A DOCTORAL DISSERTATION

# Ecology and Genetic Identification of Alien Cervid in Jeju Island, South Korea

Faculty of Science Education

GRADUATE SCHOOL
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# 제주도에 이입된 사슴류의
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# Ecology and Genetic identification of Alien Cervid in Jeju Island, South Korea

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A thesis submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Biology

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# Abstract

This study investigated the ecology of alien sika deer on Jeju Island, with particular reference to their distribution, activity pattern, diet, and phylogeny using direct, indirect, and molecular analysis methods from Oct. 2018 – Dec. 2020. Research on the distribution range using direct and indirect survey methods identifies that the alien sika on Jeju Island have expanded their ranges towards the eastern and western parts of Hallasan National Park, taking into regard that they were initially released into the northern part. The southward expansion of their range has not yet been observed. This expansion into new ranges is driven by the availability of water, food, and climatic conditions. Sika deer were observed to prefer altitudes ranging from 550-1,100m, as this range is less preferred by native roe deer and has a less anthropogenic disturbance. Some illegal translocations, deliberate releases, or escapes of farmed individuals account for the increased deer population. Due to a lack of predatory or anthropogenic disturbance, the population will continue to increase beyond the current distribution range, hence a management strategy needs to be implemented. Results on activity patterns using camera traps revealed that sika deer were active throughout both day and night, but had activity peaks towards dawn and dusk. On the seasonal scales, they showed variation in their activity pattern (dawn and dusk in spring-autumn; day and night in winter) that could be linked to marked changes in temperatures and stress established by weather conditions. Sika deer displayed a higher to the lower extent of activity overlap with eight other species, including Siberian roe deer, red deer, wild boar, Asian badgers, Jeju weasels, domestic cattle, humans, and dogs. The native Siberian roe deer showed a higher incidence of activity overlap with sika deer in both summer and winter seasons, suggesting that the two ungulates had a common dietary preference. In contrast to summer, Jeju weasels and domestic cattle were not observed during winter seasons. In both of the seasons, activity overlap was lowest for humans and dogs. Higher activity overlap among morphologically different species causes conflicts, hence

displacement of weak/small species cannot be negated. A proactive management strategy is recommended. Sika deer are dynamic in the procurement of food resources, as they fit into both the browsing and grazing categories. In this study, sika deer were found to consume 95 plant taxa of four categories (forb-climbers, graminoids, ferns and browse) using morphological and molecular analysis. Plant species belonging to the family Poaceae were consumed in a higher proportion. A higher number of dietary plants was found in summer (47 taxa) and lower in winter (27 taxa). Graminoids and forb-climbers primarily made up the spring and summer diets, whereas browse and ferns served as autumn and winter diets. This study revealed that the dietary preference of sika deer changes with the seasons. From the analysis of 21 tissue samples, five distinct groups of *CytB* haplotypes were identified, which clearly shows the presence of two haplotypes of sika deer (*C. n. yakushimae* and *C. n. taiouanus*), and three haplotypes of red deer (*C. elaphus).* Phylogenetic analysis showed that the sika deer subspecies *C. n. yakushimae* represents the mitochondrial lineage to Japanese sika deer, whereas *C. n. taiouanus* corresponds with a Taiwanese lineage. The red deer (*C. elaphus*) showed its lineage with Chinese, south-eastern Tibetan and western Sichuan populations. Through the analysis of the branching pattern of the maximum likelihood tree, no sequence mutations seem to have occurred. For the possible future hybridisation between sika with sympatric species, study should be carried out using nuclear genes.

This study provides knowledge on the ecology of alien sika deer on Jeju Island. The finding suggests that integrative studies using advanced technology are highly recommended. Furthermore, an extensive survey using a large number of fecal samples and tissue samples from different locations and various individuals is required to confirm their distribution range and determine their evolutionary relationships, respectively.

# I. INTRODUCTION

## 1. General introduction

Alien species are species that occur outside their native range and have dispersal potential. The introduction of exotic species has significant economic, social and environmental impacts (Pimentel, 2002), and impacts on the conservation of local biodiversity (Allendorf *et al.,* 2010). Certain introduced species have proved to be highly invasive, modifying the natural ecosystem and has the potential to cause significant damage to the receiving environment and require control or eradication (Pysek, 2010). Whereas, some others are largely beneficial from a conservation point of view, preserving species and genetic diversity at a time when populations in the native range have decreased. Introduced cervid species are a good system for studying invasion and conservation scenarios in wild populations (Byers, 2002; Schlaepfer *et al*., 2010).

Translocation of biological species has occurred because of globalization as people and commodities move via marine shipping, consignments of wood products carrying insects, or the transport of ornamental plants intended or unintended to new places. The pet and plant trade is the significant source of animal and plant introductions, as a result of the frequent escape or release of species into the wild (Reichard and White, 2001; Corbett and winebrake, 2008). An alien invasion is the second leading cause of habitat loss, leading to species extinction and endangerment. Invasive alien species are now major international conservation concerns and are the focus of international cooperation efforts, such as the Global Invasive Species Program (GISP). As awareness increases, individuals and their communities have the opportunity to make informed choices that will have a long-lasting impact on their descendants (Lowe *et al.,* 2000; Schlaepfer *et* *al*., 2010). Variation in the composition and structure of indigenous species and ecosystem function modification (Suarez *et al.,* 1998; O'Dowd *et al*., 2003) can occur due to environmental damage from invasive species (Whitney *et al.,* 2008). In the USA, Europe, Australia, and India the cost of biological invasions is estimated to be over $US394 billion annually (Pimentel, 2002; Hoffmann and Broadhurst, 2016).

### 1) Invasion biology of mammalian species

Many animals have made their way through previously uninhabited areas around the world (Lockwood *et al.,* 2007). Compared with other animals, mammalian species (cattle, sheep, and goats) are more successful in establishing themselves in novel environments (Clout and Russell, 2007). In addition, mammals were one of the earliest organisms to be introduced by humans. More recently, many of these mammals are valued by humans for food, hunting, and controlling previously introduced species that had proved to be a pest.

In accordance with Clout and Russell (2007) carnivores (canids, bears, and cats) and lagomorphs (hares and rabbits) contain larger proportions of successful invaders. Only 1.8% of the order rodent is characterized as invaders. Around the world, a relatively small number of mammals have been successfully established (Long, 2003). It includes sika deer (*C. nippon*), red deer (*C. elaphus*), Indian mongoose (*Herpestes javanicus*), domestic animals (cattle, pig, sheep, goat, dog, cat, donkey), and rodents. Not only the mammals but also the introduced vertebrates as birds and fishes also have a high probability of being spreading and establishing (Jeschke and Strayer, 2015). It has been shown that differences in the relative size of the brain are associated with the successful establishment of non-native mammals (Sol *et al.,* 2008). Species with large brain in comparison to the size of the body are more likely to successfully establish themselves in a new environment. Sensory specializations (Barton, 1998), cognitive capabilities (Readeer and Laland, 2002), and motor functions have all been related to increased brain size (Changizi, 2003). Although the underlying neurophysiological mechanisms are unknown (Dunbar and Shultz, 2007), the existence of such correlations implies that larger brains increase the capacity for individuals to change their behaviour in potentially adaptive ways (Allman, 2000; Reader, 2003; Byrne and Corp, 2004). Similar results were obtained from a study of introduced bird species correlating larger brain size with the higher successful establishment (Sol *et al.,* 2008).

### 2) Cervids introduction

The Cervidae family, commonly referred to as the "deer family", consists of 23 genera with 47 species and comprises three subfamilies (Holmes *et al*., 2013). Cervids can be found in a wide variety of environments, from the arctic to the tropics. Humans have long exploited native and invasive deer species, having hunted them in all the geographic areas where they are found. They are frequently hunted for their skin, timber, antlers, velvet, and other products (Nunez *et al.,* 2010). Today, many ungulates species are hunted more than necessary, which caused them to decline in their native areas (Kamgaing *et al*., 2019). To fulfill the demand of decreasing population, species were introduced from other parts of the world. The mammalian family Cervidae dominate herbivore introduction in term of frequency, with 29.2% of successful establishment in the non-native environment (Nogales *et al*., 2006; Clout and Russell, 2008). On different continents, at least thirteen species of deer (Cervidae) have been introduced outside of their natural range (Dolman and Wäber, 2008). The presence of alien deer species has resulted in benefits and costs for the new environment, but there is growing concern over their impacts (Cote *et al.,* 2004; Acevedo *et al.,* 2010). These non-native deer have had significant economic impacts on agriculture and forestry because of grazing and browsing (Ratcliffe, 1987; Putman and Moore, 1998). In addition, they can compete with native species (Hemami *et al.,* 2004; Feldhamer and Demarais, 2009) and in extreme cases, they can impact native deer through hybridization (Simberloff, 1996; Senn and Pemberton, 2009).

In South Korea three species of cervids as sika deer, red deer and elk were introduced after from various countries for commercial farming purposes (Hwang *et al*., 2014; Yun *et al*., 2018). Introduction of cervids in South Korea begins after the extinction of native Manchrian sika deer towards the end of the 20th century, after World War II (1939-1945) followed by the Korean War (1950-1953) (McCullough, 2009). After the population extinction, some Taiwanese sika deer (*Cervus nippon taiouanus*) from Taiwan and Japanese sika deer (*Cervus nippon yakushimae*) from Japan were imported for domestication and more specially for antler production (Koh et al., 2010; Jin, 2013). Some sika deer individuals were also brought from North Korea in order to establishing in wild. Red deer was abundant in northeast Korea in the early 1900s, because of ongoing capture for farming, hunting and habitat destruction they become rare by the 1960s. After decline of wild population, South Korea began to import deer from China, Canada, the USA, New Zealand, and Australia for breeding purposes (McCullough, 2009). Despite no clear evolutionary relationship, the elk individuals were supposed to have introduced from USA and Canada. Because of some clinical issue and strict law against importing wildlife, finally it become hard to import cervid from other countries.

## 2. Ecological status of introduced Cervids

### 1) Sika deer (*Cervus nippon*)

Sika deer (*C. nippon*) are small to medium-sized ungulates (height, 64 - 110 cm; weight, 40 – 150 kg) (Smith and Xie, 2013). The pelage of sika deer ranges from chestnut brown to reddish olive and has a dark dorsal stripe terminating at a large rump patch used as distinguishing characteristics (Fig. 1). The coat is mottled with white spots in summer (Jo *et al*., 2018). Primarily crepuscular, sika deer forage either singly or in small groups during the day. Adult males (buck) remain solitary most of the year except for the breeding season, while females usually live with their fawns forming a group of 2 or 3 individuals. Following the situation, sika deer can be either grazers or browsers. Sika deer living in the northern cool temperate area are grazer’s feeds primarily on dwarf bamboos, graminoids, and other coarse forages, and poorly digestible foods (Kubo, 2014). Southern deer rely on fruits and other more nutritious foods. In response to food shortages, they eat bark, dead leaves, and even underground parts of plants (Hofmann, 1989; Takatsuki, 2009a). Despite the nomenclature confusion, a study on mitochondrial DNA by Goodman *et al.* (2001) showed that the sika deer broadly splits into two distinct morphotypes throughout their range as northern and southern. Sika deer living on the southern side have smaller body sizes and have similar body coloration throughout the year (similar winter and summer coats). In contrast, the deer living in south have larger body size (Takasuki, 1983). Outside the native range, the population is established in North America (Kentucky, North Carolina, Maryland, Virginia, and Texas); Europe (Austria, Denmark, Czech Republic, France, Ireland, Germany, Poland, and United Kingdom); Australasia (New Zealand) and the Philippines (Swanson and Putman, 2009). Most introductions were made from the last years of the 19th century (the 1890s) through to the 1930s, even though some of them continued until more recently (Barančeková *et al.*, 2012; Uzal *et al*., 2012).

The history of native sika deer in South Korea is not well - documented. However, the original population of sika deer was large within South Korea. During the “Joseon Dynasty”**-** kingdom existed on Korean Peninsula from 14th to 20th century – king usually operate military training that focuses gaming (Jo *et al*., 2018). Furthermore, the Joseon government ask each province to pay tribute (containing deer meat and leather) to the king. For this purpose, governments operated farms that can artificially raise sika deer. However, the number of deer’s in the wild has gradually declined because of ongoing capture for farming, continuous hunting, and the prevalence of rinderpest - an infectious disease of ruminants, caused by a paramyxovirus - since the 17th century (Yun et al., 2018). In 1910, Korea was annexed by the Empire of Japan, which further show a significant impact upon the sika deer population.

Fig. 1. Sika deer individual in summer coat at Muljangori-oreum wetland site, Jeju Island.

Date: 2019. 09.12

In 1990, by the Treaty of Eulsa (1905), Chang-gyung-won palace (former Joseons palace) was turned into an entertainment area, including a zoo, by the Japanese protectorate. Originally, the zoo had Manchurian sika deer and Manchuria wapiti, but almost all zoo animals were killed by the Japanese military as the threat of defeat in the Pacific War. Four years after Korean War in 1957, The Chang-gyung-won Zoo the predecessor of the Seoul Zoo, brought one male and two female Formosan sika deer from Taiwan (McCullough, 2009; Informal interview). In the meantime, South Korean zoos, and local governments have attempted to introduce sika deer from North Korea and overseas. However, it has failed because relations with North Korea have deteriorated or the quarantine measures of the South Korean government's against ungulates are very strict. Despite the situation, several introductions (i.e. 676 individuals) have made from Taiwan and Japan until 1997.

When South Korea gained independence from Japan, the agriculture minister brought 60 sika deer individuals from North Korea (Jo *et al*., 2018; Oh, 2019). However, South Korea has not been able to properly secure the sika deer of North Korea. Introduced deer from Taiwan and Japan were raised in farms of Jeju Island. In 2004, private farms in Jeju have raised 501 sika deer individuals, and year after in 2008, their number has reduced to 411 individuals (Oh *et al*., 2019).

### 2) Red deer *(Cervus elaphus)*

The red deer *C. elaphus*, Linnaeus, 1758 is a species of large deer (ht. 1.2 m; wt. 100 - 350 kg) considered to have evolved in foothills of the Himalaya and expand from China to Europe via the Middle East (Groves and Grubb, 1987). The coat color is characterized by the absence of spots except for the first year (Smith and Xie, 2013) (Fig. 2). Red deer are crepuscular foragers but are active throughout day and night. They feed on every edible part of trees and shrubs (bark, shoots, branches, leaves, and needle of conifers), which consists of grasses, shrub, and tree shoots and even found to consume mushroom, lichens, and fruits (Bugalho and Milne, 2003; Nugent and Fraser, 2005). Primarily found at woodlands habitats (IUCN, 2017), they live in sexually segregated herds except during the breeding season and in winter. They have a fixed home range size (2 – 4 km$^2$) depending on available forage habitats and population density (Jo *et al.*, 2018). Red deer has adapted to live in a variety of ecosystems in its introduced range, from tropical and subtropical rainforests to temperate forests in Australia; alpine grassland to temperate lowland forests in New Zealand; and rainforest to steppes in South America. The native range of red deer extended from western China to most of temperate Europe ranging from Sweden and Norway in the north to Portugal and Spain in the west (Flueck and Flueck, 1993; Flueck, 2007).

Fig. 2. Red deer with newly growing antlers observed at Noro-oreum wetland site, Jeju Island.

Date: 2020.04.08

Wild population occurs in South Africa, Peru, and Uruguay. In Asia, deer have been completely extinct in several places; in some, they have been extinct long and in others relatively recent. In some areas, (India, China and Tibet) deer are insufficiently protected and their range and population continue to decline (Heptner *et al.*, 1988; Smith and Xie, 2013).

The red deer is the largest herbivore in Korea and was abundant in northeast Korea in the early 1900s but become rare by the 1960s. Some population exists at Mt. Baekdu and its adjacent area in North Korea, but population, conservation status, and their persist at South Korea remain uncertain (Jo *et al.,* 2018). The known sub-species of red deer in Far East Asia including Korea is *C. e. xanthopygus* (Jo *et al.,* 2018). In the mid - 1940s and 2001, South Korea began to import deer from Canada, the USA, New Zealand, and Australia for breeding purposes. During this period, 13398 red deer individuals were imported and raised at various farms within South Korea.

Among them, 261 individuals were reported to raise at various farms of Jeju Island in the year 2004, but the number of farm individuals reduced to 157 until 2008 (Oh *et al.,* 2019; Jo *et al.*, 2018). After then, no individuals were reported, as they were suspected to have escaped or been released illegally. In some forested patches within Jeju, red deer were reported frequently and even were triggered in camera traps. Escapes from the farm were thought to be the source of the deer that occurred in wild. Lack of safety and ineffective management by casual breeders offered many opportunities for deer escape.

### 3) Elk (*Cervus canadensis*)

Elk (*C. canadensis)* is the largest ungulate (ht. 0.75–1.5 m; wt. 178–497 kg) and the most advanced subspecies of red deer after moose (*Alces alces*), found mostly in North America and high mountains of Asia (Robb, 2001). The colour of fur becomes reddish hue in summer while light grey in winter. The coat of elk becomes thicker with the progression of fall to protect them from cold (Smith and Xie, 2013). The young’s are dark black in color (Fig. 3). Major habitat of elk is forest and forest edges. Elk are ruminants and browser as they feed on a wide variety of vegetation, the native grasses being a year-round supplement, particularly includes bark of trees in winter and fallen leaves in autumn, and sprouts in spring (Homolka, 1989; Beck and Peek, 2005). Males engage in mating behavior during mating season, including antler wrestling, bugling (loud sound), and posturing.

Currently native to North America (the United States and Canada) and eastern Asia (China, Kazakhstan, Kyrgyzstan, Bhutan, Mongolia and Russian), but were widely distributed in the past (Carrera and Ballard, 2003; Smith and Xie, 2013). During the late Pleistocene, their range was much wider and spread throughout Europe, Asia, and far west as France (Croitor, 2020). In the United State, elk have been reintroduced in many other states (Frick *et al*., 2008). Outside their native range, elk were introduced in areas where there were little to no large native ungulates. They have adapted well in introduced countries like Argentina and Chile but have threatened endemic species and ecosystems through competition and grazing (Galende *et al.,* 2005). Elk together with red deer were introduced to Australia and Ireland (Corbet and Southern, 1996).

Based on historical records, two distinct subspecies of elk found in China and Korea (Incheon National University, 2019) are the Manchurian wapiti (*C. c. xanthopygus*) and the Alashan wapiti (*C. c. alashanicus*). Wapiti is known to have disappeared from the

Korean Peninsula since the mid - Joseon Dynasty. Elk leather and meat were a tribute to the king have caused a sharp decline in the elk population.

Furthermore, in the 17th century, rinderpest swept across the Korean Peninsula, which is believed to have led to a significant decrease in the number of deer (Informal Interview). The decline population is maintained through the importation of 1123 individuals from the USA and Canada. In 1997, chronic wasting disease (CWD) was detected in elk imported from Canada (Sohn *et al.*, 2002). After then, Korea temporarily banned the importation of live elk, deer, and their products. In Jeju, a total of 91 farms raised 1007 deer individuals for breeding and antler production. Among them, 513 elk individuals were recorded in 2004 but their number gradually decreased to 194 in 2008 (Oh *et al.*, 2019).

Fig. 3. Elks calves in their black coat, captured at Mulchat oreum, Jeju Island.

Date: 2020.04.08

As the Korean government, pass strict laws against importing wildlife, finally it becomes hard to introduce elk from the foreign county. Gradually, their demand for meat and antler also reduce, hence the remaining few farm individuals might have released illegally. This released population might have been captured in our camera trap survey.

## 3. Distribution, activity pattern, food resources and molecular study

### 1) Distribution

The spatial arrangement of a biological taxon is referred to as species distribution. Distribution patterns change depending on the size at which they are viewed (Heath *et al.*, 2012), from the arrangement of individuals within a small family unit, to patterns within a population, or the distribution of the entire species as a whole (Turner, 1979). Several studies have shown that various factors such as climate, food, shelter, and flora influence deer distribution, abundance, and habitat use, limiting them to specific areas. Various factors such as climate, food, shelter, and flora influence deer distribution, abundance, and habitat use, limiting them to specific areas (Kaji *et al.,* 2000).

Globally, in recent decades numerous region in the world have experienced increasing cervid populations and expansion of their distributions (Cote *et al.*, 2004). More importantly, the fluctuation in the ungulate population is due to the removal of predators, disease and released into unoccupied habitat (Leader-Williams, 1988). The expanding population has various negative implication such as (i) detrimental impacts on habitat and ecosystems; (ii) damage to protected environments; (iii) increased risk of collision with vehicle; and (v) increased risk of disease transmission (Böhm *et al.*, 2006; Linden *et al.*, 2010). There is concern about the dispersal of deer species by natural and human-assisted dispersal. A way to manage deer populations is by delineating where they are distributed. Anthropogenic factors can also influence the distribution, abundance, and habitat selection of ungulate species in densely populated areas. Feeding site selection, flushing or alarm responses, and changes in rutting behavior have all been identified as effects of human disturbance on deer behaviour (Miller et al., 2001; Li *et al*., 2007; Benhaiem *et al*., 2008; Clair and Forrest, 2009). As a result, certain considerations must be taken into account in ecological studies of alien ungulate populations. In South Korea, limited study have focused habitat analysis of wildlife species as roe deer *Capreolus pygargus tianschanicus* (Kim *et al.*, 2017), wild boar *Sus scrofa* (Seo and Park, 2000), water deer *Hydropotes inermis argyropus* (Song and Kim, 2012), feral goat *Naemorhedus caudatus* (Seo *et al.*, 2008), leopard cat *Prionailurus bengalensis* (Lim *et al.*, 2015) etc. but study related to invasive alien species are lacking. Like many other tropical forest ungulates, sika deer is difficult to monitor because of their elusive nature. Using presence record from camera traps and indirect methods we document the potential distribution of sika deer in Jeju Island.

### 2) Activity pattern

Investigating activity patterns is a relatively old theme in behavioural research (Munro *et al.*, 2006; Yamazaki *et al.*, 2008). Activities data represent an essential aspect of animal behaviour and ecology, offering basic details about the species' natural history and niche. Some of the first analyses of diels (or circadian) behaviour of focal species can be found in timestamped wildlife photographs (Gerber *et al.*, 2012; Bu *et al.*, 2016). It determines how species synchronize their pattern of activity to coexist with other species, such as avoiding predation, succeeding in feeding, and reducing competition (Mugerwa *et al.*, 2017). Activity patterns of species are determined by both external factors of environment and endogenous factors, that is, physiological states. Both biotic (predators, human activity, etc.) and abiotic (light, temperature, weather, precipitation, etc.) factors of the external environment equally influence the activity patterns of species.

In the past visual observation (Stelmock and Dean, 1986) or laboratory setting (Sherwin, 1998) have been used to identify the activity of the animal. Over time, the radio-collar method becomes popular since the 1980s, as well as GPS telemetry later (Munro *et al.*, 2006; Seryodkin *et al.*, 2013). Direct observation and live capture can cause findings to be distort or have a negative impact on wildlife populations. To address these impacts, camera traps have gained popularity over the past two decades mainly because of their possibility of cost-effective and non-invasive sampling of wildlife populations. Nowadays camera traps become popular as a research tool, with uses ranging from species inventories (Silveira *et al.*, 2003), the discovery of new species (Rovero *et al.*, 2008), habitat association (Cove *et al.*, 2013), population dynamics (Karanth et al. 2006), predator-prey interaction (Linkie and Ridout, 2011), density estimation (Karanth and Nichols, 1998), and even animal behavior assessment (Lashley *et al.*, 2014). Moreover, camera trapping is highly important in identifying the behavioural aspects of wildlife such as activity patterns and circadian rhythm more importantly species that are elusive (Marcon *et al*., 2017).

The use of camera traps has helped in understanding the activity pattern of various species ranging from carnivores to birds at various locations (López González and Lorenzana Piña, 2002; Weckel *et al.*, 2006; Irineo and Moreno, 2021). A survey on the daily activity of various animals was carried out in South America, where camera traps were located on trails and roads (Gomez *et al.*, 2005). A number of the survey on activity pattern of sympatric species have been conducted using camera traps in Madagascar, China and Japan (Gerber *et al.*, 2012; Bu *et al.*, 2016; Ikeda *et al.*, 2016). Furthermore, the interference of human hikers upon the daily activity of mammals has been studying at the mountain range of South Korea using camera traps (Lee *et al.*, 2018). Despite various studies on the activity pattern of native ungulates species, studies related to non-native or alien ungulates species are lacking. Moreover, the island ecosystem, habitat, and species are more prone to invasion by alien species.

### 3) Diet selection

Information on the dietary composition of wildlife species is very important to understand food ecology and developing conservation management measures (Ahrestani *et al.*, 2012). The dietary behaviour of animals is directly related to anatomical constraints and physiological needs to maintain metabolism and bear the costs of reproduction, in addition to being affected by environmental parameters (Minder, 2006). The quality and availability of food determines the distribution of ungulates, their habitat selection, or seasonal movements and therefore their environmental impact at different spatial and temporal scales (Mobæk *et al.*, 2008; Beest *et al.*, 2010). In some instances, forage supply and feeding behavior influence the social hierarchy, the physical condition of the organism, and life-cycle characteristics, such as body weight, litter size, longevity, population demography, and dynamics of an animal (Parker *et al.*, 2009).

