

# Phylogeography of co-distributed dune scorpions identifies the Amu Darya River as a long-standing component of Central Asian biogeography

(Scorpiones: Buthidae)

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**Abstract.** Although only distantly related, *Anomalobuthus* and *Liobuthus* are monotypic and sympatric scorpion genera with psammophilic phenotypes well-suited to the dune communities of the Karakum and Kyzylkum deserts of Central Asia. We predicted that this unique combination of phenotypic convergence and sympatry should have resulted in shared phylogeographic histories. We tested this hypothesis by using mitochondrial DNA data and molecular dating techniques to reconstruct the matrilineal genealogies of *A. rickmersi* and *L. kessleri*. We also developed current and late-glacial species distribution models and landscape interpolations of genetic distances to assess the influence of historical barriers and Pleistocene climates on the phylogeography of each species. Both genera exhibited signals of restricted gene flow across the Amu Darya River, supporting our prediction of mutual histories. Levels of initial genetic differentiation within each genus date to the Late Miocene to late Pliocene. Distribution models indicate that suitable habitat may have fragmented during the Pleistocene, generally in an east-west orientation. Although the observed genetic differentiation at the Amu Darya River could be a coincidental product of lineage sorting, the fact that both species display this pattern suggests that the river has been an important biogeographic element in the development of Central Asian biotas.

**Key words.** GIS, Kazakhstan, Turkmenistan, Uzbekistan, MAXENT, COI, 16S, mitochondrial DNA.

## Introduction

The remote and sparsely populated sand deserts of Central Asia contain a rich and surprisingly well-studied biota. Russian exploration of the region began as far back as the early 1800s, with some of the first biological collections conducted by the turn of the 19<sup>th</sup> century. In 1912, and remarkably ahead of its time, a field station was built in Repetek (East Turkmenistan) to study geology and later the biodiversity of the Karakum Desert. Due to the travel restrictions on Soviet scientists for most of the 20<sup>th</sup> century, many often wound up in this desert and the surrounding mountains, as they ranked among the most exotic of the destinations in which they could legally visit (FET 1994a). For these reasons, intensive research of the region made the biota of the Karakum Desert one of the most thoroughly studied desert floras and faunas in the world.

Early geologic research uncovered a complex tectonic history in Central Asia, and the modern relief of the Karakum Desert was found to be quite young (ATAMURADOV 1994). During the Paleocene, the modern Karakum did not exist as it was partially covered by the Tethys Sea. More water flooded the landscape during marine incursions of the Eocene. Oro-

genic events then caused the sea to gradually recede during the Late Miocene, facilitating aridification across the Central Asian lowlands. The Karakum was then faced with another large marine incursion in the early Pliocene, but waters receded again by the end of the Pliocene. Although an arid-adapted biota existed as early as the mid to late Neogene, it was not until the Quaternary that tectonic activity finally slowed. This relative quiescence allowed for the development of the modern aeolian dune systems and establishment of the plant communities found in the Karakum and neighboring Kyzylkum deserts today (ATAMURA-DOV 1994).

Since the floras and faunas of the region are relatively diverse, it seems reasonable to speculate that the abovementioned geologic history, intensified by historical fluctuations in climate and a complex topography, must have facilitated vicariance in many organisms across the region. Scorpions of the area exhibit exceptionally high endemism, especially at the generic level, suggesting that they may have been especially susceptible to such vicariance. Numerous monotypic genera are found scattered among desert habitats from Morocco to China, and with three such genera endemic to their own dune communities (*Anomalobuthus*, *Liobuthus*, *Pectinibuthus*), the Karakum and Kyzylkum deserts are no exception (FET 1994b). All three of the endemic scorpions are psammophilic (sand-adapted). *Pectinibuthus* is known only from one locality (Repetek) in the eastern Karakum, whereas *Anomalobuthus* and *Liobuthus* are more widespread, occurring throughout both the Karakum and the Kyzylkum. Our study addresses the two latter psammophilic taxa, represented each by a single species, *Anomalobuthus rickmersi* Kraepelin, 1900 and *Liobuthus kessleri* Birula, 1898 (family Buthidae).

Interestingly, although *A. rickmersi* and *L. kessleri* appear to be only distantly related (see phylogeny in FET et al. 2003), they are rather similar in sand-adapted morphology and ecology. The limited information that exists on morphology of these two unique genera indicates interesting derived traits. Although very rare in Buthidae, *L. kessleri* exhibits a larger number of accessory trichobothria, a phenomenon termed 'neobothriotaxy' (VACHON 1958). Similarly unique, *A. rickmersi* displays a partial loss of tibial spurs on its legs (VACHON 1974). Although a detailed morphological assessment of both taxa is currently under way (TERUEL & FET, in progress), both genera are known to be of equivalent size and share morphological characteristics; such as setal combs on the tarsi, a condition considered to be an adaptation to life in sand (FET et al. 1998). However, a small but obvious ecological distinction between the two species is that *A. rickmersi* seems to prefer sand dunes without vegetation while *L. kessleri* is more common in vegetated dune communities dominated by White Saxaul (*Haloxylon persicum*). Perhaps it is this small difference in ecological preferences that allows these somewhat convergent species to avoid competitive exclusion and occur in sympatry.

Several other scorpion species in the Karakum and Kyzylkum are also broadly sympatric with these two monotypic taxa. For instance, the scorpiofauna of the region also contains the widespread species *Mesobuthus caucasicus* (Nordmann, 1840), which also exhibits psammophile adaptations (sand combs), and generalists like *Mesobuthus eupeus* (Koch, 1839) and *Orthochirus* spp. (FET 1994b). Although genetic data on *Orthochirus* spp. is scant, phylogenetic studies of *Mesobuthus* (GANTENBEIN et al. 2003) have provided genetic sequences for a handful of samples from the Karakum and Kyzylkum regions (see Discussion).

