ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol Vol. 54, No. 2: 233-238, April 2016
http://dx.doi.org/10.3347/kjp.2016.54.2.233

▣ BRIEF COMMUNICATION

Ornithodoros sawaii (Ixodida: Argasidae) Larvae Collected from *Hydrobates monorhis* on Sogugul and Gaerin Islands, Jeollanam-do (Province), Republic of Korea

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Abstract: The 65th Medical Brigade and Public Health Command District-Korea, in collaboration with the Migratory Bird Research Center, National Park Research Institute, conducted migratory bird tick surveillance at Sogugul and Gaerin Islands (small rocky bird nesting sites), Jeollanam-do (Province), Republic of Korea (ROK), on 30 July and 1 August 2009. Breeding seabirds captured by hands in their nesting burrows were banded, identified to species, and carefully examined for ticks during the nesting season. A total of 9 *Ornithodoros sawaii* larvae were removed from 4 adult *Hydrobates monorhis* (Swinhoe's storm petrel). The identification of the larvae of *O. sawaii* collected from migratory seabirds were molecularly confirmed using mitochondrial 16S rDNA primer sets.

Key words: Argasidae, *Ornithodoros sawaii*, *Hydrobates monorhis*, Republic of Korea

Tick-borne disease surveillance has come to the forefront due to the reemergence of zoonotic tick-borne pathogens that pose worldwide medical and veterinary health risks to wild and domestic animals and birds and incidentally to humans over the past few decades [1,2]. The role of migratory seabirds in the transportation of exotic tick species to their summer breeding and winter feeding grounds is poorly documented, due to their isolated breeding sites on remote islands that are often protected by governments. These exotic ticks may become established along the migratory routes that lead to their introduction, including their associated pathogens, to bird sanctuaries as well as developed areas. In addition, tick-borne disease surveillance, including their identification, has been little studied.

Soft ticks, Family Argasidae, are commonly associated with remote rocky nesting grounds of seabirds and for that reason have not been thoroughly investigated [3,4]. The genus *Ornithodoros* consists of the largest number of described species (112 of 193) in the Family Argasidae [5] and is commonly associated with various sea bird hosts throughout the New and Old World, including Pacific Islands [6], North and South Americas [7-9], New Zealand [10-12], Australia [12], Antarctic [12], Japan [13-19], Russia [20], and Africa [3].

The Migratory Bird Research Center, National Park Research Institute, located on Heuksan Island, conducted a monitoring program on breeding seabirds at Sogugul (34˚11' N, 125˚07' E) and Gaerin Islands (34˚11' N, 125˚09' E) located 136 km southwest of Mokpo, Jeollanam-do (Province), Republic of Korea on 30 July and 1 August 2009 (Fig. 1A, B). Sogugul and Gaerin Islands are remote uninhabited islands that are protected as National Monuments (Sogugul and Gaerin Islands, No. 341, 13 August 1984) with a total land mass of 18,400 m^2, and 49,000 m^2, respectively. These islands are known to host breeding colonies of several seabirds such as *Hydrobates monorhis* (Swinhoe) (Swinhoe's storm petrel), *Synthliboramphus antiguues* (Gmelin) (ancient murrelet), *S. wumizusume*

