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Mitochondrial diversity of the widespread Central Asian steppe tortoise (*Testudo horsfieldii* Gray, 1844): implications for taxonomy and relocation of confiscated tortoises

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Abstract. Using a nearly range-wide sampling, we investigated phylogeographic differentiation and mitochondrial diversity of *Testudo horsfieldii*, the only tortoise species confined to Central Asia. We identified three major haplotype clades with mainly parapatric distribution that do not correspond well to the currently recognized three subspecies. One clade is restricted to the Fergana Valley and seems to represent a previously overlooked evolutionarily significant unit. Another clade, consisting of several largely parapatrically distributed haplotypes, occurs in the north and the central southern part of the species' range. The third clade, likewise comprising several largely parapatrically distributed haplotypes, was identified from the south-eastern corner of the Caspian Sea in the west, from Afghanistan and Pakistan in the east and from two more northerly sites in western and south-eastern Uzbekistan. It is possible that this clade also occurs in eastern Turkmenistan and adjacent Afghanistan, regions not sampled for the present study. The generally parapatric distribution of individual haplotypes, even within each of the three major clades, suggests advanced lineage sorting, either due to limited dispersal abilities, glacial isolation in distinct local microrefuges or both acting in accord. The localized distribution of endemic haplotypes in the northern and central plains as well as in the mountainous eastern and southern parts of the distribution range supports the existence of multiple microrefuges there. Records of haplotypes of distinct clades in sympatry or close geographic proximity are likely the result of Holocene range expansions. In recent years, thousands of confiscated steppe tortoises were released into the wild. The detected mitochondrial differentiation offers a powerful tool for nature conservation, as a means of determining the geographic origin of confiscated tortoises and selecting suitable reintroduction regions.

Keywords: Central Asia, phylogeography, subspecies, *Testudo horsfieldii horsfieldii*, *Testudo horsfieldii kazachstanica*, *Testudo horsfieldii rustamovi*.

Introduction

The small to medium-sized *Testudo horsfieldii* Gray, 1844 (maximum shell length approx. 30 cm) is the only tortoise species confined to Central Asia. Its range encompasses eastern Iran, Afghanistan, Turkmenistan, Uzbekistan, Tajikistan, Kyrgyzstan, southern Kazakhstan, the westernmost part of Xinjiang (China), and in Pakistan part of the region bordering Afghanistan (Bannikov et al., 1977; Iverson, 1992; Kuzmin, 2002; Fritz and Havaš, 2007). This is an area of about 2500 km in an east-west

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direction and 1500 km in a north-south direction. The natural history of the steppe tortoise differs significantly from that of its Mediterranean congeners. *Testudo horsfieldii* is a specialized burrow-dwelling steppicolous and deserticolous species with a very short activity period to cope with extreme summer heat and winter cold (Kami, 1999; Kuzmin, 2002; Lagarde et al., 2002). For instance, steppe tortoises are active for less than three months in southern Uzbekistan (Lagarde et al., 2002). As required by their burrow-digging mode of life, steppe tortoises have a distinctive morphology. Their strong fore-feet are shovel-like with reduced digits and phalanges; the shell is almost as long as broad and conspicuously depressed (Bannikov et al., 1977; Ernst et al., 2000; Kuzmin, 2002; Hitschfeld et al., 2008). Due to the morphological distinctiveness of *T. horsfieldii*, its generic allocation to *Testudo* has repeatedly been challenged. However, removal from this genus seems unwarranted since all phylogenetic analyses using a five-gene data set (mtDNA: 12S rRNA, 16S rRNA, cyt *b*; nDNA: C-mos, Rag2) of approximately two-thirds of all testudinid species consistently found the five *Testudo* species monophyletic, albeit with moderate support values (Fritz and Bininda-Emonds, 2007).

In contrast to many Mediterranean species (e.g., Weiss and Ferrand, 2006; Schmitt, 2007), little is known about the phylogeography of Central Asian species (Fritz et al., 2008), making *T. horsfieldii* an attractive object for such investigations. In the Mediterranean, phylogeographic structures of widespread reptiles generally correlate with their glacial refugia (e.g., Joger et al., 2007; Schmitt 2007; Weiss and Ferrand, 2007). Therefore, we expect that the phylogeography of a widely distributed Central Asian species like *T. horsfieldii* could be helpful in locating its refuges as well. Moreover, observed phylogeographic structures should correspond to subspecific differentiation, whereas conflicts between subspecies delineations and phylogeographic patterns often provide evidence for the need of taxonomic revisions (Avise, 2000; Zink and Barrowclough, 2008). Currently, three subspecies of *T. horsfieldii* are recognized (Fritz and Havaš, 2007), and these differ mainly in shell shape, size and coloration (Chkhikvadze, 1988; Chkhikvadze et al., 1999; Kuzmin, 2002). However, such characters proved to be of little help for revealing evolutionarily significant units in other testudinids (*Testudo*: Carretero et al., 2005; Fritz et al., 2005, 2006, 2007; Attum et al., 2007; Široký and Fritz, 2007; *Indotestudo*: Ives et al., 2008). Although the exact distribution of the three putative *T. horsfieldii* subspecies is not entirely clear, *T. h. kazachstanica* (Chkhikvadze, 1988) is thought to occur in the northern part of the range; *T. h. rustamovi* (Chkhikvadze, Amiranashvili and Ataev, 1990) in southwestern Turkmenistan, adjacent Iran and southwestern Kazakhstan; and *T. h. horsfieldii* Gray, 1844 in the rest of the species' range (Fritz and Havaš, 2007; Vasilyev et al., 2008). A few years ago, it was proposed that these three subspecies, as well as *Testudo baluchiorum* Annandale, 1906, described from what is now the Afghan-Pakistani border region and previously considered a junior synonym of *T. h. horsfieldii*, represent distinct species (Perälä, 2002; Vetter, 2002). But a recent investigation using 12S rRNA sequences of 59 samples from Iran, eastern Uzbekistan and eastern Kazakhstan found little variation and concluded that the observed variation supports conspecificity of the studied populations (Vasilyev et al., 2008). In this paper, only four haplotypes differing by 1-3 sites were identified. One haplotype occurred in all northern sites of Uzbekistan and Kazakhstan; the other three were found in four localities in Iran and south-easternmost Uzbekistan. However, the 12S rRNA gene is slowly evolving and only moderately informative in revealing phylogeographic differentiation of chelonians. For instance, low variation was found among 12S rRNA sequences of *T. graeca* (van der Kuyl et al., 2002; Harris et al., 2003), while the more variable mitochondrial cytochrome *b* gene (cyt *b*) yielded a much more detailed and clearly structured phylogeographic pattern (Fritz et al., 2007, 2009).

In the present paper, we use cyt *b* sequence variation in a nearly range-wide sampling of the steppe tortoise to address the following questions: (i) is *T. horsfieldii* a phylogeographically structured species, and if so, (ii) does the phylogeographic pattern correlate with putative glacial refuges, and (iii) does it match the ranges of the currently recognized subspecies?

