



Origin of populations of *Portulaca oleracea* on Nishinoshima, an active volcanic oceanic island

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Abstract

Nishinoshima Island, located in the Ogasawara Islands of southern Japan, underwent major volcanic eruptions in 2013, which destroyed nearly all its vegetation. Understanding the origin and characteristics of the plant populations on Nishinoshima provides valuable insights into early stages of island ecosystem development. In this study, we analysed *Portulaca oleracea* samples collected from Nishinoshima in 2019, before subsequent eruptions eradicated the island's populations. Phylogenetic and population genetic analyses of plastome sequences and genome-wide single-nucleotide polymorphisms (SNPs) were performed to compare Nishinoshima samples with those from Japan and Guam. The plastome phylogeny identified five distinct lineages across Japan and Guam, two of which were present in the Ogasawara Islands (Chichijima and Hahajima Islands), with one shared between Nishinoshima and Chichijima. Genome-wide SNP analysis revealed two major genetic groups in Japan: one in the northern and another in the southern regions, including the Ogasawara Islands. The Nishinoshima populations belonged to the southern group but exhibited unique genetic characteristics, as shown by network and STRUCTURE analyses. These characteristics are likely due to limited seed dispersal, a founder effect, and genetic bottlenecks resulting from natural disturbance such as volcanic eruptions and typhoons. These findings suggest that *P. oleracea* on Nishinoshima is genetically close to Chichijima populations but has accumulated distinct mutations. This study provides the first genetic evidence of plant origins on Nishinoshima and contributes to understanding the dynamics of ecosystem development on newly formed oceanic islands.

Keywords Genetic structure · Island ecosystem · Oceanic islands · Portulacaceae · Seed dispersal · Volcanic activity

Introduction

Oceanic islands provide ideal systems for understanding vegetation dynamics and ecological processes over geological timescales (Stuessy et al. 2022; Whittaker et al. 1989, 2023). Newly formed oceanic islands, such as Krakatoa in Indonesia and Surtsey in Iceland, are particularly valuable

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for studying the early stages of island ecosystem development (Magnússon et al. 2014, 2022; Magnússon and Magnússon 2000; Tagawa et al. 1985). Studies on these islands have shown that most colonising plants originate from nearby continental regions or neighbouring islands with wind, birds, and ocean currents facilitating seed or propagule dispersal. However, opportunities to observe island formation and subsequent ecosystem development are rare

and stochastic, underscoring the need for further research in these systems to better understand the processes driving colonisation, succession, and long-term ecosystem stability.

Nishinoshima, located at 27.2° N and 140.9° E, is an oceanic island located approximately 130 km west of the neighbouring island, Chichijima in the Ogasawara Islands south of the Japanese archipelago (Fig. 1). The island was first recorded by the Spanish ship Rosario in 1702 and

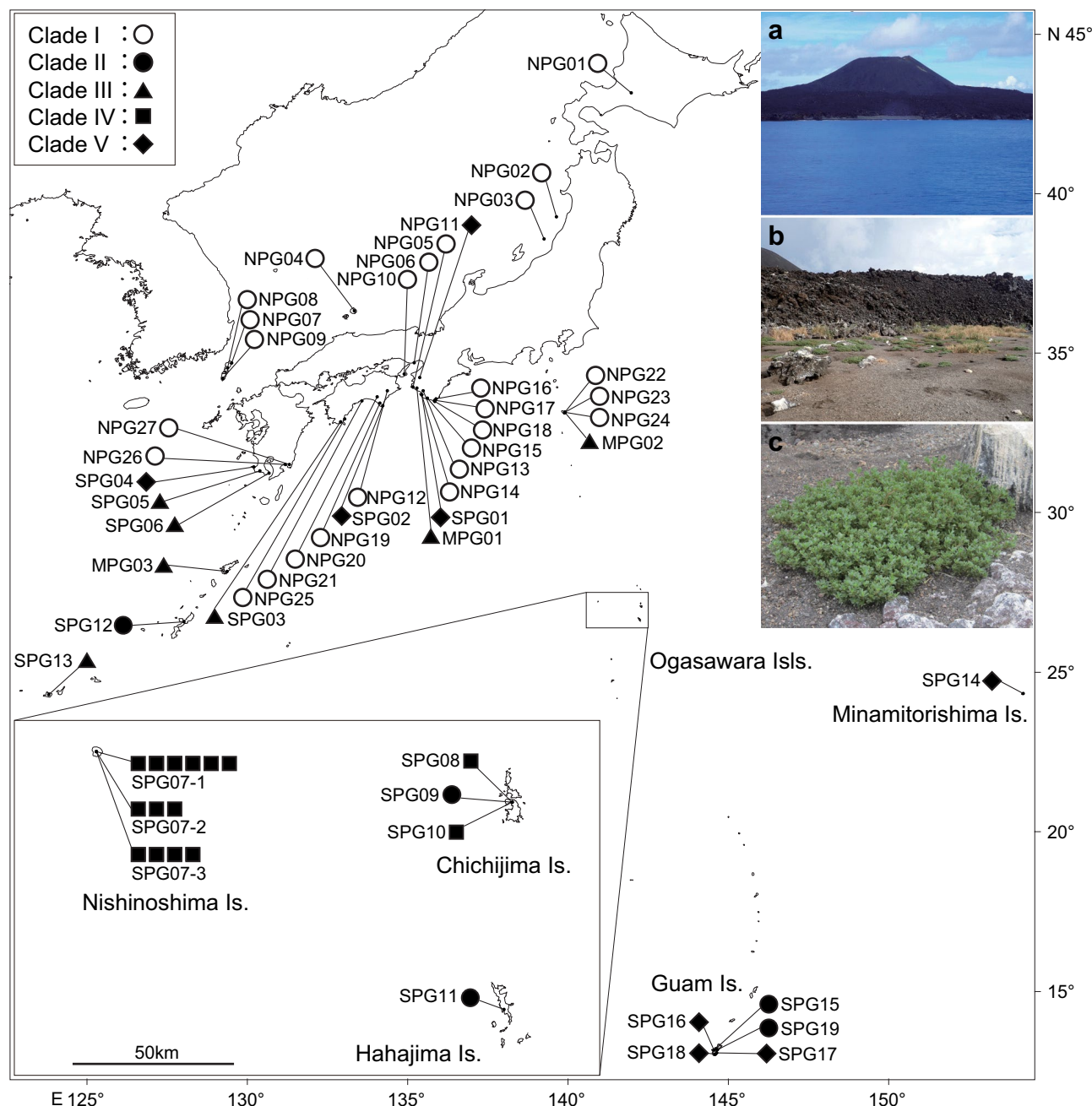


Fig. 1 Photographs, sampling location, and geographical genetic structure of *Portulaca oleracea*. Clades recognised in the plastome phylogeny are shown. The acronyms of the samples are listed in

