

1 **The complete mitochondrial genome of the Indochinese jackal (*Canis aureus cruesemanni*)**
2 **and its relationship to other subspecies of golden jackal**

3

4 Medhini S. Sosale^{1,2}, Nucharin Songsasen³, Osman Ibis^{4,5,6}, Cody Edwards^{2,7}, Henrique V.
5 Figueiró², Klaus-Peter Koepfli^{2,3}

6

7 ¹Department of Bioengineering, Volgenau School of Engineering, George Mason University,
8 Fairfax, Virginia, USA

9 ²Smithsonian-Mason School of Conservation, George Mason University, Front Royal, Virginia,
10 USA

11 ³Center for Species Survival, Smithsonian's National Zoo and Conservation Biology Institute,
12 Front Royal, Virginia, USA

13 ⁴Department of Agricultural Biotechnology, Faculty of Agriculture, Erciyes University, Kayseri,
14 Turkey

15 ⁵Genome and Stem Cell Center (GENKOK), Erciyes University, Kayseri, Turkey

16 ⁶Vectors and Vector-Borne Diseases Implementation and Research Center, Erciyes University,
17 Kayseri, Turkey

18 ⁷Department of Biology, George Mason University, Fairfax, Virginia, USA

19

20

21

22

23

24 **Abstract**

25 The Indochinese jackal (*Canis aureus cruesemanni*) is a subspecies of the golden jackal (*Canis*
26 *aureus*) found in Southeast Asia. While this species has been genetically studied in Europe, the
27 Middle East, and India, current research is lacking on the population(s) in Southeast Asia. Using
28 a genome skimming approach, we assembled the first complete mitochondrial genome for an
29 Indochinese jackal from Thailand. The annotated mitogenome contained 37 genes and is 16,729
30 bps in length. Phylogenetic analysis with 21 additional canid mitogenomes, along with analyses
31 of a cytochrome *b* gene-only data set, supports the Indochinese jackal as a distinct lineage, and
32 therefore subspecies, among golden jackals.

33
34 **Keywords:** *Canis aureus*; subspecies; phylogeny; mitochondrial genome; golden jackal
35

36
37 The golden jackal, *Canis aureus* (Linnaeus, 1758), a member of the family Canidae, is found
38 across the Eurasian continent and is generally thriving (Krofel et al., 2017). It has been
39 designated as a species of Least Concern by the IUCN Red List and has received CITES
40 Appendix III protection in India (Hoffmann et al., 2018). The taxonomy of this species has been
41 in flux in recent years due to genetic and genomic evidence supporting the recognition of golden
42 jackal populations in Africa as a distinct species, the African wolf, *Canis lupaster* (Hemprich and
43 Ehrenberg, 1832; Koepfli et al., 2015; Krofel et al., 2022). Within Eurasia, up to eight subspecies
44 have been described, although their delimitations are not well defined (Moehlman and Hayssen,
45 2018). Among these is the Indochinese jackal, *Canis aureus cruesemanni* (Matschie, 1900), first
46 described by German zoologist Paul Matschie from living specimens in the Berlin Zoological
47 Garden that came from southwest Siam (now Thailand). In Thailand, this jackal inhabits dry

48 dipterocarp forests and other open landscapes, and like other golden jackals, it is omnivorous and
49 active at twilight and night (Parr, 2003).

50
51 Recent studies on golden jackals employing microsatellites and/or partial mitochondrial DNA
52 sequences have focused on analyzing genetic diversity and structure within regional populations
53 (e.g., India, Yumnam et al., 2015; Iran, Yusefi et al., 2021) or across multiple populations, often
54 with the aim to understand the origin of the population(s) that have been rapidly expanding in
55 western Europe (Fabbri et al., 2014; Rutkowski et al., 2015; Spassov and Acosta-Pankov, 2019).
56 Phylogeographic analyses have generally revealed only little to moderate genetic structuring,
57 depending on the scale of the geographic sampling. However, none of these studies have so far
58 included samples from the part of the species' range in Southeast Asia. The Indochinese jackal
59 likely represents the easternmost population of the golden jackal (Moehlman and Hayssen,
60 2018). Therefore, we predicted that it may be genetically differentiated from western populations
61 in the species' range.

