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**Epidemic of venereal treponematosi s in wild monkeys:  
a paradigm for syphilis origin**

Oleg MEDIANNIKOV<sup>1,2</sup>, Florence FENOLLAR<sup>2,3</sup>, Bernard DAVOUST<sup>1,2</sup>, Nadia AMANZOUGAGHENE<sup>1,2</sup>, Hubert LEPIDI<sup>1,2</sup>, Jean-Pierre ARZOUNI<sup>1,2</sup>, Georges DIATTA<sup>4</sup>, Cheikh SOKHNA<sup>2,3</sup>, Jeremy DELERCE<sup>1,2</sup>, Anthony LEVASSEUR<sup>1,2</sup>, Didier RAOULT<sup>1,2\*</sup>

<sup>1</sup> Aix Marseille Univ, IRD, AP-HM, MEΦI, Marseille, France

<sup>2</sup> IHU-Méditerranée Infection, Marseille, France

<sup>3</sup> Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

<sup>4</sup> VITROME, Campus International UCAD-IRD, Dakar, Senegal

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\*Corresponding author: Didier RAOULT

Address: Institut Hospitalo-Universitaire - Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France

Phone: +33413732401; Fax: +33413732402; e-mail: didier.raoult@gmail.com

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## **Author contributions**

Conceptualization: OM, BD, DR

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1                                   **Epidemic of venereal treponematosi s in wild monkeys:**

2                                   **a paradigm for syphilis origin**

3   **ABSTRACT**

4   **Background**

5   *Treponema pallidum* infections have been primarily known as slightly contagious  
6   mucocutaneous infections, called yaws (tropical Africa and America) and bejel (subtropical  
7   North Africa). *T. pallidum* emerged as a highly infectious venereal syphilis agent in South  
8   America, probably about 500 years ago, and because of its venereal transmission, quickly  
9   caused a worldwide pandemic. The disease presents three stages: lesions including a chancre,  
10   then antibodies become detectable when or slightly after the chancre appears and prior to the  
11   development of the rash and other systemic manifestations. Venereal diseases were previously  
12   poorly known in monkeys.

13   **Methodology/Principal Findings**

14   During a fieldwork in Senegal, we discovered an epizootic outbreak of venereal disease that  
15   we explored. We detected a venereal form of *T. pallidum pertenue* infection in green monkeys  
16   and observed an epizootic outbreak in Senegal during one year, and its spread among  
17   baboons. Comparative analysis of *T. pallidum* genomes from the monkeys' chancres and  
18   other *Treponema* genomes shows an acceleration of SNPs number comparable to that  
19   observed in syphilis.

20   **Conclusions/Significance**

21   Identified *T. pallidum* clones seem to be epizootic through the mutation rate acceleration  
22   linked to their larger diffusion.

23 **AUTHOR SUMMARY**

24 All three subspecies of *Treponema pallidum* are pathogenic for humans: *T. p. pallidum*  
25 causes syphilis, *T. p. endemicum*, causes bejel or endemic syphilis and *T. p. pertenue* which  
26 causes yaws. The relationship among these species and their natural history is the subject of  
27 discussions. It is generally thought that about 500 years ago, *T. pallidum* has emerged as a  
28 highly infectious venereal syphilis agent in South America and quickly caused a worldwide  
29 pandemic because of its venereal transmission.

30 During a fieldwork in Senegal, we discovered an outbreak of venereal disease in green  
31 monkeys. We detected a venereal form of *T. pallidum pertenue* infection in green monkeys. In  
32 one year, we also observed the spread of the infection to all green monkey population and its  
33 dissemination among baboons. Comparative analysis of *T. pallidum* genomes from the  
34 monkeys' chancres and other *Treponema* genomes shows an acceleration of SNPs number  
35 comparable to that observed in syphilis. We believe that we evidenced a developing epizootic  
36 outbreak in monkeys caused by a specific clone of *T. pallidum* . It may be linked to the  
37 mutation rate acceleration.

38           **INTRODUCTION**

39           *Treponema pallidum* infections have been primarily presented as slightly contagious  
40 mucocutaneous infections, called yaws (tropical Africa and America) and bejel (subtropical  
41 North Africa). Overall, the origin of treponematoses and relations between the pandemic  
42 venereal syphilis and endemic treponematoses (yaws, bejel, and pinta) are not yet clear and  
43 even palaeontological evidences are not confirmative (1). Although endemic treponematoses  
44 have undergone a mass eradication campaign in the 1950-1960 (2), now, all treponematoses  
45 are coming back (3) and there is an urgent need to understand the pathogens that cause these  
46 diseases in order to eradicate them.

47           Moreover, treponemes cannot easily be cultured in traditional laboratory settings (4;5)  
48 and this is one of the reasons why we know little about these bacteria. Indeed, comparative  
49 genomics is one of the means to understand the biology of these bacteria better.

50           Whole genome sequencing of agents of syphilis (6), yaws (7), and bejel (8), showed  
51 very subtle differences (mostly, single nucleotide polymorphism) among these agents with  
52 almost the same genome structure and less than 0.2% divergence (7). The current dominating  
53 theory is that syphilis appeared as a venereal epidemic caused by a clone derived from the  
54 endemic yaws agent, *Treponema pallidum pertenue*, in pre-Columbian America (9) and  
55 quickly caused a worldwide pandemic following Columbus's discovery.

