

**Table S1.** Seasonal occurrence of four *Ixodes* species collected from ears of 243 red foxes harvested during the fox-hunting seasons (June 1st to March 31st) between 2009 and 2011 in west-central Poland

| Tick<br>species     | No. (%) infested hosts /no. ticks/no. ticks per host/n. ticks per infested host |                         |                         |
|---------------------|---|-------------------------|-------------------------|
|                     | Spring/Summer (VI-VIII)   |                         | Autumn (IX-XI)          |
|                     | (n = 70)*   | (n = 96)                | (n = 77)                |
| <i>I. ricinus</i>   | 29 (41.4)/102/1.5/3.5   | 17 (17.7)/43/0.4/2.5    | 10 (13.0)/17/0.2/1.7    |
| <i>I. kaiseri</i>   | 21 (30.0)/383/5.5/18.2  | 28 (29.2)/290/3.0/10.4  | 33 (42.9)/532/6.9/16.1  |
| <i>I. canisuga</i>  | 4 (5.7)/14/0.2/3.5  | 21 (21.9)/65/0.7/3.1    | 20 (30.0)/109/1.4/5.5   |
| <i>I. hexagonus</i> | 3 (4.3)/3/0.1/1.0   | 8 (8.3)/12/0.1/1.5      | 8 (10.4)/13/0.2/1.6     |
| Total               | 33 (47.1) /502/7.2/15.2   | 41 (42.7) /410/4.3/10.0 | 46 (59.7) /671/8.7/14.6 |

\*In square brackets: number of hosts sampled during a season.

**Table S2.** Detection of LD *Borrelia* species concurrently in two isolate types derived from red foxes. BA – *B. afzelii*, BG – *B. garinii*

| Fox' no. | Isolate type |       |       |
|----------|--------------|-------|-------|
|          | Blood        | Skin  | Liver |
| 1        | BA           | BA/BG | -     |
| 2        | BA/BG        | BA    | -     |
| 3        | BG           | BG    | -     |
| 4        | BG           | BG    | -     |
| 5        | BA           | BA    | -     |
| 6        | BG           | BA    | -     |
| 7        | BG           | -     | BA    |
| 8        | BG           | -     | BA    |
| 9        | BG           | -     | BA    |
| 10       | -            | BG    | BA    |

**Table S3.** Comparison of the selected 112 partial *flaB* gene sequences of *Borrelia* species amplified from red foxes (blood, and skin samples: n = 13, and 2, respectively) and their engorged *Ixodes* ticks (n = 97) sampled in this study.

| Identified<br><i>Borrelia</i><br>species | Strain   | Accessio<br>n number | Reference sequence<br>(species, strain) | Accession<br>number | Query<br>coverage | E value | Identity         |
|--|----------|----------------------|---|---------------------|-------------------|---------|------------------|
| <i>B. garinii</i>                        | 9N1L-IC  | KF422759             | <i>B. garinii</i> 20047                 | CP028861            | 100.0%            | 0.0     | 789/789 (100.0%) |
|  | 1N1N-IH  | KF422762             |   |                     | 100.0%            | 0.0     | 789/789 (100.0%) |
|  | 4C1F-IH  | KF422765             |   |                     | 100.0%            | 0.0     | 788/789 (99.87%) |
|  | K2-1F-IR | KF422774             |   |                     | 100.0%            | 0.0     | 788/789 (99.87%) |