Several methods are available for the determination of diet selection of ungulates which includes direct observation (Skoog, 1968; White and Trudell, 1980; Boertje, 1984), feeding site observation (Mysterud *et al.*, 1997; Oh *et al.*, 2015), bite count (Ortega *et al.*, 1995), stomach content analysis (Skoog, 1968), and oesophageal and rumen fistula technique (Oslon, 1991; Argunov & Stepanova, 2011). Direct animal observations generally depend upon feeding bouts. The primary benefit of direct observation is the use of minor equipment and their ease of use. Direct observation generates quantitative information when tame animals are used in the experiment (Kossak, 1983). Direct observation is possible on only a small group of animals and, despite the immense work involved, it does not generally provide accurate estimations of the numbers and proportions of the species consumed, particularly for various ungulate diets (Holechek *et al.*, 1982; Litvaitis, 2000). One of the limitations of these types of studies is that only open habitats are suitable because wild animals must be observed from a distance, attempts to quantify results can be biased (Wallmo *et al.*, 1973). As Jeju Island contains larger pasture and grassland areas hence, direct observation of feeding sites and plant species from distance can be possible. Over the past two decades, fecal analysis has been used more than any other procedure due to its unique advantages as for the collection and preservation of samples require very little equipment, it does not interfere with the normal habitat of the animals, and the only feasible method use in studying protected species. The use of fecal samples makes it possible to estimate the composition of the diet, without any direct interference (Putman, 1984). These techniques may not always true measure of diet composition since the amount of non-identifiable items can be high and the food identified may be biased in favor of less digestible matters in the diet (Litvaitis, 2000).

Plant-based materials were analyzed using a molecular technique for species identification (Czernik *et al.*, 2013; Raggi *et al.*, 2015). Chloroplast DNA sequences are used in identifying plants as they contain a high number of single cells, which allow for highly preserved sequences for primer development and nucleotide polymorphisms (Taberlet and Coissac, 2007).

### 4) Molecular study of sika deer

Molecular techniques are widely used methods in biochemistry, molecular biology, and genetics, which typically involve manipulating and analysing DNA, RNA, protein, and lipid (Tan and Yiap, 2009; Li *et al*., 2020). Molecular technologies are becoming a more important and potentially powerful tool in studying biodiversity and nature conservation (Taylor and Gemmell, 2016). Recent compilations of theoretical and applied studies in molecular genetics show the tremendous potential of this discipline to address the issue of conservation (Ungerer *et al.*, 2008). Molecular techniques can reveal how plants and animals disperse, the relationship between species and ancestor assignment. This provides the evolutionary history, order of branching, phylogeny, and taxonomic position of the specimen under study (Almeida *et al.*, 2014; Tkach *et al.*, 2020). Molecular technology can be used not just to study the genetic variability of populations, but also to features population characteristics such as the number of individuals, dynamics, taxonomy, and so on (Loewe and Hill, 2010). This technique has great advantages, that we do not need to capture animals, but can proceed to analyze through their antlers, faces, hair, and even feathers. A molecular marker of mtDNA is a useful tool for population genetic analysis and is particularly accurate for monitoring the history of the population of small species (Vaseeharan *et al.*, 2013). Despite the wide array of molecular markers (Nusser *et al.*, 1996; Kark *et al.*, 1999) to investigate the population-level genetic studies, mitochondrial genes (e.g. *CytB*, Barber, 1999) and nuclear gene (e.g. allozymes, Mahy *et al.*, 1999) are widely used. Mitochondrial DNA is a small circular molecule that consists almost entirely of a coding sequence and has a relatively high mutation rate. These features make mtDNA useful for population structure and phylogenetic studies (Taanman, 1999; Ladoukakis and Zouros, 2017).

### 5) Phylogeny of sika deer

Sika deer *C. nippon* was once common across northeastern Asia, from the Ussuri region to Vietnam, including mainland China, Japan, the Korean Peninsula, and Taiwan (Feldhamer, 1980; Kenneth, 1993). Over their range, sika deer are divided into four species i.e. *C. yesoensis* of central and northern Japan, *C. nippon* of southern Japan, *C.*

*taiouanus* of Taiwan, and *C. hortulorum* of the mainland range (Groves, 2006) but division at subspecies level is problematic as they are incompatible with previous studies (Cook *et al.,* 1999; Randi *et al.,* 2001; Grubb, 2005). Until recently, 16 sika deer subspecies were identified in their native range based on body size, color pattern, and antler color (Groves, 2006). Six of these subspecies are found only in Japan (Ohtaishi, 1986): *C. nippon centralis* (Honshu Island), *C. n. yesoensis* (Hokkaido Island), *C. n. mageshimae* (Mageshima Island), *C. n. yakushimae* (Yakushima), *C. n. nippon* (Shikoku Island, and Goto Island), and *C. n. keramae* (Ryukyu Island). The historic range of *C. n. hortulorum* (Russian sika deer) now extending its range towards the China border. In China, up to the 19th century, five subspecies were abundant (Sheng & Ohtaishi, 1993). In Vietnam, only captive populations survive with low genetic diversity. However, Won (1967) enlisted the existence of two subspecies in Korea, *C. n. mantchuricus* in Jeju Island and *C. n. hortulorum* in the Korean Peninsula. The wild population of sika deer from South Korea become decline and become extinct (Won and Smith, 1999) during the Japanese occupation in the country, though little information but the small wild population still exists in North Korea (McCullough *et al.,* 2009). Most importantly, subspecies of sika deer that were live in Mainland Asia, Korea, and China remains largely unknown and most (*C. n. mantchuricus* and *C. n. hotrulorum)* are treated as the same subspecies, as they lack DNA samples (Cook *et al.,* 1999; Randi *et al.,* 2001).

## 4. Research purposes

Cervids are widely distributed in the world and were in Korean Peninsula including Jeju Island. Hunting for meat and antlers production, they become extinct from South Korea. After the population extinction, some sika deer (*C. n. taiouanus*) from Taiwan and from Japan (*C. n. yakushimae*) were imported for domestication and antler production (Koh *et al.*, 2010; Jin, 2013). These sika deer were released on purpose and for some religious events in 1987, 1988, 2001 and 2002 as they became invasive at Mt. Songnisan National Park of South Korea (Kaji *et al.*, 2010). Similarly, in the 1990s, some sika deer were commercially farmed in Jeju Island and illegally released to Mt. Hallasan National Park for reintroduction without proper management (Jo *et al.*, 2018). Sika deer being selective foragers (Lammertsma *et al*., 2012) established themselves as successful feral populations at Jeju Island (Oh *et al.*, 2019). Since the last decade, they were frequently observed in-group but until now, study on population ecology and origin has not been conducted. Identification of species distribution range, their impact upon native species, dietary resources and their origin helps in the establishment of guidelines for sustainable management of this alien species.

Through the application of camera traps, the distribution sites and activity pattern of sika deer was identified in Jeju Island. Furthermore, diet and the phylogeny of the species has been studied through molecular technique.

The specific objective is to:

1. Identify the current distribution.
2. Analyse the seasonal activity pattern and overlap with sympatric species in summer and winter.
3. Understand the seasonal difference in diet selection.
4. Identify the genetic variability and phylogeny of alien sika deer.

# II. MATERIALS AND METHODS

## 1. Study area

### 1) Jeju Island

Jeju Island is a typical volcanic island, formed about two million years ago, by a volcanic eruption and is located on the most southerly portion of the Korean Peninsula (Fig. 4). Its topography is smooth and its oval form extends in an east-north-east direction (Banjade *et al*., 2019). The topography is composed of the larva plateau developed on the lowland shore areas, with monogenetic volcanoes on the top. There is a wide range of volcanic topography and about 360 small volcanoes known as “Oreum”. Oreums are distributed mainly in the middle mountain zones of Mt. Hallasan and are an important element that forms a unique landscape of Jeju Island (Jeong *et al*., 2015). The island contains about 120 small and large lava tubes distributed all over the island (Kim et al., 2020). The tube were formed when the basaltic lava, with low viscosity and high liquidity from the monogenetic volcanoes, hardened after eruption and the lower liquid layer of lava poured and cooled. Larva rocks including basalt and trachyte are widely distributed on the surface of the Island (Brenna, 2012). It has long believed that the volcanic activities were ignited by heat, but some other believe that activity resulted from the decompression melting of weak areas. A total of 830.94 km$^2$ (about 45%) land area was designated as a “Biosphere Reserve” by UNESCO (United Nations Education Scientific Cultural Organization) in 2002 and is an island area with diverse plant and animal species (JSSGP, 2012). Jeju contains a total of 253 inland wetlands among them five are registered as Ramsar wetlands (Kim *et al.*, 2012). These five Ramsar wetlands of biological importance are further designated as wetland protection areas by the Ministry of Environment and protected and managed by law. Due to the limited and narrow habitat area, when faced with sudden environmental changes, a group of organisms vulnerable to external threats will suffer great damage, such as destruction or extinction. The island has a wet subtropical climate with cool, dry winters and warm, wet summers. The hottest month is August, with an average temperature of 26.5°C, and the coldest is January, with an average temperature of 6°C. However, since Mt. Hallasan's climate varies with altitude, the geographical distribution of subtropical and Arctic plants and animals differs.

### Flora and fauna

The land flora of Jeju Island contains 4,764 species. Jeju Island contains various habitat types ranging from evergreen broadleaf forest, deciduous forest, and coniferous forest to grassland and wetland habitats (MoE, 2011). The evergreen forest is characterized by valley and contains *Cymbidium kanran*, *Elaeocarpus sylvestris* var. *ellipticus, and Cymbidium* goeringii found therein. The deciduous forest includes *Carpinus laxiflora*, and *Sasa quelpaertensis* species. Dominant tree species in the Coniferous forest include *Juniperus chinensis* var. *sargentii*, *Pinus densiflora*, and *Taxus cuspidate*. Jeju Island has 4,136 species of insects (Kim and Jeong, 2006). Vertebrate species includes 43 species of mammals (including sea mammals), 384 bird species, 7 amphibians and 14 species of reptiles. Large mammalian species are absent, but small mammalian species as Siberian roe deer, wild boar, Asian badger, Jeju weasel, and squirrel are present (Jo *et al*., 2012). Bird’s species as peregrine falcons, common buzzards, jays, and great tits are common in most of these habitats. Towards coastal region in summer and winter, flocks of migratory birds including white storks, black-faced spoonbills, plover, ducks, seagulls and white herons are commonly observed. The coastal wetland and the freshwater wetlands are very important as stopover sites most of these migratory birds (MoE, 2011).

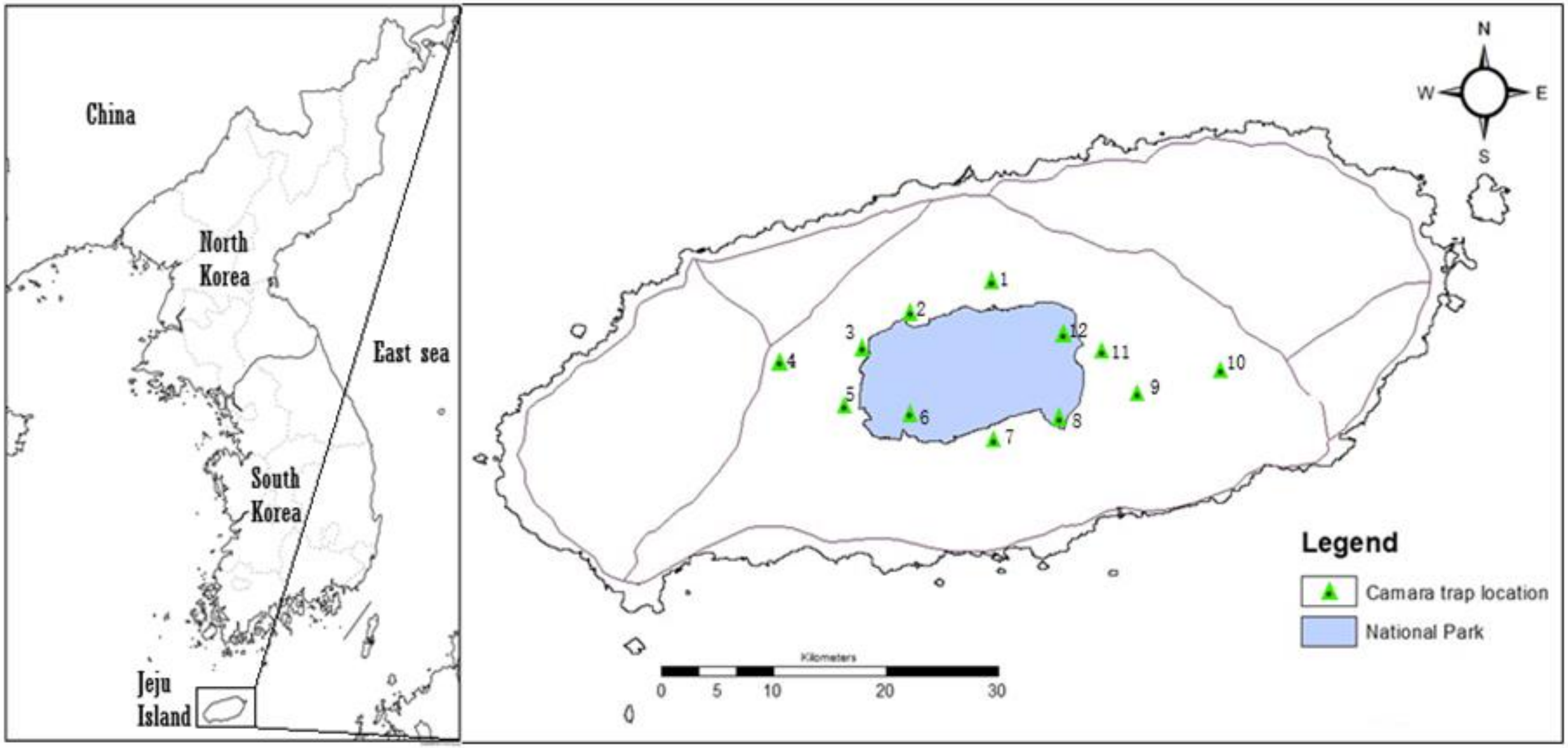

Fig. 4. Map of study area with camera trap locations.

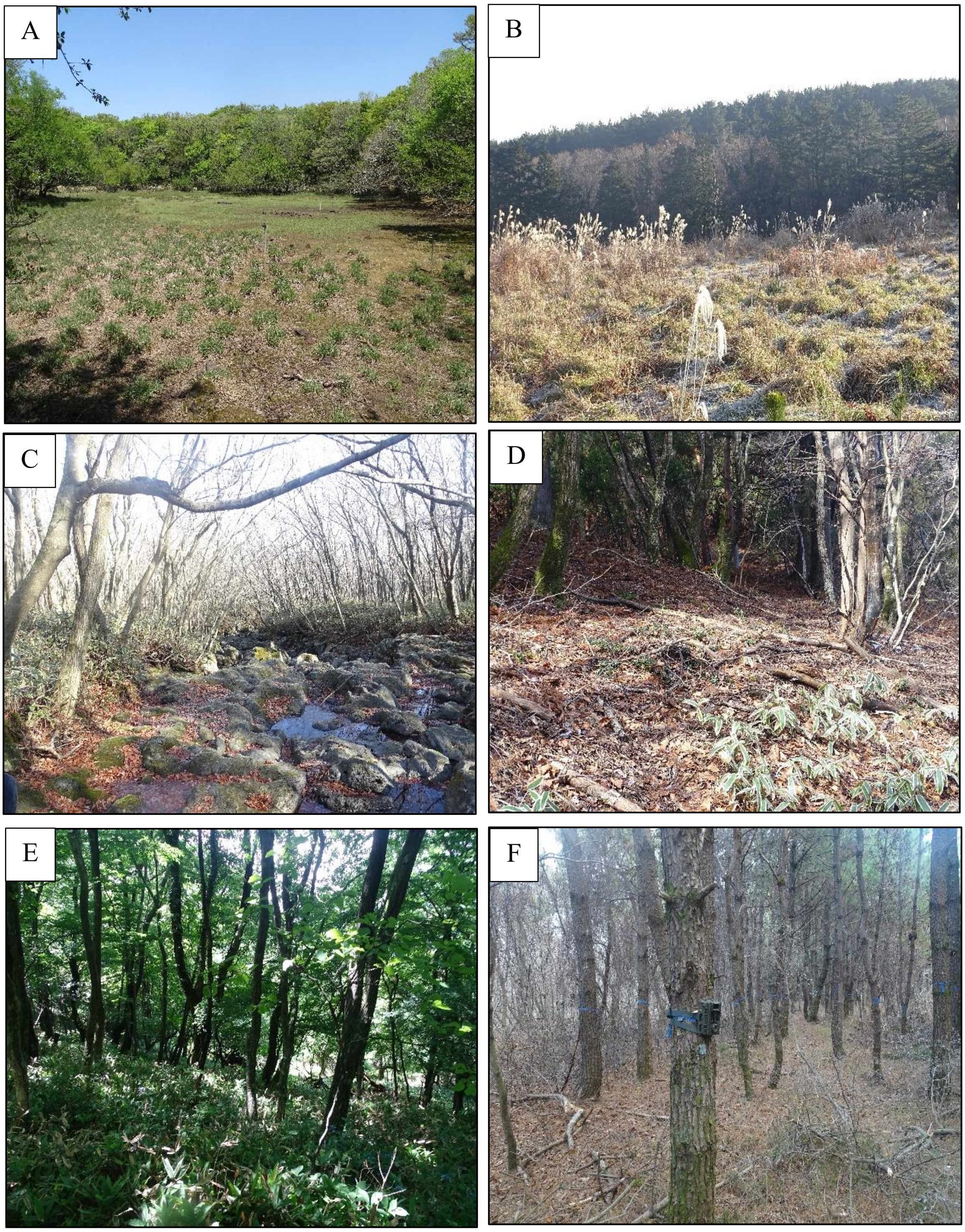

Fig. 5. Habitat types: A, wetland; B, grassland with adjacent forest; C, water bodies; D, deciduous forest; E, evergreen forest; and F, coniferous forest.

### 1) Endangered and threatened species

There are 276 species designated and protected by the Ministry of Environment as endangered species which includes sea mammals, fish and invertebrates. The Class I protected species at Jeju Island includes a species of sea lion, 10 species of birds and 2 species of insects. Class II protected species includes single species of seal and wildcat, 40 species of birds, 2 species of amphibian and reptiles including boreal digging frog, 5 species of insects, 17 species of invertebrates including *Dendronephthya suensoni*. In addition, five species of wild plants as *Euchresta japonica*, *Cymbidium lancifolium* and *Cymbidium kanran* are categorized as class I protected species in Jeju Island (Environmental white paper, 2020). Following the IUCN red list of wild plants on Jeju Island, 536 taxonomic groups were evaluated at the regional level. The groups include Extinct (1 species), Extinct in the Wild (2 species), Regional Extinct (2 species), Critically Endangered (61 taxonomic groups), Endangered (13 taxonomic groups), Vulnerable (83 taxonomic groups), and Least Concern (374 taxonomic groups). A total of 103 species of animals were listed in IUCN Red list category. They are 1 Pisces, 6 Amphibia, 5 Reptilia, 86 Aves, and 5 Mammalia by taxonomic group (Jeong *et al.*, 2015).

### 2) Hallasan National Park

Hallasan National Park (153 km$^2$) is the only National park of Jeju Island located 73 km southernmost to the Korean peninsula. Established in 1970, the park was designated as UNESCO Biosphere Reserve in 2002 and a world heritage site in 2007 (Jeong *et al.*, 2015). It is located on the central part of the Island, above 600m asl on the northern slope and above 1,000 asl on the southern slope of Mt. Hallasan. The park is famous for its vertical plant ecosystem because its climate varies from warm to cold zone. The vegetation is composed of temperate deciduous and evergreen forests. Deciduous trees (e.g. *Prunus maximowczii* Rupr., *Betula ermanii* Cham., *Quercus mongolica* Fischer) together with some coniferous trees (e.g. *Quercus serrata*, *Carpinus laxiflora*, *Sasa quelpaertensis*, *Pinus densiflor*) represent the mixed habitats, where the understory is sparse. Predominantly *Castanopsis cuspidate var. seiboldii* being the major species in evergreen forest. This mosaic habitat provides a wide range of habitat elements that can support a comparatively high number of wildlife species. The dominant large ungulate species in this region are roe deer *Capreolus pygargus*, wild boar *Sus scrofa*, sika deer *C. nippon*, and the occasional sighting of red deer *C. elaphus*. It is inhabited by fairy pitta *pitta nympha* designated as a natural monument, great spotted woodpeckers *Dendrocopos major*, Korean paradise flycatcher *Terpsiphone atrocaudata*, and cuckoos *Cuculus canorus* (Jo *et al*., 2018).

## 2. Camera traps and direct observation

### 1) Camera trapping method

To investigate the sika deer distribution range, diet, and activity pattern camera trapping (Bushwhacker, ROBOT D30, Shenzhen, China) was conducted during October 2018 - December 2020. A total of six habitat types were surveyed (both inside and outside the Hallasan National Park) using scouting cameras (Fig. 4 and 5). Cameras were strapped to trees (with permanent unique numbers) approximately 1 m above the ground by observing the permanent route of animals' activity, dirt roads, where signs of cervids were evident. To obtain an accurate activity pattern, all cameras were operational for 24 hours and programmed to take consecutive images when triggered. To reduce bias caused by multiple records of the same species at the same camera station, one record of each species at least 30-min apart was considered as an independent detection (O'Brien *et al*., 2003; Linkie and Ridout, 2011). Camera traps were checked monthly for species recorded by exchanging SD cards and, if necessary, batteries. The year was divided into four seasons: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November). The diel activity pattern was estimated as the total number of animals photographed for each season.

### 2) On-field survey for species verification

On-field survey was carried out further to confirm the presence of focal species and their distribution range. On 28 Aug 2018 for the first time, two sika deer individuals (male and female) were observed near the entrance of Mulchangori-oreum wetland, Jeju Island. The sika deer was confirmed by observing morphological structure and body colour. Their size is comparatively bigger than native roe deer and contains a white spot on their body with a black dorsal line. To identify the current extent of the sika deer distribution range within Jeju Island, information about the first released site and their current distribution was collected from books, atlas, published/unpublished reports, and journals. In every visit, fecal samples were collected for identifying the presence of species beyond the camera range limit. Distribution area investigated in 2006 and 2019 (Oh, 2006; Oh *et al*., 2019) were resampled for signs of red deer and private landowners were consulted about the present distribution of sika deer. Supplementary records were validated by exports through visual observations in the field and from digital images similar to Ni (1979) survey in 1978.

### 3) Direct observation for dietary items identification

The browsing pattern and browsed plant specimens nabbed by sika deer were collected after morphological identification and field recording. The plant was considered to be eaten by sika deer if footprints and fecal samples were seen next to the eaten plant. Furthermore, direct observation of sika deer feeding was recorded using Olympus binoculars (10 × 50 DPSI, USA) from distance. The specimen collected was identified following Lee (2003). The conservation status of all identified species has been confirmed on the Korean Red List of Threatened Species (NIBR, 2012). Furthermore, in each visit, the fecal samples were collected and try to identify the field sign to confirm their distribution.

### 4) Data analysis

To estimate the seasonal and diel activity patterns of sika deer along with sympatric species, the Kernel density estimation approach was used (Ridout and Linkie, 2009; Ikeda *et al.*, 2016; Marcon *et al.*, 2017; Ogurtsov *et al.*, 2018). It is a non-parametric approach to determining the probability density function of random variables (Worton, 1989). The coefficient of overlapping (Δ) was quantified to identify the differences in the activity patterns of sika deer with other observed species, which ranges from 0 (non-overlap) to 1 (complete overlap) (Ridout and Linkie, 2009). The precision of the estimator of overlap was estimated by 95% confidence interval using bootstrap with 10,000 resamplings (Foster *et al.*, 2013; Meredith and Ridout, 2018). The package 'Activity' (Rowcliffe *et al.*, 2014) was used to statically test the probability that two sets of observations come from the same distribution. Among the choice of various estimators, Dhat4 and Dhat1 were used which fits best for our sample sizes which are >

75 and <75 (Meredith and Ridout, 2018). The number of bootstrap iterations were set to 10,000. To determine the diel activity patterns among four time periods, photographic frequencies was calculated (the number of photos/hr) and were compared using $\chi^2$ test. All these procedures were implemented in R software (R Development Core Team, 2015) with the 'CamtrapR'(Niedballa *et al.*, 2016), 'Overlap' (Meredith and Ridout, 2018), and 'Circular'(Lund *et al.*, 2017) packages. Based on sample size, smoothing parameter of 1.0 or 0.5 was used to improve the performance of circular Kernel density by reducing biases (Rowcliffe *et al.*, 2014). In order to calculate the abundance of sika deer, total number of trap nights was calculated as $\sum_{\mathrm{i=1}} \mathrm{TN_i}$ and the number of animal passes/registrations was calculated as $\sum_{\mathrm{i=1}} \mathrm{TE_i}$ , where TN and TE as trap night and trap events at *i*th location respectively (Mohd-Azlan and Sharma, 2006; Rovero *et al.*, 2014; Ogurtsov *et al.*, 2018). The relative abundance index (RAI or Trap success) for each species was calculated as:

$$RAI = \left( \sum_{i=1} TE_i \,/ \sum_{i=1} TN_i \right) * 100$$

The RAI provides an estimate of abundance based on a total number of photographs and effort (i.e. events per 100 days of camera tapping), and hence facilitates comparisons between different sites and studies. Each photograph's time, date, and temperature were all captured by all cameras. Each photo reported time was divided into four categories: day (activity predominantly between 1 hr after sunrise and 1 hr before the sunset); night (activity predominantly between 1 hr after sunset and 1 hr before sunrise); cathemeral (activity peaks both at diurnal and nocturnal period) and crepuscular i.e., dawn and dusk (activity peaks 1 hr before and after sunrise and sunset respectively) (Bennie *et al.*, 2014; Porfirio *et al.*, 2016). The exact time of sunset and sunrise was predicted using the software Moonrise 3.5 (Sidell, 2002), and converted each photo time to solar time, as similar to Foster *et al*. (2013). The current distribution map was generated by combining the data of deer presence in Oh (2006) and Oh *et al*. (2019). Camera trap data in addition to indirect signs were used to plot the distribution map. The camera trap location and distribution range of deer were digitized in ArcMap (ArcGIS 10.1 for Desktop, ESRI software) and all calculations in Statistica 8.0 software (Statsoft, Inc.).