62
63 To test this, we generated the first complete mitochondrial genome of a male Indochinese jackal,
64 which was obtained from a specimen live-trapped in the Salakpra Wildlife Sanctuary,
65 Kanchanaburi Province, Thailand (latitude = 14.309912, longitude = 99.256454;
66 permit#0907.4/17810). A 3 ml whole blood sample was collected and stored at -80°C (NZCBI
67 acuc # 14-01) in the personal collection of N. Songsasen (SongsasenN@si.edu) at the
68 Smithsonian's National Zoo and Conservation Biology Institute
69 (<https://nationalzoo.si.edu/center-for-species-survival>). An aliquot of whole blood was delivered
70 to Psomagen, Inc. (Rockville, MD) for DNA extraction, library preparation, and sequencing.

71 Genomic DNA was extracted using the Mag-Bind Blood and Tissue Kit (Omega Bio-Tek Inc.,
72 Norcross, GA) and evaluated for quality and concentration with a Picogreen and Victor X2
73 fluorometry assay (Life Technologies, Carlsbad, CA), an Agilent 4200 TapeStation (Agilent
74 Technologies, Santa Clara, CA), and 1% gel electrophoresis. DNA was sheared into 350 bp
75 fragments with a Covaris S220 ultrasonicator (Woburn, MA) and used to prepare a library with
76 the TruSeq DNA PCR-free library kit (Illumina, San Diego, CA). The library was quality
77 checked on an Agilent 4200 TapeStation, quantitated via quantitative PCR using a Lightcycler
78 (Roche Life Science, St. Louis, MO), and then paired-end sequenced (2 x 150 bp) on an Illumina
79 NovaSeq 6000 instrument to a depth of 20x. A total of 374,172,520 reads were generated, 92.2%
80 of which had a \geq Q30 score.

81
82 Raw reads were evaluated using FastQC (Andrews, 2010) and then subsampled to 40 million
83 reads using BBDMap version 38.96 (Bushnell, 2014). Subsampled reads were trimmed and
84 filtered using AdapterRemoval (Lindgreen, 2012) within PALEOMIX version 1.3.6 (Schubert et
85 al, 2014). The read set was then mapped to the reference mitochondrial genome of the gray wolf
86 (*Canis lupus*, Linnaeus, 1758; GenBank: NC_008092.1) with the Geneious mapper using
87 medium-low sensitivity and five iterations of fine-tuning in Geneious Prime version 2022.0.2
88 (<https://www.geneious.com>). We extracted the 16,729 bp consensus sequence, which had an
89 average coverage of 231x. Annotation using MITOS (Bernt et al., 2013) resulted in 13 protein-
90 coding genes, 2 rRNAs, 22 tRNAs, and the control region.

91
92 We also sequenced the complete mitochondrial genome of a female young adult golden jackal
93 from Dagbeli, Antalya, Turkey (N 37°15'26.04'' – E 30° 29' 36.89'', altitude 785 meters),

94 representing the subspecies *C. a. moreotica*. This sample was obtained via muscle tissue
95 collected from a road-killed specimen on 26 July 2021 (Ericeyes University collection number
96 1886). The mitogenome was generated following the same methods described in Ibis et al.
97 (2020) using mitogenome-specific primers. A total of 542,318 raw reads with 88.1% having a
98 score over Q30 were generated. Read processing, mapping, and assembly were performed using
99 the same methods described above for the Indochinese jackal, except 25 iterations of fine-tuning
100 were used during mapping in Geneious Prime. A 16,669 bp consensus sequence was extracted
101 with an average coverage of 4,125x.

102

103 Mitogenomes of 18 other canid species and two golden jackals putatively representing *C. a.*
104 *syriacus* (Israel) and *C. a. indicus* (India) were downloaded from GenBank and imported into
105 Geneious Prime 2022.0.2, along with the mitogenomes of the Indochinese jackal and golden
106 jackal from Turkey. Putative subspecies designations of golden jackals used in this study
107 followed Moehlman and Hayssen (2018). We generated a multiple sequence alignment using
108 MAFFT version 7.450 (Kato and Standley, 2013) with default options (algorithm = AUTO,
109 scoring matrix = 200 PAM/k=2, gap open penalty = 1.53, offset value = 0.123). Due to poor
110 alignment in the repetitive regions, we trimmed the control region from the alignment. The
111 resulting 15,958 bp alignment was used to construct a maximum-likelihood phylogeny with
112 RAxML version 8.2.11 (Stamatakis, 2014) using the rapid hill-climbing algorithm and
113 GTRGAMMA model of substitution. One hundred (100) bootstrap replicates were subsequently
114 employed to calculate node support (bootstrapping using rapid hill-climbing, random seed = 1
115 setting in Geneious Prime).

