**Supplementary Table S2**

**Table S2.1** Primers used for amplification and sequencing of six loci included in the pan-family MLSA scheme

| Locus name | Putative function                        | Primer direction | Primer sequence (5’-3’)         |
|------------|------------------------------------------|------------------|---------------------------------|
| *gpd*      | glyceraldehyde 3-phosphate dehydrogenase | F                | TGGAATGCACCGGCATYTTCA           |
|            |                                          | R                | TCGSACATRCGGYTGAGGAAGC          |
| *dnaK*     | chaperone protein                        | F                | TCGARGTGAAGTCSCAACCAYG          |
|            |                                          | R                | ACTCTCTSGTCGGGATCGTSG           |
| *trpE*     | anthranilate synthase                     | F                | GIGIGACCRAARCTSTGGGC            |
|            |                                          | R                | CGRTGCGIGAIGGGTSCC              |
| *csdB*     | cysteine desulferase                      | F                | GGMGGATSRTRRTAGGTGAC            |
|            |                                          | R                | GARTAYGCAAYGTCATCG              |
| *leuA*     | 2-isopropylmalate synthase                | F                | CGCTGTTCGGAATGCGAGC             |
|            |                                          | R                | GTCGACGAGTGYTCGGAAT             |
| *acnA*     | aconitate hydratase                       | F                | CTGYYGYYTTCTTCCCGGT             |
|            |                                          | R                | GTCCAKGCRTAGGTCTG              |

**Table S2.2** Characteristics of the six loci included in the pan-family MLSA scheme, across the 43 *Brucellaceae* type species analysed

| Locus | Length (bp) | Mean % GC content | Polymorphic sites (%)<sup>a</sup> | π          |
|-------|-------------|-------------------|-----------------------------------|------------|
| *gpd* | 589         | 57.8              | 290 (49.24)                       | 0.1403     |
| *dnaK*| 470         | 57.9              | 199 (42.34)                       | 0.1256     |
| *trpE*| 486         | 57.6              | 260 (53.50)                       | 0.1975     |
| *csdB*| 487         | 54.5              | 250 (51.33)                       | 0.1780     |
| *leuA*| 482         | 54.5              | 237 (49.17)                       | 0.1602     |
| *acnA*| 490         | 59.4              | 219 (44.69)                       | 0.1412     |
| Concatenated | 3004 | 57.0         | 1453 (48.37)                      | 0.1565     |

<sup>a</sup> Polymorphic sites observed amongst 43 *Brucellaceae* type strains analysed; π = nucleotide diversity