

Molecular characterisation of ‘*Candidatus Borrelia tachyglossi*’ (family Spirochaetaceae) in echidna ticks, *Bothriocroton concolor*

Supplementary Materials

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Supplementary Table 1. Primers used for the amplification and sequencing of *flaB*, *glpQ*, *groEL*, and *gyrB* gene sequences.

| Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Annealing temperature (°C) | Expected size (bp) | References |
|-------------------------------------|---------------------------|---------------------------|----------------------------|--------------------|-----------------|
| <i>flaB</i> fragment 1 (Primary) | TCTGATGATGCTGCTGGTATGG | CAATCGCCTCATCCTGAT | 50 | 439 | This study |
| <i>flaB</i> fragment 1 (Secondary) | TCTGATGATGCTGCTGGTATGG | GAAGCTTGCGCACCAGTTAG | 57 | 392 | This study |
| <i>flaB</i> fragment 2 (Primary) | CTGAAGAGCTTGGAATGCAAC | AGGTACTTGATTGCTTGTGC | 52 | 545 | [1] |
| <i>flaB</i> fragment 2 (Secondary) | CTGAAGAGCTTGGAATGCAAC | GCAATCATAGCCATTGCAGATTGT | 52 | 526 | [1, 2] |
| <i>glpQ</i> fragment 1 (Primary) | CATTAATTATAGCTCACAGAG | AACAAGCATTATCAATTTTCC | 48 | 599 | [1] |
| <i>glpQ</i> fragment 1 (Secondary) | AGTGGATATTTACCAGAACA | AACAAGCATTATCAATTTTCC | 48 | 573 | This study; [1] |
| <i>glpQ</i> fragment 2 (Primary) | GCACGACCCAGAAATTGACAC | GTTGCYCCYCCRCCAACAT | 57 | 894 | This study |
| <i>glpQ</i> fragment 2 (Secondary) | CTGGCATAAGCAACAAGGCA | GTTGCYCCYCCRCCAACAT | 57 | 603 | This study |
| <i>groEL</i> fragment 1 (Primary) | TGGCTAAGGACATATATTTTA | GCTCCAACATTAATAACAGC | 48 | 1,141 | This study; [1] |
| <i>groEL</i> fragment 1 (Secondary) | GATGCKAGAAAGAGYYTGCT | GCTCCAACATTAATAACAGC | 48 | 1,115 | This study |
| <i>groEL</i> fragment 2 (Primary) | ATTGCTATACTTACTGGAGG | TTACATCATTCCCATTCCAG | 48 | 768 | [1] |
| <i>groEL</i> fragment 2 (Secondary) | GGTGTGCTTATTAGTGAGGA | TTACATCATTCCCATTCCAG | 50 | 750 | This study; [1] |
| <i>gyrB</i> fragment 1 (Primary) | ATTGAYGARGCYTTRGCRGG | GACCACTCCTGAAACCAGCA | 57 | 727 | This study |
| <i>gyrB</i> fragment 1 (Secondary) | ATTGAYGARGCYTTRGCRGG | CYTCYGTCCAYTTAAGTCCA | 51 | 643 | This study |
| <i>gyrB</i> fragment 2 (Primary) | CAARGAGRCTTAGRGAACCTGC | CCATCAACATCAGCATCAGC | 57 | 949 | This study; [3] |
| <i>gyrB</i> fragment 2 (Secondary) | CAARGAGRCTTAGRGAACCTGC | CCAACACCAGCWCCAAGAGA | 57 | 880 | This study |
| <i>gyrB</i> fragment 3 (Primary) | CTTTGGGAAACTACTATGAAYCCTG | ACATCCAGATTTACTACATCAAGYG | 51 | 764 | This study |
| <i>gyrB</i> fragment 3 (Secondary) | CTTTGGGAAACTACTATGAAYCCTG | GTTCAACWTCATCYCCCAT | 51 | 713 | This study |

