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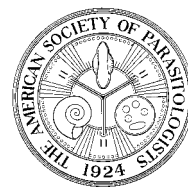
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FIRST REPORT OF NEWLY IDENTIFIED *ORNITHODOROS* SPECIES IN THE REPUBLIC OF KOREA

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KEY WORDS

ABSTRACT

Ornithodoros spp.
Larus crassirostris
Family Argasidae
New *Ornithodoros* sp.

Ticks and tick-borne diseases are important issues worldwide because of their effects on animal and human health. The genus *Ornithodoros*, which is included in the family Argasidae, is typically associated with wild animals, including seabirds. In this study, samples from the nests of seabirds and surrounding soil were collected to investigate *Ornithodoros* spp. from 9 uninhabited islands in the western, eastern, and southern parts of Korea from April 2017 to October 2018. The islands are known as the breeding places of migratory and resident birds. Ticks were collected from soil and nest material of seabirds using a Tullgren funnel and identified using *16S rRNA* and the cytochrome c oxidase 1 gene (*COI*), and host animals of soft ticks were identified using the mitochondrial DNA cytochrome *b* gene by a polymerase chain reaction. In the sequence identity of the *16S rRNA* gene fragment of *Ornithodoros* sp., *Ornithodoros sawaii* was identified as the closest homologous sequence, and the new *Ornithodoros* sp. was newly identified. We found that the newly identified *Ornithodoros* sp. in the Republic of Korea was located in uninhabited islands used as breeding places by the black-tailed gull, *Larus crassirostris*.

Ticks and tick-borne diseases are a major health threat to both humans and animals and cause significant economic losses worldwide. The Family Argasidae, including the genera *Argas*, *Ornithodoros*, *Nothoaspis*, and *Otobius*, does not have a scutum and is mainly distributed in tropical and subtropical regions in seabird colonies. *Ornithodoros* spp., which are part of the family Argasidae, are ectoparasites, and their hosts are mainly migratory birds and mammals; their environmental distribution may be affected by bird migration (Kawabata et al., 2006), or they can be spread by bats (*Anoura caudifer*) or members of the family Suidae such as warthogs (*Phacochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*) (Anderson et al., 1998). *Ornithodoros* spp. can transmit various pathogens, including *Coxiella* sp. (Reeves, 2008), *Rickettsia* spp. (Reeves et al., 2006), and *Borrelia lonestari* from seabirds, African swine fever virus (ASFV) from members of Suidae, and others. Among the *Ornithodoros* genera, the *Ornithodoros capensis* complex contains a large number of described Argasidae species and is the most widespread species around the equator, affecting seabird populations along the coasts of the Pacific, Atlantic, and Indian Oceans (Guglielmone et al., 2010; Dietrich et al., 2014). *Ornithodoros capensis* was first

reported in 1901 by Neumann and has been reported in numerous avian nests in Africa (Gómez-Díaz et al., 2012), Europe (Dupraz et al., 2016), Oceania (Ramsay, 1968; Humphery-Smith and Moorhouse, 1981), North America (Eggert and Jodice, 2008), and Asia (Kawabata et al., 2006). *Ornithodoros sawaii*, which is phylogenetically closely related to *O. capensis*, was first identified in Japan (Kitaoka and Suzuki, 1973) and subsequently reported only in Japan (Kawabata et al., 2006; Takano et al., 2009) and Korea in the nest soil and litter of *Synthliboramphus antiquus*, *Hydrobates monorhis*, and *Larus crassirostris* (Kim et al., 2015, 2016, 2017).

Various pathogens, including *Rickettsia* spp., *Coxiella* sp., *Borrelia* spp., and the Johnston Atoll and Abal viruses, have been detected in *O. capensis* globally distributed around the equator line (Dietrich et al., 2011). In Japan, *Rickettsia* was detected in *O. capensis* captured from the black-footed albatross (Kawabata et al., 2006). Additionally, a few pathogens, such as *Rickettsia* sp. and *Borrelia* sp., were detected in *O. sawaii* collected from the migratory seabirds Swinhoe's storm petrel and streaked shearwater only in Japan (Kitaoka and Suzuki, 1973; Kawabata et al., 2006; Takano et al., 2009). This study was conducted to determine



the distribution and identity of soft ticks collected from the soil of nests of migratory birds that are potential hosts in Southeast Asia.

In this study, we discovered a new *Ornithodoros* species from the nests and soil material of *L. crassirostris* and detected evidence of soft tick habitation in the uninhabited (by humans) islands of the Republic of Korea.

MATERIALS AND METHODS

Tick collection

The sites of tick collection were selected based on the habitat of migratory birds in uninhabited islands (Nan-do, 36°39'34.01"N, 125°49'24.58"E, in Chungcheongnam-do province; Chilsan-do, 35°19'20.20"N, 126°16'37.01"E; Chilbal-do, 34°47'16.46"N, 125°47'18.99"E, and Gugul-do, 34°6'59.44"N, 125°5'7.39"E; Gaerin-do, 34°6'57.81"N, 125°5'37.80"E; Sogukhol-do, 34°7'4.60"N, 125°4'40.53"E in Jeollanam-do province; Sasu-do, 33°55'15.08"N, 126°38'16.85"E, in Jeju-do province; Hong-do, 34°32'14.35"N, 128°43'58.14"E, in Gyeongsangnam-do province; and Dok-do, 37°14'24.38"N, 131°52'13.75"E in Gyeongsangbuk-do) from July to October 2017 and April to October 2018 (Fig. 1). Among these islands, Chilbal-do, Nan-do, Hong-do, and Chilsan-do are known as breeding sites of *L. crassirostris* (Fig. 2a, b), and Chilbal-do, Chilsan-do, Gaerin-do, Sogukhol-do, and Gugul-do are known as breeding sites of *H. monorhis* (Fig. 2c, d). Samples were collected in zipper bags from nest litter and soil material of migratory birds on the islands. The collected nests with soil were moved to the laboratory. Soil and litter samples from each nest site were placed separately into Tullgren funnels using a scoop. A Tullgren funnel with an incandescent heating light bulb was set up with an Erlenmeyer flask below containing 70% ethanol (Fig. 2e, f) (Kim et al., 2015). After 24 hr of radiating heat, the soft ticks entered the flask. These ticks were removed from the flask using a filter and forceps, placed in 2-ml tubes, and labeled with their collection data. Subsequently, all ticks were identified to determine their genus levels and developmental stages using taxonomic identification keys based on microscopic examination (Yamaguti et al., 1971). After identification, the ticks were placed individually into 1.5-ml tubes containing 70% ethanol.

Scanning electron microscopy (SEM)

For morphological analysis, collected tick specimens were placed in Karnovsky's fixative for 4 hr for primary fixation. The ticks were washed with 0.05-M sodium cacodylate buffer 3 times for 10 min each time. After fixation, the ticks were treated with 9.1-M cacodylate buffer containing 2% osmium tetroxide for 2 hr and then dehydrated by immersion in increasing concentrations of ethanol from 30% to 100%. Specimens were dried using a Critical Point Dryer (Leica, Wetzlar, Germany) and examined by field-emission SEM (Carl Zeiss, Oberkochen, Germany) (Fig. 3).

Molecular identification of tick species and host gene

DNA extraction: To perform molecular identification of tick species, the ticks were homogenized using a Beadbeater Tissue-Lyser II (Qiagen, Hilden, Germany) according to the manufacturer's instructions with 200 µl of lysis buffer, 40 µl of proteinase K, and 2-mm-diameter stainless beads at 30 Hz/sec for 7 min, followed by lysis at 56°C for 60 min. Genomic DNA was extracted

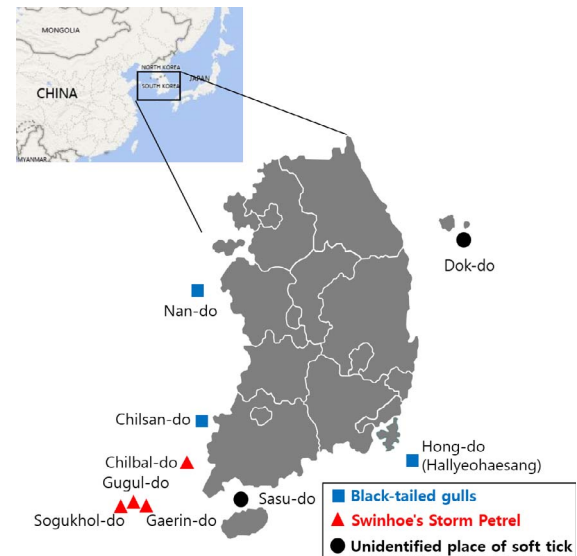


Figure 1. Samples were collected from 9 uninhabited islands that comprise the breeding grounds of soft ticks in Korea. Square icons indicate uninhabited islands of *Larus crassirostris* breeding place, triangle icons indicate uninhabited islands of *Hydrobates monorhis* breeding place, and circle icons indicate an unidentified island. Color version is available online.

according to the protocol of the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) and stored at -20°C until use.

Polymerase chain reaction (PCR) amplification: PCR was performed using primer sets based on the mitochondrial 16S *rRNA* gene (mt-rrs) fragment (Black and Piesman, 1994) and using 3 primer sets designed in this study (GenBank accession number KJ133587) based on the mitochondrial 16S *rRNA* full-length gene (Fig. 4). For precise molecular identification, mitochondrial gene fragments of cytochrome c oxidase 1 (*COI*) were also amplified by PCR (Table I).

To analyze the host animals of soft ticks, the mitochondrial DNA cytochrome *b* gene was amplified by conventional PCR (Kocher et al., 1989). PCR amplification of the host animal DNA was conducted using primer sets L14841: 5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3', and H15149: 5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3'. PCR assays were performed in a 50-µl reaction mixture with Takara Taq DNA polymerase (Takara, Shiga, Japan) at 94°C for 1 min, followed by 30 cycles for 45 sec at 94°C, 45 sec at 50°C, and 45 sec at 72°C, and a final extension step of 5 min at 72°C.

