

## CORRECTED PROOF

## Research Article

## First record and origin of the non-native Peking gecko *Gekko swinhonis* Günther, 1864 (Squamata: Gekkonidae), newly discovered in Incheon Metropolitan City, South Korea

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### Abstract

Listing alien species is becoming more important due to the global spread of infectious diseases such as COVID-19, chytrid fungus, and ranavirus. We determined the identity and origin of newly discovered geckos (hereafter, Incheon gecko) near Incheon Port, South Korea, in September 2021, through morphological and genetic studies. The number of interorbitals of the Incheon geckos overlapped only with those of *Gekko swinhonis*, among four closely allied species. In addition, the cytochrome *c* oxidase subunit 1 (*COI*, 658 bp) sequence of Incheon geckos had a close genetic *p*-distance (0.00–5.47%) with that of *G. swinhonis* in China. Furthermore, Incheon geckos shared major mitochondrial cytochrome *b* (*Cytb*, 1,140 bp) haplotypes with *G. swinhonis* samples collected in coastal cities along the Bohai Bay in northeast China, including Huludao, Shedao, Laiyang, and Tianjin, where regular car-ferry routes connect them to Incheon. These results suggest that the geckos, newly discovered in Incheon, South Korea, are *G. swinhonis* and were recently introduced from China via ocean cargo, showing a clear case of human-mediated introduction of non-native geckos.

**Key words:** Bohai Bay, car-ferry, introduction, invasive gecko, reptile

### Introduction

A non-native species refers to a species that has been introduced to another area outside its natural habitat by humans, regardless of intention (Kraus 2009). The number of non-native species introduction is increasing due to the development of transportation methods, increased international trade, and continuous economic growth (Pili et al. 2023). Invasive species, which negatively impact endemic ecosystems, cause the decline of endemic species through food and habitat competition, disrupt the ecological food web, and cause various negative impacts, including disease or parasite transmission (Casey et al. 2015; Bradley et al. 2019; González-Sánchez et al. 2021), within the introduced ecosystem. Given the global spread of infectious diseases such as COVID-19, chytrid fungus, and ranavirus through international exchanges in modern times, it is becoming increasingly important to fully document cases of alien species invasions (Borzée et al.

2021). Invasion by alien species has been observed in various taxa, and the cases of reptile invasions are also increasing (Kraus 2009; Duenas et al. 2021; Pili et al. 2023).

Reptile including geckos, anoles, and sliders are well-known taxon for non-native species (Kraus 2009; Weterings and Vetter 2018; Nania et al. 2020; González-Sánchez et al. 2021). In particular, geckos, which have well-developed toe pads that can easily attach to goods, are often introduced through aircraft or ocean cargo between countries (Behm et al. 2019; Schneider et al. 2022). They also have hard-shelled, adhesive eggs, which are also important for facilitating introduction (Rocha et al. 2022). In addition, due to their popularity as pets, geckos are accidentally or intentionally released into the nonnative ecosystem after moving through the international pet trade (Casey et al. 2015; Pili et al. 2023). Introduced geckos are known to be highly adaptable to new environments, resulting in the disruption of native gecko populations (Perella and Behm 2020; Nordberg and Schwarzkopf 2022). They may also introduce new parasites into the endemic population (Barnett et al. 2018; Borzée et al. 2021).

Two anthropogenically-introduced gecko species, *Gekko japonicus* (Schlegel, 1836) and *Hemidactylus frenatus* (Schlegel, 1836), have been reported in South Korea. *Gekko japonicus*, also found in China and Japan, was first reported in South Korea by Stejneger (1907) in Busan and inhabits several southern coastal cities, including Busan, Gimhae, and Mokpo (Kim et al. 2019b). Although *Gekko japonicus* in Busan was likely naturally dispersed from China or indirectly through Japan, those in Mokpo were likely introduced by humans directly from Japan (Kim et al. 2019b; Kim et al. 2020). Another species, *H. frenatus*, has globally dispersed from Southeast Asia as a non-native species and is now found in Central America, Northern Australia, and many Pacific islands (Weterings and Vetter 2018). In South Korea, there are observation records of *H. frenatus* in Busan (Stejneger 1907) and Incheon (Jang *personal observation*), but established populations have never been identified.

In September 2021, during a field survey, a new gecko population was discovered at the Incheon Customs History Park, near Incheon Port, located in Sinpo-dong, Incheon Metropolitan City, South Korea. In this study, we morphologically and genetically identified the species of the geckos, verified whether they were non-native species, and determined the genetic status of the population and possible dispersal or introduction routes to Incheon.

## Materials and methods

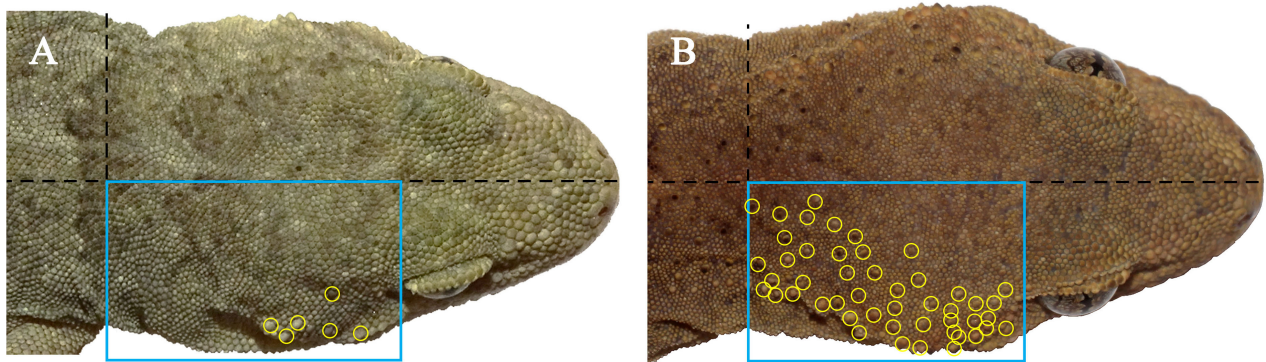
### *Field survey and sampling*

We conducted three field surveys during the three hours after sunset. We specifically targeted residential and commercial areas within a 1.5 km radius



**Figure 1.** Survey sites (circles, A) and the building in the Incheon Customs History Park (B), indicated by a yellow arrowhead, near the Incheon Port, where many geckos were found. Red and white circles indicate the presence and absence of geckos, respectively.