Table 1. Photo **a** A distant view of Nishinoshima Island in 2019; **b** a population of *P. oleracea* on Nishinoshima; **c** *P. oleracea* on Nishinoshima

given its namesake, Rosario Island, until it was renamed (Aoki and Osaka 1974; Center for Research and Promotion of Japanese Islands 2004). Nishinoshima is estimated to have formed through volcanic activity about 10 million years ago (Aoki and Osaka 1974). Since its formation, the island has frequently been impacted by large-scale disturbances, including volcanic activity and typhoons. During the Meiji period (1868–1912), workers were sent multiple times to the island collect guano (Aoki and Osaka 1974). Then in 1953, a fishing boat registered in Shizuoka Prefecture reported volcanic smoke rising from the island, signalling ongoing volcanic activity (Center for Research and Promotion of Japanese Islands 2019).

Nishinoshima underwent considerable change following a volcanic eruption on the island in 1973. By 1974, a newly formed landmass merged with the older island from the accumulation of sand and sediment (Nakano 2013). Before the eruption, Nishinoshima was originally a small landmass of about 0.07 km², but by 2024, the combined island had expanded to approximately 3 km².

The first botanical survey of Nishinoshima conducted in 1969 recorded only three species: *Eleusine indica* (L.) Gaertn. (Poaceae), *Echinochloa crus-galli* (L.) P. Beauv. (Poaceae), and *Portulaca oleracea* L. (Portulacaceae) (Asami et al. 1970). A subsequent survey in 1983 identified *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae), and by 2004, *Vitex rotundifolia* L. f. (Lamiaceae) and *Tetragonia tetragonioides* (Pall.) Kuntze (Aizoaceae) (Abe 2006; Ohsawa and Kurata 1983). Since 2013, subsequent large-scale volcanic eruptions have covered about 95% of the island with lava (Ono et al. 2015). By 2018, when volcanic activity had subsided, a follow-up survey in 2019 found that only *E. indica*, *E. crus-galli*, and *P. oleracea* survived in the remaining 5% of the uncovered land (Kamijo et al. 2020). However, further volcanic eruptions in 2020 covered this remaining area with lava. As of 2024, no plant life has been confirmed on the island (Kawakami et al. 2022).

Due to these recent eruptions and the complete reset of its vegetation, Nishinoshima provides a unique opportunity to study the processes of colonisation and succession in island systems, much like Krakatoa and Surtsey. However, Nishinoshima presents a distinct case due to its extreme isolation, located nearly 100 km from the nearest island. As a result, only three species have successfully established on the island over the past 40 years (1973–2013), reflecting a much slower rate of species colonisation compared to Krakatoa (Whittaker et al. 1989). Such extreme isolation makes Nishinoshima an ideal study site of oceanic island ecosystem development. Investigating the origin and characteristics of plant populations before eruptions provides valuable information on subsequent regeneration processes of natural vegetations and the initial stage of island ecosystem formation.

Portulaca oleracea is a cosmopolitan annual herb found across temperate and tropical regions worldwide (Akiyama 2006; Kokubugata 2017). Although the exact mode of seed dispersal for *P. oleracea* remains unclear, its seeds are nearly flat and oblate with a diameter of less than 1 mm, suggesting high-dispersal capacity by wind, birds, or ocean currents. The plant has high salt tolerance and drought resistance (Alam et al. 2014; Yazici et al. 2007), making it well adapted to coastal environments. In addition, germination temperature exceeds 20 °C (Noguchi et al. 1975), allowing for multiple generations per year especially in warm climates. While two varieties are globally recognised (POWO), a recent study by Ocampo (2012) identified seven subspecies. One of the two varieties, *P. oleracea* var. *oleracea* commonly found in open fields and along roadsides in Japan (Akiyama 2006).

In this study, we aimed to investigate the origin and diversification of *P. oleracea* populations that once grew on Nishinoshima through phylogenetic and population genetic approaches. We analysed *P. oleracea* samples collected from Nishinoshima in 2019 prior to complete disappearance of the species due to volcanic eruptions on the island, in addition to other samples collected from sites throughout Japan and Guam, another remote oceanic island located more than 1500 km south of the Ogasawara Islands. To explore the origin of pre-eruption *P. oleracea* populations on Nishinoshima, we addressed three key questions: (1) What is the genetic diversity of the populations on Nishinoshima? (2) Which populations are mostly closely related to those on Nishinoshima? (3) Is there evidence for genetic differentiation among *P. oleracea* populations in Japan and Guam? By answering these questions, we discuss plant colonisation and vegetation succession during the early stages of ecosystem development on oceanic islands. These findings provide critical insights into the mechanisms of plant dispersal and establishment in isolated environments, informing strategies for biodiversity conservation and restoration in fragile island ecosystems.

Materials and methods

Plant materials and DNA extraction

Leaf samples were collected from 254 individuals of *Portulaca oleracea* across 51 different populations spanning approximately 3500 km in Japan and Guam. Leaf samples were stored in plastic bags containing silica gel beads until further processing. Voucher specimens were deposited in the Herbarium of the Kyoto University Museum (KYO). Total DNA was extracted from dried leaves using the Plant Genomic DNA Extraction Kit (Favorgen Biotech, Taiwan) and further purified using Sera-Mag SpeedBeads (Cytiva, Tokyo, Japan) in PEG/NaCl buffer (Rohland and Reich

2012), if necessary. DNA concentration and quality were measured using a Qubit 2.0 fluorometer (ThermoFisher Scientific, CA, USA) and a NanoPhotometer N60 (Implen, Germany), respectively.

