



Population and landscape genetic analysis of the Malayan sun bear *Helarctos malayanus*

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Abstract

Conservation genetics can provide data needed by conservation practitioners for their decisions regarding the management of vulnerable or endangered species, such as the sun bear *Helarctos malayanus*. Throughout its range, the sun bear is threatened by loss and fragmentation of its habitat and the illegal trade of both live bears and bear parts. Sharply declining population numbers and population sizes, and a lack of natural dispersal between populations all threaten the genetic diversity of the remaining populations of this species. In this first population genetics study of sun bears using microsatellite markers, we analyzed 68 sun bear samples from Cambodia to investigate population structure and genetic diversity. We found evidence for two genetically distinct populations in the West and East of Cambodia. Ongoing or recent gene flow between these populations does not appear sufficient to alleviate loss of diversity in these populations, one of which (West Cambodia) is characterized by significant inbreeding. We were able to assign 85% of sun bears of unknown origin to one of the two populations with high confidence (assignment probability $\geq 85\%$), providing valuable information for future bear reintroduction programs. Further, our results suggest that developed land (mostly agricultural mosaics) acts as a barrier to gene flow for sun bears in Cambodia. We highlight that regional sun bear conservation action plans should consider promoting population connectivity and enforcing wildlife protection of this threatened species.

Keywords Sun bear · *Helarctos malayanus* · Microsatellite · Population genetics

Introduction

Determining the genetic status of wild animal populations (and captive stock) using nuclear genetic markers can provide conservation practitioners with valuable information for ex situ and in situ conservation management (Arif et al. 2011). For example, data generated using nuclear microsatellite loci can be used to determine population structure

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and aid in identifying potentially threatened sub-populations (Goossens et al. 2013; Moura et al. 2017). During the last decades microsatellite markers have become a powerful genetic tool to inform ex situ breeding programs (Witzenberger and Hochkirch 2011), they can be used for genetic fingerprinting (Mishra et al. 2017) and they can be used in the development of forensic markers to combat illegal trade (Arif et al. 2011). Most importantly, microsatellites provide in situ practitioners with valuable information about the genetic diversity of wild populations, the extent of gene flow between these populations (i.e. population connectivity), and help determine how viable wild populations are in fragmented and anthropogenic disturbed environments (Goossens et al. 2013; Frankham 2015).

Habitat fragmentation is a conservation concern because it reduces the ability of individuals to traverse to other populations, and can result in the isolation of breeding populations (Bender et al. 1998; Fahrig 2003; Lancaster et al. 2016). Isolated populations are subjected to an overall loss of genetic diversity (Paetkau et al. 1995), and potentially face problems related to inbreeding (Frankham 2005). This is particularly problematic for small populations, as this can ultimately lead to local population extinction (Frankham 2015). Inbreeding can have significant consequences for fitness, for example by reducing sperm quality and quantity (Fitzpatrick and Evans 2009), by reducing competitive abilities (Haag et al. 2002), by compromising mating ability (Frankham 2005; Liberg et al. 2005), by increasing developmental time (Fox and Reed 2010) and/or by reducing disease resistance and immune competence (Ilmonen et al. 2008). These consequences endanger populations, particularly in conjunction with the loss and disturbance of habitat (Frankham 2015). Furthermore, a reduction in genetic diversity can be detrimental to the adaptability of a species to a changing environment (Lande 1988). Therefore, the International Union for Conservation of Nature (IUCN) acknowledges the importance of understanding the genetic resources of a target species to optimize its conservation management plan (Reed and Frankham 2003) and to preserve genetic biodiversity worldwide.

Southeast (SE) Asia is recognized as one of the most biodiverse regions in the world, encompassing habitat for a range of endemic flora and fauna (Myers et al. 2000). It is also one of the areas of the world that has experienced the most severe anthropogenic disturbance (Hughes 2017; Scotson 2019), making it a region of global conservation concern (Sodhi et al. 2010). Located on the SE Asian mainland, Cambodia holds an outstanding amount of protected wildlife reserves, covering approximately 20% of the land mass (Hsu et al. 2016). However, Cambodia's wildlife is threatened by poaching (Gray et al. 2012, 2017), and has one of the highest rates of deforestation worldwide (Kapos et al. 2010; Avtar et al. 2013; Hughes 2017). Large scale clearing for

agriculture and construction projects extends into wildlife sanctuaries and national parks, creating a highly-fragmented landscape of vital wildlife habitat (Le Billon 2002). Habitat fragmentation directly reduces suitable habitat, and creates barrier-like patches of hostile and uninhabitable landscape between remaining fragments (Bender et al. 1998; Franklin et al. 2002). Furthermore, habitat fragmentation leads to reduced resource availability, which in turn lowers the carrying capacity necessary to maintain large wildlife populations of animals that require larger home ranges such as carnivores (Bender et al. 1998).

One of Cambodia's carnivores threatened by habitat loss is the sun bear *Helarctos malayanus*, a small Ursid historically distributed across most of SE Asia (Fig. 1). Although very little research has been conducted to evaluate the status of this species (Meijaard and Nooteboom 1999; Wong and Linkie 2013), the sun bear is threatened to a degree that warrants the classification 'vulnerable' on the IUCN red list (Scotson et al. 2017). It is also protected under Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2017). Throughout their range, sun bears have lost much of their habitat to land conversion. Large-scale logging of forests to establish agriculture, plantations and human settlements have left the remaining habitat fragmented, thereby fragmenting a once contiguous sun bear population into smaller, relatively isolated subpopulations (Scotson et al. 2017). Increasing human encroachment also causes more frequent human-wildlife conflicts (Suba et al. 2017) that result in either the translocation of the bear (by wildlife officials) or in the killing of the animal by local people. For example, Scotson et al. (2014) showed that many plantation workers and owners did not mind sun bears in their plantations, because they could then opportunistically kill bears and sell their organs and other bear parts. In almost all countries throughout their range, adult sun bears are killed and used in traditional Asian medicine or sold for meat (Nijman et al. 2017). Sun bear cubs are commercially traded as pets (Rog et al. 2015). This uncontrolled trade of live bears and bear parts has contributed to a steep population decline (Augeri 2005; Scotson et al. 2017; Wong et al. 2015) and as the trade is illegal, many bears are now confiscated and handed over to wildlife officials or non governmental organisations (NGOs) (Willcox et al. 2016). These confiscated sun bears are subsequently released back into their natural habitat, but due to the current lack of knowledge regarding their genetic background such releases occur opportunistically and not by informed decisions (Huber 2010). However, translocation, augmentation or rehabilitation of bears that are released into an existing population can endanger the genetic and physiological health of these populations (Van Dijk 2005; Fredriksson 2001; Huber 2010). Cambodia has

