyzed sequences 113
Table 34: Overview of the species and accession numbers used as outgroups in phylogenetic analysis 114

Table of figures

Figure 1 Onchocercid clades as proposed by Lefoulon, E. et al 3
Figure 2: Giemsa-stained blood smears of *Mansonella* sp. "DEUX" and *M. perstans* 5
Figure 3: Overview of types of genetic variants after Kockum et al., 2023.........6
Figure 4: Life cycle of *M. perstans* 9
Figure 5: Overview over the workflow of the phylogenetic analysis 22
Figure 6: 12S rDNA primer positions on the *M. perstans* reference sequence .24
Figure 7: Optimization process of the *12S* rDNA PCR 26
Figure 8: *Cox1* primer positions on *M. perstans* reference sequence 28
Figure 9: Optimization process of the *cox1* PCR...29
Figure 10: *28S* rDNA primer positions on the gene on the *M. perstans* reference sequence 31
Figure 11: Optimization process of the *28S* rDNA PCR 32
Figure 12: *12S* rDNA screen gel final protocol 36
Figure 13 *Cox1* screen gel final protocol 37
Figure 14: *28S* rDNA primer combinations 1 and 2 in nested PCR..................39
Figure 15: *28S* rDNA screen gel final protocol 40
Figure 16: Workflow of the phylogenetic analysis...41
Figure 17: *12S* rDNA alignment of the *Mansonella* sequences generated in this thesis to the *M. perstans* reference sequence..42
Figure 18: *Cox1* alignment of the *Mansonella* sequences generated in this thesis to the *M. perstans* reference sequence..43
Figure 19: *28S* rDNA alignment of sequences generated in this thesis to the *M. perstans* reference sequence. 45
Figure 21: *Cox1 M. perstans* samples generated in this thesis aligned to the *M. perstans* reference sequence 47
Figure 22: *28S* rDNA *M. perstans* samples generated in this thesis aligned to the *M. perstans* reference sequence 47
Figure 23: *Cox1* and *28S* rDNA genotype distribution for *M. perstans* sequences generated in this thesis..47

Figure 24:*12S* rDNA, *cox1* and *28S* rDNA genotype distribution for *Mansonella* sp. “DEUX” sequences generated in this thesis 48
Figure 26: *12S* rDNA phylogenetic maximum likelihood tree 53
Figure 27: *Cox1* phylogenetic maximum likelihood tree. 54
Figure 28: *28S* rDNA phylogenetic maximum likelihood tree 55
Figure 29: Questionnaire for study participants 85
Figure 30: Symptoms reported by participants 86
Figure 31: *12S* rDNA phylogenetic bayesian tree....115
Figure 32: *Cox1* phylogenetic bayesian tree 115
Figure 33: *28S* rDNA phylogenetic bayesian tree....116

List of abbreviations

bp	base pair
CDS	coding sequence
Fwd	forward
H_2O	nuclease free water
M. ozzardi	*Mansonella ozzardi*
M. perstans	*Mansonella perstans*
Mansonella. sp. “DEUX”	*Mansonella* sp. « DEUX »
M. streptocerca	*Mansonella streptocerca*
NHP	non-human primate
O. volvulus	*Onchocerca volvulus*
PCR	polymerase chain reaction
Rev	reverse
SNP	single nucleotid polymorphism
Temp.	temperature
Tm	melting temperature
V	volt
WB	DNA extracted from non-*Mansonella*-infected human whole blood

1 Introduction

1.1 *Mansonella* species

First described by Sir Patrick Manson in 1891 in New Guinea (Manson, 1891), species of the genus *Mansonella* are filarial nematodes of the genus *Onchocerca* with possibly the greatest worldwide prevalence among the agents of human filariosis (Downes & Jacobsen, 2010; Simonsen, Onapa, & Asio, 2011; Ta-Tang, Crainey, Post, Luz, & Rubio, 2018) – and simultaneously the causative agents of one of the most neglected tropical diseases. Until recently, three *Mansonella* species infecting humans were known: *M. perstans, M. ozzardi* and *M. streptocerca*. Another *Mansonella* species called *M. rodhaini* has been described to infect humans, although it primarily infects primates (Richard-Lenoble, Kombila, Bain, Chandenier, & Mariotte, 1988). Recently, the existence of a possibly distinct blood-dwelling *Mansonella* species called *Mansonella* sp. "DEUX" was confirmed in a *M. perstans* – endemic region in Gabon (Mourembou et al., 2015; Sandri et al., 2020).

Mansonella species are endemic in wide parts of the world. *M. perstans* has been described in 33 countries in Sub-Saharan Africa and is assumed to affect around 114 million people in Africa (J. L. Crainey, Ribeiro da Silva, & Luz, 2016; Simonsen et al., 2011). *M. streptocerca* has predominately been found in Central Africa with prevalence rates between 0.5% - 89% while *M. ozzardi* is spread in tropical regions of North and South America with an estimated prevalence of 3% - 61% (Downes & Jacobsen, 2010; Lima, Veggiani Aybar, Dantur Juri, & Ferreira, 2016). The lack of reliable prevalence data reflects the neglect of mansonellosis research.

1.2 Phylogenetic relationships within the genus *Onchocerca*

Species designation and the classification of phylogenetic relationships between onchocercid species has historically been difficult and current classifications are constantly challenged. Before the development of molecular methods, taxonomy of the *Onchocercidae* family relied predominantly on the morphology of microfilariae, their first filarial larval stage found in subcutis and blood, since the

recovery of adult specimen is rare. The homoplastic nature of those microfilariae across the *Onchocercidae* family, with some species being hardly distinguishable by microscopy (McCall, Townson, & Trees, 1992) has not only contributed to phylogenetic controversies and the discovery of new species, but also to controversial findings regarding geographical distribution, clinical symptoms and efficiency of treatments regarding species belonging to this family (Dukes, Gelfand, Gadd, Clarke, & Goldsmid, 1968; Ta-Tang et al., 2018). With the development of molecular methods, a more standardized assessment of species diversity has been introduced (E. Ferri et al., 2009; Xie, Bain, & Williams, 1994).

Onchocercidae are currently divided in eight subfamilies and 88 genera (Bain et al., 2015), although recent multi-locus analysis challenges this phylogeny (E. Lefoulon et al., 2015). Figure 1 demonstrates the classification of *Onchocercidae* in five sister clades according to the most recent phylogenetic analysis proposed by Lefoulon et al. (E. Lefoulon et al., 2015) and supported by others (Gaillard et al., 2020; Mirzaei et al., 2018). Both traditional and modern approaches place the genus *Mansonella* in close phylogenetic relationships with *Loa, Brugia* and *Wuchereria.*

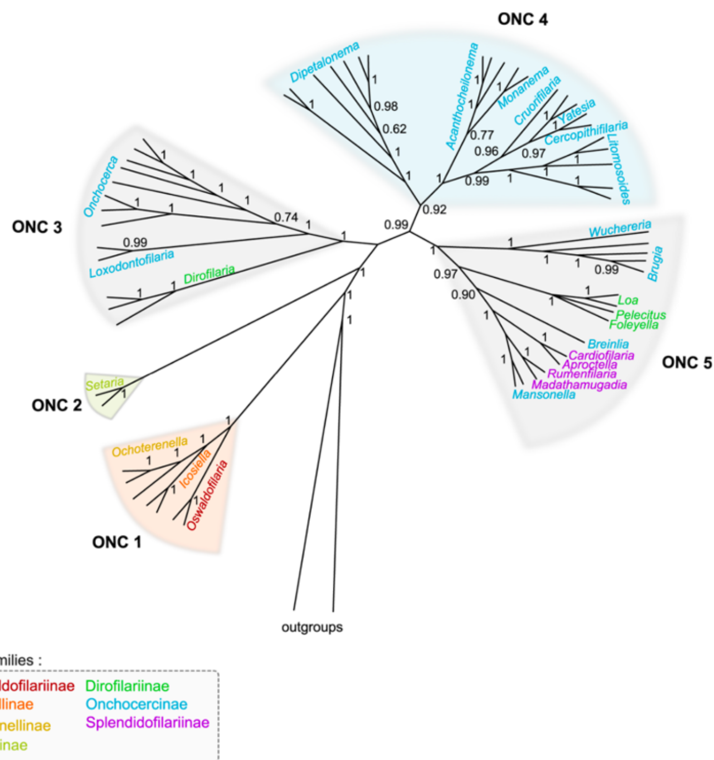

Figure 1 Onchocercid clades as proposed by Lefoulon, E. et al. (E. Lefoulon et al., 2015). Mansonella *species can be found in clade ONC5.*

Apart from morphological and molecular approaches, other species concepts propose different factors for species designation (Aldhebiani, 2018). The widely known biological species concept defines a species via the incapability of its members to reproduce with members of another species (Mayr, 1999), whereas the ecological species concept defines species over ecological competition (Van Valen, 1976), e.g. through shared hosts, vectors, life cycle or habitat. Other species concepts highlight the importance of evolution or phylogenetic relationships. In *Mansonella* spp., the homoplastic morphology, shared host species, vectors and life cycle as well as the similar pathogenicity of *M. perstans* and *M. ozzardi* complicates species designation based on morphological and ecological aspects and shifts the focus for species designation towards molecular and phylogenetic factors.

1.3 Mansonella sp. "DEUX"

The aforementioned application of new molecular methods has led to the discovery of a potential new human blood-dwelling *Mansonella* species: *Mansonella* sp. "DEUX". This distinct *Mansonella* species was observed in febrile children within the frame of a prevalence study investigating *M. perstans* and *L. loa* in Gabon in 2015 (Mourembou et al., 2015). *Mansonella sp.* "DEUX" was detected by molecular methods when they targeted a part of the ITS1-region which is highly conserved between filarial species, but the target sequence could not be amplified with *M. perstans* specific primers and probes. This observation implied a genetic difference between the species, and since the 5S rRNA sequence of this species differed from *M. streptocerca*, *Mansonella* sp. "DEUX" was thus considered a distinct *Mansonella* species (Mourembou et al., 2015). In further research, *Mansonella* sp. "DEUX" has been found to differ on a molecular level from other known *Mansonella* species such as *M. perstans* in marker regions such as ITS1, *12S* rDNA and *cox1* (Fischer, Büttner, Bamuhiiga, & Williams, 1998; Mourembou et al., 2015; Rodi et al., 2023). Whole genome analysis in 2023 further supported its characterization as a distinct species to *M. perstans* (Rodi et al., 2023).

Mansonella sp. "DEUX" has been found to have a previously unsuspected and, contrarily to the first observation, mostly asymptomatic high prevalence in Gabon. A cross-sectional study conducted in 2015 in rural areas of Gabon found 35% of the study population infected with *Mansonella* sp. "DEUX", which makes it the most prevalent filarial infection in this area compared to 32% *L. loa* infections and 9% *M. perstans* infections (Sandri et al., 2020). Detection of this species was based on molecular methods, more specifically PCR, since light microscopy did not allow the determination of species-specific morphological characteristics distinguishing *Mansonella* sp. "DEUX" from *M. perstans* as demonstrated in Figure 2. No data is yet available regarding the morphology of adult worms. Both *M. perstans* and *Mansonella* sp. "DEUX" have been found to infect non-human primates (NHPs) as well as humans (Gaillard et al., 2020; Rodi et al., 2023). While this species thus seems to share many characteristics with *M. perstans,* little further description exists about *Mansonella* sp. "DEUX".

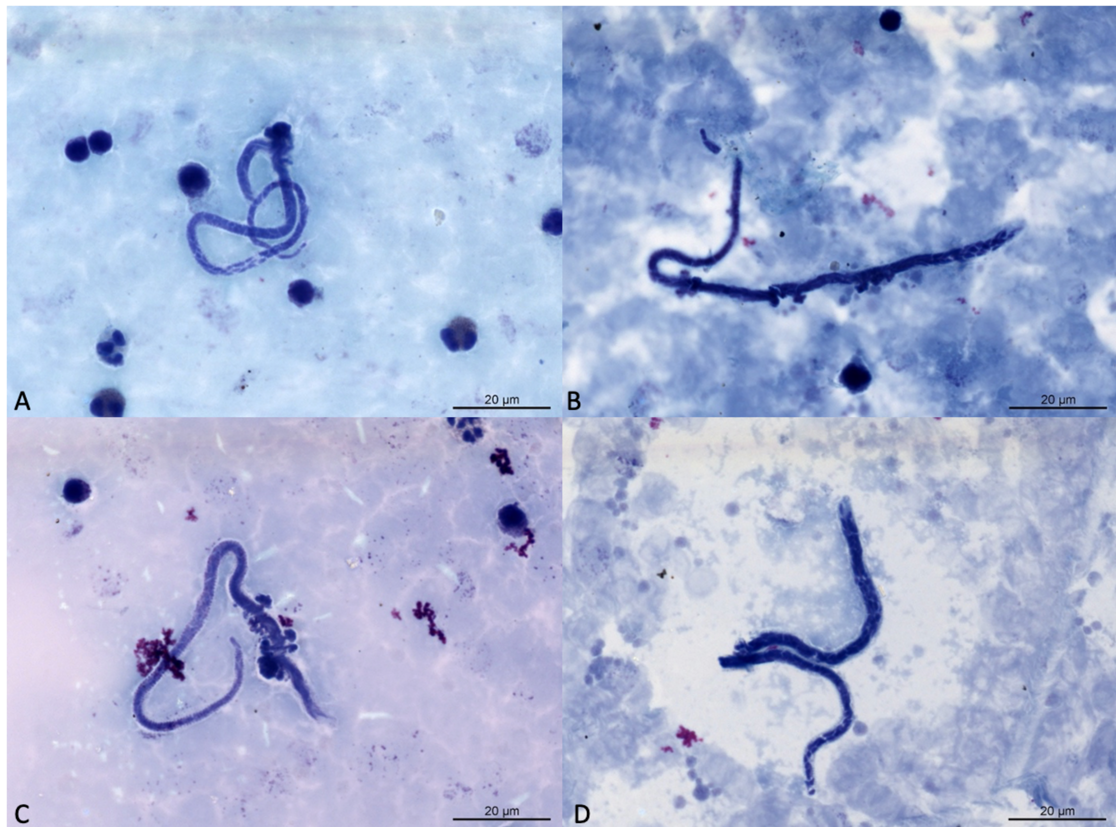

Figure 2: Giemsa-stained blood smears, 1000x magnification. Scale as indicated. Mansonella sp. "DEUX" *(A) and M. perstans (B-D)*

The actual burden of disease from *Mansonella* sp. "DEUX" is unknown so far. *Mansonella* infections usually have a low microfilaremia, which makes molecular methods such PCR due to their increased sensitivity the superior diagnostic tool. However, traditional molecular methods widely failed to detect *Mansonella* sp. "DEUX" within the frame of *Mansonella* diagnostics. (Sandri et al., 2020). Thus, it is likely that the burden of mansonellosis has thus so far been widely underreported. The publication of its whole genome and further investigation into the genetics of *Mansonella* sp. "DEUX" could improve detection methods and is thus crucial to determine its disease burden.

1.4 Genotyping assays for *Mansonella* spp.

The introduction of PCR and the development of molecular markers and molecular methods such as sequencing allows for a more standardized assessment of species diversity (Bain et al., 2015; Tang et al., 2010). Genotyping refers to the description of an individual's genetic information (Kockum, Huang,

& Stridh, 2023). Genetic markers based on single nucleotide polymorphisms (SNPs), microsatellites, insertions, deletions, copy number variations and restriction fragment length polymorphisms can be used to characterize diversity between and within species (Krueger, Fischer, & Morales-Hojas, 2007; Morales-Hojas et al., 2001). Figure 3 gives an overview over different types of genetic variants.

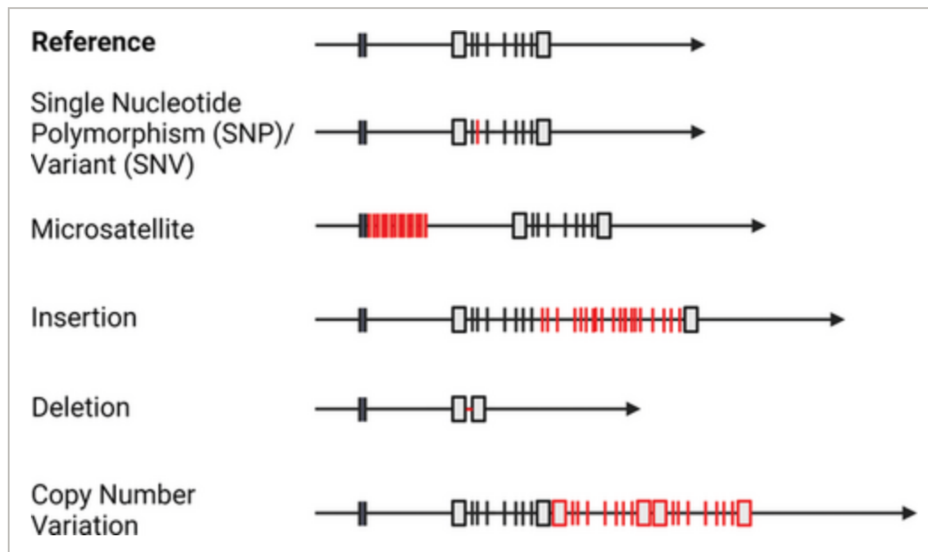

Figure 3: Overview of types of genetic variants after (Kockum et al., 2023). The red lines demonstrate variants diverging from the given sequence in black.

Several marker regions are used for the genotyping of *Onchocercidae.* For the most part, mitochondrial genes, which tend to be rather conserved among species and high copy numbers (Hedtke et al., 2019; Yilmaz et al., 2016), are used. The ITS1 region, a highly conserved nuclear region, is a frequently used marker for differentiating a wide range of species, among those onchocercid species in general and *Mansonella* spp. in particular. (Jiménez et al., 2011; Mulyani, 2022; Sandri et al., 2020).. Other, more diverse marker regions include the mitochondrial gene *cox1*, which is widely used for phylogenetic analysis in metazoans. While it contains some strongly conserved regions which can be used as primer regions for most metazoan species (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), it also shows regions with a high incidence of base substitutions which allows for discrimination of closely allied species or even subgroups within a species (Cox & Hebert, 2001; E. Ferri et al., 2009; Hebert, Cywinska, Ball, & deWaard, 2003; Wares & Cunningham, 2001). The ribosomal

mitochondrial gene *12S* rDNA and the nuclear gene *28S* rDNA, which both encode ribosomal RNA, are also under investigation for their potential as marker regions. While *12S* rDNA has more frequently been investigated than *28S* rDNA, both have previously been used for phylogenetic research on *Onchocercidae* and can thus be compared to and linked with previous research on *Mansonella* and other *Onchocercidae* (E. Ferri et al., 2009; E. Lefoulon et al., 2015; Mirzaei et al., 2018; systems). Other molecular approaches to onchocercid taxonomy include genes such as 5S rRNA*, rbp1, hsp70, myoHC* and *18S rDNA* (Binkienė, Chagas, Bernotienė, & Valkiūnas, 2021; E. Lefoulon et al., 2015; Mirzaei et al., 2018). For *Mansonella* spp., whole genomes have been published recently, facilitating the development of novel genotyping approaches (Rodi et al., 2023; Sinha A & Fombad FF, 2023). The shift from a microscopical to a more molecular approach for species designation, as demonstrated by the current phylogeny shown in Figure 1 based on multi-locus sequencing, underlining the significance of molecular methods in onchocercid phylogeny.

The applications of genotyping are very diverse. In *Onchocercidae*, genotyping has been used to improve diagnostics (Norice-Tra et al., 2017) and to identify strains from different origins or with varying pathogenicity (Meredith, Lando, Gbakima, Zimmerman, & Unnasch, 1991; Ogunrinade, Boakye, Merriweather, & Unnasch, 1999). It is now revealing to be crucial in the success of mass drug administration (MDA) (Patra, Ramu, Hoti, Pragasam, & Das, 2007) (Osei-Atweneboana, Boakye, Awadzi, Gyapong, & Prichard, 2012) to eliminate filarial species such as *W. bancrofti* and *O. volvulus* and potentially others (Hedtke et al., 2019). Investigating the diversity of parasites enables, among other, the distinction between persistent infections and reinfections. Genotyping could thus be a powerful tool within the context of *Mansonella* infections with their high transmission rates, difficult diagnostic and insufficiently investigated treatment (Ta-Tang et al., 2018).

1.5 Life cycle, vectors and transmission

All known *Mansonella* species infecting humans share a similar life cycle and vector. Figure 4 demonstrates the life cycle of *M. perstans*. Biting midges of the *Culicoides* species (*M. ozzardi* can additionally be transmitted via *Simulium* spp.) carrying filarial larvae transmit those into the human host through a bite (Higgs, Beaty, & Marquardt, 2005). The larvae mature into adult worms of 2-8 cm x 10-150 µm which live in body cavities (*M. perstans*) and subcutaneous tissues (*M. ozzardi, M. streptocerca*) (J. Kevin Baird, Neafie, Lanoie, & Connor, 1987; Downes & Jacobsen, 2010). Female adult worms produce unsheated microfilariae of 100-240 µm x 2.5-5 µm which circulate in the blood (*M. perstans, M. ozzardi)* or the skin (*M. streptocerca*) of the human host (Downes & Jacobsen, 2010). Vectors ingest microfilariae from an infected human host during a blood meal. The microfilariae migrate and mature within the insect until they can be injected in the next human host during the next blood meal (Black & Moore, 2005).

Mansonella sp. “DEUX” is characterized as a blood-dwelling, sympatric species to *M. perstans.* It might share similarities in the life cycle or vector species to *M. perstans.* even though none of these characteristics have been investigated so far in *Mansonella* sp. “DEUX”,

Mansonella perstans

Mansonella perstans

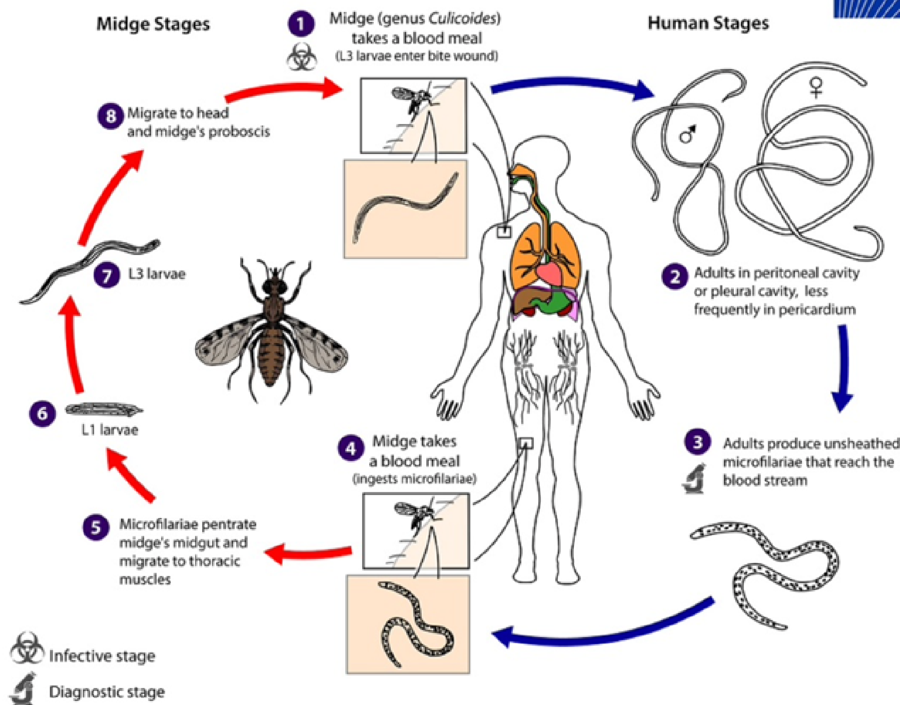

Figure 4: Life cycle of M. perstans from CDC, 04.08.2022 (CDC, 04.08.2022)

1.6 *Mansonella* diagnostics

Mansonella diagnostics is based on the detection of microfilariae in blood. To this day, no specific indirect detection methods for *Mansonella* spp. exist (Ta-Tang et al., 2018). Microfilariae of *M. perstans, Mansonella* sp. "DEUX" and *M. ozzardi* can be found in blood smears of venous or capillary blood (Mischlinger et al., 2021; Moraes, Shelley, Dias, & Silva, 1983). Unlike for other blood-dwelling parasites like *L. loa* or *W. bancrofti*, there seems to be no significant periodicity for the presence of *Mansonella* microfilariae in blood (Santa Maria Asio, Paul E Simonsen, & Ambrose W Onapa, 2009; Campillo et al., 2022; Manson, 1891).

Microscopy-based detection simultaneously enables the diagnostic of other co-endemic filariae and other blood-dwelling parasites such as *Plasmodium* species (Mourembou et al., 2015; Sandri et al., 2020; Tang et al., 2010) and is routinely used in endemic countries. Limited sensitivity of microscopy-based detection due

to the low parasitemia common in *Mansonella* infections might be a contributing factor to the presumed underreporting of *Mansonella* infections (Sandri et al., 2020). Furthermore, expertise is needed to distinguish *Mansonella* microfilariae from microfilariae of other co-endemic filarial species. Microfilariae from *M. streptocerca* can be found in skin snips from infected individuals and emerge when the snips are incubated in physiological saline solution (Mathison, Couturier, & Pritt, 2019). Differentiation of *M. streptocerca* microfilariae from those of other co-endemic tissue-dwelling filarial species, such as *O. volvulus*, can be performed via microscopy (Bamuhiiga, 1998; Noma et al., 2014). Thus, the high versatility of microscopy-based detection techniques contributes to their wide use in endemic countries.

With an increase in laboratory equipment and simplification of protocols, highly sensitive and specific molecular diagnostic based on polymerase chain reaction is becoming more prevalent especially in the context of epidemiological surveys. Currently available assays target primarily the ITS1-region (Jiménez et al., 2011; J. F. Medeiros et al., 2015; Pilotte et al., 2022; Sandri et al., 2020; Tang et al., 2010). However, access to this method is limited due to infrastructural demands. Other methods such as the development of DNA-detecting loop-mediated isothermal amplification filarial assays hold potential for *Mansonella* research in areas with limited resources (Alhassan et al., 2016; Poole et al., 2017).

1.7 Clinical presentation

Despite its broad global distribution and high prevalence, few reliable data exist about the clinical presentation of *Mansonella* infections. Mansonellosis is largely seen as an innocuous disease which is largely asymptomatic or only causes mild clinical symptoms (Anderson & May, 1991; Cook & Zumla, 2008; J. Medeiros, Crainey, Pessoa, Luz, & Marcondes, 2017). Studies have found infrequent and contradictory relationships between filarial infections and different symptoms (Ta-Tang et al., 2018) (J. K. Baird, Neafie, & Connor, 1988; Bartoloni et al., 1999). If symptoms could be associated with *Mansonella* infections, itching, joint pains, enlarged lymph nodes and abdominal symptoms were usually mentioned (Martins, Pessoa, de Medeiros, de Andrade, & Medeiros, 2010; Ta-Tang et al.,

2018). *M. ozzardi* has additionally been inconsistently associated with corneal lesions, but the impact of these lesions on visual acuity has not been investigated (Garrido & Campos, 2000; Vianna, Martins, Cohen, Cohen, & Belfort, 2012). Due to high co-endemicity with various other pathogens, those symptoms could also potentially be caused by other pathogens, which further complicates defining the clinical presentation of mansonellosis. Contrarily, there have been rare reports of *M. perstans* infections being associated with severe symptoms (Fux et al., 2006). Thus, the clinical significance of mansonellosis remains unclear.

The absence of severe symptoms strongly connected to mansonellosis does not mean it is an innocuous infection. It has been postulated that, like other filarial species, *Mansonella* species are able to influence other infections like malaria and increase their pathogenicity, and might also influence the effectivity of vaccines (Borkow & Bentwich, 2000, 2008; Dolo et al., 2012). Due to its high global prevalence, it is very likely that mansonellosis has a significant impact on global health apart from its direct and indirect morbidity. Further research into their direct and indirect pathogenic potential and burden of disease studies with control groups could give further insights.

1.8 Endosymbiosis with *Wolbachia* and its implications for treatment

The alpha-proteobacterium *Wolbachia* is a mutualistic bacterial endosymbiont commonly found in arthropods, members of the *Onchocercidae* family (E. Ferri et al., 2011; Hilgenboecker, Hammerstein, Schlattmann, Telschow, & Werren, 2008) and inconsistently reported in *M. perstans* (Gehringer et al., 2014; Grobusch, Kombila, Autenrieth, Mehlhorn, & Kremsner, 2003). Doxycycline treatment, which is active against *Wolbachia*, has shown to have an impact on development, embryogenesis, fertility, and viability of adult filarial worms harboring *Wolbachia*. Since it was found to suppress microfilarial levels in *M. perstans* infections (Batsa Debrah et al., 2019; Coulibaly et al., 2009; Gehringer et al., 2014; Keiser et al., 2008), it is likely that *Wolbachia* is indeed present in *M. perstans*. The inconsistent findings of *Wolbachia* in *M. perstans* can thus rather be attributed to different detection limits due to technical or methodical differences

of previously conducted studies than to the existence of different *M. perstans*-strains which carry or do not carry *Wolbachia* (Keiser et al., 2008).