Beyond science, a better knowledge of the phylogeography of *T. horsfieldii* could also directly improve future conservation strategies. In the former Central Asian Soviet Republics, the species is massively harvested for the pet-trade. As corollary of the implementation of CITES, thousands of confiscated tortoises have been released into the wild, often without knowledge of their geographic origin (M.A. Chirikova, T.N. Duysebayeva, V.K. Eremchenko, R.D. Kashkarov, own observ.; T. Harder, pers. comm.) and risking 'genetic pollution' of native populations. Given that a clear phylogeographic structure exists, mitochondrial haplotyping of confiscated tortoises would help to determine their geographic origin and to select suitable reintroduction regions.

Materials and methods

Sampling

Eighty blood or saliva samples of *Testudo horsfieldii* were field-collected, representing populations of most of the species' range and all three currently recognized subspecies (samples were assigned to subspecies according to their geographic origin). In addition, nine cyt *b* sequences from GenBank and 11 tissue samples of pet-trade tortoises from the collection of the Museum of Zoology Dresden were used. This total sample corresponds to 58 sampling sites (Appendix).

DNA extraction, PCR and sequencing

Total genomic DNA was extracted by overnight incubation at 55°C in lysis buffer (6% DTAB, 1.125 M NaCl, 75 mM Tris-HCl, 37.5 mM EDTA, pH 8.0) including 0.5 mg of proteinase K (Merck, Whitehouse Station, NJ) and subsequent purification following the DTAB method (Gustincich et al., 1991). DNA was precipitated from the supernatant with 0.2 volumes of 4 M LiCl and 0.8 volumes of isopropanol, centrifuged, washed, dried and resuspended in TE buffer.

Our target sequence was an mtDNA fragment containing the complete cyt *b* gene and approximately 20 bp of the adjacent tRNA-Thr gene. For polymerase chain reaction (PCR) and sequencing, the primers CytbG (Spinks et al., 2004), mt-c-For2, mt-f-na3, and mt-E-Rev2 (Praschag et al., 2007) were used. PCR amplification, PCR product purification and sequencing followed Praschag et al. (2007). Sequencing was performed on an ABI 3130 (Applied Biosystems, Foster City, CA). None of the sequences contained internal stop codons, and nucleotide frequencies corresponded to those of coding mtDNA; therefore we conclude to have amplified and sequenced mtDNA and not nuclear copies of mitochondrial genes.

Sequence analyses

The 100 *Testudo horsfieldii* sequences were manually collapsed into haplotypes, resulting in 26 distinct haplotypes. Six of these haplotypes are GenBank sequences and 15 haplotypes were found only once (Appendix). Five of the GenBank haplotypes were represented by 429-bp-long to 1063-bp-long sequences only, while the sixth GenBank haplotype and our own sequences were 1167 bp long. Phylogenetic analyses were run for two data sets, one including all 26 haplotypes and another with the shorter GenBank haplotypes removed. Data were analysed under the optimality criteria Maximum Parsimony (MP; equal weighting, command: hs add = cl) and Maximum Likelihood (ML) using PAUP*4.0b10 (Swofford, 2002) and under Bayesian inference of phylogeny (BA) using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003; settings: ngen = 10 000 000 nchains = 4 nrun = 1 sample = 500 temp = 0.2 savebrlens = yes startingtree = random; burn-in set to sample only the plateau of most likely trees). Two Genbank sequences of a closely and a distantly related testudinid species served as outgroups (AM230496, *Testudo h. hermanni*; DQ497301, *Stigmochelys p. pardalis*). *Testudo hermanni* is the sister species of *T. horsfieldii* (Fritz and Bininda-Emonds, 2007). For the data set of 26 haplotypes, 1103 of 1167 aligned characters were constant in the ingroup sequences; 31 characters were variable and parsimony-informative; 33 variable characters were singletons. In the smaller data set, 1122 sites were constant; 25 were variable and parsimony-informative and 20 variable characters were parsimony-uninformative. The best evolutionary model was established using Modeltest 3.06 (Posada and Crandall, 1998; AIC best-fit model: TrN + I). Bootstrap support values were obtained in PAUP*4.0b10 (MP: nreps = 1000, maxtree = 100; ML: nreps = 100, maxtree = 1). Uncorrected *p* distances were calculated using MEGA 3.1 (Kumar et al., 2004).

Within the same species or between closely related species, relationships of haplotypes are likely to be reticulate and ancestral haplotypes may persist, which is why intraspecific gene evolution may be only imperfectly reflected by dichotomous trees (Posada and Crandall, 2001). Therefore, we also calculated a parsimony network using TCS 1.21 (Clement et al., 2000). This software is based on statistical parsimony and connects haplotypes via a minimal number of mutational steps and allows for alternative pathways. A further advantage of network analyses is that information about the age of haplotypes may be obtained. Interiorly located haplotypes, having more than one mutational connection, are thought to be ancestral to and older than tip haplotypes (Posada and Crandall, 2001). To avoid ambiguities caused by much shorter GenBank sequences, we included in network analysis only the 91 sequences of 1167 bp length produced in this investigation, the corresponding fragment of the complete mitochondrial genome from GenBank (DQ080045), and in the last position of the data set, the 1140-bp-long GenBank sequence DQ497322.

Further, population-based analyses were performed for the 1167-bp-long sequences to better elucidate past demographic events as well as population structure. The software DnaSP 4.1 (Rozas et al., 2003) was used to carry out mismatch distribution analyses (Rogers and Harpending, 1992) as well as Fu and Li's (1993) tests for neutrality. A Mantel regression of a pairwise geographic distance matrix (that is, great-arc distances obtained from Cartesian co-ordinates) with a pairwise genetic matrix of uncorrected *p* distances, obtained in MEGA 3.1, was performed to ascertain if the distribution of genetic diversity across the species was spatially correlated.

Results

For both data sets, all phylogenetic analyses revealed the same three major clades (indicated in red, blue and black in fig. 1). The basal branching pattern of these three major clades varied between the tree-building methods, however. While ML suggested, with weak support, that the 'red clade' forms the sister group of a clade comprising the 'blue clade' and the 'black clade', Bayesian analysis placed all of the three major clades in a basal polytomy. Under MP,

