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Dokdo sea lion *Zalophus japonicus* genome reveals its evolutionary trajectory before extinction

Jungeun Kim[1†], Asta Blazyte[2,3†], Jae-Pil Choi[1], Changjae Kim[4], Fedor Sharko[5,6], Sungwon Jeon[4,7], Eun-Mi Kim[8], Hawsun Sohn[8], Jong Hee Lee[8], Hyun Woo Kim[8], Mi Hyun Yoo[9], Kyunglee Lee[8], Artem Nedoluzhko[6*] and Jong Bhak[1,2,7,10*]

Abstract

Background The Dokdo sea lion (*Zalophus japonicus*), commonly referred to as Gangchi in Korea and the Japanese sea lion internationally, was endemic to the Northwest Pacific before its extinction in the 1950s. However, its origins, speciation, and genetic diversity remain poorly understood.

Results To address this, we sequenced DNA from 16 *Z. japonicus* bone fragments, obtained from Dokdo and Ulleungdo islands in Korea. Our genome-wide SNP analyses reveal *Z. japonicus* as the earliest diverged species within its genus, redefining its evolutionary relationship with the California (*Z. californianus*) and Galapagos (*Z. wollebaeki*) sea lions. Our research further elucidates the phylogeny of *Z. japonicus*, shedding light on the complexity of the genetic isolation process within its genus that was prompted by the geographic isolation of the three populations of *Zalophus* ancestral stock. Conversely, the genetic signature of the Dokdo sea lion genome can be modeled as an evolutionary pathway involving gene flow from Otariidae species with shared range. In addition, we discovered that the population decline of *Z. japonicus* started already over 100,000 years ago; however, *Z. japonicus* genome maintained a relatively high heterozygosity despite nearing extinction.

Conclusions Our genome-scale analysis sheds light on the phylogeny of *Z. japonicus*, the evolutionary pathways underlying its speciation, and its genetic diversity before extinction. Broadly, we elucidate *Zalophus* gene flow complexity and genetic diversities among extant species. Furthermore, this study offers retrospective genomic insights into the extinction process of a carnivorous marine mammal, information that could aid conservation efforts for extant Otariidae species.

Keywords *Zalophus japonicus*, Dokdo sea lion, Japanese sea lion, Otariidae, Speciation, Whole genome sequencing, Extinction, Marine mammal, Introgression, Paleogenomics

[†]Jungeun Kim and Asta Blazyte contributed equally to this work.

*Correspondence:
Artem Nedoluzhko
nedoluzhko@gmail.com
Jong Bhak
jongbhak@genomics.org
Full list of author information is available at the end of the article

Background

The Dokdo sea lion, *Zalophus japonicus* (Otariidae: Carnivora), also known as the Japanese sea lion, was a species native to East Asia, inhabiting the Dokdo and Ulleungdo islands within Korean territorial waters (Fig. 1A). Known locally as Gangchi, this species, along with other *Otariids* such as the Northern fur seal (*Callorhinus ursinus*) and Steller sea lion (*Eumetopias jubatus*), thrived in the coastal habitats of the northwest Pacific Ocean, spanning from Russia to the coastal waters of Korea and Japan [1]. Despite the absence of dated fossil records for *Z. japonicus*, the discovery of other extant pinniped fossils, such as *E. jubatus*, suggests a long-standing pinniped habitation in these regions, potentially since the Pliocene [2]. Historical sources indicate that *Z. japonicus* was hunted for human consumption in Hokkaido since the Jomon period, but it is widely believed that this subsistence hunting did not significantly impact their population dynamics [3]. Over the past two centuries, the *Z. japonicus* population experienced a drastic decline. In the mid-nineteenth century, estimates suggested a healthy population of 30,000 to 50,000, comparable to later counts of Galapagos and California sea lions [3, 4]. However, by the 1950s, their number plummeted to just 50–60, leading to their classification as extinct by the International Union for Conservation of Nature (IUCN) in 1990 [3]. This sharp decrease was largely due to extensive hunting for meat, skin, and oil between 1904 and 1925, particularly targeting females and pups [5].

Z. japonicus is recognized as a member of the genus *Zalophus*. This genus comprises three distinct species: the western Dokdo sea lion (*Z. japonicus*), the eastern California sea lion (*Z. californianus*), and the Galapagos sea lion (*Z. wollebaeki*), which inhabits the Galapagos archipelago located along the equator in the eastern Pacific (Fig. 1B) [4]. *Z. japonicus* was initially classified as a subspecies of the California sea lion, but it was reclassified as a separate species based on unique cranial features [6] and mitochondrial DNA (mtDNA) differences [7]. Evolutionary studies suggest a divergence of 2.2 million years between *Z. japonicus* and *Z. californianus*, indicating a close genetic relationship [7]. The specific evolutionary pathways leading to the isolation of these sea lion species remain unclear. Moreover, pinnipeds exhibit a wide range of individual cranial variations [8] and documented interspecific sexual behaviors [9–12]. These behaviors may have facilitated pinniped morphological diversity. While such behaviors have not been directly observed in *Z. japonicus*, the possibility is supported by observed introgression events in other pinniped species [12, 13]. To date, genomic research on *Z. japonicus* has predominantly relied on mtDNA, which captures only a limited scope of the species' genomic diversity. In these years, whole genome data has become popular to investigate the evolution of marine mammal species with low genetic diversity, such as the Indo-Pacific humpback dolphin (*Sousa chinensis*) [14], and pinnipeds [15], especially in light of their high rates of hybridization [16, 17]. Thus, comprehensive whole-genome sequencing is essential to fully understand the Dokdo sea lion's genetic makeup and its phylogenetic relationships within the Otariidae [18].

Dokdo island, a vital habitat for *Z. japonicus* in the 1900s and one of their last rookery locations, preserved biological materials that may offer novel insights. In this study, 16 bone fragments of Dokdo sea lions were unearthed from Dokdo and the nearby Ulleungdo islands, situated 87.4 km apart in the East Sea of Korea. We sequenced and compared the genomes of extinct *Z. japonicus* with those of other sequenced Otariidae species to reveal the genetic diversity and detailed phylogeny of *Z. japonicus*. Additionally, we aimed to shed light on the evolutionary processes of speciation and introgression within this species and its genus.

Results

Dokdo sea lion samples and genomic data

A total of 16 Dokdo sea lion bone samples (Z1, Z3–Z9, Z11–Z18) were excavated from Dokdo and Ulleungdo islands (Fig. 1A, Additional file 1: Figs. S1 and S2). Genetic analysis was therefore performed, utilizing available bones such as ribs and small limb bones. DNA extraction and deep sequencing via next-generation

(See figure on next page.)