|                   |             |          |                        |          |                  |     |                  |
|-------------------|-------------|----------|------------------------|----------|------------------|-----|------------------|
|                   |             |          |                        |          |                  |     |                  |
| 1N1L-IC           | KF422779    |          | 100.0%                 | 0.0      | 787/789 (99.75%) |     |                  |
| 3N5L-IC           | KF422780    |          | 100.0%                 | 0.0      | 787/789 (99.75%) |     |                  |
| 7N5L-IC           | KF422764    |          | 100.0%                 | 0.0      | 786/789 (99.62%) |     |                  |
| 15Z13L-IC         | KF422781    |          | 100.0%                 | 0.0      | 786/789 (99.62%) |     |                  |
| 33NM1934-LC       | MG94499     | 6        | 100.0%                 | 0.0      | 786/789 (99.62%) |     |                  |
| 1N2N-IC           | KF422763    |          | 100.0%                 | 0.0      | 781/789 (98.99%) |     |                  |
| 3A2F-IC           | KF422758    |          | 100.0%                 | 0.0      | 777/789 (98.48%) |     |                  |
| 8N1L-IC           | KF422782    |          | 100.0%                 | 0.0      | 777/789 (98.48%) |     |                  |
| 1A1F-IR           | KF422766    |          | 100.0%                 | 0.0      | 775/789 (98.23%) |     |                  |
| 2Z2F-IR           | KF422767    |          | 100.0%                 | 0.0      | 772/789 (97.85%) |     |                  |
| 3A2N-IH           | HM80218     | 4        | 100.0%                 | 0.0      | 602/604 (99.67%) |     |                  |
| AM10g-BL*         | KF422841    |          | 100.0%                 | 0.0      | 601/604 (99.50%) |     |                  |
| 28K2N2-IC         | KF918603    |          | 100.0%                 | 0.0      | 601/604 (99.50%) |     |                  |
| 9PD12L1-IR        | KF918612    |          | 100.0%                 | 0.0      | 600/604 (99.34%) |     |                  |
| 34M11g-BL*        | KF422840    |          | 100.0%                 | 0.0      | 598/604 (99.01%) |     |                  |
| 30C10g-BL*        | KF422844    |          | 100.0%                 | 0.0      | 598/604 (99.01%) |     |                  |
| 22W56L1-IC        | KF918609    |          | 100.0%                 | 0.0      | 598/604 (99.01%) |     |                  |
| 12M10g-BL*        | KF422838    |          | 100.0%                 | 0.0      | 597/604 (98.84%) |     |                  |
| 39PA2L10g-IK      | KF422846    |          | 100.0%                 | 0.0      | 596/604 (98.68%) |     |                  |
| 32M11-BL*         | KF422839    |          | 100.0%                 | 0.0      | 595/604 (98.51%) |     |                  |
| 8N2L-IC           | KF918607    |          | 100.0%                 | 0.0      | 595/604 (98.51%) |     |                  |
| 4M4L-IC           | HM80218     | 2        | 100.0%                 | 0.0      | 594/604 (98.34%) |     |                  |
| 7N4L-IC           | KF422817    |          | 100.0%                 | 0.0      | 594/604 (98.34%) |     |                  |
| 10C10g1-BL*       | KF422842    |          | 100.0%                 | 0.0      | 594/604 (98.34%) |     |                  |
| 5N17L1-IC         | KF918606    |          | 100.0%                 | 0.0      | 594/604 (98.34%) |     |                  |
| 8N9L2-IC          | KF918608    |          | 100.0%                 | 0.0      | 594/604 (98.34%) |     |                  |
| 29C4N1-IC         | KF918611    |          | 100.0%                 | 0.0      | 594/604 (98.34%) |     |                  |
| 3N13L2-IC         | KF918604    |          | 100.0%                 | 0.0      | 593/604 (98.18%) |     |                  |
| 5P1N-IR           | HM80218     | 8        | 100.0%                 | 0.0      | 592/604 (98.01%) |     |                  |
| 10C10g2-BL*       | KF422843    |          | 100.0%                 | 0.0      | 592/604 (98.01%) |     |                  |
| 28K2N1-IC         | KF918602    |          | 100.0%                 | 0.0      | 592/604 (98.01%) |     |                  |
| 22W56L2-IC        | KF918610    |          | 100.0%                 | 0.0      | 592/604 (98.01%) |     |                  |
| 7Z8L-IH           | HM80218     | 5        | 100.0%                 | 0.0      | 591/604 (97.85%) |     |                  |
| 2Z-SK**           | KF918600    |          | 100.0%                 | 0.0      | 591/604 (97.85%) |     |                  |
| 6A1N-IC           | KF918601    |          | 100.0%                 | 0.0      | 590/604 (97.68%) |     |                  |
| 15Z2L-IC          | KF918605    |          | 100.0%                 | 0.0      | 589/604 (97.52%) |     |                  |
| 36K3L10-IC        | KF422845    |          | 100.0%                 | 0.0      | 585/604 (96.85%) |     |                  |
| <i>B. afzelii</i> | 15Z1L-IC    | KF422794 | <i>B. afzelii</i> BO23 | CP018262 | 100.0%           | 0.0 | 789/789 (100.0%) |
|                   | 22NM1779-LC | MG94496  |                        |          | 100.0%           | 0.0 | 788/789 (99.87%) |
|                   | 34MR2062-FR | MG94496  |                        |          | 100.0%           | 0.0 | 788/789 (99.87%) |
|                   | 15Z8L-IC    | KF422795 |                        |          | 100.0%           | 0.0 | 787/789 (99.75%) |
|                   | 20MR2041-LR | MG94496  |                        |          | 100.0%           | 0.0 | 787/789 (99.75%) |
|                   | 7N1N-IC     | KF422789 |                        |          | 100.0%           | 0.0 | 786/789 (99.62%) |
|                   | 28K1N-IH    | KF422790 |                        |          | 100.0%           | 0.0 | 786/789 (99.62%) |
|                   | 3A1F-IK     | KF422787 |                        |          | 100.0%           | 0.0 | 784/789 (99.37%) |