56           First evidences of endemic *T. pallidum* monkey infection were reported from Guinea  
57 baboons *Papio papio* (without genital lesions) in Senegal and Guinea (10-13) using serology.  
58 An isolate (Fribourg-Blanc) was also obtained from a popliteal lymph node. This strain is  
59 pathogenic for hamsters (12) and humans (14), and is genetically very similar to human yaws  
60 (15). Since then, monkeys and apes were found to suffer from endemic treponematoses  
61 comparable to yaws (16). Genital lesions caused by *T. pallidum* in non-human primates  
62 including *Papio anubis*, *Papio cynocephalus*, *Chlorocebus pygerythrus*, and *Cercopithecus*  
63 *mitis* were reported in Tanzania (12;17-19) suggesting sexual transmission. Serological

64 evidences of treponemal infection without lesions were also reported in *Papio papio* in  
65 Senegal in 2013-2014 (18) and in Tanzania (19;20).

66 During a fieldwork in Senegal, for the collection of monkey specimens originally  
67 planned for a study on Simian immunodeficiency viruses (SIV), we discovered by chance an  
68 epidemic of venereal disease that we explored. Thus, we report herein the first observed  
69 epidemic of venereal treponematosi in wild monkeys.

## 70 **MATERIALS AND METHODS**

### 71 ***Study population***

72 Green monkeys and baboons were studied in two different natural parks in Senegal  
73 (West Africa). The Niokolo-Koba national park is a World Heritage Site and one of the oldest  
74 natural protected areas in Africa. It is situated in southeastern Senegal near the Guinean  
75 border. The reserve of Bandia is a small private natural reserve situated 65 km south of Dakar,  
76 the capital of Senegal. One population in each park was studied in 2015: the green monkeys  
77 group living in the proximity of the Welcome center of Bandia natural reserve (14°33'35.5"N  
78 17°00'30.6"W) and the group of monkeys in the Niokolo forestry guardhouse in Niokolo-  
79 Koba national park (13°04'28.6"N 12°43'18.2"W). In 2016 in Niokolo-Koba national park we  
80 studied the same population of green monkeys in Niokolo forestry guardhouse as well as  
81 Guinea baboons living in sympatry and another population of green monkey from Simenti site  
82 (13°01'34.5"N 13°17'40.9"W).

### 83 ***Ethics Statement***

84 The study was approved by the Senegalese Ministry of the Environment (Direction of  
85 the National Parks, number 1302, October, 16, 2015 and number 904, July, 12, 2016) and  
86 performed in accordance with the recommendations of the Weatherall report.

### 87 ***Sample collection***

88 Three clinically healthy male green monkeys were captured in the reserve of Bandia and  
89 13 (11 males and 2 females) in Niokolo-Koba national park in November 2015. Among

90 animals collected in Niokolo-Koba national park, four male monkeys presented chancre-like  
91 lesions on the penis. None of the two female green monkeys showed clinically evident  
92 lesions. Animals were not tagged. In 2016, 21 green monkeys (including 14 male and 7  
93 female monkeys) were captured in Niokolo-Koba (two populations); among male monkeys,  
94 four presented chancres. Besides, among 14 Guinea baboons (13 male and 1 female) living in  
95 sympatry; 6 males presented with chancres (Supplementary Table 1). All individuals were  
96 anaesthetized using ketamine (10 mg/kg), which was administered intramuscularly via  
97 blowpipe or tranquilizer gun.

98 Cotton swab (Copan Diagnostics, Murrieta, California, USA) samples were taken from  
99 the edge of a lesion in clinically affected individuals and from normal unaffected tissues in  
100 healthy individuals and immediately frozen in liquid nitrogen. Anal swabs were taken from all  
101 monkeys. Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes from the  
102 femoral vein. Plasma and erythrocytes were separated by sedimentation during 3 hours and  
103 then frozen in liquid nitrogen. Samples were exported to France at -20°C and then transferred  
104 to a -80°C freezer. A tissue biopsy was taken from the chancre-like lesion of the penis of one  
105 baboon.

#### 106 ***Histologic analysis and immunohistochemical detection of *T. pallidum****

107 Formalin-fixed, paraffin-embedded tissue specimen obtained from baboon was cut to 3  
108 µm in thickness and stained with routine haematoxylin and eosin (H&E) staining. In order to  
109 detect the spirochetes, serial sections were also obtained for Warthin-Starry staining and  
110 immunohistochemical investigations. The immunohistochemical procedure used a rabbit  
111 polyclonal antibody specifically directed against *T. pallidum* diluted 1:100 as recommended  
112 by the manufacturer (Biocare Medical, Pacheco, CA, USA) with the Ventana Benchmark  
113 autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). A negative control was  
114 performed using an irrelevant rabbit polyclonal antibody.

