

1 **Trace DNA from kill sites identifies predating tigers**

2 Himanshu Chhattani<sup>1,2,3\*</sup>, Abishek Harihar<sup>2,4</sup>, Rounak Dean<sup>1</sup>, Ajay Yadav<sup>1</sup>, Kaushal Patel<sup>1</sup>,

3 Divyashree Rana<sup>1</sup>, Awadhesh Pandit<sup>1</sup>, Sanjay Kumar Shukla<sup>5</sup>, Vincent Rahim<sup>5</sup>, Uma

4 Ramakrishnan<sup>1\*</sup>

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7 <sup>1</sup> National Centre for Biological Sciences, TIFR, GKVK Campus, Bangalore 560065, India

8 <sup>2</sup> Panthera, 8 West 40th Street, 18th Floor, New York, NY 10018, United States

9 <sup>3</sup> Sastra University, Tirumalaisamudram, Thanjavur, India

10 <sup>4</sup> Nature Conservation Foundation, 1311, "Amritha", 12th Main, Vijayanagar 1st Stage,

11 Mysore 570 017, India

12 <sup>5</sup> Madhya Pradesh Forest Department, Bhopal, India

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15 \*corresponding authors: [himanshuchhattani@gmail.com](mailto:himanshuchhattani@gmail.com), [uramakri@ncbs.res.in](mailto:uramakri@ncbs.res.in)

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18 Running title: Predating tigers are identified by trace DNA

## 19 **Abstract**

20 Predation ecology and evidence-based conflict management strategies require reliable and  
21 accurate identification of individual predators. Identifying predators is, however, complex,  
22 as they are secretive and individual identification is difficult. Trace DNA that predators leave  
23 behind at kill sites might provide an effective strategy to identify them but remains poorly  
24 evaluated at scale. We use non-invasive genetic samples from kill sites to assess their utility  
25 for predator identification. We systematically investigated 198 livestock kills in two critical  
26 source tiger populations in central India: Kanha and Bandhavgarh Tiger Reserves. We  
27 collected 342 salivary swabs from carcasses, 33 scat and 395 shed hair samples as potential  
28 sources of predator DNA, and individual tigers were identified using up to 123 SNP markers.  
29 All three sample sources identified predator species with high success (>95%). We identified  
30 individuals (with at least one sample per kill site, based on >40 SNPs) at 86% of all kill sites  
31 where tigers were detected. Shed hair samples were most effective for individual  
32 identification, followed by saliva and scat. Sample source and sampling season were the  
33 primary determinants of the number of SNPs typed per sample and the success of individual  
34 identification. Based on the site and type of sample collection, we classify species and  
35 individuals into three categories: true predator (high confidence as predator), circumstantial  
36 predator (medium confidence) and predator uncertain (low confidence). Individuals were  
37 classified as a true predator at 72 sites, circumstantial predator at 34 sites and predator  
38 uncertain at 49 sites. Our protocol allowed us to differentiate between predators and  
39 scavengers, even when multiple tigers were detected at the same kill site. Surprisingly,  
40 ~40% of Bandhavgarh's tigers were identified at at least one kill site. We suggest that when  
41 paired with systematic kill site investigation and sample collection, these methods can be

- 42 effectively used to understand predation ecology better and facilitate evidence-based
- 43 conflict management.
- 44 **Keywords:** human-wildlife conflict, predation ecology, central India, non-invasive genetic
- 45 sampling, tiger conservation

## 46 **1. Introduction**

47 Variation in predation behaviour within a population can be significantly influenced by  
48 specific traits, such as sex, age, morphology/phenotype as well as individual behavioural  
49 specialisation (Berezowska-Cnota et al., 2023; Bolnick et al., 2003; Dickman & Newsome,  
50 2015; Estes et al., 2003; Scholz et al., 2020; Voigt et al., 2018). Understanding these  
51 processes at the individual level is crucial but challenging because of difficulties in reliably  
52 establishing which individuals are involved in predation events. Large carnivores often range  
53 over vast home ranges, have overlapping territories (both with individuals of the same  
54 species as well as other carnivore species), exhibit sociality and engage in scavenging and  
55 kleptoparasitism (Balme et al., 2017; Chundawat et al., 2016; Périquet et al., 2015). These  
56 traits and their elusive nature and absence of distinct individual markings in some species  
57 make it difficult to attribute a predation event to a specific individual.

58 Human carnivore conflicts, such as large carnivores' attacks on livestock and humans,  
59 present another case where reliable predator identification is critical (Goodrich, 2010; Swan  
60 et al., 2017; Treves & Karanth, 2003). Globally, conflict mitigation involves the removal of  
61 individuals through translocation or lethal measures (Linnell et al., 1999; Swan et al., 2017).  
62 However, a challenge with these approaches is unreliable identification and the consequent  
63 removal of non-target individuals (Treves & Karanth, 2003). This can not only leave conflict  
64 unsolved but can also cause social disruptions in carnivore communities, increase conflict  
65 frequency, and invite further public criticism (Athreya et al., 2011; Sinha, 2018; Woodroffe &  
66 Frank, 2005). Therefore, for such management interventions to be effective, it is important  
67 to make evidence-based decisions starting with reliable individual identification.

68 Predation events are rarely witnessed, and accurate identification during such events is  
69 even more arduous. Conventionally used methods, such as visual analysis of prey remains  
70 and deployment of camera traps at kill sites, can lead to unreliable identification. Field  
71 assessments are restricted to identifying species, are highly dependent on the field expertise  
72 of the examiner, and limit retrospective use of data (Mumma et al., 2014). They can suffer  
73 from inaccuracy when species overlap in killing and feeding characteristics (Verzuh et al.,  
74 2018). Camera traps can be highly efficient in identification if deployed at a predation site  
75 before a predation event, such as when monitoring bird nests (Steffens et al. 2012);  
76 however, their use is limited in post-predation events where they might capture visiting,  
77 scavenging species/individuals (Steffens et al., 2012). Further, identifying individual  
78 predators using images becomes a challenge when individuals lack unique natural marks for  
79 identification.

