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RESEARCH ARTICLE

Genetic diversity and structure of Eurasian otters on Kinmen Island

Nian-Hong Jang-Liaw[1] · Yu-Chun Tan[1] · Chia-Jui Chang[1] · Chung-Hao Juan[2,3] · Hsuan-Yi Hou[1] · Li-Wai Chung[4] · Huan-Song Cao[5] · Daisuke Waku[6] · Shih-Wei Chang[7] · Ling-Ling Lee[3]

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Abstract
Here we present an analysis of the genetic diversity and structure of wild Eurasian otters (*Lutra lutra*) on Kinmen Island, off the coast of eastern-southern China, as derived from high-quality DNA samples from 40 individuals. Mitochondrial DNA was sequenced at the ND5, CYTb and control regions, revealing zero nucleotide diversity within our wild-sampled individuals. In contrast, genotyping at up to 12 autosomal microsatellite loci determined no deviation from Hardy–Weinberg equilibrium and no indication of inbreeding depression ($F_{IS} = 0.010$). Based on phylogenetic analyses of the mtDNA sequences, alongside those from one individual from Taiwan, four spraint samples from northern Fujian, China (this study), and 11 published sequences, the Kinmen otter population is most genetically similar to a now-extinct population in Taiwan, and forms a monophyletic group with southern Chinese populations. These results may guide a framework for implementing Eurasian otter conservation programs in and around the Kinmen region.

Keywords Conservation · *Lutra lutra* · Microsatellites · Mitochondrial DNA

Introduction

The Eurasian otter *Lutra lutra*, a semiaquatic carnivore belonging to the family Mustelidae, has undergone a dramatic global decline throughout the twentieth century. In Europe, where water pollution caused major population losses in the 1960s and 1970s, many Eurasian otter populations have successfully recovered to nearly their previous levels (Roos et al. 2015). In contrast, in many areas of Asia, Eurasian otters remain a species of conservation concern (Roos et al. 2015; Zhang et al. 2016; Li and Chan 2017; Li et al. 2017; Jang-Liaw 2021). In Japan, for example, these otters once lived throughout the four main Japanese islands, but have not been observed in the wild since 1979 and were declared extinct in 2012 (Waku et al. 2016). Nonetheless, the species has expanded its range in South Korea since 2010 by colonizing urban areas because of successful conservation efforts on this species (Hong et al. 2017; Jo et al. 2020). This recovery on the Korean Peninsula is thought to have led to the subsequent recolonization of neighboring islands. Notably, in 2017, Eurasian otters were recorded on Tsushima Island, a small island located between Japan and the Korean Peninsula, and are considered a colony population dispersal from the mainland across the strait (Nakanishi and Izawa 2019).

Historically Eurasian otters were widely distributed in Southeast Asia, China (Gao et al. 1987; Zhang et al. 2016; Cheng et al. 2017; Li and Chan 2017; Li et al. 2017; Han & Shi 2019), and Taiwan (Lee 1996; Chang et al. 2019). Currently, the only stable Eurasian otter population in this

✉ Nian-Hong Jang-Liaw
taco.tw@gmail.com

1 Conservation Genetics Laboratory, Conservation and Research Center, Taipei Zoo. No. 30, Section 2, Xinguang Road, Wenshan District, Taipei City 116016, Taiwan

2 Kinmen Wildlife Rehabilitation and Conservation Association, No. 30, Nanxiong, Jinhu Township 891005, Kinmen County, Taiwan

3 Institute of Ecology and Evolutionary Biology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd. Daan District, Taipei City 106319, Taiwan

4 Forestry Bureau, Kinmen County. No. 31, Dongshan, Jinsha Township 89002, Kinmen County, Taiwan

5 Free Researcher. No. 5, Sanmufang, Dongjie Street, Gulou District, Fuzhou City 350000, Fujian Province, China

6 Faculty of International Agriculture and Food Studies, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

7 Endemic Species Research Institute, Jiji, Nantou, Taiwan

area can be found on Kinmen Island, a 151.6 km^2 island offshore of Fujian Province, China (Lee 1996; Hung et al. 2004; Jang-Liaw 2021; Okamoto et al. 2021). Compared with nearby areas in China, this small island provides a refuge for Eurasian otters and other wildlife; due to its battlefront position in the Taiwan Strait, land use by local people was strictly limited in the decades prior to 1992 (You et al. 2013). The island was returned to the civilian government from military control in the mid-1990s, after which travel to and from it was allowed. An increasing number of tourists, combined with the wishes of many local people to expand the urban area, are causing rapid and serious environmental degradation. Today, the native animals of Kinmen Island are incurring the stressors of human activities, such as deforestation, land and water overuse, pollution, road traffic collisions, and competition from introduced (invasive) species. Eurasian otter is now nationally classified as a critically threatened species by the Taiwanese government (Cheng et al. 2017).

The dispersal distance from Kinmen to other islands or the mainland is not an impediment to Eurasian otters because the strait between them is narrow (the narrowest distance is 1,800 m), but currently Kinmen is surrounded by many large anthropogenic barriers, such as large cities, busy harbors and shipping lines or heavy traffic construction, and the nearest known Eurasian otter population is ca. 400 km away from this island (Han and Shi 2019). Habitat fragmentation due to spatially disconnected suitable territories or migration barriers can have disadvantageous effects on genetic variability. One key feature to guarantee population survival is to maintain genetic variability within populations. This is most efficiently done by ensuring migration, which will lead to genetic exchange between populations and thus contribute to their genetic variability (Frankham et al. 2004; Allendorf and Luikart 2007; Honnen et al. 2014).

According to the absence of potential Eurasian otter populations on the mainland around Kinmen (Han & Shi 2019), the Kinmen otter is observed to form small, isolated populations with no–or severely reduced–gene flow between populations. A decline in genetic diversity could thus present an important threat to the long-term survival of the species on this island. Data on the genetic structure of natural populations are important precursors to the design and implementation of any conservation plan but are currently lacking. Although some surveys of Kinmen otter population structure and dynamics have been performed since 1992 (see Lee 1996; Hung et al. 2004), genetic data are limited. Our aim was therefore to evaluate the genetic variation of the otter population on Kinmen Island using 12 autosomal microsatellites and 3 mitochondrial (mtDNA) genes (3,220 bp in total) to (1) estimate the genetic diversity and the population structure of Kinmen otters, (2) compare the genetic variation between Kinmen and available adjacent otter populations, and (3) develop an informed commentary on the Kinmen otter conservation strategy. Other specimens from Fujian and Taiwan are analysed in this study to improve our understanding over broader taxonomic levels.

Materials and methods

Sampling

Tissue or blood samples were collected from 40 Eurasian otter individuals, as detailed in Table 1. All individuals were sampled on Kinmen Island and most were from deceased specimens (found in fields or as road kill). In addition, we analysed four spraint samples collected in northern Fujian, China (Fig. 1), and a taxidermy specimen collected in Gongliao, New Taipei City, Taiwan, in approximately 1974. This Gongliao specimen is accessed at the National Museum of Natural Sciences, Taichung, Taiwan, under specimen number NMNS20986. The samples examined in this study and applied sequence data from GenBank are summarized in Table 1.

