**Phylogeography of red muntjacs reveals three distinct mitochondrial lineages**

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## Additional file 1

**Table S1.** Comparison of number of accepted red muntjac species and subspecies among three different recent publications. Number of recognized species range from 1 to 6 and number of recognized subspecies range from none to 10.

| | Mattioli (2011)[10] | Grubb and Groves (2011)[13] | Timmins *et al*. (2016)[7] |
|---|---|---|---|
| Species | *M. muntjak* | *M. muntjak*<br>*M. vaginalis*<br>*M. malabaricus*<br>*M. aureus*<br>*M. nigripes*<br>*'M. guongdongensis'** | *M. muntjak*<br>*M. vaginalis* |
| Subspecies | *M. m. muntjak*<br>*M. m. annamensis*<br>*M. m. aureus*<br>*M. m. curvostylis*<br>*M. m. malabaricus*<br>*M. m. mengalis*<br>*M. m. montanus*<br>*M. m. nigripes*<br>*M. m. vaginalis*<br>*M. m. yunnanensis* | *M. v. vaginalis*<br>*M. v. curvostylis* | not applicable |

* Identified as *Incertae sedis*, but referring to the different form described by Li J-x &Xu, 1996

**Table S2**. Complete dataset used for analyses, with information on origin, location and contact.

| Collection ID | Sample name | Name in Figures | Genbank | Source material | Geographic origin | Geographic origin | Year | Contact |
|---|---|---|---|---|---|---|---|---|
| 46238 | MMU1 | BAL1 | KY052120 | nasal bone | West Bali | West Bali | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 46239 | MMU2 | JAV13 | KY052121 | nasal bone | West Java | Java | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 17534 | MMU3 | THA2 | KY052091 | nasal bone | Thailand | Thailand | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 91131 | MMU6 | IND5 | KY052092 | nasal bone and tissue from skull | North India | North India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 91143 | MMU8 | IND2 | KY052093 | nasal bone | North India | North India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 40556 | MMU10 | CHI3 | KY052094 | nasal bone | Yunnan China | China | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 4837 | MMU12 | IND10 | KY052095 | nasal bone | East India | East India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 34074 | MMU16 | BOR2 | KY052127 | nasal bone | Southeast Borneo | Borneo | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 92292 | MMU17 | LOM2 | KY052122 | bone from skull | Lombok | Lombok | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 70927 | MMU18 | SRI1 | KY052116 | nasal bone | East Sri Lanka | Sri Lanka | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 33459 | MMU20 | CHI1 | KY052096 | nasal bone | Yunnan China | China | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 92296 | MMU21 | LSI1 | KY052123 | tissue from skull | Lesser Sunda Islands | Lesser Sunda Islands | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 70928 | MMU22 | BOR7 | KY052124 | nasal bone | Borneo | Borneo | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| ZMA 10490 | MMU24 | SUM6 | KY052125 | nasal bone | Sumatra | Sumatra | 1950 | Pepijn Kamminga, Naturalis Leiden |
| ZMA 10491 | MMU25 | SUM1 | KY052126 | nasal bone | Sumatra | Sumatra | 1951 | Pepijn Kamminga, Naturalis Leiden |
| 16699 | MMU27 | JAV17 | KY052128 | nasal bone and tissue from skull | Java | Java | 1856 | Dr. Stefan Merker, Staatliches Museum für Naturkunde Stuttgart |
| 16701 | MMU28 | JAV15 | KY052129 | nasal bone | Java | Java | 1878 | Dr. Stefan Merker, Staatliches Museum für Naturkunde Stuttgart |
| 50998 | MMU29 | JAV5 | KY052130 | nasal bone | East Java | Java | 1950 | Dr. Stefan Merker, Staatliches Museum für Naturkunde Stuttgart |
| 15974 | MMU30 | BOR5 | KY052131 | antler drill | Borneo | Borneo | 1902 | Dr. Stefan Merker, Staatliches Museum für Naturkunde Stuttgart |
| 15968 | MMU31 | BOR6 | KY052132 | antler drill | Borneo | Borneo | 1902 | Dr. Stefan Merker, Staatliches Museum für Naturkunde Stuttgart |

