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Source: Systematic and Applied Acarology, 22(11) : 1899-1909

Published By: Systematic and Applied Acarology Society

URL: https://doi.org/10.11158/saa.22.11.9

Systematic & Applied Acarology 22(11): 1899–1909 (2017) ISSN 1362-1971 (print)
http://doi.org/10.11158/saa.22.11.9 ISSN 2056-6069 (online)

Article http://zoobank.org/urn:lsid:zoobank.org:pub:AFE04DCC-8E4F-4F92-ABB1-3E89564EC921

New distribution and host records for *Ornithodoros capensis* Neumann and *Ornithodoros sawaii* Kitaoka and Suzuki (Acari: Ixodida: Argasidae) collected from Black-tailed Gull, *Larus crassirostris*, nestlings and nest soil and litter on Hong and Nan Islands, Republic of Korea

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Abstract

The 65th Medical Brigade and Medical Department Activity-Korea, in collaboration with the Migratory Birds Research Center, National Park Research Institute, conducted a migratory bird tick-borne disease surveillance program during 2014–2015 on two small, remote, uninhabited islands, Hong (Gull) Island, southern Gyeongnam Province, and Nan Island, western Chungnam Province, Republic of Korea (ROK). Argasid ticks were collected from Black-tailed Gull (*Larus crassirostris*) nestlings that had recently died and associated nest soil/litter, and all tick life history stages were identified morphologically. Because morphological keys are unreliable for the identification of adult and nymphal argasid ticks, identifications were confirmed by genotyping using polymerase chain reaction techniques. A total of 29 *Ornithodoros capensis* larvae and 2 *Ornithodoros sawaii* larvae were collected from 4 of 7 (57.1%) Black-tailed Gull nestlings that had recently died. An additional five *O. capensis* (2 males, 1 nymph, and 2 larvae) were collected from nest soil/litter. Only *O. sawaii* larvae (2/41, 4.9%) were collected from dead Black-tailed Gull nestlings on Nan Island. This is the first report of *O. capensis* from these seabird breeding islands.

Key words: Argasidae, *Ornithodoros capensis*, *Ornithodoros sawaii*, *Larus crassirostris*, Hong Island, Nan Island, Korea

Introduction

Migratory seabirds are hosts to both soft (argasid) and hard (ixodid) ticks and may transport exotic tick species and associated tick-borne pathogens to non-endemic areas during their long migrations

between summer breeding and winter feeding grounds (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; Takano *et al*. 2009; Dietrich *et al*. 2011; Kang *et al*. 2013). However, tick ecology at seabird nesting sites is poorly understood because such sites are often located on remote, uninhabited or sparsely populated islands that are under government protection, making them inaccessible to the general public.

The genus *Ornithodoros* Koch contains the largest number of described argasid species (112/193), and many of these are commonly associated with various seabird hosts throughout the world (Asanuma *et al*. 1955; Asanuma 1960, 1965; Denmark & Clifford 1962; Yamaguti *et al*. 1971; Jonkers *et al*. 1973; Kitaoka & Suzuki 1973, 1974; Hoogstraal *et al*. 1976; Heath 1987, 2006; Murray *et al*. 1990; Keirans *et al*. 1992; Kawabata *et al*. 2006; Guglielmone *et al*. 2010; Gomez-Diaz *et al*. 2012; Vander Velde & Vander Velde 2013; Dupraz *et al*. 2016; Muñoz-Leal *et al*. 2017), including Korea (Kim *et al*. 2015, 2016a, b). However, the taxonomy of argasid ticks is confused because reliable morphological characters for identifying nymph and adult stages are often obscure or nonexistent. This report presents new host and distribution records for two ornithodorine ticks, *Ornithodoros capensis* Neumann, 1901, and *Ornithodoros sawaii* Kitaoka and Suzuki, 1973, based on collections from soil/litter at nesting sites of the Black-tailed Gull, *Larus crassirostris* Vieillot, 1818, and from dead chicks of this gull at two seabird breeding islands in the Republic of Korea (ROK).

Materials and methods

Survey area

The Migratory Birds Research Center, National Park Research Institute, conducted tick surveillance during the breeding season of Black-tailed Gull colonies at Hong (Gull) Island on 14 June and 17 July 2014, and 22 Jun 2015, and at Nan Island on 7 July 2015. Hong Island (Maejuk-ri, Hansan-myeon, Tongyeong-si, Gyeongnam Province; 34° 30' N, 128° 50' E) is a small (98,380 m^2), remote, uninhabited island 50 km southeast of Tongyeong, a major mainland port city, and has been protected as a National Monument (No. 335) since 4 November 1982 (Fig. 1A, B). Nan Island (Gauido-ri, Geunheung-myeon, Taean-gun, Chungnam Province; 36°41' N, 126°6' E) is also a small (47,603 m^2), remote, uninhabited island located 30 km west of Mohang, a major mainland port city, and has been a protected National Monument (No. 334) since 16 November 1982.

