Genetic analysis and ecological niche modeling delimit species boundary of the Przewalski's scorpion (Scorpiones: Buthidae) in arid Asian inland

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Abstract. Although venoms of scorpions have been subjected to extensive characterization, species status of venomous scorpions still remains to be critically and robustly assessed. Reliable delimitation of venomous scorpions is not only consequential to toxicological studies but also instructive to conservation and exploration of these important medical resources. In the present study, we delimited species boundary for the the Przewalski's scorpion from arid northwest China through a combined approach employing phylogenetic analysis, ecological niche modeling and morphological comparison. Our results indicate that the the Przewalski's scorpion represent an independent taxonomic unit and should be recognized as full species rank, *Mesobuthus przewalsii* **stat. n.**. This species and the Chinese scorpion *M. martensii* represent the eastern members of the *M. caucasicus* species complex which manifests a trans-Central Asia distribution across the Tianshan Mountains range. We also discussed the likely geographic barrier and climatic boundary that demarcate distributional range of the the Przewalski's scorpion.

Keywords: *Mesobuthus*, species complex, mitochondrial DNA, ecological niche modeling, distribution range,

Reliable delimitation of species is crucial to researches in ecology, biodiversity, evolution and conservation biology as species are fundamental units of these disciplines. While different ideas are prominent in various naturalists' minds when speaking of species, a common element is that species are 'separately evolving metapopulation lineages' (De Queiroz 2007). Following such a unified species concept, species can be delimited in an integrative way based on operational criteria that reflect contingent, but not necessary, properties of the common element, such as genetic divergence and ecological distinctiveness besides morphological difference that is traditionally used to recognize species (Leaché et al. 2009; Padial et al. 2010).

Genetic and ecological properties appear particular relevant to species delimitation for groups that suffer taxonomic confusion in the traditional morphological framework. It has widely acknowledged that genetic approaches have revolutionized our ability for species delimitation (Tautz et al. 2003; Godfray et al. 2004; Savolainen et al. 2005; Vogler & Monaghan 2007). DNA sequences, providing highly variable and stable characters, have become the indispensable data for resolving any kinds of taxonomic problems. DNA-based approaches play increasing role in the recognition of diversity of invertebrates, especially in groups that are hard to be recognized using classical morphological means. DNA data not only clarify taxonomic confusions but also greatly facilitate biodiversity inventory. Ecological approaches, particularly the ecological niche modeling

(ENM), associate environmental variables with known species' occurrence localities to define abiotic conditions within which populations can survive (Guisan & Thuiller 2005). ENM makes it possible to map the environmentally suitable areas for a species based on the abiotic conditions, even when species distribution is known from very limited occurrence points (Pearson et al. 2007). ENM has been already integrated into a broad variety of research disciplines which contribute to in depth taxonomic insights. In particular, ENM can provide compelling evidence for geographic isolation between allopatric populations, which has important practical implications for species delimitation (Wiens & Graham 2005). Clearly, combining genetic analysis of DNA sequences and ENM together with the traditional morphological evidences would warrant robust delimitation of species boundaries. However, such an integrated approach remains to be fully employed in the taxonomic studies of arachnids, particularly scorpions which are known for their morphological conservatism and taxonomically difficult.

Although venoms of scorpions have been subjected to extensive characterization, species status of venomous scorpions remain to be critically and robustly recognized. Scorpions of the *Mesobuthus* Vachon 1950 (Scorpiones: Buthidae) are poisonous to human beings and several species have subjected to intensive toxicological studies (Goudet et al. 2002; Cao et al. 2006; Zhu et al. 2012; Diego-García et al. 2013). Species of this genus occur widely in the temperate Palaearctic region from Balkan in the west to coastal China in the east (Shi & Zhang 2005; Shi et al. 2013). Despite their medical importance and pharmaceutical significance, however, taxonomy of this group of scorpions still lags behind. Since establishment of the genus in nearly 70 years ago (Vachon 1950), no new species has been discovered before 2000 (Shi 2007). In addition, a plethora intraspecific units, such as subspecies, forms and types, have been recognized within widely ranging species (Fet et al. 2000). Taxonomic status of these intraspecific forms is questionable. Recent treatments, particularly analyses of DNA sequences, of widespread polytypic species described earlier have led to recognition more than ten new species (Gantenbein et al. 2000; Fet et al. 2018; Mirshamsi et al. 2010, 2011). It has been also demonstrated that climatic niches are an important determinant of geographic distributions of *Mesobuthus* scorpions (Shi et al. 2007, 2015), and species differentiation is associated with significant divergence in their ecological niches (Mirshamsi 2013). These results suggest that ENM can provide additional independent support for the taxonomic validity of species recognized based on morphological features, genetic differences and other operational criteria. However, taxonomic status of the taxon in the genus *Mesobuthus* still remains to be evaluated using a combining approach based on both DNA-based analysis and ecological niche modeling.