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|---|---|---|---|---|---|---|---|---|
| 15979 | MMU32 | BOR4 | KY052133 | antler drill | Borneo | Borneo | 1902 | Dr. Stefan Merker, Staatliches Museum für Naturkunde Stuttgart |
| 97.679 | MMU34 | THA6 | KY052097 | nasal bone | Thailand | Thailand | 1900 | Dr. Rainer Hutterer, Zoologisches Forschungsmuseum Alexander Koenig Bonn |
| 2886 | MMU35 | JAV6 | KY052134 | nasal bone | West Java | Java | 1930 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 2887 | MMU36 | JAV11 | KY052135 | nasal bone | West Java | Java | 1930 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 1468 | MMU38 | SUM4 | KY052136 | nasal bone | Sumatra | Sumatra | 1904 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 2120 | MMU40 | SUM2 | KY052137 | nasal bone | Sumatra | Sumatra | 1852 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 3022 | MMU41 | JAV14 | KY052138 | nasal bone | West Java | Java | 1858 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 2931 | MMU42 | JAV1 | KY052139 | nasal bone | West Java | Java | 1929 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 2119 | MMU43 | JAV16 | KY052140 | nasal bone | West Java | Java | 1858 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 3178 | MMU44 | JAV3 | KY052141 | nasal bone | West Java | Java | 1929 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 2052 | MMU46 | JAV8 | KY052142 | nasal bone | West Java | Java | 1858 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 2117 | MMU48 | SUM5 | KY052143 | tissue | Sumatra | Sumatra | 1886 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| B3399 | MMU49 | JAV7 | KY052144 | skin | Java | Java | 1919 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| B3400 | MMU50 | JAV2 | KY052145 | skin | Java | Java | | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| B3402 | MMU51 | JAV4 | KY052146 | skin | Java | Java | | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 1497 | MMU53 | SUM3 | KY052147 | bone drill | Sumatra | Sumatra | 1904 | Dr. Frank Zachos, Naturhistorisches Museum Wien |
| 35585 | MMU59 | LOM1 | KY052148 | tissue | Lombok | Lombok | | Dr. Irina Ruf, Senckenberg Forschungsinstitut Mammalogie Frankfurt |
| 34878 | MMU60 | LOM3 | KY052149 | tissue | Lombok | Lombok | | Dr. Irina Ruf, Senckenberg Forschungsinstitut Mammalogie Frankfurt |
| 15953 | MMU62 | IND6 | KY052098 | tissue | India | India | | Dr. Irina Ruf, Senckenberg Forschungsinstitut Mammalogie Frankfurt |
| M122 | MMU64 | JAV9 | KY052150 | tissue from skeleton | Java | Java | | Mogens Andersen, Natural History Museum of Denmark Copenhagen |
| M123 | MMU65 | NEP1 | KY052099 | nasal bone | Nepal | Nepal | 1838 | Mogens Andersen, Natural |