## 3. Molecular study

### 1) Samples collection and DNA extraction

Tissue samples of deer were obtained in the form of small pieces of muscle from culled Cervids (sika and roe deer) from the World Natural Heritage Headquarters in Jeju on special request. No Cervids were killed for this study but culled under invasive deer population control programs of the local governments in collaboration with the World Natural Heritage Centre. Culling was performed by experts at various locations within Jeju Island. Altogether, 21 tissue samples of deer were obtained. Only tissue samples were used for phylogenetic study of invasive deer species but no fecal samples were used for this purpose. The selection of tissue samples over fecal samples for genetic identification because fecal materials inadequately preserved source of DNA because it is exposed to the environmental conditions that degrade DNA. All standard precautions were taken to minimize the risk of contamination. Tissue samples were stored either at -20°C or in tubes containing 99% ethanol. Genomic DNA was extracted from tissue samples using DNeasy Blood & Tissue Kit (Qiagen, Germany), and stored at 4℃ until further analysis. In addition, fecal samples of sika deer were collected from various locations for diet identification. Upon collection, the sample were stored in a -20°C freezer prior to DNA extraction. Fecal samples were promptly removed from the frozen sample immediately prior to DNA extraction. Fecal DNA was extracted from about 20mg of fecal, obtained from 1-3 pellets, using DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions with slight modification.

### 2) PCR amplification of *CYTB* gene

Genomic DNA samples were amplified using Cytochrome b (*CYTB*). The primers used for PCR amplification were directly determined by comparing the mtDNA sequence of deer (NCBI, https://www.ncbi.nlm.nih.gov) under accession no. AB245427 (Table 1). The PCR mixture consisted of 1 µl genomic DNA, 1 µl of each primer, and distilled water to the Maxime PCR PreMix Kit (i-StarTaq) to make the final volume of 20µl per sample. MiniAmp Plus Thermal cycler (Thermo Fisher Scientific, USA) was used for PCR. The PCR cycling protocol for amplification of the *CYTB* involved an initial denaturation at 95℃ for 3 min followed by 35 cycles of denaturation at 95℃ for 30 sec, aneling at 50℃ for 1 min, and elongated at 72℃ for 1 min, with a final extension at 72℃ for 5 min. The PCR amplified products were run in a 1% agarose gel stained with Safe Shine Green in 0.5×TBE buffer and visualized under UV light. Successful amplifications were purified using QIAex II Gel Extraction Kit (Qiagen, USA) and were sequenced.

Table 1. Primer sequences used for *CYTB* gene analysis of sika deer.

| Primer name | Nucleotide sequence 5'→3' |
|---|---|
| SCytB _F | CAAGAACACTAATGACCAATATCC |
| SCytB _R | TACAAGACCAGTGTATTGAGTAT |

### 3) DNA sequencing and phylogeny

The determined sequences were analyzed by the sequence alignment editing software BioEdit version 7.0.5.3 (Hall, 1999). The determined *CYTB* gene sequences and the 14 DNA sequences collected from National Center for Biotechnology Information (NCBI - https://www.ncbi.nlm.nih.gov) database were multi-aligned using Clustal W (Larkin *et al*., 2007) as implemented in the software MEGA-X (Kumar *et al*., 2018). Initial sequence comparison and measure of variation were performed using MEGA-X. Sequences were identified by utilizing species identification tools accessible in the Barcode of Life Data system (BOLD) and through BLAST search in the NCBI nucleotide database to approve their identification (Naseem, 2020). Moreover, sequence analysis tools of BOLD were additionally used for assessing intra and interspecific divergences, nearest neighbor distance as well as barcode gap. A maximum likelihood phylogenetic tree was produced utilizing the Tamura-Nei model (Tamura and Nei, 1993) with gamma distribution (T93+G). The *CYTB* segment of Siberian roe deer (*C. pygargus*) was used as an outgroup for rooting the phylogenetic tree. The pairwise genetic distance was calculated between two haplotypes determined in this study and reference sequences taken from the NCBI database. The barcode data provided by the sequences were submitted to the NCBI database using the barcode submission tool, and their accession numbers are listed in Table 10.

### 4) Meta-barcoding for dietary items

A molecular identification technique was applied for those plants that were too damaged and difficult to identify morphologically. DNA was extracted from the damaged sample using Wizard Genomic DNA kit (Promega, Wisconsin, USA). PCR reaction was done using primer designated by Taberlet *et al*. (2007) for the chloroplast DNA trnL-trnF intergenic spacer (IGS) and those designated by Chen *et al*. (2010) for internal transcribed spacer 2 (ITS2) region of nuclear ribosomal RNA genes. Using genomic DNAs isolated from the fecal samples, further molecular analysis was carried out at the expertise company Macrogen Inc. (Seoul, South Korea) by next generation DNA sequencing (NGS). All sequences determined from the NGS were compared with those previously recorded at the DNA sequence database of NCBI by Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) search program. The plant species were categorized into four categories (graminoids, forb-climber, fern, and browse) based on plant morphology and growth forms to compare the dietary selection of sika deer across seasons.

# III. RESULTS

## 1. Distribution

Current distribution maps summarize inventories on the distribution of alien sika deer, on Jeju Island (Fig. 6). There were more than 402 sighting records of sika deer on Jeju Island allocating direct (camera traps and direct sighting) and indirect (fecal samples, footprints, hair, and shed antlers) events. An increase in the distribution range was observed in this study as compared to the previous distribution range. This study shows that the current distribution range of sika deer was towards the eastern and western parts of Hallasan Nation Park as they were originally released towards the northern part i.e. near Gwaneumsa temple and its adjacent areas. Until recently, no sika deer were reported from the southern part of the national park i.e., more at Seogwipo-si (Table 2; Fig. 7).

Table 2. Observation records of sika deer at camera trap locations

| Site No. | Locations | Sika deer observation | | | | Activity |
|---|---|---|---|---|---|---|
| | | Camera trap | | Direct observation | | |
| | | 2019 | 2020 | 2019 | 2020 | |
| 1 | Ara-dong, Jeju-si | √ | √ | - | - | Moving |
| 2 | Yeon-dong, Jeju-si | √ | √ | - | √ | Grazing/Resting |
| 3 | Saekdal-dong, Seogwipo-si | - | √ | √ | - | Grazing |
| 4 | Aewol-eup, Jeju-si | √ | - | - | √ | Moving |
| 5 | Saekdal-dong, Seogwipo-si | - | - | - | - | - |
| 6 | Saekdal-dong, Seogwipo-si | - | - | - | - | - |
| 7 | Topyeong-dong, Seogwipo-si | - | - | - | - | - |
| 8 | Namwon–eup, Seogwipo-si | - | - | - | - | - |
| 9 | Jocheno-eup, Jeju-si | √ | √ | √ | √ | Grazing/Running |
| 10 | Namwon-eup, Seogwipo si | √ | √ | - | √ | Moving |
| 11 | Jocheno-eup, Jeju-si | √ | √ | √ | √ | Grazing/Resting |
| 12 | Bonggae-dong, Jeju-si | √ | √ | √ | √ | Grazing/Moving |

Some sika deer individuals were recorded from Saekdal-dong and Namwon–eup of Seogwipo-si (Table 2), but these are areas adjacent to Jeju-si. Compared to direct observation, infrared camera traps have served well at sighting sika deer individuals.

The present distribution of sika deer shows their preference to an altitude between 550m and 1,100m. They were frequently recorded at the highest altitude of 1,100m wetland and Sumeunmulbaengdui wetland sites. The distribution map shows several locations where sika deer were recorded, but were found to concentrate more in some specific areas, such as Muljangori oreum, Mulchat oreum, Noro oreum, Gwaneumsa, and Eoseungsaeng horse riding farm (Table 3). These are areas containing water bodies and pasturelands which provide good habitat to sika deer.

Sika deer were recorded not only at the camera trapping sites, but were also recorded from various habitat types, such as temperate deciduous broadleaf forests, alpine coniferous forests, temperate deciduous forests, warm temperate evergreen forests, grasslands, wetlands and pasturelands. Fecal pellets, footprints, hair, shed antlers and their territorial markings were observed at various sites. The detail-recording sites with geographical co-ordinates and their habitat types are given in Table 4. Some recorded sika deer individuals from various study locations and observed field signs are given in Fig. 8 and 9.

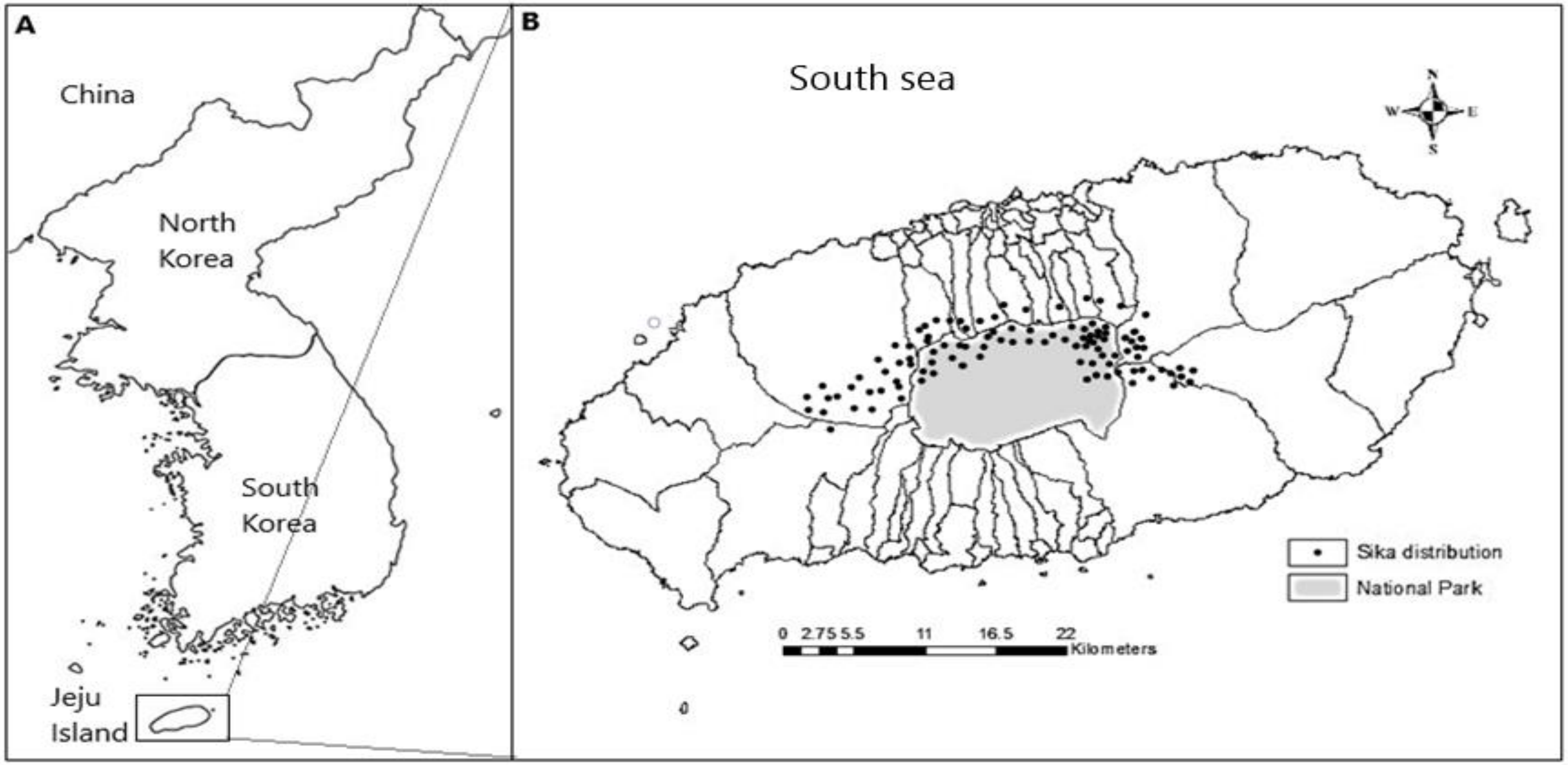

Fig. 6. Current distribution of sika deer in Jeju Island. A, Location of study area; B, geographical distribution of sika deer in this study. Dots on the map indicate finding site of sika deer. All information of each finding site are detailed in Table 3.

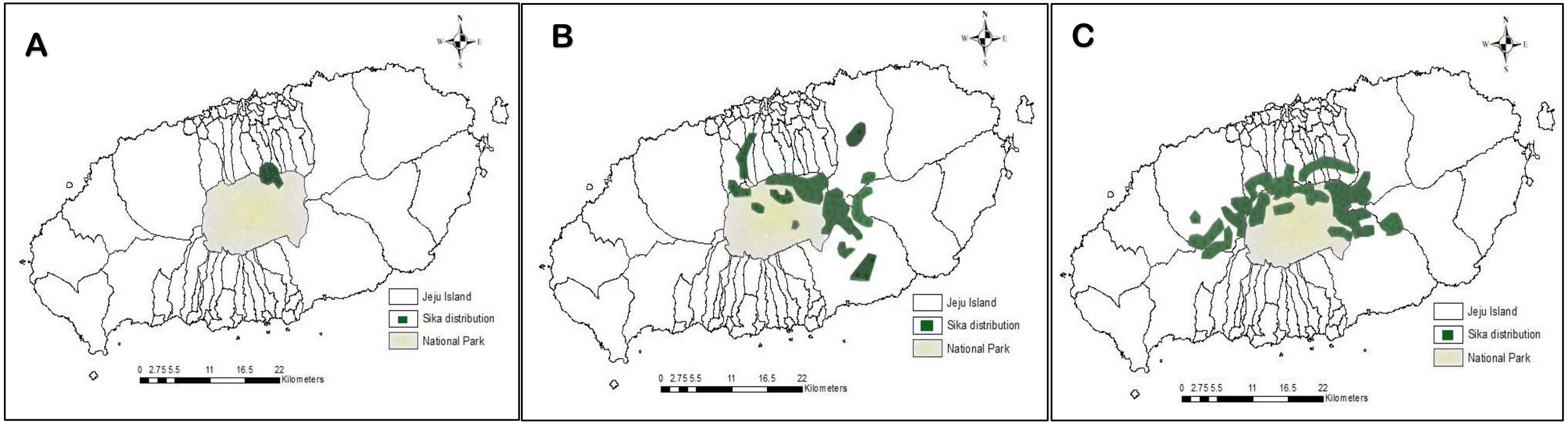

Fig. 7. Distribution of sika deer in Jeju Island. Comparison of previous literatures and current study in Jeju Island. A: Oh (2006); B: Oh *et al*. (2019); C: current study.

Table 3. Most frequent visiting sites by sika deer.

| No. | Location | No. of observation | Co-ordinate | Animal sign | Forest type |
|---|---|---|---|---|---|
| 1 | Muljangori oreum | 112 | N 33° 24’ 47.53”<br>E 126° 36’ 30.61” | Photo, feces, direct sighting | Temperate evergreen forest |
| 2 | Mulchat oreum | 56 | N 33° 23’ 52.58”<br>E 126° 39’ 04.05” | Photo, shed antlers | Temperate evergreen forest |
| 3 | Noro oreum | 106 | N 33° 22’ 09.68”<br>E 126° 26’ 25.80” | Photo, feces, footprints | Alpine coniferous and temperate deciduous forest |
| 4 | Gwaneumsa | 41 | N 33° 25’ 29.59”<br>E 126° 33’ 14.51” | Feces, hair, shed antlers | Warm temperate deciduous broadleaf forest and pastureland |
| 5 | Eoseungsaeng horse riding farm | 39 | N 33° 25’ 31.60”<br>E 126° 29’ 40.04” | Photo, footprints | Temperate deciduous broadleaf and evergreen forest |

Table 4. Observation records of the sika deer in Jeju Island in this study.

| Site no. | Co-ordinate | | Province | Species confirm by | | | | Habitat types |
|---|---|---|---|---|---|---|---|---|
| | Latitude (N) | Longitude (E) | | Photo | Fecal pellet | Footprints | Others* | |
| S01 | 33°24'29.32" | 126 36'21.20" | Bonggae-dong, Jeju-si | o | | o | | TDBF |
| S02 | 33° 24'28.72" | 126°36'21.72" | Bonggae-dong, Jeju-si | o | o | | o | TDBF |
| S03 | 33°24'29.01" | 126°36'22.20" | Bonggae-dong, Jeju-si | o | o | | | TDBF |
| S04 | 33°24'32.92" | 126°36'12.95" | Bonggae-dong, Jeju-si | o | o | | o | TDBF |
| S05 | 33°24'33.30" | 126°36'13.98" | Bonggae-dong, Jeju-si | | o | | | TDBF |
| S06 | 33°24'34.19" | 126°36'13.84" | Bonggae-dong, Jeju-si | o | | | | TDBF |
| S07 | 33°24'33.80" | 126°36'13.56" | Bonggae-dong, Jeju-si | o | o | o | o | TDBF |
| S08 | 33°24'33.66" | 126°36'13.54" | Bonggae-dong, Jeju-si | o | o | o | | TDBF |
| S09 | 33°24'28.54" | 126°36'21.65" | Bonggae-dong, Jeju-si | o | o | o | o | TDBF |
| S10 | 33°24'29.12" | 126°36'28.89" | Bonggae-dong, Jeju-si | | o | | | TDBF |
| S11 | 33°24'13.09" | 126°36'35.08" | Bonggae-dong, Jeju-si | o | o | | | TDBF |
| S12 | 33°24'14.25" | 126°36'33.11" | Bonggae-dong, Jeju-si | o | | | o | TDBF |
| S13 | 33°24'13.48" | 126°36'35.26" | Bonggae-dong, Jeju-si | o | o | | | TDBF |
| S14 | 33°24'46.63" | 126°36'37.50" | Bonggae-dong, Jeju-si | o | o | | | TDBF |
| S15 | 33°25'24.40" | 126°36'22.72" | Bonggae-dong, Jeju-si | | o | | | TDBF |
| S16 | 33°24'57.28" | 126°36'21.00" | Bonggae-dong, Jeju-si | o | o | o | o | TDBF |
| S17 | 33°24'48.06" | 126°36'28.11" | Bonggae-dong, Jeju-si | o | o | | | TDBF |
| S18 | 33°24'38.40" | 126°36'26.31" | Bonggae-dong, Jeju-si | o | | o | o | TDBF |
| S19 | 33°22 15.17" | 126°26'53.78" | Saekdal-dong, Seogwipo-si | o | o | o | | AC/TDF |
| S20 | 33°22 15.04" | 126°26'53.92" | Saekdal-dong, Seogwipo-si | o | o | | | AC/TDF |
| S21 | 33°22 13.36" | 126°26'54.27" | Saekdal-dong, Seogwipo-si | o | o | | | AC/TDF |
| S22 | 33°22 12.84" | 126°26'53.95" | Saekdal-dong, Seogwipo-si | o | o | | o | AC/TDF |
| S23 | 33°22 14.30" | 126°26v53.77" | Saekdal-dong, Seogwipo-si | o | o | o | | AC/TDF |
| S24 | 33°22 14.95" | 126°26'54.41" | Saekdal-dong, Seogwipo-si | | o | | | AC/TDF |
| S25 | 33°22 15.88" | 126°26'53.94" | Saekdal-dong, Seogwipo-si | o | o | o | | AC/TDF |
| S26 | 33°22 12.58" | 126°26'53.90" | Saekdal-dong, Seogwipo-si | o | o | o | | AC/TDF |
| S27 | 33°22'10.66" | 126°27'11.87" | Saekdal-dong, Seogwipo-si | o | o | o | | AC/TDF |
| S28 | 33°21'58.23" | 126°27'9.20" | Saekdal-dong, Seogwipo-si | o | o | | o | AC/TDF |
| S29 | 33°22'12.16" | 126°26'57.39" | Saekdal-dong, Seogwipo-si | | o | | | AC/TDF |
| S30 | 33°22'4.15" | 126°26'56.69" | Saekdal-dong, Seogwipo-si | o | o | | | AC/TDF |
| S31 | 33°21'38.99" | 126°27'47.34" | Saekdal-dong, Seogwipo-si | o | o | | | AC/TDF |
| S32 | 33°21'45.22" | 126°27'47.90" | Saekdal-dong, Seogwipo-si | o | o | | | AC/TDF |
| S33 | 33°23'44.02" | 126°39'0.99" | Jocheon -eup, Jeju-si | o | o | | | WTEDF |
| S34 | 33°23'47.86" | 126°39'2.54" | Jocheon -eup, Jeju-si | | | | o | WTEDF |
| S35 | 33°23'46.69" | 126°39'3.83" | Jocheon -eup, Jeju-si | o | | o | | WTEDF |
| S36 | 33°23'45.77" | 126°39'4.90" | Jocheon -eup, Jeju-si | o | | o | | WTEDF |
| S37 | 33°23'46.87" | 126°39'8.80" | Jocheon -eup, Jeju-si | o | | | | WTEDF |
| S38 | 33°23'41.22" | 126°39'2.73" | Jocheon -eup, Jeju-si | | | o | | WTEDF |
| S39 | 33°23'47.56" | 126°39'0.46" | Jocheon -eup, Jeju-si | o | | | | WTEDF |
| S40 | 33°25'23.42" | 126°29'35.47" | Yeon-dong, Jeju-si | o | o | | | TDBF |

Note: TDBF- Temperate deciduous broadleaf forest; AC/TDF, Alpine coniferous and temperate deciduous forest; WTEDF, Warm temperate evergreen deciduous forest; PZ/WTEF, Pasture zone and warm temp evergreen forest. *, represent antlers, bones, hair etc.