80 Trace DNA of the predator left at the kill sites in the form of its saliva, shed hair, urine, scat,  
81 etc., can often be the only evidence for conclusive predator identification. Non-invasive  
82 genetic sampling, therefore, presents a promising approach to reliable and accurate  
83 identification of predators from kill sites (Blejwas et al., 2006; Fotedar et al., 2019; Nichols et  
84 al., 2012; Sundqvist et al., 2008). This approach has advantages over conventional methods  
85 as it is more sensitive and safer (for researchers and animals), can distinguish individuals of  
86 a species even without natural markings, and, most importantly, can distinguish a predator  
87 from a scavenger when systematically sampled. While being practical, the success of  
88 genetic samples can strongly be influenced by factors such as abiotic conditions (e.g.,  
89 temperature, rainfall, light), biotic factors (e.g., microbial activity, prey and predator species,  
90 maggot infestation), as well as sample collection procedures and storage methods (Harms et

91 al., 2015; Nakamura et al., 2017; Piaggio et al., 2020; Reddy et al., 2012). If not collected  
92 systematically, genetic samples may also lead to incorrect attribution of predation events to  
93 a scavenger.

94 In this study, we attempt to evaluate and use non-invasive genetic samples from kill sites for  
95 predator identification. We do this by systematically sampling suspected tiger kill sites at  
96 two source populations in the central Indian tiger landscape. By collecting saliva, shed hair  
97 and scat samples of potential predators, we identify individual tigers using next-generation  
98 sequencing methods. We chose to sample livestock kills in these tiger reserves to evaluate  
99 molecular techniques for the following reasons: (i) livestock kills are frequently reported for  
100 financial compensation to the forest department, thereby providing opportunities to collect  
101 samples in comparison to wild prey kills, which are challenging to detect, (ii) livestock kills  
102 are quickly reported (usually <24 - 48 hours) providing an opportunity to collect fresh  
103 samples, (iii) multiple sample sources (saliva, shed hair, scat) can be collected from kill sites  
104 for comparison and, (iv) importantly, predator identification from livestock kill sites has  
105 management importance.

106 Specifically, we examined the influence of environmental factors such as season and kill site  
107 conditions on the number of SNPs typed and individual identification success. Based on our  
108 results, we propose a framework to identify and classify individual predators identified from  
109 a kill site and recommend sampling strategies for individual identification. Finally, we  
110 discuss the significance of our results, both methodological and conceptual, for conservation  
111 and management.

112

## 113 **2. Materials and Methods**

114 Fieldwork for this study was carried out in two phases. The first phase was conducted in  
115 Kanha Tiger Reserve (hereafter, Kanha) in 2017, during which we explored the availability  
116 and abundance of genetic samples along with varied approaches to kill site investigation,  
117 including sample collection. In the second phase, we conducted extensive sampling in  
118 Bandhavgarh Tiger Reserve (hereafter, Bandhavgarh) from April 2021 to March 2022. In  
119 both reserves, we systematically investigated livestock kills reported to be made by tigers by  
120 the livestock owners and forest department staff. We collected saliva, shed hair and scat  
121 samples as potential sources of predator DNA.

### 122 **2.1 Study area**

123 Kanha and Bandhavgarh have been classified as tropical moist deciduous forests with four  
124 main habitat types: grasslands, pure sal forest, miscellaneous forest and bamboo mixed  
125 forest (Awasthi et al., 2016; Champion & Seth, 1968). Kanha has an area of 2,074 km<sup>2</sup> and is  
126 divided into two management units: the inviolate core (940 km<sup>2</sup>) with minimum human  
127 presence and a multiple-use buffer (1134 km<sup>2</sup>). Bandhavgarh spans 1,537 km<sup>2</sup>, with a core  
128 of 717 km<sup>2</sup> and a buffer of 820 km<sup>2</sup>. The reserves harbour over 100 tigers each (Kanha: 129,  
129 Bandhavgarh: 165) and are critical source populations for tigers in the central Indian  
130 landscape (Qureshi et al., 2023). The local economy is largely dependent on agriculture,  
131 livestock rearing and tourism. Kanha reserve has an estimated population of 137,600  
132 humans along with 91,900 cattle in the core and buffer area (Negi & Shukla, 2011).  
133 Bandhavgarh has at least 130 villages in the park's buffer area with ~110,000 cattle heads.  
134 The resulting overlap of high human use area with that of high tiger density often results in  
135 conflicts mainly in the form of livestock depredation (annual livestock kills in Kanha range

136 from 400-600 (Negi & Shukla, 2011), while in Bandhavgarh range from 2500-2800) and,  
137 sometimes, human and tiger mortalities. Addressing conflict, therefore, is a key imperative  
138 for reserve management.

## 139 **FIELD METHODS**

### 140 **2.2 Kill site information**

141 We investigated livestock kill sites that were reported by the livestock owners to the forest  
142 department for financial compensation. Both reserves have a similar, established system of  
143 reporting kills. When a livestock carcass is located and reported to the beat guard of the  
144 area by the livestock owner, the guard then verifies the claim by physically visiting the kill  
145 site and confirming the involvement of a carnivore in making the kill. Predator identification  
146 by the forest department from the kill site is based on field signs. Once verified, the beat  
147 guard passes the message to the concerned range office, which then shares the information  
148 with the park field director's office to initiate compensatory payment. This process, from  
149 initial detection by the livestock owner to final reporting to the field director's office, usually  
150 takes 24 to 48 hours.

