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Host, season, habitat and climatic factors as drivers of Asian rodent tick (*Ixodes granulatus*) (Acari: Ixodidae) occurrence and abundance in Southeast Asia

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ABSTRACT

The Asian rodent tick (*Ixodes granulatus*) occurs throughout much of Asia, it frequently bites humans, and zoonotic pathogens, such as *Borrelia burgdorferi* (sensu lato) and *Rickettsia honei,* have been detected within it. Unfortunately, the ecology of *I. granulatus* remains poorly known, including drivers of its abundance and the interaction ecology with its sylvatic hosts. To elucidate the ecology of this medically important species, the habitat preferences of *I. granulatus* were assessed in Singapore and Malaysia. *Ixodes granulatus* showed strong associations with old forest habitats, though across different age classes of old forest there was limited variation in abundance. *Ixodes granulatus* was absent from other habitats including young forest, scrubland, and parks/gardens. Within its sylvatic rodent hosts, a range of factors were found to be statistically significant predictors of *I. granulatus* load and/or infestation risk, including sex and body condition index. Male rodents were significantly more likely to be infested and to have higher loads than females, similarly, animals with a lower body condition index were significantly more likely to be infested. Proactive public health efforts targeted at preventing bites by this tick should carefully consider its ecology to minimise ecological overlap between humans and *I. granulatus*.

1. Introduction

Ticks and tick-borne diseases pose a major global threat to the health of both humans and animals (Dantas-Torres et al., 2012). This is, in part, because of their role as vectors of pathogenic microbes, but also due to their ability to directly induce paralysis and allergic reactions, and in some cases anaemia (Graves and Stenos, 2017). Southeast and East Asia are home to a rich diversity of tick taxa, many of which are known to infest humans and act as vectors of zoonotic pathogens (Petney et al., 2019). Although a range of drugs are available to treat tick-borne infection, by the time treatments are administered, patients are often already suffering serious morbidity in addition to the myriad of sequelae associated with these infections (Madison-Antenucci et al., 2020). This reactive (treatment-focused) response to ticks and tick-borne diseases by many public health bodies adds undue burdens to the medical sector caused by patients suffering from tick-borne diseases. Instead, a proactive outlook towards ticks and tick-borne diseases must be fostered in which increased attention and resources are directed at prevention. However, prevention efforts require ecological data to inform them, and throughout much of Asia, particularly Southeast Asia, ecological data on local ticks are relatively scarce (Petney et al., 2007).

One of the most widespread tick species in Asia is *Ixodes granulatus*, which has been recorded in Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Philippines, Singapore, South Korea, Taiwan, Thailand and Vietnam (Guglielmone and Robbins, 2018). A range of important bacterial pathogens have been detected in *I. granulatus* (e.g., Kollars et al., 2001; Chao et al., 2009) and the tick species has been recorded infesting humans in numerous countries

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throughout its range (Guglielmone and Robbins, 2018). Therefore, *I. granulatus* represents a species of particular medical interest in the region and efforts should be made to prevent infestation by this species. However, despite its wide distribution and propensity to infest humans, relatively little is known about the ecology and habitat preferences of *I. granulatus* or the factors which drive its abundance (and by extension, infestation risk). The findings of Paperna (2006) and Kwak et al. (2021) provided some limited insights into habitat use by *I. granulatus*. However, host-tick interaction ecology is also an important factor to consider when assessing drivers of *I. granulatus* abundance. Some host species have been shown to act as ecological amplifiers by boosting the abundance of particular tick species (Perkins et al., 2006). Therefore, understanding the host species and host factors which are associated with higher abundance may provide further insight into the ecological drivers of *I. granulatus* abundance.

In Singapore the relationship was examined between (i) probability of tick presence (infestation risk) and (ii) count (tick load) with season, habitat, host species, and a range of host factors (age, sex, and body condition index). In Malaysia, the relationship between age classes of old forest and *I. granulatus* counts was examined along with the relationship between climatic and vegetative variables (temperature, humidity, leaf litter depth, and canopy cover) and tick counts.

2. Materials and methods

2.1. Sampling

Our study was undertaken in two locations, Pulau Tioman, Malaysia and Singapore Island, Singapore (Fig. 1). Three main age classes of old forest exist across Pulau Tioman: primary forest, intermediate forest, and secondary forest. Primary forest constitutes old growth tree communities which have not been subjected to logging and were dominated by the genera *Shorea* and *Dipterocarpus*. Intermediate forest constituted primary forest which has been partly cleared within the last century except for some large veteran trees after which secondary tree regeneration had occurred around these old growth trees. Secondary forest constituted tree communities which had naturally regenerated following complete deforestation (usually for the creation of rubber plantations) within the last century. Sampling was carried out between 8th and 14th July 2019 to coincide with the dry season so that flagging activity would not be disrupted by rainstorms. A total of 36 sites were sampled once each during the study period, representing 12 in each forest type (Fig. 2). GPS coordinates were determined using a GPSMAP® 66 s (Garmin, USA). At each site a central point was selected, and flagging was undertaken by 8 researchers within a 20 m radius around the central site point continuously for 20 min. Researchers each used a white towel (140 cm x 70 cm) attached to a 1.2 m pole for flagging. Tick flags were checked every 2 min and any ticks were collected and fixed in 80% ethanol. Climatic and vegetative variables were measured at each site including humidity, temperature, leaf litter depth, and canopy cover. Humidity and temperature were measured at 4 locations within every site using a Fluke 971 Temperature Humidity meter (Fluke, Everett, Washington, USA) and the final value was averaged based on the 4 measures. Leaf litter depth was measured at 4 locations within every site using a 30 cm ruler and the final value was based on the average of these 4 measures. Canopy cover was measured at 4 locations within every site determined using a Spherical Crown Densiometers (Forestry Suppliers, Jackson, Mississippi, USA), and the final value was based on the average of these 4 measures. Tick specimens collected on Pulau Tioman were preserved in 70% ethanol prior to being identified.