of the Incheon Customs History Park (37.468681°N; 126.622807°E), where the geckos were first observed (Figure 1B). On September 21, 2021, we surveyed only the Incheon Customs History Park, and on April 28, 2022, surveyed 30 potential habitat sites, including the Incheon Customs History Park, old historical buildings, churches, cathedrals, schools, and city parks in the northern parts of the park (Figure 1A). On August 18, 2022, in mid-summer, we surveyed 60 potential habitat sites, including the previous 30 sites, in the northern and southeastern parts of the park (Figure 1A). For specimens, we sampled only adult geckos (Snout-vent length, SVL > 45 mm; Kim et al. 2019a), which have fully developed characteristics and matured features, from the Incheon Customs History Park. We collected 7 males and 6 females (Kangwon National University Herpetology Laboratory [KNUHL] voucher numbers: G01572GS–G01584GS) on September 21, 2021 and additional 5 males and 3 females (KNUHL voucher numbers: G04001GS–G04008GS) on April 22, 2022 when the geckos (hereafter, Incheon gecko) were discovered. Geckos were determined to be male when preanal pores were present (Zhao et al. 1999). The SVL of the captured geckos was measured in units of 0.1 mm using a Vernier caliper (CD15CPX, Mitutoyo Korea Corporation, Gunpo, South Korea). We took pictures of the head (dorsal, lateral, and ventral views) and body trunk (dorsal and ventral views). After sampling the tip of the tail, approximately 1 cm long, with scissors, we released all the geckos at the capture sites. The obtained tail tissues were preserved in 99.5% ethanol at 4°C until DNA extraction. We used the first 13 adult geckos only for the morphological study and the whole group of 21 geckos for the genetic study. To collect geckos in South Korea, special permits are not required, but we followed the recommendations in the ARRIVE guidelines in this study (Percie du Sert et al. 2020).



**Figure 2.** Number of tubercles on the right-side occiput (blue boxes) in *Gekko* sp. (Kangwon National University Herpetology Laboratory [KNUHL] voucher number: G01580GS, female), newly discovered in Incheon, South Korea (A) and in *G. japonicus* (KNUHL voucher number: YenC28, male) collected in Yancheng, China (B). Tubercles are indicated by yellow circles.

### *Morphological study*

For the 13 adult geckos sampled in September 2021, we measured the SVL and counted the number of supralabials (SPL), infralabials (IFL), interorbitals (IO) between the anterior corners of the eyes, ventrals (V) at the middle of the body trunk as scale variables, and preanal pores (PP) in male (Zhao et al. 1999; Kim et al. 2019b). In addition, we counted the number of tubercles on the occiput (between the end of the eye and the thinnest part of the neck; Figure 2), which is known as a distinguishing morphological feature between *G. japonicus* and *G. swinhonis* Günther, 1864 (Rösler et al. 2011). For comparison, we counted the number of tubercles in 21 *G. japonicus* collected in Yancheng, China (Kim et al. 2020), which we had photographs of taken in the same way. All counts were performed on the right side of the gecko. For the comparison of SVL and the number of scales, we used four closely allied species, *G. swinhonis*, *G. hokouensis* Pope, 1928, *G. scabridus* Liu and Zhou, 1982, and *G. japonicus* (Zhao et al. 1999; Hoehn et al. 2007; Kim et al. 2019b). We included *G. japonicus* because the species currently inhabits South Korea, and we selected the remaining three species based on the genetic *p*-distance result of the mitochondrial cytochrome *c* oxidase subunit 1 (*COI*) gene (see the Results section) and a previous study (Rösler et al. 2011). Since the number of tubercles was normally distributed (Kolmogorov–Smirnov test,  $P > 0.05$ ), we used an independent sample T test to compare the tubercle data between Incheon geckos and Chinese *G. japonicus*. All statistical analyses were performed using SPSS v. 26 (IBM Corp, Armonk, USA).

### *Genetic Study*

#### DNA extraction, amplification, and sequencing

For genetic analysis, genomic DNA was extracted from the tail tissues of 21 adult geckos sampled in September 2021 and April 2022 with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. We quantified the extracted genomic DNA with a Qubit 4 fluorometer

(Thermo Fisher Scientific Solutions, Seoul, South Korea). To identify the species of the Incheon geckos and to verify the origin of the population, we selected the mitochondrial *COI*, which is often used in DNA barcoding, and the cytochrome b (*Cytb*) genes, which have been studied in many gecko species (Yan et al. 2010; Jeong et al. 2013; Behm et al. 2019; Sun et al. 2019; Kim et al. 2020). The partial sequences of the *COI* gene were amplified using LC01490 and HC02198 primers (Folmer et al. 1994). The complete sequences of the *Cytb* gene were amplified by Gs\_cytb\_F (5'-ATC AAG CAC AAG CAG TAA CAA A-3') and Gs\_cytb\_R (5'-ACA GGG CTT GTC CGT TTC TG-3') primers, designed with Primer3web (<https://primer3.ut.ee/>) for this study.

The polymerase chain reaction (PCR) was performed using a SimpliAmp Thermal Cycler (Applied Biosystems, California, USA) in a volume of 20  $\mu$ l, which consisted of 1  $\mu$ l of template DNA (5 ng/1  $\mu$ l), 0.5  $\mu$ l each of 10 pmol forward and reverse primers, and 10  $\mu$ l of 2X TOPsimple™ PreMIX-nTaq (Enzynomics, Daejeon, South Korea), and finally adjusted with molecular biology grade water (HyClone, Massachusetts, USA). The PCR products were confirmed on a 1% agarose gel and sequenced at Macrogen (Seoul, South Korea).

#### Identification of the species

We visually inspected and aligned the obtained sequences using MUSCLE (Edgar 2004) and trimmed the sequences in Geneious Prime v.2022.0.2 (Biomatters Ltd., Auckland, New Zealand). We finally used 658 bp for *COI* and 1,140 bp for *Cytb* in the analyses. For species identification, we analyzed the *COI* gene. Using DnaSP v.6 (Rozas et al. 2017), we identified the nucleotide mutations in the *COI* (658 bp) sequence and determined the haplotype of 21 Incheon geckos. Newly identified haplotypes of *COI* were deposited in GenBank. To identify the species based on genetic differentiation between the sequences of Incheon geckos and those of closely allied species in the genus *Gekko*, we calculated the genetic *p*-distance with MEGA v.6 (Tamura et al. 2013). For the analysis, we selected four allied species of *G. swinhonis* (GenBank accession numbers: HM802938, HM802939, HM802943, and MZ049223; Yan et al. 2010; Zhang et al. 2021), *G. hokouensis* (KT005801; Hao et al. 2016), *G. scabridus* (HM802950; Yan et al. 2010), and *G. japonicus* (KR996131; Kim et al. 2016), based on previous phylogenetic studies (Rösler et al. 2011; Sun et al. 2019), which had available *COI* sequences in GenBank.