Fig. 1 Dokdo sea lion habitat with sampling locations and sequencing (mapping) overview. **A** The map shows the Dokdo sea lion distribution range (striped area), Ulleungdo and Dokdo islands (red dots), and sample sites in Ulleungdo (latitude 37.51° and longitude 130.79°) and Dokdo (latitude 37.24° and longitude 131.86°) islands (red markers). Blue markers mark the two locations where Dokdo sea lions were last seen before being declared extinct and denote the year of last sighting in the location. **B** The species ranges and geographic distribution of *Zalophus* and other Otariidae species relevant in this study. Dark red denotes the species range of the Dokdo sea lion (*Z. japonicus*), pink denotes species range of California sea lion (*Z. californianus*), and yellow denotes Galapagos sea lion (*Z. wollebaeki*). Purple semi-transparent area denotes the species range of the Steller sea lion (*E. jubatus*), which partially overlaps the ranges of Dokdo sea lion and California sea lion. The species distribution range of Northern fur seal (*C. ursinus*) is not shown because it overlaps the range of the Steller sea lion almost entirely. **C** Dokdo sea lion (*Z. japonicus*) genome sequencing coverage (breadth of coverage; of protein coding regions—CDS, green, and whole genome—WGS, blue, respectively) comparison with California sea lion (*Z. californianus*) and Northern fur seal (*C. ursinus*), utilizing Steller sea lion (*E. jubatus*) genome as a reference

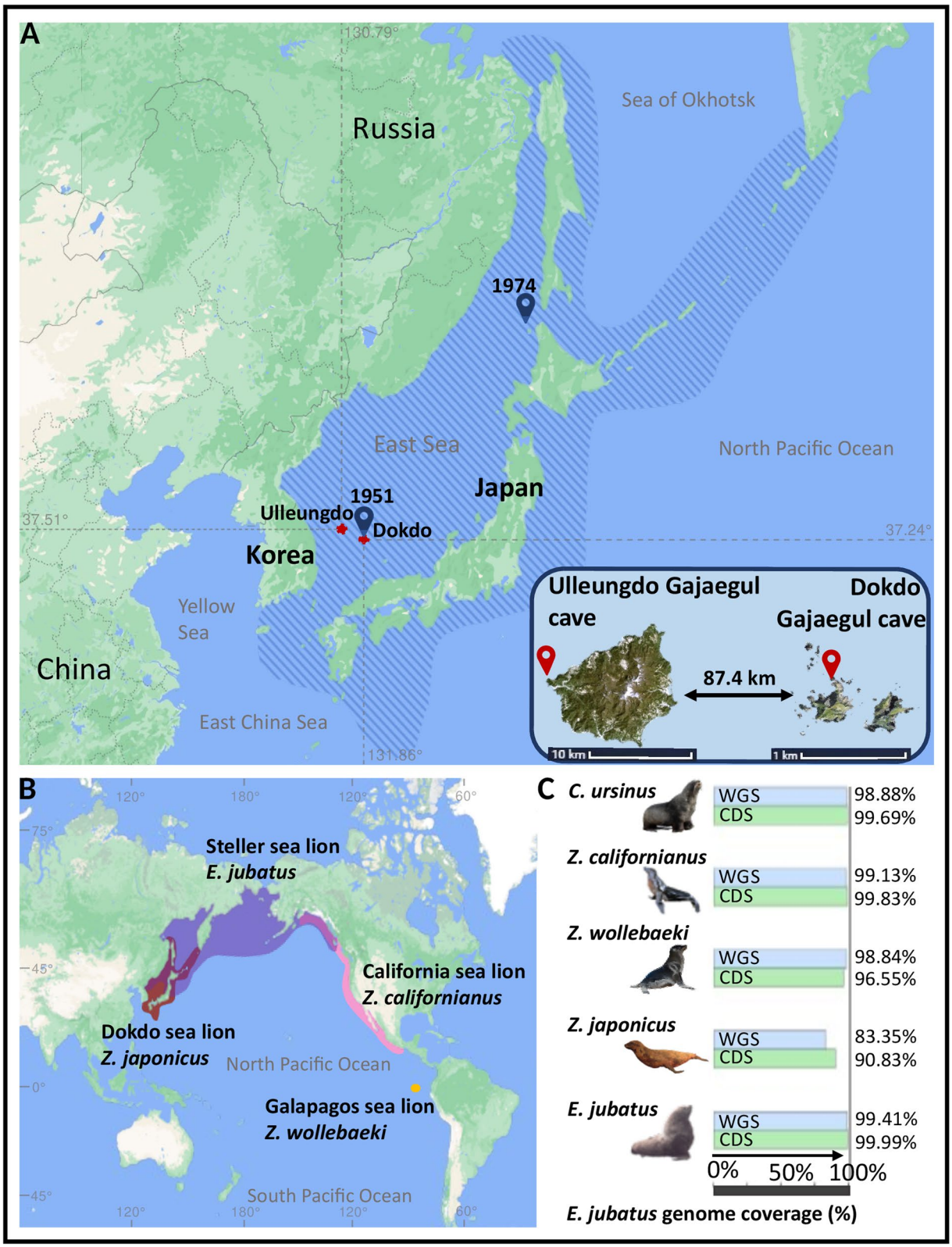


Fig. 1 (See legend on previous page.)

sequencing (NGS) were successfully conducted, allowing for further genomic analysis despite the challenges posed by the sample condition (Additional file 2: Data S1). Detailed information about laboratory techniques used is presented in Supplementary Material. Initial tests on sample Z3 using single-end (SE) and paired-end (PE) NGS revealed higher mapping rates with PE against a *Z. californianus* reference genome (ver. ZalCal 2.2; GCA_900631625.1) [19], leading us to construct PE NGS libraries for all samples. We generated 8.4 Tb of data with individual mapping rates ranging from 0.1% to 1.3% (ver. ZalCal 2.2; GCA_900631625.1) [19]. We analyzed the distribution of read lengths of *Z. japonicus* mapped to the *Z. californianus* reference genome (Additional file 1: Fig. S3). The median lengths of these reads, across 16 libraries, ranged from 85 to 151 bp; however, the presence of much shorter reads suggested endogenous DNA damage. The PALEOMIX pipeline [20] was used for bioinformatics analysis, aligning 47G reads to the *Z. californianus* genome (Additional file 1: Table S1). During this process, overlapped PE reads merged, resulting in a recovered short read length of 139 bp (Additional file 1: Fig. S4) and an average genome-wide coverage of 1.55× (Additional file 1: Table S1). DNA misincorporation levels were low (Additional file 1: Fig. S5), consistent with the species' fairly recent extinction [21].

Additionally, we sequenced modern *Z. californianus* and obtained DNA from other pinniped species for a comprehensive comparative analysis (Additional file 2: Data S2). Initially, we compared genome coverage of *Z. japonicus* with closely related Otariidae species (*Z. californianus, Z. wollebaeki*, *E. jubatus*, and *C. ursinus*) by aligning the reads to the *E. jubatus* reference genome (ASM402803v1; GCF_004028035.1) [22]. The *Z. japonicus* reads covered 83.35% of the *E. jubatus* genome and 90.83% of its protein-coding genes (Fig. 1C, Additional file 2: Data S2). In comparison, genome coverage for modern Otariidae species ranged from 98 to 99%, with protein-coding gene coverage between 91 and 99% (Fig. 1C). These data serve as a foundation for further analyses of the evolutionary history and phylogenetics of Otariidae species, contributing to a broader understanding of their genetic relationships.

