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1 **Nonhuman primates across sub-Saharan Africa are infected with the yaws**  
2 **bacterium *Treponema pallidum* subsp. *pertenue***

3

4 Running title: Yaws bacterium in wild nonhuman primates

5

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68

69 Dear Editor,

70 The bacterium *Treponema pallidum* (*TP*) causes human syphilis (subsp. *pallidum*; *TPA*), bejel  
71 (subsp. *endemicum*; *TEN*) and yaws (subsp. *pertenue*; *TPE*)<sup>1</sup>. While syphilis reached a world-wide  
72 distribution<sup>2</sup>, bejel and yaws are endemic diseases. Bejel is found in dry areas in Sahelian Africa and  
73 Saudi Arabia, whereas yaws is present in the humid tropics<sup>1</sup>. Yaws is currently reported endemic in 14  
74 countries and an additional 84 countries have a known history of yaws but lack recent epidemiological  
75 data<sup>3,4</sup>. The disease was subject to global eradication efforts in the mid-20<sup>th</sup> century but reemerged in  
76 West Africa, Southern Asia, and the Pacific region<sup>5</sup>. New large-scale treatment options triggered the  
77 ongoing second eradication campaign, which aims to eradicate yaws globally by 2020<sup>5</sup>.

78 *TPE* is usually considered as a strictly human pathogen. This perception may however partly  
79 result from the lack of detailed data on nonhuman primate (NHP)-infecting treponemes. Indeed, a  
80 number of African NHPs show skin ulcerations suggestive of treponemal infection and antibodies  
81 against *TP* have been detected in wild NHP populations<sup>6,7</sup>. While genetic studies confirmed monkeys  
82 and great apes are infected with *TP* strains<sup>8-10</sup>, most of these analyses only determined short DNA  
83 sequences. The small number of polymorphic sites examined largely precludes assignment of these  
84 strains to a particular *TP* subspecies<sup>9</sup>, especially considering that sporadic recombination events  
85 between subspecies have been reported<sup>11</sup>. The only simian strain whose whole genome was sequenced  
86 - Fribourg-Blanc, isolated from a Guinea baboon (*Papio papio*) in 1966<sup>7</sup> - unambiguously clustered  
87 with human-infecting *TPE* strains<sup>12</sup>.

88 A fundamental question with regard to yaws evolution and possibly yaws eradication is whether  
89 humans and NHPs are commonly infected with the same pathogen, *TPE*, and whether transmission  
90 between NHPs and humans occurs. To determine which pathogen causes treponematoses in NHPs  
91 across sub-Saharan Africa, we collected samples from symptomatic wild individuals belonging to three  
92 NHP species (*Cercocebus atys*, *Chlorocebus sabaesus*, and *Papio anubis*) from four independent  
93 populations in West and East Africa (**Fig 1, Supplementary Table S1, Supplementary Materials**).  
94 Samples were collected at Taï National Park (TaïNP; Côte d'Ivoire), Bijilo Forest Park (BFP, the  
95 Gambia), Niokolo-Koba National Park (NKNP, Senegal), and Lake Manyara National Park (LMNP,

96 Tanzania). Monkeys presented yaws-like orofacial and limb lesions (TaiNP, BFP) or ulcerative  
97 anogenital skin lesions (BFP, NKNP, LMNP)<sup>9</sup>.

98         Using PCR, we showed the presence of *TP* in skin lesion biopsies or swabs from NHPs at  
99 TaiNP (*C. atys*), BFP, and NKNP (*C. sabaesus*). *TP* infection in olive baboons (*P. anubis*) at LMNP had  
100 previously been confirmed<sup>6</sup>. Two samples per NHP population were selected for whole genome  
101 sequencing based on high *TP* copy number or the ability to amplify long PCR fragments  
102 (**Supplementary Table S2**). To overcome the background of host genomic DNA, we used targeted  
103 DNA capture coupled with NGS to reconstruct whole *TP* genomes<sup>2,8</sup>. Following quality filtering,  
104 removal of PCR duplicates, merging of different sequencing runs from the same sample, and mapping  
105 against the *TPE* strain Fribourg-Blanc reference genome, we obtained a range of 22,886-470,303 DNA  
106 sequencing reads per sample. All samples showed at least 80% coverage of the reference genome with  
107 depth coverage of three or higher; average genome coverage depth was between 6.1 and 121.0-fold  
108 (**Supplementary Table S3**).

