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Ticks collected from Chilbal Island, Jeollanam Province, Republic of Korea, during 2014–2015

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Abstract

The 65th Medical Brigade and Public Health Command District-Korea, in collaboration with the Migratory Birds Center, National Park Research Institute, conducted a migratory bird tick-borne disease surveillance program on Chilbal Island, a small, remote, uninhabited island in southwestern Jeollanam Province, Republic of Korea (ROK), during 2014–2015. Ticks were collected by dragging vegetation and from nest soil and litter of the Ancient Murrelet, *Synthliboramphus antiquus*, and Swinhoe's Storm Petrel, *Hydrobates monorhis*, using Tullgren funnels. A total of 115 ticks belonging to three genera and three species were collected. *Ornithodoros sawaii* (98.3%, 113 ticks) was the most frequently collected tick species, followed by *Ixodes signatus* (0.9%, 1 nymph) collected from nest soil and litter, and *Haemaphysalis flava* (0.9%, 1 male) collected by tick drag.

Key words: *Ornithodoros sawaii*, *Ixodes signatus*, *Haemaphysalis flava*, *Synthliboramphus antiquus*, *Hydrobates monorhis*, Korea

Introduction

Over the past few decades there has been a worldwide reemergence of zoonotic tick-borne pathogens that pose medical and veterinary health risks to wild and domestic animals, birds, and incidentally to humans (Jongejan & Uilenberg 2004, Heath & Hardwick 2011, Dantas-Torres *et al*. 2012). The role of migratory seabirds in the transportation of exotic tick species to their summer breeding and winter feeding grounds is poorly documented; most avian breeding sites are not accessible to the general public since they are often situated on remote and uninhabited or sparsely populated islands that are under government protection. It has been demonstrated that migratory birds transport ectoparasites, including ticks and associated tick-borne pathogens, along their migratory routes between breeding and overwintering grounds. These annual migrations result in the potential for exotic tick species to be transported over long distances to diverse environments in non-endemic regions (Kohls 1957, Hughes *et al*. 1964, Amerson 1968, Nuttall 1984, Heath 1987, 2006, Hutcheson *et al*. 2005, Kawabata *et al*. 2006, Kim *et al*. 2009, Dietrich *et al*. 2011, Kang *et al*. 2013). Recently, Kim *et al*. (2015) reported collecting ticks from nest soil and litter of the Ancient Murrelet, *Synthliboramphus antiquus* (Gmelin), and Swinhoe's Storm Petrel, *Hydrobates monorhis* (Swinhoe), on Chilbal Island, a small, remote, uninhabited island of southwestern Jeollanam Province, Republic of Korea (ROK), using Tullgren funnels (WHO model, Yonsei University, Korea; height 60 cm, diameter 23 cm). A total of 12 *Ornithodoros sawaii* Kitaoka and Suzuki (2 females, 4 males, 6 nymphs) were collected from 3/36 nest soil and litter samples obtained on 1 May and 30–31 May 2014 during the nesting season of *S. antiquus* and from 6/20 soil and litter samples collected on 14–15 September 2014 during the nesting season of *H. monorhis*. While *H. monorhis* occupies the same nesting sites as *S. antiquus*, the breeding seasons of these two species do not overlap and resident *Ornithodoros* larvae feed on young and adult birds of both species during their breeding season.

This report summarizes tick collections from nest soil and litter conducted on Chilbal Island during 2014 (Kim *et al*. 2015), with additional tick records obtained from tick drags of *S. antiquus* and *H. monorhis* breeding sites on Chilbal Island during 2014–2015.

Materials and methods

Survey area

The Migratory Birds Center, National Park Research Institute, located on Heuksan Island, Heuksan-myeon (district), Sinan-Gun (county), Jeollanam Province, ROK, conducted conservation and breeding status surveys of *S. antiquus* and *H. monorhis* during 2014–2015 on Chilbal Island (34º47' N, 125º48' E), a protected National Monument (No. 332, 14 November 1982) with a total land area of 36,900 m^2 located in Goseo-ri, Bigeum-myeon, Sinan-gun, Jeollanam Province, 64 km west of Mokpo, a major mainland port city (Fig. 1 and Fig. 2A, B).