| 8N10L-IC                                 | KF422796        |                      | 100.0%                                  | 0.0                 | 784/789 (99.37%)  |         |                  |
|--|-----------------|----------------------|---|---------------------|-------------------|---------|------------------|
| 1A10N-IR                                 | KF422791        |                      | 100.0%                                  | 0.0                 | 781/789 (98.99%)  |         |                  |
| 3N13L1-IC                                | KF918614        |                      | 100.0%                                  | 0.0                 | 601/604 (99.50%)  |         |                  |
| 9PD12L2-IR                               | KF918616        |                      | 100.0%                                  | 0.0                 | 601/604 (99.50%)  |         |                  |
| 3A3F-IC                                  | HM80219<br>3    |                      | 100.0%                                  | 0.0                 | 600/604 (99.34%)  |         |                  |
| 30C10a-BL*                               | KF422863        |                      | 100.0%                                  | 0.0                 | 600/604 (99.34%)  |         |                  |
| 42DG2N10-<br>IK                          | KF422865        |                      | 100.0%                                  | 0.0                 | 600/604 (99.34%)  |         |                  |
| 8N9L1-IC                                 | KF918615        |                      | 100.0%                                  | 0.0                 | 600/604 (99.34%)  |         |                  |
| AM10a-BL*                                | KF422861        |                      | 100.0%                                  | 0.0                 | 599/604 (99.17%)  |         |                  |
| 39PA2L10a-<br>IK                         | KF422864        |                      | 100.0%                                  | 0.0                 | 596/604 (98.68%)  |         |                  |
| 9M10-BL*                                 | KF422858        |                      | 100.0%                                  | 0.0                 | 594/604 (98.34%)  |         |                  |
| 28C10-BL*                                | KF422862        |                      | 100.0%                                  | 0.0                 | 593/604 (98.18%)  |         |                  |
| 34M11a-BL*                               | KF422860        |                      | 100.0%                                  | 0.0                 | 591/604 (97.85%)  |         |                  |
| 12M10a-BL*                               | KF422859        |                      | 100.0%                                  | 0.0                 | 589/604 (97.52%)  |         |                  |
| Identified<br><i>Borrelia</i><br>species | Strain          | Accessio<br>n number | Reference sequence<br>(species, strain) | Accession<br>number | Query<br>coverage | E value | Identity         |
| <i>B. burgdorferi</i><br>s.s.            | 22NM1783-<br>LC | MG94497<br>8         | <i>B. burgdorferi</i> ZS7               | CP001205            | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 22NM1798-<br>LC | MG94497<br>9         |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 24NM1809-<br>FR | MG94498<br>0         |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 31NM1824-<br>NH | MG94498<br>1         |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 32NM1835-<br>LH | MG94498<br>2         |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 32NM1843-<br>LC | MG94498<br>3         |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 5N11N-IC        | KF422802             |   |                     | 100.0%            | 0.0     | 786/789 (99.62%) |
|  | 1A3L-IR         | KF422799             |   |                     | 100.0%            | 0.0     | 778/789 (98.61%) |
|  | 3A2F-IC         | HM80219<br>1         |   |                     | 100.0%            | 0.0     | 601/604 (99.50%) |
|  | 5N17L2-IC       | KF918617             |   |                     | 100.0%            | 0.0     | 601/604 (99.50%) |
|  |                 |                      |   |                     |                   |         |                  |
| <i>B. valaisiana</i>                     | 22NM1795-<br>LK | MT11897<br>9         | <i>B. valaisiana</i> VS116              | NZ_ABCY0200<br>0001 | 100.0%            | 0.0     | 789/789 (100.0%) |
|  | 38MR2081-<br>NK | MT11898<br>0         |   |                     | 100.0%            | 0.0     | 789/789 (100.0%) |
|  |                 |                      |   |                     |                   |         |                  |
| <i>B. spielmanii</i>                     | 6MR2001-FR      | MG94497<br>6         | <i>B. spielmanii</i> A14S               | ABKB02000003        | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 43MR2086-<br>LK | MG94497<br>7         |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |
|  | 22NM1790-<br>LK | MT11898<br>1         |   |                     | 100.0%            | 0.0     | 786/789 (99.62%) |
|  | 30NM1817-<br>LK | MT11898<br>2         |   |                     | 100.0%            | 0.0     | 786/789 (99.62%) |
|  | L32-EA**        | JF732881             |   |                     | 100.0%            | 0.0     | 604/604 (100.0%) |
|  |                 |                      |   |                     |                   |         |                  |
| <i>B. bissetiae</i>                      | 22NM1781-<br>LC | MG94496<br>4         | <i>B. bissetiae</i> DN127               | CP002746            | 100.0%            | 0.0     | 788/789 (99.87%) |
|  | 3A5N-IK         | KF422807             |   |                     | 100.0%            | 0.0     | 787/789 (99.75%) |

