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An evaluation of noninvasive sampling techniques for Malayan sun bears

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Abstract: Traditional mark–recapture studies to estimate abundance and trends of Malayan sun bear (*Helarctos malayanus*) populations are impeded by logistics of live-trapping wild individuals. The development of noninvasive sampling techniques for monitoring sun bear populations is therefore crucial for targeted conservation action. Sun bears have short fur, and conventional hair-snagging devices are ineffective. Moreover, scats are rapidly decomposed by the warm, humid environment, as well as by invertebrates. In combination with camera-sampling, we tested 2 designs of hair traps ($n = 45$) in situ at Tabin Wildlife Reserve in Sabah, Malaysia, during April–October 2017, to obtain hair samples from wild sun bears. We also deployed 4 types of hair traps in rainforest enclosures with captive sun bears to evaluate hair-capture success and the effects of weathering, lure, and adhesive on polymerase chain reaction (PCR) amplification success. Wild adult male sun bears displayed back-rubbing behavior at hair traps and 6 individuals were identified based on unique chest marks. We collected 30 hair samples from wild sun bears, including 15 chest mark images of 6 individuals over 1,260 trap-nights. We detected adult males at hair traps more frequently than females and subadults. We obtained 39 hair samples in the captive trials. Extracted DNA from hair roots successfully amplified with mitochondrial (wild bears: 95%; captive bears: 97%) and microsatellite primers (wild bears: 100%; captive bears 87%). Adhesive and lure type did not affect PCR amplification, but weathering reduced amplification of microsatellite loci. This study is the first successful attempt to obtain genetic samples from wild sun bears using inexpensive, readily available materials such as duct tape, polybutyl glue, and locally sourced lures. The quality of genetic material from these genetic samples should be suitable for studies of population size and gene flow.

Key words: duct tape, genetic samples, hair-sampling, *Helarctos malayanus*, individual identification, Malayan sun bear, noninvasive sampling, population estimation, remote camera surveys

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The Malayan sun bear (*Helarctos malayanus*) is native to Southeast Asia and its distribution is closely tied to tropical forest, including tropical evergreen rainforest in the Sundaland subregion and more seasonal forests of mainland Southeast Asia (Scotson et al. 2017a). Sun bears are classified as "Vulnerable" following an estimated population decline of nearly 30% in the past 30 years, primarily because of habitat loss and poaching associated with illegal trade in bears and bear parts (Foley et al. 2011, Scotson et al. 2017a).

Apart from a study in Thailand (Ngoprasert et al. 2012), few reliable estimates of sun bear population sizes exist, and data on population trends are altogether lacking (Scotson et al. 2017a). Sun bear occupancy is closely tied to forest cover; thus, broad-scale declines in their distribution may be inferred from remote sensing data (Scotson et al. 2017b), but these cannot provide information on population status. Reliable methods for monitoring sun bear populations are therefore critical for focusing conservation efforts.

Studying the population dynamics, spatial ecology, or habitat use of bears requires considerable effort and expense. Research involving live capture and marking

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of sun bears is particularly challenging because of trap avoidance, low densities, and the difficulty of deploying live traps in often rugged, roadless areas (Wong et al. 2004, Fredriksson 2012, Cheah 2013, Guharajan 2016). The highest success rate was reported by Normua et al. (2004), but capture success was still low, at 117 trap-nights/capture. Noninvasive sampling methods, such as remote camera surveys and genetic sampling, require less field effort and have been used successfully in studies of species that are difficult to observe or capture (Long and Zielinski 2008, du Preez et al. 2014, Dumond et al. 2015, Zemanova 2020). However, these techniques have not been applied extensively to studies on sun bears.

With remote cameras, density estimation via a capture–recapture framework can be effective when based on unique morphological features, individual markings, or coat patterns. This method has been used in population studies of several elusive mammals, such as tigers (*Panthera tigris;* Karanth 1995) and wolverines (*Gulo gulo*; Magoun et al. 2011), and a previous remote camera study of sun bears identified individuals and estimated abundance using the highly variable chest marks (Ngoprasert et al. 2012).

Abundance or density estimation is also possible when individuals can be identified from genetic samples obtained from hair or feces, which can additionally be used to examine geographic isolation and connectivity, inbreeding, and parentage and kin structure (Taberlet et al. 1997; Woods et al. 1999; Wilson et al. 2003; Schwartz et al. 2007; Kendall et al. 2009, 2019; Dumond et al. 2015; Palomares et al. 2017). Although successfully applied in numerous bear studies worldwide (e.g., Zhan et al. 2006, Kendall et al. 2009, Dutta et al. 2015, Murphy et al. 2017), genetic sampling of sun bears is fraught with challenges. Fresh scats of this species are difficult to locate in tropical regions, where precipitation and insects rapidly decompose feces and genetic material may degrade rapidly from exposure to sunlight, warm temperatures, and humidity (Stetz et al. 2014, Dumond et al. 2015). Furthermore, commonly used hair-sampling devices, such as barbed-wire corrals (Kendall and McKelvey 2012), may be ineffective for sun bears that typically possess short guard hairs (S.T. Wong, unpublished data).