M. perstans infections are regarded as the most difficult *Mansonella* infection to treat (Asgeirsson, Harling, & Botero-Kleiven, 2017; Ta-Tang et al., 2018) and no international guidelines on mansonellosis treatment are currently available (Ferreira, Crainey, & Gobbi, 2023). While classical filarial treatment with ivermectin and albendazole alone has shown to be ineffective (S. M. Asio, P. E. Simonsen, & A. W. Onapa, 2009), other treatment options include a combination of diethylcarbamazine (DEC) and mebendazole (Bregani, Rovellini, Mbaïdoum, & Magnini, 2006) or a combination of DEC and ivermectin (Basano Sde et al., 2014; Fischer, Bamuhiiga, & Büttner, 1997; Fischer, Tukesiga, & Büttner, 1999). However, the use of the latter is limited by its severe adverse reactions in individuals which are co-infected with the frequently co-endemic *L. loa* (Wanji et al., 2017). Further research into the relationship of *Mansonella* spp. and *Wolbachia* and the subsequent implications for the use of doxycycline could have significant implications for a safe and effective treatment of these infections.

1.9 Aim of this thesis

This thesis aimed to collect further evidence whether or not *Mansonella* sp. “DEUX” is a distinct species from *M. perstans*.

As previously described, species differentiation based on traditional methods is complicated within *Mansonella* species due to many shared characteristics. With the growing importance of DNA-based species classification in onchocercids, a molecular point of view for species differentiation was approached in this thesis.

So far, the genetic differences between these species have been investigated based on the ITS1 region (Mourembou et al., 2015; Sandri et al., 2020) as well as in two whole genomes of *Mansonella* sp. “DEUX” and one whole genome of *M. perstans (Rodi et al., 2023)*. As opposed to previously used markers such as the ITS1 region, which are highly conserved between species, the identification of polymorphic markers will allow for better investigation of the different species through the genotyping of distinct parasites from the same *Mansonella* species. In this thesis, three marker regions were chosen: *12S* rDNA, *cox 1* and *28S* rDNA.

The primary objective of this study was to determine single nucleotide polymorphisms (SNPs) between *M. perstans* and *Mansonella* sp. “DEUX” in these 3 marker regions and to investigate the potential of these regions to be used for species differentiation.

Secondary objectives within the frame of this thesis were as follows:

2. To investigate intraspecific differences in these 3 regions and to evaluate their potential for genotyping
3. To compare the sequences analyzed in this thesis with published sequences from *M. perstans* and *Mansonella* sp. “DEUX” previously deposited in the gene bank, and to determine possible differences based on country of origin and host species
4. To determine the phylogenetic relationship of blood-dwelling *Mansonella* species in Gabon based on these three marker regions

To reach this aim, mono-infected *M. perstans* and *Mansonella* sp. “DEUX” individuals from Lambaréné and surrounding villages in Gabon were sampled and filarial DNA was extracted. Additional samples from other studies were included as well. Then, a PCR for each of the three genes was established to allow for amplification and subsequent sequencing of the genes. The sequenced gene regions were then subject to investigation as in accordance with the aims mentioned above.

2 Materials

2.1 Chemicals

Name	Manufacturer	Article number
DNA away	Roth	X996.1
nuclease free water	QIAGEN	129114
taq dNTP mix (10 mM)	Qiagen	201900
kapa dNTP mix (10 mM)	ThermoFisher Scientific Inc.	R0191
Bovine serum albumin (20 mg/ml)	Sigma Aldrich	B8667-5ML
Primer	Integrated DNA Technologies	NA
ExoSAP-ITTM *Express* PCR Product Cleanup Reagent	ThermoFisher Scientific Inc.	75001.1.ML
Sephadex G50 Fine Grade	SigmaAldrich	GE17057302
SeaKem ® Agarose	Biozym	50004
SYBR ® Green I Nucleic Acid Stain	Lonza Bioscience	50513
Loading buffer	in-house recipe	NA
100 bp DNA ladder	NEB	N3231
QX alignment marker 15bp/3kb	QIAGEN	929522
QX alignment marker 15bp/5kb	QIAGEN	929524
QX DNA size marker 50-800bp	QIAGEN	929561
QX DNA size marker 250-2.5kb	QIAGEN	929559
S-Monovette K3 EDTA	Sarstedt	02.1066.001
RNA later	ThermoFisher Scientific	AM7021
Safety-Multifly ® 21G	Sarstedt	85.1638.235
Giemsa's azur-eosin methylene blue solution	Merck	109203
Objektträger	R. Langenbrinck GmbH	03 0004

2.2 Kits

Name	Manufacturer	Article number
GFX TM PCR DNA and Gel Band Purification Kit	GE Healthcare	16834032
BigDye™ Terminator v1.1 Cycle Sequencing Kit	Applied Biosystems™	4337449
illustra GFX MicroSpin	Cytiva	314143-10
DNeasy ® QIAgen blood & tissue kit (250)	QIAgen	157051919
QIAmp ® Blood Mini Kit	QIAgen	51104
KAPA HiFi Hotstart plus dNTPs	Roche	KK1508
SuperScript III RT Platinum taq DNA polymerase kit	ThermoFisher	12574026
taq DNA polymerase kit including $MgCl_2$	Qiagen	201203
QIAxcel DNA Screening Kit	Qiagen	929004

2.3 Laboratory equipment

Name	Manufacturer
Mastercycler ® nexus gradient	Eppendorf
Eppendorf Mastercycler EP Gradient S	Eppendorf
QIAxcel Advanced System	Qiagen

Einkanal-Mikroliterpipette Transferpette®	Brand
Microscope model Eclipse E200	Nikon

2.4 Software

Name	**Application**	**Source**
QIAxcel screen gel software version 1.6.0	PCR analysis	Qiagen
geneious prime version 2022.1.0.	Sequence analysis	Dotmatics
PhyML 3.3.20280621	Phylogenetic tree building	(Guindon et al., 2010)
MrBayes 3.2.6	Phylogenetic tree building	(Huelsenbeck & Ronquist, 2001)
Species Delimitation Plugin	Phylogenetic calculations	(Masters, Fan, & Ross, 2011)
Blast	Primer design	NCBI
ModelFinder (IQtree)	Phylogenetic analysis	(Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017)
IDT oligo analyzer	Primer design	IDT DNA

3 Methods

3.1 Study area and acquisition of *Mansonella* DNA

A total of 84 samples, consisting of 22 *M. perstans* – mono-infections and 62 *Mansonella* sp. “DEUX” – mono-infections were included. 83 samples were derived from studies conducted in Gabon and one sample was collected in Togo. Their status as mono-infection had been assessed by a *Mansonella* species specific qPCR according to a previously published assay (Sandri et al., 2020). Six of those samples were collected within the frame of this thesis (= study cohort A), and 78 samples were from previously conducted studies (= study cohort B). An overview is given in Table 1.

Study cohort	*M. perstans* mono-infections	*Mansonella* sp. “DEUX” mono-infections	Reference	Ethics approval number(s)
Study cohort A	2	4	unpublished data	CEI-001/2022
Study cohort B	20	*58*	(Sandri et al., 2020) and unpublished data	CEI-007/2014 CEI-020/2018 #015/2012/CBRS
Total	22	62		

Table 1: Overview of samples analyzed in this study, reference sources and ethic approval

3.1.1 Study cohort A

Within the frame of this thesis, blood samples from individuals mono-infected with either *M. perstans* or *Mansonella* sp. “DEUX” were collected. A total of 12 healthy participants aged 18 and older whose previously performed blood smear indicated a microscopical Mansonella species mono-infection were included. At the time of sampling, further information regarding basic demographic data as well as clinical symptoms were gathered (see Figure 27 the supplements). The samples from this study had been collected between May – October 2022 in rural areas of Gabon (Fougamou and its surrounding villages). DNA was extracted from 100 µl fresh whole blood obtained by venipuncture in an EDTA tube using the QIAmp blood mini kit. The DNA was stored at - 20 °C until further use. After assessment by qPCR, a total of 6 samples (2 *M. perstans* mono-infections and *4 Mansonella* sp. “DEUX” mono-infections) from this study were included in this thesis.

3.1.2 Study cohort B

Study cohort B consisted of a total of 76 samples.

64 samples (6 *M. perstans* – mono-infections and 58 *Mansonella* sp. *"*DEUX" – mono-infections) were collected within the frame of a cross-sectional study conducted in February/March 2016 in rural areas of Gabon (Fougamou and its surrounding villages). Participants were healthy and aged 1 – 96 years. 500 µl whole blood obtained by venipuncture in an EDTA tube were frozen mixed with 1,3 ml RNALater. The samples were stored at – 20 °C until the extraction of nucleic acid was performed according to the publication (Woldearegai et al., 2019).

12 additional *M. perstans* - mono-infections were collected between January 2019 and August 2021 around Lambaréné from healthy participants aged 1 – 78 years. 500 µl whole blood obtained by venipuncture in an EDTA tube were frozen mixed with 1,3 ml RNALater. Additionally, two *M. perstans* mono-infected samples from healthy individuals aged 18 years and older previously collected in Togo were included. For those 14 samples, DNA extraction was performed according to 3.1.3.

3.1.3 Additional DNA extraction

Additional DNA extraction was necessary for several samples from study cohort A stored in RNA later and was conducted as follows. To remove the RNA later, samples were centrifuged for 3 minutes at 252 g and the supernatant was removed. The sample was re-suspended in 50 µl 1x PBS. Then, the DNA extraction was performed according to the QIAmp ® Blood Mini Kit protocol with a deviation in step 9: instead of adding 100 µl buffer AE and centrifuging for 1 minute, 50 µl buffer were added, centrifuged for 30 seconds, then another 50 µl buffer were added and the sample was again centrifuged for 30 seconds.

DNA from *Mansonella spp*. naïve human whole blood was used as a negative control. Blood was collected in an EDTA tube. DNA extraction was performed according to the DNeasy QIAGEN blood & tissue kit protocol with a deviation in step 9: instead of adding 200 µl Buffer AE and then centrifuging for 1min, adding 100 µl buffer and centrifuging for 30 seconds was performed twice.

DNA from *O. volvulus* and *L. loa* was extracted as described in Sandri et al (Sandri et al., 2020) and used as positive controls.

3.2 PCR setup

In this thesis, the optimization of nested PCRs was chosen as a suitable method to sensitively and specifically amplify *Mansonella* DNA. In each run, two negative controls (nuclease-free H_2O and DNA extracted from non-infected human whole blood) to exclude cross-contaminations or unspecific primer binding to the human genome as well as two positive controls (DNA from *O. volvulus,* or DNA extracted from *L. loa* or *Mansonella spp*. infected human whole blood) to distinguish failure of the PCR from amplification failure in samples were included.

All PCR components and samples were stored at -20 °C. With exception of the polymerase, the components were thawed at 4 °C and kept on ice during pipetting work to prevent DNA degradation. Before use, the thawed DNA and all PCR ingredients apart from the polymerase were suspended by vortexing and subsequent centrifugation at around 1000 g for 10 seconds to ensure even substance distribution in the liquid. The polymerase was removed from its storage at -20 °C immediately before use, gently mixed and centrifuged at around 3000 rpm for 10 seconds before use. Amplicons were stored at 4 °C for up to one week or at -20 °C until further use.

3.3 Electrophoresis

1x TBE buffer was manufactured using 100 ml of 10x TBE buffer and 900ml distilled water. To create 10x TBE buffer, 108 g TRIS, 55,6 g boric acid and 7,44 g EDTA (Na^{2+}) were mixed with 1 l distilled water. 1.5 g agarose were added for each 100 ml TBE buffer to create 1.5 % agarose gels.

Sample and marker preparation was conducted on ice and used as described in Table 2.

A

Component	Volume
Loading buffer 5x	5 µl
PCR product	10 µl
SYBR green	2 µl

B

Component	Volume
Loading buffer 5x	2 µl
marker	4 µl
SYBR green	1 µl

Table 2 Sample (A) and marker preparation (B) for gel electrophoresis

For the electrophoresis, the gel was placed into the chamber and covered with 1x TBE buffer, before markers and samples were filled into the pockets and the electrophoresis was started. Gels were run for 90 minutes at 90 V.

3.4 Amplicon purification for sequencing

3.4.1 Amplicon purification via exoSAP

After successful PCR, amplicons were purified before sequencing using exoSAP clean up. 1 µl exoSAP was added to 6 µl amplicon in a 0,2 ml softstripe and both components were mixed thoroughly before the stripe was placed in a cycler and subjected to 15 minutes at 37 °C followed by 15 minutes at 80 °C as visualized in Table 3. The products were kept on ice until further processing.

A

Component	Volume
exoSAP	1 µl
amplicon	6 µl

B

Temperature	duration
37 °C	15 min
80 °C	15 min

Table 3: Reaction mix composition (A) and cycling conditions (B) for exoSAP

3.4.2 Amplicon purification via gel

Purification via gel was conducted with the GFX TM PCR DNA and Gel Band Purification Kit. Agarose bands containing the band of interest were processed according to the manufacturer's protocol. At least 300 µl capture buffer were added regardless of weight for better purification. The DNA was stored at -20 °C until further use.

3.5 Sequencing

For each sample, the target region was sequenced with the forward primer as well as the reverse primer. Commercial sequencing was performed by Eurofins Genomics. After sample purification with exoSAP, 5 µl of sample were added to 5 µl of reverse and forward primer in 5 µM concentration respectively. Sequencing primers were used according to table 4.

Gene	name	sequence	product size(bp)	CG content	Tm (°C)
12S rDNA	12SF/	GTTCCAGAATAATCGGCTA	450	42.1 %	52.4
	12SdegR	ATTGACGGATGRTTTGTACC		42.5 %	54.2
	Mansonella species Fwd	GTTCCAGAATAATCGGCTAT	with *12SdegR*: 505	40 %	52.2
cox1	COlintF	TGATTGGTGGTTTTGGTAA	650	36.8 %	50.2
	ColintR	ATAAGTACGAGTATCAATATC		28.6 %	50.1

28S rDNA	28s 3,407 IF	GCAAACAAGTACCGTGA	636	40 %	54.7
	F28SintdR1	TCTTYACTTTCATTAYGCTT		27.8 %	47

Table 4: Sequences and further characteristics of sequencing primers. Tm = melting temperature

3.6 Phylogenetic analysis

3.6.1 Reference sequences

Table 5 summarizes the reference sequences used in this thesis. For *12S* rDNA and *cox1*, separate reference sequences for *M. perstans* and *Mansonella* sp. “DEUX” were used. For *28S* rDNA, no reference sequence for *Mansonella* sp. “DEUX” was available and thus, the *M. perstans* sequence was used. A comprehensive list of sequences and outgroups as accessed in the GenBank on 20.04.2023 and utilized in this thesis can be found in Table 33 and Table 34 in the appendix.

Gene	Species	CDS position on reference genome	Accession number	Reference
12S rDNA	*M. perstans*	7.473 – 7.866	OQ633017.1	(Ta-Tang et al., 2018)
	Mansonella sp. “DEUX”	7.477 – 7.868	OQ633020.1	
cox1	*M. perstans*	2.509 – 3.127	OQ633017.1	
	Mansonella sp. “DEUX”	2.514 – 3.132	OQ633020.1	
28S rDNA	*M. perstans*	3.467 - 3990	MN432520	(J. Crainey et al., 2020)
	Mansonella sp. “DEUX”	NA	NA	NA

Table 5: Overview of utilized reference sequences. NA = not applicable CDS = coding sequence.

3.6.2 Phylogenetic analysis of sequences

Phylogenetic analysis was performed using geneious prime software version 2022.0.1. Sequences with a %HQ of >65% were included in analysis, and consensus sequences consisting of both forward and reverse sequence were built when possible. Sequences were mapped to the respective reference sequences and trimmed and edited according to base call quality. Sequences with a minimum length of 394 bp for *12S* rDNA (with exception of one *M. perstans*-sequence with 193 bp), 622 bp for *cox1* and 605 bp for *28S* rDNA after trimming were analyzed. Nucleotide divergences were considered real if they had a Q value >30, which equals a likelihood of 99.9% for a correct call, otherwise the nucleotide was considered as similar to the reference sequence. Sequences had to be excluded when they were significantly more similar to outgroup reference

sequences than to *Mansonella* reference sequences or due to an unclear infection status. The excluded sequences were aligned with outgroups to investigate potential matches.

Subsequently, the ModelFinder subcategory of IQtree was used to determine the ideal model for phylogenetic analysis. The models were chosen based on the corrected Akaike Information Criterion (cAIC). Subsequently, maximum likelihood trees were built using geneious prime with the geneious PhyML plugin with a bootstrap support of 1000 bootstraps replicates. To compare, Bayesian trees using the geneious Mr Bayes plugin, the MCMC method, a chain length of 1 100 000 and a burn-in length of 100 000 were built.

The calculation of intra- and interspecific differences as well as of the mean pairwise identity was performed using geneious prime software version 2022.0.1 considering the respective model and tree building method. For the calculation of the closest pairwise distance, the geneious species delimitations plugin as referenced in 2.4 was used. It was investigated whether the genotypes were specific to a host species or a country of origin by comparing these characteristics between the sequences of a genotype. Expected heterozygosity (H) was calculated as

$$H = \frac{N}{(N-1)} \left(1 - \sum_{i=1\rho i^2}^{k} \right)$$

where p corresponds to the ith of k genotypes and N to the number of positive samples.

Subsequently, the sequences generated in this thesis were compared with sequences previously deposited in the gene bank to investigate potential association of genotypes with a parasite host species or country of origin. For this purpose, a search for *M. perstans* sequences from the three investigated genes was conducted, as well as sequences classified as *M. perstans* in the GenBank but assumed to be *Mansonella* sp. “DEUX” by Gaillard et al (Gaillard et al., 2020). The GenBank was accessed to acquire these additional sequences as well as outgroups for the phylogenetic analysis on 20.04.2023.

Figure 5 gives an overview over the workflow of the phylogenetic analysis.

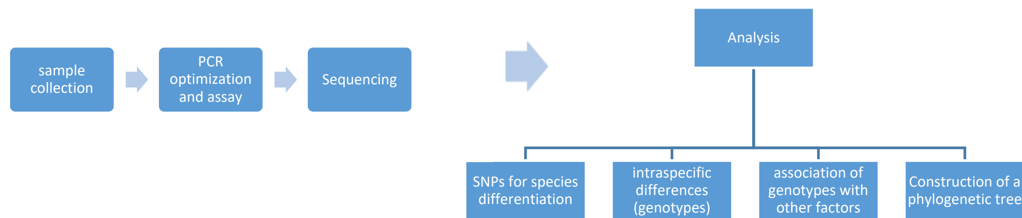

Figure 5: Overview over the workflow of the phylogenetic analysis from sample collection to analysis

3.7 PCR optimization

In order to allow for analysis of the sequences, the region of interest was first amplified by polymerase chain reaction (PCR). To increase product specificity and amplicon yield, a nested or semi-nested approach was chosen for all subsequent PCRs. To optimize a PCR, optimal sets of primers and their optimal annealing temperatures must be found to generate the desired amplicon. Moreover, cycling conditions and master mix composition can have an influence on PCR specificity and effectivity and need to be optimized as well. For the *12S* rDNA and *cox1* protocol, first optimization steps had been performed prior to this thesis.

3.7.1 Automated capillary gel electrophoresis

Amplicons were analyzed using automated capillary electrophoresis. To limit false positive results, a threshold of 5% was set in electropherogram view as recommended by the manufacturer.

A screening cartridge with a resolution of 20 - 50 bp and a detection limit of 0.1 ng/µl was used. The AM_420 method was used with a size marker concentration of 30 ng/µl. The size markers were diluted accordingly with water and the same PCR buffer concentration as in the respective nested PCR. Two different size markers were used according to the expected amplicon size. Table 6 gives an overview over the size markers and reference markers used in this thesis. Markers were stored at 4°C.

Gene	Size marker	Alignment marker
12S rDNA	50 - 800 bp	15 – 3000 bp
cox1	50 - 800 bp	15 – 3000 bp

28S rDNA	100 – 2500 bp	15 – 5000 bp

Table 6: QIAxcel size markers and alignment markers used for PCR optimization

3.7.2 Primer design

Primers were taken from previous publications or previous unpublished work as designated and otherwise designed using the geneious prime version 2022.1 primer design tool. Their specificity for Mansonella species was assessed with the NCBI BLAST tool against the NCBI data base. Further characteristics of the primer and their pairings including expected melting temperature mismatches and the potential for secondary structures were investigated using the oligo analyzer tool from IDT DNA technologies.

3.7.3 12S rDNA PCR optimization

3.7.3.1 Primer

Name	Primer sequence 5' -> 3'	Product size (bp)	CG content	Tm in °C	Source
12SdegF2/	ATTACYTATTYTTAGTTTA	600	15.8%	41.6	(E. Lefoulon et al., 2015)
12SnemR2	CTACCATACTACAACTTACGC		42.9%	55.9	
Pan filaria Fwd	ACTGGTAGTTTTTTGATTGTTTTA	with 12SnemR2: 566	25%	52.2	in-house
12SF/	GTTCCAGAATAATCGGCTA	450	42.1%	52.4	(Casiraghi, Anderson, Bandi, Bazzocchi, & Genchi, 2001)
12SdegR	ATTGACGGATGRTTTGTACC		42.5%	54.2	
Mansonella spp. Fwd	GTTCCAGAATAATCGGCTAT	with 12SdegR: 505	40%	52.2	in-house
12S_Mdeux_ perst_Rev	TTATAATAGTAATACATGATTAATA	with Mansonella spp. Fwd: 395	16%	49.3	
12S_Mperstans _Fwd	TAACTCTAATTGTTGTAATGTTTTC	328	28%	54.3	
12S_Mperstans _Rev	GCCAAATATATATCTGTTTTTAAT		25%	51.2	
12S_ Mperstans _Fwd2	ATTGTTTTACTAGTGTTCCAGAATA	with 12S_ Mperstans _Rev: 375	28%	54.5	
12S_Mdeux_ Fwd	ATTGTTTTATTAGTGTCCCAGAATA	375	28%	54.5	
12S_Mdeux_Rev	GCCAAGTATATATCTGTTTTAAAT		25%	51.2	

Table 7: 12S rDNA primer sequences and further characteristics. Tm = melting temperature. Bp = base pairs

Table 7 summarizes the primers used during the process of *12S* rDNA optimization. Figure 6 visualizes their placement on the reference sequence.

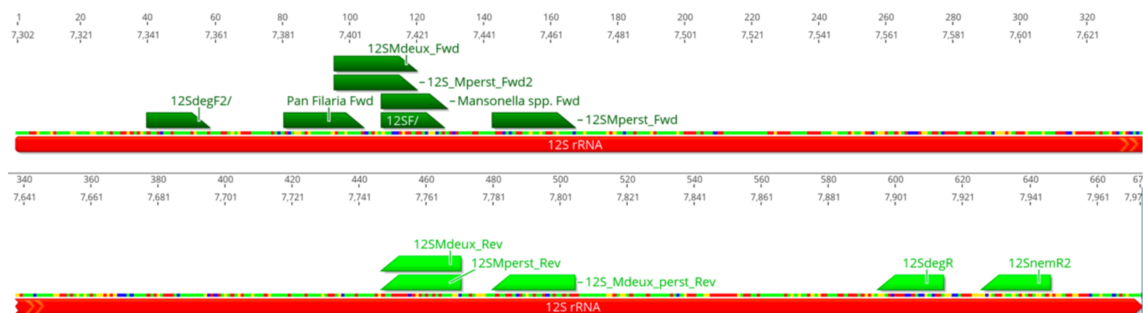

Figure 6: 12S rDNA primer positions on the M. perstans *reference sequence OQ633017 visualized with geneious prime version 2022.1.0*

3.7.3.2 Process

First, a previously established protocol was tested. The PCR products were purified through both gel electrophoresis (200 ml of a 1.5% agarose gel) with subsequent purification as well as exoSAP to compare both methods and sequenced with 12SF/ and 12SnemR2 as sequencing primers.

Subsequently, the PCR was optimized as visualized in figure 7.

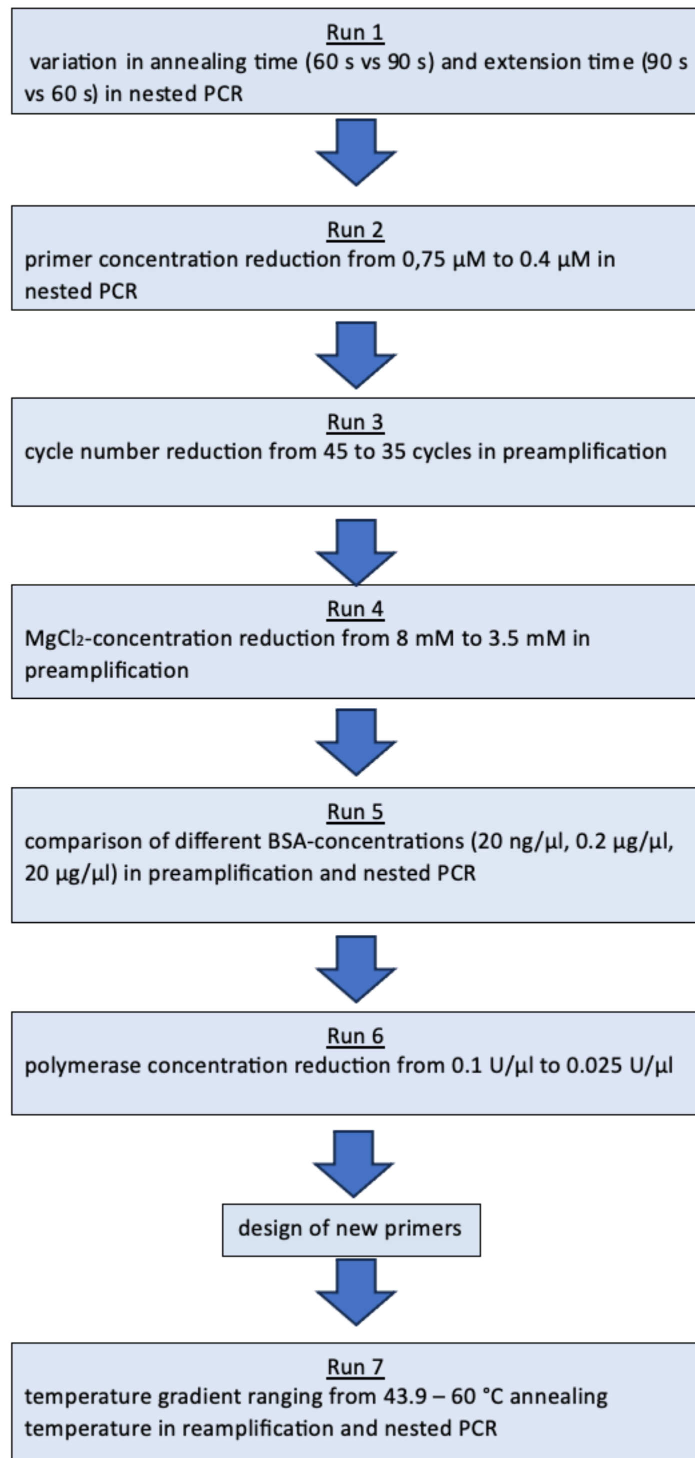

Figure 7: Optimization process of the 12S *rDNA PCR*

3.7.3.3 Final *12S* rDNA protocol

The final protocols for preamplification and nested PCR are visualized in Table 8 and Table 9.

<u>Preamplification</u>

A

Master Mix		
Component	final concentration	Volume for 15 µl
H_2O		5.475 µl
BSA [2 µg/µl]	0.5 µg/µl	3.75 µl
$MgCl_2$ [25 mM]	3.5 mM	1.2 µl
10x QIAGEN PCR buffer	1x	1.5 µl
Pan Filaria Fwd primer [10 µM]	0.4 µM	0.6 µl
12SnemR2 primer [10 µM]	0.4 µM	0.6 µl
dNTP mix [10 mM]	0.2 mM	0.3 µl
taq DNA polymerase	0.025 U/µl	0.075 µl
template		1.5 µl

B

Cycling conditions			
Step	Temperature in °C	Duration	Cycles
Initial denaturation	94	3 min	1x
Denaturation	95	30 sec	35x
Annealing	45.2	45 sec	
Extension	72	90 sec	
Final extension	72	10 min	1x

Table 8: 12S *rDNA preamplification final reaction mix composition (A) and cycling conditions (B)*

<u>Nested PCR</u>

A

Master Mix		
Component	final concentration	Volume for 15 µl
H_2O		6.675 µl
BSA [2 µg/µl]	0.5 µg/µl	3.75 µl
10x QIAGEN PCR buffer	1x	1.5 µl
12S Mansonella spp. Fwd primer [10 µM]	0.4 µM	0.6 µl
12SdegR primer [10 µM]	0.4 µM	0.6 µl
dNTP mix [10 mM]	0.2 mM	0.3 µl
taq DNA polymerase	0.025 U/µl	0.075 µl
template		1.5 µl

B

Cycling conditions			
Step	Temperature in °C	Duration	Cycles
Initial denaturation	94	3 min	1x
Denaturation	95	30 sec	40x
Annealing	63	30 sec	
Extension	72	60 sec	
Final extension	72	10 min	1x

Table 9: 12S *rDNA nested PCR final reaction mix composition (A) and cycling conditions (B)*

3.7.4 cox1 *PCR optimization*

3.7.4.1 Primer

Name	Primer sequence 5' -> 3'	Product size (bp)	GC-content	Tm in °C	Source
FCo1extdF1	TATAATTCTGTTYTDACTA	970	20.2%	43.4	(E. Lefoulon et al., 2015)
FCo1extdR1	ATGAAAATGAGCYACWACATAA		29.5%	51.9	
COlintF	TGATTGGTGGTTTTGGTAA	650	36.8%	50.2	(Casiraghi et al., 2001)
COlintR	ATAAGTACGAGTATCAATATC		28.6%	50.1	

Table 10: cox1 *primer sequences and further characteristics. Tm = melting temperature. Bp = Base pairs*

The primers used for the optimization process of the *cox1* gene are summarized in Table 10. Figure 8 visualizes their placement on the reference sequence.