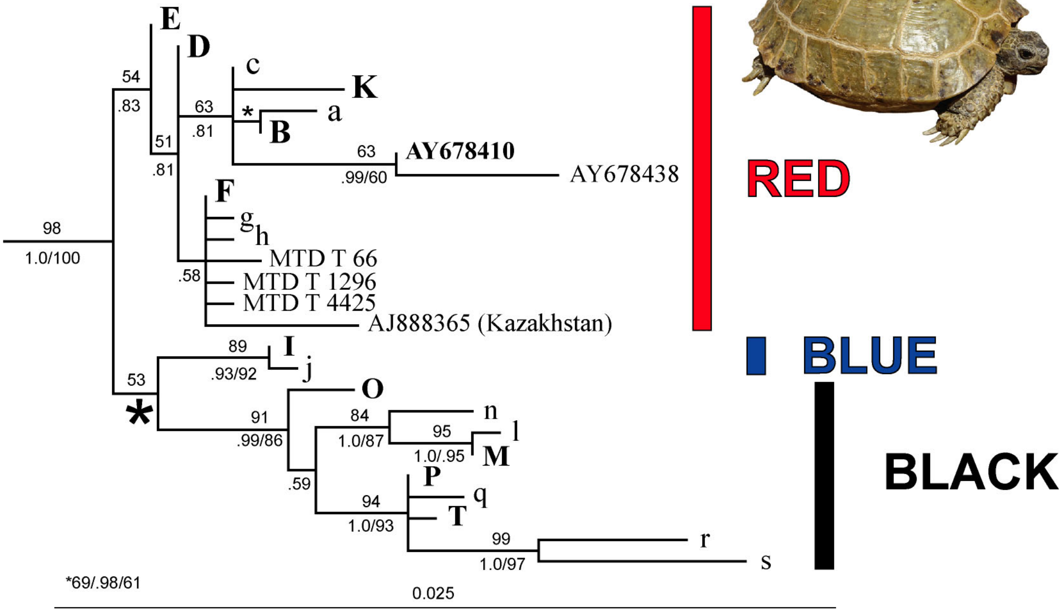

Figure 1. One of nine ML trees for 26 mtDNA haplotypes of *Testudo horsfieldii* (1167 bp, cyt *b* and partial tRNA-Thr genes; $-\ln L = 2990.5257$). The other eight ML trees differ only in the branching pattern within the 'red clade'. Major haplotype clades indicated on the right. Individual known-locality haplotypes are indicated by letters; haplotypes identified in more than one individual in upper case and bold; haplotypes identified only once, lower case. For haplotypes of unknown geographic origin, GenBank accession numbers or voucher numbers are given (MTD T = Museum of Zoology Dresden, tissue collection). AJ888365, AY678410, AY678438, 'r', and 's' are haplotypes represented by shorter GenBank sequences (429-1063 bp). Numbers above nodes are ML bootstrap values; below nodes, Bayesian posterior probabilities and MP bootstraps. The large asterisk indicates this branch was not found under BA and MP (see text). For the data set of 21 haplotypes, one ML tree with the same major topology was obtained ($-\ln L = 2815.0805$). Support values were for this data set (ML/BA/MP): red clade, 72/0.85/70; blue clade, 92/0.93/92, black clade, 95/1.0/95; under ML: (blue clade + black clade), 52; under MP: (red clade + black clade), 53.

Table 1. Uncorrected *p* distances (percentages) of the three *Testudo horsfieldii* clades (all 26 haplotypes). Below diagonal, distances between clades; on the diagonal, within-clade distances in bold (average and ranges).

	Red	Blue	Black
Red	**0.50 (0.09-1.37)**		
Blue	1.01 (0.60-1.65)	**0.09 (–)**	
Black	1.57 (0.72-2.79)	1.31 (0.94-2.23)	**1.02 (0.09-2.03)**

24 equally parsimonious trees were obtained for the 26 haplotype data set (tree length = 294; CI = 0.9252, RI = 0.8866); the 21 haplotype data set resulted in two equally parsimonious trees (tree length = 272; CI = 0.8105, RI = 0.8896). In all MP trees, a basal position of the 'blue clade' and a sister group relationship of the 'red clade' and the 'black clade' were favoured, although with weak bootstrap support (<50% in the 26 haplotype data set, 53% in the 21 haplotype data set); differences occurred in the arrangement of the branches within the 'red clade' and the 'black clade'. Average uncorrected *p* distances between the three clades ranged from 1.01% to 1.57% (table 1); the mean distance among all haplotypes of *T. horsfieldii* was 1.09%.

For all methods and both data sets, two of the major clades were moderately to well-supported ('blue' and 'black clades'). The third clade ('red') obtained only weak support in the 26 haplotype data set, due to the short GenBank sequences clustering here. In the smaller data set with GenBank haplotypes removed, its support values raised to 72/0.85/70 (ML, BA, MP; fig. 1). This 'red clade' corresponds to 15 haplotypes of the larger data set and includes some sequences of unknown geographic origin. The known-locality samples yielding haplotypes of this clade originate from the northern part of the range, except the Fergana Valley, and in the south from north-eastern and eastern Iran and the Kopet Dag region of Turkmenistan (fig. 2). In western and south-eastern Uzbekistan and in Iran haplotypes of this 'red clade' occur in sympatry or close proximity with haplotypes of the better supported, more southerly distributed 'black clade' (figs 1 and 2). The 'black clade' contains nine haplotypes that were mainly identified from the southern distribution range (north-eastern and eastern Iran, Afghanistan, Pakistan). Within the 'black clade', all tree-building methods found two well-supported subclades consisting of haplotypes (l, M, n) or (P, q, r, s, T). Haplotype O was placed as basal to these subclades in ML and Bayesian analyses, while MP found for both data sets equally parsimonious solutions suggesting haplotype O as basal or sister to the subclade (P, q, r, s, T). Haplotypes l, M, n, and O originate in Pakistan, Afghanistan, and south-eastern Uzbekistan, while haplotypes P, q, r, s, and T were identified from western Uzbekistan, north-eastern and eastern Iran. The third major clade (blue in figs 1 and 2) comprises only two closely related haplotypes from the Fergana Valley.

In parsimony network analysis (fig. 3), the three haplotype clades appear as distinct clusters without reticulations. Haplotypes of the 'red clade' are separated from the 'blue clade' by a minimum of seven mutational steps and from the 'black clade' by a minimum of ten steps. Haplotypes of the 'blue' and the 'black clade' are distant by a minimum of eleven steps. Within the 'red clade' occurs a maximum number of nine steps; within the 'blue clade', one step; and within the 'black clade', twelve steps. Haplotype O, having an ambiguous position within the 'black clade' in phylogenetic analyses, branches off basally in the subnet of the 'black clade'.

The mismatch distributions of the entire data set of 92 sequences, as well as for each of the three clades described above, are shown in figure 4. Under a coalescent model, a sudden

