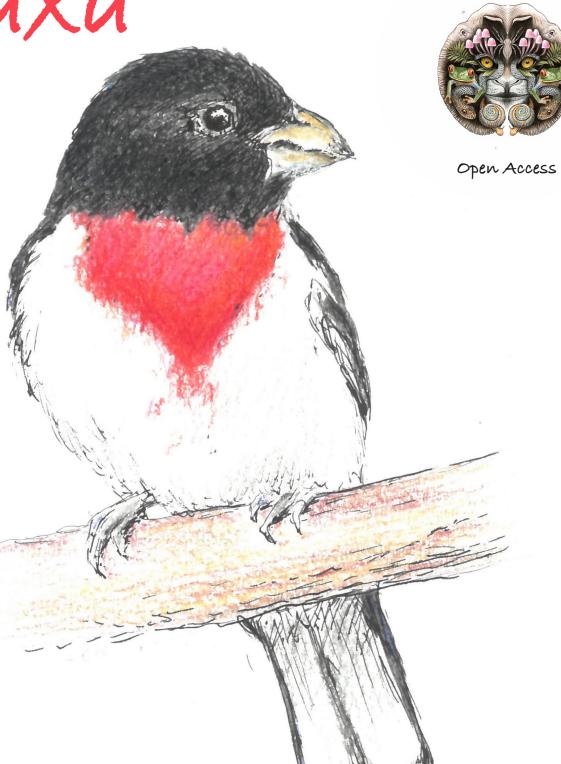
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ARTICLE

Small Wild Cats Special Series

Diet of Rusty-spotted Cat *Prionailurus rubiginosus*(I. Geoffroy Saint-Hilaire, 1831) (Mammalia: Carnivora: Felidae) in Sanjay Gandhi National Park, Mumbai, India

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Abstract: The 103.68 km² Sanjay Gandhi National Park (SGNP), Mumbai, exists amidst human densities that figure among the highest in the world. The rich biodiversity of SGNP includes the Rusty-spotted Cat *Prionailurus rubiginosus*, endemic to India, Sri Lanka, and Nepal, and categorised as 'Near Threatened' on the IUCN Red List. Little is known about its ecology and the dynamics of its coexistence with the other small carnivores in SGNP. We conducted a study with citizen volunteers to explore the diet of the Rusty-spotted Cat and other sympatric small carnivores in SGNP and in the adjoining human-dominated areas of Yeur village, Shivaji Nagar, Dahisar Quarry, and Aarey Milk Colony. After initial training, the volunteers collected scat samples from all forest ranges in SGNP and the surrounding areas outside, following defined protocols. Seventy-eight scat samples were analysed for species assignments using standardised molecular techniques, felid-specific primers, and DNA sequencing, and 24 were identified as of the Rusty-spotted Cat. The contents of the samples were examined under a microscope to identify prey remains. Results were presented as the mean number of scat samples containing remains of specific taxa with 95% Confidence Intervals. Diet estimated from 22 Rusty-spotted Cat scat samples and 52 samples of other small carnivores revealed rodents to be the major prey of the entire group. However, a higher proportion of Rusty-spotted Cat scat samples had remains and none with remains of rodents (95%) and reptiles (6%) as compared to samples of other small carnivores, i.e., 79% with rodent remains and none with remains of reptiles. On the other hand, a lower proportion of Rusty-spotted Cat scat samples had remains of insects (14%), plant matter (9%), and birds (5%) than samples of other small carnivores (40% plant matter, 38% insects, 17% birds). Our results highlight the role of small carnivores, especially Rusty-spotted Cat in regulatory services through pest control.

Keywords: Ecosystem services, molecular tools, rodent prey, scat analysis, small carnivores.

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Author contribution: SM, NK and PVK conceptualised the study. PK, ARG, KP, SM and PVK analysed and mapped the samples. SM wrote the manuscript. PVK, PK and NK reviewed and edited the manuscript.