Supplementary Table 2. Genetic distance matrix of partial *flaB* gene sequences showing percentage nucleotide identities between ‘*Candidatus* *Borrelia* tachyglossi’ genotypes A, B, and C, other *Borrelia* species, and the outgroup, *Brachyspira pilosicoli*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype B (KY586964) | | | | | | | | | | | | | | | | | |
| 2. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype C (KY586965) | 99.9 | | | | | | | | | | | | | | | | |
| 3. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype A (KY586966) | 99.6 | 99.7 | | | | | | | | | | | | | | | |
| 4. <i>Borrelia turcica</i> IST7 (KF422815) | 88.0 | 87.9 | 87.9 | | | | | | | | | | | | | | |
| 5. <i>Borrelia anserina</i> BA2 (CP005829) | 85.9 | 86.0 | 85.8 | 87.2 | | | | | | | | | | | | | |
| 6. <i>Borrelia hermsii</i> (NC_010673) | 84.7 | 84.8 | 84.6 | 86.7 | 92.9 | | | | | | | | | | | | |
| 7. <i>Borrelia parkeri</i> (CP007022) | 84.6 | 84.7 | 84.5 | 87.3 | 93.2 | 93.3 | | | | | | | | | | | |
| 8. <i>Borrelia miyamotoi</i> (CP006647) | 82.3 | 82.4 | 82.3 | 85.1 | 91.9 | 91.1 | 91.7 | | | | | | | | | | |
| 9. <i>Borrelia crocidurae</i> (CP003426) | 83.3 | 83.2 | 83.2 | 86.1 | 90.1 | 89.0 | 90.3 | 89.0 | | | | | | | | | |
| 10. <i>Borrelia duttonii</i> Ly (CP000976) | 82.8 | 82.6 | 82.6 | 85.8 | 90.1 | 88.8 | 90.3 | 89.0 | 98.8 | | | | | | | | |
| 11. <i>Borrelia recurrentis</i> (CP000993) | 82.6 | 82.5 | 82.5 | 85.8 | 90.1 | 88.8 | 90.3 | 89.0 | 98.8 | 99.7 | | | | | | | |
| 12. <i>Borrelia hispanica</i> (U28498) | 80.0 | 79.9 | 79.9 | 82.3 | 87.1 | 85.3 | 86.7 | 85.2 | 93.1 | 93.9 | 93.8 | | | | | | |
| 13. <i>Borrelia afzelii</i> Tom3107 (CP009212) | 83.2 | 83.1 | 82.8 | 85.6 | 85.5 | 84.1 | 84.2 | 82.8 | 84.1 | 83.5 | 83.5 | 81.3 | | | | | |
| 14. <i>Borrelia garinii</i> (CP003151) | 83.0 | 82.8 | 82.6 | 85.3 | 84.5 | 83.3 | 84.1 | 81.7 | 83.2 | 82.6 | 82.6 | 80.0 | 94.7 | | | | |
| 15. <i>Borrelia valaisiana</i> (CP009117) | 82.7 | 82.6 | 82.3 | 86.2 | 84.6 | 84.1 | 84.5 | 83.0 | 83.9 | 83.7 | 83.7 | 81.2 | 95.7 | 95.2 | | | |
| 16. <i>Borrelia chilensis</i> (CP009910) | 83.1 | 83.0 | 82.7 | 86.0 | 84.7 | 83.7 | 84.3 | 83.0 | 83.7 | 83.5 | 83.3 | 81.4 | 95.1 | 93.8 | 94.9 | | |
| 17. <i>Borrelia burgdorferi</i> B31 (AE000783) | 82.4 | 82.3 | 82.1 | 85.7 | 84.3 | 83.3 | 83.7 | 82.0 | 83.7 | 83.5 | 83.4 | 81.4 | 94.4 | 93.6 | 94.7 | 94.9 | |
| 18. <i>Brachyspira pilosicoli</i> (AY241832) | 46.5 | 46.7 | 46.7 | 46.8 | 47.8 | 47.3 | 47.2 | 47.0 | 46.7 | 46.6 | 46.5 | 45.8 | 46.3 | 47.4 | 46.9 | 47.4 | 47.2 |

