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Article

Ixodes nipponensis Kitaoka and Saito and *Amblyomma testudinarium* Koch (Acari: Ixodida: Ixodidae) Collected from Reptiles (lizards, skinks, and snakes) in the Republic of Korea, 2016

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Abstract

A survey of reptile-associated ticks was conducted from March–October 2016 to determine their relative abundance, stage of development, and geographic and host distributions among lizards, skinks, and snakes in the Republic of Korea. A total of 30 lizards (3 species), 5 skinks (1 species), and 63 snakes (10 species) were collected. A total of 66 ixodid ticks belonging to two species (*Ixodes nipponensis* and *Amblyomma testudinarium*) were collected from 11/30 (36.7%) lizards, 2/5 skinks (40.0%), and 6/63 snakes (9.5%). *Ixodes nipponensis* was collected only from lizards and skinks, while *A. testudinarium* was collected only from snakes. The Amur Grass lizard, *Takydromus amurensis,* had the highest tick index (3.0) (total number ticks/total number hosts) among lizards and skinks, while the Short-tailed Mamushi (Viperidae), *Gloydius brevicaudus*, had the highest tick index (0.57) among snakes. *Ixodes nipponensis* larvae and nymphs accounted for 46.4% and 53.6% of all ticks collected from lizards and skinks, respectively, while only *A. testudinarium* nymphs were collected from snakes. Nymphs of both species of ticks were collected from lizards, skinks, and snakes from March-September, while *I. nipponensis* larvae were collected only from June-September. *Ixodes nipponensis* larvae and nymphs were preferentially attached on the foreleg axillae (66.1%), followed by lateral trunk (23.2%) and head and near the eye (10.7%) of lizards and skinks. None of the ticks collected from lizards, skinks or snakes were positive for severe fever with thrombocytopenia syndrome virus (SFTSV).

Key words: *Ixodes nipponensis, Amblyomma testudinarium,* lizards, skinks, snakes, Korea

Introduction

Ixodid ticks are ectoparasites of a broad range of hosts, including reptiles, *e.g.*, lizards, skinks, tortoises, and snakes (Yoneda 1981, Bauwens *et al*. 1983, Krinsky 1983, Hammond & Dorsett 1988, Fujita & Takada 1997, Durden *et al*. 2002, Eisen *et al*. 2004, Fajfer 2012). Noh (1965) first reported *Ixodes granulatus* Supino blood feeding on a Tsushima Ground Skink, *Scincella vandenburghi* (Schmidt), in the Republic of Korea (ROK), and later Ra *et al*. (2011) reported *Ixodes nipponensis*

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Kitaoka and Saito nymphs on four lizard species collected at 22 sites in the ROK. More recently, *I. nipponensis* nymphs and larvae and *Amblyomma testudinarium* Koch nymphs were reported blood feeding on three species of lizards and three species of snakes, respectively, collected in 6 provinces and one metropolitan city of the ROK (Suh *et al*. 2016).

The role of reptiles as hosts of ticks and as reservoir hosts of bacterial pathogens, *e.g.*, *Borrelia* spp. and spotted fever group rickettsiae, has been investigated in some detail (Wright *et al*. 1998, Dsouli *et al*. 2006, Reeves *et al*. 2006), while studies of their role as reservoir hosts of arboviruses, *e.g.*, severe fever with thrombocytopenia syndrome virus (SFTSV), have been limited (Reisen *et al*. 2007, Suh *et al*. 2016). SFTSV, a tick-borne Bunyavirus, was first identified in China in 2009 and retrospectively in a patient that died in Korea in 2012. With better recognition, the number of SFTS cases increased from 36 (17 deaths; 47.2%) in 2013 (including 1 retrospective case in a patient that died in 2012), 55 (16 deaths; 29.1%) in 2014, 79 (21 deaths; 26.6%) in 2015, 165 (19 deaths; 11.5%) in 2016, and >250 (54 deaths; 20.0%) in 2017 (KCDC 2017). To better understand the relationship between reptilian hosts and associated ticks and pathogens affecting human and veterinary health, a program was developed to determine tick relative abundance and infestation rates, as well as the stage(s) of development associated with particular host species, and the geographical distributions of both reptiles and associated ticks.