|                          |             |          |                                |          |        |     |                  |
|--------------------------|-------------|----------|--------------------------------|----------|--------|-----|------------------|
|                          | 12N1N-IK    | KF422806 |                                |          | 100.0% | 0.0 | 785/789 (99.49%) |
|                          | 3N2L-IC     | KF918618 |                                |          | 100.0% | 0.0 | 603/604 (99.83%) |
|                          |             |          |                                |          |        |     |                  |
| <i>B. carolinensis</i>   | 22NM1786-LC | MG944970 | <i>B. carolinensis</i> SCW-22  | KF422810 | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 30NM1816-LC | MG944971 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 30NM1818-LK | MG944972 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 33NM1932-LC | MG944973 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 33MR2061-LC | MG944974 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 35MR2063-NK | MG944975 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          |             |          |                                |          |        |     |                  |
| <i>B. californiensis</i> | 23NM1807-LC | MG944984 | <i>B. californiensis</i> CA446 | KF422809 | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 5MR1999-LR  | MG944986 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 6MR2005-NR  | MG944987 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 13MR2025-LR | MG944988 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 20MR2034-NK | MG944989 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 20MR2038-LK | MG944991 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 30MR2046-LK | MG944992 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 37MR2080-LK | MG944995 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 20MR2035-NK | MG944990 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 33MR2048-LK | MG944993 |                                |          | 100.0% | 0.0 | 788/789 (99.87%) |
|                          | 30NM1820-LC | MG944985 |                                |          | 100.0% | 0.0 | 785/789 (99.49%) |
|                          | 33MR2051-LK | MG944994 |                                |          | 100.0% | 0.0 | 784/789 (99.37%) |
|                          |             |          |                                |          |        |     |                  |
| <i>B. americana</i>      | 29C4N2-IC   | KF918619 | <i>B. americana</i> CA8        | KF422811 | 100.0% | 0.0 | 604/604 (100.0%) |
|                          | 30C4F1-IR   | KF918620 |                                |          | 100.0% | 0.0 | 603/604 (99.83%) |
|                          | 30C4F2-IR   | KF918622 |                                |          | 100.0% | 0.0 | 602/604 (99.67%) |
|                          | 32C7N-IK    | KF918621 |                                |          | 100.0% | 0.0 | 600/604 (99.34%) |
|                          |             |          |                                |          |        |     |                  |
| <i>B. lanei</i>          | 33NM1921-LC | MG944965 | <i>B. lanei</i> CA28           | KF422812 | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 33NM1953-LC | MG944966 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 5MR1995-LK  | MG944967 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 20NM2032-NK | MG944968 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |
|                          | 43MR2088-LK | MG944969 |                                |          | 100.0% | 0.0 | 789/789 (100.0%) |

|                   |             |          |                        |          |        |     |                  |
|-------------------|-------------|----------|------------------------|----------|--------|-----|------------------|
| <i>B. turcica</i> | 33NM1854-LC | MG944997 | <i>B. turcica</i> IST7 | CP028884 | 100.0% | 0.0 | 787/789 (99.75%) |
|-------------------|-------------|----------|------------------------|----------|--------|-----|------------------|

\* blood sample, \*\* skin sample

**Table S4.** MEGA X results of mean distance between *Borrelia* species obtained on the basis of *flaB* gene sequence fragment comparison.

|                            | BB    | BLN   | BAM   | BCL   | BCR   | BBI   | BA    | BSP   | BG    | BV    | BTC |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| <i>B. burgdorferi</i> s.s. |       |       |       |       |       |       |       |       |       |       |     |
| <i>B. lanei</i>            | 0.023 |       |       |       |       |       |       |       |       |       |     |
| <i>B. americana</i>        | 0.035 | 0.025 |       |       |       |       |       |       |       |       |     |
| <i>B. californiensis</i>   | 0.037 | 0.023 | 0.039 |       |       |       |       |       |       |       |     |
| <i>B. carolinensis</i>     | 0.037 | 0.020 | 0.043 | 0.030 |       |       |       |       |       |       |     |
| <i>B. bissettiae</i>       | 0.040 | 0.024 | 0.046 | 0.033 | 0.006 |       |       |       |       |       |     |
| <i>B. afzelii</i>          | 0.066 | 0.056 | 0.069 | 0.067 | 0.058 | 0.065 |       |       |       |       |     |
| <i>B. spielmanii</i>       | 0.064 | 0.051 | 0.071 | 0.066 | 0.055 | 0.062 | 0.057 |       |       |       |     |
| <i>B. garinii</i>          | 0.075 | 0.054 | 0.067 | 0.069 | 0.061 | 0.062 | 0.066 | 0.064 |       |       |     |
| <i>B. valaisiana</i>       | 0.059 | 0.041 | 0.057 | 0.052 | 0.045 | 0.051 | 0.053 | 0.062 | 0.062 |       |     |
| <i>B. turcica</i>          | 0.230 | 0.213 | 0.225 | 0.227 | 0.219 | 0.226 | 0.236 | 0.240 | 0.245 | 0.210 |     |