The aim of the present study is thus to liquidate the taxonomic status of *Mesobuthus* species from China. We focused specially on the Przewalski's scorpion, *M. caucsicus przewalsii*, of which taxonomic status is unclear and has never been subjected to genetic and ecological assessment. Up to date, six *Mesobuthus* species have been reported from China, viz. *M. bolensis*, *M. caucasicus*, *M. eupeus*, *M. karshius*, *M. longichelus* and *M. martensii*. Three of them (*M. caucasicus*, *M. eupeus*, and *M. martensii*) are geographically widespread, each having two subspecies been recorded in China. Other three species (*M. bolensis*, *M. karshius* and *M. longichelus*) was descripted recently and narrowly distributed (Sun & Sun 2011; Sun & Zhu 2010; Sun et al. 2010). The species *M. caucasicus* is one of the most morphologically diverse and geographically wide-spread species in the genus. It ranges from Caucasian Mountains in the west to the north west China in the east. Historically, six subspecies have been recognized (Fet et al. 2000). Recently revision based on morphology and mitochondrial DNA sequences revealed that this taxon represents a species complex; four subspecies have been elevated to species rank and six additional new species was described from Central Asia (Fet et al. 2018). However, the only subspecies which occurs to the east of the Tianshan Mountains and the Pamir Plateau (Birula 1897), the Przewalski's scorpion *M. caucsicus przewalsii* was not examined. Here we present our results from genetic analysis, ecological niche modeling and morphology that liquidate the taxonomic status of the Przewalski's scorpion and its relationship with to other member of the *M. caucasicus* complex and other Chinese species, and geographical distribution boundaries.

METHODS

Scorpion sampling and morphological examination.---Scorpions were collected through habitat searching with UV-light. The sampling localities were geo-positioned using a GPS receiver (Garmin International). Morphological observation was performed under a Nikon SMZ1500 stereomicroscope. Samples are preserved in 99.7% ethanol and deposited at Institute of Zoology, Chinese Academy of Sciences, Beijing.

Molecular phylogenetic analysis.---One specimen from each of collection site was used for sequencing the mitochondrial cytochrome c oxidase subunit I (mtCOI). Genomic DNA was extracted from preserved tissues using a modified phenol-chloroform extraction procedure (Zhang & Hewitt 1998). The primers, PCR profiles and sequencing protocols followed Shi et al. (2013). The unique haplotypes have been deposited in GenBank under accession numbers xxxxxxxx-xxxxxxxx. Sequences for other species of the *M. caucasicus* complex recognized by Fet et al. (2018) were downloaded from GenBank. We also included six sequences which represented the major mitochondrial lineages of the Chinese scorpion *M. martensii* (Shi et al. 2013), and seven sequences of the mottled scorpion *M. eupeus monglicus* from the north side of the Tianshan Mountains (Table 1). Sequences were aligned together with the sequences generated in the present study using CLUSTAL X 1.83 (Thompson et al. 1997) and further inspected by eye. Phylogenetic analyses were performed using both maximum likelihood (ML) and Bayesian methods. ML analysis was carried out using IQ-TREE v1.6.10 (Nguyen et al. 2015) with DNA substitution model selected by ModelFinder (Kalyaanamoorthy et al. 2017). Branch supports were obtained with the ultrafast bootstrap with 1000 replicates (Hoang et al. 2018). Bayesian analysis was carried out with MrBayes 3.2 (Ronquist et al. 2012). Analyses were initiated with random starting trees and run for 2×10^6 generations with four Markov chains employed. Trees were sampled every 200 generations and the 'temperature' parameter was set to 0.2. The first 25% trees were discarded as burn-in after checking for stationary and the convergence of the chains. Genetic distances between morphologically and/or phylogenetically recognized species were calculated using Kimura 2-parameter model (K2P distance) with MEGA 5.05 (Tamura et al. 2011).

Ecological niche modeling.---We predicted potentially suitable distribution area through ecological niche modeling (ENM). ENM was performed using MaxEnt version 3.4.1 (Phillips & Dudik 2008) based on scorpion occurrence points and bioclimatic variables. For he Przewalski's scorpion, a total of 11 GPS points were recorded during field survey for this study and additional 19 occurrence records were georeferenced from literatures. The bioclimatic variables were download from the WorldClilm database (http://www.worldclim.org/). These climatic variables represent a set of measurements that summarize temperature and precipitation at a 2.5 arc-minute resolution (c. 5×5 km). We masked the climatic variable to known range of the *M. caucasicus* complex, spanning from 30 to 50 °N and from 40 to 105 °E, to avoid sampling unrealistic background data and thus inflating the strength of predictions. We removed highly correlated (Pearson's correlation, $|r| \geqslant 0.80$) climatic variables before performing ENM. MaxEnt was run with a convergence threshold of 10^{-5} and maximum number of iterations of 10,000 with cross validation. Model performance was assessed via the area under the ROC (receiver operating characteristic) curve (AUC) statistic and the importance of variables was assessed by jackknife tests. We employed the maximum training sensitivity plus specificity threshold for converting continuous models to binary predictions. We did not confine the background sampling of ENM within the minimum convex polygon of the occurrence points so that the suitable distributional region was slightly over-predicted. Ecological niche models for the geographically neighboring species, the Chinese scorpion *M. martensii* (Shi et al. 2007) were projected together with the Przewalski's scorpion to check range overlap visually. To test whether ecological niche of the Przewalski's scorpion overlaps with other species of the *M. caucasicus* complex, we also performed independent ENM for these species collectively since the phylogenetically validated occurrence data for each individual species was very few. A total 74 occurrence points for nine species (Fet et al. 2018), all of which occur to the west of the Tianshan Mountains, were used to construct a single distribution model. Such a lumping prediction will cause over-prediction for individual species due to sampling background points in a wider and unrealistic region for the relevant species. Given the over-prediction of both models, we considered they gave over-estimate of distributional overlap, in another words, a conservative prediction of distribution separation.