Tick collections

Dead Black-tailed Gull nestlings were individually sealed in plastic Ziploc® bags (30 x 40 cm) at the collection site (Fig. 2A, B), then placed in a Styrofoam cooler and transported to the Migratory Birds Research Center. Approximately 24 hrs after the nestlings were placed in the Ziploc bags, they were removed, leaving the detached ticks in the Ziploc bags, which were returned with the ticks to the Styrofoam cooler. In addition, Black-tailed Gull nest soil/litter (50–100 g) was collected using a small scoop and samples placed separately in Ziploc bags (25 x 28 cm) that were then transferred to a Styrofoam cooler and returned to the Migratory Birds Research Center. All ticks from the dead nestlings and the soil/litter samples were transported to the 5th Medical Detachment, Yongsan U.S. Army Garrison, Seoul, ROK. The soil and litter samples from each nest site were placed separately, according to date of collection, in 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 hrs, the ticks were removed, placed individually in 2 ml cryovials containing 80% EtOH, and labeled with a unique nest identification number, as

described by Kim *et al*. (2015). Ticks from the dead nestlings also were placed in 70% EtOH and labeled with a unique collection number. All ticks were subsequently identified to stage of development and species using standard keys (Kohls 1957, Yamaguti *et al*. 1971, Kitaoka & Suzuki 1973). Three larvae (2014) and one male and eight larvae (2015) were submitted to the Korea National Institute of Health for specific identification by polymerase chain reaction (PCR).

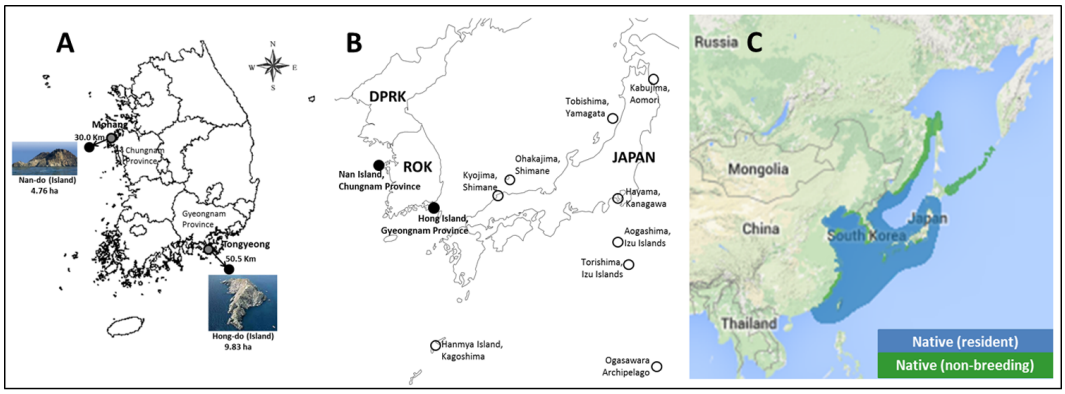

FIGURE 1. (A) Collection sites (●) of *Ornithodoros capensis* from nest soil/litter and recently dead nestlings of the Black-tailed Gull (*Larus crassirostris* Vieillot, 1818) on Hong (Gull) Island, Gyeongnam province, and Nan Island, Chungnam province, Republic of Korea [Tongyeong and Mohang (●), mainland port city]. (B) Collection records of *O. capensis* in Japan and Korea [previously reported (**o**) and this survey (●)]. (C) Distribution map of the Black-tailed gull (*Larus crassirostris*) (image from *http://maps.iucnredlist.org/map.html?id=22694289*).

PCR and sequencing analysis

Total DNA was prepared from individual ticks using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with minor modification, and stored at -20°C until used. PCR was performed using primer sets (mt-rrs1: 5-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3 and mt-rrs2: 5-CCG GTC TGA ACT CAG ATC AAG TA-3) based on the mitochondrial 16S rDNA gene (mt-rrs) fragment, as previously described by Black and Piesman (1994) and Ushijima *et al*. (2003). PCR assays were performed using 50 µL of reaction mixture with TaKaRa ExTaq™ DNA polymerase (Takara, Shiga, Japan) at 94°C for 5 min, followed by 35 cycles for 10 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C, and final extension for 5 min at 72°C. PCR products were then purified using a QIAquick® Gel Extraction Kit (Qiagen, Hilden, Germany), cloned using pCR4-TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA), and sequenced using ABI Prism BigDye™ Terminator v3.1Cycle Sequencing Kits and an ABI 3730xl sequencer (Applied Biosystems®, Foster City, CA, USA) at Macrogen, Inc. (Daejeon, ROK). Sequencing results were assembled using the SeqMan program implemented in DNASTAR software (version 5.0.6; DNASTAR Inc., Madison, WI, USA) to determine consensus sequences. Sequence data for the mt-rrs gene fragment were deposited in GenBank under Accession Numbers KY825206-KY825217.