Tick collections

On Chilbal Island the Ancient Murrelet nests in early spring, whereas Swinhoe's Storm Petrel nests in fall. Although the nesting seasons of the two species do not overlap, they occupy the same nests. Using a small scoop, 50–100 g of soil and nest litter were collected from nesting areas during the nesting season of the Ancient Murrelet (1 and 31 May 2014, 9–10 April, 9–10 June 2015) and Swinhoe's Storm Petrel (15 September 2014; 9 October 2015) (Fig. 2A, B). Soil and litter samples were placed in plastic Ziploc® bags (25 x 28 cm) and sent to the 5th Medical Detachment, Yongsan U.S. Army Garrison, Seoul, ROK. Soil and litter samples from each nest site were placed separately inside Tullgren funnels equipped with a 52W incandescent light bulb (heat source) at the top and a collection bottle (120 ml urine specimen container) at the base containing 50 ml of 70% ethanol (EtOH). After 24 hours of exposure, the material in each collection bottle was examined for arthropods and the ticks removed and placed individually in 2 ml cryovials containing 70% EtOH, labeled with a unique nest identification number, and then microscopically examined to determine developmental stage and genus using taxonomic identification keys (Kohls 1957, Kitaoka & Suzuki 1973). Additional tick collections were conducted by tick drag, as previously described by Chong *et al*. (2013), on 30 April and 1 May 2014, and ticks removed and similarly placed in 2 ml labeled cryovials of 70% EtOH. Ticks, excluding *Ornithodoros* spp., were identified to species using dissecting stereomicroscopes (x100) and keys developed by Yamaguti *et al*. (1971).

Polymerase chain reaction (*PCR*) *and sequencing analysis*

Because morphological identification of *Ornithodoros* spp. nymphs and adults is often unreliable, samples of the *Ornithodoros* spp. collected on Chilbal Island were identified by PCR using partial mitochondrial 16S ribonucleic acid gene (mt-rrs) primer sets developed for the identification of soft ticks and sequence analysis of *O. capensis* Neumann and *O. sawaii* in Japan and other countries (Ushijima *et al*. 2003, Kim *et al*. 2015). Total DNA was prepared from individual ticks using a DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with minor modification and stored at -20°C until used. The resulting product consisted of 475 base pairs, including the primer sets. PCR assays were performed using 50 µL of reaction mixture with Takara *Ex Taq*™ DNA polymerase (Takara, Shiga, Japan) at 94℃ for 5 min, followed by 35 cycles of 10 sec at 94ºC, 30 sec at 55ºC, 30 sec at 72ºC, and a final extension step of 5 min at 72ºC. PCR products were then purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and sequenced after cloning into pCR®4-TOPO® plasmid (Invitrogen, Carlsbad, California, USA), using Applied Biosystems (ABI) (Applied Biosystems, Foster City, California, USA) Prism® BigDye™ Terminator v3.1 Cycle Sequencing Kit, with an ABI 3730xl sequencer at Cosmogenetech, Co., Ltd. (Daejeon, ROK). Sequencing results were assembled using the DNASTAR® SeqMan v5.0.6 software, (DNASTAR Inc., Madison, Wisconsin, USA) to determine consensus sequences.