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INTRODUCTION

Small carnivores have demonstrated their value in controlling pests, drawing attention to the larger effort required to monitor their responses to changes in their environment, in order to effectively plan their conservation (Marneweck et al. 2021; Bandyopadhyay et al. 2024). Further, Marneweck et al. (2022) argue that small carnivores are an ideal group to study for understanding the effects of global change due to their higher diversity, intermediate trophic position, wider ecological niches, and higher reproductive rates than of large carnivores.

Sanjay Gandhi National Park (SGNP), spread over an area of 103.68 km², is unique in being located within one of the world's most densely populated cities and is popularly referred to as the lungs of Mumbai (Everard 2019). However, the protected area faces severe threats from human encroachments and rapid development along its boundary (Zérah & Landy 2013; Shinde 2017; Engineer 2018). Although several studies on various taxa have been undertaken in SGNP, the Leopard Panthera pardus has received most research attention, largely due to severe conflict issues (Munde & Limaye 2013; Surve et al. 2022). Eight other carnivore species have been reported in SGNP, including the Rusty-spotted Cat Prionailurus rubiginosus and Jungle Cat Felis chaus, Small Indian Civet Viverricula indica, Asian Palm Civet Paradoxurus hermaphroditus, Indian Grey Mongoose Herpestes edwardsii, Ruddy Mongoose Herpestes smithii, Golden Jackal Canis aureus and Striped Hyena Hyaena hyaena (Surve et al. 2015; Mukherjee et al. 2020). All these species are placed in Schedule I of the Wildlife (Protection) Amendment Act, 2022. Among these, the Rusty-spotted Cat is a species of conservation priority in India and SGNP, as the larger part of its relatively restricted global distribution falls within the country (Munde & Limaye 2013; Mukherjee et al. 2016a).

The Rusty-spotted Cat is categorised as 'Near Threatened' on the IUCN Red List, and is endemic to India, Sri Lanka, and Nepal (Mukherjee et al. 2016a). It is the smallest member of the cat family, weighing 2 kg on average (Pocock 1939; Nowell & Jackson 1996; Sunquist & Sunquist 2002). Based on preliminary information on habitat requirements, a population decline of up to 25% is predicted in the next decade, largely due to habitat loss associated with large-scale expansion of agriculture, development and urbanisation (Mukherjee et al. 2016a; Sharma & Dhakad 2020). Some observations on the cat suggest that it largely feeds on small mammals (Patel 2006; Athreya 2010; Langle 2019). Although SGNP has a

captive breeding facility for the Rusty-spotted Cat, there is very little information available on its ecology within SGNP. The same applies to all the other small carnivores with only sporadic reports from by-catch data on camera traps placed for the Leopard.

Dietary studies can provide useful insights into several aspects of small carnivore ecology, e.g., community dynamics, competition, and niche spaces, and provide information on ecosystem services and functioning (McNab 2002; Ćirović et al. 2016; Everard 2019; Müller et al. 2022). A reason for this low volume of information on small carnivores is perhaps the difficulty in studying their ecology, especially diet and behaviour due to their largely cryptic habits. With molecular techniques, these aspects can now be explored through non-invasive means (Piggott & Taylor 2003). Available literature on the diet of some small cats suggests that rodents form the major prey of the Jungle Cat (Mukherjee et al. 2004; Majumdar et al. 2011), of the Leopard Cat Prionailurus bengalensis (Rabinowitz 1990; Grassman et al. 2005; Rajaratnam et al. 2007; Shezad et al. 2012; Lorica & Heany 2013, Parchizadeh et al. 2023) and of the Caracal Caracal caracal (Mukherjee et al. 2004; Braczkowski et al. 2012). In contrast, viverrids and herpestids feed largely on insects and plant matter (Su & Sale 2007; Kalle et al. 2012; Akrim et al. 2023).

We involved citizen volunteers in our research and exposed them to the scientific methods used in studying the diets of small carnivores (Mukherjee et al. 2021). In this paper, we present results from our study on the diets of Rusty-spotted Cat and other co-occurring small carnivores in SGNP and the adjoining areas in Yeur village, Shivaji Nagar, Dahisar Quarry, and Aarey Milk Colony.