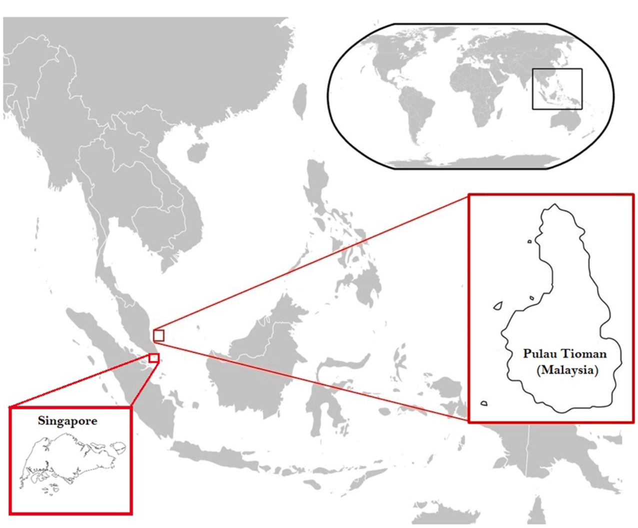

Fig. 1. location of the areas (Pulau Tioman, Malaysia; Singapore) were *Ixodes ganulatus* were sampled.

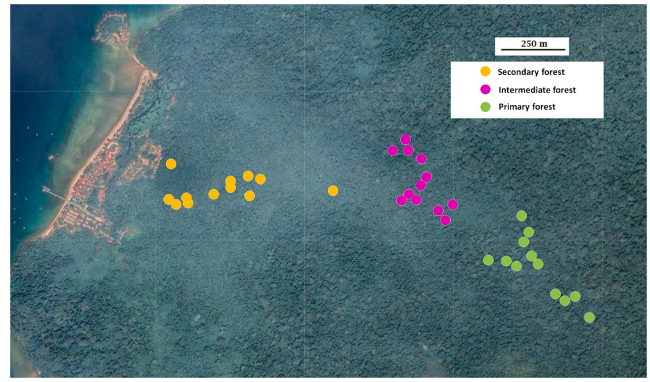

Fig. 2. sampling sites on Pulau Tioman (Malaysia) at which flagging was undertaken to survey the abundance of *Ixodes granulatus*. (orange markers = sampling sites in old secondary forest, pink markers = sampling sites on intermediate forest, green markers = sampling sites in primary forest).

In Singapore, small mammals were collected and sampled for *I. granulatus* ticks over 4.5 years (October 2011 – March 2016) and all small mammal work was approved by the National University of Singapore Institutional Animal Care and Use Committee (IACUC protocol approval B01/12), National Parks (Permit no NP/RP12- 004) and Agri-Food and Veterinary Authority of Singapore (Permit no AV16.01.004.0004). Small mammals were trapped across four habitat types: old forest, young forest, scrubland, and parks/gardens (Fig. 3). Old forests consist of tall and species-rich tree communities which represent regrowth from deforestation activities >50 years ago. Young

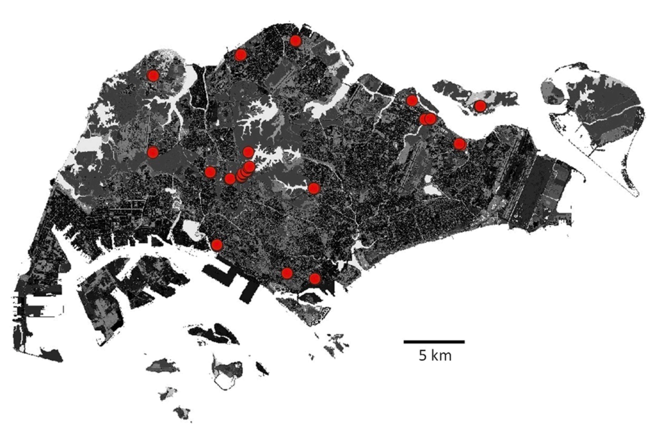

Fig. 3. sampling sites in Singapore at which small mammal trapping was undertaken to survey the presence and abundance of *Ixodes granulatus*.

forests all had a simpler structure, with dominant shrubs such as *Adinandra dumosa*, and are found mostly in small sized patches and represent regrowth from deforestation activities <50 years ago. Scrublands were characterized by an open undergrowth without many trees. Parks/gardens represent intensively managed vegetation which was largely composed of exotic plant species in close proximity to man-made structures. The classification of the vegetation cover for each site was determined using the map generated by Yee et al. (2011). In total, 33 sites were sampled including 10 in old forests, 14 in young forests, 5 in scrubland habitats, and 4 in parks/gardens (Fig. 3). Small mammals were caught using Tomahawk and Sherman live traps baited with peanut butter, rolled oats, banana, and sweet potato. Due to access restrictions from the government and military, equal numbers of sampling sites could not be established in all habitat types (Fig. 4). Small mammals were identified to species based on Francis and Barrett (2008). Each captured host was assessed and was designated as male or female and either an adult, subadult, or juvenile to provide an age classification. Body condition index (BCI) was calculated based on Labocha et al. (2014) (body mass/body length). Ticks were removed with fine forceps, placed into individual vials with virus transport media and stored at −80 °C prior to being identified. Seasons are somewhat variable in the region, and the monsoons and dry seasons can commence and end at somewhat different times depending on the year, so for our analysis we classified the seasons based on a simplified version of the season in Singapore outlined by Hassim and Timbal (2019). All rodents were released after capture.

2.2. Specimen fixation and identification

Tick specimens were examined using a M205C stereomicroscope and identified using keys by Kwak (2020) for the ticks of Pulau Tioman and Anastos (1950) for Singapore. All ticks identified as *Ixodes granulatus* were included in the analysis (Fig. 5). Specimens were deposited in the Singapore National Tick Reference Collection (SNTRC).

