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Review

A review of the genus *Rusa* in the indo-malayan archipelago and conservation efforts

Nur Alizati Nabila Giarat Ali ^{a,b}, Mohd Lutfi Abdullah ^a, Siti Azizah Mohd Nor ^b, Tan Min Pau ^b,
Noor Azleen Mohd Kulaimi ^a, Darlina Md Naim ^{c,*}

^a Department of Wildlife and National Parks, 56000 Cheras, Kuala Lumpur, Malaysia

^b Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

^c School of Biological Sciences, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia

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ABSTRACT

Genus *Rusa*, belonging to the deer family Cervidae is native to the Indo-Malaya Archipelago (IMA). However, detailed information on the *Rusa* genus in the IMA is limited. This review provides comprehensive information on the *Rusa* genus in the IMA including, threats and conservation efforts. There are four species of deer in *Rusa* genus, which is Sambar deer (*Rusa unicolor*), Javan deer (*Rusa timorensis*), Visayan spotted deer (*Rusa alfredi*) and Philippine deer (*Rusa marianna*). Despite their wide distribution in the South Asian and Southeast Asian regions, they are under serious threats. Some conservation efforts that are being done to protect and conserve them among others are; (1) facilities protection, (2) habitat enrichment programme, (3) *Ex-situ* conservation, (4) legislations, and (5) captive breeding. Conservation through genetics is also an important step in conserving these species. Recommendations for conservation of the genus are also discussed; 1. maintenance of ecosystem. 2. more effective monitoring system on the existing protected area. 3. *ex-situ* conservation, and 4. habitat monitoring.

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* Corresponding author.

E-mail address: darlinamdn@usm.my (D.M. Naim).

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1. Introduction

The deer, family Cervidae similar to many other wildlife is facing an enormous threat of extinction in many parts of their distributional range. The family is distributed throughout the northern hemisphere, South America and Southeast Asia (Gilbert et al., 2006). Comprising of 55 species, it is the second most speciose family of artiodactyls after the Bovidae (International Union for the Conservation of Nature - IUCN). Being a herbivore, deer is the middle link of the food chain between its carnivorous predators and its plant food resources. Their decline or disappearance in the ecosystem will disrupt the food chain, resulting in other animals being targeted and therefore impacting the wildlife balance (Does, 2018).

The genus *Rusa* belongs to family Cervidae in the superorder of even-toed ungulates, Cetartiodactyla. This family was first described in 1820 by the German zoologist, Georg August Goldfuss in Handbuch der Zoologie. Genus *Rusa* refers to four species of deer which are endemic to several regions of South Asia and Southeast Asia; the Sambar deer, *R. unicolor*, Javan deer, *R. timorensis*, Philippine deer, *R. marianna*, and Visayan spotted deer, *R. alfredi*. *Rusa unicolor* are native in the Asian region, inhabiting the forests of China, India, Sri Lanka, Nepal, in addition to the Indo-Malay Archipelago (IMA). The other three are endemic to specific areas within the IMA biogeographic hotspot which comprises of Indonesia, Malaysia, Brunei and the Philippines. The Javan rusa, *R. timorensis* is native to Indonesia where it is still found in good numbers. It has however also found its way not only to adjacent regions but most interestingly to more distant countries such as Australia, New Zealand, South Africa and the United States as introductions for managed game animals, trophy hunting and commercial meat and antler production (Hedges et al., 2015). In many of these areas, they have been well adapted and some have escaped into the wild and are established feral populations (Hedges et al., 2015). Based

on the IUCN (2008), both *R. unicolor* and *R. timorensis* are classified as Vulnerable (Hedges et al., 2015) while *R. marianna* and *R. alfredi* have been classified as Endangered since 2008 (MacKinnon et al., 2015; Brook, 2016).

Considering its threatened status, a comprehensive understanding of several pertinent aspects of the *Rusa* spp. is critical. Here we describe a comprehensive scientific literature on this genus in the Indo-Malay Archipelago (IMA). A limited number of reviews of this genus are available (see e.g. Jain et al., 2018; Davis et al., 2016; Moriarty, 2004; Spaggiari and Garine-Wichatitsky, 2005) but these have been based mainly on information outside the IMA. Here, we attempt to expand these earlier investigations with more extensive search. This review could serve as reference for future research in the *Rusa* spp. of the IMA biodiversity hotspot region but which unfortunately is experiencing the highest decline rate in biodiversity globally. Description of some general aspects for the four *Rusa* spp. that includes the taxonomy and distribution will be explained in section one. Threats that is affecting the *Rusa* spp. and efforts to conserve the *Rusa* spp. in the Indo-Malay Archipelago will be discussed in section two. Information on genetic studies will be explained in section three, and ways to improve will be discussed further in section four.

2. *Rusa* genus

2.1. Taxonomy and distribution

2.1.1. Sambar deer, *Rusa unicolor*

Rusa unicolor was initially referred as *Cervus unicolor* up until the 20th century (IUCN, 2015). However, Grubb (1990) resurrected the genus *Rusa* and this was supported by Groves (2003) and Grubb (2005). *Rusa unicolor* is the largest species of the Cervidae

family (Leslie, 2011). It has been recognized into eight subspecies; *R. unicolor boninensis*, *R. u. brookei*, *R. u. cambojensis*, *R. u. dejeani*, *R. u. equinus*, *R. u. swinhoei*, *R. u. hainana* and *R. u. unicolor*. These subspecies of *Rusa* inhabit various areas of the Asian continent: *R. u. cambojensis* in mainland Southeast Asia; *R. u. dejeani* in Southern China; *R. u. equinus* in Sumatra; *R. u. hainana* in Hainan, China; *R. u. swinhoei* in Taiwan and *R. u. unicolor* in India, Bangladesh and Sri Lanka. Reported that *R. u. boninensis* that once existed in Bonin Island, Japan is now extinct (thainationalpark.com).

2.1.2. Javan rusa, *Rusa timorensis*

Javan rusa is comprised of seven subspecies: *R. timorensis timorensis*, *R. t. djonga*, *R. t. floresiensis*, *R. t. macassaricus*, *R. t. moluccensis*, *R. t. renschi* and *R. t. russa*. These subspecies are endemic to several localities in Indonesia, with some being introduced to other locations within Indonesia or abroad. Javan rusa is believed to be native only to Java and Bali in Indonesia (Corbet and Hill, 1992; Heinsohn, 2003; Grubb, 2005; Hedges et al., 2015). However, it has been introduced to many other places in the Indo-Pacific region (Grubb, 2005; Groves & Grubb, 2011; Hedges et al., 2015) including Sunda Island, Maluku Island (Moluccan), Celebes and Timor where secondary introductions have also taken place and populations have become established as feral populations. These include *R. t. timorensis* in Timor (West Timor and Timor Leste); *R. t. djonga* in Muna and Buton (Southeast Sulawesi); *R. t. macassaricus* in Celebes Island; *R. t. moluccensis* in Moluccan Island; *R. t. renschi* in Bali and *R. t. russa* in Java. The Javan rusa, of Moluccan origin which was introduced originally from Java, was secondarily introduced into West Papua in the first quarter of the 20th century; and now its range include the southern coastal plains of New Guinea Papua of Gulf of Papua to the FakFak Peninsula and Doberai Peninsula (Hedges et al., 2015). Javan deer has been recorded as Vulnerable since 2008 in IUCN as the absolute populace has been assessed to number less than 10,000 individuals, and evaluated to be declining.

2.1.3. Visayan spotted deer, *Rusa alfredi*

The Visayan spotted deer or 'Philippine spotted deer', *Rusa alfredi* is also known as 'Prince Alfred's deer' in honour of Prince Alfred (Prince of Wales), who sent the first known specimen to the scientist, Sclater. P. L. (Nowak, 1991; Whitehead, 1993). It was initially classified as a subspecies of *R. unicolor* (Grubb and Groves, 1983). In 1983, this species was separated as a valid species although several authors had earlier considered it as a subspecies of the Philippine brown deer, *R. marianna* (Grubb and Groves, 1983; Whitehead, 1993). Visayan spotted deer is listed as Endangered under the IUCN since 2008 as their population size was estimated to be fewer than 2500 mature individuals in 1996 (Brook, 2016). The numbers continue to decline and there is no local population that contains more than 250 mature individuals (Brook, 2016). Visayan spotted deer is endemic to the Western Visayan Island and in the Negros-Panay Faunal Region in central Philippines. This species is one of the three endemic species in the Philippines and once roamed Guimaras, Negros, Cebu, Masbate and Ticao Islands (Oliver, 1993, 1996; Heaney et al., 1998; Grubb, 2005) but has disappeared since mid-20th century. Presently, Visayan spotted deer is only restricted to Mount Madja and Mount Baloy, the area of west Panay (the Philippines) and a forest area in Negros (Oliver et al., 1992). In the mid-20th century, Visayan spotted deer was extirpated in Cebu. A few individuals were reported in Masbate (a region in the Philippines) between 1991 and 1993 but has since become extinct in that area (Brook et al., 2016).

