

Ectoparasites: immature Japanese hard ticks (*Ixodes nipponensis*; Acari: Ixodidae) on Korean lizards

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Abstract

Although lizards are important hosts for hard ticks (Ixodidae), very few studies have been conducted in South Korea. To determine whether or not hard ticks can infest lizards endemic to South Korea, we examined 77 lizards of four species (*Eremias argus*, *Sincella vandenburghi*, *Takydromus amurensis*, and *Takydromus wolteri*) that were collected at 22 different sites between April and October 2010. We confirmed that all four lizard species can be infested by *Ixodes nipponensis* larvae or nymphs. Of the 62 *E. argus* examined, we found an average of 12.5 larvae on two lizards and an average of one nymph on one lizard. We found seven nymphs on one *S. vandenburghi*. We found an average of two nymphs on two of the five *T. amurensis* and an average of one nymph on four of the nine *T. wolteri*. *Ixodes nipponensis* larvae and nymphs were found most frequently on the foreleg axillae (87.8%), followed by the forelegs (7.3%), the eyelids (2.4%), and the ears (2.4%) of the lizards. To the best of our knowledge, this is the first report of *I. nipponensis* infestations of lizards endemic to South Korea.

Key words: classification, ectoparasite, hard tick, Ixodidae, Japanese hard tick, lizard

INTRODUCTION

Various ectoparasites have been found on reptiles, including ticks, mites, mosquitoes, flies, and leeches (Pough et al. 2004). Because ectoparasites suck lymph and blood from their hosts, they are capable of spreading viruses and bacteria and causing secondary infestations. The typical negative effects of ectoparasites on their hosts include decreased energy, impaired locomotor skills, diminished social activity, reproductive failure, and an elevated risk of mortality for the hosts themselves (Schall and Sarni 1987, Dunlap and Schall 1995, Oppliger et al. 1996, Sorci et al. 1996).

A well-known group of ectoparasites on snakes and lizards are hard ticks (e.g., Ixodidae) (Durden et al. 2002). Larvae and nymphs of the tick species *Ixodes* have been reported on skinks (*Eumeces* spp., *Scincella* spp.), glass lizards (*Ophisaurus* spp.), spiny lizards (*Sceloporus* spp.), and grass lizards (*Takydromus* spp.) (Fujita and Takada 1978, Yoneda 1981, Bauwens et al. 1983, Oliver et al. 1993, Fujita and Takada 1997, Lane and Quistad 1998, Eisen et al. 2004). Prior research has focused on the ectoparasitic patterns of hard ticks on lizards (Bauwens et al. 1983, Oliver et al. 1993), the role of lizards as hosts for hard ticks

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(Matuschka et al. 1991, Eisen et al. 2004), the function of lizards in the spread and life cycle of Lyme disease (*Borrelia burgdorferi*) (Lane and Quistad 1998, Ostfeld and Keesing 2000, Tjisse-Klasen et al. 2010), and the effects of hard ticks on the behavior and reproduction of lizards (Main and Bull 2000, Fujimoto 2002, Václav et al. 2007).

The life cycle of hard ticks consists of egg, larva, nymph, and adult stages (Sonenshine 1991, Noda et al. 2004, Lee 2009). Hard ticks depend on various terrestrial vertebrates (i.e., rodents, birds, and reptiles) to act as hosts, so that they may feed on their lymph and blood. They also play a key role spreading fungi, viruses, rickettsiae, bacteria, and protozoa among their hosts (Sonenshine 1991). In South Korea, hard ticks infest domestic and wild animals, particularly rodents, birds, skinks (Arthur 1957, Noh 1965, Kim 1970, Kim and Lee 1989, Shim et al. 1992, 1993, 1994, Lee et al. 1997, Eum et al. 2006, Kim et al. 2009), and humans (Kang et al. 1982, Cho et al. 1991, Yoon et al. 1996, Yun et al. 2001). Only a single study has been conducted that relates the *Ixodes granulatus* ectoparasite to the Korean skink (*Scincella vandenburghi*) host (Noh 1965).