Cloning, sequencing, and phylogenetic analysis

The amplified PCR products were purified using a Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. After purification, the PCR products were cloned into the pGEM®-T Easy Vector Systems (Promega, Madison, Wisconsin), followed by transformation into *Escherichia coli* DH5a cells, and then the cells were plated onto LB agar containing 100 µg/ml of ampicillin. Recombinant clones were selected by blue-white screening. Plasmid DNA for sequencing was purified using the

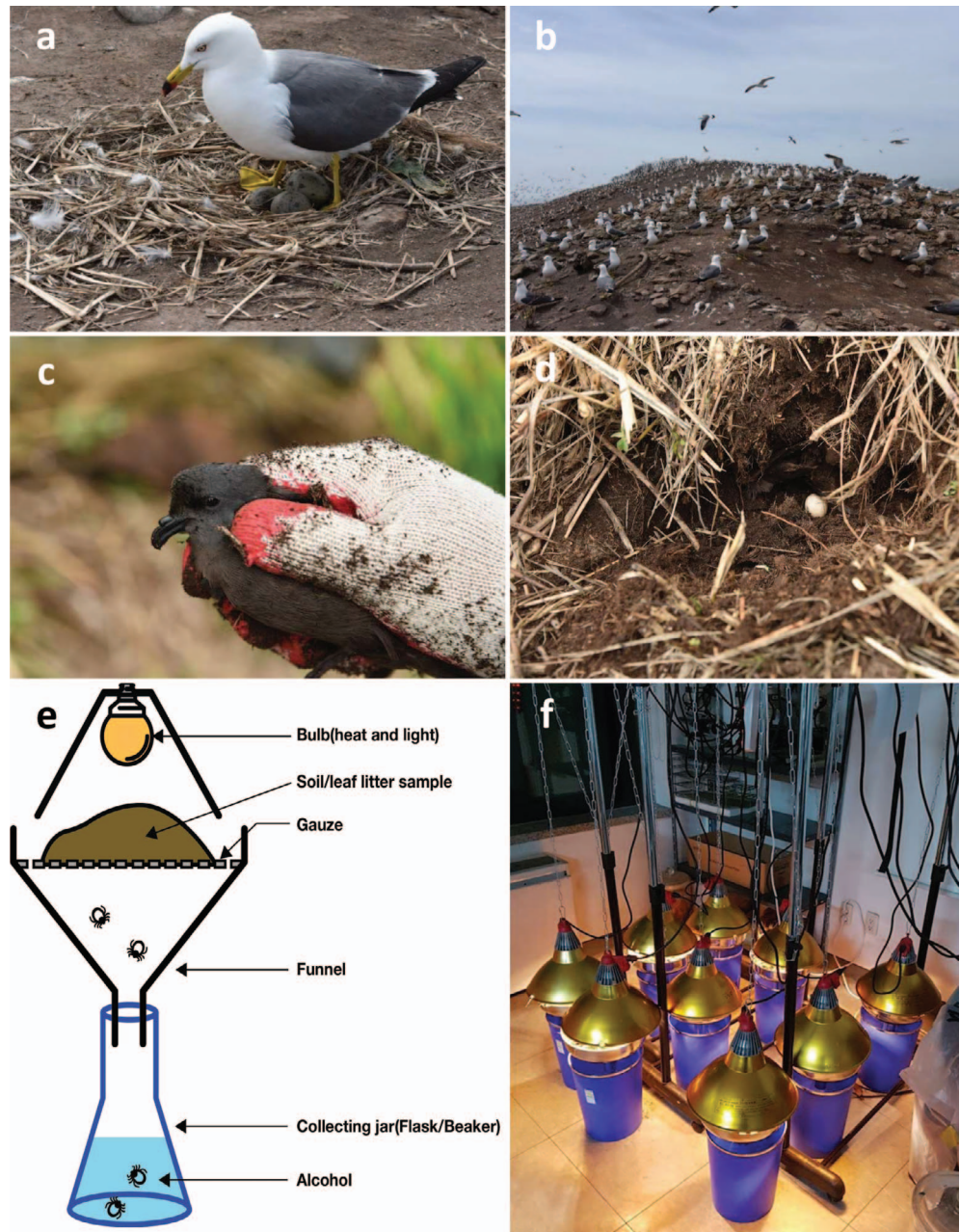


Figure 2. Investigation of (a) *Larus crassirostris* and their (b) nests in Chilsan-do and (c) *Hydrobates monorhis* and their (d) nests in Chilbal-do. (e, f) Tullgren funnel for tick collection. *Larus crassirostris* and *Hydrobates monorhis* are the dominant birds in Chilsan-do and Chilbal-do, respectively. Several *Calonectris leucomelas* individuals were detected in Chilbal-do, and *Egretta eulophotes* and *Haematopus ostralegus* individuals were detected in Chilsan-do. Sixty nest litter and soil samples were collected from Chilsan-do and Chilbal-do. Color version is available online.

MG™ Plasmid SV Miniprep kits (Macrogen, Seoul, Korea). Purified recombinant plasmid DNA was sequenced using a T7 and SP6 promoter primer set by dideoxy termination with an automatic sequencer (ABI 3730xl capillary DNA sequencer, Applied Biosystems, Foster City, California). The obtained sequences were evaluated with Chromas software (Ver. 2.6.2, <http://technelysium.com.au/wp/chromas/>) and aligned by Clustal X2 (Ver 2.0, <http://www.clustal.org/>). Phylogenetic trees were constructed using the maximum-likelihood method in the Kimura 2-parameter model with the MEGA 7 program (1,000 bootstrap replicates) (Kimura, 1980; Kumar et al., 2016). The nucleotide sequences generated were deposited into GenBank under accession numbers MK389598–MK389641, MK605995–MK606024,

MK606031–MK606060, and MK613790–MK613833 for the *16S rRNA* gene and MK836055–MK836059 and MT040624 for *COI*.

RESULTS

In this study, tick surveillance was conducted on Korean islands during the breeding season of migratory birds, including *L. crassirostris* and *H. monorhis*, from July to October 2017 and from April to October 2018. Nests and soil material of migratory birds were collected to capture argasid ticks from uninhabited islands. In total, 77 ticks were collected from 410 nests with soil: 30 ticks from Chilsan-do, 19 ticks from Chilbal-do, 8 ticks from

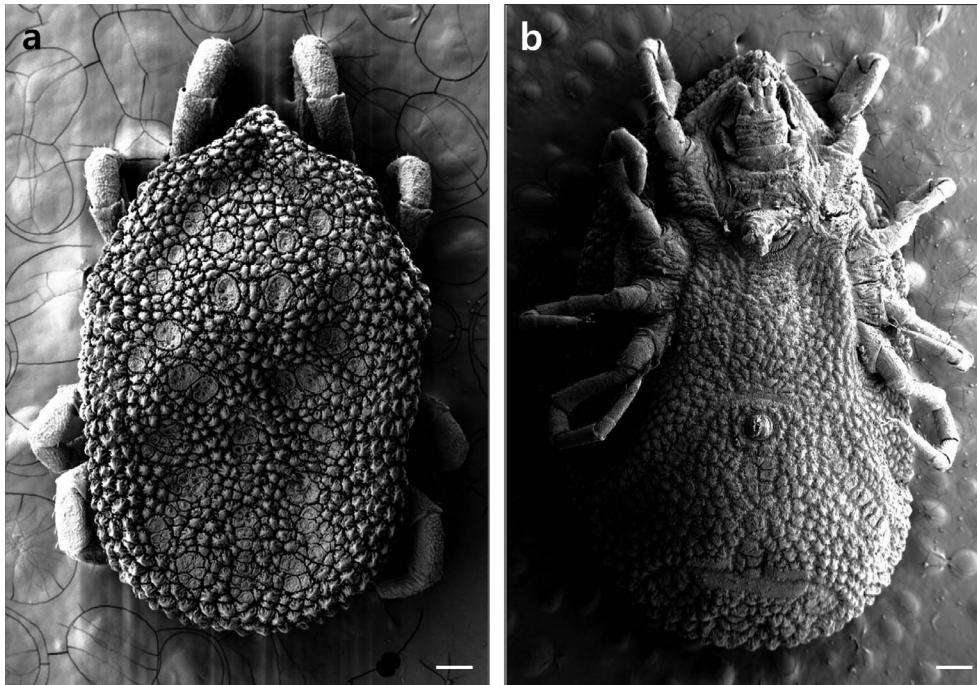


Figure 3. Images of *Ornithodoros* spp. ticks from Chilbal-island acquired by SEM. (a) Dorsal view. (b) Ventral view. Bars = 100 μ m.

Gugul-do, 2 ticks from Sogukhol-do, 16 ticks from Gaerin-do, 1 tick from Nan-do, and 1 tick from Hong-do (Table II).

To morphologically identify the argasid ticks, we evaluated a total of 77 Argasidae ticks by optical microscopy; however, significant differences between species were not observed in the images. Therefore, three randomly selected soft tick specimens were used for SEM analysis. SEM images of the argasid tick specimens were acquired, but no significant differences were observed between species. Thus, PCR was conducted using 74 argasid ticks, and phylogenetic analysis was conducted for the mitochondrial gene *16S rRNA* by PCR. To determine the host animals of ticks, mitochondrial DNA cytochrome *b* gene sequences were amplified from the tick samples (Suppl. Data, Fig. S1).

Phylogenetic analysis showed that the Argasidae ticks detected in this study were grouped in 2 clades, which showed 89.1% identity to *O. sawaii* (GR4) and a new *Ornithodoros* sp. (CS1) in the *16S rRNA* full gene (Fig. 5). Table III shows 88.4 to 89.0% identity in the *16S rRNA* full gene when comparing *Ornithodoros* sp. (domestic isolate, MK613790), *O. sawaii* (domestic isolate, MK606044), *O. capensis* (South Africa, KR907245), *O. capensis* (South Africa, KJ133586), and *O. capensis* (Japan, AB075953) sequences. The sequence identity matrix in Table IV was generated from the 74.4 to 94.4% range of identities between the sequences of the new *Ornithodoros* sp. and *Ornithodoros* spp. from the GenBank database, where only more than 80.0% identities were selected. Table IV shows 80.1 to 94.4% identity in partial *16S rRNA* when comparing *Ornithodoros* sp. (domestic

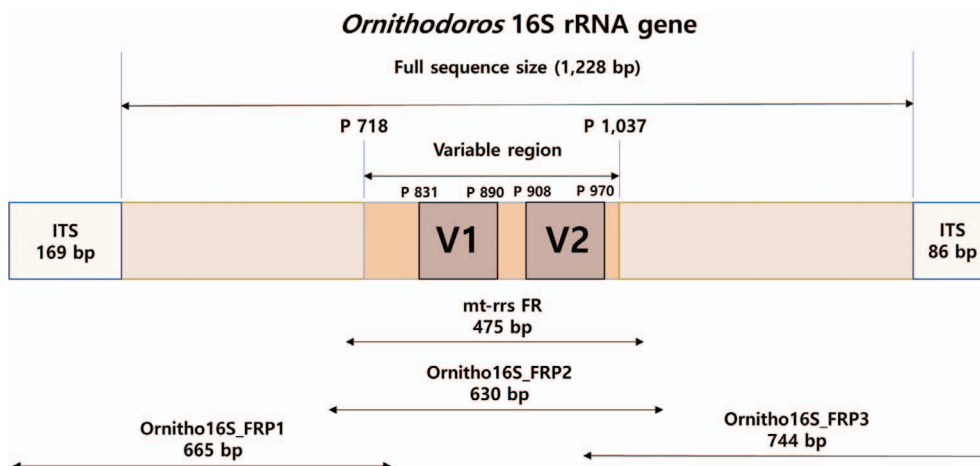


Figure 4. Schematic diagram of gene and primers to identify the *16S rRNA* full sequence of the *Ornithodoros* species (1,228 bp). P: position. Color version is available online.

Table I. Polymerase chain reaction (PCR) primers and conditions for identifying partial and full gene of *16S rRNA* and cytochrome c oxidase 1 gene (*COI*).