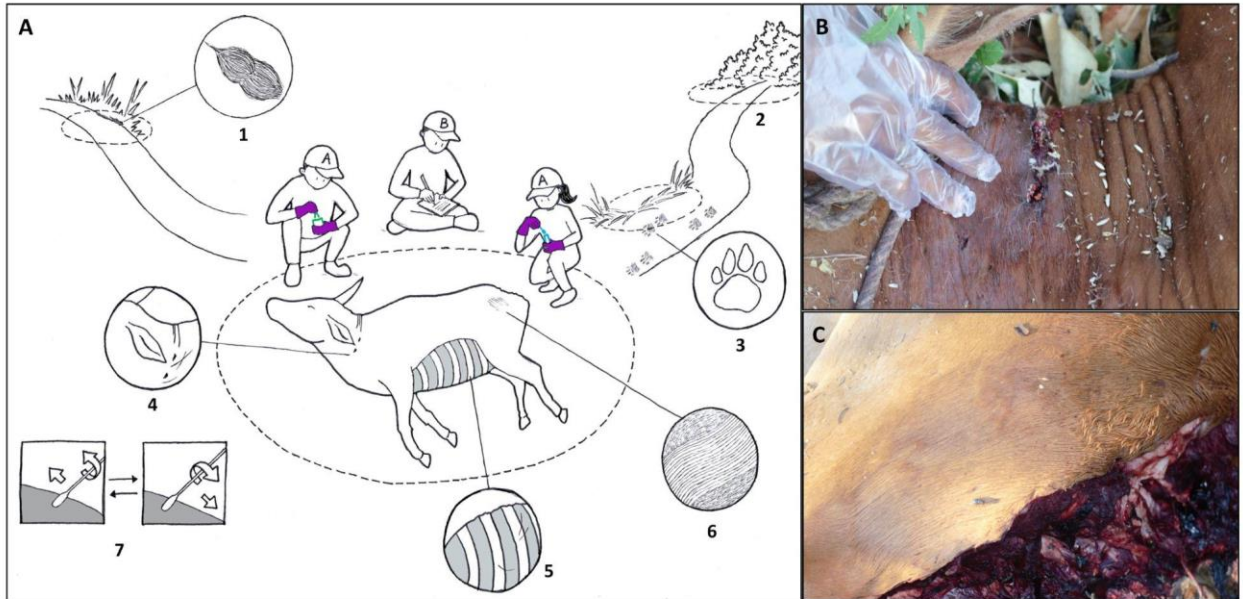
151 At each kill site, we first noted the GPS location, date and time of sample collection, and  
152 identity of the investigating team and the sample collector. We collected information on  
153 prey species, age and kill date, time and rainfall from the livestock owner. We recorded the  
154 forest department's assessment of the predating species. This was done before our  
155 investigation to avoid influencing the department's assessment. Additional observations  
156 such as drag distance (in metres, from the inferred kill location to the location of the  
157 carcass, wherever visible), percentage consumption (based on visual estimate), feeding



158 pattern (parts consumed, removal of tail and intestines, etc.), maggot infestation (visual  
159 estimate, categorised as high/medium/low), canopy cover over the carcass (visual estimate  
160 in %) were noted. Photographs of the carcass were taken at the site.

161 At each kill site, we began our examination by conducting a quick 'walk through' to gain an  
162 overview, identify sites with evidence and inferred kill location. An intensive search for  
163 evidence was carried out along the drag marks between the carcass location and the  
164 inferred kill location. We scanned animal trails and potential carnivore resting areas for  
165 shed hair and faecal (scat) samples. Our search around the kill site and on the trails was  
166 limited to a distance of 30 meters. This was done to reduce the chances of encountering  
167 other carnivore samples since both reserves are high carnivore density areas. Carnivore  
168 resting areas were identified based on anomalous vegetation and impressions on soil.  
169 Pugmarks, scrape marks and scats detected near the kill sites were assigned to carnivore  
170 species based on characteristics like shape, size, etc., following published field manuals  
171 (Karanth & Nichols, 2002). Active care was taken not to disturb evidence during this search.  
172 However, the forest department/livestock owner had, in some cases, cleared the  
173 surrounding vegetation to ease access to the carcass before our arrival. Two people  
174 conducted searches independently to maximise sample collections and reduce detection  
175 bias. Sites where carnivore pugmarks of different characteristics (shape, size), multiple  
176 resting sites (of varying sizes), irregular lick marks and multiple scat samples were found,  
177 were noted as kill sites with multiple carnivore presence. Once initial scene documentation  
178 was completed, we decided on the collection order and initiated sample collection. We  
179 collected saliva samples first (starting from predation wounds) and then scat and shed hair

180 to reduce the chances of sample contamination. We tried to decrease the time spent and  
181 disturbance caused at a kill site so as not to disturb the kill site.



182  
183 **Figure 1:** A) Field sampling scheme illustrating various types of genetic samples of potential  
184 predators collected at a kill site: 1) scat sample collected using swab 2) inferred kill location  
185 is a source of shed hair sample 3) resting sites are a source of shed hair samples, 4)  
186 predation marks in the neck region are a source of saliva samples, 5) feeding area source of  
187 saliva and shed hair samples, 6) lick marks characterised by unidirectional bend of hair are  
188 source of saliva samples and 7) showing swabbing technique. Samples collected from 2 and  
189 4 were classified as predation samples, whereas the rest were classified as post-predation  
190 samples. B) Photograph of predation wound in neck region with visible saliva deposit around  
191 the puncture region (darker colour) and C) Photograph of carnivore lick areas with saliva  
192 deposit.

193

### 194 **2.3 Non-invasive genetic sampling**

195 We collected genetic evidence from salivary marks, shed hair, and scats at each kill site.

196 Sampling kits were not opened until all ancillary information was collected. To minimise the  
197 risk of on-field sample contamination, we changed gloves while collecting different samples  
198 and stored samples in different zip lock bags. Shed hair samples were collected  
199 independently from the various locations using sterile forceps and stored separately in zip  
200 lock bags or sterile 2 ml tubes to avoid sample contamination. Traces of potential carnivore  
201 saliva from the carcass and scat samples found near the kill site were collected using sterile  
202 polyester swabs. Efforts were made to collect at least three saliva swabs (one each from  
203 different parts of the carcass with at least one swab from the predation bite marks  
204 whenever available), multiple shed hair samples and all the scat samples detected at the kill  
205 site. The number and type of samples collected from each site also varied based on kill site  
206 conditions such as the presence of carnivores, scavengers and proximity to villages.

207 For swab collections, swabs were first briefly soaked in Longmire lysis buffer (Longmire et  
208 al., 1997) and then rolled over the target area for 10-12 seconds following Mumma et al.,  
209 2014. For scats, swabs were rolled over the outer, shiny areas preferred for host DNA.