2.1.4. Philippine deer, *Rusa marianna*

The Philippine deer or 'Philippine brown deer', *R. marianna*, is a medium-sized deer native to the Philippines and was first described

from introduced populations on Guam in the Mariannas Islands (MacKinnon et al., 2015). Four subspecies have been described: *R. marianna marianna* from Luzon Island; *R. m. barandana* from Mindoro; *R. m. nigella* from upland sites of Mindanao; and *R. m. nigricans* from lowland sites of Mindanao and adjacent island of Basilan (Grubb and Groves, 1983; Oliver et al., 2008; Heaney et al., 2010). However, taxonomic relationships between *R. m. nigella* and *R. m. nigricans* are not fully understood as they display variation of body size, pelage colour and several other characters (Heaney et al., 1998; Oliver et al., 2008). It had previously been recorded at Biliran Island, Catanduanes Island, Bohol Island, Marinduque Island, Dinagat Island and Siargao Island but are now either possibly or confirmed extinct in some of these locations (MacKinnon et al., 2015). Present distribution is highly fragmented across the country and is much reduced from its historical distribution mainly due to illegal hunting and habitat loss (Oliver et al., 2008). The Philippine deer is closely related to the Visayan spotted deer, *R. alfredi*, but they do not overlap geographically (Meijaard & Groves, 2004) as the latter is now restricted to Panay and Negros in west-central Philippines.

The Philippine deer was introduced to Guam, Micronesia in 1771 for recreational hunting. However, over the years they became established at other sites on the island namely, Rota, Pohnpei and Saipan through secondary introductions (Wiles et al., 1999; Wiles, 2012). In the late 18th and early 19th, the Philippine deer was introduced to Ogasawara Island, Japan by Spanish sailors, but it went extinct in 1925 (MacKinnon et al., 2015). After World War II, Philippine deer was reintroduced to Ogasawara Island from Guam, but presently, it has ceased to exist on the island (Miura & Yoshihara, 2002; Grubb, 2005). Currently only Rota and Saipan (Northern Mariana Islands) and Guam and Pohnpei (Micronesia) support feral populations (Wiles et al., 1999, Wiles, 2012). These feral populations have well adapted to their new homes and in the absence of a natural predator, they had reportedly caused serious agricultural damage in Ogasawara Island in the 1880s to 1940s and Pohnpei in 1940s to 1960s as well as ecological damage in Pohnpei, Rota and Guam since 1940s (Wiles et al., 1999; Wiles, 2012). The Bagobo-Tagabawa tribe in Davao City identifies the Philippine deer as a "cultural keystone species" as it plays a vital role in their culture.

2.2. Morphological description

2.2.1. Sambar deer, *Rusa unicolor*

Sambar has predominantly grey-brown skin that is covered by dark brown hair, which is sometimes slightly reddish and darker along the midline. The tail is bushy, mainly blackish with whitish underside and also around the rump area (Francis, 2008). The male Sambar is darker in colour than the female Sambar. The male has long coarse hair on the neck, abdomen and back. Measuring an imposing two metres in height from head to tail and weighing up to 320 kg. The antlers are usually rusine, the brow tines are simple and the beams forked at the tip into three tines. The antlers typically reach up to 110 cm in full length (Leslie, 2011).

2.2.2. Javan rusa, *Rusa timorensis*

Male Javan rusa are larger than females. The males weigh approximately 150 kg, whereas the females weigh about 70 kg. Males often have a lyre-shaped and three-tined antlers. Both females and males have a rough greyish brown coat that is often coarse in appearance. Both their ears are rounded and broad. The animals have short legs, giving an appearance of being short and stubby (Cranbrook, 1991; Huffman, 1990).

2.2.3. Visayan spotted deer, *Rusa alfredi*

The Visayan spotted deer are small, the shoulder height of mature deer is between 75 cm and 80 cm, and body length is

128 cm. Females are usually much smaller than males whereas ears and tails are relatively short (Kurt, 1990; Whitehead, 1993). They have fine, dense and soft dark-brown coat on their upper body. Some spots are also seen on the backs and flanks. Pale white fur is spotted on the underside as well on the chin and lower lip. This species is distinguished from other native deer by the presence of nominal spots; all other Philippine deer are solid in colour (Whitehead, 1993). The legs are paler than the body, especially below the hock and carpus (Rabor, 1977; Grubb and Groves, 1983). The muzzle and forehead are dark while preorbital gland is surrounded by black hairs (Grubb and Groves, 1983). Male deer has three tined antlers, including a small brow tine. Their antlers are short, stout and rugose (Grubb and Groves, 1983; Whitehead, 1993).

2.2.4. Philippine deer, *Rusa marianna*

The Philippine deer is much smaller than the Sambar deer although *R. m. nigricans* is bigger than *R. m. nigella* (Grubb and Groves, 1983; Oliver et al., 2008). Their relative weight is between 40 and 96 kg, body length of 100–170 cm, shoulder height 55–95 cm and tail length 8–14 cm (Nowak, 1999; Wiles et al., 1999). Most of the populations feature medium to dark brown pelage with coarse hair, undersides and legs may be paler sometimes. Underside of the tail and insides of the ears are whitish or light brown. Some of the populations may be either darker or paler overall (Grubb and Groves, 1983; Nowak, 1999; Oliver et al., 2008). Antlers of mature bucks are slender typically and three-tined, featuring a rear-facing terminal fork and a single brow tine (Wiles et al., 1999). Antlers usually measure 16–40 cm but sometimes it can reach up to 53 cm (Wheeler, 1979; Grubb and Groves, 1983; Nowak, 1999).

2.3. Habitat and feeding habit

2.3.1. Sambar deer, *Rusa unicolor*

The Sambar deer is adapted to a wider variety of forest types compared to its other sister species in the Cervidae family (Schaller, 1967; Timmins et al., 2015). In Sabah, Borneo, Sambar was recorded in camera-trapping in both mature and young forests stand (Matsubayashi and Sukor, 2005; Timmins et al., 2015). In other areas of Borneo, Sambar commonly inhabits secondary forests of gently sloping terrain, and can also be found in dipterocarp forests on steep terrain and swamp forests (Payne et al., 1985; Timmins et al., 2015). In general, however, Sambar usually avoids steep terrain, and prefers open habitat (Trisurat et al., 2010) and dense shrubs which are close to water sources and grassland (Bagchi et al., 2003). Simbharoen et al. (2014) studied the ecological factor (s) based on nine ecological variables that influence (s) Sambar distribution and abundance in western Thailand. These variables were measured by sambar pellet abundance (Kruuk et al., 1994). Their study revealed that Sambar distribution and abundance indices in the Huai Kha Khaeng Wildlife Sanctuary were related to distance from the Huai Kha Khaeng River. The abundance of Sambar was greater in the areas closest to the main river which have lower elevations, comprising mainly of mixed deciduous forest. This was attributed to the high food availability supported by the constant water supply (Budke et al., 2008). Another study by Simcharoen et al. (2014) at the Huai Kha Khaeng Wildlife Sanctuary was based on the female tiger's, *Panthera tigris*, home range size and prey abundance: important metrics for management. This sanctuary is a mixed of deciduous forest, dry evergreen forest, hill evergreen forest and dry deciduous dipterocarp forest. This place had abundance of the tiger's prey such as Sambar and barking deer with a large enough prey biomass of >5000 kg km², to support the prey requirement for female tiger and their cubs.

According to McKay and Eisenberg (1974), Sambar does not shift their ranges seasonally and they are sedentary. Studies in non-IMA regions have shown that Sambar can adapt to various habitats; in thorny and arid forests of Gujarat and Rajasthan, dry deciduous as well as moist forests along the Indian Peninsular, in pine and oak forests in the Himalayan foothills while in the Western Ghats, they inhabit evergreen and semi-evergreen forests (Sankar and Acharya, 2004; Timmins et al., 2015). In Thailand, however, Sambar was recorded in shallower slopes, open habitat and near to streams with lower amount of rainfall (Lynam et al., 2012; Timmins et al., 2015).

Being herbivores (Matsubayashi et al., 2007) Sambar feed on herbs, young leaves of woody plants and fallen fruits, and also on shrubs. In areas where they inhabit near to human settlements, they enter gardens and plantations to feed and consequently are often in human conflict. Adult male Sambar often visits natural mineral sources, such as wildlife salt licks, as they need it for antler growth (Francis, 2008). Natural salt licks are not only important for their daily supplements, but also for reproductive nutrition (Matsubayashi et al., 2007). In the Deramakot Forest Reserve, Sabah, the salt licks are very popular for female deer in late pregnancy and during lactation to meet their high requirement for calcium for the newborn and infants (Hays and Swenson, 1984; Kovacs, 2005). In the wet season they forage on the tree bark which contains higher concentration of calcium than other foods (Matsubayashi et al., 2007). Sambars often visit the natural licks in the dark to avoid predators.

2.3.2. Javan rusa, *Rusa timorensis*

Like the Sambar, they are highly adaptable with successful populations in the mountains, shrublands, marshes and forests (Whitehead, 1993; Oka, 1998). They are non-selective in their diet, facilitated by their mouth shape and dentition (Dryden & Bisseling, 1999). These grazers have taller molar teeth which gives them a hypsodontic index (HI) (height of third molar/length of second molar) of about 1.8, compared to 1.4 and 1.3 for intermediate feeders and concentrated selectors, respectively (Dryden and Bisseling, 1999). Hypsodont is a pattern of dentition with elongated crown and body of the tooth and open roots that continue to grow as fast as they are worn down, an added advantage to the ungulates. Most herbivorous grazers have developed tall molars that can cope better with fibrous plant material which are sometimes impregnated with silica and often contaminated with soil. Javan rusa diet includes herbs, leaves, bark of shrubs and even seaweed (Kitchener et al., 1990; Oka, 1998; Keith and Pellow, 2005).