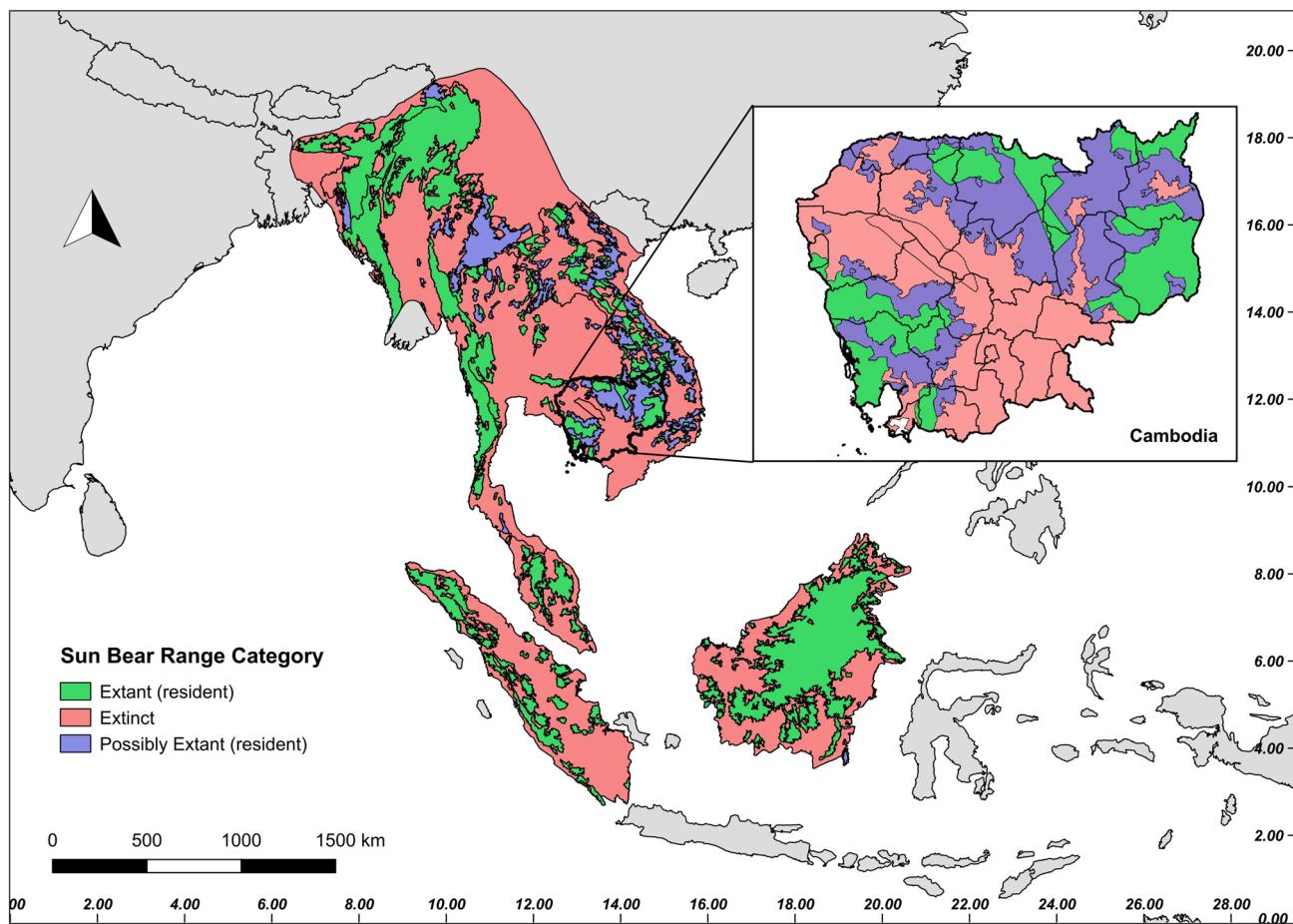


Fig. 1 Sun bear (*Helarctos malayanus*) distribution map, altered from the IUCN Red List map (Scotson et al. 2017). The colours represent the confirmed present (green) and probable present (blue) distribution

ranges, as well as the areas where the sun bear is assumed to have gone extinct (red)

one of the highest confiscation rates of illegally traded sun bears (Foley et al. 2011). Therefore, identifying the genetic variability and population structure of the Cambodian sun bear can provide conservation practitioners with valuable information regarding which populations are targeted by illegal trade and therefore require stricter enforcement of legal protection. Furthermore, knowing the population structure in Cambodia will allow local sun bear managers to make informed decisions about which population is a genetic match for the bears in their care, and where they can potentially release these bears.

Here we present (to our knowledge) the first genetic study for sun bears using nuclear genetic markers (microsatellites). The aims of this study were to (1) evaluate genetic diversity and population structure of Cambodia's sun bears, and to (2) identify the population of origin of confiscated bears that have been traded illegally and are now housed in rescue centers. This study provides a first step in understanding the genetic viability, connectivity and conservation status of the wild sun bear populations in Cambodia.