Many phylogenetic studies have investigated the patterns and processes that generate convergent ecotypes (e.g. LOSOS 1992, 1998, MATOS 2002, JOHANNESSON 2003, REDING 2009), but few have compared the phylogeographies of such ecotypes after they have converged,

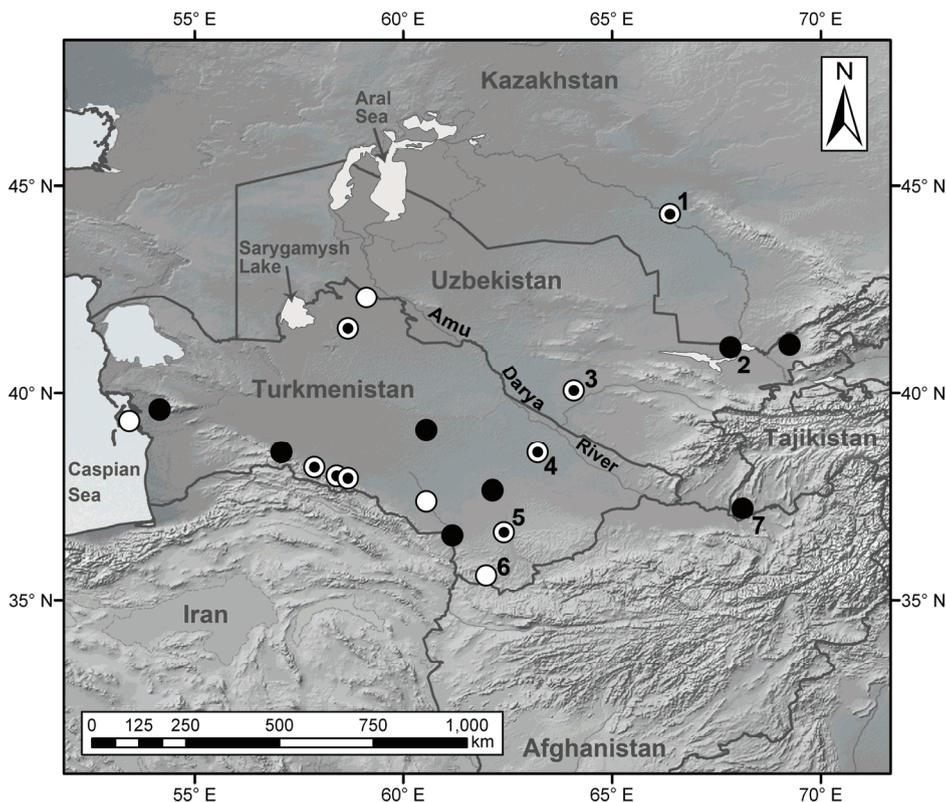


Fig. 1. Distribution map of *Anomalobuthus rickmersi* (white circles) and *Liobuthus kessleri* (black circles) samples used in this study. White circles with black dots signify locations where both species were found in sympatry. Numbers indicate localities of samples used in genetic analyses: 1) Baigakum, Kazakhstan; 2) Chardara, Uzbekistan; 3) Bukhara, Uzbekistan; 4) Repetek, Turkmenistan; 5) Oktyabrsky, Turkmenistan; 6) Chainury, Turkmenistan; 7) southwest Tajikistan. Coordinates of all depicted localities were used for species distribution modelling.

especially of co-distributed species. We examine the phylogeographies of *L. kessleri* and *A. rickmersi* to test our prediction that the unique combination of phenotypic convergence and sympatry should cause organisms to experience analogous biogeographic histories. By sequencing mitochondrial genes commonly used to detect structure at the intraspecific level, we tested this hypothesis by reconstructing the maternal genealogies of sympatric populations of *L. kessleri* and *A. rickmersi* in the eastern portion of the Karakum and Kyzylkum deserts. Due to the stochasticity of the lineage sorting process, our null expectation was idiosyncratic patterns within each independent genealogy. Any topological congruencies between the two genealogies would therefore constitute departures from the null, instead supporting our hypothesis of a common biogeographic history (AVISE & BALL 1990, AVISE 2000).

To evaluate whether the histories are temporally concordant, we used a rate-calibrated molecular dating approach to estimate the time to the most recent common ancestor (TMRCA)

for each species. We then assessed whether genetic patterns could be attributed to fluctuations in climate during the Pleistocene by constructing species distribution models (SDM) and projecting them onto reconstructions of climates during the last glacial maximum (LGM, c. 21 Ka). Finally, we examined whether the phylogeographic patterns could have alternatively been influenced by the Amu Darya River – a long-standing, large river that flows east to west between the two deserts – by visually assessing variation in genetic distance values along a latitudinal transect for *A. rickmersi* and with a landscape interpolation of genetic distances for *L. kessleri*.