Consequently, this study determined which types of hard ticks infest which endemic lizards. Additionally, we classified the hard tick species with their associated lizard species.

MATERIALS AND METHODS

Lizard and tick collection

To collect ectoparasitic ticks from lizards, we first captured lizard specimens by hand from the following regions: Gyeonggi-do (Kwangju, Yeosu, Yangpyeong, and Icheon), Gangwon-do (Chuncheon, Wonju, Yeongwol, Pyeongchang, and Gangneung), Chungcheong-do (Jecheon, Chungju, Danyang, Taean, Boryeong, and Seocheon), and Gyeongsang-do (Cheongsong, Gunwei, Sungju, Kimcheon, Gumi, Goryeong, and Hapcheon). This specimen collection period lasted from April to October 2010. Upon catching a lizard, we determined its species and examined it for the presence of hard ticks. In the event that we found ectoparasitic ticks on a lizard's body, we recorded their number and the locations where they were attached. Next, we measured the lizard's snout-vent length (in increments of 0.1 cm) and its body weight (in increments of 0.1 g) using a digital vernier caliper (CD-15CPX; Mitutoyo, Kawasaki, Japan) and a digital balance (TMB 120-1; Kern, Balingen, Germany),

respectively. Finally, we released the lizards at the site where they were caught. Additionally, we also obtained a sample of Korean skinks (*S. vandenburghi*) infested by ticks that were caught in Sinan, Jeonnam in April 2009 by a researcher at Jeju National University.

Observation and classification of ticks

Before conducting tick observations, we cleaned the ticks by submerging them in a 10% KOH solution for 1-3 hours. Afterwards, we observed the ticks with either one or both of the following light microscopes: a dissecting microscope (#5424 Stereo Crystal-Pro; Konus, Verona, Italy), or an Eclipse 50i (Nikon, Tokyo, Japan). To investigate the level of detail necessary to construct a classification key for the ticks, we used a scanning electron microscope (SEM). Before this set of observations, the ticks were treated following the method of Park et al. (2008). The ticks were fixed using a 4% glutaraldehyde solution (in 0.2 M phosphate buffer, pH 7.4), washed three times using the same buffer, then serially dehydrated in 50, 60, 70, 80, 90, and 100% ethanol for 30 min at each concentration. Next, the medium was exchanged three times (for 30 min each time) with isoamyl acetate. The ticks were dried using critical-point drying, mounted on a specimen stub, coated with Au-Pd (E-1010; Hitachi, Tokyo, Japan), and observed under a S-3500N low-vacuum SEM (Hitachi) at the Korea Basic Science Institute, Chuncheon, Kangwon. By employing the SEM in our observations, we observed a total of 12 larvae on the Mongolian racerunner (*Eremias argus*) and a combined total of four nymphs on the Korean skink (*S. vandenburghi*) and the white-striped grass lizard (*Takydromus wolteri*). To locate classification keys for the *Ixodes* ticks, we employed Yamaguti et al. (1971) and Lee (2009). To help determine the species of larval and nymphal ticks found, classification keys by Ono (1962), Kitaoka and Saito (1967), and Yamaguti et al. (1971) were also used.

RESULTS

We collected 77 lizards representing four species (*E. argus*, *S. vandenburghi*, *Takydromus amurensis*, and *T. wolteri*) from 22 sites. Of the 77 lizards collected, 10 lizards from Chuncheon, Yeongwol, Taean, and Sinan were infested by a combined total of 41 hard ticks (Table 1). These ticks were found mainly on the foreleg axillae (87.8%), followed by the forelegs (7.3%), the eyelids (2.4%), and the ears (2.4%) (Fig. 1).