Table 4. Continued

| Site no. | Co-ordinate | | Province | Species confirm by | | | | Habitat types |
|---|---|---|---|---|---|---|---|---|
| | Latitude (N) | Longitude (E) | | Photo | Fecal pellet | Footprints | Others* | |
| S41 | 33°25'25.19" | 126°29'34.63" | Yeon-dong, Jeju-si | o | | o | | TDBF |
| S42 | 33°25'25.89" | 126°29'36.97" | Yeon-dong, Jeju-si | o | o | | o | TDBF |
| S43 | 33°25'29.03" | 126°29'35.42" | Yeon-dong, Jeju-si | o | o | | | TDBF |
| S44 | 33°25'25.50" | 126°29'34.06" | Yeon-dong, Jeju-si | o | | | | TDBF |
| S45 | 33°25'23.59" | 126°29'34.72" | Yeon-dong, Jeju-si | o | o | | | TDBF |
| S46 | 33°25'30.24" | 126°33'4.20" | Ara-dong, Jeju-si | | o | | o | PZ/WTEF |
| S47 | 33°25'28.24" | 126°32'58.90" | Ara-dong, Jeju-si | | o | | | PZ/WTEF |
| S48 | 33°25'23.85" | 126°33'1.93" | Ara-dong, Jeju-si | | o | | o | PZ/WTEF |
| S49 | 33°25'20.55" | 126°33'9.34" | Ara-dong, Jeju-si | | o | | | PZ/WTEF |
| S50 | 33°25'23.36" | 126°33'35.36" | Ara-dong, Jeju-si | | o | | | PZ/WTEF |
| S51 | 33°25'28.12" | 126°33'32.62" | Ara-dong, Jeju-si | | | | o | PZ/WTEF |
| S52 | 33°25'38.19" | 126°33'30.20" | Ara-dong, Jeju-si | | o | | | PZ/WTEF |
| S53 | 33°25'29.11" | 126°33'19.14" | Ara-dong, Jeju-si | | o | | | PZ/WTEF |
| S54 | 33°25'23.53" | 126°33'28.16" | Ara-dong, Jeju-si | | o | | o | PZ/WTEF |
| S55 | 33°24'36.07" | 126°28'46.92" | Haean-dong, Jeju-si | | o | | o | WTEDF |
| S56 | 33°23'59.36" | 126°27'42.12" | Haean-dong, Jeju-si | | o | | | WTEDF |
| S57 | 33°25'50.41" | 126°26'10.24" | Haean-dong, Jeju-si | | | | o | WTEDF |
| S58 | 33°24'52.68" | 126°29'1.27" | Haean-dong, Jeju-si | | o | | o | WTEDF |
| S59 | 33°24'56.58" | 126°28'32.87" | Haean-dong, Jeju-si | | o | | | WTEDF |
| S60 | 33°24'50.63" | 126°28'15.93" | Haean-dong, Jeju-si | | | o | | WTEDF |
| S61 | 33°20'10.25" | 126°36'39.68" | Namwon-eup, Seogwipo-si | | | | o | TDBF |
| S62 | 33°22'3.49" | 126°38'1.35" | Namwon-eup, Seogwipo-si | | o | | o | TDBF |
| S63 | 33°22'58.81" | 126°37'45.13" | Namwon-eup, Seogwipo-si | | o | | o | TDBF |
| S64 | 33°22'45.99" | 126°37'28.25" | Namwon-eup, Seogwipo-si | | o | | | TDBF |
| S65 | 33°22'42.96" | 126°37'30.68" | Namwon-eup, Seogwipo-si | | o | | | TDBF |
| S66 | 33°22'12.62 | 126°41'34.42" | Namwon-eup, Seogwipo-si | o | o | o | o | TDBF |
| S67 | 33°22'90.50" | 126°41'30.15" | Namwon-eup, Seogwipo-si | o | o | o | | TDBF |
| S68 | 33°21'51.74" | 126°41'26.48" | Namwon-eup, Seogwipo-si | | | o | o | TDBF |
| S69 | 33°21'47.20" | 126°41'18.87" | Namwon-eup, Seogwipo-si | | o | | o | TDBF |
| S70 | 33°25'30.17" | 126°31'45.13" | Ora il-dong, Jeju-si | | o | | | WTEDF |
| S71 | 33°25'41.83" | 126°31'50.89" | Ora il-dong, Jeju-si | | o | | | WTEDF |
| S72 | 33°25'35.23" | 126°32'19.82" | Ora il-dong, Jeju-si | | | | | WTEDF |
| S73 | 33°25'14.87" | 126°31'14.17" | Ora il-dong, Jeju-si | | o | | | WTEDF |
| S74 | 33°25'60.93" | 126°31'16.33" | Ora il-dong, Jeju-si | | o | | | WTEDF |
| S75 | 33°25'8.87" | 126°31'24.88" | Ora il-dong, Jeju-si | | o | | | WTEDF |
| S76 | 33°23'11.83" | 126°23'32.76" | Aewol-eup, Jeju-si | | o | o | o | WTEDF |
| S77 | 33°23'70.58" | 126°23'30.66" | Aewol-eup, Jeju-si | | o | | | WTEDF |
| S78 | 33°23'34.12" | 126°23'44.44" | Aewol-eup, Jeju-si | | o | | | WTEDF |
| S79 | 33°23'41.35 | 126°37'0.58" | Jocheon -eup, Jeju-si | | | | o | TDBF |
| S80 | 33°23'7.13" | 126°37'10.51" | Jocheon -eup, Jeju-si | | o | | | TDBF |
| S81 | 33°24'12.76" | 126°37'17.62" | Jocheon -eup, Jeju-si | | o | | | TDBF |
| S82 | 33°22'3.12" | 126°37'37.61" | Jocheon -eup, Jeju-si | o | o | | o | TDBF |

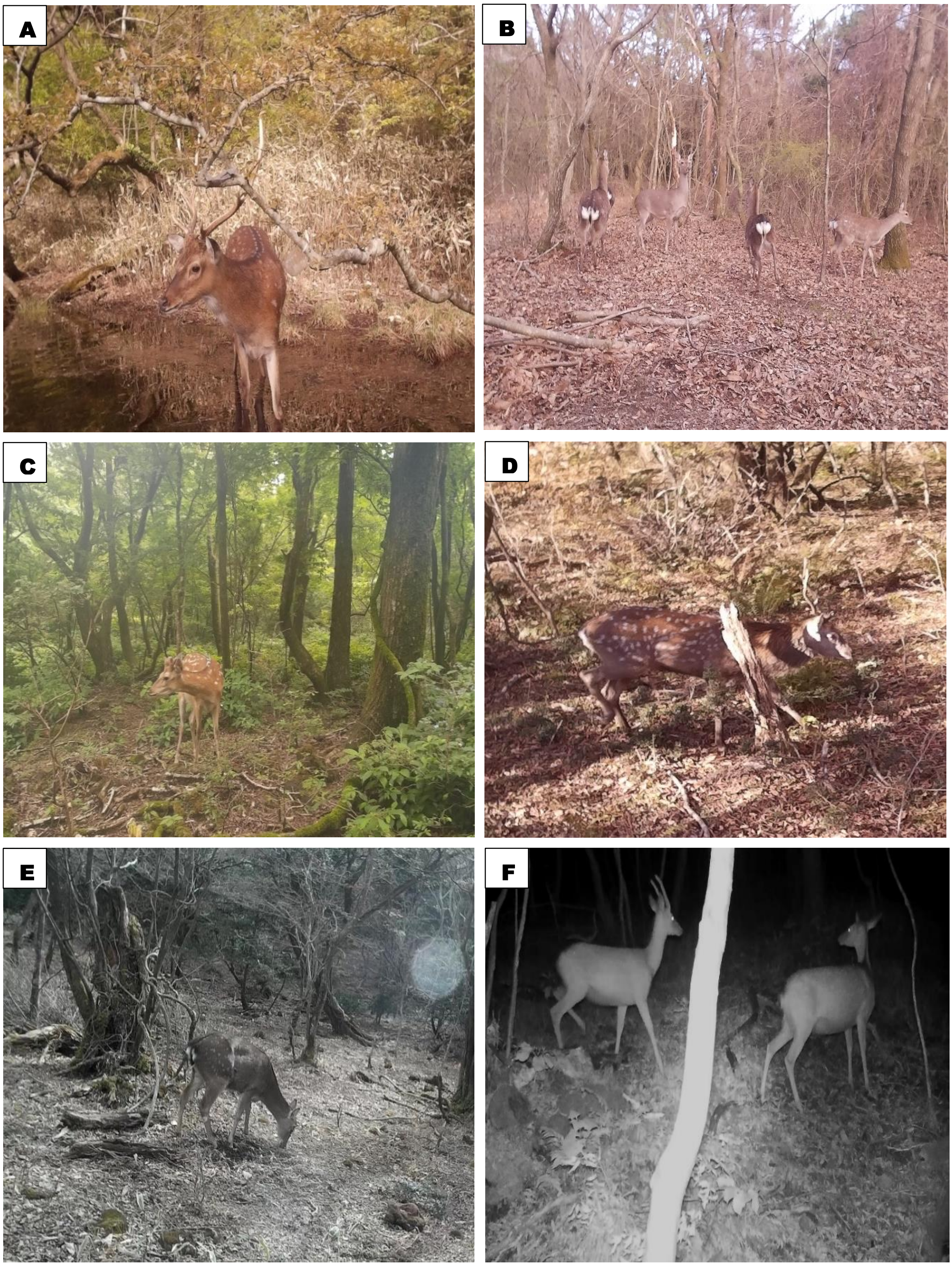

Fig. 8. Observed sika deer individuals from various locations within Jeju Island: A, Noro oreum; B, Eoseungsaeng horse riding farm; C, Muljangori oreum; D, Mulchat oreum; E, Sumeunmulbaengdui wetland; and F, Mulyeongari-oreum.

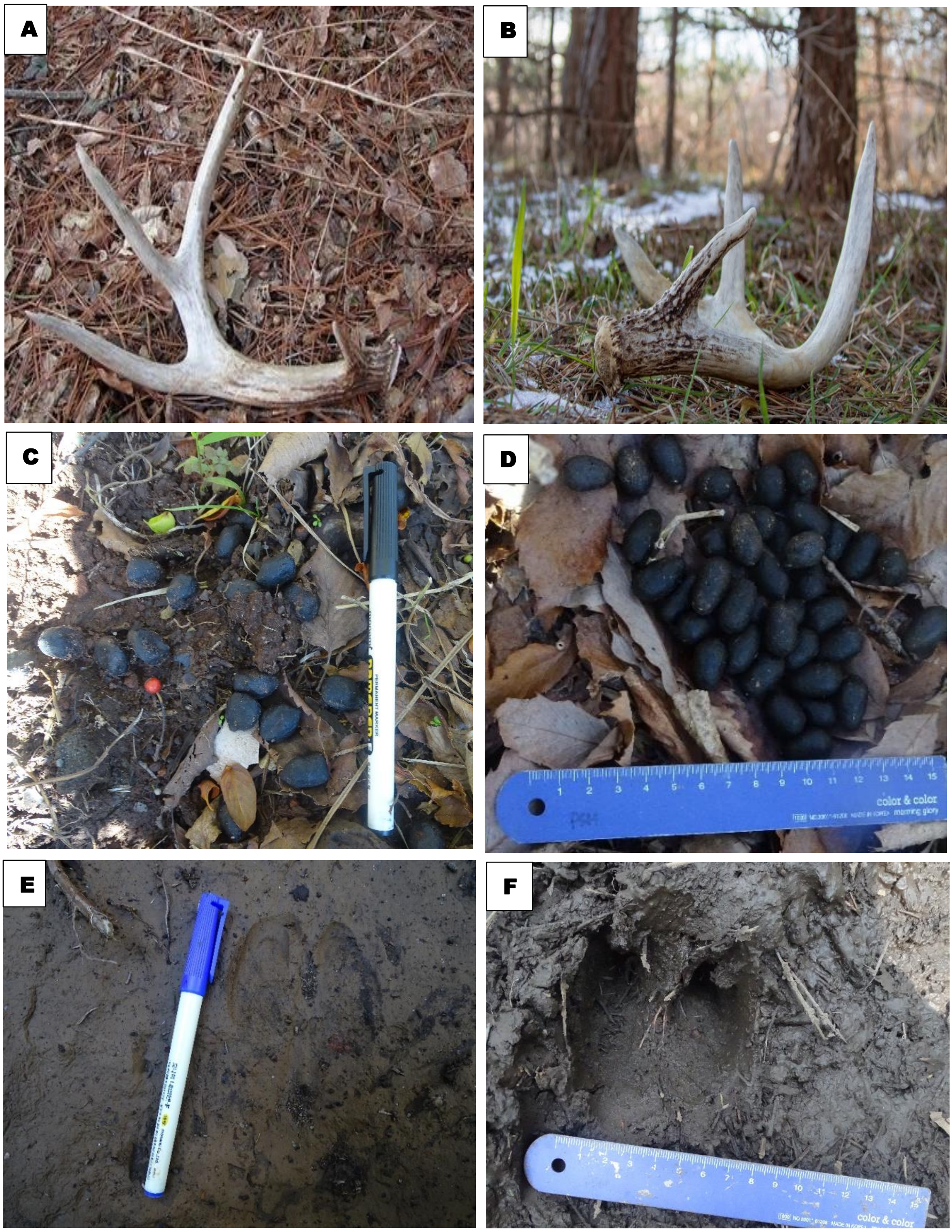

Fig. 9. Observed field sign of sika deer within Jeju Island: A and B – Shed antlers; C and D; fecal pellets; E and F; Footprints.

## 2. Activity pattern

Within 6,840 trap nights, a total of 2,497 trap events for nine species were recorded (Fig. 10) from 12 locations, after subtracting the number of trap nights when cameras were nonfunctional (Fig. 4). The trap events for Siberian roe deer (68.95%) was highest followed by sika deer (10.45%), human (5.26%), wild boar (4.99%); Asian badger (3.43%), cow (2.05%), red deer (1.20%), Jeju weasel (0.85%) and dog (0.74%). The maximum value of the abundance index noted for the roe deer (RAI = 25.84) followed by sika deer (RAI = 3.91) (Table 5).

Table 5. Camera traps events of nine species from twelve locations of Jeju Island

| Animal species | Trap events (TE) | Relative abundance index (RAI) | No. of picture | % of picture |
|---|---|---|---|---|
| Roe deer | 1,768 | 25.84 | 6,712 | 49.18 |
| Sika deer | 268 | 3.91 | 3,514 | 25.75 |
| Human | 135 | 1.97 | 458 | 3.35 |
| Wild boar | 128 | 1.87 | 1,928 | 14.12 |
| Badger | 88 | 1.28 | 315 | 2.30 |
| Cow | 38 | 0.55 | 72 | 0.51 |
| Red deer | 31 | 0.45 | 54 | 0.39 |
| Weasel | 22 | 0.32 | 46 | 0.33 |
| Dog* | 19 | 0.27 | 78 | 0.57 |
| Total | 2,497 | - | 13,177 | |

Note: For camera trap locations, refer to Materials and Methods section.

*, Dog, - feral and/or domesticated.

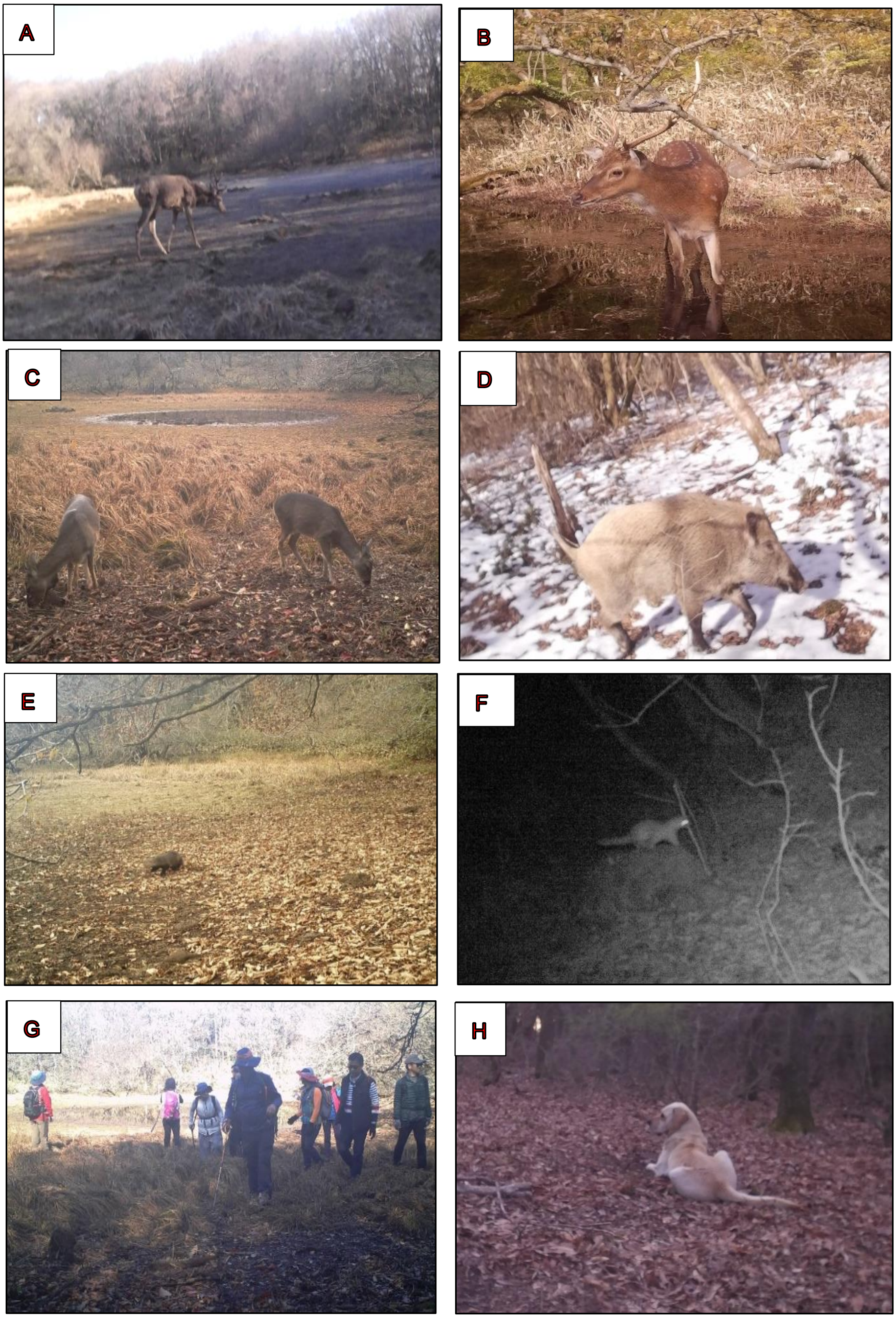

Fig. 10. Mammalian species photographed by camera trap at various stations: A, red deer; B, sika deer; C, roe deer; D, wild boar; E, badger; F, weasel; G, human; and H, dog.

### 1) Seasonal activity pattern of sika deer

Sika deer of Jeju Island varies in their activity patterns seasonally but the peak in activity time within a day is largely constant except for winter. The change of weather and climatic condition (Fig. 11) affects sika deer being photographed in each seasons from 93 (34.70%) in summer to the least 34 (12.68%) during winter. Most notably, the probability of beginning their activity peaks to dawn and dusk during spring-autumn to day and night times in winter (Fig. 12).

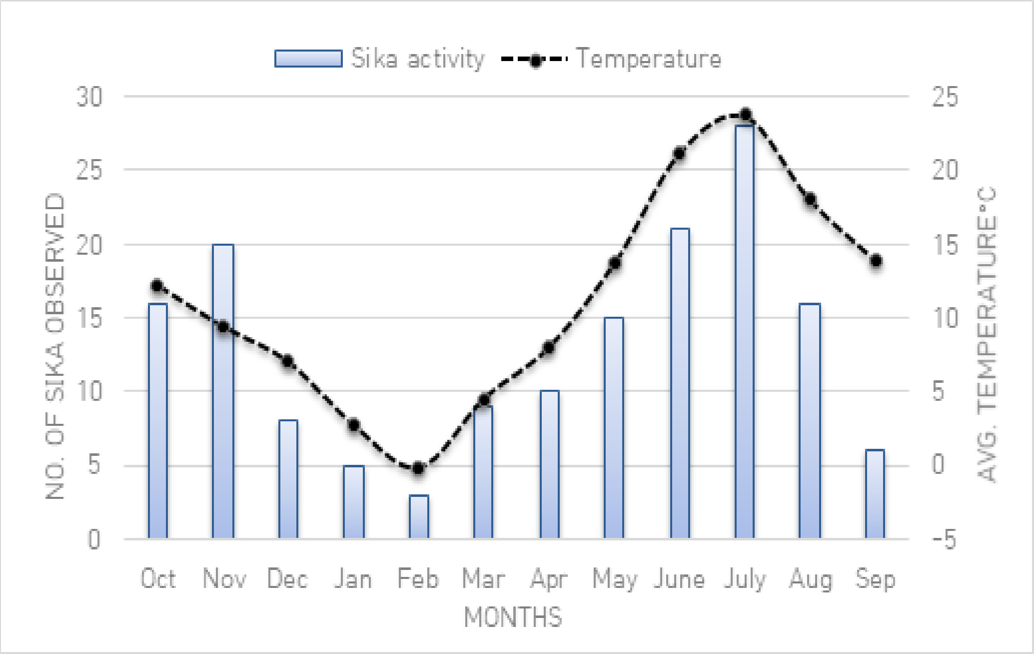

Fig. 11. Number of sika deer observed in relation to mean monthly temperature fluctuation.

Table 6. Seasonal pattern of diel activity pattern in sika deer

| Seasons | Category* | | | | $\chi^2$ - value | *P*-value |
|---|---|---|---|---|---|---|
| | Dawn | Day | Dusk | Night | | |
| Spring | 5.0±1.15 | 3.0±0.57 | 4.3±2.0 | 2.0±0.54 | 7.80 | 0.25 |
| Summer | 6.32±0.88 | 3.0±1.15 | 8.3±1.45 | 4.3±1.20 | 6.91 | 0.32 |
| Autumn | 3.0±0.57 | 2.0±0.57 | 4.0±1.52 | 2.0±1.00 | 3.91 | 0.68 |
| Winter | 1.0±0.0 | 1.6±0.33 | 1.0±0.03, | 3.6±0.33 | 0.50 | 0.09 |
| $\chi^2$ – value | 2.71 | 4.45 | 12.22 | 2.72 | | |
| *P* – value | 0.84 | 0.61 | 0.57 | 0.84 | | |

These values indicate the average photographic frequencies and SE. Differences in the frequencies among four time periods were tested using $\chi^2$ test.

*abbreviations of each category were given in Materials and Methods section.

Temperature showed negative effects on the number of deer photographed during winter but the photographic frequencies were not different among the four time period in winter (Table 6). Despite least observed species during winter the activity curves peaks more towards night hours, showing its nocturnal behavior (Fig. 12).

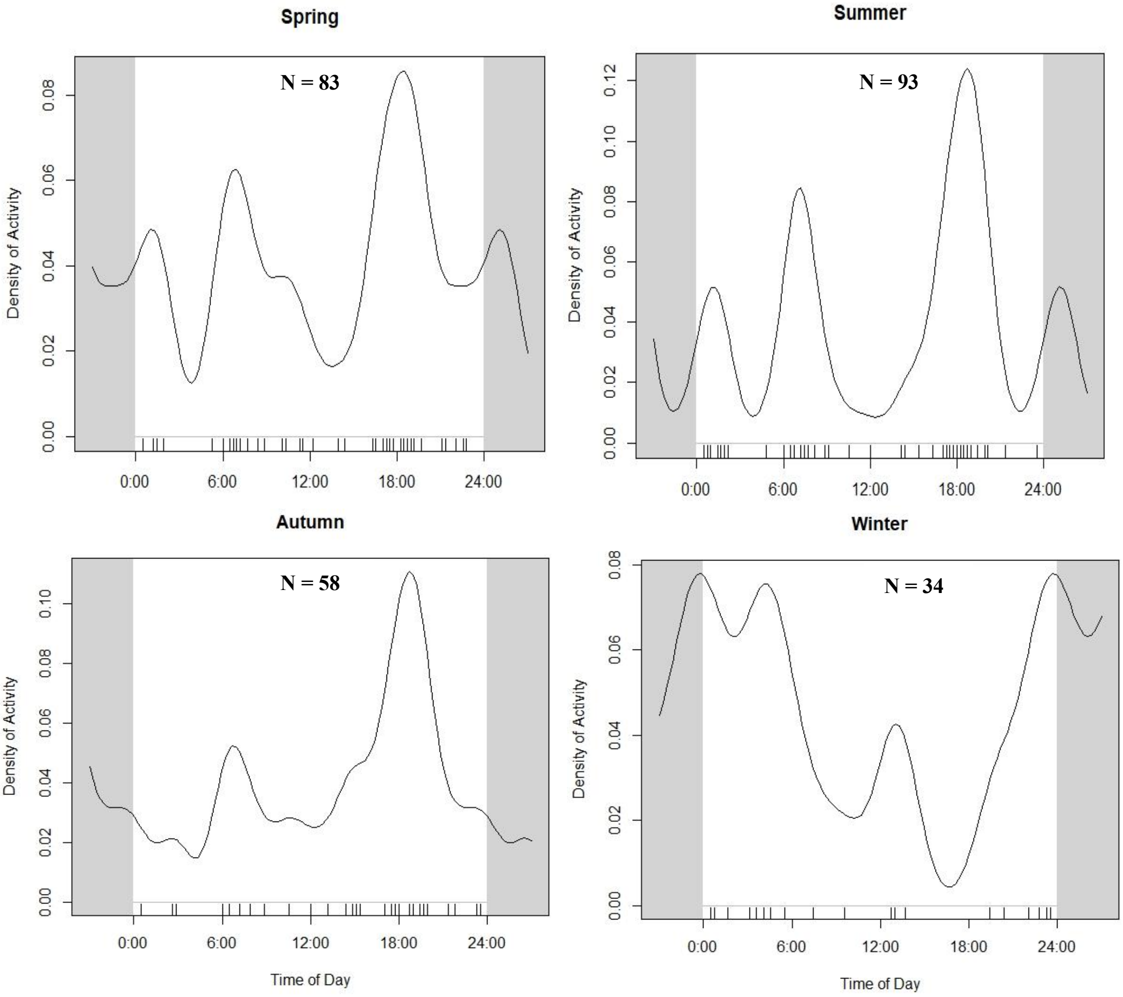

Fig. 12. Seasonal pattern of daily activity of sika deer in Jeju Island. Black lines and bars indicate Kernel density and trap events respectively: X-axis represents time of day in hours, Y-axis represents density of activity.

## 2) Diel temporal overlap of sika deer with sympatric species in summer

When considering the activity overlap between sika deer and other observed sympatric mammalian species in summer (Fig. 13), it was observed that roe deer clearly expressed a high degree of overlap ($\Delta 4 = 0.70$; CI = 0.64-0.84). Both species active throughout the day and night, with two distinct peaks of activity in the twilight hours. Twilight hour activity is more pronounced for sika deer compared to roe deer (Fig. 13b).