Materials and methods

Tick collections

The National Institute of Biological Resources (NIBR), Incheon Metropolitan City, collaborated with the Korea National Institute of Health (KNIH), Korea Centers for Disease Control and Prevention (KCDC), Cheongju-si, Chungbuk Province, Republic of Korea, and the Medical Department Activity-Korea (MEDDAC-K)/65th Medical Brigade, Yongsan US Army Garrison, Seoul, ROK, to conduct a tick-borne disease surveillance program as it relates to reptilian hosts (lizards, skinks, and snakes) in five provinces [Chungbuk (Boeun and Goesan counties), Chungnam (Yaesan County), Jeonbuk (Wanju and Buan counties), Jeonnam (Haenam, Gangjin, Shinan and Yeongam counties, and Yeosu City), and Gyeongnam (Hapcheon and Goseong counties)] from March-October, 2016 (Figure 1). Lizards, skinks and snakes that were not infested with ticks were released at the capture site, while those infested with ticks were necropsied under an institutionally approved animal use protocol. Prior to necropsy, ticks were carefully removed with a fine forceps, placed in 2-ml cryovials containing 80% ethanol, and sent to the Entomology Section, Force Health Protection and Preventive Medicine, MEDDAC-K, where they were identified to species and developmental stage under a dissecting microscope using standard keys and current nomenclature (Yamaguti *et al*. 1971, Guglielmone *et al*. 2014).

Detection of SFTSV

Following identification, all ticks (40 nymphs and 26 larvae) were placed in a secure Styrofoam container of dry ice and transported to the KNIH, where they were stored at -80°C until assayed for the detection of the partial medium (M) gene segment of SFTSV by reverse transcription-polymerase chain reaction (RT-PCR). Tick samples were homogenized individually in 600 µl of phosphate-buffered saline (pH 7.0) using a Precellys® 24 high-throughput tissue homogenizer (Bertin Technologies, Bretonneux, France) and 2.8-mm stainless steel beads. A viral RNA extraction kit (iNtRON Biotechnology, Seongnam, ROK) was used to extract RNA from the supernatant of the tick homogenates. To detect the partial M segment of SFTSV, a 1-step RT-PCR was performed using a

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DiaStar™ 2X OneStep RT-PCR Pre-Mix Kit (SolGent, Daejeon, ROK) via a previously described method (Yun *et al*. 2014).