BB: *B. burgdorferi* s.s., BLN: *B. lanei*, BAM: *B. americana*, BCL: *B. californiensis*, BCR: *B. carolinensis*, BBI: *B. bissettiae*, BA: *B. afzelii*, BSP: *B. spielmanii*, BG: *B. garinii*, BV: *B. valaisiana*, BTC: *B. turcica*

**Table S5.** Comparison of the selected 33 partial *p66* gene sequences of *B. burgdorferi* s.l. species amplified from engorged *Ixodes* ticks removed from red foxes sampled in this study.

| Identified <i>Borrelia</i> species | Strain      | Accession number | Reference sequence (species, strain) | Accession number | Query coverage | E value | Identity         |
|------------------------------------|-------------|------------------|--------------------------------------|------------------|----------------|---------|------------------|
| <i>B. garinii</i>                  | 4C1F-IH     | MT118983         | <i>B. garinii</i> 20047              | CP028861         | 100.0%         | 0.0     | 599/599 (100.0%) |
|                                    | 33NM1934-LC | MT118984         |                                      |                  | 100.0%         | 0.0     | 586/599 (97.83%) |
| <i>B. afzelii</i>                  | 22NM1779-LC | MT118985         | <i>B. afzelii</i> BO23               | CP018262         | 100.0%         | 0.0     | 589/599 (98.33%) |
|                                    | 34MR2062-FR | MT118987         |                                      |                  | 100.0%         | 0.0     | 592/599 (98.83%) |
|                                    | 20MR2041-LR | MT118986         |                                      |                  | 100.0%         | 0.0     | 592/599 (98.83%) |
| <i>B. burgdorferi</i>              | 22NM1783-LC | MT118988         | <i>B. burgdorferi</i> ZS7            | CP001205         | 100.0%         | 0.0     | 589/596 (98.83%) |
|                                    | 22NM1798-LC | MT118989         |                                      |                  | 100.0%         | 0.0     | 594/596 (99.66%) |
|                                    | 24NM1809-FR | MT118990         |                                      |                  | 100.0%         | 0.0     | 589/596 (98.83%) |