Congeneric Zalophus speciation involved introgression

Dokdo sea lion genetic ancestry and relationship with other Otariidae species is not yet well understood and has not yet been studied outside the morphological classification and mtDNA analysis [7, 23, 24]. To elucidate the genomic composition of *Zalophus* sea lions, *f4*-statistics *Zalophus* and with other pinnipeds.

Firstly, the *f4*-statistics unsurprisingly show that congeneric *Zalophus* species share the highest genetic affinities among each other compared to *E. jubatus*. This genetic evidence supports all *Zalophus* sea lion classification within the same genus despite their geographically distinct species ranges (Additional file 1: Table S2). Secondly, *Z. japonicus* exhibits a distinct genetic makeup from its closest relatives, *Z. californianus* and *Z. wollebaeki* (Fig. 2A and Additional file 1: Table S3). The *f4*-statistics, *f4*(*C. ursinus*, *E. jubatus*; *Z. japonicus, Z. wollebaeki*) and *f4*(*C. ursinus*, *E. jubatus*; *Z. japonicus, Z. californianus*), show significantly positive values, indicating that *Z. wollebaeki* and *Z. californianus* share excess alleles with *E. jubatus* (or a closely related lineage), whereas *Z. japonicus* does not. These findings suggest asymmetric allele sharing, consistent with gene flow between *E. jubatus* and the other two *Zalophus* species, but not with *Z. japonicus*, indicating its distinct evolutionary path. In contrast to the *f4*-statistics results, the *f3*-statistics revealed strong admixture signals involving *Z. japonicus*. When *Z. japonicus* was used as the target population, we observed significantly negative *f3*-statistics with various combinations of source populations including *E. jubatus*, *C. ursinus*, and the other *Zalophus* species (Fig. 2B). This pattern suggests that *Z. japonicus* harbors genetic components derived from multiple pinniped lineages, even though such signals were not evident in the *f4*-statistics. Notably, no such admixture signals were detected when *Z. californianus* or *Z. wollebaeki* were used as target populations, suggesting that gene flow from *Z. japonicus* into these species did not occur. These contrasting patterns support the notion that *Z. japonicus* followed a distinct evolutionary trajectory. While neither *f3*- nor *f4*-statistics can determine the direction or timing of gene flow, qpGraph analysis (Fig. 2C) offers a plausible admixture model that fits the observed allele sharing patterns. The qpGraph results suggest that *Z. japonicus* diverged early in the *Zalophus* lineage and subsequently retained genetic components from admixture events involving both *C. ursinus* (64%) and *E. jubatus* (about 10%) (Fig. 2C). This model reflects a unique admixture pattern in *Z. japonicus*, likely influenced by the historical range and migratory behaviors of *C. ursinus*, which facilitated gene flow from these distinct lineages. Given *C. ursinus*'s extensive range across the North Pacific, it is plausible that seasonal migrations and overlapping habitats with *Z. japonicus* created opportunities for admixture. These environmental and geographical factors may have enabled *Z. japonicus* to retain genetic components from both *C. ursinus* and *E. jubatus*. In contrast, *Z. californianus* and *Z. wollebaeki* appear in the qpGraph analysis as more independently evolving lineages after their divergence, maintaining a greater degree of genetic isolation from other species. This indicates that while *Z. japonicus* was influenced by multiple