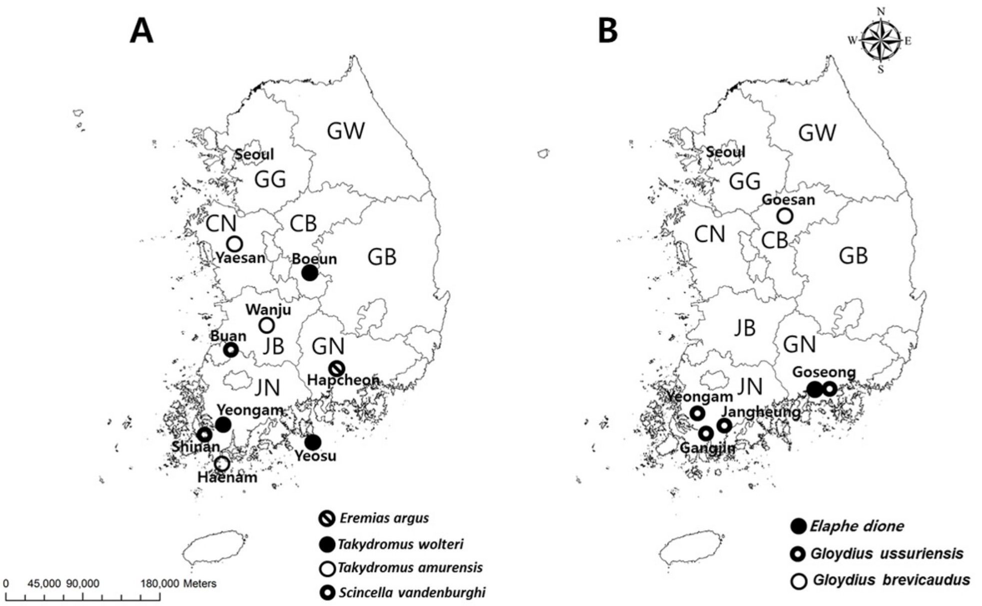

FIGURE 1. Map showing collection sites of lizards and skinks infested with *Ixodes nipponensis* nymphs and larvae (A): *Takydromus wolteri*, *Takydromus amurensis*, *Eremias argus* (Squamata: Lacertidae), and *Scincella vandenburghi* (Squamata: Scincidae); and snakes infested with *Amblyomma testudinarium* nymphs (B): *Elaphe dione* (Squamata: Colubridae), *Gloydius brevicaudus*, and *Gloydius ussuriensis* (Squamata: Viperidae). (GG = Gyeonggi Province; GW = Gangwon Province; CB = Chungbuk Province; CN = Chungnam Province; JB = Jeonbuk Province; JN = Jeonnam Province; GB = Gyeongbuk Province; GN = Gyeongnam Province).

Results

Tick collections

A total of 98 reptiles, including lizards (30, 3 species belonging to two genera), skinks (5, 1 species), and snakes (63, 10 species belonging to 7 genera) were collected (Table 1). The Mountain Grass Lizard (*Takydromus wolteri* Fischer) (14, 14.3% of all hosts) and the Steppe Rat Snake (*Elaphe dione* (Pallas)) (14, 14.3%) were the most frequently collected species, followed by the Ussuri Mamushi (*Gloydius ussuriensis* (Emelianov)) (13, 13.3%), the Mongolia Racerunner (*Eremias argus* Peters) (11, 11.2%), the Amur Rat Snake (*Elaphe schrenckii* (Strauch)) (8, 8.2%), the Short-tailed Mamushi (*Gloydius brevicaudus* (Stejneger)), the Tiger Keelback (*Rhabdophis tigrinus* (Boie)) (7, 7.1%), the Tsushima Ground Skink (*S. vandenburghi*), the Amur Grass Lizard (*Takydromus amurensis* Peters) (5, 5.1%), the Rock Mamushi (*Gloydius saxatilis* (Emelianov)) (4, 4.1%), the Red-banded Snake (*Lycodon rufozonatus* Cantor) (3, 3.1%), the Frog-eating Rat Snake (also called the Red-backed Rat Snake) (*Oocatochus rufodorsatus* (Cantor)) (3, 3.1%), the Japanese Keelback (*Hebius vibakari* (Boie)) (2, 2.0%), and the Slender Racer (*Orientocoluber spinalis* (Peters)) (2, 2.0%).

A total of 66 ixodid ticks belonging to two genera and two species, *I. nipponensis* and *A. testudinarium*, were collected from 11/30 (36.7%) lizards, 2/5 (40.0%) skinks, and 6/63 (9.5%) snakes. *Takydromus amurensis* (4/5, 80.0%) was the most frequently infested reptile, followed by *S. vandenburghi* (2/5, 40.0%), *T. wolteri* (5/14, 35.7%), *G. ussuriensis* (4/13, 30.8%), *E. argus* (2/11,

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18.2%), *G. brevicaudus* (1/7, 14.3%), and *E. dione* (1/14, 7.1%), while the remaining less frequently collected species of snakes were negative for ticks (Table 1).

Ixodes nipponensis larvae and nymphs were collected only from lizards (21 nymphs, 26 larvae) and skinks (9 nymphs), while *A. testudinarium* nymphs (10) were collected only from snakes (Table 1). Among the lizards and skinks, *T. amurensis* had the highest tick index (total number ticks/total number hosts) (3.00), followed by *E. argus* (1.91), *S. vandenburghi* (1.80), *T. wolteri* (0.79) (Table 1). *Ixodes nipponensis* larvae and nymphs were preferentially attached to the foreleg axillae (66.1%), followed by the lateral trunk (23.2%), and head and eye (10.7%) body region of lizards and skinks (Figure 2).