109         We generated maximum likelihood, Bayesian and maximum parsimony trees based on the  
110 genomes reconstructed in our study and all available reference genomes (total sequence length:  
111 1,133,379 nucleotides). In all trees, *TPE* and *TPA* strains formed reciprocally monophyletic groups,  
112 with a mean *TPE/TPA* strain divergence of 0.099%. NHP-infecting *TP* strains all clustered with human-  
113 infecting *TPE* strains (**Fig 1; Supplementary Figure S1**). The *TPE* clade exhibited a star-like branching  
114 pattern whereby all basal branches were very short and received low statistical support. Importantly,  
115 this pattern does not support a clear reciprocal monophyly of the *TPE* strains infecting humans and  
116 NHPs. In line with this, the minimum divergence between strains infecting humans and NHPs was  
117 lower than the maximum divergence amongst human and NHP-infecting strains (0.011% versus  
118 0.015% and 0.024%). Human-infecting *TPE* strains Samoa D, CDC-2, CDC-2575, Ghana-051, and  
119 Gauthier, which span a broad geographic and temporal range (at least four decades), were less divergent  
120 from each other than the two strains infecting sooty mangabeys from a single social group at TaiNP  
121 (0.011% versus 0.017% sequence divergence, respectively). While intra-group strain divergence was  
122 low for the two African green monkey populations and the olive baboons (0.0003% and 0.0017%,  
123 respectively), intra-species strain divergence for African green monkeys was relatively high when

124 compared to the divergence observed between the two most divergent human strains (0.0094% versus  
125 0.015%).

126 For the sample LMNP-1, we determined the complete genome sequence and structure (average  
127 depth of coverage: 169x; GenBank: CP021113; **Supplementary Tables S5-6**)<sup>12</sup>. The LMNP-1 genome  
128 showed the same structure as published complete genomes of human-infecting *TPE* strains and the  
129 simian strain Fribourg-Blanc. It was more similar to the human-infecting *TPE* Gauthier strain than the  
130 simian isolate Fribourg-Blanc, showing differences at 266 and 325 chromosomal positions,  
131 respectively. Most differences were single nucleotide substitutions or small indels (**Supplementary**  
132 **Table S7**). The LMNP-1 and Gauthier strains exhibited the same number of the 24-bp repeats in the  
133 *TP\_0470* gene (n=25) and Gauthier had only one 60-bp repeat more than LMNP-1 strain in the *arp*  
134 gene (LMNP-1 n=9 vs. Gauthier n=10). All 60-bp repeats in the *arp* gene of LMNP-1 were of Type II  
135 and were identical to other *TPE* strains<sup>13</sup>. The *tprK* gene of LMNP-1 only had three variable regions,  
136 V5-V7, when compared to other *TPE* strains. In addition to differences in *TP\_0433*, *TP\_0470*, and *tprK*  
137 genes, relatively large indels were determined in *TPEGAU\_0136* (33-nt long deletion; specific for  
138 strains Gauthier and Samoa D), in *TPFB\_0548* (42-nt long deletion; specific for strain Fribourg-Blanc),  
139 in *TPEGAU\_0858* (79-nt long deletion; specific for strain Gauthier), in the intergenic region (IGR)  
140 between *TPEGAU\_0628* and *TPEGAU\_0629* (302-nt long deletion; specific for strain Gauthier), and  
141 in IGR between *TPFB\_0696* and *TPFB\_0697* (430-nt long insertion; specific for strain Fribourg-  
142 Blanc); the length of other sequence differences ranged between 1-15 nts. RNA operons structure of  
143 the LMNP-1 genome (coordinates 231,180-236,139; 279,584-284,533; according to *TPE* strain  
144 Gauthier: NC\_016843.1) was similar to strains Gauthier, CDC-2, and Fribourg-Blanc, but different to  
145 the strains Samoa D, Samoa F, and CDC-1. The LMNP-1 16S-5S-23S was identical in both operons  
146 and 23S rRNA sequences were identical to other *TPE* strains except for strain Fribourg-Blanc (having  
147 a single nucleotide difference at position 458). We did not find any mutations associated with macrolide  
148 resistance (e.g. A2058G, A2059G)<sup>14</sup>. When the two NHP-infecting *TPE* strains, Fribourg-Blanc and  
149 LMNP-1, were compared to the closest human-pathogenic *TPE* strains CDC-2 and Gauthier,  
150 respectively, only 7.2% and 9.1% of all coding sequences (77 and 97 coding sequences out of 1065)



151 contained amino acid substitutions, suggesting limited functional divergence (**Supplementary Tables**  
152 **S7-9**).