2.3.3. Visayan spotted deer, *Rusa alfredi*

Visayan spotted deer are most common in the dense interior of the islands in the Philippines. This species was reported in the primary and secondary growth forests, which is from sea level to at least 2000 m above sea level. Their habitat is now restricted to steep, rugged slopes of dipterocarp forest for protection, since these are less accessible to humans (Cox, 1987; Brook, 2016). They rely on dense forest for refuge (Rabor, 1977; Brook, 2016), but grassy patches and secondary communities are also favoured by this species to forage. Visayan also favour areas that have undergone natural disturbance, such as landslides and fires. This helps to open up the canopy which allows the growth of tender plants that are close to ground for ease of feeding (Cox, 1987; Heaney and Regalado, 1998). Visayan can also persist in degraded habitats, for example cogon grassland.

The herbivorous Visayan diet includes a variety of vegetation. They prefer succulent vegetation which emerges after a natural disaster (Whitehead, 1993; Heaney and Regalado, 1998). Visayan eat young shoots of cogon grass and young leaves and bud that

can be found near the forest floor (Rabor, 1977). They will also lick the ashes after a fire outbreak for their mineral content.

2.3.4. Philippine deer, *Rusa marianna*

The Philippine deer inhabit areas from sea level to 2900 m in primary and secondary forests and grasslands (Sanborn, 1952; Rabor, 1986; Heaney et al., 1998, 2006, 2010; Oliver et al., 2008). They inhabit lowland and montane moist forest (including mossy forest), dry forest, seasonally wet/flooded grasslands, and montane grasslands (Heaney et al., 1998; Oliver et al., 2008; Balete et al., 2011). They actually prefer to forage in grasslands under primary and secondary forests but due to loss of habitats, they have often move uphill. In Micronesia (introduced) and the Philippines (native), this species can occupy most habitat types (Wiles et al., 1999; Wiles, 2012). These include moist secondary forest and primary limestone and volcanic woods, secondary scrubby growths, grasslands, freshwater wetlands and farmlands. In both regions, this species has readily entered burned grasslands to feed on new plant growth (Wheeler, 1979; Wiles et al., 1999; Heaney et al., 2010; Wiles, 2012).

The Philippine deer consume a diverse diet comprised of at least 82 plant species, which include both wild and agricultural trees, shrubs, grasses, herbaceous plants, vines, ferns, and mushrooms (Wheeler, 1979; Wiles et al., 1999; Wiles, 2012). All parts of the plants are eaten - shoots, fruits, foliage, and tree bark. However, there are differences in diet between the northern versus southern Guam as evident based on faecal concentrations of diaminopimelic acid (Conry, 1986).

2.4. General behavior

2.4.1. Sambar deer, *Rusa unicolor*

Sambar deer are mainly nocturnal; they will rest during the day in the heavy forest cover (Medway, 1969). They are typically found in small familial groups during the mating season. A single female often dominates her young, and also perhaps her female yearling; mature males >6 years old are solitary, with young males grouping together, close to females, or as satellites to the solitary mature males (Schaller, 1967; Eisenberg and Lockhart, 1972; Khan et al., 1995). Males will establish territories primarily during the breeding seasons as they are nomadic. In areas that have plentiful of rummage and water accessibility, Sambar deer may roam in a larger group of 30–40 (Geist, 1998) as observed in a study conducted in Sri Lanka (Kurt, 1978).

All Sambar deer are proficient swimmers (Nowak, 1999; Payne et al., 1985) where they swim with the body fully submerged and only the head above the water (Prater, 1980) to avoid insects and to forage (Richardson, 1972; Shea et al., 1990; Shukla and Khare, 1998). Insects can enter and occupy the nasal cavity of the deer. The nasal cavity serves as a perfect environment for their maggots to thrive and metamorphose into adults (Schmidt, 2018). The invasion does not only cause extreme discomfort to the host but can cause mortalities due to the blocked airway passage.

2.4.2. Javan rusa, *Rusa timorensis*

Javan rusa are nocturnal, although they do browse and graze during the day. When the mating season arrives, males usually decorate their antlers with grass and twigs to attract females and intimidate competitors. Javan males are often aggressive towards one another and extremely vocal. Males and females live separately, except during mating season. The young calves will stay with their mother until they reach sexual maturity. Javan normally associate in herds (Cranbrook, 1991; Huffman, 1999). Javan are generalists in their diet, assisted by their flexible dentition and mouth shape (Dryden and Bisseling, 1999) and eat herbs, leaves and bark of shrubs, and even seaweed too (Kitchener et al., 1990;

Oka, 1998). Most of their fluid requirement comes from the food they consume as they hardly drink (Kitchener et al., 1990).

2.4.3. Visayan spotted deer, *Rusa alfredi*

Visayan spotted deer are nocturnal feeders, as such they are often hunted at night with a headlight. The beam from the headlight evokes a frightened reaction and the animal would freeze in its tracks, instead of attempting to escape (Rabor, 1977). Visayan spotted deer are considered social animals although found in small groups in the wild, generally up to three animals. Males are frequently seen alone, and females are often accompanied by single young (Oliver et al., 2008). Even so, larger groups have been successfully maintained in captivity for long periods. Thus, the smaller groups in the wild may be due to human pressures (Oliver et al., 2008). During the rutting season (Whitehead, 1993), males will produce a roar-like vocalization. The vocalizing which is performed from a raised ground, resembles a barking dog if heard from a distance (Rabor, 1977). This vocalization is likely some form of visual and chemical communication between females and males, indicating their estrous status (Whitehead, 1993).

2.4.4. Philippine deer, *Rusa marianna*

Philippine deer are nocturnal, they will look for food such as grasses, leaves, berries and fallen fruits at night while foods in the dense forest thickets are foraged during the daytime. Mating season is usually between September to January. During the mating season, the females organize in small groups composed of eight individuals at most, whereas males are solitary and aggressive. After approximately six months gestation, females give birth to a single fawn with light colored spots that eventually disappear after several weeks.

2.5. Population densities

Sambar deer has a widespread distribution in southern Asia, as well as in the IMA, but the densities are low and no longer abundant throughout most of its native range, except in some protected areas (Sankar and Acharya, 2004; Timmins et al., 2008). Density varies depending on season, grouping behavior, habitat conditions, competition, predation and also degree of protection. In the Bukit Barisan Selatan National Park, Sumatra, Indonesia, Sambar deer was estimated at 0.62–1.42 individuals/km² in lowland rain forest (O'Brien et al., 2003). Its relative abundance indices are 5.6 times higher in low human density area than high human density area. In a dry tropical forest in Thailand, their relative abundance is approximately 1.9–4.2 individuals/km² (Srikosamatara, 1993). Densities also vary considerably outside of the IMA. Moist and dry deciduous tropical forests in India support 0.24–10.70 individuals/km² (Berwick and Jordan, 1971) while lowland dry-zone scrub jungle in Sri Lanka has 0.70–1.17 individuals/km² (Eisenberg and Lockhart, 1972). In Nepal, 2.0–11.5 individuals/km² have been documented in the riverine and Shorea forest and tall-grass habitats (Srikosamatara, 1993); whereas, the feral populations in Florida has a density of 1.76–6.01 individuals/km² (Flynn et al., 1990).

In Malaysia, estimates of population densities in their natural habitats have been conducted in several protected and reserve areas (DWNP, 2015). These studies have revealed that in the last decade, the Sambar deer populations have declined drastically. Camera-trapping surveys have shown absence or low densities of the species in several of the major remaining forest areas (Kawanishi and Sunquist, 2004). During a decade of study from 1998 to 2008, camera trapping studies (68 photographs) detected Sambar deer in only three out of the 18 Permanent Reserved Forests (PRF) from over more than 20,000 combined camera trapped days. However, all three protected areas (PAs) (Taman Negara Pahang, Krau Wildlife Reserve and Endau-Rompin) and two PRFs (ULU



Fig. 1. Range map of Sambar deer (*Rusa unicorn*) Source: IUCN (2015).