Study area

SGNP is credited with providing several ecosystem services, including provisioning of water to the metropolis, recreation to tourists who visit daily and supporting services for maintaining biodiversity (Everard 2019) (Figure 1). Tulshi and Vihar lakes are located within SGNP and provision part of the city's water requirements; several streams and rivers flow through SGNP and into the Arabian Sea (Munde & Limaye 2013).

Due to its proximity to the coastal region, SGNP experiences a mean humidity of 75% (Munde & Limaye 2013). The southwest monsoon occurs from June to September with an average of 2,000 mm of rain (Munde & Limaye 2013). The mean annual temperature is 27 °C, occasionally soaring up to 40 °C, and January is generally the coolest month with a mean minimum temperature



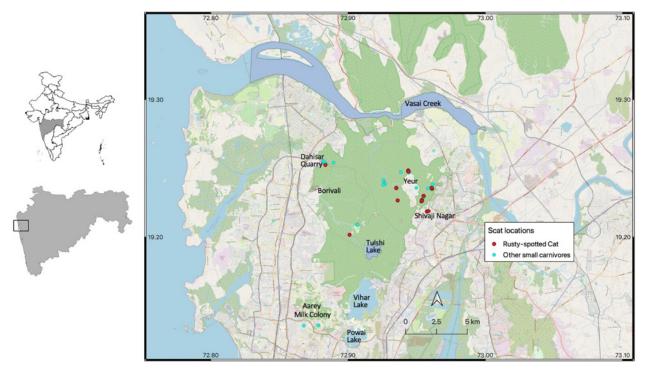


Figure 1. Locations of scat samples of the Rusty-spotted Cat and small carnivores in Sanjay Gandhi National Park and adjoining areas.

of 19 °C (Munde & Limaye 2013).

The major forest types in SGNP are Southern Moist Teak-bearing, Southern Moist Mixed Deciduous, Mangrove Scrub, and Western Subtropical Hill forests (Champion & Seth 1968). SGNP falls within the 5A-Malabar Plains Biogeographic Zone (Rogers et al. 2002).

The faunal diversity list of SGNP includes 172 species of butterflies, 50 species of herpetofauna, 286 species of birds, and around 43 mammalian species (Kasambe 2012). Apart from the Leopard and smaller carnivores, mammals occurring in SGNP include Sambar Rusa unicolor, Chital Axis axis, Southern Red Muntjac Muntiacus muntjac, Indian Chevrotain Moschiola indica, Wild Pig Sus scrofa, Northern Plains Gray Langur Semnopithecus entellus, Bonnet Macaque Macaca radiata, Rhesus Macaque M. mullata, Black-naped Hare Lepus nigricolis, Indian Crested Porcupine Hystrix indica, palm squirrels Funambulus and several species of murid rodents (Edgaonkar & Chellam 1998; Pradhan 2002; Surve et al. 2015). Due to numerous human settlements within SGNP, several domestic mammals are also present, including Domestic Dog Canis familiaris, Domestic Cat Felis catus, Goat Capra hircus, Water Buffalo Bubalus bubalis and Cattle Bos taurus (Surve et al. 2015).

MATERIALS AND METHODS

In March and April 2017, volunteers from Mumbai were trained in field techniques which included locating and collecting scats, using field instruments and software such as hand-held GPS, mobile phone applications for marking coordinates and uploading data, monitoring water bodies and streams, camera trapping, and the basics of GIS applications (Mukherjee et al. 2021). Soon after the training, from 16 April 2017 to 15 May 2018, volunteers formed three groups, one for each of the three Forest Ranges closest to their residences and visited various locations within SGNP to collect scat samples. Each group had a team leader who prepared a schedule for sampling, which was restricted to weekends and holidays. Each team comprised two to four volunteers who walked trails within the Forest Range and collected scat samples following a specific protocol. Only intact samples were collected. Once a scat was located, it was photographed along with a labelled vial with the date, geographic coordinates and sample number, a scale and GPS unit or android phone with the geo-coordinates visible, placed next to it. This photograph, along with the names of members of the sampling team, date, time, name of the Forest Range, and geographic coordinates were uploaded onto the android application Epicollect 5 (Aanenson et al. 2009),



which could be accessed by the investigators.