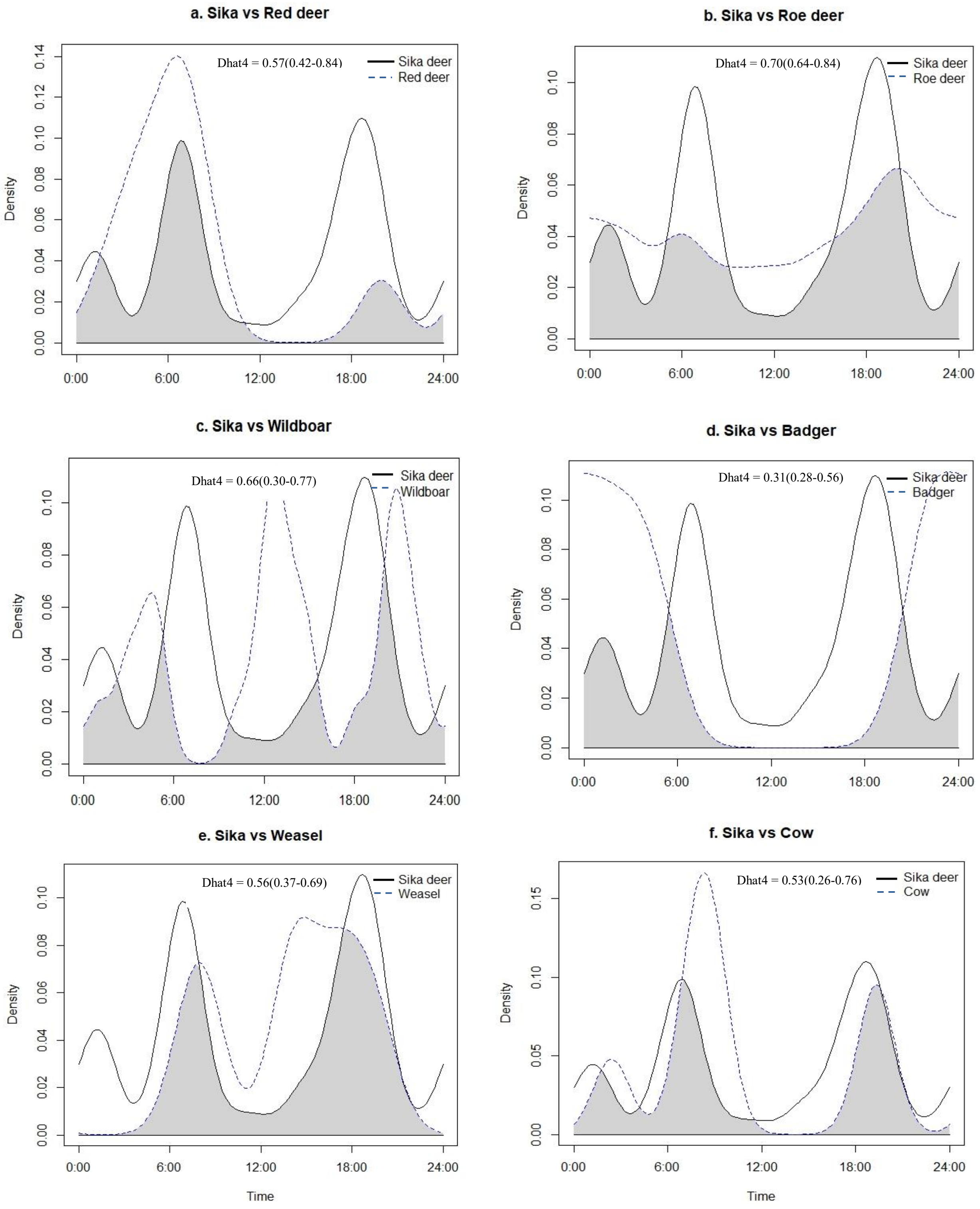

Fig. 13. Daily activity overlaps and their estimators (Dhat4 and CI) between sika deer and associated mammalian species in summer. Solid and dashed lines, grey shaded area indicates Kernel densities of two species, overlap coefficient respectively; X-axis- time of day in hours, Y-axis- density of activity

Wild boar shows cathemeral activity (activity peaks both at nocturnal and diurnal period). Lower activity was observed at dusk compared to dawn activity. The coefficient of activity overlap between the wild boar and sika deer is lower (Δ4 = 0.66; CI = 0.30-0.77; Fig. 13c) compared to that of sika deer and roe deer (Δ4 = 0.70; CI = 0.64-0.84). The red deer, the largest mammals' species observed at Jeju Island showed medium activity overlap with sika deer (Δ4 = 0.57; CI = 0.42-0.84) at twilight hours. Red deer showed its higher activity level at dawn compared to dusk (Fig. 13a).

Badger is the only species, which shows predominantly nocturnal behavior with activity peaks towards midnight. The coefficient of activity overlap between badger and sika deer seems weaker (Δ4 = 0.31; CI = 0.28-0.56; Fig. 13d). In, opposite to badger behavior, the weasel shows higher diurnal activity. Moring activity begins at dawn and cease towards noon. Following noon, activity increase dramatically and abruptly ceased at night (Fig. 13e). Cows show two activity peaks, one in the morning and the other in the evening. Activity peaked latest in the morning and in the evening with pronounces activity in the morning (Fig. 13f).

### 3) Temporal response of sika deer to human activity in summer

Humans and wildlife have a negative interaction, since animals' behavior is fully distracted by human activity. Human activity was diurnal, with a peak around 12:00 hr. (Fig. 14a). Most wildlife species together with sika deer ($\Delta_4$ = 0.29; CI = 0.19-0.51) significantly reduced their activity in response to human activity. In some incidences dogs together with humans, were observed, which threatened the wildlife activity (Fig. 14b).

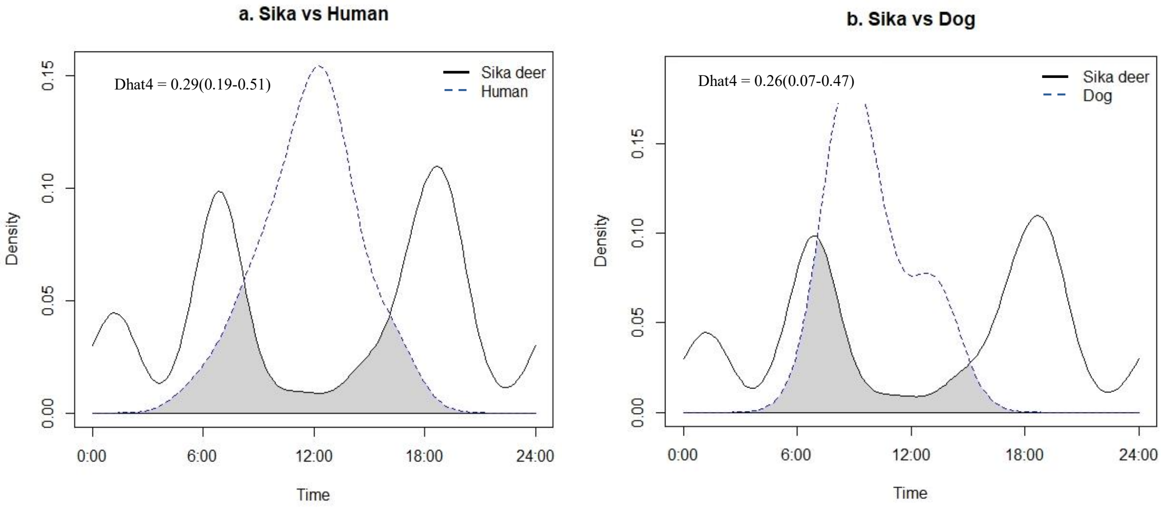

Fig. 14. Daily activity overlaps and their estimators (Dhat4 and CI) between sika deer and associated human activity in summer. Solid and dashed lines, grey shaded area indicates Kernel densities of two species, overlap coefficient respectively; X-axis represent time of day in hours, Y-axis represent density of activity.

### 4) Diel temporal overlap of sika deer with sympatric species in winter

Sika deer in winter shows its cathemeral behavior (activity peaks both at nocturnal and diurnal period). Despite limited number of observed species (only 6), most species shows their activity towards day hours. Similar to summer activity, roe deer in winter expressed a high degree of overlap with sika deer (Δ4 = 0.73; CI = 0.52-0.87) compared to other observed species. Both of this species have their activity overlap more towards night hours (Fig 15b). Despite few trap events, red deer in our study shows its cathermeral behavior. Activity begins after dusk, abruptly increase at night and cease towards dawn. The day time activity begins towards noon (Fig. 15a). Compared to other sympatric species, red deer also shows greater degree of overlap with sika deer (Δ4 = 0.70; CI = 0.62-0.86; Fig 15a).

As similar to summer activity, the activity of badge is nocturnal (Fig. 15c). The coefficient of activity overlap between badger and sika deer seems stronger compared to wild boar (Δ4 = 0.61; CI = 0.28-0.80; Fig. 13d). Wild boar in this study shows cathemeral behavior (activity peaks both at nocturnal and diurnal period) in winter. Lower activity was observed at twilight hours compared to daylight hours. The coefficient of activity overlap between the wild boar and sika deer is lower (Δ4 = 0.34; CI = 0.09-0.63; Fig. 15c) compared to that of sika deer and roe deer (Δ4 = 0.73; CI = 0.52-0.87).

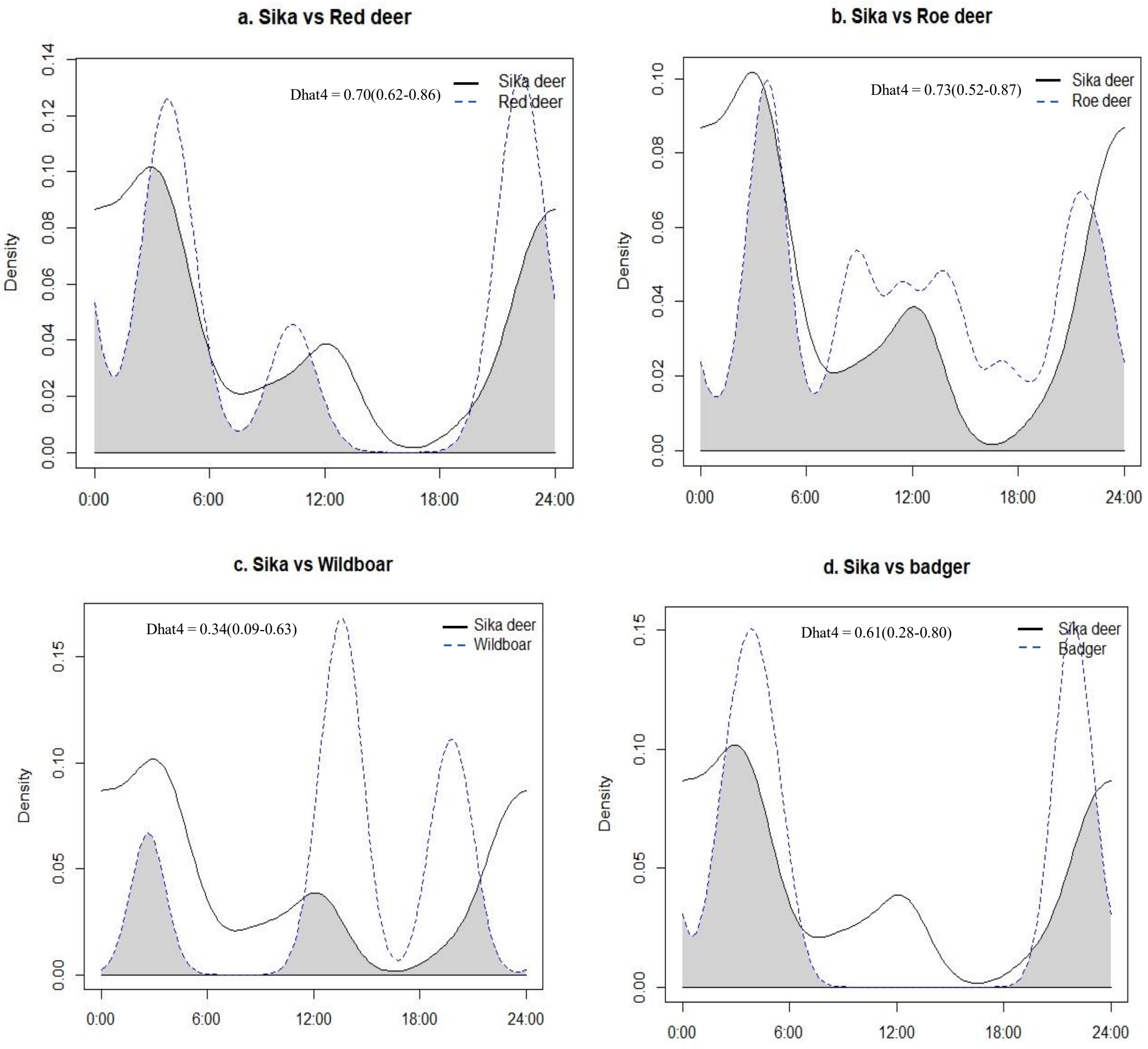

Fig. 15. Daily activity overlaps and their estimators (Dhat4 and CI) between sika deer and associated mammalian species in winter. Solid and dashed lines, grey shaded area indicates Kernel densities of two species, overlap coefficient respectively; X-axis- time of day in hours, Y-axis- density of activity

### 5) Temporal response of sika deer to human activity in winter

Despite lower photographic events for human in winter, but their activity peaks more towards daylight hours (Fig 16a). In addition, dogs have their higher activity towards day and night hours but have lower degree of overlap with sika deer (Δ4 = 0.37; CI = 0.27-0.79; Fig 16b).

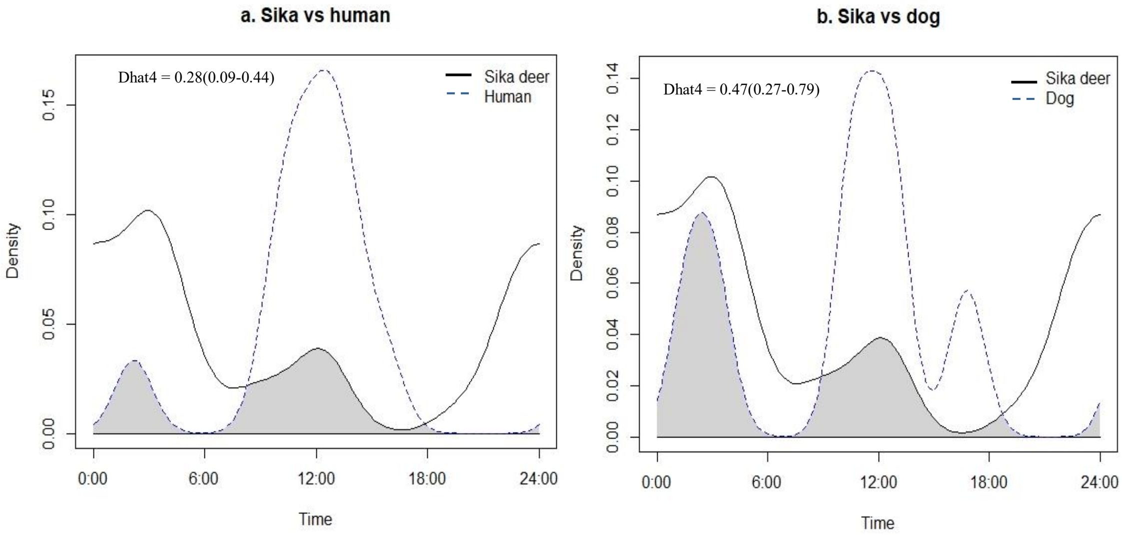

Fig. 16. Daily activity overlaps and their estimators (Dhat4 and CI) between sika deer and associated human activity in winter. Solid and dashed lines, grey shaded area indicates Kernel densities of two species, overlap coefficient respectively; X-axis represent time of day in hours, Y-axis represent density of activity.

## 2. Dietary resource used

A total of 95 plant taxa were identified as composing their diet, and were classified into 34 families, 23 orders and 4 classes (Table 7). A total of 68 plant taxa were identified using morphological analysis, but 27 plant taxa could not be identified through morphological or anatomical comparison, hence we used a molecular technique for identification. Dicotyledonae (65 taxon) was observed in the highest proportion, followed by Monocotyledonae (21 taxon), Polypodiopsida (5 taxon) and Coniferopsida (4 taxon) (Fig. 15). In general, sika deer fed on species of at least 75 forb-climbers, 24 graminoids, 8 ferns and 38 browse (Appendix 1.) (Fig. 17). Plant species belonging to the family Poaceae were consumed in higher proportions (10.52%), followed by Asteraceae (9.47%), Rosaceae (8.42%), Fabaceae (6.31%) and others (Table.7).

### 1) Seasonal diet

The composition of plant species in the diet varies seasonally (higher in summer: 47 taxon and lower in winter: 26 taxon) on Jeju Island. Graminoids and forb-climbers primarily made up the spring and summer diets (approximately 49.6%), whereas browse and ferns constituted the greatest portion of the diet in autumn and winter (21.3%). Conversely, the number of browse and ferns decreased with the progression of the growing season and later increased in autumn and reached the maximum in winter (Fig. 18).

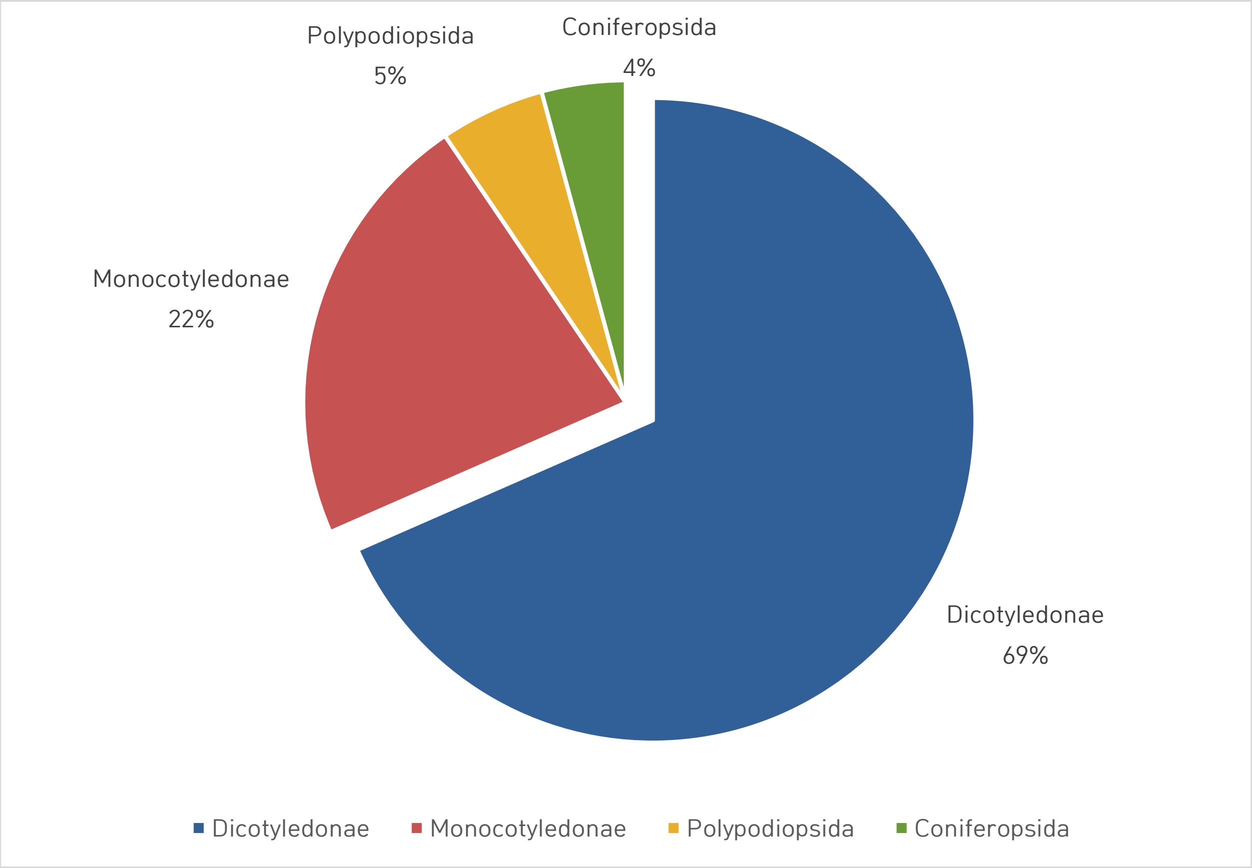

Fig. 17. Pie chart showing consumed plants taxa of various class.

Table 7. List of higher taxonomic level of dietary plant species of sika deer.

| Class | Order | Family | No. of taxa |
|---|---|---|---|
| Polypodiopsida | Polypodiales | Dryopteridaceae | 3 |
| | | Pteridaceae | 2 |
| Coniferopsida | Cupressales | Cupressaceae | 1 |
| | Pinales | Pinaceae | 3 |
| Monocotyledonae | Asparagales | Asparagaceae | 2 |
| | | Orchidaceae | 1 |
| | Liliales | Colchicaceae | 1 |
| | | Melanthiaceae | 1 |
| | | Smilacaceae | 1 |
| | Poales | Cyperaceae | 5 |
| | | Poaceae | 10 |
| Dicotyledonae | Apiales | Apiaceae | 4 |
| | | Araliaceae | 2 |
| | Asteriales | Asteraceae | 9 |
| | Caryophyllales | Caryophyllaceae | 3 |
| | Cornales | Cornaceae | 1 |
| | | Hydrangeaceae | 1 |
| | Cucurbitales | Cucurbitaceae | 3 |
| | Dipsacales | Adoxaceae | 3 |
| | Fabales | Fabaceae | 6 |
| | Fragales | Fragaceae | 1 |
| | Gentianales | Rubiaceae | 4 |
| | Geraniales | Apocynaceae | 1 |
| | | Geraniaceae | 1 |
| | Lamiales | Acanthaceae | 2 |
| | | Oleaceae | 3 |
| | | Plantaginaceae | 2 |
| | Myrtales | Onagraceae | 1 |
| | Oxalidales | Oxalidaceae | 2 |
| | Ranunculales | Ranunculaceae | 4 |
| | Rosales | Rosaceae | 8 |
| | Saxifragales | Daphniphyllaceae | 1 |
| | | Haloragaceae | 1 |
| | Vitales | Vitaceae | 2 |

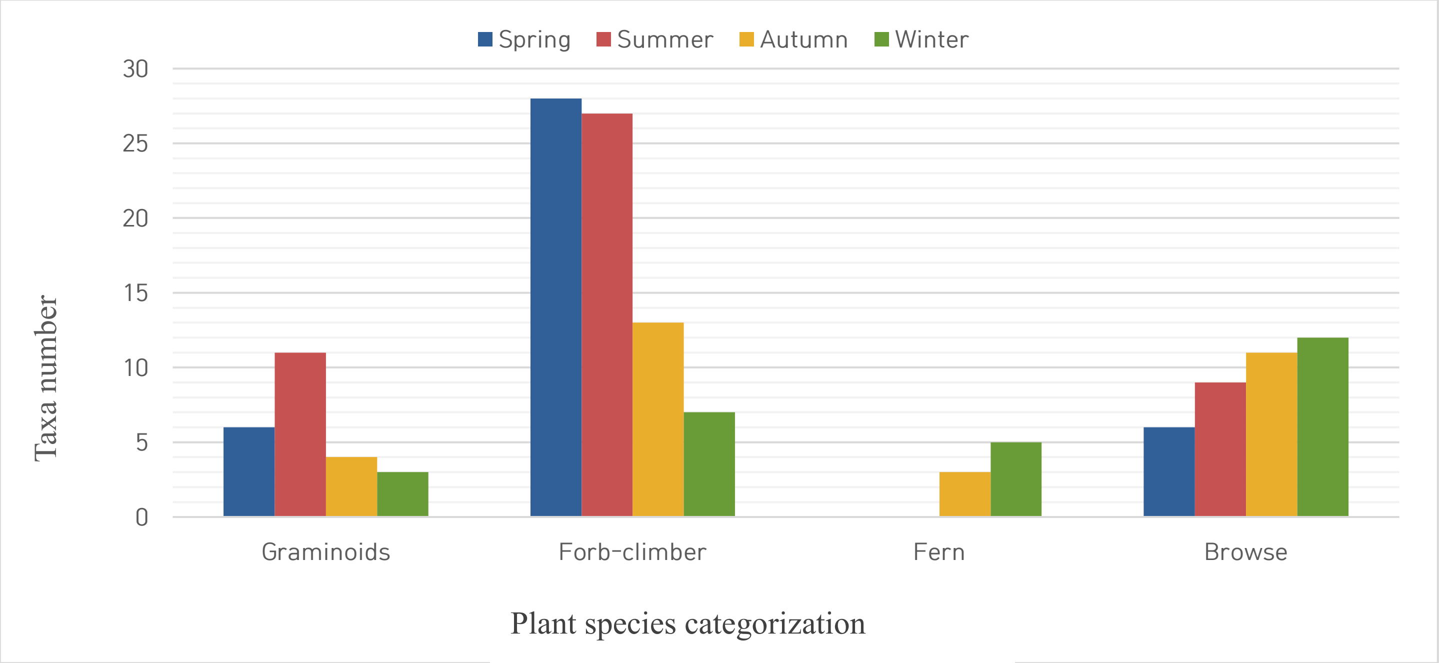

Fig. 18. Consumption of food categories in different seasons. Forbs-climbers were appears to consume more compared to other food categories.

## 3. Molecular study

### 3.1 Molecular study of alien Cervids using tissue samples

All collected tissue samples were successfully amplified and sequenced to evaluate their phylogenetic relationship. The analysis of all samples yielded 21 complete *CytB* sequences of 1140 bp. All the haplotype sequences found in this study were submitted to the GenBank database with the accession numbers MW169432 to MW169452 (Table 9).

The phylogenetic analysis was done for *CytB* to evaluate the phylogenetic relationships among the haplotypes found. In this analysis, the haplotypes of several sika deer subspecies (from Japan and continental Asia) and red deer obtained from the Gene Bank database were included with our own samples (Fig. 17). The resulting tree is divided into three major clades, which clearly show five distinct groups of haplotypes (Table 8). Among these groups, CyG1 includes five samples of *C. n. yakushimae,* representing the mitochondrial lineage of Japanese sika deer, whereas CyG2 includes four samples of *C. n. taiounaus* that corresponded almost perfectly to the Taiwanese lineage. Similarly, CyG3, CyG4, and CyG5 contain 12 samples of *C. elaphus* (Sichuan deer), which radiated from the Sichuan Province of China and the southeast. In the genetic distance index of the *CytB* gene sequence, CyG1 was the closest to AB218689 (*C. n. yakushimae*), CyG2 to DQ985076 (*C. n. taiouanus*) and CyG5 to KX449334 (*C. e. macneilli*) which displayed as 0.0000. The genetic distance index of CyG2 and CyG3, CyG3 and DQ985076 (*C. n. taiouanus*) was found to be the most distant relationship at 0.0504 (Table 10). The base composition in the sequences of sika deer was on average T (28.7%), C (27.4%), A (30.6%), and G (13.3%) in *CytB* (Appendix. 2). The maximum likelihood tree with 17 *CytB* haplotypes of deer (5 haplotypes from this study and 12 haplotypes from the GeneBank database) was shown in Fig. 17. All *CytB* gene sequences used in this study to analyse the *CytB* gene are shown in Table 9.