Target genes	PCR primers and conditions	Primer sequences (5'–3')			Cycles	Size of PCR products	References
		Denaturation (C/sec)	Annealing (C/sec)	Extension (C/sec)			
<i>16S rRNA</i> fragment	mt-rrs1	CTG CTC AAT GAT TTT TTA AAT TGC TGT GG				475 bp	Black and Piesman (1994)
	mt-rrs2	CCG GTC TGA ACT CAG ATC AAG TA					
<i>16S rRNA</i> + <i>ITS</i> gene	Ornitho16S_FP1	TTA TTA GGC CCC TTC CGA AT			30	Total	Designed in this study
	Ornitho16S_RP1	TGG GAC AAG AAG ACC CTA TGA				1,551 bp	
	Ornitho16S_FP2	AGG GTC TTC TTG TCC CAA AA				- <i>16S rRNA</i> gene	
	Ornitho16S_RP2	TTC CCG AAA AAT TTT GAG CTA C				(1,229	
	Ornitho16S_FP3	AAG CAT CAT TGA GCA GGT TTT				–1,233 bp)	
	Ornitho16S_RP3	TGT ACC GGA AGG TGC CTT TA					
	Conditions	94/10	55/30	72/30			
<i>COI</i>	TickCO1 F	ATT TTA CCG CGA TGA HTW TTY T			35	676 bp	Binetruy et al. (2019)
	TickCO1 R	CWG GRT GRC CAA ARA ATC AAA ATA					
	Conditions	94/30	50/30	72/60			

isolate, MK613790), *O. sawaii* (domestic isolate, MK606044), and *Ornithodoros* spp. from NCBI. The identity between the new *Ornithodoros* sp. and *O. capensis* ranged from 90.0 to 94.4% (Table V). All sequences of *O. capensis* were analyzed, and the overlapping data were deleted. Thirty *O. sawaii* (40.5%) samples were collected from the *H. monorhis* nests with soil from Chilbal-do, Chilsan-do, Gaerin-do, Sogukhol-do, and Gugul-do, and 44 new *Ornithodoros* sp. (59.5%) samples were collected from *L. crassirostris* nests with soil from Chilbal-do, Nan-do, Hong-do, and Chilsan-do. To evaluate the genetic relationship between *O. sawaii* and the new *Ornithodoros* sp., both *16S rRNA* gene nucleotide sequences were independently analyzed with other reference sequences available in the GenBank database. Additionally, randomly selected soft tick specimens were molecularly analyzed for *COI* by PCR for more precise identification.

Phylogenetic analysis of *COI* revealed 2 clusters with new *Ornithodoros* sp. and *O. sawaii* (Fig. 6). Table VI shows 73.3 to 84.4% identity in *COI* when comparing new *Ornithodoros* sp. (domestic isolate, MK836055) and *Ornithodoros* spp. from the

GenBank database, and Table VII shows 86.8 to 88.1% identity in *COI* when comparing new *Ornithodoros* sp. (domestic isolate, MK836055) and *O. capensis* from the GenBank database. All sequences of *O. capensis* were analyzed, and the overlapping data were deleted. *Ornithodoros sawaii* could not be analyzed due to the lack of sequence. Tables VIII and IX show the alignment of the variable regions V1 and V2 of the *16S rRNA* gene sequences from the new *Ornithodoros* sp. and other *Ornithodoros* species, respectively. Aligned sequences were edited to the overlapped length, and a sequence identity matrix was generated for the alignments. The percentage of sequence identity was calculated on the alignments from the 332-bp segment of the *16S rRNA* gene containing both the V1 and V2 regions (Fig. 4).

The cytochrome *b* gene fragment was sequenced to identify the host animals. In total, 16 sequences were acquired from 74 samples, including 9 samples from Gaerin-do, 1 sample from Chilbal-do, and 6 samples from Chilsan-do. According to sequencing analysis of the 16 amplicons, black-tailed gulls (*L. crassirostris* = 6) were detected in samples from Chilsan-do, and Swinhoe's storm petrel (*H. monorhis* = 10) were detected in samples from Gaerin-do and Chilbal-do. The results showed that the sequences collected from Gaerin-do and Chilbal-do had 100% identity with the *H. monorhis* isolate from the United Kingdom (HG975295), and the sequences collected from Chilsan-do had 99% identity with *L. crassirostris* from South Korea (KM507782). The sequence analyses of the new *Ornithodoros* sp. and *O. sawaii* were aligned using multiple sequence alignment with hierarchical clustering (<http://multalin.toulouse.inra.fr/multalin/>) to compare their differences (Fig. 7) (Corpet, 1988). Figure 7 shows invariant sequences except for positions at A529, G589, and C659 in the all-new *Ornithodoros* sp. from this study. This shows the exact locality of the invariant but unique sites for the new *Ornithodoros* sp. and *O. sawaii*. The sequence analyses of the new *Ornithodoros* sp., *O. sawaii*, and *O. capensis* were also aligned to compare their differences, and these results are shown in Figure 8.

Table II. The number of nests and soft ticks from uninhabited islands (2017–2018).

Name of islands	Number of nests with soil collection	Number of nests positive for soft ticks (%)	Number of collected soft ticks (%)
Nan-do	60	1 (1.70%)	1 (1.30%)
Chilsan-do	60	10 (16.70%)	30 (38.97%)
Chilbal-do	60	10 (16.70%)	19 (24.68%)
Gaerin-do	30	6 (20.00%)	16 (20.78%)
Sogukhol-do	30	2 (6.70%)	2 (2.59%)
Gugul-do	60	5 (8.30%)	8 (10.39%)
Sasu-do	50	0	0
Hong-do			
(Hallyeohaesang)	50	1 (2.00%)	1 (1.30%)
Dok-do	10	0	0
Total	410	35 (8.50%)	77

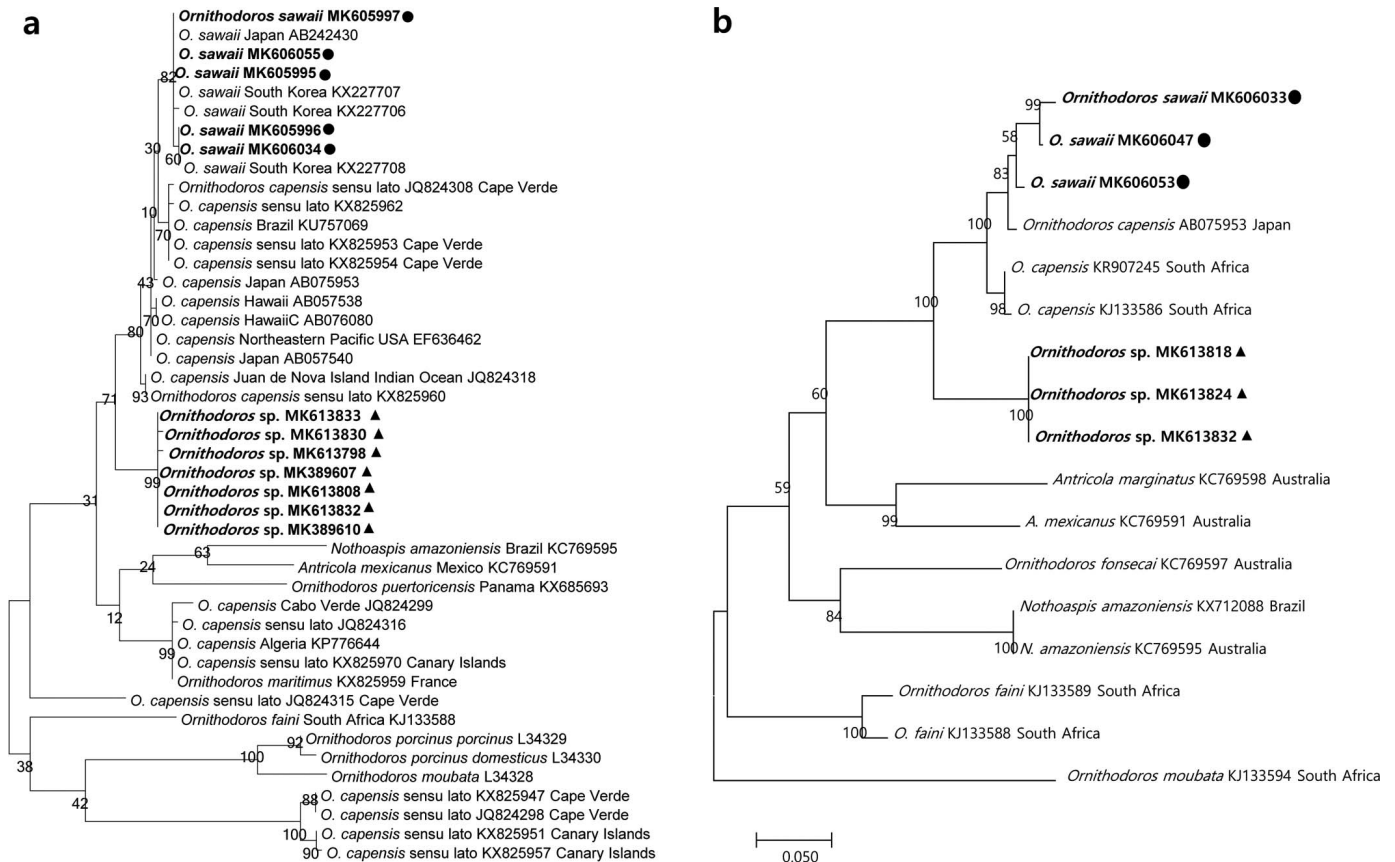


Figure 5. Phylogenetic tree of *Ornithodoros* spp. ticks collected in this study. The maximum-likelihood method was used to construct the phylogenetic tree. The phylogenetic tree was constructed based on (a) partial sequences (475 bp) and (b) full-length sequences (1,233 bp) of *16S rRNA*. Circle icons indicate *Ornithodoros sawaii*, and triangle icons indicate new *Ornithodoros* sp. GG: Gugul-do; GR: Gaerin-do; CB: Chilbal-do; CS: Chilsan-do; ND: Nan-do.

DISCUSSION

As described above, Gaerin-do, Sogukhol-do, Gugul-do, Chilbal-do, Chilsan-do, Hong-do (Hallyeohaesang), and Nan-do are uninhabited islands that are used as mass breeding places for migratory birds. Chilsan-do, Hong-do (Hallyeohaesang), and Nan-do are well known as the mass breeding place for *L. crassirostris* colonies, which are common resident birds in the western, southern, and eastern seas around the Republic of Korea and distributed mostly in the Far East, including in Japan, southwestern Russia, and eastern China (Kwon et al., 2006); these birds have also been infrequently observed in Mexico (Garrett and Molina, 1998). Although these resident seabirds are often observed in mainland coastal areas, they mate and raise their chicks from early April to late August on nearby uninhabited islands (Lee and Yoo, 2005; Kwon et al., 2006).

There are currently 12 described species of soft ticks associated specifically with seabirds, all of which belong to the *Argas* and *Ornithodoros* genera (Dietrich et al., 2011), including *O. sawaii* collected in this survey and the newly identified new *Ornithodoros* sp., which is associated with migratory birds including seabirds. The samples of nests with soil containing *Ornithodoros* spp. soft ticks were collected from June to October 2017 and April to October 2018. Therefore, the nests formed on the ground by

migratory birds may create a habitat for *Ornithodoros* spp. Among the uninhabited islands investigated, the new *Ornithodoros* sp. was identified on the western islands, which are the breeding sites of *L. crassirostris*. We selected western and

Table III. The matrix of genetic identity for the *16S rRNA* full sequence (1,229 bp) of the newly identified *Ornithodoros* sp. in the Republic of Korea and other *Ornithodoros* spp.*

16S rRNA	1	2	3	4	5
1	100 (1,228)	89.0	88.7	88.6	88.4
2	135 (1,228)	100 (1,224)	99.5	95.9	96.3
3	139 (1,228)	7 (1,224)	100 (1,224)	95.6	95.9
4	140 (1,229)	51 (1,226)	55 (1,226)	100 (1,225)	96.7
5	143 (1,230)	46 (1,227)	50 (1,227)	40 (1,227)	100 (1,225)

* 1, new *Ornithodoros* sp. (MK613790); 2, *Ornithodoros sawaii* (domestic isolate, MK606044); 3, *Ornithodoros capensis* (South Africa, KR907245); 4, *O. capensis* (South Africa, KJ133586); 5, *O. capensis* (Japan, AB075953). Percent identity between sequences is shown as the upper matrix. The lower matrix shows the number of nucleotide differences in bold print and the number of overlapping nucleotides in parentheses.