116

117 To place the Indochinese jackal into a wider phylogeographic context, we also analyzed a data
118 set comprised of complete cytochrome *b* (*CYTB*) gene sequences. A total of 18 *CYTB* sequences
119 were downloaded from GenBank and imported into Geneious Prime. The final taxon set included
120 *Canis aureus* (n=13), with sequences representing animals sampled from Afghanistan, Egypt,
121 India, Israel, and Serbia, plus the *CYTB* sequences from the jackals from Thailand and Turkey;
122 *Canis lupaster* (n=4), and *Canis lupus* (n=3). As with the mitogenome sequences, the *CYTB*
123 sequences were aligned with MAFFT version 7.450 (Kato and Standley, 2013) using default
124 settings. We employed RAxML version 8.2.11 (Stamatakis, 2014) using the rapid hill-climbing
125 algorithm and GTRGAMMA model of substitution to estimate the maximum-likelihood
126 phylogeny from the 1,140 bp alignment. Node support was calculated using 100 bootstrap
127 replicates as described above for the mitogenome data set. Finally, we used the *CYTB* sequences
128 to construct a haplotype network using TCS with the program PopART (Leigh and Bryant,
129 2015).

130

131 The phylogenetic tree in Figure 1 based on the mitogenome alignment shows that the
132 Indochinese jackal is grouped with other golden jackals with 100% bootstrap support. The four
133 golden jackal sequences comprise four unique haplotypes. The Indochinese jackal forms a
134 distinct lineage and shows a 2.8% sequence divergence from other putative subspecies of golden
135 jackal, supporting the former's distinctiveness and subspecific status. The phylogenetic tree in
136 Figure 2 based on the *CYTB* alignment shows a similar pattern of relationships despite the larger
137 number of sequences and wider geographic representation, with the Indochinese golden jackal
138 forming the earliest branching lineage and showing a 4.1% sequence divergence from other
139 golden jackals. The grouping of sequences from India and more western regions is consistent

140 with the results reported by Yumnam et al. (2015). Interestingly, the topology within golden
141 jackals shows a pattern of branching from east to west, suggesting that Southeast Asia may be
142 the region of origin for this species. However, additional studies using more samples and data
143 from the nuclear genome are needed to test this hypothesis. The haplotype network analysis of
144 the *CYTB* gene sequences in Figure 3 places the Indochinese jackal sample separately from the
145 remaining golden jackal samples by 46 substitutions, which is only slightly less than the number
146 of substitutions (57) separating golden jackals from the African wolf. The figure also
147 demonstrates that the Indochinese jackal shares a common ancestor with other populations of
148 golden jackal, further supporting the information obtained from the phylogenetic trees. Our
149 results provide a foundation for further studies of the Indochinese jackal, which will help inform
150 the evolutionary history and conservation status of this little-known subspecies.