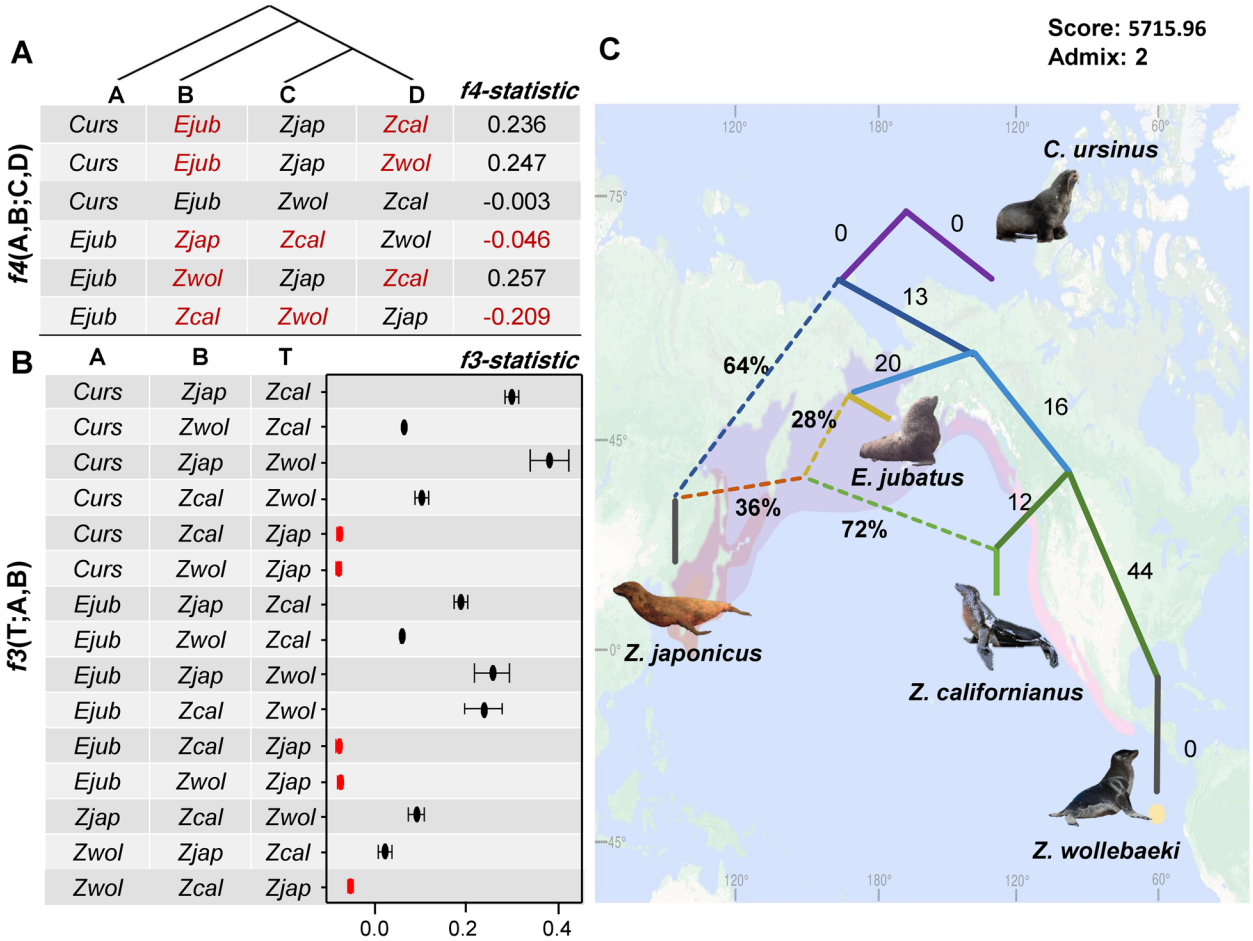


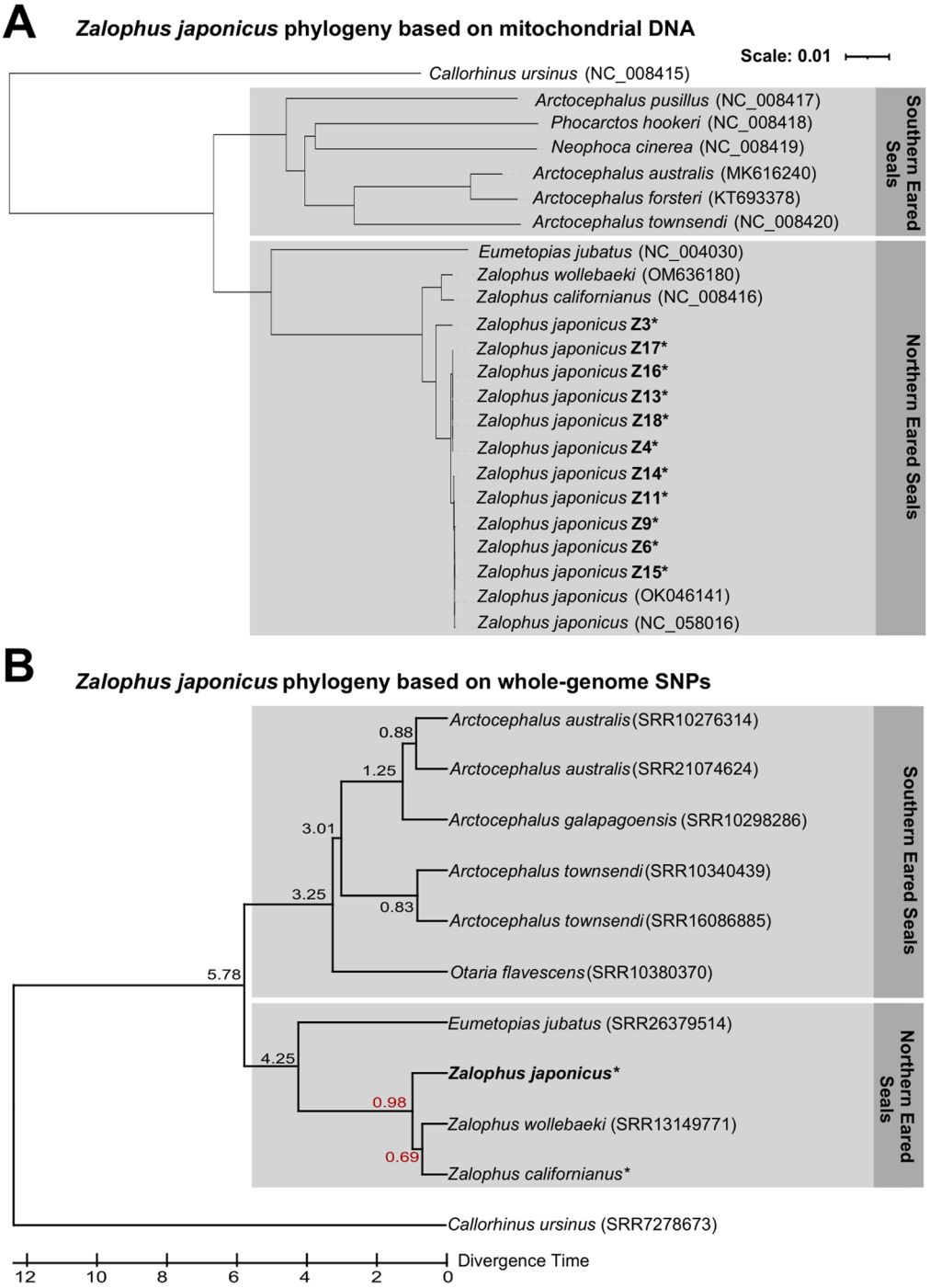
Fig. 2 Genetic ancestry of Dokdo sea lion. **A** Genetic ancestry of Otariidae species based on *f4*(A,B;C,D) statistics. The B genome has a higher genetic affinity with the D genome when the Z-score is > 3. On the other hand, the B genome has a higher genetic affinity with the C genome when the Z-score is < −3. The red text in the table indicates statistically identical genetic ancestry. The raw data for *f4*-statistics is presented in Additional file 1: Table S3. B Admixture *f3* statistics using notation *f3*(T;A,B). In this statistic, negative Z-scores < −3 indicate A and B genomes were admixed in the target genome (T). Instances with negative *f3*-statistics are presented in red color. The raw data for *f3*-statistics is presented in Table S4. **C** Genetic ancestry of *Zalophus* species based on a qpGraph algorithm [25]

gene flow events, *Z. californianus* and *Z. wollebaeki* were shaped primarily by isolation and independent evolutionary trajectories.

Complex introgressive speciation of Zalophus species explains their phylogenetic ambiguities

Our study for the first time validated *Z. japonicus* phylogeny using 68,383,858 autosomal SNVs (Additional file 2: Data S5) and compared it with whole-mtDNA-based classification (Fig. 3, Additional file Fig. S6 and S7). The mtDNA phylogeny of *Z. japonicus* specimens revealed a total of three mtDNA haplotypes: two genetically uniform and nearly indistinguishable as well as one highly distinct, represented by sample Z3 (Fig. 3A, and Additional file 1: Fig. S8 and S9). The genetic distances between the two closer haplotypes were sufficient to differentiate them as separate mtDNA haplotypes but subtle enough to be possibly derived from the same maternal *Z. japonicus* ancestor (0.0008 vs 0.0004) (Fig. 3A). However, the mtDNA-based genetic distance between Z3 and the common *Z. japonicus* ancestor (0.0038) suggested a separate maternal lineage (Additional file 1: Fig. S8). Moreover, this phylogenetic distance for Z3 was comparable to the distance between *Z. wollebaeki* and *Z. californianus* and their common maternal ancestor (0.0026 and 0.0029, respectively) (Fig. 3C).