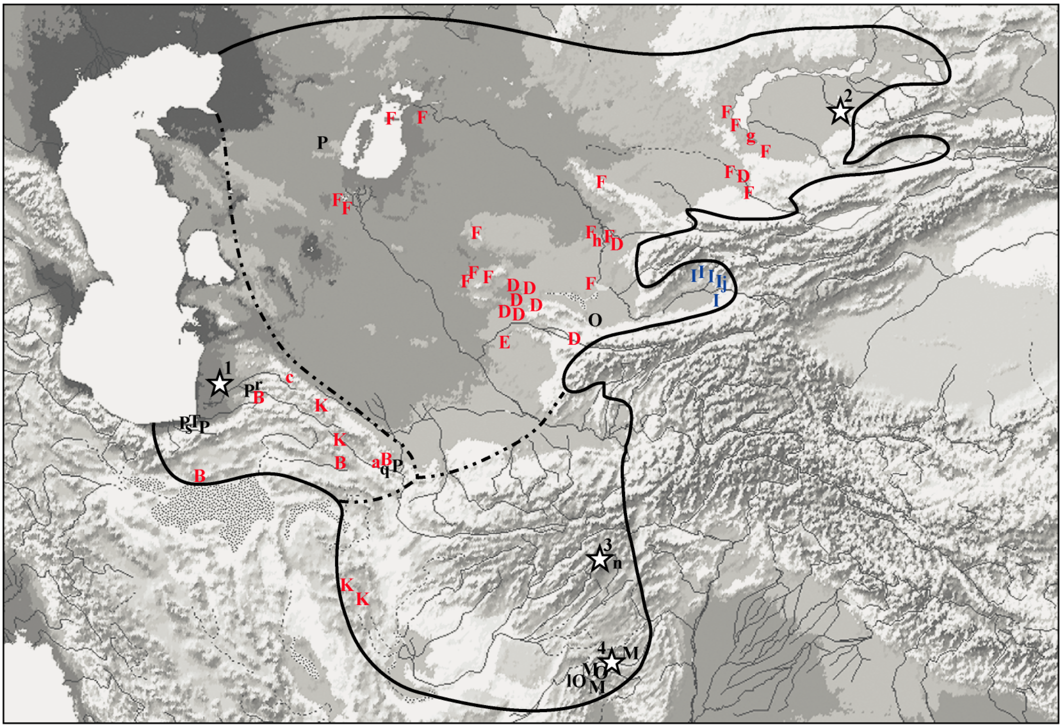

Figure 2. Geographic distribution of mtDNA haplotypes of *Testudo horsfieldii*. Colours correspond to other figures. Note occurrence of haplotypes of the 'red clade' and the 'black clade' in the same sites or in close geographic proximity. Simplified range borders of the three currently recognized subspecies indicated. Stars represent type localities: 1 – *T. h. rustamovi* (Chkhikvadze, Amiranashvili and Ataev, 1990); 2 – *T. h. kazachstanica* (Chkhikvadze, 1988); 3 – *T. h. horsfieldii* Gray, 1844; 4 – '*T. baluchiorum* Annandale, 1906'.

population is expected to result in a peak in the distribution of pairwise differences (plotted red lines) between sequences (Rogers and Harpending, 1992), whereas a population maintaining a constant size through time is expected to follow a trend similar to that plotted by blue lines. Analyses of the entire data set, as well as for each clade, showed a large proportion of closely related haplotypes. However, a past sudden population expansion is most likely to have occurred in the 'black clade', while the 'red clade' shows moderate signs of demographic expansion. The 'blue clade' perfectly fits the model of constant population size. Fu and Li's (1993) D* and F* indices of neutrality were all slightly negative for all tested groups (fig. 4), and in the case of a neutral marker such as mitochondrial DNA, this indicates a greater number of pairwise differences relative to segregating sites and a possibly expanding population. However, none of these indices were significant. A Mantel regression found that only 24% of the variation in the genetic data could be attributed to geographic variation, precluding the assumption of a clinal population structure of isolation-by-distance.

Discussion

Although we found only moderate variation in the studied mtDNA fragment, our results provide evidence that *Testudo horsfieldii* is a phylogeographically structured species. We identified three major clades of haplotypes with mainly parapatric ranges. Even within each of the three major clades, the more frequent haplotypes have largely parapatric distributions, suggestive of advanced lineage sorting, either due to limited dispersal abilities, glacial isolation in dis-

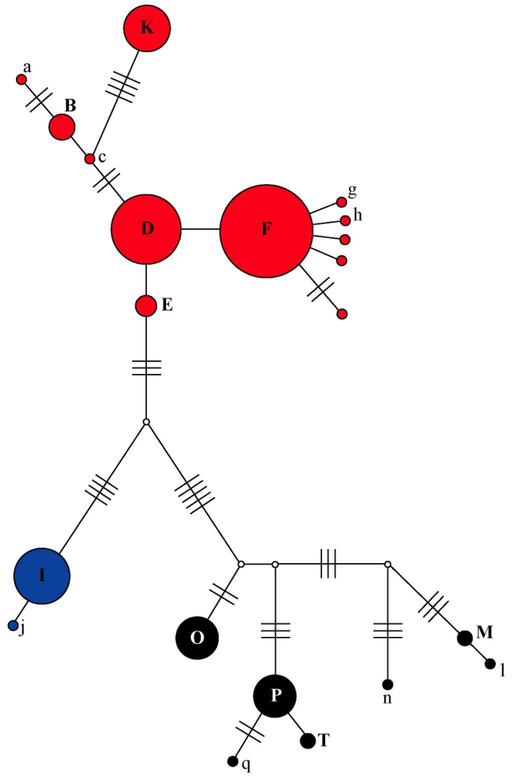

Figure 3. Parsimony network of 93 mtDNA sequences of *Testudo horsfieldii* (1167 bp, cyt *b* and partial tRNA-Thr genes; spring tree). Colours correspond to other figures; symbol size, approximate haplotype frequency; missing node haplotypes, open circles. Each line joining haplotypes indicates one substitution except when hatchmarks across lines are present; then each mark indicates one step. Only known-locality haplotypes bear letters. Haplotype frequencies are B: $n = 4$; D: $n = 14$; E: $n = 3$; F: $n = 27$; I: $n = 12$; K: $n = 7$; M: $n = 2$; O: $n = 6$; P: $n = 6$; T: $n = 2$. Other haplotypes were identified only once.

tinct local microrefuges or both acting in concert. The average uncorrected *p* distances of the three clades (table 1) fall within the range observed between North African subspecies of *T. graeca*, arguing for Early to Middle Pleistocene differentiation in both cases and in favour of repeated glacial fragmentation (cf. Fritz et al., 2009). Furthermore, the localized distribution of endemic haplotypes in the northern and central plains as well as in the mountainous eastern and southern parts of the distribution range of *T. horsfieldii* supports the existence of multiple microrefuges. This is in line with our results of the Mantel test, indicating discontinuous divergence in geographic distribution.

The three major clades of *T. horsfieldii* do not agree well with its currently recognized three subspecies and also do not support the validity of *T. baluchiorum* Annandale, 1906, described from the Afghan-Pakistani border region (fig. 2). One of our three major clades, the 'blue clade' from the Fergana Valley within the range of *T. h. kazachstanica*, seems to represent a previously overlooked evolutionarily significant unit. Its occurrence in the Fergana Valley suggests that microrefuges were located not only within the extant southern range, but also fairly far north. The 'blue clade', isolated in the Fergana Valley, has maintained a constant population size throughout its demographic history, while the other clades show at least some evidence of recent population expansion that seems to be related to Holocene warming and range extension.