RESULTS

Genetic divergence and phylogenetic relationship.---A best-fit model of TIM3+F+R3 was selected by all three information criteria implemented in ModelFinder. Based on this DNA evolution model, IQ-TREE inferred a maximum likelihood tree with optimal log-likelihood of -3984.05 from mtCOI DNA sequences (Fig. 1). In the maximum likelihood tree, most species of the *M. caucasicus* complex formed strongly supported (bootstrap support: 100) monophyletic clade except for *M. elenae* which appeared paraphyletic with respect to *M. parthorum* (Fig. 1). All samples for the Przewalski's scorpion clustered in a fully supported monophyletic clade, which was clearly separated from other members of the complex. The Przewalski's scorpion was most closely related to a lineage composed of *M. nenilini and M. kaznakovi*. Although the inter-relationships among the member of the *M. caucasicus* complex were largely unresolved, all species of the complex formed a moderately supported clade (bootstrap support: 85). The Chinese scorpion *M. martensii* was nested within this clade while the mottled scorpion *M. eupeus* coalesced with the common ancestor of the *M. caucasicus* complex (Fig. 1). Bayesian MCMC sampling was converged after 2×10^{6} generations as indicated by the value of the potential scale reduction factor (PSRF) approaching 1.00 and the average standard deviation of split frequencies less than 0.0075. All clades corresponding to species recognized in the maximum likelihood tree were resolved in the Bayesian majority role consensus tree (Fig. S1). However, the inter relationships among species of the *M. caucasicus* complex were unresolved, forming a multifurcating clade harboring subclades for *M. przewalskii* and *M. martensii*.

The interrelationships among species of the *M. caucasicus* complex is shown by phylogenetic network to illustrate the phylogenetic uncertainty (Fig. 2). The result was completely in congruence with the maximum likelihood tree, for which poorly supported internodes were indicated by reticulations. Although the interrelationships between species is poorly resolved, no reticulations have occurred in the most recent common ancestors for each species of the complex. The

Przewalski's scorpion is clearly diverged from other member of the species complex and warrants a species rank. The divergence of the Chinese scorpion *M. martensii* is comparable to the divergences among the members of the species complex. Estimates of net evolutionary divergences in the mtCOI sequences among species of the *M. caucasicus* complex are shown in Table 2. The interspecies genetic distances (based on K2P model) among member of the *M. caucasicus* complex ranges from 4.25% (*M. elenae* vs. *M. parthorum*) to 11.18% (*M. intermedius* vs. *M. fuscus*). The genetic distances between the Przewalski's scorpion other species of the complex ranges 7.41% to 10.33%. Genetic distances of 8.78% to 13.61% were observed between the *M. caucasicus* complex and *M. eupeus*.

Geographic distribution and ecological niche modeling.---We assembled a total of 30 geographic points, 11 recorded from field survey and 19 georeferenced from literatures, for occurrence of the Przewalsk's scorpion. These occurrence points cover a large geographic space, stretching about 2000 km from east to west. All the occurrence sites are to the south of the Tianshan Mountains range and mostly in the Tarim Basin. After removed highly correlated ($|r| \geq 0.80$) climatic variables, six variables were used in ecological niche modeling. These include Bio3 = isothermality, Bio8 = Mean Temperature of Wettest Quarter, Bio9 = Mean Temperature of Driest Quarter, Bio13 = Precipitation of Wettest Month, Bio14 = Precipitation of Driest Month, and Bio15 = Precipitation Seasonality. We also performed ENM using only the 11 occurrence sites from which samples were sequenced for phylogenetic analysis. The predicted potential distribution areas almost overlapped that predicted with all occurrence sites. The ENM performed very well with AUC value of 0.983. Thus we only report the ENM results based on the full distributional records here. The relative contributions of these six climatic variables to the model ranged from 1.3% (Bio3) to 54.0% (Bio13). The ENM performed very well with an AUC value of 0.976.

The predicted suitable distribution areas for the Przewalski's scorpion are shown in Figure 3. The entire Tarim basin and adjacent Gobi regions are suitable for survival of *M. przewalsii*, however, no areas to the west of the Tianshan Mountains and the Pamir Plateau is predicted suitable. The large suitable areas were predicted in the Junggar basin to the north of the Tianshan Mountains. However, our field survey suggested that this species does not occur in these regions. On the contrary, another species *M. eupeus mongolicus* is very common and these regions represent the core distribution range of the later (Shi et al. 2015). We assume these areas are over predictions of the ecological niche model. There are limited overlaps in predicted suitable distribution areas between *M. przewalsii* and and *M. martensii* along the northeast edge of the Qinghai-Tibet Plateau. Field survey also implicates that contact between the Przwewalsi's scorpion and the *M. martensii* is unlikely. There is at least 500 km distribution gap between these two species along the Hexi corridor, which is currently distributed by *M. eupeus mongolicus* (Shi et al. 2015). The predicted suitable distribution areas for all other nine species of the *M. caucasicus* complex lumped together effectively restricted to the west of the Tianshan Mountains (Figure 3). The predicted distribution ranges for these scorpions were clearly separated by unsuitable areas composed of the Tianshan Mountains from the suitable range of the Przwewalsi's scorpion in the Tarim Basin. No overlap was observed in potential distribution range between the Przwewalsi's scorpion and other species of the complex (Fig. 3). We define the Tainshan Mountains as the northern boundary, the western entrance of the Hexi corridor as the eastern boundary, the Pamir as the western boundary and the Qing-Tibet Plateau as the southern boundary, respectively, for the Przwewalsi's scorpion.