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|---|---|---|---|---|---|---|---|
| M505 | MMU67 | LSI2 | KY052151 | nasal bone | Belitung Island | Belitung Island | 1887 | History Museum of Denmark Copenhagen Mogens Andersen, Natural |
| M537 | MMU68 | IND7 | KY052100 | nasal bone and tissue from skull | India | India | 1887 | History Museum of Denmark Copenhagen Mogens Andersen, Natural |
| M1267 | MMU72 | THA4 | KY052101 | tissue from skull | Thailand | Thailand | 1926 | History Museum of Denmark Copenhagen Mogens Andersen, Natural |
| M125 | MMU74 | THA1 | KY052102 | Tissue in alcohol | South Thailand | Thailand | 1877 | History Museum of Denmark Copenhagen |
| 46109 | MMU75 | IND9 | KY052103 | skin | South India | South India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 91138 | MMU81 | IND3 | KY052104 | skin | India | India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 91130 | MMU82 | IND4 | KY052105 | nasal bone | India | India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 70927 | MMU83 | SRI2 | KY052117 | nasal bone | Sri Lanka | Sri Lanka | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 11959 | MMU86 | THA5 | KY052106 | nasal bone | Thailand | Thailand | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| 91133 | MMU87 | IND8 | KY052107 | nasal bone | India | India | | Dr. Frieder Mayer, Naturkundemuseum Berlin |
| RMNH.MAM.33818 | MMU90 | JAV10 | KY052152 | nasal bone | West Java | Java | 1930 | Pepijn Kamminga, Naturalis Leiden |
| RMNH.MAM.36981 | MMU91 | THA3 | KY052108 | tissue from skull | North Thailand | Thailand | 1989 | Pepijn Kamminga, Naturalis Leiden |
| RMNH.MAM.33814 | MMU95 | LSI3 | KY052153 | tissue from skull | Bangka Island | Banka | | Pepijn Kamminga, Naturalis Leiden |
| RMNH.MAM.4929 | MMU100 | JAV12 | KY052154 | nasal bone | Java | Java | | Pepijn Kamminga, Naturalis Leiden |
| RMNH.MAM.4916 | MMU102 | IND1 | KY052109 | nasal bone | North India | India | | Pepijn Kamminga, Naturalis Leiden |
| RMNH.MAM.29758.a | MMU105 | BOR3 | KY052155 | tissue from skull | Central Borneo | Borneo | 1894 | Pepijn Kamminga, Naturalis Leiden |
| RMNH.MAM.29754.a | MMU107 | BOR1 | KY052156 | tissue from skull | Borneo | Borneo | | Pepijn Kamminga, Naturalis Leiden |
| 74035/009957 | C.2 | CHI2 | KY052110 | nasal bone | Yunnan China | China | | Douglas Yu, The Ecology, Conservation, and Environment Center |
| RJT118 | RJT118 | LAO1 | KY052082 | | Laos | Laos | | R. J. Timmins |
| M2.3 | M2.3 | VIE3 | KY052083 | tissue from skull | North C. Vietnam | North Vietnam | 2011 | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| M2.9 | M2.9 | VIE7 | KY052084 | bone | North C. Vietnam | North Vietnam | 2010 | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| M2.14 | M2.14 | VIE2 | KY052085 | bone | Central Vietnam | Central Vietnam | 2011 | Dr. Minh Le, Center for Natural Resources & Environmental Studies |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| M3.8 | M3.8 | VIE11 | KY052086 | bone | North E. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| M5.11 | M5.11 | VIE13 | KY052087 | dry skin | North Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| M6.5 | M6.5 | VIE8 | KY052088 | bone | North C. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| M6.12 | M6.12 | VIE1 | KY052089 | bone | Central Vietnam | Central Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| M6.17 | M6.17 | VIE4 | KY052090 | bone | South Vietnam | South Vietnam | 2012 | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| x15 | x15 | VIE9 | KY052111 | bone | North C. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| x17 | x17 | VIE12 | KY052112 | bone | North C. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| x19 | x19 | VIE10 | KY052113 | bone | North C. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| x20 | x20 | VIE6 | KY052114 | bone | North C. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| x39 | x39 | VIE5 | KY052115 | bone | North C. Vietnam | North Vietnam | | Dr. Minh Le, Center for Natural Resources & Environmental Studies |
| mm13 | mm13 | MAL1 | KY052118 | | Peninsular Malaysia | Peninsular Malaysia | | Monash University, Malaysia |
| mm20 | mm20 | MAL2 | KY052119 | | Peninsular Malaysia | Peninsular Malaysia | | Monash University, Malaysia |