Except the Fergana Valley, the distribution of our 'red clade' in the north matches the range of *T. h. kazachstanica* and the distribution of the northern 12S rRNA haplotype of Vasilyev et al. (2008). However, in the south the same 'red clade' penetrates deep into the putative ranges of *T. h. horsfieldii* and *T. h. rustamovi*, proposing a distinctly larger range of *T. h. kazachstanica* than thought before. The other haplotypes identified from the ranges of *T. h. horsfieldii* and *T. h. rustamovi* represent another major clade ('black clade'), suggesting weak differentiation and synonymy of both subspecies. In some sites in Iran and western and south-eastern Uzbekistan, haplotypes of the 'black clade' were recorded together with or in close proximity to haplotypes of the 'red clade'. This, as well as the record of a southern 12S rRNA haplotype in south-eastern Uzbekistan (Vasilyev et al., 2008), may indicate the existence of secondary contact zones due to Holocene range expansions and a wider distribution of the 'black clade' in Turkmenistan and northern Afghanistan, regions not sampled for the present study.

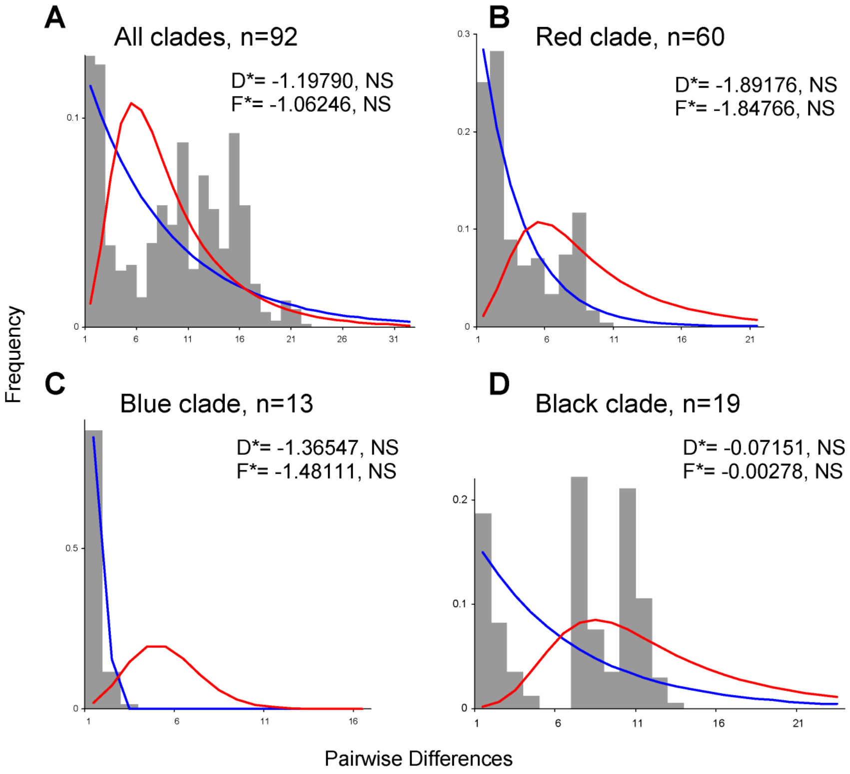

Figure 4. Mismatch distributions and demographic indices for the entire data set (panel A) and for each of the three phylogenetic clades (panels B-D). Observed mismatch distributions are graphed in grey columns, whereas line distributions correspond to those expected under a model of constant population size (blue) and a model of population expansion (red). NS, not significant.

In the former Central Asian Soviet Republics, steppe tortoises have been heavily exploited for the pet-trade for decades. From 1976 to 1993, more than one million steppe tortoises were collected in Kazakhstan alone (United Nations Environment Programme, World Conservation Monitoring Centre, 2004). With the implementation of CITES, collection and export quotas were introduced (Rhodin, 2003; Bykova et al., 2007). For instance, in 2002 Kazakhstan, Tajikistan and Uzbekistan had export quotas of 40 000, 20 000 and 30 000 live tortoises, respectively (United Nations Environment Programme, World Conservation Monitoring Centre, 2004). Because of such harvest quotas and poaching, the number of confiscated tortoises increased considerably and thousands of confiscated tortoises were released into the wild, often without knowledge of their geographic origin (M.A. Chirikova, T.N. Duysebayeva, V.K. Eremchenko, R.D. Kashkarov, own observ.; T. Harder, pers. comm.). While we excluded from sampling any sites that could harbour such released tortoises, it is obvious that the current repatriation practice bears the risk of admixture of distinct genetic lineages and should not be continued.

Conclusions

Testudo horsfieldii is a phylogeographically structured species. Its differentiation pattern strongly suggests that the current diversity was shaped by glacial range interruptions. Refuges and microrefuges were most probably not only located in the south of the extant range, but also fairly far north. The weak agreement between subspecies and phylogroups of *T. hors-*

fieldii suggests that the morphological characters used for subspecies delineation are of limited taxonomic value, as in other *Testudo* species (Carretero et al., 2005; Fritz et al., 2005, 2006, 2007, 2009; Attum et al., 2007; Široký and Fritz, 2007). This is also underlined by the fact that some allegedly diagnostic osteological characters of *T. horsfieldii* subspecies (Perälä, 2002) were found to be ontogenetic variation (Hitschfeld et al., 2008). One of the three mtDNA clades identified in the present paper seems to represent a previously overlooked evolutionarily significant unit. Future investigations should re-analyze morphological variation in the light of phylogeographic differentiation to unravel the impact of ecological constraints and ancestry.

The phylogeographic pattern of *T. horsfieldii* argues for great caution with further releases of confiscated steppe tortoises without knowledge of their origin. However, our study offers a highly conservation-relevant application of phylogeography. Mitochondrial haplotyping of confiscated tortoises would allow for a much better selection of suitable releasing sites, based on the largely allopatric distribution of individual haplotypes.

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References

Attum, O., Baha el Din, S., Carranza, S., Earley, R., Arnold, E.N., Kingsbury, B. (2007): An evaluation of the taxonomic validity of *Testudo werneri*. Amphibia-Reptilia **28**: 393-401.

Avise, J.C. (2000): Phylogeography. The History and Formation of Species. Cambridge, MA and London, Harvard University Press.

Bannikov, A.G., Darevskii, I.S., Ishchenko, V.G., Rustamov, A.K., Shcherbak, N.N. (1977): Opredelitel' zemnovodnykh i presmykayushchikhsya fauny SSSR. Moscow, Prosveshcheniye.

Bykova, E.V., Sorochinsky, V.G., Golenkevich, A.V., Peregontsev, E.A., Sorochinskaya, I.N., Sorochinsky, G.Y. (2007): Ranching as a method of conservation of the wild population of Horsfield's tortoise, *Agrionemys horsfieldii*. Russ. J. Herpetol. **14**: 232-236.

Carretero, M.A., Znari, M., Harris, D.J., Macé, J.C. (2005): Morphological divergence among populations of *Testudo graeca* from west-central Morocco. Animal Biol. **55**: 259-279.

Chkhikvadze, V.M. (1988): O sistematicheskom polozhenii sovremennykh sukhoputnykh cherepakh Srednei Azii i Kazakhstana. Izv. Akad. Nauk GSSR, Ser. biol. **14**: 110-113.