Materials and methods

Study area and sample collection

We collected samples from sun bears housed at the 'Free the Bears' sanctuary in Phnom Tamao Zoo and Wildlife Rescue Centre, Cambodia. The sun bears included in this study were confiscated or donated from 16 different provinces in Cambodia: Kampot ($n=1$), Kandal ($n=7$), Koh Kong ($n=5$), Kompong Cham ($n=2$), Kompong Som ($n=1$), Kompong Speu ($n=1$), Kompong Thom ($n=3$), Kratie ($n=4$), Oddar Meancheay ($n=3$), Phnom Penh ($n=22$), Preah Vihear ($n=4$), Pursat ($n=1$), Ratanakiri ($n=13$), Siem Reap ($n=7$), Stung Treng ($n=13$), and Takeo ($n=3$) (Fig. 2). One sample was from a sun bear born in captivity.

We collected samples non-invasively, primarily through the collection of the mucus layer of scat samples. We further sampled saliva, tissue and blood as opportunity allowed. Over the course of 10 days in August 2013 we collected 99 saliva, 81 scat, two claws, one blood, one phlegm, and one

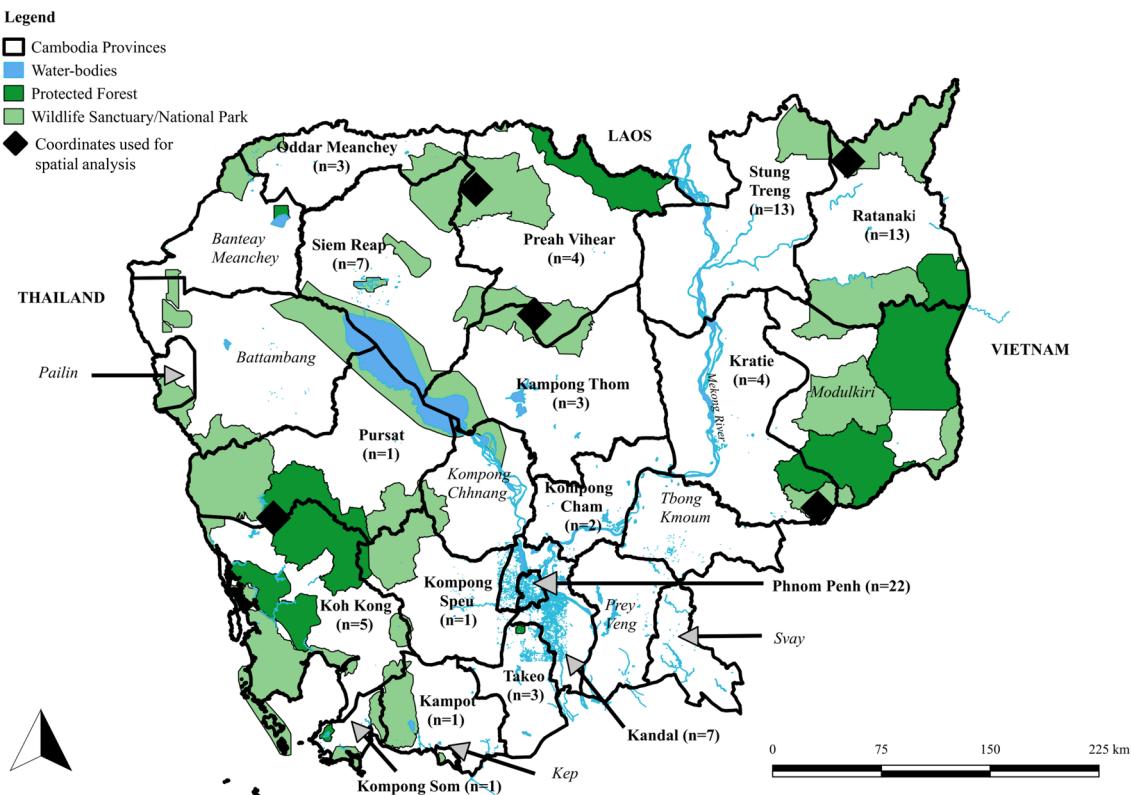


Fig. 2 Map of Cambodia indicating geographic origin of sun bear samples (see also Suppl. Table S1). Forests and national parks are also displayed. Coordinates used for spatial analysis are indicated by black diamonds

tissue sample (a total of 185 samples) from 94 individual bears. From these 185 samples, we used samples for 91 sun bears for further analysis (those for which two samples were available; Supplementary Table S1). Additionally, we used blood samples from two deceased zoo animals (IZW ID# 2449 and IZW ID# 2462) to test and optimize primers for the microsatellite loci (below).

These samples comprised two categories of accuracy in their stated origin: (1) ‘*good origin accuracy*’, for sun bears with good record keeping and for which the origin of the individual sample could be traced back to the location of capture; and (2) ‘*uncertain origin*’, for sun bears whose exact origin was not traceable (e.g. subjected to wildlife trafficking, or born in captivity). We considered samples of uncertain origin as samples that required population assignment.

Laboratory procedures

We extracted DNA from all sample types (above) using the GEN-IAL First DNA All-tissue DNA extraction Kit (GEN-IAL GmbH, Troisdorf, Germany), following the manufacturer’s protocol.