The scat samples were shipped to the Indian Institute of Science Education and Research (IISER) Tirupati for further analysis. Due to the large number of scat samples collected and the limited time to analyse them, they were initially assigned to cats because of their compact shape that is segmented and with tapering ends (Chame 2003) and based on personal observations by the first author. A small portion of the samples that was most intact and smooth, and visually assigned to cats, was kept aside for molecular analysis and assignment to a predator species. These samples were weighed and analysed for diet remains. Prey remains such as teeth, bones, feathers and other undigested matter were observed under a microscope. The percentage of samples containing specific prey remains was determined (Klare et al. 2011). Data were analysed using R version 3.2.3 (R Development Core Team 2016), package "boot" version 1.3-24 (Canty & Ripley 2019). Sub-samples equalling original sample sizes (n=22 for Rusty-spotted Cat and n=52 for other unidentified small carnivores) were analysed using non-parametric bootstrap analysis with 6,000 simulations. Results were presented as the mean number of scat samples containing remains of specific taxa with basic 95% Confidence Intervals.

We used a commercially available stool DNA extraction kit from HiMedia Laboratories following the manufacturer's protocols, with a control in each set of extractions to detect any contamination. We targeted the 16s rRNA region of the mitochondrial DNA for assigning the samples to predators, using primers designed by Mukherjee et al. (2016b). The primers amplified a region of 200 bp and their sequences were as follows:

Felid16srRNA Forward: 5' AATTGACCTTCCCGTGAAGA 3' Felid16srRNA Reverse: 5' TCCGACTGGTTAGTCTAGAT 3'

The T_m of both primers was 58 °C, and we used an annealing temperature of 50 °C in the Polymerase Chain Reaction (PCR) programme. PCR reactions were set up in volumes of 20 μ l with a PCR Master Mix (MM) (Origin Diagnostics, Kerala). Bovine Serum Albumin (BSA) (Sigma-Aldrich) was added to the reactions for better results. The volumes and concentrations of the reagents used were as follows: 5 ml of MM, 2 μ l of 2 mM primers, 2 μ l of 4 mg BSA, 7 μ l of Mili-Q water, and 4 μ l of DNA extract. Specifications of the PCR program used were initiation at 94 °C for 10 minutes, denaturation at 94 °C for 30 seconds, amplification at 50 °C for 45 seconds, elongation at 72 °C for 50 seconds, and final elongation 72 °C for 10 seconds. The 2nd, 3rd and 4th steps were repeated for 59 cycles.

We used UV-treated hoods and had PCR negative

controls to detect any contamination during the PCR stage.

We viewed the PCR products through gel electrophoresis on a 2% agarose gel (HiMedia laboratories) with Orange G loading dye from Sigma-Aldrich and GelRedTM DNA stain (Life Technologies, India). We loaded a 100 bp ladder (HiMedia laboratories) along with the PCR products as reference. The PCR products that amplified with the felid primers were sent to Chromgene Biotech Private Limited for forward and reverse reaction sequencing. We used Chromas version 2.6.5 (Technelysium Pty Ltd.) to view and clean sequences, and then used the BLAST analysis (Basic Local Alignment Search Tool) on NCBI (McGinnis & Madden 2004) for identifying species. We aligned sequences using ClustalW in MEGA 6.0 (Tamura et al. 2013) for alignments. For sequences that were identified as Rusty-spotted Cat, we constructed a Neighbour Joining phylogenetic tree in MEGA 6.0 (Tamura et al. 2013), rooted with members of the genus Felis (Domestic Cat, Accession Number: AF006453.1; Afro-Asiatic Wildcat F. lybica, Accession Number: AF006395.1; Jungle Cat, Accession Number: AF006393.1). We also included Leopard Cat P. bengalensis (Accession Number: AF006437.1), Fishing Cat P. viverrinus (Accession Number: AF006451.1) and an existing sequence of Rusty-spotted Cat (Accession Number: NC_028304.1) to depict the accuracy of the assignments. All existing sequences were obtained from NCBI (Clark et al. 2016). We mapped the locations of Rusty-spotted Cat and other small carnivore scat samples used in the diet analysis using QGIS Version 2.8.2-Wien (QGIS 2015).