• Received 28 November 2015, revised 25 January 2016, accepted 14 February 2016.
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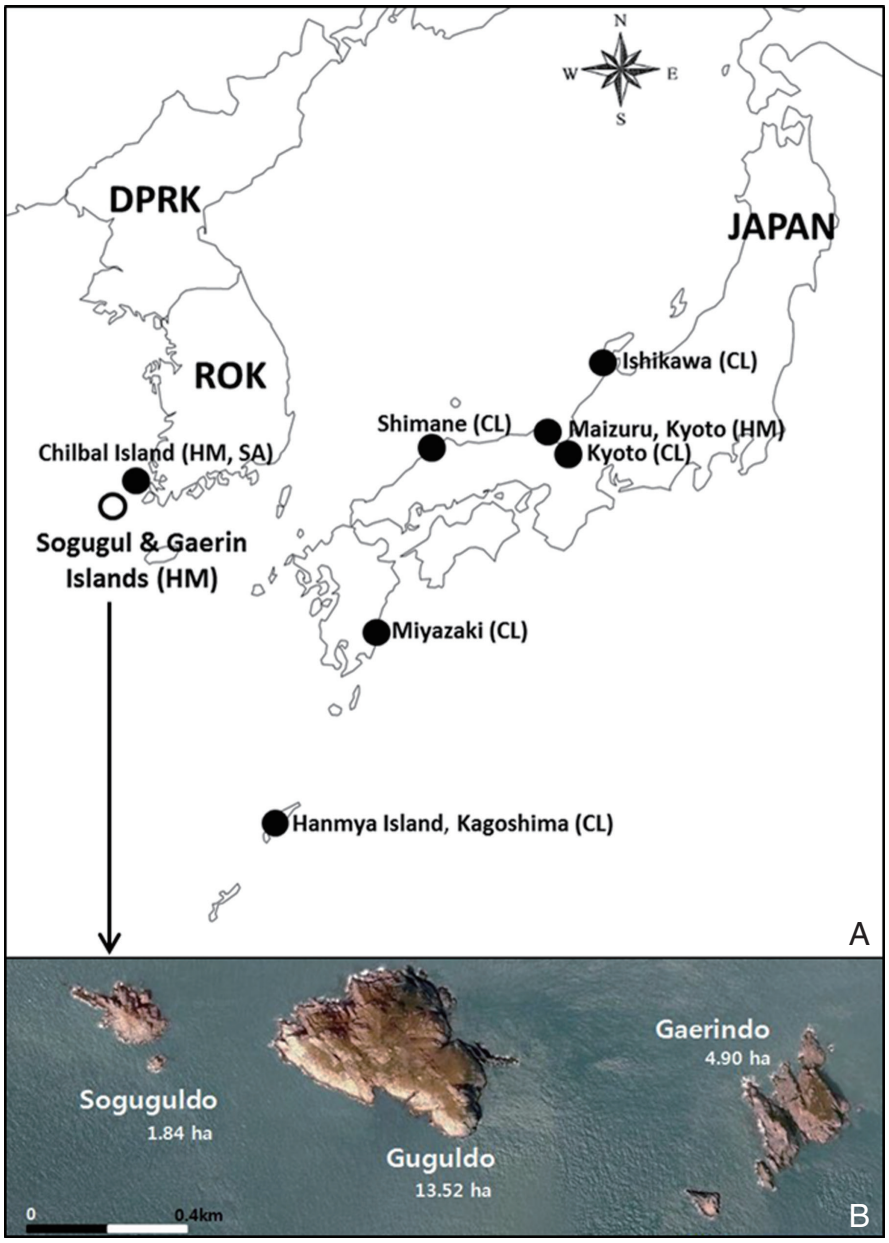

Fig. 1. Collection sites and host records of *Ornithodoros sawaii* in Japan and Korea [closed circle (previously reported) and open circle (this survey)] (A), at Sogugul (left) and Gaerin (right) Islands, Jeollanam-do (Province), Republic of Korea (B) (HM, *Hydrobates monornis*; CL, *Calonectris leucomelas*; SA, *Synthliboramphus antiquus*) (image from *http://map.daum.net*).

Temminck (crested murrelet), and *Calonectris leucomelas* Temminck (streaked shear-water).

Nesting seabirds were captured by hand from their nest burrows under vegetation covers. Breeding adults captured from nests were carefully removed, identified to species, and then banded with a unique identification number. Prior to release, they were examined for ticks, particularly around the head, neck, wings, and abdomen, and ticks were carefully removed with a forceps to prevent injury to the bird. After removal, ticks were placed individually in cryovials containing 70% ethanol and labeled with a unique identification number that corresponded to the migratory bird collection data. Ticks were microscopically examined, developmental stages determined, and then identified to genus and species using morphological keys [16,17,21-23]. Field data that included the unique bird identification number, species, and other pertinent information were