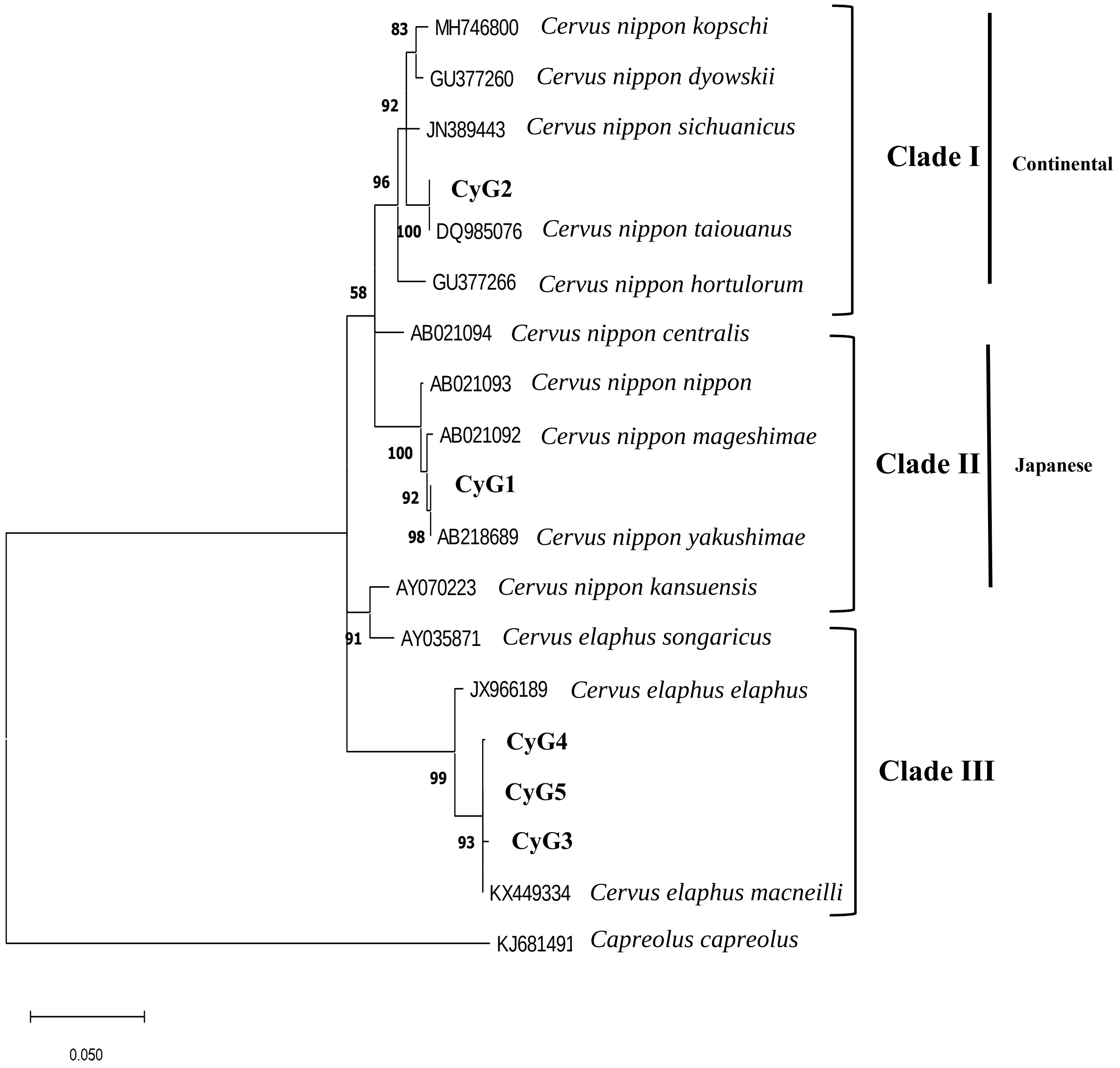

Fig. 19. Phylogenetic tree for the *CytB* haplotype of *C. nippon* and *C. elaphus*. The Maximum likelihood phylogenetic tree based on the mtDNA *CytB* gene sequences for two haplotypes of *C. nippon* and three haplotype of *C. elaphus* collected from Jeju Island and references sequences of various Cervids taken from NCBI database. Genetic distance was calculated using Tamura-Nei's model (Tamura and Nei, 1993). *CytB* sequence of 13 Cervid species and *Capreolus capreolus* were used as outgroups.

Table 8. Similarity search for *CytB* sequence of deer

| Groups | Specimen | Species | Accession no. | Identity (%) | Reference |
|---|---|---|---|---|---|
| CyG1 | SCY01, SCY09, SCY18, SCY20, SCY21 | *C. n. yakushimae* | AB218689 | 100 | Wada *et al*., 2005 |
| CyG2 | SCY06, SCY07, SCY13, SCY14 | *C. n. taiouanus* | EF139156 | 100 | Han *et al*., 2006 |
| CyG3 | SCY03-05, SCY08, SCY10, SCY12, SCY15-17 | *C. elaphus* | AB001612 | 100 | Matsunaga *et al*. 1997 |
| CyG4 | SCY02, SCY19 | *C. elaphus* | KM410147 | 100 | Krojerova *et al*., 2014 |
| CyG5 | SCY11 | *C. elaphus* | MN746793 | 100 | Koehler *et al*., 2019 |

Table 9. Accession number and information of the *CYTB* sequences for *C. nippon* and *C. elaphus* species used in this study.

| Species* | Origin Country | *CytB* | | |
|---|---|---|---|---|
| | | Specimen | Accession no. | Reference |
| *Cervus nippon yakushimae* | Korea | SCY01 | MW169432 | This study |
| *Cervus elaphus macneilli* | Korea | SCY02 | MW169433 | This study |
| *Cervus elaphus macneilli* | Korea | SCY03 | MW169434 | This study |
| *Cervus elaphus macneilli* | Korea | SCY04 | MW169435 | This study |
| *Cervus elaphus macneilli* | Korea | SCY05 | MW169436 | This study |
| *Cervus nippon taiouanus* | Korea | SCY06 | MW169437 | This study |
| *Cervus nippon taiouanus* | Korea | SCY07 | MW169438 | This study |
| *Cervus elaphus macneilli* | Korea | SCY08 | MW169439 | This study |
| *Cervus nippon yakushimae* | Korea | SCY09 | MW169440 | This study |
| *Cervus elaphus macneilli* | Korea | SCY10 | MW169441 | This study |
| *Cervus elaphus macneilli* | Korea | SCY11 | MW169442 | This study |
| *Cervus elaphus macneilli* | Korea | SCY12 | MW169443 | This study |
| *Cervus nippon taiouanus* | Korea | SCY13 | MW169444 | This study |
| *Cervus nippon taiouanus* | Korea | SCY14 | MW169445 | This study |
| *Cervus elaphus macneilli* | Korea | SCY15 | MW169446 | This study |
| *Cervus elaphus macneilli* | Korea | SCY16 | MW169447 | This study |
| *Cervus elaphus macneilli* | Korea | SCY17 | MW169448 | This study |
| *Cervus nippon yakushimae* | Korea | SCY18 | MW169449 | This study |
| *Cervus elaphus macneilli* | Korea | SCY19 | MW169450 | This study |
| *Cervus nippon yakushimae* | Korea | SCY20 | MW169451 | This study |
| *Cervus nippon yakushimae* | Korea | SCY21 | MW169452 | This study |
| *Cervus nippon taiouanus* | Taiwan | DQ985076 | DQ985076 | Chen *et al.*, 2006 |
| *Cervus nippon hortulorum* | Korea | GU377266 | GU377266 | Chung *et al.*, 2010 |
| *Cervus nippon dybowskii* | Korea | GU377260 | GU377260 | Chung *et al.*, 2010 |
| *Cervus nippon yakushimae* | Japan | AB218689 | AB218689 | Wada *et al.*, 2005 |
| *Cervus nippon mageshimae* | Japan | AB021092 | AB021092 | Kuwayama *et al.*, 2016 |
| *Cervus nippon kopschi* | China | MH746800 | MH746800 | Xu *et al.*, 2018 |
| *Cervus nippon sichuanicus* | China | JN389443 | JN389443 | Yang *et al.*, 2016 |
| *Cervus nippon nippon* | Japan | AB021093 | AB021093 | Kuwayama *et al.*, 1988 |
| *Cervus nippon centralis* | Japan | AB021094 | AB021094 | Kuwayama *et al.*, 1988 |
| *Cervus elaphus macneilli* | China | KX449334 | KX449334 | Qin *et al.*, 2016 |
| *Cervus elaphus kansuensis* | Germany | AY070223 | AY070223 | Ludt *et al.*, 2016 |
| *Cervus elaphus elaphus* | Russia | JX966189 | JX966189 | Kuznetsova, M.V. 2016 |
| *Cervus elaphus songaricus* | China | AY035871 | AY035871 | Ludt *et al.*, 2001 |
| *Capreolus capreolus* | Korea | KJ681491 | KJ681491 | Hassanin *et al.*, 2016 |

*Species name of the specimen tested in this study was determined from the BLAST search results of *CytB* gene sequences.

Table10. Pairwise genetic distance between the haplotypes of *C. nippon* and *C. elaphus*.

| Haplotype | CyG1 | CyG2 | CyG3 | CyG4 | CyG5 | Cny | Cnm | Cnn | Cnk | Cnc | Cnd | Cnt | Cns | Cem | Cek | Ces | Cee |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CyG1 | | | | | | | | | | | | | | | | | |
| CyG2 | 0.0413 | | | | | | | | | | | | | | | | |
| CyG3 | 0.0647 | 0.0789 | | | | | | | | | | | | | | | |
| CyG4 | 0.0624 | 0.0740 | 0.0036 | | | | | | | | | | | | | | |
| CyG5 | 0.0634 | 0.0751 | 0.0027 | 0.0009 | | | | | | | | | | | | | |
| Cny | **0.0000** | 0.0413 | 0.0647 | 0.0624 | 0.0634 | | | | | | | | | | | | |
| Cnm | 0.0036 | 0.0424 | 0.0634 | 0.0587 | 0.0598 | 0.0044 | | | | | | | | | | | |
| Cnn | 0.0046 | 0.0380 | 0.0657 | 0.0610 | 0.0621 | 0.0053 | 0.0062 | | | | | | | | | | |
| Cnk | 0.0405 | 0.0178 | 0.0756 | 0.0707 | 0.0718 | 0.0425 | 0.0424 | 0.0381 | | | | | | | | | |
| Cnc | 0.0344 | 0.0342 | 0.0688 | 0.0640 | 0.0650 | 0.0355 | 0.0313 | 0.0312 | 0.0335 | | | | | | | | |
| Cnd | 0.0405 | 0.0151 | 0.0704 | 0.0653 | 0.0664 | 0.0405 | 0.0416 | 0.0368 | 0.0079 | 0.0307 | | | | | | | |
| Cnh | 0.0393 | 0.0247 | 0.0767 | 0.0718 | 0.0729 | 0.0403 | 0.0381 | 0.0360 | 0.0241 | 0.0291 | 0.0225 | | | | | | |
| Cnt | 0.0413 | **0.0000** | 0.0789 | 0.0740 | 0.0751 | 0.0413 | 0.0424 | 0.0380 | 0.0178 | 0.0342 | 0.0151 | 0.0247 | | | | | |
| Cns | 0.0416 | 0.0149 | 0.0718 | 0.0670 | 0.0680 | 0.0416 | 0.0427 | 0.0382 | 0.0140 | 0.0303 | 0.0100 | 0.0208 | 0.0149 | | | | |
| Cem | 0.0634 | 0.0751 | 0.0027 | 0.0009 | **0.0000** | 0.0634 | 0.0598 | 0.0621 | 0.0718 | 0.0650 | 0.0664 | 0.0729 | 0.0751 | 0.0680 | | | |
| Cek | 0.0410 | 0.0431 | 0.0610 | 0.0564 | 0.0574 | 0.0419 | 0.0440 | 0.0419 | 0.0429 | 0.0378 | 0.0353 | 0.0450 | 0.0431 | 0.0357 | 0.0574 | | |
| Ces | 0.0445 | 0.0465 | 0.0635 | 0.0588 | 0.0599 | 0.0454 | 0.0476 | 0.0453 | 0.0453 | 0.0404 | 0.0378 | 0.0412 | 0.0465 | 0.0401 | 0.0599 | 0.0173 | |
| Cee | 0.0649 | 0.0744 | 0.0177 | 0.0158 | 0.0149 | 0.0676 | 0.0628 | 0.0650 | 0.0727 | 0.0599 | 0.0657 | 0.0723 | 0.0744 | 0.0649 | 0.0149 | 0.0572 | 0.0576 |

Note: CyG = Cytochrome B Group, Cny, *C. n. yakushimae*; Cnm, *C. n. mageshimae*; Cnn, *C. n. nippon*; Cnk, *C .n .kopschi*; Cnc, *C. n. centralis*; Cnd, *C. n. dybowski*; Cnh, *C. n. hortulorum*; Cnt, *C. n. taiouanus*; Cns, *C. n. sichuanicus*; Cem, *C. e. macneilli*; Cek, *C. e. kansuensis*; Ces, *C. e. songaricus*; Cee, *C. e. elaphus.*

# IV. DISCUSSION

## 1. Distribution

This study provided an analysis of the distribution range of sika deer on Jeju Island. Sika deer in Jeju have expanded considerably over the 25-year span from 1992 to 2020 (Fig. 3). The parts of the recent expansion have resulted from frequent releases in 1992 (2 males and 4 females); 1993 (1 male and 4 females); 1994 (1 male and 1 female) and several illegal releases and escapes from a farm (Oh, 2006). Between 1993 and 2004, only 16 deer individuals and some signs of deer presence were reported in the wild.

Sika deer were initially released at the north-facing slope of Hallasan National Park on Jeju Island (Oh, 2019), the same area where several individuals were observed in 2006 (Fig. 4A). A survey 13 to 14 years after their introduction showed that sika deer have greatly increased their range towards the eastern and western sides of the national park (this study). This range expansion could be the result of several factors, as: i) the species was originally released into unsuitable habitat; ii) interspecific competition with native or high-density populations, in which one species may be forced to occupy suboptimal habitats, as similar to Iberian Ibex (Acevedo *et al*., 2010), and iii) it could be the result of human-induced factors. Originally, sika deer were released at Gwaneumsa and its peripheral areas, which are covered with deciduous broadleaf forests and pastureland areas. These habitat types support sika deer during the hot summer months, but are less likely to support them during the winter months, as available food resources are less abundant and covered by deep snow. Temperate evergreen forests at the eastern and western parts of the national park during winter support ungulates by providing sufficient food and good cover. The habitat types with adequate forage and good canopy cover are highly preferred by ungulate species in winter (Hundertmark *et al*., 1990), as the canopy limits the snow deposition and limits energy costs. This might have caused sika deer to expand their range towards the eastern and western parts of Hallasan National Park.

In addition, the presence of water sources and climatic conditions can also cause range expansion. Wildlife generally requires a good supply of freshwater around their habitats. Well known inland wetlands on Jeju Island are located at the eastern (i.e. Mulchat oreum, Muljangori-oreum wetland, and Mulyeongari-oreum wetland) and the western parts (i.e. 1,100m wetland and Sumeunmulbaengdui wetland) of the national park. The availability of water encourages sika deer to concentrate at these wetland sites (eastern and western sides) to quench their thirst. In particular, ungulates prefer areas with wetlands and streams (Zhang *et al.,* 2006). In Japan, sika deer are reported to concentrate more in areas where there is the presence of wetland sites (Takafumi, 2017). Furthermore, the climatic condition at their first released site may have influenced sika's behavioral or activity pattern. The strong wind and less sunlight in the north have made it hard for sika deer to stay at their original released site, hence they have radiated towards the east and west. Several studies have found that alien mammals (Winter *et al.,* 2010) and bird species (Dyer *et al.,* 2017) are limited by climatic conditions. Korean water deer (*Hydropotes inermis argyropus*) have also been observed resting or keeping warm on a south-facing slope during the day (Kim *et al.,* 2010). Similarly, other deer species, such as white-tailed deer in Canada (Wetzel, 2012) and moose in Minnesota (Gao, 2013), showed their avoidance of north-facing slopes.

Following the result of 2004, sika deer were found to be isolated in one specific area, and later the growing population forced them to disperse into their new habitat.

Although the population size of sika deer on Jeju Island remains unknown, the population is on the rise. The sighting of hinds with very young spotted fawns indicates that the habitat is favourable for the species. The habitat characteristics and lack of natural and anthropogenic barriers suggest that the increasing population will expand their range and the impact upon biodiversity will continue unless there are sufficient management efforts to maintain density at a level that limits negative impacts. Of particular concern is the potential negative impact on endemic and floristic plant species, such as *Carpinus laxiflora, Abis koreana*, *Lygodium japonicum (Kim et al., 2020).*

The sika deer distribution range identified in this study can be applied to the conservation and management of sika deer on Jeju Island. For conservation efforts to be viable, conservation agencies must consider not only the current presence of sika deer, but also their future dispersal. The formation of these population groups in ecosystems beyond their biological range and dispersion capacity relates them to non-native invasive species (Falk-Petersen *et al*., 2006), which may cause serious ecological problems in the host environment, and should be continuously monitored, if not eradicated (Moriarty, 2004). Similarly, significant parasite abundances might be predicted when high population densities are attained, along with restricted food supply, leading to a loss of fitness and increased aggregations (Gortázar *et al*., 2006, Acevedo *et al*., 2010).

If distribution range increases towards the southern part of the national park, the likely impact on agricultural land natural forest ecosystems, as well as deer-vehicle collisions, cannot be negated. The likely effects of sika deer on native sympatric species and bird communities are also of concern. Hence, frequent monitoring of their distribution range is highly important and steps should be taken to manage deer within suitable core areas.

## 2. Activity pattern

This intensive camera trap survey provides detailed activity patterns of multiple sympatric mammals and identified their seasonal and diel activity patterns. Surprisingly, to date, no study on the seasonal and diel activity behavior of sika deer with sympatric species has been conducted in South Korea, neither any camera-trap-based species inventory on Jeju Island. Animal trails were good candidates for this type of camera-traps survey, and the infrared camera becomes able to capture the majority of species therein and missed in capturing small animals like squirrel, nutria, and chipmunk.

### 1) Seasonal activity pattern of sika deer

Seasonal and diel activity pattern of species are determined both by external factor of environment, and endogenous factors themselves, physiological state. Both biotic (predator, human activity, etc.) and abiotic (light, temperature, weather, precipitation, etc.) factors of the external environment highly influence the activity pattern of species. When measured over 24 hr., sika deer were active all day, with peaks at dawn and dusk and troughs in the middle of the day and the middle of the night. This study is consistent with studies conducted on white-tailed deer *Odocoileus virginianus* (Paul and Mccullough, 1990), elk *C. canadensis* (Green and Bear, 1990), and moose *Alces alces gigas* (Gillingham and Klein, 1992). The seasonal activity profile shows two different activity peaks at dawn and dusk, which are driven by the 2-3 hr of twilight period at dawn and dusk rather than the circadian timing mechanism.

Similar reports for red deer and elk (Ensing *et al.*, 2014), reindeer (Stokkan *et al.*, 2007; Oort *et al.*, 2007) as well as horse (Murphy *et al.*, 2011), physiological and behavioral data suggest ungulates may generally have weak circadian organization and follow 2-3 hr. of twilight time for their activity peak at dawn and dusk.

The climatic condition can also affect animal activity patterns. Sika deer activity in warmer months peaks earlier and their evening activity later, because of their lower level of heat tolerance. Bourgoin *et al.* (2011) and Brivio *et al.* (2019) reported that most ungulates during hot summer months reduced their daytime activity. In Jeju Island, during cold seasons sika deer were less detected, and their activity shift towards day and night hours. Because of low temperature and heavy snowfall towards morning and evening, sika deer shift their activity towards mid-day and night, the warmest part of the day. During extreme winter, ungulates are subjected to nocturnal hypometabolism to conserve energy (Signer *et al.*, 2011; Turbill *et al.*, 2011), indicating that this phenomenon might have resulted in the low activity levels of sika deer just before sunrise (dawn) and just after sunset (dusk) in this study.

### 2) Diel and temporal overlap of sika deer in summer and winter seasons

Activity overlap when compared with sympatric species, roe deer shows a high degree of overlap in summer and in winter, as roe has a higher abundance index (25.84). The activity of both species peaks at dawn and dusk in summer and day and night time in winter. Roe deer is a native species to Jeju Island whose activity is closely synchronized to that with sika deer. The study on the dietary items of both species refers that they exhibit highly similar resource demands in diets and habitat use in this survey area, which may intensify competition under certain circumstances such as resource shortage and/or deficiency. The inter-species competition between morphologically different species (larger sika deer and smaller roe deer) is expected to displace native roe deer from formerly occupied sites, which eventually affected their activity patterns. Similarly, many sympatric native species are displaced from their core habitats (Desbiez *et al.*, 2009) and even exhibit differences in activity patterns (Ilse *et al.*, 1995; Focardi *et al.*, 2006; Galetti *et al.*, 2015) in response to exotic or feral species. Although less detected, red deer activity in summer peaks at twilight hours as similar to most European red deer (Carranza *et al.*, 1991). The daily activity of ungulates is typical (Turner, 1979; Berger *et al.*, 2002), and consists of feeding phases alternating with resting phases (for chewing and rumination). Thus, red deer activity in Jeju Island likely to have been influenced by feeding and ruminating bout and some other external factors. During winter, because of harsh climatic condition towards morning and evening, red deer shifted their activity activity towards mid-day and night, the warmest part of the day as similar to sika deer in this study.

The wild boar usually exhibits cathemeral behaviour whose activity peak more towards mid-day and at night hours in both the seasons. Wild boars in Australia (Caley, 1997) and southern Texas (Campbell and Long, 2010) show nocturnal activity whereas, in Tennessee and Texas their behaviour is predominantly crepuscular (Ilse *et al.*, 1995). The plasticity of the activity has been noted because this species is primarily nocturnal, but may be able to shift its activity to the day when that is advantageous. Contrary to wild boar behaviour, sika deer do not show individual flexibility except that in winter because of cold weather. This difference in activity pattern between sika deer and wild boar most likely arises from the difference in the digestive systems of ruminants and monogastrics.

In both the seasons badger showed crepuscular-nocturnal activity similar to the activity of most European badger (Goszczyński *et al.*, 2005; Rosalino *et al.*, 2005).

Interestingly, activity begins at dusk with the peak at night and the day passivity begins just after dawn. During this study, Jeju weasel - the smallest mammals photographed – was only observed during summer months but not in winter season. The summer activity pattern of Jeju weasel was mostly diurnal and reduce their activity towards night hours. A study on radio - collared weasels from the National Park of Poland (Jedrzejewski *et al.*, 2000), and the man - made coniferous forest of the UK (Brandt and Lambin, 2005), showed higher activity during the day, reduced at dark, and were completely inactive during night hours. Asian badger and Jeju weasel are the two sympatric mustelids that exhibit great dietary overlap (Seki *et al.*, 2014; Law, 2018), suggesting little interspecific competition for food. Despite the similar diets (i.e., earthworms, insects, rats), badger exhibit exclusively nocturnal activity patterns, whereas weasel is almost exclusively diurnal, suggesting that these two mustelids limit completion by avoiding each other. Although there was no clear evidence to prove the relationship between sika deer and these small carnivores, the increased sika deer population modify the understory vegetation and soil composition (through dung deposition) which probably increase the number of earthworms and insects (Seki *et al.*, 2014), which also increase the population of badger and weasel.

### 3) Effect of human interference on sika deer in summer and winter seasons

In most animal studies, activity overlap is primarily related to the predator-prey relationship (Linkie and Ridout, 2011; Foster *et al.*, 2013; Noor *et al.*, 2017) but the overlap pattern of sika deer in this study area is limited by secondary factors (i.e., human activity and available resources), due to the absence of potential predator. Human activity during daylight hours in both seasons, may lead sika deer to shift its activity towards dawn and dusk. The active presence of humans switched sika deer into a more alert behavioural mode, i.e., increased vigilance, which frequently interrupted foraging bouts, decreased feeding time, and increased traveling time. Continuous disturbance of behavioral modes during the day led to more of an activity shift. Several studies in sika deer (Takahashi *et al.*, 2012), red deer (Jayakody *et al.,* 2008; Boyce *et al.,* 2010; Ciuti *et al.,* 2012), roe deer (Krop-Benesch *et al.,* 2013; Pagon *et al.,* 2013) have shown changes of their behaviour to avoid human disturbances. Lethal human activities (e.g. hunting) are strictly prohibited but some legal hunters are allowed to hunt if the wildlife conflict or exceed the carrying capacity. These legal hunters take dogs, which become a threat to wildlife. The compounding effect of dogs on the disturbance of wildlife has been found on mammals (Miller *et al*., 2001).

The introduction of alien species disrupts the ecosystem by negatively affecting native species through competition and predation (Long, 2003; Blackwell, 2005). In addition to this, invasive alien species are also a threat to crop and pastureland and through the transmission of zoonosis (Pejchar and Mooney, 2009). According to IUCN (2000), preventing the introduction of potentially harmful organisms is the first and only way to limit the effects of biological invasion. However, sika deer in this region is already introduced but is still in the earlier stage of their invasion process. Until recently no significant impacts were reported, as sika deer were observed residing in only a few locations. No doubt, the increasing population will rapidly expand to other locations and can cause damage to agricultural or/and forestry habitats similar to the result of sika deer in Japan (Takatsuki, 2009; Nagaike, 2012; Ohashi *et al.*, 2014).