Muda and Temenggor) surveys have supported Sambar occurrence (346 photographs in 19,900 days). Of these sightings, Sambar was commonly detected in two sites, Endau Rompin and the Temenggor PRFs. In an earlier study from 1997 to 1999, Sambar was camera captured at only one of the nine areas surveyed, with the most frequently photographed in Temenggor. In more recent surveys spanning from 2009 to 2013, focusing on the three national tiger conservation areas of Endau-Rompin, Royal Belum State Park, and Temenggor, Sambar were detected in only four out of ten units surveyed through observational surveys. Of these, only the Royal Belum State Park had regular sightings (Kawanishi et al., 2014). In the case of Taman Negara Pahang also known as the Pahang National Park, a general density estimates based on photographic capture-recapture analysis showed a decline from 0.20 to 0.01 animals per km² over this period of study. This observation paralleled with the observational survey and camera-trapping studies between the year 2000 and 2010 (Kawanishi et al., 2014). Camera-trapping of eight forest sites in Sabah and Sarawak between 2010 and 2012 recorded Sambar in each site (Brodie et al., 2014). However, they were commonly found only in three out of the eight sites, Maliau Basin and Danum Valley in Sabah and Hose Mountains in Sarawak. Sadly, based on this survey, Sambar has apparently been extirpated in the Lambir Hills National Park, Sarawak. Another survey in Batang Ai National Park, Sarawak found only a single Sambar, attributed to the unfortunate conse-

quence of intensive hunting at salt licks (Meredith, 1995). In general, across Sabah and Sarawak, the number of Sambar has declined drastically due to illegal hunting (Bennett et al., 2000). The Department of Wildlife and National Parks Peninsular Malaysia (DWNP) now conducts an annual population distribution survey of ungulates, Sambar and the Barking deer, *Muntiacus muntjac* as one of the main agendas for camera trapping programme. In 2018, this programme was conducted at two locations; Krau Wildlife Reserve and Berkelah Forest Reserve in Pahang which is along the Main Range. Only a single Sambar was observed in the Berkelah Forest Reserve within the three months' programme based on 16 camera traps at eight camera stations. The area has logging activities and there are future plans for a hydroelectric dam along its three main streams - Sg. Tekai, Sg. Pertang, and Sg. Ong. None was observed in the Krau Forest despite a greater camera trapping effort (28) at 14 stations in the year 2018. In the previous tiger monitoring survey at Sungai Yu Ecological Corridor (Kuala Lipis, Pahang), several Sambar footprints were observed and believed to have been a victim of a tiger attack (DWNP, 2013). Range map of Sambar are shown in Fig. 1.

There is limited information on the Philippine deer population size and densities in the Philippines and their introduced areas (Wiles, 2012). Most remaining Philippine deer populations are small, although still common in some remote sites (Oliver et al., 2008; Heaney et al., 2010). Densities of deer are variable on the



Fig. 2. Range map of Javan rusa (*Rusa timorensis*). Source: IUCN (2015).

island, the deer are high in forested areas especially on military lands, but lower in the area with more human habitation (probably 0–0.5 km²) (Wiles, 2012). In another area of dense forest at the Andersen Air Force Base (AAFB), especially in the Munitions Storage Area (MSA) that has never been opened for intensive legal hunting, Wiles et al. (1999) estimated a minimum density of 60–80 deer/km² in 1990s. Likewise, based on a survey conducted in 2001 at the same area, higher densities of deer (183 deer/km²) was suggested (Vogt, U.S Navy, Hawaii, USA, unpublished data, 2005). Fig. 4 shows the range map of Philippine deer.

Reliable records on population densities of the Javan and Visayan deer are scarce or unavailable and therefore not described here. Both Figs. 2 and 3 are the range map of Javan and Visayan deer.

2.6. Threats

2.6.1. Sambar deer, *Rusa unicolor*

Despite its worldwide distribution, the Sambar deer faces a high risk of extinction at the local or regional scales based on the small population census size of various local populations (Timmins et al., 2008). As revealed through several surveys, Sambar populations have declined dramatically in the last few decades in many parts of its distribution where conservation efforts are not in place or have not been successful. The main factors connected to this are illegal hunting, poaching, over exploitation of natural forests, niche deterioration and urbanization of habitat (Timmins et al., 2015).

Furthermore, Sambar deer are the main prey of tigers. Its wide overlapping distribution with tiger populations such as in Myanmar, Thailand, Taiwan, China, and Cambodia have made it highly vulnerable to predation (Corbett and Hill, 1992). According to Kawanishi et al. (2014), the decline has reached 50% or higher in Peninsular Malaysia and the species has been listed as a Vulnerable species in the IUCN Red list since 2008 (Timmins et al., 2015).

Poaching is a primary threat to this wildlife in this region, especially since the meat is a delicacy throughout the Sundaic region (Timmins et al., 2008), and extremely highly prized. They are hunted for personal consumption, as trophies or sold to restaurants (DWNP, 1992a). Sambar meat is considered a premium product and often sold to only a selected group of customers. Illegal hunting activities are not confined to locals but also poachers from other neighbouring countries (MYCAT, 2012a), as their own wildlife supplies are drastically diminishing (MYCAT, 2012a).

According to an interview-based survey conducted between 2006 and 2007 by the wildlife trade monitoring network, TRAFFIC, more than 80% of the respondents from 18 villages across Peninsular Malaysia opined that overhunting is the primary reason for depletion of sambar. Habitat loss and degradation is also another contributory factor due to rapid development in recent times. Logging roads have provided much easier unwarranted access for the poachers to intrude into the forests. Furthermore, the agriculture and construction industries which contribute to a sizeable 4% and 16% respectively of Malaysia's national GDP (Economic Planning Unit, EPU 2013) have led to rapid habitat declines and

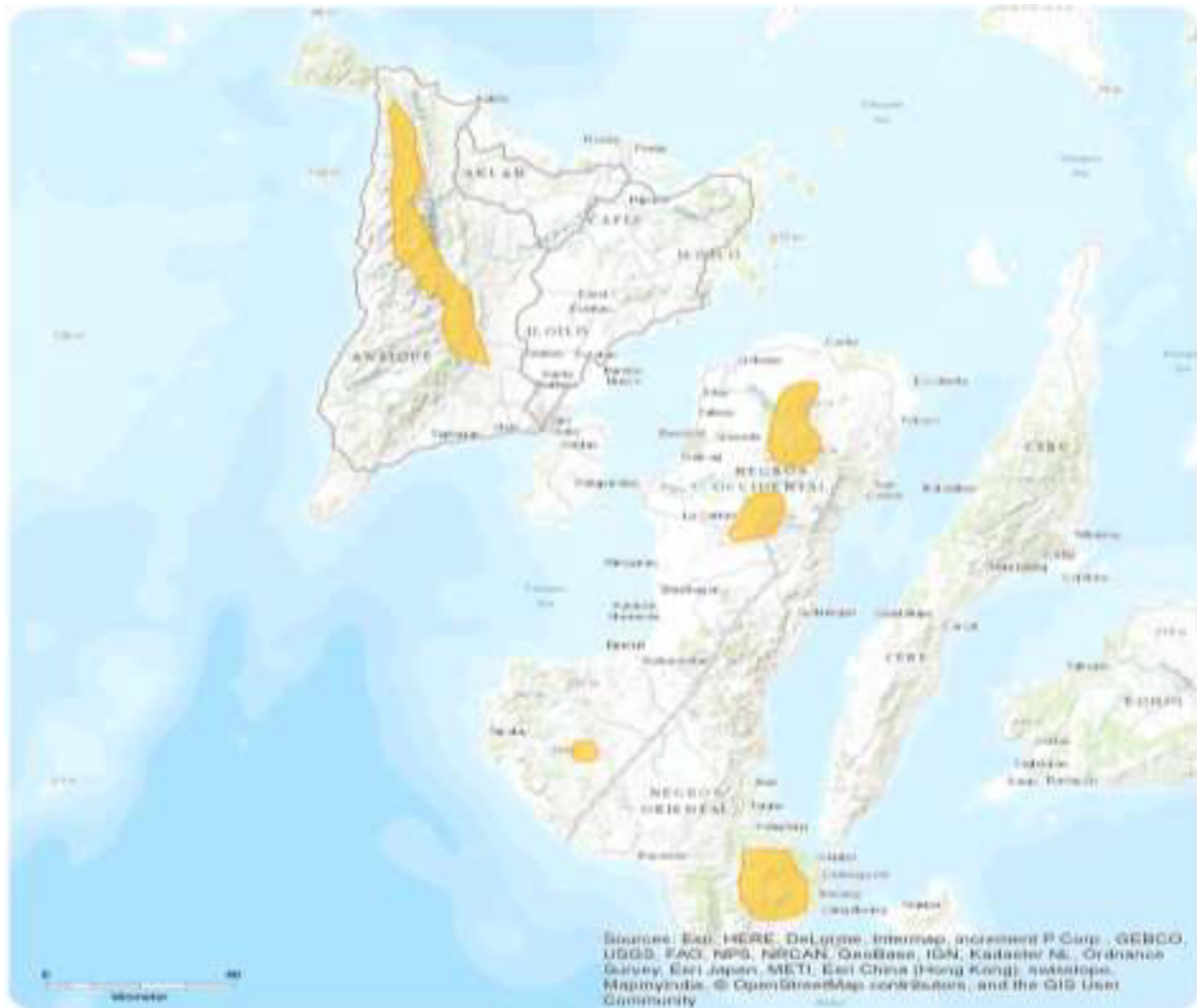


Fig. 3. Range map of Visayan Spotted deer (*Rusa alfredi*). Source: IUCN (2016).

degradation due to massive establishment and expansion of large-scale monoculture plantations and forest fragmentation resulting from road and railway constructions (MYCAT, 2012b). Almost all the surrounding areas outside of the protected areas and permanent reserve forests have been drained and cleared for conversion to agriculture plantations (DWNP, 2010) for rubber and oil palm. These areas are under the jurisdiction of each state with lower priority for biodiversity conservation, and no requirement for consultation with the federal government or conservation NGOs prior to the conversion.