Plastome sequencing and phylogenetic analyses

Plastome sequences were obtained from 61 representative *P. oleracea* samples (Table 1), with at least one sample per population. DNA libraries for shotgun sequencing were prepared using the xGen™ DNA Library Prep EZ UNI Kit with xGen™ Stubby Adapter and UDI Primer Pairs (IDT, Iowa, USA). The pooled libraries were sequenced with 150-bp paired-end reads using an Illumina HiSeqX (Macrogen, South Korea) and a DNBSEQ-G400 (BGI, Shenzhen, China). The raw sequence data were trimmed using Trimmomatic with default setting (Bolger et al. 2014). The resulting high-quality reads were assembled using GetOrganelle (Jin et al. 2020). All plastome sequences from the 64 samples were aligned using MAFFT ver.7 (Katoh and Standley 2013). Assembly errors were manually corrected using Geneious Prime 2020.1.2. (Geneious, Boston, MA, USA). Gene annotation was performed in Geneious prime using the *P. oleracea* plastome genome (MK397889) as a reference. The finalised plastome sequences and their annotations were deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers PV658073-PV671030).

A maximum likelihood (ML) tree was constructed using RAxML-NG (Kozlov et al. 2019), based on sequences from the small single-copy (SSR), large single-copy (LSC), and one inverted repeat (IR) regions, excluding inversion sites. The analysis was performed with 20 independent ML searches and 1000 bootstrap replicates using the TVM+I+G4 model, which was selected using ModelTest-NG (Darriba et al. 2020). The resulting tree was visualised using FigTree Version 1.4.4 (Rambaut and Drummond 2018).

Genome-wide SNPs

Genome-wide SNPs were identified using multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq, Suyama and Matsuki 2015). Following the protocol described by Suyama et al. (2022), a pooled DNA library of 254 samples was prepared and sequenced on a MiSeq instrument using a MiSeq Reagent Kit Version 3, 150 cycles (Illumina, CA, USA), and a DNBSEQ-G400 sequencer (MGI Tech, Shenzhen, China) for paired-end 150 cycles.

Low quality reads and adapter sequences were removed using fastp (Chen et al. 2018). After trimming the last base in both R1 and R2 from MiSeq, we obtained 79 bp sequences for both paired-end reads. To standardise the read lengths across sequencing platforms, at least 71 bases were

trimmed from both R1 and R2 of the DNBSEQ-G400 data. The cleaned reads were filtered against a reference plastome sequence of *P. oleracea* (NC036236) to exclude plastome reads using BOWTIE 2 (Langmead and Salzberg 2012). SNPs were called from the resulting nuclear reads using Stacks 2 (Rochette et al. 2019) with default settings, except for the following populations options: `-min-maf 0.05`; `-max-obs-het 0.7`; `-write-single-snp`; and `-min-samples-overall` (or `-R`, minimum percentage of individuals across populations required to process a locus), which was varied from 0.1 to 0.9 in increments of 0.1. As detailed in the Results section, a data matrix with $R=0.8$ was used for subsequent population genetic analysis. Population genetic statistics were calculated in Stacks 2 (Rochette et al. 2019). Neighbor-Net analysis was conducted using SplitsTree4 v4.20.2 (Huson and Bryant 2006), treating an ambiguous site as MatchStates. Bayesian clustering analysis was performed using STRUCTURE 2.3.4 (Pritchard et al. 2000). Markov chain Monte Carlo (MCMC) searches consisted of 1×10^6 burn-in steps followed by 1×10^6 iterations. Ten replicate runs were performed for each K from 1 to 11, and the mean log-likelihood probabilities and delta K were calculated using the Evanno method (Evanno et al. 2005) using StructureSelector (Li and Liu 2018).

Results

Plastome sequencing

We obtained an average of 20,220,226 paired-end reads (ranging from 12,860,895 to 53,589,779) of 150 bp from 61 samples. The plastome size of *Portulaca oleracea* ranged from 156,514 to 156,581 bp and contained 93 unique genes and 19 duplicated genes within the IR regions, totalling 131 genes. Furthermore, eight ribosomal RNAs (rRNA) and 37 transfer RNAs (tRNAs) genes were identified. No structural differences were observed among the plastomes of *P. oleracea*.

Plastome phylogeny

The alignment length of the SSC, LSC, and IR regions (without inversions) was 132,315 bp across 64 samples, including two reference *P. oleracea* (NC036236 and MK397889) and an outgroup taxon *P. umbraticola* Kunth (MT231887). The resulting ML tree revealed five major clades among the *P. oleracea* samples analysed. Clade I comprised samples from Hokkaido (NPG01) to Kagoshima (NPG27), excluding Osaka (NPG11). Clade II included samples from the Ryukyu Islands (SPG12), Ogasawara Islands (Chichijima and Hahajima) (SPG09 and SPG11), and Guam (SPG15 and SPG19). Clade

Table 1 Sources of materials of *Portulaca oleracea* L. used in the study, locality, voucher, longitude, and latitude information

