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RESEARCH ARTICLE

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Behaviour and activity pattern of a Kanchanaburi viper *Trimeresurus kanburiensis*

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ABSTRACT – Several conservation assessments have proposed that the Kanchanaburi pit viper *Trimeresurus kanburiensis* is threatened with extinction due to limited distribution, few documented sites and the collection of mature individuals for the international pet trade. No previous study has evaluated these threats comprehensively, nor investigated the natural history of the species in the wild. To address these knowledge gaps, we began with a brief review of wildlife trade reports and then during September–November 2020 we undertook a field study (nearly 100 surveyor hours) using fixed camera recording. We located only a single specimen, a male, whose behaviour, activity pattern and natural history appeared similar to those of other green pit viper species. The general habitat selected by this individual conformed to prior observations although close proximity to a paved road and use of a termite mound were novel. The review of wildlife trade reports did not reveal any seizures or observations, which was likely due to *T. kanburiensis* not receiving formal international protection and exclusion from stringent local legislation. We recommend further field study, identification of sites of occurrence, and investigation on popular websites and social media platforms that trade in *T. kanburiensis* and phenotypically similar Thai species. For a Thai language summary of this article see Supplementary Material on the BHS website.

INTRODUCTION

Viper diversity is comparatively high in Thailand, with 23 species currently recorded in the country (Thai National Parks, 2023). Much attention and study has been devoted to finding and describing species, taxonomy and nomenclature, and venomics, with very little work being published describing basic natural history beyond initial species descriptions and personal observations supplementing peripheral topics. Conservation measures based on data from studies prioritising these topics tend to be unreliable and ineffective without a strong ecological foundation. In this study of the Kanchanaburi pit viper *Trimeresurus kanburiensis* Smith 1943, which has been categorised by the International Union for the Conservation of Nature (IUCN) as Endangered (Chan-Ard et al., 2022), we address a significant conservation knowledge gap with a novel natural history field study complemented by a discussion of conservation threats.

Two of the three justifications presented within the IUCN ENB1ab(v) listing for *T. kanburiensis* are habitat-related restricted-distribution of little more than 3,000 km² and presence in fewer than five locations. These have been indirectly addressed through surveys, but the third justification for listing the viper as endangered, trade (either wild-caught or captive), has not been investigated. Interestingly, *T. kanburiensis* was initially proposed to be of 'Least Concern' and 'Endemic' in the Thailand Red Data: Vertebrates list (Office of Natural Resources and Environmental Policy and Planning, 2007), in 2017 this was conservatively increased to 'Endangered' (Office of Natural Resources and Environmental Policy and Planning, 2017). The Thailand Red Data List does not provide justifications for each species assessed.

MATERIALS AND METHODS

Field observations

Field surveys (14 visual-encounter hikes plus 8 by road) to find *T. kanburiensis* were conducted during 4-day sampling sessions per month between September–November 2020 for a total of 96.9 surveyor hours at Khao Laem National Park (KLNP), Thailand (Fig. 1). This is a previously unpublished but locally well-known site for the Kanchanaburi pit viper. Local national park management were notified a minimum of two weeks in advance of every survey session, a ranger from the Department of National Parks, Wildlife and Plant Conservation (DNP) was present during every survey, and no animals were handled/captured nor were they or their habitat intentionally disturbed as per research and ethical permission requirements. The viper field research adhered to Guidelines for the Treatment of Animals in Behavioural Research (developed by the Association for the Study of Animal Behaviour; ASAB, 2012) and Guidelines for Research on Live Amphibians and Reptiles (American Herpetological Animal Care and Use Committee; HACC, 2004) ethical standards.