#### Origin of the Incheon population

To evaluate the genetic diversity of the Incheon geckos, we analyzed the mitochondrial *Cytb* gene. Using DnaSP v.6. (Rozas et al. 2017), we identified the nucleotide mutations in the *Cytb* (1,140 bp) sequence and determined

the haplotypes of 21 Incheon geckos. Newly identified haplotypes of *Cytb* were deposited in GenBank. We subsequently calculated the number of haplotypes (Nh), haplotype diversity (h), and nucleotide diversity ( $\pi$ ) of the *Cytb* sequence of the Incheon geckos using the same program.

To determine the origin of the Incheon population, we produced a haplotype table and compared the *Cytb* haplotypes of 21 Incheon geckos with those of 301 *G. swinhonis* (GenBank accession numbers: GQ131337–GQ131373; Yan et al. 2010), which were collected from 27 Chinese populations across its distribution range in a previous study by one of our authors (Yan et al. 2010). We also produced a median-joining haplotype network of the *Cytb* haplotypes using PopART v.1.7 (<https://popart.maths.otago.ac.nz/>) to evaluate the genetic relationships between the Incheon population and the Chinese populations. In addition, to investigate the phylogenetic relationships between the Incheon population and the Chinese populations, we constructed a Bayesian inference (BI) tree with the 38 whole *Cytb* sequence (1,140 bp) haplotypes of 41 individuals (4 from Incheon and 37 from China). As outgroups, we included the *Cytb* sequence haplotypes of *G. japonicus* (GenBank accession number: KR996131; Kim et al. 2016), *G. auriverrucosus* Zhou and Liu, 1982 (GQ857615; Yan et al. 2010), and *G. hokouensis* (KT005801; Hao et al. 2016). As a phylogenetic analysis model, we selected the TIMef model out of 56 models as the most suitable model using ModelTest 3.7 (Posada 2003). The BI tree was constructed through 2,000,000 model generations with the Markov chain Monte Carlo (MCMC) method in MrBayes v.3.2.7 (Ronquist et al. 2012). The first 5% of the initial results (100,000 generations in total) were removed as burn-in. We used FigTree v. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) to visualize the phylogenetic tree. We also constructed the BI tree using *Cytb* sequence data of 322 whole *G. swinhonis* individuals, including the same 3 outgroups, and provided the result as Supplementary material Figure S1.

## Results

### *Morphological and genetic identification of the species*

At the Incheon Customs History Park, we found a total of 179 geckos (54 in September 2021, 15 in April 2022, and 110 in August 2022). Additionally, we found five geckos in a residential area located in the southeastern part of the Incheon Customs History Park in August 2022 (Figure 1A). However, we did not find any geckos in other residential and commercial areas in the northern and eastern parts of the park during the surveys (Figure 1A).

We provided detailed morphological characteristics of the 13 adult geckos collected in Incheon in Table 1. Among the six measured variables, SVL, SPL, IFL, V, and PP overlapped with those of the four allied species,

**Table 1.** Comparison of morphological features of *Gekko* sp., newly discovered at Incheon Customs History Park, South Korea, with those of four closely allied species, *G. swinhonis*, *G. japonicus*, *G. hokouensis*, and *G. scabridus*. Data are presented as mean  $\pm$  s.d. (range). SVL, snout-vent length; SPL, supralabials; IFL, infralabials; IO, interorbitals; V, ventrals; PP, preanal pores in males.

Parameters		SVL	SPL	IFL	IO	V	PP	Reference
<i>Gekko</i> sp.	Male (n = 7)	54.6 $\pm$ 4.7 (49.0–61.3)	10.6 $\pm$ 0.5 (10–11)	9.7 $\pm$ 0.5 (9–10)	23.4 $\pm$ 0.8 (23–25)	40.0 $\pm$ 1.2 (38–41)	8.0 $\pm$ 0.6 (7–9)	This study
	Female (n = 6)	59.6 $\pm$ 4.1 (53.0–63.4)	11.0 $\pm$ 0.0 (11)	10.5 $\pm$ 0.6 (10–11)	23.8 $\pm$ 1.2 (22–25)	40.8 $\pm$ 1.5 (39–43)	NA	
<i>Gekko swinhonis</i>		< 66	7–12	7–11	23–24	40	7–9	Zhao et al. 1999; Rösler et al. 2011
<i>Gekko hokouensis</i>		< 70	10–14	8–11	30–33	36–43	5–9	Zhao et al. 1999; Rösler et al. 2011
<i>Gekko scabridus</i>		< 77	9–11	9–11	30	NA	10–15	Zhao et al. 1999; Rösler et al. 2011
<i>Gekko japonicus</i>		43.6–75.0	9–14	7–13	26–44	35–58	2–9	Zhao et al. 1999; Rösler et al. 2011; Kim et al. 2019b

**Table 2.** Uncorrected pairwise genetic  $p$ -distances (%) of the *COI* (658 bp) haplotypes between the *Gekko* sp. (Types 1–3), newly discovered at Incheon Customs History Park, South Korea, and four closely allied species, *G. swinhonis*, *G. hokouensis*, *G. scabridus*, and *G. japonicus*.

Species (GenBank accession number)	1	2	3	4	5	6	7	8	9	10
1 Type 1 (OQ326914)										
2 <i>Gekko</i> sp. Type 2 (OQ326915)	5.32									
3 Type 3 (OQ326916)	0.15	5.47								
4 <i>Gekko swinhonis</i> (HM802938)	0.00	5.32	0.15							
5 <i>Gekko swinhonis</i> (MZ049223)	0.15	5.47	0.00	0.15						
6 <i>Gekko swinhonis</i> (HM802939)	0.15	5.47	0.30	0.15	0.30					
7 <i>Gekko swinhonis</i> (HM802943)	5.32	0.00	5.47	5.32	5.47	5.47				
8 <i>Gekko hokouensis</i> (KT005801)	16.87	17.48	16.87	16.87	16.87	17.02	17.48			
9 <i>Gekko scabridus</i> (HM802950)	18.84	19.15	19.00	18.84	19.00	19.00	19.15	20.67		
10 <i>Gekko japonicus</i> (KR996131)	21.43	21.58	21.28	21.43	21.28	21.58	21.58	20.67	22.34	

while IO overlapped only with *G. swinhonis* but not with the other three allied species. The number of tubercles on the occiput ( $7.1 \pm 2.9$ ,  $n = 13$ ) of Incheon geckos was significantly less than that of Chinese *G. japonicus* ( $32.8 \pm 8.3$ ,  $n = 21$ ) ( $t = 10.78$ ,  $df = 32$ ,  $P < 0.001$ ; Figure 2).

