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Authors: Kim, Heung-Chul, Park, Chang-Uk, Kim, Miran, Kim, Yang-Mo, Yeo, Juhan, et al.

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Ornithodoros sawaii (Acari: Ixodida: Argasidae) collected from nest soil/litter of *Synthliboramphus antiquus*, *Synthliboramphus wumizusume*, and nest soil/litter and nestlings of *Hydrobates monorhis* on Gugul and Sogugul Islands, Jeollanam Province, Republic of Korea

HEUNG-CHUL KIM[1]*, CHANG-UK PARK[2]*, MIRAN KIM[2], YANG-MO KIM[2], JUHAN YEO[2], YOUNG-SOO KWON[2], SEOK-MIN YUN[3], WON-JA LEE[3], SUNG-TAE CHONG[1], TERRY A. KLEIN[4] & RICHARD G. ROBBINS[5,6]

[1] *5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, USA*

[2] *Migratory Bird Research Center, Korea National Park Research Institute, Korea National Park Service, Jinmaeul-gil, Heuksan-myeon, Sinan county, Jeollanam Province 58863, Republic of Korea*

[3] *Division of Arboviruses, Center for Immunology and Pathology, National Institute of Health, Korea Centers for Diseases Control and Prevention, Cheongju-si, Chungbuk Province 28159, Republic of Korea*

[4] *Medical Department Activity-Korea/65th Medical Brigade, Unit 15281, APO AP 96205-5281, USA*

[5] *Walter Reed Biosystematics Unit, Department of Entomology, Smithsonian Institution, MSC, MRC 534, 4210 Silver Hill Road, Suitland, MD 20746-2863, USA*

[6] *Corresponding author. E-mail: richard.g.robbins@gmail.com*

** These authors contributed equally to this work.*

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 on Gugul and Sogugul Islands, two small, remote, uninhabited islands in southwestern Jeollanam Province, Republic of Korea (ROK) from 2015–2016. Ticks were collected from nest soil/litter of the Ancient Murrelet, *Synthliboramphus antiquus*, Japanese Murrelet, *Synthliboramphus wumizusume*, and Swinhoe's Storm Petrel, *Hydrobates monorhis*, using Tullgren funnels. A total of 100 *Ornithodoros sawaii* (25 females, 56 males, 17 nymphs, and 2 larvae) were collected from 2/33 (6.1%) Ancient Murrelet, 3/11 (27.3%) Japanese Murrelet, and 23/94 (24.5%) Swinhoe's Storm Petrel nest sites. In addition, 3 larvae were collected from Swinhoe's Storm Petrel nestlings. Ticks were identified morphologically and species determination was confirmed using polymerase chain reaction techniques. This is the first record of *O. sawaii* collected from nest soil/litter of the Japanese Murrelet during this bird's nesting season in the ROK.

Key words: Argasidae, *Ornithodoros sawaii*, *Synthliboramphus antiquus*, *S. wumizusume*, *Hydrobates monorhis*, Korea

Introduction

Worldwide, there has been a resurgence of interest in zoonotic tick-borne pathogens that affect the health of wild and domestic animals, birds, and humans (Heath & Hardwick 2011, Dantas-Torres *et al*. 2012). In the Republic of Korea (ROK), tick-borne pathogenic bacteria [*e.g.*, *Ehrlichia* and *Anaplasma* (Chae *et al*. 2003, Kang *et al*. 2013), *Bartonella* (Kim *et al*. 2005, Kang *et al*. 2013), *Borrelia* (Park *et al*. 1992, Kee *et al*. 1994), and *Rickettsia* spp. (Jang *et al*. 2004, Lee *et al*. 2013,

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Noh *et al*. 2017)] and viruses [*e.g.*, tick-borne encephalitis (Kim *et al*. 2009b, Ko *et al*. 2010, Yun *et al*. 2013) and severe fever with thrombocytopenia syndrome (SFTS) (Kim *et al*. 2013, Park *et al*. 2014, Yun *et al*. 2014, 2016)] manifest varying degrees of disease nationwide.

The role of migratory seabirds in the transport of exotic tick species to and from their summer breeding and winter feeding grounds has been poorly documented, in part because their breeding sites are often located on remote, uninhabited or sparsely populated islands that are usually under government protection and inaccessible to the general public. Annual migrations of migratory seabirds to and from their breeding and feeding grounds may result in the importation of exotic tick species that are transported over long distances and 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*. 2009a, Dietrich *et al*. 2011, Kang *et al*. 2013).