DISCUSSION

The Chinese scorpion is a member of species complex.---An interesting finding of the present study is that the Chinese scorpion *M. martensii* appears to be a member of the *M. caucasicus* complex. In the phylogenetic analyses using both ML method and Bayesian method, a subclade of *M. martensii* clustered with subclades representative of the *M. caucasicus* complex in a major clade that was reciprocally monophyletic with respective to the mottled scorpion *M. eupeus* (Fig. 1). This result is consistent with our earlier finding that *M. caucasicus* s. l. is paraphyletic with respective to *M. martensii* (Shi et al. 2013). The mtCOI sequences distances between *M. martensii* and *M. caucasicus* s. l. range from 7.2% to 8.4% which is significantly smaller than the genetic distances between some members (*i.e.* subspecies recognized earlier) of the *M. caucasicus* species complex. For instance, genetic distances between the Przewalskii scorpion and seven Central Asian species (*M. elenae, M. kreuzbergi*, *M. caucasicus, M. nemilini*, *M. parthorum*, *M. intermedius* and *M. fuscus*) were larger than 8.9%. As mentioned earlier, there is not clear geography barrier exist between *M. martensii* and the Przewalskii scorpion. Thus the *M. caucasicus* complex represent a phylogenetically and geographically coherent lineage that include 11 species spreading from the Caucasian region by *M. caucasicus* in the west, across the Tianshan mountains, and to the East China Plain by *M. martensii*. Besides the highly similar genetic distances among species and poorly resolved phylogeny suggest that this species complex might have undergone radiative speciation that gave rise to multiple species in a short time interval in early evolutionary history. Speciation and biogeographic history of the species complex is high likely linked to the regional tectonic evolution and climatic changes. Clearly extensive sampling of genomic sequences is required to fully resolve the inter-relationships among species within the complex so that such a biogeography hypothesis can be rigorously tested.

The Przewalski's scorpion represents an independent species.---According the unified species concept, species represent separately evolving metapopulation lineages (De Queiroz 2007), which possess many contingent, but not necessary, properties making them be reciprocally monophyletic, ecologically divergent, or morphologically distinctive (Leaché et al. 2009). Here we adopt the unified species concept and use these contingent properties as the operational criteria to delimit species for the Przewalski's scorpion. Firstly, the mitochondrial DNA data revealed that the Przewalski's scorpion represents monophyletic taxonomic unit. In the phylogenetic analyses of mtCOI DNA sequences, all sample of the Przewalski's scorpion clustered together and formed a strongly supported monophyletic clade in both ML and Bayesian phylogenies (Fig. 1 & S1). Its monophyly was also supported by the network analysis. Although heavy reticulation have formed in the ancestry nodes, the clade of the Przewalski's scorpion was connected to the network with a single branch (Fig. 2). In addition, we found deep genetic divergences of 7~10% between the Przewalski's scorpion and other member of the *M. caucasicus* complex. Assuming a divergence rate of 1.7% for *Mesobuthus* scorpions (Shi et al. 2013), this genetic distance corresponds to a divergence time of 4~6 million years. Such a long-term period would allow enough time for sorting of ancestral polymorphisms so that species become reciprocally monophyletic.

Secondly, our results of ENM suggested that the Przewalski's scorpion represents an distinctive ecological unit. The core potential distributional areas for the Przewalski's scorpion confined in the Tarim Basin and the surrounding high mountain ranges were not suitable for survival of scorpions (Fig. 3). This observation implies that the high mountains, particularly the Tianshan, have constituted an important geophysical barrier. The central range of Tianshan Mountains appeared impermeable and isolated the Przewalski's scorpion from other Central Asian species of the *M. caucasicus* complex in the west and the mottled scorpion *M. eupeus mongolicus* in the north. On the contrary, there appears no significant physical barrier that would isolate the Przewalski's scorpion from other *M. martensii* and *M. eupeus mongolicus* outside of the Tarim basin in the east. Consistent with studies on other congeneric species (Mirshamsi 2013; Shi et al. 2007, 2015), it appeared that climatic variables were also a determinative factor in define geographic distribution of the Przewalski's scorpion. This point was particularly supported by its eastern distribution boundary, where no prominent geophysical barrier exist. Its potential distributional range was clearly separated from that of its geographically neighboring species, *M. eupeus mongolicus* in the north (Shi et al. 2015) and *M. martensii* in the east (Shi et al. 2007).

Finally, several morphological studies have revealed that the Przewalski's scorpion was also morphological distinct. The Przewalski's scorpion is most morphologically similar to *M. interdedius* (Birula 1897; Sun & Zhu 2010). However, two species can be distinguished by the number of pectinal teeth. Pectines of the Przewalski's scorpion have 15-19 teeth in females and 19-23 teeth in males, while those of *M. interdedius* have 20-25 teeth in females and 26-30 teeth in males, repectively (Sun & Zhu 2010). In comparison with two parapatric species, the movable and fixed fingers possess 11 and 10 oblique rows of granules respectively (Sun & Zhu 2010), while both the movable and fixed fingers of *M. martensii* possess 12-13 rows of oblique granules and *M. eupeus mongolicus* posess 11 and 10 rows respectively (Shi et al. 2007).