## Material and methods

**Taxon Sampling.** *Anomalobuthus rickmersi* – KAZAKHSTAN: Baigakum, 44°20'37" to 44°20'29" N, 66°27'09" to 66°27'07" E, 127-143 m asl, 25.v.2002 (V. Fet), n=3. TURKMENISTAN: Chainury, Badghyz, Serhetabad District, Mary Province, 35°40'52" N, 62°01'34" E, 458 m asl, 6.iv.2002 (V. Fet, A. Gromov), n=2; near Oktyabrsky Well, Serhetabad District, Mary Province, 36°41'58" N, 62°24'04" E, to 36°41'42" N, 62°23'57" E, 382-380 m asl, 13.iv.2002 (V. Fet, A. Gromov), n=1; Repetek, 38°33'54" N, 63°10'51" E, 201 m asl, 15-18.iv.2002 (V. Fet), n=1. UZBEKISTAN: between Bukhara & Gazli, 40°05'03" N, 64°04'29" E, 206 m, 11.v.2002 (V. Fet), n=2. – *Liobuthus kessleri* – KAZAKHSTAN: Chardara, 41°08'19" N, 67°47'58" E to 41°08'17" N, 67°47'57" E, 288-309 m, 24.v.2002 (V. Fet), n=2; Baigakum, 44°20'37" to 44°20'29" N, 66°27'09" to 66°27'07" E, 127-143 m asl, 25.v.2002 (V. Fet), n=2. TURKMENISTAN: near Oktyabrsky Well, Serhetabad District, Mary Province, 36°41'58" N, 62°24'04" E to 36°41'42" N, 62°23'57" E, 382-380 m, 13.iv.2002 (V. Fet, A. Gromov), n=1; Repetek, 38°33'54" N, 63°10'51" E, 201 m, 15-18.iv.2002 (V. Fet), n=1. UZBEKISTAN: between Bukhara & Gazli, 40°05'03" N, 64°04'29" E, 206 m, 11 May 2002 (V. Fet), n=2. TAJIKISTAN: Kurdzhalalkum Sands, 5-6 km SW of Kurdzhalou, 30 km NNW from Aivadgi, Shaartuz District, Khatlong Province (former Kurgan-Tyube Province), 37°08'11" N, 68°09'46" E, 2.v.2002 (A. Feodorov), n=2.

**Laboratory methods.** We extracted total genomic DNA using a DNeasy extraction kit (Qiagen Inc., Valencia, CA). To amplify and sequence a portion of the cytochrome oxidase subunit I (COI) gene, we used the primer Nancy and a modified version of primer C1-J-1517 (5'-AAT CAT AAG GAT ATT GGG AC-3') (SIMON et al. 1994; THOMAS & HEDIN 2008). Scorpion-specific primers from GANTENBEIN et al. (1999) were also used to amplify and sequence a portion of the 16S ribosomal RNA. Amplifications were conducted by polymerase chain reactions using AmpliTaq Gold (Applied Biosystems, Inc., Foster City, CA, USA) and annealing temperatures of 48°-54° C with 34 cycles for COI, and 50° C with 30 cycles for 16S. Amplified products were purified using ExoSap-IT (USB Corp., Cleveland, OH, USA). Fluorescence-based cycle sequencing was performed using a BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Qiagen Inc., Valencia, CA, USA), with electrophoresis on an ABI 3130 automated sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequence data for *Mesobuthus martensii* (Karsch, 1879) and *Buthus occitanus* (Amoreux, 1789) were downloaded from GenBank as outgroups (accession numbers NC\_009738.1 and NC\_010765.1). Sequences were aligned against the outgroups using SEQUENCHER v. 4.9 (Gene Codes Corp., Inc., Ann Arbor, MI, USA) and alignments were verified by eye.

**Phylogenetic analyses and divergence dating.** We assessed phylogenetic patterns using the criterion of Bayesian inference (BI) implemented in MRBAYES v. 3.1.2 (RONQUIST & HUELSENBECK 2003) using concatenated COI and 16S sequences for *A. rickmersi* and *L. kessleri*. For preliminary runs we selected appropriate models for unpartitioned and partitioned data sets

using jModeltest (POSADA & CRANDALL 1998) under the Akaike Information Criterion (POSADA & BUCKLEY 2004). We then determined the best fit partitioning scheme using Bayes factors on the harmonic mean marginal likelihood values (NYLANDER et al., 2004). Final analyses were run on data partitioned by gene and codon for 50 million generations using the appropriate substitution models. Trees were sampled every 5000 generations with the first 10 million generations of sampled trees discarded as burn-in after confirming chain stationarity using TRACER v. 1.5 (RAMBAUT & DRUMMOND 2007).

We estimated TMRCAs for the *A. rickmersi* and *L. kessleri* samples in BEAST v. 1.5.3 (DRUMMOND & RAMBAUT 2007) with the alignments partitioned by gene. We applied the Yule tree prior and mutation rates of 0.005 substitutions/site/million years for 16S (GANTENBEIN & LARGIADÈR 2002) and 0.007 substitutions/site/million years for COI (GANTENBEIN et al. 2005). Analyses were conducted as two independent runs of 20 million Markov Chain Monte Carlo generations each, sampling every 1000 generations, and with the first 10% of the generations discarded as burn-in. We used LOGCOMBINER v.1.6.1 (DRUMMOND & RAMBAUT 2007) to combine trees and parameter estimates, and TRACER was used to examine the estimated sample sizes (ESS) to avoid poor estimates of the parameters (ESS < 200).

**Landscape genetics.** We calculated pairwise genetic distances (uncorrected *p*-distance and Kimura 2-parameter distance) separately among locations of *A. rickmersi* and *L. kessleri* using MEGA v.5 (TAMURA et al. 2011). To better visualize patterns of genetic variation across the landscape, we generated a Delaunay triangulation-based connectivity network and calculated residual genetic distances at midpoints for *L. kessleri* using the program ALLELES IN SPACE (MILLER 2005). We then used ARCGIS v. 9.3 (ESRI, Redlands, CA, USA) to interpolate genetic distances across the landscape using the inverse distance weighted interpolation procedure (WATSON & PHILIP 1985; WATSON 1992) with default parameters.