Camera-sampling and hair-sampling each have their technical and logistical advantages and limitations (Janečka et al. 2011). In this study, we tested and compared noninvasive sampling methods using remote cameras and 2 different designs of hair traps in situ at Tabin Wildlife Reserve, Sabah, Malaysia. We also conducted an independent assessment of hair trap designs in rainforest enclosures at the Bornean Sun Bear Conservation Centre. Specifically, our objectives were to evaluate and compare 1) the effectiveness of hair-sampling and remote camera-sampling for individual identification; and 2) the effects of different lures, adhesives, and hair-snagging designs on polymerase chain reaction (PCR) amplification success.

Study area

We conducted in situ sampling during April–October 2017 at Tabin Wildlife Reserve (5°12.51′N, 118°43.11′E), Sabah, Malaysia. The reserve comprises 112,200 ha of selectively logged forest. Warm, humid conditions characterize the climate and daily rain showers are typical. Annual mean rainfall is approximately 3,000 mm with an annual mean temperature of 27°C (Mitchell 1995, Turner and Foster 2006). Permits for research were obtained from the Sabah Biodiversity Council (JKM/MBS.1000-2/2 JLD.5 [114]).

We conducted tests on captive bears during April–May 2019 at the Bornean Sun Bear Conservation Centre, Sabah, Malaysia, where 2.47 ha of rainforest served as a semi-natural environment for sun bears rescued primarily from the illegal trade in pets and from illegal ownership for commercial or personal reasons. Large chain-linked pens averaging approximately 0.2 ha subdivided the forest enclosure and contained 1–5 individual bears from 0900 to 1530 hours each day.

Methods

Hair-sampling and remote camera-sampling: wild bears

We established 45 sample sites that were spaced >1 km apart and distributed to represent a variety of habitat types and different levels of anthropogenic influence (Fig. 1). We tested 2 hair-sampling designs, each using duct tape (3M Scotch, Maplewood, Minnesota, USA) as the snagging device. The first design was a modification of the corral method developed by Woods et al. (1999); but, rather than barbed wire, we used one strand of steel cable wrapped with duct tape, with the adhesive side facing outward (hereafter, 'wire trap'; Fig. 2a). We positioned the cable 0.5 m from the ground to create a small enclosure around 3–4 corner trees. We baited these sites with 200 g shrimp paste and 2 pieces of salted fish (*Decapterus* spp.) wrapped in black shading net for protection from heavy rain. We hung bait 1.5–1.7 m above ground level to discourage removal, and at the approximate center of the enclosure to encourage bears to step over or crawl under the cable. Based on barbed-wire sam-