210 Samples were categorised as either predation or post-predation samples (Figure 1).

211 Predation samples were samples that we suspected to be deposited during a predation  
212 event. These included saliva samples collected from predation wounds (distinguished from  
213 other bite marks based on the occurrence of haemorrhage) and shed hair samples collected  
214 from the inferred kill location (where the predation event occurred). Post-predation  
215 samples included saliva samples from lick and feeding marks, shed hair samples from  
216 suspected resting sites and sites near the carcass. Scat and uncategorised samples were

217 categorised as post-predation samples. Swab samples were stored in 2 ml tubes with 1.5 ml  
218 Longmire lysis buffer.

## 219 **GENETIC ANALYSIS**

### 220 **2.4 DNA extraction and species identification**

221 DNA extraction was done in a dedicated low-concentration DNA extraction facility at the  
222 National Centre for Biological Sciences (NCBS), Bangalore. DNA extraction was done using  
223 QIAGEN® DNA Tissue and Blood extraction kits, following the protocol suggested by the  
224 manufacturer with modifications. Extraction controls (negatives) were set to detect any  
225 contamination. DNA extraction of different sample types was done separately to avoid  
226 contamination. All samples were screened using tiger-specific primers to identify positives  
227 (Bhagavatula & Singh, 2006). PCR controls were set to detect contamination while setting  
228 up the PCR reaction.

### 229 **2.5 Individual identification**

230 Samples genetically confirmed to be tigers were genotyped using a panel of 123 SNPs, as  
231 Natesh et al. (2019) described. This mainly involved a three-step process – a multiplex PCR,  
232 an indexing PCR, and library preparation and sequencing. Multiplex PCR of Kanha samples  
233 was performed using a unified pool of 123 primers. Primers were split into two pools (based  
234 on amplicon size) for Bandhavgarh samples. Indexing PCR was performed to add a unique  
235 combination of i5 and i7 Illumina indexes to samples. The indexed products were pooled  
236 and bead-purified to retain only amplicons of targeted size. The resulting library was then  
237 sequenced on an Illumina Miseq platform to obtain 75x2 paired-end reads. All the samples  
238 were processed in replicates.

239 Data filtering and subsequent individual identification were done independently for both  
240 study sites. Raw reads were filtered using the program TrimGalore  
241 ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) with a quality value of  
242 30 on phred33 scale to remove adapters and short reads. Filtered reads were mapped to  
243 the tiger genome assembled in the lab (Armstrong et al., 2021) using bwa mem (Li, 2013)  
244 with a penalty value of 3. Variant calling was done using bcftools (Li, 2011). Variants were  
245 filtered to retain only the targeted loci, following which genotype quality (10 or above) and  
246 depth (10 or above) filters using GATK (DePristo et al., 2011). Missing data filters were  
247 applied to remove loci missing in >15% of samples and samples with less than 40 SNPs.  
248 After filtering, we retained 95 and 101 SNPs for Bandhavgarh and Kanha, respectively.  
249 Individuals were identified based on PI-HAT values using Plink (Purcell et al., 2007). PI-HAT  
250 values of known replicate pairs were used to determine the cut-off for recapture  
251 identification, as suggested by Natesh et al., 2019 and Sagar et al., 2021. Data of known  
252 replicates were merged to reduce missingness and re-analysed to obtain pairwise  
253 relatedness between unique samples after determining the relatedness cut-off.  
254 Samples with pairwise relatedness greater than the recapture cut-off were identified as the  
255 same individual. Unique samples that had pairwise relatedness greater than 0.5 and lesser  
256 than the recapture cut-off with any sample were categorised as uncertain and discarded to  
257 avoid ambiguity. All other samples were identified as unique individuals without  
258 recaptures. Individual identification was done using Program R v 4.0.2.

## 259 **2.6 Predicting SNP typing and individual identification success**

260 We conducted standard Generalised Linear Models (GLM) analysis using Program R v 4.0.2  
261 to assess how season and sample type influence the number of SNPs typed and individual

262 identification success. We also evaluated the impact of sampling lag (number of days from  
263 predation event till sampling) and canopy cover (in percentage), but they were excluded  
264 from the final model as there was insufficient coverage. Rainfall and maggot infestation  
265 showed association with season and were therefore excluded. We used only Bandhavgarh  
266 samples to develop our models since there weren't enough samples from Kanha across the  
267 seasons, and the lab protocols for individual identification were different from those of  
268 Bandhavgarh. The SNP count used for the analysis was the number of SNPs retained  
269 following filtering for genotype quality and depth. Similarly, any sample having more than  
270 40 SNPs following filtering (depth (10), quality (10) and removing missing SNPs) were  
271 classified as successful for individual identification.

## 272 **2.7 Predator assignment**

273 Based on the location and type of sample used for identification, we classified tigers into  
274 three categories: True predator, circumstantial predator, and predator uncertain. Each of  
275 these assignments was at both species and individual levels. True Predators are species,  
276 individuals that were solely identified using predation samples, i.e. saliva samples from  
277 predation wounds on the carcass and shed hair samples collected from the location of kill.  
278 Circumstantial predators are species and/or individuals that were likely to be predators but  
279 were not identified from the predation samples either because predation samples were not  
280 collected or were ineffective in identification. Circumstantial predators were identified only  
281 when multiple samples were collected from a site, and all resulted in the same  
282 identification. Any sample where species and/or individuals identified from a kill site that  
283 did not fit the above categories were assigned as predator uncertain (schematic flow chart  
284 in Supplementary Figure S1). The highest confidence was assigned to true predator,

285 followed by circumstantial predator and the least for predator uncertain, when ranking the  
 286 likelihood of a species or individual being involved in the predation event.