Locality	Voucher	Population								Accession number of plastome
		Longitude	Latitude	code	N_{MIG}	H_e	P_i	N_{pl}		
Sapporo-shi, Hokkaido, Japan	Nishimura_230813-01	43.1	141.3	NPG01	3	0.052	0.067	1	PV671014	
Tobishima, Sakata-shi, Yamagata Pref., Japan	Kawakami_221027-01	39.2	139.5	NPG02	5	0.133	0.158	1	PV671015	
Awashimaura-mura, Niigata Pref., Japan	Kawakami_221013-01	38.5	139.3	NPG03	6	0.110	0.123	1	PV671016	
Okinoshima-cho, Oki-gun, Shimane Pref., Japan	Kawakami_221128-498	36.3	133.3	NPG04	1	0.062	0.124	1	PV671017	
Iwaya, Nada-ku, Kobe-shi, Hyogo Pref., Japan	Nakano_220923-141	34.7	135.2	NPG06	5	0.068	0.077	1	PV671019	
Mine, Mine-cho, Tsushima-shi, Nagasaki Pref., Japan	Nakano_230926-651	34.5	129.3	NPG07	5	0.042	0.049	1	PV671020	
Nishidomari, Kamitsushima-cho, Tsushima-shi, Nagasaki Pref., Japan	Nakano_230927-662	34.7	129.5	NPG08	3	0.040	0.049	1	PV671021	
Tsutsu, Izuhara-cho, Tsushima-shi, Nagasaki Pref., Japan	Nakano_230928-672	34.1	129.2	NPG09	5	0.038	0.045	1	PV671022	
Fukurahei, Minamiawaji-shi, Hyogo Pref., Japan	Nakano_220923-151	34.2	134.7	NPG10	6	0.043	0.048	1	PV671023	
Uchihata-cho, Kishiwada-shi, Osaka Pref., Japan	Nakano_220922-131	34.5	135.4	NPG11	5	0.049	0.057	1	PV671024	
Shioya-cho, Gobo-shi, Wakayama Pref., Japan	Takayama_220731-01	33.9	135.2	NPG05	1	0.056	0.111	1	PV671018	
Naka-cho, Naka-gun, Tokushima Pref., Japan	Nakano_220925-221	33.9	134.5	NPG12	5	0.059	0.068	1	PV671025	
Nada-cho, Gobo-shi, Wakayama Pref., Japan	Nakano_220903-50	33.8	135.2	MPG01	5	0.198	0.225	1	PV671011	
Sandanbeki, Shirahama-cho, Higashimuro-gun, Wakayama Pref., Japan	Nakano_220912-120	33.7	135.3	SPG01	5	0.075	0.086	1	PV658083	
Omezu, Minabe-cho, Hidaka-gun, Wakayama Pref., Japan	Nakano_220903-60	33.8	135.3	NPG13	5	0.078	0.088	1	PV671026	
Ezura, Shirahama-cho, Higashimuro-gun, Wakayama Pref., Japan	Nakano_220912-110	33.7	135.3	NPG14	5	0.039	0.044	1	PV671027	
Mirozu, Susami-cho, Nishimuro-gun, Wakayama Pref., Japan	Nakano_220911-70	33.5	135.6	NPG15	6	0.071	0.079	1	PV671028	
Hashiguiwa, Kushimoto-cho, Higashimuro-gun, Wakayama Pref., Japan	Nakano_220911-90	33.5	135.8	NPG16	5	0.068	0.077	1	PV671029	
Kushimoto, Kushimoto-cho, Higashimuro-gun, Wakayama Pref., Japan	Nakano_220911-100	33.5	135.8	NPG17	5	0.067	0.076	1	PV671030	
Kushimoto-cho, Higashimuro-gun, Wakayama Pref., Japan	Nakano_220911-80	33.5	135.8	NPG18	5	0.073	0.083	1	PV658073	
Yasuda-cho, Aki-gun, Kochi Pref., Japan	Nakano_220925-201	33.5	134.0	NPG19	5	0.070	0.080	1	PV658074	
Saiwai-cho Aki-shi Kochi Pref., Japan	Nakano_220925-181	33.5	133.9	NPG20	5	0.075	0.087	1	PV658075	
Kochi port, Niida, Kochi-shi, Kochi Pref., Japan	Nakano_220925-191	33.5	133.6	NPG21	5	0.117	0.133	1	PV658076	
Nanbara Senjojiki, Hachijo Island, Hachijo-cho, Tokyo Pref., Japan	Nakano_220827-01	33.1	139.8	NPG22	6	0.047	0.053	1	PV658077	
Yokoma beach, Hachijo Island, Hachijo-cho, Tokyo Pref., Japan	Nakano_220828-11	33.1	139.8	NPG23	5	0.044	0.051	1	PV658078	
Shueyoshi, Hachijo Island, Hachijo-cho, Tokyo Pref., Japan	Nakano_220829-29	33.1	139.8	NPG24	5	0.048	0.054	1	PV658079	
Ohgago, Hachijo Island, Hachijo-cho, Tokyo Pref., Japan	Nakano_220829-17	33.1	139.8	MPG02	5	0.272	0.306	1	PV671012	

Table 1 (continued)