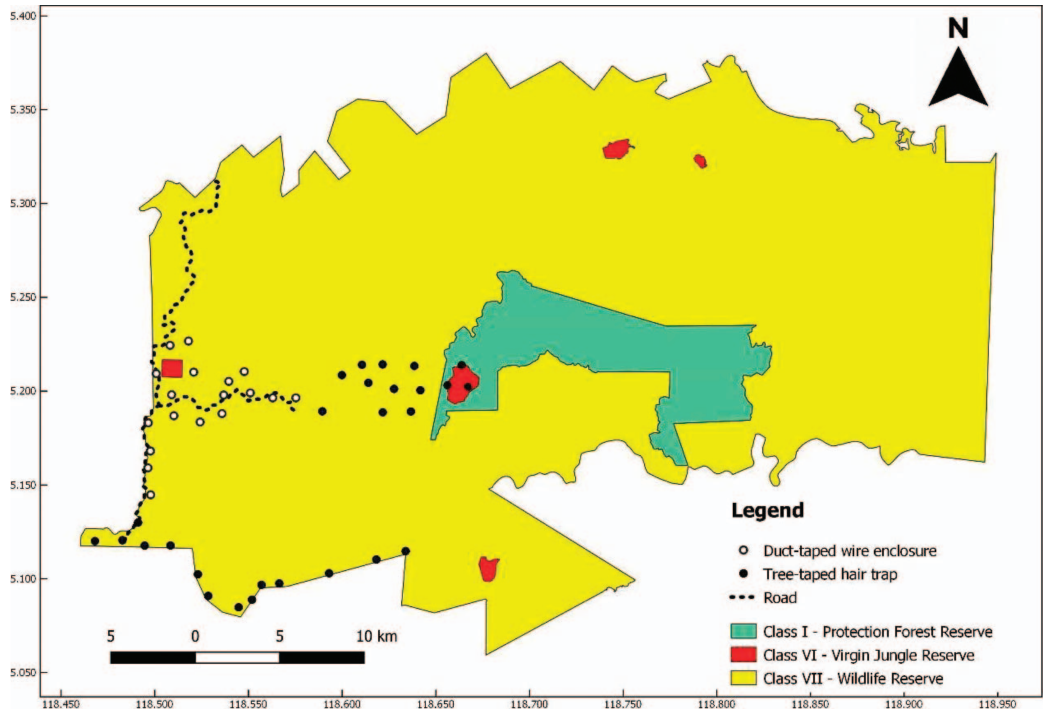

Fig. 1. Distribution of 45 sample sites for noninvasive sampling of sun bears (*Helarctos malayanus*) in Tabin Wildlife Reserve, Sabah, Malaysia, April–October 2017. White circles indicate 18 sites with duct-taped wire enclosures, and black circles indicate 27 sites with tree-taped hair traps. Areas classified as Class VI Virgin Jungle Reserve had no previous history of logging, Class I Protection Forest Reserves had not been logged since 1980, and Class VII Wildlife Reserve Forest was selectively logged in the 1980s (Reynolds et al. 2011).

pling design used in other bear studies, we treated hairs deposited within a 30-cm section of duct tape as an independent sample (Woods et al. 1999, Tredick et al. 2007). We placed a remote camera (Moultrie M-999i or S-50i; EBSCO Industries, Birmingham, Alabama, USA) at each wire trap, approximately 3.5 m from the center of the enclosure to verify whether hair samples were from >1 bear. Remote cameras were mounted on trees approximately 0.5 m above ground, set in motion-detect mode for 24-hour operation, with 3 photos/trigger and 10 seconds of video with no delay.

The second sampling design consisted of duct tape wrapped around a tree with the adhesive side of the tape facing out (hereafter, 'tree trap'; Fig. 2b). The length of the tree trunk covered by tape was 1.5–1.7 m, with the bait tied to the tree above the tape, and the camera placed about 5 m from the baited tree. We spread shrimp paste on the side of the tree facing the remote camera. At each site, we taped 2–3 trees that were positioned <2 m apart. The intent of this design was to encourage sun bears to deposit hair by climbing the tree to reach the bait, or to stand bipedally while rubbing their back against the tree, depositing hair and providing an image of the chest mark for the remote camera. We assumed all hairs collected from a single tree were from one bear, and tested that assumption based on the camera records.

The 45 sample sites consisted of 18 wire traps and 27 tree traps (Fig. 1). We deployed 13–18 sample sites at a time for 28 consecutive days. We visited each site every 7 days to collect hair samples, renew duct tape and bait, and check camera function. We verified sun bear visitation from camera images and videos and claw marks on tree trunks. We collected hairs with visible root bulbs using forceps, depositing each independent sample in a separate envelope labeled with site number, date of bear visit, and date of hair collection. Envelopes were stored in a cabinet away from light and moisture. We used one-sample chi-squared goodness-of-fit tests to assess whether frequencies of obtaining hair samples and chest mark images differed by trap type, with expected frequencies based on the ratio of wire traps to tree traps.

Fig. 2. (a) A hair trap consisting of a duct-taped wire enclosure with bait hung at >1.5 m height to encourage sun bears (*Helarctos malayanus*) to enter the enclosure and make contact with the duct tape; (b) a hair trap consisting of inversed duct tape on a 1.5–1.7-m section of tree where shrimp paste was spread on the tree before wrapping with duct tape. This design encouraged sun bears to stand up on their hind legs to reach the bait, or rub their backs on the tree, exposing their chest mark to the remote camera and depositing hair on the duct tape.

Hair-sampling: captive bears

Our sampling in the captive trials consisted of 4 hair traps (Fig. S1, Supplemental material): barbed wire with barbs coated with polybutyl-based rat glue; a brush-hook design using rat glue; duct tape with metal mesh; and duct tape with plastic mesh. We tested 2 types of lure, applied directly to the tree trunk at the same level as the sampling material: 100 g fermented shrimp paste dissolved in 1.5 L water and an oil in which salted, dried fish had been fried (600 g fish: 1.5 L palm oil).

We deployed hair traps over a 27-day period, distributing the 2 types of lure so that each was used on all 4 trap types. We set up traps before bears were released into forest enclosures each morning and checked traps each evening. All hairs collected from a single trap were considered one sample, after which the trap was reset with fresh materials. We placed hair samples in labeled paper envelopes with forceps, and stored them in a sealed, dry box containing silica. We used half the hair samples to examine the effects of weathering on PCR amplification success; these were placed in petri dishes perforated with holes and exposed to ambient temperature, rainfall, and sunlight for 7 days before storage. We categorized amount of hair recovered in samples based on whether or not they contained $\geq$15 hairs with roots (the threshold at which our DNA elution protocol had to be adjusted).

Table 1. Number of sun bear (*Helarctos malayanus*) hair samples and chest mark images captured using wire traps and tree traps, Tabin Wildlife Reserve, Sabah, Malaysia, April–October 2017. We considered ≥1 sun bear photo at a trap site during a 24-hour period to be an independent detection.