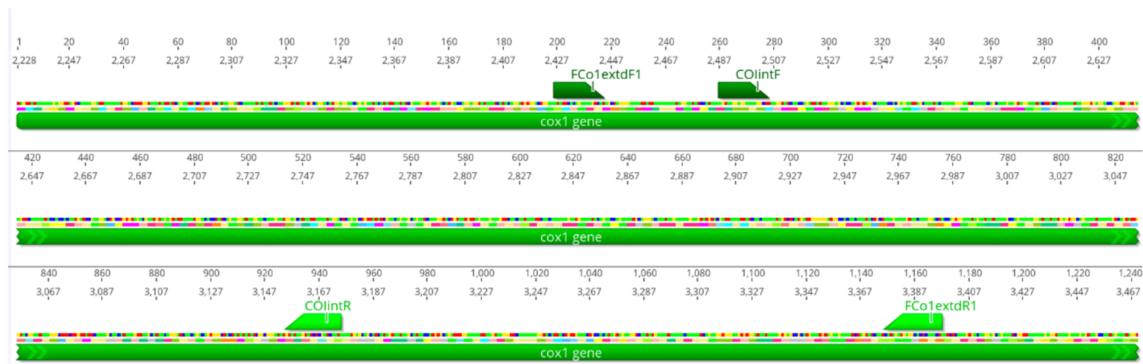

Figure 8: cox1 *primer positions on* M. perstans *reference sequence OQ633017 visualized with geneious prime version 2022.1.0*

3.7.4.2 Process

A previously established protocol was tested and subsequently optimized. The optimization process is visualized in Figure 9.

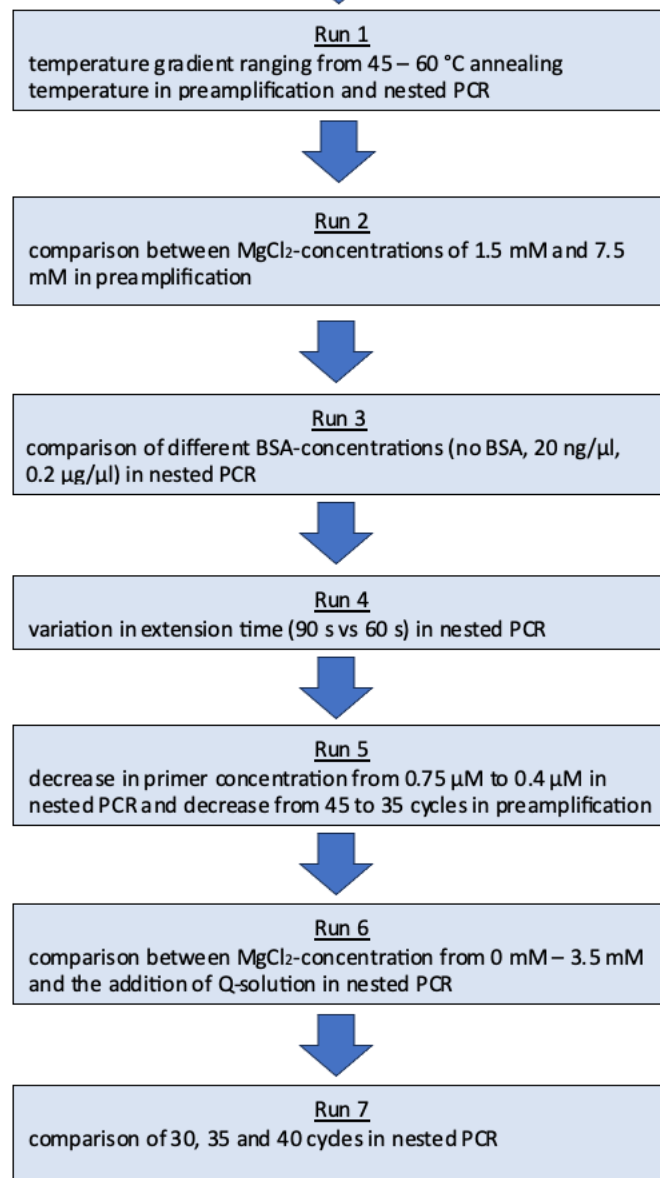

Figure 9: Optimization process of the cox1 *PCR*

3.7.4.3 Final *cox1* protocol

Table 11 and Table 12 summarize the final protocol for the *cox1* amplification.

<u>Preamplification</u>

A

Master Mix		
component	final concentration	Volume for 15 µl
H_2O		7.125 µl
10x QIAGEN PCR buffer	1x	2.5 µl
FCo1extdF1 primer [10 µM]	0.4µM	1 µl
FCo1extdR1 primer [10 µM]	0.4µM	1 µl
dNTP mix [10 mM]	0.2mM	0.3 µl
$MgCl_2$ [25 mM]	1.5mM	0 µl
taq DNA polymerase	0.025 U/µl	0.075 µl
template		3 µl

B

Cycling conditions			
Step	Temperature in °C	Duration	Cycles
Initial denaturation	94	3 min	1x
Denaturation	95	30 sec	45x
Annealing	50	30 sec	
Extension	72	60 sec	
Final extension	72	10 min	1x

Table 11: cox1 *preamplification final reaction mix composition (A) and cycling conditions (B)*

<u>Nested PCR</u>

A

Master Mix		
component	final concentration	Volume for 15µl
H_2O		6.825 µl
$MgCl_2$ 25 mM	1 mM	0.6 µl
BSA (0.2 µg/µl)	20 ng/µl	1.5 µl
10x QIAGEN PCR buffer	1x	1.5 µl
10 µM *COlintF* primer	0.4 µM	0.6 µl
10 µM *COlintR* primer	0.4 µM	0.6 µl
dNTP mix (10 mM)	0.2 mM	0.3 µl
taq DNA polymerase	0.025 U/µl	0.075 µl
template		3 µl

B

Cycling conditions			
Step	Temperature in °C	Duration	Cycles
Initial denaturation	94	3 min	1x
Denaturation	95	30 sec	30x
Annealing	52	30 sec	
Extension	72	60 sec	
Final extension	72	10 min	1x

Table 12: cox1 *preamplification final reaction mix composition (A) and cycling conditions (B)*

3.7.5 28S rDNA optimization

3.7.5.1 Primer

Name	**Primer sequence 5' -> 3'**	**Product size (bp)**	**CG content**	**Tm in °C**	**Source**
F28SF1	CCTCAACTCAGTCGTGATTACC	1150	50%	55	(E. Lefoulon et al., 2015)
F28SR2	CTCTGGCTTCATCCTGCCTCA		57.1%	56.9	
28S_3.139 OFwd	TTAGTAACGGCGAGTGAAAA	with F28SR2: 1093	40%	51.7	in-house
F28SintdR1	TCTTYACTTTCATTAYGCTT	with F28SF1: 970	30%	47	(E. Lefoulon et al., 2015)
28S_3,319IF	AAGTTATTCCTTGGAGTCGG	with F28SintdR1: 722	45%	51.9	in-house
28S_3,398IF	ATGAGACCGATAGCAAACAA	with F28SintdR1: 646	40%	51.6	
28S_3,407IF	GCAAACAAGTACCGTGA	with F28SintdR1: 635	40%	49.8	

Table 13: 28SrDNA primer sequences and further characteristics. Tm = melting temperature. Bp = Base pairs

Table 13 summarizes the primers used during the optimization process. Figure 10 visualizes their placement on the *28S* rDNA gene on the reference sequence.

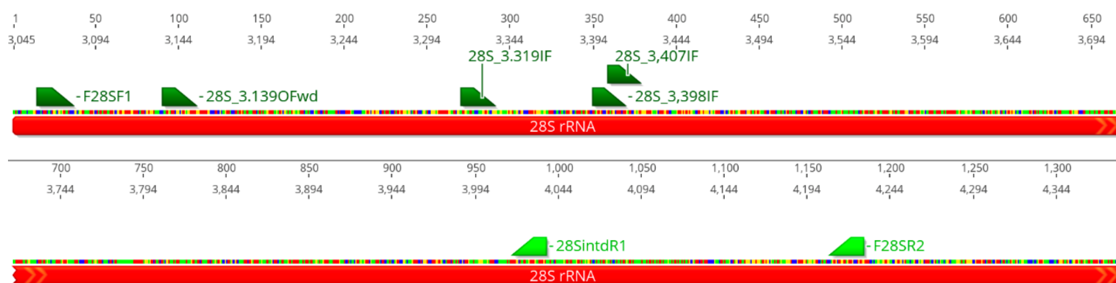

Figure 10: 28S rDNA primer positions on the gene on the M. perstans *reference sequence MN432520 visualized with geneious prime version 2022.1.0*

3.7.5.2 Process

The optimization of the *28S* rDNA PCR was based on a protocol published by Lefoulon, E. et al (E. Lefoulon et al., 2015). The PCR optimization process is visualized in Figure 11.

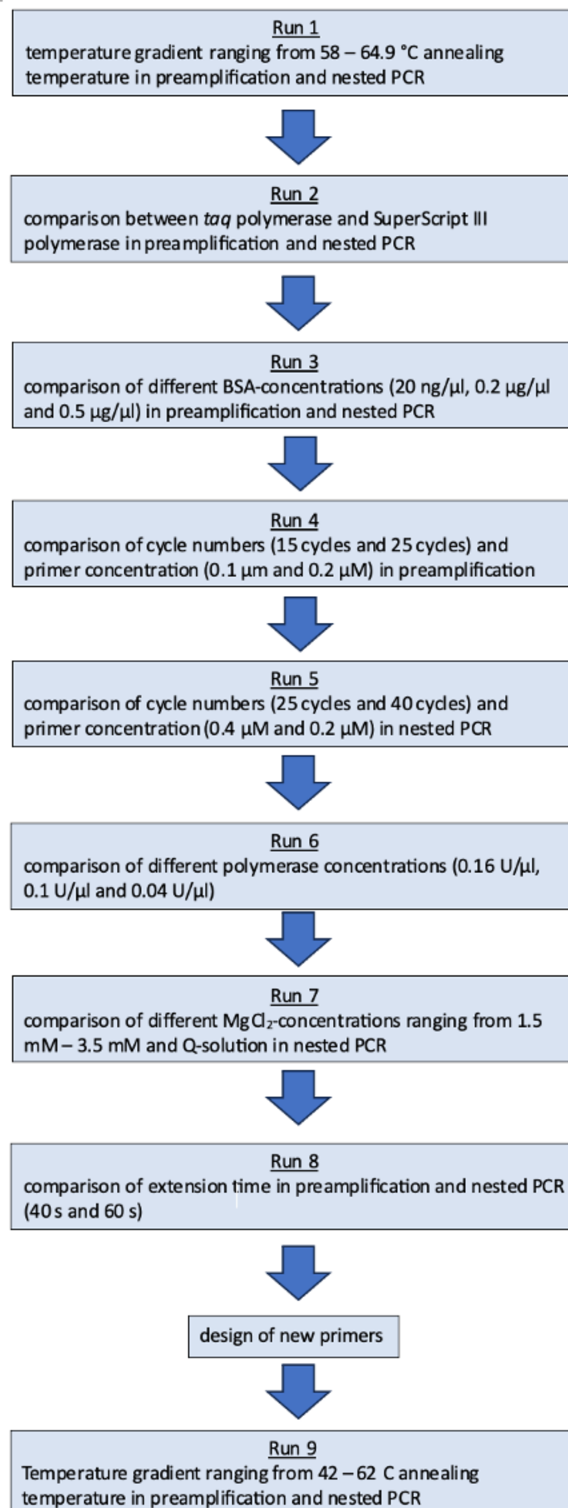

Figure 11: Optimization process of the 28S *rDNA PCR*

3.6.3.4 Final protocol

The final protocols for the *28S* rDNA PCR are visualized in Table 14 and Table 15.

Preamplification

A

Master Mix		
component	final concentration	Volume for 15 µl
2x Reaction mix	1x	7.5 µl
H_2O		5.1 µl
28s 139.2F primer [10 µM]	0.1 µM	0.15 µl
F28SR2 primer [10 µM]	0.1 µM	0.15 µl
Super Script III RT Platinum Taq Mix	2 U	0.6 µl
template DNA		1.5 µl

B

Cycling conditions			
Step	Temperature in °C	Duration	Cycles
reverse transcriptase	55	30 min	1x
initial denaturation	94	2 min	1x
denaturation	94	15s	40x
annealing	53.4	30s	
extension	68	1 min	
final extension	68	5 min	1x

Table 14: 28S *rDNA preamplification final protocol reaction mix composition (A) and cycling conditions (B)*

Nested

A

Master Mix		
component	final concentration	Volume for 15 µl
H_2O		10.425 µl
10x QIAGEN PCR buffer	1x	1.5 µl
28s 407F primer [10 µM]	0,4 µM	0.6 µl
F28SintdR1primer [10 µM]	0,4 µM	0.6 µl
dNTP mix [10 mM]	0,2 mM	0.3 µl
taq DNA polymerase	0.025 U/µl	0.075 µl
template DNA		1.5 µl

B

Cycling conditions			
Step	Temperature in °C	Duration	Cycles
initial denaturation	94	3 min	1x
denaturation	95	30 s	40x
annealing	41.9	30s	
extension	72	1 min	
final extension	72	10 min	1x

Table 15: 28S *rDNA nested PCR final protocol reaction mix composition (A) and cycling conditions (B)*

4 Results

To analyze the chosen marker regions in *M. perstans* and *Mansonella* sp. “DEUX”, PCR assays were developed to amplify and further sequence the regions of interest: *12S* rDNA, *cox1* and *28S* rDNA. Results of the steps undertaken for development and optimization of these assays are summarized in the following chapter. Subsequently, the regions were compared between both species. Moreover, different specimen of the same species were compared to analyze their potential as genotyping markers, additional sequences deposited in the gene bank were investigated to determine possible differences based on country of origin and host species, and phylogenetic trees were constructed with those sequences to visualize the phylogenetic relationship of blood-dwelling *Mansonella* species in Gabon based on these three marker regions.

4.1 Sample collection of study cohort A and clinical presentation

Based on thick blood smear screening, 12 participants (five females, seven males) aged 18 – 83 years living in rural areas diagnosed with microscopic *Mansonella* mono-infections were sampled. Out of those, two participants were mono-infected with *M. perstans* and four were mono-infected with *Mansonella* sp. “DEUX” at the time of sampling as determined with an ITS1 qPCR. No filarial DNA could be found in two participants and four participants were either infected with *L. loa* or coinfected with more than one filarial species. Further characterization of symptom distribution within study cohort A can be found in Figure 28 in the appendix.

4.2 PCR optimizations

The goal of the PCR optimizations was to amplify the target gene region and generate a single amplicon with a high enough concentration to allow for sequencing. Changes of conditions in each run were evaluated regarding their effectiveness towards this goal and subsequently adapted or rejected.

4.2.1 12S rDNA

Based on the primers by Casiraghi et al. as described in Table 7, the expected amplicon size for the *12S* rDNA gene was 600 bp for preamplification and 450 bp in the nested PCR. First, a protocol based on previous work was tested, which

showed additional bands in the positive controls. Sequencing the amplicons from this PCR proved futile regardless of purification method. Subsequently, the PCR protocol was optimized. As a first step, extension and annealing time in nested PCR were varied and the primer concentration was reduced. The combination of these changes led to the elimination of one additional band in the positive controls. Decreasing the primer concentration in nested PCR, the cycle number in preamplification and the $MgCl_2$-concentration in preamplification reduced unspecific binding. However, the amplicon could not be sequenced. Thus, a BSA-concentration of 2 µg/µl BSA in preamplification and nested PCR was used to increase the amount of target amplicon but it did not eliminate the unspecific bands. The polymerase concentration was reduced to conform with manufacturer's instructions. Since no modification succeeded in creating one single amplicon, different primers were designed and evaluated. Since especially the primer pair previously chosen for preamplification had a considerable difference in melting temperature of approximately 14 °C (see Table 7), special importance was given to create primer pairs with similar melting temperatures. Different sets of forward and reverse primers were combined and compared to each other. Using the primers Pan Filaria Fwd and 12SnemR2, an annealing temperature of 45.2 °C in preamplification resulted in a clean negative control and a band with approximately expected amplicon size of 600 bp in the positive control. In the nested PCR, the combination of Mansonella species Fwd primer and 12SdegR was chosen as it led to the longest amplicon with a size of 505 bp. The band with the expected amplicon size had the highest and very little unspecific binding at 63 °C annealing temperature. Figure 12 demonstrates the QIAxcel screen gel image of the final protocol.

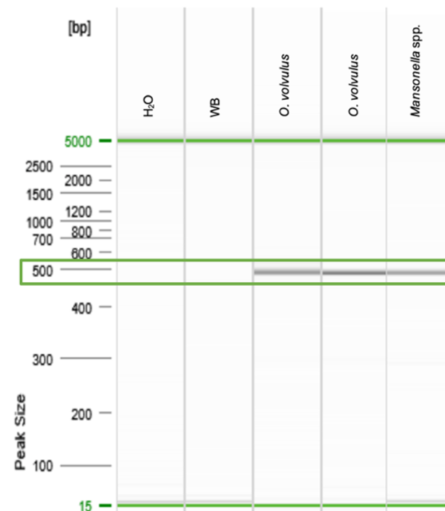

Figure 12: 12S *rDNA screen gel final protocol. H_2O and WB = negative controls;* O. volvulus + Mansonella *spp. = positive controls. Green box: expected amplicon size of approximately 450 bp*

4.2.2 cox1

The expected amplicon sizes for the *cox1* were 970 bp for preamplification and 650 bp for the nested amplicon as listed in Table 10. Upon testing the previously optimized protocol, multiple bands around the expected amplicon size appeared in the gel electrophoresis and the protocol was thus optimized. At first, a temperature gradient ranging from 45 – 60 °C was run to determine the optimal annealing temperature in preamplification and nested PCR. An annealing temperature of 50 °C in preamplification and 52 °C in nested PCR led to the highest amplicon concentration around the expected amplicon size and the least unspecific binding. The evaluation of different $MgCl_2$ concentrations of 1.5 mM and 7.5 mM in preamplification showed no difference in unspecific binding or amplicon concentrations. A comparison of different BSA-concentrations in nested PCR revealed a slight improvement in PCR specificity when a final BSA concentration of 20 ng/ µl was used. Neither increasing the extension time in nested PCR to 90 seconds nor reducing the cycle number in preamplification and the primer concentration in nested PCR to 0.4 µM reduced the unspecific bands. However, a combination of the original 45 cycles number in preamplification in combination with a primer reduction in nested PCR resulted in a single clear peak at 650 bp in all *Mansonella* controls. The evaluation of the effect of Q-solution and different $MgCl_2$-concentrations between 1.5 mM and 3.5 mM on nested PCR revealed that a final $MgCl_2$-concentration of 2.5 mM increased amplicon

concentration without causing additional bands to sufficient levels even in samples diluted to 1:10. The optimal cycle number in nested PCR was set to 30, as this number led to an increase in target amplicon concentration of approximately 650 bp without additional bands in the positive controls. The gel image of the final protocol is visualized in Figure 13.

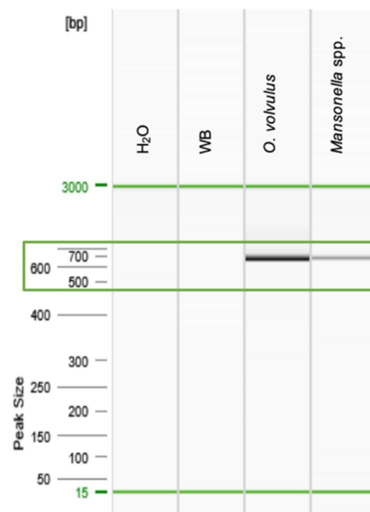

Figure 13 Cox1 *screen gel final protocol.* H_2O *and WB = negative controls;* O. volvulus *and* Mansonella *spp. = positive controls. Green box: expected amplicon size of approximately 650 bp*

4.2.3 28S rDNA

The first version of the *28S* rDNA protocol was based on a publication by Lefoulon, E. et al (E. Lefoulon et al., 2015). As listed in Table 13, the expected amplicon sizes for this PCR were 1150 bp for the preamplification and 970 bp for nested PCR.

First, the optimal annealing temperature for the preamplification was determined at 64.9 °C, as this resulted in the highest concentration of the expected amplicon in the *O. volvulus* positive control. The *Mansonella* positive controls remained negative in all temperatures. Using *SuperScript III RT* in preamplification and *taq* polymerase at 58 °C annealing temperature in nested PCR produced the highest concentration of target amplicon in the positive control. Bands with a size around 500 and 600 bp could be found in low concentrations regardless of annealing temperature in WB and all positive controls. The most pronounced increase in target band concentration occurred with a final BSA-concentration of 0.5 ug/µl in

both preamplification and nested PCR. The variation and combination of different primer concentrations and cycle numbers in preamplification revealed no decrease in unspecific binding with cycle reduction, however a reduction of the target amplicon concentration could be observed. Cycle number reduction did not improve unspecific binding, but a reduction of the primer concentration to 0.4 µM did. The decrease in *taq* polymerase concentration in nested PCR to correspond with manufacturer's instructions showed no impact on the PCR. Adding Q-solution led to no bands in any of the positive controls, however the target amplicon concentration increased with the $MgCl_2$-concentration and a final $MgCl_2$-concentration of 3.5 mM was determined. Comparing and combining different extension times in preamplification and nested PCR showed no improvement of unspecific binding.

Since all previous methods proved futile in eliminating unspecific binding, different primers were designed to replace the forward primers and to use two separate primer sets. The annealing temperature gradient performed in preamplification showed no amplicon in any samples, so the amplicon processed at 53.4 °C was evaluated as template for nested PCR. In nested PCR, three different forward primers in combination with F28SintdR1 were tested on an annealing temperature gradient. QIAxcel screening gel results of this PCR are visualized in Figure 14. Using combination 1 (with 28S_3,319IF) resulted in a thin band around the expected amplicon size of 722 bp. Samples processed with combination 3 (with 28S_3.398IF) remained negative regardless of annealing temperature. The negative controls in combination 2 (with 28S_3.407IF) were positive around the expected amplicon size of 635 bp, indicating a contamination.

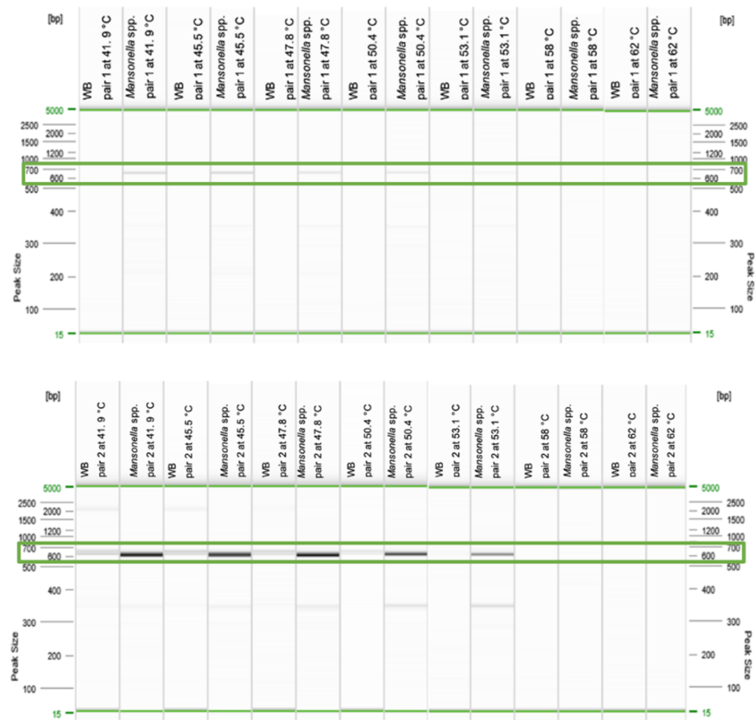

Figure 14: 28S *rDNA primer combinations 1 and 2 in nested PCR. WB = negative control;* Mansonella *spp. = positive control. Green boxes: expected amplicon sizes of 722 bp (pair 1) and 635 bp (pair 2). Pair 1 demonstrates a weak band around the expected amplicon size in the positive controls. Pair 2 demonstrates a strong band around the expected amplicon size in the positive controls and a weak one in the negative controls. The bands disappear under higher temperatures.*

Repetition of combination 2 with a nested annealing temperature of 41.9°C resulted in a highly concentrated single band around the expected amplicon size in the *Mansonella* spp. positive control as visualized in Figure 15. In this PCR,

Mansonella spp. and *L. Loa* positive controls could be amplified but not the previously used *O. volvulus* positive control.

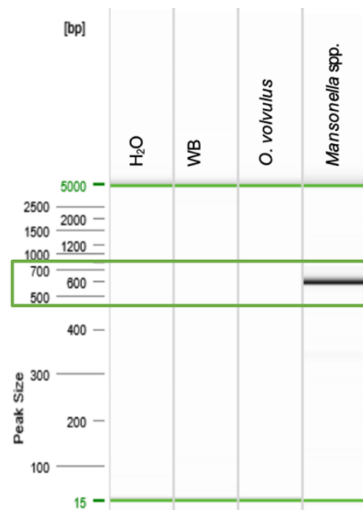

Figure 15: 28S *rDNA screen gel final protocol.* H_2O *and WB = negative controls*; O. volvulus *and* Mansonella *spp. = positive controls. Green box: expected amplicon size of approximately 635 bp*

4.2.4 PCR amplification and sequencing

After thorough adaption of PCR conditions, optimized PCR protocols could be applied and used for amplification and sequencing. A total of 84 samples were analyzed for *cox1* and *28S* rDNA, while only 64 samples could be analyzed for *12S* rDNA due to persisting unspecific bands in the negative controls. In case of amplification failure, samples were re-run up to two times. In-house sequencing failed in generating high quality sequences for any of the three genes, hence a commercial sequencing service was employed. While PCR positivity was overall >80%, significant differences regarding the percentages of retrieved sequences could be observed for the different genes. For *12S* rDNA, 21 of 64 sequences could be successfully sequenced, while 70 and 72 sequences respectively could be retrieved from 84 samples for *cox1* and *28S* rDNA. Table 16 gives an overview of the amplification results.

Gene	**Total samples**	**Amplification**			**Retrieved sequences**			**% retrieved sequences in total**
		Amplified samples	**Amplification failures**	**PCR positivity**	**Single sequences**	**Con-sensus**	**Total**	
12S rDNA	64	56	8	87.5%	21	0	21	32.8

cox1	84	71	13	84.5%	22	48	70	83.3
28S rDNA	84	71	13	84.5%	34	38	72	85.7

Table 16: Overview of amplified and sequenced samples

4.3 Phylogenetic analyses

In order to qualify and quantify the genetic differences between *M. perstans* and *Mansonella* sp. "DEUX", sequences from three different genes were compared both between the species and between individuals of the same species. Figure 16 demonstrates the workflow with which the sequences were analyzed.

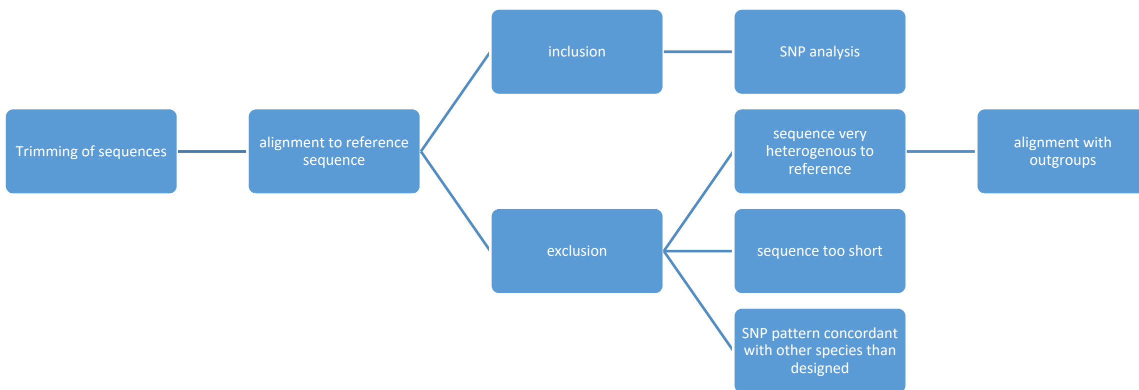

Figure 16: Workflow of the phylogenetic analysis

For each of the three genes, sequences had to be excluded from analysis, as they did not meet the quality criteria as described in the method section. Additionally, three samples had to be excluded from analysis as they were determined to be *M. perstans* by qPCR while their SNP pattern resembled *Mansonella* sp. "DEUX" in *cox1* and *28S* rDNA sequences. Table 17 gives an overview of the analyzed sequences.

