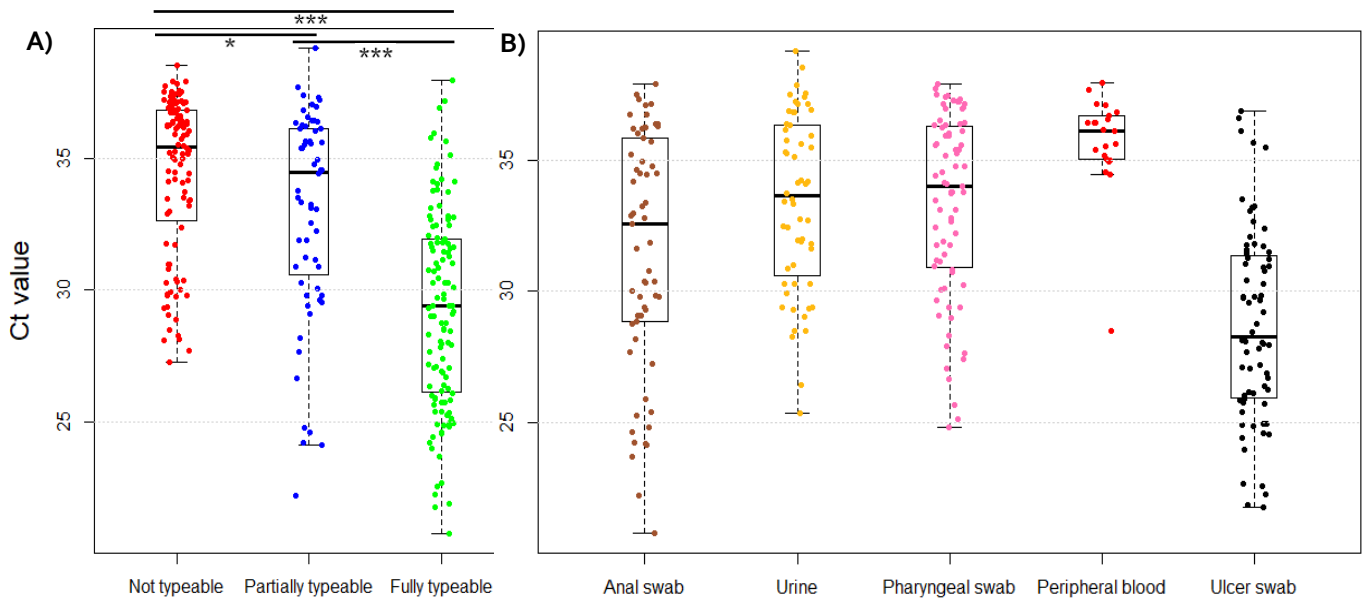


Supplementary Figure 1



Supplementary Figure 1. Distribution of cycle threshold (Ct) values of all TP DNA positive samples in this study. A) Ct values grouped and colored by typeability. B) Ct values by sample type, of which 61 anal swabs, 56 urine samples, 75 pharyngeal swabs, 22 peripheral blood samples and 73 ulcer swabs. * $p < 0.05$, *** $p < 0.0001$ (using one-way ANOVA)

Supplementary Table 1

Supplementary Table 1. Overview of TP DNA positive samples deriving from patients with infectious syphilis and their proportions within each syphilis stage, overall and the number of samples of which a TP type was obtained.

Type of sample	Primary syphilis 69 patients (%)	Secondary syphilis 64 patients (%)	Early latent syphilis 29 patients (%)	Total #typed / Total #samples (%)
Anal swab	13 (19)	37 (58)	11 (38)	10 / 61 (16)
Urine	24 (35)	26 (41)	6 (21)	23 / 56 (41)
Peripheral blood	2 (3)	15 (23)	5 (17)	1 / 22 (5)
Pharyngeal swab	7 (10)	47 (73)	21 (72)	35 / 75 (47)
Ulcer swab	63* (91)	10 (16)	0 (0)	50 / 73 (68)
Total	109	135	43	119 / 287 (42)

**Remaining 6 patients with primary syphilis did not have an ulcer swab available for the study.*

Supplementary Table 2

Supplementary Table 2. Overview of all (partial) allelic profiles of samples per body locations of 182 patients with at least one allelic variant. X refers to an undetermined allelic variant. "Unknown" lineages lack typing information of the *tpo136* and the *tpo548* locus.

Allelic profiles	Lineage	Anus	Urogenital	Peripheral blood	Pharynx	Total
1.3.1	SS14	8	25		9	42
1.1.1	SS14	1	9		9	19
9.7.3	Nichols	2	5	1	8	16
1.1.8	SS14	2	3		3	8
3.2.3	Nichols		4		3	7
1.1.9	SS14		2		1	3
1.66.1	SS14	1			1	2
1.64.1	SS14		1			1
1.52.1	SS14		1			1
1.17.9	SS14		1			1
1.43.1	SS14				1	1
1.65.1	SS14				1	1
30.3.1	SS14				1	1
29.7.3	Nichols		1			1
1.X.1	SS14	3	3		3	9
X.3.1	SS14	2	1		2	5
X.1.1	SS14	1	1	1		3
1.3.X	SS14		1	1		2
1.X.8	SS14			1	1	2
X.1.8	SS14	1				1
X.7.3	Nichols	1				1
29.X.3	Nichols			1		1
1.X.9	SS14				1	1
9.X.3	Nichols		1			1
X.2.3	Nichols		1			1
X.63.1	SS14		1			1
6.X.1	SS14				1	1
X.X.1	Unknown	1		3	4	8
X.1.X	SS14	1	2	2	2	7
1.X.X	SS14	3	2		1	6
X.X.9	Unknown			1	1	2
9.X.X	Nichols			1		1
19.X.X	SS14				1	1
29.X.X	Nichols				1	1
X.66.X	SS14		1			1
X.7.X	Nichols				1	1
X.X.8	Unknown				1	1
X.X.3	Unknown	1				1

Supplementary Table 3

Supplementary Table 3. Chi-squared or Fisher's Exact Tests of distribution of lineages (Nichols and SS14) by (A) syphilis stage, (B) age, (C) HIV status and (D) number of TP DNA detected at different sites of the 93 patients with at least one TP type.

A)

		Lineage (N = 93 patients)		Total
		Nichols n (%)	SS14 n (%)	
Syphilis stage	Primary	7 (15)	41 (85)	48
	Secondary	11 (31)	24 (69)	35
	Early latent	1 (10)	9 (90)	10
	Total	19 (20)	74 (80)	93

Fisher's Exact Test 0.13

B)

		Nichols n (%)	SS14 n (%)	Total
Age (in years)	≤34	10 (26)	28 (74)	38
	35-44	4 (15)	23 (85)	27
	45-54	3 (20)	12 (80)	15
	55+	2 (15)	11 (85)	13
	Total	19 (20)	74 (80)	93

Fisher's Exact Test 0.72

C)

		Nichols n (%)	SS14 n (%)	Total
HIV status	Negative	4 (15)	23 (85)	27
	Positive	15 (23)	51 (77)	66
	Total	19 (20)	74 (80)	93

Pearson's χ^2 test 0.56

D)

	#anatomical locations	Nichols n (%)	SS14 n (%)	Total
TP DNA detected	1	9 (47)	48 (64)	57
	2	6 (31)	10 (14)	16
	3	2 (11)	10 (14)	12
	4	2 (11)	6 (8)	8
	Total	19	74	93

Fisher's Exact Test 0.26