151

152

153

154

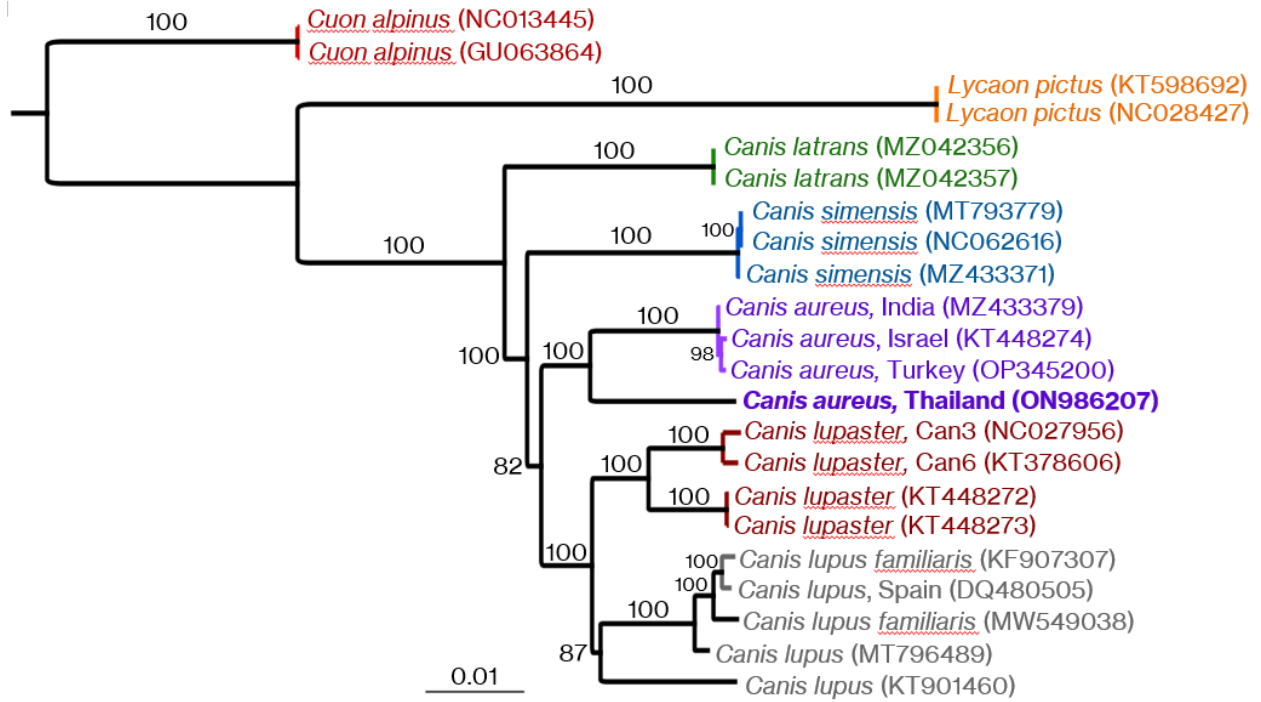
155

156

157

158

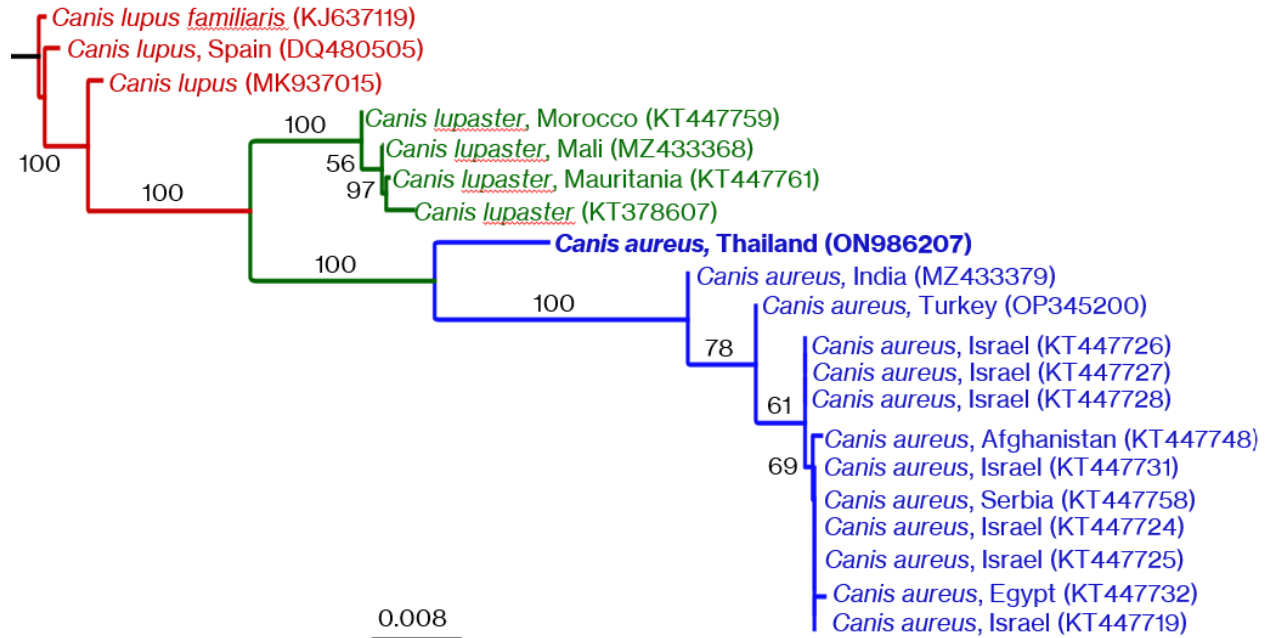
159



160

161

162 **Figure 1:** Maximum-likelihood phylogenetic tree based on the 15,958 bp mitogenome alignment
 163 showing the relationship of the Indochinese jackal (*Canis aureus*, Thailand) to other golden
 164 jackals and species within the genus *Canis*. African painted dog (*Lycaon pictus*) and dhole (*Cuon*
 165 *alpinus*) were used to root the tree. Numbers represent bootstrap values (%) and the GenBank
 166 accession number for each sequence is shown in parentheses.