Table IV. The matrix of genetic identity for the *16S rRNA* sequence fragment (320 bp) for the newly identified *Ornithodoros* sp. in the Republic of Korea and other *Ornithodoros* spp.*

16S rRNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	100 (320)	94.4	93.4	93.4	90.7	87.3	87.2	86.6	86.3	86.0	85.9	85.9	85.9	85.7	85.6
2	18 (320)	100 (319)	96.9	96.9	90.9	86.9	87.5	87.8	87.5	86.3	86.2	86.3	87.5	85.0	86.2
3	21 (320)	10 (319)	100 (319)	100	89.7	87.2	88.1	86.5	87.5	86.9	86.5	85.3	87.8	84.4	86.5
4	21 (320)	10 (319)	0 (319)	100 (319)	89.7	87.2	88.1	86.5	87.5	86.9	86.5	85.3	87.8	84.4	86.5
5	30 (321)	29 (320)	33 (320)	33 (320)	100 (320)	84.4	86.6	86.9	85.3	85.6	85.9	84.4	85.3	84.7	85.9
6	41 (322)	42 (321)	41 (321)	41 (321)	50 (321)	100 (321)	86.3	84.4	85.0	86.0	85.0	82.6	86.6	83.9	84.4
7	41 (320)	40 (320)	38 (320)	38 (320)	43 (320)	44 (322)	100 (319)	86.9	87.1	87.5	84.7	83.1	88.1	88.1	85.0
8	43 (320)	39 (319)	43 (319)	43 (319)	42 (320)	50 (321)	42 (320)	100 (319)	86.2	84.7	84.6	82.2	84.6	85.4	85.3
9	44 (320)	40 (319)	40 (319)	40 (319)	47 (320)	48 (321)	41 (319)	44 (319)	100 (318)	84.4	86.2	83.8	85.3	84.7	86.5
10	45 (321)	44 (320)	42 (320)	42 (320)	46 (320)	45 (322)	40 (320)	49 (320)	50 (320)	100 (320)	85.6	83.8	85.3	81.3	86.3
11	45 (320)	44 (319)	43 (319)	43 (319)	45 (320)	48 (321)	49 (320)	49 (319)	44 (319)	46 (320)	100 (313)	84.0	83.7	82.2	97.1
12	45 (320)	44 (320)	47 (320)	47 (320)	50 (320)	56 (321)	54 (320)	57 (320)	52 (320)	52 (320)	51 (320)	100 (319)	82.2	83.2	83.8
13	45 (320)	40 (319)	39 (319)	39 (319)	47 (320)	43 (321)	38 (320)	49 (319)	47 (319)	47 (320)	52 (319)	57 (320)	100 (319)	86.3	84.0
14	46 (322)	48 (321)	50 (321)	50 (321)	49 (321)	52 (322)	38 (320)	47 (321)	49 (320)	60 (321)	57 (321)	54 (321)	44 (321)	100 (319)	82.2
15	46 (320)	44 (319)	43 (319)	43 (319)	45 (320)	50 (321)	48 (320)	47 (319)	43 (319)	44 (320)	9 (313)	52 (320)	51 (319)	57 (321)	100 (313)
16	47 (321)	49 (320)	51 (320)	51 (320)	47 (320)	52 (321)	40 (321)	55 (320)	53 (320)	53 (321)	53 (320)	58 (320)	48 (320)	33 (321)	55 (320)
17	47 (321)	48 (320)	46 (320)	46 (320)	60 (320)	56 (321)	63 (319)	56 (320)	60 (319)	62 (320)	60 (320)	62 (320)	57 (320)	60 (319)	54 (321)
18	47 (320)	49 (319)	52 (319)	52 (319)	52 (320)	46 (321)	47 (319)	47 (319)	45 (318)	54 (320)	53 (317)	62 (320)	53 (319)	58 (320)	53 (317)
19	48 (322)	52 (321)	50 (321)	50 (321)	52 (321)	48 (321)	56 (321)	55 (321)	56 (321)	60 (321)	54 (321)	56 (321)	59 (321)	72 (322)	58 (321)
20	48 (320)	51 (319)	52 (319)	52 (319)	53 (320)	46 (321)	48 (319)	52 (319)	55 (318)	48 (320)	48 (318)	61 (320)	54 (319)	56 (320)	50 (318)
21	50 (321)	47 (320)	47 (320)	47 (320)	41 (321)	53 (321)	48 (321)	54 (320)	54 (320)	56 (320)	59 (319)	48 (320)	53 (320)	59 (322)	59 (319)
22	52 (320)	53 (319)	51 (319)	51 (319)	50 (320)	52 (321)	48 (319)	51 (319)	57 (318)	53 (320)	56 (317)	62 (319)	44 (319)	44 (320)	54 (317)
23	55 (321)	60 (319)	59 (320)	59 (320)	55 (320)	62 (321)	54 (319)	62 (320)	60 (318)	63 (320)	65 (317)	67 (321)	63 (320)	64 (319)	64 (317)
24	57 (321)	53 (321)	58 (321)	58 (321)	53 (321)	57 (322)	56 (321)	57 (321)	58 (321)	51 (321)	54 (321)	56 (321)	59 (321)	68 (321)	54 (321)
25	59 (321)	56 (320)	55 (320)	55 (320)	64 (321)	47 (321)	55 (321)	63 (320)	60 (321)	55 (322)	59 (320)	69 (323)	67 (320)	66 (323)	59 (320)
26	61 (320)	57 (319)	59 (319)	59 (319)	63 (320)	59 (321)	62 (319)	65 (319)	63 (318)	57 (320)	54 (321)	60 (319)	65 (319)	66 (319)	61 (319)
27	63 (320)	57 (320)	54 (320)	54 (320)	61 (320)	60 (321)	63 (320)	67 (320)	60 (321)	58 (320)	63 (320)	73 (320)	61 (320)	69 (321)	62 (320)
28	63 (320)	68 (319)	68 (319)	68 (319)	70 (320)	65 (321)	62 (319)	61 (320)	61 (319)	57 (321)	61 (318)	75 (319)	69 (319)	77 (319)	60 (318)
29	64 (322)	62 (321)	67 (321)	67 (321)	75 (322)	70 (322)	63 (322)	69 (321)	66 (321)	67 (322)	67 (318)	73 (322)	70 (321)	77 (321)	70 (318)
30	64 (322)	69 (321)	67 (321)	67 (321)	69 (322)	66 (323)	63 (321)	69 (321)	66 (320)	64 (322)	63 (320)	82 (322)	72 (321)	76 (321)	63 (320)

* 1, NIOSK (MK613790); 2, *Ornithodoros capensis* (South Africa, KR907245); 3, *Ornithodoros sawaii* (domestic isolate, MK606044); 4, *O. sawaii* (Japan, AB242430); 5, *Ornithodoros maritimus* (Spain, KX825970); 6, *Ornithodoros rondoniensis* (Brazil, JQ951962); 7, *Ornithodoros atacamensis* (Chile, KT894586); 8, *Ornithodoros* sp. (JX455899); 9, *Ornithodoros lahillei* (Chile, KP403288); 10, *Ornithodoros rietcorraei* (Brazil, KX130782); 11, *Ornithodoros amblyus* (Peru, KX825971); 12, *Ornithodoros fonsecai* (Brazil, MG970562); 13, *Ornithodoros kohlsi* (Bolivia, KX130783); 14, *Ornithodoros rioplatensis* (Uruguay, EU283343); 15, *Ornithodoros denmarki* (Mexico, KX825972); 16, *Ornithodoros hasei* (Brazil, KX781698); 17, *Ornithodoros vigerasi* (JQ397634); 18, *Ornithodoros yumatensis* (Mexico KX668415); 19, *Ornithodoros faccinii* (Brazil, KP861242); 20, *Ornithodoros brodyi* (Mexico, KY454706); 21, *Ornithodoros dyeri* (Mexico, KU551919); 22, *Ornithodoros guaporensis* (Brazil, KT781507); 23, *Ornithodoros knoxjonesi* (Mexico, KU663027); 24, *Ornithodoros faini* (Uganda, KU295468); 25, *Ornithodoros coriaceus* (USA, NC039857); 26, *Ornithodoros quilensis* (Argentina, JN255575); 27, *Ornithodoros peruvianus* (Chile, HQ111351); 28, *Ornithodoros turicata* (L34327); 29, *Ornithodoros occidentalis* (Spain, KC311537); 30, *Ornithodoros parkeri* (EU009925). Percent identity between sequence is shown as the upper matrix. The lower matrix shows the number of nucleotide differences in bold print and the number of overlapping nucleotides in parentheses.

Table V. The matrix of genetic identity for the *16S rRNA* partial (320 bp) sequence for the the newly identified *Ornithodoros* sp. in the Republic of Korea and other *Ornithodoros capensis* occurrences.*

16S rRNA	1	2	3	4	5	6	7	8	9	10	11
1	100 (320)	94.4	94.4	94.1	94.1	94.1	93.8	93.8	92.5	90.7	90.0
2	18 (320)	100 (319)	97.5	96.9	98.1	97.5	96.9	97.8	92.8	90.9	90.9
3	18 (320)	8 (319)	100 (319)	98.1	98.8	98.1	96.9	99.1	91.9	91.6	91.5
4	19 (320)	10 (319)	6 (319)	100 (319)	98.8	98.8	98.1	99.1	91.6	90.9	91.5
5	19 (320)	6 (319)	6 (319)	4 (319)	100 (319)	98.8	98.1	99.7	92.2	90.9	91.5
6	19 (320)	8 (319)	6 (319)	4 (319)	4 (319)	100 (319)	97.5	99.1	92.8	91.9	92.9
7	21 (320)	10 (319)	10 (319)	6 (319)	6 (319)	8 (319)	100 (319)	97.8	92.2	89.7	90.3
8	20 (320)	7 (319)	3 (319)	3 (319)	1 (319)	3 (319)	7 (319)	100 (319)	91.9	91.2	91.9
9	24 (320)	23 (320)	29 (319)	30 (319)	25 (320)	26 (319)	25 (320)	29 (319)	100 (318)	89.1	89.7
10	30 (321)	29 (320)	27 (320)	29 (320)	29 (320)	26 (320)	33 (320)	28 (320)	37 (320)	100 (320)	97.5
11	32 (320)	29 (319)	27 (319)	27 (319)	27 (319)	24 (319)	32 (319)	26 (319)	36 (319)	8 (320)	100 (319)

* 1, new *Ornithodoros* sp. (MK613790); 2, *Ornithodoros capensis* (South Africa, KR907245); 3, *O. capensis* (France, KX825960); 4, *O. capensis* (French Guiana, MH513308); 5, *O. capensis* (Japan, AB076082); 6, *O. capensis* (JQ824319); 7, *O. capensis* (JQ824322); 8, *Ornithodoros sawaii* (domestic isolate, MK606044); 9, *O. capensis* (Japan, AB242432); 10, *O. capensis* (France, KX825969); 11, *O. capensis* (Algeria, KP776644).