The sampling scheme of *A. rickmersi* was not well-enough dispersed to construct a landscape interpolation, but formed a nearly linear arrangement of sample sites running southwest to northeast, somewhat comprising a latitudinal transect. Therefore, we used this transect to assess the change in genetic distances within *A. rickmersi* in relation to the Amu Darya River. Genetic distances were plotted against the latitude of the Euclidean midpoint between each pair of geographically adjacent samples. This was then compared to the latitude of the Amu Darya River where it crossed the transect.

**Species distribution modelling.** The program MAXENT 3.3.2 (PHILLIPS et al. 2006) was used to develop current (0 Ka) and LGM (c. 21 Ka) reconstructions of the climatic niche of *A. rickmersi* and *L. kessleri*. MAXENT was chosen because it has been demonstrated to consistently outperform other modelling approaches (ELITH et al. 2006). The program uses presence-only occurrence records, random background points, and environmental variables (GIS layers) as input data to approximate an unknown distribution that maximizes entropy, subject to the constraints imposed by the known occurrences. In other words, the values of the environmental variables act as constraints on the unknown distribution, forcing the mean and variance of the output SDMs to approximate that of the occurrence data.

Our occurrence data comprised of 12 georeferenced *A. rickmersi* and 18 *L. kessleri* localities. Although these sample sizes are low, the localities are well dispersed, capturing a large extent of the climatic variation in the known distributions of each species. Furthermore, MAXENT performs well with small sample sizes, even as low as five (PHILLIPS et al. 2006; HERNANDEZ et al. 2006). Historic records were georeferenced using Google Earth (<http://earth.google.com>) to estimate coordinates from detailed collecting records. Coordinate estimations with uncertainty greater than 5 km were excluded. We then used 19 bioclimatic layers representing climatic trends, seasonality, and extremes of temperature and precipitation, and clipped them to an extent covering the Karakum and Kyzylkum deserts and a small portion of the surrounding regions.

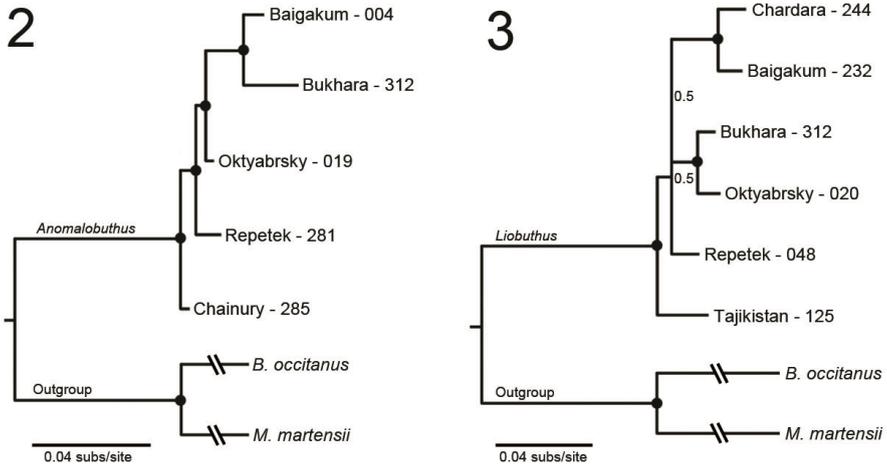
Table 1. Pairwise genetic distance matrices by uncorrected  $p$ -distance (below the diagonals) and Kimura 2-parameter distance (above the diagonals) for *Anomalobuthus* and *Liobuthus*.

<i>Anomalobuthus</i>							
	Sample Site	1	2	3	4	5	
1	Baigakum	-	0.036	0.032	0.025	0.040	
2	Bukhara	0.035	-	0.051	0.051	0.066	
3	Chainury	0.031	0.049	-	0.007	0.014	
4	Oktyabrsky	0.024	0.049	0.007	-	0.014	
5	Repetek	0.038	0.063	0.014	0.014	-	
<i>Liobuthus</i>							
	Sample Site	1	2	3	4	5	6
1	Tajikistan	-	0.045	0.043	0.048	0.045	0.040
2	Baigakum	0.044	-	0.036	0.024	0.042	0.032
3	Bukhara	0.042	0.035	-	0.033	0.019	0.027
4	Chardara	0.046	0.023	0.032	-	0.036	0.036
5	Oktyabrsky	0.044	0.040	0.019	0.035	-	0.036
6	Repetek	0.038	0.031	0.027	0.035	0.035	-

We ran MAXENT using logistic output, default settings (regularization multiplier = 1; convergence threshold = 0.00001; maximum iterations = 500; duplicate records removed), and random seeding. To test the robustness of MAXENT models we used cross-validation, dividing presence points into five groups and ran five iterations, using a different group each for each run. Thus, 20% of presence points were used as test points and 80% were used for training (NOGUÉS-BRAVO 2009). The SDMs were then projected onto simulated past climate data representing the LGM derived using the Community Climate System Model (CCSM; OTTO-BLIESNER et al. 2006) and Model for Interdisciplinary Research on Climate (MIROC; HASUMI & EMORI 2004). Habitat suitability was displayed in ARCGIS using two thresholds: the lowest training presence threshold (LPT) and a threshold that rejects the lowest 10% of predicted values (PEARSON et al. 2002). The former threshold is considered conservative as it only predicts grid cells at least as suitable as those where presence record have been recorded (PEARSON et al. 2002).