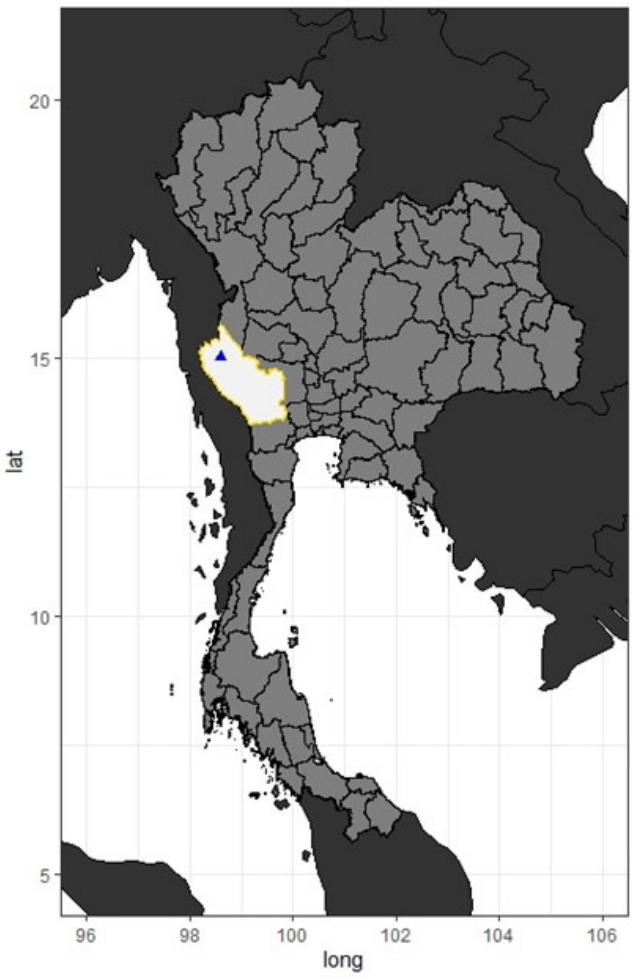

Figure 1. Kanchanaburi pit viper *Trimeresurus kanburiensis* study location at Khao Laem National Park (indicated by a blue triangle) study area within Thailand which is shaded in light grey. Within Thailand, provinces are partitioned in black, with Kanchanaburi outlined in yellow.

A continuous feed 'security video camera' (Hikvision model DS-2CE16C0T-IRF), set to record at a rate of 29 frames per second and mounted on a tripod, was used to monitor the behaviour, natural history and activity patterns of *T. kanburiensis*. The ethogram for this study, a set of terms and descriptions of the behaviours of an animal (Lehner, 1987), comprised behavioural states and events (ambush, rest and move states; headbob, probe, strike, tail undulation and gape events; as defined in Barnes & Knierim, 2019). Non-parametric circular kernel-density function analysis was employed to delineate temporal differences and similarities of behaviours to each other and also specifically to relate the presence of prey on camera to ambush behaviour displayed by *T. kanburiensis*. A coefficient of overlap (Δ) was used to measure the extent of overlap between two kernel-density estimates in the 'overlap' R package (Ridout & Linkie, 2009; Linkie & Ridout, 2011). General microhabitat notes were made, and canopy cover (%) was estimated using the CanopyApp smartphone application (Landert, 2016).

Review of trade threats

We sought to understand the threat (reflected by the IUCN listing) that international trade may pose to *T. kanburiensis*.

Figure 2. An adult male Kanchanaburi pit viper *Trimeresurus kanburiensis* observed initially moving in limestone karst habitat interspersed with bamboo in Khao Laem National Park, Kanchanaburi province, Thailand. Neither the viper nor its habitat was disturbed and just this one photograph was taken with flash, a second photo was taken without flash immediately after and then again later in the sampling session during the day.

A review of trade literature post-2001 was undertaken by accessing records publicly available from the Convention on International Trade in Endangered Species (CITES) and Law Enforcement Management Information System (LEMIS) using R packages, 'rcites' (Geschke et al., 2021) and 'lemis' (Eskew et al., 2020), and from the Thailand CITES Enforcement Division, DNP through their website (https://portal.dnp.go.th/p/WildlifeConserve), trade data files archived on OSF with other *T. kanburiensis* publication project code and data. Results from program R were confirmed through review of records publicly available on the LEMIS and CITES websites, and it is worth noting that no Thai pit viper species (including *T. kanburiensis*) are specifically listed in CITES Appendices 1–3.