Locality	Voucher	Population							Accession number of plastome
		Longitude	Latitude	code	<i>N</i> _{MIG}	<i>He</i>	<i>Pi</i>	<i>N</i> _{pl}	
Kotsuka, Shimanto-shi, Kochi Pref., Japan	Nakano_220924-161	33.0	133.0	NPG25	6	0.045	0.050	1	PV658080
Misaki, Tosashimizu-shi, Kochi Pref., Japan	Nakano_220924-171	32.7	133.0	SPG02	5	0.104	0.120	1	PV658084
Muroto cape, Muroto-shi, Kochi Pref., Japan	Nakano_220925-211	33.2	134.2	SPG03	6	0.081	0.092	1	PV658085
Honjo, Kushima-shi, Miyazaki Pref., Japan	Nakano_221014-245	31.4	131.3	NPG26	6	0.068	0.076	1	PV658081
Shibushi port, Shobisho-cho, Shibushi-shi, Kagoshima Pref., Japan	Nakano_221014-255	31.5	131.1	NPG27	5	0.081	0.092	1	PV658082
Iwato, Makurazaki-chi, Kagoshima Pref., Japan	Nakano_221012-236	31.3	130.3	SPG04	5	0.089	0.103	1	PV658086
Yamakawa port, Yamakawakanebu-cho, Ibusuki-chi, Kagoshima Pref., Japan	Nakano_221013-242	31.2	130.6	SPG05	3	0.096	0.125	1	PV658087
Kataura, Kasasa-cho, Minamisatuma-shi, Kagoshima Pref., Japan	Nakano_221012-226	31.4	130.1	SPG06	6	0.094	0.105	1	PV658088
Koniya, Setouchi-cho, Oshima-gun, Kagoshima Pref., Japan	Nakano_220727-40	28.2	129.3	MPG03	5	0.251	0.287	1	PV671013
Nishinoshima Island, Ogasawara-mura, Tokyo Pref., Japan	Kamijo_2019-559	27.2	140.9	SPG07-1	7	0.070	0.078	6	PV658089 PV658090 PV658091 PV658092 PV658105 PV658106
Nishinoshima Island, Ogasawara-mura, Tokyo Pref., Japan	Kamijo_2019-559	27.2	140.9	SPG07-2	7	0.081	0.091	3	PV658107 PV658108 PV658109
Nishinoshima Island, Ogasawara-mura, Tokyo Pref., Japan	Kamijo_2019-559	27.2	140.9	SPG07-3	7	0.073	0.085	4	PV658110 PV658111 PV658112 PV658113
Chuoizan, Chichijima Island, Ogasawara-mura, Tokyo Pref., Japan	Takayama_181016-499	27.1	142.2	SPG08	1	0.051	0.102	1	PV658114
Ohomura, Chichijima Island, Ogasawara-mura, Tokyo Pref., Japan	Nishimura_230322-01	27.1	142.2	SPG09	12	0.136	0.144	1	PV658115
Nishimachi, Chichijima Island, Ogasawara-mura, Tokyo Pref., Japan	Takayama_230716-01	27.1	142.2	SPG10	4	0.068	0.084	1	PV658116
Oki port, Hahajima Island, Ogasawara-mura, Tokyo Pref., Japan	Nishimura_230318-01	26.6	142.2	SPG11	6	0.039	0.044	1	PV658117
Kin, Kin-cho, Kunigami-gun, Okinawa Pref., Japan	Nakano_221111-491	26.5	127.9	SPG12	6	0.099	0.112	1	PV658118
Ishigaki-shi, Okinawa Pref., Japan	Takayama_230829-04	24.3	124.2	SPG13	3	0.058	0.074	1	PV658119
Minamitorishima, Ogasawara-mura, Tokyo Pref., Japan	Kawakami_220526-398	24.3	154.0	SPG14	6	0.039	0.044	1	PV658120
Marine Corps Dr, Asan, Guam, USA	Michael_PO-AB	13.5	144.7	SPG15	5	0.090	0.104	1	PV658121
Emerald valley, Guam, USA	Michael_PO-EV	13.5	144.7	SPG16	5	0.047	0.054	1	PV658122
Ipan beach park, Guam, USA	Michael_PO-TB	13.4	144.7	SPG17	5	0.083	0.098	1	PV658123
Benny Espinosa avenue, Guam, USA	Michael_PO-SS	13.3	144.8	SPG18	5	0.103	0.120	1	PV658124
Unnamed road, University of Guam, Guam, USA	Michael_PO-UG	13.4	144.8	SPG19	5	0.083	0.099	1	PV658125
Average						0.081	0.096		
Total						258		61	

Population codes were set to identify each individual. N_{MIG} , number of samples for MIG-seq analysis; N_{pl} , number of samples for plastome analysis; H_e , expected heterozygosity; P_i , estimate of nucleotide diversity

III included samples from Hachijojima Island (Tokyo) (MPG02) to Ryukyu Islands (SPG13). Clade IV included samples from Nishinoshima (SPG07) and Chichijima (SPG08 and SPG10). Clade V comprised samples from Osaka (NPG11), Wakayama (SPG01), Kochi (SPG02), Kagoshima (SPG06), Minamitorishima Island (SPG14), and Guam (SPG16, SPG17, and SPG18), along with the two reference *P. oleracea* accessions (Fig. 2).

Geographically, Clade I was found across the Japanese archipelago, from Hokkaido to mainland Kagoshima Prefecture. Clade II occurred in subtropical to tropical islands, spanning Chichijima to Guam (approximately 27°–13° N latitude). Clade III was found along the Pacific side from Ryukyu Islands to Hachijojima Island. Clade IV was exclusive to Nishinoshima and Chichijima Islands, with identical cpDNA sequences. Clade V had the broadest distribution, from Osaka Prefecture to Guam. Sympatric distribution of different clades was observed on Hachijojima in Tokyo, Wakayama, Kochi, and Kagoshima Prefectures. A map illustrating these five clades is shown in Fig. 1.

Population genetic analyses by genome-wide SNPs

Based on an optimal genotyping rate and more than 1000 SNPs, an *R* value of 0.8 was selected for subsequent population genetic analyses. The number of SNPs and the genotyping rate for the other *R* values tested is provided in Table 2. The results of the Neighbor-Net and STRUCTURE analyses were consistent across the different *R* values, except for *R*=0.9. The population network tree revealed three distinct clusters: the Northern Population Group (NPG) cluster, which includes populations from Hokkaido to Kagoshima; the Southern Population Group (SPG) cluster consisting of populations from Wakayama to Guam, including the Ogasawara and Ryukyu Islands; and the Middle Population Group (MPG) cluster which includes populations from Nada-cho, Wakayama Prefecture (MPG01); Ohgago, Tokyo Prefecture (MPG02), and Koniya, Kagoshima Prefecture (MPG03). The Nishinoshima populations formed a distinct subcluster within the SPG, closely related to those from the nearby Chichijima (Fig. 3).