We used the following 17 microsatellite loci for genotyping: *Umar01*, *Umar02*, *Umar03*, *Umar04*, *Umar05*,

Umar06, *Umar07*, *Umar08*, *Umar09*, *Umar10* (Poissant and Davis 2011), *G10X* (Straka et al. 2012), *Mu05*, *G1D* (Andreassen et al. 2012), *UarD3139*, *UarT739*, *UarD1585* (Kleven et al. 2012), *MU50* (Bellemain and Taberlet 2004) (Table 1). PCRs were conducted as follows: 5 µL 2x Qia- gen multiplex Master Mix (Qiagen), 1 µL Q-Solution (Qiagen), 1 µL primer mix (0.5 µL primer F + 0.5 µL primer R; at 10 pmol/µL), 1 µL template DNA (from a 50 µL elution volume) and 2 µL RNase-free water. We amplified the PCR product under the following conditions: initial denaturing at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing with a touchdown protocol for the first five cycles (from 63 to 55 °C in 2 °C increments) followed by 30 cycles at 55 °C for 90 s, and extension at 72 °C for 30 s. The final extension was at 60 °C for 30 min. For hair and scat samples, we increased the number of cycles (up to 10 additional cycles). We used the A3130xl DNA sequencer (ABI Applied Biosystems, Foster, CA, USA) to separate the amplified fragments and scored allele sizes using the GeneMapper v 4.0 application (ABI) based on a GeneScan™ 500 ROX (both Thermo Fischer Scientific Darmstadt, Germany) internal size standard.

Table 1 Microsatellite loci employed in the study

Locus	Dye	Repeat motif	Size range*	Source
UarD1585	6 FAM	(GT)16	121–131	Kleven et al. (2012)
UarD3139	6FAM	(GT)22	107–121	Kleven et al. (2012)
UarT739	6FAM	(TATC)10	108–132	Kleven et al. (2012)
Mu50	Ned		106–136	Bellemain and Taberlet (2004)
Umar 01	Hex	(CA)17	225–233	Poissant and Davis (2011)
Umar 02	Hex	(GT)14	200–212	Poissant and Davis (2011)
Umar 03	6FAM	(TG)14	232–240	Poissant and Davis (2011)
Umar 04	6FAM	(TG)19TTTGA(GT)4	117–127	Poissant and Davis (2011)
Umar 05	6FAM	(GT)3C(TG)13	202–218	Poissant and Davis (2011)
Umar 06	Hex	(TG)2CG(TG)2CT(TG)3C(GT)11	247–249	Poissant and Davis (2011)
Umar 07	Hex	(CA)14	184–192	Poissant and Davis (2011)
Umar 08	6FAM	(TA)3(TG)6CGTGTA(TG)8	202–204	Poissant and Davis (2011)
Umar 09	Hex	(CA)19	229–243	Poissant and Davis (2011)
Umar 10	Hex	(CA)16	226–234	Poissant and Davis (2011)
G10X	Hex		132–156	Straka et al. (2012)
G1D	Ned		123–139	Andreassen et al. (2012)
Mu05	Ned		109–133	Andreassen et al. (2012)

*As given in source

Population genetic analyses

For non-invasively collected samples, genotypes may either be incomplete or may suffer from allele dropout or false alleles. For this reason, we applied a maximum likelihood approach (Miller et al. 2002) to ensure reliable genotypes: we genotyped each sample in duplicates and only retained genotypes that had consistent allele calls between replicates. If we observed a mismatch, we re-extracted DNA of the respective sample and re-genotyped it (in duplicate). If there was no material left for a second extraction, or if the second round of parallel amplifications showed mismatches again, the sample was discarded. Furthermore, we removed all samples that had data missing for more than one locus.

We used MICRO-CHECKER v.2.2 (van Oosterhout et al. 2004) to check for genotyping errors and the likelihood of null alleles being present. We examined deviations from HWE and tested for linkage disequilibrium (LD) using GENEPOL v.4.6 (Raymond and Rousset 1995). Observed (H_O) and expected (H_E) heterozygosity were estimated using POPGENE v.1.32 (Yeh et al. 1997). F statistics were calculated with FSTAT v.2.9.3.2 (Goudet 2002) and corrected P -values were applied for multiple comparisons when appropriate (Bonferroni correction).

For inferences about the population structure in Cambodia, we used Bayesian inference, implemented in the program STRUCTURE v.2.3.4 (Pritchard et al. 2000), to estimate the likely number of genetic clusters (K) for a range of K values from $K=1$ to $K=5$. Each number of K clusters was assessed in 10 independent runs, with a burn-in of 100,000 MCMC steps, followed by an additional 200,000 MCMC

steps. The most likely K in this dataset was calculated using the ΔK approach of Evanno et al. (2005) as implemented in STRUCTUREHARVESTER (Earl and von Holdt 2012; <http://taylor0.biology.ucla.edu/structureHarvester/>).

We also used Bayesian inference to infer population structure using spatial data, as implemented in the software TESS (v.2.3; Chen et al. 2007). Only sun bear samples with ‘good origin accuracy’ were used in this analysis. Due to the uncertainty of the *exact origins* of many of the confiscated bears (despite good knowledge regarding province and capture locality), we determined the *most likely origin* of sun bears for every province. These coordinates were based on areas of known sun bear occupancy as determined by the IUCN sun bear specialist group, as well as the knowledge of local sun bear experts. As some bears were confiscated in highly developed areas (e.g. in a city), we also considered excluding such samples. The analysis of population structure was thus conducted with the following two datasets: (i) all sun bears with good origin accuracy ($N=35$), and (ii) excluding samples from highly developed areas ($N=30$). For each dataset, we ran TESS 100 times for each K in a range from $K=2$ to $K=5$, and averaged results (per K) over the 20% runs with the lowest Deviance Information Criterion (DIC), as suggested by the developers (Chen et al. 2007). Each run consisted of 100,000 sweeps, of which the first 50,000 were discarded as burn-in.

We used a landscape genetics approach to determine which landscape features impact genetic exchange between sun bear populations. For this, we used the resistance surface optimisation routine (Peterman et al. 2014), implemented in the R package *ResistanceGA* (Peterman 2018).