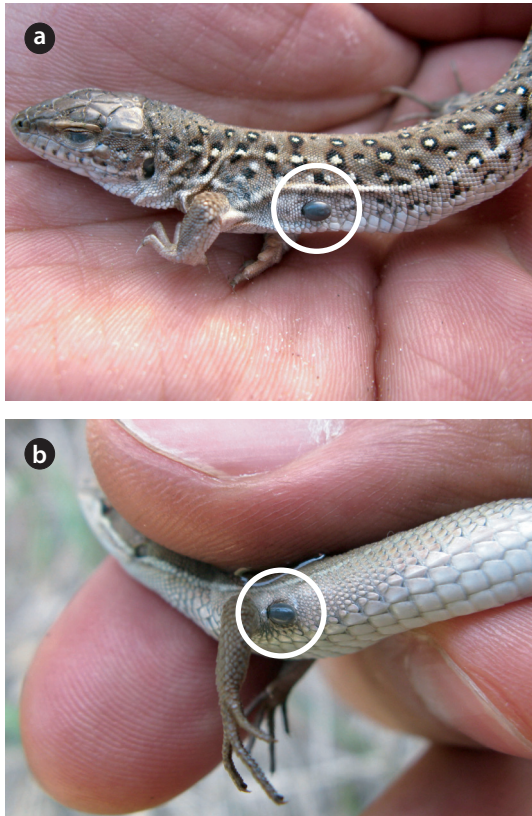


Fig. 1. *Ixodes nipponensis* nymphs on (a) the Mongolian racerunner (*Eremias argus*) and (b) the white-striped grass lizard (*Takydromus wolteri*).

Because the hard ticks had a serrated hypostome on the capitulum at the anterior end of the body, as well as a scutum and an anal groove that rounded the anus anteriorly and was without a festoon, we classified these hard ticks as *Ixodes* ticks (Fig. 2). Of the 41 ticks, 16 were nymphs with four pairs of legs but no genital aperture, whereas 25 ticks were larvae and had three pairs of legs. In both larvae and nymphs, the cornua and auricula protruded in a triangular shape from the left and right bottom end of the capitulum and were visible in both dorsal and ventral views, respectively (Figs. 3a, 3b, 4a, and 4b).

The end of the larvae hypostome was blunt, whereas that of nymphs was sharp. On the hypostome, both larvae and nymphs had 3/3 upper tooth rows and 2/2 lower tooth rows (Figs. 3c and 4c). Both larvae and nymphs had relatively long external and internal spurs on the first coxal plate, whereas the external spurs on the remaining coxae were not extended to the next coxal plate (Figs. 3d and 4d). The postscutal setae were forked, and the forked setae were often observed on other parts of the body such as legs (Figs. 3e, 3f, 4e, and 4f). Based on these classification characteristics, the hard ticks collected were all identified as *Ixodes nipponensis* larvae or nymphs.

The 16 ticks collected from Chuncheon, Yeongwol, and Sinan between April and May were all nymphs, whereas the 25 ticks collected in July from *E. argus* were all larvae. Of the 62 *E. argus* collected, we found an average of 12.5 larvae on two lizards and an average of one nymph on one lizard. Additionally, we found seven nymphs on one *S. vandenburghi*, an average of two nymphs on two of five *T. amurensis*, and an average of one nymph on four of nine *T. wolteri* examined (Table 1).

DISCUSSION

This study identified Japanese hard tick (i.e., *I. nipponensis*) larvae and nymphs on the bodies of four lizard species endemic to South Korea (*E. argus*, *S. vandenburghi*, *T. amurensis*, and *T. wolteri*). To our knowledge, this is the first report of *I. nipponensis* infestation of lizards endemic to South Korea.

The infestation rates by larval and nymphal *I. nipponensis* depended on the species of lizard infested. In general, *Ixodes* tick (Bauwens et al. 1983) infestation rate is affected by body size, home range size, activity periods, and use of different habitats. Previous studies have shown that skinks and grass lizards, which live mainly on the ground in grasslands and on the floors of forests, respectively, have more ticks on their bodies than those of anole lizards, which live mainly in the upper stories

Table 1. Japanese hard tick (*Ixodes nipponensis*) larvae and nymphs found on lizard species endemic to South Korea