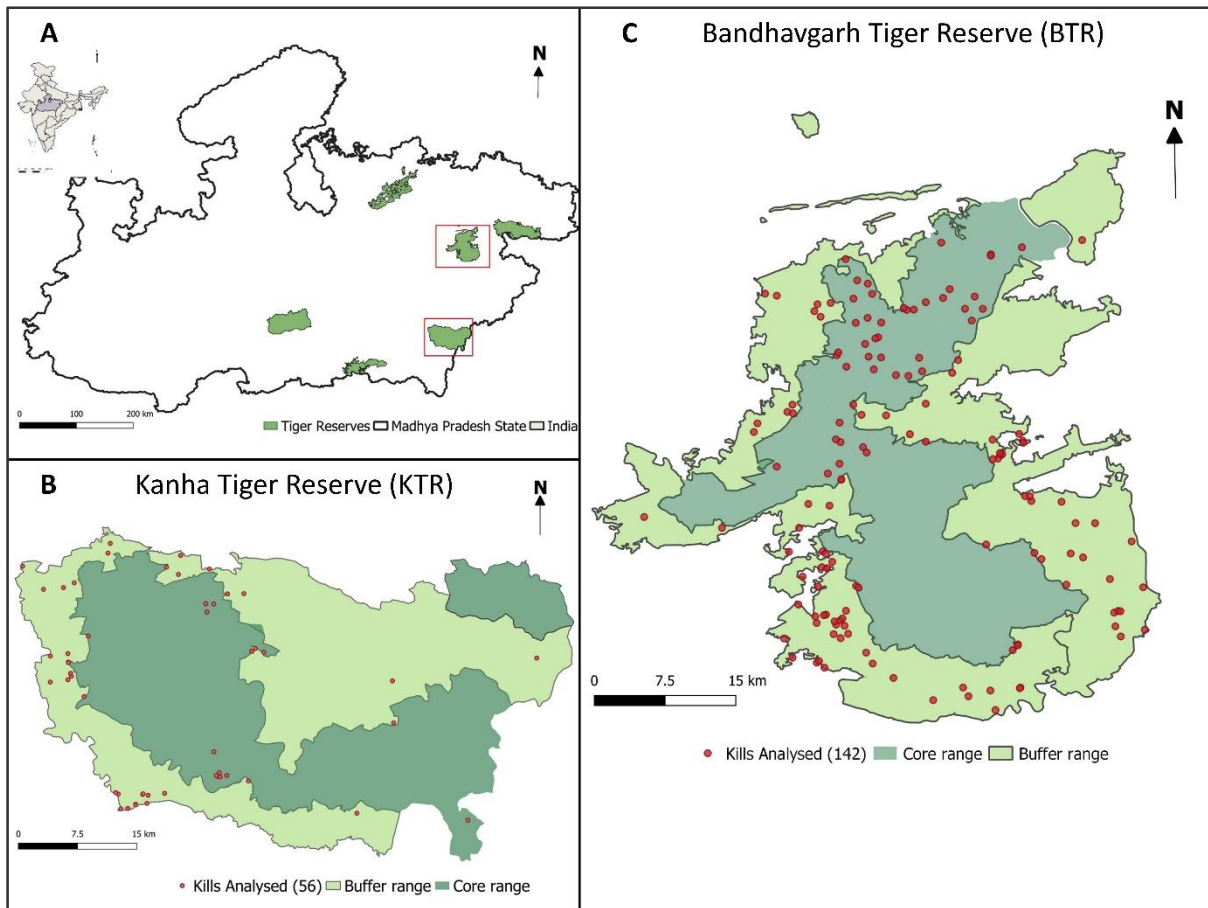
Classification	Level	Sample used for identification	Condition	Confidence Assignment
True predator (TP)	Species	Predation	1. Species identified using only saliva samples from predation marks or shed hair samples from inferred kill location.	High
	Individual	Predation	1. Individual identified using only saliva samples from predation marks or shed hair samples from inferred kill location.	
Circumstantial predator (CP)	Species	Post-predation	1. No predation mark/inferred kill location sample 2. All/multiple samples from the kill site should identify only one species from the kill site.	Moderate
	Individual	Post-predation	1. No predation mark/ inferred kill location sample for species identification. 2. Individual identified from samples at the kill site, but not	

			<p>predation mark/ inferred kill location.</p> <p>3. All/multiple samples identify a single individual from the kill site.</p>	
Predator uncertain	Species	Post-predation	<p>1. Criteria for either TP or CP cannot assign species.</p> <p>2. More than one potential predator species is identified at the kill site using samples other than predation samples.</p> <p>3. When the species has been identified from just one sample, another than the predation sample.</p>	Low
	Individual	Post-predation	<p>1. Individual cannot be identified using criteria for TP or CP.</p> <p>2. More than one potential predator individual identified at the kill site using samples other than from predation mark/inferred kill location .</p> <p>3. Has been identified from just one sample other than from the predation mark/ inferred kill location.</p>	

287 **Table 1:** Predator classification scheme with conditions for assignment.



288 **3. Results:**



289

290 **Figure 2:** Study area map with sampling locations. A) Boundary of Madhya Pradesh state in  
291 India with study sites highlighted in red box. B) Kills sampled in Kanha Tiger Reserve (N = 56)  
292 and, C) Kills sampled inside Bandhavgarh tiger reserve (N = 142).

293 **3.1 Sample availability and species identification**

294 We collected sampled 342 saliva swabs, 395 shed hair and 33 scat samples from 198 kill  
295 sites (Bandhavgarh: 142 and Kanha: 56) (Supplementary Table S1). Shed hair and saliva  
296 samples were the most abundant source of DNA and were collected from 85% and 72% of  
297 kill sites sampled respectively. Shed hair samples were the only source of DNA for 52 kill  
298 sites, while saliva samples were the only source of DNA for 27 sites. Scat samples were

299 found at 10% of the kill sites. At least one predation sample (saliva from the predation  
300 wound or shed hair from the inferred kill location) was collected from 153 (78%) kill sites.  
301 Season influenced the availability of saliva samples from predation wound, with samples  
302 collected from fewer kill sites in monsoon (28%) compared to winter (60%) and summer  
303 (60%).

304 Tigers were detected at all kill sites with species identification success with 98.5% saliva  
305 swabs, 98.2% shed hair and 96.9% scat samples. Using tiger-specific primers limited our  
306 detections to tigers; therefore, no amplification doesn't indicate the absence of carnivore  
307 DNA. Season did not significantly influence the species identification success of any sample  
308 types.

### 309 **3.2 Impact of season and sample type on number of SNPs typed:**

310 Using saliva samples from the winter season as the reference group, our models indicate  
311 a 30% and 53% decline in SNPs typed in summer and monsoon, respectively (Table 1). For  
312 shed hair (again, hair in winter as reference), an 11% and 37% decline in SNPs typed was  
313 observed in summer and monsoon, respectively. Shed hair samples typed more SNPs in  
314 comparison to saliva samples across seasons. In winter, the number of SNPs typed was  
315 highest for saliva and shed hair samples. A stronger seasonal impact was observed for saliva  
316 samples than shed hair samples.

