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 tp0136 and the tp0548 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       |       |
| 1.52.1           | SS14     | 1    |            | 1                | 1       |       |
| 1.17.9           | SS14     | 1    |            | 1                | 1       |       |
| 1.43.1           | SS14     | 1    |            | 1                | 1       |       |
| 1.65.1           | SS14     | 1    |            | 1                | 1       |       |
| 30.3.1           | SS14     | 1    |            | 1                | 1       |       |
| 29.7.3           | Nichols  |      |            | 1                | 1       |       |
| 1.1.1            | 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       |       |
| 1.68.1           | SS14     | 1    |            | 1                | 1       |       |
| X.7.3            | Nichols  |      |            | 1                | 1       |       |
| 29.X.3           | Nichols  |      |            | 1                | 1       |       |
| 1.X.9            | SS14     | 1    |            | 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.1.1            | Unknown  | 1    | 3          | 4                | 8       |       |
| X.1.X            | SS14     | 1    | 2          | 2                | 7       |       |
| 1.X.3            | SS14     | 3    | 2          | 1                | 6       |       |
| X.9.9            | Unknown  | 1    | 1          | 2                |         |       |
| 9.XX             | Nichols  | 1    |            | 1                | 1       |       |
| 19.XX            | SS14     | 1    |            | 1                | 1       |       |
| 29.XX            | Nichols  | 1    |            | 1                | 1       |       |
| X.66.X           | SS14     | 1    |            | 1                | 1       |       |
| X.7.X            | Nichols  | 1    |            | 1                | 1       |       |
| X.9.X            | Unknown  | 1    |            | 1                | 1       |       |
| X.3.X            | Unknown  | 1    |            | 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) |
|---|---|
|   | Nichols n (%) | SS14 n (%) | Total |
| **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 χ² 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** |