Gene	**Sequenced samples**			**Analyzed samples**			**Length of analyzed sequences in bp**
	M. perstans	***Mansonella* sp. "DEUX"**	**Total**	***M. perstans***	***Mansonella* sp. "DEUX"**	**Total**	
12S rDNA	2	19	21	1	13	14	199 (*M. perstans)* or 394 (*Mansonella* sp. "DEUX")
cox1	14	56	70	9	46	54	618

28S rDNA	20	52	72	16	44	60	523

Table 17: Overview of analyzed samples. bp = base pairs

4.3.1 Primary objective: Single nucleotide polymorphisms between M. perstans *and Mansonella sp. "DEUX"*

4.3.1.1 *12S* rDNA

After excluding all unsuitable sequences, a total of 14 sequences remained for analysis. Figure 17 below visualizes this analysis of a single *M. perstans* sequence, which had to be trimmed to 199 bp due to its low quality, together with 13 *Mansonella* sp. "DEUX" sequences with a length of 394 bp aligned to the *M. perstans* reference sequence OQ633017.1.

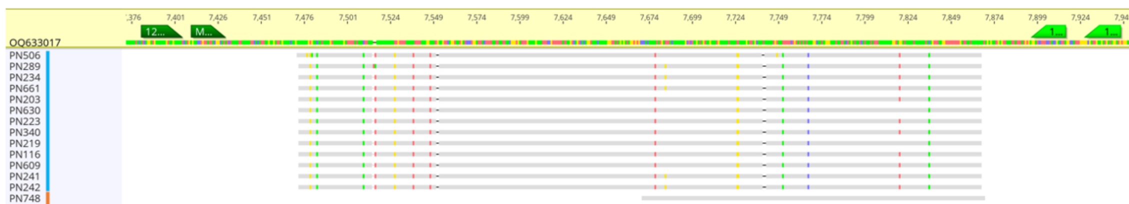

Figure 17: 12S *rDNA alignment of the Mansonella sequences generated in this thesis to the* M. perstans *reference sequence OQ633017.1. Green boxes = primer regions. Blue line =* Mansonella *sp. "DEUX" sequences. Orange line =* M. perstans *sequences.*

Table 18 lists 13 SNPs consistent in all *M. perstans* sequences and *Mansonella* sp. "DEUX" sequences generated in this thesis and their placement on the reference sequence.

CDS position	***M. perstans***	***Mansonella* sp. "DEUX"**
7.479	A	G
7.483	C	T
7.510	C	T
7.516	-	Insertion W
7.526	A	G
7.537	T	A
7.547	T	A
7.551	G	Deletion G
7.725	A	G
7.740	A	Deletion A
7.751	A	T
7.766	T	C
7.836	C	T

Table 18: 12S *rDNA SNPs in* Mansonell*a sp. "DEUX" sequences generated in this thesis and their placement on the* M. perstans *reference sequence OQ633017. CDS = coding sequence*

Additionally, two SNPs could be found in 12 out of 13 *Mansonella* sp. “DEUX” respectively which are visualized in Table 19.

CDS position	*M. perstans*	*Mansonella* sp. “DEUX”
7.677	T	A
7.741	T	Deletion T

Table 19: Additional 12S *rDNA SNPs present in a majority of* Mansonella *sp. "DEUX" sequences generated in this thesis and their placement on the* M. perstans *reference sequence OQ633017. CDS = coding sequencce*

Interspecific differences between both species ranged from 3.7% - 4.6%.

4.3.1.2 *cox1*

Figure 18 visualizes the alignment of the 9 *M. perstans* samples and 46 *Mansonella* sp. “DEUX" samples analyzed in this thesis.

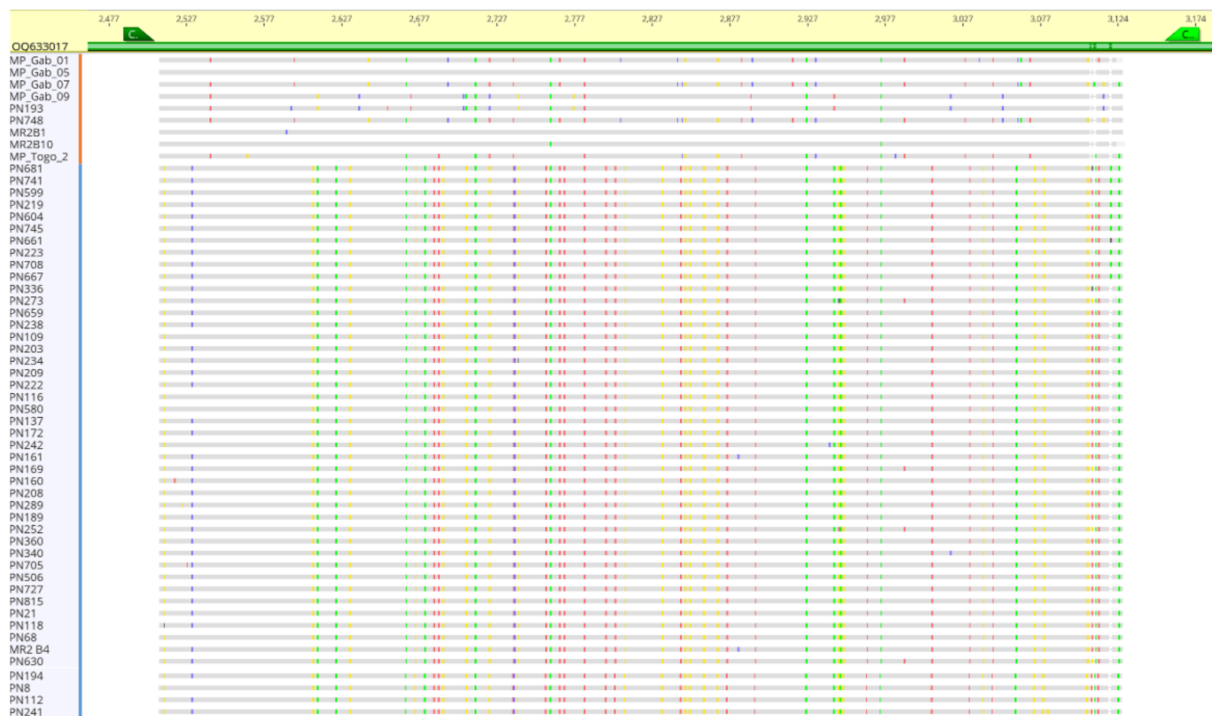

Figure 18: Cox1 *alignment of the* Mansonella *sequences generated in this thesis to the reference sequence OQ633017.1. Green boxes = primer binding sites. Orange line =* M. perstans *sequences. Blue line =* Mansonella *sp. “DEUX” sequences.*

gives an overview over the 34 SNPs found between *M. perstans* and *Mansonella* sp. “DEUX” sequences analyzed in this thesis with their placement on the *M. perstan*s reference sequence OQ633017.

CDS position	***M. perstans***	***Mansonella* sp. “DEUX"**
2.512	A	G (97.8%) or S (2.2%)
2.608	A	G
2.611	A (7/9) or G (2/9)	T
2.623	A	T
2.632	T	G
2.674	T	G
2.680	A	T
2.686	G	A
2.707	A (7/9) or T (2/9)	G
2.722	T (3/9), A (4/9) or C (2/9)	G
2.738	T	C
2.758	G	A
2.770	G	A
2.797	G	A
2.803	G	A
2.809	A	G
2.833	A	G
2.845	G	A
2.851	T	G
2.860	T	G
2.875	G	A
2.893	G	A
2.944	G (7/9) or A (2/9)	T
2.947	T	G (95.6%) or A (2.2%) or R (2.2%)
2.948	C	T
2.950	T	G
2.965	G	A
3.007	G	A
3.031	G	A
3.040	A	G
3.061	G	T
3.073	A	G
3.079	A	G
3.109	T	A (89.1%) or G (10.9%)

Table 20: cox1 *SNPs in* Mansonella *sp. “DEUX” sequences generated in this thesis and their placement on the* M. perstan*s reference sequence OQ633017. CDS = coding sequence*

The sequences between the species differed in 8.1% - 9.1%.

4.3.1.3 28S *rDNA*

The alignment of the 16 analyzed *M. perstans* sequences and 46 *Mansonella* sp. “DEUX” sequences to the *M. perstans* reference sequence MN432520 is visualized in Figure 19.

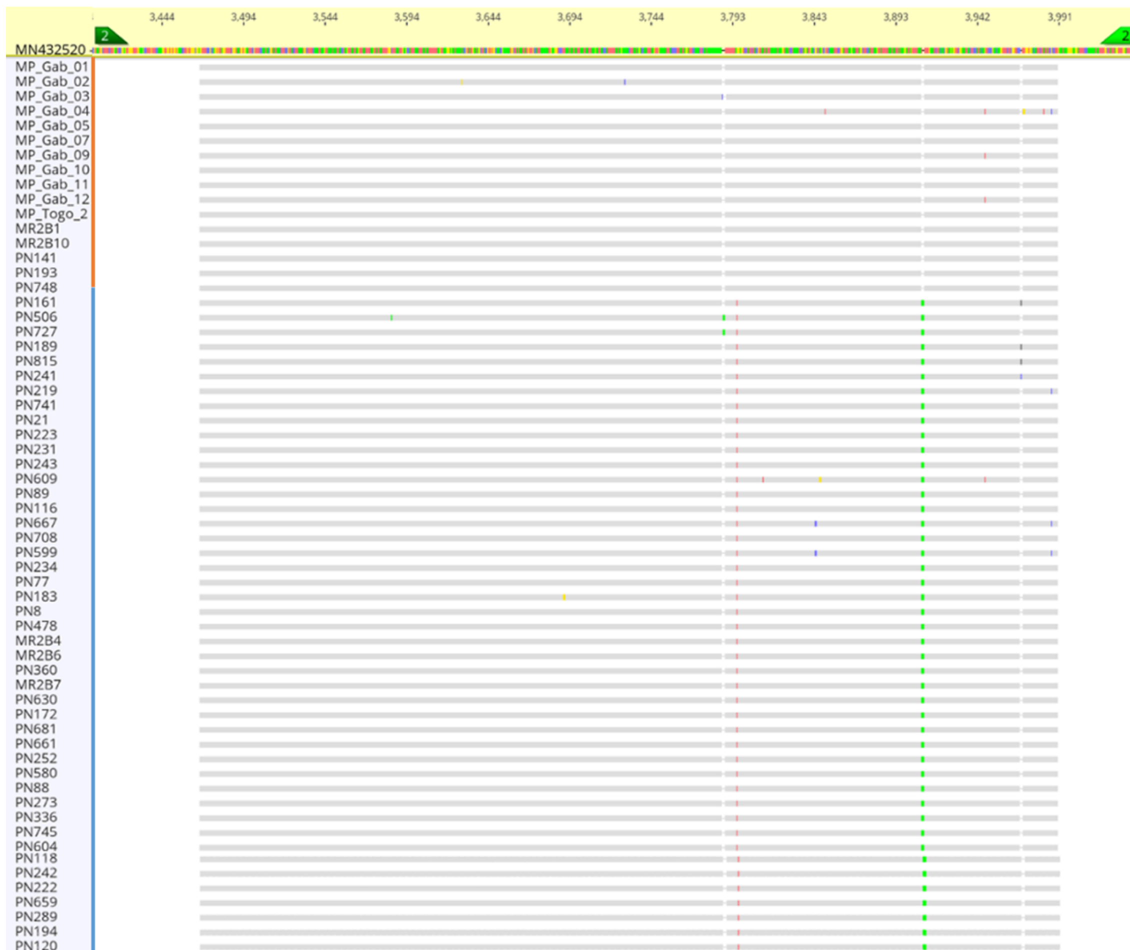

Figure 19: 28S rDNA alignment of sequences generated in this thesis to reference sequence MN432520. Green boxes = primer binding sites. Orange line = M. perstans *sequences. Blue line =* Mansonella *sp. "DEUX" sequences.*

Three SNPS could be observed as listed in Table 21 with their placement on the *M. perstans* reference sequence MN432520.

CDS position	***M. perstans***	***Mansonella* sp. "DEUX"**
3.795	G	A
3.908	A	T
3.908	-	Insertion T

Table 21: 28S rDNA SNPs in Mansonella *sp. "DEUX" sequences generated in this thesis and their placement on the* M. perstans *reference sequence MN432520. CDS = coding sequence*

Interspecific divergence in this region was 0.6% - 1.4%.

4.3.1.4 Overview of the single nucleotide polymorphisms between *M. perstans* and *Mansonella* sp. "DEUX" and comparison to interspecific nucleotid divergences between other onchocercid species

The three analyzed genes showed different degrees of divergence and SNP patterns. Table 22 gives an overview over the length of the analyzed sequences

and the number of consistent SNPs found between *M. perstans* and *Mansonella* sp. “DEUX”. *Cox1* shows the most interspecific SNPs, reaching a maximum of 9.1% difference in this region, while the *28S* rDNA gene seems to be highly conserved between both species with a maximum 1.4% difference.

Gene	Length of the analyzed sequence	Number of SNPs between *M. perstans* and *Mansonella* sp. “DEUX”	% SNPs between *M. perstans* and *Mansonella* sp. “DEUX”	Interspecific differences in %	Previously observed nucleotide divergences between onchocercid species in %
12S rDNA	394 bp	13	3.8	3.7 – 4.6	> 2 (Mirzaei et al., 2018)
cox1	617	34	5.6	8.1 – 9.1	4.5 – 13 (G. Ferri, Alù, Corradini, Licata, & Beduschi, 2009; Emilie Lefoulon et al., 2017) (E. Ferri et al., 2009; Mirzaei et al., 2018)
28S rDNA	605	3	0.5	NA	NA

Table 22: Overview of SNPs between Mansonella *species in sequences generated in this thesis and comparison with interspecific nucleotide divergences between other onchocercid species. BP = base pairs.*

Moreover, the diversity in the *cox1* gene between *M. perstans* and *Mansonella* sp. “DEUX” is within previously observed nucleotide diversity in this gene region in other onchocercid species, and the *12S* rDNA diversity is even greater than in other species.

4.3.2 *Secondary objective 1: Investigation of the intraspecific differences in* **M. perstans** *and* **Mansonella** *sp. “DEUX” in* **12S** *rDNA, cox1 and* **28S** *rDNA*

4.3.2.1 *M. perstans*

For the *12S* rDNA gene, the single analyzed *M. perstans* sequence was identical to the reference sequence. No comment on intraspecific diversity is thus possible.

For *cox1*, a total of 9 genotypes could be identified within the 9 analyzed *M. perstans* sequences. Figure 20 visualizes the diversity in the *M. perstans* sequences, demonstrating that one sequence was identical to the reference genome, and the other 8 samples differed in at least one SNP.

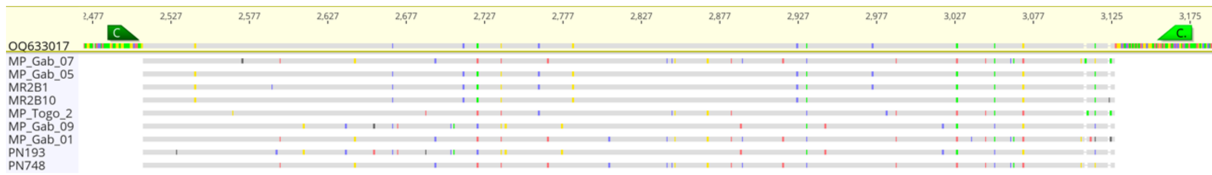

Figure 20: Cox1 M. perstans *samples generated in this thesis aligned to reference sequence OQ633017.1. Green boxes = primer binding sites*

In the 16 *M. perstans* sequences generated for *28S* rDNA, three different genotypes could be found, consisting of a major genotype comprising 14 sequences and two individual sequences. The alignment of these sequences is visualized in Figure 21. Mean pairwise identity across the *cox1* sequences was 96.2%, ranging from 93.6% - 99.8%.

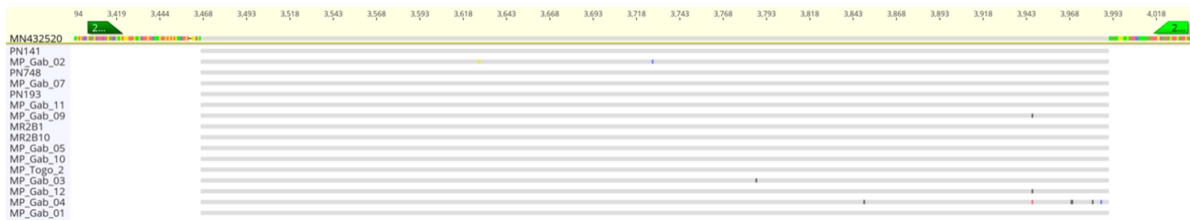

Figure 21: 28S rDNA M. perstans *samples generated in this thesis aligned to reference sequence MN432520. Green boxes = primer binding sites*

Figure 22 visualizes the previously stated observation that the *cox1* region is more diverse within *M. perstans* than the *28S* rDNA region. Mean pairwise identity for the *28S* rDNA region was 99.8% ranging from 98.9% – 100%.

cox1 **genotype distribution for 9** ***M. perstans*** **sequences**

28S **rDNA genotype distribution for 16** ***M. perstans*** **sequences**

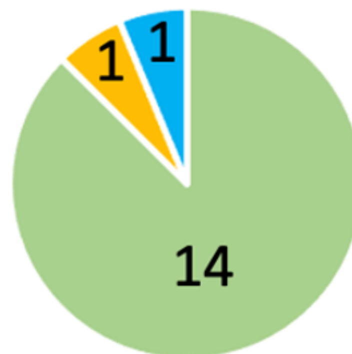

Figure 22: cox1 *and* 28S *rDNA genotype distribution for* M. perstans *sequences generated in this thesis. More different genotypes can be found in the* cox1 *region than in the* 28S *rDNA region. No genotype distribution was visualized for* 12S *rDNA, since only one sequence could be analyzed.*

4.3.2.2 *Mansonella* sp. “DEUX”

For the *12S* rDNA gene, a total of 5 genotypes within 13 sequences could be determined. Two major genotypes with five and four sequences respectively as well as three minor genotypes with one or two sequences could be determined. The mean pairwise identity of the *Mansonella* sp. “DEUX" samples was 99.4%, with intraspecific identity ranging from 97.9% - 100%.

Within the 46 *cox1* sequences for *Mansonella* sp. “DEUX”, a total of 19 genotypes could be identified. The most prevalent genotype included 13 sequences, which corresponds to almost a third of the total sequences. Additionally, several minor genotypes with two to five sequences could be observed, as well as 12 individual sequences. A mean pairwise identity of 99.7% ranging from 98.8% - 100% could be observed.

For the *28S* rDNA gene, a total of 4 genotypes could be observed within the 44 *Mansonella* sp. “DEUX” sequences. A major genotype including 37 sequences identical to the SNP pattern demonstrated in Table 21 could be observed, the other genotypes consisted of one to four sequences. Mean pairwise identity was 99.9%, ranging from 99.5% – 100%.

Figure 23 visualizes the genotype distribution within the regions.

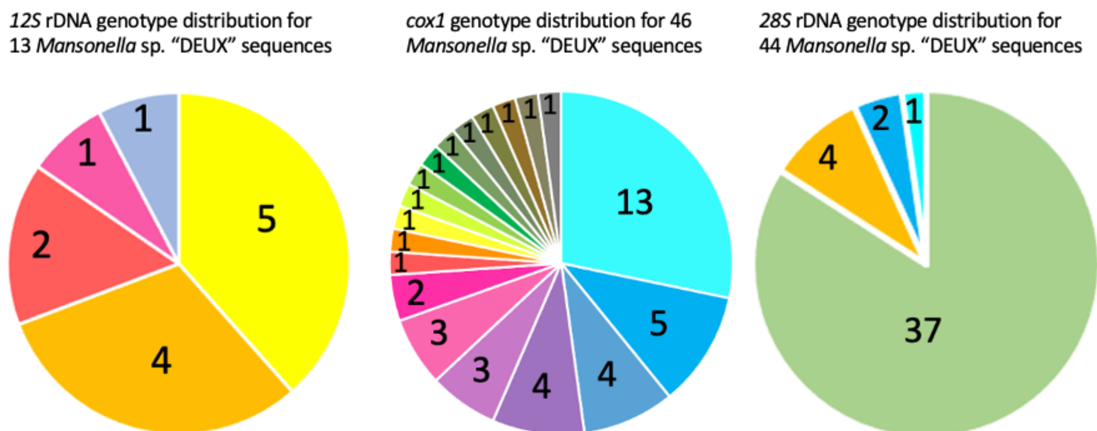

Figure 23: 12S *rDNA,* cox1 *and* 28S *rDNA genotype distribution for* Mansonella *sp. “DEUX” sequences generated in this thesis. Overall, the* cox1 *region has the most genotypes, while the* 28S *rDNA region is the most identical region.*

4.3.2.3 Overview over the intraspecific differences in *M. perstans* and *Mansonella* sp. “DEUX” in *12S* rDNA, *cox1* and *28S* rDNA

The *12S* rDNA and *cox1* regions appear to be highly polymorphic marker regions, although not individual for the parasites, while the *28S* rDNA region appears rather conserved within the species. Table 23 gives an overview over the analyzed sequences and genotypes. The expected heterozygosity has also been calculated, revealing a high expected heterozygosity especially for the *cox1* region. The most diverse marker region overall observed in this thesis was the *cox1* marker region in *M. perstans*, followed by the same region in *Mansonella* sp. “DEUX”. It can be observed that intraspecific identity is higher within *Mansonella* sp. “DEUX” than within *M. perstans,* and that the *cox1* sequence in *M. perstans* demonstrates the highest intraspecific diversity overall.

Marker region	***M. perstans***			***Mansonella* sp. “DEUX”**		
	Sequences	**Genotypes**	**Expected heterozygosity**	**Sequences**	**Genotypes**	**Expected heterozygosity**
12S rDNA	1	1	NA	13	5	0.85
cox1	9	9	1	46	19	0.89
28S rDNA	16	3	0.24	44	4	0.28

Table 23: Overview of the number of sequences and genotypes from samples investigated in this thesis, including the expected heterozygosity. NA = not applicable

4.3.3 Secondary objective 2: Comparison with previously deposited sequences

To enhance the validity of this analysis, previously deposited sequences from Gen-bank were compared to the sequences generated within the frame of this thesis. Several unclassified *12S* rDNA sequences deposited by Gaillard et al. (Gaillard et al., 2020) clustered together with *Mansonella* sp. “DEUX” in a recent analysis (Rodi et al., 2023) and were thus presumed to be *Mansonella* sp. “DEUX. No additional sequences could be found for the *28S* gene. Table 24 gives an overview over the additionally analyzed samples. A comprehensive overview over the analyzed sequences can be found in Table 33 in the appendix.

Gene	Species	This thesis	Available in NCBI database	Total
12S rDNA	*M perstans*	1	19	20
	Mansonella sp. “DEUX"	13	24	27

cox1	*M. perstans*	9	18	27
	Mansonella sp. “DEUX"	46	48	94

Table 24: overview of analyzed sequences including previously published sequences

The goal of this comparison was to investigate whether the SNPs between species which were found in this thesis could also be found in previously deposited sequences. The additional sequences originated from both human and non-human primate (NHPs) hosts, and from Cameroon as well as from Gabon. Thus, possible genomic correlations between sequences originating from different host species and countries of origin were investigated.

Table 25 summarizes the origin of the analyzed sequences.

Source	**Country of origin**	**number of sequences**		
		***12S* rDNA**	***cox1***	***28S* rDNA**
Human blood	Gabon	16	61	59
	Cameroon	6	7	0
	Togo	0	1	1
	Other	2	3	0
Stool from NHP	Gabon	9	15	0
	Cameroon	23	38	0

Table 25: overview of host species, source material and country of origin of analyzed sequences including previously published sequences

4.3.3.1 *M. perstans*

Including 19 additional *12S* rRNA *M. perstans* sequences lead to the identification of 10 additional genotypes. In all 20 sequences, one major genotype in 11 sequences including the sequence generated in this thesis could be identified in addition 9 individual sequences were seen. The major genotype included sequences from different countries and different host species.

For the *cox1* region, an additional 16 genotypes could be found within the 18 *M. perstans* sequences, leading to a total of 25 genotypes in 27 sequences. The genotypes were predominantly individual. Two genotypes consisted of two sequences each, among which sequences from different countries and host species shared a genotype.

All sequences from the *28S* rDNA region originated from human hosts. The sequence originating from Togo shared a major genotype with 13 other *M. perstans* sequences from Gabon.

4.3.3.2 *Mansonella* sp. “DEUX”

Within the additional 24 sequences for *12S* rDNA, 5 additional genotypes could be found. A total of 10 genotypes could thus be identified in the 37 sequences with one major genotype comprised of 23 sequences, several minor genotypes comprising two to four sequences and six individual genotypes. Genotypes were shared between sequences from different countries of origin and host species.

In the 48 additional *cox1* sequences, 21 new individual genotypes could be observed. Thus, in 94 sequences, a total of 41 genotypes could be observed. One major genotype consisting of 39 sequences could be observed, as well as several minor genotypes comprising one to five sequences. The major genotype included sequences from different countries of origin and host species.

4.3.3.3 Overview over the comparison with previously deposited sequences

The inclusion of sequences from different countries and host species could confirm the previously described SNP pattern between *M. perstans* and *Mansonella* sp. “DEUX”. With exception of the *cox1* marker region in *M. perstans,* which was predominantly individual, the genotypes for the different marker regions usually consisted of one or two major genotypes comprising at least a third of the total sequences and several minor genotypes. Table 26 summarizes the number of sequences and genotypes analyzed.

Species	**Marker region**					
	***12S* rDNA**		***cox1***		***28S* rDNA**	
	Sequences	Genotypes	Sequences	Genotypes	Sequences	Genotypes
M. perstans	20	10	27	25	16	3
Mansonella sp. “DEUX”	37	10	94	40	44	4

Table 26: Overview of the number of sequences and genotypes with the inclusion of additional sequences deposited in GenBank by other studies

Since not all genotypes are individual in either region and genotypes are shared between sequences from different countries and host species, no association of genotypes with their country of origin or host species could be observed.

4.3.4 Secondary objective 3: Visualization of the phylogenetic relationship

Phylogenetic trees were constructed to visualize the relationship of filarial species to each other. Genotypes from all analyzed *Mansonella* spp. sequences collected

within this thesis as well as the additional sequences found in the data bank and analyzed in 4.4.4 were included. Outgroups from other filarial species as listed in Table 34 in the appendix were included. Additional to other onchocercid species, another potential new *Mansonella* species, *M. cermeli*, postulated by Rodi et al. (Rodi et al., 2023) was included as outgroup.

Three sequences for *cox1* had to be excluded since they were more diverse to the reference sequences than to the outgroups, Three further samples had to be excluded due to an unclear infection status.

Optimal substitution models and tree models varied between the genes and were individually optimized for each data set. The optimal substitution model for the datasets according to corrected AIC criteria was the HKY + F model for *12S* rDNA and the GTR + F + G4 model for *cox1* and *28S* rDNA. Additional Bayesian trees were included for comparison, which can be found in the appendix in Figure 29 - Figure 31.

4.3.4.1 12S *rDNA*

In the phylogenetic tree based on *12S* rRNA visualized in Figure 24, a distinct branching of *Mansonella sp. "DEUX"* sequences in blue within the *M. perstans* cluster in orange could be observed with a bootstrap support of 53.2. Two *M. perstans* sequences branched within the *Mansonella* sp. "DEUX" with a low bootstrap support (25.5 respective 8.8). Another M. perstans genotype clustered outside of the specific *M. perstans* cluster but closer to it than *M. ozzardi*.