Both autosomal SNV and mtDNA-based phylogenetic trees presented the same topology with two distinct Otariidae clades and Northern fur seal (*C. ursinus*) as an outgroup: one of Northern pinnipeds composed of *Zalophus* and *Eumetopias* sea lions, and one of Southern pinnipeds composed of *Phocarctos* and *Neophoca* sea lions with *Arctocephalus* fur seals (Fig. 3A, B). In this context, *Z. japonicus* showed almost equal phylogenetic



C **Comparison of *Zalophus japonicus* phylogenetic distances based on A) and B)**

		A. australis (average)	*C. ursinus* (average)	*E. jubatus* (average)	*Z. californianus* (average)	*Z. wollebaeki* (average)
mtDNA	***Z. japonicus****	0.0670	0.0908	0.0512	0.0100	0.0099
WGS	***Z. japonicus****	0.3857	0.8369	0.2424	0.0470	0.0488

Fig. 3 Dokdo sea lion phylogeny. **A** mtDNA-based phylogenetic tree of Otariidae in the context of sea lions and other pinnipeds. **B** Autosomal SNV-based (WGS) phylogenetic tree of Otariidae in the context of sea lions and other pinnipeds (Additional file 2: Data S5). **C** Phylogenetic distance comparison between mtDNA and autosomal SNV-based (WGS) phylogeny (Additional file 2: Data S6)

distance to *Z. wollebaeki* and *Z. californianus* (mtDNA, 0.0099 and 0.014; WGS, 0.0488 and 0.0470, respectively) (Fig. 3C, Additional file 2: Data S6). Even though *Z. japonicus* was similarly related to its congenerics, the genetic distance between *Z. wollebaeki* and *Z. japonicus* was about 35% greater compared to the distance between *Z. wollebaeki* and *Z. californianus* based on WGS, and about 61% greater based on mtDNA (Additional file 2: Data S6). Interestingly, while mtDNA suggested that *Z. wollebaeki* was marginally phylogenetically closer to *Z. japonicus,* the whole-genome SNPs indicated a closer relationship between *Z. japonicus* and *Z. californianus* (Fig. 3C). Despite this discrepancy, our and previously reported [26] intrageneric *Zalophus* phylogeny aligns with *f4*-statistic that showed higher genetic affinity between *Z. wollebaeki* and *Z. californianus* compared to the affinity each of them had with *Z. japonicus*.

Heterozygosity of extinct Dokdo sea lion

Our subsequent objective was to elucidate the genetic diversity of the *Z. japonicus* samples within the context of population analyses. To accurately estimate heterozygosity (theta) from low depth *Z. japonicus* data, we calculated individual heterozygosity values for *Z. japonicus* and represented the minimum and maximum theta values as confidence intervals (Additional file 2: Data S7). The moderate heterozygosity value of *Z. japonicus* (0.00267) was greater than that of any other *Zalophus* species and several marine mammal species that are recognized as "Least concern" by the International Union for Conservation of Nature and Natural Resources (IUCN) (Fig. 4). Interestingly, the heterozygosity levels of *C. ursinus* and *E. jubatus* ("Vulnerable" and "Near threatened", respectively) differed drastically from each other (Fig. 4). Despite *C. ursinus* having been extirpated from most of its range over the past 200–800 years due to hunting and environmental factors, its heterozygosity remains at a relatively high level (0.001416), which corresponds with the historical DNA analysis previously published [27]. In contrast, the heterozygosity estimate for *E. jubatus* (0.0005) was almost as low as that of *Z. wollebaeki* (0.00039) ("Endangered") (Fig. 4). This finding may reflect the ongoing population decline of *E. jubatus*, which began in the 1980s and persists across its distribution range [28, 29]. Notably, *Z. japonicus* shares a similar pattern with other marine species that experienced

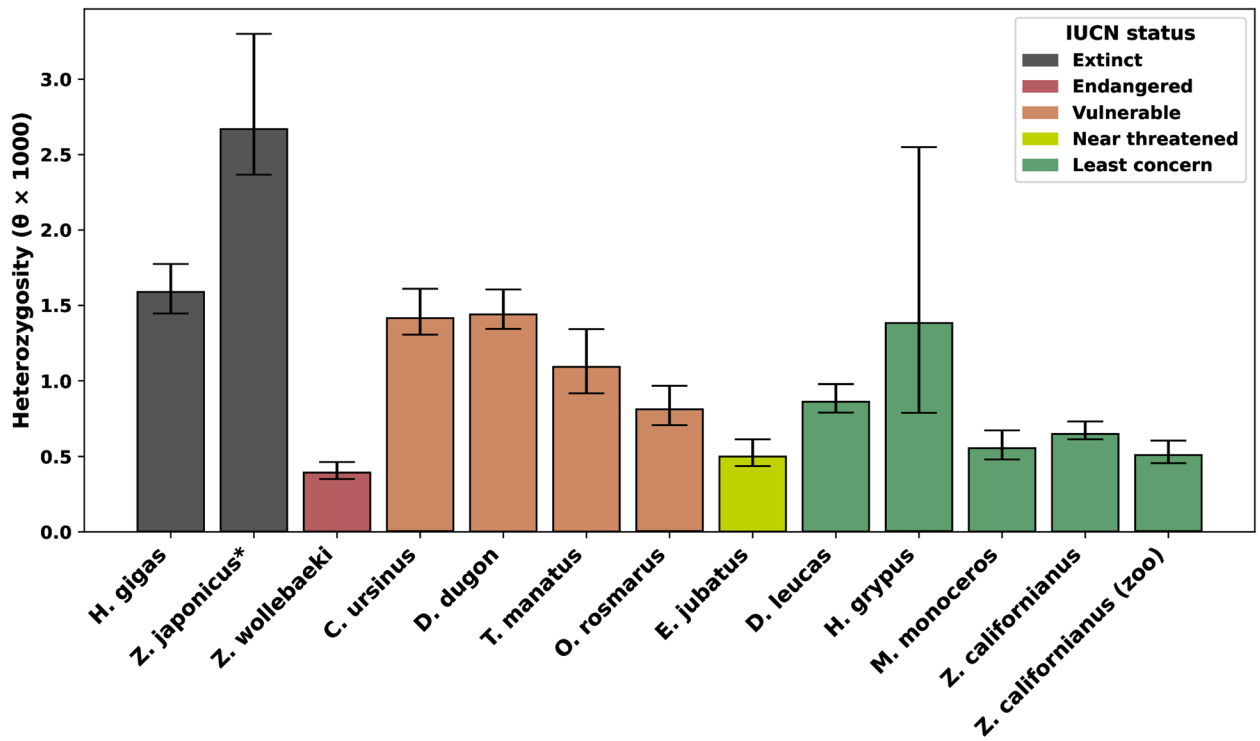


Fig. 4 Heterozygosity of Dokdo sea lion in the context of other marine mammals. The average heterozygosity values are multiplied by 1000 for visualization purposes. The species included in the comparison are listed with their common and scientific names as follows: Steller's sea cow (*H. gigas*), Dokdo sea lion (*Z. japonicus*), Galapagos sea lion (*Z. wollebaeki*), Northern fur seal (*C. ursinus*), dugong (*D. dugon*), West Indian manatee (*T. manatus*), walrus (*O. rosmarus*), Steller sea lion (*E. jubatus*), beluga whale (*D. leucas*), grey seal (*H. grypus*), narwhal (*M. monoceros*), and California sea lion (*Z. californianus*), including a zoo sample of the latter. The colors indicate IUCN conservation status (2024)

rapid extinction due to human activities, such as *H. gigas* (0.001589) (*Steller's sea cow*). High heterozygosity levels in these species may reflect their historically large population sizes before human exploitation. For *H. gigas*, which was rapidly driven to extinction due to overhunting and human activities, its large historical population size likely contributed to elevated genetic diversity before its decline [30]. Therefore, this analysis indicates that the heterozygosity of *Z. japonicus* (0.00267) is not alarmingly low and does not point to severe inbreeding. It is unlikely that genetic diversity alone would be a significant factor contributing to the extinction risk of this species. Similar to *H. gigas*, which was rapidly driven to extinction by human activities, the decline of *Z. japonicus* may have been heavily influenced by overexploitation, habitat destruction, and other anthropogenic pressures rather than genetic factors such as lack of heterozygosity.

