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Source: Mammal Study, 47(4) : 275-282

Published By: Mammal Society of Japan

URL: https://doi.org/10.3106/ms2022-0022

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Mammal Study 47: 275–282 (2022) DOI: 10.3106/ms2022-0022
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Rapid communication

Differential tick-infestation rate between *Rattus norvegicus* and *R. rattus*, with the first records of the ixodid tick *Ixodes granulatus* and its infestation in rodents, free-ranging cats, and humans from Mikura-shima Island, Japan

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Abstract. Relatively few studies have investigated host-tick relationships in island environments. To assess the relationships, we captured a total of 51 rodents (Norway rat, $n = 29$; black rat, $n = 22$) from which a total of 56 ticks were collected to assess host-tick relationships on Mikura-shima Island, Izu Islands, Japan. We also collected two ticks from a free-ranging cat and a tick from a human resident on the island. The 53 morphologically identifiable ticks were *Ixodes granulatus*, whose distribution on Mikura-shima Island was not previously reported. Interestingly, the tick infestation rates significantly differed between two rodent species: 44.8% ($n = 29$) in the Norway rats and 4.5% ($n = 22$) in the black rat. This insight prompts consideration of the mechanisms behind the differential tick-infestation rate in wildlife hosts and infectious disease transmission in rodent-dominated ecosystems. This study is also the first record of this ixodid infesting domestic cats and a human from the Izu Islands and is the second case of this ixodid biting a human in Japan. Future work should characterize the tick fauna of the island environment and the potential risks of tick-borne diseases.

Key words: Mikura Island, on-host copulation, rodent trapping, tick-host relationships, zoonoses.

Abstract in Japanese（要旨）．ドブネズミとクマネズミにおける異なるマダニ寄生率，および御蔵島からのミナミネズミマダニ *Ixodes granulatus* の分布とそのネズミ，イエネコ，ヒトへの刺咬事例の初記録．島嶼域における移入ネズミ類とマダニの関係を評価した研究は比較的少ない．本研究では，伊豆諸島の御蔵島におけるマダニとホストの関係を明らかにするために，51 頭のネズミ（ドブネズミ 29 頭，クマネズミ 22 頭）を捕獲し，これらから 56 頭のマダニを採集した．また，ノネコから 2 頭と島民から 1 頭のマダニを採集した．種同定が可能であった 53 頭のマダニのすべてがミナミネズミマダニ *Ixodes granulatus* と同定され，御蔵島において本種の初めての分布記録となった．興味深いことに，2 種のネズミの間でマダニの寄生率が異なり，ドブネズミが 44.8%（$n = 29$），クマネズミが 4.5%（$n = 22$）であった．この結果は，ネズミ類が優占する生態系における宿主間の寄生率の違いや感染症伝播プロセスのメカニズムの解明に示唆を与えるであろう．また，本研究は伊豆諸島においてミナミネズミマダニがイエネコとヒトを刺咬した初めての報告になる．特に，ヒトの刺咬例としては私たちが知る限り日本で 2 例目となる．島嶼におけるマダニの基本的な情報についてはまだ不足しており，今後のマダニ相の把握やマダニ媒介感染症の潜在的リスクの評価が求められる．

Ticks are one of the primary vectors of pathogens that burden humans and animals worldwide (Randolph 2009; Colwell et al. 2011). The vector tick population feeds on the blood of host animals, whose abundance can determine the tick abundance and therefore the frequency of tick–human encounters (Wilson 1994). However, not all

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host animals equally contribute to tick abundance. For example, the anti-tick behavior of hosts can decrease the intensity of tick infestation (Keesing et al. 2009; Doi et al. 2021). On the other hand, due to the host preference of ticks, the presence or increase of one host can lead to an increase in a particular tick species (Keesing et al. 2013; Hofmeester et al. 2017; Valcárcel et al. 2017). Thus, the host-tick relationship is essential for vector control, providing fundamental insights into tick population dynamics and tick-borne disease transmission (Randolph 2004; Ostfeld et al. 2018).