In summary, all above evidences indicate that the Przewalski's scorpion is genetic differentiated, phylogenetically independent, ecologically divergent and morphologically distinctive from other species. These contingent properties collectively support that this scorpion is a separately evolving lineage and should be recognized as a full species.

TAXONOMY

Family Buthidae C. L. Koch, 1837

Genus *Mesobthus* Vachon, 1950

Mesobuthus przewalskii (Birula, 1897) **stat. n.**

Buthus caucasicus przewalskii, Birula, 1897:337-338.

Buthus przewalskii: Kishida, 1939:44.

Mesobuthus caucasicus intermedius: Vachon, 1958: 150, Fig. 31.

Olivierus caucasicus intermedius: Farzanpay, 1987: 156; Fet et al. 2000: 191; Zhu et al. 2004:113.

Mesobuthus caucasicus przewalskii: Shi & Zhang 2005:475; Sun & Zhu 2010:4-5; Sun & Sun 2011:60-61; Di et al. 2015:111

Type locality.---CHINA (near Lob-nor and Qiemo in the east edge of the Tarim Basin, Xinjiang)

Distribution.---China (Tarim Basin, Xinjiang; Ejina Qi, Inner Mongolia; Guanzhou and Donghuang, Gansu)

Remarks.--- Based on results and discussions presented above, we propose to elevate the taxonomic status of Przewalskii scorpion to full species. Refer to Sun and Zhu (2010) for full morphological description and key to species. The occurrence of this species outside of China can be tentative excluded (Fig. 3).

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Table 1. Genbank accession numbers of *Mesobuthus* scorpions and outgroup sequences.

Species	Label	GenBank Nos.	Location	References
M. przewalskii	XJTP1501		China (Turpan, Xinjiang)	This study
	GSDH1801		China (Dunhuang, Gansu)	This study
	GSGZ1501		China (Guazhou, Gansu)	This study
	NMEJ01		China (mesozoic-cenozoic, Inner Mongolia)	This study
	XJKE1801		China (Korla, Xinjiang)	This study
	XJHM1501		China (Hami, Xinjiang)	This study
	XJSS1502		China (Shanshan, Xinjiang)	This study
	TMSK1802		China (Tumushuke, Xinjiang)	This study
	XJTrM1501		China (Tarim, Xinjiang)	This study
	XJKC1801		China (Kuche, Xinjiang)	This study
M. martensii	MmaCN01	KC141981	China	Shi et al. 2013
	MmaCN10	KC141990	China	Shi et al. 2013
	MmaCN20	KC142000	China	Shi et al. 2013
	MmaCN40	KC142020	China	Shi et al. 2013
	MmaCN35	KC142015	China	Shi et al. 2013
	MmaCN44	KC142024	China	Shi et al. 2013
	Buthid002			Fet et al. 2018
M. kaznakovi		MG586944	Uzbekistan (Jizzakh)	
M. nenilini	UZi1	AJ783522	Uzbekistan (Namangan)	Gantenbein et al. 2005
	UZf1	AJ783518	Uzbekistan (Namangan)	Gantenbein et al. 2005
	VF3023		Turkmenistan (Akhal Province)	Fet et al. 2018
M. gorelovi		MG586946		
	TUo1			Gantenbein et al. 2005
		AJ783515	Turkmenistan (Lebap)	
	VF3012	MG586939	Turkmenistan	Fet et al. 2018
	VF3017	MG586945	Turkmenistan	Fet et al. 2018
	TUi2	AJ783614	Turkmenistan (Mary)	Gantenbein et al. 2005
	VF3002	MG586938	Kazakhstan	Fet et al. 2018
	UZc1	AJ550696	Uzbekistan (Ferghana)	Gantenbein et al. 2003
	UZh1	AJ783521	Uzbekistan (Bukhara)	Gantenbein et al. 2005
M. kreuzbergi	MRG1640	MG586942	Turkmenistan	Fet et al. 2018
	UZd1	AJ550697	Uzbekistan (Babatag Mts)	Gantenbein et al. 2003
M. fuscus	MRG1636	MG586937	Tajikistan	Fet et al. 2018
	MRG1637	MG586935	Tajikistan	Fet et al. 2018
	MRG1639	MG586934	Tajikistan	Fet et al. 2018
	MRG1638	MG586936	Tajikistan	Fet et al. 2018
M. intermedius	MRG1635	MG586941	Turkmenistan	Fet et al. 2018
	MRG1634	MG586940	Turkmenistan	Fet et al. 2018
M. caucasicus	MRG1632	MG586931	Turkey	Fet et al. 2018
M. elenae	UZl2	AJ783525	Uzbekistan (Surkhandarya)	Gantenbein et al. 2005
	VF3033	MG586933	Tajikistan	Fet et al. 2018

