**Supplemental Tables**

Supplemental Table 1: POCP values for all pairs of strains show that *Elizabethkingia* strains typically share 90% or more of their proteins with other strains in the genus, but a lower percent with strains from closely related genera.

Supplemental Table 2: Supplemental Table 1: Phenotypic characteristics of strains originally described as *E. meningoseptica* (1= KC1913, 2 = G4076, 3 = G4120), *E. anophelis* (5 = R26), *E. endophytica* (6 = JM-87), *Elizabethkingia* genomospecies 1 (7 = 0422, 8 = 3375, 9 = E6809, 10 = F3543, 11 = F3201), *E. miricola* (12 = DSM 14571), *Elizabethkingia* genomospecies 2 (13 = G4071, 14 = G4074, 15 = G4121), *Elizabethkingia* genomospecies 3 (16 = G0146, 17 = G4075, 18 = G0153) and *Elizabethkingia* genomospecies 4 (19 = G4122, 20 = G4123, 21 = G4070). All strains were negative for acid production from inositol, raffinose, rhamnose, salicin, sorbitol, and sucrose.

Supplemental Table 3: Phenotypic data for strains in CDC’s strain collection that were assigned to one of three major groups by MALDI-TOF.

Supplemental Table 4: In-silico analysis yields essentially the same result regardless of whether the genome being compared is in draft form (contigs) or complete and circularized. For ANIb, results can vary based on which genome is subject and which is queried, so data is reported for both (ie, “ANIb A=S” uses the genome A as the subject, while “ANIb A=Q” uses genome A as the query).

**Supplemental Figures**

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Supplemental Figure 1: Scatter plots showing the *in silico* calculated values of ANIb, predicted DDH using each of the GGDC formulas, and amino acid identity (AAI), as compared to the DNA-DNA hybridization determined by traditional techniques (tDDH), at 55°C (A) and 70°C (B). All *in silico* methods show a high degree of correlation with tDDH measurements, with GGDC formula 2 being the best proxy for tDDH.

Supplemental Figure 2: Scatter plots of the predicted DDH from each of the three GGDC formulas, compared to ANIb for all strain combinations. Both the predicted DDH for formulas 1 and 3 are linearly correlated with ANIb values, but the predicted DDH for formula 2 shows a non-linear relationship.

Supplemental Figure 3: SNP tree of all strains from Table 1, generated by ParSNP and Gingr, based on a core genome alignment of 4%.

Supplemental Figure 4: SNP tree of strains that are not *E. meningoseptica* or *E. anophelis*, generated by ParSNP and Gingr, based on a core genome alignment of 31%. Phylogenetic relationships between *E. miricola* strains and strains from the species that are most closely related to *E. miricola* are emphasized.

**Supplemental text**

**Complete circularized genomes are not necessary for WGS-based species determination.**

For 11 strains, we had complete circularized genomes as well as contigs, and we included both assemblies in our analysis. This produced 715 genome-pairs for which we had both complete and draft results (Supplemental Table 4). For the one analytical method that can yield differing results depending on which genome is the query and which is the subject (ANIb), results were compared in both directions. For this analysis, we are fortunate that all *Elizabethkingia* genomes are a similar size, as the ANIb of genomes that differ significantly by size can vary depending on which genome is used as the query. In all cases, the results correlated almost perfectly and the difference between the calculated results for contigs vs. the complete genome was minimal. The largest absolute difference is shown on the table, and the absolute difference between the measurements was plotted as a function of the value determined for the complete genome (not shown). The difference between measurements made in the range that is informative for species delimitation (predicted DDH of 60 to 80 for GGDC formula 2, and ANIb between 93 and 97) is less than 0.3 or 0.09, respectively. WGS contigs alone can therefore reliably be used for species determination of *Elizabethkingia* strains.

**MALDI-TOF spectrum for Identification of *Elizabethkingia***

MALDI-TOF classification of *Elizabethkingia* is available through CDC’s MicrobeNet database, an online database that allows public health laboratories anywhere in the world to match their diagnostic tests results against the CDC’s unique collection of pathogens. Species within MicrobeNet are represented with pictures, biochemical panel results, 16S rRNA sequence information, MALDI-TOF mass spectrum and eventually whole genome sequence. MicrobeNet’s MALDI-TOF module was designed in collaboration with Bruker Daltonics to deliver a familiar interface, while offering access to spectrum from CDC’s bacterial collections.

Unlike most species identifications, the spectra of all *Elizabethkingia* strains score above a 2.0 (green score) in the Bruker MBT software, when compared to any known *Elizabethkingia* strain. This does not preclude identification as the scores allow three clear taxonomic distinctions can be made: *E. meningoseptica*, *E. anophelis* and all other *Elizabethkingia*. The distinctions are additionally clarified through the numerical scores of MBT results, with all MSP’s of the correct identification clustering together above the other species identifications. Unfortunately it is not possible to distinguish between *E. miricola*, E. *ursingii*, *E. bruuniana*, and E. *occulta* using direct-transfer protocols.

It is additionally worth noting that the Bruker database at present has one *Elizabethkingia meningoseptica* MSP that will score with *Elizabethkingia anophelis* and must be discounted when using the Bruker and MicrobeNet databases together.