167

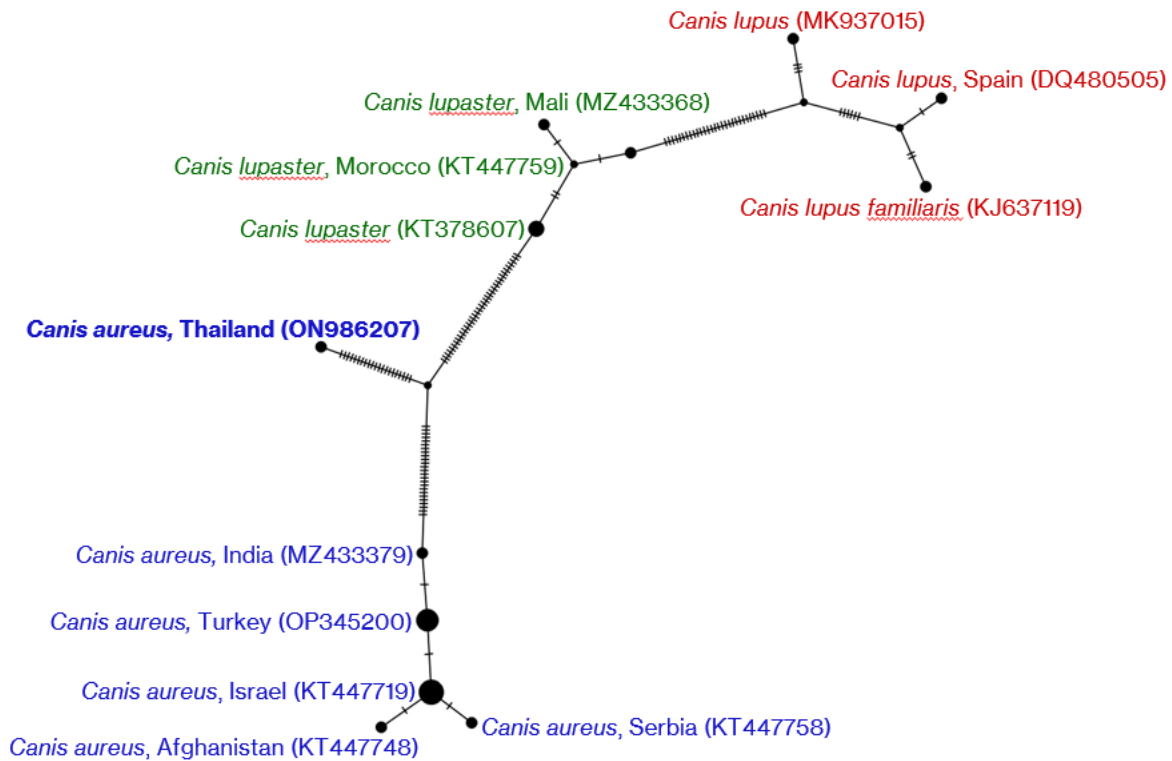
168 **Figure 2:** Maximum-likelihood phylogenetic tree based on the 1,140 bp *CYTB* alignment. The

169 Indochinese jackal from Thailand is shown in bold blue font. Gray wolf (*Canis lupus*) and

170 domestic dog (*Canis lupus familiaris*) samples were used to root the tree. Numbers represent

171 bootstrap values (%) and the GenBank accession number for each file is shown in parentheses.

172



173

174

175 **Figure 3:** Haplotype network based on the 1,140 bp *CYTb* alignment. The Indochinese jackal
 176 from Thailand is shown in bold blue font. Each individual haplotype and/or ancestor is
 177 represented by a black dot, and each dash in between the dots corresponds to a substitution. Dot
 178 size corresponds to the frequency of a particular haplotype.