**Table S3.** Long-range PCR primer sequences and annealing temperatures used for bait development.

| | Position on ref. | Sequence 5'-3' | Annealing Temp.[a] |
|---|---|---|---|
| Fragment 1 | 2478 | F: CGATTAAAGTCCTACGTGATCTGAG | 58 °C |
| | 6926 | R: GTTATGATGTTGGCTTGAAACCAG | |
| Fragment 2 | 6506 | F: GCTATYATRGGAGGATTTGTTCAC | 58 °C |
| | 12903 | R: GATTAGGGCTGTTGTRGTAAATG | |
| Fragment 3 | 12339 | F: TTACAAATCTTAACGCCTGAGACTTC | 58 °C |
| | 2548 | R: TAGATAGAAACCGACCTGGATTACTC | |

[a] PCR was done with MyFi™ Mix (Bioline GmbH, Germany) with 1x MyFi Mix, 0.4 µM each primer and water to the final volume of 50 µL for each of the fragments separately, in a total of 35 amplification cycles.

**Table S4.** Sequencing results from all samples used in this study, with indication of sequencing platform, percentage of reads on target, average base coverage depth and percentage of reference genome covered with at least 3x coverage.

| Sample Name | Captured | Sequencing platform | Reads on target | Average read depth | % genome covered ≥ 3x |
|---|---|---|---|---|---|
| BAL1 | yes | PGM | 53.65% | 109.24 | 100 |
| JAV13 | yes | PGM | 45.29% | 294.49 | 100 |
| THA2 | yes | PGM | 20.92% | 45.73 | 99.9 |
| IND5 | no | Illumina | 0.54% | 90.76 | 100 |
| IND2 | yes | PGM | 18.15% | 16.17 | 99.4 |
| CHI3 | no | Illumina | 0.06% | 11.13 | 92.4 |
| IND10 | yes | PGM | 46.65% | 257.54 | 100 |
| BOR2 | yes | Illumina | 2.21% | 14.07 | 93.7 |
| LOM2 | yes | Illumina | 36.22% | 124.63 | 100 |
| SRI1 | yes | PGM | 20.46% | 112.53 | 99.2 |
| CHI1 | yes | PGM | 4.30% | 186.14 | 100 |
| LSI1 | yes | Illumina | 45.26% | 172.8 | 100 |
| BOR7 | yes | PGM | 10.17% | 39.69 | 99.9 |
| SUM6 | yes | PGM | 36.05% | 96.74 | 100 |
| SUM1 | yes | PGM | 47.57% | 64.02 | 99.8 |
| JAV17 | yes | PGM | 71.62% | 65.65 | 99.8 |
| JAV15 | yes | PGM | 65.44% | 88.36 | 100 |
| JAV5 | yes | PGM | 55.88% | 163.68 | 100 |
| BOR5 | yes | PGM | 6.24% | 6.87 | 88.1 |
| BOR6 | yes | PGM | 31.17% | 7.97 | 93.9 |
| BOR4 | yes | PGM | 18.20% | 27.78 | 99.9 |
| THA6 | yes | PGM | 26.33% | 178.63 | 100 |
| JAV6 | yes | PGM | 6.35% | 187.35 | 100 |
| JAV11 | yes | PGM | 40.60% | 36.44 | 99 |
| SUM4 | yes | PGM | 44.44% | 278.49 | 100 |
| SUM2 | yes | PGM | 61.77% | 27.59 | 99 |
| JAV14 | yes | PGM | 30.97% | 144.77 | 100 |