Lizard species	No. of lizards examined	Lizards infested by larva (No. of larvae found)	Lizards infested by nymphs (No. of nymphs found)
<i>Sincella vandenburghi</i>	1	0	1 (7)
<i>Takydromus amurensis</i>	5	0	2 (4)
<i>Takydromus wolteri</i>	9	0	4 (4)
<i>Eremias argus</i>	62	2 (25)	1 (1)

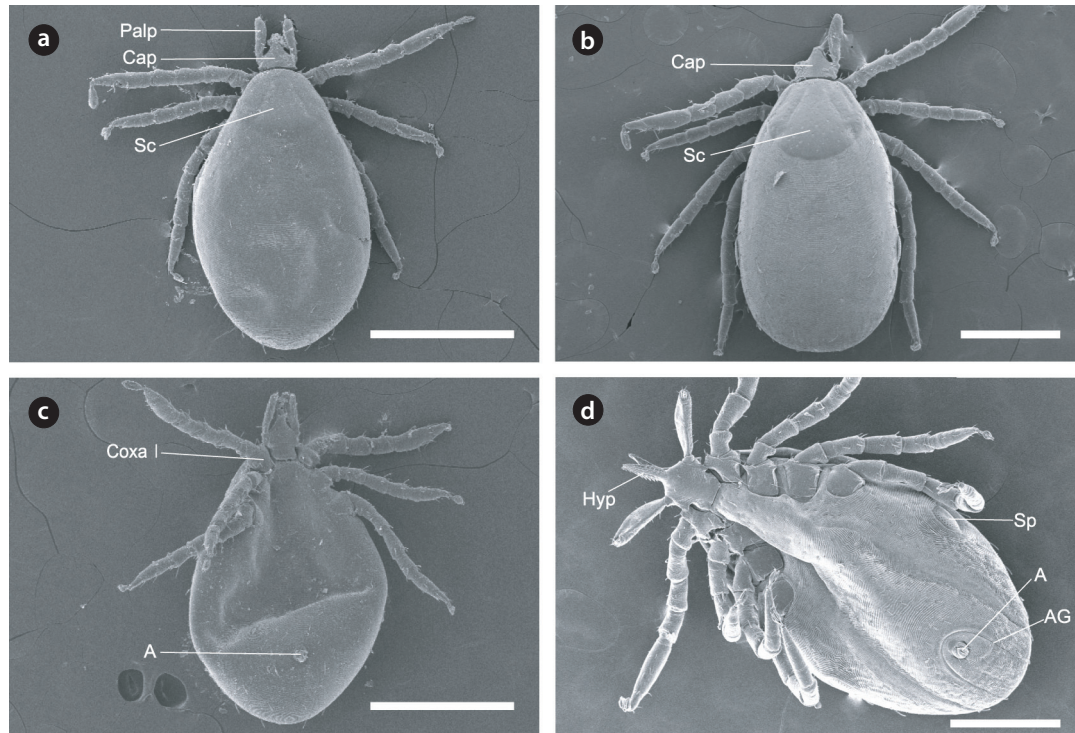


Fig. 2. Dorsal and ventral views of a typical larva (a, c) and nymph (b, d) of *Ixodes nipponensis* collected from the Mongolian racerunner (*Eremias argus*) and the white-striped grass lizard (*Takydromus wolteri*), respectively. A, anus; AG, anal groove; Cap, capitulum; Hyp, hypostome; Sc, scutum; Sp, spiracle. Scale bars = 0.5 mm.

of plants (Apperson et al. 1993, Durden et al. 2002). Such studies have also indicated that there is a higher chance of infestation by hard ticks when potential hosts are on the ground. Unfortunately, the sample size of each lizard species examined in this study varied widely; consequently, additional studies with larger, more comparable lizard samples will be necessary to compare the *Ixodes* tick infestation rate in different lizard species.