2.3. Statistical analysis

We fit hierarchical Bayesian models for all data. Priors were chosen to be weakly regularizing to control for both under- and overfitting of the model to the data. Convergence criteria, such as effective sample

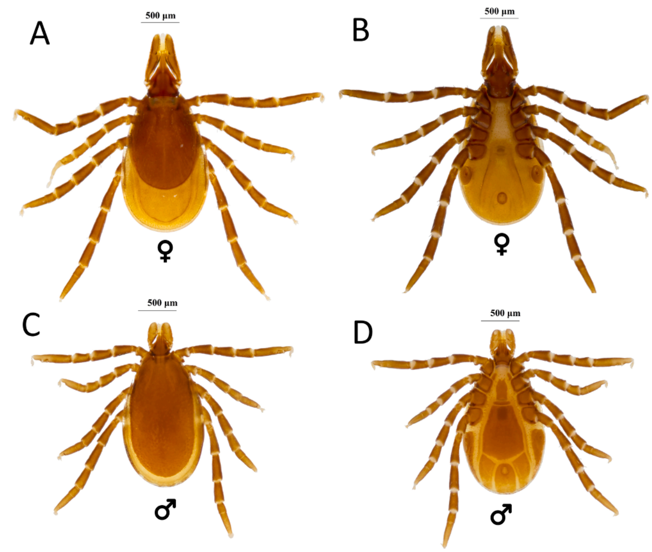

Fig. 5. Adult *Ixodes granulatus* ticks (A) female dorsal aspect, (B) female ventral aspect, (C) male dorsal aspect, (D) male ventral aspect.

sizes and R-hat values, were used to check for appropriate model convergence throughout, and trace plots were inspected for signs of incomplete mixing when necessary. All models were fit with the 'rethinking' (McElreath, 2023) package, model results and visualizations were conducted using the 'tidybayes-rethinking' (Kay, 2023) and 'ggplot2' (Wickham, 2016). All computing was conducted in R Core Team (2022).

To predict the relationship between tick count, forest type, and climatic and vegetative variables in Tioman, we fit two additive two-level models with Poisson distributions, one with climatic variables and one other with vegetative characteristics where forest type was the primary level. All continuous predictors were scaled to have a mean of zero and a standard deviation of one. To assess the significance between forest type and (i) humidity, (ii) leaf litter depth, (iii) canopy cover, Kruskal-Wallis rank sum tests were used.

We fit a three-level Bayesian Bernoulli distribution model to predict the relationship between season, habitat type, species and infestation

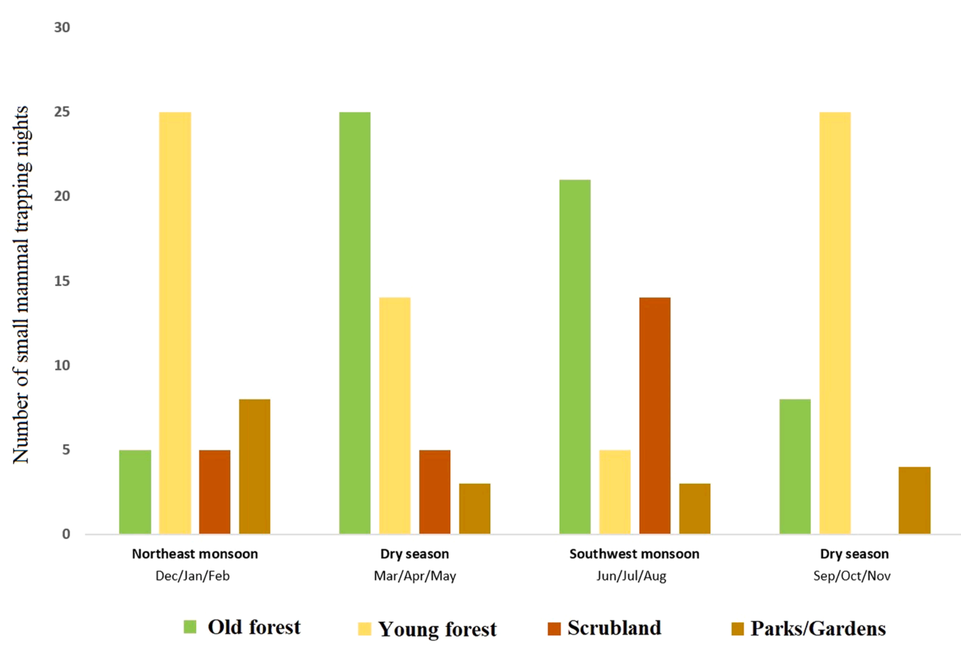

Fig. 4. number of small mammal trapping nights per habitat type per season in Singapore from October 2011 – March 2016.

risk in Singapore. The first of these models used a Poisson distribution to predict the relationship between season, forest type, host species and *I. granulatus* load. Next, a two-level Bayesian Bernoulli distribution model was used to predict the relationship between season, small mammal age, sex, body condition and probability of *I. granulatus* presence. Following this a two-level Bayesian Bernoulli distribution model was employed with a Poisson distribution to predict the relationship between season, small mammal sex, age, body condition, and *I. granulatus* load. A Logistic regression was performed to assess the significance of each rodent host for *I. granulatus*, and a Chi-square test for independence was also performed as an alternative to the above. Fisher's exact test was performed to assess the relationship between infestation risk and age. Fisher's exact test was also performed to assess the relationship between infestation risk and sex. A Wilcoxon rank sum test was performed to assess the relationship between infestation risk and BCI. Then, a Kruskal-Wallis rank sum test was performed to assess the relationship between tick load and age. Following this, a Wilcoxon rank sum test was performed to assess the relationship between tick load and sex.

3. Results

3.1. Relationships between tick counts, forest type, climatic, and vegetative variables

On Pulau Tioman, a total of 36 ticks were collected comprising 17 males and 9 females. In Singapore 99 ticks were collected comprising 84 females, 11 males, and 4 nymphs. Although the credible intervals overlapped, estimated counts for intermediate forest types was higher than the other forest types (Fig. 6). While there was no relationship between tick count and humidity (Fig. 7a), a slightly positive relationship between tick count and temperature was identified (Fig. 6b). Canopy cover and tick count were shown to have a negative relationship (Fig. 7c) and a slightly negative relationship was also identified between tick count and leaf litter depth (Fig. 7d). Kruskal-Wallis rank sum tests were used to assess whether humidity, leaf litter depth, or canopy cover had any statistically significant difference between the three forest types. However, no statistically significant difference was found for humidity ($p = 0.1$), leaf litter depth ($p = 0.8$), or canopy cover ($p = 0.1$) between the three forest types.

3.2. Relationships between risk of tick infestation, season, habitat, and host species

There were 594 small mammals included in this analysis and of the 26 infested by *I. granulatus* (Table 1), of these 21 were *Sundamys annandalei,* three were *Callosciurus notatus,* one was *Rattus tanezumi,* and one was *Tupaia glis*. Although credible intervals overlapped for all seasons within habitats, probability of infestation was lower in the first dry season then in other seasons in old forest habitat (Fig. 8). Credible intervals overlapped for most species (Fig. 9). Unsurprisingly, infestations of *C. notatus, R. tanezumi*, and *T. glis* by *I. granulatus* were not statistically significant (p= >0.05), while infestations of *S. annandalei* by *I. granulatus* were highly significant ($p = 0.0005$). This suggests that *S. annandalei* is an important host for *I. granulatus* in Singapore.