This method optimises resistance surfaces by attempting to maximise the relationship between pairwise resistance distances and genetic distances in an iterative process. Initially single resistance surfaces are independently optimised before combinations of these are optimised in composite resistance surfaces. The maximum number of iterations for each surface was 1000. Resistance distances were calculated using *gdistance* (van Etten 2017), and genetic distances were calculated as ‘1—proportion of shared alleles’ (Bowcock et al. 1994) using *PopGenReport* (Adamack and Gruber 2014). Following optimisation of surfaces, maximum likelihood population effects (MLPE) mixed models (Clarke et al. 2002), implemented in *lme4* (Bates et al. 2015), were fitted and compared using AICc. Furthermore, pseudo bootstrap analyses were conducted by subsampling observations to identify top ranked surfaces. The routine was repeated 5 times to determine the prevailing resistance surface transformations and model rankings (details in Suppl. File S1). The initial single resistance surfaces were created using a categorical land cover map developed within the ESA GlobCover 2009 Project (Arino et al. 2012). The land cover map was reclassified into 5 broader classes (Suppl. File S1): agricultural mosaics, natural mosaics, water and wetland, settlements, and ‘other’. Each land cover class was separated into individual rasters by assigning pixel values 1 = not class and 100 = class. These were then aggregated (15 km resolution) into continuous resistance layers by averaging pixel values (Suppl. File S1) before being optimised as outlined above. We used the same two datasets as in the spatial analysis of population structure in TESS (see above).

Results

We analyzed a total of 17 microsatellite loci in 91 samples. Among these 17 microsatellites, three were invariant (Umar04, Umar06 and Umar08) and two others yielded inconsistent results between the genotyping replicates (Umar07 and Umar10) and were thus excluded from further analysis. We also had to exclude 23 samples due to missing data at more than one locus, with the result that 68 samples remained with data for at least 11 of the 12 microsatellite loci; these comprised 35 samples with ‘good origin accuracy’, and 33 of ‘uncertain origin’.

Population structure

To determine the population structure in Cambodian sun bears, we analyzed the full dataset of 68 samples using STRUCTURE. The ΔK statistic (Evanno et al. 2005) indicated the presence of two genetic clusters ($K=2$; see Suppl. File S2), visualized in a STRUCTURE bar plot (Fig. 3a; samples with good origin accuracy are indicated by filled

circles above the bars). We then projected the STRUCTURE results for samples with good origin accuracy ($N=35$) onto the map of Cambodia (Fig. 3b). However, when this reduced dataset was used, neither a non-spatial (software STRUCTURE) nor a spatial (software TESS) approach was able to detect population structure (see Suppl. File S2). When we excluded samples from highly developed areas ($N=5$; see Methods), spatial analysis of population structure using TESS ($N=30$) gave highly congruent results with the STRUCTURE analysis at $K=2$ (Fig. 3c, d). Higher values of K resulted in few, if any, samples with population assignment $Q \geq 85\%$ to a genetic cluster (Suppl. File S2).

The two genotypic clusters appeared geographically separated, one in the West, the other one in the East of the country (Fig. 3b, d), although many provinces had individuals with a genetic background from both (i.e. admixed individuals). Out of the 35 samples of good origin accuracy, 10 could be assigned to the ‘West population’ and 17 to the ‘East population’ ($Q \geq 85\%$); 8 samples were considered admixed (Table 2). Samples of uncertain origin ($N=33$) comprised many individuals that could be assigned with high probability ($Q > 85\%$, Table 2) to one of the two populations using STRUCTURE (Fig. 3a). Of the 33 samples with uncertain origins, 17 could be assigned to the West population, 9 to the East population, and 7 samples were considered admixed (Table 2). Thus, in total 53 out of the 68 samples (78%) could be assigned with high probability to one of the two populations (27 to West, 26 to East).

We then examined whether the two populations were significantly differentiated using two datasets: (i) only samples of good origin accuracy that had been assigned with high probability to either one of the two populations ($N=27$), and (ii) all samples ($N=53$) that had been assigned with high probability to the two populations (admixed individuals were excluded). Considering only samples of good origin accuracy, the F_{ST} value of 0.108 was highly significant ($P < 0.001$), as was the F_{ST} value of 0.133 when considering all samples ($P < 0.001$).

Summary statistics

Measures of diversity were calculated for the two populations (West and East) as well as for the total dataset (Table 3). While the West population displayed greater heterozygosity ($H_O=0.494$, $H_E=0.557$) and a higher mean number of alleles ($N_A=5.6$) than the East population ($H_O=0.476$, $H_E=0.524$, $N_A=4.2$), it nevertheless displayed signs of inbreeding ($F_{IS}=0.13$; $P < 0.05$). One locus in this population (UarD3139) was not in HWE ($P=0.04$), and one pair of loci showed significant LD (UarD1585 and UarT739). In the East population all loci were at HWE and no loci pairs showed signs of LD.