Supplementary Table 3. Genetic distance matrix of partial *glpQ* gene sequences showing percentage nucleotide identities between ‘*Candidatus* *Borrelia* tachyglossi’ genotypes A, B, and C, other *Borrelia* species, and the outgroup, *Escherichia coli*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----|------|------|
| 1. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype B (KY586967) | | | | | | | | | | | | | | | | | | |
| 2. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype A (KY586968) | 98.6 | | | | | | | | | | | | | | | | | |
| 3. <i>Borrelia</i> sp. tAG17M (AB529398) | 84.3 | 84.4 | | | | | | | | | | | | | | | | |
| 4. <i>Borrelia</i> sp. tAG88 (AB529404) | 84.4 | 84.8 | 99.7 | | | | | | | | | | | | | | | |
| 5. <i>Borrelia</i> sp. tAG66M (AB529402) | 84.1 | 84.4 | 99.2 | 99.5 | | | | | | | | | | | | | | |
| 6. <i>Borrelia</i> sp. tAG85 (AB529403) | 84.3 | 84.7 | 99.2 | 99.5 | 99.1 | | | | | | | | | | | | | |
| 7. <i>Borrelia turcica</i> (AB529430) | 84.1 | 84.2 | 95.5 | 95.4 | 95.0 | 95.0 | | | | | | | | | | | | |
| 8. <i>Borrelia</i> sp. Tortoise14H1 (AB529431) | 85.4 | 85.7 | 94.1 | 94.2 | 93.7 | 93.7 | 93.6 | | | | | | | | | | | |
| 9. <i>Borrelia</i> sp. Tick98M (AB529432) | 83.9 | 84.0 | 86.5 | 86.3 | 85.9 | 86.3 | 85.6 | 86.6 | | | | | | | | | | |
| 10. <i>Borrelia anserina</i> BA2 (CP005829) | 83.8 | 83.8 | 83.2 | 83.3 | 83.1 | 83.3 | 84.4 | 83.6 | 82.0 | | | | | | | | | |
| 11. <i>Borrelia coriaceae</i> Co53 (CP005745) | 84.1 | 84.8 | 83.0 | 83.0 | 82.7 | 82.8 | 83.3 | 83.1 | 82.8 | 87.6 | | | | | | | | |
| 12. <i>Borrelia parkeri</i> (CP007022) | 84.6 | 84.6 | 82.4 | 82.3 | 82.0 | 82.3 | 84.1 | 83.0 | 83.3 | 89.6 | 89.2 | | | | | | | |
| 13. <i>Borrelia hermsii</i> (CP005680) | 84.1 | 84.3 | 83.0 | 82.8 | 82.8 | 82.7 | 84.4 | 83.0 | 83.5 | 88.3 | 87.9 | 90.3 | | | | | | |
| 14. <i>Borrelia miyamotoi</i> (CP006647) | 83.1 | 82.8 | 83.1 | 82.7 | 82.5 | 82.7 | 83.3 | 82.7 | 83.2 | 87.0 | 86.7 | 87.9 | 88.2 | | | | | |
| 15. <i>Borrelia theileri</i> (KF569938) | 80.5 | 80.8 | 82.2 | 81.8 | 81.6 | 81.8 | 81.8 | 80.8 | 81.7 | 84.4 | 85.4 | 85.0 | 85.9 | 90.6 | | | | |
| 16. <i>Borrelia crocidurae</i> (CP003426) | 80.8 | 80.9 | 80.4 | 80.5 | 80.3 | 80.5 | 80.5 | 80.7 | 81.1 | 84.4 | 84.6 | 84.6 | 84.8 | 83.2 | 82.6 | | | |
| 17. <i>Borrelia duttonii</i> Ly (CP000976) | 80.7 | 81.0 | 80.3 | 80.4 | 80.2 | 80.2 | 80.4 | 81.0 | 80.9 | 84.1 | 84.9 | 84.4 | 85.0 | 83.2 | 82.0 | 97 | | |
| 18. <i>Borrelia recurrentis</i> (CP000993) | 80.5 | 80.9 | 80.2 | 80.3 | 80.1 | 80.1 | 80.3 | 80.9 | 81.0 | 84.0 | 85.0 | 84.6 | 85.1 | 83.1 | 82.2 | 97 | 99.7 | |
| 19. <i>Escherichia coli</i> (X56907) | 54.0 | 54.1 | 55.2 | 55.3 | 55.1 | 55.3 | 55.8 | 54.5 | 54.9 | 56.4 | 56.3 | 56.2 | 54.9 | 55.4 | 55.8 | 54 | 54.5 | 54.4 |