Laboratory procedures

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufacturer's instructions for tissue samples and the modified protocol of Jang-Liaw (2021) for the spraint samples (no. 42–45 in Table 1). We sampled dried muscle tissue from the inner part of the paw pad of the Taiwanese otter taxidermy specimen. All equipment for sampling was sterilized in a UVP CL-1000 Ultraviolet Crosslinker (UVP, Upland, CA, USA). Ancient DNA extraction following the protocol by Gamba et al. (2014) was used for the taxidermy specimen, for which we modified the volume of EDTA and Proteinase K. This extraction step was carried out with all persons wearing protective clothing in the experiment room, which can be irradiated with UV light, in the Taipei Zoo.

The three mitochondrial segments (ND5, CYTb and D-loop) were sequenced for 40 Kinmen otter specimens and three spraint specimens collected from northern Fujian Province, China. Taq DNA Polymerase 2 × Master Mix RED (AMPLIQON, Denmark) was used for polymerase chain reaction (PCR) amplification of the mtDNA segments, with the oligonucleotide primer pairs KCND5F (5'-CAA GAA CTG CTA ACT CGT GCT C-3') and KCND5R (5'-CAG TGT GAT GGT TTG TAT GGT CT-3') for ND5; KCCBF (5'-CCC GCC GTA GAT AGG AGA AGG TT-3') and KCCBR (5'-GAA TCA GGG AAT AGT TTA GTT AGA-3') for CYTb; and KCCRF (5'-CAA AAA GCC CCA CCA TCA GC-3') and KCCRR (5'-CTC TTC TAG GCA TTT TCA GTG-3') for the D-loop. All PCR amplifications were

Table 1 Detailed information on the Eurasian otter samples used in this study

No	Sample ID	Collection Date	Locality	Latitude	Longitude	Sex	Sample type	Lab approach	Haplotypes*	Accession No	Citation
1	KC-02	4/27/2014	Kinmen, Taiwan	24.48504	118.4204	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464990, 5032, 5074	This study
2	KC-03	1/22/2014	Kinmen, Taiwan	24.48396	118.4149	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464991, 5033, 5075	This study
3	KC-04	9/30/2014	Kinmen, Taiwan	24.45645	118.3694	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464992, 5034, 5076	This study
4	KC-05	10/6/2014	Kinmen, Taiwan	24.48104	118.4018	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464993, 5035, 5077	This study
5	KC-06	10/17/2014	Kinmen, Taiwan	24.43621	118.4387	F	Muscle	3 mtDNA segments + microsatellites	H1	MW464994, 5036, 5078	This study
6	KC-07	1/18/2015	Kinmen, Taiwan	24.47561	118.3969	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464995, 5037, 5079	This study
7	KC-08	2015/8/19	Kinmen, Taiwan	24.44064	118.4149	M	Muscle	3 mtDNA segments	H1	MW464996, 5038, 5080	This study
8	KC-09	11/7/2015	Kinmen, Taiwan	24.45978	118.4324	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464997, 5039, 5081	This study
9	KC-10	2016/2/4	Kinmen, Taiwan	24.46217	118.3333	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464998, 5040, 5082	This study
10	KC-11	3/22/2016	Kinmen, Taiwan	24.4585	118.3789	M	Muscle	3 mtDNA segments + microsatellites	H1	MW464999, 5041, 5083	This study
11	KC-12	4/9/2016	Kinmen, Taiwan	24.5148	118.4149	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465000, 5042, 5084	This study
12	KC-13	10/11/2016	Kinmen, Taiwan	24.50692	118.4131	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465001, 5043, 5085	This study
13	KC-14	10/13/2016	Kinmen, Taiwan	24.45878	118.4314	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465002, 5044, 5086	This study
14	KC-15	12/20/2016	Kinmen, Taiwan	24.45355	118.3816	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465003, 5045, 5087	This study

Table 1 (continued)

No	Sample ID	Collection Date	Locality	Latitude	Longitude	Sex	Sample type	Lab approach	Haplotypes*	Accession No	Citation
15	KC-16	1/20/2017	Kinmen, Taiwan	24.48947	118.4064	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465004, 5046, 5088	This study
16	KC-17	1/28/2017	Kinmen, Taiwan	24.51136	118.4126	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465005, 5047, 5089	This study
17	KC-18	3/5/2017	Kinmen, Taiwan	24.47526	118.3965	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465006, 5048, 5090	This study
18	KC-20	9/7/2017	Kinmen, Taiwan	24.45734	118.3676	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465007, 5049, 5091	This study
19	KC-21	12/29/2017	Kinmen, Taiwan	24.42181	118.4384	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465008, 5050, 5092	This study
20	KC-22	2/28/2018	Kinmen, Taiwan	24.4858	118.4082	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465009, 5051, 5093	This study
21	KC-24	2018/9/30	Kinmen, Taiwan	24.46188	118.3349	–	Muscle	3 mtDNA segments + microsatellites	H1	MW465010, 5052, 5094	This study
22	KC-277	2019/8/21	Kinmen, Taiwan	24.46889	118.3982	–	Muscle	3 mtDNA segments + microsatellites	H1	MW465011, 5053, 5095	This study
23	KC-29	2020/3/21	Kinmen, Taiwan	24.484	118.389	–	Muscle	3 mtDNA segments + microsatellites	H1	MW465012, 5054, 5096	This study
24	KC-30	2020/4/15	Kinmen, Taiwan	24.39283	118.3154	–	Muscle	3 mtDNA segments + microsatellites	H1	MW465013, 5055, 5097	This study
25	KNP-002	2/17/2013	Kinmen, Taiwan	24.43979	118.4261	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465014, 5056, 5098	This study
26	KNP-003	–	Kinmen, Taiwan	–	–	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465015, 5057, 5099	This study
27	KNP-006	–	Kinmen, Taiwan	–	–	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465016, 5058, 5100	This study

Table 1 (continued)

No	Sample ID	Collection Date	Locality	Latitude	Longitude	Sex	Sample type	Lab approach	Haplotypes*	Accession No	Citation
28	KNP-009	12/19/2010	Kinmen, Taiwan	24.49109	118.4034	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465017, 5059, 5101	This study
29	KNP-010	10/15/2012	Kinmen, Taiwan	24.4284	118.3603	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465018, 5060, 5102	This study
30	NTU-001	1996/08	Kinmen, Taiwan	24.39439	118.3157	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465019, 5061, 5103	This study
31	NTU-004	7/24/1997	Kinmen, Taiwan	24.453	118.408	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465020, 5062, 5104	This study
32	NTU-005	6/22/1998	Kinmen, Taiwan	24.4386	118.3303	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465021, 5063, 5105	This study
33	NTU-006	–	Kinmen, Taiwan	–	–	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465022, 5064, 5106	This study
34	T-19165	4/30/2010	Kinmen, Taiwan	24.43679	118.4268	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465023, 5065, 5107	This study
35	T-19166	9/24/2010	Kinmen, Taiwan	24.4907	118.404	F	Muscle	3 mtDNA segments + microsatellites	H1	MW465024, 5066, 5108	This study
36	T-8896	7/29/2003	Kinmen, Taiwan	–	–	M	muscle	3 mtDNA segments + microsatellites	H1	MW465025, 5067, 5109	This study
37	T-8897	10/29/2003	Kinmen, Taiwan	24.46045	118.4331	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465026, 5068, 5110	This study
38	XiaoXing	2006/7/4	Kinmen, Taiwan	24.472	118.315	M	Muscle	3 mtDNA segments + microsatellites	H1	MW465027, 5069, 5111	This study
39	DaKin (DK)	2014/4/2**	Kinmen, Taiwan (Rescued)	24.467	118.315	M	Blood	Complete mtDNA + microsatellites	H1	MW316682	This study
40	JinSha	2014/6/18**	Kinmen, Taiwan (Rescued)	24.52	118.42	F	Blood	3 mtDNA segments + microsatellites	H1	MZ502394, 2401, 2407	This study
41	TG (NMNS20986)	c.a. 1974	Gongliao, Taiwan	25.05784	121.8945	–	Stuffed specimen	5 mtDNA segments	–	MW582866-2870	This study