With exceptions, ticks belonging to the family Ixodidae (hard ticks) are more commonly found on so-called perching birds (order Passeriformes) (Morshed *et al*. 2005), while members of the family Argasidae (soft ticks) are usually collected from the nests and burrows of non-passeriform ground-dwelling/nesting birds, including migratory seabirds (Hoogstraal *et al*. 1976, Gomez-Diaz *et al*. 2012). The genus *Ornithodoros* Koch contains the largest number of described species of soft ticks (112/193) (Guglielmone *et al*. 2010), and these are frequently associated with various seabird hosts in both the Eastern and Western Hemispheres, including the Pacific Islands (Vander Velde & Vander Velde 2013), North and South America (Denmark & Clifford 1962, Jonkers *et al*. 1973, Keirans *et al*. 1992), New Zealand (Heath 1987, 2006, Murray *et al*. 1990), Australia (Murray *et al*. 1990), Antarctica (Murray *et al*. 1990), Japan (Asanuma *et al*. 1955, Asanuma & Fukuda 1957, Asanuma 1960, 1965, Yamaguti *et al*. 1971, Kawabata *et al*. 2006), Russia (Akhundova *et al*. 1971), and Africa (Hoogstraal *et al*. 1976).

This report describes collections of *Ornithodoros sawaii* Kitaoka and Suzuki, 1973 from nest soil/litter of the Ancient Murrelet, *Synthliboramphus antiquus* (Gmelin, 1789), the Japanese Murrelet, *Synthliboramphus wumizusume* (Temminck, 1835), and both nest soil/litter and nestlings of Swinhoe's Storm Petrel, *Hydrobates monorhis* (Swinhoe, 1867), in the ROK. The collections from *S. wumizusume* nest soil/litter represent a probable new host record for *O. sawaii*.

Materials and methods

Survey area

The Migratory Birds Research 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 the Ancient Murrelet, Japanese Murrelet, and Swinhoe's Storm Petrel on Gugul and Sogugul Islands (34º04' N, 125º07' E), two small, remote, uninhabited islands that are protected National Monuments (No. 341, 13 August 1984); the islands have a total land area of 13.52 ha and 1.84 ha, respectively, and are located in Gageodo-ri, Heuksan-myeon, Sinan-gun, Jeollanam Province, 136 km west of Mokpo, a major mainland port city (Fig. 1).

Tick collections

Tick surveys were conducted on Gugul and Sogugul Islands during the Ancient Murrelet and Japanese Murrelet breeding season (March–early May), and Swinhoe's Storm Petrel breeding season (June–October). Although the Murrelet and Storm Petrel breeding seasons do not overlap, they occupy the same nest sites. Using a small scoop, 50–100 g of soil and litter were collected from nests occupied by Ancient Murrelets and Japanese Murrelets on 22 April, 21 May 2015, and 7 May 2016, and from nests occupied by Swinhoe's Storm Petrel on 11 August, 12 September, and 15 October

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2016 (Fig. 2). Soil and litter samples were placed in plastic Ziploc® bags (25 x 28 cm) that were then transferred to a Styrofoam cooler and transported 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. After 24 hours of exposure, the material in the collection bottle was examined for arthropods. Ticks were removed and placed individually in cryovials containing 80% ethanol, labeled with a unique nest identification number, and later microscopically examined to determine developmental stage and genus using taxonomic identification keys (Kohls 1957, Yamaguti *et al*. 1971, Kitaoka & Suzuki 1973). Breeding season Swinhoe's Storm Petrel nestlings were carefully removed from their nests, 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, carefully removed with a forceps to prevent injury to the bird, were placed individually in 2 ml cryovials containing 70% EtOH and then labeled with a unique nest identification number, as described by Kim *et al*. (2016a). Two females and four males (2015 samples) and one female, three males, three nymphs, and one larva (2016 samples) were submitted to the Korea National Institute of Health for specific identification by polymerase chain reaction (PCR). 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.