| JAV1 | no | Illumina | 0.92% | 74.02 | 100 |
| JAV16 | yes | PGM | 30.47% | 36.33 | 99.5 |
| JAV3 | no | Illumina | 0.18% | 14.49 | 92.5 |
| JAV8 | yes | Illumina | 4.88% | 25.39 | 96.1 |
| SUM5 | no | Illumina | 0.05% | 100.91 | 100 |
| JAV7 | yes | Illumina | 3.88% | 79.14 | 98.2 |
| JAV2 | yes | Illumina | 9.26% | 89 | 99.6 |
| JAV4 | yes | PGM | 50.03% | 10.66 | 89.7 |
| SUM3 | yes | PGM | 24.66% | 72.7 | 100 |
| LOM1 | yes | PGM | 28.73% | 37.86 | 99.1 |
| LOM3 | yes | PGM | 33.09% | 35.93 | 99.3 |
| IND6 | yes | PGM | 50.73% | 47.47 | 99.9 |
| JAV9 | yes | PGM | 27.96% | 20.03 | 98.4 |
| NEP1 | yes | PGM | 59.64% | 31.76 | 100 |
| LSI2 | yes | PGM | 16.87% | 17.46 | 98.8 |
| IND7 | yes | PGM | 47.52% | 146.08 | 100 |
| THA4 | no | Illumina | 0.03% | 8.43 | 86.1 |
| THA1 | yes | Illumina | 14.59% | 92.01 | 100 |
| IND9 | yes | PGM | 33.76% | 7.29 | 92.6 |
| IND3 | yes | Illumina | 1.76% | 22.85 | 98.17 |
| IND4 | yes | Illumina | 8.41% | 56.76 | 99.65 |
| SRI2 | yes | PGM | 61.03% | 51.33 | 98.6 |
| THA5 | yes | Illumina | 29.52% | 155.25 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| IND8 | yes | Illumina | 64% | 218.42 | 100 |
| JAV10 | no | Illumina | 0.07% | 78.4 | 99.7 |
| THA3 | yes | PGM | 39.65% | 17.13 | 99.8 |
| LSI3 | yes | Illumina | 18.50% | 147.87 | 100 |
| JAV12 | yes | Illumina | 3.52% | 34.46 | 99.4 |
| IND1 | yes | PGM | 74.84% | 108.69 | 100 |
| BOR3 | yes | Illumina | 19.56% | 88.28 | 97.9 |
| BOR1 | yes | Illumina | 39.98% | 115.74 | 99.3 |
| CHI2 | no | Illumina | 0.19% | 49.41 | 100 |
| LAO1 | no | Illumina | 0.18% | 53.8 | 99.05 |
| VIE3 | no | Illumina | 0.04% | 12.66 | 96.7 |
| VIE7 | no | Illumina | 0.03% | 11.42 | 95.4 |
| VIE2 | no | Illumina | 0.07% | 16.19 | 92.5 |
| VIE11 | no | Illumina | 0.06% | 21.06 | 97 |
| VIE13 | no | Illumina | 0.08% | 19.42 | 99.7 |
| VIE8 | no | Illumina | 0.10% | 29.03 | 96.8 |
| VIE1 | no | Illumina | 0.03% | 8.19 | 90.6 |
| VIE4 | no | Illumina | 0.03% | 11.25 | 94.1 |
| VIE9 | no | Illumina | 0.12% | 37.95 | 99.9 |
| VIE12 | no | Illumina | 0.20% | 70.42 | 100 |
| VIE10 | no | Illumina | 0.07% | 31.10 | 100 |
| VIE6 | no | Illumina | 0.03% | 9.38 | 94.2 |
| VIE5 | no | Illumina | 0.06% | 30.30 | 99.9 |
| MAL1 | no | Illumina | 0.93% | 66.63 | 100 |
| MAL2 | no | Illumina | 0.58% | 57.01 | 100 |