Nucleotide mutations were found in a total of 36 variable sites in the *COI* gene (658 bp) of Incheon geckos, generating 3 haplotypes (GenBank accession numbers: OQ326914–OQ326916). The genetic  $p$ -distance of the *COI* haplotypes of Incheon geckos was the closest to those of *G. swinhonis*, ranging from 0.00 to 5.47%, followed by *G. hokouensis* (16.87–17.48%), *G. scabridus* (18.84–19.15%), and *G. japonicus* (21.28–21.58%, Table 2).

#### Origin of the Incheon *G. swinhonis* population

Nucleotide mutations were found in a total of 73 variable sites in the *Cytb* gene (1,140 bp) of 21 Incheon geckos, generating 4 haplotypes (GenBank accession numbers: OQ326593–OQ326596). The first three haplotypes, H1, H4, and H24, were previously found in Chinese *G. swinhonis* populations (Yan et al. 2010), while the fourth haplotype, H38, was newly observed in the Incheon population (Figure 3, Table S1). The haplotype diversity ( $h \pm$  s.d.)

of the Incheon population's *Cytb* gene was  $0.776 \pm 0.038$ , and the nucleotide diversity ( $\pi \pm$  s.d.) was  $0.0214 \pm 0.0065$ .

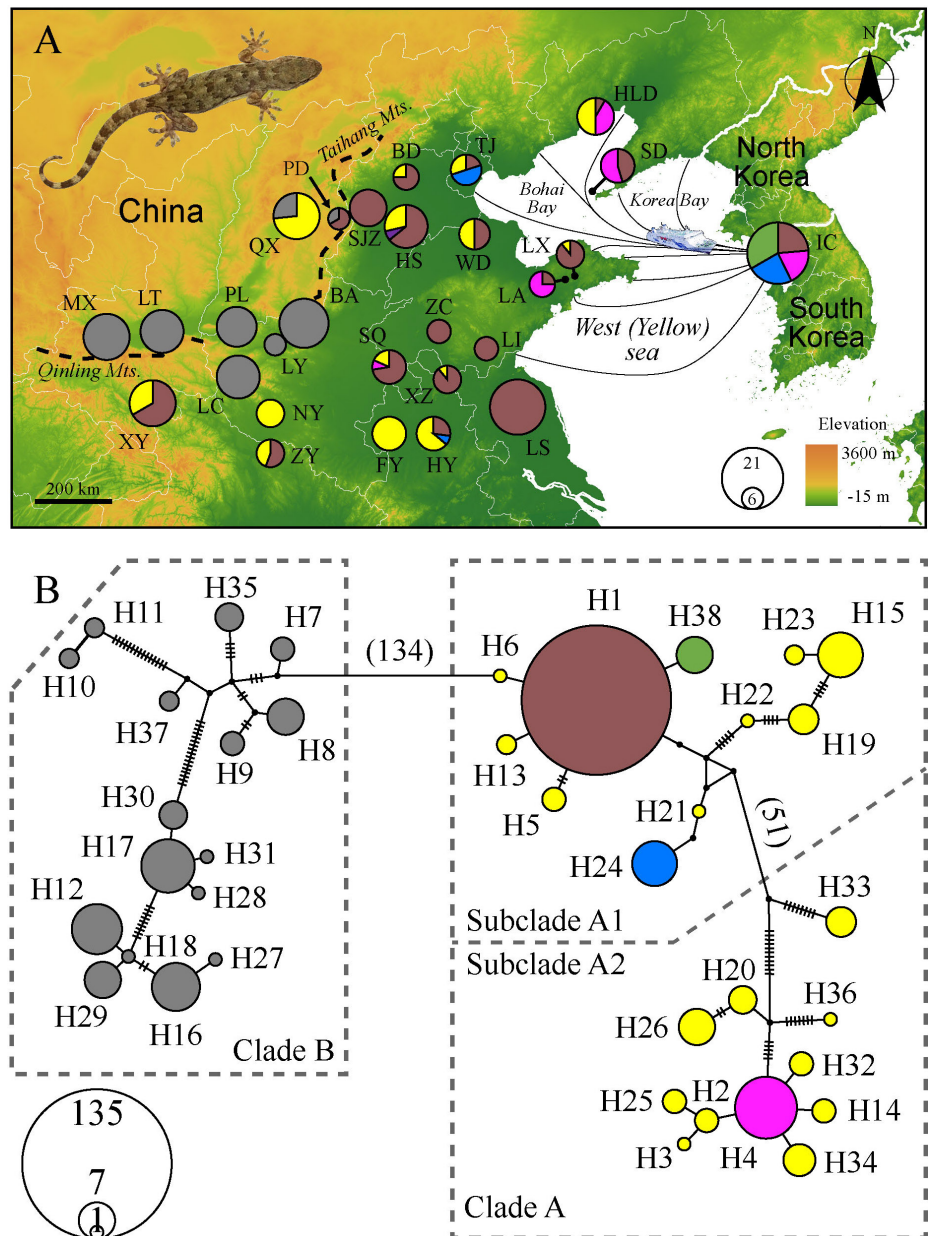
We compared the *Cytb* haplotypes of 322 whole geckos from Incheon and 27 Chinese populations (Table S1). H1, which was found in five Incheon geckos out of 21 (23.8%), was found in 19 Chinese populations (70.4%). H4, which was found in four Incheon geckos (19.1%), was the main haplotype in Huludao (41.7% of the geckos), Shedao (54.5%), and Laiyang (75.0%) in China. In addition, a few geckos in Hengshui (7.1%) and Shangqiu (9.1%) had the H4 haplotype. H24 haplotype, which was found in five Incheon geckos (23.8%), was the main haplotype of Tianjin (50.0%) and was a minor haplotype in Huaiyuan (9.1%) in China. The remaining seven Incheon geckos (33.3%) had the H38 haplotype. Interestingly, no Chinese population simultaneously had H4 and H24, which were found together in the Incheon population (Figure 3). In the haplotype network, haplotypes that were found in the Incheon population were scattered at different locations (Figure 3B). The H38 haplotype was derived from H1, H24 was from H21, and H4 was the origin for four different haplotypes. In the BI phylogenetic tree, which was constructed with the 38 whole *Cytb* sequence haplotypes of 41 geckos, Incheon geckos all belonged to clade A of *G. swinhonis* in China, which was defined in a previous study (Yan et al. 2010) (Figure 4). Incheon geckos were separately placed at four different locations in two subclades of clade A.