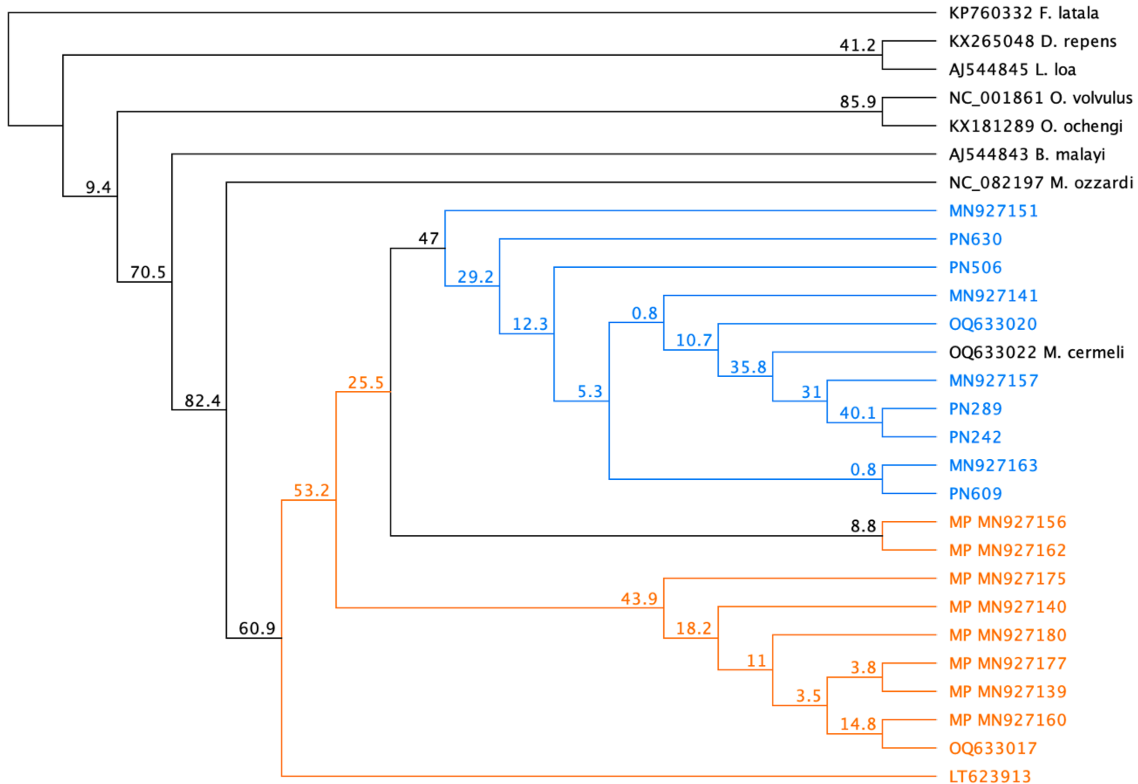

Figure 24: 12S rDNA phylogenetic maximum likelihood tree with 1000 bootstrap replicates . Orange = M. perstans. Blue = Mansonella sp. "DEUX". Numbers: bootstrap support

The closest pairwise tree distance between both species was 0.050, which was smaller than the closest pairwise tree distance between *M. ozzardi* and *M. perstans* (0.143). Compared to interspecific distances, branches within the species cluster were short with average pairwise tree distances of 0.004 for *M. perstans* and 0.026 for *Mansonella* sp. "DEUX".

4.3.4.2 *cox1*

In the phylogenetic tree based on the *cox1* region as demonstrated in Figure 25, a distinct clustering of *M. perstans* in orange and *Mansonella* sp. *"DEUX*" in blue with moderate-high bootstrap support (61.7 for the clade, and 98.9 and 70.1 for the species respectively) could be observed. While *M. ozzardi* and *M. cermeli* branched with *M. perstans* and *Mansonella* sp. "DEUX" with high bootstrap support (90.1 for the clades), both *Onchocercae* and *D. repens* clustered within the same branch as *L. loa* and *B. malayi* with lower bootstrap support.

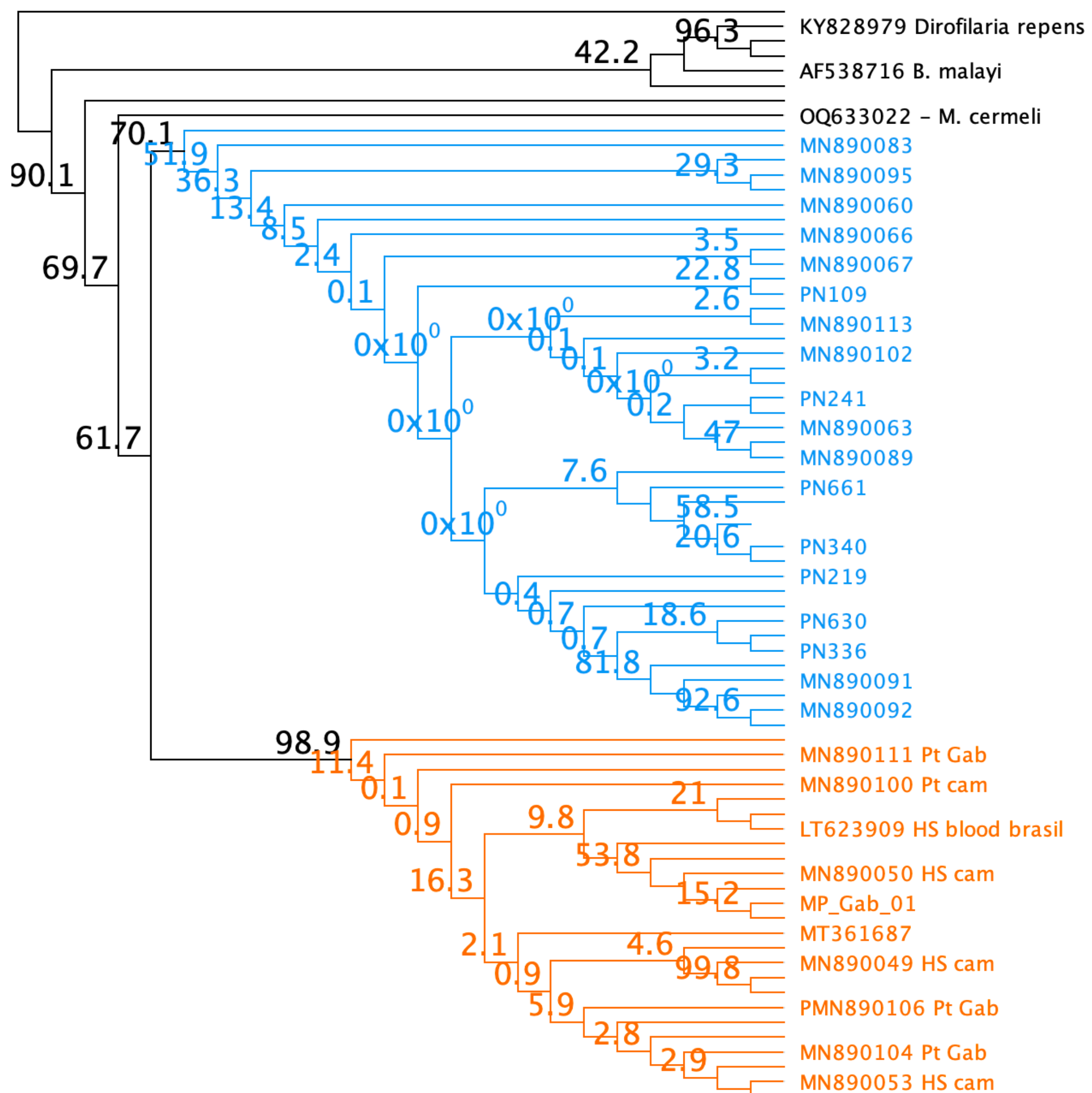

Figure 25: cox1 *phylogenetic maximum likelihood tree with 1000 bootstrap replicates. Orange* = M. perstans*. Blue* = Mansonella *sp. "DEUX".*

The closest pairwise tree distance between both species was 0.149, which was larger than the closest pairwise tree distance between both *Onchocerca* species (0.029) but smaller than the closest pairwise tree distance between *M. ozzardi* and *Mansonella* sp. “DEUX” (0.243). Branches between individual genotypes within the species were short (average pairwise tree distance within genotypes was 0.033 for *M. perstans* and 0.012 for *Mansonella* sp. “DEUX”).

4.3.4.3 28S *rDNA*

The construction of a phylogenetic tree based on the *28S* rDNA region as demonstrated in Figure 26 revealed distinct clustering of both *Mansonella* species with high bootstrap support for *M. perstans* (84.1) in orange and lower bootstrap support for *Mansonella* sp. “DEUX” (58.6) in blue, the clade itself had a high bootstrap support of 83.8. While the onchocercid family branched apart from *Filaria latala, M. ozzardi* branched separately from the other *Mansonella* species. *O. lupus* and *O. fasciata* brached with *B. malayi* and *L. loa*, while *D. immitis* branched apart. Bootstrap support for the outgroups was low.

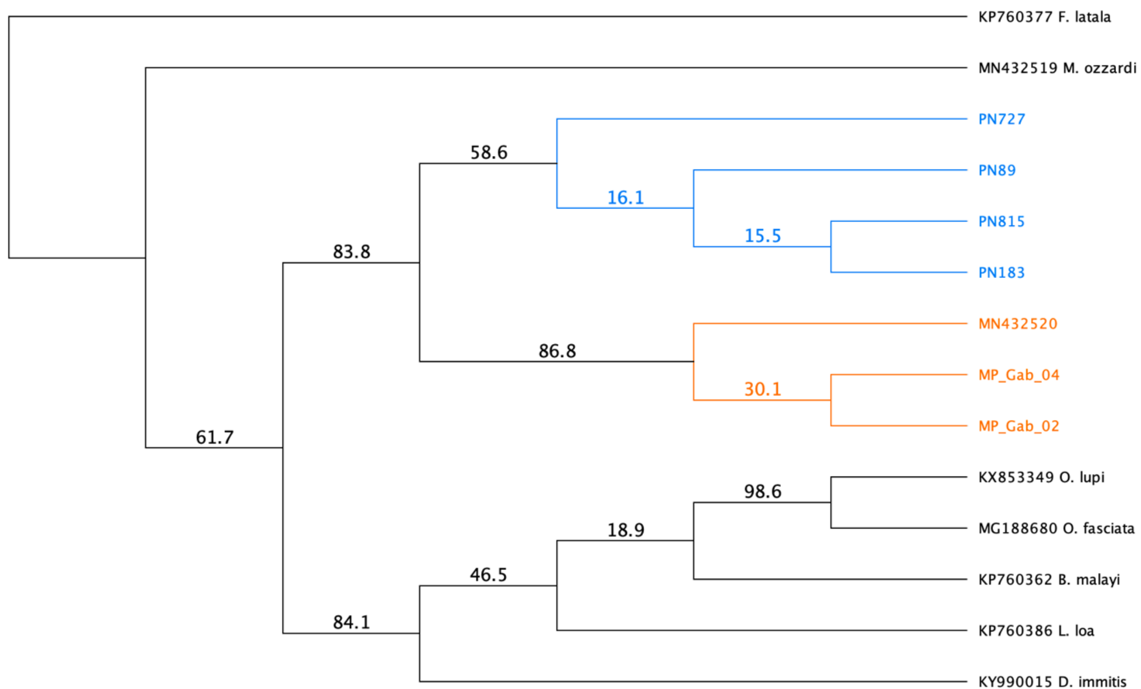

Figure 26: 28S *rDNA phylogenetic maximum likelihood tree with 1000 bootstrap replicates. Orange =* M. perstans. *Blue =* Mansonella *sp. “DEUX”*

The closest pairwise tree distance between both species was 0.007, which was larger than the closest pairwise tree distance between both *Onchocerca* species (0.022) but smaller than the closest pairwise tree distance between *M. ozzardi* and *Mansonella* sp. “DEUX” (0.021). Branches between individual genotypes within the species were short (average pairwise tree distance within genotypes was 0.005 for *M. perstans* and less than 0.001 for *Mansonella* sp. “DEUX”).

4.3.5 Clustering ouf the previously excluded sequences

A total of three samples and three additional individual sequences for *cox1* had to be excluded due to high divergence to the reference sequence or an unclear infection status. Comparing the highly diverse sequences to the reference sequences lead to the detection of >99% similarity with a positive control sequence (*L. loa* or *O. volvulus* respectively). The third sequence showed >90% similarity with different filarial species, such as *Onchocerca* species or Dirofilaria repens. The samples with unclear infection status had been determined *M. perstans* monoinfected in the original qPCR and mono- or coinfected with *Mansonella* sp. “DEUX” in the repetition. For these sequences, the *cox1* region showed predominantly *Mansonella* sp. “DEUX” SNPs next to *M. perstans* SNPs, the SNPs in the *28S* rDNA gene could not be determined due to low base call quality and the one sequence that could be analyzed for 12S rDNA had partially *M. perstans* SNPs and partially *Mansonella* sp. “DEUX” SNPs.

4.3.6 Overview of the phylogenetic analysis

4.3.6.1 Suitable marker regions for species differentiation

For all three genes, complex SNP patterns with high discriminatory power were observed and are thus suitable for species differentiation. While *12S* rDNA and *cox1* sequences demonstrated a significant overall divergence between the species, *28S* rDNA was highly conserved. All phylogenetic trees showed distinct clustering according to species with varying bootstrap support. Due to the higher number of SNPs, the greater length of the retrieved sequences and the higher interspecific difference between both *Mansonella* species, the *cox1* region is the

most suitable marker region to distinguish between *M. perstans* and *Mansonella* sp. DEUX". However, the use of more conserved gene regions with less SNPs is also highly suitable for species differentiation due to the relative greater analytical conciseness.

4.3.6.2 Intraspecific differences and potential for genotyping

Apart from the *cox1* region in *M. perstans*, one or two major genotypes with several minor genotypes could be observed in the marker regions. Most genotypes differed in only few SNPs and mean intraspecific identity was high. Table 27 summarizes the intraspecific differences within the marker regions. Similarity overlaps occurred between the most diverse intraspecific sequences (with a minimum for intraspecific identity of 97.1% for *12S* rDNA, 93% for cox1, and 98.9% for *28S* rDNA respectively) and the most similar interspecific sequences (with a maximum of interspecific identity of 97.9% for *12S* rDNA, 91.9% for *cox1* and 99.4% for *28S* rDNA respectively) in all three regions.

Gene	**Intraspecific genetic difference in %**			
	in sequences from this thesis		**with additional sequences**	
	M. perstans	*Mansonella* sp. "DEUX"	*M. perstans*	*Mansonella* sp. "DEUX"
12S rDNA	NA	99.4 (97.9 – 100)	99.4 (97.1 – 100)	99.5 (98.6 – 100)
cox1	96.2 (93.6 – 99.8)	99.7 (98.8 – 100)	97.3 (93.6 – 100)	99.4 (93 – 100)
28S rDNA	99.8 (98.9 – 100)	99.9 (99.5 – 100)	NA	NA

Table 27: Intraspecific differences. The number indicates the average difference, the numbers in parentheses indicate the range.

4.3.6.3 SNPs as markers between different sources or countries of origin

The sequences utilized in this thesis originated from different host species (humans or NHPs) and country of origin (Gabon, Cameroon, Togo) and a correlation of these factors to specific genotypes were analyzed. Neither could SNP patterns be observed depending on these factors nor could clustering in the phylogenetic trees be observed. Genotypes were shared between sequences regardless of host species or country of origin.

4.3.6.4 Determination of the phylogenetic relationship based on *12S* rDNA*, cox1* and *28S* rDNA

The phylogenetic analysis based on those three genes revealed distinct SNP patterns, high intraspecific identity within both species and the comparably high divergence of sequences between species and thus supports the status of *M. perstans* and *Mansonella* sp. “DEUX” as two distinct species. While species differentiation is possible with all three investigated gene regions, the *cox1* gene was found to be the most suitable gene to differentiate between species due to the complex SNP pattern.

5 Discussion

The discovery of *Mansonella sp.* "DEUX", a potentially new *Mansonella* species divergent from *M. perstans* in diagnostically relevant genetic regions with high prevalence in Gabon draws attention to the highly neglected field of tropical filariasis (Sandri et al., 2020). The morphological similarity of *Mansonella* microfilariae contributes to underdiagnosing *Mansonella* infections, confusion with other members of the *Onchocercidae* family and thus contradictory characterization of species, and to the description of several potential *Mansonella* species (Dukes et al., 1968; Ta-Tang et al., 2018). The true clinical relevance of *Mansonella* infections is thus difficult to characterize. Investigating species on a molecular level is a powerful approach which allows for a standardized classification of species, the investigation of their phylogenetic relationship and potentially even the recognition of individual parasites within the frame of a genotyping approach (Hebert et al., 2003; E. Lefoulon et al., 2015). Molecular methods have gained importance over traditional approaches when investigating onchocercid species differentiation. The aim of this thesis was to investigate the three genes *12S* rDNA*, cox1* and *28S* rDNA in *Mansonella* sp. "DEUX" and its sympatric species *M. perstans* as potential genotyping regions to distinguish between those species and identify individual parasites or parasite strains. The *12S* rDNA and *cox1* regions have previously been important pillars in the description of *Onchocercidae* phylogeny (E. Lefoulon et al., 2015).

5.1 Sample collection

From 12 microscopically *Mansonella* - mono-infected participants, a total of 6 mono-infections could be retrieved (2 *M. perstans* and 4 *Mansonella* sp. "DEUX"). Although these numbers are too low for significant analysis, it was observed that that *Mansonella* sp. "DEUX" is more prevalent than *M. perstans* in this area in Gabon, which is in line with previous observations (Sandri et al., 2020). Since all samples which tested positive in the pan filarial qPCR also tested positive for at least one of the tested filarial species, there is no evidence for previously undiscovered species based on investigation of the ITS1 region. Further ongoing investigations will be able to validate or dispute these observations in a greater sample size.

5.2 Polymerase chain reaction

5.2.1 PCR optimization

To successfully amplify a specific target region on a gene via PCR, it is necessary to determine the optimal master mix and cycling conditions for this PCR. Primer design and primer concentration as well as the concentration of all other ingredients (polymerase, nucleotides, and additives like BSA or Mg^{2+}) are important parameters (Farell & Alexandre, 2012; Lorenz, 2012; Staff, 2019). The cycling conditions are, apart from annealing temperature and cycle number, optimized for use with the polymerase kit, though it can be helpful to evaluate a variation of these conditions.

Within the frame of these PCR optimizations, the most important component was clearly the primer design and the optimal primer annealing temperature (see Figure 14 as observed before (Lorenz, 2012; Staff, 2019). The correct annealing temperature is also a crucial factor for amplification (see Figure 14). The addition of Mg^{2+} ions can increase both specific and unspecific binding, as Mg^{2+} ions can increase the activity of the polymerase by acting as a cofactor and stabilize the annealing of primers to both correct and incorrect binding sites in DNA (Lorenz, 2012). Therefore, it is necessary to find the optimal Mg^{2+} concentration for each PCR, which usually varies between 0.5 mM and 5 mM (Lorenz, 2012). In the polymerase buffer of the kits used, a $MgCl_2$-concentration of 1.5 mM in the *taq* polymerase buffer and a $MgSO_4$-concentration of 1.6 mM in the *SuperScript III* buffer sufficed for satisfactory amplification in most protocols. BSA can alleviate the effect of PCR inhibitors by binding to those (Farell & Alexandre, 2012) and was thus evaluated for effectiveness, however, similar to the variation of primer concentration, polymerase concentration, extension time and cycle number, it showed only little influence on PCR amplification in this thesis.

5.2.2 Primer design

The design of new primers proved crucial in two of the three PCRs in this thesis, such as in the final run of the *28S* rDNA protocol, where newly designed primers with the optimal annealing temperature sufficed to amplify the target region without further PCR optimization. The greatest limitation regarding primer design

in this thesis was the availability of sequences, as there was only one reference sequence for *Mansonella 28S* rDNA, and none for *O. volvulus* DNA, which hinders the search for a constant site for primer binding. It can be assumed that the amplification failure for *O. volvulus* was due to divergences in the selected area.

5.2.3 PCR amplification

Amplification rates of the PCRs were generally high (>84%) and comparable between the gene regions. The likelihood of these divergences being due to errors in sample handling, misdistribution of master mix ingredients in the samples, or pipetting errors were reduced by twice repeating the PCR in case of failed amplification. Factors such as DNA degradation, low DNA quality, low DNA purity, the presence of strong PCR inhibitors and divergent primer regions are to be considered as well. For the three samples which did not amplify in any PCR, a false positive result in the original qPCR is also possible.

The use of primers covering a different conserved portion of the genes might be an option for future experiments to increase amplification rates. Especially in *Mansonella* sp. “DEUX” *12S* rDNA showed notable divergences in the sequenced areas close to the 3’ end of the inner forward primer used in this thesis. While single internal primer mismatches usually hardly impact amplification, possible mismatches in the 3’ region of the primer region are able to significantly reduce amplicon yield (Kwok et al., 1990). *O. volvulus*, which was used as positive control, usually showed stronger bands than the *Mansonella* positive control. The most likely explanation for this observation is a higher DNA concentration in the *O. volvulus* positive control, which was extracted from an adult worm, than in the *Mansonella* positive control, which was extracted from whole blood from infected individuals.

5.2.4 Sequencing

While the sequencing of *cox1* sequences and *28S* rDNA sequences generally proved successful, only reverse sequences could be retrieved for *12S* rDNA. Neither the use of previously published sequencing primers nor the creation of different sequencing primers led to improved sequencing results even though the

PCR showed clear, single bands. Since the sequencing workflow itself was under the same conditions as for *cox1* and *28S* rDNA, an issue within the workflow was unlikely. Since the *12S* rDNA gene is widely used in genetic barcoding across metazoan species with its first set of universal primers designed in 1989 (Kocher et al., 1989), and has previously been sequenced for *Mansonella* and other members of the *Onchocercidae* (E. Lefoulon et al., 2015; Mirzaei et al., 2018), general unsuitability of this gene for sequencing cannot be assumed. However, regarding the deposited sequences for *Mansonella*, it can be observed that they map more towards the 3' end of the reference sequence than the sequences analyzed in this thesis. Since only a limited number of sequences were available for primer design, it is possible that the region chosen for the inner primer was too diverse to allow for sufficient binding. However, in this case, an amplification failure would most likely also have occurred. A more likely explanation is the formation of secondary structures within the AT-rich regions such as the region not covered by most of the sequences deposited in the Genbank which can hinder the sequencing process. Unexplained difficulties with retrieving sequences from the *Mansonella 12S* rDNA gene had previously been observed (Gaillard et al., 2020). Meanwhile, additional sequences of the entire *12S* rDNA gene have become available for *M. perstans* and *Mansonella* sp. "DEUX" (Rodi et al., 2023) as well as the sequences generated in this thesis which can be taken in account for primer design regarding possible polymorphisms in the primer binding regions. For future experiments, the design of primers further towards the 3' end of the *12S* rDNA sequence could be considered.

5.3 Phylogenetic analysis

The three chosen marker regions were investigated for differences between *Mansonella* parasites designated as *M. perstans* and *Mansonella* sp. "DEUX" by ITS1 qPCR. Moreover, intraspecific polymorphic qualities were analyzed and the results yielded in this thesis were compared to previous species classification proposed by Lefoulon (E. Lefoulon et al., 2015) and supported by others and evidence for the existence of different *Mansonella* strains based on their geographical origin and host species was investigated.

5.3.1 Species differentiation based on **12S** *rDNA,* **cox1** *and* **28S** *rDNA*

In all three marker regions, distinct SNP patterns between *M. perstans* and *Mansonella* sp. “DEUX” could be observed. Interspecific distances and nucleotide divergences were in accordance with or even greater than those between other onchocercid species for *12S* rDNA and *cox1,* as demonstrated in the results, thus supporting the species delimitation between *M. perstans* and *Mansonella* sp. “DEUX”. The *cox1* marker region has previously been found suitable to discriminate between different onchocercid species (E. Ferri et al., 2009), as can be observed in this thesis. No previous observations could be found for nucleotide divergence between species in the *28S* rDNA region.

Moreover, the findings from this thesis based on SNP analysis and phylogenetic clustering also support the classification of the not further classified *12S* rDNA sequences deposited by Gaillard et al. as *Mansonella* sp. “DEUX” as suggested by Rodi et al. (Rodi et al., 2023).

5.3.2 Intraspecific differences and the potential for genotyping

The three gene regions *12S* rDNA, *cox1* and *28S* rDNA were investigated for their genetic diversity in both species. The intraspecific nucleotide divergences observed for *12S* rDNA and *cox1* were in line with previous research, as visualized in Table 28.

Gene region	**maximal intraspecific nucleotide divergences**			
	In this thesis in %		**previously observed within *M. perstans***	
	M. perstans	***Mansonella* sp. “DEUX”**	**Value in %**	**source**
12S rDNA	2.9%	1.4%	10%	(E. Ferri et al., 2009)
cox1	6.8%	7%	7.06%	
28S rDNA	1.1%	0.5%	NA	NA

Table 28: comparison of intraspecific nucleotide divergences to previous results

The resulting overlap between inter- and intraspecific nucleotide distance for both regions had also been observed before (Emilie Lefoulon et al., 2017).

In both species, the *12S* rDNA region and the *cox1* region were diverse within the species, while the *28S* rDNA region was rather conserved, as summarized in Table 23. The expected heterozygosity is thus highest for the *cox1* region in *M. perstans,* and this region is also the region with the highest expected

heterozygosity regarding both species with values of 1 for *M. perstans* and 0.89 for *Mansonella* sp. “DEUX”. While both the *12S* rDNA region as well as the *cox1* region are thus the better markers for genotyping investigated in this thesis based on genetic diversity alone, considering other factors such as amplicon length and the rate of the retrieved sequences (compare Table 16 and Table 17), the *cox1* region can thus be considered the most suitable marker region for genotyping.

5.3.3 Association of genotypes with country of origin or host species

Sequences were compared within the species regarding their country of origin (Gabon, Cameroon, Togo or not closer specified) and their host species (humans or NHPs). In none of the investigated regions, specific genotypes could be associated with a country of origin or host species. Sequences with the same country of origin or host species did not branch together on any phylogenetic tree. Therefore, the existence of different *Mansonella* strains per country cannot be confirmed.

While differences due to geographic distributions could have been expected regarding the sequences from Togo, it is not surprising that no or little genetic distinction could be observed between sequences collected in Gabon and Cameroon, since the countries are neighboring. *M. perstans* is able to infect NHPs as well as human hosts (Gaillard et al., 2020), and while the existence of different parasite strains infecting humans and NHPs is possible, this hypothesis could not be confirmed in this thesis. Moreover, the analysis of sequences deposited in the gene bank by Gaillard et al. (Gaillard et al., 2020) supports the classification of these sequences as *Mansonella* sp. “DEUX”. Thereby, the data of this thesis supports the hypothesis that also Mansonella sp “DEUX is able to infect both NHPs and humans previously proposed (Rodi et al., 2023) as well as the suggestion that *Mansonella* sp. “DEUX” has previously been described in NHPs, as previously postulated (Mourembou et al., 2015).. So far, *M. perstans* and *Mansonella* sp. “DEUX” were the only two *Mansonella* species which could be detected in the stool of NHPs and in human blood (Gaillard et al., 2020).

5.3.4 Sequences excluded from analysis

A total of three samples and three *cox1* sequences were excluded. Three *cox1* sequences were excluded due to high divergence to the Mansonella reference sequences in the *cox1* region and three samples were excluded due to an unclear infection status by *ITS1* qPCR. The highly divergent *cox1* sequences showed high similarity with other onchocercid species, thus a contamination of the samples with positive controls is possible. The three excluded samples could be identified as *Mansonella* sp. “DEUX” in the *28S* rDNA region as well as during the initial *ITS1* qPCR, thus contaminations during the PCR amplification seem more likely than true divergences in the SNP pattern between species. These sequences showed a mixed SNP pattern in *cox1* and *12S* rDNA, which was considered more likely to be an expression of different parasitemias in coinfected samples, qPCR failure, contaminated samples or sequencing errors than a recombination between species. While incongruent species designation between the markers analyzed in this thesis and the widely used *ITS1* region cannot be excluded, the reasons for excluding sequences from analysis do not point to this.

5.3.5 Interpretation of the incongruences between Bayesian trees and maximum likelihood trees and the clustering of the outgroups

Different approaches to phylogenetic reconstruction had to be undertaken to find the most suitable models for phylogenetic representation. Two different phylogenetic reconstruction methods were used: one based on maximum likelihood and a Bayesian phylogenetic method. Maximum likelihood trees differed from Bayesian trees in *12S* rDNA and *28S* rDNA. For *12S* rDNA, divergences were small (two *M. perstans* sequences that clustered with *Mansonella* sp. “DEUX” in the maximum likelihood tree but not in the Bayesian tree, and the clustering of *M. cermeli* within *Mansonella* sp. “DEUX” in the maximum likelihood tree but not the Bayesian tree, slightly distinct clustering of the outgroups). However, all these changes occurred in regions with low bootstrap support or low posterior probability respectively. Thus, divergent results in different models are not surprising. Overall and based on sequence divergences, the Bayesian tree depicted a more congruent version of phylogenetic relationships for the *12S* rDNA region. For *28S* rDNA, all

Mansonella sp. “DEUX” sequences clustered apart from each other in the Bayesian tree while *M. perstans* as well as most of the outgroups clustered together. Since previous analysis confirmed low intraspecific divergences within *Mansonella* sp. “DEUX” and distinct differences between the outgroups, and bootstrap support for the two distinct *Mansonella* clades was high in the maximum likelihood tree, the Bayesian model might not be suitable for this dataset. The maximum likelihood tree and the Bayesian tree were mostly identical for *cox1*, thereby underlining the general suitability of this region to reliably differentiate between species. Moreover, the use of different outgroups was necessary for the *12S* rDNA phylogenetic tree: *O. ochengi* had to be replaced by *M. perforata* to avoid long-branch attraction which placed *M. cermeli* within the *Mansonella* sp. “DEUX” cluster despite considerable nucleotide divergences. The necessity of this measure underlines the lower suitability of the *12S* rDNA region for phylogenetic analysis compared to other regions such as *cox1.* To conclude, a distinct clustering of both species could be observed in both models and the slight variations between models does not contradict the general statement of this phylogenetic analysis.