	TJd2	AJ783605	Tajikistan (Khatlong)	Gantenbein et al. 2005
	TJd1	AJ783604	Tajikistan (Khatlong)	Gantenbein et al. 2005
	VF3028	MG586932	Uzbekistan	Fet et al. 2018
	UZl1	AJ783524	Uzbekistan (Surkhandarya)	Gantenbein et al. 2005
	TUg1	AJ783612	Turkmenistan (Mary)	Gantenbein et al. 2005
	TUn1	AJ783514	Turkmenistan (Mary)	Gantenbein et al. 2005
	TUp1	AJ783516	Turkmenistan (Mary)	Gantenbein et al. 2005
M. parthorum	TUb1	AJ783606	Turkmenistan (Mary)	Gantenbein et al. 2005
	TUh1	AJ783613	Turkmenistan (Mary)	Gantenbein et al. 2005
M. eupeus	MeuKZ1201		Kazakhstan	This study
	MeuCN06	KC142030	China	Shi et al. 2013
	MeuCN15	KC142039	China	Shi et al. 2013
	MeuMN01	KC142041	Mongolia	Shi et al. 2013
	MeuCN10	KC142034	China	Shi et al. 2013
	MeuTJ01	KC142042	Tajikistan	Shi et al. 2013
	MeuCN01	KC142025	China	Shi et al. 2013
A. australis	Aau	AF370829	outgroup	Giribet et al. 2001
B. occitanus	BocMt	EU523755	outgroup	Masta and Boore 2008

Table 2. **Estimates of net evolutionary divergence in the mtCOI sequences between species of *Mesobuthus caucasicus* complex.** Show here are the mean ± standard error of net genetic distances calculated using the Kimura 2-parameter model (lower triangle). Entries on the diagonal present means of within species genetic distances and their standard errors.

	M. przewalskii	***M. martensii***	***M. gorelovi***	***M. kaznakovi***	***M. elenae***	***M. kreuzbergi***	***M. caucasicus***	***M. nemilini***	***M. parthorum***	***M. intermedius***	***M. fuscus***
M. przewalskii	**0.0127 ±0.0038**										
M. martensii	0.0741 ±0.0103	**0.0439 ±0.0022**									
M. gorelovi	0.0778 ±0.0100	0.0748 ±0.0113	**0.0301 ±0.0048**								
M. kaznakovi	0.0793 ±0.0111	0.0832 ±0.0120	0.0817 ±0.0124	**N.A**							
M. elenae	0.0891 ±0.0112	0.0717 ±0.0116	0.0702 ±0.0100	0.0811 ±0.0111	**0.0208 ±0.0040**						
M. kreuzbergi	0.0897 ±0.0135	0.0737 ±0.0108	0.0813 ±0.0125	0.0876 ±0.0134	0.0870 ±0.0120	**0.0291 ±0.0069**					
M. caucasicus	0.0900 ±0.0113	0.0835 ±0.0119	0.0808 ±0.0112	0.0898 ±0.0130	0.0923 ±0.0121	0.0916 ±0.0126	**N.A**				
M. nemilini	0.0910 ±0.0130	0.0761 ±0.0136	0.0748 ±0.0128	0.0449 ±0.0094	0.0705 ±0.0123	0.0955 ±0.0151	0.1003 ±0.0144	**0.0315 ±0.0071**			
M. parthorum	0.0976 ±0.0139	0.0795 ±0.0136	0.0570 ±0.0108	0.0820 ±0.0139	0.0425 ±0.0093	0.0809 ±0.0139	0.0863 ±0.0148	0.0884 ±0.0156	**0.0736 ±0.0012**		
M. intermedius	0.0998 ±0.0110	0.0839 ±0.0108	0.0776 ±0.0116	0.1056 ±0.0129	0.1068 ±0.0150	0.0959 ±0.0123	0.1084 ±0.0124	0.1072 ±0.0170	0.0911 ±0.0143	**0.0000 ±0.0000**	
M. fuscus	0.1033 ±0.0131	0.0834 ±0.0126	0.0746 ±0.0105	0.0909 ±0.0125	0.0915 ±0.0137	0.0747 ±0.0121	0.1066 ±0.0143	0.0886 ±0.0132	0.0902 ±0.0148	0.1118 ±0.0147	**0.0026 ±0.0014**
M. eupeus	0.1269 ±0.0122	0.1152 ±0.0118	0.0969 ±0.0140	0.1151 ±0.0125	0.1089 ±0.0146	0.1075 ±0.0123	0.1236 ±0.0127	0.1171 ±0.0155	0.0878 ±0.0147	0.1361 ±0.0146	0.1231 ±0.0149