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Model	Estimate	Std. Error	Pr(> z )
Saliva * Winter (intercept)	3.71379	0.01469	<2e-16***
Saliva * Summer	-0.35052	0.02816	<2e-16***
Saliva * Monsoon	-0.75672	0.04481	<2e-16***
Shed Hair * Winter	0.42914	0.01788	<2e-16***
Shed Hair * Summer	0.23086	0.23086	<2.52e-12***
Shed Hair * Monsoon	0.29070	0.04850	2.05e-09***

317 **Table 2:** Results of Generalised Linear Models (GLM) examining the influence of sample type  
318 (saliva and shed hair) and season (winter, summer and monsoon) on the number of SNPs  
319 typed.

320 **3.3 Individual identification success**

		KILL SITES		SAMPLES	
Source	Season	Processed	Success (in %)	Processed	Success (in %)
Saliva	Winter	53	64	134	53.7
	Summer	44	56.8	100	47
	Monsoon	45	57.8	103	43.7
Shed hair	Winter	57	79.5	157	78.3
	Summer	50	90	108	70.4
	Monsoon	59	69.5	123	54.5
Scat	Winter	7	57.1	11	72.7
	Summer	5	20	8	37.5
	Monsoon	7	85.7	13	61.5

321 **Table 3:** Summary of individual identification success for various sample types (saliva, shed  
 322 hair and scat) across seasons (winter, summer and monsoon).

323 After applying missing data filters, we set 40 SNPs as a cut-off for individual identification.  
324 Data filtering and subsequent individual identification were conducted independently for  
325 each study site. Of 757 unique tiger-positive samples processed, 449 samples (59%) had  
326 more than 40 SNPs and were used for individual identification (Table 2). PI-HAT scores of  
327 known replicates ranged from 0.64 to 1 for Kanha and from 0.69 to 1 for Bandhavgarh  
328 (Supplementary Figure S2). A recapture cut-off of 0.81 was set for Kanha and Bandhavgarh  
329 based on the 97.5% percentile of the relatedness distribution (between replicates).  
330 Seventy-two tigers (Kanha 19 and Bandhavgarh 53) were identified with varying numbers of  
331 recaptures (pairwise relatedness of Bandhavgarh individuals in Supplementary Figure S3).  
332 Four individuals from Kanha and 11 from Bandhavgarh had no recaptures and were  
333 detected only once. Seventy-one samples were classified as uncertain as they didn't have  
334 more than 0.81 relatedness with any sample and/or had greater than 0.55 relatedness with  
335 multiple individuals. These samples were discarded to avoid ambiguity.

336 At least one tiger was identified from 86% of all kill sites sampled. Shed hair samples were  
337 the most successful source (82%) for individual identification across seasons, followed by  
338 saliva (59%) and scat (58%) at kill sites (Table 2). Shed hair and saliva samples were the sole  
339 source of individual identification at 80 and 29 sites, respectively. Predation samples  
340 identified individuals at 53% of the sites where they were collected. Notably, both types of  
341 predation samples (saliva associated with predation mark and shed hair associated with  
342 predation mark) identified the same individual at 10 of 11 kill sites.

343 Our models indicate a higher probability of individual identification with shed hair samples  
344 when compared to saliva samples. Using the winter season as the reference group, the  
345 probability of individual identification declined by 10% and 30% in summer and monsoon,

346 respectively, for saliva samples (Table 3). Similarly, for shed hair samples, the probability of  
347 individual identification decreases by 21% and 34% in summer and monsoon compared to  
348 winter.

349 Based on the estimated mean confidence interval, we infer that the number of saliva  
350 samples required for confident individual identification from a kill site (with 90% probability)  
351 is 16, six and four samples in monsoon, summer, and winter, respectively (Supplementary  
352 Table S2). For shed hair samples, the numbers are lower overall, with five, three and two  
353 samples required in the monsoon, summer and winter, respectively.