## Results

**Phylogenetic analyses and divergence dating.** The sequencing procedure resulted in 1045 bp (653 bp COI, 392 bp 16S) sequences for *A. rickmersi*, and 1031 bp (722 bp COI, 309 bp 16S) sequences for *L. kessleri*. All clades of *A. rickmersi* were strongly supported by high Bayesian posterior probabilities (=1.0; Fig. 2) in the phylogenetic analyses. A genetic break is evident between the northern (Baigakum and Bukhara) and southern localities (Oktyabrsky, Repetek, and Chainury) separated by the Amu Darya River (Figs 1-2). A sample from Chainury, which is the most southern sampled location, has a basal position on the tree. The three southern sites seem to have evolved earlier than the two sites north of the river, which form a clade.



Figs 2-3. Bayesian phylogenetic trees of 2) *Anomalobuthus rickmersi* and 3) *Liobuthus kessleri* constructed using concatenated COI and 16S mitochondrial sequence data. Black dots represent nodes supported with 100% Bayesian posterior probability. Hatch marks indicate outgroup branches that have been truncated.

The matrilineal phylogeny for *L. kessleri* (Fig. 3) placed the rare sample from southwestern Tajikistan as basal. The remaining samples formed a poorly resolved polytomy, with the two northernmost samples (Chardara and Baigakum; Fig. 1, 2) again forming a clade supported by high posterior probabilities. A relationship between two of the more centrally positioned samples (Bukhara and Oktyabrsky) is also well-supported.

The estimated TMRCA for the *L. kessleri* sequences was between 4.6 Ma and 9.4 Ma, with a mean estimate of 6.8 Ma. A slightly younger age was estimated for *A. rickmersi*, ranging from 2.9 Ma to 7 Ma with a mean of 5 Ma.

**Landscape genetics.** Uncorrected genetic *p*-distances ranged from 0.007 to 0.063 for *A. rickmersi* and from 0.019 to 0.046 in *L. kessleri* (Table 1). Interpolations of genetic distances of *L. kessleri* revealed a conspicuous pattern of elevated genetic distances between the southeastern sites and northeastern sites, forming an elongated area of high genetic distance paralleling the border of Turkmenistan and Uzbekistan, roughly following the drainage of the Amu Darya River (Fig.4). In all other areas, such as between Turkmenistan sites and the three northeastern sites, the interpolations display low genetic distances. Between adjacent samples sites for *A. rickmersi*, genetic distances are low (<0.035) except for a nearly two-fold increase (0.063) where the transect crosses the Amu Darya River (Fig. 5).

**Species distribution modelling.** Climate-based SDMs performed well, as operating characteristic curve values were high (>0.95). Models of current climate suggested that suitable habitat for *A. rickmersi* (Fig. 6a) and *L. kessleri* (Fig. 6b) existed throughout the Karakum and Kyzylkum deserts from the Caspian Sea through Turkmenistan and eastern Uzbekistan, and north just into Kazakhstan. Although these regions are isolated by major mountain barriers, suitable climate for these species was also predicted in disjunct patches in Iran and southern Afghanistan, highlighting areas that could be inhabited by related or ecologically equivalent species.

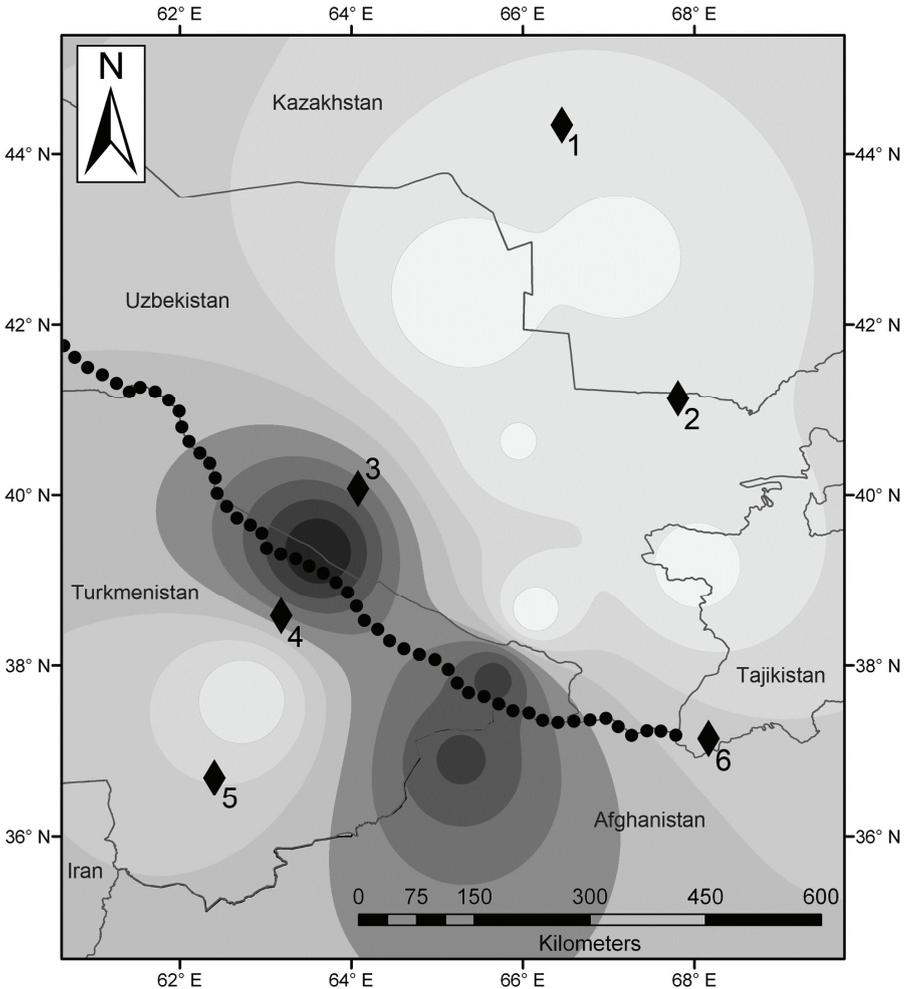


Fig. 4. Interpolated pairwise genetic distances within *Liobuthus kessleri*. Diamonds indicate the sampling locations. Shading represents genetic distances ranging from small (light) to large (dark). The Amu Darya River is demarcated by the dotted line.