|                          |             |          |                                |                 |        |     |                  |
|--------------------------|-------------|----------|--------------------------------|-----------------|--------|-----|------------------|
|                          | 32NM1843-LC | MT118991 |                                |                 | 100.0% | 0.0 | 595/596 (99.83%) |
| <i>B. valaisiana</i>     | 22NM1795-LK | MT118992 | <i>B. valaisiana</i> VS116     | NZ_ABCY02000001 | 100.0% | 0.0 | 594/599 (99.17%) |
|                          | 38MR2081-NK | MT118993 |                                |                 | 100.0% | 0.0 | 599/599 (100.0%) |
| <i>B. spielmanii</i>     | 6MR2001-FR  | MT118996 | <i>B. spielmanii</i> A14S      | ABKB02000003    | 100.0% | 0.0 | 598/599 (99.83%) |
|                          | 43MR2086-LK | MT118997 |                                |                 | 100.0% | 0.0 | 596/599 (99.50%) |
|                          | 22NM1790-LK | MT118994 |                                |                 | 100.0% | 0.0 | 597/599 (99.67%) |
|                          | 30NM1817-LK | MT118995 |                                |                 | 100.0% | 0.0 | 597/599 (99.67%) |
| <i>B. bissetiae</i>      | 22NM1781-LC | MT118998 | <i>B. bissetiae</i> DN127      | CP002746        | 100.0% | 0.0 | 590/599 (98.50%) |
| <i>B. carolinensis</i>   | 30NM1816-LC | MT118999 | <i>B. carolinensis</i> SCW-22  | MT119003        | 100.0% | 0.0 | 597/599 (99.67%) |
|                          | 30NM1818-LK | MT119000 |                                |                 | 100.0% | 0.0 | 589/599 (98.33%) |
|                          | 33NM1932-LC | MT119001 |                                |                 | 100.0% | 0.0 | 598/599 (99.83%) |
|                          | 33MR2061-LC | MT119002 |                                |                 | 100.0% | 0.0 | 591/599 (98.66%) |
| <i>B. californiensis</i> | 23NM1807-LC | MT119004 | <i>B. californiensis</i> CA446 | MT119008        | 100.0% | 0.0 | 595/599 (99.33%) |
|                          | 30NM1820-LC | MT119005 |                                |                 | 100.0% | 0.0 | 595/599 (99.33%) |
|                          | 6MR2005-NR  | MT119006 |                                |                 | 100.0% | 0.0 | 593/599 (99.00%) |
|                          | 33MR2051-LK | MT119007 |                                |                 | 100.0% | 0.0 | 593/599 (99.00%) |
| <i>B. americana</i>      | 29C4N2-IC   | MT119015 | <i>B. americana</i> CA8        | MT119019        | 100.0% | 0.0 | 593/599 (99.00%) |
|                          | 30C4F1-IR   | MT119016 |                                |                 | 100.0% | 0.0 | 592/599 (98.83%) |
|                          | 30C4F2-IR   | MT119017 |                                |                 | 100.0% | 0.0 | 595/599 (99.33%) |
|                          | 32C7N-IK    | MT119018 |                                |                 | 100.0% | 0.0 | 598/599 (99.83%) |
| <i>B. lanei</i>          | 33NM1921-LC | MT119009 | <i>B. lanei</i> CA28           | MT119014        | 100.0% | 0.0 | 596/599 (99.50%) |
|                          | 33NM1953-LC | MT119010 |                                |                 | 100.0% | 0.0 | 596/599 (99.50%) |
|                          | 5MR1995-LK  | MT119011 |                                |                 | 100.0% | 0.0 | 596/599 (99.50%) |
|                          | 20NM2032-NK | MT119012 |                                |                 | 100.0% | 0.0 | 595/599 (99.33%) |
|                          | 43MR2088-LK | MT119013 |                                |                 | 100.0% | 0.0 | 597/599 (99.67%) |

**Table S6.** MEGA X results of mean distance between *B. burgdorferi* s.l. species obtained on the basis of *p66* gene sequence fragment comparison.

|                            | BB    | BLN   | BAM   | BCL   | BCR   | BBI   | BA    | BSP   | BG    | BV |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| <i>B. burgdorferi</i> s.s. |       |       |       |       |       |       |       |       |       |    |
| <i>B. lanei</i>            | 0.046 |       |       |       |       |       |       |       |       |    |
| <i>B. americana</i>        | 0.051 | 0.053 |       |       |       |       |       |       |       |    |
| <i>B. californiensis</i>   | 0.069 | 0.068 | 0.096 |       |       |       |       |       |       |    |
| <i>B. carolinensis</i>     | 0.079 | 0.075 | 0.092 | 0.074 |       |       |       |       |       |    |
| <i>B. bissettiae</i>       | 0.073 | 0.068 | 0.086 | 0.070 | 0.033 |       |       |       |       |    |
| <i>B. afzelii</i>          | 0.082 | 0.086 | 0.089 | 0.103 | 0.104 | 0.086 |       |       |       |    |
| <i>B. spielmanii</i>       | 0.082 | 0.084 | 0.088 | 0.105 | 0.099 | 0.083 | 0.012 |       |       |    |
| <i>B. garinii</i>          | 0.088 | 0.096 | 0.100 | 0.119 | 0.108 | 0.097 | 0.054 | 0.056 |       |    |
| <i>B. valaisiana</i>       | 0.088 | 0.096 | 0.104 | 0.112 | 0.121 | 0.101 | 0.070 | 0.075 | 0.070 |    |

BB: *B. burgdorferi* s.s., BLN: *B. lanei*, BAM: *B. americana*, BCL: *B. californiensis*, BCR: *B. carolinensis*, BBI: *B. bissettiae*, BA: *B. afzelii*, BSP: *B. spielmanii*, BG: *B. garinii*, BV: *B. valaisiana*, BTC: *B. turcica*

**Table S7.** Comparison of the selected 44 partial intergenic spacer (IGS) of 3-methyladenine glycosylase (*mag*) and tRNA-Ile (*trnI*) genes sequences of *Borrelia* species amplified from engorged *Ixodes* ticks removed from red foxes sampled in this study.