Although credible intervals for median counts overlapped for all seasons within habitats, tick counts were higher overall in old forest habitats (Fig. 9). Estimated median tick counts were highest for *S. annanadalei.* Estimated median tick counts were similar for all other species.

3.3. Singapore results on probability of tick presence and tick counts on host age, sex and body condition

Credible intervals overlapped between small mammal ages and sex (Fig. 10a and b). Individuals with lower BCI values showed a higher probability of tick presence (Fig. 10c). A Wilcoxon rank sum test suggested that BCI was a statistically significant predictor of infestation by *I. granulatus*, with animals that had lower BCI values being significantly more likely to be infested ($p = 0.01$). Individuals with lower BCI values had higher tick counts (Fig. 11c). Credible intervals overlapped with adults showing higher tick counts than juveniles or subadults (Fig. 11a). However, Fisher's exact test suggested that age was not a statistically significant predictor of *I. granulatus* infestation risk ($p = 0.31$). Kruskal-Wallis rank sum test also suggested that age is not a statistically significant predictor of *I. granulatus* load ($p = 0.2$). Males had higher tick counts than females (Fig. 11b). Fisher's exact test suggested that sex was a statistically significant predictor of infestation by *I. granulatus* ($p =$ 0.02), with males being more likely to be infested than females. A Wilcoxon rank sum test also suggested that sex was also a statistically significant predictor of *I. granulatus* load ($p = 0.01$), with males once again more likely to have higher loads than females (Figs. 12 and 13).

4. Discussion

4.1. Ecology of Ixodes granulatus

Based on the field data collected in this study we regard *I. granulatus* as a non-nidicolous species as it is readily sampled via flagging. *Ixodes granulatus* typically dwells on the forest floor (Masuzawa et al., 2004). This is likely a behavioral trait developed to maximise ecological overlap with the rodents which constitute its primary hosts. Based on our

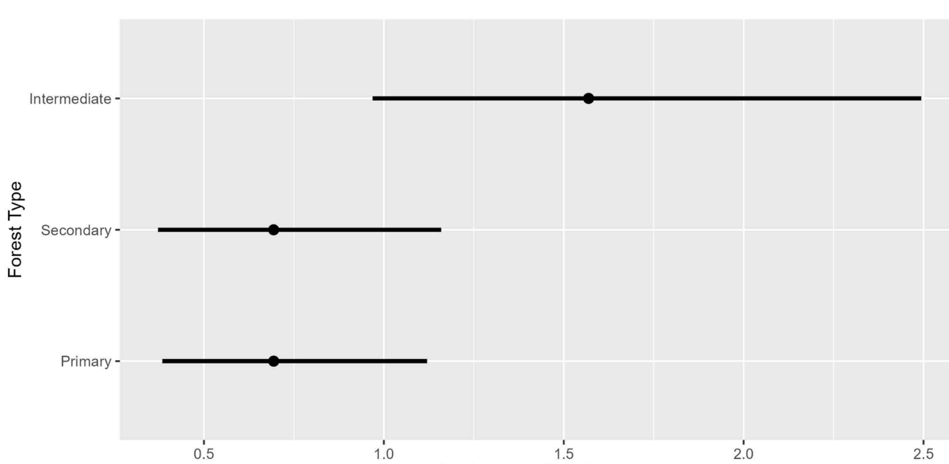

Fig. 6. Estimated median counts with 89% credible intervals of *I. granulatus* among 3 forest types in Tioman.

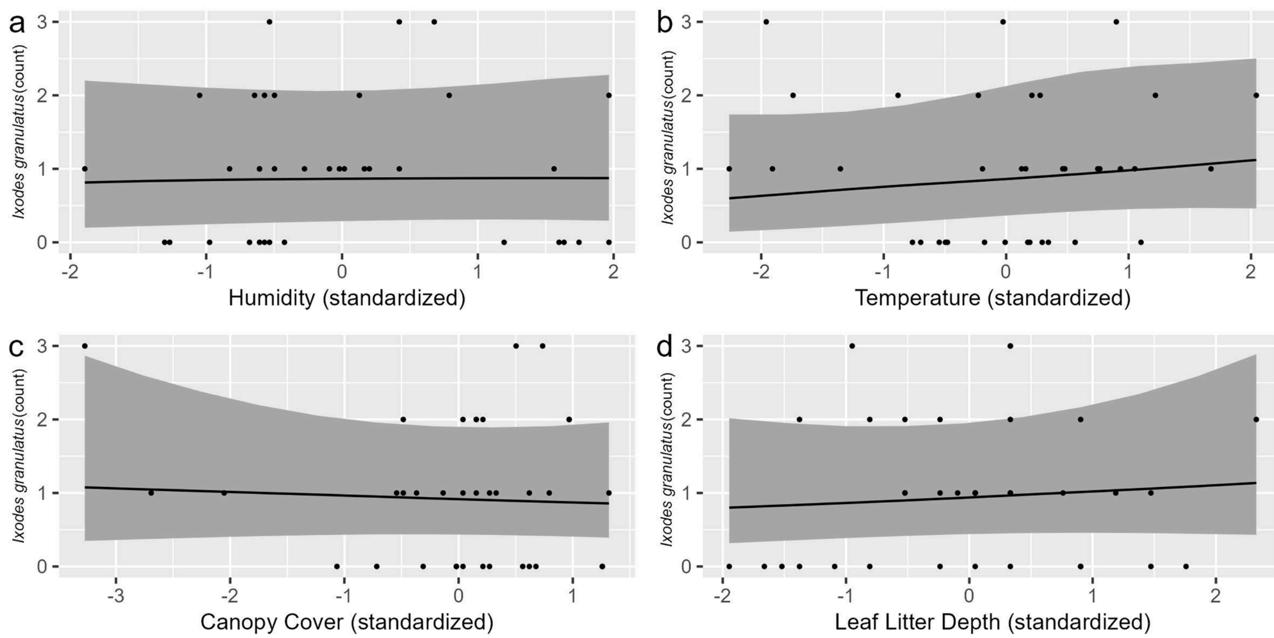

Fig. 7. Linear prediction results of counts of *I. granulatus*; (a) humidity, (b) temperature, (c) canopy cover, and (d) leaf litter depth with grey shaded 89% credible intervals.