2.6.2. Javan rusa, *Rusa timorensis*

During 1980s–1990s, the setting up of national park for Javan rusa managed to control poaching and land conversion activities. Yet, large numbers of Javan rusa in the Baluran National Park, Indonesia were lost due to a decrease in grazing areas caused by the invasion of the Acacia tree which converted the original open grassland into dense thorny scrub-forest. This plant was introduced to create a natural fire-break around the park, but instead became a hazard to the park's monsoon forests as they contained resin and flammable oils. Furthermore, repeated cutting of the acacia to manage its growth instead led to coppicing into very dense thickets that contained little or no grass or other herbal food source and led to difficulty for the deer to penetrate. The habitat loss and poaching due to limited enforcement resources have become serious threats to the Baluran National Park (Hedges et al., 2015).

In Java, the Javan rusa was poached with snares and dogs in the early days, but later guns became widely used in the late 1990s and 2000s (Hedges et al., 2015). Poaching of Javan are normally for the meat, medicinal applications (usually antler scrapings are utilised to treat various ailments), handicraft products and also as pets (Hedges et al., 2008). Javan rusa is seen as a source of extra income and of animal protein by many local communities despite being legally protected (Semiadi, 2006).

2.6.3. Visayan spotted deer, *Rusa alfredi*

This species has declined primarily due to habitat conversion such as logging, agriculture and hunting (Cox, 1987; Oliver et al., 1991; Oliver, 1992). Local farmers and recreational hunters from larger cities have been intensively hunting this species for meat to be sold to speciality restaurant and trophies (Cox, 1987; Oliver et al., 1992a, 1992b; Evans et al., 1993; Oliver, 1994) despite being fully protected by legislations. Highly skilful local hunters from upland communities hunt the Visayan to meet a strong demand for the species as pets (Brook, 2016). Furthermore, illegal logging and agricultural expansion have led to habitat fragmentation and reduction of populations (Brook, 2016), similar to other *Rusa* spp..

2.6.4. Philippine deer, *Rusa marianna*

The Philippine deer is threatened as a result of continuing and severe loss of habitat due to illegal logging, human encroachment for agricultural development and mining (Villamor, 1991). The

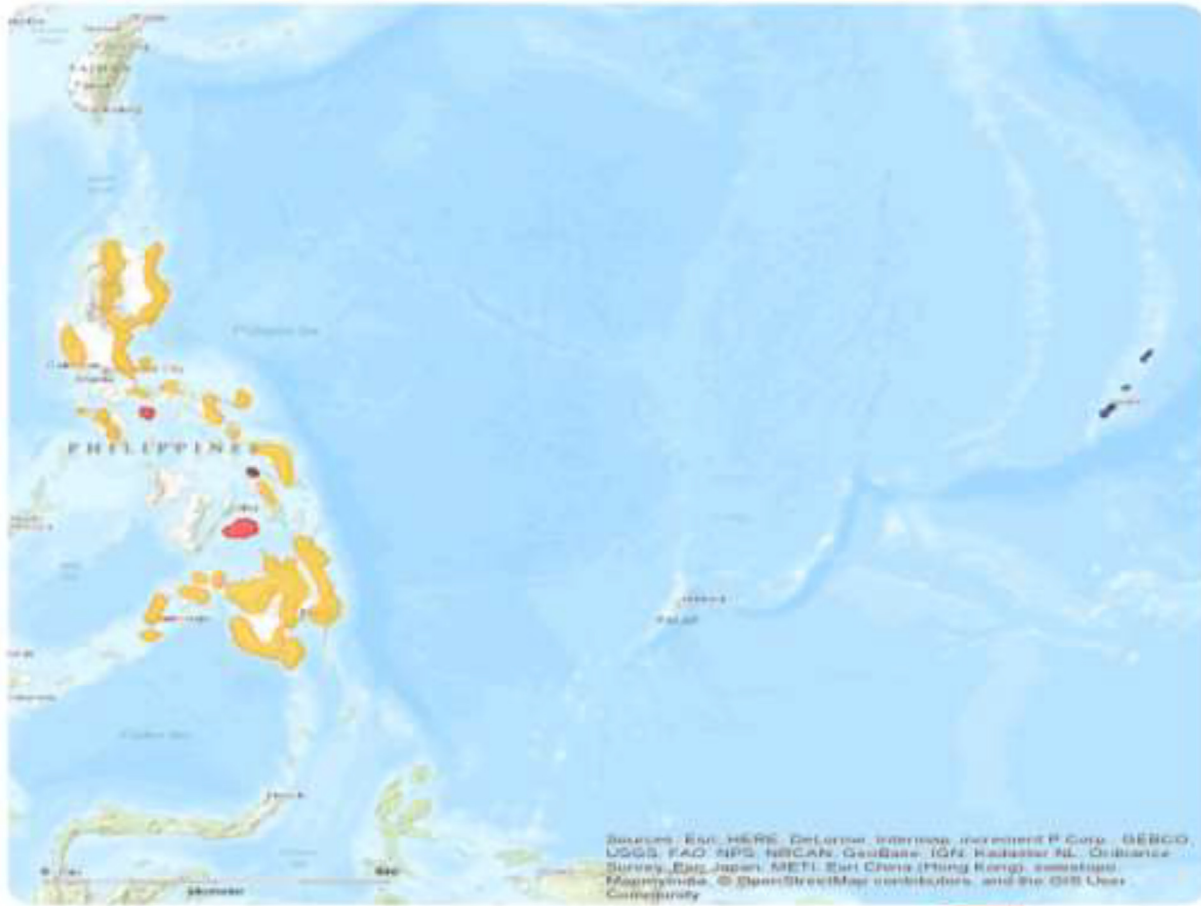


Fig. 4. Range map of Philippine deer (*Rusa marianna*). Source: IUCN (2015).

Philippine deer is hunted for meat, trophies, trade and hides. Legal protective measures are often not enforced (Villamor, 1991). Animal removal from the wild for commercial purposes and establishment of deer farms near protected areas pose potential threats as they can spread diseases and also hybridise with local populations. In particular, the Mindoro subspecies (*R. m. barandana*) are highly threatened due to its small population size. Poaching from Tagalog Mindorenos area and hunting by indigenous people are the main threats to *R. m. barandana* (MacKinnon et al, 2015).

2.7. Deer-ecological balance

Wildlife conservation is important for balancing the ecosystem and ecology, especially in the food chain. A reduction in one of the species in the link will affect the ecological balance. Wildlife also plays an important role in keeping the environment healthy and clean; as illustrated by the natural behavior of eagles to clean up carcasses (Kotiyal, 2019). The herbivorous deer is the intermediate link in the food chain. Herbivores obtain energy from consuming grass or leaves, but are in turn become the food source for predators. The energy absorbed by deer from consuming the organisms at the bottom of food chain was then transferred to the carnivore, the animal at the top food chain. However, an over-abundance of deer in the habitat will also have a negative impact on the ecosystem. Unregulated deer population will lead to over-browsing as they compete over the same preferred food supplies of other wildlife, such as songbirds. Deer browsing may also harm undergrowth, shrubs and seedlings and could also disrupt the ground nesting of

certain species, such as amphibians (Does, 2018). Additionally, limited vegetation will put other wildlife at risk.

3. Conservation efforts

3.1. Protection facilities

3.1.1. Sambar deer, *Rusa unicolor*

Much has been said about the alarming decline of the Sambar deer species, but conservation pursuits are being undertaken by various countries to address this. One of the main conservation efforts of the *Rusa* spp. in Malaysia was the setting up of three deer sanctuaries to maintain, protect and conserve the Sambar. The first deer sanctuary is the Wildlife Conservation Centre (WCC), Sungkai, Perak set up by DWNP with cooperation of the Perak State Government. The Sungkai WCC was approved under the Second Malaysia Plan (a 5-yearly development plan for the nation) and is located in the Wildlife Reserve, Sungkai, Perak. The Reserve covers a land area of 8060 ha and provides a suitable habitat for the *Rusa* sp. with its flowing rivers, hills and swamps. To set up the WCC, in 1977, the government cleared 10 ha of the Reserve and dedicated eight plots as grazing land. Each plot was planted with three grass varieties namely guinea grass, molasses grass, and pangola grass. The Sambar deer were brought in from Taiping Zoo, Melaka Zoo and Johor Zoo while a few were gifts from the Sultan of Perak, three months after the grass planting. In total, there are three males and eight females made up the initial population. Subsequently, there were new introductions from the National Zoo, Kuala Lumpur (3), Sabah Zoo (2), while another

five deer were brought in from the Singapore Zoo, although these had originated from Indonesia. Most of the deer in WCC Gua Musang, Kelantan originated from WCC Sungkai, and the deer in WCC Kemahang, Kelantan were all brought from WCC Gua Musang and WCC Sungkai.

As of December 2018, the total Sambar in Sungkai reached 79 individuals, while in Gua Musang the total Sambar was 66 individuals (DWNP, 2018). According to DWNP (2012), there were 185 Sambar kept in WCC Sungkai and WCC Gua Musang in year 2012, compared to 151 individuals in previous years which showed a slight improvement over the previous number.