Supplementary Table 4. Genetic distance matrix of partial *groEL* gene sequences showing percentage nucleotide identities between ‘*Candidatus* *Borrelia* tachyglossi’ genotypes A, B, and C, other *Borrelia* species, and the outgroup, *Treponema pallidum*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype B (KY586969) | | | | | | | | | | | | | | | | |
| 2. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype A (KY586971) | 100 | | | | | | | | | | | | | | | |
| 3. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype C (KY586970) | 99.9 | 99.9 | | | | | | | | | | | | | | |
| 4. <i>Borrelia</i> anserina (CP005829) | 88.9 | 88.9 | 89.0 | | | | | | | | | | | | | |
| 5. <i>Borrelia</i> miyamotoi (CP006647) | 88.8 | 88.8 | 88.9 | 91.6 | | | | | | | | | | | | |
| 6. <i>Borrelia</i> parkeri (CP007022) | 89.0 | 89.0 | 89.1 | 92.8 | 92.9 | | | | | | | | | | | |
| 7. <i>Borrelia</i> hermsii (NC_010673) | 89.3 | 89.3 | 89.4 | 92.9 | 92.5 | 94.3 | | | | | | | | | | |
| 8. <i>Borrelia</i> crocidurae (CP003426) | 88.2 | 88.2 | 88.3 | 91.9 | 91.8 | 91.9 | 92.2 | | | | | | | | | |
| 9. <i>Borrelia</i> duttonii Ly (CP000976) | 87.9 | 87.9 | 88.0 | 91.9 | 91.5 | 91.7 | 91.9 | 99.2 | | | | | | | | |
| 10. <i>Borrelia</i> recurrentis (CP000993) | 88.0 | 88.0 | 88.2 | 92.0 | 91.7 | 91.9 | 91.9 | 99.3 | 99.6 | | | | | | | |
| 11. <i>Borrelia</i> hispanica (GU357581) | 87.6 | 87.6 | 87.8 | 91.6 | 91.1 | 91.7 | 91.6 | 98.2 | 98.0 | 98.0 | | | | | | |
| 12. <i>Borrelia</i> afzelii Tom3107 (CP009212) | 84.3 | 84.3 | 84.3 | 84.4 | 85.6 | 84.9 | 85.2 | 84.7 | 84.8 | 84.9 | 84.5 | | | | | |
| 13. <i>Borrelia</i> garinii (CP003151) | 83.9 | 83.9 | 83.9 | 84.3 | 85.2 | 84.8 | 84.4 | 84.3 | 84.5 | 84.6 | 84.2 | 96.2 | | | | |
| 14. <i>Borrelia</i> valaisiana (CP009117) | 84.0 | 84.0 | 83.9 | 84.3 | 85.4 | 84.6 | 84.8 | 84.4 | 84.5 | 84.6 | 84.3 | 96.1 | 95.7 | | | |
| 15. <i>Borrelia</i> burgdorferi B31 (AE000783) | 84.3 | 84.3 | 84.3 | 84.4 | 85.4 | 85.0 | 84.8 | 84.5 | 84.6 | 84.7 | 84.1 | 95.8 | 95.6 | 94.5 | | |
| 16. <i>Borrelia</i> chilensis VA1 (CP009910) | 85.0 | 85.0 | 84.9 | 84.3 | 85.1 | 85.0 | 84.7 | 84.5 | 84.5 | 84.6 | 84.5 | 93.4 | 92.8 | 93.3 | 93.6 | |
| 17. <i>Treponema</i> pallidum (NZ_CP010566) | 63.7 | 63.7 | 63.7 | 62.5 | 61.6 | 62.1 | 62.1 | 62.6 | 62.7 | 62.7 | 62.1 | 62.7 | 62.4 | 62.7 | 62.4 | 63.2 |

Supplementary Table 5. Genetic distance matrix of partial *gyrB* gene sequences showing percentage nucleotide identities between ‘*Candidatus* *Borrelia* tachyglossi’ genotypes A, B, and C, other *Borrelia* species, and the outgroup, *Spirochaeta lutea*.