Although our study was limited by the relatively low number of camera traps and survey sites, but will shed light on diel and seasonal activity patterns of sika deer. Management of invasive sika deer can be well planned when the activity pattern of this species is well understood. Therefore, further studies are needed to investigate the exact diel and seasonal variation on activity pattern in addition to activity overlap with sympatric species.

## 2. Diet composition

Assessment of dietary choices of a species is critical to understand its foraging plasticity and inform subsequent habitat and forage management measures. The dietary study of sika deer identified 95 plant taxa, which is comparatively lower than the diet of native roe deer on Jeju Island (Kim, 2000; Oh *et al*., 2015; Adhikari *et al*., 2016). Lower plant taxa in the diet of sika deer could be aligned with the lower availability of plant species at their habitat range. It is well known that the sika deer on Jeju Island were observed only at an altitude range of 550-1100m, which may contain a low diversity of plant species. In contrast to this study, roe deer were investigated in a variety of habitats and seasons (shrub lands, forests of various altitudes, agricultural lands, semi urban regions and pasturelands). Climatic variation is caused by changes in height and latitude, which influences the richness, evenness, and variety of plant species (Willig et al 2003). Other environmental elements, such as geography, climate, and quality of soil also have a role in causing a large difference in plant species.

Sika deer are known to be intermediate eaters, with seasonal food preferences that fall into both the browsing and grazing categories, and they are extremely dynamic in their use of resources (Kobayashi and Takatsuki, 2012). Diet was seasonally assessed in order to determine changes in dietary patterns between seasons and to determine preferences for particular plant species, relative to their availability, at different times of the year (Seto *et al.,* 2015). On Jeju Island, sika deer were found to consume various plant types (graminoids, forb-climbers, ferns and browse), but concentrate mainly on forb-climbers when the resource is plentiful. The combination of a small stomach and high metabolic demands of the species requires sika deer to choose high quality plants. Newly grown and highly nutritious forb-climbers and graminoids in spring and summer support these findings. Dietary preferences for these food categories were reported for sika deer in Japan (Yokoyama *et al.,* 2000) and New Zealand (Husheer and Robertson, 2005; Ramsey *et al.,* 2012). In autumn, herbaceous plants gradually reduced, and sika deer were found to feed more on twigs and leaves of deciduous trees together with forbs and graminoids, as reported in the diet of roe deer on Jeju Island (Adhikari *et al.,* 2016). This result shows that dicots had a higher percentage of occurring in sika deer diet compared to monocot and other plant species. Variation in the consumption of different plant types by herbivores have been related to changes in the amount of chemical compositions of food plants (Sukumar, 1989). In general, diet selection by ungulates depends upon the amount of protein and fiber content (Klaus-Hugi *et al.*, 1999). In dry seasons, the abundance of herbaceous plants reduces, and graminoids, if available, also lose their palatability and nutritive quality in comparison to wet seasons (Sukumar, 1989; Baskaran, 1998). This could probably explain why monocots are less preferred over dicots. Higher preferences of dicotyledonous plants over monocotyledonous were reported for native roe deer (Oh *et al*., 2015; Adhikari *et al.*, 2016).

The volume of browse, ferns and *Sasa quelpaertensis* (evergreen - dwarf bamboo) increases significantly in winter when the availability of previously abundant food sources (forb-climbers and graminoids) decreases. Because of heavy snow and low humidity in winter, the areal parts of herbaceous plants and graminoids usually die, and therefore sika deer are constrained to eat readily available browse, bark and *S. quelpaertensis* (Yokoyama *et al.,* 2000). Nagaike (2020) reported that bark stripping by sika deer increased under conditions of high snow accumulation. Higher consumption of woody plants was observed for other deer species, such as roe deer (Nevřelová & Ružičková, 2015) and red deer (Zhong *et al.,* 2020) during winter, because snow cover prevented the deer access to understory vegetation (Heggberget *et al.,* 2002; Seto *et al.,* 2015).

In the spring, summer, and autumn, dietary items were found to be sufficient for sika deer, but not in the winter. Despite a lack of natural food resources during winter, sika deer likely have less impact upon agriculture compared to sympatric roe deer (Jeong et al., 2001; Yoon, 2003). The difference in behavioral patterns between these two sympatric ungulate species might depend upon the altitude variations of their habitat ranges. A study on distribution patterns of sika deer on Jeju Island (this study) identifies that sika deer were only observed at altitudes ranging from 550-1,100m, i.e., a range that contains few cultivated lands. On the contrary, roe deer were commonly observed at a low altitude (Kim, 2017) where most of the cultivated lands are located. Hence it is reasonable to expect that during the resource-deficient period, cultivated lands are more probable to be raided by roe deer than by sika deer.

### 4. Molecular study of Cervids using tissue samples

Mitochondrial genes are a powerful molecular method for inferring phylogenies (Kern *et al*., 2020). Molecular phylogenetic analysis was used to provide accurate classification of alien deer species, as well as their native geographical origin. This study represents the first population genetics study of alien sika deer using *CytB* on Jeju Island.

The phylogenetic analysis results showed that the alien deer living on Jeju Island are of the genus Cervus, but are of two distinct species, i.e., *C. nippon* and *C. elaphus,* affirming that they are derived from different maternal lineages. Through the analysis of the branching pattern of the maximum likelihood tree, no sequence mutation seems to have occured. Analysis revealed that there are two distinct lineages of sika deer (Clade I and Clade II), with one of these lineage (Clade I) clusters aligning with a continental sika deer subspecies, whereas the other (Clade II) with a Japanese sika deer subspecies. Among five distinct groups of haplotypes, CyG1 shows similarity in gene sequence with the Japanese subspecies, whereas CyG2 with the subspecies of continental Asia (Fig. 19). CyG1 was identified as having the closest relation with gene sequences of AB218689 (*C. n. yakushimae*). Therefore, it suggests that a portion of the sika deer living on Jeju Island was introduced from the Yakushima region of Southern Japan. The *C. n. yakushimae* is the smallest subspecies of Japanese deer, and is endemic to Yakushima and Kuchinoerabu-jima Islands (Kuroiwa *et al.,* 2017). The population of *C. n. yakushimae* in Japan has increased since the 1970s (Takatsuki, 2009), which had reached a density of 43-78 head/km$^2$ until 2001 (Agetsuma *et al*., 2011), which has caused serious damage to agricultural and forest lands as a consequence of grazing and browsing pressure (Koda *et al.,* 2008; Onoda and Yahara, 2015). To mitigate further damage, the expanding population was culled and/or translocated to other countries (Kyushu Regional Forest Office, 2014), as was the case of the sika deer introduction in South Korea.

Furthermore, sequences from CyG2 show their close relationship with the gene sequence of DQ985076 (*C. n. taiouanus*). This is the gene sequence of Formosan sika deer reported in Taiwan. Therefore, it is highly probable that the species living on

Jeju Island were introduced from Taiwan. The EF139156 and EF058308 (*C. n. taiouanus*) sequences, which showed 100% concordance in the similarity search results of the *CytB* sequence (Table 9), are the *CytB* gene sequence of Formosan sika deer living in Mt. Songnisan National Park, South Korea. It can be said that the group had been introduced to Jeju Island shortly after having been introduced into mainland Korea, not having inhabited the area long enough to have gone through the evolutionary process.

The genetic analysis based on mitochondrial DNA showed that the species observed in clade III were identified as *C. elaphus,* and were closely related to the Sichuan deer (*C. e. macneilli*). These three groups (CyG3, CyG4 and CyG5) were not divided into monolines due to the relatively short genetic distance between them. The Sichuan deer is a subspecies of red deer only distributed in the western Sichuan Province of China and south-eastern Tibet (Ma *et al.,* 1998). *C. elaphus* living on Jeju Island are highly likely to have been introduced from China. Although some polymorphisms of red deer individuals were confirmed in the *CytB* gene sequences analysed in this study, they do not show a branching pattern that is distinct from the previously reported sequence. Therefore, it is predicted that the red deer have not inhabited the area for a long enough time to have gone through the evolutionary process, but were instead recently introduced from China.

The Dybowski's sika deer (*C. n. hortulorum*) is considered the largest native subspecies of sika deer in South Korea (McCullough, 2009), but this study could not find this species. Similarly, *C. n. hortulorum* was not reported in the deer farms of mainland Korea or Jeju Island (Koh *et al.*, 2010). Due to poaching and overhunting, this species seems to be either extinct from the wild, or there is a lack of any relevant information (Harris, 2008).

Sika deer and red deer are closely related species, and hybrids are known to be formed in the regions where these two species were introduced (Goodman *et al.,* 1999; Senn *et al.,* 2009). No change in nucleotide sequences was observed in this study, but the possibility of hybridization between related species in the future cannot be completely excluded. Sika deer are experiencing serious genetic pollution in many groups in Japan (Ohmura *et al.,* 1983; Tamate, 2009), and hybrids with red deer have been reported in the United States (Mungall and Sheffield, 2000).

Jeju Island, as a "Biosphere Reserve", contains unique and diverse species. Due to the limited and narrow land area, when faced with sudden environmental changes, a group of organisms vulnerable to external threats will suffer great damage, such as destruction or extinction (Jeong *et al*., 2015). When alien species are introduced into the natural ecosystem, competition for food and habitat with indigenous species occurs frequently, causing habitat destruction and biodiversity reduction. Over the past 100 years, island biodiversity has been rapidly changing due to invasive species, habitat change, excessive land use, climate change and pollution. Overall, the most representative cause of the extinction of island organisms is the influx of alien species (Russell and Kueffer, 2019).

Hwang *et al.* (2014), in their study, identified the presence of two alien sika deer subspecies in mainland South Korea. However, on Jeju Island, there have not been authentic reports of the presence of alien ungulate species before this study. Two sika deer subspecies and red deer were confirmed through this study. Although not confirmed through molecular study, elk were photographed in camera traps. Hence, a molecular study using large sample sizes is recommended for species identification. Molecular genetic methods could provide information on the exact species identification and inflow paths of deer living in Hallasan National Park, which could provide a sustainable management plan.

# CONCLUSION AND FUTURE IMPLICATIONS

The integrated knowledge of the ecology and genetics of alien species is important for predicting invasion success and determine the influences of introduction, range expansion. This study has investigated the history of invasive species and examined their genetics to determine their evolution range. Sika deer in Jeju Island was initially released to the northern part of Hallsan National Park but have expanded their range more towards the eastern and western part. The present distribution of sika deer showed their preferences to altitude ranging from 550m-1,100m. A study on activity patterns revealed that sika deer exhibits a bimodal activity pattern towards dawn and dusk except during winter seasons. When considering activity overlap with other sympatric species, the activity of sika deer overlaps with that of roe deer more than other sympatric species. Sika deer are dynamic in the use of resources. Altogether 95 plant taxa of four categories (graminoids, forb-climber, fern and browse) were identified using morphological and molecular analysis. A higher number of dietary plants was found in summer (47 taxa) and lower in winter (27 taxa). Some endemic and threatened taxa are included in the diet. Graminoids and forb-climber primarily made up the spring and summer diets whereas browsing and fern as autumn and winter diets. The molecular technique was used for species identification. This study identified five distinct groups of haplotypes from the analysis of 21 samples. Among five haplotypes, two haplotypes are of sika deer (*C. n. yakushimae* and *C. n. taiouanus*), and three is of red deer (*C. elaphus).* Phylogenetic analysis through Maximum likelihood showed that the sika deer subspecies has been introduced from Japan and Taiwan whereas red deer form China.

Knowledge of whether an alien species poses a risk to the host environment and native species based on scientific data and proper identification of the results and discussion is critical to its management. This investigation sheds light on the ecology and phylogenetic relationship of alien sika deer in Jeju sland. Management innervations can be well planned when the distribution and activity patterns of target species are well understood. In case, if population control measures are planned, the use of this knowledge can help target the place/spot and time of day for implementation of the actions.

Deer population management is a difficult task. It is necessary to have a thorough understanding of the deer population and their distribution range in order to manage them effectively. Knowledge of deer diet and activity patterns is required for predictive management of deer populations and their influence on biodiversity (Mayle 1996). The first step in dealing with the problem of invasive species is to control the import of new possible invaders and to prevent the escape of species. The control of invasion is only possible when species are at their earlier stage of establishment or their low density (IUCN, 2000). However, sika deer in this region are already introduced but are still in the earlier stage of their invasion process as they were observed in some isolated patches within Jeju Island. The expanding population can cause substantial damage to agriculture, forestry, and the natural forest ecosystem, and steps should be taken to manage deer (Nagaike, 2012). Additionally, the alien sika deer can threaten the genetic characteristics of native roe deer as has happened with sika deer in Britain (Ratcliffe, 1989). Given the current situation, we believed the most urgent action regarding invasive sika deer is the control of population rather than eradication efforts. Similarly, it can be predicted that in near future the impact of sika deer would increase in Jeju Island causing a significant effect on the natural ecosystem and biodiversity.

Method of control available includes continuous monitoring, surveillance, and control methods. For effective control, the following management methods need to be implemented (Butchko *et al*., 3003; Lowney *et al*., 2005). The cost and the effectiveness vary considerably among the methods.

1. Monitoring and surveillance are the important components for effective management of introduced population. It aids in the collection of baseline population data and the identification of habitat used by target species. It will be easier to monitor and classify their effects once the habitat location is determined.
2. Trapping is the most effective way to control wild or alien ungulates. Depending on herd size, single-animal and multi-capture traps can be used. Traps need to be set in narrow passage to be effective. The use of radio-transmitter on remote traps can help to determine from a distance, that a trap has been triggered. Nets such as rocket nets and drop nets are effective in capturing deer and other ungulates (Schemnitz, 1994). Usually ungulates can be effectively baited with food containing high-energy during winter months when natural food are scarce.
3. Ground-based shooting is an important method of managing wild or alien ungulate populations (McCann *et al*., 2004) that can control population growth. Trained technicians can easily and economically kill a large number of ungulates. Shooting from the field has proven to be the most effective method of ungulate eradication. When ungulates are in low densities, shooting them with dogs is more successful.

4. For large animals, shooting from the air is accurate. Deer or other ungulates can be removed with the aid of helicopter shooting (Butchko *et al*., 2003). This technique may not be effective in dense forest habitat of Jeju Island, but can be effective at large pastureland areas.

5. Population management necessitates the use of fencing. After locating the habitat range, the area can be fenced. Fencing may be used to partition the land into small parcels to facilitate eradication and to keep animals from returning to an area once it is cleared (Butchko *et al*., 2003).
6. There have been technical advancements in ungulate management and eradication equipment. Night vision goggles, forward-looking infrared (FLIR) thermal imagers, and suppressed rifles are examples of technical advancements that help ungulate removal programs work effectively. In a field test in Pennsylvania, FLIR found up to 70% more deer in thick vegetation.

For control approaches to be successful, a number of issue must be addressed. Challenges include adequate monitoring of ungulate populations to measure progress, maintaining the support of decision-makers who approve funding, etc. This study recommend the census work using appropriate methodology. The density of deer can only be achieved by annual or bi-annual census work. With the integration of all information the successful decision on the management and control of all deer population can be reasonably made.

This study sheds light on the distribution range and activity pattern of sika deer in Jeju Island.. In general camera traps do not provide detailed activity patterns of wildlife species but can only reflect the activity of animal movements within camera location, hence the use of a radio collar is required for identifying the exact activity pattern of elusive and alien species.

As shown in this study, the introduced species and native sympatric ungulates sharing functional traits such as activity pattern, diet selection, which may lead to competition due to similarity in habitat and foraging locations. Throughout the year, the native roe deer tends to congregate with alien sika deer. All of this may lead to native populations being excluded (because of small size) from preferred resource usage, particularly during summer drought. It may be appropriate to take measures such as cultivating part of pastures as supplementary grazing to meet dietary requirements and keep animals in good condition during drought conditions. The information acquired from this study objectives could assist the conservation and management plan for the alien sika deer population in Jeju Island, South Korea.

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# 적 요

이 연구는 2018년 10월부터 2020년 12월까지 제주도에 이입된 외래사슴류 종을 분자유전학적 방법으로 정확하게 동정하고, 분포 현황, 활동 패턴, 먹이원 등을 밝히기 위하여 이루어졌다. 연구결과, 제주도에 서식하는 사슴류는 한라산국립공원의 동·서부로 분포범위가 확대된 것으로 확인되었고, 남쪽으로의 확장은 관찰되지 않았다. 이러한 분포범위의 확대는 물, 먹이, 기후 조건의 가용성에 따른 결과로 보여진다. 꽃사슴은 550~1100 m의 고도를 선호하는 것으로 관찰되었는데, 이는 노루와의 경쟁을 피하기 위한 수단으로 판단된다. 제주도 내 사슴 개체수의 증가는 수입된 개체들의 불법 방사 또는 사육장 탈출이 원인이 되며, 현재 제주도에는 대형 포식자가 없기에 분포범위가 더욱 확장될 가능성이 있어 개체수 조절을 위한 포획은 이루어져야 할 것이다. 카메라 트랩을 이용한 꽃사슴의 일일 활동 패턴을 분석한 결과, 새벽과 해질녘에 빈번하게 활동하며, 계절별로는 봄, 여름, 가을에는 새벽과 해질녘에, 겨울에는 정오 무렵과 자정 무렵에 활발한 활동을 하는 것으로 나타났는데, 이러한 결과는 계절별 기후와 관련이 있는 것으로 판단된다. 꽃사슴은 노루와 활동시간대가 가장 비슷하였고, 그 다음으로 붉은사슴, 멧돼지, 오소리, 제주족제비, 새, 인간, 개 등의 순으로 나타났다. 노루와 꽃사슴의 유사한 활동시간은 두 종의 식이 선호도가 유사하다는 것을 보여주는 것이다. 형태학적으로 다른 종들 사이의 활동시간 중복은 종간경쟁을 유발하게 되어 경쟁에 약한 종들의 도태가 나타날 수 있어 이를 막기 위한 관리가 필요할 것이다.

꽃사슴은 이용 가능한 먹이 자원에 따라 먹이 선호도를 쉽게 바꿀 수 있다.

꽃사슴의 먹이원을 분석한 결과, 화본식물, 초본-덩굴식물, 양치식물, 목본식물 중 95분류군의 식물을 섭이하는 것으로 나타났다. 이 중 벼과를 가장 선호하며, 겨울철(27 분류군)보다는 여름철(47 분류군)에 더 다양한 식물을 섭이하는 것으로 나타났다. 봄과 여름에는 주로 화본식물과 초본-덩굴식물을, 가을과 겨울에는 양치식물과 목본식물을 주 먹이원으로 이용하는 것으로 나타났는데, 이러한 결과는 꽃사슴이 영양분이 많고, 섬유질이 적은 먹이를 선호하며, 계절에 따른 식이 선호도의 변화를 보여주는 것이다.

제주도 사슴류 21개체의 mtDNA *CytB* 유전자 서열을 분석한 결과, 꽃사슴(*C. nippon*)은 2가지의 haplotype으로 구분되었고, 붉은사슴(*C. elaphus*)은 3가지 haplotype으로 구분되어 총 5가지 haplotype이 있는 것이 확인되었다. 계통분지도 상에서 꽃사슴은 야쿠시마꽃사슴 (*C. n. yakushimae*) 과 대만꽃사슴 (*C. n. taiouanus*) 등 2개의 아종으로 분지하였으며, 붉은사슴 (*C. elaphus*) 은 중국 쓰촨지방 및 티베트 남동부에 분포하는 집단과 근연인 것을 확인할 수 있었다. 이 연구에서 분석한 사슴 집단은 교잡에 의한 염기서열의 변화는 없는 것으로 확인되었으나, 향후 제주도에 서식하는 집단에서 근연종 간의 교잡 가능성을 완전히 배제할 수 없기에 교잡종의 확인을 위한 핵DNA 분석 연구는 수행되어야 할 것이다. 이 연구를 통해 제주도에 이입된 외래사슴류의 생태학적 정보를 제공할 수 있었으며, 첨단기술을 활용한 추가적인 연구 수행의 필요성을 제안한다. 또한, 다양한 서식지에서 사슴류의 분변 및 조직 시료를 수집하여 추가적인 분석이 이루어진다면 제주도 사슴류의 분포 및 진화 유연관계에 대한 보다 명확한 규명이 이루어질 것이 기대된다.

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Appendix 1. List of dietary plant taxa found in different seasons, and their food categories

| Class | Order | Family | Species | Season | | | | Forage category | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Spring | Summer | Autumn | Winter | G | Fc | f | B |
| Polypodiopsida | Polypodiales | Dryopteridaceae | *Arachniodes festina* | | | | + | | | √ | |
| | | | *Polystichum polyblepharum* | | | | + | | | √ | |
| | | | *Dryopteris blanfordii* | | | + | + | | | √ | |
| | | Pteridaceae | *Coniogramme intermedia* | | | + | + | | | √ | |
| | | | *Pteris multifida* | | | + | + | | | √ | |
| Coniferopsida | Cupressales | Cupressaceae | *Cryptomeria japonica* | | | | + | | | | √ |
| | Pinales | Pinaceae | *Abies koreana* | | | | + | | | | √ |
| | | | *Pinus thunbergii* | + | | + | | | | | √ |
| | | | *Pinus pungens* | | | | + | | | | √ |
| Monocotyledonae | Asparagales | Asparagaceae | *Hosta clausa var. normalis* | + | | | | | √ | | |
| | | | *Hosta minor* | + | | | | | √ | | |
| | | Orchidaceae | *Cephalanthera erecta* | | | + | | | √ | | |
| | Liliales | Colchicaceae | *Disporum sessile* | + | | | | | √ | | |
| | | Melanthiaceae | *Veratrum maackii var. parviflorum* | + | | | | | √ | | |
| | | Smilacaceae | *Smilax china* | | + | | + | | | | √ |
| | Poales | Cyperaceae | *Carex lenta* | | | + | | √ | | | |
| | | | *Carex dimorpholepis* | + | | + | | √ | | | |
| | | | *Cyperus exaltatus* var. *iwasakii* | | + | | | √ | | | |
| | | | *Cyperus iria* | | + | | | √ | | | |
| | | | *Kyllinga brevifolia* | | | | + | √ | | | |
| | | Poaceae | *Arthraxon hispidus* | | | + | | √ | | | |
| | | | *Chascolytrum uniolae* | + | + | | | √ | | | |
| | | | *Cynodon dactylon* | | + | | | √ | | | |

Continued

Appendix 1.

| Class | Order | Family | Species | Season | | | | Forage category | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Spring | Summer | Autumn | Winter | G | Fc | f | B |
| | | | *Imperata cylindrica* | + | + | | + | √ | | | |
| | | | *Lolium multiflorum* | | + | | | √ | | | |
| | | | *Oryza sativa* | + | + | | | √ | | | |
| | | | *Poa annua* | + | + | + | | √ | | | |
| | | | *Poa sphondylodes* | | + | | | √ | | | |
| | | | *Sasa quelpaertensis* | | + | | + | √ | | | |
| | | | *Zoysia japonica* | + | + | | | √ | | | |
| Dicotyledonae | Apiales | Apiaceae | *Angelica dahurica* | + | | | + | | | | √ |
| | | | *Cryptotaenia japonica* | | + | | | | √ | | |
| | | | *Ostericum grosseserratum* | | | + | | | √ | | |
| | | | *Torilis scabra* | + | + | | | | √ | | |
| | | Araliaceae | *Dendropanax morbiferus* | | + | | + | | | | √ |
| | | | *Kalopanax environmental* | + | | | | | √ | | |
| | Asteriales | Asteraceae | *Ambrosia artemisiifolia* | + | | | | | √ | | |
| | | | *Artemisia japonica* | | + | | | | √ | | |
| | | | *Artemisia stolonifera* | | + | + | | | √ | | |
| | | | *Aster scaber* | + | | | | | √ | | |
| | | | *Aster yomena* | + | | | | | √ | | |
| | | | *Cirsium vulgare* | + | | + | | | √ | | |
| | | | *Erigeron janivultus* | + | + | | | | √ | | |
| | | | *Hypochaeris radicata* | + | + | | | | √ | | |
| | | | *Taraxacum platycarpum* | + | | | | | √ | | |
| | | Campanulaceae | *Adenophora remotiflora* | + | | | | | √ | | |
| | | | *Peracarpa carnosa var.circaeoides* | + | | | | | √ | | |

Continued

Appendix 1.