Phylogenetic analysis

Sequence data for amplicons of the mt-rrs gene fragment were analyzed using MEGA 6.0 software (http://www.megasoftware.net) (Tamura *et al*. 2013). Sequences for *Ornithodoros* spp. collected from nesting sites were aligned and compared with previously published *Ornithodoros* spp. and

facilitated using the CLUSTALW method (Lasergene program version 5, DNASTAR Inc. Madison, WI). 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. Multiple alignments were performed using the same values. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (pairwise deletion).

Results and discussion

Black-tailed Gulls build their nests on the ground on isolated islands that are difficult to access in the ROK. They are moderately large birds with a white body, gray wings, black tail, and yellow bill with red and black spots near the tip (Fig. 2A, B). They are distributed throughout much of East Asia, including the Democratic People's Republic of Korea, ROK, Japan, maritime Russia and eastern China (Won & Kim 2012, BirdLife International 2016) (Fig. 1C), but have also infrequently been observed in North America (Heinl 1997), Mexico (Garrett & Molina 1998) and the Philippines (Redman 1993). Although they are resident seabirds that are often seen in coastal seashore areas, they mate and raise their chicks from April-July on nearby isolated islands (Kwon *et al*. 2006), where they feed on small marine fish (Kwon *et al*. 2013).

In this study, tick surveillance was conducted on Hong and Nan Islands during the Black-tailed Gull breeding season (Fig. 2). A total of 29 *Ornithodoros capensis* larvae and two *O. sawaii* larvae were collected from 4/7 (57.1%) Black-tailed Gull nestlings that had recently died. Additionally, five *O. capensis* (2 males, 1 nymph, and 2 larvae) were collected from associated nest soil and litter (Table 1). While the morphological identification of *Ornithodoros* spp. nymphs and adults is often difficult or impossible, larval morphological characters are reported to be reliable (Jones & Clifford 1972, Kitaoka & Suzuki 1973). Larvae were morphologically determined as *O. capensis* based on the following taxonomic characters: dorsal plate pyriform, widest posteriorly, dorsal setae 22–25 pairs, dorsolateral setae 18–21 pairs, central setae 4 pairs, posthypostomal setae 2 pairs, hypostome with apex blunt and dentition 5/5 in the anterior third, 4/4 near mid-length, and 2/2 toward base. Because similarly reliable characters and keys for the identification of *Ornithodoros* nymphs and adults are unavailable, these stages were identified using PCR and amplicon cloning and sequencing for comparison with *O. capensis* and *O. sawaii* specimens collected in Japan, Korea, and other countries (Fig. 3).

TABLE 1. Number and stage of development of *Ornithodoros capensis* and *O. sawaii* collected from recently dead nestlings and associated nest soil and litter of the Black-tailed Gull (*Larus crassirostris*) on Hong Island, Gyeongnam Province and Nan Island, Chungnam Province, Republic of Korea, 2014–2015.

Year	Sites	Samples	Month	Infested/ tested soil and litter	Infestation rates (%)	*Ornithodoros capensis*				*Ornithodoros sawaii*				Total
						F	M	N	L	F	M	N	L	
2014	Hong Island	Dead nestlings	JUN	3/5	60.0	0	0	0	18	0	0	0	0	18
		Nest soil and litter	JUL	0/6	0.0	0	0	0	0	0	0	0	0	0
2015	Hong Island	Nest soil and litter	JUN	3/14	21.4	0	1	0	2	0	0	0	0	3
	Nan Island	Dead nestlings	JUL	1/2	50.0	0	0	0	11	0	0	0	2	13
		Nest soil and litter	JUL	2/19	10.5	0	1	1	0	0	0	0	0	2
	Total			8/46	17.4	0	2	1	31	0	0	0	2	36

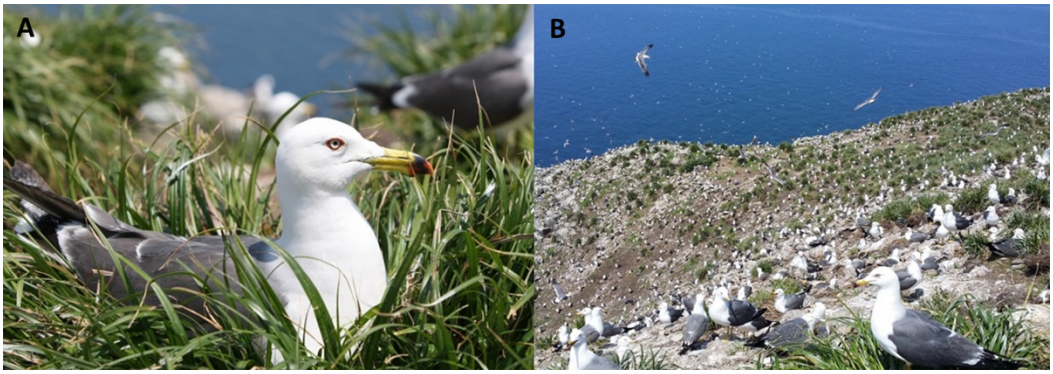

FIGURE 2. (A) Black-tailed Gull (*Larus crassirostris*) nest, and (B) breeding site on Hong Island, Gyeongnam Province, Republic of Korea (photographed by Chang-uk Park).