Hair trap type	No. of traps deployed	Sites visited by sun bears	Combined effort (trap-nights)	Independent detections	Hair samples collected	Chest mark captures
Wire trap	18	3	504	7	5	1
Tree trap	27	15	756	34	25	14
Total	45	18	1,260	41	30	15

We used one-sample chi-squared goodness-of-fit tests to assess whether frequencies of obtaining hair samples differed by trap type, or lure type; expected frequencies were based on proportions of different types of trap deployed or types of lure.

Extraction and amplification of DNA from hair samples

We extracted DNA from hair samples using the Qiagen Investigator Kit (Qiagen Company, Hilden, Germany). Samples with ≥15 hairs were eluted twice at 20 μL and samples with <15 hairs were eluted twice at 15 μL. DNA concentration (μg/μL), and absorbance readings at different wavelengths (A 260/230 and A 260/280) were determined using Biodrop μlite (Biodrop UK Ltd, Cambridge, UK). We used 2 sets of primers designed specifically for sun bears (W. Ling Lai, Sunway University, unpublished data): a mitochondrial marker (MtCR1, F: ACCTACTAACACTAACATGA, R: CATGACACCACAGTTATGTG) targeting a 764-basepair (bp) portion of the sun bear mitochondrial genome, including a part of the CYTB gene, tRNA-Thr, tRNA-Pro and the partial 5' end of the control region; and a nuclear microsatellite marker with a trinucleotide repeat (GAA; MS38, F: AAGACGGCTCAGAACAGAGG, R: GCAAGGCCTGAGACAGATGT) with an expected amplification product of 282 bp.

We conducted amplification in a total volume of 20 μL, containing 30 ng of total DNA, 10 μL of 2× ExPrime Taq Premix (with ExPrime Taq DNA Polymerase 1 unit/10μL, 2× reaction buffer, 4mM $MgCl_2$, enzyme stabilizer, sediment loading dye, pH 9.0, 0.5 mM of each dNTP; GeNet Bio Company Ltd, Daejeon, Republic of Korea), and 1 μM each of MtCR1 forward and reverse primers. The PCR cycling conditions started with an initial denaturation at 95°C for 6 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing temperature at 55°C for 30 seconds, and extension at 72°C for 1 minute. The last step was final extension at 72°C for 10 minutes and holding at 4°C. For microsatellite markers, we adjusted the annealing temperature to 64°C with 34 repeat cycles. We subjected amplified DNA samples to gel electrophoresis to view the amplification products (2% Agarose gel, 80 V, 70 min).

Results

Hair-sampling and remote camera-sampling: wild bears

Three of 18 wire traps and 15 of 27 tree traps were visited by sun bears in 1,260 trap-nights of sampling. We obtained 5 hair samples from wire traps (including 3 from the same bear during a single visit) and 25 hair samples from tree traps (Table 1). Based on sampling effort, tree traps were significantly more successful than wire traps at capturing hair samples ($\chi^2 = 6.81$, 1 df, $P = 0.009$, $n = 45$). Camera images confirmed visits by adult males 21 times, 2 visits by an adult female with a cub, 6 visits by 1 bear or a pair whereby sex could not be verified, and 1 visit where the camera malfunctioned but claw marks confirmed a bear visit. Overall, cameras confirmed 1.4 times more visits to sites than did hair samples.

Tree traps also were more successful than wire traps at capturing images of a bear's chest mark (Table 1; $\chi^2 = 6.94$, 1 df, $P = 0.008$, $n = 45$). At tree traps, some bears stood up to rub their backs on the trunk, depositing hair on the tape and exposing their chest mark to the camera (Fig. 3). We obtained 26 independent detections of this back-rubbing behavior, solely involving adult males, with 20 yielding hair samples. Sun bears also deposited hair by climbing the tree to reach the bait, hugging the tree, or rubbing other parts of their body (e.g., head and neck) against the tree. Male and female bears also were recorded rolling where liquefied bait had dripped on the ground, but this behavior produced no clear images of chest marks. Eight hair samples were from sites visited by multiple bears or were from multiple visits during a 7-day period, and we treated those as mixed samples. Assuming that the remaining 22 hair samples could be successfully

Fig. 3. Individual sun bears (*Helarctos malayanus*) identified at tree-taped hair traps, exposing their chest mark while rubbing on a bait-smeared tree trunk, Tabin Wildlife Reserve, Sabah, Malaysia, April–October 2017. Images showed that ≥4 of the 5 unique individuals (a, b, c, and e) were males based on genitalia.

genotyped, hair samples were 1.5 times more likely to provide individual identification than chest mark images.

We identified 1 individual from the wire traps based on a single image, and 5 individuals from the tree traps based on 14 chest mark images from video capture (Fig. 3). Tree trap images confirmed 8 recaptures of 1 individual at 4 different locations and 2 recaptures of a second individual at the same location (Fig. 4). At least 4 of the 5 individual bears identified at the tree traps were males (Fig. 3).