179

180 **Acknowledgments**

181 The authors would like to thank Dr. Anneke DeLuycker and Dr. Joshua Davis at the
 182 Smithsonian-Mason School of Conservation in Front Royal, VA for their support of this research
 183 project through the Spring 2022 Endangered Species Conservation course. The authors would
 184 like to thank the Faculty of Forestry, Kasetsart University, the Department of National Parks,
 185 Wildlife and Plant Conservation, Thailand, and the Zoological Park Organization of Thailand for
 186 their support in sample collection.

187

188 **Disclosure Statement**

189 The content of this paper was developed solely by the authors. We declare no conflict of interest.

190

191 **Data Availability Statement**

192 The raw read data for the Indochinese jackal from Thailand were deposited in the NCBI Short
193 Read Archive under a BioProject (accession: PRJNA847318) and with a BioSample ID of
194 SAMN29334461. The new mitogenomes sequenced and assembled from golden jackals from
195 Thailand and Turkey were deposited into NCBI's GenBank under the accession numbers
196 ON986207 and OP345200, respectively.

197

198

199 **References**

200 Andrews, S. (2010). FastQC: A Quality Control Tool for High Throughput Sequence Data.

201 Babraham Bioinformatics. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

202 Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritsch, G., Pütz, J.,

203 Middendorf, M., & Stadler, P. F. (2013). MITOS: Improved de novo metazoan

204 mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, 69(2), 313–

205 319. <https://doi.org/10.1016/j.ympev.2012.08.023>

206 Bushnell, B. (2014). BBMap: A Fast, Accurate, Splice-Aware Aligner. No. LBNL-7065E.

207 Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA.

208 Fabbri, E., Caniglia, R., Galov, A., Arbanasić, H., Lapini, L., Bošković, I., Florijančić, T.,

209 Vlasseva, A., Ahmed, A., Mirchev, R. L., & Randi, E. (2014). Genetic structure and

210 expansion of golden jackals (*Canis aureus*) in the north-western distribution range
211 (Croatia and eastern Italian Alps). *Conservation Genetics*, 15(1), 187–199.
212 <https://doi.org/10.1007/s10592-013-0530-7>

213 Hemprich, F.G., & Ehrenberg, C.G. (1832). *Symbolae Physicae quae ex Itinere Africam*
214 *Borealem et Asoam Occidentalem Decas Secunda*. Ex Officina Academica.

215 İbiş, O., Selçuk, A. Y., Sacks, B. N., Yıldız, B., Özcan, S., Kefelioglu, H., & Tez, C. (2020).
216 Whole mitochondrial genome of long-clawed mole vole (*Prometheomys*
217 *schaposchnikowi*) from Turkey, with its phylogenetic relationships. *Genomics*, 112(5),
218 3247–3255. <https://doi.org/10.1016/j.ygeno.2020.06.008>

219 Hoffmann, M., Arnold, J., Duckworth, J.W., Jhala, Y., Kamler, J.F. & Kropfel, M. 2018. *Canis*
220 *aureus* (errata version published in 2020). *The IUCN Red List of Threatened Species*
221 2018: e.T118264161A163507876

222 Katoh, K. & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7:
223 Improvements in Performance and Usability. *Molecular Biology and Evolution*, 30(4),
224 772–780. <https://doi.org/10.1093/molbev/mst010>

225 Koepfli, K.-P., Pollinger, J., Godinho, R., Robinson, J., Lea, A., Hendricks, S., Schweizer, R. M.,
226 Thalmann, O., Silva, P., Fan, Z., Yurchenko, A. A., Dobrynin, P., Makunin, A., Cahill, J.
227 A., Shapiro, B., Álvares, F., Brito, J. C., Geffen, E., Leonard, J. A., ... Wayne, R. K.
228 (2015). Genome-wide Evidence Reveals that African and Eurasian Golden Jackals Are
229 Distinct Species. *Current Biology: CB*, 25(16), 2158–2165.
230 <https://doi.org/10.1016/j.cub.2015.06.060>

231 Kropfel, M., Giannatos, G., Čirovič, D., Stoyanov, S., & Newsome, T. M. (2017). Golden jackal
232 expansion in Europe: A case of mesopredator release triggered by continent-wide wolf

233 persecution? *Hystrix, the Italian Journal of Mammalogy*, 28(1), 9–15.
234 <https://doi.org/10.4404/hystrix-28.1-11819>