In previous analysis, a multitude of phylogenetic models has been used to evaluate the relationship between *M. perstans* and *Mansonella* sp. “DEUX” (Casiraghi et al., 2001; Gaillard et al., 2020; E. Lefoulon et al., 2015; Mourembou et al., 2015). Both Bayesian models and Maximum likelihood models with different substitution parameters have been used. While the use of different models leads to difficult comparability between results, it is important to consider that different models may be suitable for different datasets, which is why a model selection analysis was performed within the frame of this thesis. Moreover, since evidence for two distinct species could be found in a multitude of models, the differences between *M. perstans* and *Mansonella* sp. “DEUX” seem robust to model selection. This strongly supports the status of these two species as truly divergent from another.

In all three regions, the chosen outgroups did not cluster as according to multi-locus sequencing originally proposed by Lefoulon and confirmed by others visualized in Figure 1. In the *12S* rDNA maximum likelihood tree, the clustering

of *M. cermeli*, a potential new *Mansonella* species, within the *Mansonella* sp. “DEUX” as visualized in suggests that this model might not be suitable for differentiation between *Mansonella* species, since this does not occur in the Bayesian tree*.* In the *cox1* region, all included *Mansonella* species branched together, but further branching as according to the classification by Lefoulon could not be observed and bootstrap support for the clades was low (42.2 for the clade representing the outgroups). In the *28S* rDNA region, *M. ozzardi* and the rest of the ONC5 clade clustered apart from *Mansonella* spp. However, the *Mansonella* species were more similar to the sequence from *M. ozzardi* than to the sequences supposedly closer related to it, so unsuitability of this model to correctly represent this particular phylogenetic relationship is probable. Other factors such as evolutionary rate variations in different genes should also be taken in account to explain different clustering of outgroups in different genetic regions . When taken into consideration that Lefoulon’s classification was based on multi-locus sequencing of a total of seven genes, including the genes investigated in this thesis, it becomes apparent that the individual genes do not necessarily need to demonstrate the same phylogenetic relationships without invalidating the classification*.*

5.3.6 *The status of* **Mansonella** *sp. “DEUX” as a distinct species within the onchocercid family*

Species differentiation in onchocercids is increasingly based on molecular methods. Apart from genomic differences, resulting characteristics such as morphology, pathogenicity and host specificity can be important characteristics when considering species differentiation as well. *Mansonella* sp. “DEUX” was first pronounced a distinct species based on its genetic difference to *M. perstans* in the *ITS1* region, which is highly conserved between filarial species (Mourembou et al., 2015) and further research including the results from this thesis have supported its status as a distinct species. As discussed above, *M. perstans* and *Mansonella* sp. “DEUX” have similar intra- and interspecific genetic differences than other sister species within the onchocercid species. Not all onchocercid species are morphologically distinctive (McCall et al., 1992), and while the microfilariae of *M. perstans* and *Mansonella* sp. “DEUX” are indistinguishable

(Sandri et al., 2020), no data is yet available regarding the morphology or behavior of adult worms. This limitation is also applicable to other factors such as pathogenicity, host specificity or vector specificity. However, *M. perstans* and *M. ozzardi* cause similar symptoms, can both be transmitted by *Culicoides* spp. and infect humans, which demonstrates that these factors are not obligatory for species delimitation. *Mansonella* sp. “DEUX” is believed to cause similar symptoms as *M. perstans*, as previously observed (Sandri et al., 2020) and seen in this thesis, shares humans and NHPs as host species with *M. perstans,* and possibly shares the same *Culicoides* vector. Despite their shared habitat, *M. perstans* and *Mansonella* sp. “DEUX” have been found to be consistently distinct in certain gene regions. Thus, it can be presumed that no mating occurs between the two species. Since reproductive incompatibility is frequently considered as a factor for species delimitation (McCall et al., 1992), this observation strongly supports the status of *Mansonella* sp. “DEUX” as a distinct species.

5.4 Outlook

The rather recent discovery and delimitation of the highly endemic *Mansonella* sp. “DEUX” underlines the status of mansonellosis as one of the most neglected tropical diseases (Simonsen et al., 2011) with possible other species, a hardly characterized vector-host-relationship, questionable pathogenicity and many open questions pertaining to basic characteristics such as prevalence, distribution, and burden of disease. This thesis validates the existence of *Mansonella* sp. “DEUX” as a distinct *Mansonella* species and the use of the *ITS1* region to detect and differentiate these species. Based on these observations, further research into the epidemiology of *Mansonella* sp. “DEUX” outside of Gabon and Cameroon and its possible hosts is possible and warranted. Moreover, with improved diagnostics and further knowledge about *Mansonella* species, a better characterization of their impact on their hosts becomes possible, as well as the possibility to answer many more open questions pertaining to basic characteristics such as prevalence, distribution, and burden of disease regarding *Mansonella* species in general and *Mansonella* sp. “DEUX” in particular.

The improvement of molecular markers could also contribute to better characterization of the burden of disease caused by *Mansonella* species and evaluate possible therapeutic options. The observations in this thesis regarding unclear symptoms in infected and non-infected participants underlines the persisting difficulty of characterizing *Mansonella* infections in a non-clinical setting. Apart from improving molecular diagnostic tools, standardized testing and ideally a non-infected control group from the same environment are necessary to further investigate the clinical presentation of *Mansonella* infections. Reliable species differentiation could also help characterize *Wolbachia* carriage in *Mansonella* sp. “DEUX”, which has implications on *Mansonella* treatment with Doxycyline.

As further supported with the results from this thesis which support the existence of *Mansonella* sp. “DEUX” as a distinct *Mansonella* species, it is possible that other previously undiscovered *Mansonella* species exist. Thus, the underreporting of *Mansonella* infections in areas where molecular methods specific to *M. perstans* are used is probable, which in turn impacts our current knowledge of *Mansonella* epidemiology. The development of diagnostic methods is necessary for a better assessment of *Mansonella* epidemiology, which could help recognize the direct and indirect burden of disease from mansonellosis.

5.5 Conclusion from this thesis

The primary objective of this thesis was to investigate genetic differences between *M. perstans* and *Mansonella* sp. “DEUX” in three marker regions: *12S* rDNA, *cox1* and *28S* rDNA. The genetic differences found in this thesis characterize *M. perstans* and *Mansonella* sp. “DEUX” as two separate species. They also validate the widely used *ITS1* region as suitable for species differentiation. While distinct SNP patterns between both species could be found in all three investigated regions, the *cox1* region was the most polymorphic region followed by the *12S* rDNA region, while the *28S* rDNA region was highly conserved. Within the species, the *cox1* region was again the most polymorphic region followed by the *12S* rDNA region, while the *28S* rDNA region was highly

conserved. No association of certain genotypes with geographical location or host species could be observed. The construction of phylogenetic trees supported the species delimitation through predominantly distinct clustering of both species in all three regions, and the *cox1* region was shown to be highly robust to alterations in the phylogenetic models.

To further solidify the status of *Mansonella* sp. “DEUX” as a distinct species apart from its molecular characterization, characteristics it presumably shares with *M. perstans* such as pathogenicity, host species and the *Culicoides* vector should be investigated. Further research into genetical epidemiology, the carriage of *Wolbachia* and clinical importance of mansonellosis is necessary to characterize the impact of infections with *M. perstans* and *Mansonella* sp. “DEUX” and its treatment for millions of people at risk.

6 Summary

M. perstans is a blood-dwelling human filarial parasite widely endemic on the African continent. Recently, a potential new *Mansonella* species has been discovered in Gabon: *Mansonella sp. "DEUX".* In this thesis, genetic differences between *M. perstans* and *Mansonella* sp. "DEUX" were investigated and compared to explore the delimitation of *Mansonella* sp. "DEUX" as its own species.

For this purpose, blood samples from mono-infected participants were collected in Gabon. Species were designated as *M. perstans* or *Mansonella* sp. "DEUX" by *ITS1* qPCR. PCRs were optimized to amplify three selected gene regions: *12S* rDNA, *cox1*, and *28S* rDNA. A total of 84 samples could be used for amplification.

PCR optimization depended majorly on suitable primers and their optimal annealing temperature. The investigation of SNPs between species revealed distinct SNP patterns between *M. perstans* and *Mansonella* sp. "DEUX" in all three regions which were congruent with their species designation by *ITS1* qPCR. Inter- and intraspecific divergences between the two investigated species were comparable to other species of the onchocercid family. While the *cox1* region in *M. perstans* had individual genotypes, the other regions showed predominantly a major genotype and several minor genotypes, which were shared regardless of country of origin or host species of the parasite, as determined by the comparison with previously published sequences. Clustering according to species in phylogenetic trees could be observed in all regions. Overall, the *cox1* region was considered the most suitable region for species differentiation, as has been observed in other onchocercid species.

Results found in this thesis support the delimitation of *M. perstans* and *Mansonella* sp. "DEUX" as distinct species within the onchocercid family and the use of PCRs targeting the *ITS1* region to distinguish them. Further research into pathogenicity is necessary not only for further species delimitation but also for better characterization of mansonellosis.

7 Zusammenfassung

M. perstans ist eine im Blut lebende Filarianart, die auf dem afrikanischen Kontinent endemisch ist. Vor kurzem wurde in Gabun eine potenzielle neue *Mansonella*-Spezies entdeckt: *Mansonella* sp. "DEUX". In dieser Arbeit wurden die genetischen Unterschiede zwischen *M. perstans* und *Mansonella* sp. "DEUX" analysiert, um die Abgrenzung von *Mansonella* sp. "DEUX" als eigene Art zu untersuchen.

Zu diesem Zweck wurden in Gabun Blutproben von mono-infizierten Teilnehmern entnommen. Die Unterscheidung zwischen *M. perstans* oder *Mansonella* sp. "DEUX" wurde anhand einer qPCR der *ITS1*-Region getroffen. PCRs wurden optimiert, um drei ausgewählte Genregionen zu amplifizieren: *12S* rDNA, *cox1* und *28S* rDNA. Insgesamt konnten 84 Proben für die Amplifikation verwendet werden.

Die PCR-Optimierung hing in erster Linie von den geeigneten Primern und deren optimaler Annealing-Temperatur ab. Kongruent mit ihrer Bestimmung als *M. perstans* oder *Mansonella* sp. "DEUX" durch die *ITS1*-PCR konnten in allen drei Regionen unterschiedliche SNP-Muster gezeigt werden. Die inter- und intraspezifische Divergenz zwischen den beiden untersuchten Arten war vergleichbar mit anderen Arten der Onchocercid-Familie. Während die *cox1*-Region bei *M. perstans* individuelle Genotypen aufwies, zeigten die anderen Regionen überwiegend einen Haupt-Genotyp und mehrere kleinere Genotypen, die unabhängig von Herkunftsland oder Wirtsart des Parasiten waren, auch im Vergleich mit vorher publizierten Sequenzen. In allen Regionen konnte eine Clusterbildung nach Arten in phylogenetischen Bäumen beobachtet werden. Insgesamt wurde die *cox1*-Region als die am besten geeignete Region für die Artdifferenzierung angesehen, wie dies auch bei anderen Onchocercid-Arten beobachtet wurde.

Die in dieser Arbeit gefundenen Ergebnisse unterstützen die Unterscheidung von *M. perstans* und *Mansonella* sp. "DEUX" als unterschiedliche Arten innerhalb der Onchocercid-Familie und die Verwendung von *ITS1*-basierten PCRs, um sie zu unterscheiden. Weitere Untersuchungen zur Pathogenität sind nicht nur zur

weiteren Abgrenzung der Arten, sondern auch zur besseren Charakterisierung der Mansonellose erforderlich.

8 Bibliography

Aldhebiani, A. Y. (2018). Species concept and speciation. *Saudi J Biol Sci, 25*(3), 437-440. doi:10.1016/j.sjbs.2017.04.013

Alhassan, A., Osei-Atweneboana, M. Y., Kyeremeh, K. F., Poole, C. B., Li, Z., Tettevi, E., . . . Carlow, C. K. (2016). Comparison of a new visual isothermal nucleic acid amplification test with PCR and skin snip analysis for diagnosis of onchocerciasis in humans. *Mol Biochem Parasitol, 210*(1-2), 10-12. doi:10.1016/j.molbiopara.2016.07.006

Anderson, R. M., & May, R. M. (1991). *Infectious diseases of humans: dynamics and control*: Oxford university press.

Asgeirsson, H., Harling, A., & Botero-Kleiven, S. (2017). Successful treatment of 2 imported cases of Mansonella perstans infection. *PLoS Negl Trop Dis, 11*(5), e0005452. doi:10.1371/journal.pntd.0005452

Asio, S. M., Simonsen, P. E., & Onapa, A. W. (2009). Analysis of the 24-h microfilarial periodicity of Mansonella perstans. *Parasitology research, 104*(4), 945-948.

Asio, S. M., Simonsen, P. E., & Onapa, A. W. (2009). Mansonella perstans: safety and efficacy of ivermectin alone, albendazole alone and the two drugs in combination. *Annals of Tropical Medicine & Parasitology, 103*(1), 31-37. doi:10.1179/136485909X384929

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. doi:10.11646/zootaxa.3918.2.1

Baird, J. K., Neafie, R. C., & Connor, D. H. (1988). Nodules in the conjunctiva, bung-eye, and bulge-eye in Africa caused by Mansonella perstans. *Am J Trop Med Hyg, 38*(3), 553-557. doi:10.4269/ajtmh.1988.38.553

Baird, J. K., Neafie, R. C., Lanoie, L., & Connor, D. H. (1987). Adult Mansonella perstans in the Abdominal Cavity in Nine Africans. *The American Journal of Tropical Medicine and Hygiene, 37*(3), 578-584. doi:10.4269/ajtmh.1987.37.578

Bamuhiiga, J. T. (1998). Mansonella streptocerca: another filarial worm in the skin in Western Uganda. *Community Eye Health, 11*(26), 28.

Bartoloni, A., Cancrini, G., Bartalesi, F., Marcolin, D., Roselli, M., Arce, C. C., & Hall, A. J. (1999). Mansonella ozzardi infection in Bolivia: prevalence and clinical associations in the Chaco region. *Am J Trop Med Hyg, 61*(5), 830-833. doi:10.4269/ajtmh.1999.61.830

Basano Sde, A., Fontes, G., Medeiros, J. F., Aranha Camargo, J. S., Souza Vera, L. J., Parente Araújo, M. P., . . . Aranha Camargo, L. M. (2014). Sustained clearance of Mansonella ozzardi infection after treatment with ivermectin in the Brazilian Amazon. *Am J Trop Med Hyg, 90*(6), 1170-1175. doi:10.4269/ajtmh.13-0410

Batsa Debrah, L., Phillips, R. O., Pfarr, K., Klarmann-Schulz, U., Opoku, V. S., Nausch, N., . . . Hoerauf, A. (2019). The Efficacy of Doxycycline Treatment on Mansonella perstans Infection: An Open-Label, Randomized Trial in Ghana. *Am J Trop Med Hyg, 101*(1), 84-92. doi:10.4269/ajtmh.18-0491

Binkienė, R., Chagas, C. R. F., Bernotienė, R., & Valkiūnas, G. (2021). Molecular and morphological characterization of three new species of avian Onchocercidae

(Nematoda) with emphasis on circulating microfilariae. *Parasites & Vectors, 14*(1), 137. doi:10.1186/s13071-021-04614-8

Black, W., & Moore, C. (2005). Biology of disease vectors. In: Marquardt WC: Burilington, MA: Elsevier Academic Press.

Borkow, G., & Bentwich, Z. (2000). Eradication of helminthic infections may be essential for successful vaccination against HIV and tuberculosis. *Bull World Health Organ, 78*(11), 1368-1369.

Borkow, G., & Bentwich, Z. (2008). Chronic parasite infections cause immune changes that could affect successful vaccination. *Trends Parasitol, 24*(6), 243-245. doi:10.1016/j.pt.2008.02.009

Bregani, E. R., Rovellini, A., Mbaïdoum, N., & Magnini, M. G. (2006). Comparison of different anthelminthic drug regimens against Mansonella perstans filariasis. *Trans R Soc Trop Med Hyg, 100*(5), 458-463. doi:10.1016/j.trstmh.2005.07.009

Campillo, J. T., Louya, F., Bikita, P., Missamou, F., Pion, S. D. S., Boussinesq, M., & Chesnais, C. B. (2022). Factors associated with the periodicity of Loa loa microfilaremia in the Republic of the Congo. *Parasites & Vectors, 15*(1), 417. doi:10.1186/s13071-022-05541-y

Casiraghi, M., Anderson, T. J., Bandi, C., Bazzocchi, C., & Genchi, C. (2001). A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of Wolbachia endosymbionts. *Parasitology, 122 Pt 1*, 93-103. doi:10.1017/s0031182000007149

CDC. (04.08.2022). Mansonellosis. Retrieved from https://www.cdc.gov/dpdx/mansonellosis/index.html

Chung, M., Aluvathingal, J., Bromley, R. E., Nadendla, S., Fombad, F. F., Kien, C. A., . . . Dunning Hotopp, J. C. (2020). Complete Mitochondrial Genome Sequence of Mansonella perstans. *Microbiol Resour Announc, 9*(30). doi:10.1128/mra.00490-20

Cook, G. C., & Zumla, A. (2008). *Manson's tropical diseases*: Elsevier Health Sciences.

Coulibaly, Y. I., Dembele, B., Diallo, A. A., Lipner, E. M., Doumbia, S. S., Coulibaly, S. Y., . . . Klion, A. D. (2009). A randomized trial of doxycycline for Mansonella perstans infection. *N Engl J Med, 361*(15), 1448-1458. doi:10.1056/NEJMoa0900863

Cox, A. J., & Hebert, P. D. (2001). Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. *Mol Ecol, 10*(2), 371-386. doi:10.1046/j.1365-294x.2001.01188.x

Crainey, J., Costa, C., Leles, L., Silva, T., Narzetti, L., Santos, Y., . . . Luz, S. (2020). Deep Sequencing Reveals Occult Mansonellosis Coinfections in Residents From the Brazilian Amazon Village of São Gabriel da Cachoeira. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71*. doi:10.1093/cid/ciaa082

Crainey, J. L., Ribeiro da Silva, T. R., & Luz, S. L. B. (2016). Historic accounts of Mansonella parasitaemias in the South Pacific and their relevance to lymphatic filariasis elimination efforts today. *Asian Pacific Journal of Tropical Medicine, 9*(3), 205-210. doi:https://doi.org/10.1016/j.apjtm.2016.01.040

Dolo, H., Coulibaly, Y. I., Dembele, B., Konate, S., Coulibaly, S. Y., Doumbia, S. S., . . . Klion, A. D. (2012). Filariasis attenuates anemia and proinflammatory responses associated with clinical malaria: a matched prospective study in children and young adults. *PLoS Negl Trop Dis, 6*(11), e1890. doi:10.1371/journal.pntd.0001890

Downes, B. L., & Jacobsen, K. H. (2010). A Systematic Review of the Epidemiology of Mansonelliasis. *Afr J Infect Dis, 4*(1), 7-14. doi:10.4314/ajid.v4i1.55085

Dukes, D. C., Gelfand, M., Gadd, K. G., Clarke, V. D., & Goldsmid, J. M. (1968). Cerebral filariasis caused by acanthocheilonema perstans. *Cent Afr J Med, 14*(2), 21-27.

Farell, E. M., & Alexandre, G. (2012). Bovine serum albumin further enhances the effects of organic solvents on increased yield of polymerase chain reaction of GC-rich templates. *BMC Res Notes, 5*, 257. doi:10.1186/1756-0500-5-257

Ferreira, M. U., Crainey, J. L., & Gobbi, F. G. (2023). The search for better treatment strategies for mansonellosis: an expert perspective. *Expert Opinion on Pharmacotherapy, 24*(15), 1685-1692. doi:10.1080/14656566.2023.2240235

Ferri, E., Bain, O., Barbuto, M., Martin, C., Lo, N., Uni, S., . . . Casiraghi, M. (2011). New insights into the evolution of Wolbachia infections in filarial nematodes inferred from a large range of screened species. *PLoS One, 6*(6), e20843. doi:10.1371/journal.pone.0020843

Ferri, E., Barbuto, M., Bain, O., Galimberti, A., Uni, S., Guerrero, R., . . . Casiraghi, M. (2009). Integrated taxonomy: traditional approach and DNA barcoding for the identification of filarioid worms and related parasites (Nematoda). *Front Zool, 6*, 1. doi:10.1186/1742-9994-6-1

Ferri, G., Alù, M., Corradini, B., Licata, M., & Beduschi, G. (2009). Species identification through DNA "barcodes". *Genet Test Mol Biomarkers, 13*(3), 421-426. doi:10.1089/gtmb.2008.0144

Fischer, P., Bamuhiiga, J., & Büttner, D. W. (1997). Treatment of human Mansonella streptocerca infection with ivermectin. *Trop Med Int Health, 2*(2), 191-199. doi:10.1046/j.1365-3156.1997.d01-233.x

Fischer, P., Büttner, D. W., Bamuhiiga, J., & Williams, S. A. (1998). Detection of the filarial parasite Mansonella streptocerca in skin biopsies by a nested polymerase chain reaction-based assay. *Am J Trop Med Hyg, 58*(6), 816-820. doi:10.4269/ajtmh.1998.58.816

Fischer, P., Tukesiga, E., & Büttner, D. W. (1999). Long-term suppression of Mansonella streptocerca microfilariae after treatment with ivermectin. *J Infect Dis, 180*(4), 1403-1405. doi:10.1086/315014

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol, 3*(5), 294-299.

Fux, C. A., Chappuis, B., Holzer, B., Aebi, C., Bordmann, G., Marti, H., & Hatz, C. (2006). Mansonella perstans causing symptomatic hypereosinophilia in a missionary family. *Travel Med Infect Dis, 4*(5), 275-280. doi:10.1016/j.tmaid.2005.07.001

Gaillard, C. M., Pion, S. D., Hamou, H., Sirima, C., Bizet, C., Lemarcis, T., . . . Locatelli, S. (2020). Detection of DNA of filariae closely related to Mansonella perstans in

faecal samples from wild non-human primates from Cameroon and Gabon. *Parasites & Vectors, 13*(1), 313. doi:10.1186/s13071-020-04184-1

Garrido, C., & Campos, M. (2000). First report of presumed parasitic keratitis in Indians from the Brazilian Amazon. *Cornea, 19*(6), 817-819. doi:10.1097/00003226-200011000-00011

Gehringer, C., Kreidenweiss, A., Flamen, A., Antony, J. S., Grobusch, M. P., & Bélard, S. (2014). Molecular Evidence of Wolbachia Endosymbiosis in Mansonella perstans in Gabon, Central Africa. *The Journal of Infectious Diseases, 210*(10), 1633-1638. doi:10.1093/infdis/jiu320

Grobusch, M. P., Kombila, M., Autenrieth, I., Mehlhorn, H., & Kremsner, P. G. (2003). No evidence of Wolbachia endosymbiosis with Loa loa and Mansonella perstans. *Parasitol Res, 90*(5), 405-408. doi:10.1007/s00436-003-0872-z

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology, 59*(3), 307-321. doi:10.1093/sysbio/syq010

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proc Biol Sci, 270*(1512), 313-321. doi:10.1098/rspb.2002.2218

Hedtke, S. M., Kuesel, A. C., Crawford, K. E., Graves, P. M., Boussinesq, M., Lau, C. L., . . . Grant, W. N. (2019). Genomic Epidemiology in Filarial Nematodes: Transforming the Basis for Elimination Program Decisions. *Front Genet, 10*, 1282. doi:10.3389/fgene.2019.01282

Higgs, S., Beaty, B., & Marquardt, W. (2005). Biology of disease vectors.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H. (2008). How many species are infected with Wolbachia?--A statistical analysis of current data. *FEMS Microbiol Lett, 281*(2), 215-220. doi:10.1111/j.1574-6968.2008.01110.x

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*(8), 754-755. doi:10.1093/bioinformatics/17.8.754

Jiménez, M., González, L. M., Carranza, C., Bailo, B., Pérez-Ayala, A., Muro, A., . . . Gárate, T. (2011). Detection and discrimination of Loa loa, Mansonella perstans and Wuchereria bancrofti by PCR-RFLP and nested-PCR of ribosomal DNA ITS1 region. *Exp Parasitol, 127*(1), 282-286. doi:10.1016/j.exppara.2010.06.019

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods, 14*(6), 587-589. doi:10.1038/nmeth.4285

Keiser, P. B., Coulibaly, Y., Kubofcik, J., Diallo, A. A., Klion, A. D., Traoré, S. F., & Nutman, T. B. (2008). Molecular identification of Wolbachia from the filarial nematode Mansonella perstans. *Mol Biochem Parasitol, 160*(2), 123-128. doi:10.1016/j.molbiopara.2008.04.012

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci U S A, 86*(16), 6196-6200. doi:10.1073/pnas.86.16.6196

Kockum, I., Huang, J., & Stridh, P. (2023). Overview of Genotyping Technologies and Methods. *Current Protocols, 3*(4), e727. doi:https://doi.org/10.1002/cpz1.727
Krueger, A., Fischer, P., & Morales-Hojas, R. (2007). Molecular phylogeny of the filaria genus Onchocerca with special emphasis on Afrotropical human and bovine parasites. *Acta Tropica, 101*(1), 1-14. doi:https://doi.org/10.1016/j.actatropica.2006.11.004
Kwok, S., Kellogg, D. E., McKinney, N., Spasic, D., Goda, L., Levenson, C., & Sninsky, J. J. (1990). Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. *Nucleic Acids Res, 18*(4), 999-1005. doi:10.1093/nar/18.4.999
Lefoulon, E., Bain, O., Bourret, J., Junker, K., Guerrero, R., Canizales, I., . . . Martin, C. (2015). Shaking the Tree: Multi-locus Sequence Typing Usurps Current Onchocercid (Filarial Nematode) Phylogeny. *PLoS Negl Trop Dis, 9*(11), e0004233. doi:10.1371/journal.pntd.0004233
Lefoulon, E., Giannelli, A., Makepeace, B. L., Mutafchiev, Y., Townson, S., Uni, S., . . . Martin, C. (2017). Whence river blindness? The domestication of mammals and host-parasite co-evolution in the nematode genus Onchocerca. *International Journal for Parasitology, 47*(8), 457-470. doi:https://doi.org/10.1016/j.ijpara.2016.12.009
Lima, N. F., Veggiani Aybar, C. A., Dantur Juri, M. J., & Ferreira, M. U. (2016). Mansonella ozzardi: a neglected New World filarial nematode. *Pathog Glob Health, 110*(3), 97-107. doi:10.1080/20477724.2016.1190544
Lorenz, T. C. (2012). Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *J Vis Exp*(63), e3998. doi:10.3791/3998
Manson, P. (1891). THE FILARIA SANGUINIS HOMINIS MAJOR AND MINOR, TWO NEW SPECIES OF HÆMATOZOA. *The Lancet, 137*(3514), 4-8.
Martins, M., Pessoa, F. A., de Medeiros, M. B., de Andrade, E. V., & Medeiros, J. F. (2010). Mansonella ozzardi in Amazonas, Brazil: prevalence and distribution in the municipality of Coari, in the middle Solimões River. *Mem Inst Oswaldo Cruz, 105*(3), 246-253. doi:10.1590/s0074-02762010000300002
Masters, B., Fan, V., & Ross, H. (2011). Species Delimitation - a Geneious plugin for the exploration of species boundaries. *Molecular ecology resources, 11*, 154-157. doi:10.1111/j.1755-0998.2010.02896.x
Mathison, B. A., Couturier, M. R., & Pritt, B. S. (2019). Diagnostic Identification and Differentiation of Microfilariae. *J Clin Microbiol, 57*(10). doi:10.1128/jcm.00706-19
Mayr, E. (1999). *Systematics and the origin of species from the viewpoint of a zoologist*. Cambridge, Massachusetts: Havard University Press.
McCall, P. J., Townson, H., & Trees, A. J. (1992). Morphometric differentiation of Onchocerca volvulus and O. ochengi infective larvae. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 86*(1), 63-65. doi:https://doi.org/10.1016/0035-9203(92)90443-G
Medeiros, J., Crainey, J., Pessoa, F., Luz, S., & Marcondes, C. (2017). Arthropod borne diseases.