RESULTS

Over approximately five months, 126 scat samples were collected in Yeur, Tulshi and Borivali Forest Ranges within SGNP and surrounding areas in Yeur village, Shivaji Nagar, Dahisar Quarry and Aarey Milk Colony (Figure 1). From these, 78 were visually assigned to small cats based on their shape. These 78 were subjected to DNA analysis, of which 30 samples (38%) gave positive results with the felid primers and were sent for sequencing. Results from BLAST revealed that 24 of these were of Rusty-spotted Cat, comprising 20 from Yeur Range, two from Shivaji Nagar, and one each from Tulshi Range and Dahisar Quarry area. Five were not of felids but most similar to mongoose species. One scat did not generate a good enough sequence for assignment. The average weight of Rusty-spotted Cat scat was 4 g (range: 1.2–16.5

Contents of scat samples

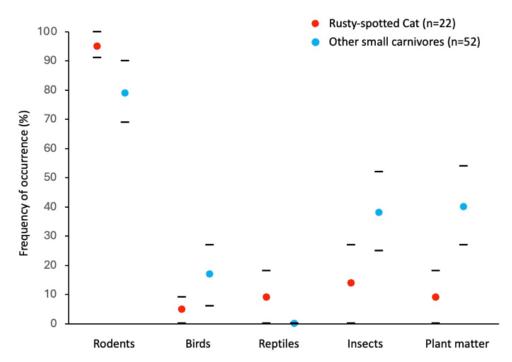


Figure 2. Diet of Rusty-spotted Cat and other small carnivores in SGNP showing bootstrap means with confidence Intervals.

g). No Jungle Cat scat was reported through molecular analysis.

Seventy-four scat samples with discernible food remains were analysed for diet and showed a predominance of rodent remains (Figure 2). Of the 24 scat samples assigned to Rusty-spotted Cat, two from Yeur Range had no identifiable remains and were not included in the diet analysis. The phylogeny of the sequences supported the BLAST results of assignments to Rusty-spotted Cat (Figure 3). A comparison of diets revealed a higher presence of rodents in the diet of the Rusty-spotted Cat (Table 1).

We presume that the rodents consumed by the Rusty-spotted Cat belong to the *Mus* genus, based on size and morphological characteristics of rodent molars found in the sample (Image 1).

DISCUSSION

Our study is the first to involve citizen volunteers in sampling scat of small carnivores in India and to systematically document Rusty-spotted Cat diet. Our results of murid rodents forming the predominant diet of the Rusty-spotted Cat corroborate earlier observations

Table 1. Mean percentage frequency of prey items in scat of Rustyspotted Cat and other unidentified small carnivores from Sanjay Gandhi National Park and surrounding areas in Mumbai, India, with bootstrap 95% confidence intervals (CI)

Prey	Rusty-spotted Cat (n = 22) Mean (%), (95% CI)	Other small carnivores (n = 52) Mean (%), (95% CI)
Rodents	95, (91–100)	79, (69–90)
Birds	5, (0–27)	17, (6–27)
Reptiles	6, (4–18)	0
Insects	14, (0–27)	38, (25–52)
Plant matter	9, (0–18)	40, (27–54)

(Patel 2006; Athreya 2010; Langle 2019). Systematic studies on the diets of other small cat species in varied habitats reiterate the role of small cats as rodent control agents and highlight their ecosystem services (Rabinowitz 1990; Mukherjee et al. 2004; Grassman et al. 2005; Rajaratnam et al. 2007; Majumdar et al. 2011; Braczkowski et al. 2012; Shezad et al. 2012; Lorica & Heany 2013, Mukherjee et al. 2016b; Parchizadeh et al. 2023).