Table 1
small mammals sampled across four habitat types (old forest, young forest, scrubland, parks/gardens) for *Ixodes granulatus* in Singapore.

Host species	Hosts sampled	Infested hosts	Infestation rate (%)	Mean burden ± 95% CI
Callosciurus notatus	100	3	3%	2**
Maxomys rajah	1	0	0%	–
Mus castaneus	37	0	0%	–
Sundamys annandalei	97	21	22%	4 ± 3.38
Rattus exulans	5	0	0%	–
Rattus norvegicus	33	0	0%	–
Rattus tanezumi	116	1	0.8%	1*
Rattus tiomanicus	23	0	0%	–
Suncus murinus	78	0	0%	–
Tupaia g	106	1	0.9%	1*

* = singleton infestations;.
** = confidence intervals not calculated due to small host samples (three or fewer hosts); infestation rate and burden rounded to nearest whole number; mean tick burden reported with a 95% confidence interval.

dataset from Singapore we showed that *I. granulatus* has strong habitat preferences, and was abundant in old forests, but was absent from scrubland, parks/gardens, and young forest. Interestingly, Paperna (2006) reported *I. granulatus* from young secondary forest on an offshore island in Singapore (Pulau Semakau). However, none of Paperna's specimens were available for examination in this study to confirm the identification. Therefore, it is unclear whether the record by Paperna (2006) constitutes *I. granulatus* or a morphologically similar species of *Ixodes*. However, if *I. granulatus* does indeed occur on Pulau Semakau it may support the hypothesis by Paperna (2006) that the loss of original habitat and natural hosts can drive rapid evolution in *I. granulatus* resulting in greater ecological plasticity than presently recognised. However, within the main island of Singapore and in Malaysia, *I. granulatus* appears to be relegated to old forest habitats. In addition to the findings on habitat preferences, our Malaysia dataset indicated that there was only limited difference in the abundance of *I. granulatus* within the age classes of old forest (Table 2). While the age of trees within these forest types varies markedly, we detected no significant difference in humidity, leaf litter depth, or canopy cover between them. Humidity and canopy cover have been suggested to be important factors which may influence the diversity and abundance of ticks in Southeast Asian forests (Kwak et al., 2021). Therefore, the relative uniformity in humidity, leaf litter depth, or canopy cover between the three forest types may account for the relative uniformity in abundance of *I. granulatus* between them. The slightly negative relationship that was found between leaf litter depth and tick abundance may be due to the fact that ticks could find it harder to attach to collecting flags in deeper leaf litter. Therefore, this relationship may reflect a weakness of the sampling method rather than a true negative relationship between tick abundance and leaf litter depth.

Host interactions are also a crucial factor which influence the ecology of ticks. We hypothesise that *I. granulatus* is a three-host tick given that all other members of the genus *Ixodes* (for which the life cycle has been studied) are three-host ticks (Apanaskevich and Oliver, 2014). We also suggest this because two-host and one-host life cycles in ixodid ticks are rare and appear to be a derived character in the Ixodidae rather than an ancestral one (Apanaskevich and Oliver, 2014). Across its range, *I. granulatus* predominantly infests rodents, particularly murids; though it has also been recorded from other mammals, birds, and reptiles (Guglielmone et al., 2014). Within our study sites in Singapore, *I. granulatus* was recorded from *S. annandalei, C. notatus, R. tanezumi*, and *T. glis*. However, *S. annandalei* was the most consequential host, a pattern which reflects that reported by Kwak et al. (2021) and Paperna (2006). Within this study, *S. annandalei* males were significantly more likely to be infested than conspecific females. This pattern may be explained by the fact that *S. annandalei* males have been shown to have significantly larger home ranges (~2 ha) than females (~0.5 ha) and exhibit greater movement across their ranges than females (Thillagavathy, 1968). This would likely bring them into contact with questing *I. granulatus* more frequently than the more sedentary females. More interesting though is the relationship between lower body condition and elevated risk of infestation and tick count by *I. granulatus*. Although a correlation is sometimes recorded between hosts with a reduced body condition and high parasite load, the causal direction between the two variables is difficult to determine; do more parasites produce weak

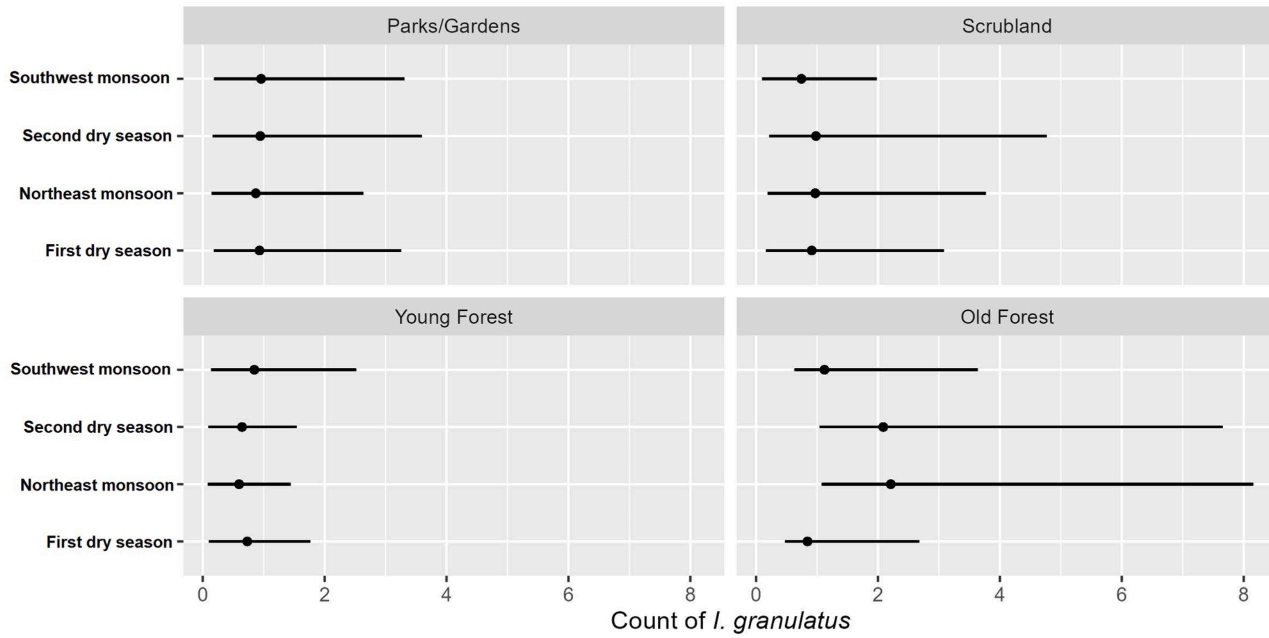

Fig. 8. Median tick count estimates and 89% credible intervals from the hierarchical Bayesian model for habitat and season in Singapore.