| Class | Order | Family | Species | Season |  |  |  | Forage category |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Spring | Summer | Autumn | Winter | G | Fc | f | B |
|  | Caryophyllales | Caryophyllaceae | *Cerastium fontanum* | + | + |  |  |  | √ |  |  |
|  |  |  | *Cerastium holosteoides* |  | + |  |  |  | √ |  |  |
|  |  |  | *Stellaria media* | + | + |  |  |  | √ |  |  |
|  | Cornales | Cornaceae | *Cornus controversa* |  | + | + |  |  |  |  | √ |
|  |  | Hydrangeaceae | *Hydrangea serrata* |  | + | + |  |  |  |  |  |
|  | Cucurbitales | Cucurbitaceae | *Cucurbita moschata* | + |  | + |  |  | √ |  |  |
|  |  |  | *Gynostemma pentaphyllum* |  |  | + |  |  | √ |  |  |
|  |  |  | *Trichosanthes kirilowii* |  | + |  |  |  | √ |  |  |
|  | Dipsacales | Adoxaceae | *Viburnum dilatatum* | + |  |  | + |  |  |  | √ |
|  |  |  | *Viburnum erosum* | + |  | + |  |  |  |  | √ |
|  |  |  | *Sambucus nigra* | + | + | + |  |  |  |  | √ |
|  | Fabales | Fabaceae | *Albizia julibrissin* |  |  | + | + |  |  |  | √ |
|  |  |  | *Lespedeza cuneata* |  |  | + |  |  |  |  | √ |
|  |  |  | *Mimosa pudica* | + |  |  |  |  | √ |  |  |
|  |  |  | *Pueraria montana* |  | + |  | + |  | √ |  |  |
|  |  |  | *Sophora flavescens* |  | + |  |  |  | √ |  |  |
|  |  |  | *Vicia sativa* | + | + |  |  |  | √ |  |  |
|  | Fragales | Fragaceae | *Quercus serrata* |  | + |  | + |  |  |  | √ |
|  | Gentianales | Rubiaceae | *Damnacanthus indicus* |  | + |  |  |  |  |  | √ |
|  |  |  | *Galium aparine* |  | + |  |  |  | √ |  |  |
|  |  |  | *Paederia scandens* | + | + | + |  |  | √ |  |  |
|  |  |  | *Rubia chinensis* |  |  | + |  |  | √ |  |  |
|  | Geraniales | Apocynaceae | *Metaplexis japonica* |  |  |  | + |  | √ |  |  |
|  |  | Geraniaceae | *Geranium thunbergii* | + | + |  |  |  | √ |  |  |
|  | Lamiales | Acanthaceae | *Justicia procumbens* | + |  |  |  |  | √ |  |  |

Continued

Appendix 1.

| Class | Order | Family | Species | Season |  |  |  | Forage category |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Spring | Summer | Autumn | Winter | G | Fc | f | B |
|  |  |  | *Strobilanthes oliganthus* | + |  |  |  |  | √ |  |  |
|  |  | Oleaceae | *Ligustrum obtusifolium* |  |  | + | + |  |  |  | √ |
|  |  |  | *Ligustrum japonicum* |  |  | + |  |  |  |  | √ |
|  |  |  | *Osmanthus heterophylla* |  |  |  | + |  |  |  | √ |
|  |  | Plantaginaceae | *Veronica persica* |  | + | + |  |  | √ |  |  |
|  |  |  | *Veronica arvensis* |  | + | + |  |  | √ |  |  |
|  | Myrtales | Onagraceae | *Oenothera biennis* |  | + | + |  |  | √ |  |  |
|  |  |  | *Viola phalacrocarpa* |  | + |  |  |  | √ |  |  |
|  | Oxalidales | Oxalidaceae | *Oxalis corniculata* |  |  | + |  |  | √ |  |  |
|  | Ranunculales | Ranunculaceae | *Clematis brevicaudata* |  | + |  |  |  | √ |  |  |
|  |  |  | *Clematis mandshurica* |  |  |  | + |  | √ |  |  |
|  |  |  | *Ranunculus cantoniensis* | + |  | + |  |  | √ |  |  |
|  |  |  | *Ranunculus sceleratus* | + | + |  |  |  | √ |  |  |
|  | Rosales | Rosaceae | *Agrimonia pilosa* |  | + |  |  |  | √ |  |  |
|  |  |  | *Aruncus aethusifolius* |  | + |  |  |  | √ |  |  |
|  |  |  | *Duchesnea chrysantha* |  | + |  |  |  | √ |  |  |
|  |  |  | *Geum japonicum* |  |  |  | + |  | √ |  |  |
|  |  |  | *Prunus conradinae* |  | + | + |  |  |  |  | √ |
|  |  |  | *Potentilla indica* | + | + |  |  |  | √ |  |  |
|  |  |  | *Rosa rugosa* |  | + | + |  |  |  |  | √ |
|  |  |  | *Rubus coreanus* |  | + | + |  |  |  |  | √ |
|  | Saxifragales | Daphniphyllaceae | *Daphniphyllum macropodum* | + |  | + |  |  |  |  | √ |
|  |  | Haloragaceae | *Haloragis micrantha* |  |  |  | + |  | √ |  |  |
|  | Vitales | Vitaceae | *Ampelopsis heterophylla* | + | + |  | + |  | √ |  |  |
|  |  |  | *Cayratia japonica* |  |  |  | + |  | √ |  |  |

Continued

Appendix 2. Nucleotide composition of mitochondrial *CYTB* gene sequences in genus Cervus used in this study

| **Sample** | **Nucleotide** | | | |
|---|---|---|---|---|
| | **T (%)** | **C (%)** | **A (%)** | **G (%)** |
| SCY01, SCY09, SCY18, SCY20, SCY21 | 27.6 | 28.6 | 30.4 | 13.4 |
| SCY06, SCY07, SCY13, SCY14 | 28.6 | 27.6 | 30.5 | 13.3 |
| SCY03-05, SCY08, SCY10, SCY12, SCY15-17 | 29.2 | 26.8 | 30.7 | 13.3 |
| SCY02, SCY19 | 29.3 | 26.7 | 30.8 | 13.2 |
| SCY11 | 29.3 | 26.7 | 30.7 | 13.3 |
| *Cervus nippon yakushimae* | 27.6 | 28.6 | 30.4 | 13.4 |
| *Cervus nippon taiouanus* | 28.6 | 27.6 | 30.5 | 13.3 |
| *Cervus nippon hortulorum* | 28.6 | 27.6 | 30.7 | 13.1 |
| *Cervus nippon dybowskii* | 29.1 | 27.8 | 29.9 | 13.2 |
| *Cervus nippon yesoensis* | 28.6 | 27.6 | 30.5 | 13.3 |
| *Cervus nippon kopschi* | 28.6 | 27.6 | 30.6 | 13.2 |
| *Cervus nippon sichuanicus* | 28.2 | 27.8 | 30.8 | 13.2 |
| *Cervus nippon centralis* | 28.6 | 27.6 | 30.6 | 13.2 |
| *Cervus elaphus kansuensis* | 29.1 | 26.8 | 30.8 | 13.3 |
| *Cervus elaphus hippelaphus* | 29.2 | 26.7 | 30.7 | 13.4 |
| *Cervus elaphus songaricus* | 28.9 | 27.1 | 30.8 | 13.2 |
| *Cervus elaphus yarkandensis* | 28.9 | 27.1 | 30.7 | 13.2 |
| Mean | 28.7 | 27.4 | 30.6 | 13.3 |

Note: T, Thymine; C, Cytosine; A, Adenine; G, Guanine.

Appendix 3. Multiple sequence alignment of *CYTB* gene sequences used in this study

```
SCY01       ATAAAAATTG TAAACAACGC ATTCATTGAC CTCCCCGCCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCCTTACT
SCY02       ATAAAAATTG TAAACAACGC ATTTATTGAC CTCCCAGCCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCATTACT
SCY03       ATAAAAATTG TAAACAACGC ATTTATTGAC CTCCCAGCCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCATTACT
SCY06       ATAAAAATTG TAAACAACGC ATTTATTGAC CTCCCCACCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCCTTACT
SCY11       ATAAAAATTG TAAACAACGC ATTTATTGAC CTCCCAGCCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCATTACT
DQ985076    ATAAAAATTG TAAACAACGC ATTTATTGAC CTCCCCACCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCCTTACT
AB218689    ATAAAAATTG TAAACAACGC ATTCATTGAC CTCCCCGCCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCCTTACT
AB001612    ATAAAAATTG TAAACAACGC ATTTATTGAC CTCCCAGCCC CATCAAATAT TTCATCCTGA TGAAATTTCG GCTCATTACT
Consensus   ********** ********** *** ****** *****  *** ********** ********** ********** **** *****

SCY01       AGGAATTTGC CTAATCCTAC AAATCCTCAC AGGCCTATTC CTAGCAATAC ACTATACATC CGACACAATA ACAGCATTCT
SCY02       AGGAGTCTGT CTAATCCTAC AAATCCTCAC AGGCCTATTC CTAGCGATAC ACTATACATC TGATACAATA ACAGCATTCT
SCY03       AGGAGTCTGC CTAATCCTAC AAATCCTCAC AGGCCTATTC CTAGCGATAC ACTATACATC TGATACAATA ACAGCATTCT
SCY06       AGGAATTTGT CTAATCCTAC AAATTCTCAC AGGCCTATTC CTAGCAATAC ACTATACATC CGACACAATA ACAGCATTCT
SCY11       AGGAGTCTGT CTAATCCTAC AAATCCTCAC AGGCCTATTC CTAGCGATAC ACTATACATC TGATACAATA ACAGCATTCT
DQ985076    AGGAATTTGT CTAATCCTAC AAATTCTCAC AGGCCTATTC CTAGCAATAC ACTATACATC CGACACAATA ACAGCATTCT
AB218689    AGGAATTTGC CTAATCCTAC AAATCCTCAC AGGCCTATTC CTAGCAATAC ACTATACATC CGACACAATA ACAGCATTCT
AB001612    AGGAGTCTGC CTAATCCTAC AAATCCTCAC AGGCCTATTC CTAGCGATAC ACTATACATC TGATACAATA ACAGCATTCT
Consensus   **** * **  ********** **** ***** ********** ***** **** **********  ** ****** **********

SCY01       CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGTTG AATTATTCGA TACATACACG CAAACGGGGC ATCAATATTT
SCY02       CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGCTG AATTATTCGA TATATACACG CAAACGGGGC ATCAATATTT
SCY03       CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGCTG AATTATTCGA TATATACACG CAAACGGGGC ATCAATATTT
SCY06       CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGTTG AATTATTCGA TACATACACG CAAACGGGGC GTCAATATTC
SCY11       CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGCTG AATTATTCGA TATATACACG CAAACGGGGC ATCAATATTT
DQ985076    CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGTTG AATTATTCGA TACATACACG CAAACGGGGC GTCAATATTC
AB218689    CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGTTG AATTATTCGA TACATACACG CAAACGGGGC ATCAATATTT
AB001612    CCTCTGTCAC CCATATCTGT CGAGATGTCA ATTATGGCTG AATTATTCGA TATATACACG CAAACGGGGC ATCAATATTT
Consensus   ********** ********** ********** ******* ** ********** ** ******* **********  ******** 

SCY01       TTCATCTGCC TATTCATACA CGTAGGACGA GGTCTGTACT ACGGATCATA TACTTTTCTA GAGACATGAA ACATCGGAGT
SCY02       TTCATCTGTC TATTCATACA TGTAGGGCGA GGCCTGTACT ACGGATCATA TACTTTTCTA GAGACGTGAA ACATCGGAGT
SCY03       TTCATCTGTC TATTCATACA TGTAGGGCGA GGCCTGTACT ACGGATCATA TACTTTTCTA GAGACGTGAA ACATCGGAGT
SCY06       TTCATCTGCC TATTCATACA TGTAGGACGA GGCCTGTACT ACGGATCATA TACTTTTCTA GAGACATGAA ACATCGGAGT
SCY11       TTCATCTGTC TATTCATACA TGTAGGGCGA GGCCTGTACT ACGGATCATA TACTTTTCTA GAGACGTGAA ACATCGGAGT
DQ985076    TTCATCTGCC TATTCATACA TGTAGGACGA GGCCTGTACT ACGGATCATA TACTTTTCTA GAGACATGAA ACATCGGAGT
AB218689    TTCATCTGCC TATTCATACA CGTAGGACGA GGTCTGTACT ACGGATCATA TACTTTTCTA GAGACATGAA ACATCGGAGT
AB001612    TTCATCTGTC TATTCATACA TGTAGGGCGA GGCCTGTACT ACGGATCATA TACTTTTCTA GAGACGTGAA ACATCGGAGT
Consensus   ******** * **********  ***** *** ** ******* ********** ********** ***** **** **********

SCY01       AATTCTCCTA TTTACAGTCA TAGCCACAGC ATTCGTAGGA TACGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
SCY02       AATTCTTCTA TTTACAGTTA TAGCCACAGC ATTCGTAGGA TATGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
SCY03       AGTTCTTCTA TTTACAGTTA TAGCCACAGC ATTCGTAGGA TATGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
SCY06       AATCCTCCTA TTTACAGTTA TAGCCACAGC ATTCGTAGGA TATGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
SCY11       AGTTCTTCTA TTTACAGTTA TAGCCACAGC ATTCGTAGGA TATGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
DQ985076    AATCCTCCTA TTTACAGTTA TAGCCACAGC ATTCGTAGGA TATGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
AB218689    AATTCTCCTA TTTACAGTCA TAGCCACAGC ATTCGTAGGA TACGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
AB001612    AGTTCTTCTA TTTACAGTTA TAGCCACAGC ATTCGTAGGA TATGTCCTAC CATGAGGACA AATATCATTC TGAGGAGCAA
Consensus   * * ** *** ******** * ********** ********** ** ******* ********** ********** **********

SCY01       CAGTCATTAC CAACCTTCTC TCAGCAATTC CATACATTGG CACAAACCTA GTCGAATGGA TCTGAGGAGG CTTTTCAGTA
SCY02       CAGTCATCAC CAACCTTCTC TCAGCAATTC CATATATTGG GACAAACCTA GTCGAATGGA TCTGAGGAGG CTTTTCAGTA
SCY03       CAGTCATCAC CAACCTTCTC TCAGCAATTC CATATATTGG GACAAACCTA GTCGAATGGA TCTGAGGAGG CTTTTCAGTA
SCY06       CAGTCATCAC CAACCTCCTC TCAGCAATTC CATACATTGG CACAAACCTA GTCGAATGAA TCTGAGGGGG CTTTTCAGTA
SCY11       CAGTCATCAC CAACCTTCTC TCAGCAATTC CATATATTGG GACAAACCTA GTCGAATGGA TCTGAGGAGG CTTTTCAGTA
DQ985076    CAGTCATCAC CAACCTCCTC TCAGCAATTC CATACATTGG CACAAACCTA GTCGAATGAA TCTGAGGGGG CTTTTCAGTA
AB218689    CAGTCATTAC CAACCTTCTC TCAGCAATTC CATACATTGG CACAAACCTA GTCGAATGGA TCTGAGGAGG CTTTTCAGTA
AB001612    CAGTCATCAC CAACCTTCTC TCAGCAATTC CATATATTGG GACAAACCTA GTCGAATGGA TCTGAGGAGG CTTTTCAGTA
Consensus   ******* ** ****** *** ********** **** *****  ********* ******** * ******* ** **********

SCY01       GATAAAGCAA CCCTAACCCG ATTTTTCGCC TTCCACTTTA TTCTTCCATT TATCATCACA GCACTCGCTA TAGTACACTT
SCY02       GACAAAGCAA CCCTAACCCG ATTTTTCGCT TTCCACTTTA TTCTCCCATT TATCATCGCA GCACTCGCTA TAGTACACTT
SCY03       GACAAAGCAA CCCTAACCCG ATTTTTCGCT TTCCACTTTA TTCTCCCATT TATCATCGCA GCACTCGCTA TAGTACACTT
SCY06       GATAAAGCAA CCCTAACCCG ATTTTTCGCT TTCCACTTTA TTTTTCCATT CATCATCGCA GCACTCGCTA TAGTACATTT
SCY11       GACAAAGCAA CCCTAACCCG ATTTTTCGCT TTCCACTTTA TTCTCCCATT TATCATCGCA GCACTCGCTA TAGTACACTT
DQ985076    GATAAAGCAA CCCTAACCCG ATTTTTCGCT TTCCACTTTA TTTTTCCATT CATCATCGCA GCACTCGCTA TAGTACATTT
AB218689    GATAAAGCAA CCCTAACCCG ATTTTTCGCC TTCCACTTTA TTCTTCCATT TATCATCACA GCACTCGCTA TAGTACACTT
AB001612    GACAAAGCAA CCCTAACCCG ATTTTTCGCT TTCCACTTTA TTCTCCCATT TATCATCGCA GCACTCGCTA TAGTACACTT
Consensus   ** ******* ********** *********  ********** ** * *****  ****** ** ********** ******* **

SCY01       ACTCTTCCTT CACGAGACAG GATCCAACAA CCCAACAGGA ATCCCATCGG ACGCAGACAA AATCCCCTTC CATCCTTACT
SCY02       ACTCTTCCTT CACGAAACAG GATCTAATAA CCCAACAGGA ATTCCATCAG ACGCAGACAA AATCCCCTTT CATCCTTATT
SCY03       ACTCTTCCTT CACGAAACAG GATCTAATAA CCCAATAGGA ATTCCATCAG ACGCAGACAA AATCCCCTTT CATCCTTATT
SCY06       ACTCTTCCTT CACGAGACAG GATCCAACAA CCCAACAGGA ATCCCATCGG ACGCAGACAA AATCCCCTTC CATCCTTACT
SCY11       ACTCTTCCTT CACGAAACAG GATCTAATAA CCCAACAGGA ATTCCATCAG ACGCAGACAA AATCCCCTTT CATCCTTATT
DQ985076    ACTCTTCCTT CACGAGACAG GATCCAACAA CCCAACAGGA ATCCCATCGG ACGCAGACAA AATCCCCTTC CATCCTTACT
AB218689    ACTCTTCCTT CACGAGACAG GATCCAACAA CCCAACAGGA ATCCCATCGG ACGCAGACAA AATCCCCTTC CATCCTTACT
AB001612    ACTCTTCCTT CACGAAACAG GATCTAATAA CCCAATAGGA ATTCCATCAG ACGCAGACAA AATCCCCTTT CATCCTTATT
Consensus   ********** ***** **** **** ** ** ***** **** ** ***** * ********** *********  ******** *

SCY01       ATACCATTAA AGATATCCTA GGCATCTTAC TTCTAGTACT CTTCCTGATA TCACTAGTAT TATTCGCACC AGACCTGCTT
SCY02       ATACCATTAA AGATATCTTA GGCATCTTAC TTCTTGTACT CTTCTTAATA TTACTAGTAT TATTCGCACC AGACCTACTT
SCY03       ATACCATTAA AGATATCTTA GGCATCCTAC TTCTTGTACT CTTCTTAATA TTACTAGTAT TATTCGCACC AGACCTACTT
SCY06       ATACTATCAA AGATATCTTA GGCATCTTAC TTCTAATACT CTTCCTAATA TTACTAGTAT TATTCGCACC AGACCTGCTT
SCY11       ATACCATTAA AGATATCTTA GGCATCTTAC TTCTTGTACT CTTCTTAATA TTACTAGTAT TATTCGCACC AGACCTACTT
DQ985076    ATACTATCAA AGATATCTTA GGCATCTTAC TTCTAATACT CTTCCTAATA TTACTAGTAT TATTCGCACC AGACCTGCTT
AB218689    ATACCATTAA AGATATCCTA GGCATCTTAC TTCTAGTACT CTTCCTGATA TCACTAGTAT TATTCGCACC AGACCTGCTT
AB001612    ATACCATTAA AGATATCTTA GGCATCCTAC TTCTTGTACT CTTCTTAATA TTACTAGTAT TATTCGCACC AGACCTACTT
Consensus   **** ** ** ******* ** ****** *** ****  **** **** * *** * ******** ********** ****** ***
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Continued

Appendix 3.

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SCY01         GGAGATCCAG ACAACTACAC CCCAGCAAAT CCGCTCAACA CACCCCCTCA CATCAAACCT GAATGATATT TCCTATTTGC
SCY02         GGAGATCCAG ATAACTACAC CCCAGCAAAC CCACTCAACA CACCCCCTCA TATTAAACCT GAATGATATT TCCTATTTGC
SCY03         GGAGATCCAG ATAACTACAC CCCAGCAAAC CCACTCAACA CACCCCCTCA TATTAAACCT GAATGATATT TCCTATTTGC
SCY06         GGAGATCCAG ACAACTATAC CCCAGCAAAT CCACTCAGCA CACCCCCTCA CATCAAACCC GAATGATACT TCCTATTTGC
SCY11         GGAGATCCAG ATAACTACAC CCCAGCAAAC CCACTCAACA CACCCCCTCA TATTAAACCT GAATGATATT TCCTATTTGC
DQ985076       GGAGATCCAG ACAACTATAC CCCAGCAAAT CCACTCAGCA CACCCCCTCA CATCAAACCC GAATGATACT TCCTATTTGC
AB218689       GGAGATCCAG ACAACTACAC CCCAGCAAAT CCGCTCAACA CACCCCCTCA CATCAAACCT GAATGATATT TCCTATTTGC
AB001612       GGAGATCCAG ATAACTACAC CCCAGCAAAC CCACTCAACA CACCCCCTCA TATTAAACCT GAATGATATT TCCTATTTGC
Consensus     ********** * ***** ** *********  ** **** ** **********  ** *****  ******** * **********

SCY01         ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC TATCCTAATC CTGATCCTCA
SCY02         ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC CATCCTAGTC TTAATTCTCA
SCY03         ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC CATCCTAGTC TTAATTCTCA
SCY06         ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC TATCCTAATC TTGATTCTCA
SCY11         ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC CATCCTAGTC TTAATTCTCA
DQ985076       ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC TATCCTAATC TTGATTCTCA
AB218689      ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC TATCCTAATC CTGATCCTCA
AB001612      ATACGCAATC CTACGATCAA TTCCCAACAA ACTAGGAGGA GTCTTAGCCC TAGTCTCATC CATCCTAGTC TTAATTCTCA
Consensus      ********** ********** ********** ********** ********** **********  ****** **  * ** ****

SCY01         TGCCTCTTCT TCACACGTCC AAACAACGCA GCATGATATT CCGACCATTC AGCCAATGCC TGTTCTGAAT CTTAGTAGCA
SCY02         TGCCTCTTCT TCACACATCC AAACAACGCA GCATGATATT CCGACCATTC AGTCAATGCC TATTCTGAAT CTTAGTAGCA
SCY03         TGCCTCTTCT TCACACATCC AAACAACGCA GCATGATATT CCGACCATTC AGTCAATGCC TATTCTGAAT CTTAGTAGCA
SCY06         TGCCTTTTCT TCACACGTCC AAACAACGCA GCATGATATT CCGACCATTC AGCCAATGCC TATTCTGAAT CTTAGTAGCA
SCY11         TGCCTCTTCT TCACACATCC AAACAACGCA GCATGATATT CCGACCATTC AGTCAATGCC TATTCTGAAT CTTAGTAGCA
DQ985076       TGCCTTTTCT TCACACGTCC AAACAACGCA GCATGATATT CCGACCATTC AGCCAATGCC TATTCTGAAT CTTAGTAGCA
AB218689       TGCCTCTTCT TCACACGTCC AAACAACGCA GCATGATATT CCGACCATTC AGCCAATGCC TGTTCTGAAT CTTAGTAGCA
AB001612       TGCCTCTTCT TCACACATCC AAACAACGCA GCATGATATT CCGACCATTC AGTCAATGCC TATTCTGAAT CTTAGTAGCA
Consensus      ***** **** ****** *** ********** ********** ********** ** ******* * ******** **********

SCY01         GACCTACTAA CACTCACATG AATTGGAGGA CAACCAGTTG AATACCCCTT TATTATTATT GGACAACTAG CATCTGTCCT
SCY02         GATCTACTAA CACTTACATG AATCGGAGGA CAACCAGTCG AATACCCCTT TATCATTATT GGACAACTAG CATCTGTCTT
SCY03         GATCTACTAA CACTTACATG AATCGGAGGA CAACCAGTCG AATACCCCTT TATCATTATT GGACAACTAG CATCTGTCTT
SCY06         GACCTACTAA CACTCACATG AATTGGAGGA CAACCAGTTG AGTACCCTTT TATTATTATT GGACAACTAG CATCTATCCT
SCY11         GATCTACTAA CACTTACATG AATCGGAGGA CAACCAGTCG AATACCCCTT TATCATTATT GGACAACTAG CATCTGTCTT
DQ985076      GACCTACTAA CACTCACATG AATTGGAGGA CAACCAGTTG AGTACCCTTT TATTATTATT GGACAACTAG CATCTATCCT
AB218689      GACCTACTAA CACTCACATG AATTGGAGGA CAACCAGTTG AATACCCCTT TATTATTATT GGACAACTAG CATCTGTCCT
AB001612      GATCTACTAA CACTTACATG AATCGGAGGA CAACCAGTCG AATACCCCTT TATCATTATT GGACAACTAG CATCTGTCTT
Consensus      ** ******* **** ***** *** ****** ******** * * ***** ** *** ****** ********** ***** ** *

SCY01         ATACTTCTTC ATTATCCTAG TCCTTATACC GATCACCAGC ACAATCGAAA ACAACCTCCT AAAATGAAGA
SCY02         ATATTTCTTC ATTATCCTAG TCCTCATACC AATCACCAGC ACAATCGAAA ACAACCTCCT AAAATGAAGA
SCY03         ATATTTCTTC ATTATCCTAG TCCTCATACC AATCACCAGC ACAATCGAAA ACAACCTCCT AAAATGAAGA
SCY06         ATACTTTTTC ATTATTCTGG TCCTTATACC GATCACCAGC ACAATCGAAA ATAACCTCCT AAAATGAAGA
SCY11         ATATTTCTTC ATTATCCTAG TCCTCATACC AATCACCAGC ACAATCGAAA ACAACCTCCT AAAATGAAGA
DQ985076       ATACTTTTTC ATTATTCTGG TCCTTATACC GATCACCAGC ACAATCGAAA ATAACCTCCT AAAATGAAGA
AB218689       ATACTTCTTC ATTATCCTAG TCCTTATACC GATCACCAGC ACAATCGAAA ACAACCTCCT AAAATGAAGA
AB001612       ATATTTCTTC ATTATCCTAG TCCTCATACC AATCACCAGC ACAATCGAAA ACAACCTCCT AAAATGAAGA
Consensus      *** ** *** ***** ** * **** *****  ********* ********** * ******** **********
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