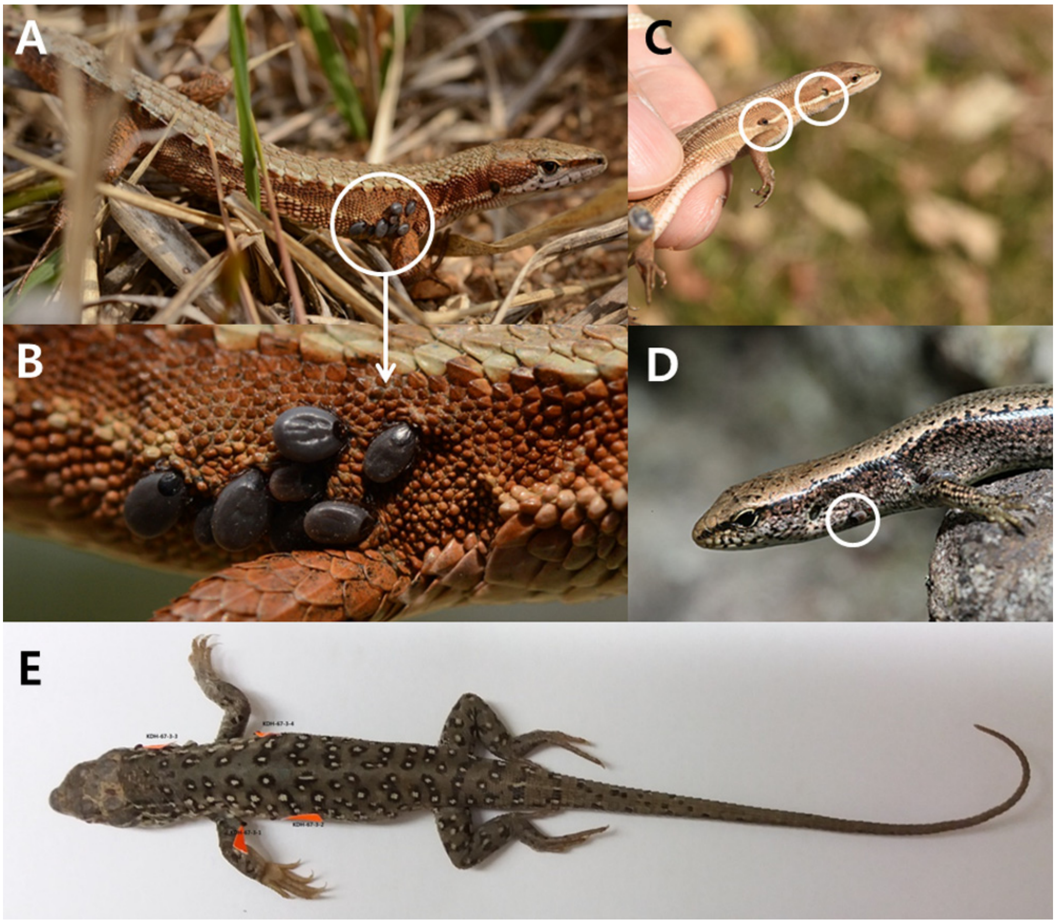

FIGURE 2. *Takydromus amurensis* (A, B), *Takydromus wolteri* (C), *Scincella vandenburghi* (D), and *Eremias argus* (E) with *Ixodes nipponensis* nymphs and larvae.

Amblyomma testudinarium was the only species of tick collected from snakes – none were collected from lizards and skinks. A total of 10 *A. testudinarium* nymphs were collected from three of ten snake species (*E. dione*, *G. brevicaudus*, and *G. ussuriensis*). *Gloydius ussuriensis* was the snake most frequently infested with ticks (4/13 snakes; 30.8%), followed by *G. brevicaudus* (1/7; 14.3%), and *E. dione* (1/14; 7.1%). *Gloydius brevicaudus* had the highest tick index (0.57) among the snakes, followed by *G. ussuriensis* (0.38), and *E. dione* (0.07). *Amblyomma testudinarium* nymphs were preferentially attached to the head and eye (40.0%), followed by the lateral trunk (60.0%) of snakes (Table 1, Figure 3). In two other recent studies of amblyommine ticks collected

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from East Asian snakes, three females of *Amblyomma helvolum* Koch were removed from the head of a Taiwan Stink Snake, *Elaphe carinata* (Günther), in southern Taiwan (Chao *et al*. 2013), while two and three *Amblyomma* nymphs were collected, respectively, from the lateral sides of the body of a Taiwanese Rat Snake (also called the Beauty Rat Snake), *Orthriophis taeniurus friesi* (Werner) and of a Chinese Cobra, *Naja atra* Cantor, in west-central Taiwan (Norval *et al*. 2009).