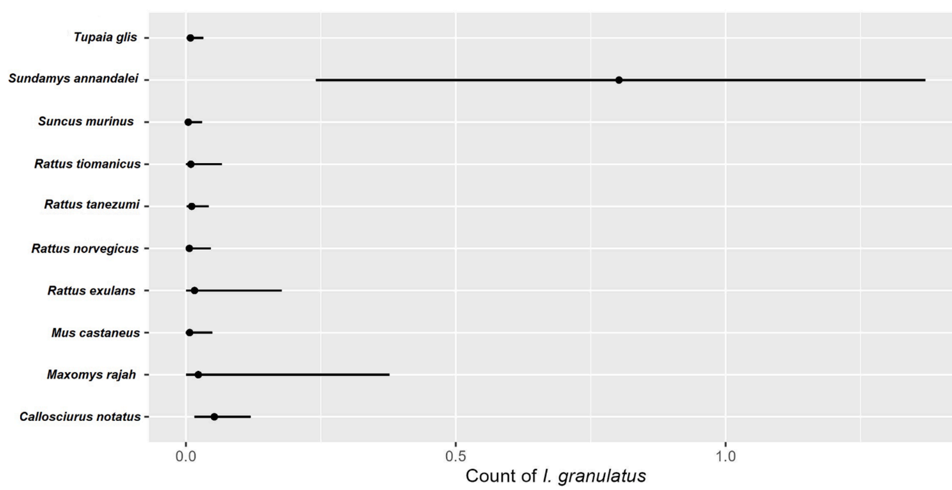

Fig. 9. Median tick count estimates and 89% credible intervals from the hierarchical Bayesian model for species in Singapore.

hosts, or do weak hosts contract more parasites? The conventional wisdom states that more parasites cause hosts to have lower body conditions. However, Coulson et al. (2018) provide strong arguments for a more nuanced view of relationships between parasite load and body condition. One hypothesis which may explain the case of high *I. granulatus* infestation risk in *S. annandalei* with lower body condition is that hosts with lower body condition may spend more time foraging in an effort to ameliorate their low body condition and in doing so increase their probability of encountering questing *I. granulatus*. However, without carefully planned field studies it's simply not possible to determine the direction of the causality between *I. granulatus* infestation and body condition in *S. annandalei*. Therefore, further study is required to elucidate the cause(s) of this relationship.

4.2. Medical importance of *I. granulatus*

Human infestation by *I. granulatus* has been recorded in numerous countries including Singapore (Paperna, 2006), Malaysia (Audy et al., 1960), Thailand (Tanskul et al., 1983), Vietnam (Grokhovskaya and Nguyen, 1968), South Korea (Yun et al., 2014), and Japan (Doi et al., 2022). Adult female *I. granulatus* are most commonly encountered either crawling on, or attached to, humans (e.g., Guglielmone and Robbins, 2018; Kwak, 2020; Doi et al., 2022), though infestations caused by larvae have also been described (Paperna, 2006). Infestations by *I. granulatus* reportedly cause lesions at the site of the bite, though no other impacts have yet been reported (Paperna, 2006).

Tick-borne pathogens can be a source of unidentified acute febrile illness in humans, many of which are underdiagnosed (Belongia et al., 2001). A wide range of microbial genera with pathogenic members have been detected in *I. granulatus*. These include *Ehrlichia* (Takano et al., 2009), *Borrelia* (Chao et al., 2010; Khoo et al., 2018; Chao et al., 2012), and *Rickettsia* (Fujita et al., 2008; Tsai et al., 2008; Shih et al., 2021). However, while many of these are closely related to known pathogens, they themselves have not yet been shown to be pathogenic in humans at the time of writing. Therefore, their public health risk remains largely unknown. Despite this, two microbial species known to be pathogenic in humans have been detected in *I. granulatus*, namely *Rickettsia honei* (Kollars et al., 2001) and *Borrelia burgdorferi* s.l. (Chao et al., 2009).

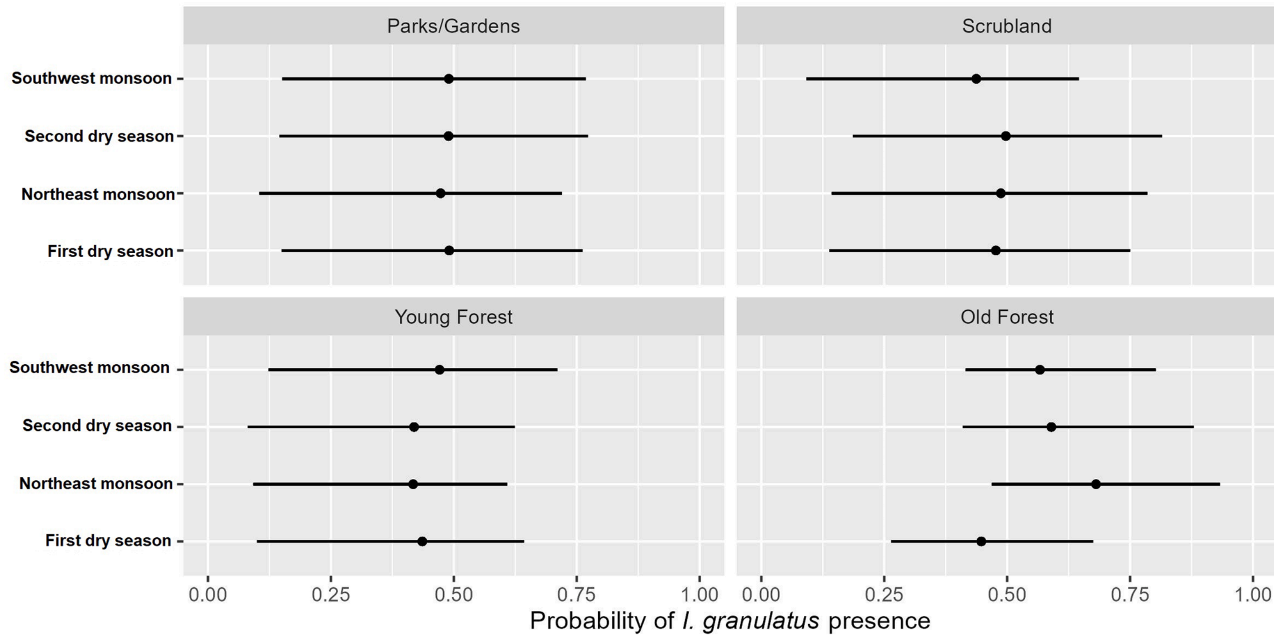

Fig. 10. Median probability estimates and 89% credible intervals from the hierarchical Bayesian model for habitat and season in Singapore.