#### 115 ***Western-blotting***

116 The Euroimmun anti-*T. pallidum* Western-blotting tests (Euroimmun, Lübeck,  
117 Germany) were performed according to the manufacturer's instructions. Samples and negative  
118 controls (human sera previously tested negative for syphilis) were diluted in serum diluent  
119 (1:51), and 1.5 mL of the diluted samples was dispensed in each channel with a strip. After  
120 incubation for 30 minutes on a rocking shaker at room temperature, the diluted samples were  
121 aspirated off and washed 3 times for 5 minutes with 1.5 mL of diluted universal buffer. Strips  
122 were then incubated with 1.5 mL of the enzyme conjugate (alkaline phosphatase conjugated  
123 antihuman IgG) solution for 30 minutes with agitation, pipetted out and washed 3 times for 5  
124 minutes with 1.5 mL of diluted buffer. Strips were further incubated on a rocking shaker with  
125 1.5 mL of substrate solution for 10 minutes at room temperature. The substrate solution was  
126 pipetted out, and strips were rinsed 3 times with cold deionised or distilled water and air-  
127 dried.

128 The development of colored bands on a strip indicated the presence of anti *T. pallidum*  
129 IgG antibodies. Strips with these bands were compared against a positive control strip with  
130 bands identifying TpN15, TpN17, p22 (unspecific), TpN45, and TpN47 protein antigens. A  
131 sample was considered positive if the test strip displayed more than one of the 4 specific  
132 bands (TpN15, TpN17, TpN45, and TpN47).

### 133 ***Molecular detection***

134 The genomic DNA was extracted from cotton swabs by using a QIAamp Tissue Kit  
135 (Qiagen, Hilden, Germany) with the EZ1 apparatus following the manufacturer's protocols.  
136 The genomic DNA was stored at -20°C under sterile conditions until the next stage of the  
137 investigation. DNA was stored at -20°C until assayed by PCR. The qPCR assays were  
138 performed to screen all samples using *polA*-based and *flaA*-based primers and probes  
139 (Supplementary Table 1) (21;22). All qPCRs were performed using a CFX96™ Real-Time  
140 system (Bio-Rad Laboratories, Marnes-La-Coquette, France) and the Eurogentec Master Mix  
141 Probe PCR kit (Eurogentec, Seraing, Belgium). We included *T. pallidum* DNA extracted from

142 a syphilis patient's swab as positive control and master mixtures as a negative control for each  
143 assay. Samples were considered positive when the cycle's threshold (Ct) was lower than 35  
144 Ct. In order to identify the subspecies of *T. pallidum* infecting monkeys, we chose two genes  
145 (TENDBA\_0136 and TENDBA\_0859 based on *T. pallidum endemicum* genome) (8) that  
146 would enable us to characterize identified treponema (Supplementary Table 1).

#### 147 ***Treponemal genome sequencing by in-solution hybridization capture***

148 DNA extracts were fragmented using a Covaris S220 Focused-ultrasonicator® in a  
149 volume of 130 µl low EDTA TE buffer using settings to generate a modal 400 bp fragment  
150 size (Intensity = 4, Duty cycle = 10%, Cycles per burst = 200, Treatment time = 55 s,  
151 Temperature = 7°C). Fragmented extracts were concentrated using the MinElute PCR  
152 purification kit and eluted into 2x10ul low EDTA TE buffer (Qiagen). DNA concentrations of  
153 all extracts were then measured using a Qubit dsDNA High Sensitivity kit (Thermo Fischer  
154 Scientific, Waltham, MA, USA). Libraries were prepared using the Accel-NGS 2S DNA PCR  
155 free library kit following the standard protocol, with unique single indices (Swift Biosciences,  
156 Ann Arbor, MI, USA). Libraries were quantified using the KAPA HiFI library quantification  
157 kit (Roche, Basel, Switzerland) and, when needed, subsequently amplified using the KAPA  
158 Hot Start Library Amplification Kit (Roche), with Illumina adaptor specific primers, and then  
159 re-quantified. Samples were pooled according to species: i) for baboon libraries, 100 ng per  
160 library was used (total pool mass: 700 ng), ii) and for African green monkey libraries, 140 ng  
161 per library was used (total pool mass: 700 ng).

162 RNA baits covering the entire *T. pallidum pertenuae* Fribourg-Blanc genome were  
163 designed with 2-fold tiling of 120mer baits. We essentially followed the myBaits Sequence  
164 Enrichment for Targeted Sequencing protocol (Version 2.3.1) but only used 125 ng bait per  
165 capture round and a hybridization time of 20 hours (Arbor Biosciences, Ann Arbor, MI).  
166 Following this first round of capture, the capture product was amplified using the KAPA Hot  
167 Start Library Amplification Kit for 20 cycles. DNA was concentrated using a MinElute PCR

168 Purification Kit and 1 µg of DNA was used for the second round of hybridization capture,  
169 performed under the same conditions as for the first round of capture. The final capture  
170 product was amplified using the KAPA Hot Start Library Amplification Kit for 12 cycles,  
171 purified using the MinElute PCR Purification Kit, quantified using the KAPA HiFi Library  
172 Quantification Kit, and diluted to 4 nM as input for an Illumina MiSeq run (v3; 2x300 bp).