Discussion

The deep DNA sequencing and advances in bioinformatics allowed us to understand the basics of evolution better, and to describe traces of genetic introgression and the events that accompanied them, e.g., rapid speciation, multiple ecological radiations, and rapid adaptation to the changing environment [31–33]. The extinct Dokdo sea lion (*Zalophus japonicus*) represents a unique evolutionary lineage within Otariidae, and its genome provides a rare opportunity to explore the genetic makeup and demographic history of a recently lost marine mammal. Despite the limitations associated with degraded and low-yield ancient DNA, we merged non-overlapping variants from the *Z. japonicus* individuals and applied stringent filtering to minimize spurious signals. Our analysis revealed that *Z. japonicus* was genetically distinct from its extant congeners (Fig. 2), underscoring its evolutionary singularity. Intergeneric fertile hybridization in pinnipeds is a well-documented phenomenon, which adds an additional layer of complexity analyzing their speciation, phylogeny, and ancestry [12, 34]. Our study sheds light on the evolution of extant Otariidae species inhabiting the Northern Pacific Ocean, with a special focus on the genus *Zalophus*, especially the Dokdo sea lion, an extinct member of this genus (Fig. 1). Through *f3*- and *f4*-admixture statistics, we describe an introgression from *C. ursinus* and/or *E. jubatus* to *Z. californianus* and *Z. wollebaeki* (Fig. 2). Moreover, we inferred possible ancient gene flow involving the extinct Dokdo sea lion and other Otariidae species. While there are no remaining historical or scientific records on *Z. japonicus* hybridization, *Z. californianus* as a species has a rich hybridization history in zoo enclosures with mixed-species pinniped exhibits. On a larger scale, recently, compelling evidence has emerged suggesting that smaller wild *E. jubatus* body size found specifically in the Oregon population in the USA could be attributed to a paternal genetic input from male *Z. californianus* that opportunistically mate with *E. jubatus* females during their seasonal migrations [35]. Furthermore, it is well established that there was a significant overlap not only in the species ranges (Fig. 1B) but also an ecological niche between extinct *Z. japonicus* and extant *C. ursinus* and *E. jubatus* [3, 4, 36], providing plausible scenarios for past gene flow. Although the precise timing and evolutionary consequences of these admixture events remain unclear, our findings raise the possibility that interspecific gene flow may have enhanced the genetic diversity and adaptive potential of *Zalophus* species.

The Dokdo sea lion is one of the species that is historically considered a victim of theriocide [3]. Our genomic analysis confirms that *Z. japonicus*, a recently extinct iconic species in Korea and Japan, is genetically and evolutionarily very distinct from *Z. californianus* and *Z. wollebaeki* (Fig. 2). Importantly, the results also support historical account suggesting that its extinction was unlikely to have resulted from a natural evolutionary dead end. Instead, we found that *Z. japonicus* exhibited relatively high levels of genomic heterozygosity, even when compared to other endangered or extinct marine mammals (Fig. 4). To account for low sequencing depth, we applied a window-based heterozygosity estimation approach (window size, 5000 bp; step size, 1000 bp) and calculated the median θ value across individuals. Inter-individual variation in heterozygosity was minimal, suggesting that these specimens shared consistently high genetic diversity at the time of extinction. A similar observation was made in another extinct species, *Hydrodamalis gigas* [30], indicating that high heterozygosity alone is not necessarily protective against rapid population collapse driven by humans. Among the lines of evidence we report, the relatively high heterozygosity suggests that the theriocide inflicted on the *Z. japonicus* population was faster than the pace at which inbreeding could accumulate deleterious effects. These findings reinforce our interpretation that *Z. japonicus* was a genetically viable population that was driven to extinction primarily due to human pressures.

We acknowledge that our study has additional limitations, including a small sample number and low sequencing depth, which precluded us from performing certain analyses. These constraints were largely due to the degraded state of all *Z. japonicus* bone specimens. Furthermore, we could not comprehensively validate relative sample dating and DNA misincorporation estimates with other methods due to expected sample age estimates falling within the modern-historical range. Despite these limitations, we made deliberate efforts to

mitigate the limitations inherent to low-depth ancient DNA. In gene flow (Fig. 2) and phylogenetic analyses (Fig. 3), we maximized the representation of *Z. japonicus* genotypes to ensure their inclusion in evolutionary inferences. For demographic and heterozygosity analyses, we avoided potential biases from sample merging by treating each individual separately and applying consistent, stringent filtering. Window-based heterozygosity estimation (Fig. 4) allowed us to extract meaningful historical signals despite low sequencing depth. We believe these efforts enabled a reliable reconstruction of the genetic features of *Z. japonicus* even under constrained conditions.

The case of *Z. japonicus* illustrates the vulnerability of even genetically viable populations to anthropogenic pressures. As one of the few pinniped species to go extinct in historical times, its genome offers a window into past biodiversity and informs our understanding of evolutionary trajectories disrupted by human activity. Future work should prioritize the recovery of higher-coverage genomes from additional *Z. japonicus* individuals, targeted ancient DNA capture approaches, and comparative studies with both extant and other extinct pinniped lineages. These efforts will be instrumental in reconstructing the complete evolutionary history of *Z. japonicus* and in contextualizing its extinction within broader patterns of marine mammal biodiversity loss.

Conclusions

This study provides novel genomic, evolutionary, and demographic insights based on the whole genome sequencing and analysis of an extinct sea lion from East Asia, *Z. japonicus.* The *Z. japonicus* genome fills a significant gap in the collective knowledge on the sea lion genus *Zalophus* and, more broadly, the eared seal family Otariidae. Our phylogenetic and admixture analyses suggest that (1) *Z. japonicus* represents the earliest diverging lineage within *Zalophus.* (2) *Z. japonicus* exhibited a complex history of admixture within the Otariidae family, highlighting its unique evolutionary trajectory. (3) The three *Zalophus* species, though geographically separated, share strong genetic and evolutionary affinities. Moreover, the combination of relatively high heterozygosity and historical demographic decline in *Z. japonicus* raises the possibility that its extinction was influenced more by anthropogenic impacts than by genetic factors alone. These estimates provide an important complementary line of evidence to the sparsely documented knowledge on the *Z. japonicus* extinction. This work underscores the power of paleogenomics to reconstruct lost biodiversity and highlights the importance of integrating genomic data into conservation and historical ecology.