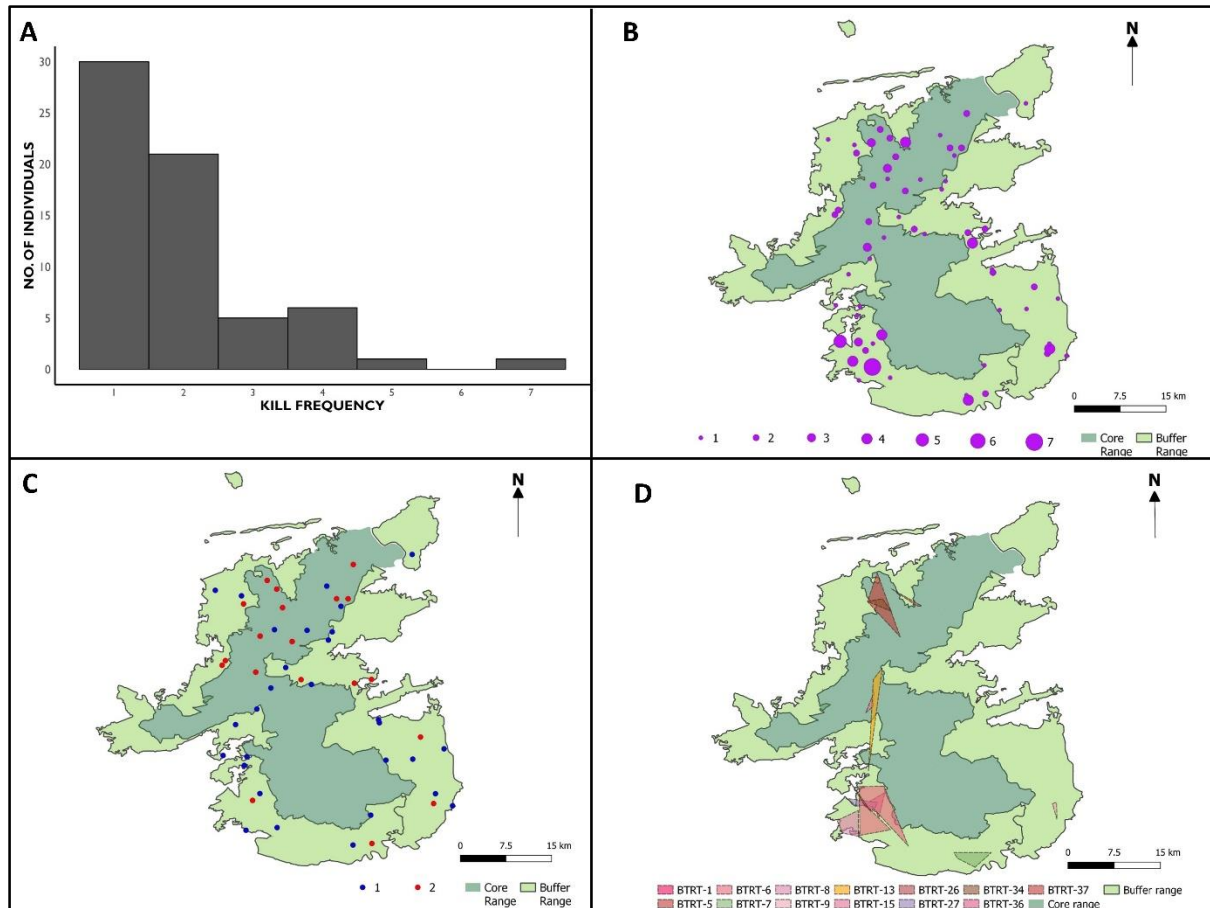
---

<b>Model</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>Pr(&gt;  z )</b>
<b>Saliva * Winter (intercept)</b>	0.05311	0.18821	0.18821
<b>Saliva * Summer</b>	-0.90041	0.33880	0.00787**
<b>Saliva * Monsoon</b>	-1.62173	0.52639	0.00206**
<b>Shed Hair * Winter</b>	1.16223	0.26920	1.58e-05***
<b>Shed Hair * Summer</b>	0.34647	0.44633	0.43760
<b>Shed Hair * Monsoon</b>	0.46355	0.59352	0.43479

354 **Table 4:** Generalised Linear Models (GLM) results examining the influence of sample type  
355 (saliva and shed hair) and season (winter, summer and monsoon) on individual identification  
356 success.

### 357 **3.4 Preliminary insights on individual engagement in depredation**

358 While samples from 169 kill sites had more than 40 SNPs, individual tigers were identified at  
359 155 of the 198 kill sites where tigers were detected. Eighty-seven unique individuals were  
360 identified from these sites. Multiple individuals were detected at 19 sites. Forty-six per  
361 cent of the individuals were detected in only one kill site, while almost a third (29%) were at  
362 two kill sites. In other words, 75% of all tigers found at kill sites were at only one or two kill  
363 sites. Twenty individuals (23%) were detected at three to five kill sites, and only two  
364 individuals (2%) were detected at seven kills. Our sampling of kill sites was more intensive in  
365 Bandhavgarh, and we attempted to investigate spatial distributions of individual predators  
366 here (Figure 3).



367

368 **Figure 3:** Summary of individuals identified across kills in Bandhavgarh Tiger Reserve. A)

369 Histogram of individuals found across kill sites indicating majority of individuals were

370 identified at one or two sites, B) frequency of individual occurrence across all kills, C)

371 individuals identified (N = 51) at one or two kills D) occurrence polygon of individuals (N =

372 13) identified at 3 or more kills.

### 373 3.5 Predator assignment

374 We compiled predator assignments for both Bandhavgarh and Kanha. Tigers were detected

375 at all 198 kill sites. By our defined categories, we were able to identify the 'true predator' at

376 151 kills, the 'circumstantial predator' and 'predator uncertain' at the species level for 34

377 and 12 sites, respectively. Individual tigers were assigned as 'true predators' for 72 kills and



378 as 'circumstantial predators' at 34 sites. Individuals were identified using multiple predation  
379 samples at 11 sites. All samples identified a unique individual in ten of these 11 sites. Two  
380 unique individuals were detected at one site using different predation samples, including  
381 saliva and shed hair. One of these individuals was detected at three other sites, while the  
382 second wasn't recaptured at any other site. At 49 kill sites, individuals were identified as  
383 predator uncertain. Within these sites, individuals were classified as predator uncertain  
384 when identification was obtained from only one sample (38 kills) or when multiple  
385 individuals were detected (11 kills). Moreover, using the predation sample we successfully  
386 differentiated between predator and predator uncertain individuals at seven sites where  
387 multiple individuals were detected.

#### 388 **4. Discussion**

389 Reliable identification of predating individuals is fundamental to understanding predation  
390 ecology, as well as in the management of human-wildlife conflict. Our study assessed the  
391 utility of trace DNA samples in predator identification while examining factors that influence  
392 success. We successfully identified tigers at all sampled sites, achieving a 100% success rate  
393 in species identification, and identified individual tigers at 86% of all kill sites. We find that  
394 the sample source and the sampling season are determinants of SNP typing and individual  
395 identification success. We develop a framework to assign confidence in predator  
396 assignments based on sample sources for individual identification. At 46% of kills, we  
397 identified true predator (high confidence), circumstantial predator (medium confidence) at  
398 22% of sites, and predator uncertain (low confidence) at 32% of sites at individual level. We  
399 conclude that trace genetic samples, combined with systematic kill investigation, can  
400 successfully identify predators.