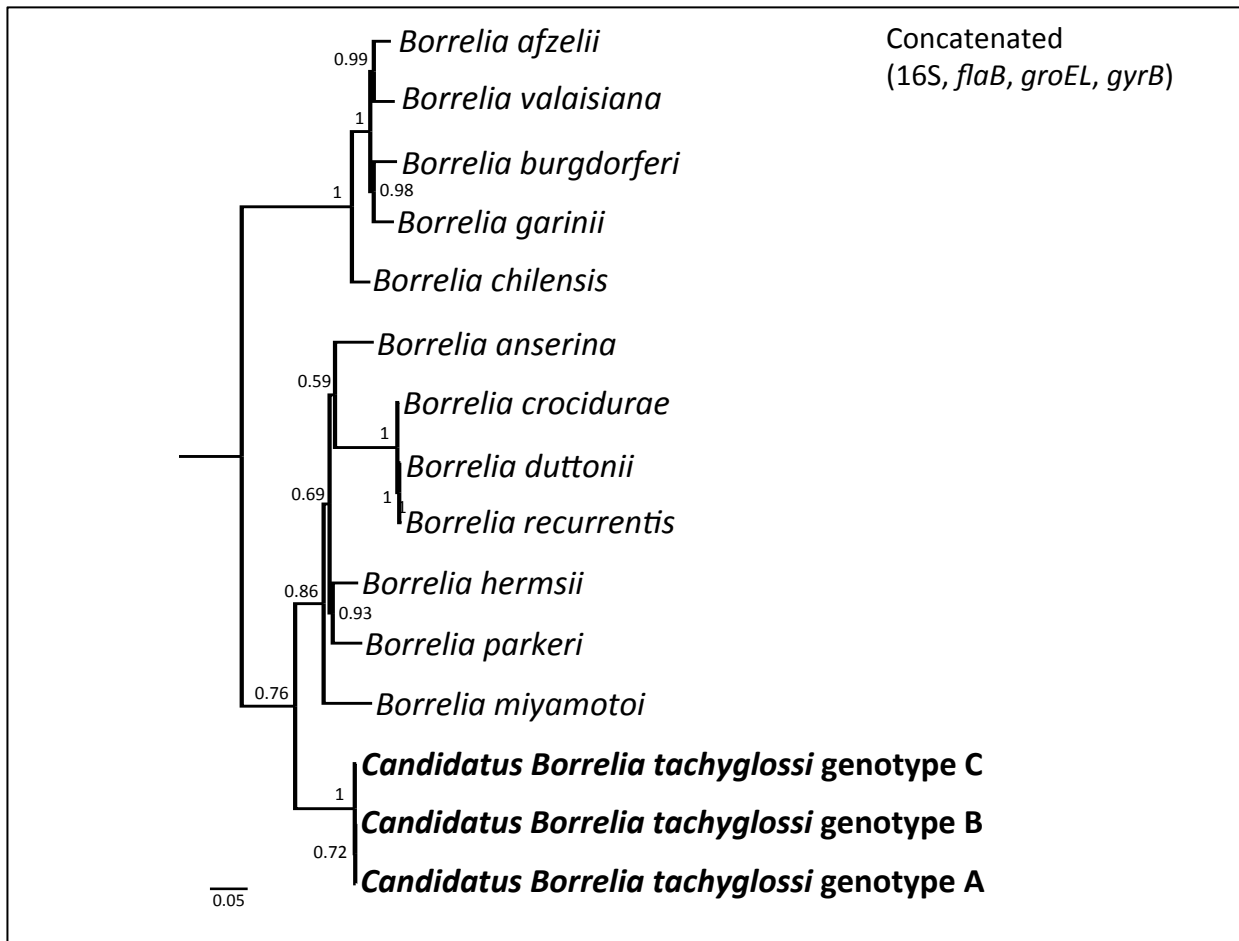
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype B (KY586972) | | | | | | | | | | | | | | | | |
| 2. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype A (KY586974) | 99.9 | | | | | | | | | | | | | | | |
| 3. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype C (KY586973) | 99.6 | 99.7 | | | | | | | | | | | | | | |
| 4. <i>Borrelia</i> anserina BA2 (CP005829) | 86.0 | 86.1 | 86.1 | | | | | | | | | | | | | |
| 5. <i>Borrelia</i> parkeri (CP007022) | 86.9 | 87.0 | 86.8 | 91.1 | | | | | | | | | | | | |
| 6. <i>Borrelia</i> hermsii (NC_010673) | 88.1 | 88.2 | 88.2 | 90.7 | 92.8 | | | | | | | | | | | |
| 7. <i>Borrelia</i> miyamotoi (CP006647) | 86.8 | 87.0 | 86.9 | 88.1 | 89.0 | 90.0 | | | | | | | | | | |
| 8. <i>Borrelia</i> crocidurae (CP003426) | 85.3 | 85.4 | 85.1 | 86.7 | 87.3 | 88.3 | 86.7 | | | | | | | | | |
| 9. <i>Borrelia</i> duttonii Ly (CP000976) | 85.4 | 85.4 | 85.2 | 86.7 | 87.4 | 88.4 | 86.6 | 99.6 | | | | | | | | |
| 10. <i>Borrelia</i> recurrentis (CP000993) | 85.0 | 85.1 | 84.8 | 86.5 | 87.3 | 88.3 | 86.4 | 99.3 | 99.5 | | | | | | | |
| 11. <i>Borrelia</i> turcica IST7 (AB473535) | 87.9 | 88.0 | 87.8 | 85.0 | 86.4 | 86.7 | 86.2 | 85.7 | 85.6 | 85.4 | | | | | | |
| 12. <i>Borrelia</i> afzelii Pko (CP002933) | 80.8 | 81.0 | 80.8 | 79.6 | 80.8 | 79.7 | 80.3 | 79.8 | 79.8 | 79.7 | 80.8 | | | | | |
| 13. <i>Borrelia</i> garinii (CP003151) | 81.2 | 81.3 | 81.2 | 79.4 | 80.8 | 80.1 | 79.6 | 80.2 | 80.3 | 80.1 | 80.5 | 94.4 | | | | |
| 14. <i>Borrelia</i> valaisiana (CP009117) | 81.3 | 81.4 | 81.3 | 80.0 | 80.9 | 80.2 | 80.5 | 80.1 | 80.3 | 80.1 | 80.7 | 94.4 | 93.5 | | | |