| Identified <i>Borrelia</i> species | Strain      | Accession number | Reference organism (species, strain) | Accession number | Query coverage | E value | Identity           |
|------------------------------------|-------------|------------------|--------------------------------------|------------------|----------------|---------|--------------------|
| <i>B. garinii</i>                  | 4C1F-IH     | MT119020         | <i>B. garinii</i> 20047              | CP028861         | 100.0%         | 0.0     | 1179/1183 (99.66%) |
|                                    | 15Z13L-IC   | MT119021         |                                      |                  | 100.0%         | 0.0     | 1176/1183 (99.41%) |
|                                    | 33NM1934-LC | MT119023         |                                      |                  | 100.0%         | 0.0     | 1162/1183 (98.22%) |
|                                    | 8N1L-IC     | MT119022         |                                      |                  | 100.0%         | 0.0     | 1173/1183 (99.15%) |
| <i>B. afzelii</i>                  | 22NM1779-LC | MT119027         | <i>B. afzelii</i> BO23               | CP018262         | 100.0%         | 0.0     | 784/793 (98.87%)   |
|                                    | 34MR2062-FR | MT119029         |                                      |                  | 100.0%         | 0.0     | 784/793 (98.87%)   |
|                                    | 20MR2041-LR | MT119028         |                                      |                  | 100.0%         | 0.0     | 784/793 (98.87%)   |
|                                    | 7N1N-IC     | MT119025         |                                      |                  | 100.0%         | 0.0     | 783/793 (98.74%)   |
|                                    | 28K1N-IH    | MT119026         |                                      |                  | 100.0%         | 0.0     | 783/793 (98.74%)   |
|                                    | 3A1F-IK     | MT119024         |                                      |                  | 100.0%         | 0.0     | 783/793 (98.74%)   |
|                                    | 22NM1783-LC | MT119030         | <i>B. burgdorferi</i> ZS7            |                  | 100.0%         | 0.0     | 530/531 (99.81%)   |
| <i>B. burgdorferi</i>              | 22NM1798-LC | MT119031         |                                      | CP001205         | 100.0%         | 0.0     | 529/531 (99.62%)   |
|                                    | 24NM1809-FR | MT119032         |                                      |                  | 100.0%         | 0.0     | 527/531 (99.25%)   |

|                          |             |              |                                |                     |        |     |                  |
|--------------------------|-------------|--------------|--------------------------------|---------------------|--------|-----|------------------|
|                          | 31NM1824-NH | MT11903<br>3 |                                |                     | 100.0% | 0.0 | 530/531 (99.81%) |
|                          | 32NM1843-LC | MT11903<br>4 |                                |                     | 100.0% | 0.0 | 529/531 (99.62%) |
| <i>B. valaisiana</i>     | 22NM1795-LK | MT11903<br>5 | <i>B. valaisiana</i> VS116     | NZ_ABCY020000<br>01 | 100.0% | 0.0 | 861/861 (100.0%) |
|                          | 38MR2081-NK | MT11903<br>6 |                                |                     | 100.0% | 0.0 | 861/861 (100.0%) |
| <i>B. spielmanii</i>     | 6MR2001-FR  | MT11903<br>9 | <i>B. spielmanii</i> A14S      | ABKB02000003        | 100.0% | 0.0 | 497/498 (99.80%) |
|                          | 43MR2086-LK | MT11904<br>0 |                                |                     | 100.0% | 0.0 | 498/498 (100.0%) |
|                          | 22NM1790-LK | MT11903<br>7 |                                |                     | 100.0% | 0.0 | 496/498 (99.60%) |
|                          | 30NM1817-LK | MT11903<br>8 |                                |                     | 100.0% | 0.0 | 498/498 (100.0%) |
| <i>B. bissetiae</i>      | 22NM1781-LC | MT11904<br>2 | <i>B. bissetiae</i> DN127      | CP002746            | 100.0% | 0.0 | 509/514 (99.03%) |
|                          | 3A5N-IK     | MT11904<br>1 |                                |                     | 100.0% | 0.0 | 514/514 (100.0%) |
| <i>B. carolinensis</i>   | 22NM1786-LC | MT11904<br>3 | <i>B. carolinensis</i> SCW-22  | MT119049            | 100.0% | 0.0 | 450/456 (98.68%) |
|                          | 30NM1816-LC | MT11904<br>4 |                                |                     | 100.0% | 0.0 | 451/456 (98.90%) |
|                          | 30NM1818-LK | MT11904<br>5 |                                |                     | 100.0% | 0.0 | 456/456 (100.0%) |
|                          | 33NM1932-LC | MT11904<br>6 |                                |                     | 100.0% | 0.0 | 455/456 (99.78%) |
|                          | 33MR2061-LC | MT11904<br>7 |                                |                     | 100.0% | 0.0 | 452/456 (99.12%) |
|                          | 35MR2063-NK | MT11904<br>8 |                                |                     | 100.0% | 0.0 | 450/456 (98.68%) |
| <i>B. californiensis</i> | 23NM1807-LC | MT11905<br>0 | <i>B. californiensis</i> CA446 | MT119055            | 100.0% | 0.0 | 402/403 (99.75%) |
|                          | 30NM1820-LC | MT11905<br>1 |                                |                     | 100.0% | 0.0 | 402/403 (99.75%) |
|                          | 6MR2005-NR  | MT11905<br>2 |                                |                     | 100.0% | 0.0 | 403/403 (100.0%) |
|                          | 33MR2051-LK | MT11905<br>3 |                                |                     | 100.0% | 0.0 | 402/403 (99.75%) |
|                          | 37MR2080-LK | MT11905<br>4 |                                |                     | 100.0% | 0.0 | 403/403 (100.0%) |
|                          |             |              |                                |                     |        |     |                  |
| <i>B. americana</i>      | 29C4N2-IC   | MT11906<br>2 | <i>B. americana</i> CA8        | MT119066            | 100.0% | 0.0 | 534/536 (99.63%) |
|                          | 30C4F1-IR   | MT11906<br>3 |                                |                     | 100.0% | 0.0 | 536/536 (100.0%) |
|                          | 30C4F2-IR   | MT11906<br>4 |                                |                     | 100.0% | 0.0 | 530/536 (98.88%) |
|                          | 32C7N-IK    | MT11906<br>5 |                                |                     | 100.0% | 0.0 | 532/536 (99.25%) |