Percent identity between sequence is shown as the upper matrix. The lower matrix shows the number of nucleotide differences in bold print and the number of overlapping nucleotides in parentheses.

Table IV. Extended.

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
85.4	85.4	85.3	85.1	85.0	84.4	83.8	82.9	82.2	81.6	80.9	80.3	80.3	80.1	80.1
84.7	85.0	84.6	83.8	84.0	85.3	83.4	81.2	83.5	82.5	82.1	82.2	78.7	80.7	78.5
84.1	85.6	83.7	84.4	83.7	85.3	84.0	81.6	81.9	82.8	81.5	83.1	78.7	79.1	79.1
84.1	85.6	83.7	84.4	83.7	85.3	84.0	81.6	81.9	82.8	81.5	83.1	78.7	79.1	79.1
85.3	81.3	83.8	83.8	83.4	87.2	84.4	82.8	83.5	80.1	80.3	80.9	78.1	76.7	78.6
83.8	82.6	85.7	85.0	85.7	83.5	83.8	80.7	82.3	85.4	81.6	81.3	79.8	78.3	79.6
87.5	80.3	85.3	82.6	85.0	85.0	85.0	83.1	82.6	82.9	80.6	80.3	80.6	80.4	80.4
82.8	82.5	85.3	82.9	83.7	83.1	84.0	80.6	82.2	80.3	79.6	79.1	80.9	78.5	78.5
83.4	81.2	85.8	82.6	82.7	83.1	82.1	81.1	81.9	81.3	80.2	81.3	80.9	79.4	79.4
83.5	80.6	83.1	81.3	85.0	82.5	83.4	80.3	84.1	82.9	82.2	81.9	82.2	79.2	80.1
83.4	81.3	83.3	83.2	84.9	81.5	82.3	79.5	83.2	79.6	83.2	80.3	80.8	78.9	80.3
81.9	80.6	80.6	82.6	80.9	85.0	80.6	79.1	82.6	78.6	81.2	77.2	76.5	77.3	74.5
85	82.2	83.4	81.6	83.1	83.4	86.2	80.3	81.6	79.1	79.6	80.9	78.4	78.2	77.6
89.7	81.2	81.9	77.6	82.5	81.7	86.3	79.9	78.8	79.6	79.3	78.5	75.9	76.2	76.3
82.8	83.2	83.3	81.9	84.3	81.5	83.0	79.8	83.2	81.6	80.9	80.6	81.1	78.0	80.3
100 (320)	80.3	83.1	79.1	81.6	83.2	85.3	79.7	79.1	79.6	76.9	77.8	76.3	78.6	76.4
63 (320)	100 (318)	80.3	78.2	79.0	83.2	79.3	79.2	79.1	80.7	82.4	79.8	81.6	73.8	80.1
54 (320)	63 (319)	100 (316)	82.2	89.0	82.8	82.6	82.9	81.6	79.1	78.9	79.7	78.9	76.8	77.4
67 (321)	70 (321)	57 (321)	100 (321)	81.9	82.9	81.4	78.9	82.9	79.5	79.1	78.2	78.5	75.5	78.0
59 (320)	67 (319)	35 (317)	58 (321)	100 (317)	84.1	82.3	83.3	81.0	82.2	78.9	81.3	80.8	77.5	78.4
54 (322)	54 (322)	55 (320)	55 (321)	51 (320)	100 (318)	81.8	81.8	81.9	80.6	79.9	80.7	78.4	75.9	77.9
47 (320)	66 (319)	55 (316)	60 (322)	56 (317)	58 (319)	100 (316)	79.4	79.8	82.2	81.8	80.9	78.9	74.9	79.7
65 (320)	66 (318)	54 (316)	68 (322)	53 (317)	58 (319)	65 (316)	100 (314)	77.6	79.1	78.2	79.1	76.9	77.7	77.0
67 (321)	67 (321)	59 (321)	55 (322)	61 (321)	58 (321)	65 (321)	72 (321)	100 (321)	81.7	81.0	78.6	80.1	77.1	80.8
66 (323)	62 (322)	67 (320)	66 (322)	57 (320)	62 (320)	57 (320)	67 (320)	59 (322)	100 (317)	78.1	81.6	83.4	78.2	83.6
74 (320)	56 (318)	67 (318)	67 (321)	67 (318)	64 (319)	58 (318)	69 (317)	61 (321)	70 (320)	100 (317)	81.7	78.2	77.3	77.4
71 (320)	65 (322)	65 (320)	70 (321)	60 (320)	62 (321)	61 (320)	67 (320)	69 (322)	59 (321)	59 (322)	100 (320)	76.3	76.4	77.0
76 (321)	59 (321)	67 (317)	69 (321)	61 (318)	69 (319)	67 (318)	73 (316)	64 (321)	53 (320)	69 (317)	76 (320)	100 (314)	76.3	88.4
69 (323)	84 (321)	74 (319)	79 (322)	72 (320)	77 (320)	80 (319)	71 (319)	74 (323)	70 (321)	73 (321)	76 (322)	76 (320)	100 (317)	78.3
76 (322)	64 (322)	72 (319)	71 (323)	69 (320)	71 (321)	65 (320)	73 (318)	62 (323)	53 (323)	72 (319)	74 (322)	37 (318)	70 (322)	100 (317)

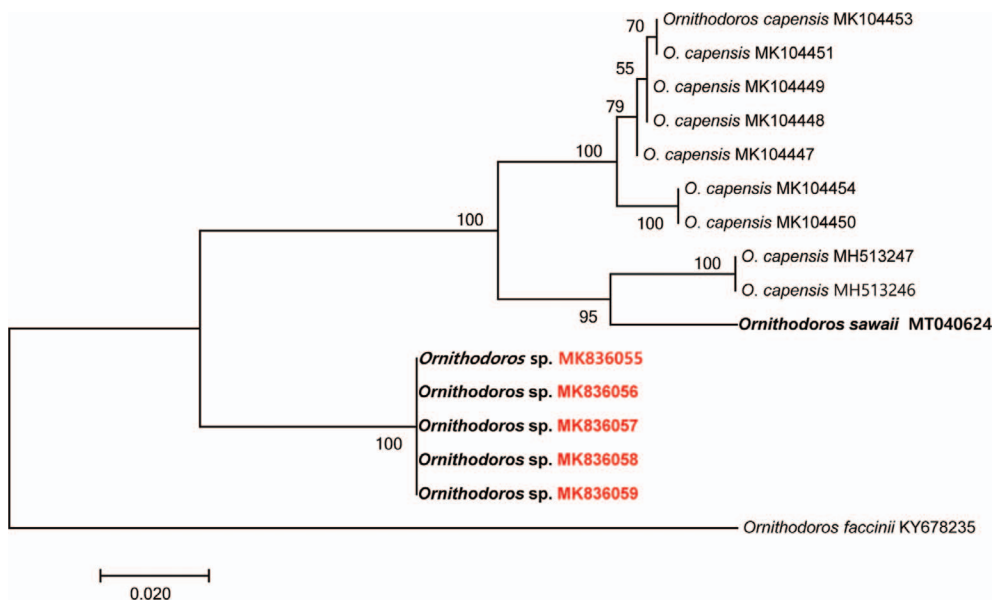


Figure 6. Phylogenetic tree derived from the nucleotide sequences of *COI*. The maximum-likelihood method was used to construct the phylogenetic tree. The phylogenetic tree was constructed based on partial sequences (653 bp). CB: Chilbal-do; CS: Chilsan-do; ND: Nan-do. Color version is available online.

Table VI. The matrix of genetic identity for the *COI* (385 bp) sequence for the newly identified *Ornithodoros* sp. in the Republic of Korea and other *Ornithodoros* spp.*

<i>COI</i>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	100	84.4	84.4	84.2	84.2	82.9	82.6	81.8	81.6	81.3	81.3	81.0	81.0	79.5	76.6	75.6	75.6	75.6	75.3	74.8	73.3
2	60	100	99.2	98.7	99.5	82.9	82.6	83.4	83.6	83.4	87.3	83.1	87.5	77.9	77.7	76.6	76.6	77.1	77.4	75.8	73.5
3	60	3	100	99.0	99.7	82.6	82.3	82.9	83.6	83.4	87.0	83.1	87.3	77.4	77.4	75.8	75.8	76.9	77.7	76.1	73.3
4	61	5	4	100	99.2	81.8	81.6	83.1	84.2	83.9	87.5	83.6	87.8	77.7	76.6	76.1	76.1	76.1	77.4	75.6	73.5
5	61	2	1	3	100	82.3	82.1	83.1	83.9	83.6	87.3	83.4	87.5	77.7	77.1	76.1	76.1	76.6	77.4	75.8	73.5
6	66	66	67	70	68	100	99.7	82.9	81.8	82.1	81.8	81.3	81.6	77.7	74.8	74.6	74.6	74.0	79.0	75.1	73.0
7	67	67	68	71	69	1	100	82.9	81.8	82.1	81.8	81.3	81.6	77.4	74.6	74.3	74.3	73.8	78.7	75.1	72.7
8	70	64	66	65	65	66	66	100	94.0	93.8	82.6	93.5	82.6	75.6	73.5	72.7	72.7	72.7	74.0	74.8	69.9
9	71	63	63	61	62	70	70	23	100	99.7	83.4	99.5	83.1	77.1	74.0	73.3	73.0	73.3	74.3	74.8	70.9
10	72	64	64	62	63	69	69	24	1	100	83.1	99.2	82.9	76.9	73.8	73.0	72.7	73.0	74.6	75.1	71.2
11	72	49	50	48	49	70	70	67	64	65	100	82.9	99.7	74.8	74.3	73.5	73.8	73.8	73.0	74.0	70.1
12	73	65	65	63	64	72	72	25	2	3	66	100	82.6	76.6	73.5	72.7	72.5	73.0	73.8	74.3	70.4
13	73	48	49	47	48	71	71	67	65	66	1	67	100	75.1	74.6	73.8	74.0	74.0	72.7	73.8	70.4
14	79	85	87	86	86	86	87	94	88	89	97	90	96	100	75.8	75.6	75.3	74.8	73.0	73.0	74.8
15	90	86	87	90	88	97	98	102	100	101	99	102	98	93	100	97.1	97.4	99.0	74.6	75.6	75.8
16	94	90	93	92	92	98	99	105	103	104	102	105	101	94	11	100	99.7	96.1	74.0	75.3	75.1
17	94	90	93	92	92	98	99	105	104	105	101	106	100	95	10	1	100	96.4	73.8	75.6	75.1
18	94	88	89	92	90	100	101	105	103	104	101	104	100	97	4	15	14	100	73.8	74.6	75.3
19	95	87	86	87	87	81	82	100	99	98	104	101	105	104	98	100	101	101	100	77.4	77.7
20	97	93	92	94	93	96	96	97	97	96	100	99	101	104	94	95	94	98	87	100	75.1
21	103	102	103	102	102	104	105	116	112	111	115	114	114	97	93	96	96	95	86	96	100

* 1, new *Ornithodoros* sp. (MK836055); 2, *Ornithodoros yunker* (Chile, MN027577); 3, *O. yunker* (Peru, MN027576); 4, *O. yunker* (Peru, MN027582); 5, *O. yunker* (Peru, MN027581); 6, *Ornithodoros maritimus* (France, KX826017); 7, *O. maritimus* (France, KX826016); 8, *O. maritimus* (Chile, MN027573); 9, *Ornithodoros spheniscus* (Chile, MN027574); 10, *O. spheniscus* (Chile, MN027575); 11, *Ornithodoros amblus* (Peru, MN027585); 12, *O. spheniscus* (Chile, MN027572); 13, *O. amblus* (Peru, MN027586); 14, *Ornithodoros yumatensis* (USA, KY922348); 15, *Ornithodoros* sp. (Spain, KX825994); 16, *Ornithodoros* sp. (Cape Verde, KX826008); 17, *Ornithodoros* sp. (Cape Verde, KX826007); 18, *Ornithodoros* sp. (Spain, KX825993); 19, *Ornithodoros canestrinii* (Iran, KX589252); 20, *Ornithodoros asperus* (Iran, MK048332); 21, *Ornithodoros lahorensis* (Iran, MK318148).