Table 1 (continued)

No	Sample ID	Collection Date	Locality	Latitude	Longitude	Sex	Sample type	Lab approach	Haplotypes*	Accession No	Citation
42	FD-28	3/28/2019	Fuzhou, China	27.35	120.2	–	Spraint	3 mtDNA segments	H2	MW465028, 5070, 5112	This study
43	FD29-1 (CF)	3/29/2019	Fuzhou, China	27.35	120.2	–	Spraint	complete mtDNA	H2	MW344881	This study
44	FD29-2	3/29/2019	Fuzhou, China	27.35	120.2	–	Spraint	3 mtDNA segments	H2	MW465030, 5072, 5114	This study
45	FD29-3	3/29/2019	Fuzhou, China	27.35	120.2	–	Spraint	3 mtDNA segments	H2	MW465031, 5073, 5115	This study
46	CS	–	Sichuan, China	–	–	–	NCBI	Almost complete mtDNA	H3	LC049952	Waku et al. 2016
47	CU1	–	Unknown, China	–	–	–	NCBI	Almost complete mtDNA	H4	LC049377	Waku et al. 2016
48	CU2	–	Unknown, China	–	–	–	NCBI	Almost complete mtDNA	H5	LC049378	Waku et al. 2016
49	JK	–	Kanagawa, Japan	–	–	–	NCBI	Almost complete mtDNA	H6	LC049955	Waku et al. 2016
50	LK	–	Khammouane, Laos	–	–	–	NCBI	Almost complete mtDNA	H7	LC094961	Waku et al. unpublished
51	UK	–	Wincanton, UK	–	–	M	NCBI	Complete mtDNA	H8	LR822067	Wellcome Sanger Institute Data Sharing
52	DM	–	Unknown, Denmark	–	–	–	NCBI	Almost complete mtDNA	H9	MN122838	Margaryan unpublished
53	RS	–	Sakhalin, Russia	–	–	–	NCBI	Almost complete mtDNA	H10	LC049954	Waku et al. 2016
54	SK1	–	Unknown, South Korea	–	–	–	NCBI	Complete mtDNA	H11	EF672696	Ki et al. 2010
55	SK2	–	Unknown, South Korea	–	–	–	NCBI	Complete mtDNA	H10	FJ236015	Jang et al. 2009
56	Japanese otter	–	Kochi, Japan	–	–	–	NCBI	Almost complete mtDNA	H12	LC050126	Waku et al. 2016

*Haplotypes of 3,220 bp-in-length alignment mitochondrial genome

**Date of entering Taipei Zoo

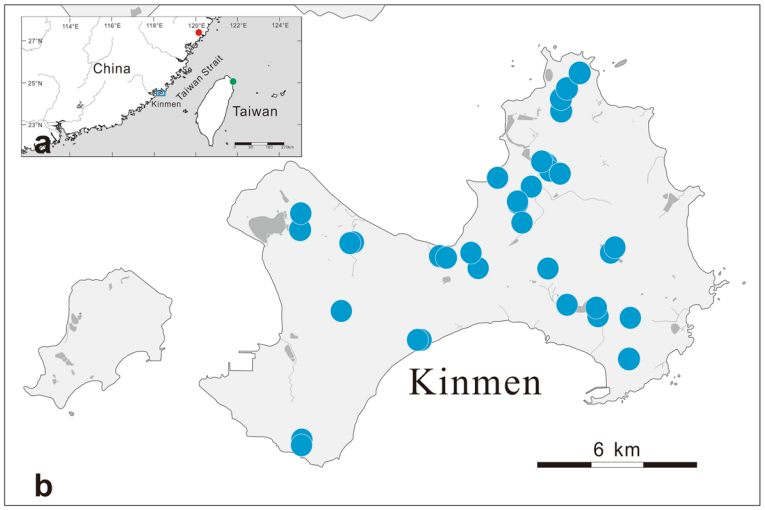

Fig. 1 Locations of sampling sites with accurate GPS records of collected samples used in this study. **a** A map depicting the location of mainland China, Kinmen Island and Taiwan. The red site indicates the collecting site of four spraint samples (No. 42–45 in Table 1), and the green site indicates the sampling location of a Taiwanese otter (No. 41). The blue rectangle indicated the location of Kinmen which is shown in more detail in b. **b** Larger map of Kinmen Island. Blue solid circles indicate known sampling locations of Kinmen otters. See Table 1 for detail information

performed in a 25 µl reaction volume. The PCR conditions consisted of 5 min at 94 °C, 35 cycles of denaturation (at 94 °C for 50 s), annealing (at 57 °C for D-loop, at 50 °C for ND5 and CYTb; all for 50 s), and extension (at 72 °C for 1 min), and a final step of 5 min at 72 °C, using a Biometra TRIO 48 Thermal Cycler (Analytik Jena, Jena, Germany). All the aforementioned primers were designed with reference to the complete mtDNA sequences (e.g. GenBank accession no. LC049952, LC049955, EF672696 and FJ236015) in GenBank for this study. Furthermore, we used the primers L-Pro (5'-CGT CAG TCT CAC CAT CAA CCC CCA AAG C -3') and H-Phe (5'-GGG AGA CTC ATC TAG GCA TTT TCA GTG -3') for PCR and sequencing of D-loop segments (Mucci et al. 1999). PCR products were purified using ExoSAP-It (USB Corp., Cleveland, OH, USA) and Sanger-sequenced on an Applied Biosystems 3730XL DNA Analyser bidirectionally using the primers for PCR and the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit ver. 3.1 (Applied Biosystems). This protocol for the amplification of mtDNA was used on all samples of extracted DNA. All obtained DNA sequences were deposited in GenBank (accession codes listed in Table 1).

Additionally, we generated two whole mitochondrial genome sequences from one Kinmen otter (Dakin, no.39 in Table 1) and from one spraint specimen from northern Fujian (FD29-1, no.43 in Table 1) using either long-range PCR following Chiang et al. (2017) and NGS sequencing for the former or the Multiplex PCR method (MPCR) following Krause et al. (2006) and Waku et al. (2016) for the latter. Dakin's mitogenome sequencing was achieved with two pairs of long-range PCR primers (Ll#1-F: 5'-CTC AAC ACT TCT TAG GCC TAT CGG GTA T-3' and Ll#1-R: 5'-TGT ATT GAG CGG GTT GGC AGG AGT GTA GTT GTC-3'; Ll#3-F: 5'-ACA CAA CCA CAG CCT TCT CAT CAG TCG-3' and Ll#3-R: 5'-GCT AAG TGC AGG GAA AAG GTT GTC AGG T-3') designed for this study based on the complete sequence of *Lutra lutra* from Korea (GenBank accession no. FJ236015). The two mtDNA segments were sequenced on the Illumina MiSeq platform with 300 bp paired-end reads. The target genomic DNA was interrupted by the random shotgun approach to construct a DNA library. The output sequences with low-quality bases were cut off (using Q20 as the standard) at the end of each sample's clean reads, and we discarded sequences shorter than 50 bp in length after excision, or sequences containing more than two n bases. We then used CLC Genomics Workbench (version 7.5) software for de novo assembly. Two additional primers (Ll#S-F: 5'-AAG CGG GTG CAG GGA CAG G-3' and Ll#S-R: 5'-AGT GGT TAT GGC ATT GGC TTG AAA-3') were used for PCR amplification and Sanger sequencing to complete the last part of Dakin's mitogenome as surplus to the MiSeq results. Meanwhile, the mitogenome of the FD29-1 spraint sample was divided into 46 fragments with overlapping regions in neighboring fragments with 167 outer (for MPCR) and inner (for simplex PCR) primers following Waku et al. (2016). The positions of RNA genes were predicted using MITOS (Bernt et al. 2013), and the locations of protein-coding genes were identified by comparing the annotations of another Korean Eurasian otter (Ki et al. 2010; GenBank accession no. EF672696).