153 Our findings unambiguously indicate that at least three African NHP species (representing four  
154 populations) from West and East Africa currently suffer from treponematosi s caused by *TPE*. Taking  
155 into account the isolation of the Fribourg–Blanc strain from Guinea baboons in 1966 and its recent  
156 sequencing and identification as a member of the *TPE* clade<sup>12</sup>, this represents four African NHP species  
157 and five populations whose symptoms can be explained by *TPE* infections. Coupled with a growing  
158 number of clinical and serological observations<sup>6,7,9,10</sup>, this suggests infection of NHPs with *TPE* is  
159 common throughout sub-Saharan Africa. Humans are not the exclusive host for the yaws bacterium and  
160 NHPs are infected with the same bacterial agent.

161 *TPE* strains in NHPs exhibit considerable genetic diversity, which at least equals that found  
162 among published human-infecting *TPE* strains. Importantly, we found no evidence for a clear sub-  
163 differentiation of NHP- and human-infecting *TPE* strains, i.e. these strains did not form well-supported  
164 reciprocally monophyletic groups. Rather, the star-like topology of our phylogenomic tree suggests a  
165 rapid initial radiation of the ancestor of *TPE* which may have involved transmission across primate  
166 species barriers in a relatively distant past (with respect to the *TPE* clade depth). These results neither  
167 support, nor allow us to exclude, a possible recent transmission of *TPE* between NHPs and humans,  
168 especially due to the large geographic and temporal separation between the two groups of samples being  
169 compared. A major hurdle in identifying such potential transmission events is the availability of enough  
170 bacterial genomes. Despite large numbers of human cases, very few genomes have been determined  
171 from human-infecting *TPE* strains and only from a very limited geographic range. Generating additional  
172 human-infecting *TPE* genomes represents an important area of research that, coupled with the genomes  
173 of *TPE* strains infecting NHPs presented here, could now enable the detection of recent zoonotic  
174 transmission events, would any exist.

175 Since yaws has not been reported for several decades in humans in countries where we find  
176 NHPs to be infected with *TPE*, we expect that if transmission happens, it is only at very low frequency  
177 (as is the case for many zoonotic diseases). Of course, such low frequency zoonotic transmission does  
178 not explain the reemergence of yaws, which is the consequence of continued human-to-human

179 transmission. However, now that eradication of yaws seems within reach<sup>15</sup>, the finding that *TPE* strains  
180 circulate in NHPs certainly calls for more research into their diversity and zoonotic potential.

181

## 182 **Data availability**

183 All raw read files have been deposited in NCBI as part of the BioProject PRJNA343706.

184

## 185 **Competing interests**

186 The authors declare that they have no competing interests.

187

## 188 **Supporting information**

189 **Supplementary Materials.** This document comprises supporting methods and additional results.

190 **Supplementary Figure S1.** Phylogenetic trees of *TP* whole genome sequences.

191 **Supplementary Table S1.** Nonhuman primates anesthetized for this study.

192 **Supplementary Table S2.** Molecular analyses (PCR and sequencing) performed on blood samples,  
193 skin tissue samples, and lesion swabs.

194 **Supplementary Table S3.** Read mapping and genotyping results.

195 **Supplementary Table S4.** Published genomes used for phylogenetic analyses.

196 **Supplementary Table S5.** List of primers used for long-range PCR amplification of *TP* intervals of  
197 the East African baboon genome (strain LMNP-1).

198 **Supplementary Table S6.** Summary of the PSGS sequencing results of four genomic DNA (gDNA)  
199 pools of the East African baboon genome (strain LMNP-1).

200 **Supplementary Table S7.** Number of nucleotide differences (i.e. indels and SNVs) of various  
201 lengths between the genome of the baboon (strain LMNP-1) and the published *TPE* genome of strains  
202 Gauthier and Fribourg-Blanc.