|                   |             |              |                        |          |        |     |                  |
|-------------------|-------------|--------------|------------------------|----------|--------|-----|------------------|
| <i>B. lanei</i>   | 33NM1921-LC | MT11905<br>6 | <i>B. lanei</i> CA28   | MT119061 | 100.0% | 0.0 | 485/488 (99.39%) |
|                   | 33NM1953-LC | MT11905<br>7 |                        |          | 100.0% | 0.0 | 485/488 (99.39%) |
|                   | 5MR1995-LK  | MT11905<br>8 |                        |          | 100.0% | 0.0 | 486/488 (99.59%) |
|                   | 20NM2032-NK | MT11905<br>9 |                        |          | 100.0% | 0.0 | 487/488 (99.80%) |
|                   | 43MR2088-LK | MT11906<br>0 |                        |          | 100.0% | 0.0 | 487/488 (99.80%) |
| <i>B. turcica</i> | 33NM1854-LC | MT11906<br>7 | <i>B. turcica</i> IST7 | CP028884 | 100.0% | 0.0 | 308/309 (99.68%) |

**Table S8.** MEGA X results of mean distance between *Borrelia* species obtained on the basis of intergenic spacer (IGS) of 3-methyladenine glycosylase (*mag*) and *tRNA-Ile* genes sequence fragment comparison.

|                            | BB    | BLN   | BAM   | BCL   | BCR   | BBI   | BA    | BSP   | BG    | BV    | BTC |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| <i>B. burgdorferi</i> s.s. |       |       |       |       |       |       |       |       |       |       |     |
| <i>B. lanei</i>            | 0.080 |       |       |       |       |       |       |       |       |       |     |
| <i>B. americana</i>        | 0.087 | 0.074 |       |       |       |       |       |       |       |       |     |
| <i>B. californiensis</i>   | 0.144 | 0.103 | 0.136 |       |       |       |       |       |       |       |     |
| <i>B. carolinensis</i>     | 0.130 | 0.133 | 0.153 | 0.128 |       |       |       |       |       |       |     |
| <i>B. bissettiae</i>       | 0.155 | 0.131 | 0.163 | 0.129 | 0.091 |       |       |       |       |       |     |
| <i>B. afzelii</i>          | 0.258 | 0.240 | 0.273 | 0.190 | 0.276 | 0.299 |       |       |       |       |     |
| <i>B. spielmanii</i>       | 0.278 | 0.235 | 0.280 | 0.186 | 0.270 | 0.310 | 0.113 |       |       |       |     |
| <i>B. garinii</i>          | 0.225 | 0.203 | 0.202 | 0.146 | 0.224 | 0.255 | 0.178 | 0.144 |       |       |     |
| <i>B. valaisiana</i>       | 0.222 | 0.183 | 0.230 | 0.170 | 0.251 | 0.270 | 0.275 | 0.186 | 0.290 |       |     |
| <i>B. turcica</i>          | 0.539 | 0.570 | 0.543 | 0.575 | 0.597 | 0.542 | 0.581 | 0.631 | 0.544 | 0.578 |     |

BB: *B. burgdorferi* s.s., BLN: *B. lanei*, BAM: *B. americana*, BCL: *B. californiensis*, BCR: *B. carolinensis*, BBI: *B. bissettiae*, BA: *B. afzelii*, BSP: *B. spielmanii*, BG: *B. garinii*, BV: *B. valaisiana*, BTC: *B. turcica*