DNA extraction yielded approximately 300 ng of DNA per sample (30 μL of elution with DNA concentration of approx. 10 ng/μL). We extracted and amplified DNA from 22 unmixed hair samples. Mitochondrial primers produced a single band at the expected molecular weight for all but one of the 22 samples (95%; Fig. 5). The microsatellite primer amplified all 22 samples, producing a band at the expected position. Three samples were sequenced, confirming amplification of the correct locus of the targeted mitochondrial region and microsatellite regions.

Hair-sampling: captive sun bears

We recovered hair with roots from 39 of 51 (76.5%) deployed hair traps over 27 days (Table S1, Supplemental material). We obtained 24, 13, and 2 hair samples, respectively, from hair-trapping trials in pens containing bears of both sexes ($n = 27$ trials), females only ($n = 19$ trials), and 1 adult male ($n = 5$ trials). Although male–female pens yielded a larger proportion of samples (88%) than female-only pens (68%), female-only pens had fewer bears on average. Bears responded to hair traps by sniffing and manipulating them with paws or rubbing their heads and necks on the baited surface. Based on sampling effort (no. of traps of each type deployed), no single trap design surpassed the others in snagging hair ($\chi^2 = 0.23$, 3 df, $P = 0.970$; Fig. 6), or obtaining ≥15 hairs with roots in a sample ($\chi^2 = 3.68$, 3 df, $P = 0.300$). However, traps using duct tape ($n = 15$, 29%) were twice as likely as those with polybutyl glue ($n = 7$, 14%) to trap ≥15 hairs with roots. Success rates of obtaining hair with roots at traps using shrimp paste (81%) and fish oil (76%) did not differ ($\chi^2 = 0.037$, 1 df, $P = 0.850$).

Amplification of extracted DNA with mitochondrial primers was successful for 38 of the 39 samples (97%); each sample produced a single bright band on agarose gels at the expected position with no noticeable difference in band brightness between fresh and weathered samples, or among trap types (Fig. S2, Supplemental material).

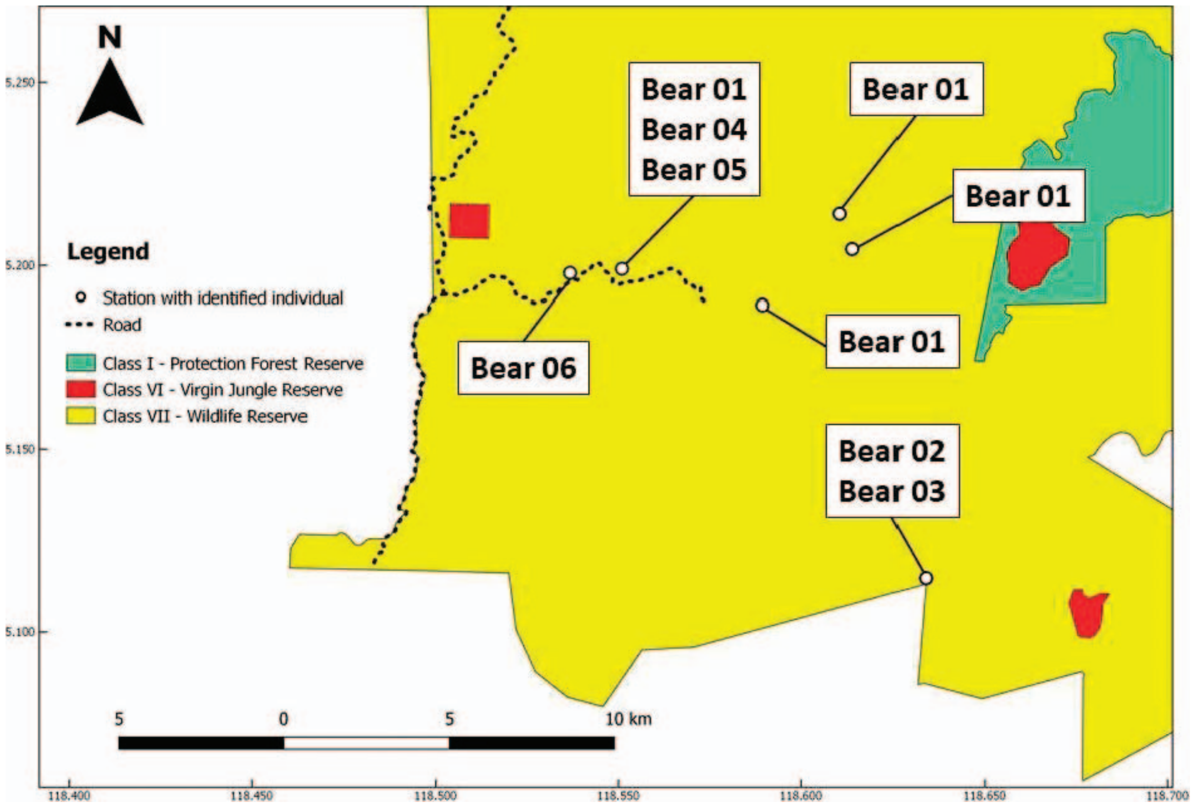

Fig. 4. Locations of identified sun bears (*Helarctos malayanus*) based on chest markings in Tabin Wildlife Reserve, Sabah, Malaysia, April–October 2017. Bear 01, Bear 02, Bear 03, Bear 04, and Bear 05 were identified using tree-taped hair traps. Bear 06 was identified using a duct taped-wire enclosure.