Consistent with previous findings, most of the ticks found in this study were located on the lizards' foreleg axillae. Hard ticks are known to be found principally on areas where the skin is weak and well-lined, although they can also be occasionally collected from other parts of the body (Durden et al. 2002). Among the skinks (genus *Eumeces*), *Ixodes* ticks were frequently attached near the shoulders, legs, and armpits, whereas they were attached mostly at the lateral grooves on the glass lizard (genus *Ophisaurus*) (Apperson et al. 1993, Oliver et al. 1993). Moreover, the lizards' armpits, which are particularly difficult to reach and are well protected from the outside, are also recognized as the most susceptible place for *Ixodes* tick infestation (Bauwens et al. 1983, Oliver et al. 1993).

The larval and nymphal *Ixodes* ticks collected in this

study displayed several distinctive classification characteristics matching those of *I. nipponensis*. First, the scutal setae were distinctively shorter than the dorsal setae. Second, the external spurs on the coxae (except the first coxa) were not extended to the next coxa plate. Third, the end of the larvae's hypostome was blunt, whereas that of nymphs was sharp. Fourth, the postscutal setae were forked. Fifth, forked setae were often observed on other parts of the body such as legs. Based on these previously described characteristics (Ono 1962, Kitaoka and Saito 1967, Yamaguti et al. 1971), we identified the hard ticks collected as larval or nymphal Japanese hard ticks (*I. nipponensis*).

Although immature larval and nymphal *Ixodes* ticks use both small mammals and other reptiles as hosts, *Ixodes* ticks such as *I. ricinus* (Matuschka et al. 1991), *I. scapularis* (Apperson et al. 1993), and *I. pacificus* (Eisen et al. 2004) are known to prefer lizards as hosts. In South Korea, small mammals, including the black-striped field mouse (*Apodemus agrarius*) and the Ussuri white-toothed shrew (*Crocodyura lasiura*), are frequently infested with *Ixodes* ticks (Shim et al. 1992, 1993, Kim et al. 2009). However, our research is the first to show that lizards endemic to South Korea are an important host for immature *Ixodes*

