

## Phylogeographical Analysis of *Eremias argus* in Yoobu-do Island and Sohwang Sand Dune, Korea

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**Abstract:** *Eremias argus*, which specifically inhabits riverside or coastal sand dune, is an endangered species designated by the Ministry of Environment of Korea because it has been drastically decreasing due to the destruction of habitat and the human activities. Therefore, it is required to prepare a countermeasure for the strategic and systematic solutions for the conservation of the *E. argus*. This study analyzed mtDNA cytochrome oxidase subunit I (COI) gene of *E. argus* inhabiting Yoobu-do island and Sohwang sand dune. This study aims to provide fundamental genetic information through phylogeographical analysis between two regions. Of 11 individuals of *E. argus* used in the analysis, the total 652~656 bp of mtDNA COI genes for 3 individuals of *Takydromus wolteri* as 'out group' were analyzed. Intraspecific genetic distance for *E. argus* was shown to be the same result of 0.000. As for *T. wolteri*, it was 0.005, showing very similar result. *T. wolteri* inhabiting Gapcheon stream in Daejeon metropolitan city was found to be the highest intraspecific genetic distance (0.008). The NJ tree of *E. argus* and *T. wolteri* indicated there was almost no genetic difference between habitat for *E. argus*. For *T. wolteri*, however, there was slight difference between the individuals inhabiting Yoobu-do island and those inhabiting Gapcheon stream in Daejeon metropolitan city. Therefore, it is necessary to prepare conservation strategies to increase genetic diversity of *E. argus* in Yoobu-do island in the future because it is highly presumed that the population of *E. argus* in Yoobu-do island have been flown from nearby coastal sand dune.

**Keywords:** Yoobu-do Island, Sohwang Sand Dune, Phylogeographical analysis, *Eremias argus*, Korea

### Introduction

According to SSC (Species Survival Commission) experts of IUCN (International Union for Conservation of Nature) and reports published by ZSL (Zoological Society of London), 19% of total reptiles around the world are an endangered species (www.iucn.org). Reptile is vulnerable to environmental changes due to its long lifespan, late sexual maturation, and low rate of breeding success (Scott and Seigel, 1992). The destruction and fragmentation of habitat is known to be the most significant cause resulting in the decrease of reptile population and its number of individuals (Ballinger & Congdon 1996; Vega *et al.* 2000). Therefore, the number of reptile individuals around the world has been consistently decreased (Whitfield *et al.*, 2007).

Reptile shows the apparent phylogeographical pattern because it is mostly small size, has slow mobility, and

highly relies on habitat (Avice, 2000). *Eremias* genus in China shows phylogeographical differences depending on the boundary areas of land (Qun Zhao *et al.*, 2011). Lambetaz (1989) reported that the east group and the west group of *Xerobates agassizi* with the Colorado River between had different group structures because of the limitation part of mtDNA and the difference in haplotype.

Family Lacertidae in Korea is reported to be 4 species including *Takydromus auroralis*, *T. wolteri*, *T. amurensis*, and *Eremias argus* (Lee, 2010). Among these species, which is a protected species as an endangered species assigned by the Ministry of Environment of Korea (NIBR, 2011), usually inhabits sand dune along the coast and it also inhabits several inland mountain areas (Chang *et al.*, 2006; Song, 2007). In particular, *E. argus* is characterized by inhabiting the vegetation of coastal sand dune, herbaceous inland vegetation, and regular size of sandy areas (Kim 2012; Song, 2007; Kim and Song, 2010). However, the coastal areas and grasslands in Korea are highly in demand for land usage. Moreover the large scale of stream development can result in the wide destruction of habitat, which can highly lead to declining populations of *E. argus*

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**Table 1.** Number of Individuals and sampling site of Lacertidae

No.	Species	Location	No. of Individuals
1	<i>Eremias argus</i>	Yoobu-do island, Seocheon-gun, Chungcheongnam-do, Korea	4
		Sohwang sand dune, Seocheon-gun, Chungcheongnam-do, Korea	7
2	<i>Takydromus wolteri</i>	Yoobu-do island, Seocheon-gun, Chungcheongnam-do, Korea	2
		Gap-cheon stream, Yuseong-gu, Daejeon metropolitan city, Korea	1
Total	2 species	3 sampling area	14

(NIBR, 2011).

Consequently, this study aims to present the conservation measures for *E. argus* population which is highly phylogeographically endemic and endangered species. For this reason, this study investigates the genetic diversity between two areas by analyzing mtDNA cytochrome oxidase subunit I (COI) gene of *E. argus* inhabiting Yoobu-do island and Sohwang sand dune. Moreover, this study provides fundamental genetic information through phylogeographical analysis.

## Material and Methods

### Specimen collection and DNA extraction

*E. argus* used in this study is total 11 individuals of specimens owned by National Science Museum of Korea. 4 and 7 individuals were sampled from Yoobu-do island and Sohwang sand dune, respectively. As for the out group, 3 individuals of *T. wolteri* collected from Yoobu-do island and Daejeon areas were used.