Sequence analysis showed that *O. capensis* larvae [KOR-H1406Y-1-8 (KY825206), KOR-H1406Y-2-2 (KY825207), KOR-H1407Y-53-1 (KY825208), and KOR-H1506-14 (KY825209)] collected from Black-tailed Gull nestlings on Hong Island and nymphs [KOR-N1507Y-14 (KY825215)] and larvae [KOR-N1507Y-6-6 (KY825210), KOR-N1507Y-6-7 (KY825211), KOR-N1507Y-6-9 (KY825212), KOR-N1507Y-6-11 (KY825213), KOR-N1507Y-6-13 (KY825114)] collected from Black-tailed Gull nestlings and nest soil and litter on Nan Island aligned most closely with *O. capensis* in GenBank. *Ornithodoros capensis* from Korea demonstrated 22–25 base pair (bp) differences and 94.1–95.4% identity in nucleotide (nt) sequences with *O. capensis* from breeding bird colonies in Japan (423 or 475 bp) (Table 2). These results constitute new distribution and host records for *O. capensis* in the ROK.

Sequence analysis of *O. capensis* collected in Korea, compared with *O. capensis* collected in other regions of the world (*e.g.*, Japan, which is geographically close to Korea, the Pacific Ocean, Indian Ocean, Atlantic Ocean, Mediterranean Sea, and South America), demonstrates genetic differences that may be due to geographic isolation or preferred/available hosts. However, Dupraz *et al*. (2016) demonstrated that morphological variation, analyzed genetically and on the basis of tick size and shape, of at least five different species within the *O. capensis* complex varied strongly in relation to five different host types (shearwater, booby, gull, tern, and penguin) and weakly with geographical distribution. *Ornithodoros capensis* was collected only from the Black-tailed Gull in Korea, but in Japan it has been collected from this species and three others: Streaked Shearwater [*Calonectris leucomelas* (Temminck, 1835)], Black-footed Albatross [*Phoebastria* (=*Diomedea*) *nigripes* (Audubon, 1849)], and Ancient Murrelet [*Synthliboramphus antiquus* (Gmelin, 1789)] (Asanuma *et al*. 1955, Yamaguti *et al*. 1971, Tsurumi *et al*. 2002).

Sequence analysis (Table 2) of *O. capensis* collected on Hong and Nan Islands of Korea demonstrated 94.1–95.5% (mean 94.8%) identity with *O. capensis* from other areas and from the Japanese Islands. However, *O. capensis* from Peru, the Galapagos Islands (No. 28–30 in Table 2), and the Cape Verde and Canary Islands (No. 34–39) demonstrated 90.3–91.7% identity. These differences may be due to geographic isolation or preferred/available hosts. Additional molecular and morphological studies of immature and mature specimens are needed to determine the status of *O. capensis* throughout its geographic and host range (Fig. 3, Table 2).

Ornithodoros sawaii larvae [KOR-N1507Y-6-8 (KY825216), and KOR-N1507Y-6-10 (KY825217)], collected from a single dead Black-tailed Gull nestling on Nan Island, aligned most closely with *O. sawaii*. Comparatively, there were only 0–7 nt differences with 98.5–100% nt sequence identity between *O. sawaii* collected from Chilbal and Gugul Islands, ROK (Kim *et al*.

2015, 2016a, b, 2017), and 98.3–100% identity (0–7 nt differences) with *O. sawaii* collected from seabirds and nest soils in Japan (Tsurumi *et al*. 2002; Kawabata *et al*. 2006; Takano *et al*. 2014) (Fig. 3). Our collection therefore represents a new distribution record for *O. sawaii* in the ROK.

As seabirds migrate to the mainland, there is potential for the dispersal of soft ticks and associated pathogens. *Rickettsia, Borrelia,* and *Coxiella* spp. have been identified from *O. capensis* collected from *C. leucomelas* and *L. crassirostris* in Japan (Kawabata *et al*. 2006), from Brown Pelican (*Pelecanus occidentalis* Linnaeus, 1766) in the U.S. (Reeves *et al*. 2006), and from Sooty Tern [*Onychoprion fuscatus* (Linnaeus, 1766)], Wedge-tailed Shearwater [*Puffinus* (= *Ardenna*) *pacificus* (Gmelin, 1789)], Masked Booby (*Sula dactylatra* Lesson, 1831), and Red-footed Booby [*S. sula* (Linnaeus, 1766)] on Indian Ocean Islands (Dietrich *et al*. 2014). Recently, Kang *et al*. (2013) reported *Anaplasma*, *Bartonella*, and *Borrelia* spp. from *Ixodes* spp. collected from migratory birds on Hong (Red) Island (34º 41' N, 125º 11' E) 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 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 to determine the geographical distribution and host range of these ticks and, importantly, the specific pathogens carried by ticks associated with resident and migratory seabirds inhabiting islands and coastal areas of the ROK.