The trade (CITES and LEMIS) program R code, list and pictures of reptile and amphibian species observed during KLNP field surveys can be found at osf.io/2sh3m. Video recordings have also been reposited at Movie Archives of Animal Behavior (http://movspec.mus-nh.city.osaka.jp/ethol/title-e.php) and on Youtube (https://www.youtube.com/channel/UCdRlzXz9YbUR2eWyEAEGU4g).

RESULTS

Field observations

Only a single adult male *T. kanburiensis* was observed during the study period, which by its colouration, body plan and size (> 300 mm by visual estimation) was identified by reference to Cox et al. (2012). The observation was made on 3 November at 19:40 h when the snake was moving slowly as if searching for a potential ambush site (Fig. 2). The viper was spotted with a headlamp, one photograph was taken without flash and one with flash from a distance of >2 m, neither of which appeared to disturb the slowly moving viper as pace and direction did not appear to change. Surveyors moved away and briefly checked whether the viper was moving using the lowest headlamp settings (10 minutes

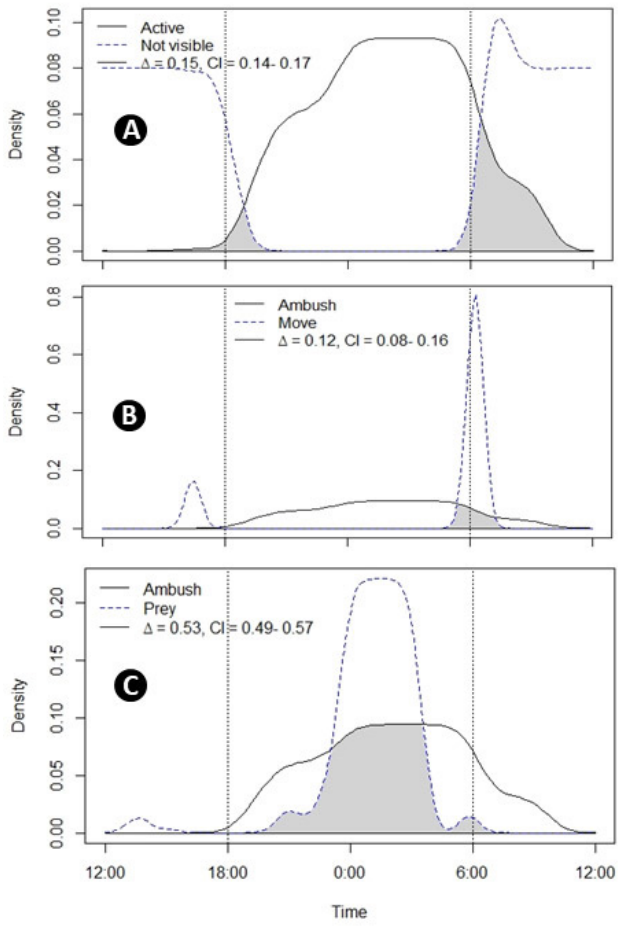

Figure 3. Temporal activity patterns observed for an adult male Kanchanaburi pit viper *Trimeresurus kanburiensis* observed on a continuous feed camera at Khao Laem National Park, Thailand, with the overlapping coefficient (Δ) represented by the grey shaded area - **A.** The viper was primarily active during the night (between 18:00–06:00 h) and usually not visible during the day, **B.** Move behaviour was primarily observed in the early morning and early evening close to the overlap periods of ambush and when the viper was not visible, **C.** Prey was primarily observed during the night, this matched the *T. kanburiensis* ambush foraging pattern.

and 35 minutes after initial observation). The video camera was then set (lights never being pointed directly at the viper during this process) about 2.5 m horizontal ground distance away from the viper at approximately 55 minutes after initial observation when the viper was observed in ambush posture on a termite mound. The camera recorded from then until 6 November morning (09:45 h, 61.5 hours), with SD card failure experienced the first night. When the camera was retrieved on the last morning the Kanchanaburi viper was observed in ambush posture and left undisturbed.