Figures

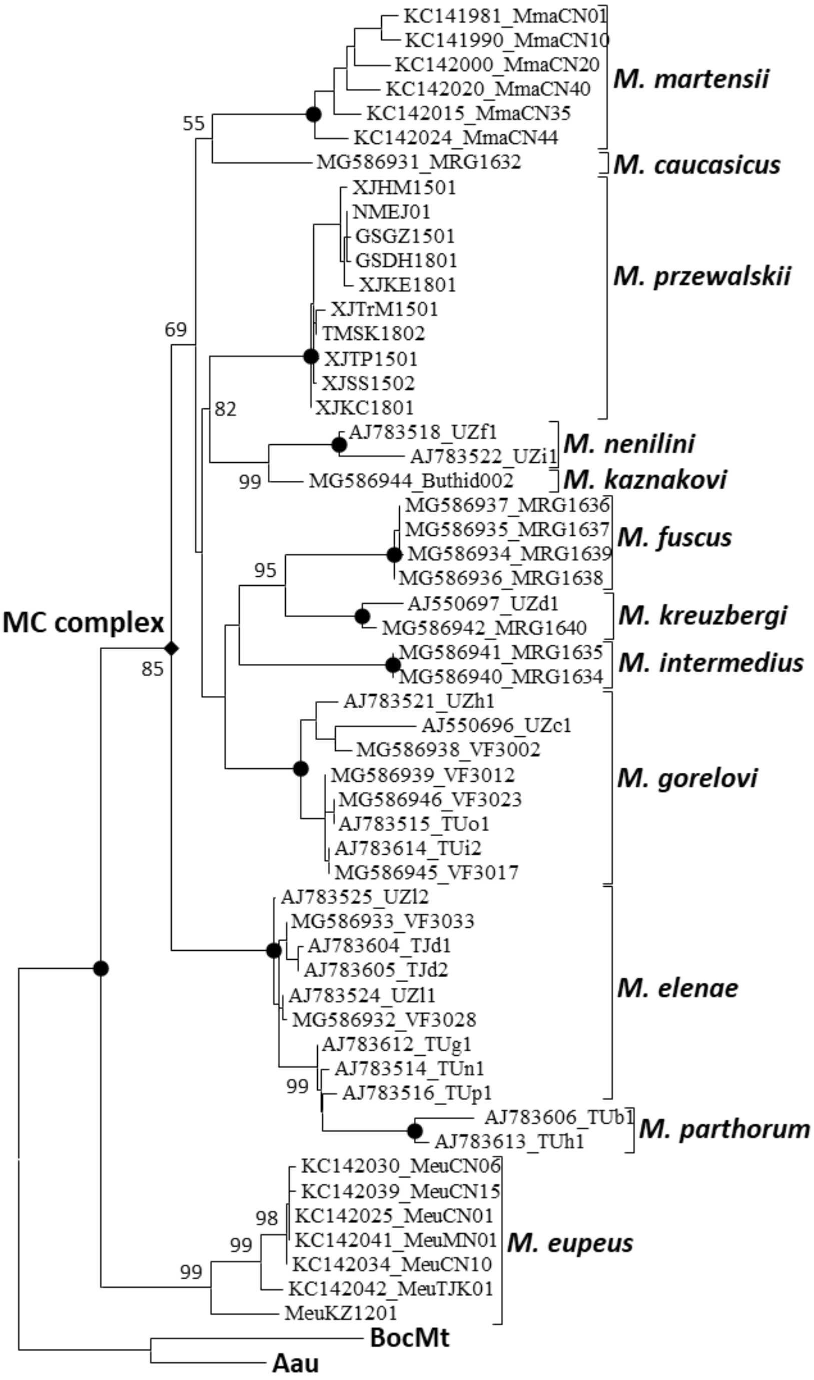

Figure 1. Maximum likelihood phylogeny of the *Mesobuthus caucasicus* species complex reconstructed using mitochondrial DNA sequences. The Przewalski's scorpion (*M. przewalskii*) is deeply diverged from other species and the Chinese scorpion (*M. martensii*) belongs to the species complex. Node supports are shown by filled cycles for 100 and otherwise by the exact numbers for bootstrapping probabilities from 1000 replicates. The node demoted by a diamond indicates the most recent common ancestor for the *M. caucasicus* complex (MC complex).