### 173 ***Bioinformatic and evolutionary analyses***

174 The draft genome sequences of *T. pallidum* A10, *T. pallidum* A12, *T. pallidum* BNK07,  
175 and *T. pallidum* SNK05 have been deposited at European Bioinformatics Institute (EBI),  
176 European Nucleotide Archive (ENA) under the accession numbers: OMPM01000001-  
177 OMPM01000006; OMHU01000001-OMHU01000103, OMHW01000001-OMHW01000017,  
178 OMHZ01000001-OMHZ01000216, respectively.

179 All other *Treponema* genomes (CP002103, CP016062, CP016065, CP010422,  
180 CP007548, CP002375, CP002376, and CP003902) were downloaded from NCBI  
181 ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). After sequencing, reads from BNK07, A10, and A12 were mapped  
182 on the reference *T. pallidum* Fribourg Blanc using CLC Genomics workbench 7 with the  
183 following thresholds: 0.7 for coverage and 0.8 for similarity. Consensus sequences were  
184 extracted from the mapped reads (>3 reads threshold).

185 As for single nucleotide polymorphisms (SNPs), SNPs were calculated using CLC  
186 Genomics workbench 7 based on consensus sequences. Two hypervariable regions (positions  
187 230-240 kb and 280-290 kb) were excluded from the analyses. Genomes were annotated  
188 using Prokka (23) with default parameters. Regarding ortholog detection, we applied  
189 Proteinortho with default values (24). All orthologous genes were aligned using Muscle (25),  
190 then concatenated. Phylogenetic reconstruction (for Figure 4) was performed using RaxML  
191 (Randomized Axelerated Maximum Likelihood) with the GTRCAT model and a bootstrap  
192 value of 100 (26).

193 In order to investigate the possible relationships among strains, the neighbor-joining  
194 network using the Bandelt method was constructed using the NETWORK5.0  
195 program([www.fluxus-engineering.com/sharenet.htm](http://www.fluxus-engineering.com/sharenet.htm)) using equal weights for all mutations  
196 (27).

## 197 **RESULTS**

198 In November 2015, in order to study SIV, we investigated and sampled two populations  
199 of green monkeys (*Chlorocebus sabaues*) in Senegal: one from Bandia private nature reserve  
200 and one from Niokolo-Koba National park (Figure 1). In Niokolo, we discovered  
201 serendipitously typical chancrous lesions looking like human syphilis on four of fourteen  
202 monkey penises (Figure 2 and supplementary Figure 1) that were positive for *T. pallidum*  
203 using two different specific qPCR assays (Supplementary Tables 1 and 2). Specific Western-  
204 blot serology (Figure 2) was also positive for the four symptomatic monkeys. Among the  
205 eight asymptomatic monkeys, only three were Western-blot positive, but two females and  
206 three juvenile males were negative. All monkeys from Bandia reserve were negative.

207 During a second mission in August 2016, we observed the development of this venereal  
208 disease in various monkey populations, the natural evolution of this venereal disease  
209 mimicking primary syphilis in humans. We studied two populations of green monkeys in  
210 Niokolo-Koba, including the same population studied in 2016. All captured monkeys were  
211 seropositive in both populations, with a significantly higher prevalence than in 2015 (7/12 in  
212 2015 versus 21/21 in 2016, Fisher exact test = 0.003,  $p < 0.05$ ). Moreover, five out of twenty-  
213 one monkeys were qPCR-positive: three males with chancre-like genital lesions (genital  
214 swabbing), one female without lesions (genital swabbing), and one male without genital  
215 lesions (anal swabbing) (Supplementary Figure 1). Although animals captured in 2015 were  
216 not tagged, serendipitously, in 2016 we captured remarkable one-eyed male, the same already  
217 tested one year ago. His chancre spontaneously regressed to a qPCR-negative scar  
218 (Supplementary Figure 1), mimicking the natural history of primary syphilis in humans.

219 In August 2016, we also captured fourteen Guinea baboons living in sympatry with  
220 green monkeys. We detected penis chancres on six baboons (Supplementary Figure 1), all  
221 qPCR-positive. Moreover, six out of eleven sampled sera were Western-blot positive  
222 including three without genital lesions. One PCR-positive monkey was seronegative,  
223 suggesting very recent infection. Spirochetes were stained in great number in the connective  
224 tissue of the foreskin using Warthin-Starry stain and by immunohistochemistry (Figure 2 and  
225 Supplementary Figure 2). Bacteria were mixed with an inflammatory infiltrate composed of  
226 lymphocytes, plasma cells, and neutrophils (Supplementary Figure 2). All monkeys with  
227 genital lesions observed in 2016 were then treated by injections of prolonged penicillin.

228 Firstly, we selected two gene fragments presenting differences among most known *T.*  
229 *pallidum* subspecies. A 589-bp portion of TENDBA\_0136 gene coding for virulence-  
230 associated *Treponema* conserved hypothetical proteins that binds human fibronectin (28) was  
231 found to be identical for all qPCR-positive monkeys (both green monkeys and baboons). It  
232 was also identical to all strains except SamoaF strains of yaws agent and Fribourg-Blanc  
233 *Treponema*. A 464-bp portion of TENDBA\_0859 gene coding for a protein of unknown  
234 function was identical in all green monkeys sampled in 2015 and 2016 but also in one baboon  
235 (BNK14, Supplementary Table 2) sampled in 2016 and infected by the same *Treponema* as  
236 green monkeys. All other baboons had two identical point mutations differentiating them from  
237 all known *Treponema* strains, evidencing the circulation of another clone. Closest identity  
238 was, however, with Fribourg-Blanc *Treponema* (462/464) (Supplementary Figure 3).