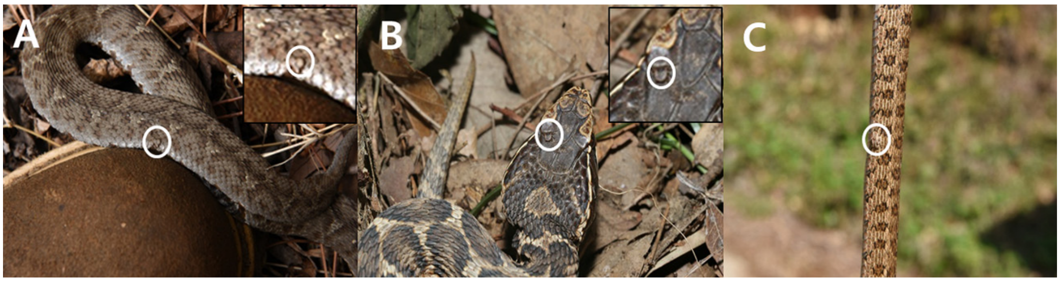

FIGURE 3. *Gloydius ussuriensis* (A), *Gloydius brevicaudus* (B), and *Elaphe dione* (C) with *Amblyomma testudinarium* nymphs.

TABLE 1. Numbers of lizards, skinks, and snakes captured, numbers infested with ticks, and numbers of ticks (*Ixodes nipponensis* and *Amblyomma testudinarium*) collected, by stage of development and species from March to October 2016, Republic of Korea.

Host		No. hosts captured	No. hosts infested	Infestation rate (%)	No. ticks collected	Tick Index[a]	Tick species	Developmental stages	
								Nymphs	Larvae
Skink	*Scincella vandenburghi*	5	2	40.0	9	1.80	*I. nipponensis*	9	0
Lizard	*Takydromus amurensis*	5	4	80.0	15	3.00	*I. nipponensis*	15	0
	Takydromus wolteri	14	5	35.7	11	0.79	*I nipponensis*	5	6
	Eremias argus	11	2	18.2	21	1.91	*I nipponensis*	1	20
Snake	*Elaphe dione*	14	1	7.1	1	0.07	*A. testudinarium*	1	0
	Elaphe schrenckii	8	0	0.0	0	0.00	-	-	-
	Gloydius brevicaudus	7	1	14.3	4	0.57	*A. testudinarium*	4	0
	Gloydius saxatilis	4	0	0.0	0	0.00	-	-	-
	Gloydius ussuriensis	13	4	30.8	5	0.38	*A. testudinarium*	5	0
	Hebius vibakari	2	0	0.0	0	0.00	-	-	-
	Lycodon rufozonatus	3	0	0.0	0	0.00	-	-	-
	Rhabdophis tigrinus	7	0	0.0	0	0.00	-	-	-
	Oocatochus rufodorsatus	3	0	0.0	0	0.00	-	-	-
	Orientocoluber spinalis	2	0	0.0	0	0.00	-	-	-
	Total	98	19	19.4	66	0.67		40	26

[a] Tick Index = Total numbers of ticks collected/total numbers of skinks, lizards, or snakes collected.

Seasonal distribution

Ixodes nipponensis nymphs were collected from lizards and skinks during March (5/30; 16.7%), April (20/30; 66.7%), May (1/30; 3.3%) and June (4/30; 13.3%), while larvae were collected only during June (22/26; 84.6%), August (3/26; 11.5%) and September (1/26; 3.8%) (Table 2). *Amblyomma testudinarium* nymphs were collected from snakes during April (2/10; 10.0%), May (4/10; 40.0%), June (3/10; 30.0%) and August (1/10; 10.0%) (Table 2).

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TABLE 2. Reptile species, month of collection, province of collection, and number of larvae and nymphs of *Ixodes nipponensis* and *Amblyomma testudinarium* collected from reptiles from March to October 2016, Republic of Korea.