Analyses of the selected mtDNA segment sequences

Haplotype diversity (h), average pairwise nucleotide substitutions (k), and other phylogenetic and genetic diversity statistics were conducted using MEGA X version 10.2.4 (Kumar et al. 2018) and DNA SP version 6 (Rozas et al. 2017) inferred from 3 mtDNA segments of all Kinmen specimens, the complete mitogenome of FD29-1 and the available complete mitogenomes of *Lutra lutra* from GenBank (Table 1). The complete mitogenomes used in this study were trimmed to the same length as the 3 mtDNA segments

sequenced from the Kinmen population after alignment, and the segments were concatenated together for subsequent analyses. The pairwise genetic distance among those sequences was calculated in MEGA X based on the Kimura 2-Parameter model of substitution (Kimura 1980). jModelTest2.1.10 was used to find the best-fit substitution model of sequence evolution for constructing phylogenetic trees (Posada 2008). Unrooted haplotype networks were drawn to infer haplotype relationships with the median-joining network procedure using the software POPART v. 1.7 (Leigh and Bryant 2015), and the median-joining (MJ) algorithm was applied (Bandelt et al. 1999).

Phylogenetic analysis among populations

In addition to the analyses at the interpopulation level, we constructed phylogenetic trees of Kinmen otter and other available populations using neighbor-joining (NJ), maximum likelihood (ML), and Bayesian inference (BI) analyses based on complete mtDNA genome (mitogenome) sequences. The sequence of the Taiwanese otter taxidermy specimen was aligned with these complete mitogenomes, considering the unsequenced parts as missing data, as well as other incomplete mitogenomes downloaded from GenBank (LC049377, LC049378, LC049952, LC049954, LC049955, MN122838, LC050126, LC094961, and LR822067; for the aligned FASTA file, see S1). The NJ and ML analyses were performed in MEGA X (Kumar et al. 2018). The best-fit model selected by jModelTest 2.1.7 (Darriba et al. 2012) using the Bayesian Information Criterion was HKY + I (Hasegawa et al. 1985). However, as the model was not available in NJ in MEGA X, as well as for the genetic distance analysis, we used the simpler Kimura 2-Parameter model (Kimura 1980) to estimate genetic distance, as it is the most widely used model of molecular evolution for estimating genetic differences and phylogenetic relationships (Nishimaki and Sato 2019). Nodal support of the NJ and ML trees was evaluated using 1000 bootstrap replicates. The Bayesian analysis was conducted with 2,000,000 generations with likelihood settings from the best-fitting model HKY + I (rates = equal; pinvar = 0.7920) with base frequencies of A = 0.3225, C = 0.2744, G = 0.1456, T = 0.2575 and a transition/transversion ratio of 17.7336, selected by hLRTs (hierarchical likelihood ratio tests), which were obtained from jModelTest with MrBayes 3.2.7 (Ronquist et al. 2012). Approximately 25% of the sampling trees were discarded (as burn-in). Trees were sampled every 100 generations. A 50% majority-rule consensus tree was calculated using the remaining 15,000 trees (with log-likelihoods converged to stable values). Two separate runs with four Markov chains were performed.

Microsatellite genotyping analysis

Twelve microsatellite markers (Lut701, Lut715, Lut717, Lut733, Lut782, Lut832, Lut833, 04OT04, 04OT05, 04OT07, 04OT14 and 04OT22; Dallas and Piertney 1998; Dallas et al. 1999; Huang et al. 2005; see Table 2) were applied in this work to amplify the tissue specimens, as well as four spraint specimens collected from northern Fujian. To improve the reliability of allele scoring, we only used tetranucleotide-repeat loci to avoid the stutter bands that are frequently seen in dinucleotide-repeat loci. PCR was

Table 2 The genetic diversity of 39 Eurasian otter samples collected from Kinmen Island as estimated from 12 microsatellite loci

Locus	N_a	A_r	H_o	H_e	F_{IS}	HWE	F(null)	r
Lut701	4	2.505	0.462	0.387	− 0.195	NS	− 0.2457	–
Lut715	6	4.278	0.821	0.775	− 0.06	NS	− 0.0368	–
Lut717	4	2.697	0.641	0.577	− 0.112	NS	− 0.0628	–
Lut733	5	3.104	0.564	0.600	0.06	NS	0.0249	–
Lut782	3	2.24	0.436	0.531	0.181	NS	0.0834	–
Lut832	3	2.756	0.513	0.531	0.035	NS	− 0.0156	–
Lut833	4	2.955	0.667	0.637	− 0.047	NS	− 0.0354	–
04OT04	5	3.924	0.718	0.737	0.027	NS	0.0141	–
04OT05	5	3.646	0.513	0.705	0.275	NS	0.1283*	–
04OT07	4	2.691	0.605	0.534	− 0.135	NS	− 0.1075	–
04OT14	5	3.565	0.605	0.645	0.063	NS	0.0308	–
04OT22	3	2.94	0.641	0.659	0.028	NS	0.0038	–
Mean	4.25	3.108	0.599	0.610	0.010			− 0.029

N_a mean number of alleles per locus, A_r allelic richness, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient, HWE the number of loci departed from Hardy–Weinberg equilibrium, NS Non-significance, F(null) the frequency of null alleles (*shows signs of a null allele), r relatedness

performed in 25 µl volumes with Quick Taq HS DyeMix (TOYOBO, Japan). The samples successfully amplified were diluted at a 1:100 ratio and genotyped on an ABI PRISM 3730 XL DNA Analyser (Applied Biosystems).

The initial microsatellite alleles were scored and determined using GeneMarker 2.20 (SoftGenetics LLC, State College, PA, USA). Population genetic analysis was performed with GenAlEx 6.51 (Peakall and Smouse 2006, 2012), determining the number of alleles (*Na*), expected heterozygosity (H_e), observed heterozygosity (H_o) and relatedness (r) values. The inbreeding coefficient (F_{IS}) and allelic richness (Ar) were calculated by Fstat 2.9.4 (Goudet 2003). Deviations from Hardy–Weinberg equilibrium (HWE) for loci were tested in Genepop v. 4.7.3 (Rousset 2008) using 100,000 dememorization, 1,000 batches, and 10,000 iterations. A Bonferroni correction for multiple comparisons was applied for the pairwise p values from the HWE tests. Micro-Checker v.2.23 (Van Oosterhout et al. 2004) was used to detect null alleles among loci. To evaluate if the occurrence of a recent bottleneck appeared in the population, a heterozygosity excess test was conducted with the software BOTTLENECK 1.2 (Piry et al. 1999). The two-phase model (TPM) with 95% single-step mutation and a 12% variance was executed. We used the sign test and one-tailed Wilcoxon test to determine the statistical significance. In addition, a mode shift test was performed to evaluate potential distortion of the allele frequency distribution (Luikart et al. 1998), which was also implemented in BOTTLENECK 1.2. We conducted different statistical approaches according to the genetic index's type. For the relatedness (r) and inbreeding coefficient (F_{IS}), we used the permutation test implemented in GenAlEx 6.51 and Fstat 2.9.4, respectively.