239 The clinical natural history and dynamics (2015 to 2016) of the discovered disease  
240 resembles that of syphilis: apparition of a chancre harboring *Treponema* before serological  
241 response, then the chancre heals spontaneously; finally, anti-treponemal antibodies appear and  
242 persist (Figure 2). Here, we are facing an epizootic outbreak of a bacterial infection. Such  
243 outbreaks are often caused by a single clone (29-32), whereas endemic diseases are caused by  
244 several genotypes. To evaluate the evolutionary ratio of this putative clone we sequenced it.

245 We succeeded in sequencing almost entirely the genomes of *Treponema* from lesions of  
246 baboons (BNK07) and green monkeys (SNK05) sampled in 2016 and from two green  
247 monkeys (A10 and A12) sampled in 2015, and compared them to other open source complete  
248 genomes. We found that they were nearly identical (Figure 3, Table 1). We have obtained the  
249 sequence of the treponeme genome sequences from the geographical region where Fribourg-  
250 Blanc isolated his strain in 1966. This strain is thought not to have evolved very much in the  
251 laboratory because it remained most of the time frozen since the 1960s. The relationships  
252 between different strains of subspecies of *T. pallidum* based on SNPs are also represented on  
253 the Median Joining Network (MJN) (Figure 4).

254

255 **Table 1. Pairwise comparison of the number of SNPs between different strains.**

	A10	A12	BNK07	SNK05	<i>T. pallidum</i> str Fribourg- Blanc CP003902	<i>T. pallidum</i> subsp <i>endemicum</i> str Bosnia CP007548	<i>T. pallidum</i> subsp <i>pallidum</i> str PTSIF1200 CP016062	<i>T. pallidum</i> subsp <i>pallidum</i> str PTSIF1261 CP016065	<i>T. pallidum</i> subsp <i>pallidum</i> str Seattle Nichols	<i>T. pallidum</i> subsp <i>pertenue</i> str CDC2 CP002375	<i>T. pallidum</i> subsp <i>pertenue</i> str Gauthier CP002376
A12	53										
BNK07	64	108									
SNK05	112	155	145								
<i>T. pallidum</i> str Fribourg-Blanc CP003902	66	108	63	146							
<i>T. pallidum</i> subsp <i>endemicum</i> str Bosnia CP007548	371	397	368	439	372						
<i>T. pallidum</i> subsp <i>pallidum</i> str PTSIF1200 CP016062	801	837	797	863	802	848					
<i>T. pallidum</i> subsp <i>pallidum</i> str PTSIF1261 CP016065	798	834	790	853	795	842	3				
<i>T. pallidum</i> subsp <i>pallidum</i> str Seattle Nichols	830	862	822	884	824	857	167	167			
<i>T. pallidum</i> subsp <i>pertenue</i> str CDC2 CP002375	101	145	105	185	94	342	777	769	799		
<i>T. pallidum</i> subsp <i>pertenue</i> str Gauthier CP002376	117	158	119	196	111	357	791	781	808	67	
<i>T. paraluisuniculi</i> str Cuniculi CP002103	6,269	6,225	6,237	6,139	6,28	6,291	6,278	6,281	6,316	6,27	6,268

256 Strains (str) A10 and A12 are from green monkeys sampled in November 2015, SNK05 from a green monkey sampled in August 2016 and BNK07 from a baboon sampled in

257 August 2016.

258 Thus, the single nucleotide polymorphism between *Treponema* from BNK07 (found in  
259 2016) baboons and Fribourg-Blanc strain (isolated 50 years ago) in only 63 base pairs may  
260 correspond to the natural evolution of the genome of *T. pallidum*, *i. e.* 1.26 mutations per year.  
261 Interestingly, this is comparable with the generally accepted mutation rate in unicellular  
262 eukaryotes and prokaryotes of roughly 1/300 mutations ( $10^{-6}$  MB/generation) per effective  
263 genome per cell generation (33) applied to one *T. pallidum* generation in 30 hours (34).  
264 Moreover, the average number of 2111.7 nucleotide differences between syphilis and yaws  
265 strains (7) may be easily obtained by applying this mutation rate for the 500-year-old  
266 documented history of treponematoses in the Old World (7;8;15). Therefore, the forgotten and  
267 neglected now Hudson's Unitarian hypothesis (35;36), stating that all human treponematoses  
268 are caused by the same agent and that differences are due to climatic conditions and social  
269 habits of the targeted population, may have a second life. Other authors, however, estimate  
270 the evolution time between yaws and syphilis agents in around 10,000 years (37), although  
271 the most recent common ancestor of human syphilis strains evolved after 15<sup>th</sup> century,  
272 precisely around 1744 (38). The numbers of SNPs detected between strains isolated during  
273 rapidly evolving outbreak in 2015 (A10, A12) and 2016 (SNK05) were 112 and 155,  
274 respectively. Although it may be explained by sampling existing genetic variations in this  
275 bacterial population, these numbers of SNPs are particularly elevated while only one year  
276 separated both samplings and, consequently, reflected the epidemic nature of these clones.  
277 The long branch regrouping *Treponema* strains of the current epizooty in monkeys on the  
278 phylogenetic tree (Figure 4) also resembles that of the *Treponema* of syphilis.