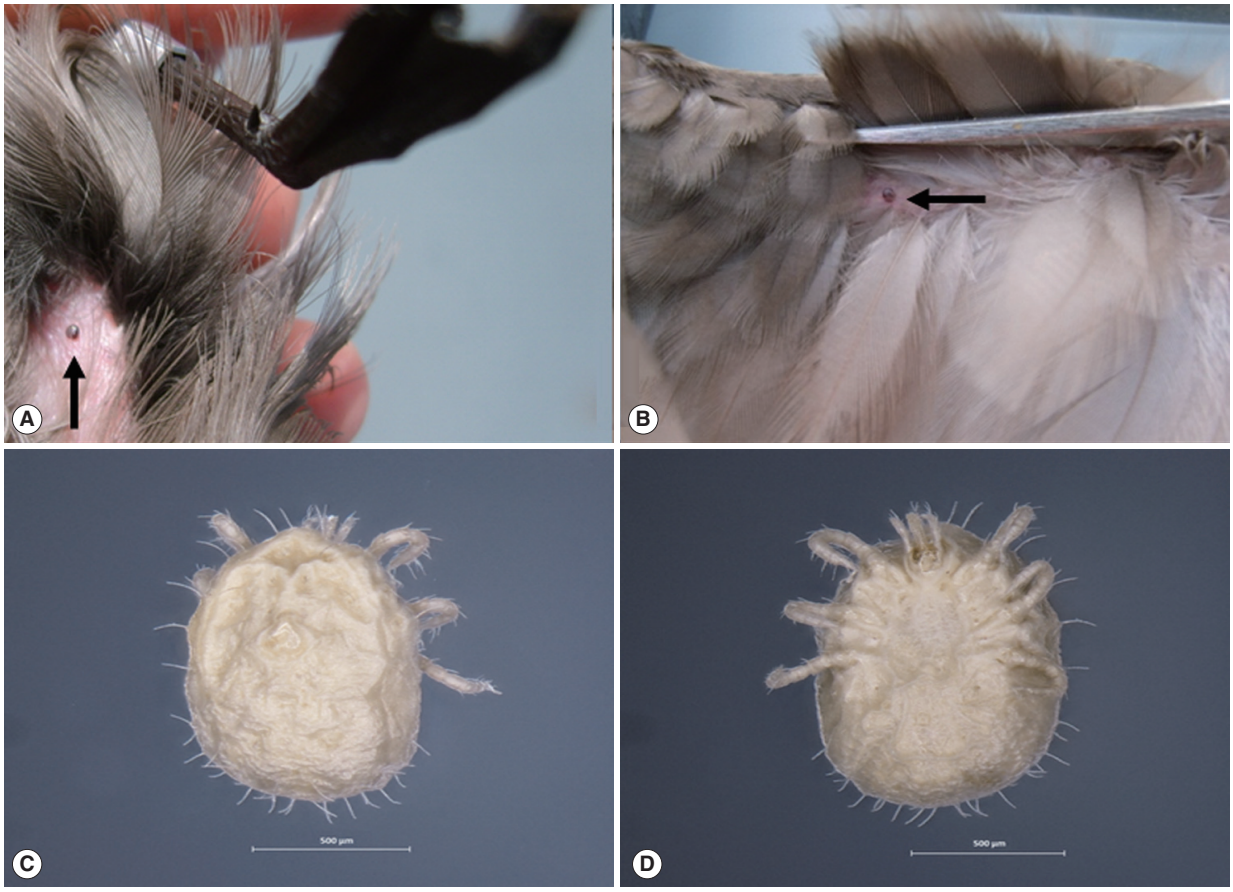

Fig. 2. *Ornithodoros sawaii* larvae located on the brood patch of the abdomen (A) and on the left under wing between the ulnar and radius (B) of *Hydrobates monorhis*. Dorsal (C) and ventral (D) views of an *O. sawaii* larva with scale bars of 500 μm.

archived for future references.

PCR was performed using mt-rrs primer sets based on the mitochondrial 16S rDNA gene (mt-rrs) fragment previously described by Black and Piesman [24], Ushijima et al. [25], and Kim et al. [26]. The resulting product consisted of 475 base pairs, including the primer sets. Sequencing results were assembled using the SeqMan program implemented in DNASTAR software (version 5.0.6; DNASTAR Inc., Madison, Wisconsin, USA) to determine the consensus sequences. Sequence data were analyzed using the MEGA 6.0 software (http://www.megasoftware.net) [27]. For phylogenetic analysis, the neighbor-joining (NJ) and bootstrap tests were carried out according to the Kimura's 2-parameter distance method [28,29].