| 15. <i>Borrelia</i> burgdorferi B31 (AE000783) | 80.8 | 80.9 | 80.8 | 79.4 | 80.7 | 80.0 | 79.8 | 80.3 | 80.4 | 80.3 | 80.9 | 93.9 | 93.9 | 93.6 | | |
| 16. <i>Borrelia</i> chilensis VA1 (CP009910) | 81.9 | 82.0 | 82.0 | 80.1 | 81.6 | 81.2 | 80.6 | 81.0 | 80.9 | 80.7 | 80.7 | 92.9 | 92.9 | 92.7 | 92.7 | |
| 17. <i>Spirochaeta</i> lutea (JNUP01000064) | 59.3 | 59.4 | 59.3 | 57.8 | 59.1 | 58.8 | 58.2 | 58.7 | 58.6 | 58.6 | 59.7 | 58.3 | 58.6 | 58.5 | 58.5 | 58.1 |

Supplementary Table 6. Genetic distance matrix of concatenated 16S, *flaB*, and *gyrB* gene sequences showing percentage nucleotide identities between ‘*Candidatus* *Borrelia* *tachyglossi*’ genotypes A, B, and C, other *Borrelia* species, and the outgroup, *Spirochaeta* *lutea*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1. <i>Candidatus</i> <i>Borrelia</i> <i>tachyglossi</i> genotype B | | | | | | | | | | | | | | | | |
| 2. <i>Candidatus</i> <i>Borrelia</i> <i>tachyglossi</i> genotype A | 99.8 | | | | | | | | | | | | | | | |
| 3. <i>Candidatus</i> <i>Borrelia</i> <i>tachyglossi</i> genotype C | 99.7 | 99.8 | | | | | | | | | | | | | | |
| 4. <i>Borrelia</i> <i>turcica</i> | 91.2 | 91.2 | 91.1 | | | | | | | | | | | | | |
| 5. <i>Borrelia</i> <i>anserina</i> | 89.6 | 89.6 | 89.6 | 89.3 | | | | | | | | | | | | |
| 6. <i>Borrelia</i> <i>hermsii</i> | 90.7 | 90.8 | 90.8 | 90.2 | 93.6 | | | | | | | | | | | |
| 7. <i>Borrelia</i> <i>parkeri</i> | 89.9 | 90.0 | 89.9 | 90.1 | 93.9 | 94.9 | | | | | | | | | | |
| 8. <i>Borrelia</i> <i>miyamotoi</i> | 89.2 | 89.3 | 89.3 | 89.3 | 91.9 | 92.8 | 92.4 | | | | | | | | | |
| 9. <i>Borrelia</i> <i>crocidurae</i> | 88.8 | 88.9 | 88.7 | 89.6 | 90.8 | 91.6 | 91.4 | 90.5 | | | | | | | | |