## Discussion

Based on morphological and genetic findings, geckos recently discovered in Incheon, South Korea, were identified as non-native *G. swinhonis*, which was introduced from China in modern times. The number of interorbitals of the Incheon geckos overlapped only with that of *G. swinhonis*. The number of tubercles on the occiput was significantly lower than that of *G. japonicus*, which inhabits in South Korea. In addition, the Incheon geckos had the closest *p*-distance with *G. swinhonis* for *COI* sequences. These results confirm that the Incheon geckos are *G. swinhonis*. Furthermore, both in the haplotype network and in the BI tree (Figures 3, 4), Incheon haplotypes and geckos were all intermixed with those of Chinese *G. swinhonis*. In addition, Incheon geckos shared their major *Cytb* haplotypes mainly with the geckos inhabiting coastal cities (Huludao, Shedao, Tianjin, and Laiyang) along the Bohai Bay in northeast China, where regular car-ferry routes operate between Incheon and those cities (Park and Ahn 2018). These results suggest that Incheon geckos are non-native *G. swinhonis* and were recently introduced from China via ocean cargo.

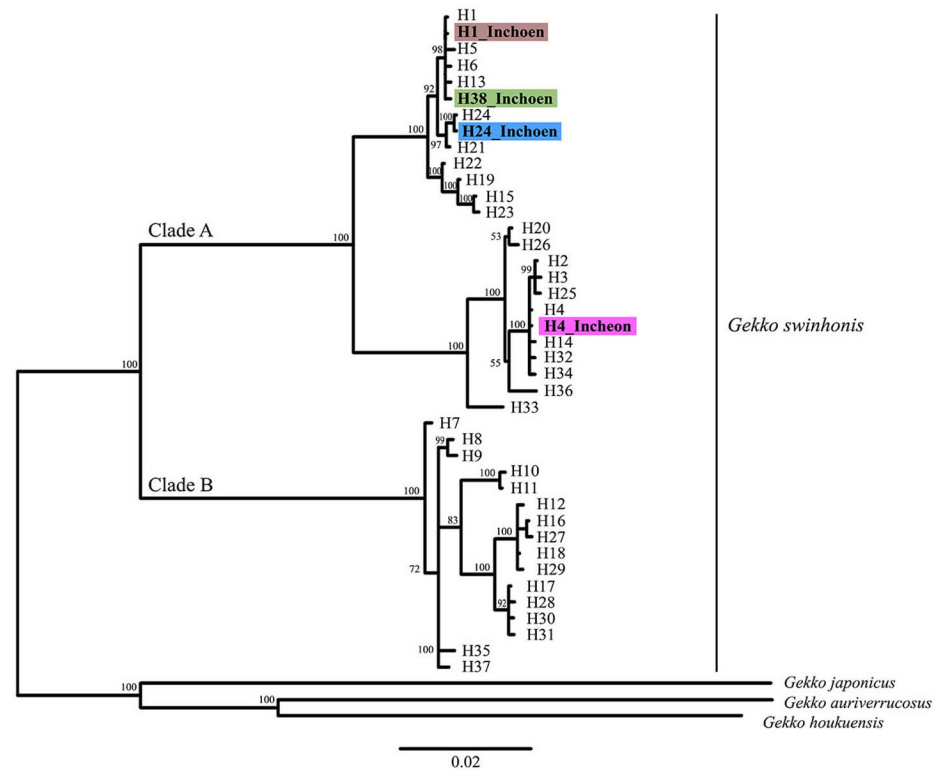
If Incheon *G. swinhonis* were naturally dispersed from China, three possible routes can be considered. The first is a route from northeastern China. If this is correct, *G. swinhonis* should also be distributed in North





**Figure 3.** Distribution map (A) and a median-joining network (B) of 38 *Cytb* (1,140 bp) haplotypes of *Gekko swinhonis* across the Incheon population in South Korea and 27 populations in China. The brown, pink, blue, and green colors indicate H1, H4, H24, and H38 haplotypes, respectively. The remaining other haplotypes were combined for clarity and indicated by yellow for the phylogenetic clade A group and gray for the clade B group (Yan et al. 2010). The sizes of the nodes (circles, B) are proportional to the number of individuals. Solid lines (A) are regular car-ferry routes between Incheon Port and 10 coastal cities in China. Population names are abbreviated: IC, Incheon, South Korea; HLD, Huludao, Liaoning; SD, Shedao, Liaoning; TJ, Tianjin; BD, Baoding, Hebei; SJZ, Shijiazhuang, Hebei; HS, Hengshui, Hebei; WD, Wudi, Shandong; LX, Laixi, Shandong; LA, Laiyang, Shandong; LI, Linyi, Shandong; ZC, Zoucheng, Shandong; QX, Qingxu, Shanxi; PD, Pingding, Shanxi; PL, Pinglu, Shanxi; MX, Meixian, Shaanxi; LT, Lintong, Shaanxi; XY, Xunyang, Shaanxi; BA, Boai, Henan; LY, Luoyang, Henan; LC, Luanchuan, Henan; SQ, Shangqiu, Henan; NY, Nanyang, Henan; XZ, Xuzhou, Jiangsu; LS, Lianshui, Jiangsu; FY, Fuyang, Anhui; HY, Huaiyuan, Anhui; ZY, Zaoyang, Hubei, China.

Korea, which is located in the middle of the dispersal route. However, there is no report on *G. swinhonis* in North Korea (Kim and Han 2009). In addition, *G. swinhonis* would be found in Ganghwa, Paju, and Gimpo, which are northern cities of Incheon in South Korea. However, there have



**Figure 4.** Bayesian inference (BI) phylogenetic tree constructed with the 38 *Cytb* sequence (1,140 bp) haplotypes of 41 *Gekko swinhonis* (4 from Incheon and 37 from China), and 3 outgroup sequences (*Gekko japonicus*, *G. auriverrucosus*, and *G. hokouensis*). The brown, green, blue, and pink colors indicate the specimens, which were collected in Incheon, South Korea, and had H1, H38, H24, and H4 *Cytb* haplotypes, respectively. Bayesian posterior probabilities (%) are reported above tree branches. The clades A and B were as defined in Yan et al. (2010).