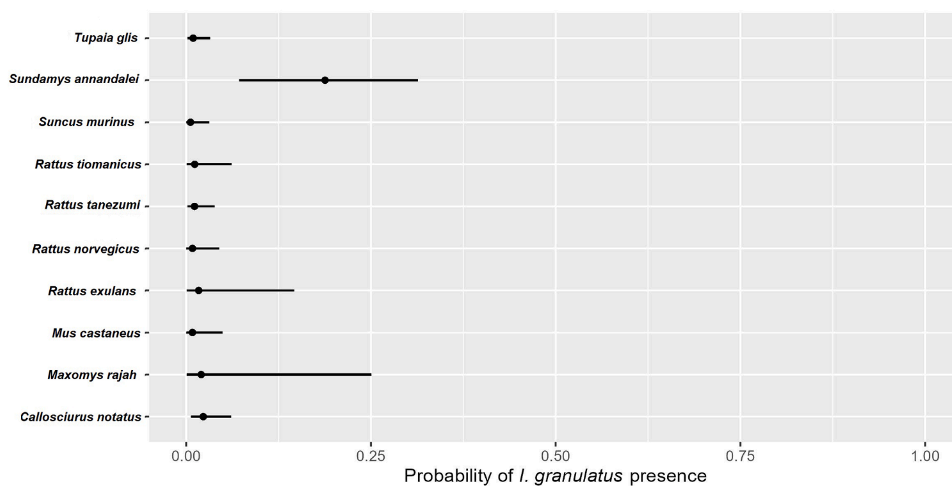

Fig. 11. Median probability estimates of tick presence and 89% credible intervals from the hierarchical Bayesian model for species in Singapore.

Rickettsia honei is an important and widespread spotted fever group *Rickettsia* known to cause serious morbidity in humans (Graves and Stenos, 2003). It has been detected in a range of reservoir hosts including both mammals and reptiles as well as from several different tick genera including *Amblyomma, Bothriocroton*, and *Ixodes* (Graves and Stenos, 2003). The *Borrelia burgdorferi* species complex is perhaps the most widely known (and studied) lineage of tick-borne pathogens and represent a major cause of tick-borne disease in humans (Rudenko et al., 2011). The *B. burgdorferi* complex is transmitted by a number of tick species, predominantly members of the genus *Ixodes*. (Rudenko et al., 2011). *Ixodes granulatus* represents a species of major public health concern in Asia and increased research should be focused on the relationships between this tick and the microbial species associated with it; particularly the competency of *I. granulatus* to act as a vector of *R. honei* and *Borrelia burgdorferi* (s.l.).

4.3. Tick ecology and infestation prevention efforts

Abiotic environmental factors contribute significantly to the abundance and diversity of ticks. This is largely because ticks spend the vast majority of their lives off the host, in the environment. Temperature and humidity are major drivers of desiccation rate which is a major factor ticks must contend with. Bull and Smyth (1973) showed that desiccation rate was a significant driver of range partitioning between three tick species with abutting allopatric distributions. In contrast to this, Ehrmann et al. (2017) showed that in other cases microclimate only plays a minimal role while habitat structure acted as a major driver of tick abundance and occurrence. Dobson et al. (2011) further highlighted the importance of habitat structure on tick abundance. A more detailed understanding of the ecological drivers of tick abundance provides the foundations for more active infestation prevention efforts. However, the focus of such prevention efforts can vary markedly. Ehrmann et al. (2017) suggest that tick infestation risk could be partially addressed through actions taken to manage forest to minimise the availability of habitat favourable to ticks. However, efforts to mitigate the ecosystem disservices imposed by ticks may undermine important ecosystem services provided by other species which share their habitats. Therefore, a more ecologically sensible approach to addressing the risks associated