The *T. kanburiensis* was primarily observed in an ambush posture during the night and was usually not visible during the day, with limited movement between day and night (Fig. 3A& B). On 4 November, the viper was seen on the camera entering the termite mound for shelter. Behavioural events were infrequently observed, as were prey. No conspecifics or predators were visible on the camera. The most frequent behavioural event displayed by the viper was headbobbing, which was observed 85 times between 19:03–06:28 h. Only

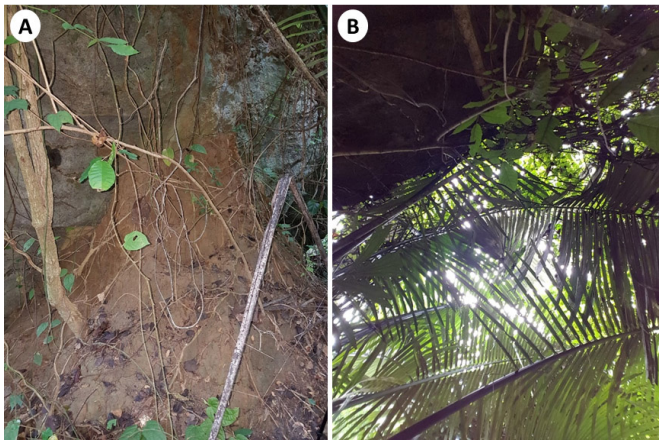

Figure 4. Microhabitat where a *Trimeresurus kanburiensis* was observed in the Khao Laem National Park, Kanchanaburi province, Thailand - **A.** Location where the viper was observed in an ambush posture on an inactive termite mound, **B.** The canopy almost (approximately 1.5 m) directly above the foraging site

four probes (at approximately the following times 20:02 h, 03:24 h, 03:25 h and 03:26 h) and two gapes were observed (approximately 21:25 h and 01:07 h). One strike was observed (approximately 20:19 h), but no prey was visible for more than four hours prior nor any other organism which could have elicited a defensive strike.

Potential prey observed on camera included geckos (*Cyrtodactylus* and *Hemidactylus* sp.), a squirrel, a bird (passerine) and rats. No previous predation observations of wild *T. kanburiensis* have been published, but captive specimens have been successfully fed lizards and pinky mice (Cox et al., 2012). Potential prey was primarily observed at night which largely overlapped the viper's foraging period (Fig. 3C), although it is worth noting that the squirrel, bird and rats were likely too large for the viper to ingest. Nine species of snake (including a road-killed adult female *Trimeresurus albolabris*) and six species of lizards (including signs of monitor lizards *Varanus nebulosus*) were observed in Khao Laem National Park during the study; adult *Cyrtodactylus* sp. (undescribed), a juvenile *Acanthosaura crucigera* and an adult *Lycodon davidsonii* were observed within 15 m of the *T. kanburiensis* (summarised in the Table at osf.io/2sh3m), and frog *Micryletta erythropoda* was observed within 5 m.

The immediate habitat of the *T. kanburiensis* matched previous descriptions for the species, i.e. limestone karst interspersed with bamboo. However, the viper's ambush site was within 30 m of a well-traveled paved road with one lane going in each direction (total width of the road approximately 10 m) which has not been previously reported. Similar habitat was also present on the opposite side of the road. The termite mound (Fig. 4A) where the *T. kanburiensis* was sheltering and waiting in ambush appeared to be old or abandoned, and no termites were visible; the overall appearance of the mound indicated that the termites might be of the genus *Macrotermes*. Two potential species are *Macrotermes cabonarius* or *Macrotermes annadalia*, which can be differentiated by soldier head color (black or red, respectively; personal communication, Dr. Warin Boonriam).

Canopy cover at the microhabitat level (Fig. 4B) was 66.2% approximately 1.5 m away from the viper. It was not possible to make an estimation directly above the viper due to it being present during monitoring at the same site; attempts would have potentially disturbed the viper, which was prohibited under the study research and ethics permissions. The canopy cover at the point of measurement appeared to be fairly similar to that directly overhead the viper.