been no reports, despite three nationwide reptile surveys (Jeon et al. 2023). Considering these points, this route is unlikely. A second possible route is from the Shandong Peninsula in China through the West (or Yellow) Sea, which was land during the last glacial maximum period (25,000 ~ 15,000 years Before Present; Othman et al. 2022). If this is the case, Incheon geckos should have dominantly shared genes with geckos in cities such as Laiyang, Laixi, Linyi, and Lianshui in the Shandong Peninsula, but our results do not support this. Additionally, *G. swinhonis* should be found in Siheung, Ansan, Hwaseong, and Pyeongtaek, the westernmost coastal cities in South Korea, which are the closest to the Shandong Peninsula. However, as far as we know, Incheon Customs History Park is the only known population of *G. swinhonis* in South Korea. Considering these results, the second route is also unlikely. Finally, geckos might also have rafted to South Korea from the east coast of China on flotsam, jetsam, or vegetation (Siler et al. 2012; Kim et al. 2020). However, this is not a likely explanation considering that there are no major oceanic currents such as the Kuroshio current in the West (or Yellow) Sea. Also, except for Incheon, no reports of *G. swinhonis* are known from other eastern coastal cities in South Korea.

If the population is not established by natural dispersal, possible anthropogenic introduction via ocean cargo can be considered. Overseas

trade between the cities on the Korean Peninsula and those in Bohai Bay in northeastern China was actively carried out during the Three Kingdoms period (the 4<sup>th</sup>–5<sup>th</sup> centuries) on the Korean Peninsula (Moon 2015). If *G. swinhonis* was introduced to Incheon at this time, it should be found in trade ports in South Korea such as Deokjeokdo Island, Yeongheungdo Island, and Hwaseong City. However, there have been no reports of finding any *G. swinhonis* in these areas thus far, so the introduction at that time is unlikely. Incheon Port opened in 1883, and since then, overseas trades with eastern coastal cities along Bohai Bay and the West (Yellow) Sea in China have been conducted in earnest (Kim 2017). Since 1990, a car-ferry (a bulk carrier), has been in service, and currently, car-ferries are regularly operating to transport passengers and cargo between Incheon and 10 Chinese coastal cities (Park and Ahn 2018). If the Incheon *G. swinhonis* was introduced from China via ocean cargo, the haplotype similarity would be related to these car-ferry routes. Except for the common *Cytb* H1 and Incheon private H38 haplotypes, the H4 haplotype of Incheon geckos is the main haplotype in Huludao, Shidao, and Laiyang, and H24 is the main haplotype in Tianjin in China. Surprisingly, all of these cities or nearby cities, such as Yingkou, Dalian, Yantai, and Qingdao, have a regular car-ferry route to Incheon Port. These results strongly support that *G. swinhonis* in Incheon was introduced from China via ocean cargo in modern times.

Since recently introduced gecko populations suffered bottleneck effects and/or founder effects in the establishment process, it is possible for them to have low genetic diversity (Kim et al. 2020). However, the Incheon *G. swinhonis* population showed higher haplotype diversity of the *Cytb* gene at 0.776 than the 0.629 of the populations of clade A in China (Yan et al. 2010). This result may appear when a small number of geckos are introduced from several different populations or repeatedly introduced from a single population (Kolbe et al. 2013; Rocha et al. 2022). This process has been well studied in introduced gecko populations in North and Central America and Hong Kong (Perella and Behm 2020; Fieldsend et al. 2021; González-Sánchez et al. 2021; Dufour et al. 2022). In this study, none of the 27 populations in China had both the H4 and H24 *Cytb* haplotypes, while the Incheon *G. swinhonis* population had both simultaneously. This result indicates that geckos in the Incheon population were introduced from at least two regions in China (Kim et al. 2020; Schneider et al. 2022). In addition, the fact that Incheon geckos were placed in multiple subclades of Chinese *G. swinhonis* in the haplotype network and the BI tree also supports this explanation.

In the survey of other commercial and residential areas except for the Incheon Customs History Park, we found five additional *G. swinhonis* in only one residential area approximately 850 m southeast of the park. Although a two-lane road has to be crossed to approach the park, the residential area is

locally connected to the park by dense buildings and street trees. On the other hand, we did not find any geckos in the remaining large areas, which have potential gecko habitats in schools, cathedrals, parks, and residences (Kim et al. 2019a) and are separated by six-lane roads from the park. These results might be explained by the fact that the urban environment, isolated by wide roads, greatly reduces the spread of introduced geckos (Hoehn et al. 2007; Hibbitts et al. 2017). However, it might simply imply that there were not enough opportunities or time to spread from the original settled site to other areas because they were recently introduced. Cargo transportation is very frequent between the Incheon Port and other nearby areas, such as the container storage area. The potential for further introduction and expansion of existing *G. swinhonis* populations is very high and requires immediate attention. On the other hand, *G. swinhonis* is known as an endemic species to China and was recently listed as a vulnerable species in the IUCN red list of threatened species due to its population decline (Yan 2021). Incheon population might serve as an *ex-situ* population for its long-term conservation. Nevertheless, the possibility of disrupting Korean urban ecosystems by decreasing native moths and nocturnal insects and introducing new parasites or diseases (Borzée et al. 2021; González-Sánchez et al. 2021) should be carefully investigated in the near future.

The geckos discovered at the Incheon Customs History Park in South Korea were identified as unrecorded non-native *G. swinhonis* based on morphological and genetic results. They could have arrived in modern times from at least two different populations in China along regular car-ferry routes between the coastal cities of Incheon and Bohai Bay. Our findings may be one of the clear cases of the human-mediated introduction of non-native geckos. In addition, this is the first foreign record of the Chinese endemic *G. swinhonis* and the third gecko species reported from South Korea. Given the potential for non-native species to disrupt the native ecosystem, their designation as an invasive species and the development of management plans are urgently necessary.

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### Authors' contribution

D. Park contributed to the study conception and design. H. Kwon, J. Yan, H. Jang, JS. Kim, J. Park, and IK. Park performed material preparation, data collection, and analysis. H. Kwon wrote the first draft of the manuscript. D. Park and J. Yan edited the manuscript. All authors read and approved the final manuscript.

## Ethics and permits

Sampling, handling, and other experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Kangwon National University (KW-211104-1).