TABLE 2. Matrix of genetic similarity for the 16S rRNA partial sequence of *Ornithodoros capensis* used in this survey.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
1	***	99.2	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.4	94.6	94.1	94.3	94.9	94.9	94.7	95.2	94.9	94.7	94.4	94.4	94.2	94.2	94.2	94.8	94.6	94.4	90.0	90.0	89.8	94.4	94.5	93.9	92.3	90.9	92.1	91.4	90.8	91.4	1
2	0.8	***	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.4	94.6	94.1	94.3	94.9	94.9	94.7	95.2	94.9	94.7	94.4	94.4	94.2	93.9	93.9	94.5	94.6	94.4	90.0	90.0	89.8	94.4	94.2	93.6	92.3	90.9	92.1	91.4	90.5	91.1	2
3	0.4	0.4	***	100	100	100	100	100	100	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	3
4	0.4	0.4	0.0	***	100	100	100	100	100	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	4
5	0.4	0.4	0.0	0.0	***	100	100	100	100	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	5
6	0.4	0.4	0.0	0.0	0.0	***	100	100	100	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	6
7	0.4	0.4	0.0	0.0	0.0	0.0	***	100	100	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	7
8	0.4	0.4	0.0	0.0	0.0	0.0	0.0	***	100	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	8
9	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	***	99.8	95.0	94.6	94.8	95.4	95.4	95.2	95.6	95.4	95.2	94.8	94.9	94.6	94.5	94.5	95.1	95.1	94.9	90.5	90.4	90.3	94.9	94.8	94.2	92.8	91.4	92.5	91.8	91.0	91.6	9
10	0.6	0.6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	***	94.8	94.3	94.6	95.2	95.2	94.9	95.4	95.2	94.9	94.6	94.6	94.4	94.2	94.2	94.8	94.9	94.6	90.3	90.2	90.0	94.6	94.5	93.9	92.5	91.2	92.3	91.6	90.8	91.4	10
11	5.6	5.6	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.4	***	99.3	99.3	99.1	99.1	99.1	98.8	99.1	98.8	98.6	98.6	98.3	98.0	98.0	100	100	99.8	90.0	90.9	89.6	97.9	93.9	93.9	92.7	92.7	92.4	92.2	92.4	92.2	11
12	6.2	6.2	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.9	0.7	***	99.8	99.8	99.8	99.8	99.5	99.8	99.5	99.3	99.3	99.1	98.8	98.8	99.1	99.3	99.1	89.1	90.0	89.1	98.6	93.9	93.9	92.4	92.4	92.2	92.4	91.3	91.9	12
13	5.9	5.9	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.6	0.7	0.2	***	99.5	99.5	99.5	99.3	99.5	99.3	99.1	99.1	98.8	98.6	98.6	99.1	99.3	99.1	89.3	90.2	89.3	98.3	93.6	93.6	92.2	92.2	92.0	92.2	91.0	91.6	13
14	5.2	5.2	4.8	4.8	4.8	4.8	4.8	4.8	4.8	5.0	1.0	0.2	0.5	***	99.6	99.6	99.8	100	99.8	99.1	99.1	98.8	98.6	98.6	98.8	99.1	98.8	90.8	90.4	90.8	98.4	94.2	94.2	92.3	90.9	92.1	92.3	91.0	91.6	14
15	5.2	5.2	4.8	4.8	4.8	4.8	4.8	4.8	4.8	5.0	1.0	0.2	0.5	0.4	***	99.6	99.8	99.6	99.4	99.1	99.1	98.8	98.6	98.6	98.8	99.1	98.8	90.3	90.0	90.3	98.4	94.2	94.2	92.3	90.9	92.1	92.3	91.0	91.6	15
16	5.5	5.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.2	1.0	0.2	0.5	0.4	0.4	***	99.4	99.6	99.4	99.3	99.3	99.1	98.8	98.8	98.8	99.1	98.8	90.5	90.2	90.5	98.6	93.9	93.9	92.5	91.2	92.3	92.5	91.3	91.9	16
17	5.0	5.0	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.8	1.2	0.5	0.7	0.2	0.2	0.6	***	99.8	99.6	98.8	98.8	98.6	98.3	98.3	98.6	98.8	98.6	90.5	90.2	90.5	98.1	94.5	94.5	92.1	90.7	91.8	92.1	90.8	91.4	17
18	5.2	5.2	4.8	4.8	4.8	4.8	4.8	4.8	4.8	5.0	1.0	0.2	0.5	0.0	0.4	0.4	0.2	***	99.8	99.1	99.1	98.8	98.6	98.6	98.8	99.1	98.8	90.8	90.4	90.8	98.4	94.2	94.2	92.3	90.9	92.1	92.3	91.0	91.6	18
19	5.5	5.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.2	1.2	0.5	0.7	0.2	0.6	0.6	0.4	0.2	***	98.8	98.8	98.6	98.3	98.3	98.6	98.8	98.6	90.5	90.2	90.5	98.1	93.9	93.9	92.1	90.7	91.8	92.1	90.8	91.4	19
20	5.9	5.9	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.6	1.4	0.7	1.0	0.9	0.9	0.7	1.2	0.9	1.2	***	99.8	99.5	99.7	99.7	98.3	98.4	98.1	89.8	90.2	89.8	98.6	94.8	94.8	92.7	93.0	92.5	92.7	91.9	92.5	20