**Table S5:** Number of Variable sites (V) and Parsimony informative sites (Pi) per coding gene, throughout the full mitogenomes obtained.

| | **ND1** | **ND2** | **Cox1** | **Cox2** | **Cox3** | **ND3** | **ND4L** | **ND4** | **ND5** | **ND6** | **Cytb** | **D-loop** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **V** | 126 | 147 | 163 | 76 | 108 | 43 | 38 | 170 | 277 | 64 | 182 | 157 |
| **Pi** | 102 | 101 | 111 | 54 | 79 | 31 | 27 | 132 | 191 | 45 | 127 | 120 |

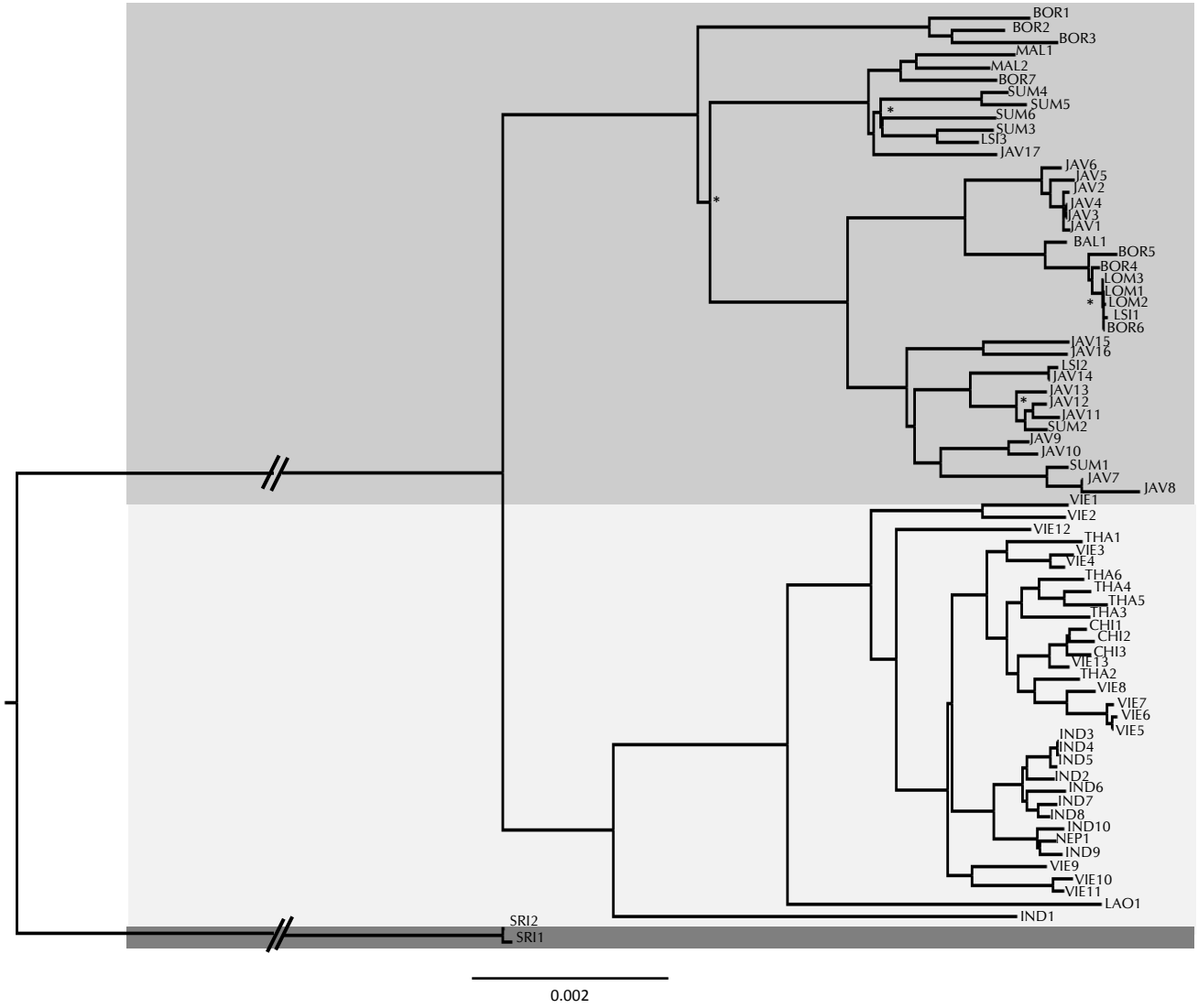


**Figure S1.** Best tree obtained with Maximum Likelihood analyses with RAxML. The same topology is recovered as with Bayesian inferences, with three distinct mitochondrial clades. All branches are supported with at least 90% bootstrap support, except when indicated otherwise with *.