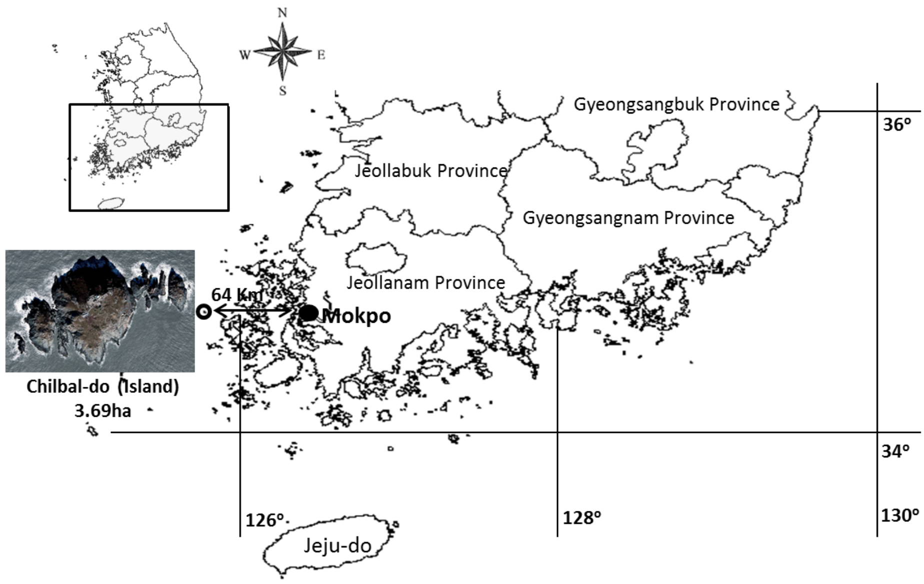

FIGURE 1. Collection site of *Ornithodoros sawaii* and *Ixodes signatus* (○) from Ancient Murrelet (*Synthliboramphus antiquus*) and Swinhoe's Storm Petrel (*Hydrobates monorhis*) nest soil and litter and *Haemaphysalis flava* taken by tick drag on Chilbal Island, Jeollanam Province, Republic of Korea, 2014–2015 [Mokpo (●), mainland port city] (image from *http://map.daum.net*).

Phylogenetic analysis

Sequence data were analyzed using MEGA 6.0 software (http://www.megasoftware.net) (Koichiro *et al*. 2013). Alignment and comparisons of the partial mt-rrs segment sequences for *Ornithodoros* spp., amplified from specimens of previously published *Ornithodoros* spp., were facilitated using the CLUSTALW DNASTAR Laser gene program, version 5. For phylogenetic analysis, neighbor-joining (NJ) and bootstrap tests were carried out according to the Kimura 2-parameter distance method (Kimura 1980, Saitou & Nei 1987). Pairwise alignments were performed with an open-gap penalty of 15 and a gap extension penalty of 6.66 and multiple alignments performed using the same values. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (pairwise deletion).

FIGURE 2. Chilbal Island. Lighthouse at apex of the island (A), steep rocky slope (B), nest burrow used by Ancient Murrelet (*Synthliboramphus antiquus*) with eggs (C), Swinhoe's Storm Petrel (*Hydrobates monorhis*) burrow under roots of *Carex bootiana*, predominant grass species on Chilbal Island (D), Ancient Murrelet incubating eggs during nesting season (E), Swinhoe's Storm Petrel in rock crevices during nesting season (F).

Results and discussion

A total of 113 *O. sawaii* (7 females, 11 males, 95 nymphs) were collected from nest soil and litter samples. A total of 8/78 (10.3%) samples were positive for *O. sawaii* (3 females, 1 male, 6 nymphs) during the breeding season of *S. antiquus* in the spring, and 11/50 (22.0%) were positive for *O. sawaii* (4 females, 10 males, 89 nymphs) during the breeding season of *H. monorhis* in the fall. Additionally, one *Ixodes signatus* Birula nymph was collected from nest soil and litter during the nesting season of *S. antiquus* in June 2015, and one *Haemaphysalis flava* Neumann male was collected from dragging grasses near the lighthouse during May 2014 (Table 1, Fig. 3).

In 2014, DNA was detected from 10/12 *Ornithodoros* adults and nymphs and the mt-rrs sequenced. Previously submitted GenBank accession numbers were: females, KP730690 (KOR-C1405-5), and KT372792 (KOR-C1409-20); males, KP730691 (KOR-C1409-2), KP730692 (KOR-C1409-10), KT372787 (KOR-C1405-5-2), and KT372790 (KOR-C1409-4); nymphs, KP730693 (KOR-C1405-E4), KT372788 (KOR-C1405-5-3), KT372789 (KOR-C1405-5-4), and KT372791 (KOR-C1409-18) collected from Chilbal Island (Kim *et al*. 2015); and larvae, KP899267 (KOR-G0908-100) collected from Gaerin Island, Jeollanam Province, ROK (Kim *et al*. 2016). In 2015, DNA was detected from 82/101 adults and nymphs and the mt-rrs gene sequenced and confirmed as

O. sawaii. Two females, 2 males, and 6 nymphs were maintained as voucher specimens at the 5th Medical Detachment, and 9 samples were negative by PCR. Accession numbers from 9/82 *O. sawaii* positive samples submitted to GenBank were: females, KX227701 (KOR-C1506-A4), KX227703 (KOR-C1510-8-40), and KX227704 (KOR-C1510-8-41); males, KX227705 (KOR-C1510-8-42), KX227706 (KOR-C1510-8-43), and KX227707 (KOR-C1510-8-44); nymphs, KX227700 (KOR-C1506-A3), KX227702 (KOR-C1510-7-1), and KX227708 (KOR-C1510-9-2). Only 0–3 base differences were observed with 99.4-100% similarity in nucleotide sequences between *O. sawaii* collected from Gaerin Island in 2009, Chilbal Island in 2014, and other collection sites (Miyazaki, Shimane, Kyoto, Kutsujima, and Ishikawa) in Japan (Fig. 4). The mt-rrs gene sequences of ticks identified in this study collected in 2015 were deposited in GenBank under accession numbers (KX227700-KX227708).