| 10. <i>Borrelia</i> <i>duttonii</i> | 88.7 | 88.8 | 88.6 | 89.4 | 90.8 | 91.6 | 91.4 | 90.4 | 99.6 | | | | | | | |
| 11. <i>Borrelia</i> <i>recurrentis</i> | 88.5 | 88.5 | 88.4 | 89.3 | 90.7 | 91.4 | 91.3 | 90.3 | 99.3 | 99.6 | | | | | | |
| 12. <i>Borrelia</i> <i>afzelii</i> | 86.0 | 86.0 | 86.0 | 86.7 | 86.0 | 85.7 | 86.4 | 85.6 | 85.7 | 85.6 | 85.4 | | | | | |
| 13. <i>Borrelia</i> <i>valaisiana</i> | 86.2 | 86.2 | 86.2 | 86.8 | 85.9 | 86.0 | 86.5 | 85.7 | 85.8 | 85.8 | 85.7 | 96.2 | | | | |
| 14. <i>Borrelia</i> <i>garinii</i> | 86.2 | 86.2 | 86.2 | 86.4 | 85.5 | 85.8 | 86.3 | 85.1 | 85.6 | 85.6 | 85.4 | 95.8 | 95.5 | | | |
| 15. <i>Borrelia</i> <i>burgdorferi</i> | 85.8 | 85.8 | 85.9 | 86.8 | 85.6 | 85.8 | 86.3 | 85.1 | 85.9 | 86.0 | 85.8 | 95.6 | 95.5 | 95.5 | | |
| 16. <i>Borrelia</i> <i>chilensis</i> | 86.7 | 86.7 | 86.7 | 86.9 | 86.2 | 86.6 | 87.0 | 85.9 | 86.3 | 86.3 | 86.0 | 95.2 | 95.1 | 94.9 | 95.1 | |
| 17. <i>Spirochaeta</i> <i>lutea</i> | 63.1 | 63.2 | 63.1 | 63.1 | 62.0 | 62.8 | 62.8 | 62.1 | 62.6 | 62.5 | 62.4 | 62.4 | 62.7 | 62.5 | 62.7 | 62.6 |

Supplementary Table 7. Genetic distance matrix of concatenated 16S, *flaB*, *groEL*, and *gyrB* gene sequences showing percentage nucleotide identities between ‘*Candidatus* *Borrelia* tachyglossi’ genotypes A, B, and C, other *Borrelia* species, and the outgroup, *Spirochaeta lutea*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|--|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype B | | | | | | | | | | | | | | | |
| 2. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype C | 99.8 | | | | | | | | | | | | | | |
| 3. <i>Candidatus</i> <i>Borrelia</i> tachyglossi genotype A | 99.9 | 99.8 | | | | | | | | | | | | | |
| 4. <i>Borrelia</i> anserina | 89.4 | 89.4 | 89.4 | | | | | | | | | | | | |