Chkhikvadze, V.M., Amiranashvili, N.G., Ataev, Ch. (1990): Novyi podvid sukhoputnoi cherepakhi iz yugozapadnogo Turkmenistana. Izv. Akad. Nauk Turkmensk. SSR, Ser. biol. **1**: 72-75.

Clement, M., Posada, D., Crandall, K.A. (2000): TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9**: 1657-1660.

Ernst, C.H., Altenburg, R.G.M., Barbour, R.W. (2000): Turtles of the World. World Biodiversity Database, CD-ROM Series, Windows Version 1.2. Amsterdam, Biodiversity Center of ETI.

Fritz, U., Bininda-Emonds, O.R.P. (2007): When genes meet nomenclature: Tortoise phylogeny and the shifting generic concepts of *Testudo* and *Geochelone*. Zoology **110**: 298-307.

Fritz, U., Havaš, P. (2007): Checklist of chelonians of the world. Vertebr. Zool. **57**: 149-368.

Fritz, U., Široký, P., Kami, H., Wink, M. (2005): Environmentally caused dwarfism or a valid species – Is *Testudo weissingeri* Bour, 1996 a distinct evolutionary lineage? New evidence from mitochondrial and nuclear genomic markers. Mol. Phylogenet. Evol. **37**: 389-401.

Fritz, U., Auer, M., Bertolero, A., Cheylan, M., Fattizzo, T., Hundsdörfer, A.K., Martín Sampayo, M., Pretus, J.L., Široký, P., Wink, M. (2006): A rangewide phylogeography of Hermann's tortoise, *Testudo hermanni* (Reptilia: Testudines: Testudinidae): implications for taxonomy. Zool. Scr. **35**: 531-543.

Fritz, U., Hundsdörfer, A.K., Široký, P., Auer, M., Kami, H., Lehmann, J., Mazanaeva, L.F., Türkozan, O., Wink, M. (2007): Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). Amphibia-Reptilia **28**: 97-121.

Fritz, U., Ayaz, D., Buschbom, J., Kami, H.G., Mazanaeva, L.F., Aloufi, A.A., Auer, M., Rifai, L., Šilić, T., Hundsdörfer, A.K. (2008): Go east: Phylogeographies of *Mauremys caspica* and *M. rivulata* – Discordance of morphology, mitochondrial and nuclear genomic markers and rare hybridization. J. Evolution. Biol. **21**: 527-540.

Fritz, U., Harris, D.J., Fahd, S., Rouag, R., Graciá Martínez, E., Giménez Casalduero, A., Široký, P., Kalboussi, M., Jdeidi, T.B., Hundsdörfer, A.K. (2009): Mitochondrial phylogeography of *Testudo graeca* in the Western Mediterranean: Old complex divergence in North Africa and recent arrival in Europe. Amphibia-Reptilia **30**: 63-80.

Fu, Y.-X., Li, W.-H. (1993): Statistical tests of neutrality of mutations. Genetics **133**: 693-709.

Gustincich, S., Manfioletti, G., del Sal, G., Schneider, C., Carninci, C. (1991): A fast method for high-quality genomic DNA extraction from whole human blood. BioTechniques **11**: 298-302.

Harris, D.J., Znari, M., Macé, J.-C., Carretero, M.A. (2003): Genetic variation in *Testudo graeca* from Morocco estimated using 12S rRNA sequencing. Rev. Esp. Herpetol. **17**: 5-9.

Hitschfeld, E., Auer, M., Fritz, U. (2008): Phalangeal formulae and ontogenetic variation of carpal morphology in *Testudo horsfieldii* and *T. hermanni*. Amphibia-Reptilia **29**: 93-99.

Iverson, J.B. (1992): A Revised Checklist with Distribution Maps of the Turtles of the World. Richmond, IN, Privately Printed.

Ives, I., Spinks, P.Q., Shaffer, H.B. (2008): Morphological and genetic variation in the endangered Sulawesi tortoise *Indotestudo forstenii*. Conserv. Genet. **9**: 709-713.

Joger, U., Fritz, U., Guicking, D., Kalyabina-Hauf, S., Nagy, Z.T., Wink, M. (2007): Phylogeography of western Palaearctic reptiles – Spatial and temporal speciation patterns. Zool. Anz. **246**: 293-313.

Kami, H.G. (1999): On the biology of the Afghan Tortoise, *Testudo horsfieldi*, in north-eastern Iran. Zool. Middle East **19**: 43-54.

Kumar, S., Tamura, K., Nei, M. (2004): MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief. Bioinform. **5**: 150-163.

van der Kuyl, A.C., Ballasina, D.L.P., Dekker, J.T., Maas, H., Willemsen, R.E., Goudsmit, J. (2002): Phylogenetic relationships among the species of the genus *Testudo* (Testudines: Testudinidae) inferred from mitochondrial 12S rRNA gene sequences. Mol. Phylogenet. Evol. **22**: 174-183.

Kuzmin, S.L. (2002): The Turtles of Russia and Other Ex-Soviet Republics. Frankfurt am Main, Chimaira.

Lagarde, F., Bonnet, X., Nagy, K., Henen, B., Corbin, J., Naulleau, G. (2002): A short spring before a long jump: the ecological challenge in the steppe tortoise (*Testudo horsfieldi*). Can. J. Zool. **80**: 493-502.

Perälä, J. (2002): Biodiversity in relatively neglected taxa of *Testudo* L., 1758 s. l. Chelonii **3**: 40-53.

Posada, D., Crandall, K.A. (1998): Modeltest: testing the model of DNA substitution. Bioinformatics **14**: 817-818.

Posada, D., Crandall, K.A. (2001): Intraspecific gene genealogies: trees grafting into networks. Trends Ecol. Evol. **16**: 37-45.

Praschag, P., Hundsdörfer, A.K., Fritz, U. (2007): Phylogeny and taxonomy of endangered South and Southeast Asian freshwater turtles elucidated by mtDNA sequence variation (Testudines: Geoemydidae: *Batagur*, *Callagur*, *Hardella*, *Kachuga*, *Pangshura*). Zool. Scr. **36**: 429-442.

Rhodin, A.G.J. (2003): Conservation and Trade of Asian Freshwater Turtles and Tortoises: Updated Review of Status and Threats with Recommendations for CITES Listings. Geneva, CITES. Available at http://www.cites.org/eng/com/ac/19/E19-15-1.doc.

Rogers, A.R., Harpending, H. (1992): Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. **9**: 552-569.

Ronquist, F., Huelsenbeck, J.P. (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**: 1572-1574.

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., Rozas, R. (2003): DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19**: 2496-2497.

Schmitt, T. (2007): Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. Front. Zool. **4** (11): 1-12.

Široký, P., Fritz, U. (2007): Is *Testudo werneri* a distinct species? Biologia **62**: 228-231.

Spinks, P.Q., Shaffer, H.B., Iverson, J.B., McCord, W.P. (2004): Phylogenetic hypotheses for the turtle family Geoemydidae. Mol. Phylogenet. Evol. **32**: 164-182.

Swofford, D.L. (2002): PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sunderland, MA, Sinauer Associates.