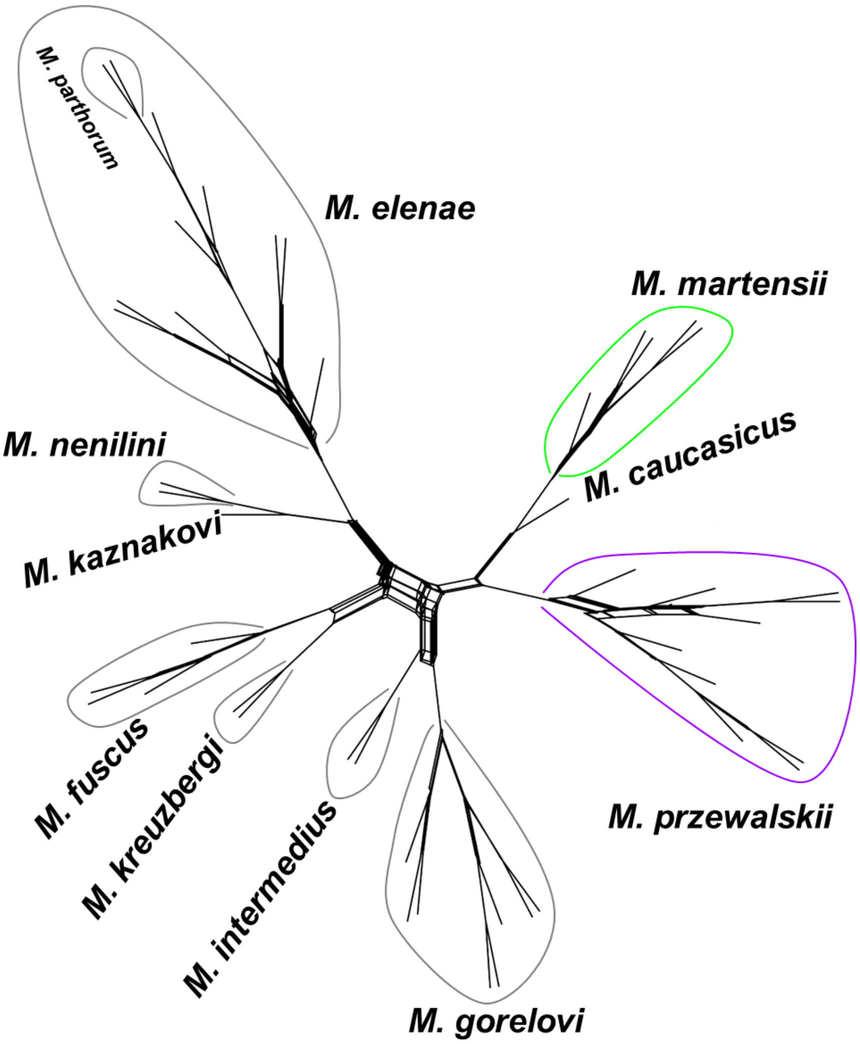

Figure 2. Phylogenetic network for the *Mesobuthus caucasicus* species complex. Although the interrelationships between species is poorly resolved, no reticulations have occurred in the most recent common ancestors for each species. The Przewalski's scorpion is clearly diverged from other member of the species complex and warrants a species rank. The divergence of the Chinese scorpion M. martensii is comparable to the divergences among the members of the species complex.

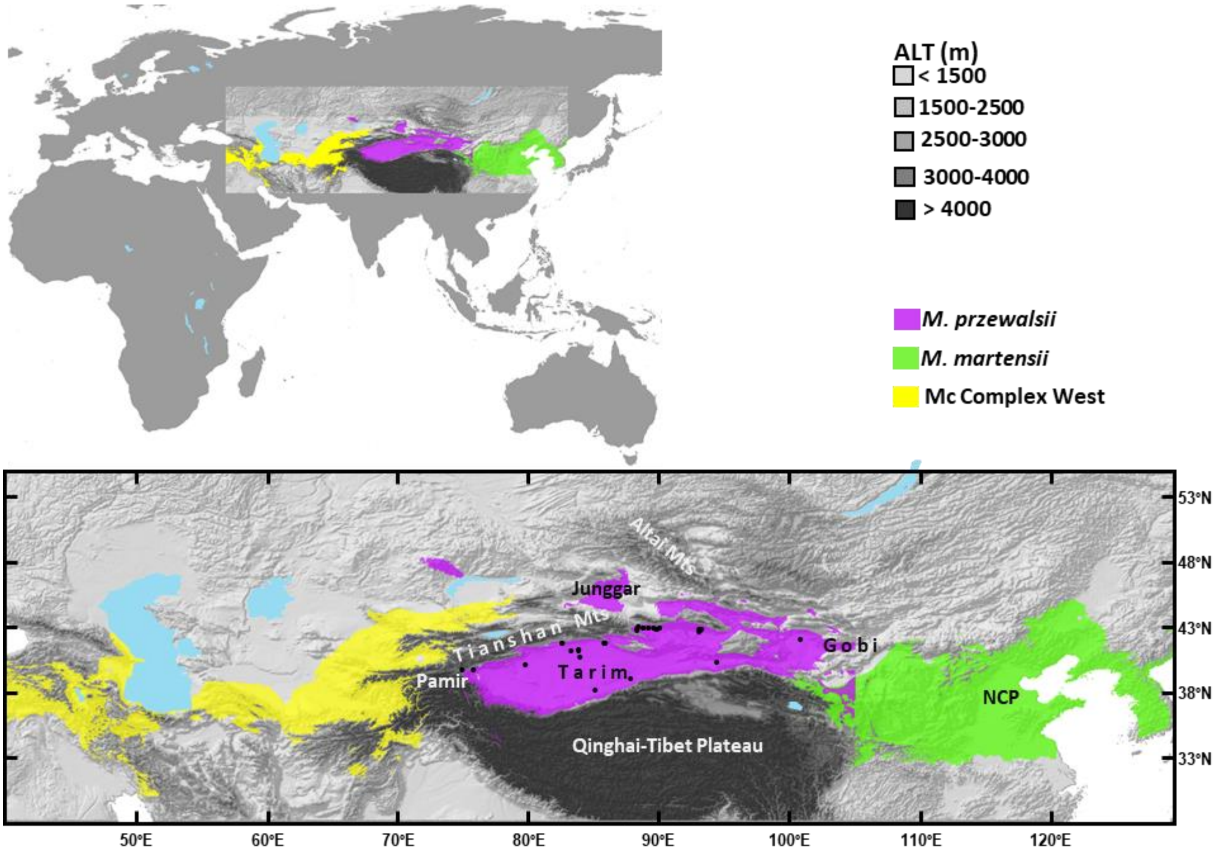

Figure 3. Ecological niche models of *Mesobuthus* scorpions. Potential distribution areas for the Przewalski's scorpion (*M. przewalsii*, purple) is shown together the Chinese scorpion (*M. martensii*, light green) and other species of the *M. caucasicus* complex (Mc Complex West, yellow). The entire Tarim Basin and adjacent Gobi region are suitable for survival of *M. przewalsii*. No area to the west of the Tianshan Mts and the Pamir Plateau is suitable for *M. przewalsii*, and similarly no area to the east of the Tianshan Mts and Pamir Plateau is suitable for other species of the *M. caucasicus* complex. There are overlaps in predicted suitable distribution areas between *M. przewalsii* and *M. martensii* along the northeast edge of the Qinghai-Tibet Plateau. The suitable areas in the Junggar Basin and two the north of the Tianshan Mts are due to over prediction of the model, because *M. przewalsii* does not occur in these regions. Ecological niche model for *M. martensii* was adopted from Shi et al. 2007.