Under the assumption of niche conservatism, the tendency for the niche of a species to change little over time (Peaerman et al. 2008), which may or may not be true for psammophilic scorpions, we can use LGM projections of the SMDs to make inferences about the historical distributions of *A. rickmersi* (Fig. 6c-e) and *L. kessleri* (Fig. 6d-f). Primarily, the LGM models suggested that the range of both species was restricted to southern refugia during the LGM. Three of the four LGM models (Fig. 6c, d, f) implied that these refugia were fragmented and arranged latitudinally along the southern margin of the Karakum Desert.

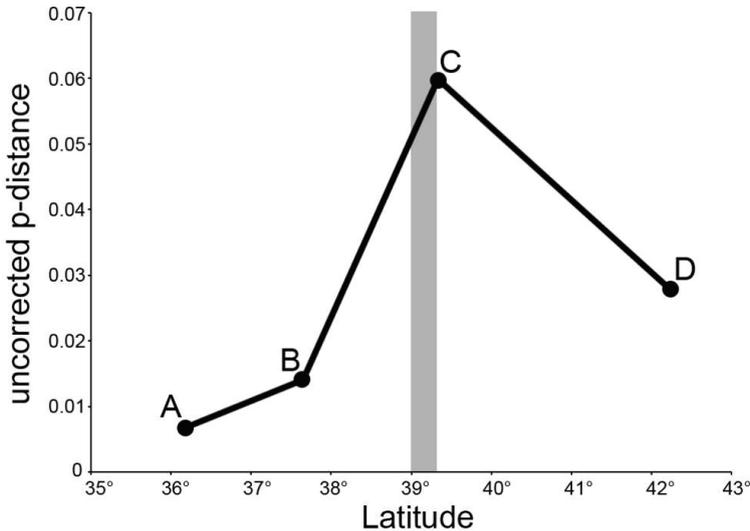


Fig. 5. Graph showing change in genetic distance (Y axis) between five sites where *Anomalobuthus rickmersi* were collected, depicted as a linear latitudinal transect (X axis). The gray bar represents the area where the transect crosses the Amu Darya River drainage. Dots represent latitudinal midpoints between collecting sites as follows: (A) between Chainury and Oktyabrsky, (B) between Oktyabrsky and Repetek, (C) between Repetek and Bukhara, and (D) between Bukhara and Baigakum.

## Discussion

Our results provide one of only a handful of molecular studies of Central Asian taxa, and the first molecular investigation specifically addressing the relationships between scorpions from the Karakum and Kyzylkum deserts. LGM reconstructions of the distributions of *A. rickmersi* and *L. kessleri* suggested that populations within both species may have been fragmented during the Pleistocene, especially in an east/west orientation. However, molecular dating techniques placed the TMRCA for each species in a much older timeframe, between the Late Miocene and late Pliocene. Pleistocene fragmentation can therefore be rejected as the initial cause of divergence between populations in *A. rickmersi* and *L. kessleri*, although it may have still been responsible for some of the more shallow patterns recovered in the matrilineal genealogies of each species. Intriguingly, landscape assessments reveal a prominent signal of genetic discontinuity across the Amu Darya River in both species, supporting our hypothesis that co-distributed organisms with similar phenotypes and ecologies are likely to have also experienced mutual biogeographic histories. While we caution that the observed patterns could just be a coincidental byproduct of the stochastic nature of the lineage sorting process, the geographic congruence between the river and the locations of increased genetic differentiation in both *A. rickmersi* and *L. kessleri* makes it more parsimonious to surmise causation by the Amu Darya River.

The Amu Darya has long been an element in Central Asian deserts, thus it is not surprising that the river appears to have also been a barrier to scorpions. However, based on its history, we did not expect genetic divergences across the river to be so deep. The Amu Darya is