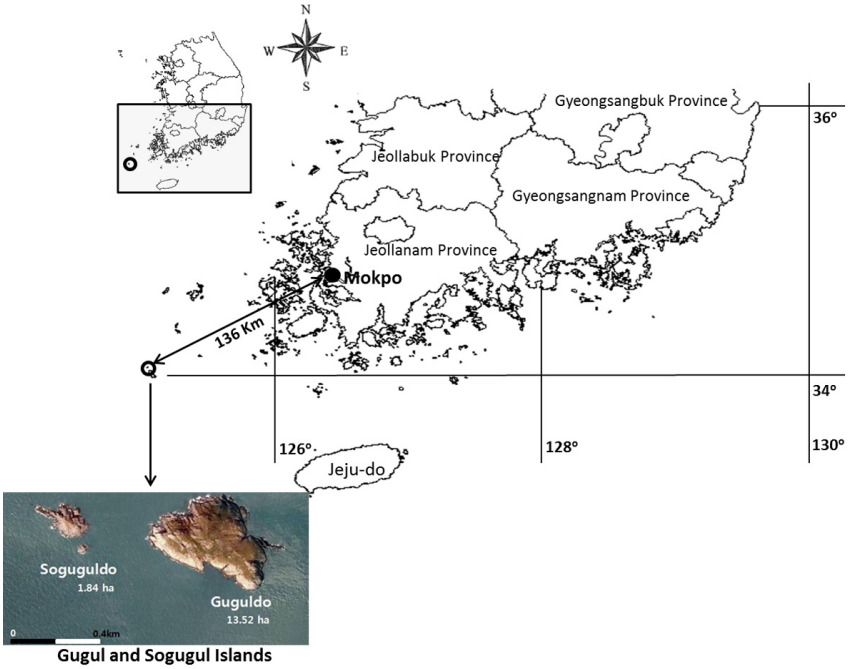


FIGURE 1. Collection sites (○) of *Ornithodoros sawaii* from the Ancient Murrelet (*Synthliboramphus antiquus*), Japanese Murrelet (*Synthliboramphus wumizusume*) nest soil/litter, and Swinhoe's Storm Petrel (*Hydrobates monorhis*) nest soil/litter and nestlings on Gugul and Sogugul Islands, Jeollanam Province, Republic of Korea [Mokpo (●), mainland port city] (map image from *http://map.daum.net*).

PCR and sequencing analysis

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 previously described by Black and Piesman (1994) and Ushijima

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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 then 30 sec at 72°C, with a final extension step of 5 min at 72°C. PCR products were then purified using a QIAquick® Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and sequenced after cloning into pCR®4-TOPO® plasmid (Invitrogen, Carlsbad, USA), using ABI Prism BigDye™ Terminator v3.1 Cycle Sequencing Kits and an ABI 3730xl sequencer (Applied Biosystems®, Foster City, 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. The mt-rrs sequences of ticks identified in this study have been deposited in GenBank under accession numbers KY654988-KY655001.



FIGURE 2. Three avian hosts of *Ornithodoros sawaii*: (A) Japanese Murrelet (*Synthliboramphus wumizusume*), (B) Ancient Murrelet (*Synthliboramphus antiquus*), and (C) Swinhoe's Storm Petrel (*Hydrobates monorhis*) (photos by Chang-Uk Park, co-author).

Phylogenetic analysis

Sequence data were analyzed using MEGA 6.0 software (http://www.megasoftware.net) (Tamura *et al*. 2013). The amplified partial mt-rrs segment sequences for *Ornithodoros* spp. collected from the nesting sites were aligned and compared with previously published *Ornithodoros* spp., a process facilitated using the CLUSTALW method (Lasergene program version 5, DNASTAR, Inc.). 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 also performed using the same values. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (pairwise deletion).

Results and Discussion

A total of 100 *Ornithodoros sawaii* (25 females, 56 males, 17 nymphs, and 2 larvae) were collected from 2/33 (6.1%) Ancient Murrelet, 3/11 (27.3%) Japanese Murrelet, and 23/94 (24.5%) Swinhoe's Storm Petrel nest sites. In addition, 3 larvae were collected from Swinhoe's Storm Petrel nestlings (Table 1).

Because the morphological identification of *Ornithodoros* spp. nymphs and adults is often unreliable, specimens collected on Gugul and Sogugul Islands 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 with *O. sawaii* in Japan and other countries (Fig. 3). Sequence data for

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O. sawaii collected from nest soil and litter on Gugul Island during the breeding season of the Ancient Murrelet [2 males (KOR-G1505-E2-1 and KOR-G1505-E2-2)] , the Japanese Murrelet [2 females (KOR-G1505W-Z1-7 and KOR-G1505W-Z1-8) and 2 males (KOR-G1505W-Z3-3 and KOR-G1505W-Z3-4)], and Swinhoe's Storm Petrel [1 female (KOR-G1608-40-1), 2 males (KOR-G1608-2-2 and KOR-G1608-2-3), 2 nymphs (KOR-G1610-1-3 and KOR-G1610-1-4), and 1 larva (KOR-G1609-4-3)] aligned closely with *O. sawaii* sequence data submitted to GenBank (Fig. 3). Similarly, sequence data for *O. sawaii* collected from nest soil and litter on Sogugul Island during Swinhoe's Storm Petrel breeding season [1 male (KOR-S1610-13-1), and 1 nymph (KOR-S1610-2-2)] aligned closely with *O. sawaii* in the ROK (Fig. 3). Comparatively, there were just 0-2 base differences, with 99.5–100% nucleotide sequence identities between *O. sawaii* collected from Gugul and Sogugul Islands and other collection sites in Japan (Miyazaki, Shimane, Kyoto, Kutsujima, and Ishikawa Islands) (Kawabata *et al*. 2006, Takano *et al*. 2014). In Japan, *O. sawaii* has been recorded from two migratory seabirds, the Streaked Shearwater, *Calonectris leucomelas* (Temminck, 1836), and Swinhoe's Storm Petrel (Kitaoka and Suzuki 1973, 1974, Kawabata *et al*. 2006, Takano *et al*. 2014). Our survey showed that *O. sawaii* is associated with nests of the Ancient Murrelet and Japanese Murrelet, as well as Swinhoe's Storm Petrel. This is the first record of *O. sawaii* collected from nest soil/litter of the Japanese Murrelet.