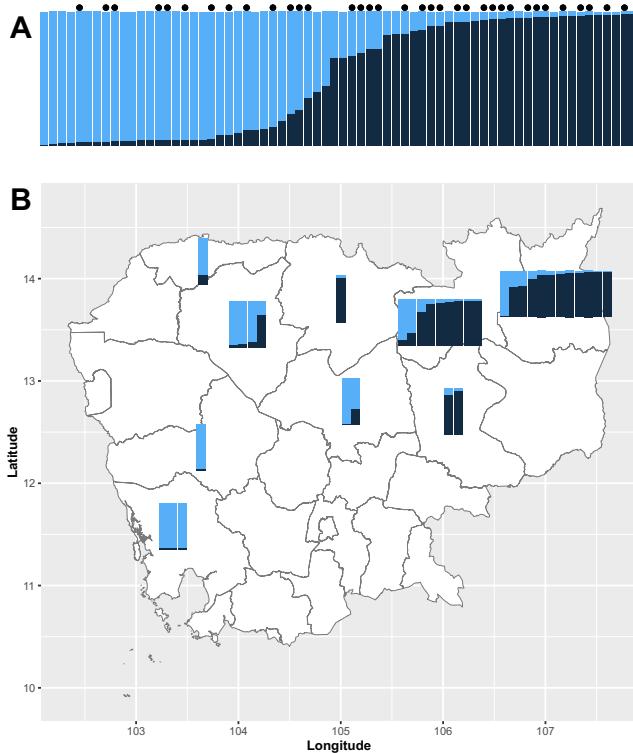
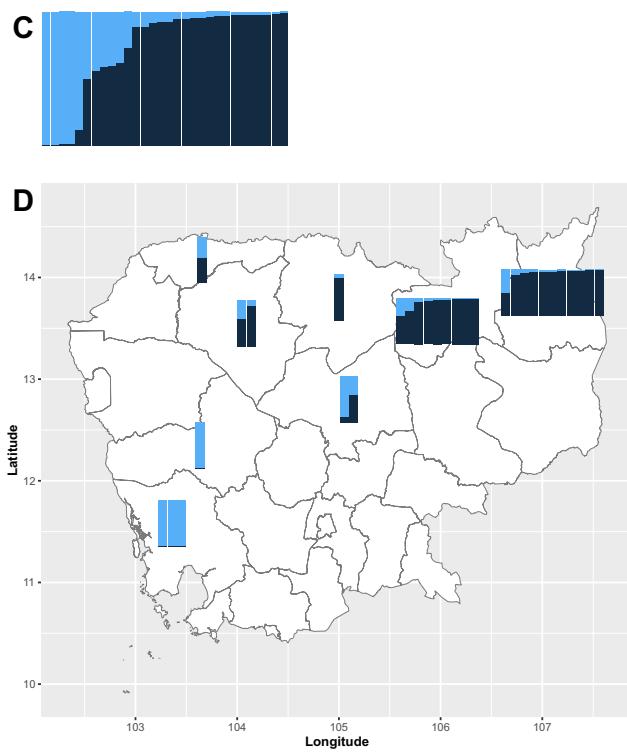
STRUCTURE

Fig. 3 Analysis of population structure among Cambodian sun bears. **a** STRUCTURE bar plot for $K=2$ ($N=68$); samples with 'good origin accuracy' are indicated by a filled circle above the bar of the respective individuals. **b** STRUCTURE bar plots for samples with

TESS

'good origin accuracy' ($N=35$) projected onto a map of Cambodia. **c** TESS bar plot for $K=2$ ($N=30$), excluding samples with origin in highly developed areas. **d** TESS bar plots ($N=30$) projected onto a map of Cambodia

Table 2 Assignment of Cambodian sun bear samples to the West and East populations (assignment in STRUCTURE, $Q \geq 85\%$)

Sample origin	West Pop	East Pop	Admixed	N
Good accuracy	10	17	8	35
Uncertain origin	17	9	7	33
Total	27	26	15	68

Table 3 Summary statistics

Samples	N	H_E	H_O	N_A	F_{IS}
West population [†]	27	0.557	0.494	5.6	0.13*
East population [†]	26	0.524	0.476	4.2	0.11
All samples	68	0.582	0.480	6.3	0.18*

N number of samples, H_E expected heterozygosity, H_O observed heterozygosity, N_A mean number of alleles, F_{IS} inbreeding coefficient (* $P < 0.05$)

[†]Assignment of samples to population ($Q \geq 85\%$) using STRUCTURE, see Table 2

Landscape genetics

Similar to the spatial analysis using TESS, the landscape genetic analysis was affected by the inclusion of samples from highly developed areas ($N=5$). When these samples were excluded, MLPE model selection by AICc ranking (Table 4) suggested that geographic distance best describes the spatial distribution of genetic variation among Cambodian sun bears ($N=30$). In the pseudo-bootstrap analysis (Table 5), geographic distance was also ranked highest. Besides geographic distance, natural and agricultural mosaics, and composites thereof including water, also ranked highly. This was also the prevailing result for analyses conducted for all samples of good origin accuracy ($N=35$). However, the inclusion of the five individuals from highly developed areas shifted the model ranking by reducing the ranking of geographic distance, instead ranking agricultural mosaics highest in model selection, with and without pseudo-bootstrapping, closely followed by composite surfaces including water. The null model was not favoured for either dataset, indicating a higher likelihood that spatial distribution of

Table 4 Summarised MPLE comparison, including the top 5 models ranked by AICc, null models, and full composite models

Dataset	Surface ^a	k	AICc	Weight	R2m	R2c	Delta.AICc
<i>N</i> =35	Agricultural mosaic	4	−1223.988	0.232	0.069	0.339	0
	Natural mosaic	4	−1223.879	0.22	0.067	0.337	0.109
	Distance	2	−1223.57	0.188	0.034	0.289	0.418
	Agricultural mosaic & water	5	−1221.335	0.062	0.065	0.336	2.653
	Natural mosaic & water	5	−1221.275	0.06	0.062	0.331	2.713
	Null	1	−1209.198	0	0	0.252	14.789
	Agricultural mosaic & natural mosaic & water & artificial & other	16	−1171.115	0	0.067	0.338	52.873
<i>N</i> =30	Distance	2	−878.279	0.413	0.041	0.312	0
	Natural mosaic	4	−876.243	0.149	0.068	0.357	2.036
	Agricultural mosaic	4	−876.207	0.146	0.068	0.358	2.071
	Natural mosaic & water	5	−873.774	0.043	0.068	0.359	4.505
	Agricultural mosaic & water	5	−873.767	0.043	0.067	0.359	4.511
	Null	1	−867.29	0.002	0	0.269	10.989
	Agricultural mosaic & natural mosaic & water & artificial & other	16	−812.371	0	0.068	0.359	65.908