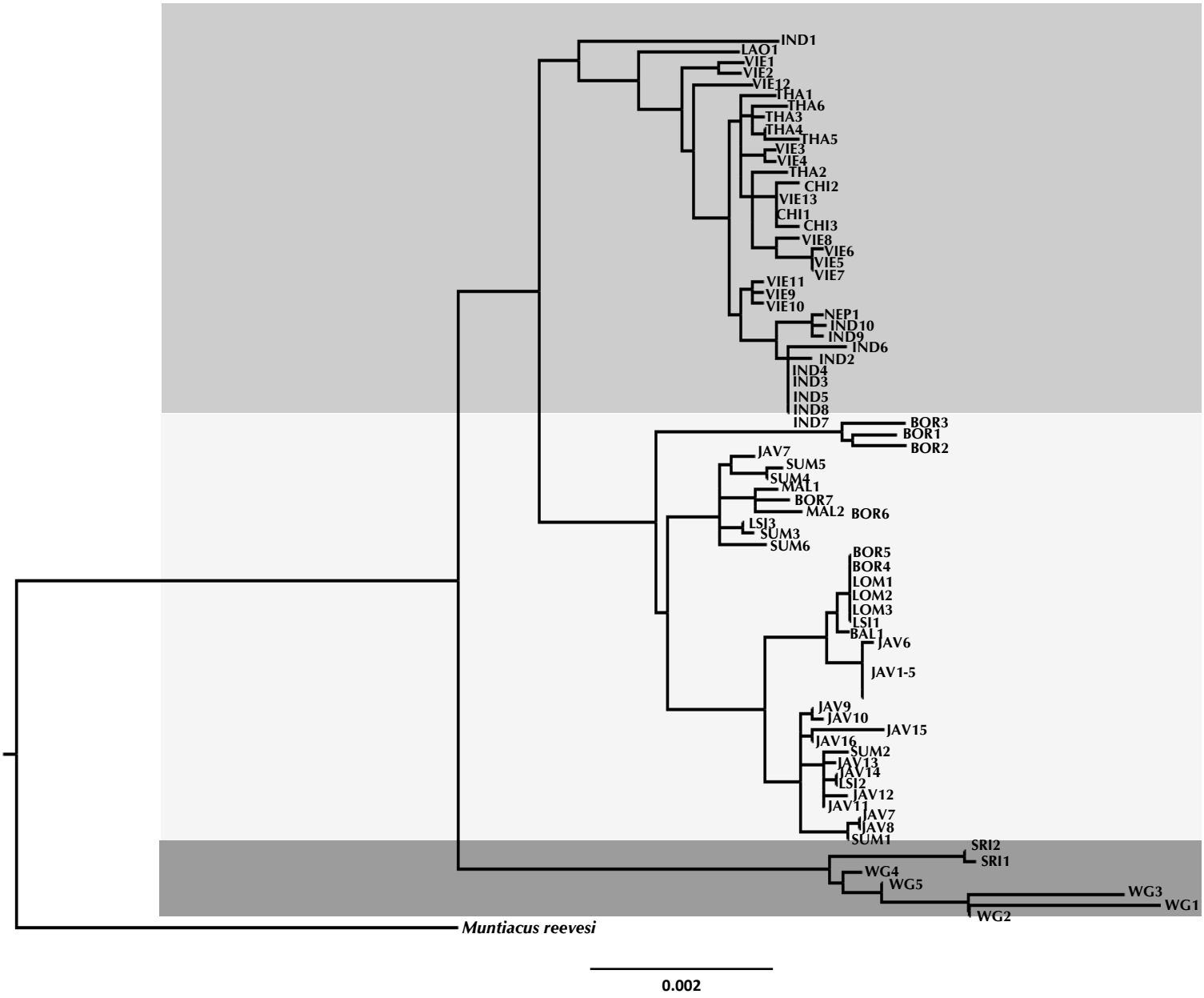


**Figure S2**. Best tree obtained with Maximum Likelihood analyses of the Cytochrome B gene of all red muntjac samples included in this study and five additional Genbank sequences from the Western Ghats. This tree was obtained with RAxML and shows similar topology as the trees with full mitogenome, although bootstrap values are generally lower. This result shows the relationship between Sri Lanka and Western Ghats populations, as they cluster together in the same clade.