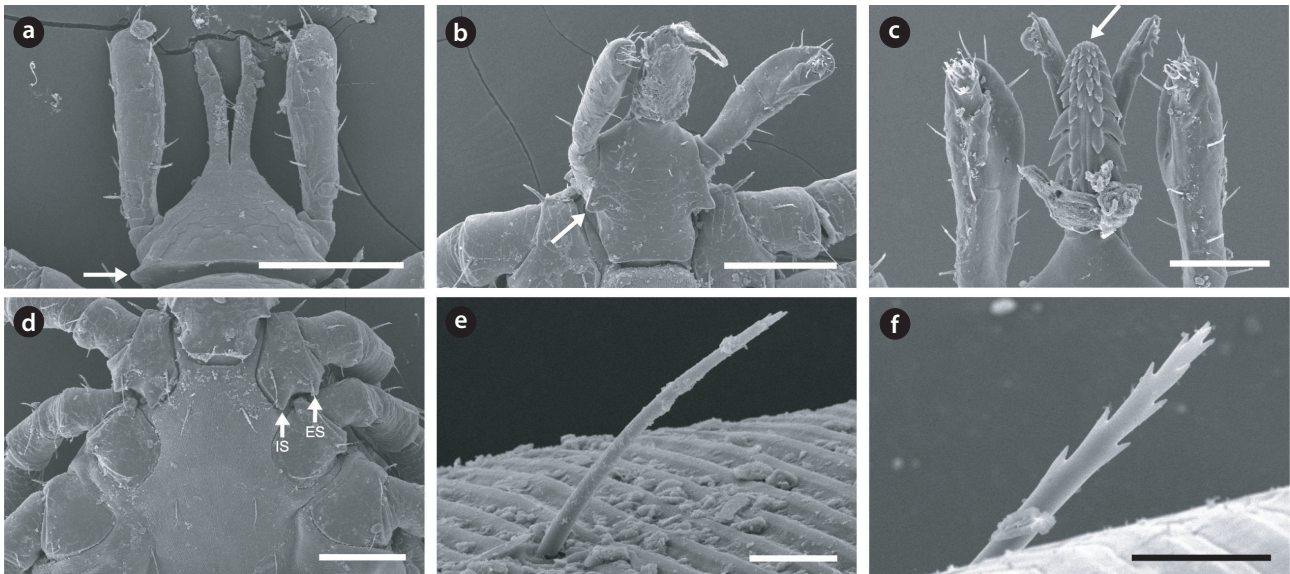


Fig. 3. Classification keys for larval *Ixodes nipponensis* are indicated by the following arrows: (a) cornua on the dorsal capitulum, (b) auricula on the ventral capitulum, (c) hypostome, (d) external spur (ES) and internal spur (IS) on the coxa I, (e) postscutal seta, and (f) the tip of the postscutal seta. Scale bars = 100 μ m (a, b, d), 50 μ m (c), 10 μ m (e, f).

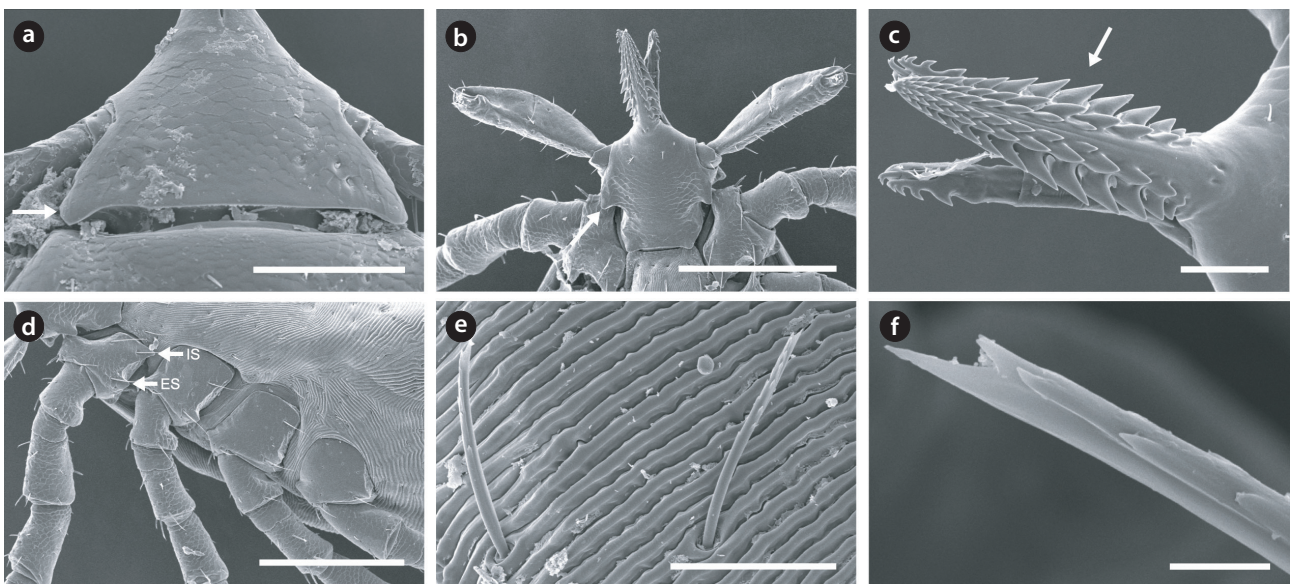


Fig. 4. Classification keys for nymphal *Ixodes nipponensis* are indicated by the following arrows: (a) cornua on the dorsal capitulum, (b) auricula on the ventral capitulum, (c) hypostome, (d) external spur (ES) and internal spur (IS) on the coxa I, (e) postscutal seta, and (f) the tip of the postscutal seta. Scale bars = 100 μ m (a), 300 μ m (b, d), 50 μ m (c, e), 5 μ m (f).

ticks. As ectoparasites, ticks negatively affect a host's health and reproduction (Sonenshine 1991, Main and Bull 2000). It seems reasonable to assume that Korean lizards would be negatively impacted by ticks in the field as well. Finally, it is important to pay close attention to such ectoparasites on lizards in captivity, for purposes related to keeping of pets and to conservation.

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