Two datasets were used: (i) all individuals with ‘good origin accuracy’ (*N*=35), and (ii) excluding sun bears from highly developed areas (*N*=30)

The prevailing results are represented here by repetition 1 and 4, respectively. Full results of all 5 repetitions and all combinations in Suppl. File S3

^aDetails in Suppl. File S1

Table 5 Summarised pseudo-bootstrap analysis (1000 bootstraps), including the 5 top ranked models, and full composite models

Dataset	Surface ^a	k	Avg.AICc	Avg.weight	Avg.rank	Avg.R2m	Percent.top
<i>N</i> =35	Agricultural mosaic	4	−656.321	0.043	6.992	0.061	30.5
	Natural mosaic & water	5	−656.481	0.047	7.71	0.057	30.4
	Agricultural mosaic & water	5	−656.412	0.045	7.679	0.059	13.2
	Distance	2	−654.623	0.027	19.436	0.035	8.7
	Other	4	−654.9	0.029	16.901	0.037	7.9
	Agricultural mosaic & natural mosaic & water & artificial & other	16	−654.723	0.019	27.527	0.06	0
<i>N</i> =30	Distance	2	−454.018	0.041	13.064	0.043	34.6
	Agricultural mosaic	4	−454.656	0.043	9.506	0.062	24.1
	Agricultural mosaic & water	5	−454.786	0.043	6.373	0.061	13.3
	Natural mosaic & water	5	−454.782	0.043	6.546	0.061	8.5
	Natural mosaic & other	5	−454.687	0.043	8.863	0.062	6.4
	Agricultural mosaic & natural mosaic & water & artificial & other	16	−452.476	0.013	29.669	0.061	0

Two datasets were used: (i) all individuals with ‘good origin accuracy’ (*N*=35), and (ii) excluding sun bears from highly developed areas (*N*=30)

The prevailing results are represented here by repetition 1 and 4, respectively. Full results of all 5 repetitions and all combinations in Suppl. File S3

^aDetails in Suppl. File S1

genetic variation is explained by geographic distance and/or resistance models based on land cover classes.

The transformations of the single resistance surfaces using both datasets indicated higher resistance due to agricultural and artificial mosaics, low resistance in

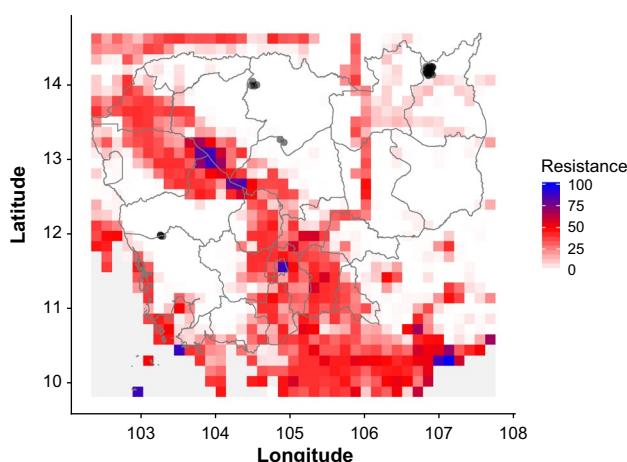


Fig. 4 Composite resistance surface (i.e. all land cover classes) optimized using *resistanceGA*, for the dataset ($N=30$) excluding samples with origins in highly developed areas. Depicted is repetition 4 of the analysis (others are presented in Suppl. File S3)

natural mosaics, and high resistance due to water bodies (Suppl. File S3). When considering all landscape classes, this describes a spatial band of high resistance bisecting Cambodia from northwest to the south (Fig. 4; see also Suppl. File S3).

Discussion

For rare, elusive, and data deficient species, conservation genetics provides valuable information that is difficult or impossible to gain by other means (Frankham, 2015; Schwart et al. 2007). For example, by elucidating population structure, population connectivity and genetic variability, genetic studies provide data to identify conservation priorities and to make informed management decisions aimed at maintaining viable and variable populations (De Salle and Amato 2004; Nunney and Campbell 1993; Pérez et al. 2014).

In this study, we provide (to our knowledge) the first population genetic data for the sun bear *Helarctos malayanus* using nuclear genetic markers. We elucidate the population structure of this data deficient species in Cambodia, where the sun bear is threatened by habitat loss and fragmentation, as well as by human persecution (Scotson et al. 2017; Scotson 2019). Furthermore, we were able to evaluate if there is ongoing gene flow among populations, and considered the ability of the employed markers to assign confiscated bears to their source population. Lastly, we considered what landscape features, both natural and anthropogenic, impact gene flow in Cambodian sun bears.

Population structure in Cambodia

Based on allele frequencies at microsatellite loci we identified two distinct sun bear populations in Cambodia, which we refer to as the West and East populations based on their spatial distribution. This spatial pattern was obvious, despite having reliable information about the geographic origin for only half of our samples, owing to the fact that these were derived from confiscated or donated sun bears ('Free the Bears' sanctuary in Phnom Tamao Zoo and Wildlife Rescue Centre, Cambodia). Trafficking of sun bears also poses a challenge in this respect, as we observed that individuals 'originating' from highly developed areas (e.g. cities) hampered our spatial analyses.

Despite these limitations, the combination of spatial and non-spatial analyses enabled us to obtain a picture of the population structure of Cambodian sun bears, and the microsatellite markers we used allowed us to assign most of the sun bears to one of the two populations, even when information regarding the origin of an individual was lacking. The majority of sun bears (78%) had a high probability of being assigned ($Q \geq 85\%$) to one of the two populations. The degree of admixture of the remaining individuals suggests that there is, or at least has been in the recent past, some genetic exchange between the West and East populations. We encourage further work to address this important issue of population connectivity in Cambodia, and advocate extending this to neighbouring countries as natural dispersal and illegal trade are not restricted by political boundaries. For example, the forests and national parks in Thailand that border Cambodia (e.g. Thap Lan National Park) may be important for connecting Cambodian sun bear populations.