Microsatellite primers produced clear bands at the expected position for all fresh samples ($n = 19$) and 10 of 20 weathered samples, with the remainder of weathered samples showing faint ($n = 5$) or no bands ($n = 5$), for a total amplification success of 34 out of 39 samples (87%).

Discussion

Although camera detections confirmed more visits by sun bears than did hair samples, our results suggest that hair samples may provide more data for identifying individuals. The combination of remote camera and genetic data enhanced our ability to identify unique individuals and determine recaptures. We successfully extracted and amplified DNA from the hair samples using mitochondrial and microsatellite primers developed for this species. The tree trap was the most effective design and most practical for field application, but our findings also suggest that snaring devices deploying duct tape or polybutyl glue may produce sufficient quantities of hair and yield amplifiable DNA. Designs using shrimp paste and fish oil as lures were similarly successful. Traps using duct tape may yield more hair with root bulbs on average, possibly because the adhesive surface area may be easily increased, unlike polybutyl glue, which is viscous and difficult to spread.

In our in situ tests, the wire corrals were less effective than tree traps, which may be due in part to hesitation among bears to enter the cable enclosure. For example, we had 2 photos of sun bears at the perimeter of the wire trap that did not subsequently enter the enclosure. We did not observe such hesitation with tree traps and had greater success with obtaining hair samples. Tree traps only require a small quantity of bait, duct tape, and a remote camera; therefore, they present a promising technique for field deployment, particularly in roadless and rugged study areas. However, we note that one drawback to the use of duct tape as a hair-sampling device is that