## 279 **DISCUSSION**

280 We believe that the emergence of an outbreak of venereal clonal treponematoses in  
281 monkeys may be a paradigm of human syphilis. A non-venereal *Treponema* infection due to  
282 the agent of human yaws, *T. pallidum pertenuis*, is known in monkeys in Western Africa (39).  
283 We occasionally identified in 2015 is what we think to be the beginning of the outbreak in

284 green monkeys of West Senegal. The population of green monkeys in Niokolo-Koba is  
285 estimated as 30,000 individuals (40); in Bandia reserve that is situated in about 550 km to the  
286 north-west, no estimation is available and may be around several thousands. Within a few  
287 months, the tested population of green monkeys in Niokolo-Koba was infected. Moreover, it  
288 was found in baboons in which we identified 2 genotypes including one almost identical to  
289 that of green monkeys. We think that it is compatible with cross-species transmission of *T.*  
290 *pallidum pertenu*e that was already reported in Côte d'Ivoire (41). Although only few green  
291 monkey were studied in Bandia reserve, the absence of venereal treponematosi (both acute  
292 and serological) may mean that the presumable outbreak didn't yet reach here. We speculate  
293 that this outbreak resembles the emergence of syphilis that started as a local venereal disease,  
294 stemming from endemic yaws in America, and became rapidly pandemic following  
295 Columbus's arrival (42).

296         The difference between yaws and syphilis is that syphilis is a very contagious, venereal  
297 disease, which develops in three stages (43), an inoculation chancre where treponema is found  
298 and where antibodies are not found, spontaneous healing of chancre where antibodies are  
299 found for life. First, we detected these antibodies in monkeys by Western-blot, and then we  
300 have seen a dramatic extension of syphilis in one year from a part of the population to entire  
301 population (based on serology). The late clinical manifestations, secondary (rash) and tertiary  
302 syphilis (gum, neurological, and vascular involvements) are currently poorly described in  
303 monkeys (41;44) and must be investigated. The rash, however, may be invisible in monkeys.

304         In addition, genome studies show that the bacterium is very close to the agent of yaws  
305 (once endemic in Senegal) with a number of SNPs compatible to a rapid and epidemic spread  
306 of syphilis observed elsewhere. In addition, we hypothesize the transfer of this clone to  
307 baboons (based on identification of a very close strain found in one baboon). It suggests that  
308 the disease could spread rapidly to different primates. Thus, this strain of *Treponema* may  
309 become epidemic and this outbreak may originate from the emergence of a clone. In

310 treponemes, this has already happened once in humans with the global syphilis epidemic. The  
311 genomic era allows a paradigm change. It becomes clear now that in most cases such as here  
312 but also for tuberculosis with the Beijing clone (29), with *Clostridium difficile* clones (30),  
313 with typhoid (31) or with cholera (32), the epidemics are caused by clones emerging among  
314 numerous endemic strains following usually little genetic modifications. Currently, genomic  
315 studies carried out on these bacteria have made it possible to show, with respect to endemic  
316 strains, an increase in the number of SNPs which makes possible to identify the epidemic  
317 clones because their multiplication number is greater than that of the endemic species. We  
318 believe that we evidenced the beginning of an epizooty of treponematoses. Its risk of  
319 dissemination to other populations of monkeys and apes requires an active surveillance in  
320 these primate populations.

321         These results lead to at least two important questions regarding emergence of an  
322 epidemic of treponematoses: i) If this zoonosis threatens to become the reservoir of a new  
323 disease in humans as it has already happened with HIV (45), ii) If prevention could be  
324 considered with systematic treatment of monkey populations in which one or more cases were  
325 found to be positive, for example by penicillins *per os* in the food.

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442  
443

444 **Figure legends.**

445 **Figure 1.** Map of Senegal with the locations of collection sites (Map created from Wikimedia  
446 Commons: [https://commons.wikimedia.org/wiki/Atlas\\_of\\_the\\_world](https://commons.wikimedia.org/wiki/Atlas_of_the_world)).

447 **Figure 2.** Parallel in the clinical course between human syphilis and treponematoses of green  
448 monkeys. The chancre was on the penis of a green monkey (Niokolo forestry guardhouse)  
449 sampled in November 2015 (A09) and was PCR positive for *T. pallidum*. The visualization of  
450 *T. pallidum* (brown spirochetes) using immunohistochemical detection (original magnification  
451 x 400) was based on the biopsy of a penis lesion found on a baboon sampled in August 2016  
452 (BNK14). The positive western blotting serology was obtained from a green monkey sampled  
453 in August 2016 (SNK21).