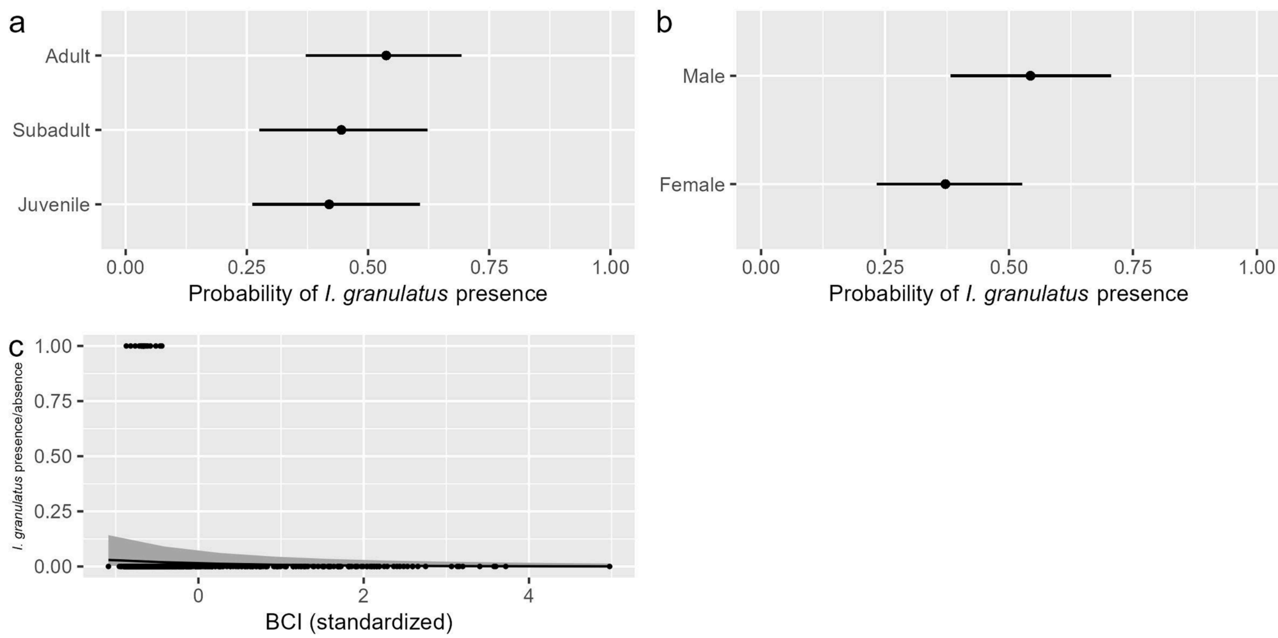

Fig. 12. Median probability estimates of tick presence and 89% credible intervals and linear predictions of *I. granulatus* presence for small mammal; (a) age, (b) sex, and (c) body condition in Singapore.

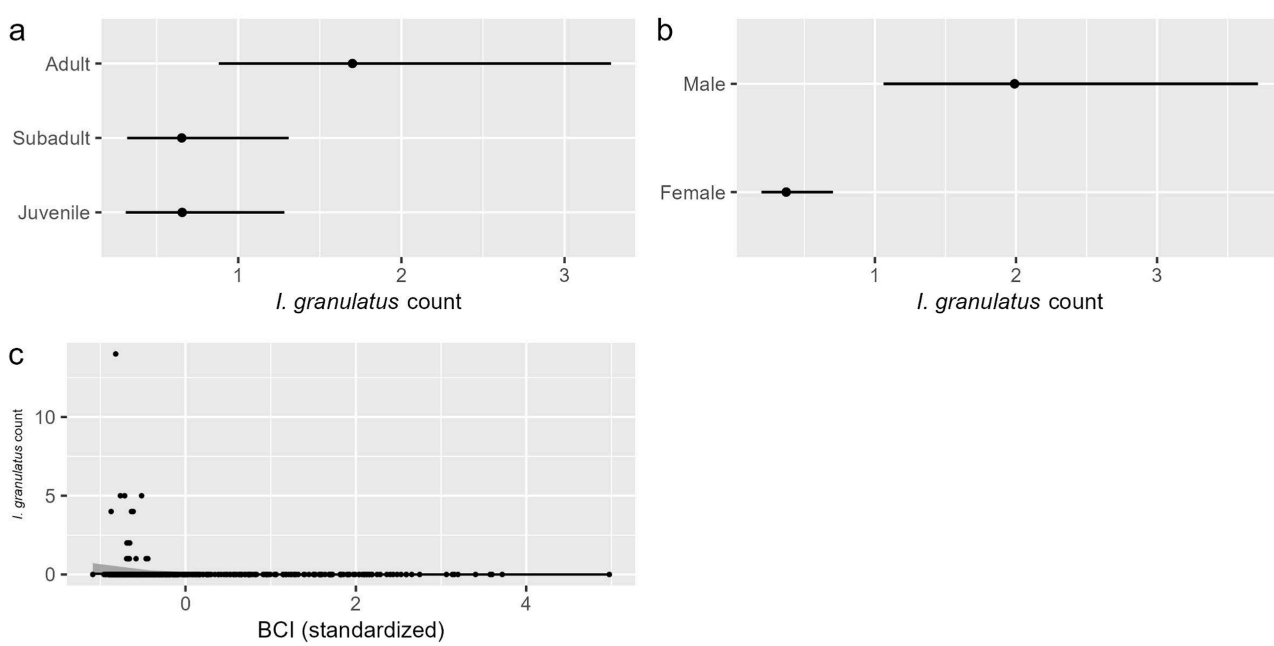

Fig. 13. Median tick count estimates and 89% credible intervals and linear predictions of *I. granulatus* counts for small mammal; (a) age, (b) sex, and (c) body condition in Singapore.

Table 2
Ixodes granulatus flagged from different forest types in Malaysia.

Forest type	Number samples per forest type	Mean number of ticks per site
Old secondary forest	9	0.75
Intermediate forest	19	1.5
Primary forest	8	0.6

with ticks may not be to reduce favorable habitat areas but instead to reduce the ecological overlap between humans and *I. granulatus* within these areas. Plowright et al. (2017) highlight that pathogens and parasites must overcome numerous ecological thresholds for spillover to occur, and that adequate management of any single one of these thresholds has the potential to prevent spillover. Human exposure and structural barriers are two key thresholds ticks must overcome to bite humans. However, both of these can be subjected to high levels of control by public heath bodies to significantly reduce tick infestation risk. The first of these thresholds (human exposure to ticks) can be minimized through environmental risk mapping (e.g., Swart et al., 2014) which can help planners to designate no-access areas (in areas with high tick abundance), design hiking trails through areas of habitat with lower tick abundances, and provide the public with forecasts on seasonal tick risk. The second of the aforementioned thresholds (structural barriers) are those designed to reduce the risk of biting if a human is exposed to a tick. This includes protective clothing, regular tick checks, and repellents which planners can advocate for among the public (Evans et al., 1990).

5. Conclusions

Understanding the ecology of the medically important tick *I. granulatus* is crucial to mitigate human infestation by this species throughout much of Asia. This study highlighted the importance of old forest habitats and rodents for the persistence of *I. granulatus* populations. It also provided evidence for the non-nidicolous ecology of *I. granulatus* as evidenced by the successful surveying of this species from the forest floor via flagging, and the significantly higher loads of *I. granulatus* on male *S. annandalei* (which are far more active than female conspecifics). Efforts aimed at reducing *I. granulatus* infestations should focus on minimising contact between humans and ticks in the old forest habitats which this tick species prefers.

Ethical statement

No experiments were performed on animals or human subjects and all ethical guidelines for research in Japan, Singapore, and Malaysia were adhered to. All small mammal work was approved by the National University of Singapore Institutional Animal Care and Use Committee (IACUC protocol approval B01/12), National Parks (Permit no NP/RP12- 004) and Agri-Food and Veterinary Authority of Singapore (Permit no AV16.01.004.0004).

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data availability

Data will be made available on request.

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