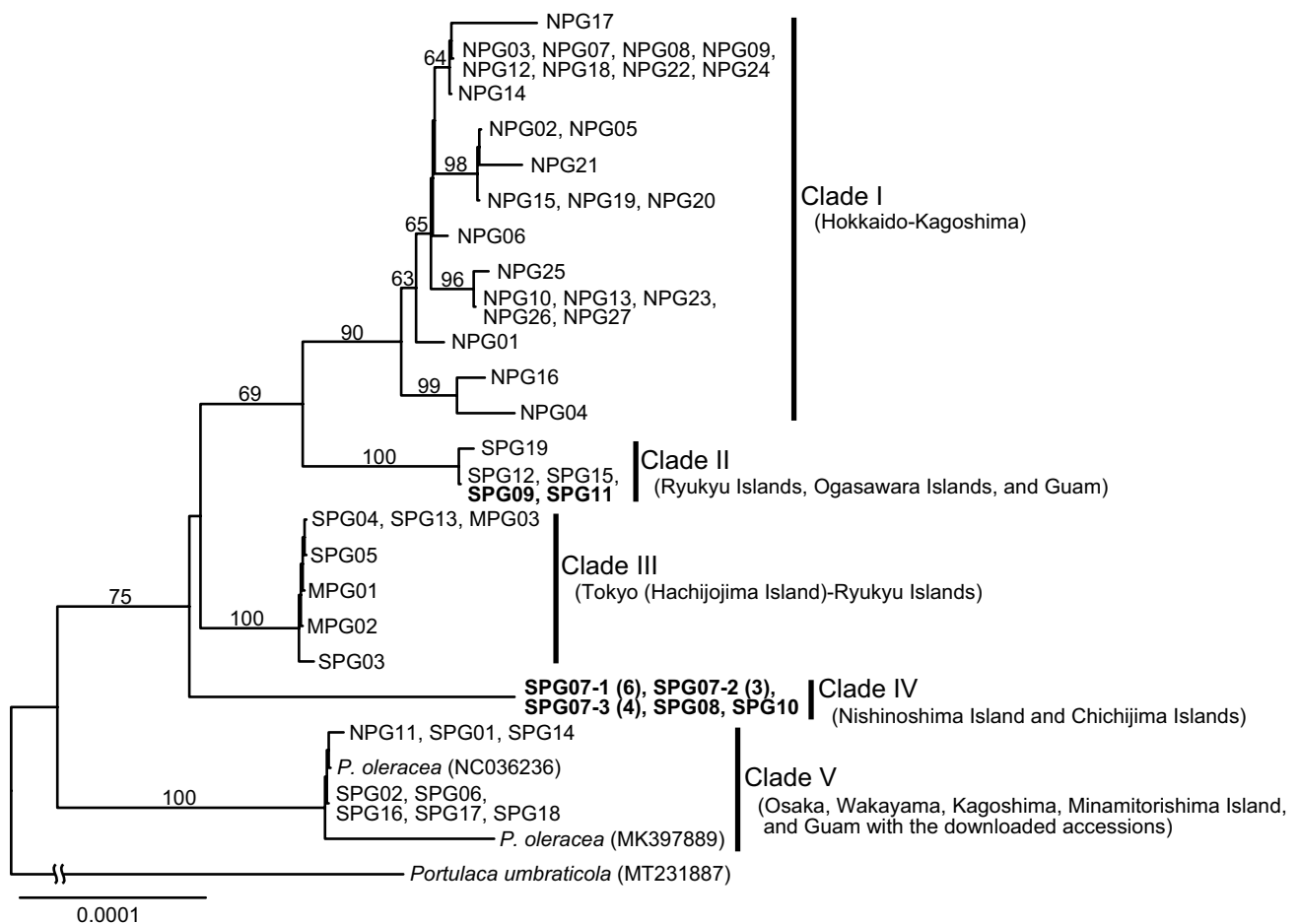


Fig. 2 Plastome phylogeny of *Portulaca oleracea*. Bootstrap probabilities are shown on branch. The acronyms of the samples are listed in Table 1. The acronyms in bold are samples from the Ogasawara Islands

Table 2 Number of SNPs and genotyping rate for different R values

	Number of SNPs	Genotyping rate (%)
0.1	10,128	37.5
0.2	6836	48.8
0.3	5067	57.5
0.4	3867	64.9
0.5	2881	72.2
0.6	2131	78.7
0.7	1537	84.5
0.8	1003	89.9
0.9	524	94.6

$R=0.8$ (in bold) was selected for the data matrix used in this study

In the STRUCTURE analysis, the highest delta K was observed at $K=2$ which was identified as the optimal number of clusters for the dataset (Evanno et al. 2005). However, the likelihood value stabilised after $K=5$ (Fig. 4a). The clustering results from $K=2$ to 5 are shown in Fig. 4b. At $K=2$, the clusters corresponded to the NPG (cluster I), and SPG (cluster II) identified in the Neighbor-Net analysis. Among the three populations on Nishinoshima, cluster II comprised approximately 80% while cluster I comprised approximately 20%. At $K=3$, cluster III emerged, which was dominant among the Nishinoshima populations and also observed on nearby Chichijima to a lesser degree. At $K=4$, cluster IV replaced cluster III, which corresponds to the NPG of $K=3$. At $K=5$, cluster V predominated in parts of the Chichijima and Hahajima Islands. The average t values from $K=2$ to 5 are presented in Table 3. At $K=3$, the F value of cluster III was 0.777, which was higher than clusters I and II.

In samples from Nishinoshima Island, heterozygosity and nucleotide diversity were slightly lower than average.

Discussion

The origin and genetic diversity of *P. oleracea* populations on Nishinoshima offer valuable insights into plant colonisation and vegetation succession on newly formed oceanic islands. This study addressed three key questions: (1) What is the genetic diversity of the populations on Nishinoshima? (2) Which populations are mostly closely related to those on Nishinoshima? (3) Is there evidence for genetic differentiation among *P. oleracea* populations in Japan and Guam? By investigating these questions, we aim to shed light on the processes driving early ecosystem development and the mechanisms underlying plant dispersal and establishment in isolated environments.

Origin and genetic diversity of *Portulaca oleracea* on Nishinoshima Island

The plastome sequences of *Portulaca oleracea* on Nishinoshima Island are identical to those of Chichijima Island, strongly suggesting a genetic connection between populations on Nishinoshima and the Ogasawara Islands. Given Nishinoshima's relatively young age and small size, it is likely that *P. oleracea* populations in the Ogasawara Islands served as the sources for those on Nishinoshima.

Although the exact mode of seed dispersal for *P. oleracea* remains unclear, its seeds are nearly flat and oblate with a diameter of less than 1 mm, suggesting high-dispersal capacity by wind, birds, or ocean currents. Five other plant species have historically been observed on Nishinoshima