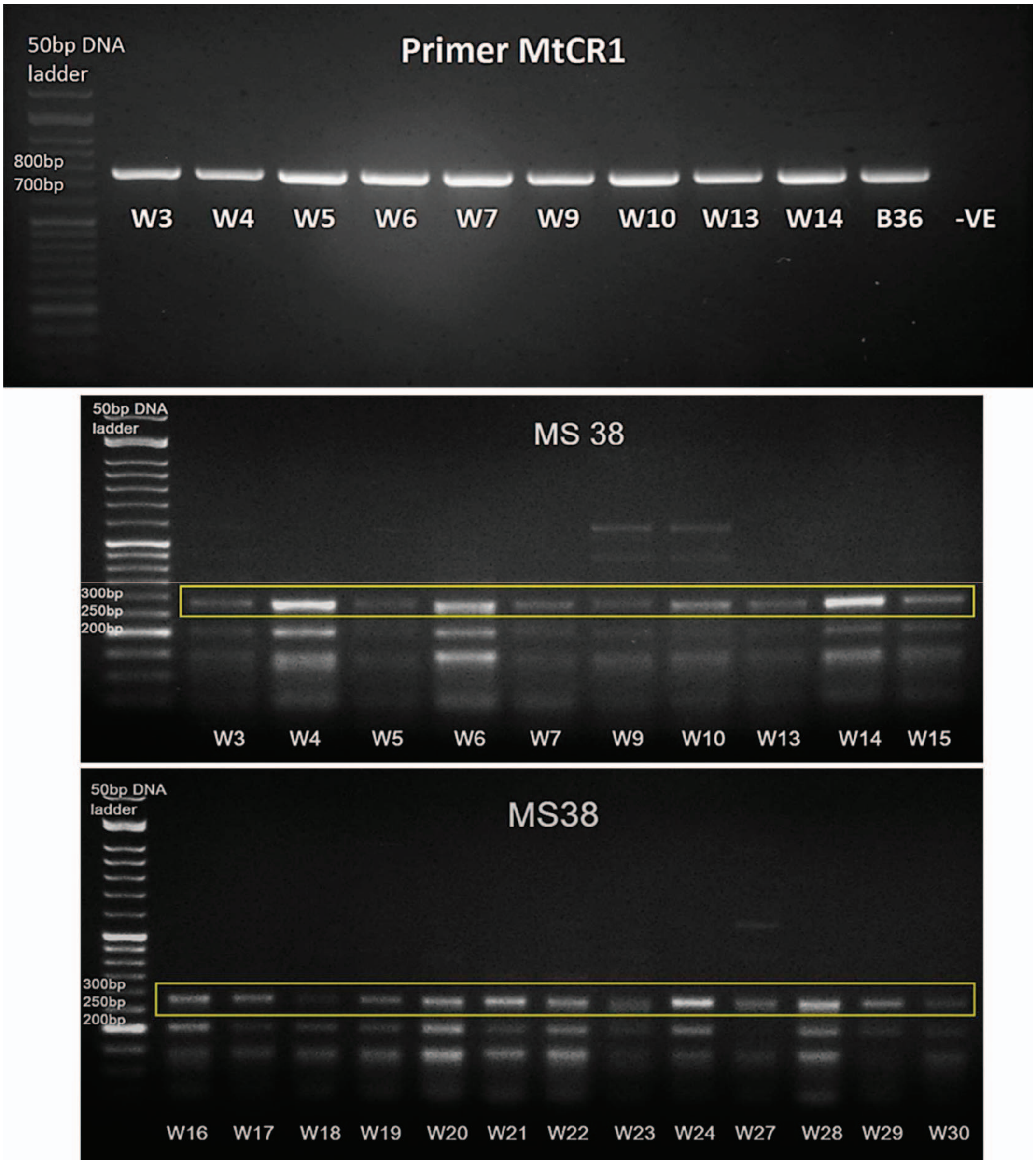

Fig. 5. Examples of polymerase chain reaction (PCR) amplification of DNA from hair roots collected from wild bears in Tabin Wildlife Reserve, Sabah, Malaysia, April–October 2017. The mtCR1 fragment is a 764-basepair (bp) portion of the mitochondrial control region; MS 38 is a nuclear microsatellite marker with a trinucleotide repeat (GAA) and an expected amplification product of 282 bp; B36 was a positive control using hairs plucked from a captive sun bear (*Helarctos malayanus*); -VE was a negative control. Expected fragment of the microsatellite is outlined in yellow.

we unintentionally trapped small reptiles on 3 occasions; in such situations, a small amount of mineral oil may be used to release the animal from the adhesive surface of the tape (Parkhurst 2009). The shrimp paste and salted fish bait remained intact for ≥14 days, despite daily showers and humid conditions, continuing to emit a detectable odor. In comparison, Ngoprasert et al. (2012) used 6 kg of raw meat and 3 remote camera units per trap site to

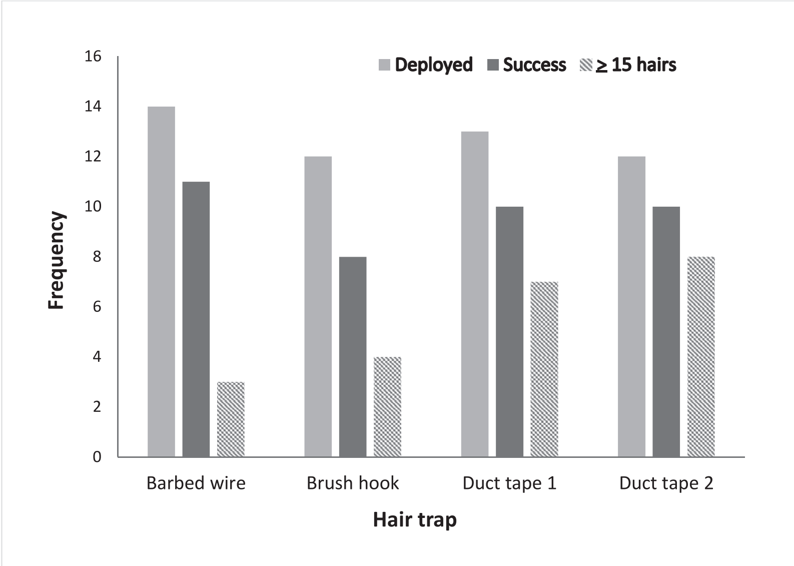

Fig. 6. Number and type of hair traps deployed at the Bornean Sun Bear Conservation Centre, Sabah, Malaysia, April–October 2017, and success rates of obtaining hairs from sun bears (*Helarctos malayanus*). Barbed wire = barbed wire with barbs coated with polybutyl-based rat glue; Brush hook = brush-hook design using rat glue; Duct tape 1 = duct tape with metal mesh; and Duct tape 2 = duct tape with plastic mesh (see Fig. S1 for more detailed description).

obtain photos of chest marks. Sun bears display their unique chest mark when they are standing upright on their hind legs, and our tree-trap design effectively captured chest mark images. We identified 5 individual sun bears from 14 chest mark images using tree traps (with 10 images representing recaptures of 2 individuals), whereas we identified only 1 individual based on a single image at a wire trap. All bears that exhibited back-rubbing behavior subsequently climbed the tree to reach the bait. This behavior allowed confirmation of sun bear presence when cameras malfunctioned, as evidenced by claw marks. Captive bears at the Bornean Sun Bear Conservation Centre do not display the classic tree-rubbing behavior of other bear species. However, they do rub their heads and upper body against substances with strong odors, such as durian (*Durio* spp.) fruit pulp. Similar to the captive bears, our data indicate that tree-rubbing in wild sun bears may be stimulated by substances with strong scents, such as shrimp paste, which enhanced the effectiveness of the tree-trap design. It is unknown whether tree-rubbing by wild adult male sun bears may also be stimulated by the presence of estrous females (e.g., as in American black bears [*Ursus americanus;* Taylor et al. 2015]). If so, this behavior is unlikely to be seasonal in sun bears, where estrus and births occur throughout the year.