The rodent is a reservoir of various tick-borne pathogens, such as *Borrelia burgdorferi* sensu lato, *Babesia microti*, *Ehrlichia* sp., *Rickettsia helvetica*, and *R. honei* (Hu et al. 1997; Karbowiak 2004; Fujita et al. 2008; Takano et al. 2009; Gassner et al. 2013; Burri et al. 2014). Norway rat (*Rattus norvegicus*) and black rat (*R. rattus*) are common rodents residing both near human residences (Feng and Himsworth 2014) and in natural habitats, such as the islands where they were introduced. Indeed, non-native rodents often dominate island environments lacking mammalian species (Harper 2006; Harper and Bunbury 2015); thus, they may constitute the main host of ticks in the island environment. Although previous studies conducted on islands have frequently focused on rodents as reservoirs of tick-borne pathogens (e.g., Smith et al. 1993; Fujita et al. 1996; Yamauchi et al. 2013; Werden et al. 2014; Cicculli et al. 2019), relatively few studies have focused on the ecological evaluation of the host-parasite relationship between ticks and multiple introduced rodents in the island environments (Rand et al. 2004; Kwak et al. 2021). Island environments are important systems in terms of human health risk, as they often lack medical facilities and have weak health care systems. On the other hand, the island environment has simpler fauna in both host wildlife and ticks than the mainland, making it a suitable study site for elucidating the mechanisms of host-tick relationships.

Here, we investigated the host-tick relationships on human-inhabited Mikura-shima Island, Izu Islands, Japan, where there is no distribution of native mammals except for bats, and introduced rodents, Norway rats and black rats (Takada et al. 1999; Azumi et al. 2019), and free-ranging cats (Oka 2019; Tokuyoshi et al. 2020; Azumi et al. 2021; Nagata et al. 2022) have been established. In addition, to the best of our knowledge, the only ixodid ticks collected from this island were *Ixodes philipi* from the breeding burrows of the streaked shearwaters (*Calonectris leucomelas*) (Takahashi et al. 2005; Mitani et al. 2007) (Supplementary Table S1). In our preliminary research, we were unable to collect any other ticks by flagging, which is a common sampling method in tick surveys. To describe the present composition of host-tick relationships, we conducted trapping rodents, the dominant mammalian species of this island, throughout the island, and identify the tick species infesting captured rodents. We also quantified tick infestation rates in the rodents, which has not been evaluated in previous studies on Izu Islands.

Furthermore, we report ticks collected from a free-ranging cat and a resident in this study. Together with the results on rodents, we have obtained tick-infestation information from all terrestrial mammals on Mikura-shima Island, all of which are the first records of host-tick relationships on Mikura-shima Island.

Materials and methods

Study area

Mikura-shima Island (33°52'N, 139°36'E) (also described as Mikura-jima Island or Mikura Island) is located on the Izu Islands, Japan (Fig. 1). The island is 20.54 km^2 with 851 m peak elevation. The broad-leaved evergreen forest covers the lower to middle altitude, and bamboo bush and shrub cover the higher altitude (Kamijo et al. 2001). The climate is temperate (Kawamoto 2006). Approximately 300 humans live on the island, mainly settled in the northwestern part of the island (Mikura-shima Village 2022) (Fig. 1). The two species of introduced rodents are the main terrestrial mammalian species of Mikura-shima Island (Azumi et al. 2019), and the free-ranging cat is the only carnivorous mammal on the island (Oka 2019; Tokuyoshi et al. 2020; Azumi et al. 2021; Nagata et al. 2022).

Rodent sampling

The rat traps were set for a total of 496 trap nights to capture Norway and black rats from July to September of 2021. The HOGA S-B141023 box trap (HOGA, Kyoto, Japan) was used with dried sausages. Each capturing site consisted of 16 traps arranged in a 4 × 4 grid with 20 m intervals. The nine capturing sites were selected at the nesting sites of streaked shearwater (Fig. 1). The traps were checked every day. The captured rodents were euthanized using carbon dioxide gas.