21	5.8	5.8	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.6	1.4	0.7	1.0	0.9	0.9	0.7	1.2	0.9	1.2	0.0	***	99.8	99.7	99.7	98.3	98.6	98.4	90.5	90.2	90.5	98.8	94.8	94.8	93.0	93.0	92.8	93.0	91.9	92.5	21
22	6.1	6.1	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.8	1.7	1.0	1.2	1.2	1.2	0.9	1.4	1.2	1.4	0.2	0.2	***	99.7	100	98.0	98.4	98.1	90.3	90.0	90.3	99.1	94.5	94.5	92.8	92.8	92.5	92.8	91.6	92.2	22
23	6.0	6.3	5.7	5.7	5.7	5.7	5.7	5.7	5.7	6.0	2.1	1.2	1.5	1.5	1.5	1.2	1.8	1.5	1.8	0.3	0.3	0.3	***	99.7	98.0	98.0	97.7	87.9	87.9	87.6	98.8	94.5	94.5	92.5	92.5	92.2	92.8	91.6	92.5	23
24	6.0	6.3	5.7	5.7	5.7	5.7	5.7	5.7	5.7	6.0	2.1	1.2	1.5	1.5	1.5	1.2	1.8	1.5	1.8	0.3	0.3	0.0	0.3	***	98.0	98.0	97.7	87.9	87.9	87.6	99.1	94.5	94.5	92.5	92.5	92.2	92.8	91.6	92.5	24
25	5.4	5.7	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.4	0.0	0.9	0.9	1.2	1.2	1.2	1.5	1.2	1.5	1.8	1.8	2.1	2.1	2.1	***	100	99.7	89.0	89.0	88.2	97.7	93.9	93.9	92.5	92.5	92.2	92.2	91.3	92.5	25
26	5.6	5.6	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.3	0.0	0.7	0.7	0.9	0.9	0.9	1.2	0.9	1.2	1.4	1.4	1.7	2.1	2.1	0.0	***	99.8	91.0	91.1	90.5	97.9	93.9	93.9	92.8	92.8	92.5	92.3	91.3	92.2	26
27	5.8	5.8	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.6	0.2	1.0	1.0	1.2	1.2	1.2	1.4	1.2	1.4	1.7	1.7	1.9	2.4	2.4	0.3	0.2	***	90.8	90.9	90.3	97.7	93.6	93.6	92.5	92.5	92.3	92.1	91.0	91.9	27
28	10.7	10.7	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.4	10	10.3	10.5	9.9	10.4	10.1	10.1	9.9	10.1	10.2	10.1	10.4	11.4	11.4	10.7	9.9	10.1	***	100	98.1	90.0	89.9	89.6	90.8	90.8	90.5	90.0	87.6	88.2	28
29	10.8	10.8	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.5	10	10.3	10.5	10	10.5	10.2	10.2	10.0	10.2	10.2	10.2	10.5	11.4	11.4	10.7	10.0	10.2	0.0	***	98.1	90.0	89.9	89.6	90.7	90.7	90.4	90.0	87.6	88.2	29
30	11.0	11.0	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.7	10.5	10.3	10.5	9.9	10.4	10.1	10.1	9.9	10.1	10.2	10.1	10.4	11.8	11.8	11.8	10.4	10.7	1.9	1.9	***	90.0	88.4	88.1	89.8	89.8	89.6	89.6	86.4	87.0	30
31	5.8	5.8	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.6	2.2	1.4	1.7	1.7	1.7	1.4	1.9	1.7	1.9	1.2	1.2	0.9	1.2	0.9	2.4	2.1	2.4	10.4	10.5	10.4	***	93.6	93.6	92.1	92.1	91.8	92.1	91.0	91.6	31
32	5.4	5.7	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.4	6.1	6.4	6.4	6.1	6.1	6.4	5.7	6.1	6.4	5.4	5.4	5.7	5.7	5.7	6.1	6.1	6.4	9.4	9.4	10.4	6.7	***	99.4	91.6	91.6	91.6	91.9	91.3	91.6	32
33	6.1	6.4	5.7	5.7	5.7	5.7	5.7	5.7	5.7	6.1	6.1	6.4	6.4	6.1	6.1	6.4	5.7	6.1	6.4	5.4	5.4	5.7	5.7	5.7	6.1	6.1	6.4	9.8	9.8	10.8	6.7	0.6	***	91.6	91.6	91.6	91.9	91.3	91.6	33
34	8.1	8.1	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.9	7.7	8.0	8.2	8.1	8.1	7.9	8.4	8.1	8.4	7.4	7.4	7.6	7.9	7.9	7.9	7.6	7.9	9.9	10.0	11.0	8.4	9.0	9.0	0.0	***	100	99.8	98.1	98.8	99.7	34
35	9.1	9.1	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.9	7.7	8.0	8.2	9.1	9.1	8.9	9.3	9.1	9.3	7.4	7.4	7.6	7.9	7.9	7.9	7.6	7.9	9.9	10.0	11.0	8.4	9.0	9.0	0.0	0.0	***	99.8	98.1	98.8	100	35
36	8.4	8.4	7.9	7.9	7.9	7.9	7.9	7.9	7.9	8.1	8.0	8.2	8.5	8.4	8.4	8.1	8.7	8.4	8.7	7.7	7.6	7.9	8.2	8.2	8.2	7.9	8.1	10.1	10.2	11.2	8.7	9.3	9.3	0.2	0.2	***	97.9	98.6	99.4	36
37	9.2	9.2	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.9	8.2	8	8.2	8.1	8.1	7.9	8.4	8.1	8.4	7.4	7.4	7.6	7.6	7.6	8.2	8.1	8.4	10.7	10.8	11.2	8.4	8.6	8.6	1.9	1.9	2.1	***	97.1	97.7	37
38	9.9	10.2	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.9	9.2	9.2	9.5	9.5	9.5	9.2	9.9	9.5	9.9	8.6	8.6	8.9	8.9	8.9	9.2	9.2	9.5	11.8	11.8	13.1	9.5	9.3	9.3	1.2	1.2	1.5	3.0	***	98.8	38
39	8.9	9.2	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.9	7.9	8.2	8.6	8.6	8.6	8.2	8.9	8.6	8.9	7.6	7.6	7.9	7.9	7.9	7.9	7.9	8.2	10.7	10.7	12.1	8.6	9.0	9.0	0.0	0.0	0.3	2.1	1.2	***	39
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	