United Nations Environment Programme, World Conservation Monitoring Centre (2004): Review of Significant Trade. Analysis of Trade Trends with Notes on the Conservation Status of Selected Species. Annex C: Reptiles and Amphibians. Geneva, CITES. Available at http://www.cites.org/common/com/ac/20/E20-inf-12.pdf.

Vasilyev, V.A., Bondarenko, D.A., Peregontsev, E.A., Voronov, A.S., Ryskov, A.P., Semenova, S.K. (2008): Polimorfism gena 12S rRNK i filogeografiya sredneaziatskoy cherepakhi *Agrionemys horsfieldii* Gray, 1844. Genetika **55**: 784-788.

Vetter, H. (2002): Terralog: Turtles of the World, Vol. 1. Africa, Europe and Western Asia. Frankfurt am Main, Chimaira.

Weiss, N., Ferrand, N. (2007): Phylogeography of Southern European Refugia. Berlin, Heidelberg, New York, Springer.

Zink, R.M., Barrowclough, G.F. (2008): Mitochondrial DNA under siege in avian phylogeography. Mol. Ecol. **17**: 2107-2121.

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Appendix. Samples and GenBank sequences of *Testudo horsfieldii* and their haplotypes. Sample numbers are either MTD T numbers (Museum of Zoology Dresden, tissue collection) or GenBank accession numbers. Haplotypes identified in more than one case in bold. Accession numbers of haplotypes identified in this study are FM883673-FM883692.

Sample	Subspecies	Locality	Latitude	Longitude	Haplotype
5034	*horsfieldii*	Afghanistan: Ghazni	33°33′N	68°26′E	n
3049	*horsfieldii*	Iran: Khorasan: Sarbisheh	32°35′N	59°48′E	**K**
3048	*horsfieldii*	Iran: Khorasan: vicinity of Birjand	32°53′N	59°12′E	**K**
5362	*horsfieldii*	Pakistan: Hazarganji	30°02.820′N	66°52.945′E	l
5359	*horsfieldii*	Pakistan: Hazarganji	30°02.994′N	66°52.872′E	**O**
5361	*horsfieldii*	Pakistan: Hazarganji	30°03.033′N	66°53.983′E	**O**
5355	*horsfieldii*	Pakistan: Karkhasa	30°10.942′N	66°57.251′E	**O**
5356	*horsfieldii*	Pakistan: Kuchlack	30°22.489′N	66°58.416′E	**M**
5358	*horsfieldii*	Pakistan: Quetta	30°08.957′N	66°56.931′E	**O**
5360	*horsfieldii*	Pakistan: Quetta	30°08.957′N	66°56.931′E	**O**
5357	*horsfieldii*	Pakistan: Torghar	31°11.888′N	68°27.359′E	**M**
AJ888365	*kazachstanica*	Kazakhstan	–	–	AJ888365
5416	*kazachstanica*	Kazakhstan: Kyzylkum sands, WNW Chardara village	41°14′N	67°42′E	**F**
5418	*kazachstanica*	Kazakhstan: Kyzylkum sands, WNW Chardara village	41°15′N	67°42′E	**F**
5415	*kazachstanica*	Kazakhstan: S Arys sands	42°09′N	68°25′E	**F**
5414	*kazachstanica*	Kazakhstan: Arys sands	42°13′N	68°19′E	**D**
5417	*kazachstanica*	Kazakhstan: Arys sands	42°13′N	68°19′E	**D**
5413	*kazachstanica*	Kazakhstan: Arys sands	42°13′N	68°19′E	h
5412	*kazachstanica*	Kazakhstan: E Kyzylkum sands; 197 m elevation	42°16′N	67°56′E	**F**
5409	*kazachstanica*	Kazakhstan: Mt. Karatau	43°40′N	69°01′E	**F**
3064	*kazachstanica*	Kazakhstan: Jambyl region: Merke District: vicinity of Kenesh village	43°57.666′N	73°34.217′E	**D**
3062	*kazachstanica*	Kazakhstan: Jambyl region: Merke District: vicinity of Kenesh village	43°57.666′N	73°34.217′E	**F**
5407	*kazachstanica*	Kazakhstan: Zhusandala valley, S Taukum sands (southern Lake Balkhash region); 377 m elevation	44°30.266′N	75°03.697′E	**F**
5408	*kazachstanica*	Kazakhstan: Zhusandala valley, S Taukum sands (southern Lake Balkhash region); 377 m elevation	44°30.266′N	75°03.697′E	**F**
5405	*kazachstanica*	Kazakhstan: coast of Alakul lake (southern margin of Lake Balkhash); 340 m elevation	44°50.249′N	74°12.778′E	g
5406	*kazachstanica*	Kazakhstan: 10 km SE Kashkateniz village (western coast of Lake Balkhash); 359 m elevation	45°42.883′N	73°24.305′E	**F**
5419	*kazachstanica*	Kazakhstan: SW coast Lake Balkhash, 5 km to Minaral village	45°24.067′N	73°38.280′E	**F**
5411	*kazachstanica*	Kazakhstan: Aral Sea: Barsakelmes island	45°40′N	59°55′E	**F**
5410	*kazachstanica*	Kazakhstan: E coast Aral Sea, 30 km SE Karateren village	45°47′N	61°19′E	**F**
3060	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: Kyzyk-Kiyya District, 18 km NE Kyzyl-Kiyya town, vicinity of Khauz village	40°23′N	72°15′E	**I**
3061	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: Kyzyk-Kiyya District, 18 km NE Kyzyl-Kiyya town, vicinity of Khauz village	40°23′N	72°15′E	**I**
3056	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: Mayli-Suu canyon, 7 km from Kok-Tash village	41°14′N	72°27′E	**I**

Appendix. (Continued).