TABLE 1. Number and stage of *Ornithodoros sawaii* collected from nest soil/litter of Ancient Murrelet (*Synthliboramphus antiquus*), Japanese Murrelet (*Synthliboramphus wumizusume*), and nest soil/litter and nestlings of Swinhoe's Storm Petrel (*Hydrobates monorhis*) on Gugul and Sogugul Islands, Jeollanam Province, Republic of Korea, 2015–2016.

Year	Collection Island	Host bird	Month	Infested/ tested soil and litter	Infestation rates (%)	*Ornithodoros sawaii*					
						F	M	N	L	L*	Total
2015	Gugul Island	Ancient Murrelet (*Synthliboramphus antiquus*)	APR	0/5	0	0	0	0	0		0
			MAY	2/25	8.0	0	3	2	0		5
		Japanese Murrelet (*Synthliboramphus wumizusume*)	APR	0/1	0	0	0	0	0		0
			MAY	3/10	30.0	13	19	3	0		35
2016	Gugul Island	Ancient Murrelet (*Synthliboramphus antiquus*)	MAY	0/3	0	0	0	0	0		0
		Swinhoe's Storm Petrel (*Hydrobates monorhis*)	AUG	12/46	26.1	8	29	1	0		38
			SEP	2/12	16.7	1	0	1	2	1	5
			OCT	3/22	13.6	1	0	5	0	2	8
	Sogugul Island	Swinhoe's Storm Petrel (*Hydrobates monorhis*)	OCT	6/14	42.9	2	5	5	0		12
	Total			28/138	20.3	25	56	17	2	3	103

*Larvae collected from nestlings of Swinhoe's storm petrel, *Hydrobates monorhis*.

Japanese Murrelets are medium-sized blackish seabirds (Fig. 2A) that are endemic to regions of warm ocean currents near central and southern Japan, Korea, and Russia. Following the breeding season, these birds move to the southeastern coastal margins of Hokkaido, Honshu and Kyushu, where they overwinter. Japanese Murrelet populations are declining in many localities, particularly the Izu Islands (BirdLife International 2016a). 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 (Fig. 2B). Their range extends from the Yellow Sea (islands off China and Korea),

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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 2016b). On Gugul and Sogugul Islands, Japanese and Ancient Murrelets arrive concurrently at their feeding/nesting grounds one month before egg-laying from March–early May, and after rearing their chicks, they move to their coastal summer feeding grounds. Swinhoe's Storm Petrels migrate to Gugul and Sogugul Islands and other nearby islands from early June–October and occupy the same nest sites as the Ancient and Japanese Murrelets (Lee *et al*. 2009, Park & Takeshi 2011). Swinhoe's Storm Petrels are small seabirds that range from southern Indonesia and the Indian Ocean to northeastern Asia [Russia (south of Vladivostok), Japan, Korea, and Taiwan] (Lee *et al*. 2009, BirdLife International 2016c) (Fig. 2C).

Ornithodoros sawaii is closely related to *O. capensis*, a species with a broad distribution that includes North Atlantic, Pacific, and Indian Ocean islands and coastal areas of South Africa, New Zealand, and southern Australia (Kohls 1957, Heath 1987). *Ornithodoros sawaii* was first described in 1973, but in Japan it is known only from nests of the Streaked Shearwater and Swinhoe's Storm Petrel located on the Japanese islands of Hanmya, Miyazaki, Shimane, Maizuru, Kyoto, and Ishikawa (Kitaoka & Suzuki 1973, 1974, Kawabata *et al*. 2006, Takano *et al*. 2014). In Korea, *O. sawaii* larvae were recovered from the wing and abdomen of Swinhoe's Storm Petrel on Sogugul and Gaerin Islands (Kim *et al*. 2016a), and nymphs and adults were collected from nest soil/litter during the nesting season of the Ancient Murrelet and Swinhoe's Storm Petrel on Chilbal Island (Kim *et al*. 2015, 2016b).