Table VII. The matrix of genetic identity for the *COI* (385 bp) full sequence for the newly identified *Ornithodoros* sp. in the Republic of Korea and other *Ornithodoros capensis* occurrences.*

<i>COI</i>	1	2	3	4	5	6	7	8	9	10
1	100	88.1	87.8	87.5	87.5	87.5	87.5	87.3	87.0	86.8
2	50	100	99.7	98.2	99.2	99.5	93.8	98.4	99.0	97.9
3	51	3	100	98.4	99.5	99.7	93.5	98.7	99.2	98.2
4	50	2	1	100	98.4	98.7	93.3	99.7	97.7	99.2
5	50	9	10	9	100	99.2	93.3	98.7	98.7	98.2
6	48	6	7	6	5	100	93.3	99.0	99.0	98.4
7	48	7	8	7	4	3	100	93.5	92.7	93.5
8	46	6	7	6	4	3	2	100	97.9	99.5
9	48	28	25	26	28	26	26	24	100	97.4
10	48	29	26	27	30	27	28	26	13	100

* 1, new *Ornithodoros* sp. (domestic isolate, MK836055); 2, *Ornithodoros capensis* (South Africa, MK104397); 3, *O. capensis* (South Africa, MK104449); 4, *O. capensis* (South Africa, MK104427); 5, *O. capensis* (South Africa, MK104417); 6, *O. capensis* (South Africa, MK104453); 7, *O. capensis* (Brazil, MN027570); 8, *O. capensis* (South Africa, MK104441); 9, *O. capensis* (South Africa, MK104436); 10, *O. capensis* (South Africa, MK104443).

Table VIII. Variable region 1 in the 16S rRNA sequence for the newly identified *Ornithodoros* sp. in the Republic of Korea and *Ornithodoros capensis*.*

Position	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132
1	T	T	T	A	T	A	A	T	T	-	-	T	T	A	T	T	T	A	A	A
2	•	•	A	G	G	•	•	G	•	T	•	•	•	•	•	A	A	•	G	T
3	•	•	A	•	A	•	G	G	-	•	A	A	A	T	•	•	•	T	•	•
4	•	•	•	T	A	T	•	A	-	T	G	•	•	•	•	•	•	•	T	T
5	A	•	•	•	A	•	•	A	•	T	A	A	•	T	•	•	•	T	•	•
6	•	•	•	T	G	T	•	A	A	T	•	•	•	•	•	•	•	•	T	T
7	•	•	•	-	A	T	•	A	C	T	G	•	-	•	•	•	•	T	T	•
8	•	•	•	•	A	•	•	A	-	•	-	-	-	T	•	•	A	T	T	T
9	•	•	•	•	A	G	T	A	•	T	•	•	A	T	•	•	•	•	•	•
10	•	•	•	T	A	G	T	A	A	A	•	A	•	•	•	•	•	•	T	T
11	•	•	•	T	A	T	•	A	•	T	•	•	•	•	•	•	•	T	•	•
12	•	•	•	G	•	G	•	•	•	•	•	•	•	•	•	•	•	G	T	•
13	•	•	•	•	•	G	•	C	•	•	•	•	•	G	•	•	•	•	•	•
14	•	•	•	•	•	G	•	C	•	•	•	•	•	G	•	•	•	•	•	•
15	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•
16	•	•	•	•	A	•	•	A	G	•	•	•	•	•	•	•	•	•	T	T
17	•	•	•	T	G	•	•	A	A	A	•	•	A	T	•	•	A	T	•	•
18	•	•	•	G	•	•	T	A	A	•	•	•	•	•	•	•	•	•	T	T
19	•	•	•	•	A	G	T	•	•	•	•	•	•	T	•	A	•	T	T	•
20	•	C	•	T	A	•	G	A	A	A	•	•	•	T	•	•	•	•	•	•
21	A	•	•	T	•	•	•	A	A	T	•	•	•	C	•	•	A	•	C	G
22	•	•	•	-	A	•	•	A	-	•	•	-	•	T	•	•	A	T	T	T
23	•	•	•	T	A	•	•	A	-	•	•	-	A	T	A	•	A	T	T	T
24	•	•	•	-	A	T	•	A	-	•	•	-	•	•	A	•	A	T	T	T
25	•	•	•	-	G	T	G	A	-	•	•	-	A	•	A	•	A	T	T	T
26	•	•	•	T	A	•	G	•	C	•	•	•	G	G	•	•	•	•	•	T
27	•	•	•	T	A	•	T	•	•	•	•	G	•	G	•	A	•	•	G	T
28	A	•	•	T	A	•	•	A	A	A	•	-	-	T	•	•	A	G	G	T
29	•	A	•	G	A	•	G	A	-	•	•	A	A	T	•	G	•	T	•	G
30	•	A	•	•	A	T	T	A	•	•	•	A	A	•	A	•	•	T	T	•
1	T	T	T	A	T	A	A	T	T	-	-	T	T	A	T	T	T	A	A	A
31	C	A	A	•	A	•	G	•	-	•	•	G	A	T	G	•	C	•	T	T
32	•	•	A	•	G	•	•	•	-	•	•	-	A	G	G	•	A	T	T	T
33	•	•	A	•	A	•	•	A	-	•	•	•	A	T	A	•	•	T	T	T
34	•	•	•	•	A	•	G	-	-	•	•	•	•	T	•	•	A	T	T	T
35	•	C	A	•	A	G	•	C	C	•	•	•	•	T	C	•	•	G	T	T
36	•	•	A	•	A	G	•	C	•	•	•	•	•	T	C	•	•	T	T	T
37	•	•	A	•	A	G	•	C	•	•	•	•	•	T	C	•	•	T	T	T
38	•	•	A	•	A	G	•	C	•	•	•	•	•	T	C	•	•	T	C	T
39	•	•	A	•	A	•	•	G	C	•	•	•	•	T	•	•	•	T	T	C
40	•	•	A	•	A	•	•	G	C	•	•	•	•	T	•	•	•	T	T	•
41	•	•	A	•	A	•	•	•	-	•	•	•	A	T	•	•	•	G	G	•
42	C	•	A	•	A	•	T	•	-	•	•	•	A	T	•	•	•	•	G	•
43	•	A	A	•	A	•	•	A	•	•	•	•	A	T	•	•	•	T	•	G
44	•	C	G	G	A	G	G	•	-	•	•	•	A	T	•	•	•	T	•	G
45	C	A	•	T	A	G	T	G	-	•	•	-	A	G	-	G	•	T	•	T
46	C	A	•	T	A	•	•	A	-	•	•	-	G	•	-	•	•	T	•	T
47	C	A	•	T	A	•	•	A	-	•	•	-	A	G	A	•	•	T	•	T
48	C	A	•	T	A	•	•	-	-	•	•	-	A	•	A	•	•	C	•	T
49	C	•	•	T	•	•	-	-	-	•	•	•	C	C	C	•	•	T	•	G
50	C	•	•	T	A	C	•	-	-	•	•	-	A	G	•	•	•	T	T	G
51	•	•	•	T	•	G	•	G	A	A	•	A	•	T	A	G	•	•	T	T
52	C	A	•	•	A	G	T	•	•	•	•	A	•	T	•	A	•	•	T	•
53	A	C	A	T	A	G	T	•	•	T	•	A	G	T	•	G	A	G	T	G
54	•	•	G	•	A	•	T	•	•	T	•	•	•	T	•	•	G	•	G	•
55	•	•	A	•	A	G	-	-	-	•	•	-	•	•	A	•	•	T	•	•
56	G	A	A	•	A	•	-	-	-	•	•	-	•	•	A	•	•	T	•	•

* 1, new *Ornithodoros* sp. (MK613790); 2, *Ornithodoros vignerasi* (JQ397634); 3, *Ornithodoros coriaceus* (USA, NC039857); 4, *Ornithodoros faini* (Uganda, KU295468); 5, *Ornithodoros rioplatensis* (Uruguay, EU283343); 6, *Ornithodoros hasei* (Brazil, KX781698); 7, *Ornithodoros atacamensis* (Chile, KT894586); 8, *Ornithodoros guaporensis* (Brazil, KT781507); 9, *Ornithodoros faccinii* (Brazil, KP861242); 10, *Ornithodoros rondoniensis* (Brazil, JQ951962); 11, *Ornithodoros maritimus* (Spain, KX825970); 12, *Ornithodoros kohlsi* (Bolivia, KX130783); 13, *Ornithodoros sawaii* (Republic of Korea, MK606044); 14, *O. sawaii* (Japan, AB242430); 15, *Ornithodoros capensis* (South Africa, KR907245); 16, *Ornithodoros rostratus* sp. (JX455899); 17, *Ornithodoros rietcorraei* (Brazil, KX130782); 18, *Ornithodoros lahillei* (Chile, KP403288); 19, *Ornithodoros dyeri* (Mexico, KU551919); 20, *Ornithodoros peruvianus* (Chile, HQ111351); 21, *Ornithodoros fonsecai* (Brazil, MG970562); 22, *Ornithodoros yumatensis* (Mexico, KX668415); 23, *Ornithodoros brodyi* (Mexico, KY454706); 24, *Ornithodoros denmarki* (Mexico, KX825972); 25, *Ornithodoros amblyus* (Peru, KX825971); 26, *Ornithodoros xerophylus* (Argentina, KP403287); 27, *Ornithodoros quilinensis* (Argentina, JN255575); 28, *Ornithodoros rostratus* (Brazil, KC769592); 29, *Ornithodoros brasiliensis* (Brazil, KC769593); 30, *Ornithodoros hermsi* (NC039832); 31, *Ornithodoros gurneyi* (Australia, AY436767); 32, *Ornithodoros turicata* (L34327); 33, *Ornithodoros parkeri* (EU009925); 34, *Ornithodoros knoxjonesi* (Mexico, KU663027); 35, *Ornithodoros porcinus domesticus* (L34330); 36, *Ornithodoros phacochoerus* (South Africa, MF415624); 37, *Ornithodoros moubata* (L34328); 38, *Ornithodoros waterbergensis* (South Africa, MF415616); 39, *Ornithodoros huajianensis* (China, MK208994); 40, *Ornithodoros compactus* (South Africa, MF415614); 41,

Table VIII. Extended.