1: **KY825211** (KOR-N1507Y-6-7), 2: **KY825209** (KOR-H1506-14), 3: **KY825215** (KOR-N1507Y-14), 4: **KY825210** (KOR-N1507Y-6-6), 5: **KY825212** (KOR-N1507Y-6-9), 6: **KY825208** (KOR-H1407Y-53-1), 7: **KY825213** (KOR-N1507Y-6-11), 8: **KY825214** (KOR-N1507Y-6-13), 9: **KY825206** (KOR-H1406Y-1-8), 10: **KY825207** (KOR-H1406Y-2-2), 11: *O. capensis* Hanmya A, 12: *O. capensis* Hanmya B, 13: *O. capensis* OC-A, 14: O. *capensis* SC1, 15: O. capensis hawaii1, 16: *O. capensis* hawaii2, 17: *O. capensis* HawaiiC, 18: *O. capensis* TexasA, 19: *O. capensis* TexasB, 20: KU757079 (*O. capensis*, QGI, Brazil), 21: JQ824309 (*O. capensis* O185), 22: JQ824313 (*O. capensis* O361), 23: KX825955 (*O. capensis* O1077), 24: KX825956 (*O. capensis* O1086), 25: KX825960 (*O. capensis* O798), 26: JQ824318 (*O. capensis* O426), 27: JQ824319 (*O. capensis* O429), 28: JQ824301 (*O. capensis* O121), 29: JQ824302 (*O. capensis* O123), 30: JQ824303 (*O. capensis* O128), 31: JQ824305 (*O. capensis* O135), 32: KX825966 (*O. capensis* O915), 33: KX825967 (*O. capensis* O916), 34: JQ824297 (*O. capensis* O107), 35: KP776644 (*O. capensis* tube 5/tick 19), 36: JQ824316 (*O. capensis* O412), 37: JQ824323 (*O. capensis* O5), 38: KX825949 (*O. capensis* O874), 39: KX825970 (*O. capensis* O425).