#### 401 **4.1 Season and sample source determine identification success**

402 Shed hair samples consistently typed more SNPs and had higher individual identification  
403 success than saliva and scat samples, regardless of the season. Winter was observed to be  
404 the most favourable season for sampling. As predicted, the individual identification success  
405 rate per sample was lower during the monsoon season. We could not test the independent  
406 impact of rainfall and sampling time lag on success as rainfall was strongly associated with  
407 season, and we had a low sample size for delayed sampling events (> three days). While  
408 earlier studies in controlled settings have shown the negative impact of rainfall and time lag  
409 on DNA quality (Harms et al., 2015; Piaggio et al., 2020; Reddy et al., 2012), testing  
410 independent effects in field studies becomes challenging due to deposition of fresh saliva  
411 across feeding bouts. Despite declining individual identification success per sample, the  
412 success per kill remained consistent across seasons. This implies that collecting multiple  
413 samples from various sources at a single kill could help alleviate the seasonal impact. We  
414 therefore recommend the collection of more samples in monsoon followed by summer and  
415 winter. We endorse previous suggestions to collect samples promptly to prevent  
416 degradation and minimise the influence of scavengers (Ganz et al., 2023; Mumma et al.,  
417 2014). Additionally, we strongly recommend collecting multiple sources of samples,  
418 including predation samples (such as saliva from fatal wounds and shed hair from the point  
419 of kill), at all sites, as these samples aid in differentiation between predators and  
420 scavengers, ultimately strengthening confidence assignment in identification. We recognise  
421 that processing more samples can increase costs and recommend prioritising sample  
422 processing, such as predation samples, over others to reduce costs.

423

## 424 **4.2 High proportion of individuals engage in depredation**

425 Human-wildlife conflict poses a significant threat to conservation initiatives, and targeted  
426 removal of individuals is widely used as a strategy for conflict management. This approach,  
427 however, assumes unequal contributions of specific "problem" individuals in conflict  
428 engagement, essentially drawing from intra-species variations (Swan et al., 2017). We  
429 identified 87 individual tigers from 155 sites in Kanha and Bandhavgarh that were present at  
430 livestock kills. Considering the recent findings from the All India Tiger Estimation (Qureshi et  
431 al., 2023), which estimated Bandhavgarh's tiger population at 165 individuals, our study  
432 identified ~40% of these individuals at livestock kills. 75% of the individuals identified, in both  
433 Kanha and Bandhavgarh, were involved in less than three kills while accounting for 58% of  
434 kills. Establishing presence of problem individuals would require information on intra-  
435 population variation in diet and livestock depredation frequencies by individual tigers,  
436 resulting economic losses and the determinants of this variation. We acknowledge the  
437 limitations of our study to comment on the existence of "problem" individuals, and further  
438 research is required to validate their presence, especially in systems with large numbers of  
439 livestock kills such as ours. Moreover, we propose differential assessments of cases involving  
440 livestock depredation and human attacks when investigating potential problem individuals.  
441 Removal of individuals has consequences on population dynamics and raises ethical  
442 considerations. Therefore, for such interventions to be effective, it is crucial to establish the  
443 existence of problem individuals in the system and accurately identify the predator before  
444 resorting to targeted removal. Otherwise, targeted removal will be a tool for balancing  
445 conservation and political goals without serving its intended purpose of conflict reduction.

446

447 **4.3 Conclusive identification of predator will require an adaptive and systematic sampling**  
448 **design**

449 Genetic methods are more effective in predator identification than field-based methods  
450 (Ganz et al., 2023; Mumma et al., 2014). Samples can also be collected from human  
451 predation sites where use of conventional methods becomes a challenge and has ethical  
452 considerations (Pandey & Sharma, 2016). They are advantageous in identifying the  
453 individual predator even when visually unique individual markings are lacking. Trace DNA  
454 deposited during a predation event (such as saliva from predation wounds and shed hair at  
455 kill locations), essentially serves as evidence, whereas camera traps are typically deployed  
456 after the event. However, genetic and conventional methods are prone to misidentification,  
457 especially when multiple potential predators and scavengers are at the kill site (Ganz et al.,  
458 2023; Steffens et al., 2012; Verzuh et al., 2018). It is, therefore, crucial to triangulate  
459 information from various sources, including field investigations, camera trapping, genetic  
460 data, and data from collared individuals to identify predators reliably. Once a predation  
461 event is confirmed (see Cristescu et al., 2022), the kill site should be systematically explored  
462 and documented, followed by meticulous sample collection. To enhance the reliability of  
463 assessments, we recommend assigning confidence levels based on the information source  
464 and associated errors. We provide a framework for confidence assignment using genetic  
465 data by categorising samples according to their source and collection site. The highest  
466 confidence is attributed to samples likely deposited during a predation event, such as saliva  
467 samples from fatal wounds (indicated by haemorrhage) and shed hair samples from the  
468 inferred kill location. This framework can be adapted and modified in future studies to  
469 encompass various other sources of information like camera trap images from kill sites,

470 prior knowledge of individual territories and radio telemetry data. We align with Cristescu  
471 et al. (2022) in recognising that reporting such evidence and subsequent assessments will  
472 enhance transparency and foster public support in decision-making.

#### 473 **4.4 Integration of molecular approaches into management**

474 The broader acceptance and utilisation of genetic tools face challenges due to cold storage,  
475 extended processing times, and high sample processing costs (Khan & Tyagi, 2021).  
476 However, lysis buffers allow for sample storage at room temperature, addressing the cold  
477 storage requirement. Advancements in next-generation sequencing methods can further  
478 diminish processing time and costs (see Natesh et al., 2019). These methods also enable the  
479 real-time generation of high-quality data using non-invasive samples (Urban et al., 2023).  
480 We propose establishing a reference genetic database, particularly for individuals involved  
481 in conflicts. Furthermore, acknowledging that genetic information does not align with the  
482 visual identification typically required for management actions, this database can be linked  
483 to the physical identification of individuals. This can be achieved by simultaneously  
484 sampling kills for trace DNA and deploying camera traps at kill sites in addition to  
485 opportunistic sampling during animal sightings and the capture of individuals. This  
486 comprehensive database of individuals will enable a better understanding of predation  
487 ecology through robust individual identification and promote evidence-based conflict  
488 management.

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## 504 **Conflict of interest**

505 The authors declare no conflict of interest.

## 506 **Author Contributions**

507 HC, AH, and UR conceived the ideas and designed methodology; HC, RD, AY and KP  
508 conducted field sampling; HC, DR and AP did laboratory work and NGS data analysis; SS and  
509 VR provided essential support for field sampling; HC wrote the first draft. All authors gave  
510 final approval for publication.

511

512 **Data availability**

513 Raw sequence data and scripts for variant calling will be deposited at NCBI and Github,

514 respectively, and will be made available upon acceptance.

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