[illegible]

Table IX. Variable region 2 in the 16S *rRNA* sequence for the newly identified *Ornithodoros* sp. in the Republic of Korea and *Ornithodoros capensis*.*

P	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
1	A	A	T	A	T	T	T	A	T	C	T	T	T	A	A	A	A	G	T	T
2	•	•	•	G	G	•	G	•	•	•	•	•	•	•	•	•	•	T	•	•
3	•	T	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	T	•	•
4	•	T	•	G	A	G	A	•	•	•	•	•	•	•	•	•	•	T	G	A
5	•	•	•	G	•	•	•	•	A	•	•	•	•	•	•	•	•	T	A	G
6	•	•	•	G	•	•	•	•	A	•	•	•	•	•	•	•	•	A	G	A
7	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	T	•	A
8	G	T	•	T	•	•	•	•	A	•	•	•	•	•	•	•	•	A	G	G
9	•	T	•	T	A	A	A	•	•	•	•	•	•	•	G	T	•	A	A	A
10	•	•	•	T	A	•	•	•	•	•	•	•	•	•	T	•	•	A	•	•
11	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	A	-
12	•	T	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•
13	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•
14	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•
15	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
16	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	A	A	A
17	•	T	•	•	•	G	A	•	•	•	•	•	•	•	•	•	G	T	•	G
18	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	T	A	-
19	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	A	•	A
20	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	T	T	•	•
21	•	-	•	•	•	G	•	•	A	•	•	•	•	•	G	•	•	A	•	-
22	•	•	•	G	A	G	•	•	•	•	•	•	•	•	•	•	•	A	G	•
23	•	T	G	•	A	A	A	•	•	•	•	•	•	•	•	•	•	A	•	A
24	•	T	•	•	A	A	A	•	A	•	•	•	•	•	•	•	•	T	•	G
25	•	T	•	•	A	A	A	•	A	•	•	•	•	•	•	•	•	A	•	G
26	•	T	•	T	A	•	•	•	•	•	•	•	A	•	G	T	•	C	A	-
27	•	T	•	•	A	•	A	•	•	•	•	•	A	•	•	•	•	T	G	G
28	•	•	•	T	A	A	•	•	•	•	•	•	•	•	•	•	•	T	A	•
29	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	A	G
30	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•
31	•	T	•	G	A	G	A	•	•	•	•	•	•	•	•	•	T	T	G	A
32	•	-	•	•	G	G	A	•	•	•	•	•	•	•	•	•	•	T	-	-
33	•	T	•	C	A	G	A	•	•	•	•	•	•	•	•	•	•	A	G	A
34	•	G	•	•	A	A	G	•	•	•	•	•	•	•	•	•	•	A	A	G
35	T	T	•	•	A	A	A	•	A	•	•	•	•	•	G	•	•	•	•	-
36	T	T	•	T	A	A	A	•	A	•	•	•	•	•	•	•	•	A	•	-
37	T	T	•	T	A	A	A	•	A	•	•	•	•	•	•	•	•	A	•	-
38	T	T	•	•	A	A	A	•	A	•	•	•	•	•	•	•	•	A	•	-
39	T	T	•	T	A	G	A	•	A	•	•	•	•	•	•	•	G	A	•	-
40	T	T	•	T	A	G	A	•	A	•	•	•	•	•	•	•	G	A	•	-
41	C	T	•	G	A	G	A	•	A	•	•	•	•	•	G	•	•	A	•	-
42	T	T	•	G	A	G	A	•	A	•	•	•	•	•	G	•	•	A	•	-
43	T	T	•	G	G	•	•	•	A	•	•	•	•	•	•	•	•	-	-	-
44	T	T	A	G	-	G	•	•	A	•	•	•	•	•	•	•	•	•	•	-
45	•	T	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	A	G	-
46	•	T	•	•	•	G	A	•	•	•	•	•	•	•	•	•	•	C	A	-
47	•	T	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	A	A	A
48	•	C	•	•	•	A	G	•	•	•	•	•	•	•	•	•	•	A	A	A
49	•	T	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	A	A	A
50	•	T	•	G	•	•	G	•	•	•	•	•	•	T	T	•	•	•	G	A
51	•	T	•	G	C	A	-	•	•	•	•	•	•	•	•	•	•	A	A	G
52	C	T	•	•	G	G	A	•	•	•	•	•	•	•	•	•	•	T	G	-
53	C	T	•	•	A	A	A	•	•	•	•	•	•	•	•	T	G	A	•	-
54	C	T	•	G	•	G	A	•	•	•	•	•	•	•	G	G	T	A	•	A
55	•	T	•	•	A	G	C	•	•	•	•	•	A	•	•	•	•	•	A	A
56	•	G	•	G	•	G	A	•	•	•	•	•	G	•	G	•	•	A	A	-

* 1, new *Ornithodoros* sp. (MK613790); 2, *Ornithodoros viguerasi* (JQ397634); 3, *Ornithodoros coriaceus* (USA, NC039857); 4, *Ornithodoros faini* (Uganda, KU295468); 5, *Ornithodoros rioplatensis* (Uruguay, EU283343); 6, *Ornithodoros hasei* (Brazil, KX781698); 7, *Ornithodoros atacamensis* (Chile, KT894586); 8, *Ornithodoros guaporensis* (Brazil, KT781507); 9, *Ornithodoros faccinii* (Brazil, KP861242); 10, *Ornithodoros rondoniensis* (Brazil, JQ951962); 11, *Ornithodoros maritimus* (Spain, KX825970); 12, *Ornithodoros kohlsi* (Bolivia, KX130783); 13, *Ornithodoros sawaii* (Republic of Korea, MK606044); 14, *O. sawaii* (Japan, AB242430); 15, *Ornithodoros capensis* (South Africa, KR907245); 16, *Ornithodoros* sp. (JX455899); 17, *Ornithodoros rietcorrei* (Brazil, KX130782); 18, *Ornithodoros lahillei* (Chile, KP403288); 19, *Ornithodoros dyeri* (Mexico, KU551919); 20, *Ornithodoros peruvianus* (Chile, HQ111351); 21, *Ornithodoros fonsecai* (Brazil, MG970562); 22, *Ornithodoros yumatensis* (Mexico, KX668415); 23, *Ornithodoros brodyi* (Mexico, KY454706); 24, *Ornithodoros denmarki* (Mexico, KX825972); 25, *Ornithodoros amblus* (Peru, KX825971); 26, *Ornithodoros xerophylus* (Argentina, KP403287); 27, *Ornithodoros quilinensis* (Argentina, JN255575); 28, *Ornithodoros rostratus* (Brazil, KC769592); 29, *Ornithodoros brasiliensis* (Brazil, KC769593); 30, *Ornithodoros hermsi* (NC039832); 31, *Ornithodoros gurneyi* (Australia, AY436767); 32, *Ornithodoros turicata* (L34327); 33, *Ornithodoros parkeri* (EU009925); 34, *Ornithodoros knoxjonesi* (Mexico, KU663027); 35, *Ornithodoros porcinus domesticus* (L34330); 36, *Ornithodoros phacochoerus* (South Africa, MF415624); 37, *Ornithodoros moubata* (L34328); 38, *Ornithodoros waterbergensis* (South Africa, MF415616); 39, *Ornithodoros huajianensis* (China, MK208994); 40, *Ornithodoros compactus* (South Africa, MF415614); 41, *Ornithodoros kalahariensis* (GU355919); 42, *Ornithodoros noorsveldensis* (South Africa, MF415644); 43, *Ornithodoros pavementosus* (South Africa,

Table IX. Extended.

211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231
G	A	A	T	A	-	-	A	A	T	T	A	G	A	A	-	-	-	C	C	A
•	T	•	-	-	•	•	•	•	•	A	T	•	•	•	•	•	•	•	•	•
T	T	T	A	G	•	T	•	•	•	•	•	•	T	T	•	•	•	•	•	•
A	G	•	•	G	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	G	•	A	-	•	•	-	•	•	•	G	•	•	•	•	•	•	•	•	•
A	G	•	A	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
A	T	G	A	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
A	•	G	G	-	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•
A	G	•	A	G	•	T	•	•	G	•	•	•	•	•	•	•	•	•	•	•
T	T	T	A	T	•	T	•	•	•	•	•	•	T	•	•	•	•	•	•	•
•	G	•	A	G	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	G	•	G	G	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•
•	T	•	G	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	T	•	G	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	T	•	G	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	T	T	G	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•
•	•	T	G	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
-	G	•	A	G	•	A	•	•	•	•	•	•	T	•	•	•	•	•	•	•
•	G	•	A	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	G	T	G	G	•	•	•	•	•	•	T	•	•	C	•	•	•	•	•	•
A	G	•	A	G	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•
A	•	-	A	T	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	T
•	•	-	G	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	T
•	•	•	G	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	•	•	G	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
A	G	G	A	G	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	T
T	G	G	A	-	•	•	-	•	•	•	•	•	•	•	•	•	•	•	•	•
A	•	T	•	G	•	A	•	G	•	•	•	•	T	T	•	•	•	•	•	•
-	-	T	•	G	•	T	•	•	•	•	•	•	•	T	•	•	•	•	•	•
-	-	•	•	•	A	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•
T	T	•	•	T	•	•	•	•	A	A	•	•	•	•	•	•	•	•	•	•
-	-	G	A	-	•	•	•	•	A	•	T	•	•	•	•	•	•	•	•	•
A	•	-	-	-	•	•	•	-	•	•	T	•	•	T	•	•	•	•	T	T
-	-	G	•	•	•	T	T	•	C	•	•	•	•	T	•	•	•	•	•	•
-	-	G	•	•	•	T	T	•	•	•	G	•	•	T	•	•	•	•	•	•
-	-	G	•	•	•	T	T	•	•	•	G	•	•	T	•	•	•	•	•	•
-	-	•	•	G	A	T	•	-	•	•	G	•	•	•	•	•	•	•	•	•
-	-	•	•	G	•	A	T	•	•	•	G	•	•	T	•	•	•	•	•	•
-	-	•	•	G	•	T	T	T	•	•	T	•	•	T	•	•	•	•	•	•
•	T	G	•	G	•	T	T	T	•	•	G	•	•	T	•	•	•	•	•	•
-	-	•	•	G	•	T	T	•	•	•	G	•	•	T	•	•	•	•	•	•
A	T	T	A	T	•	A	•	•	A	•	•	•	•	•	•	•	•	•	•	G
A	T	T	A	T	•	A	•	•	G	A	•	•	•	C	•	•	•	•	•	•
A	•	•	•	•	T	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•
T	T	•	•	•	•	•	•	•	A	•	•	•	•	T	•	•	•	•	•	G
T	T	•	•	•	C	A	•	•	A	•	•	•	•	C	•	•	•	•	•	•
A	T	T	A	T	•	A	•	•	G	•	•	•	•	T	•	•	•	•	•	•
A	G	G	A	G	•	G	•	•	G	•	•	•	•	T	•	•	•	•	•	T
A	•	•	A	•	•	T	•	•	G	A	•	•	•	•	•	•	•	•	•	T
A	G	•	A	T	•	G	•	•	G	•	•	•	•	T	•	•	•	•	•	G
A	-	-	-	-	•	C	•	•	•	A	•	•	•	•	•	•	•	•	•	•
•	T	T	•	-	•	•	-	•	•	•	•	•	G	C	•	•	•	•	•	•
-	T	T	•	T	•	T	•	•	•	•	G	•	•	T	•	•	•	•	•	•

←

MF415634); 44, *Ornithodoros savignyi* (Egypt, KU163242); 45, *Ornithodoros sonrai* (Senegal, KP644222); 46, *Ornithodoros rupestris* (Algeria, KC311545); 47, *Ornithodoros maroccanus* (Algeria, KC311535); 48, *Ornithodoros costalis* (Morocco, KC311532); 49, *Ornithodoros occidentalis* (Spain, KC311537); 50, *Ornithodoros merionesi* (Morocco, KC311538); 51, *Ornithodoros cavernicolous* (Brazil, KX139436); 52, *Ornithodoros normandi* (Tunisia, KC311544); 53, *Ornithodoros erraticus* (Algeria, KC311541); 54, *Ornithodoros zumpti* (South Africa, KR907254); 55, *Ornithodoros tholozani* (Israel, MF615997); 56, *Ornithodoros kairouanensis* (Tunisia, KC311546). The dots denote nucleotides that are identical for that position; dashes (-) represent gaps in the sequences introduced during alignment.