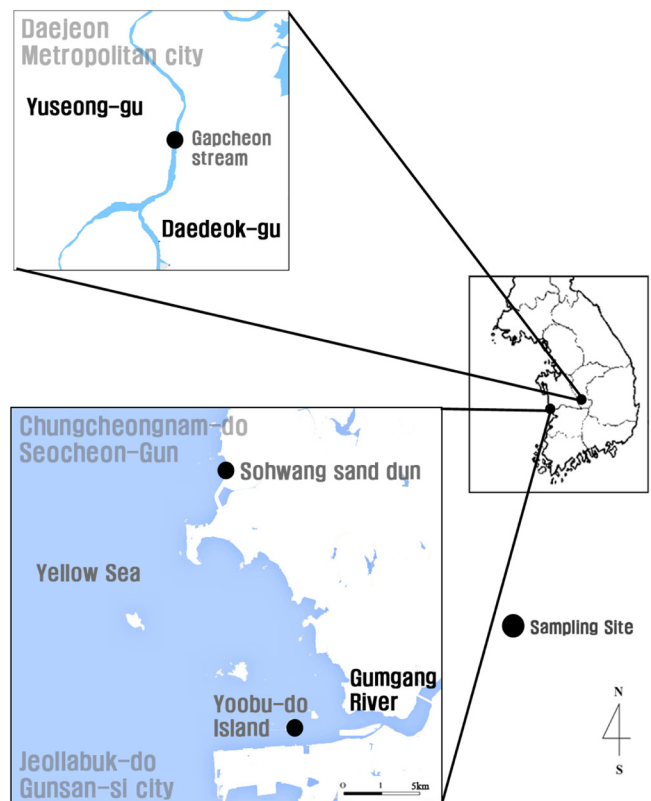
For the extraction of genomic DNA, the tissue of about 20 mg was excised for minimizing the damage of sample. The excised tissue was from caudal muscle of each individual. Genomic DNA was extracted by using Prime prep. TM Genomic DNA Isolation Kit (Genet Bio, Korea).

### PCR amplification

The PCR was amplified from the COI gene of mtDNA and primers were designed using the reference primer set (RepCOI-F 5'-TNTTMTCAACNAACCACAA-AGA-3' and 5'-ACTTCTGGRTGKCCAAARAATCA-3) (Nagy *et al.*, 2012). The PCR amplification conditions consisted and pre-denaturation, 94°C for 5 min, followed by 40 cycles of 30 sec at 94°C, 30 sec at 46.5°C, 30 sec at 72°C and a final extension at 72°C for 5 min. PCR Product was purified by using PCR Purification Kit (Bioneer, Korea). Sequence was consulted to an analysis institute (Solgent, Korea) for analyzing the sequence with direct sequencing method.

### Data analysis

The Sequence result was analyzed by using Bioedit Sequence Alignment Editor (Tom Hall, 2005) and ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The comparative analysis was conducted by multiple alignment. The MEGA5.22 (Tamura *et al.*, 2011) software was used to



**Fig. 1.** Study area

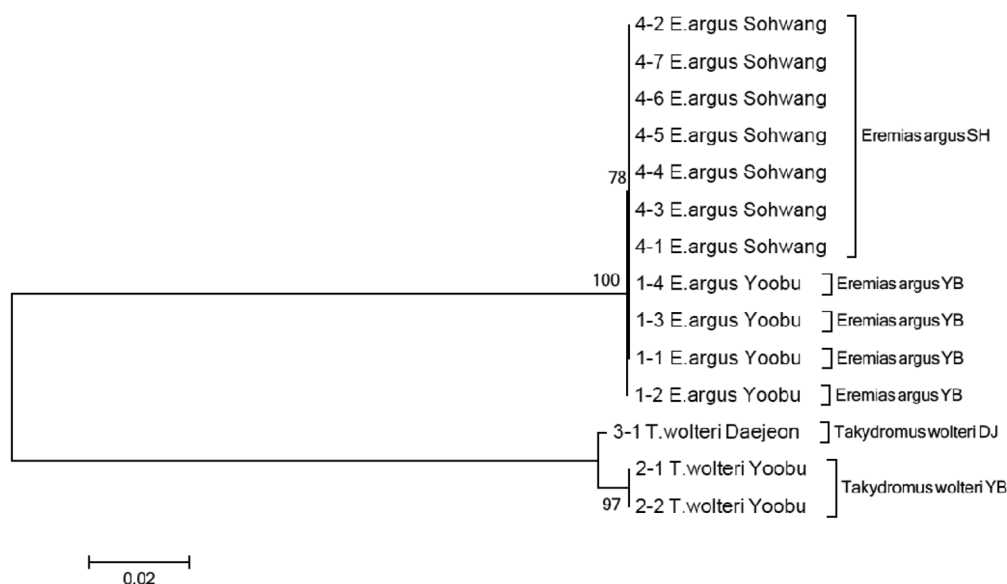
construct phylogenetic trees by Neighbor-Joining method using K2P distance (Kumar *et al.* 2004). The value of bootstrap to estimate the reliability of each intersection of the phylogenetic trees was obtained through 1,000 replications.