Host species	Collection month	Collection sites (Province)	No. reptiles infested	*Ixodes nipponensis*		*Amblyomma testudinarium*		Total
				Nymphs	Larvae	Nymphs	Larvae	
Scincella vandenburghi	MAR	Jeonbuk	1	5	0			5
	APR	Jeonnam	1	4	0			4
Takydromus amurensis	APR	Jeonnam	1	12	0			12
	APR	Chungnam	2	2	0			2
	MAY	Jeonbuk	1	1	0			1
Takydromus wolteri	APR	Chungbuk	1	1	0			1
	APR	Jeonnam	1	1	0			1
	JUN	Jeonnam	1	3	2			5
	AUG	Jeonnam	1	0	3			3
	SEP	Jeonnam	1	0	1			1
Eremias argus	JUN	Gyeongnam	2	1	20			21
Elaphe dione	APR	Gyeongnam	1			1	0	1
Gloydius brevicaudus	MAY	Jeonbuk	1			4	0	4
Gloydius ussuriensis	APR	Gyeongnam	1			1	0	1
	JUN	Jeonnam	2			3	0	3
	AUG	Jeonnam	1			1	0	1
Total			19	30	26	10	0	66

SFTSV detection

SFTSV, previously detected in snakes, lizards, and skinks during a 2015 survey (Suh *et al*. 2016), was not detected in either *I. nipponensis* or *A. testudinarium* collected from lizards and snakes during the 2016 reptile tick survey.

Discussion

Habitat restoration and alteration, including a major reforestation program initiated in the 1960s and rapid urbanization following the end of the Korean War in 1953, have led to changes in landscape ecology conducive to higher populations of wild animals, such as small mammals (*e.g.*, rodents, soricomorphs, rabbits, and weasels), larger mammals (*e.g.*, deer, wild pigs, raccoon dogs, badgers, and feral dogs and cats), local and migratory birds, and reptiles (*e.g.*, lizards, skinks, and snakes) that are hosts to various species of ticks (Kim *et al*. 2010b, 2011, 2013, Chong *et al*. 2013a, 2013b, Kang *et al*. 2013, Shin *et al*. 2013, Park *et al*. 2014). Ticks harbor numerous zoonotic pathogens, *e.g.*, viruses, bacteria and protozoa, that are of veterinary and medical importance, and as humans encroach upon the habitats of wild animals and birds, they and their pets/domestic animals may be exposed to ticks and associated tick-borne pathogens (Kang *et al*. 1982, Park *et al*. 2011, Yun *et al*. 2012). Based on recent case increases for a number of tick-borne diseases, *e.g.*, SFTS, which increased from 36 cases in 2013 to >250 cases in 2017, tick bites and illnesses due to tick-borne pathogens appear to be underreported in the ROK (Jang *et al*. 2004, Choi *et al*. 2005, Shin *et al*. 2013,

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Yi *et al*. 2016). Because human tick bites from *I. nipponensis* and *A. testudinarium* are reported more frequently for patients seen at medical clinics in the ROK, these two species may play an important role in the transmission of tick-borne pathogens to humans and domestic animals (Kang *et al*. 1982, Lee *et al.* 1989, Cho *et al*. 1994, 1995, Yamada *et al*. 1996, Ryu *et al*. 1998, Chae *et al*. 2000, Yun *et al*. 2001, Ko *et al*. 2002, Chang *et al*. 2006, Kim *et al*. 2010a, 2014b, Suh *et al*. 2013).

In order to provide a descriptive analysis of disease risks to human and domestic animal populations, it is important to identify the relative abundance of ticks, their host associations, stages of development found on various hosts, geographic distributions, and potential for the maintenance and transmission of zoonotic pathogens. Disease threat assessments and risk analyses are central to the development of disease mitigation strategies (*e.g.*, use of insecticide-impregnated uniforms) for US and ROK civilian and military populations, and to efforts to increase awareness of disease risks, thus reducing the impact of zoonotic tick-borne diseases.