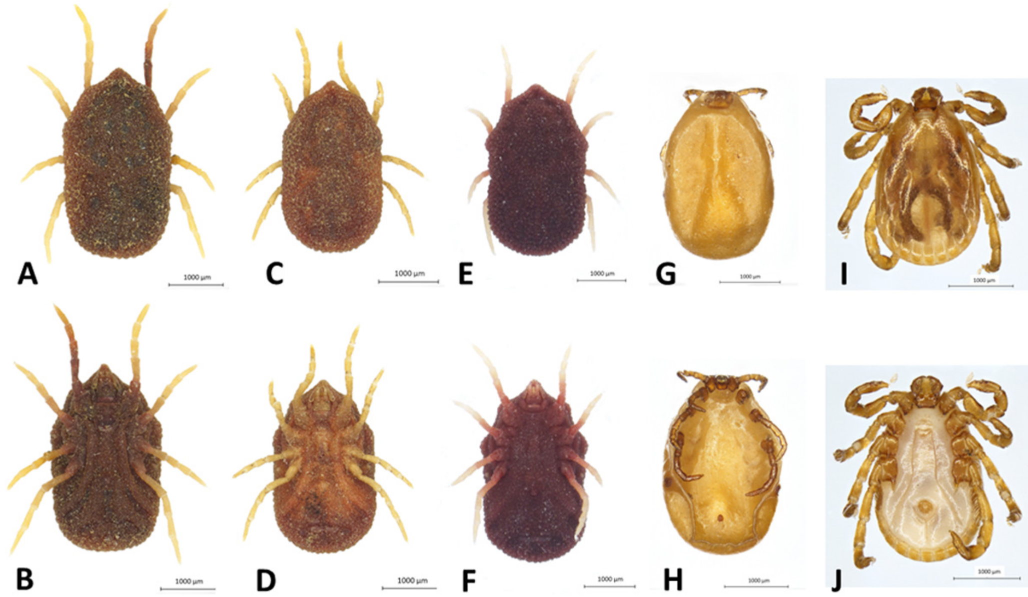

FIGURE 3. Dorsal and ventral view of *Ornithodoros sawaii* female (A, B), male (C, D), nymph (E, F), and *Ixodes signatus* nymph (G, H) collected from nest soil and litter of the Ancient Murrelet (*Synthliboramphus antiquus*) and Swinhoe's Storm Petrel (*Hydrobates monorhis*) during June and October 2015, and *Haemaphysalis flava* male (I, J) collected by tick drag on May 2014 at Chilbal Island, Jeollanam Province, Republic of Korea (all scale bars, 1,000μm).

In Japan, *O. sawaii* has been recorded from two seabird species, the Streaked Shearwater, *Calonectris leucomelas* (Temminck), and Swinhoe's Storm Petrel (Kitaoka & Suzuki 1973, 1974, Kawabata *et al*. 2006, Takano *et al*. 2014). Our survey showed that *O. sawaii* was associated with nests occupied by both *S. antiquus* and *H. monorhis* during the spring and fall, respectively. Ancient Murrelets are medium-sized blackish seabirds that nest in colonies in rock crevices and burrow under the roots of *Carex bootiana* Hooker and Arnott (predominant grass species on Chilbal Island), where the females lay eggs (Fig. 2C, E). Their range extends from the Yellow Sea (islands off China and Korea), Taiwan, Hong Kong, islands in the East Sea along the Korean Peninsula, the Russian Pacific coast and the Aleutian Islands to the Haida Gwaii (Queen Charlotte) archipelago of British Columbia, Canada, and as far south as the southern coast of California, USA (Del Hoyo *et al*. 1996, BirdLife International 2014a). Many of the islands where Ancient Murrelets nest are uninhabited and not readily accessible and are therefore infrequently surveyed.