Review of trade threats

The only record of any *Trimeresurus*/green pit vipers being reported or seized by Thai authorities (DNP) was in 2009 (56 live, 240 dead *T. trigonocephalus*; non-native to Thailand if correctly identified). Interestingly, there were 20 records provided by CITES of *Trimeresurus* between 2013–2020, one of which was listed as '*Trimeresurus* sp.' (labelled 'specimen') in 2017, the other 19 records being designated specifically as '*Trimeresurus mangshanensis*' which is listed under CITES Appendix II. LEMIS 2000–2014 records list '*Trimeresurus* sp.' (346 records), '*Trimeresurus albolabris*' (40 records), '*Trimeresurus gramineus*' (2 records), '*Trimeresurus hageni*' (2 records), '*Trimeresurus jerdoni*' (3 records), '*Trimeresurus popeiorum*' (3 records), '*Trimeresurus puniceus*' (5 records), '*Trimeresurus purpureomaculatus*' (2 records), '*Trimeresurus stejnegeri*' (1 record) and '*Trimeresurus trigonocephalus*' (4 records). Note that the nomenclature used by LEMIS does not reflect current taxonomy. Just 6 (all '*Trimeresurus* sp.') records listed Thailand as a country of origin, which were being shipped to 2 natural history museums (3 records) and a university in the United States (3 records).

Despite conservative categorisations in international (IUCN) and local (Thailand) Red Lists, it is worth pointing out that these assessments do not imply legal or enforcement capabilities. With few exceptions, Thai wildlife trade law appears to follow CITES for international trade. However possession is regulated by the Thai Wild Animal Reservation and Protection Act (WARPA B.E. 2535, 1992; and WARPA B.E. 2562, 2019)(Moore et al., 2016), which does not list *T. kanburiensis* (although there was documented discussion about it at the 2014 and 2015 CITES meetings; CITES AC27 Inf. 7; CITES AC28 Doc. 14.3). Personal communication does indicate potential inclusion in the WARPA edition currently being drafted.

DISCUSSION

Our single observation of a *T. kanburiensis* did conform to the bamboo and limestone karst habitat use of the species, but the close proximity to a paved and well-used road does present unique future study opportunities in the form of genetics, mortality and general influence of human disturbance. Camera study, a relatively non-invasive method, appeared similar in success, namely in the identification of key ecological topics such as behaviour and potential prey, to prior study of green pit vipers in natural and human-dominated areas (Barnes & Knierim, 2019; Barnes et al., 2020). This success can be attributed at least partly to the fact that these snakes are ambush foragers and relatively resilient to human proximity disturbance, factors that may be important for the design of effective conservation strategies. They may also have a bearing on local snakebite management efforts given that there is at least one documented severe bite case attributed to *T. kanburiensis* in the wild (Warrell et al., 1992).

The number of sites known to be occupied by *T. kanburiensis* has more than doubled since the last IUCN Red List assessment, although new sites (including ours) on private land, protected land, temples and even a university campus in Thailand, remain largely in the realm of local knowledge and have not been published in western journals. Furthermore, the geographical range of the Kanchanaburi pit viper may be greater than currently stated as it has been suggested that it extends into Myanmar (Malhotra & Thorpe, 2004; which was repeated in Chan-Ard, 2022), but this is unconfirmed. The survey effort required during our study to find a *T. kanburiensis* was substantial, indicating that occupancy analysis with detection probabilities and population estimation would be difficult to calculate reliably.

To date no ecological study of wild Kanchanaburi pit vipers has been published beyond initial description and subsequent expeditions for study of presence, which coupled with limited quantification of effort and descriptions of observations provide at best only partial (at worst, biased) knowledge of the basic natural history of the species. Similarly, enforcement and legal protection for *T. kanburiensis* is currently unclear or non-existent. To address these disparities, we recommend a natural history and trade study focused on *T. kanburiensis* and two phenotypically similar, and potential habitat specialist species, *Trimeresurus kuiburiensis* and *Trimeresurus venustus*. This should be followed by clear legislation for protection of the species both locally and internationally that will reflect that research.

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