## References

- Barnett LK, Phillips BL, Heath AC, Coates A, Hoskin CJ (2018) The impact of parasites during range expansion of an invasive gecko. *Parasitology* 145: 1400–1409, <https://doi.org/10.1017/S003118201800015X>
- Behm JE, van Buurt G, DiMarco BM, Ellers J, Irian CG, Langhans KE, McGrath K, Tran T, Helmus M (2019) First records of the mourning gecko (*Lepidodactylus lugubris* Duméril and Bibron, 1836), common house gecko (*Hemidactylus frenatus* in Duméril, 1836), and tokay gecko (*Gekko gecko* Linnaeus, 1758) on Curaçao, Dutch Antilles, and remarks on their Caribbean distributions. *BioInvasions Records* 8: 34–44, <https://doi.org/10.3391/bir.2019.8.1.04>
- Borzée A, Kielgast J, Wren S, Angulo A, Chen S, Magellan K, Messenger KR, Hansen-Hendriks CM, Baker A, Dos Santos MM, Kusriani M, Jiang J, Maslova IV, Das I, Park D, Bickford D, Murphy RW, Che J, van Do T, Nguyen TQ, Chuang MF, Bishop PJ (2021) Using the 2020 global pandemic as a springboard to highlight the need for amphibian conservation in eastern Asia. *Biological Conservation* 255: 108973, <https://doi.org/10.1016/j.biocon.2021.108973>
- Bradley BA, Laginhas BB, Whitlock R, Allen JM, Bates AE, Bernatchez G, Diez J, Early R, Lenoir J, Vilà M, Sorte CJB (2019) Disentangling the abundance-impact relationship for invasive species. *Proceedings of the National Academy of Sciences of the United States of America* 116: 9919–9924, <https://doi.org/10.1073/pnas.1818081116>
- Casey CL, Hernandez SM, Yabsley MJ, Smith KF, Sanchez S (2015) The carriage of antibiotic resistance by enteric bacteria from imported tokay geckos (*Gekko gecko*) destined for the pet trade. *Science of The Total Environment* 505: 299–305, <https://doi.org/10.1016/j.scitotenv.2014.09.102>
- Duenas MA, Hemming DJ, Roberts A, Diaz-Soltero H (2021) The threat of invasive species to IUCN-listed critically endangered species: A systematic review. *Global Ecology and Conservation* 26: e01476, <https://doi.org/10.1016/j.gecco.2021.e01476>
- Dufour PC, Miot EF, So TC, Tang SL, Jones EE, Kong TC, Yuan FL, Sung YH, Dingle C, Bonebrake TC (2022) Home and hub: pet trade and traditional medicine impact reptile populations in source locations and destinations. *Proceedings of the Royal Society B* 289: 20221011, <https://doi.org/10.1098/rspb.2022.1011>
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797, <https://doi.org/10.1093/nar/gkh340>
- Fieldsend TW, Krysko KL, Sharp P, Collins TM (2021) Provenance and genetic diversity of the non-native geckos *Phelsuma grandis* Gray 1870 and *Gekko gecko* (Linnaeus 1785) in southern Florida, USA. *Biological Invasions* 23: 1649–1662, <https://doi.org/10.1007/s10530-021-02463-1>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299
- González-Sánchez VH, Johnson JD, González-Solis D, Fucsko LA, Wilson LD (2021) A review of the introduced herpetofauna of Mexico and Central America, with comments on the effects of invasive species and biosecurity methodology. *ZooKeys* 1022: 79–154, <https://doi.org/10.3897/zookeys.1022.51422>
- Hao S, Yu D, Ping J, Zhou H, Zhang Y (2016) Complete mitochondrial genomes of two gecko species, *Gekko hokouensis* and *Gekko japonicus* (Squamata, Gekkonidae). *Mitochondrial DNA Part B* 1: 346–347, <https://doi.org/10.1080/23802359.2015.1137809>
- Hibbitts TJ, Fitzgerald LA, Walkup DK, Ryberg WA (2017) Why didn't the lizard cross the road? Dunes sagebrush lizards exhibit road-avoidance behavior. *Wildlife Research* 44: 194–199, <https://doi.org/10.1071/WR16184>
- Hoehn M, Sarre SD, Henle K (2007) The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? *Molecular Ecology* 16: 3299–3312, <https://doi.org/10.1111/j.1365-294X.2007.03352.x>
- Jeon JY, Lee DK, Kim JH (2023) Functional group analyses of herpetofauna in South Korea using a large dataset. *Scientific Data* 10: 1–15, <https://doi.org/10.1038/s41597-022-01924-z>
- Jeong TJ, Jun J, Han S, Kim HT, Oh K, Kwak M (2013) DNA barcode reference data for the Korean herpetofauna and their applications. *Molecular Ecology Resources* 13: 1019–1032, <https://doi.org/10.1111/1755-0998.12055>
- Kim HS (2017) The diplomatic policy of Hanabusa Yoshimoto on Joseon and the opening of Incheon Port. *Dongguk History & Culture Institute* 63: 155–186
- Kim LT, Han GH (2009) Animal of Chosun (amphibian and reptiles). Science and Technology Publishing House, Pyongyang, North Korea, 138 pp