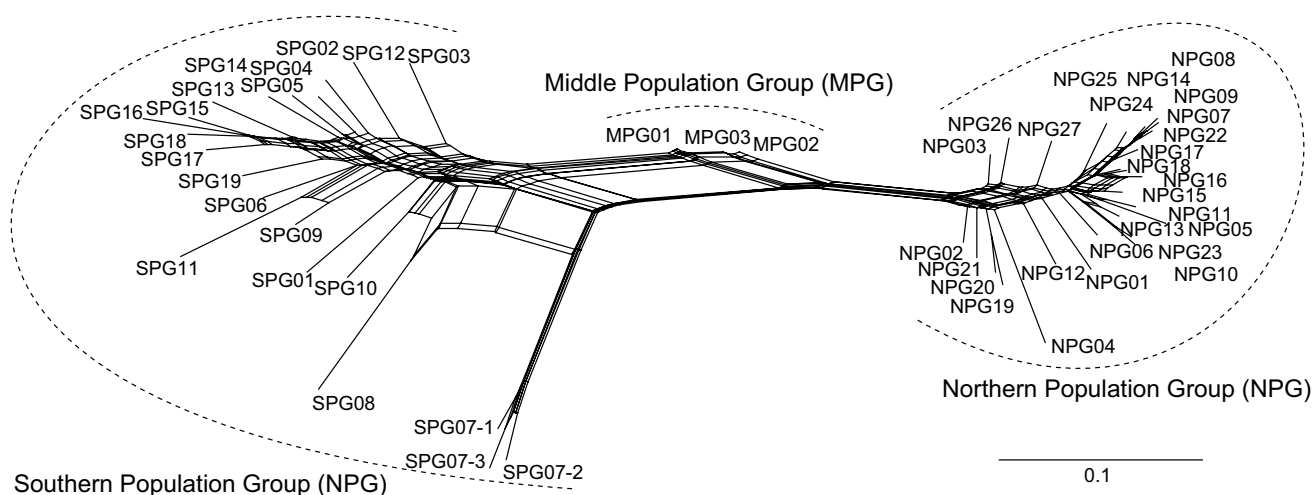


Fig. 3 Neighbor-Net tree of *Portulaca oleracea* based on 1003 SNPs obtained from MIG-seq analysis. The acronyms of the samples are listed in Table 1. The acronyms in bold are samples from the Ogasawara Islands

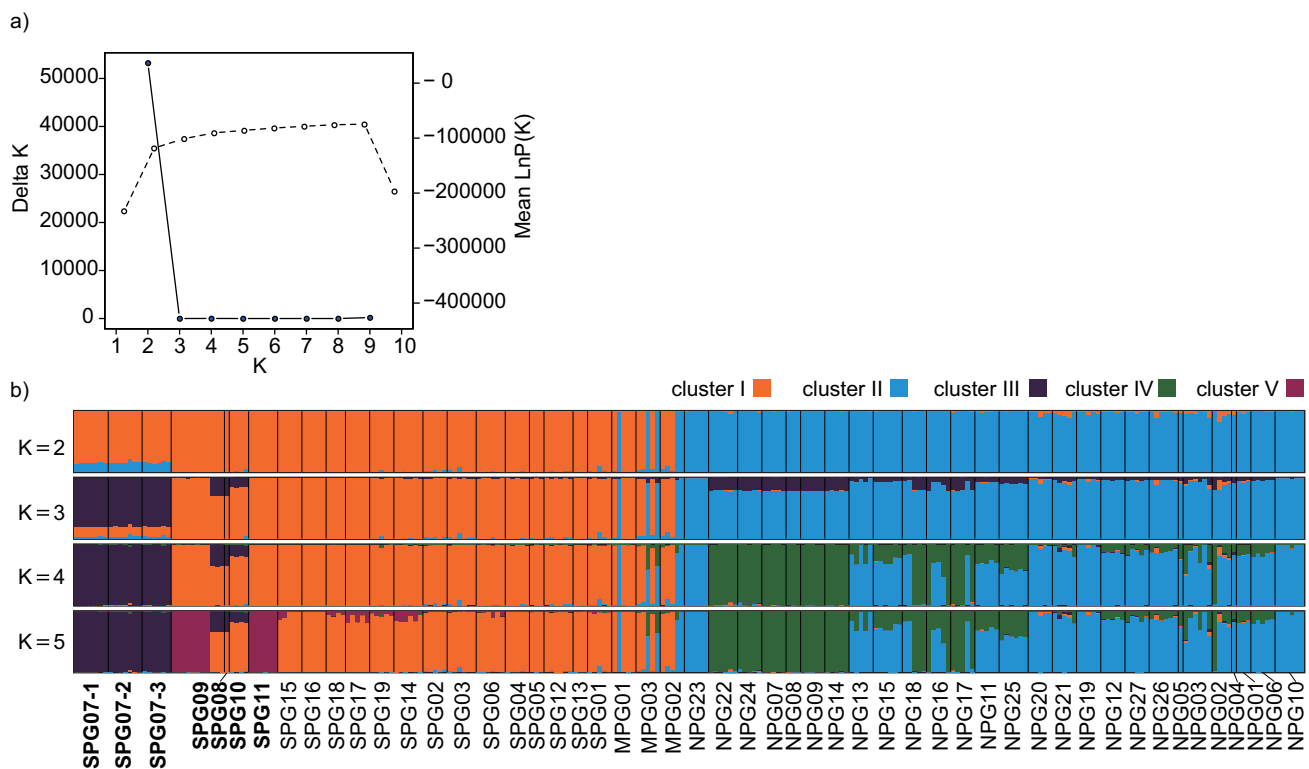


Fig. 4 **a** Values for delta K (closed circle) and mean likelihood (open circle) in Bayesian clustering analyses in STRUCTURE of *Portulaca oleracea* based on 1003 SNPs from MIG-seq data. **b** Bar plot of the

STRUCTURE analysis. Analyses from $K=2$ to 5 are shown. The acronyms of the samples are listed in Table 1. The acronyms in bold are samples from the Ogasawara Islands

Table 3 F values of each cluster in Bayesian clustering analyses in STRUCTURE of *Portulaca oleracea* based on 1003 SNPs from MIG-seq data

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
$K=2$	0.814	0.512			
$K=3$	0.728	0.720	0.777		
$K=4$	0.763	0.719	0.799	0.850	
$K=5$	0.775	0.627	0.803	0.890	0.851

Island: *Eleusine indica*, *Echinochloa crus-galli*, *Ipomoea pes-caprae*, *Vitex rotundifolia*, and *Tetragonia tetragonioides* (Abe 2006). The two grasses *E. indica* and *E. crus-galli* have small seeds that could potentially be dispersed by wind or by attaching to birds (epizoochory), as seeds of other Poaceae species have been reported to disperse via seabirds in the Ogasawara Islands (Aoyama et al. 2012). In contrast, the other three species are typically found on sandy beaches worldwide and possess relatively large seeds that are well-suited for dispersal by sea currents. Since these five species also occur on other islands in the Ogasawara Island group, which is the closest landmasses to Nishinoshima, they likely serve as a key seed source for plant colonisation on the island.