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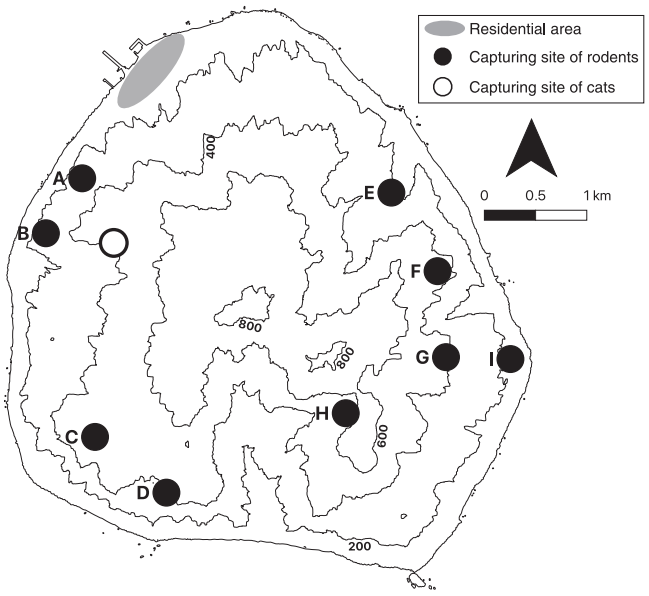


Fig. 1. Capturing sites of rodents and a feral cat on Mikura-shima Island.

Tick samples and infestation rate in rodents

The captured rodents were visually examined, and infesting ticks were collected using forceps. The collected ticks were preserved in 99.5% ethanol until morphological identification under stereo microscope (SMZ-180, Nikon, Tokyo, Japan). The ticks were identified according to the methods described by Yamaguti et al. (1971) and Fujita and Takada (2007). Then, the infestation rates were compared across rodents using the chi-squared test.

We also identified ticks collected from a free-ranging cat and a human resident of the island (one of the authors: K. Kogi). Two ticks were collected from a free-ranging cat, which was captured on December 28, 2021, approximately 1.5 km from a residential area for free-ranging cat management; it was a male juvenile with a body weight of 1.4 kg. A tick bit the resident's shoulder on July 20, 2019, and was preserved in 70% ethanol.

Results

We captured a total of 51 rodents (Norway rat, $n = 29$; black rat, $n = 22$), from which a total of 56 ticks were collected from 13 Norway rats and one black rat (Table 1). The number of ticks collected from each rodent ranged from 0 to 13. The ticks were mostly found infesting the dorsocervical and dorsolumbar regions of the rodents. The ticks were collected from rodents at all capturing sites, except for sites D and F (Fig. 1, Table 1). We also collected two ticks from a free-ranging cat and a tick from a human resident (Table 2, Fig. 2).

Of the 53 ticks collected from rodents, two ticks collected from free-ranging cat, and a tick collected from human were identified as *I. granulatus* according to the morphological characteristics of short internal and external spurs on coxa I; the male cornua is short and absent in the female and nymph, the length of dorsal setae and scutum setae are the same in nymph, anterior tip of hypostoma is brunt in female and nymph, and the female scutum is granulated and long along the antero-

Table 1. Tick *Ixodes granulatus* infestation rate in Norway rats *Rattus norvegicus* and black rats *R. rattus* on Mikura-shima Island

Capturing sites*	Norway rats			Black rats		
	Number of infested animals	Number of captured animals	Tick infestation rate (%)**	Number of infested animals	Number of captured animals	Tick infestation rate (%)**
A	4	7	57.1	0	7	0
B	1	3	33.3	0	3	0
C	1	1	100.0	1	5	20.0
D	0	2	0	0	1	0
E	4	8	50.0	–	–	–
F	–	–	–	0	2	0
G	1	2	50.0	0	3	0
H	1	3	33.3	0	1	0
I	1	3	33.3	–	–	–
Total	13	29	44.8	1	22	4.5

* Capturing sites (A–I) correspond to those in Fig. 1.
** The infestation rate was calculated as the proportion of rodents infested with one or more ticks.

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Table 2. Stage composition of *Ixodes granulatus* collected from Norway rats, black rats, free-ranging cat, and human in Mikura-shima Island

Host species	Number of infested animals investigated in this study	Number of *I. granulatus*				Number of unidentified ticks	Sampling period
		Adult male*	Adult female**	Nymph	Larva		
Norway rat	13	5	35 (n = 4, 11.4%)	0	0	6	July to September, 2021
Black rat	1	2	8 (n = 1, 12.5%)	0	0	0	July to September, 2021
Free-ranging cat	1	0	0	2	0	0	December 28, 2021
Human	1	0	1	0	0	0	July 20, 2019

* including four and one copulating males from Norway and black rats, respectively.
** Numbers and percentages of copulating female individuals were also recorded.



