**Supplementary Table S5.** BLASTN analysis of the gene markers for *S. pneumoniae* and *S. pseudopneumoniae* against additional genome sequences available in GenBank by November 2019, as well as genome sequences of 20 *S. pneumoniae* and 14 *S. pseudopneumoniae* clinical strains that were not included in the pangenome analysis. The numbers indicate number of BLASTN hits of the gene markers against a set of genome sequences.

| Gene marker | *S. pneumoniae* |  | *S. pseudopneumoniae* |  |
|-------------|----------------|---|-----------------------|---|
|              | NCBI genomes | Clinical strains<sup>a</sup> | NCBI genomes | Clinical strains<sup>b</sup> |
| Pneumo_127  | 41            | 20 | 0                     | 0 |
| Pneumo_436  | 42            | 20 | 0                     | 0 |
| Pneumo_1011 | 40            | 20 | 2                     | 0 |
| Pneumo_1012 | 40            | 20 | 2                     | 0 |
| Pneumo_1013 | 40            | 20 | 2                     | 0 |
| Pneumo_1014 | 40            | 20 | 2                     | 0 |
| Pneumo_1361 | 42            | 20 | 0                     | 0 |
| Pneumo_1362 | 42            | 20 | 0                     | 0 |
| Pneumo_1961 | 41            | 20 | 1                     | 0 |
| Pneumo_1964 | 42            | 20 | 1                     | 0 |
| Pseudo_228  | 0             | 0  | 29                    | 14 |
| Pseudo_231  | 0             | 0  | 29                    | 14 |
| Pseudo_232  | 0             | 0  | 29                    | 14 |
| Pseudo_641  | 0             | 0  | 29                    | 14 |
| Pseudo_764  | 0             | 0  | 37                    | 14 |
| Pseudo_899  | 0             | 0  | 29                    | 14 |
| Pseudo_901  | 0             | 0  | 29                    | 14 |
| Pseudo_902  | 0             | 0  | 29                    | 14 |
| Pseudo_1933 | 0             | 0  | 26                    | 14 |

<sup>a</sup>Salvà-Serra *et al.* 2017.

<sup>b</sup>This study.