Rickettsia and *Borrelia* spp. have been detected in *O. sawaii* collected from the Streaked Shearwater and Swinhoe's Storm Petrel in Japan (Kawabata *et al*. 2006, Takano *et al*. 2009). Recently, Kang *et al*. (2013) reported *Anaplasma*, *Bartonella*, and *Borrelia* spp. from *Ixodes* spp. that were collected from migratory birds during 2008–2009 on Hong Island (34º41' N, 125º11' E), located near Gugul and Sogugul Islands. Such results do not necessarily mean that migratory birds are reservoirs of these pathogens or are 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 define the geographical distribution, host range, and specific pathogens associated with ticks collected from nesting sites and from resident and migratory seabirds inhabiting islands and coastal areas of the ROK.

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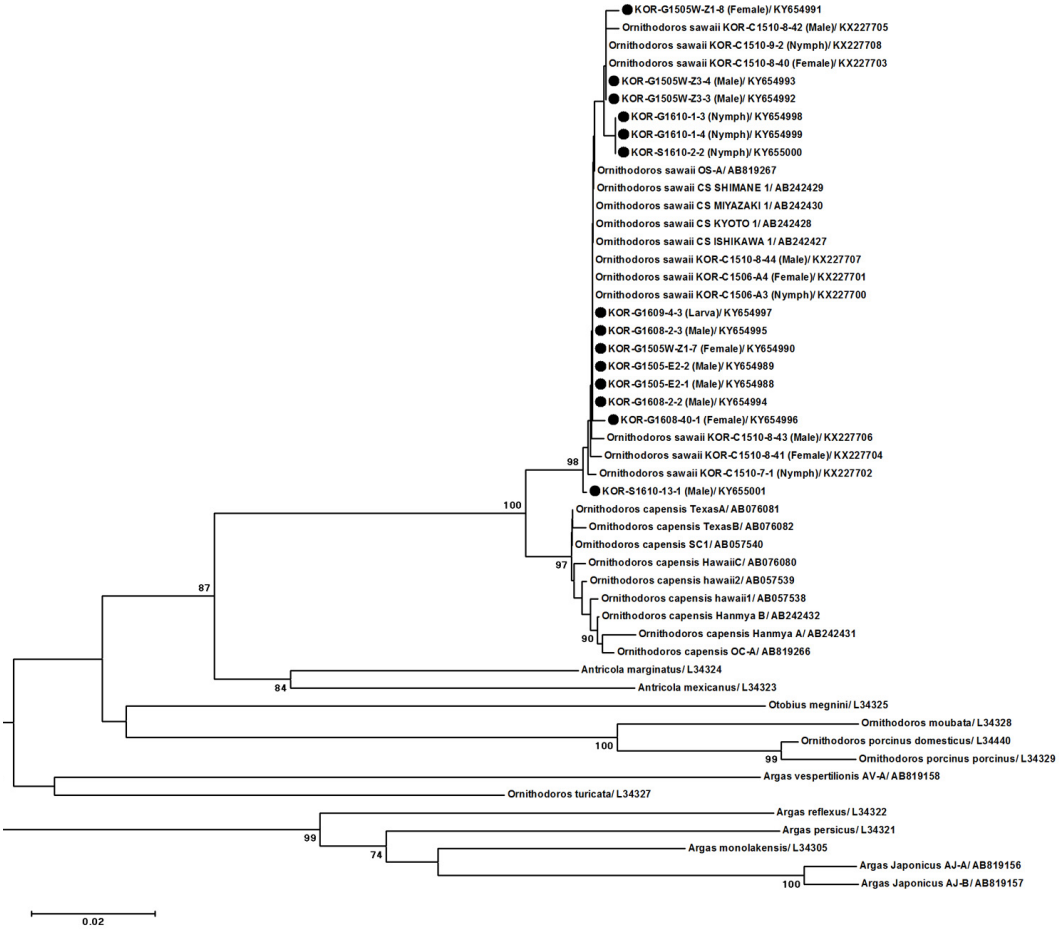


FIGURE 3. Phylogenetic analysis based on mt-rrs of *Ornithodoros sawaii* collected from the Ancient Murrelet (*Synthliboramphus antiquus*) and Japanese Murrelet (*Synthliboramphus wumizusume*) nest soil/litter, and Swinhoe's Storm Petrel (*Hydrobates monorhis*) nest soil/litter and nestlings. The phylogenetic trees were 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).

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