235 Krofel, M., Hatlauf, J., Bogdanowicz, W., Campbell, L. a. D., Godinho, R., Jhala, Y. V.,
236 Kitchener, A. C., Koepfli, K.-P., Moehlman, P., Senn, H., Sillero-Zubiri, C., Viranta, S.,
237 Werhahn, G., & Alvares, F. (2022). Towards resolving taxonomic uncertainties in wolf,
238 dog and jackal lineages of Africa, Eurasia and Australasia. *Journal of Zoology*, 316(3),
239 155–168. <https://doi.org/10.1111/jzo.12946>

240 Leigh, J.W. & Bryant, D. (2015). PopART: Full-feature software for haplotype network
241 construction. *Methods in Ecology and Evolution*, 6(9), 1110–1116.
242 <https://doi.org/10.1111/2041-210X.12410>

243 Lindgreen, S. (2012). AdapterRemoval: Easy Cleaning of Next Generation Sequencing Reads,
244 *BMC Research Notes*, 5, 337. <https://doi.org/10.1186%2F1756-0500-5-337>

245 Linnaeus, C. 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera,*
246 *species, cum characteribus, differentiis, synonymis, locis.* Editio decima, reformata.
247 Volume 1. Laurentii Salvii, Stockholm, Sweden.

248 Matschie, P. 1900. Herr Matschie sprach über den Schakal des Menam-Gebietes in Siam.
249 *Sitzungs-Berichte der Gesellschaft der Naturforschender Freunde zu Berlin* 3, 144–145.

250 Moehlman, P. D., & Hayssen, V. (2018). *Canis aureus* (Carnivore: Canidae). *Mammalian*
251 *Species*, 50(957), 14–25. <https://doi.org/10.1093/mspecies/sey002>

252 Parr, J. K. W. 2003. *A Guide to the Large Mammals of Thailand*. Sarakadee Press, Bangkok,
253 Thailand. 206 pp.

254 Rutkowski, R., Krofel, M., Giannatos, G., Ćirović, D., Männil, P., Volokh, A. M., Lanszki, J.,
255 Heltai, M., Szabó, L., Banea, O. C., Yavruyan, E., Hayrapetyan, V., Kopaliani, N.,

256 Miliou, A., Tryfonopoulos, G. A., Lymberakis, P., Penezić, A., Pakeltytė, G., Suchecka,
257 E., & Bogdanowicz, W. (2015). A European Concern? Genetic Structure and Expansion
258 of Golden Jackals (*Canis aureus*) in Europe and the Caucasus. *PLOS ONE*, *10*(11),
259 e0141236. <https://doi.org/10.1371/journal.pone.0141236>

260 Schubert, M., Ermini, L., Der Sarkissian, C., Jónsson, H., Ginolhac, A., Schaefer, R., Martin, M.
261 D., Fernández, R., Kircher, M., McCue, M., Willerslev, E., & Orlando, L. (2014).
262 Characterization of ancient and modern genomes by SNP detection and phylogenomic
263 and metagenomic analysis using PALEOMIX. *Nature Protocols*, *9*(5), 1056–1082.
264 <https://doi.org/10.1038/nprot.2014.063>

265 Spassov, N., & Acosta-Pankov, I. (2019). Dispersal history of the golden jackal (*Canis aureus*
266 moreoticus Geoffroy, 1835) in Europe and possible causes of its recent population
267 explosion. *Biodiversity Data Journal*, *7*, e34825. <https://doi.org/10.3897/BDJ.7.e34825>

268 Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of
269 large phylogenies. *Bioinformatics*, *30*(9), 1312–1313.
270 <https://doi.org/10.1093/bioinformatics/btu033>

271 Yumnam, B., Negi, T., Maldonado, J. E., Fleischer, R. C., & Jhala, Y. V. (2015).
272 Phylogeography of the Golden Jackal (*Canis aureus*) in India. *PLOS ONE*, *10*(9),
273 e0138497. <https://doi.org/10.1371/journal.pone.0138497>

274 Yusefi, G. H., Godinho, R., Khalatbari, L., Broomand, S., Fahimi, H., Martínez-Freiría, F., &
275 Alvares, F. (2021). Habitat use and population genetics of golden jackals in Iran: Insights
276 from a generalist species in a highly heterogeneous landscape. *Journal of Zoological*
277 *Systematics and Evolutionary Research*, *59*(7), 1503–1515.
278 <https://doi.org/10.1111/jzs.12519>