Singleplex PCR analysis of weathered hair samples from our captive trials provided some evidence that degradation may indeed occur and affect amplification success for microsatellite loci. Nevertheless, all except one of the in situ samples amplified successfully using one round of amplification with mitochondrial and microsatellite, which confirms that the quality of genetic material is sufficient for studies requiring individual identification. Furthermore, sufficient volumes of DNA were recovered from in situ samples to permit ≥10 singleplex reactions. Microsatellite markers with smaller fragment sizes than used in this study may successfully amplify even partially degraded samples. Given recent advances in optimized multiplex PCR protocols for samples holding very small quantities of DNA (Sharma et al. 2013, Tumendemberel et al. 2019), our findings hold much promise for future studies of genetic structure, connectivity, and population size in sun bears.

Although our trials with captive bears did not suggest that female bears avoided hair traps, data from Tabin Wildlife Reserve indicated that females and juvenile sun bears may be under-represented. Heterogeneity in detection probability is a common problem in wildlife studies, but may be addressed by adapting the sampling design (Ebert et al. 2010). In our study, capture heterogeneity may have been a function of relatively limited spatial coverage of sample sites. Sex-specific biases in detection probabilities commonly occur in bear studies (Laufenberg et al. 2013). Differences in space use between males and females often is a contributing factor, with male detection probabilities enhanced because of larger home ranges and movements. Increasing spatial sampling intensity (i.e., reducing distance among hair traps), sampling a larger portion of the population by expanding the geographic area, and increasing the number of trap-nights may improve female detection rates (Ríos-Uzeda et al. 2007). Advances in analytical techniques can also be used to account for heterogeneity in detection probabilities, but require sufficient sample sizes (Laufenberg et al. 2013). Remote camera images often cannot provide conclusive evidence regarding the sex of sampled individuals, so future analyses would be enhanced with a genetic marker for sex (Bidon et al. 2013). Behavioral responses also can affect detection probabilities and may have played a role in our study as well. For example, there may be a tendency for female sun bears (particularly those with young) to be more cautious and less likely to investigate novel objects, including lures and bait or the white surface of duct tape, which is highly conspicuous in forests. Locating natural travel corridors and strategically applying potential surfaces of contact (e.g., sides of tree trunks or the undersurface of twigs) with inconspicuous polybutyl glue may increase female and subadult representation in hair samples.

Management implications

This study was a systematic attempt to develop standardized and logistically feasible techniques for obtaining genetic samples from wild sun bears. Our results suggest that opportunities exist to enhance density estimation of sun bear populations with a technique that can simultaneously obtain hair samples and chest mark photos and the tree-trap technique presents a particularly promising approach. Equipment and supply needs for the genetic sampling are modest, thus facilitating deployment in areas where access is limited and resources to conduct population studies are scarce. This technique only requires a single remote camera for visual identification of unique individuals based on chest marks, but video recording capabilities are essential to do so reliably.

The sampling techniques we tested were not designed to obtain a single-catch sample (e.g., Beier et al. 2005). Over one-fourth of all hair samples were collected at sites visited by multiple bears during a trap session. To reduce the probability of collecting mixed hair samples, we recommend >2 sampling trees/site at approximately 2-m intervals, omitting bait, and using only shrimp paste as a scent lure. Additional investigations are needed to evaluate whether lack of bait reduces the effectiveness of sampling and to address potential sampling bias toward adult males.

Acknowledgments

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Supplemental material

Table S1. Number of hair traps of each type deployed on captive sun bears (*Helarctos malayanus*), type of lure used, number of traps yielding hairs with roots (trap success) and number of traps yielding ≥15 hairs with roots.

Fig. S1. Hair traps deployed at the Bornean Sun Bear Conservation Centre, Sabah, Borneo: (A) barbed wire with barbs trimmed to 0.5 cm and coated with polybutyl-based rat glue (Affluent Cycle Industries Sdn. Bhd, Beranang, Malaysia) wrapped around a tree trunk; (B) a brush-hook design consisting of plywood (25 × 20 × 0.9 cm) wired to a tree, to which we attached a plastic brush (14 × 6 cm) with stiff bristles and ∼10 metal hooks (3 cm) coated with rat glue; (C) duct tape design #1, with strips of inversed duct tape affixed to plywood (40 × 40 cm) over which a sheet of metal mesh (1.5 cm) was affixed to protect hair follicles from embedding in the adhesive when a sun bear (*Helarctos malayanus*) rubbed on it; and (D) duct tape design #2 with strips of inversed duct tape wrapped over black plastic mesh (120 × 30 × 60 cm; 1.5-cm mesh size), with the mesh serving to reduce residue from the lure attaching to hair follicles.

Fig. S2. Examples of polymerase chain reaction (PCR) amplification of DNA from roots of captive sun bear (*Helarctos malayanus*) hair collected from different types of hair traps, lures, and adhesives. The amplified fragment is a 764-basepair (bp) portion of the mitochondrial control region. Fresh controls (FC) and weathered controls (WC) consisted of hairs plucked from sun bears during routine veterinary examinations.