Fig. 2. Human infestation case of *Ixodes granulatus* (photo taken by Kazunobu Kogi).

posterior axis (Fig. 3). The species of the remaining six ticks collected from Norway rats could not be identified because of loss of or damage to the capitulum, coxae, and scutum.

The tick infestation rates across the two rodent species were significantly different ($\chi^2 = 4.75$, $df = 1$, $P < 0.05$): 44.8% of Norway rats (13/29) and 4.5% of the black rats (1/22) were infested by *I. granulatus* (Table 1).

We also confirmed on-host copulating *I. granulatus* collected from rodents (Fig. 4); 11.4% (4/35) and 12.5% (1/8) of the female *I. granulatus* infesting Norway rats and black rats, respectively, were copulating with males (Table 2). All the ticks collected from rodents and the resident were at the adult stage, and the tick from the free-ranging cat was at the nymph stage (Table 2).

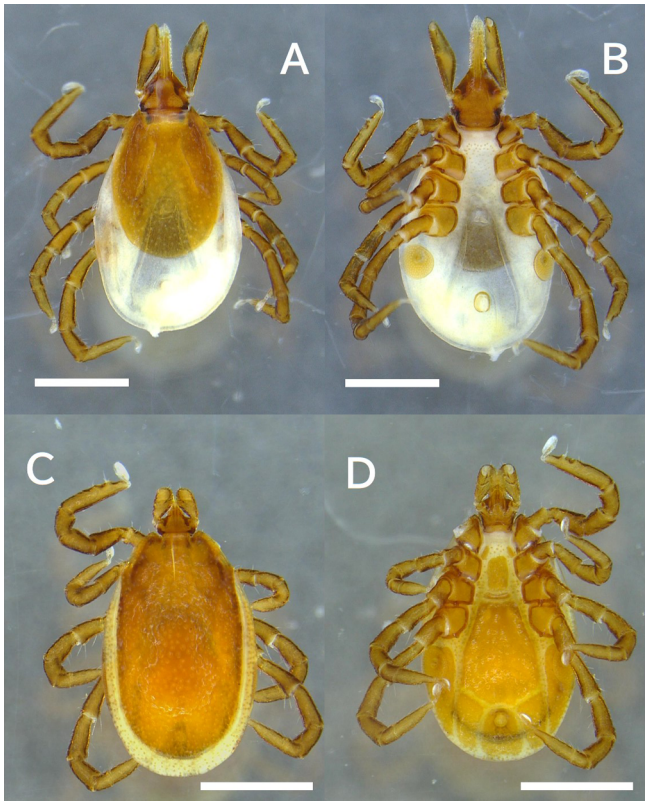


Fig. 3. Image of collected *Ixodes granulatus*. Dorsal and ventral view of female adult (A and B) and male adult (C and D). The scale bar indicates 1 mm.

Discussion

This study confirmed *I. granulatus* distribution in Mikura-shima Island for the first time based on the infestation in all terrestrial mammalian hosts on this island. In addition, *I. granulatus* was found from rodents captured throughout the island. This indicates that it is a common ixodid tick inhabiting Mikura-shima

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Fig. 4. Copulating pair of *Ixodes granulatus*. Six cases of on-host copulation of *I. granulatus* were confirmed. The scale bar indicates 1 mm.

Island. Prior to this study, the only tick species found on Mikura-shima Island was *I. philipi* (Supplementary Table S1) (Mitani et al. 2007; Takahashi et al. 2005), which mainly infests avian hosts (Guglielmone et al. 2014). Both studies of *I. philipi* on Mikura-shima Island collected the ticks from the nest burrows of the streaked shearwater. Meanwhile, *I. granulatus* is often collected from rodent hosts as well as domestic cats and small Indian mongooses (*Urva auropunctata*) on the Nansei Islands, Japan (Kitaoka and Suzuki 1974; Takada 1990; Fujita et al. 1996; Shimada et al. 2003; Fujita et al. 2008; Ishibashi et al. 2009). Thus, the lack of studies on tick infestation in mammalian hosts may explain why *I. granulatus* was not previously reported here.