| 5. <i>Borrelia</i> hermsii | 90.3 | 90.3 | 90.3 | 93.4 | | | | | | | | | | | |
| 6. <i>Borrelia</i> parkeri | 89.7 | 89.7 | 89.7 | 93.5 | 94.7 | | | | | | | | | | |
| 7. <i>Borrelia</i> miyamotoi | 89.1 | 89.2 | 89.2 | 91.8 | 92.7 | 92.6 | | | | | | | | | |
| 8. <i>Borrelia</i> crocidurae | 88.6 | 88.6 | 88.6 | 91.1 | 91.8 | 91.6 | 90.9 | | | | | | | | |
| 9. <i>Borrelia</i> duttonii | 88.5 | 88.4 | 88.5 | 91.1 | 91.7 | 91.5 | 90.8 | 99.5 | | | | | | | |
| 10. <i>Borrelia</i> recurrentis | 88.3 | 88.3 | 88.4 | 91.1 | 91.6 | 91.5 | 90.7 | 99.3 | 99.6 | | | | | | |
| 11. <i>Borrelia</i> afzelii | 85.5 | 85.5 | 85.5 | 85.5 | 85.6 | 85.9 | 85.6 | 85.4 | 85.4 | 85.3 | | | | | |
| 12. <i>Borrelia</i> valaisiana | 85.5 | 85.5 | 85.5 | 85.5 | 85.7 | 85.9 | 85.6 | 85.4 | 85.4 | 85.3 | 96.2 | | | | |
| 13. <i>Borrelia</i> garinii | 85.5 | 85.5 | 85.5 | 85.1 | 85.4 | 85.8 | 85.1 | 85.2 | 85.2 | 85.1 | 95.9 | 95.6 | | | |
| 14. <i>Borrelia</i> burgdorferi | 85.4 | 85.4 | 85.4 | 85.2 | 85.5 | 85.9 | 85.2 | 85.5 | 85.6 | 85.5 | 95.7 | 95.2 | 95.6 | | |
| 15. <i>Borrelia</i> chilensis | 86.2 | 86.2 | 86.2 | 85.7 | 86.0 | 86.4 | 85.6 | 85.7 | 85.7 | 85.6 | 94.7 | 94.6 | 94.3 | 94.6 | |
| 16. <i>Spirochaeta</i> lutea | 62.9 | 62.9 | 63.0 | 62.2 | 62.6 | 62.5 | 62.0 | 62.6 | 62.5 | 62.4 | 62.5 | 62.7 | 62.5 | 62.7 | 62.7 |



Supplementary Fig. 1. Phylogenetic reconstructions of concatenated 16S, *flaB*, *groEL*, and *gyrB* sequences of ‘*Candidatus Borrelia tachyglossi*’ genotypes A, B, and C identified in *B. concolor* ticks from echidnas. *Spirochaeta lutea* was used as outgroup. Sequences in this study are indicated in bold.

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