Many protected areas which have been set up in its distributional range support good numbers of Sambar populations. However, despite the legal protection status of these areas, it has not stemmed declines and local extinction due to illegal hunting. Kawanishi et al. (2014), suggested upgrading 'Protected' status to 'Totally Protected Species' under the wildlife legislation in Malaysia. 'Totally Protected Species' means a total ban on hunting or trade to arrest the decline (WWF-Malaysia, 2013). "Protected" gives the same terms as 'Totally Protected Species', but the amount of the wildlife left is the difference. Sambars in Malaysia is protected under Act 716, Wildlife Conservation Act 2010. Under the Act, penalties for hunting or keeping totally protected wildlife is a maximum RM 300 000 fine or/and 10 years jail.

In Malaysia, habitat enrichment programme for wildlife has been introduced under the provisions of the 11th Malaysia Plan (DWNP, 2016). The programme involves maintenance of grazing field, forest replanting, clearance of the forest for new shoots and maintenance of artificial salt licks in protected areas. To this end several sites have been designated involving; six sites as grazing field, one site for forest clearance and two sites for forest replanting in Malaysia (DWNP, 2018). The grazing area is a source of continuous food supply for wildlife such as elephant, gaur (Buffalo), Banteng (Bali cattle), Tapir, Napuh (greater mouse deer), Sambar deer, and barking deer (Muntjac). Most of these sites which are mainly in the national parks or conservation areas have been planted with the favoured grass varieties of the animals namely Napier, Signal, Setaria, Centro and Stylo (DWNP, 2011). In addition, a total of 30 artificial salt licks have been maintained at six habitat enrichment sites in national parks, wildlife reserves and sanctuaries.

Ex situ conservation effort through captive breeding was first established in East Kalimantan in 1998 to save the declining populations of Sambar deer. This pioneering effort with a starting captive herd of 223 heads in the Penajam District led to the establishment of several private facilities in the province, such as at Bearau and at Nunukan, both with more than 20 heads at the time of set up. However, the conception rates have been very low, ranging from 48.8% to 83.3%, with lower number of offspring born than the initial reproductive female (Semiadi, 2008).

3.1.2. Javan rusa, *Rusa timorensis*

In Indonesia, the Javan rusa is protected by legislations that were introduced in 1999 by the Ministry of Forestry Indonesia (Hedges et al., 2015). This conservation legislation comes under Law No 5 of 1990 on Conservation of Biodiversity and Ecosystems. Unfortunately, this legislation only focuses on the protection in designated areas but does not comprehensively provide protection for wildlife especially from negative impacts of development (Apriyani et al., 2018).

Some investment has been initiated in the commercial breeding of the Javan rusa in East Java based on the Multiple Objective Goal Programming (MOGP) approach. Through this method the resource requirements are determined so that commercial targets could be achieved within the resources available (Chuang and Lu, 1997). Thus, in the case of commercial breeding of the Javan rusa, in the first and second generations, the individuals were maintained for

conservation purpose only, where trading was prohibited. Based on this approach, commercial activity would only commence in the third generation of the breeding programme i.e. only in the third generation are the animals bred for trading (Santoso, 2012). The potential economic benefits were clearly evident through this structured programme. As the commercial deer breeding activity produced more deer numbers than required for the conservation purpose it could generate higher economic benefit compared to a conservation only strategy (Santoso, 2012).

3.1.3. Visayan spotted deer, *Rusa alfredi*

Visayan is fully protected under Philippine Forestry law, but unfortunately enforcement is lacking in most areas. A number of protected areas have been established, but management and enforcement remain ineffective in most of the protected areas (Brook, 2016). Although awareness programmes are actively conducted, they have not been effective in curbing illegal hunting due to the high monetary reward from hunting activity (Brook, 2016). The four Visayan protected areas established were Mount Canlaon National Park, North Negros Forest Reserve, Mount Talinis/Lake Balinsasayao Reserve and the proposed West Panay Mountain National Park. In 1990 the Philippines Spotted Deer Conservation Programme (PSDCP) was established through collaboration between the Department of Environment and Natural Resources (DENR) and Parc Zoologique et Botanique de la Ville Mulhouse (Mulhouse Zoo, France) to protect this species. This programme was aimed to enable a systematic conservation breeding programme from donated and confiscated animals which were previously illegally held as pests by private owners (Brook, 2016). The activities include: development of three local wildlife rescue and breeding centres at Negros and Panay (two on Negros, one on Panay); wide-ranging field surveys and other field studies; facilitating the establishment of new protected areas as well as habitat restoration; and public awareness programme to address human-deer conflicts (Brook, 2016). Through such efforts, this programme deemed the most successful of its kind in the Philippines has produced viable progeny which are now fully protected in well-established facilities worldwide. As of December 2013, 138 individuals (57 male, 81 female) were already maintained in protected captivity comprising of 29 (12 males, 17 females) individuals in the Negros captive breeding facility and the rest in 27 other institutions in Europe (Schubert and Heckel, 2013; Brook, 2016).

3.1.4. Philippine deer, *Rusa mariana*

Captive breeding of this species was promoted by the Ecosystems Research and Development Bureau (ERDB) which was established by the Department of Environment and Natural Resources of Philippines to support rural communities through research and development (MacKinnon et al., 2015). These include the setting up of a ranch with mixed agriculture set up of a large herd of deer and domestic stock (chicken and ducks) at Batangas in the Calabarzon region, deer farms in the Bicol region, and small backyard farms in Luzon (MacKinnon et al., 2015). Overall, protective regulations are already in place but enforcement is not stringent. Outside of the Philippines, there are also several protected areas in the Mariana group of islands which have a good number of deer: five protected areas in Guam; Guam National Wildlife Refuge, Anao Conservation Reserve, Cotal Conservation Reserve, Guam Territorial Seashore Park and Bolanos Conservation Reserve. There are three in Rota: Sabana Heights Wildlife Conservation Area, l'Chenchon Park Bird Sanctuary and Wedding Cake Wildlife Conservation Area and one in Pohnpei: Watershed Forest Preserves (Wiles, 2012).

3.2. Conservation genetics in deer

Genetics data are now recognized as of critical value in the conservation of animals particularly of endangered ones. The field of conservation genetics applies genetics tools to conserve and restore biodiversity. One of the fundamental components of biodiversity is genetic diversity. Understanding levels of genetic diversity provides information that can contribute to knowledge of their evolutionary history and potential, important for strategising conservation and management efforts of rare and endangered species. Genetic diversity influences the adaptive flexibility of a species to environmental changes (Vrijenhoek, 1994). Low population diversity signals risk of low fitness and hence higher extinction risk. The aim of a conservation genetics programme is towards maintenance of a healthy level of genetic content within the population. Genetic variability estimates permit assessment of relatedness among and within populations (individuals) and thus facilitate selection for a systematic breeding and wildlife management programme. These measurements could be effectively performed through the utilisation of molecular markers such as mitochondrial and nuclear genes. Therefore, prudent management decisions could be strategised based on an understanding of the population structure and variability of the biological resource in question.

3.2.1. Mitochondrial DNA studies in the taxonomy and phylogenetics of the deer

The mitochondrial genome has proven to be a very efficient marker to study the taxonomy, evolution and population genetics of animal groups (Harrison, 1989; [Avise, 2004](#); [Hurst et al., 2005](#)) including in the context of conservation genetics. It is widely accepted that taxonomy, or biological classification, should be based on phylogenetic, or evolutionary relationships ([Cronin, 2003](#)). On the whole, there is a lack of information on genetics study of the deer as a group. Very little information on genetics is available within the IMA region ([Eljaafari, 2005](#); [Martins et al., 2017](#)). The utilisation of mitochondrial DNA markers such as the control region, cytochrome *b* (*cyt b*) and cytochrome oxidase subunit 1 genes (CO1), has enabled the elucidation of the taxonomic relationships among families, genera, species and even up to subspecies level.

On a higher taxonomic level, the systematics of the deer families Cervidae, Bovidae and Moschidae and other actiodactyls was investigated by analyzing the mitochondrial genes 12S and 16S rRNA and an 828 bp of the nuclear betaspectrin gene region ([Kuznetsova et al., 2005](#)). Cervidae was found to be a sister clade to Bovidae. Moschidae is sister to this clade. Several molecular synapomorphic characteristics of Cervidae and musk deer, the only extant species of Moschidae were revealed. The Cervidae family splits into three clades which includes the genera 1. *Cervus* and *Muntiacus* ([Schaller, 2000](#)), 2. *Capreolus*, *Hydropotes*, *Alces* ([Pavlinov, 2003](#)) and *Rangifer* and 3. *Odocoileus*, and the remaining genera.

Since the introduction of the DNA barcoding technique by [Hebert et al. \(2003\)](#), this method has become the gold standard in taxonomic studies. This approach is a method of species identification using a short section of the DNA from a specific gene or genes, typically the mitochondrial COI gene in animals. [Kumar et al. \(2018\)](#) used DNA barcoding method as a tool for robust identification of cervids of India and its utility in wildlife forensics. Seven deer species which comprise of 31 cervids from genera *Cervus*, *Axis* and *Muntiacus* were barcoded using the COI gene, all of which demonstrated highly supported monophyly through discrete clustering according to genus. The data provided an accurate taxonomic tool for species identification and is of critical value in wild animal forensics for law enforcement agencies.