## Results and Discussion

This study analyzed the total 652~656 bp of mtDNA COI genes of 11 individuals of *E. argus* and 3 individuals of *T. wolteri*. Intraspecific genetic distance for *E. argus* was shown to be the same result of 0.000. As for *T. wolteri*, it was 0.005, showing very similar result. *T. wolteri* inhabiting Gapcheon stream in Daejeon metropolitan city was found to be the highest intraspecies genetic distance (0.008). The genetic distance between *E. argus* and *T. wolteri* was high (0.245) (Table 2). This result was similar to that of Chang's

**Table 2.** Investigation of genetic distance (K2P) of COI gene between Lacertidae

	1-1	1-2	1-3	1-4	2-1	2-2	3-1	4-1	4-2	4-3	4-4	4-5	4-6	4-7
1-1 <i>E. argus</i> Yoobu														
1-2 <i>E. argus</i> Yoobu	0.000													
1-3 <i>E. argus</i> Yoobu	0.000	0.000												
1-4 <i>E. argus</i> Yoobu	0.000	0.000	0.000											
4-1 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000										
4-2 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000	0.000									
4-3 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000	0.000	0.000								
4-4 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
4-5 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
4-6 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
4-7 <i>E. argus</i> Sohwang	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
2-1 <i>T. wolteri</i> Yoobu	0.246	0.246	0.246	0.246	0.246	0.247	0.246	0.246	0.246	0.246	0.246			
2-2 <i>T. wolteri</i> Yoobu	0.246	0.245	0.246	0.246	0.246	0.247	0.246	0.246	0.246	0.246	0.246	0.000		
3-1 <i>T. wolteri</i> Daejeon	0.242	0.242	0.242	0.242	0.242	0.243	0.242	0.242	0.242	0.242	0.242	0.008	0.008	

**Fig. 2.** Neighbor-Joining (NJ) tree of the mtDNA COI gene of Lacertidae

(2011) study reporting that the intraspecific genetic distance for *T. wolteri* was 0.000~0.009. The NJ tree of *E. argus* and *T. wolteri* indicated there was almost no genetic difference between habitat for *E. argus*. For *T. wolteri*, however, there was slight difference between the individuals inhabiting Yoobu-do island and those inhabiting Gapcheon stream in Daejeon metropolitan city (Fig. 1). The possible reason for no genetic difference in *E. argus* on different habitats is because the populations of *E. argus* inhabiting upstream sand dunes and coastal sand dunes in the nearby Yoobu-do island have been flown into Yoobu-do island, floating with broken branches along the river under the adverse meteorological conditions such as rainstorms. This claim can be supported by the study conducted by Samonds *et al.*, (2012) reporting that the reptiles in Madagascar islands in Africa drifted more than 400 km on floating rafts. Moreover, as it is reported that the two population groups of *T. wolteri*

in main island of Jeju and Gapado island in Seogwipo had the same DNA sequence (Chang, 2011), it is considered that the ancestors of *E. argus* in Yoobu-do island have not formulate their genetic pool only for Yoobu-do because they have somewhat recently been distributed to the coastal sand dune in nearby Yoobu-do island.

However, the difference between the individuals inhabiting Yoobu-do island and those inhabiting Gapcheon stream in Daejeon metropolitan city might result from the geographical differences in that the individuals of *T. wolteri* were collected from inland streams, instead of the coastal sand dune in nearby Yoobu-do island. By using molecular phylogenetic method by mtDNA 12S rRNA, Chang (2011) found that the population of Jeju island was different from other population of inland.

The species along the riverside have been reduced due to the recent construction project such as four major river

project, estuary dams, and reclaimed lands (NiBR, 2011). Thus, it is necessary to develop more effective protection strategies and to secure the population with consistent genetic diversity for the endangered species such as *E. argus*. Furthermore, *E. argus* inhabiting Yoobu-do island showed small population compared to that of inland and its habitats are concentrated on the west coast of Korea including the eastern part of China. In light of this, it can be presumed that *E. argus* might have been isolated in islands of the west coast over the recent glacial period. Therefore, it is required to carry out diverse genetic analysis by securing additional genes in mtDNA in the future. Moreover, the population should be managed effectively through diverse analysis methods such as genotyping using microsatellite.

### Acknowledgments

This work was supported by the “National Research Foundation of Korea (NRF)”, grant funded by Ministry of Science, ICT and Future Planning (MSIP) (NRF-2008-2004707) and “A study on characteristic of ecology and behavior of reptiles (KAERI/CM-1826/2013)” Korea Atomic Energy Research Institute.

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Received: 28. Sep. 2013

Revised: 11. Oct. 2013

Accepted: 17. Oct. 2013