454 **Figure 3.** Phylogenomic tree reconstruction based on the core genomes of *Treponema* isolates  
455 including *T. pallidum* subspecies and monkey *T. pallidum* from the present study.

456 **Figure 4.** Median Joining Network (MJN) representing the relationships between different  
457 strains of subspecies of *T. pallidum* based on SNPs. Each circle area indicates a unique  
458 genotype. The number of mutation steps between strains is indicated in red.

459 **Supporting Information Legends**

460 **Supplementary Table 1.** List of primers and probes used in the study.

461 **Supplementary Table 2.** Monkeys included in the study from Bandia reserve (BR), Niokolo-  
462 Koba National park, Niokolo Forestry Guard (NFG), and Niokolo-Koba National park,  
463 Simenti group (SG) as well as results of the detection of treponemes.

464 **Supplementary Figure 1.** Syphilis-like lesions of monkeys from Niokolo-Koba National  
465 park, Senegal. (A) Recovering penis lesions, *C. sabaesus*, Niokolo forestry guardhouse,  
466 August 2016. (B) Penis and scrotum lesions accompanied by regional lymphadenopathy  
467 (arrow), *C. sabaesus*, Simenti, August 2016. (C) Penis and scrotum lesions, *P. papio*, Simenti,  
468 August 2016.

469 **Supplementary Figure 2.** Visualization of *T. pallidum* (arrows) in penis lesions in Guinea  
470 baboons using Warthin-Starry staining (original magnification x 1,000).

471 **Supplementary Figure 3.** Molecular phylogenetic analysis of *Treponema* strains by  
472 Maximum Likelihood method based on concatenated sequences of TENDBA\_0136 and  
473 TENDBA\_0859 genes. The Genbank accession numbers of *Treponema* genomes are  
474 indicated at the beginning. The evolutionary history was inferred by using the Maximum  
475 Likelihood method based on the HKY model. The bootstrap consensus tree inferred from  
476 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. The  
477 analysis involved 23 nucleotide sequences. Positions containing gaps and missing data were  
478 eliminated. Evolutionary analyses were conducted in Topali.



Dakar

Bandia reserve, 2015



Niokolo-Koba national parc

Forestry guard, 2015 and 2016

Simenti, 2016



HUMAN



Infectious contact

Chancere

Negative serology

Clinical cure

Secondary stage

Tertiary stage

Positive serology

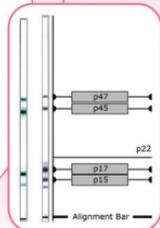
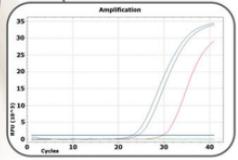
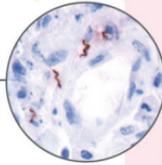
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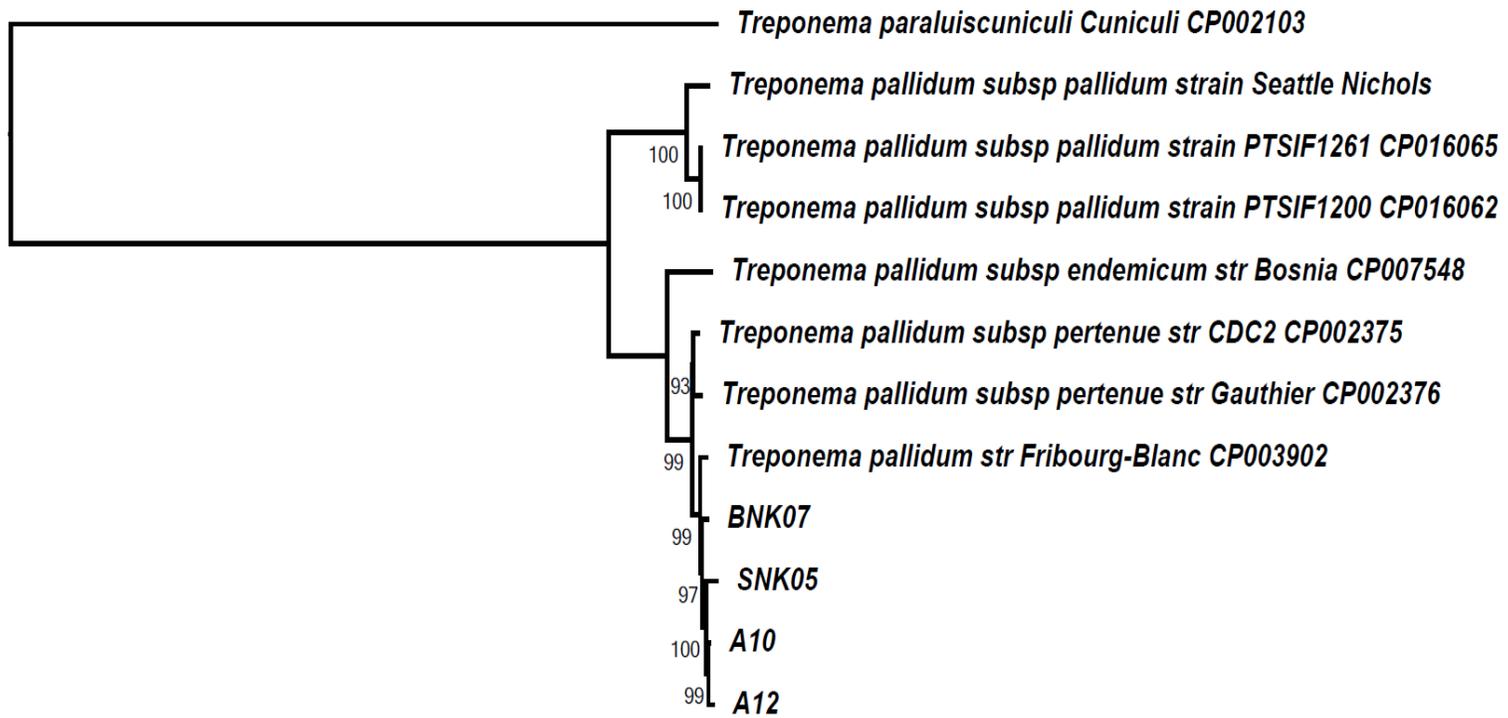
PCR negative

