**Supplementary Table S1.** List of primers used for the molecular characterization of the ticks

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Target gene | Species | Forward (5' to 3') | Binding location | Reverse (5' to 3') | Binding location | Amplicon size (bp) | Reference |
| *cox1* | *Haemaphysalis* spp. | GGAACAATATATTTAATTTTTGG | 43–65 bp on *cox1* | ATCTATCCCTACTGTAAATATATG | 868–891 bp on *cox1* | ~850 | [1] |
| *H. longicornis* | CTTGGCGGAATACCCCCATT | 351–370 bp upstream of *cox1* | CCAGGTTGCCCTAGTTCTATTCG | 109–131 bp on *cox1* | ~500 | in this study |
| CTCAGGAGGAGGAGACCCAA | 663–682 bp on *cox1* | TGGTGAATTTCTGTCGGGAA | 19–49 bp downstream of *cox1* | ~900 | in this study |
| *H. flava* | ACCACCATTATTAGGCTTCGTAAT | 350–373 bp upstream of *cox1* | AGTACCAGGTTGACCTAATTCTATT | 111–135 bp on *cox1* | ~500 | in this study |
| TGGACACCCAGAAGTTTACATTT | 711–733 bp on *cox1* | TGAGCGGCTAGTAAAATTGTTTATGA | 141–166 bpdownstream of *cox1* | ~900 | in this study |
| *H. doenitzi* | CCATAAGTAAATTTACAATTTACCGCC | 13–39 bp upstream of *cox1* | CCAATTAAGTTTCCTGGTTGTCCA | 120–143 bp on *cox1* | ~182 | in this study |
| ACCCTTATTCGTCTGATCAGTTCT | 540–563 bp on *cox1* | AATTGGTGAATTTCTATCAGGGAATGA | 26–52 bpdownstream of *cox1* | ~1090 | in this study |
| *Amblyomma*  *testudinarium* | GGTCAACAAATCATAAAGATATTGG | 20–44 bp on *cox1* | TAAACTTCAGGGTGACCAAAAAATCA | 703–728 bp on *cox1* | ~710 | [1] |
| CCCCCTTTTTAGGATTTATTGCA | 359–381 bp upstream of *cox1* | TGTTCCTGGTTGGCCTAATTCT | 114–135 bp on *cox1* | ~500 | in this study |
| TCTCCTTCTTTCTTTGCCTGT | 579–599 bp on *cox1* | TGACCTTCTATTATTGAACGACTTAAA | 191–217 bpdownstream of *cox1* | ~1300 | in this study |
| *Ixodes nipponensis* | GCCATTTTACCGCGATGA | 1–15 bp on *cox1* | GGTGGGCTCATACAATAAATCC | 847–868 bp on *cox1* | ~860 | [2] |
| ACTATGTGCCTTCAAAGTACAAAA | 143–166 bp upstream of *cox1* | ACCTGGTTGACCTAACTCAGT | 112–132 bp on *cox1* | ~300 | in this study |
| TCCCGGGTTTGGAATAGTTTCT | 741–762 bp on *cox1* | ACGTAGTGAGGGTATAGCAATAAA | 231–254 bp downstream of *cox1* | ~1200 | in this study |
| 16S rDNA | *H. longicornis* | AATTCCTCACATTTATCTGTCACTTT |  | GCCCGTCGCTCTTTCTTAAAGA |  | ~1330 | in this study |
| *H. flava* | GCTCTAAAAGAGTGAAAAATGCAACT |  | TGGAAAGTGCTTTAAAAATGAAATCA |  | ~1410 | in this study |
| *H. doenitzi* | GCTCTAGAAGAGTGAAGAAAGCTACT |  | ATTCGCCCGTCGCTCTTTAT |  | ~1460 | in this study |
| *A. testudinarium* | TAAAGGAAGCTTAAATTCCTCAC |  | ATATCGCCCGTCGCTCTTTC |  | ~1380 | in this study |
| *I. nipponensis* | CGCATTTAACTTTCTGCCACTTTAA |  | TATCGCCCGTCGCTCTCAGA |  | ~1420 | in this study |
| 18S rDNA | Eukaryote-common | AACCTGGTTGATCCTGCCAGT |  | TGATCCTTCTGCAGGTTCACCTAC |  | 1812-1816 | [3] |

1. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 1994;3(5):294-299.

2. Mayne P, Song S, Shao R, Burke J, Wang Y, et al. Evidence for *Ixodes holocyclus* (Acarina: Ixodidae) as a vector for human lyme borreliosis infection in Australia. *J Insect Sci* 2014;14(1):271. https://doi.org/10.1093/jisesa/ieu133

3. Mangold AJ, Bargues MD, Mas-Coma S. 18S rRNA gene sequences and phylogenetic relationships of European hard-tick species (Acari: Ixodidae). *Parasitol Res* 1998;84(1):31-37. https://doi.org/10.1007/s004360050352