- Kim IH, Park J, Cheon KS, Lee HJ, Kim JK, Park D (2016) Complete mitochondrial genome of Schlegel's Japanese gecko *Gekko japonicus* (Squamata: Gekkonidae). *Mitochondrial DNA Part A* 27: 3684–3686, <https://doi.org/10.3109/19401736.2015.1079855>
- Kim DI, Park IK, Kim JS, Ota H, Choi WJ, Kim IH, Park D (2019a) Spring and summer microhabitat use by Schlegel's Japanese gecko, *Gekko japonicus* (Reptilia: Squamata: Gekkonidae), in urban areas. *Animal Cells and Systems* 23: 64–70, <https://doi.org/10.1080/19768354.2018.1554542>
- Kim DI, Park IK, Ota H, Fong JJ, Kim JS, Zhang YP, Li SR, Choi WJ, Park D (2019b) Patterns of morphological variation in the Schlegel's Japanese gecko (*Gekko japonicus*) across populations in China, Japan, and Korea. *Journal of Ecology and Environment* 43: 1–9, <https://doi.org/10.1186/s41610-019-0132-5>
- Kim JS, Park J, Fong JJ, Zhang YP, Li SR, Ota H, Min SH, Min MS, Park D (2020) Genetic diversity and inferred dispersal history of the Schlegel's Japanese gecko (*Gekko japonicus*) in Northeast Asia based on population genetic analyses and paleo-species distribution modelling. *Mitochondrial DNA Part A* 31: 120–130, <https://doi.org/10.1080/24701394.2020.1742332>
- Kolbe JJ, Lavin BR, Burke RL, Rugiero L, Capula M, Luiselli L (2013) The desire for variety: Italian wall lizard (*Podarcis siculus*) populations introduced to the United States via the pet trade are derived from multiple native-range sources. *Biological Invasions* 15: 775–783, <https://doi.org/10.1007/s10530-012-0325-7>
- Kraus F (2009) Alien Reptiles and Amphibians. Springer, Dordrecht, Netherlands, 563 pp, <https://doi.org/10.1007/978-1-4020-8946-6>
- Moon AS (2015) Sea trade routes of the Baekje to East Asia and ports of call. *International Journal of Korean History* 119: 113–158
- Nania D, Flecks M, Rödder D (2020) Continuous expansion of the geographic range linked to realized niche expansion in the invasive mourning gecko *Lepidodactylus lugubris* (Duméril & Bibron, 1836). *PLoS ONE* 15: e0235060, <https://doi.org/10.1371/journal.pone.0235060>
- Nordberg EJ, Schwarzkopf L (2022) Afraid of the dark? The influence of natural and artificial light at night on the behavioral activity of a nocturnal gecko. *Frontiers in Ecology and Evolution* 10: 821335, <https://doi.org/10.3389/fevo.2022.821335>
- Othman SN, Litvinchuk SN, Maslova I, Dahn H, Messenger KR, Andersen D, Jowers MJ, Kojima Y, Skorinov DV, Yasumiba K, Chuang MF, Chen YH, Bae Y, Hoti J, Jang Y, Borzee A (2022) From Gondwana to the Yellow Sea, evolutionary diversifications of true toads *Bufo* sp. in the Eastern Palearctic and a revisit of species boundaries for Asian lineages. *eLife* 11: e70494, <https://doi.org/10.7554/eLife.70494>
- Park SE, Ahn SB (2018) Strategies of car-ferry shipping companies according to the Korea-China Free Trade Agreement. *Journal of Korea Port Economic Association* 34: 111–132, <https://doi.org/10.38121/kpea.2018.03.34.1.111>
- Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, J. Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *Journal of Cerebral Blood Flow & Metabolism* 40: 1769–1777, <https://doi.org/10.1177/0271678X20943823>
- Perella CD, Behm JE (2020) Understanding the spread and impact of exotic geckos in the greater Caribbean region. *Biodiversity and Conservation* 29: 1109–1134, <https://doi.org/10.1007/s10531-020-01939-1>
- Pili AN, Tingley R, van Winkel D, Maria L, Chapple DG (2023) The escalating global problem of accidental human-mediated transport of alien species: A case study using alien herpetofauna interceptions in New Zealand. *Biological Conservation* 12: 109860, <https://doi.org/10.1016/j.biocon.2022.109860>
- Posada D (2003) Using MODELTEST and PAUP\* to Select a model of nucleotide substitution. *Current Protocols in Bioinformatics* 6.5: 1–14, <https://doi.org/10.1002/0471250953.bi0605s00>
- Rocha S, Trinks A, Harris DJ, Larson G, Cheke AS (2022) The global and Western Indian Ocean dispersal of house geckos from Asia using historical and Mitochondrial DNA perspectives. *Frontiers in Ecology and Evolution* 10: 791762, <https://doi.org/10.3389/fevo.2022.791762>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542, <https://doi.org/10.1093/sysbio/sys029>
- Rösler H, Bauer AM, Heinicke MP, Greenbaum E, Jackman T, Nguyen TQ, Ziegler T (2011) Phylogeny, taxonomy, and zoogeography of the genus *Gekko* Laurenti, 1768 with the revalidation of *G. reevesii* Gray, 1831 (Sauria: Gekkonidae). *Zootaxa* 2989: 1–50, <https://doi.org/10.11646/zootaxa.2989.1.1>
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* 34: 3299–3302, <https://doi.org/10.1093/molbev/msx248>

- Schneider SA, Broadley HJ, Andersen JC, Elkinton JS, Hwang SY, Liu C, Noriyuki S, Park JS, Dao HT, Lewis ML, Gould JR, Hoelmer KA, Diaz R (2022) An invasive population of Roseau Cane Scale in the Mississippi River Delta, USA originated from northeastern China. *Biological Invasions* 24: 2735–2755, <https://doi.org/10.1007/s10530-022-02809-3>
- Siler CD, Oaks JR, Welton LJ, Linkem CW, Swab JC, Diesmos AC, Brown RM (2012) Did geckos ride the Palawan raft to the Philippines? *Journal of Biogeography* 39: 1217–1234, <https://doi.org/10.1111/j.1365-2699.2011.02680.x>
- Stejneger L (1907) Herpetology of Japan and Adjacent Territory. US Government Printing Office, Washington DC, USA, 577 pp, <https://doi.org/10.5479/si.03629236.58.i>
- Sun CH, Liu DW, Huang YL, Zhou YW, Hou SL, Lu CH (2019) Genetic diversity analysis of Peking gecko (*Gekko swinhonis*) in mid-Eastern China based on mitochondrial COI and Cytb gene sequences. *Mitochondrial DNA Part B* 4: 2156–2158, <https://doi.org/10.1080/23802359.2019.1623724>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729, <https://doi.org/10.1093/molbev/mst197>
- Weterings R, Vetter KC (2018) Invasive house geckos (*Hemidactylus* spp.): their current, potential and future distribution. *Current Zoology* 64: 559–573, <https://doi.org/10.1093/cz/zox067>
- Yan J (2021) *Gekko swinhonis*. *The IUCN Red List of Threatened Species* 2021: e.T178208A127944524, <https://doi.org/10.2305/IUCN.UK.2021-2.RLTS.T178208A127944524.en>
- Yan J, Wang Q, Chang Q, Ji X, Zhou K (2010) The divergence of two independent lineages of an endemic Chinese gecko, *Gekko swinhonis*, launched by the Qinling orogenic belt. *Molecular Ecology* 19: 2490–2500, <https://doi.org/10.1111/j.1365-294X.2010.04660.x>
- Zhao E, Zhao K, Zhou K (1999) *Fauna Sinica. Reptilia Vol. 2. Squamata, Lacertilia*. Science Press, Beijing, China, 394 pp

### Web sites and online databases

- Zhang W, Zhang X, Peng Z, Guo M, Yuan C (2021) Novel gene sequence from *Gekko swinhonis*. Accession number: MZ049223. GenBank. <https://www.ncbi.nlm.nih.gov/nuccore/MZ049223>

### Supplementary material

The following supplementary material is available for this article:

**Figure S1.** Bayesian inference tree constructed with the *Cytb* sequence (1,140 bp) of 322 *Gekko swinhonis*.

**Table S1.** The *Cytb* (1,140 bp) haplotypes of 322 *Gekko swinhonis*.