?

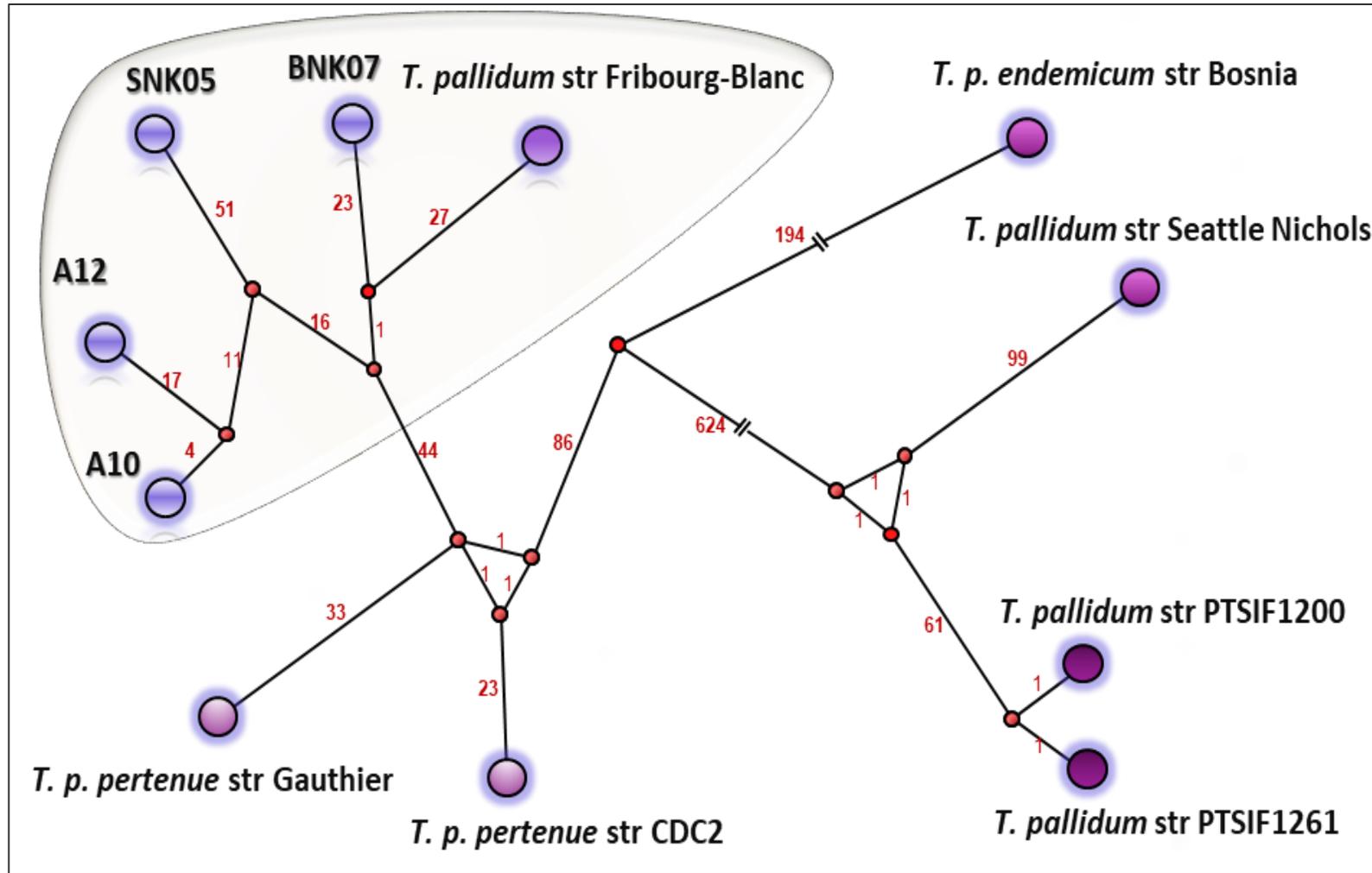
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MONKEY





0.001



A10 and A12 are from green monkeys sampled in November 2015, SNK05 from a green monkey sampled in November 2015 and BNK07 from a baboon sampled in August 2016.