[Yan et al. \(2013\)](#), conducted a forensic DNA barcoding and bio-response studies of animal horn products used in traditional medicine. They successfully identified 223 specimens representing 10 species in the Cervidae and Bovidae and illegal adulterants in the horn products. The results of the analysis indicate that it is possible to identify and discriminate between species from which the animal horns were obtained and therefore monitor incidences of illegal substituents. All haplotypes assembled into their orthologous mitochondrial DNA groupings. A further phylogenetic analysis including GeneBank dataset gathered each presumed species into monophyletic clusters of species and families.

Besides the COI gene, other markers have also been efficient in elucidating taxonomic relationships of the deer. One of the earliest conservation genetics studies of the deer was based on the mitochondrial control region, also known as the D-loop by [Kocher et al. \(1989\)](#). Their study on the endangered Brazilian Pampas deer, *Ozotoceros bezoarticus* revealed high variability attributed to the large historical population sizes of millions of individuals in contrast to the fewer than 80 000 estimated during their study.

A study on the Eld's deer, *Panolia eldii* by [Balakrishnan et al. \(2003\)](#) also supported the utility of the control region or D-loop region for genetics conservation in the deer through precise taxonomic identification at subspecies level. Their study involved three subspecies of Eld's deer across South Asia and Indochina. *Cervus eldi eldi*, *C. e. thamin* and *C. e. siamensis* were sampled from Chatthin Wildlife Sanctuary and their results showed that *C. eldi eldi* is more closely related to *C. e. thamin* than to *C. e. siamensis*, which was consistent with biogeographic considerations. There was also a strong signal of phylogeographic structure both between subspecies and among populations within subspecies. The delineation into different gene pools recommends that the subspecies and populations be treated as discrete management units. Any programme for restocking and breeding should take this into consideration.

The utilization of D-loop marker on the Formosan sambar deer (*R. unicolor swinhoei*) ([Chen et al., 2011](#)) and 13 other cervids revealed a clear demarcation and confirmed the taxonomic integrity between the Asian and European taxa. As expected, all *R. unicolor* were categorized into the Asian cluster. The Formosan sambar deer was shown to be phylogenetically close to the Japanese deer, *Cervus nippon* and also to the red deer, *Cervus elaphus*. The complete mitochondrial genome ([Chen et al., 2011](#)) of Hainan Sambar deer, *R. unicolor hainana*, also revealed the same order and arrangement, in congruence with the genetic relationship pattern observed in [Chen et al. \(2011\)](#). Three subspecies of *R. unicolor* were clustered into a single clade, *R. u. dejeani*, *R. u. swinhoei* and *R. u. hainana* with *R. timorensis* as a sister group.

Based on the mtDNA control region, [Gupta et al. \(2015\)](#) detected a 40 bp insertion that could delineate the South Indian *Rusa unicolor* (insertion mostly present) from the North and Central Indian (absent) populations. This was attributed to a potential ecological barrier that might be preventing the expansion of insertion-positive sambar and suggested that indels could have an important role in identification of genetically differentiated populations. Thus, this study provided a potential marker for molecular screening and identification of sambar population management units based on genetic consideration.

[Angom et al. \(2015\)](#) developed a protocol for the identification of two endangered cervids from decomposed and degraded samples based on mitochondrial DNA cytochrome *b* (*cyt b*) gene and the control region. Species identification was achieved for 16 unknown carcasses. Phylogenetics analysis with GenBank sequences successfully clustered all 16 samples into 14 Hog Deer, *Axis porcinus* and two Eld's deer, *Panolia eldii*. This study highlighted the reliability and rapidity of this marker to identify cervid species even in degraded samples. This detection approach is par-

ticularly beneficial for investigation of endangered species where fresh samples are often a limitation.

The origin of introduced Formosan sika deer into Okinoshima (Wakayama Prefecture, Japan) was elucidated utilizing a 423 bp *cyt b* and nuclear *Da-lactal-bumin (aLAlb)* genes (Matsumoto, 2014) based on 16 tissue samples. Three *cyt b* haplotypes were detected; two of Formosan sika deer, *C. nippon* (13 samples) and one Formosan sambar, *C. unicolor swinhoei* (3 samples) haplotype. On the other hand, the nuclear *aLAlb* also detected three haplotypes but of a different configuration: Formosan sika deer, red deer (*C. elaphus*), and an additional unknown sequence. This suggested hybridization had occurred among three deer species in the Okinoshima population.

Thus, the above studies illustrate the importance of molecular tools in complementing traditional approaches for precise and holistic understanding of the taxonomic status of the deer. Such information are critical for the management of this group as earlier described in this section.

3.2.2. Microsatellite studies in deer

Although mitochondrial markers are excellent in elucidating genetic relationships at various taxonomic hierarchy as well as spatial and temporal distributions, they are limited to only maternal inheritance information (Sato and Sato, 2013). Thus, nuclear markers are necessary tools to understand biparental inheritance for a comprehensive understanding. One of the most widely used nuclear markers are the microsatellites which consist of short, tandemly repeated DNA sequences, randomly dispersed throughout the genome with a Mendelian pattern of inheritance. In the last decades, microsatellite markers have been widely used for the study of variation in genetics, population structures and differentiation (Polzahn et al., 2000; Hu et al., 2007). Microsatellite markers are commonly used for individual identification, kinship and relationship determination, population size estimation and habitat range, and also studying ecological genetics (Bruford and Wayne, 1993; Bowcock et al., 1994; Buchanan et al., 1996; Beaumont and Bruford, 1998). As these markers are highly polymorphic, co-dominant and abundant across the genome, they is highly useful for population and conservation studies and eventually will facilitate for genotyping (Red, 1998; Kim et al., 2004). A DNA profiling approach would permit the design of increased selection regimes to create a genetically rich baseline population and thus reduce the risks of inbreeding.

Lin et al. (2014) developed eight microsatellite primers which successfully amplified 20 captive Formosan Sambar deer. Observed heterozygosity was low with a mean of 0.310, and five of the eight alleles had values below the mean. Their results showed high levels of inbreeding, and also selective mating among a small circle of individuals, which had led to dramatic losses of genetic polymorphisms. These microsatellite loci could be applied, not only in assessment of population structure and genetic variability, but also helps in monitoring the population dynamics and determine dispersal patterns of the Formosan sambar deer in Taiwan. Furthermore, they could be trialed for studies on other deer species and utilized if found suitable for cross species amplification, thus overcoming some of the issues in microsatellite development.

The establishment of a microsatellite marker system and 16S mtDNA successfully generated a barcoding inventory of almost half of the world's 55 cervid species based on high quality nuclear and mitochondrial DNA extracts from antler trophies (Hoffmann et al., 2014). Their study provided a protocol for successful amplification from archived antler samples. This technique could find wide applicability in deer species research worldwide and over a broad range of issues from taxonomy, forensic analyses and assessing trafficking of rare species. Furthermore, the non-invasive method

makes it highly amenable for population genetics and conservation studies in endangered animals.

In another study Brinkman et al. (2010) developed an efficient protocol for extracting DNA from fecal pellets from Sitka black-tailed deer, *Odocoileus hemionus sitkensis* based on previously developed microsatellite markers as well as a suite of new markers. Individual identification of Sitka black-tailed deer was achieved and could provide wildlife managers with a sensitive tool to monitor populations. In addition, the molecular approach could assist in a better understanding of social structure, paternity, kinship, sex ratios, gene flow and phylogeography (Brinkman et al., 2010). This technique may also enable mark-recapture studies that can estimate key population parameters such as abundance and survival not only in this species but be used as a model in other key deer species where data is limited.

To date, the most comprehensive comparative phylogeographic study in the IMA was the study by Martins et al. (2017) on the Javan rusa and Sambar. Archived samples were analysed using microsatellite markers and complete mitogenomes. Nuclear DNA separated the individuals into the two species, but mtDNA revealed that all *R. timorensis* sampled to the east of the Sunda shelf carried haplotypes from *R. unicolor* and a single *R. unicolor* from South Sumatra carried a *R. timorensis* haplotype. Thus, they concluded that hybridization had occurred between Javan rusa and Sambar in Sundaland during the Late Pleistocene and resulted in human-mediated introduction of hybrid descendants in all islands outside Sundaland.

A study by Eljaafari (2005) characterized three deer species from captive facilities in Malaysia, namely *C. unicolor*, *C. timorensis* and *C. nippon* based on karyotyping, biochemical polymorphisms and randomly amplified polymorphic DNA (RAPD) techniques. *Cervus timorensis* and *C. nippon* were introduced into the captive centres from their native origins. Karyotyping generally showed more similar characteristics between *C. timorensis* and *C. unicolor* than between either of this with *C. nippon*. Similarly, the biochemical analysis showed close genetic affinity between *C. timorensis* and *C. sambar* as expected based on other studies but a closer relationship of *C. timorensis* with *C. nippon* than *R. timorensis* and *R. sambar*. The discrepancy was probably due to the inconsistency of RAPD markers.