Genetic diversity of Cambodian sun bears

Understanding the structure and health of a population allows conservation scientists to evaluate the protection status of a species or subspecies and to identify populations of conservation concern (Pelletier et al. 2017). Genetic monitoring of *Ursidae* species has allowed researchers and conservationists to identify healthy and endangered populations. The brown bear (*Ursus arctos*) has a large distribution range and is one of the best studied bear species (Swenson et al. 2011; Tammeleht et al. 2010). Genetic monitoring has allowed scientists to recognize that some brown bear populations display signs of significant inbreeding, making them more vulnerable to extinction (Pérez-España et al. 2009). Consequently, these populations receive more protection and a higher conservation priority (Paetkau and Strobeck 1998).

In the absence of other sun bear studies that have examined diversity at nuclear markers, it is difficult to draw concrete conclusions about the potential consequences of the observed genetic diversity in the Cambodian sun bear

populations. While high inbreeding coefficients (significant for the West population) indicate that these populations are genetically impoverished, and are thus of conservation concern, we lack an intraspecific baseline of a ‘healthy’ sun bear population for comparative purposes. This highlights the urgent need to conduct similar studies for other sun bear populations throughout the species’ range.

Although microsatellite data obtained for other species should not be directly compared with the sun bear data, a tentative and superficial comparison to brown bear (*Ursus arctos*) studies shows that genetically diverse populations (e.g. Tammeleht et al. 2010; Straka et al. 2012; Kopatz et al. 2014; Ambarli et al. 2018) in brown bears have observed and expected heterozygosities (H_O and H_E) in the range of 0.69 to 0.83. The values measured for these parameters in the Cambodian sun bear are substantially lower ($H_O=0.48$, $H_E=0.58$). This level of genetic diversity is more similar to that found in the endangered Spanish brown bear population ‘West-Spain’ ($H_O=0.44$, $H_E=0.45$; Pérez-España et al. 2009). Thus, it could be argued (with all due caution) that Cambodian sun bears are of conservation concern and should receive a similar level of protection and conservation focus as Spanish brown bears. However, further molecular data for sun bears is clearly required to provide an intraspecific context within which to consider our results.

Developed land as barrier?

To understand what landscape features may impact the distribution of genetic variation in Cambodian sun bears, we considered the impact of agricultural and natural land cover (incl. water and wetland), as well as that of human settlements. Our results indicate that anthropogenically modified land (agricultural and other human development) has the greatest detrimental impact on genetic exchange among sun bear populations. And that conversely, natural land cover enhances genetic exchange. Our results support the conclusions of a recent study (Scotson 2019), which predicted a divergence of Cambodian sun bear populations due to the negative effects of anthropogenically modified land on sun bear occupancy and survival.

Enhancing connectivity between populations to counteract loss of diversity is a frequently recommended conservation action (e.g. for Spanish brown bears, Pérez-España et al. 2009). Expanding human-altered landscapes have caused deforestation and thus the fragmentation of sun bear habitat. Although sun bears are highly adaptable and have been found using oil palm plantations (Guharajan et al. 2018), they are often killed opportunistically if encountered (Scotson et al. 2014). Human development also includes the development of infrastructure, making the remaining habitat patches more accessible to poachers (Clements et al. 2014) or increasing incidences of roadkill in wildlife

(Cheema 2015; Scotson 2019; Schlossberg 2018). We would thus argue that it is the reduction of human persecution, in addition to halting habitat fragmentation, which needs to be a focus for conservation actions. Despite being a strictly protected species, the existing wildlife protection laws are insufficiently enforced. At the same time, the demand for sun bears and their derivatives is still thriving (INTERPOL 2014; Feng et al. 2009). Sun bear meat is sold on markets, their claws turned into lucky charms, and the bile sold for Traditional Asian medicine (TAM). If a hunted bear is a female with a cub, the cub is then illegally traded as a pet, often advertised on social media (Krishnasamy and Stoner 2016). Cubs that are not sold as pets are often supplied as stock for the bear bile farming industry (Tsai 2008).

Effective conservation actions should thus not only be focused on protecting their natural habitat and enhancing the connectivity of sun bear populations, but must also curtail the illegal trade of sun bears and their derivatives.

Conclusion

In this first study of sun bears using nuclear microsatellites, we have identified two genetically and geographically distinct populations in Cambodia. The two populations have, at best, moderate genetic diversity and show signs of inbreeding. It appears that, at least until the recent past, there has been genetic exchange between these populations, and it should be a priority to further our knowledge in this regard. Both with respect to how this exchange occurs (e.g. in which regions of Cambodia), and to what extent it can be enhanced. To put our results in the context of the entire sun bear meta population, the development of a reference database of the sun bear populations across its distribution range is required (e.g. to gain knowledge regarding the genetic exchange with sun bear populations in Vietnam, Laos and Thailand). Therefore, further microsatellite (or SNP) based research on sun bears is recommended to develop such a reference database. This reference database could also serve as a forensic tool to combat the illegal wildlife trade that is a significant threat to this species.

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Author contribution M.K., J.F. and D.W.F. conceived of the study. M.K. collected samples, obtained permits, and conducted laboratory procedures. M.K., R.M., J.P. and D.W.F. conducted analyses. M.K. and D.W.F. wrote the paper with input from all authors.

Data availability Microsatellite data and detailed information regarding sample origin were deposited in Dryad: <https://doi.org/10.5061/dryad.c2fqz614b> (will be provided upon acceptance).

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