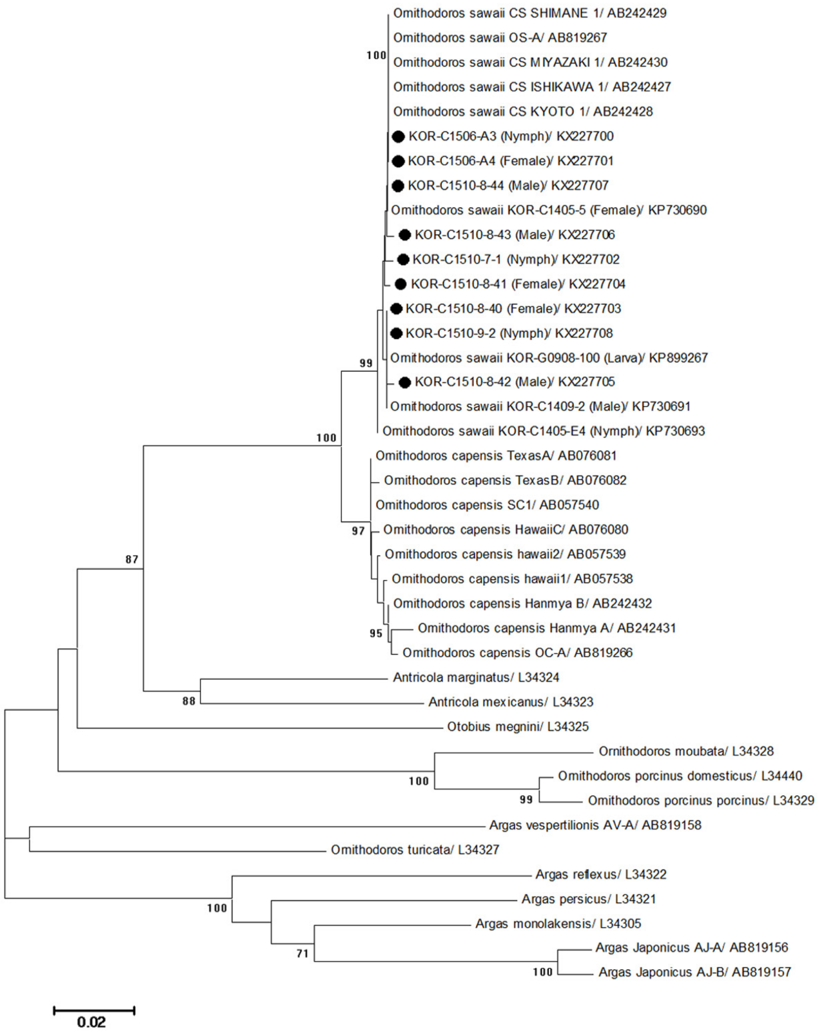

FIGURE 4. Phylogenetic analysis based on mt-rrs gene of *Ornithodoros sawaii* collected from Ancient Murrelet (*Synthliboramphus antiquus*) and Swinhoe's Storm Petrel (*Hydrobates monorhis*) nest soil and litter on Chilbal Island, Jeollanam Province, Republic of Korea, 2015. The PCR product consisted of 475 bp, including the primer sets. Phylogenetic trees were constructed based on NJ methods and bootstrap tests carried out according to the Kimura 2-parameter distances method. The percentages of replicate trees in which the associated taxa are clustered together in the bootstrap test (1,000 replicates) were calculated. In this analysis, the phylogenetic branches were supported with more than 70% bootstrap values. The length of the bar corresponds to the degree of sequence divergence. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (pairwise deletion).

Ancient Murrelets arrive on Chilbal Island from early April to June, a month before egg-laying. After rearing their chicks, they move to their coastal summer feeding grounds. Swinhoe's Storm Petrels arrive on Chilbal and other nearby islands from early June to October and occupy the same nesting sites as the Ancient Murrelet (Park & Takeshi 2011). Swinhoe's Storm Petrels are small blackish seabirds with white rumps and a range that extends from southern Indonesia and the Indian Ocean to northeastern Asia [Russia (south of Vladivostok), Japan, Korea, and Taiwan] (BirdLife International 2014b) (Fig. 2D, F). The population dynamics of *O. sawaii* in the context of this host transition merit investigation.