A total of 9 larvae of *Ornithodoros sawaii* Kitaok and Suzuki were removed from the wings and abdomen of 2 adult *H. monorhis* captured on 31 July and 1 August in 2009 at Sogugul Island (6 larvae) and 2 adult *H. monorhis* at Gaerin Island (3 larvae) (Fig. 2A, B). *O. sawaii* and *O. capensis* Neumann are closely related to each other, and morphological differentiation is based on the shape of the dorsal plate, dentition of the hypostome, and outline of the basis capituli in the ventral aspect (Fig. 2C, D). To confirm these identifications, the ticks were also identified by PCR using partial mitochondrial 16S ribonucleic acid gene primer sets designed for the identification of soft ticks, and products were cloned and sequenced, and then compared with 16S rDNA gene fragments of *O. capensis* and *O. sawaii* in Japan and other countries (Fig. 3). *Ornithodoros* larvae collected from *H. monorhis* from Gaerin (KOR-G0908-100) Island most closely aligned with *O. sawaii* with 0-1 base differences and 99.8-100% identity compared to female, male, and nymph stages of *O. sawaii* collected from Chilbal Island [26], and other collection sites of Japan (Miyazaki, Shimane, Maizuru, Kyoto, and Ishikawa).

H. monorhis breeding pairs nest in colonies among rock crevices and burrows under *Carex bootiana* Hook grass roots during the breeding season. *H. monorhis* are small blackish