To determine the number of genetically distinct units in Kinmen, we used a Bayesian clustering approach that was implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). Each individual was assigned to a genetically related cluster based on the admixture model with allele correlated frequencies. To avoid potential deviations among runs, 10 independent replicates were performed in the ancestral clusters (K) ranging from 1 to 5. Each run was performed by a Markov Chain Monte Carlo (MCMC) using 100,000 iterations following a burn-in period of 10,000 iterations. The best number of clusters was determined by the ad hoc statistic ΔK (Evanno et al. 2005), which was implemented in the web-based program STRUCTURE HARVESTER (Earl and VonHoldt 2012). All the output from each run was summarized and visualized by CLUMPAK 1.1 (Kopelman et al. 2015). In addition, A Principal Coordinates Analysis (PCoA) and AMOVA (Analysis of Molecular analysis) were performed to examine the potential clustering pattern, which was analysed by GenAlEx 6.51.

Results

Complete MtDNA sequences of Eurasian otters

In this work we contributed two complete mitogenomes of *Lutra lutra* sampled from Kinmen and northern Fujian. They were both determined to be 16,536 bp in size, including 13 typical vertebrate protein-coding genes (PCGs), 22 transfer RNA genes, 2 ribosomal RNA genes, and a control region, and the GenBank accession codes are MW316682 and MW344881, respectively. Detailed information on the mitochondrial genome comparison of the two sequences is summarized in Table 3. The divergence of whole mitochondrial genomes between them was 0.31% based on the Kimura 2-parameter distance (Kimura 1980), and the variability ranged from 0–0.59% among the 13 PCG sequences. For the combined sequences of 13 PCGs, the divergences of nucleotide and amino acid sequences between the two sequences were 0.30% (34 of 11,420 sites) and 0.36% (15 of 3,805 codons), respectively. Divergences between the two rRNA genes were 0.42% (12S rRNA) and 0.06% (16S rRNA). The control region showed the highest nucleotide sequence divergence (0.93%) among those of other genes listed in Table 3.

In addition to the two complete mitogenomes from Kinmen and northern Fujian, we sequenced partial mtDNA from a Taiwanese otter taxidermy specimen. The extracted DNA of this specimen was very low quality and highly contaminated by microbes. Only five segments could be sequenced successfully by the MPCR method (Waku et al. 2016) over the 46 sets of MPCR fragments, which totaled 2,019 bp, with lengths of 483 bp (at the position of 1,210–1,691 bp compared to the complete Kinmen otter's mitogenome MW316682), 530 bp (3,150–3,679 bp), 487 bp (8,701–9,187 bp), 424 bp (11,584–12,007 bp) and 95 bp (15,768–15,862). We also tried to amplify the extracted DNA from the Taiwanese otter sample with Illumina short-read sequencing, but almost all of the sequenced data comprised contaminated microbial sequences. The five sequences of the Taiwanese otter specimen were deposited in GenBank as well (accession numbers are MW582866-2870; Table 1).

The genetic diversity of mtDNA at the population level

We obtained an alignment of a total of 45 sequences of the partial mitochondrial genome totaling 3,220 bp, including CYTb (1,335 bp, including 33 bp of partial ND6, total trnE, CYTb and trnT, and 21 bp of partial trnP), ND5 (832 bp, including 60 bp of partial tRNAtrnL and partial

Table 3 Comparison of nucleotide length, GC content, the number of codons, start codon, stop codon, and nucleotide and/or amino acid sequence divergences (% Kimura 2-parameter distance) between the two complete mitochondrial genomes of Eurasian otters from Kinmen Island and northern Fuzhou, China

Gene	Dakin(MW316682) / FD29-1(MW344881)					Nucleotides divergence(%)	Amino acid divergence(%)
	Sequence length(bp)	GC content(%)	No. of codons	Start codon	Stop codon		
12SrRNA	964/964	–	–	–	–	0.42	–
16SrRNA	1568/1568	–	–	–	–	0.06	–
ND1	956/956	44.03/44.13	318/318	ATG/ATG	TA/TA	0.31	0
ND2	1042/1042	40.06/40.06	347/347	ATC/ATC	T/T	0.39	0.29
COI	1545/1545	43.88/43.75	515/515	ATG/ATG	TAA/TAA	0.13	0
COII	684/684	43.27/42.84	228/228	ATG/ATG	TAA/TAA	0.44	0
ATP8	201/201	35.32/35.32	67/67	ATG/ATG	TAA/TAA	0	0
ATP6	681/681	41.70/41.70	227/227	ATG/ATG	TAA/TAA	0.59	0
COIII	784/784	45.59/45.34	261/261	ATG/ATG	T/T	0.26	0
ND3	348/348	41.09/41.09	116/116	ATA/ATA	TAA/TAA	0	0
ND4L	297/297	40.07/39.73	99/99	ATG/ATG	TAA/TAA	0.34	0
ND4	1378/1378	41.61/41.83	459/459	ATG/ATG	T/T	0.36	0
ND5	1830/1830	42.51/42.62	610/610	ATA/ATA	TAA/TAA	0.22	0.49
ND6	534/534	40.45/40.64	178/178	ATG/ATG	TAA/TAA	0.19	0
CytB	1140/1140	44.21/44.30	380/380	ATG/ATG	AGA/AGA	0.44	0.26
PCG*	11,420/11420	42.52/42.52	3805/3805	–	–	0.30	0.36
control region	1090/1090	46.46/46.10	-	–	–	0.93	–
entire genome	16,536/16536	41.93/41.91	3805/3805	–	–	0.31	0.44

* Combined sequences of 13 protein-coding genes

ND5) and D-loop segments (1,053 bp), from specimens collected across Kinmen Island from 1996 to 2020, including two captive individuals in the Taipei Zoo, which were rescued and found on Kinmen Island in 2014 (no. 39 DaKin and no. 40 JinSha; see Table 1). The complete mitogenome of the rescued individual Dakin shows the same H1 haplotype across the same three mtDNA segments. In total, the pooled mtDNA alignment (3,225 aligned nucleotides/3,220 nucleotides excluding sites with gaps; 55 individuals including spraint samples and downloaded sequences used in this study but excluding the Taiwanese otter taxidermy specimen) showed 11 distinct haplotypes, defined by 75 mutations, which included 52 variable sites, as well as 22 parsimony informative sites. Surprisingly, all sequences of the 40 Kinmen otters belonged to a single haplotype, H1. Haplotype H2 was detected in four spraint samples collected from northern Fujian in March 2019. Each of the other DNA segments mined from GenBank (listed in Table 1) showed unique haplotypes across the 3,220 bp alignment, except for two sequences from Sakhalin, Russia (no. 53 in Table 1) and South Korea (no. 55), which shared the same haplotype, H10. A combined sequence of Japanese otter (*Lutra nippon*), an extinct species proposed by Waku et al. (2016) (no. 56 in Table 1), was shown to be a phylogenetically distant haplotype, H12.