Ornithodoros sawaii is closely related to *O. capensis*, a species with a broad distribution that includes North Atlantic, Pacific, and Indian Ocean islands as well as coastal areas of South Africa, New Zealand, and southern Australia (Kohls 1957, Heath 1987). *Ornithodoros sawaii* was first described in 1973, but its known distribution includes only Japan (Hanmya Island, Miyazaki, Shimane, Kyoto, Kutsujima, and Ishikawa) (Kitaoka & Suzuki 1973, 1974, Kawabata *et al*. 2006, Takano *et al*. 2014) and the Republic of Korea (Kim *et al*. 2015).

TABLE 1. Records of ticks collected by tick drag and from nest soil and litter (Tullgren funnel) of the Ancient Murrelet (*Synthliboramphus antiquus*) and Swinhoe's Storm Petrel (*Hydrobates monorhis*) on Chilbal Island, Jeollanam Province, Republic of Korea, 2014–2015.

Year	Host bird	Month	Infested/ tested soil samples	Infestation rates (%)	*Ornithodoros sawaii*			*Ixodes signatus*	*Haemaphysalis flava**
					Nymph	Male	Female	Nymph	Male
2014	Ancient Murrelet (*Synthliboramphus antiquus*)	MAY	3/36	8.3	4	1	1		
	Swinhoe's Storm Petrel (*Hydrobates monorhis*)	SEP	6/21	28.6	2	3	1		
	Drag	MAY							1
2015	Ancient Murrelet (*Synthliboramphus antiquus*)	APR	0/3	0					
		JUN	5/39	12.8	2	-	2	1	
	Swinhoe's Storm Petrel (*Hydrobates monorhis*)	OCT	5/29	17.2	87	7	3		
	Total		19/128	14.8	95	11	7	1	1
					113 (98.26%)			1 (0.87%)	1 (0.87%)

*One male of *Haemaphysalis flava* collected by dragging grasses near the lighthouse located at the apex of Chilbal Island on 1 May 2015.

Haemaphysalis flava, originally described from Japan (Yamaguti *et al*. 1971), has long been known from mainland Korea and its islands (Kishida 1936). One male *H. flava* was collected on 1 May 2015 while dragging grasses near a lighthouse located at the apex of Chilbal Island. *Ixodes signatus* was first reported in 1968–1969 (4 females, 1 male); later in 1982 (21 females, 1 male, 4 larvae) from the Daurian Starling, *Sturnia sturnia* (Pallas), Japanese Cormorant, *Phalacrocorax capillatus* (Temminck and Schlegel), and Pelagic Cormorant, *P. pelagicus* Pallas, captured in Seoul Metropolitan City, and at Namhae, Koejae, Gyeongnam Province (Noh 1969, 1983); and in 1987 (3 nymphs) from the White-tailed Sea Eagle, *Haliaeetus albicilla* L., captured at Seongsan, Jeju-do (Province) (Kang *et al*. 1987). In our survey, one nymph was collected from nest soil on Chilbal Island during the nesting period of the Ancient Murrelet (Fig. 3).

The role of migratory seabirds as reservoirs of tick-borne diseases and in transporting exotic tick species and associated pathogens to avian summer breeding and winter feeding grounds is poorly documented. *Ornithodoros* spp. have been collected from seabirds, and nymphs and adults can survive without feeding for long periods between nesting seasons, resulting in the infestation of naïve birds that return to nesting sites, as well as their chicks. Additionally, birds may become infected with novel pathogens that are transmitted while at their feeding and/or breeding sites. Recent surveys have demonstrated *Rickettsia* and *Borrelia* spp. in *O. sawaii* collected from *C. leucomelas* and *H. monorhis* in Japan (Kawabata *et al*. 2006, Takano *et al*. 2009). More recently, Kang *et al*. (2013) reported *Anaplasma*, *Bartonella*, and *Borrelia* spp. from *Ixodes* spp. collected from migratory birds on Hong Island (34º 41'N, 125º 11'E), Jeollanam Province, located near Chilbal Island, during 2008–2009. Such results do not necessarily mean that migratory birds are reservoirs of these pathogens or responsible for the introduction of exotic ticks and associated pathogens into non-endemic areas, but they hint at the potential role of migratory birds in the dispersal of ticks and tick-borne microbial agents in northeastern Asia. Further studies are needed in order to define the geographical distribution, host range, and specific pathogens associated with ticks collected from resident and migratory seabirds inhabiting islands and coastal areas of the ROK.

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The opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Departments of the Army or Defense.

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