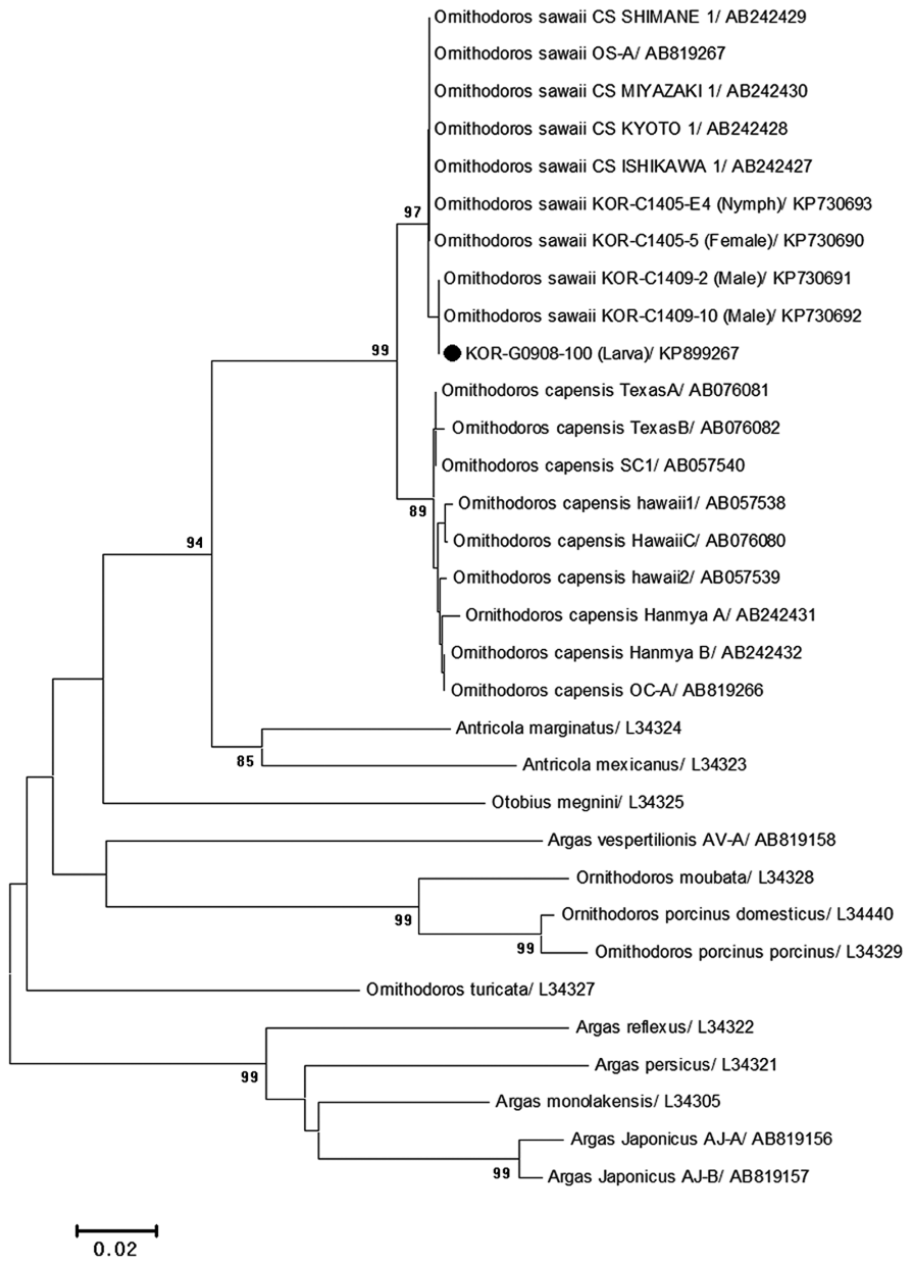

Fig. 3. Phylogenetic analysis based on mt-*rrs* of *Ornithodoros sawaii* larvae collected from the abdomen and wings of Swinhoe's storm petrels (*Hydrobates monorhis*). The phylogenetic tree was constructed based on NJ methods and bootstrap tests carried out according to the Kimura 2-parameter distances method. The percentages of replicate trees in which the associated taxa are clustered together in the bootstrap test (1,000 replicates) were calculated. The phylogenetic branches were supported with more than 70% bootstrap values in this analysis. 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).

seabirds with white rumps and distributed from southern Indonesia and Indian Ocean to northeast Asia, including the ROK [30,31]. Nesting populations on Gugul Islets (Sogugul, Gugul, and Gaerin Islands) make up approximately 75% of the global populations [31]. Nesting sites (e.g., Sogugul and Gaerin Islands) are remote small rocky volcanic outcroppings that are difficult to access and are therefore infrequently surveyed. Nesting soil and litter of the ancient murrelet, *Synthliboramphus antiquus* (Gmelin) and *H. monorhis* were collected at Chilbal Island, a small remote uninhabited island of south-

western Jeollanam-do Province, ROK, and ticks were collected from the nest soil and litter using the Tullgren funnel [26]. A total of 12 *H. sawaii* (2 females, 4 males, and 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* [26]. While *H. monorhis* occupies the same nesting sites as *S. antiquus*, the breeding seasons of these 2 species do not overlap, and resident *Ornithodoros* larvae feed on young and adult birds of both species during their breeding season. This is the first collection report of *O. sawaii* larvae recovered from the wing and abdomen of *H. monorhis* in the ROK in this survey.

In Japan, *O. sawaii* was recorded from burrow nest of hosts, *Calonectris leucomelas* (Temminck) (streaked shear-water), and *H. monorhis* at Hanmya, Miyazaki, Shimane, Maizuru, Kyoto, and Ishikawa [17,19,32,33] (Fig. 1A).

Recently, Kang et al. [34] reported tick-borne pathogens belonging to the genera *Anaplasma*, *Bartonella*, and *Borrelia* from *Ixodes* spp. collected during 2008-2009 from migratory birds on Hong Island, Jeollanam-do Province. Hong Island is near Sogugul and Gaerin Islands where *O. sawaii* were collected from *H. monorhis*. These results do not conclusively identify the role of migratory birds as reservoirs, the introduction of exotic ticks in non-endemic areas, or the role of soft ticks in the potential maintenance of tick- borne pathogens, but do provide an insight into the potential role of migratory birds in the dispersal and infectious cycles of tick-borne diseases in Northeast Asia.

This report summarizes the first collection record of *O. sawaii* larvae, based on morphological and molecular identification, from 2 remote islands in the ROK. Further studies are needed to define the geographical distribution, host range, and pathogens present in ticks collected from nesting soil and litter, and resident and migratory seabirds inhabiting islands and coastal areas of the ROK.

ACKNOWLEDGMENTS

We thank the members of the Migratory Bird Research Center, National Park Research Institute, Korea National Park Service, on Heuksan Island, Jeollanam-do Province, Republic of Korea, for collecting nesting soil and litter during seabird conservation and breeding surveys. We also appreciate Shinan County office that issued relevant permits and supported our field works. The tick-borne disease surveillance was supported by the Response System (AFHSB-GEIS) to read: Armed Forces Health Surveillance Branch, Global Emerging Infections Surveillance and Response System (AFHSB-GEIS) and Public Health Command District- Korea (Provisional). 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.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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