Methods

Experimental design

For our genomic comparison study of the extinct *Z. japonicus*, we used 16 *Z. japonicus* bones (Additional file 1: Figs. S1, S2)—three from Gajaegul in Ulleungdo (Gaze Cave, 37.51° N, 130.79° E) and 13 from Seodo Gajaegul in Dokdo islands (Gaze Cave, 37.24° N, 131.86° E) (Additional file 1: Table S1). Both sites are named "Gajaegul" meaning "sea lion cave" in Ulleungdo county dialect. The *Z. japonicus* bones were provided by Cetacean Research Institute of National Institute of Fisheries Science in Republic of Korea. Morphological examination of the bones did not provide conclusive evidence regarding whether they originated from the same individual due to their small size and fragmented nature. The collection of Dokdo sea lion bones was conducted under the permission granted by the Gyeongbuk province local government for the collection of protected marine organisms (Permit No. 2019–2). We also collected a *Z. californianus* muscle sample from the Seoul Grand Park, Republic of Korea, during its necropsy process (Permit No. Seoul Grand Park Scientific Research 2020–009). As our study involved extinct animals and cadavers, ethical approval was not required. In addition, we downloaded 12 pinniped genomes from NCBI (Additional file 2: Data S5), which were used to construct a phylogenetic tree, conduct ancestry analysis, and perform genetic diversity studies.

DNA extraction and next generation sequencing

To eliminate the potential contamination, we washed *Z. japonicus* bones with ethanol and applied UV irradiation (10 min on each side) using a UV crosslinker device. After isolating only the internal bone tissue, we subjected it to additional UV irradiation to remove any contaminants, such as bacteria and fungi. The genomic DNA from the extinct species was extracted using DNeasy Tissue & Blood Kit (Qiagen, Valencia, CA) and the Cetrimonium bromide (CTAB) manual. To generate Illumina NGS data, we constructed PE and SE libraries using the KAPA Hyper Library Preparation Kit (Kapa Biosystems, Woburn, MA, USA) and the Accel NGS 1S plus DNA kit (Swift BioSciences, Washtenaw County, Michigan, USA), respectively, according to the manufacturer's instructions. The Illumina-based NGS sequencing was performed with Illumina NovaSeq 6000 (Illumina, CA, USA) and NextSeq 500 (Illumina, CA, USA). For MGI-Seq, we constructed paired-end libraries with MGIEasy DNA Library Prep Kit (MGI, Shenzhen, China) and sequenced them on the DNBSEQ-T7 sequencing platform.

Dokdo sea lion mitochondrial genome assembly and phylogeny

Upon assessing the read quality with Trimmomatic (ver. 0.39) [37], we extracted mtDNA reads by mapping all the short DNA reads to the mitochondrial genome (mito-genome) of *Z. californianus* (Acc. NC_008416) [38]. We assembled the mito-genome of the *Z. japonicus* (Z6) sample using NOVOPlasty (ver. 4.2) [39]. Minor gaps of the mito-genome assembly were filled in by conducting Sanger sequencing (Additional file 1: Tables S5, S6) followed by assembly using Cap3 program (updated on December 21, 2007) [40]. We predicted and annotated *Z. japonicus*' mito-genome using the MITOS program (http://mitos.bioinf.uni-leipzig.de/index.py) (Additional file 1: Fig. S6, Additional file 2: Data S3) [41]. Complete mtDNA genomes were aligned with Mummer (ver. 4.0.0rc1) (Additional file 1: Fig. S7) [42] and Dendroscope (ver. 3.5.10) [43]. We additionally obtained ten mtDNA genome consensus sequences by aligning our samples' NGS reads to Z6 deep-sequenced mtDNA genome (Additional file 2: Data S4). The consensus sequences were obtained from high quality SNV data (mapping quality > 30, genotype quality > 20, and coverage > 10) by implementing samtools consensus utility (ver. 1.9) [44]. Five samples (Z1, Z5, Z7, Z8, and Z12) were excluded in the process due to significantly lower (insufficient) amount of NGS reads (Additional file 2: Data S4). We then constructed the phylogenetic tree of the ten *Z. japonicus* mtDNA genomes along with other closely related species (Fig. 3A, Additional file 1: Fig. S8 and Additional file 2: Data S6). We aligned CDS sequences using muscle program (ver. 3.8.31) [45] and constructed the phylogenetic tree using phyML (ver. 3.1) with default parameters (ver. 3.1) [46].

Variant analysis for extinct Z. japonicus

We filtered low-quality reads from our ancient sequences using Trimmomatic (version 0.39) with default settings [37]. Additionally, for DNBSEQ-T7 data, we applied the "HEADCROP 15 bp" option to remove the first 15 bases from each read. To generate BAM files, we utilized the PALEOMIX pipeline [20], employing different reference genomes according to the purpose of the analysis. During the AdapterRemoval step (version 2.2.0), we collapsed reads with an overlap longer than 11 bp (default) and set a minimum length of 20 bp (–minlength 20). We did not filter reads based on mapping quality, as the minimum mapping quality was set to 0 (MinQuality: 0). Reads were aligned to the reference genome using BWA (0.7.17-r1188) [47]. PCR duplicates were filtered, and base qualities were rescaled using mapDamage.

Phylogeny, admixture and genomic composition analyses of Dokdo sea lion and other pinnipeds

To construct a phylogenetic tree and analyze ancestry, we aligned reads to an outgroup species that is the most distantly related to *Z. japonicus*, namely the walrus, *Odobenus rosmarus* (acc. ANOP00000000) [48]. Specifically for *Z. japonicus* DNA samples, we applied the PALEOMIX pipeline [20] by mapping all *Z. japonicus* reads to the *O. rosmarus* genome [48]. We estimated deamination patterns using mapDamage (ver. 2.0) [49]; however, there are no significant deamination in the *Z. japonicus* data (Additional file 1: Fig. S5). We constructed the *Z. japonicus* consensus sequence using ANGSD's -doFasta function (ver. 0.939) after removing repetitive loci and focusing on high-confidence regions identified by SNPable (https://lh3lh3.users.sourceforge.net/snpable.shtml). We used the following options for ANGSD: -GL 2 -doFasta 2 -doCounts 1 -minMapQ 30 -minQ 30 -setMinDepth 2 -setMaxDepth 10 [50]. High-quality SNVs were identified from mappable regions determined by SNPable, which assess the uniqueness of sequences in the reference genome to exclude repetitive or low-complexity regions prone to mapping errors.

We downloaded publicly available sequencing data for 10 pinniped species from the NCBI SRA and ENA databases (Additional file2: Data S5) [10, 51]. For the modern mammal genomes, their NGS reads were aligned to the same reference genome using bwa mem (ver. 0.7.17) [47] after filtering out low-quality reads using Trimmomatic with Quality < 30 and read length < 70 (ver. 0.39) [37]. We then utilized Picard (https://broadinstitute.github.io/picard/) (ver. 2.27.5) to eliminate PCR duplicates and employed the GATK (ver. 4.1.3.0) for variant calling [52]. We constructed consensus sequence using the vcf2phylip [53] utilizing only genome-wide variants in the *Z. japonicus* bam files with read depth larger than three. A phylogenetic tree was constructed using the maximum likelihood method in the MEGA X (ver. 10.2.2) [54]. We inferred divergence time of the pinnipeds using the RelTime method [55, 56], which branch lengths calculated by the maximum likelihood method under the General Time Reversible substitution model [57]. We used the calibration constraint of 4.25 Mya for the divergence time between *Z. japonicus* and *E. jubatus*, obtained from the TimeTree database [58], to scale relative divergence times to absolute time estimates. Evolutionary analyses, including the construction of a phylogenetic tree, were conducted in MEGA X.