Sample	Subspecies	Locality	Latitude	Longitude	Haplotype
3057	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: Mayli-Suu canyon, 7 km from Kok-Tash village	41°14′N	72°27′E	**I**
3058	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: Mayli-Suu canyon, 7 km from Kok-Tash village	41°14′N	72°27′E	**I**
3059	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: Mayli-Suu canyon, 7 km from Kok-Tash village	41°14′N	72°27′E	j
3054	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: 12 km SW Tash-Komur town	41°18′N	72°08′E	**I**
3055	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: 12 km SW Tash-Komur town	41°18′N	72°08′E	**I**
3050	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: W Ala-Buka village	41°24′N	71°28′E	**I**
3051	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: W Ala-Buka village	41°24′N	71°28′E	**I**
3063	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: W Ala-Buka village	41°24′N	71°28′E	**I**
3052	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: vicinity of Kerben (Karavan) village	41°29′N	71°45′E	**I**
3053	*kazachstanica*	Kyrgyzstan: Fergana Valley: Jalal Abad: vicinity of Kerben (Karavan) village	41°29′N	71°45′E	**I**
3065	*kazachstanica*	Kyrgyzstan: Chuy region: 7.5 km N Telek village, left bank of Ak-Suu river	43°14′N	74°03′N	**F**
3066	*kazachstanica*	Kyrgyzstan: Chuy region: 7.5 km N Telek village, left bank of Ak-Suu river	43°14′N	74°03′N	**F**
3532	*kazachstanica*	Uzbekistan: Ecocenter "Djeiran"; 223 m elevation	39°34.737′N	64°41.296′E	**E**
3528	*kazachstanica*	Uzbekistan: Zaravshan Nature Reserve; 720 m elevation	39°40.292′N	67°05.492′E	**D**
3536	*kazachstanica*	Uzbekistan: 10 km S Turkmen village; 800 m elevation	39°53.520′N	68°30.000′E	**O**
3533	*kazachstanica*	Uzbekistan: 20 km W Orazjan village; 373 m elevation	40°31.773′N	65°10.315′E	**D**
3537	*kazachstanica*	Uzbekistan: 25 km E crossroads Jangigazgan-Zafarabad (southern Kyzylkum); 357 m elevation	40°36.414′N	65°16.705′E	**D**
3525	*kazachstanica*	Uzbekistan: western part of Ajakagitma depression; 100 m elevation	40°39.672′N	64°26.847′E	**D**
3529	*kazachstanica*	Uzbekistan: 20 km E Jangigazgan village; 195 m elevation	40°43.344′N	64°52.290′E	**D**
3531	*kazachstanica*	Uzbekistan: Karakata depression: vicinity of Aznek well; 193 m elevation	40°43.384′N	64°52.219′E	**D**
3534	*kazachstanica*	Uzbekistan: 2 km W Kyzylcha village (northern foothills of Nuratau ridge); 410 m elevation	40°44.271′N	66°04.034′E	**D**
3535	*kazachstanica*	Uzbekistan: 10 km N Ajakkuduk village (northern foothills of Sangruntau ridge); 450 m elevation	41°19.170′N	65°05.416′E	**D**
3538	*kazachstanica*	Uzbekistan: 40 km S Uzunkuduk village; 357 m elevation	41°23.694′N	63°10.809′E	**F**
3523	*kazachstanica*	Uzbekistan: Buchara region: Kara-kyr lake; 98 m elevation	41°24.102′N	63°14.425′E	**F**
3524	*kazachstanica*	Uzbekistan: western part of Nuratau ridge: 20 km N Nurata city; 401 m elevation	41°24.102′N	65°47.695′E	**D**
3526	*kazachstanica*	Uzbekistan: 15 km N Tamdy-truba village; 99 m elevation	41°31.885′N	64°01.906′E	**F**
3527	*kazachstanica*	Uzbekistan: Djamankum sands: vicinity of Aktakyr village; 398 m elevation	41°44.204′N	63°19.244′E	**F**
3530	*kazachstanica*	Uzbekistan: Bukantau mountain ridge: Chingildy gorge; 417 m elevation	42°41.514′N	63°25.550′E	**F**
3521	*kazachstanica*	Uzbekistan: Ustyurt plateau; 240 m elevation	43°19.201′N	58°09.710′E	**F**
3522	*kazachstanica*	Uzbekistan: Ustyurt plateau: Karakalkan, 100 km SE Kungrad; 246 m elevation	43°30.017′N	58°10.006′E	**F**
3520	*kazachstanica*	Uzbekistan: Ustyurt plateau: vicinity of Churuk well; 214 m elevation	44°59.853′N	56°54.800′E	**P**
1429	*rustamovi*	Iran: Semnan: Markouh, 60 km S Damghan	35°38′N	54°23′E	**B**
2671	*rustamovi*	Iran: Khorasan: Nishaboor (Neyshabour)	36°03′N	59°07′E	**B**
3027	*rustamovi*	Iran: Khorasan: Anjir Belag, 35 km SE Besangan, S Sharakhs; 834 m elevation	36°05.592′N	60°39.240′E	a
3026	*rustamovi*	Iran: Khorasan: Anjir Belag, 35 km SE Besangan, S Sharakhs; 834 m elevation	36°05.592′N	60°39.240′E	q

Appendix. (Continued).

Sample	Subspecies	Locality	Latitude	Longitude	Haplotype
2274	*rustamovi*	Iran: Khorasan: Besangan lake, 50 km E Masshad	36°15′N	59°00′E	**K**
2275	*rustamovi*	Iran: Khorasan: Besangan lake, 50 km E Masshad	36°15′N	59°00′E	**K**
2276	*rustamovi*	Iran: Khorasan: Besangan lake, 50 km E Masshad	36°15′N	59°00′E	**K**
5241	*rustamovi*	Iran: Khorasan: Besangan lake, 50 km E Masshad	36°15′N	59°00′E	**K**
3025	*rustamovi*	Iran: Khorasan: Ab Belutch, 17 km SE Besangan, S Sharakhs	36°16′N	60°39′E	**P**
3030	*rustamovi*	Iran: Khorasan: Khangiran, Chah Artesian, near Turkmenian border, W Sharakhs; 152 m elevation	36°34.855′N	60°45.630′E	**B**
2670	*rustamovi*	Iran: Golestan: Hezarpich village near Gorgan	36°46′N	54°28′E	**B**
1419	*rustamovi*	Iran: Golestan: vicinity of Gorgan city	36°51′N	54°28′E	**P**
AJ888366	*rustamovi*	Iran: Golestan: vicinity of Gorgan city	36°51′N	54°28′E	r
2279	*rustamovi*	Iran: Golestan: Miankaleh peninsula	36°54.337′N	54°00.413′E	**P**
AJ888367	*rustamovi*	Iran: Golestan: Miankaleh peninsula	36°54.337′N	54°00.413′E	s
1422	*rustamovi*	Iran: Golestan: Miankaleh peninsula	36°54.337′N	54°00.413′E	**T**
1423	*rustamovi*	Iran: Golestan: Miankaleh peninsula	36°54.337′N	54°00.413′E	**T**
2674	*rustamovi*	Iran: Khorasan: Bajgiran	37°36′N	58°24′E	**K**
1424	*rustamovi*	Iran: Golestan: Moraveh Tappeh	37°55′N	55°57′E	**P**
1425	*rustamovi*	Iran: Golestan: Moraveh Tappeh	37°55′N	55°57′E	**P**
DQ080045	*rustamovi*	Turkmenistan: Kopet Dag	37°50′N	58°15′E	c
AY678410	?	unknown	–	–	**AY678410**
AY678434	?	unknown	–	–	**AY678410**
AY678438	?	unknown	–	–	AY678438
DQ445847	?	unknown	–	–	**E**
DQ497322	?	unknown	–	–	**F**
66	?	unknown	–	–	MTD T 66
83	?	unknown	–	–	**F**
84	?	unknown	–	–	**F**
941	?	unknown	–	–	**D**
1296	?	unknown	–	–	MTD T 1296
1695	?	unknown	–	–	**F**
2256	?	unknown	–	–	**F**
3435	?	unknown	–	–	**E**
3436	?	unknown	–	–	**D**
4424	?	unknown	–	–	**F**
4425	?	unknown	–	–	MTD T 4425