The star-like median-joining network of 12 haplotypes did not show any obvious signal of phylogeographic structuring (Fig. 2). The haplotype H1 that is detected in all Kinmen samples could be grouped with H2-H8, which corresponded to the sequences from Kanagawa (Japan), China, Laos, and UK. The other haplotypes, corresponding to Denmark (H9), Korea and Sakhalin (H10 and H11), show more phylogenetic distance from the Kinmen population. The Japanese otter, H12, showed the most phylogenetic distance to all the others.

Genetic structure in microsatellites

For the population in Kinmen, we successfully genotyped 39 individuals (no. 1–40 in Table 1, except no. 7/KC-08, which failed in the microsatellite test due to the poor quality of this tissue sample) collected across different years at the 12 nuclear microsatellite loci. This sample collection comprises a comprehensive sampling of most habitats on Kinmen Island over a period of 24 years. The four spraint samples collected from northern Fujian failed to be genotyped at all 12 microsatellite markers used in

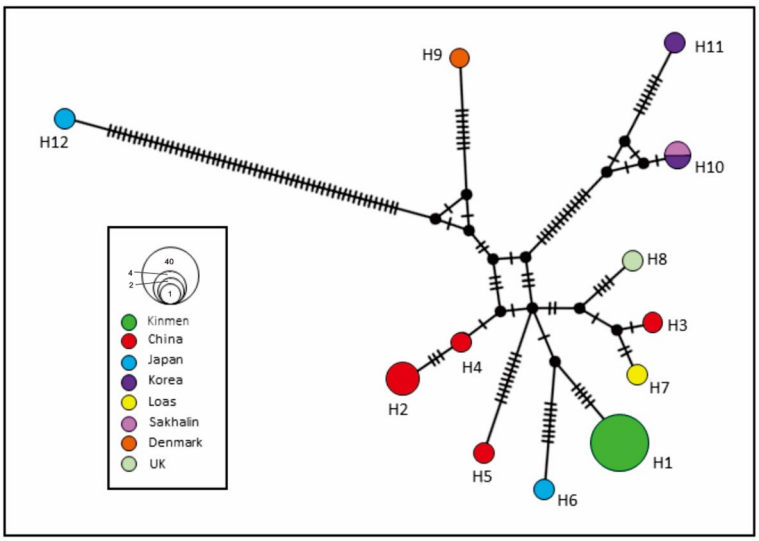

Fig. 2 A median-joining (MJ) haplotype network based on variation between haplotype sequences of Eurasian otters inferred from 3,220 bp of mitochondrial DNA sequences. The sampling location information is indicated by different colors. Circle size reflects the number of individuals having the corresponding haplotype. Each line connecting two circles represents a single mutational step between one haplotype and another. Small solid circles signify possible missing haplotypes

this study due to their poor DNA quality and thus were not included in our analysed dataset.

Most loci were polymorphic, showing moderate diversity. No loci showed departure from Hardy–Weinberg equilibrium, consistent with the fact that no inbreeding was detected, as revealed by nonsignificant F_{IS} values. (Table 2). For the bottleneck detection analysis, there were no significance values found in any of the heterozygosity excess tests (Sign test: P value = 0.412, Wilcoxon's signed-rank test: P value = 0.150), and the mode shift test was normal. The Micro-Checker v.2.23 software revealed that one locus (04OT05) was detected with null alleles, probably due to homozygote excess. The mode shift test detected a shifted distribution of alleles in the Kinmen otter population, suggesting a signature of a recent decrease in population size (Fig. S2). The STRUCTURE analysis revealed that the most likely group number is 3 according to the best ΔK value, which means that there should be 3 ancestral origins of this population. However, no clear and coherent association between geographic location and grouping pattern can be found (Fig. 3). No significant difference was detected among individuals found in different geographic locations on Kinmen (P = 0.270) based on the AMOVA (S3). Principal Coordinate Analysis (PCoA) showed no clustering between the tested Kinmen otter samples as well (Fig. 4). Generally, no distinct population structure can be found according to the results obtained from the STRUCTURE, PCoA and AMOVA analyses. That some individuals have a relatively close relationship in certain regions might suggest that potential familial lineages have been incorporated into this island population. However, the overall pattern is not significant according to the results from the AMOVA.

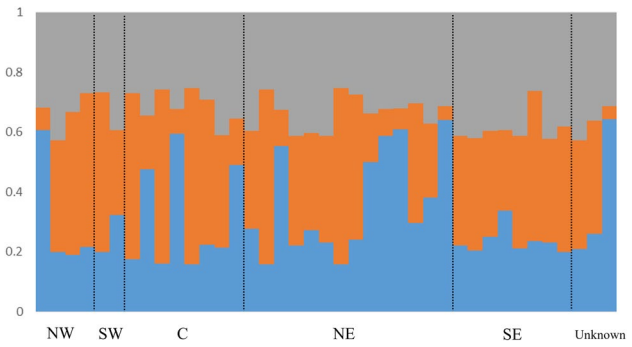

Fig. 3 The genetic clustering of 39 Kinmen otter samples based on STRCTURE analysis. The best K value was for K = 3. Each ancestral cluster is denoted in different colors. Each vertical bar represents an individual and the ratio of its color is proportional to the assignment rate to the likely cluster. The abbreviations are the geographic locations on Kinmen Island for each tested individual, which were noted as *NW* Northern West, *SW* Southern West, C Central, *NE* Northern East, *SE* (Southern East) and Unknown

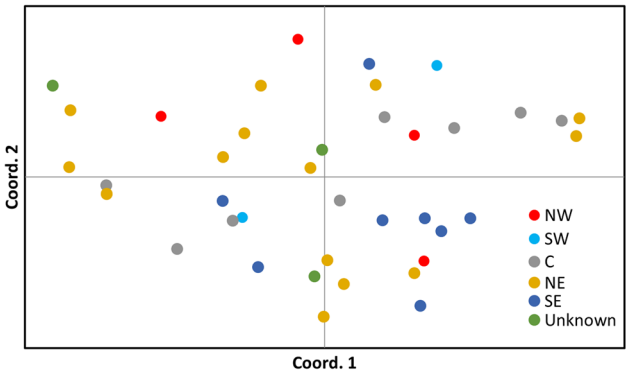

Fig. 4 Principal Coordinates (PCoA) analysis of 39 Kinmen otter samples. Each individual was coded in different colors according to its geographic location. Refer to Fig. 3 for the abbreviations of geographic location

Phylogeny of kinmen and Taiwan specimens based on mtDNA data

The phylogenetic tree reconstructed by either the NJ, ML or BI methods based on complete mitochondrial genomes produced very similar tree topologies (Fig. 5), which revealed three major groups. The first one is a Southern Asia haplogroup, in which the complete mitogenome sequence of a spraint sample from northern Fujian, China (CF in Fig. 5), a complete mitogenome sequence of captive individual from Kinmen Island (KM), and a partial mtDNA sequence of a specimen from Gongliao, Taiwan (TG), were monophyletically grouped together with two complete mitogenome sequences from China (sampling location unknown; CU1) and Kanagawa, Japan (JK). On the other hand, sequences of otters from Sichuan, China (CS) and Khammouane, Laos (LK) were grouped together, and surprisingly, the sequence of a UK individual was grouped with the CS + LK group with high node support values (99/91/100 for NJ/ML/BS analyses). The abovementioned sequences were grouped as sister groups and formed a monophyletic group with another Chinese individual (CU2). The second group formed a Northern Asia haplogroup, which included sequences from South Korea (SK1 and 2) and Sakhalin (RS). These sequences were grouped with high node support values in all tree reconstruction methods. The sequence from Denmark (DM) formed a third isolated lineage apart from the other two groups. Compared with the Denmark sequence, the Southern and Northern Asia haplogroups were grouped together with high support values (96/94/100; see Fig. 5).