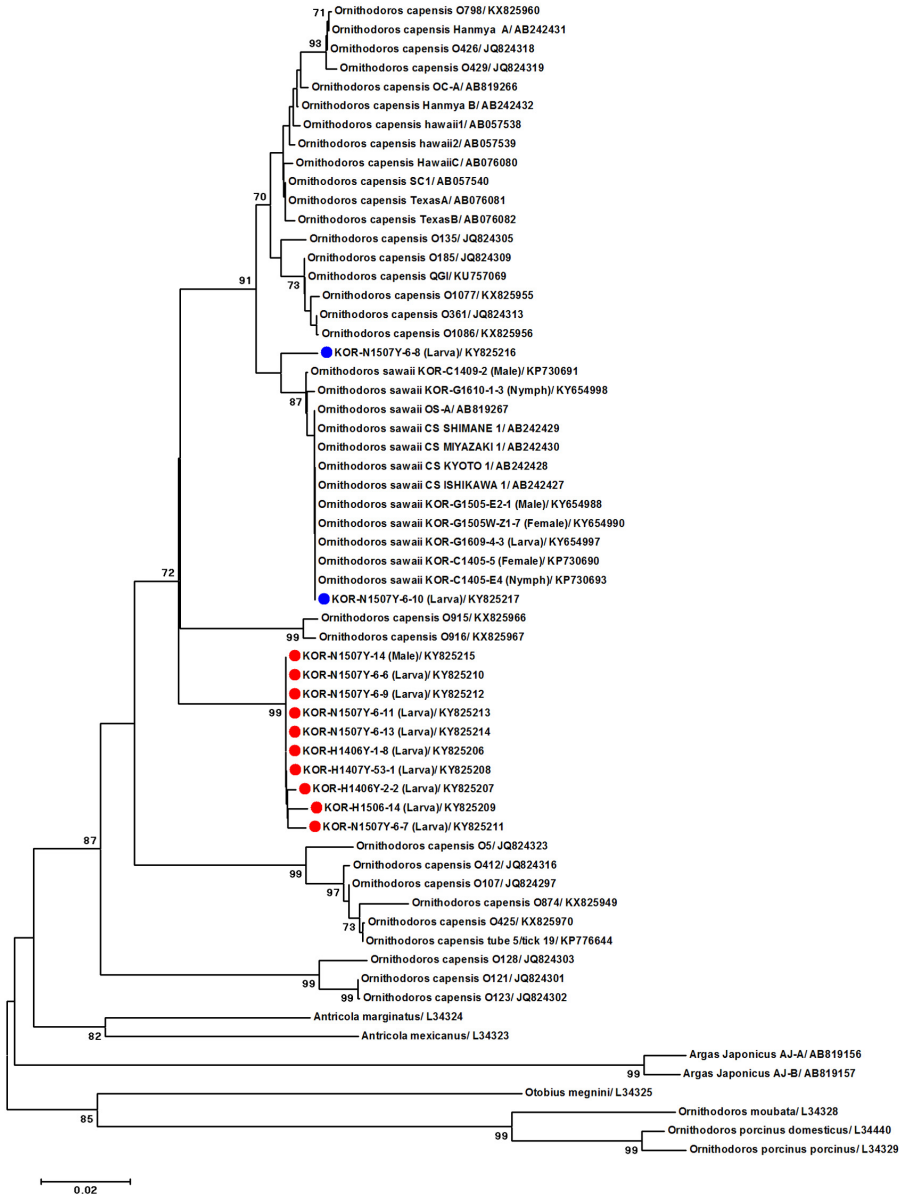

FIGURE 3. Phylogenetic analysis based on mt-rrs gene fragments of *Ornithodoros* spp. collected from nest soil/litter and recently dead nestlings of the Black-tailed Gull (*Larus crassirostris*). The phylogenetic tree was constructed based on NJ methods and bootstrap tests carried out according to the Kimura 2-parameter distances method. The percentage of replicate trees in which the associated taxa are clustered together in the bootstrap test (1,000 replicates) was 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). The *red* and *blue dots* respectively denote the *O. capensis* and *O. sawaii* ticks in this study. The label includes country (KOR, the Republic of Korea), collection site (N for Nan Island, H for Hong Island), collection year, month and collection source (*e.g.*, 1507Y for 2015 July from dead young bird, and 1507 for 2015 July from nest soil/litter sample), subject ID number for each Black-tailed Gull (*e.g.*, Y-6-10) and developmental stage of the ticks.

Acknowledgments

We thank Dr. Jong-Gil Park, Chief, Migratory Birds Research Center, National Park Research Institute, Korea National Park Service, on Hong (Gull) Island, Gyeongnam Province and Nan Island, Chungnam Province, Republic of Korea, for collecting recently dead nestlings and nest soil and litter during seabird conservation and breeding surveys. The seabird survey was supported by "Bird Survey and Research," Korea National Park Research Institute; the Armed Forces Health Surveillance Branch-Global Emerging Infections Surveillance and Response System (AFHSB-GEIS), Silver Spring, MD, USA; and the 65th Medical Brigade. The opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the US Departments of the Army or Defense.

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Submitted: 11 Sept. 2017; accepted by Lidia Chitimia-Dobler: 23 Sept. 2017; published: 24 Oct. 2017