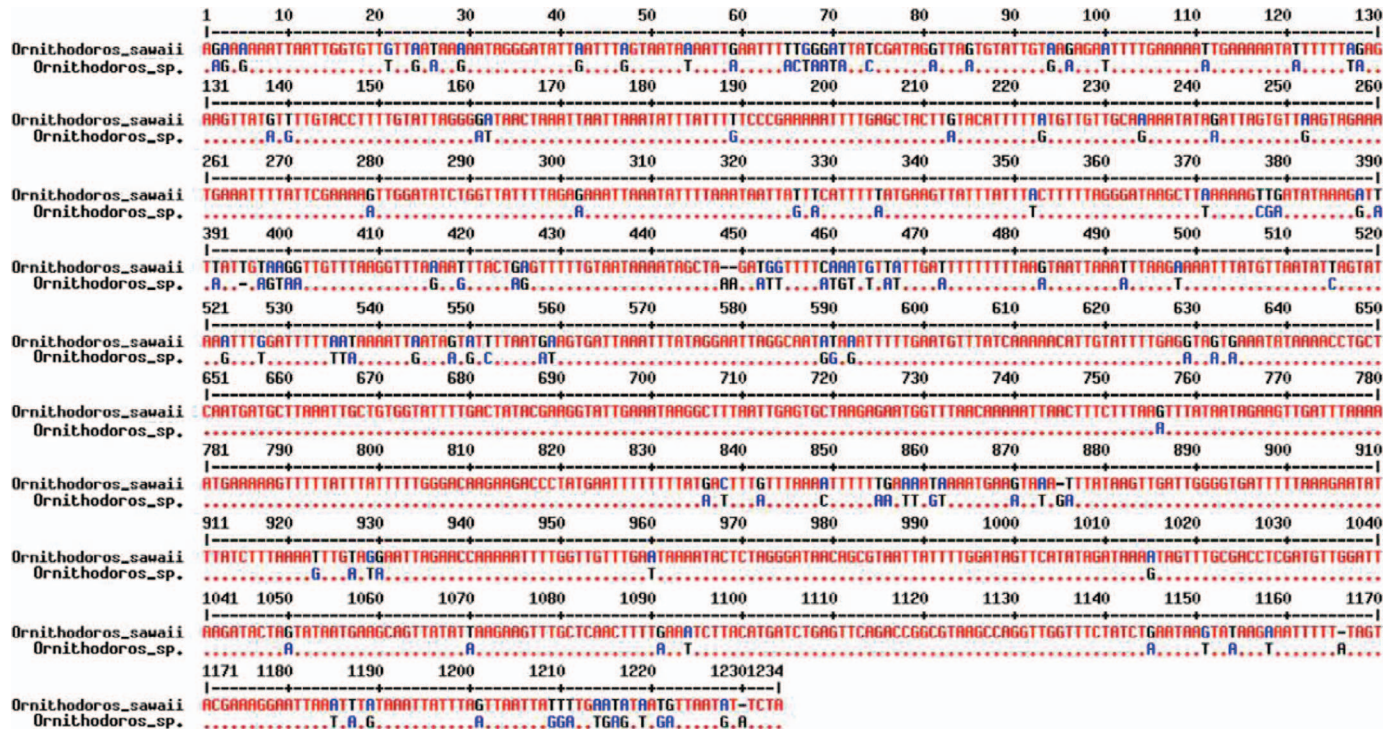


Figure 7. Alignment of the 16S rRNA full sequence of the new *Ornithodoros* sp. (domestic isolate, MK613790) and *Ornithodoros sawaii* (domestic isolate, MK606044). Color version is available online.

southern islands because migratory birds migrate to these islands from East Asia and North America to breed and raise their chicks.

In Japan, *O. sawaii* has been recorded from 2 seabird species, the streaked shearwater, *Calonectris leucomelas* (Temminck), and Swinhoe's storm petrel, *H. monorhis* (Kitaoka and Suzuki, 1973; Kawabata et al., 2006; Takano et al., 2014). In South Korea, *O. sawaii* has been reported from ancient murrelet, *Synthliboramphus antiquus*, *H. monorhis* (Kim et al., 2015, 2016), and *L. crassirostris* (Kim et al., 2017; this study). As the feeding time of soft ticks is shorter than that of hard ticks, their repletion time can be less than 1 hr (Dietrich et al., 2011), and thus the dispersal of soft ticks through the host may be more limited and occur only in a very restricted space (Heath, 1987). This suggests that the new *Ornithodoros* sp. collected in this study has lived on these uninhabited islands and formed colonies for a long time. *Ornithodoros sawaii*, however, was detected on Gugul-do and Chilbal-do near Japan and may have been transported by Japanese migratory birds. It is well known that ticks are ectoparasites; however, this does not mean that they transmit pathogens, as their locations on the uninhabited islands are not accessible to the public. The species *L. crassirostris* breeds and raises its chicks on uninhabited islands, where it remains throughout the coldest season. It then moves to the coast for feeding, living near humans; therefore, the possibility that migratory birds function as vectors of pathogens cannot be completely excluded.

This study has some limitations. Studies are needed to evaluate tick-borne pathogens from soft ticks collected in this study such

as *Borrelia* spp. and *Rickettsia* spp., among others. Whole-genome sequencing of the new *Ornithodoros* sp. and *O. sawaii* has not been conducted in South Korea; only full-length 16S rRNA gene sequencing was conducted. Thus, additional studies are needed.

In this study, we found that the new *Ornithodoros* sp. inhabited the western islands Chilsan-do, Hong-do, and Nan-do and were mainly bred and reared by the black-tailed gull. This indicates that black-tailed gull (Chilsan-do and Nan-do) and *H. monorhis* (Chilbal-do) dominate these islands (respectively). This is the first record of the new species that was found in 44 soft ticks collected from nests (with soil material) of *L. crassirostris* on the breeding islands Nan-do, Chilsan-do, Chilbal-do, and Hong-do (Hallyeo-haesang). We found molecular differences for the new *Ornithodoros* sp. via a significantly different sequence from the 16S rRNA gene and *COI* gene, but not morphological differences. Significant variability was found in the nucleotide sequences of *O. capensis*, the new *Ornithodoros* sp., and *O. sawaii* in the partial sequence of 16S rRNA. We also found that the host of the new *Ornithodoros* sp. was *L. crassirostris*. Further studies are needed to investigate the pathogens of ticks and additional inhabited western and eastern islands to collect ticks to obtain more detailed information.

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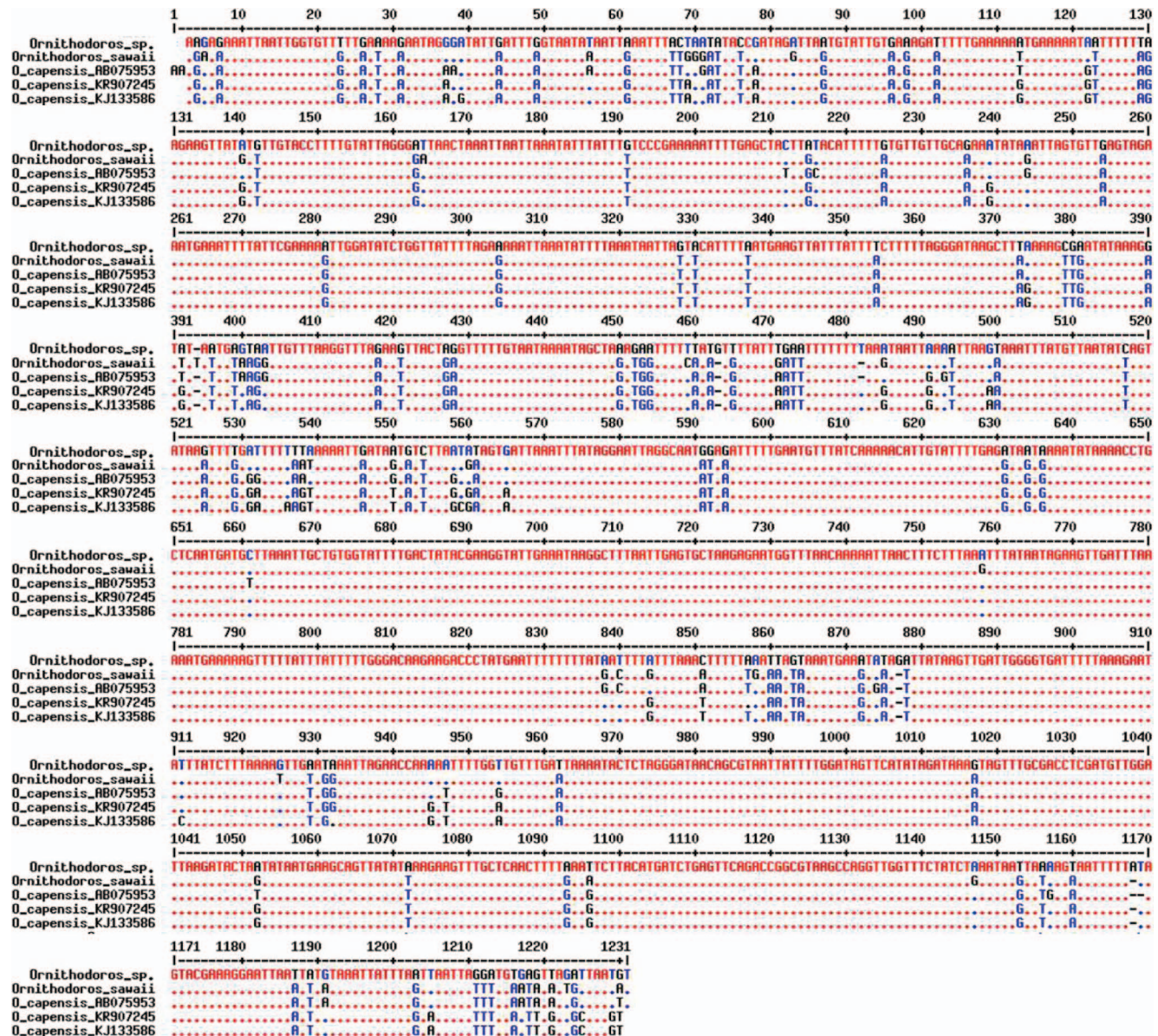


Figure 8. Alignment of the 16S rRNA full sequence of the new *Ornithodoros* sp. (domestic isolate, MK613790), *Ornithodoros sawaii* (domestic isolate, MK606044), and *Ornithodoros capensis* (Japan, AB075953; South Africa, KR907245; South Africa, KJ133586). Color version is available online.

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