Discussion

Genetic characterization of the Kinmen otter population

It is widely understood that low levels of genetic variability occur in populations of the Eurasian otter (Mucci et al. 1999; Ferrando et al. 2004; Ketmaier and Bernardini 2005; Pérez-Haro et al. 2005; Ki et al. 2010; Cohen et al. 2012; Honnen et al. 2014). The low genetic variability found in Eurasian otter studies can be explained by population fragmentation (Dallas et al. 2002; Ketmaier and Bernardini 2005; Mucci et al. 2010; Cohen et al. 2012), genetic drift, inbreeding events (Larson et al. 2002; Frankham 2005), relatively recent demographic expansion from a small population (Mucci et al. 2010; Guerrero et al. 2015), or recent drastic decline in population size (Hoelzel et al. 1994). In this study, the difference in the degree of population differentiation between the mitochondrial and nuclear markers is remarkable. At present, in Europe, the phylogenetic structures of Eurasian

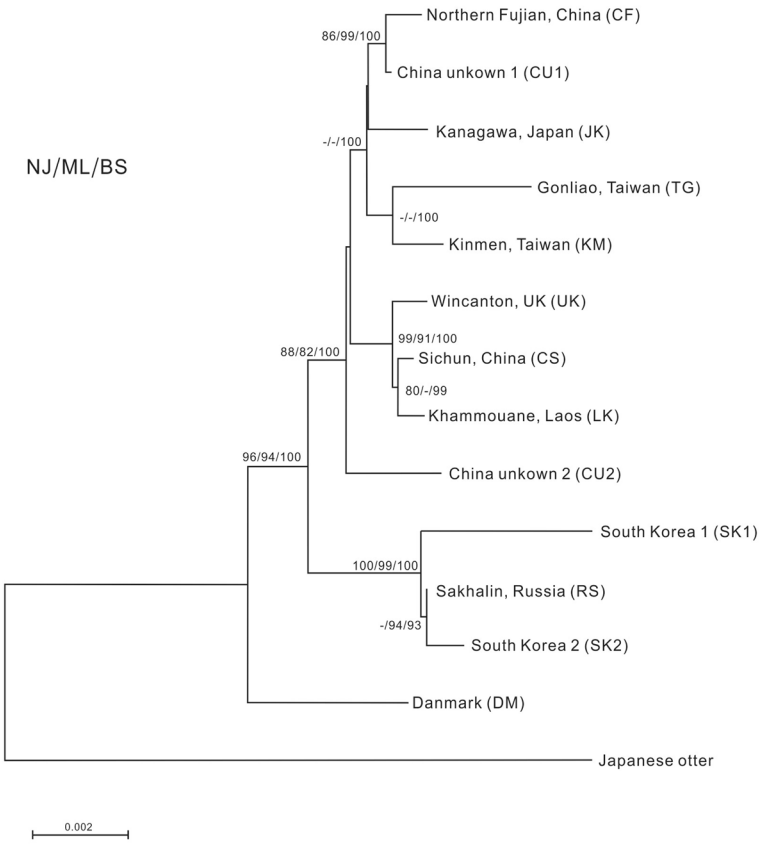

Fig. 5 Neighbor-joining tree of haplotypes inferred from complete mtgenome sequences. Branch lengths are proportional to the scale given in nucleotide substitutions per site. Numbers at major internal nodes are bootstrap support values (NJ and ML based on 1000 replicates) or posterior probabilities (BI) in the order of NJ/ML/ BI. The aligned FASTA file of this tree can be found in supplementary file S1

otters are more well known than those of Asian populations. Finnegan and Néill (2010) reported that in Irish and European populations, Eurasian otter had an *h* of 0.56 and π of 0.003 mtDNA diversities in CR segments of 299 bp (n = 407). Mucci et al. (2010) revealed that the overall haplotype diversity of European populations was high in a 1,580 bp mtDNA segment (0.79 ± 0.037, SD), but nucleotide diversity (0.0014 ± 0.00012) and the average number of pairwise differences (2.25) were low, suggesting that extant otter mtDNA lineages originated recently. Ranyuk and Ansorge (2015) also reported low epigenetic variability of the Eurasian otter from Europe to Kamchatka. Furthermore, we found no genetic variation in mtDNA level among the sampled specimens collected from Kinmen Island. The only population information provided by mtDNA data in this study is that all the tested individuals represent a single maternal ancestor, which revealed that Kinmen otters are descendants of a very small ancestral population.

Nevertheless, the results of the microsatellite analyses provided a different view. In the tested samples from Kinmen Island, the mean number of alleles per locus showed a moderate level ($N_a = 4.25$; see Table 2), which is similar to the result found in European populations, that is, 4.9 on average (Mucci et al. 2010). The observed and expected heterozygosities were moderate ($H_o = 0.60$; $H_e = 0.61$) as well, which were 0.50 and 0.58, respectively, on average across populations in Europe (Mucci et al. 2010). The 12 loci were found to be in Hardy–Weinberg equilibrium, which was consistent with the nonsignificant F_{IS} values, indicating no inbreeding in the Kinmen Island otters. In summary, Kinmen Island otters showed no evidence of a bottleneck in any of the microsatellite analyses but instead showed moderate levels of microsatellite allelic diversity. The results of the microsatellite analyses did not support the low genetic variation hypothesis suggested by the mtDNA results.

Such mito-nuclear discordance can be found in animals where males have larger daily movement distances and home-range sizes than females (Paetkau et al. 1998; Waits et al. 2000; Scribner et al. 2001; Dubey et al. 2008; Thorpe et al. 2008; Peters et al. 2012; Folt et al. 2019; Dessi et al. 2022). The limitation of female movement may initiate phylogenetic structure of the maternally inherited mtDNA, while increased male movement drives broader gene flow of the nuclear genome (Folt et al. 2019). In addition, male polygyny is known to generate strong genetic links within a population (Chesser 1991), and Eurasian otter males are considered to be polygynous (Kruuk 2006). In this study, the mito-nuclear discordance results support such a scenario in Kinmen Island otters; that is, the genetic structuring may also have been caused by the mating system. Quaglietta et al. (2013) proposed that behavioral barriers that are mainly related to dispersal behavior and mating systems are intrinsic biological mechanisms of the Eurasian otter.

In addition to mechanisms related to male/female dispersal and mating systems, the inherent evolutionary properties of the two marker types also explain the differences in genetic diversity. In general, single nucleotide polymorphisms in mitogenome sequences are expected to have a slower rate of mutation/substitution compared to microsatellite loci, which evolve via DNA slippage of tandem repeat arrays (Wierdl et al. 1997; Lai and Sun 2003; Putman and Carbone 2014). In addition, although no signal of a bottleneck was detected in Kinmen, the demographic history of the Kinmen otter population may have influenced present-day genetic diversity.