Small mammals, birds and reptiles are hosts of *I. nipponensis* larvae, which are commonly collected by tick drag in tall grasses and herbaceous vegetation bordering forested hillsides and mountains, wetland and dryland farms, and military training areas. Nymphs and adults are found on larger mammals, *e.g.*, deer, and are much less frequently collected from vegetation by tick drag (Kim *et al*. 2010b, 2013, 2014a, Ra *et al*. 2011, Kang *et al*. 2013, HC Kim personal communication). *Ixodes nipponensis* nymphs and adults are more frequently reported to bite humans, possibly because of their larger mouthparts (*i.e.*, longer hypostomes), which are probably more irritating to human hosts than the relatively smaller mouthparts of *Haemaphysalis* spp. (Cho *et al*. 1995, Ryu *et al*. 1998, Yun *et al*. 2001, Ko *et al*. 2002, Jeon *et al*. 2014). During a 2015 reptile tick survey, *I. nipponensis* nymphs accounted for 88.9% of ticks collected from lizards and skinks, while larvae accounted for only 11.1% (Suh *et al*. 2016), whereas during this survey, *I. nipponensis* larvae accounted for 46.4% of the ticks collected from lizards and skinks. This difference was due, in part, to the high numbers of *I. nipponensis* larvae (20 larvae) collected from *Eremias argus* in mid-June. However, in previous studies the number of *I. nipponensis* larvae increased in July, peaked in August, and then declined in September (Kim *et al*. 2013, Coburn *et al*. 2016, Suh *et al*. 2016) (Table 1).

Amblyomma testudinarium adults are relatively large ticks that are frequently reported to bite humans, which is not unexpected, given this species' biting behavior (blood feeding on hosts for up to 30 days) as well as its large mouthparts that are capable of producing painful bites (Kim *et al*. 2010a, 2014b, Suh *et al*. 2013). In Korea, *A. testudinarium* was infrequently collected by tick drag or from small mammals (>2,500 rodents and soricomorphs) during a comprehensive survey in various habitats, perhaps in part because of its biting behavior and preference for larger mammals (Yamaguti *et al*. 1971, Kim *et al*. 2011, 2013, 2014a, Coburn *et al*. 2016, Yun *et al*. 2016, Johnson *et al*. 2017).

In this survey, a total of 98 reptiles, including lizards, skinks, and snakes, were collected from March-October. As in the 2015 reptile survey, *I. nipponensis* nymphs were collected only from lizards and skinks, while *A. testudinarium* nymphs were collected only from snakes (Suh *et al*. 2016). Suh *et al.* (2016) collected two genera of lizards (2 species) and skinks (1 species) and five genera of snakes (8 species). Similarly, this survey resulted in the collection of two genera of lizards (3 species) and skinks (1 species), and seven genera of snakes (10 species). The lower number of *A. testudinarium* collected from snakes in 2016 (10) compared to 2015 (48) and the relatively low infectivity rate of ticks (1/48; 2.1%) may have resulted in none of the *A. testudinarium* collected during 2016 being positive for SFTS virus. While similar numbers of *I. nipponensis* nymphs were collected (2015, 32; 2016, 30), only two (6.3%) were positive for SFTS virus in 2015. Moreover, both positive *I. nipponensis* nymphs were from the same locality, indicating focal distribution of SFTSV. Although larval *I. nipponensis* were collected, and there is evidence of transovarial transmission of SFTSV, none were positive during either 2015 or 2016.

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While *Haemaphysalis longicornis* Neumann is considered the primary vector of SFTSV, this virus also has been detected in *I. nipponensis* biting humans and in specimens collected by tick drag, as well as in *A. testudinarium* biting humans (Park *et al*. 2014, Yun *et al*. 2014). On average, there are approximately 40 tick bites reported by the Korea Centers for Disease Control and Prevention annually, with many unreported based on the number of SFTSV infections identified annually from 2013-2017 (36, 55, 79, 165 and >250 cases, respectively). Although *I. nipponensis* and *A. testudinarium* collected from lizards (*T. wolteri*; Hapcheon County, Gyeongsangnam Province) and one snake (*R. tigrinus*; Wanju County, Jeollabuk Province) were positive for SFTSV in a 2015 survey (Suh *et al*. 2016), none of the ticks collected from lizards and snakes during the 2016 reptile tick survey were positive for SFTSV.

Further collections in other areas (*e.g.*, Gyeonggi, Gangwon, and Gyeongbuk provinces and Jeju Island) are necessary to better understand the geographical and host distributions of ticks associated with reptiles and the potential impact of tick-associated pathogens on human and animal health.

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