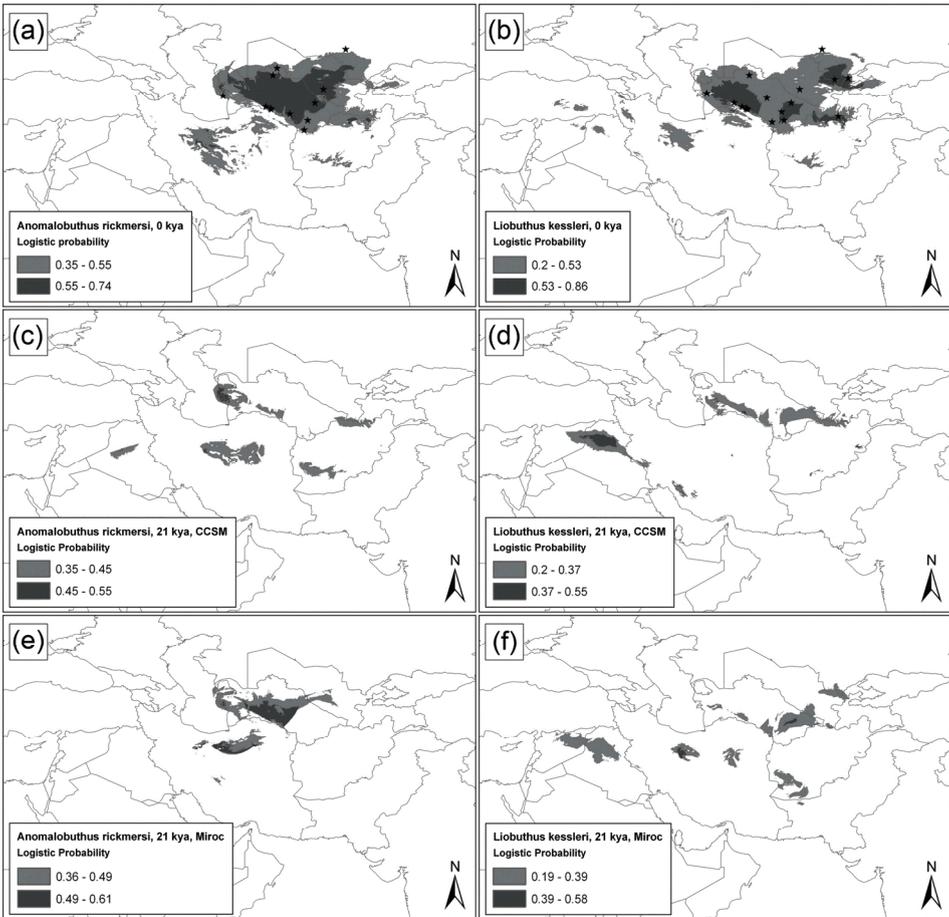


Fig. 6. Species distribution models for *Anomalobuthus rickmersi* and *Liobuthus kessleri* under current and Last Glacial Maximum (LGM, c. 21 ka) climate scenarios: Community Climate System Model (CCSM) and Model for Interdisciplinary Research on Climate (MIROC). (a) *A. rickmersi*, current; (b) *L. kessleri*, current; (c) *A. rickmersi*, LGM using CCSM; (d) *L. kessleri*, LGM using CCSM; (e) *A. rickmersi*, LGM using MIROC; (f) *L. kessleri*, LGM using MIROC. All models are presented on maps of the current configuration of land-masses and sea levels.

thought to have formed contemporaneously with the deserts, both the result of orogenesis associated with the Indian plate moving northward under the Asian plate. By the mid Pliocene, the Paleo-Amu Darya and its tributaries had formed a deep drainage to the Caspian Sea (ATAMURADOV 1994). During the late Pliocene, however, the river is thought to have been much smaller, with flow rates 3.5 to 4 times less than now (TROFIMOV 2005). Consequently we did not expect the Amu Darya to have been a major barrier to scorpions until the cooler and wetter climates of the Pleistocene. Instead, the Amu Darya appears to have acted as a biogeographic barrier even prior to the Pleistocene. During the Pleistocene, the river continued to act as a barrier as it wandered across the Karakum forming the vast alluvial plain still

present today, but veered north to the Aral Depression by the mid Quaternary, no longer flowing to the Caspian Sea (ATAMURADOV 1994). Alluvial deposits of the Karakum were then further transformed by wind action, creating the modern sand desert which could have the stage for evolution of *L. kessleri* and *A. rickmersi* into psammophilic phenotypes. The Amu Darya continued to change course, wandering across the alluvial plain and temporarily spilling into the Uzboy Corridor and filling the Sarykamysh Depression, eventually (late Khvalynian, ca. 10-13 Ka) draining entirely to the Aral Sea. While the position of the river has been unstable, it has always flowed to the west or northwest from the mountains of Tajikistan and Afghanistan, presumably isolating scorpion populations north and south of the river, even when flow rates were low. But to what extent has the Amu Darya River impacted co-occurring taxa?

In his classical treatise on zoogeography of Central Asia, KRYZHANOVSKY (1965) listed a number of endemic Karakum psammophiles; such as beetles *Remipedella* (an endemic Karakum genus), *Discoptera eylandti*, *Earophanta loudoni*, *E. serrata*, *Habrobates vernalis* (Tenebrionidae), several species of *Philothis* (Histeridae), and *Diesia sefirana* (Carabidae). Disjunct sister species have been documented from both the eastern and western Karakum (e.g. tenebrionids *Argyrophana caspia* and *A. deserti*, weevils *Mesostylus uzboicus* and *M. hauseri*), potentially a result of changes in the drainage patterns of the Amu Darya River. In addition, the Amu Darya appears to have been a barrier leading to isolation and subsequent evolution of two disjunct subspecies, *Discoptera komarovi komarovi* (Eastern Karakum) and *D. k. transoxiana* (Southern Kizylkum) (KRYZHANOVSKY 1965, KRYZHANOVSKY & ATAMURADOV 1994).

Of the small number of phylogenetic studies of Central Asian taxa, a few also recover divergences in the deserts flanking the Amu Darya. In an analysis of the Sunwatcher Toad-Headed agama species complex (*Phrynocephalus* spp.), samples from the plains of the Karakum and Kyzylkum revealed four highly divergent lineages (5% to 11.1% for COI, uncorrected *p*), leading the authors to speculate that ancient river drainages might have been a greater impact on the biogeographic history of Central Asian organisms than the more commonly invoked effects of Pleistocene climate cycles (SOLOVYEVA et al. 2011).

In the Rapid Fringe-toed Lizard (*Eremias velox*), two samples collected from the Karakum Desert in Turkmenistan and one sample from Golestan in Northern Iran were genetically similar (GUO et al. 2011). Molecular clock estimates suggest that these three samples diverged from samples from northwestern China sometime between the Late Miocene (5.9 Ma) and late Pleistocene (0.5 Ma), a timeframe that encompasses the uplift of the northwestern Tibetan Plateau (ZHENG et al. 2000). Similar patterns were found within Asian Rock Agamas, the *Laudakia caucasia* species group, where a north-south split was attributed to uplifting of the Zagros Mountains in the Late Miocene or early Pliocene (MACEY et al. 1998). What neither group of authors proposed, however, is the additional possibility that north-south divergences could also be attributed to the Amu Darya River, which nicely bisects the genetically differentiated samples in both studies.