In contrast, the diets of the other small carnivores show a much higher proportion of insects and plant matter than consumed by the Rusty-spotted Cat. This is in



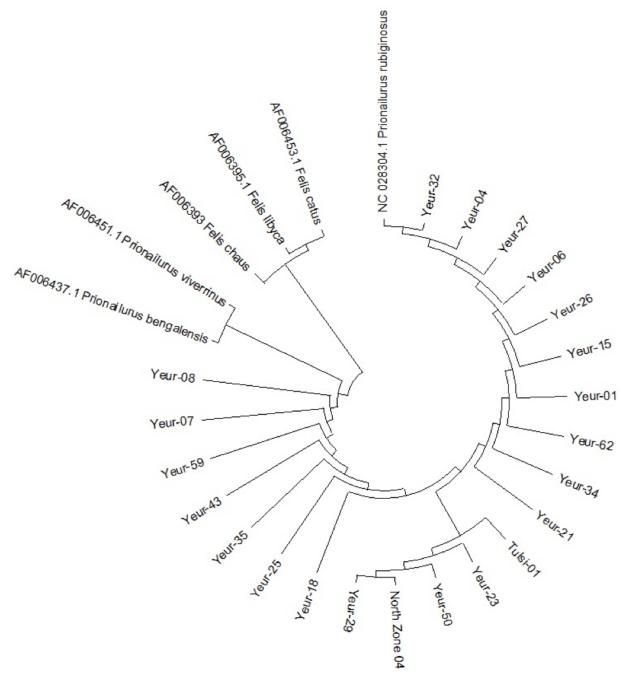


Figure 3. Neighbour Joining Tree of Rusty-spotted Cat 16s rRNA mitochondrial DNA sequences from scat samples used for diet analysis.

line with existing information (Su & Sale 2007; Kalle et al. 2012; Akrim et al. 2023).

Future studies can focus on standardising primers for other carnivore species, identifying the prey remains in scat with greater precision through Next Generation Sequencing work, quantifying diets and estimating prey abundance for more precise and meaningful results (Mukherjee et al. 2004; Klare et al. 2011; Shezad et al. 2012).

Visual assignment of scat had an error of more than 60%, where scat samples of other carnivores were assigned to small cats. The primers designed for detecting felids gave an error of 17%, where five mongoose scat samples were amplified, an error that was noticed after sequencing. Based on these, we recommend using PCR amplification followed by sequencing for assigning scat to species for obtaining reliable results. Further, there is a possibility of false negatives where the primers





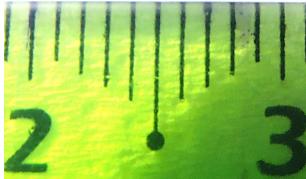


Image 1. Rodent dentition found in a scat sample of Rusty-spotted Cat identified as *Mus* sp., scale: 1 mm. © Arati Gawari & Kartik Pillai.

did not amplify small cat DNA, and some scat samples could have erroneously been placed in the unidentified carnivore group, biasing the diet results. In the future, this can be addressed by using additional primer sets designed on other regions of the DNA (Liu et al. 2023).

Studies conducted in other parts of the country had a larger proportion of 47–67% of scat assigned to felids using molecular tools (Mukherjee et al. 2010, 2016b), whereas only 38% in the current study in SGNP were detected as being felid scat. This can either be attributed to the poorer condition of the scat samples during collection or smaller populations of small cats in SGNP. A drawback of this study was that most scat samples were collected in the Yeur Range, which could reflect the possible unequal effort put in by volunteer teams, since each team was assigned to a specific forest range and adjoining areas outside.

A report by Everard (2019) listing the potential ecosystem services of SGNP includes possible regulatory services by predators that can be hampered by habitat destruction. The results of our project highlight the importance of generating information on such services, especially around the fringes and outside the perimeter of SGNP. We also found scat near human habitation outside the boundary in Yeur and Dahisar Quarry, though most of the sampling was restricted within SGNP. Unlike the Leopard, small carnivores do not pose a threat to

human lives, so the conflict with humans is unidirectional where developmental activities are directly responsible for habitat loss.

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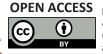
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