Relationships of Eurasian otter between Kinmen, Taiwan and Fujian

Although once widely distributed in Europe, Asia and North Africa as a flourishing carnivore species, the interpopulation genetic information about Eurasian otters is very limited. Numerous studies on the genetic structure of European populations have been published (e.g., Dallas et al. 1999; Kranz 2000; Pertoldi et al. 2001; Randi et al. 2003; Ferrando et al. 2004; Pérez-Haro et al. 2005; Prigioni 2005; Kalz et al. 2006; Prigioni et al. 2006; Hájková et al. 2007; Preston et al. 2007; Prigioni et al. 2007; Ferrando et al. 2008; Anna et al. 2009; Finnegan and Néill 2010; Koelewijn et al. 2010; Mucci et al. 2010; Sherrard-Smith and Chadwick 2010; Urban et al. 2010; Honnen et al. 2014; Pedroso et al. 2014), but information on Eurasian otters from other continents is quite lacking. This is because most Eurasian otter populations outside Europe have not recovered or have even disappeared after the global population decline in the last century, increasing the difficulty for researchers to find enough samples for study. For example, research on the Eurasian otter in China began in the 1960s. At that time, the Eurasian otter was regarded as a harmful animal that endangered fisheries. The relevant research focused on the damage of this carnivore to the fishery industry and how to control it. Sporadic thinking about otter conservation began to appear as late as the 1980s, while Eurasian otter populations across China largely disappeared due to massive hunting, and academic research reports began to appear sporadically after the mid-2010s. Although several remaining populations have been found recently in China, their detailed information, such as population size, range, and genetic structure, remains unknown (Han & Shi 2019).

Our analyses of the interpopulation relationships showed that the Kinmen Island otters and those of southern China, which are assigned as the subspecies *L. l. chinensis* (Wilson and Reeder 2005; Hung and Law 2016), share a relatively close relationship. It is also known that the Taiwanese otter population should be a branch of the subspecies *L. l. chinensis* based on our limited DNA data of a Taiwanese

individual, which formed a monophyletic group with otters from southern China, as well as otters from northern Fujian, China and Kanagawa, Japan. The distribution range of the subspecies *L. l. chinensis* might have been larger than currently thought, including those populations that once survived on the large islands of Japan. Unfortunately, both populations in Taiwan and Japan are considered extinct today.

There are 11 subspecies of Eurasian otter proposed by Wilson & Reeder (2005), and Hung & Law (2016) considered the extinct Japanese otter as the twelfth subspecies. However, there have been very few discussions on the taxonomic issue of Eurasian otters at the subspecies level (Oleynikov and Saveljev 2015; Hung and Law 2016; Waku et al. 2016). The situation of Eurasian otters in many areas of Asia remains unknown (Li and Chan 2017; Han and Shi 2019). Lacking an understanding of the detailed population differentiation at the subspecies level will make an appropriate conservation strategy for all Eurasian otters unreachable. More research using regional Eurasian otter specimens, genetic data analyses, and large-scale population comparisons using both genetic and morphologic characters are necessary for global Eurasian otter conservation actions.

Conservation and management implications for the Kinmen otter

Small et al. (2003) reported that the nuclear DNA structure in American marten *Martes americana* paralleled the mitochondrial DNA structure, indicating that the dispersal of both males and females is inhibited among most islands and between the mainland and most islands. In contrast, the mito-nuclear discordance of the genetic structure of Kinmen otters implied that this population was not isolated. However, this population is spatially isolated, and potential genetic interaction with other populations on nearby mainland seems impossible at present. No other Eurasian otter population can be found around Kinmen Island thus far (Han and Shi 2019). Therefore, the isolation of this population might have occurred very recently.

The accompanying high levels of local inbreeding can result in genetic depletion and inbreeding depression (Zachos et al. 2007; Johnson et al. 2010; Habel and Zachos 2012). Evaluation of both the genetic variability and the degree of differentiation and connectivity among populations is critical to determine the current status and future potential viability of a species (Honnen et al. 2014). Kinmen otters showed a moderate level of genetic diversity in microsatellite analyses but no mtDNA genetic diversity at all. Since mtDNA is of maternal origin, we are concerned that migration, especially for females, between Kinmen and other populations is very restricted. Although the population of Kinmen otters seems stable in number and newborn cubs can be easily observed at present (Okamoto et al. 2021), there is no evidence that they can receive new individuals from nearby populations to increase or maintain their genetic variability, as other Eurasian otter populations seem to be unavailable around Kinmen Island in recent times (Li and Chan 2017; Li et al. 2017; Chang et al. 2019; Han and Shi 2019). Migration events of other populations into Kinmen Island that maintain the moderate microsatellite variation of the Kinmen otter population might be few in number or occurred before the extirpation of Eurasian otters in southerneastern China. The conservation of Kinmen otters should therefore be more proactive to keep this population sustainable with healthy genetic diversity. The strategies for conservation activities, such as (a) rebuilding their historical populations in the suitable habitats near Kinmen in cooperation with colleagues in China; (b) maintaining an insurance population through conservation breeding programmes in zoos or by expanding the Kinmen population into suitable protected areas in Taiwan; and (c) genetic rescue by introducing individuals from other, genetically similar populations, should be discussed to achieve a consensus for such an action. Although the Taipei Zoo has good connections with conservation institutes in Korea, which can provide potential donors for translocation to Kinmen Island, our mitogenome results suggest a distant relationship between Kinmen and Korean otters. Breeding between these two populations might dilute the endemic genetic diversity of both populations and might lower the global genetic diversity of Eurasian otters overall. It is therefore critical to locate any possible residual Eurasian otter populations on or near Kinmen Island as soon as possible and to understand their kinships within and between those populations before potential recessive inheritance problems due to inbreeding occur. A broad survey of surviving Eurasian otter populations in southerneastern mainland China is therefore acutely necessary for Kinmen otter conservation.

Participating in wildlife conservation programs is an important task for modern zoos. Through conservation programs in both wild and captive populations, zoological institutions provide a valuable contribution to species conservation by safeguarding against disastrous loss in the wild and supplying a potential source of wildlife for population supplementation or reintroduction in the future (Mitchell et al. 2011). The Taipei Zoo has long been the government-assigned facility for the care of seized and rescued wild animals (Chang et al. 2018; Kao et al. 2020) and has been the key institution for both the ex situ (captive breeding and educational programs) and in situ (wild population monitoring and genetic surveys) conservation of Eurasian otters on Kinmen Island. The genetic data of the wild population in this study will contribute to the conservation strategy of Eurasian otters on Kinmen Island both ex situ and in situ and will be beneficial to the management of the possible surviving Eurasian otter populations in East Asia in the future.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-023-01525-2.

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Author contributions This study was conceptualized by NHJL and managed by YCT. YCT coordinated the fieldwork. Sample collection was completed by NHJL, YCT, CJC, CHJ, HYH, HSC and LLL. Material preparation and lab work were organized and performed by NHJL, CHJ, HYH and WD. NHJL and CHJ contributed to the analytical methods. NHJL wrote and revised the manuscript. LWC and SWC contributed to the conservation strategy. The funding for this work was acquired by NHJL and LLL. All authors read and approved the final manuscript.

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Data Availability All data generated or analysed during this study are included in this published article and its supplementary information files. The mtDNA sequence data for all samples are available from GenBank and summarized in Table 1.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

Ethical approval All tissue samples were deposited in the Wildlife Cryobank of Taipei Zoo, and were used here with permission by the Forestry Bureau, the Council of Agriculture, the Executive Yuan, Taiwan, per the following authorizations: 0961608667 (2007), 0961701136, 097011626 (2008), 0980108575 (2009), 0991603072 (2010), 1000116384 (2011), 1011700515 (2012), 1021610837 (2013), 1031700533 (2014), 1041700584 (2015–16), 1061700466 (2017–19), and 1096011590 (2020–22).

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