Medeiros, J. F., Almeida, T. A., Silva, L. B., Rubio, J. M., Crainey, J. L., Pessoa, F. A., & Luz, S. L. (2015). A field trial of a PCR-based Mansonella ozzardi diagnosis assay detects high-levels of submicroscopic M. ozzardi infections in both venous blood samples and FTA card dried blood spots. *Parasit Vectors, 8*, 280. doi:10.1186/s13071-015-0889-z

Meredith, S. E., Lando, G., Gbakima, A. A., Zimmerman, P. A., & Unnasch, T. R. (1991). Onchocerca volvulus: application of the polymerase chain reaction to identification and strain differentiation of the parasite. *Exp Parasitol, 73*(3), 335-344. doi:10.1016/0014-4894(91)90105-6

Mirzaei, M., Ghahvei, Y., Lefoulon, E., Lia, R. P., Otranto, D., Martin, C., & Sazmand, A. (2018). Morphological and molecular characterization of Onchocerca fasciata (Nematoda, Onchocercidae) from dromedary camels (Camelus dromedarius) in Iran. *Parasite, 25*, 50. doi:10.1051/parasite/2018045

Mischlinger, J., Manego, R. Z., Mombo-Ngoma, G., Ekoka Mbassi, D., Hackbarth, N., Ekoka Mbassi, F. A., . . . Ramharter, M. (2021). Diagnostic performance of capillary and venous blood samples in the detection of Loa loa and Mansonella perstans microfilaraemia using light microscopy. *PLoS Negl Trop Dis, 15*(8), e0009623. doi:10.1371/journal.pntd.0009623

Moraes, M. A., Shelley, A. J., Dias, A. P., & Silva, C. J. (1983). The concentration of Mansonella ozzardi microfilariae in skin capillaries. *Trans R Soc Trop Med Hyg, 77*(4), 463-466. doi:10.1016/0035-9203(83)90113-x

Morales-Hojas, R., Post, R. J., Shelley, A. J., Maia-Herzog, M., Coscarón, S., & Cheke, R. A. (2001). Characterisation of nuclear ribosomal DNA sequences from Onchocerca volvulus and Mansonella ozzardi (Nematoda: Filarioidea) and development of a PCR-based method for their detection in skin biopsies. *International Journal for Parasitology, 31*(2), 169-177. doi:https://doi.org/10.1016/S0020-7519(00)00156-9

Mourembou, G., Fenollar, F., Lekana-Douki, J. B., Ndjoyi Mbiguino, A., Maghendji Nzondo, S., Matsiegui, P. B., . . . Mediannikov, O. (2015). Mansonella, including a Potential New Species, as Common Parasites in Children in Gabon. *PLOS Neglected Tropical Diseases, 9*(10), e0004155. doi:10.1371/journal.pntd.0004155

Mulyani, Y., Rosidah. (2022). Internal Transcribed Spacer (ITS) Region as Molecular Marker for Parasite Identification in Aquaculture: A Review. *Asian Journal of Fisheries and Aquatic Research, 19*(1), 9-17. doi:10.9734/ajfar/2022/v19i130463

Noma, M., Zouré, H. G., Tekle, A. H., Enyong, P. A., Nwoke, B. E., & Remme, J. H. (2014). The geographic distribution of onchocerciasis in the 20 participating countries of the African Programme for Onchocerciasis Control: (1) priority areas for ivermectin treatment. *Parasit Vectors, 7*, 325. doi:10.1186/1756-3305-7-325

Norice-Tra, C. T., Ribeiro, J., Bennuru, S., Fay, M. P., Tyagi, R., Mitreva, M., & Nutman, T. B. (2017). Insights Into Onchocerca volvulus Population Biology Through Multilocus Immunophenotyping. *J Infect Dis, 216*(6), 736-743. doi:10.1093/infdis/jix394

Ogunrinade, A., Boakye, D., Merriweather, A., & Unnasch, T. R. (1999). Distribution of the blinding and nonblinding strains of Onchocerca volvulus in Nigeria. *J Infect Dis, 179*(6), 1577-1579. doi:10.1086/314784

Osei-Atweneboana, M. Y., Boakye, D. A., Awadzi, K., Gyapong, J. O., & Prichard, R. K. (2012). Genotypic analysis of β-tubulin in Onchocerca volvulus from communities and individuals showing poor parasitological response to ivermectin treatment. *Int J Parasitol Drugs Drug Resist, 2*, 20-28. doi:10.1016/j.ijpddr.2012.01.005

Patra, K. P., Ramu, T., Hoti, S. L., Pragasam, G. S., & Das, P. K. (2007). Identification of a molecular marker for genotyping human lymphatic filarial nematode parasite Wuchereria bancrofti. *Experimental Parasitology, 116*(1), 59-65. doi:https://doi.org/10.1016/j.exppara.2006.11.011

Pilotte, N., Thomas, T., Zulch, M. F., Sirois, A. R., Minetti, C., Reimer, L. J., . . . Saunders, L. J. (2022). Targeting a highly repetitive genomic sequence for sensitive and specific molecular detection of the filarial parasite Mansonella perstans from human blood and mosquitoes. *PLoS Negl Trop Dis, 16*(12), e0010615. doi:10.1371/journal.pntd.0010615

Poole, C. B., Li, Z., Alhassan, A., Guelig, D., Diesburg, S., Tanner, N. A., . . . Carlow, C. K. (2017). Colorimetric tests for diagnosis of filarial infection and vector surveillance using non-instrumented nucleic acid loop-mediated isothermal amplification (NINA-LAMP). *PLoS One, 12*(2), e0169011. doi:10.1371/journal.pone.0169011

Raele, D. A., Pugliese, N., Galante, D., Latorre, L. M., & Cafiero, M. A. (2016). Development and Application of a Loop-Mediated Isothermal Amplification (LAMP) Approach for the Rapid Detection of Dirofilaria repens from Biological Samples. *PLoS Negl Trop Dis, 10*(6), e0004789. doi:10.1371/journal.pntd.0004789

Richard-Lenoble, D., Kombila, M., Bain, O., Chandenier, J., & Mariotte, O. (1988). Filariasis in Gabon: human infections with Microfilaria rodhaini. *Am J Trop Med Hyg, 39*(1), 91-92. doi:10.4269/ajtmh.1988.39.91

Rodi, M., Gross, C., Sandri, T. L., Berner, L., Marcet-Houben, M., Kocak, E., . . . Held, J. (2023). Whole genome analysis of two sympatric human Mansonella: Mansonella perstans and Mansonella sp “DEUX”. *Frontiers in Cellular and Infection Microbiology, 13*. doi:10.3389/fcimb.2023.1159814

Sandri, T. L., Kreidenweiss, A., Cavallo, S., Weber, D., Juhas, S., Rodi, M., . . . Held, J. (2020). Molecular Epidemiology of Mansonella Species in Gabon. *The Journal of Infectious Diseases, 223*(2), 287-296. doi:10.1093/infdis/jiaa670

Simonsen, P. E., Onapa, A. W., & Asio, S. M. (2011). Mansonella perstans filariasis in Africa. *Acta Tropica, 120*, S109-S120. doi:https://doi.org/10.1016/j.actatropica.2010.01.014

Sinha A, L. Z., Poole CB, Morgan RD, Ettwiller L, Lima NF, Ferreira MU,, & Fombad FF, W. S. a. C. C. (2023). Genomes of the human filarial parasites *Mansonella perstans* and *Mansonella ozzardi*. *Front. Trop. Dis, 4*. doi:doi: 10.3389/fitd.2023.1139343

Staff, T. S. B. T. B. (2019, 25.09.2019). PCR Primer Design Tips. Retrieved from https://www.thermofisher.com/blog/behindthebench/pcr-primer-design-tips/#:~:text=Here%20are%20some%20guidelines%20for,the%20stability%20of%20the%20primer.

systems, B. o. l. d. Retrieved from http://www.boldsystems.org/index.php. http://www.boldsystems.org/index.php

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*, 9-24. doi:10.2147/rrtm.S125750

Tang, T. H., López-Vélez, R., Lanza, M., Shelley, A. J., Rubio, J. M., & Luz, S. L. (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), 823-828. doi:10.1590/s0074-02762010000600016

Tavares da Silva, L. B., Crainey, J. L., Ribeiro da Silva, T. R., Suwa, U. F., Vicente, A. C., Fernandes de Medeiros, J., . . . Luz, S. L. (2017). Molecular Verification of New World Mansonella perstans Parasitemias. *Emerg Infect Dis, 23*(3), 545-547. doi:10.3201/eid2303.161159

Van Valen, L. (1976). ECOLOGICAL SPECIES, MULTISPECIES, AND OAKS. *TAXON, 25*(2-3), 233-239. doi:https://doi.org/10.2307/1219444

Vianna, L. M., Martins, M., Cohen, M. J., Cohen, J. M., & Belfort, R., Jr. (2012). Mansonella ozzardi corneal lesions in the Amazon: a cross-sectional study. *BMJ Open, 2*(6). doi:10.1136/bmjopen-2012-001266

Wanji, S., Eyong, E. J., Tendongfor, N., Ngwa, C. J., Esuka, E. N., Kengne-Ouafo, A. J., . . . Mackenzie, C. D. (2017). Ivermectin treatment of Loa loa hyper-microfilaraemic baboons (Papio anubis): Assessment of microfilarial load reduction, haematological and biochemical parameters and histopathological changes following treatment. *PLoS Negl Trop Dis, 11*(7), e0005576. doi:10.1371/journal.pntd.0005576

Wares, J. P., & Cunningham, C. W. (2001). Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution, 55*(12), 2455-2469. doi:10.1111/j.0014-3820.2001.tb00760.x

Woldearegai, T. G., Lalremruata, A., Nguyen, T. T., Gmeiner, M., Veletzky, L., Tazemda-Kuitsouc, G. B., . . . Held, J. (2019). Characterization of Plasmodium infections among inhabitants of rural areas in Gabon. *Scientific reports, 9*(1), 9784. doi:10.1038/s41598-019-46194-9

Xie, H., Bain, O., & Williams, S. A. (1994). Molecular phylogenetic studies on filarial parasites based on 5S ribosomal spacer sequences. *Parasite, 1*(2), 141-151. doi:10.1051/parasite/1994012141

Yilmaz, E., Fritzenwanker, M., Pantchev, N., Lendner, M., Wongkamchai, S., Otranto, D., . . . Krücken, J. (2016). The Mitochondrial Genomes of the Zoonotic Canine Filarial Parasites Dirofilaria (Nochtiella) repens and Candidatus Dirofilaria (Nochtiella) hongkongensis Provide Evidence for Presence of Cryptic Species. *PLoS Negl Trop Dis, 10*(10), e0005028. doi:10.1371/journal.pntd.0005028

9 Erklärung zum Eigenanteil

Die Arbeit wurde im Institut für Tropenmedizin Tübingen unter Betreuung von Prof. Dr. Peter Kremsner und Dr. Jana Held durchgeführt.

Die Konzeption der MANREF-2 Studie geschah durch Dr. Jana Held und Capucine Sicard. Die Sammlung der Blutproben aus dieser Studie, deren DNA-Extraktion sowie die Befragung der Probanden erfolgte ausschließlich durch mich. Sammlung und DNA-Extraktion der Proben aus anderen Studien erfolgte durch andere Mitarbeitende des Instituts.

Sämtliche Versuche bezüglich der PCR-Optimierung, Anwendung der optimierten Protokolle und Vorbereitung für die Sequenzierung wurden nach Einarbeitung durch Miriam Rodi, Martha Salinas-Medina und David Weber von mir eigenständig durchgeführt. Die Primer wurden wie gekennzeichnet aus Veröffentlichungen übernommen und sonst mithilfe des geneious prime design tool von mir gestaltet, mit Ausnahme des *12S* rDNA Pan Filaria Fwd primers und des *12S* rDNA Mansonella spp. Fwd primers, welche von anderen Mitgliedern des Instituts kreiert wurden. Sequenzierung erfolgte durch die Firma eurofins genomics.

Die phylogenetische Auswertung erfolgte eigenständig durch mich.

Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Bei der Abfassung der Dissertation sind Rechte Dritter nicht verletzt worden. Ich habe diese Dissertation bisher an keiner in- oder ausländischen Hochschule zur Promotion eingereicht.

Tübingen, den 30.04.2024

Mara Fischer

10 Acknowledgements

My deepest appreciation belongs to Dr. Jana Held for her supervision, invaluable feedback and for allowing me to join the MANREF-2 study in Gabon, which was not only an incredible research opportunity but also a cherished adventure.

Furthermore, this project would not have been possible without the unwavering and uplifting support of Miriam Rodi. I am deeply grateful for the guidance I received from Martha Salinas-Medina and David Weber. Additionally, I am immensely thankful to Capucine Sicard, who shared not only her immense knowledge but also the MANREF-2 journey with me. Moreover, deserving of my deepest gratitude are my participants and the QIAxcel. I would also like to thank Pia Springmann for her valuable editing help.

Many thanks to the entire AG Held and the Institute for Tropical Medicine for supporting and guiding me in my endeavours. especially to Tina Krüger and Katarzyna Kawecka in Tübingen and Esther Mehmel, Eva Danner and Maxim Viehweg in Lambaréné. My sincere thanks also to my parents Evelyn and Martin, my sister Tessa, my grandparents, Oskar, Lilli, and my friends, whose unconditional support, motivation and belief in me and my goals were invaluable.

Finally, I would like to thank Cosima for setting my feet on this path and walking it with me. I do not know where or who I would be today without you.

11 Supplementary files

11.1 Clincal presentation

11.1.1 Questionnaire for study participants

Initiales de l'enquêteur.trice :

Questionnaire patient
Nom de l'étude : *Mansonella species reference genomes* (MANREF-2)

1) Numéro du patient : ____________________
2) Date de l'échantillonnage : ____________________
3) Lieu d'habitation du patient : ____________________
4) Données GPS : ____________________
5) Sexe :
   - □ Homme
   - □ Femme
6) Age : ____________________
7) Numéro de téléphone : ____________________
8) Profession : ____________________
9) Le patient part-il/elle régulièrement en brousse/champs/plantations ? □ Non □ Oui
10) Combien de fois par jour le patient est-il/elle piqué par des fourous ?
    - □ Tout le temps (plus de 50 par jour)
    - □ Souvent (entre 10 et 50 fois par jour)
    - □ Parfois (moins de 10 fois)
    - □ Jamais
11) Le patient présente-t-il un ou plusieurs des symptômes suivants ? Commentaires (depuis quand, à quelle fréquence, pourquoi)
    - Démangeaisons/gratti-gratta
      - Tout le temps
      - Souvent
      - Parfois
      - Jamais
    - Fatigue
      - Tout le temps
      - Souvent
      - Parfois
      - Jamais
    - Éruption(s) cutanée(s) (plaques de boutons)
      - Tout le temps
      - Souvent
      - Parfois
      - Jamais
    - Maux de tête
      - Tout le temps
      - Souvent
      - Parfois
      - Jamais
    - Maux de ventre
      - Tout le temps
      - Souvent
      - Parfois
      - Jamais
    - Douleurs articulaires
      - Tout le temps
      - Souvent
      - Parfois
      - Jamais
    - Gonflement(s) sous-cutané(s) (nodules)
      - Tout le temps
      - Souvent
      - Parfois

Etude MANREF-2 version 21.03.23 1/2

Initiales de l'enquêteur.trice :

      - Jamais
    - Symptômes oculaires (liés au filaires dans l'oeil)
      - Oui
      - Non
12) Est-il/elle atteint(e) d'une autre maladie, à sa connaissance? □ Non □ Oui, laquelle ? __________
13) À quel point les problèmes de peau ont-ils affecté la vie du patient au cours de la dernière semaine?
    - Énormément
    - Beaucoup
    - Un peu
    - Pas du tout
14) Le patient est-il atteint par un filaire (diagnostiqué par un médecin)?
    - □ Oui
    - □ Non

    Si oui, lequel/lesquels ? ____________________
15) Le patient a-t-il récemment reçu un traitement contre les filaires cette année (e.g. ivermectine, albendazole, mebendazole, traitement traditionnel) ?
    - □ Oui
    - □ Non

    Si oui, quel traitement ? Pendant combien de temps ?____________________
16) Le patient a-t-il récemment (moins d'un an) voyagé en dehors de la région d'habitation ?
    - □ Oui
    - □ Non

    Si oui, où ? ____________________
17) Le patient vit-il en présence d'animaux (chiens, chats, rats..) ?
    - □ Oui
    - □ Non

    Si oui, lesquels ?____________________
18) Quelles sont les conditions d'hébergement du patient ?
    - □ Pas d'accès à l'eau courante
    - □ Pas de mise à niveau du sol
    - □ Pas d'accès à l'électricité
    - □ Pas de toilettes à chasse d'eau / toilettes à compost / toilettes chimiques
    - □ Toit en matériau végétal ou en taule

Etude MANREF-2 version 21.03.23 2/2

Figure 27:Questionnaire for study participants containing basic demographic data and clinical symptoms

11.1.2 Clinical symptoms as reported by participants

Symptoms reported by participants of MANREF-2

CONTROL GROUP (n = 2)

	filarial infection			Gender	Occupation	Age	Symptoms							
	M.perstans	*Mansonella sp. "DEUX"*	*L. loa*				arthralgia	itching	reduced visus	abdominal complaints	headache	rash	nodules	fatigue
B3	0	0	0	0	0	59	1	1	1	0	1	0	0	0
B8	0	0	0	0	1	83	1	1	1	1	0	0	0	0

FILARIENGRUPPE (n = 3)

	filarial infection			Gender	Occupation	Age	Symptoms							
	M.perstans	*Mansonella sp. "DEUX"*	*L. loa*				arthralgia	itching	reduced visus	abdominal complaints	headache	rash	nodules	fatigue
B2	0	1	1	0	0	62	1	1	1	0	0	0	0	0
B5	0	1	1	0	1	60	1	1	1	1	1	1	1	1
B12	0	0	1	0	1	mittelalt?	1	0	0	0	0	0	0	0

MANSONELLA-GRUPPE (n = 7)

	filarial infection			Gender	Occupation	Age	Symptoms							
	M.perstans	*Mansonella sp. "DEUX"*	*L. loa*				arthralgia	itching	reduced visus	abdominal complaints	headache	rash	nodules	fatigue
B1	1	0	0	1	2	49	1	1	0	1	0	1	1	0
B10	1	0	0	1	1	43	1	1	0	1	1	0	0	1
B4	0	1	0	0	0	59	1	1	1	0	0	1	1	0
B6	0	1	0	0	3	19	0	0	0	0	0	1	0	1
B7	0	1	0	1	3	18	1	1	1	0	1	1	1	0
B9	0	1	0	1	1	48	1	0	1	0	1	1	0	0
B11	1	1	0	1	1	37	1	0	0	0	1	0	0	0

0 = negative 0 = negative 0 = negative 0 = male 0 = village representative 0 = not reported
1 = infected 1 = infected 1 = infected 1 = female 1 = field or forest work 1 = reported
2 = housework
3 = student

Figure 28: Symptoms reported by participants

11.1.3 Distributions of symptoms as reported by participants

	Number of participants (and the respective percentage) indicating the symptom							
	Arthralgia	Itching	Reduced visus	Abdominal complaints	Headaches	Rash	Nodules	Fatigue
Negative (n =2)	2/2 (100)	2/2 (100)	2/2 (100)	1/2 (50)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)
Mixed filarial infections (n = 3)	3/3 (1000	2/3 (66)	2/3 (66)	1/3 (33)	1/3 (33)	1/3 (33)	1/3 (33)	1/3 (33)
Mansonella mixed infection (n =1)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)
M. perstans infections (n = 2)	2/2 (100)	2/2 (100	0/2 (0)	2/2 (100	1/2 (50)	1/2 (50)	1/2 (50)	1/2 (50)
Mansonella sp. “DEUX” infections (n = 4)	2/4 (50)	2/4 (50)	3/4 (75)	0/4 (0)	2/4 (50)	100	2/4 (50)	1/4 (25)

Table 29: distribution of clinical symptoms in participants without infection, with infection with mixed filarial species and Mansonella mono- or coinfections

11.2 Phylogenetic analysis

11.2.1 Sequences generated in this thesis

11.2.1.1 12S rDNA

Species	Sample ID	Sequence 5’ -> 3’
M. perstans	PN748	CCTCTTTTATAGTWAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTATTT-TGTAATGCTTT- ATT-----TT---TA--G--ATTAAAA- ACAGATATATATTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATG-G- TATTTT-ATTTT-TTATCTGAAATTGGAAAAAAAAGTAATTATTTTTT
Mansonella sp. “DEUX”	PN506	TTT--TGGG--KTTTTTATTTTGAT-TT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT--TT-----TT---TA--G--GTTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT
	PN289	TTT--TTGG--GKTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAAWTTWWTTTTTT-T-T--TTGAG-- AAAATTT--TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTGAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT
	PN241	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTWATAGTGAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT
	PN242	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTGAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT
	PN609	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT-

		TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAAGTAATTATTTTTT
	PN219	TTT--TTGG--GKTTTTATTTTGAT-YT-TTT--GTAAAAWATTTTAATTT-WTTTTTT-T-T--TTGAG-- AAAATTT--TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATG-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAAGTAATTATTTTTT
	PN234	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTGAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAAGTAATTATTTTTT
	PN661	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTGAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAAGTAATTATTTTTT
	PN203	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAAGTAATTATTTTTT
	PN630	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATG-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAAGTAATTATTTTTT
	PN223	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAATATTTTAATTT-WTTTTTT-T-T--TTGAG--AAAATTT- -TAAAAATTTGGATTA-

		TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATR-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT
	PN340	TTT--TTGG--GTTTTTATTTTGAT-YT-TTT--GTAAAAWATTTTAATTT-WTTTTTT-T-T--TTGAG-- AAAATTT--TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTCGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTCTGGATTTCTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATR-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT
	PN116	TTT--TTGG--GKTTTTWWTTTKRW-YT-TTT-KKWAAAATWTTTTAATTT-WTTTTTT-T-T--TTGAG-- AAAATTT--TAAAAATTTGGATTA- TTGAACTGGATTAGTACCCAGGTAATCAAAATTAATTAATTYGGGAGTAAAGTTTTGTTTAAACCGAAAA AATATTGACTGACTYTGGATTTYTTTTTGGAATATGTGTAT---- GGAGAGCCCTCTTTAATAGTAAATCTTTTTTTGCACATGTATGATTGTTTAGTTTTTATTTTGTTT- TGTAATGCTTT---T-----TT---TA--G--ATTTAAA- ACAGATATATACTTGGCTTATGGGTTTATTAATCATGTATTACTATTATA-AATTTTTTTTGGATA-G- TATTTT-ATTTT-TTATTTGAAATTGGAAAAAAAAGTAATTATTTTTT

Table 30: 12S *rDNA sequences generated in this thesis*

11.2.1.2 *cox1*

Species	Sample ID	Sequence 5' -> 3'
M. perstans	MR2B10	ATACTTCCTGTAATATTGGGTGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGAAC TTTTTATCCTCCTCTTAGTACCGTTGGTCAGCCAGAGATGTCGTTAGATGTGATAATTTTAGGTCTCC ATACTGTTGGTATTGGTTCTTTGTTAGGTGCTATTAATTTTATGGTTACTGTGCAGAATATACGTTCT GTTGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTTTGTTAGTTT TATCTGTTCCTGTTTTAGCTGGGTCTTTATTGTTTTTGTTGATAGATCGTAATTTTAATACTTCTTTTTA TGACACTAGTAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGG TTTATGTTATTATTTTGCCTGTTTTTGGTATTATTAGTGAGGCTGTTTTATTTTTGACTGATAAGGATC GGTTATTTGGGCAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTAT-T-CTGGGTACTT-CTG
	MP_Gab_07	ATACTTCCTGTAATATTGGGTGCTCCTGAGATAGCTTTTCCTCGTGTTAATGCTTTATCTTTYTGGGTT ACTTTTTCTGCTTTGTTAATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGGAC TTTTTATCCTCCTCTTAGTACTGTTGGTCAGCCAGAGATGTCGTTAGACGTGATAATTTTAGGTCTTC ATACTGTAGGTATTGGTTCTTTATTAGGTGCTATTAATTTTATGGTTACTGTACAGAATATACGTTCTA TTGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTCTGCTGGTTTT ATCTGTTCCTGTTTTGGCTGGGTCTTTATTATTTTTGCTGATAGATCGTAATTTTAATACTTCATTTTAT GATACTAGCAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAAGT

		TTATGTTATTATTTTGCCTGTTTTTGGTATTATTAGAGAGGCTGTTTTATTTTTAACTGATAAGGATCG GCTTTTTGGACAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTGT-TTCTGGGGACTT-CTG
	PN193	ATACTTCCTGTAATATTGGGYGCTCCTGAGATAGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGCTGATGGTTTATCAATCGTTTTTTATTGGGAGTGGTCCTGGAAGCAGTTGAAC TTTTTATCCACCTCTTAGTACCGTAGGTCAGCCAGAGATGTCRTTAGATGTGATAATTCTTGGTCTTC ATACTGTCGGTATTGGTTCTTTGTTGGGTGCTATTAATTTTATGGTTACTGTGCAGAATATGCGTTCT ATTGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTTTGTTAGTTT TATCTGTTCCTGTTTTAGCTGGGTCTTTATTGTTTTTATTGATAGATCGTAATTTTAATACTTCTTTTTA TGATACTAGTAAGGGGGGTAGACCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGG TTTATGTTATTATTTTGCCTGTTTTTGGCATTATTAGTGAGGCTGTTTTATTTTTGACTGACAAGGATC GGTTATTTGGGCAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTAT-T-CTGGGCACTT-CTG
	MP_Gab_09	ATACTTCCTGTAATATTGGGTGCTCCTGAGATAGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAATCGTTTTTTATTGGGAGTGGTCCTGGAAGCAGTTGAAC TTTTTATCCWCCTCTTAGTACCGTAGGTCAGCCAGAGATGTCGTTAGATGTGATAATTCTTGGTCTTC ATACTGTCGGTATTGGTTCTTTGTTGGGTGCTATTAATTTTATGGTTACTGTGCAGAATATGCGTTCT ATTGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTTTGTTAGTTT TATCTGTTCCTGTTTTAGCTGGGTCTTTATTGTTTTTATTGATAGATCGTAATTTTAATACTTCTTTTTA TGATACTAGTAAGGGGGGTAGACCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGG TTTATGTTATTATTTTGCCTGTTTTTGGCATTATTAGTGAGGCTGTTTTATTTTTGACTGACAAGGATC GGTTATTTGGGCAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTAT-T-CTGGGCACTT-CTG
	MP_Togo_2	ATACTTCCTGTAATATTGGGTGCTCCTGAGATAGCTTTTCCTCGTGTTAATGCTTTGTCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGAAC TTTTTATCCTCCTCTTAGTACTGTTGGTCAGCCAGAGATGTCATTAGATGTGATAATTTTAGGTCTTCA TACTGTAGGTATTGGTTCTTTATTAGGTGCTATTAATTTTATGGTCACTGTGCAGAATATACGTTCTAT TGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTTTGCTGGTTTTA TCTGTTCCTGTTTTGGCTGGGTCTTTATTATTTTTGTTGATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGCAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCACCCTGAAGTT TATGTTATTATTTTGCCTGTTTTTGGTATTATTAGAGAGGCTGTTTTATTTTTAACTGATAAGGATCGG TTATTTGGACAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTAT-T-TTGGGTACTT-CTG
	PN748	ATACTTCCTGTAATATTGGGTGCTCCTGAGATAGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTAATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGGAC TTTTTATCCTCCTCTTAGTACTGTTGGTCAGCCAGAGATGTCGTTAGACGTGATAATTTTAGGTCTTC ATACTGTAGGTATTGGTTCTTTATTAGGTGCTATTAATTTTATGGTTACTGTACAGAATATACGTTCTA TTGCTGTTACTTTGGATCAGATCAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTCTGCTGGTTTT ATCTGTTCCTGTTTTGGCTGGGTCTTTATTATTTTTGCTGATAGATCGTAATTTTAATACTTCATTTTAT GATACTAGCAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAAGT TTATGTTATTATTTTGCCTGTTTTTGGTATTATTAGAGAGGCTGTTTTATTTTTAACTGACAAGGATCG GCTTTTTGGACAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTGT-T-CTGGGGACTT-CTG
	MP_Gab_01	ATACTTCCTGTAATATTGGGTGCTCCTGAGATAGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTAATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGGAC TTTTTATCCTCCTCTTAGTACTGTTGGTCAGCCAGAGATGTCGTTAGACGTGATAATTTTAGGTCTTC ATACTGTAGGTATTGGTTCTTTATTAGGTGCTATTAATTTTATGGTTACTGTACAGAATATACGTTCTA TTGCTGTTACTTTGGATCAGATCAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTCTGTTGGTTTT ATCTGTTCCTGTTTTGGCTGGGTCTTTATTATTTTTGCTGATAGATCGTAATTTTAATACTTCATTTTAT GATACTAGCAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAAGT

		TTATGTTATTATTTTGCCTGTTTTTGGTATTATTAGAGAGGCTGTCTTATTTTTAACTGATAAGGATCG GCTTTTTGGACAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTGT-T-CTAGGNACTT-CTG
	MP_Gab_05	ATACTTCCTGTAATATTGGGTGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGAAC TTTTTATCCTCCTCTTAGTACCGTTGGTCAGCCAGAGATGTCGTTAGATGTGATAATTTTAGGTCTCC ATACTGTTGGTATTGGTTCTTTGTTAGGTGCTATTAATTTTATGGTCACTGTGCAGAATATACGTTCT GTTGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTTTGTTAGTTT TATCTGTTCCTGTTTTAGCTGGGTCTTTATTGTTTTTGTTGATAGATCGTAATTTTAATACTTCTTTTTA TGACACTAGTAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTCTTTGGTCATCCTGAGG TTTATGTTATTATTTTGCCTGTTTTTGGTATTATTAGTGAGGCTGTTTTATTTTTGACTGATAAGGATC GGTTATTTGGGCAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTAT-T-CTGGGTACTT-CTG
	MR2B1	ATACTTCCTGTAATATTGGGTGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTCTGTTGATGGTTTATCAATCATTTTTTATTGGGAGTGGTCCTGGAAGTAGTTGAAC TTTTTATCCTCCTCTTAGTACCGTTGGTCAGCCAGAGATGTCGTTAGATGTGATAATTTTAGGTCTCC ATACTGTTGGTATTGGTTCTTTGTTAGGTGCTATTAATTTTATGGTCACTGTGCAGAATATACGTTCT GTTGCTGTTACTTTGGATCAGATTAGAATGTTTGTTTGAACTTCTTATTTAACTTCTTTTTTGTTAGTTT TATCTGTTCCTGTTTTAGCTGGGTCTTTATTGTTTTTGTTGATAGATCGTAATTTTAATACTTCTTTTTA TGACACTAGTAAGGGGGGTAGGCCTCTTCTTTATCAGCATTTGTTTTGATTCTTTGGTCATCCTGAGG TTTATGTTATTATTTTGCCTGTTTTTGGTATTATTAGTGAGGCTGTTTTATTTTTGACTGATAAGGATC GGTTATTTGGGCAAACAAGAATAACTTTTGCTTCTATTTGAATTGCTAT-T-CTGGGTACTT-CTG
Mansonella sp. “DEUX”	PN223	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-R-TTAGGTACTTTCTG
	PN708	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTTTCTG
	PN741	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGTAT-TTAGGTACTT-TCT
	PN667	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTAGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTTTCTG
	PN219	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-R-TTAGGTACTTTCTG
	PN604	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTTTCTG
	PN661	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTTTCTG
	PN681	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-R-TTAGGTACTTTCTG
	PN273	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCRTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAAGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-G-TTAGGTACTT-CTG
	PN360	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN252	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCATTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAAGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-G-TTAGGTACTT-CTG
	PN659	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN340	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGCATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN289	ATGCTTCCTGTAATGTTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN8	ATGCTTCCTGTAATATTGGGYGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTRTTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN116	ATGCTTCCTGTAATATTGGGTGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN336	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAARTTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTYTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGARGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-R-TTAGGTACTT-CTG
	PN241	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGKCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT
		TTATTTGGGCAGACAAGGATGACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN234	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT
		ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT
		TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA
		TACTGTGGGTATTGGTTCTTTACTKGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT
		TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA
		TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG
		ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT
		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT
		TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN161	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT
		ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT
		TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA
		TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT
		TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA
		TCTGTGCCTGTTTTGGCTGGATCTTTACTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG
		ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT
		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT
		TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN118	ATSCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT
		ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT
		TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA
		TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT
		TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA
		TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG
		ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT
		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT
		TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN194	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT
		ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT
		TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA
		TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT
		TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA
		TCTGTGCCTGTTTTGGCTGGATCTTTAYTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG
		ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT
		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT
		TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN208	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT
		ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT
		TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA
		TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT
		TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGYTA
		TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG
		ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN242	ATGCTTCCTGTAATATTGGGYGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGCAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN109	ATGCTTCCTGTAATATTGGGTGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN580	ATGCTTCCTGTAATATTGGGTGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN160	ATGCTTCCTATAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN189	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN137	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN506	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTAGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN68	ATGCTTCCTGTAATATTGGGYGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN745	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTGTTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-TCK
	PN203	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN727	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN209	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN238	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN705	ATGCTTCCTGTAATATTAGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN172	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN112	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN815	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN169	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAAGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-G-TTAGGTACTT-CTG
	MR2 B4	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTACTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN630	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAAGTT

		TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-G-TTAGGTACTT-CTG
	PN21	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN222	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-CTG
	PN599	ATGCTTCCTGTAATATTGGGCGCTCCTGAGATGGCTTTTCCTCGTGTTAATGCTTTATCTTTTTGGGTT ACTTTTTCTGCTTTGTTGATGGTTTATCAGTCTTTTTTTATTGGTAGTGGTCCGGGAAGTAGTTGAACT TTTTATCCTCCTCTTAGTACTGTTGGGCAGCCTGAGATATCATTGGATGTGATAATTTTGGGTCTTCA TACTGTGGGTATTGGTTCTTTACTGGGTGCTATTAATTTTATAGTTACTGTACAAAATATACGTTCTAT TGCTGTTACTTTAGATCAAATTAGGATGTTTGTTTGAACTTCTTATTTGACTTCTTTTTTATTGGTGTTA TCTGTGCCTGTTTTGGCTGGATCTTTATTGTTTTTGTTAATAGATCGTAATTTTAATACTTCTTTTTATG ATACTAGTAAGGGGGGTAGTCCGTTGCTTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTT TATGTTATTATTTTACCTGTTTTTGGTATTATTAGTGAAGCTGTTTTGTTTTTAACTGATAAGGATCGT TTATTTGGGCAGACAAGGATAACTTTTGCTTCTATTTGAATTGCTGT-A-TTAGGTACTT-TCK

Table 31: cox1 *sequences generated in this thesis*

11.2.1.3 *28S* rDNA

Species	**Sample ID**	**Sequence 5' -> 3'**
M, perstans	MP_Gab_01	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG

	MP_Gab_02	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTGTCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAAT TTTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTCTTCAATATGTCTTAAATGAAG TTTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	PN748	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MP_Gab_07	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MP_Gab_11	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	PN193	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG

	MP_Gab_09	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTWACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MR2B1	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MR2B10	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MP_Gab_05	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MP_Gab_10	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG

	MP_Togo_2	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MP_Gab_04	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCRACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTAACCCGTCTTGAAACACGGACCARGGAGTCTAA CAWATACCCAAG
	MP_Gab_03	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTY- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	MP_Gab_12	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTRACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG
	PN141	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATGGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTA- AAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAA CATATACGCAAG

Mansonella sp. "DEUX"	PN89	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN189	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN815	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN506	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGNAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTTAAAAAATAGTGACATACATCGATTATAATTTTAT TGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATA ATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACAC GGACCAAGGAGTCTAACATATACGCAAG
	PN241	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN161	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT-

		AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN727	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAACTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATATAAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATTTTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGTTTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTTAAAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN120	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAACTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATATAAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATTTTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGTTTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT-AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN242	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAACTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATATAAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATTTTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGTTTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT-AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN289	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAACTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATATAAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATTTTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGTTTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT-AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN219	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAACTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATATAAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATTTTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGTTTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT-AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACAGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGAACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACSCAAG
	PN609	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAACTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT

		AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATWATAATTTTATTGATTACATTTACATGTAGTCGCAKGCGACTTTAAC AGTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTG AACGTTAATCACCTATCTRACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN741	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN231	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN223	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN21	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN243	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA

		GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN667	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCSCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACSCAAG
	PN116	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN599	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCSCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACSCAAG
	MR2B6	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	MR2B4	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN708	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT

		TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN183	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAGTTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN8	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN77	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN234	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTRTTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN172	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATWATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTG AACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG

	PN252	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN88	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN194	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN273	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN659	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN630	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT-

		AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN580	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN745	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN681	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN336	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN604	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN661	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT

		AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN222	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAARATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN360	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	PN118	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG
	MR2B7	GAAACCGCTGAGATGGAAACGGATAGAGTTAACGAAACTTATCGATATTCAACCGATCAATTTAGCAAA CTTTATTGATTTATTATTCATGAATAATGAATAAAATGATATTAAAGGCAATTAAGTGCTAAATTAAATAT AAAAAATGATTGGTGTATTTATCGATAGTATGCATCGAGAGCTTCGTTTTAGCATATTATAGAAGCTAATT TTTAGTGGACCAATTATTATAATTAATAATTGTAATCCTAAAAATATGTTTTCAATATGTCTTAAATGAAGT TTTAATCCCAATTATGTAACTTTCACTATTTTTTTTTT- AAAAAATAGTGACATACATCGATTATAATTTTATTGATTACATTTACATGTAGTCGCATGCGACTTTAACA GTAAATGTTAATGCAACTAATTGATAAAATTATAATATTGTATGAAATTTTAAAGTTACATCTCGATGTGA ACGTTAATCACCTATCTGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATATACGCAAG

Table 32: 28S rDNA sequences generated in this thesis

11.2.2 Additionally analyzed sequences, accessed 20.04.2023

Gene	Accession number	Host species	Host materiel	Country	Reference
12S rDNA	MN927175	*Homo sapiens*	blood	Cameroon	
	MN927176	*Homo sapiens*	blood	Cameroon	

	MN927177	*Homo sapiens*	blood	Cameroon	(Gaillard et al., 2020)
	MN927178	*Homo sapiens*	blood	Cameroon	
	MN927179	*Homo sapiens*	blood	Cameroon	
	MN927180	*Homo sapiens*	blood	Cameroon	
	MN927142	*Gorilla g. gorilla*	stool	Cameroon	
	MN927145	*Gorilla g. gorilla*	stool	Cameroon	
	MN927146	*Gorilla g. gorilla*	stool	Cameroon	
	MN927147	*Gorilla g. gorilla*	stool	Cameroon	
	MN927149	*Gorilla g. gorilla*	stool	Cameroon	
	MN927150	*Gorilla g. gorilla*	stool	Cameroon	
	MN927151	*Gorilla g. gorilla*	stool	Cameroon	
	MN927143	*Gorilla g. gorilla*	stool	Cameroon	
	MN927144	*Gorilla g. gorilla*	stool	Cameroon	
	MN927148	*Gorilla g. gorilla*	stool	Cameroon	
	MN927159	*Gorilla g. gorilla*	stool	Cameroon	
	MN927138	*Pan t. troglodytes*	stool	Gabon	
	MN927139	*Pan t. troglodytes*	stool	Gabon	
	MN927140	*Pan t. troglodytes*	stool	Gabon	
	MN927141	*Pan t. troglodytes*	stool	Cameroon	
	MN927152	*Pan t. troglodytes*	stool	Cameroon	
	MN927153	*Pan t. troglodytes*	stool	Cameroon	
	MN927154	*Pan t. troglodytes*	stool	Cameroon	
	MN927156	*Pan t. troglodytes*	stool	Cameroon	
	MN927157	*Pan t. troglodytes*	stool	Gabon	
	MN927158	*Pan t. troglodytes*	stool	Cameroon	
	MN927160	*Pan t. troglodytes*	stool	Gabon	
	MN927161	*Pan t. troglodytes*	stool	Gabon	
	MN927162	*Pan t. troglodytes*	stool	Cameroon	
	MN927163	*Pan t. troglodytes*	stool	Cameroon	
	MN927164	*Pan t. troglodytes*	stool	Cameroon	
	MN927165	*Pan t. troglodytes*	stool	Cameroon	
	MN927166	*Pan t. troglodytes*	stool	Cameroon	
	MN927168	*Pan t. troglodytes*	stool	Cameroon	
	MN927169	*Pan t. troglodytes*	stool	Cameroon	
	MN927170	*Pan t. troglodytes*	stool	Gabon	
	MN927171	*Pan t. troglodytes*	stool	Gabon	
	MN927172	*Pan t. troglodytes*	stool	Gabon	
	MN927173	*Pan t. troglodytes*	stool	Cameroon	
	MN927174	*Pan t. troglodytes*	stool	Cameroon	
	MN927167	*Cercocebus agilis*	stool	Cameroon	
	LT623913	*Homo sapiens*	blood	Brazil	(Tavares da Silva et al., 2017)
	OQ633020	*Homo sapiens*	blood	Gabon	(Rodi et al., 2023)
	OQ633021	*Homo sapiens*	blood	Gabon	
	OQ633022	*Homo sapiens*	Blood	Gabon	
	MT361687	*Homo sapiens*	blood	Cameroon	(Chung et al., 2020)
cox1	MN890048	*Homo sapiens*	blood	Cameroon	[18]
	MN890049	*Homo sapiens*	blood	Cameroon	
	MN890050	*Homo sapiens*	blood	Cameroon	
	MN890051	*Homo sapiens*	blood	Cameroon	
	MN890052	*Homo sapiens*	blood	Cameroon	
	MN890053	*Homo sapiens*	blood	Cameroon	
	MN890054	*Homo sapiens*	blood	Cameroon	
	MN890080	*Gorilla g. gorilla*	stool	Cameroon	
	MN890081	*Gorilla g. gorilla*	stool	Cameroon	
	MN890082	*Gorilla g. gorilla*	stool	Cameroon	
	MN890083	*Gorilla g. gorilla*	stool	Cameroon	
	MN890084	*Gorilla g. gorilla*	stool	Cameroon	
	MN890085	*Gorilla g. gorilla*	stool	Cameroon	
	MN890087	*Gorilla g. gorilla*	stool	Cameroon	
	MN890089	*Gorilla g. gorilla*	stool	Cameroon	
	MN890091	*Gorilla g. gorilla*	stool	Cameroon	
	MN890092	*Gorilla g. gorilla*	stool	Cameroon	

	MN890115	*Gorilla g. gorilla*	stool	Cameroon	
	MN890116	*Gorilla g. gorilla*	stool	Cameroon	
	MN890118	*Gorilla g. gorilla*	stool	Cameroon	
	MN890119	*Gorilla g. gorilla*	stool	Cameroon	
	MN890120	*Gorilla g. gorilla*	stool	Cameroon	
	MN890121	*Gorilla g. gorilla*	stool	Cameroon	
	MN890056	*Pan t. troglodytes*	stool	Cameroon	
	MN890057	*Pan t. troglodytes*	stool	Cameroon	
	MN890058	*Pan t. troglodytes*	stool	Cameroon	
	MN890059	*Pan t. troglodytes*	stool	Cameroon	
	MN890060	*Pan t. troglodytes*	stool	Cameroon	
	MN890061	*Pan t. troglodytes*	stool	Cameroon	
	MN890062	*Pan t. troglodytes*	stool	Cameroon	
	MN890063	*Pan t. troglodytes*	stool	Cameroon	
	MN890064	*Pan t. troglodytes*	stool	Cameroon	
	MN890065	*Pan t. troglodytes*	stool	Cameroon	
	MN890066	*Pan t. troglodytes*	stool	Cameroon	
	MN890067	*Pan t. troglodytes*	stool	Cameroon	
	MN890068	*Pan t. troglodytes*	stool	Cameroon	
	MN890069	*Pan t. troglodytes*	stool	Cameroon	
	MN890095	*Pan t. troglodytes*	stool	Cameroon	
	MN890096	*Pan t. troglodytes*	stool	Cameroon	
	MN890097	*Pan t. troglodytes*	stool	Cameroon	
	MN890098	*Pan t. troglodytes*	stool	Cameroon	
	MN890099	*Pan t. troglodytes*	stool	Cameroon	
	MN890100	*Pan t. troglodytes*	stool	Cameroon	
	MN890101	*Pan t. troglodytes*	stool	Cameroon	
	MN890074	*Pan t. troglodytes*	stool	Gabon	
	MN890075	*Pan t. troglodytes*	stool	Gabon	
	MN890076	*Pan t. troglodytes*	stool	Gabon	
	MN890078	*Pan t. troglodytes*	stool	Gabon	
	MN890102	*Pan t. troglodytes*	stool	Gabon	
	MN890103	*Pan t. troglodytes*	stool	Gabon	
	MN890104	*Pan t. troglodytes*	stool	Gabon	
	MN890105	*Pan t. troglodytes*	stool	Gabon	
	MN890106	*Pan t. troglodytes*	stool	Gabon	
	MN890107	*Pan t. troglodytes*	stool	Gabon	
	MN890108	*Pan t. troglodytes*	stool	Gabon	
	MN890109	*Pan t. troglodytes*	stool	Gabon	
	MN890110	*Pan t. troglodytes*	stool	Gabon	
	MN890111	*Pan t. troglodytes*	stool	Gabon	
	MN890113	*Pan t. troglodytes*	stool	Gabon	
	MN890055	*Cercocebus agilis*	stool	Cameroon	
	KU215907	*Homo sapiens*	Blood	Italy	(Raele, Pugliese, Galante, Latorre, & Cafiero, 2016)
	LT623909	*Homo sapiens*	Blood	Brazil	(Tavares da Silva et al., 2017)
	OQ633020	*Homo sapiens*	blood	Gabon	(Rodi et al., 2023)
	OQ633021	*Homo sapiens*	blood	Gabon	
	OQ633022	*Homo sapiens*	Blood	Gabon	
	MT361687	*Homo sapiens*	blood	Cameroon	(Chung et al., 2020)

Table 33: characteristics of additionally analyzed sequences

11.2.3 Outgroups, accessed 20.04.2023

Species	**Accession number**		
	***12S* rDNA**	***cox1***	***28S* rDNA**
Filaria latala	KP760332	KP760186	KP760362
Dirofilaria spp.	KX265048	KY828979	KY990015

Onchocerca spp.	KX265048	KX181289	KX853349
	NC_001861	NC_001861	MG188680
B. malayi	AJ544843	AF538716	KP760362
L. loa	AJ544845	HQ186250	KP760362
M. cermeli	OQ633022	OQ633022	NA
M. ozzardi	NC_082197	KP760195	MN432519
Additional outgroups	AM779802 (*M. perforata*)	NA	NA

Table 34: Overview of the species and accession numbers used as outgroups in phylogenetic analysis

11.2.4

11.2.4.1 *12S* rDNA

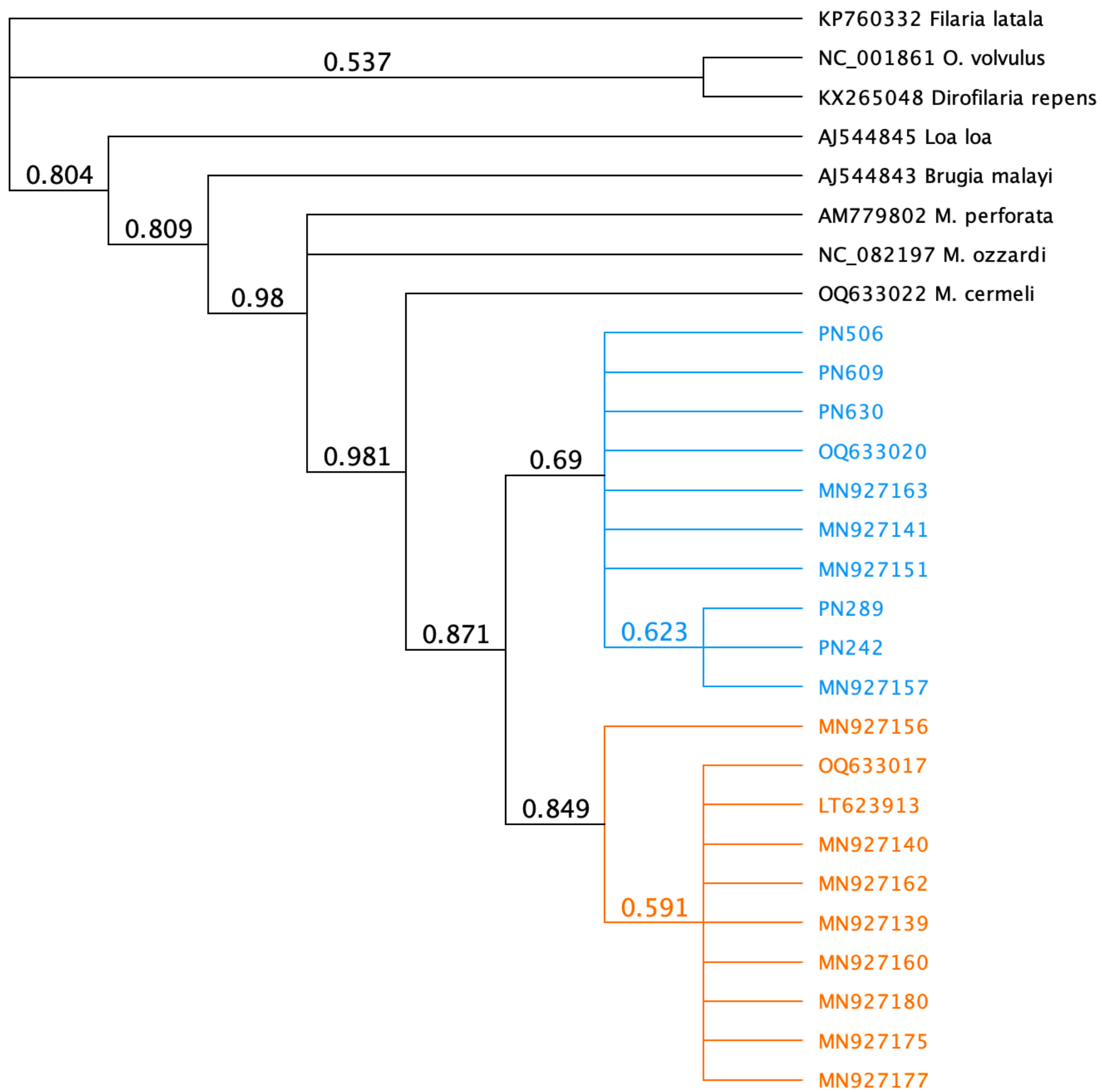

Figure 29: 12S *rDNA phylogenetic bayesian tree. Orange* = M. perstans. *Blue* = Mansonella *sp. "DEUX". Numbers: posterior probability*

11.2.4.2 cox1

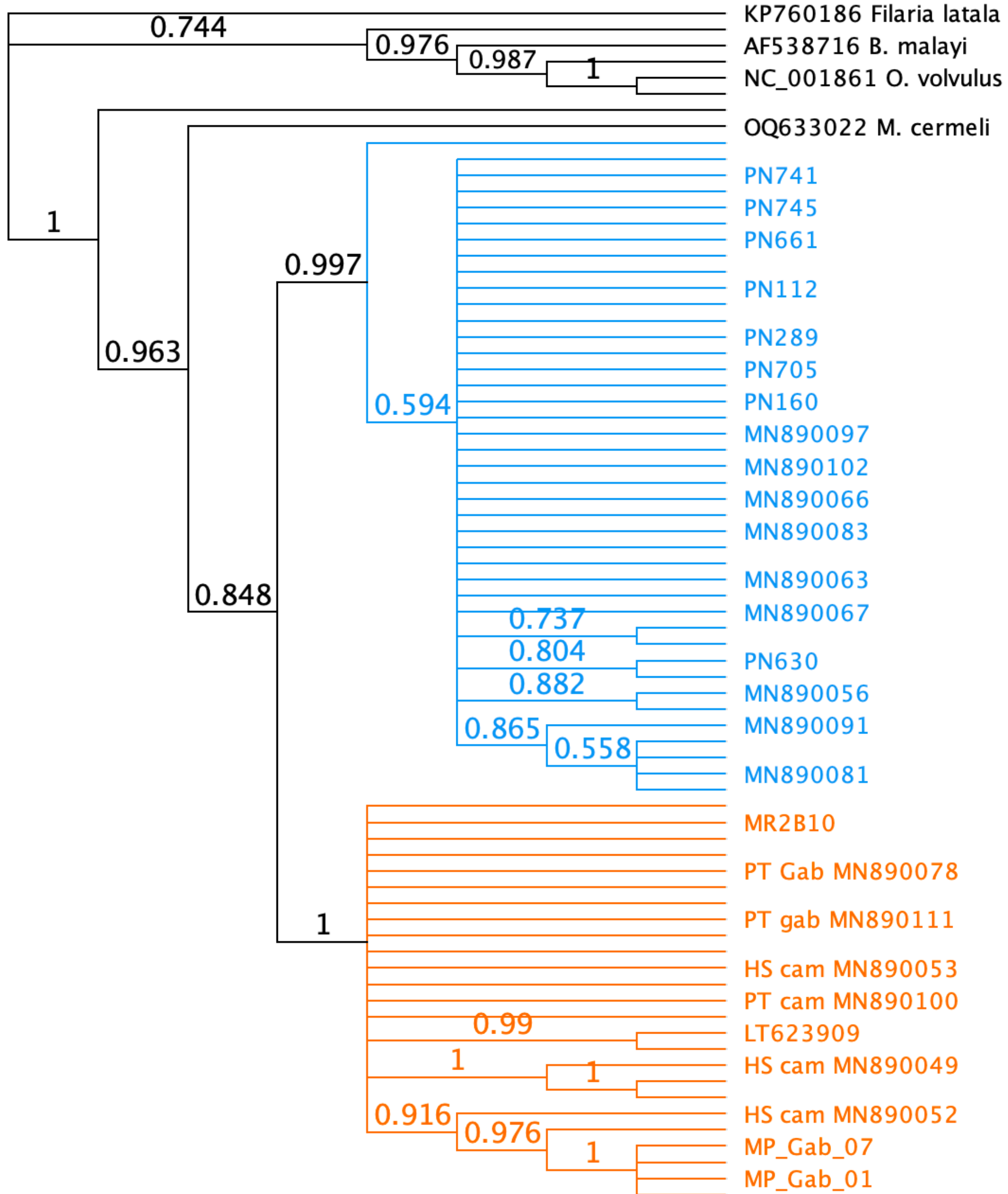

Figure 30: cox1 *phylogenetic bayesian tree. Orange* = M. perstans. *Blue* = Mansonella *sp. "DEUX". Numbers: posterior probability*

11.2.4.3 *28S* rDNA

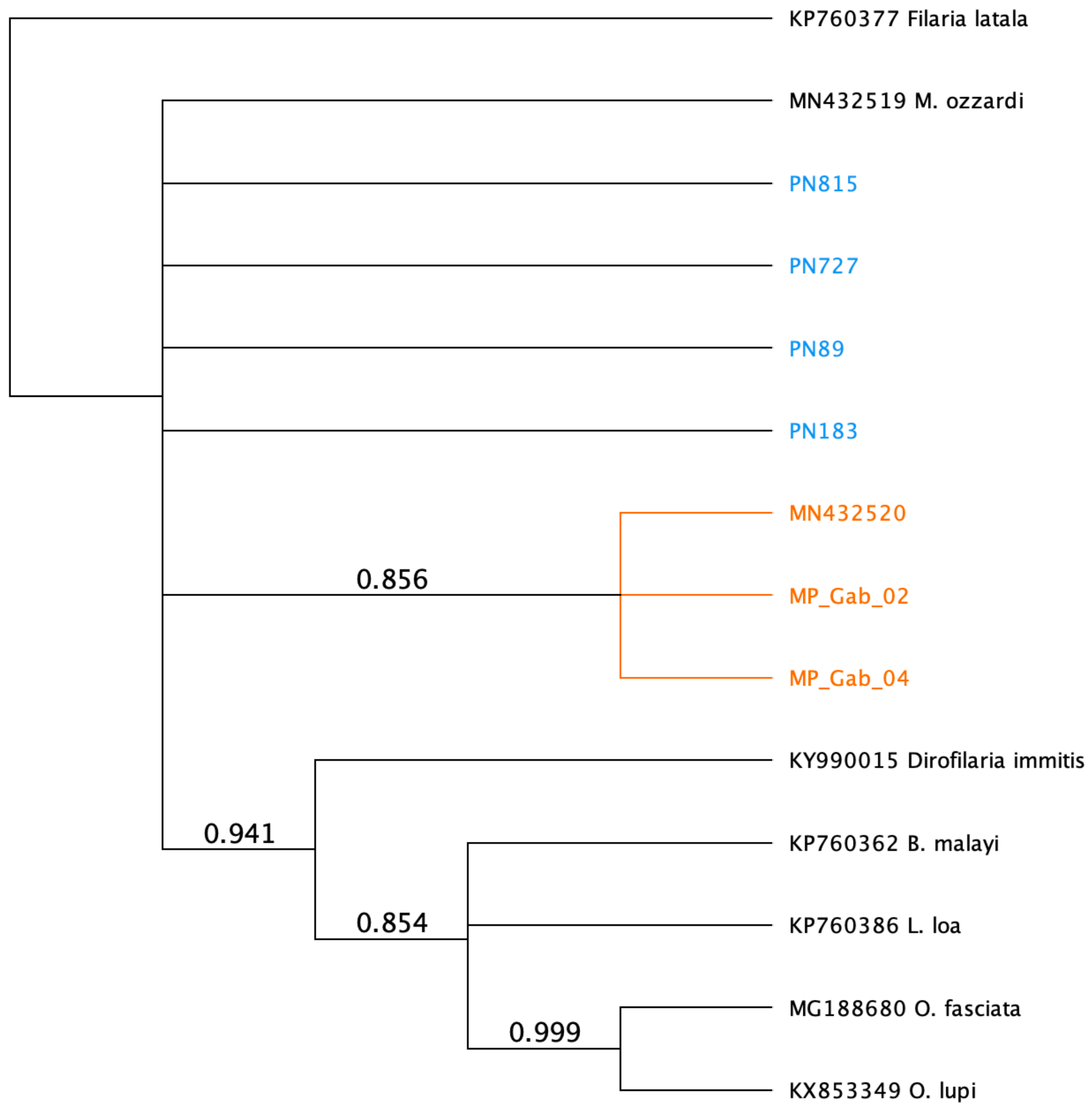

Figure 31: 28S rDNA phylogenetic bayesian tree. Orange = M. perstans*. Blue =* Mansonella *sp. "DEUX". Numbers: posterior probability*