Given its potential dispersal capacity and co-occurrence with other plant species of comparable modes of dispersal, bird dispersal serves as a likely colonisation mechanism for *P. oleracea* on Nishinoshima. Before the 2024 eruption, more than three thousand seabirds, including *Sula leucogaster* Bod., were observed on Nishinoshima (Kawakami et al. 2022). Although seeds of *P. oleracea* have not been directly identified on seabirds, they are known to inadvertently transport the seeds of other plants by attachment to their bodies (Aoyama et al. 2012). Although seabird populations declined on Nishinoshima during a 2024 survey (Kawakami unpublished), populations have rebounded across the Ogasawara Islands (Kawakami and Horikoshi 2022; Suzuki et al. 2019). If the volcanic activity on Nishinoshima subsides, seabirds may return, potentially facilitating further plant colonisation.

Although migration of seeds and propagules from Chichijima to Nishinoshima is highly probable, opportunities for *P. oleracea* to successfully colonise Nishinoshima may have been extremely limited. Genetic analyses, including STRUCTURE and Neighbor-Net based on genome-wide SNPs, revealed a distinct genotype unique to the Nishinoshima populations, suggesting a strong founder effect wherein genetic diversity was likely limited to a few initial colonisers. In the STRUCTURE analysis, the F value of cluster

III, which was dominant in Nishinoshima populations, was significantly higher compared to other clusters, suggesting the impact of pronounced genetic drift likely attributed by disturbances such as volcanic eruptions and typhoons which further reduced genetic diversity. Additionally, the presence of two distinct lineages in the plastome phylogeny and SNP analyses within the Ogasawara Islands suggests multiple colonisation events of *P. oleracea*.

The presence of two distinct lineages of *P. oleracea* in Japan

Our study identified two genetically distinct groups of *P. oleracea* in Japan. Genome-wide SNP analyses revealed a southern group (SPG), including populations from Wakayama to Guam, and a northern group (NPG) from populations spanning Hokkaido to Kyushu. The southern group exhibited relatively derived cpDNA haplotypes, forming a monophyletic clade in phylogenetic analyses. The northern group contained more basal cpDNA haplotypes, forming a paraphyletic clade. Although we were unable to determine the geographic origin of certain GenBank accessions (NC036236 and MK397889), their cpDNA haplotypes formed a basal monophyletic clade in our phylogenetic trees. This finding suggests that the northern group may have been derived from the southern group, though further sampling is required to fully understand *P. oleracea* as a cosmopolitan species.

In the MPG01 (Gobo, Wakayama), MPG02 (Ohgago, Hachijojima), and MPG03 (Koniya, Setouchi-cho, Amami Oshima Island) populations, both genetic groups coexisted, but no genetic admixture was detected in the STRUCTURE analysis. Although plastome sequences were determined from only one individual per population, genome-wide SNPs analyses and cpDNA haplotypes showed minimal genetic overlap, except in NPG11 (Uchihata-cho, Kishiwada-shi, Osaka). These findings suggest that the southern and northern genetic groups have likely been reproductively isolated. Further investigation, including morphological comparisons and cross-compatibility tests, could provide additional evidence to confirm this hypothesis.

The basic chromosome number in the genus *Portulaca* is $x=9$. While *P. oleracea* from the New World has been reported to have a chromosome number of $2n=54$ (Danin et al. 1978; Walter et al. 2015), those in Japan typically exhibit $2n=52$, with aneuploidy observed in several species (Sato et al. 2007). However, the study by Sato et al. (2007) primarily covered populations corresponding to the northern group identified in this study and did not adequately sample regions where the southern group occurs. As differences in chromosome number can act as reproductive barriers between populations, we plan to verify the chromosome numbers in the southern group in future studies.

Plant colonisation and vegetation succession on newly formed oceanic islands

In some regions, *P. oleracea* is considered an introduced species, often cultivated for horticultural purposes (Egea-Gilabert et al. 2014; Lim and Quah 2007). In the Ogasawara Islands, it is also considered introduced and is commonly found in rural areas (Toyoda 2014).

The plastome phylogeny revealed two distinct cpDNA haplotypes on the Ogasawara Islands. One haplotype, belonging to clade II, was found on the Chichijima and Hahajima islands, as well as in Guam and the Ryukyu Islands, suggesting either recent human-mediated introductions or rapid expansion from its natural range. The other cpDNA haplotypes, belonging to clade IV, were exclusively found on Nishinoshima and Chichijima and are genetically distinct from the other analysed cpDNA haplotypes. Although direct evidence for natural colonisation mechanisms such as wind, sea currents, or bird dispersal is difficult to obtain, the unique genetic characteristics of the Nishinoshima Island population suggest natural isolation over an extended period.

Given the geographic isolation of Nishinoshima Island, future plant colonisation events are likely to occur from other islands in the Ogasawara Island group. Genetic analyses confirmed a strong affinity between *P. oleracea* on Nishinoshima and Chichijima Islands, supporting this hypothesis. By preserving genetic samples and continuing flora surveys on these islands, similar analyses can be applied to other species, enhancing our understanding of early vegetation succession following volcanic events.

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Author contributions T.N. and K.T. conceived and designed the study and drafted the manuscript. N.H. and K.T. drafted the manuscript. T.N., K.K., T.K., M.M., M.A.P.F., and K.T. collected samples. H.N. performed molecular laboratory work, participated in data analysis, and performed the sequence alignments. All authors provided final approval for publication and agreed to be responsible for this study. Hiroshi Noda and Tomoyuki Nakano contributed equally as first authors.

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Data availability Complete plastid genome sequences including gene annotation are deposited in NCBI under accession numbers (PV658073-PV671030). Raw sequence reads and MIG-seq reads are available via BioProject (PRJDB20623). The plastome data matrix and MIG-seq can be provided by the authors upon request.

Declarations

Conflict of interest The authors declare no competing interests.

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