Although *I. granulatus* has already been reported from rodent hosts on neighboring islands (Asanuma and Sekikawa 1952) (Supplementary Table S1), our study is the first to quantify the tick infestation rates in this region, since Asanuma and Sekikawa (1952) have not recorded quantitative tick-infestation rates on rodents. It is noteworthy that differential tick-infestation rate between Norway rats and black rats was detected in this study: black rats were less infested by *I. granulatus* than Norway rats. Black rats are known to be more arboreal than Norway rats, which are more ground-dwelling (Key and Woods 1996; Foster et al. 2011), which may help black rats avoid infestation. However, Ishak et al. (2018) reported the opposite results, recording *I. granulatus* infestation in black rats but not in Norway rats in the mainland of Malaysia. Similarly, Mori et al. (2019) showed that black rats were infested by *I. ricinus* more intensively than Norway rats in the mainland of Italy. There is still no unifying hypothesis explaining this context dependency in these tick infestations. Thus, this study, which provides new case study on different infestation rates of *I. granulatus* in rodents in the island environment, will motivate future research on the mechanisms behind the differential tick-infestation rate in wildlife hosts and infectious disease transmission, especially in rodent-dominated ecosystems.

Infestation of *I. granulatus* in domestic cats (Shimada et al. 2003; Azama et al. 2022) and human (Azama et al. 2020) was previously reported only from Okinawa Prefecture, the Nansei Islands, in Japan. Therefore, this study is the first record of this ixodid infesting domestic cats and human on the Izu Islands (Supplementary Table S1). To the best of our knowledge, there was previously only one case of a human bite by this ixodid in Japan (Azama et al. 2020). Therefore, this study reports the second case of human tick-bite by this ixodid in Japan (Fig. 2). Because the domestic cats are one of the most familiar animals to humans, there is the potential risk to transfer the ticks and associated pathogens (for Mikura-shima Island, e.g., *R. honei*, *Candidatus* Neoehrlichia mikurensis) (Fujita et al. 1996; Kawahara et al. 2004; Fujita et al. 2008; Silaghi et al. 2016) from free-ranging domestic cats to humans (Dantas-Torres and Otranto 2014; Duplaix et al. 2021; Nguyen et al. 2021). While rodent population control would not be usually a practical attempt, human behavior changes toward domestic cats, such as keeping owned cats indoors and restrictions on direct contacts with free-ranging cats, would more readily reduce the risk of tick bite and subsequent transmission of tick-borne zoonoses.

We also note that copulation of *I. granulatus* was observed in our samples. While many ticks in genus *Ixodes* are known to mate only in off-host environments, such as on vegetation, some *Ixodes* ticks belonging to the *I. ricinus* complex and *I. angustus* engage in off- and on-host copulation (Kiszewski et al. 2001; Sonenshine and Coons 2014). Whereas *I. granulatus* is rarely collected off-host such as vegetations (Doi et al. 2020) and off-host copulation of this tick has never been observed, Ishak et al. (2018) previously reported that all adult male *I. granulatus* specimens were collected while cop-

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ulating with females on wildlife hosts. We also observed on-host copulation in this tick. *I. granulatus* is closely related to the *I. ricinus* and thus may have the same mating strategies.

Conclusion

This study reported the distribution of the *I. granulatus* on Mikura-shima Island; no tick investigations in animals had been conducted here–only from bird nest burrows. We not only reported distinct *I. granulatus* infestation rates in Norway and black rats but also collected this tick species from a free-ranging cat and human resident. However, general information about the tick fauna on Mikura-shima Island and the quantified tick burden of the rodents remain unknown. Future studies should elucidate tick fauna and the potential risks of tick-borne diseases in the island environment.

Supplementary data

Supplementary data are available at *Mammal Study* online.
Supplementary Table S1. Records of ticks from the Izu Islands from 1942 to present.

Acknowledgments: We would like to thank Nariko Oka for preparing application on field sampling and Hisayo Hayama for collecting tick samples in the field. We would also like to thank two anonymous reviewers for their constructive comments. We got permissions of conducting research and sampling on Mikura-shima Island from Mikura-shima Village office. YW completed the ethical course of Animal Care and Use, and Human Subjects Research authorized by Association for the Promotion of Research Integrity (course completion report no. AP00000626504). This study was supported by the Environment Research and Technology Development Fund (JPMEERF20204005, JPMEERF20204006) of the Environmental Restoration and Conservation Agency of Japan, and the Research Fellow of Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP22J01651.

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Received 1 June 2022. Accepted 27 June 2022.
Published online 16 September 2022.
Editor was Jun J. Sato.

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