In a study on Central Asian Tortoises (*Agrionemys horsfieldii*), identical haplotypes were found throughout all of Uzbekistan and Kazakhstan except for a basal haplotype in the Babatag Mountains of southeastern Uzbekistan (VASILYEV et al. 2008). Unfortunately, no samples were collected from south of the Amu Darya River in Turkmenistan, but several samples were collected from nearby Iran. Without samples from intervening Turkmenistan it is hard to say if the river had any impact on the mitochondrial DNA structure in *A. horsfieldii*, but the level of divergence between Iranian samples and samples north of the Amu Darya is consistent with a hypothesis of divergence at the river.

Therefore, while phylogenetic information spanning the Amu Darya River is surprisingly scant, there is at least some evidence that the river may have been a biogeographic barrier to other organisms as well. On the other hand, with the exception of SOLOVYEVA et al. (2011), we are not aware of any recent biogeographic literature that proposes the possible influence of the Amu Darya River on the structuring of biodiversity.

Additional insight can be gained by comparing our data to a parallel study of another sympatric buthid scorpion genus, *Mesobuthus* (GANTENBEIN et al. 2003). Genetic material from *Mesobuthus* was collected during the same expedition (in 2002) that produced our samples of *A. rickmersi* and *L. kessleri*, hence some of the collection localities are identical. The genus spans the same geographic range as *A. rickmersi* and *L. kessleri* within the Karakum and Kyzylkum deserts, but is much more widespread, ranging from the Balkans to eastern China. The large and numerous species in the genus are the predominant scorpions in the deserts of Central Asia, and their morphological polymorphism led to the creation of numerous subspecies, though of unclear validity (FET 1994b).

A molecular phylogeny using mitochondrial and nuclear sequence data (GANTENBEIN et al. 2003) placed a sand desert population of *Mesobuthus eupeus* from Turkmenistan (*MeuTUa1*, Repetek) in a clade separate to that of a northern clade in Uzbekistan and Kazakhstan (north of the Amu Darya). The northern clade contained a sample from Baigakum (*MeuKZb1*), forming a subclade that ranged throughout Kazakhstan and Mongolia. The genetic distance (COI, uncorrected *p*) between the two *M. eupeus* samples from north and south of the Amu Darya is 9.8%. Based on a mutation rate of 1.4% per million years (GANTENBEIN et al. 2005), the divergence between these samples would have occurred during the Late Miocene (7 Ma), a date that falls within the timeframe estimated for initial divergences within *A. rickmersi* and *L. kessleri*. Furthermore, two more COI samples of *M. eupeus* from south of the Amu Darya at Chainury (*MeTUm1-2*, GANTENBEIN et al. 2005) are 8.8% to 9.0% divergent from the Baigakum sample, also indicative of Late Miocene divergence (6.28 to 6.43 Ma). These levels of genetic divergence are consistent with the hypothesis that *M. eupeus* is most likely a complex of species (see FET 2010; MIRSHAMSI et al. 2010, 2011; KOVAŘÍK et al. 2011). In various parts of its wide range, dune habitats are populated by distantly related clades of *M. eupeus*. As in *A. rickmersi* and *L. kessleri*, *M. eupeus* seems to be susceptible to biogeographic events that promote divergence and appears to have been influenced by the Amu Darya River as early as the Late Miocene.

On the contrary, a different pattern is seen in the related and sympatric species *M. caucasicus*. Samples from south of the Amu Darya at Repetek (*McTUo1*) and north of the river at Baigakum (*McKZb1*) were 0.7% different at COI (GANTENBEIN et al. 2005), corresponding to the late Pleistocene (0.5 Ma). While this date is in agreement with our hypothesis that the Amu Darya was a stronger barrier to gene flow during the cooler and wetter Pleistocene, this low level of genetic divergence could also simply be attributed to isolation by distance, especially since the distance between the samples is nearly 700 km. Either way, it is clear that the Amu Darya has not been a strong biogeographic barrier for *M. caucasicus* as it seems to have been for *M. eupeus*, *A. rickmersi* and *L. kessleri*.

The levels of divergence within *Anomalobuthus*, *Liobuthus*, and *Mesobuthus* are somewhat deep for intraspecific studies, but consistent with phylogeographic studies of other scorpions (BORGES et al. 2010, GANTENBEIN et al. 2001, 2003, GANTENBEIN & LARGIADÈR 2003, PARMAKELIS et al. 2006a-b, SOUSA et al. 2010). Our study thereby further substantiates the idea that scorpions are ideal for tracking relationships between earth history and biotic diversification across deeper timescales than possible with most other organisms (BRYSON et al. in review). In this case, by studying the matrilineal relationships between

populations of psammophilic scorpions, we have identified a potentially vital but underappreciated biogeographic element of Central Asia, the Amu Darya River. Future studies of Central Asian deserts should explicitly test for divergence across this river to assess the degree to which this ancient river system has similarly structured genetic diversity among other arid-adapted organisms. Furthermore, added sampling along longitudinal transects in addition to sampling north and south of the Amu Darya could also address the possibility of east-west fragmentation of suitable habitats during the Pleistocene, as indicated by our SDMs (Fig. 6). Finally, our study provides evidence that when organisms undergo ecological and phenotypic convergence, then over time their biogeographic histories may begin to converge as well.

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