203 **Supplementary Table S8.** Proteins encoded by the *TPE* strain Fribourg-Blanc genome with 1 and  
204 more amino acid changes when compared to the *TPE* strain CDC-2 proteome.

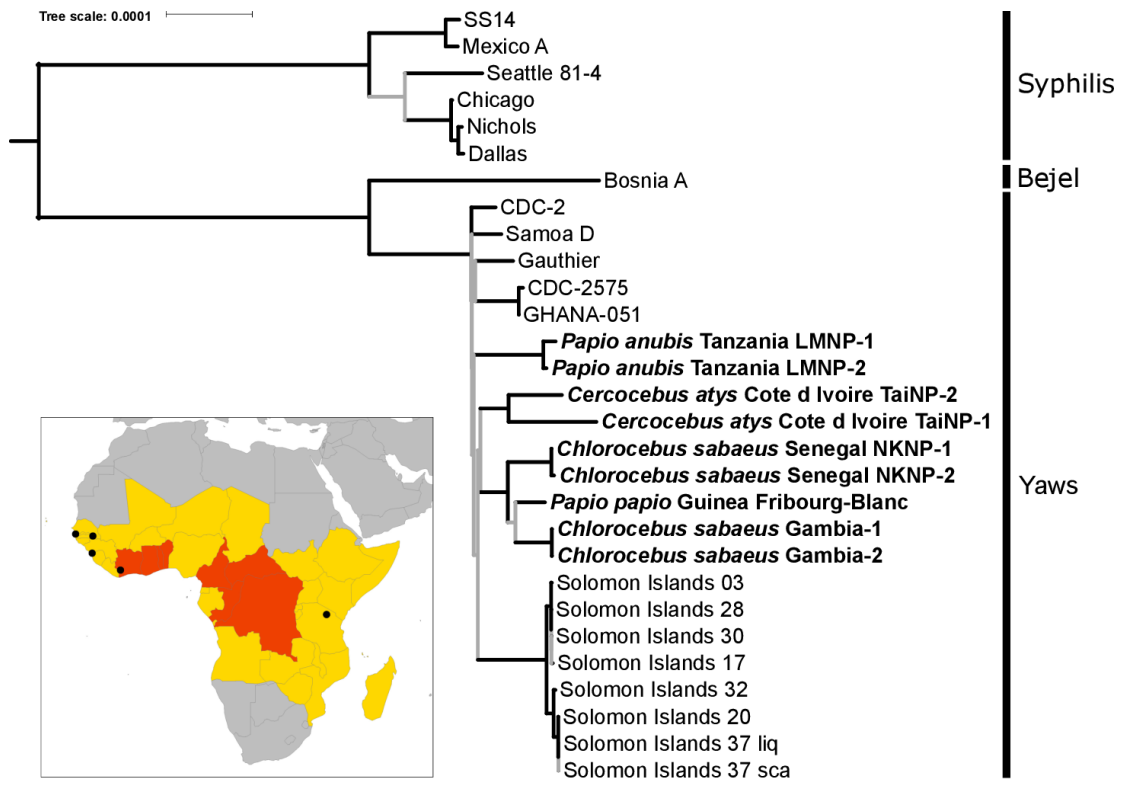
205 **Supplementary Table S9.** Proteins encoded by the LMNP-1 baboon strain with one and more amino  
206 acid changes when compared to the *TPE* strain Gauthier proteome.  
207

208 **Figure Legend**

209 **Fig 1. Phylogenomic analysis of NHP- and human-infecting *Treponema pallidum* strains.** In this  
210 maximum likelihood tree, nodes that had less than 95% ultrafast bootstrap approximation support are  
211 indicated with grey lines. Tip labels indicate the NHP species sampled, the country of origin, and the  
212 sample ID. The scale is in nucleotide substitution per site. The inset is a map of Africa where sites of  
213 origin of the NHP samples from which a *TP* genome was determined are indicated with black circles.  
214 A country's 2013 yaws status based on the World Health Organization's Global Health Observatory  
215 (<http://www.who.int/gho/en/>) is indicated by its color: grey indicates no previous history of yaws  
216 infections in humans, yellow indicates a country previously endemic for yaws though the current status  
217 is unknown, and countries in red indicate countries which are currently considered endemic for yaws.  
218

219 **Figures**

220 **Fig 1**



221

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239

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