The *f3*- and *f4*-statistics (Additional file 1: Table S2–4) [22] were conducted using an ADMIXTOOLS (ver. 2.0) algorithm [25]. An admixture graph was constructed with the qpGraph model [25] with admix = 2. The

qpGraph [25] was automatically optimized for genetic admixture of our admixture model.

The maps used for the *Otariinae* species' distribution were obtained from https://mapstyle.withgoogle.com. The graphic representations of each species in Figs. 1 and 2 as well as those of Ulleungdo and Dokdo islands were created based on royalty-free images under the Creative Commons (CC) license.

Calculation of the heterozygosity of Dokdo sea lion genomes

To accurately identify the heterozygous regions in the *Z. japonicus* genome, we calculated the distribution of heterozygous positions across the genomic loci from a bam file, wherein *Z. japonicus* reads were mapped to the most closely related reference genome, *Z. californianus* (Acc. GCF_00976235) [19] using the PALEOMIX pipeline [20]. To ensure high data quality, we removed regions corresponding to repetitive and low-complexity sequences from the BAM file using SNPable-filtered masks in combination with bedtools (ver. 2.26.0) [59]. For the modern mammal genomes, we used the bam files mapped reads to the most closely related reference genomes (Additional file 2: Data S7) [19, 22, 38, 48, 60–62]. We then implemented ROHan (ver. 1.0.1) [63] to estimate the heterozygosity only on the high-quality loci with high-quality variants. To reduce bias associated with sparse variant density in low-depth *Z. japonicus* individuals, we implemented ROHan with parameters –window 5000 and –step 1000, allowing for more stable heterozygosity estimates across genomic regions. For each individual, we calculated the median θ value across windows as a representative estimate of heterozygosity and used the standard deviation of per-window θ values as an error measure, which was visualized as error bars for *Z. japonicus* (Fig. 4). This approach helps mitigate the inflation or deflation of point estimates that may arise from localized sequencing gaps or low-confidence regions.

f-statistics analyses for Dokdo sea lion and other Otariidae species

For ancestry analysis, we used *f4*-statistics and admixture *f3*-statistics. The admixture *f3*- and *f4*-statistics were conducted using an ADMIXTOOLS (ver. 2.0) algorithm [25]. All formulas employed are detailed in Additional file 1: Table S2–S4. Our criterion for significance was set at an absolute Z-score less than three.

Abbreviations

IUCN	International Union for Conservation of Nature
mtDNA	Mitochondrial DNA
NGS	Next-generation sequencing
SE	Single-end
PE	Paired-end
Ne	Effective population size

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-025-02351-3.

Additional file 1: Fig. S1. Excavation of Dokdo sea lion bones. Fig. S2. Dokdo sea lion bones used in this study. Fig. S3. Distribution of read lengths of *Z. japonicus* mapped to the *Z. californianus* reference genome. Fig. S4. Read length distribution mapped to California sea lion (*Z. californianus*) genome.Fig. S5. Postmortem DNA damage pattern in the DNA-libraries of Dokdo sea lion generated by PALEOMIX pipeline. Fig S6. Assembly and annotation of the complete mitochondrial genome of Dokdo sea lion using the Z6 individual specimen. Fig. S7. Comparison of mitochondrial genomes between *Z. japonicus* and *Z. californianus* using mummer (ver. 4.0.0rc1). Fig. S8. Phylogenetic tree of the mitogenomes of Dokdo sea lion and related species. Fig. S9. The most informative mtDNA sites of the *Z. japonicus*. Table S1. Mapping statistics of 16 *Z. japonicus* libraries using the PALEOMIX pipeline to California sea lion (*Z. californianus*) reference genome. Table S2. Gene flow between Steller sea lion (*E. jubatus*) and *Zalophus* species with a form of *f4*(A,B;C,D). Table S3. *f4*-statistics of Otariidae species with a form of *f4*(A,B;C,D). Table S4. Genetic admixture of *Zalophus* species compared to their related species. Table S5. Primers to filling the mito-genome gap of Dokdo sea lion (Z6 individual) and melting temperature (Tm) used. Table S6. Sanger sequencing reads to fill mito-genome gap of Z6 individual.

Additional file 2: Data S1. Statistics of DNA sequencing and mapping statistics to California sea lion reference genome. Data S2. Number (No) of mapped read to Steller sea lion reference genome and coverage statistics. Data S3. Annotation of the mitochondrial genome of Dokdo sea lion. Data S4. Coverage of the mitochondrial genomes based on reads with mapping quality > 30, genotype quality > 20, and coverage >10. Data S5. Mammal genome datasets used for ancestry analysis. Data S6. Genetic distance matrix for mtDNA (A) and nuclear genome (B). Data S7. Average genome-wide autosomal heterozygosity values.

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Authors' contributions

Conceptualization: J.K., J.C., A.B, S.J., F.S., A.N., and J.B.; Methodology: C.K., J.K, F.S.; Investigation: E.K, H.-W.K., M.Y., J.-H.L., K.L., and H.S.; Visualization: A.B, J.C., and J.K.; Supervision: J.K., A.B., A.N., and J.B.; Writing—original draft: J.K., J.C., A.B; Writing—review & editing: A.N, and J.B. All authors read and approved of the final manuscript.

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Data availability

All data are publicly available for scientific research. Sequencing data have been deposited in the NCBI SRA with accession number PRJNA982545. All the other data are available either in the main text or in the supplementary materials.

Declarations

Ethics approval and consent to participate

The collection of Dokdo sea lion bones was conducted under the permission granted by the Gyeoungbuk province local government for the collection of protected marine organisms (Permit No. 2019–2). We also collected a *Z. californianus* muscle sample from the Seoul Grand Park, Republic of Korea,

obtained during the necropsy process (Permit No. Seoul Grand Park Scientific Research 2020–009). As our study involved extinct animals and cadavers, ethical approval was not required.

Consent for publication
Not applicable.

Competing interests
C.K. and S.J. were employees of Clinomics Inc. S.J. and J.B. are employees of AgingLab Inc. Other authors declare that they have no competing interests.

Author details
[1]Personal Genomics Institute, Genome Research Foundation, Cheongju 28190, Republic of Korea. [2]Korean Genomics Center (KOGIC), Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, Republic of Korea. [3]School of Medicine, Gachon University, Incheon 406-840, Republic of Korea. [4]Clinomics, Inc., UNIST, Ulsan 44919, Republic of Korea. [5]Paleogenomics Laboratory, European University at Saint Petersburg, Saint Petersburg, Russia. [6]National Research Center "Kurchatov Institute", Moscow, Russia. [7]AgingLab, Inc., UNIST, Ulsan 44919, Republic of Korea. [8]National Institute of Fisheries Science, Busan 46083, Republic of Korea. [9]Conservation and Health Center, Seoul Zoo, Gwacheon 13829, Republic of Korea. [10]Korean Genomics Center, Department of Biomedical Engineering, UNIST, Ulsan 44919, Republic of Korea.

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