Apart from these limited studies, there is no literature or reliable account of any other genetic variability studies of the *Rusa* spp. in the IMA.

3.2.3. Preliminary genetic data of captive populations in Malaysian sanctuaries

For endangered species, captive broodstock populations is a critical resource for future efforts to rebuild healthy population numbers. Thus, for conservation purposes, it is important that a broodstock management plan considers the genetic makeup of the founding individuals of the breeding population. Ideally this should include all available haplotypes to maximize the genetic variability and ensure minimal adverse genetic impacts on the farming populations due to inbreeding. In the case of restocking, released animals must be as closely related to their wild siblings to ensure genetic compatibility for mating. Preliminary data on the genetic variability of partial *cyt b* (435 bp) gene of *R. unicolor* based on a total of 145 samples from all the three deer sanctuaries (WCC Sungkai, WCC Gua Musang and WCC Kemahang) showed very low genetic variation with only five closely related haplotypes (differentiated by one or two sequences) (manuscript in preparation). It was not unexpected that there would be a considerable amount of genetic admixture among the three sanctuaries as many were of the same origin with the free movement among sanctuaries and introduction from conservation facilities such as zoos from neighbouring countries. However, what was surprising was the lim-

ited combined genetic pool available for setting up a systematic breeding programme, considering these animals had originated from various sources (see section 2). This appears to reflect the decline of the Sambar in the wild. Whether this is the current genetic status of the Sambar in the IMA can only be confirmed with a more intensive survey of wild populations. Furthermore, comparisons with GenBank data from other countries revealed the very close genetic affinity (even identical sequences) of the Malaysian samples with haplotypes of populations from Indonesia and Thailand, including an archived sample, suggesting very low genetic variability for this species in this region. However, this study was based on only a single gene analysed on a small representative population and may not be a true indication of the genetic variability of this species in the region. Application of more variable markers such as microsatellites are required in future studies. However, it should also be acknowledged that maintaining genetic diversity alone in the captive populations does not ensure survival in the wild due to possible behavioural and genetic adaptations in captivity and incompatibility with the external environment. Noel et al. (1994) reported that due to low survival rates of captive-reared birds, shows deficits in foraging and poor socialization, thus causes heavy loss to predators. Thus, research must be done along those lines.

3.2.4. Significance and importance of deer conservation

The deer plays various very important roles in the ecosystem including in maintaining ecological balance, economic values as a meat product and traditional medicine. They are a keystone species in the ecosystem as they act as both important consumer as well as prey. Their feeding activities can directly and indirectly affect many plants and animals. Hewitt (2015) emphasized that the ecological value of deer has become widely recognized with the improved understanding of ecosystems and their functioning. Deer influences vegetation communities through herbivory and seed dispersal, through which it impacts important ecological processes such as nitrogen cycling, fire regimes and plant succession (Hobbs, 1996; Myers et al., 2004; Côte, 2011).

On the other hand, deer is an important prey animal for large carnivores like tigers, leopards, and so forth (Kumar et al., 2018) and structures a necessary part to the ecosystem. For instance, the Sambar is a favoured prey species especially for tigers in South and Southeast Asia (e.g., Seidensticker and McDougal, 1993; Karanth and Sunquist, 1995; Biswas and Sankar, 2002). Deer poaching has brought about a delicate issue in the biological system since the declining number of preys affects food accessibility for carnivores. Thus, conserving *Rusa* offers parallel protection of tigers which largely inhabit overlapping habitats (Corbett and Hill, 1992).

On a commercial scale, deer meat is a much sought after commodity as is well known. Perhaps, less known is its importance in traditional medicine. Kawtikar et al. (2010) reviewed the traditional use of antlers and future perspectives in medicine. Deer antlers is believed to contain many biologically compounds in the treatment of various diseases according to medical reports and clinical observations (Kawtikar et al., 2010). Antlers are made of chemical components which consists of sugars, fatty acids, amino acids, and nucleotides as essential molecules. According to the FDA the velvet antler is useful in arthritis treatment and has been proven to enhance athletic performance. Bioactive compounds of velvet antlers is a rich source of potential materilas in boosting immunity, illness prevention and propagating longevity in humans (Walker et al., 2001). Deer antler may improve immune responses for patients with bone marrow depressions (Yang et al., 2004). Chinese and Korean medicine regards velvet antler as an effective promoter of health, which strengthens the body, healing and improving tissue function.

3.3. Recommendations for moving forward

Improved management practices are essential if the natural resource community is to reverse the declining trend in deer population that began as early as in the 19th century and has continued unabated till now. It is well known that one of the factors for the decline in *Rusa* spp. and other wildlife is illegal hunting. Currently, many legislations are already in place in the IMA countries to manage hunting. However, this practice is still rampantly occurring. Therefore, increased enforcement of wildlife legislations is critical. The DWNP (Malaysia) and the relevant authorities in other countries must step up efforts to monitor wildlife trade Active law enforcement, such as anti-poaching patrols, intelligence gathering and arrests should be increased in rural areas, especially in areas that are located close to tiger habitats. However, conducting these activities are very challenging especially in resource-strapped government agencies. Thus, public participation and 'buy in' from locals as well as NGOs are crucial. An example could be the formation of informant networks to circumvent the limited human resources of the government agencies. This could ensure a more effective monitoring system that includes mechanisms for alerting the authorities to illegal trade activities and exposing negative trends in the trade for on the ground protection. The effectiveness of these conservation interventions could be assessed through regular surveys on the status of target species.

The ecosystem concept of *in situ* ensures that all plants and animals in the ecosystem are maintained at viable levels in their native habitats and that basic ecosystem processes are perpetuated indefinitely (Simcharoen et al., 2014). The ecosystem includes all environmental variables such as forage, thermal cover and security cover, among others. Such protection areas are already available throughout the IMA, mainly in the form of national parks. However, their maintenance requires high human and financial resources. Presently most national parks in the IMA countries are fragmented and therefore require independent administration which could be very costly (Brook, 2016). We could emulate some of the successful programmes being undertaken by other countries outside the Indo-Malay Archipelago. A magnificent example of a successful *in situ* model of ecosystem management concept is the Western Forest Complex (WEFCOM) of Thailand. Forming the largest forest track in mainland Southeast Asia, it is made up of 17 contiguous protected areas; 11 national parks and 6 wildlife sanctuaries. These protected areas provide ideal habitats for Sambar deer population restoration (Steinmetz et al., 2009). The WEFCOM which has three large protected areas - Huai Khakhaeng, Thungyai Naresuan West-East, prioritise wide interior valleys as conservation sites. These valleys are the key areas in wildlife sanctuaries, and frequently support the largest habitat of tiger populations in Southeast Asia (Walston et al., 2010) and also where Sambar deer is the main source of food. The Thai government is trying to expand the government-funded park protection while the ranger training programmes are fully supported by WEFCOM. Some of the protected species in the sanctuary include the Asian elephant, wild water buffalo, jungle cat, tiger, and of course its major prey, the Sambar deer. In Vietnam innovative programme such as the WWF supported Carbon and Biodiversity Project is delivering excellent results. Through this programme, locals are employed as forest guards to patrol reserves which have recorded successful annual removal of 14 000 snares (WWF.Panda.org, 2013). In the US, the government has introduced incentive programs for land managers to be directly involved in wildlife conservation (California Department of Fish and Wildlife, 2015). These programme increases the opportunity for private land owners to engage in activities to enhance management practices for wildlife such as the deer. For instance, the California Legislature enacted the Private Lands Management (PLM) program and the Shared

Habitat Alliance for Recreational Enhancement (SHARE) program for private landowners and delegated authority department to implement these programmes cooperatively. Similar programmes could be introduced in this region which could also reduce conflict between deer and humans. Coordinated management efforts among federal wildlife and other relevant agencies, state (in Malaysia forests are under the purview of state department) and private landowners would ensure mutual benefits (including for the animals). For the Department to manage deer at a landscape level, outreach to public land management agencies and private landowners are important. Outreach actions have become extremely important to solicit the opinions of stakeholders and will also serve as a medium for the Department to provide general and specific information on deer management needs and issues to agencies, landowners and the general public. Therefore, enhanced management and protection of existing protected areas could be achieved through co-management between the relevant national and local government agencies and NGOs, that would enable increased allocation of budget for conservation purposes (Brook et al., 2016) and 'unmanaged' and 'unprotected' areas which do not receive (or minimal) national budget resource allocations could also be assisted.

4. Research, monitoring and adaptive management

Ex situ conservation is a necessary complement to *in situ* for biodiversity conservation. As discussed earlier several sanctuaries and zoos have been established in the region. These *ex situ* set ups could be the focal point of research to improve the survivorship of the deer. Coordinated effort is a necessity to improve the well-being of the *Rusa* spp. Zoos could work together to create a stable population of the target taxa across the region. There are many examples of animals that have been saved from the brink of extinction, some have even formed viable populations and reintroduced to its natural habitat through concerted efforts and research at these facilities. Examples include the Arabian oryx, *Oryx leucoryx*, or white oryx, which was extinct in the wild as of 1972, but was reintroduced to the wild starting in 1982 from breeding work at Phoenix Zoo in 1963. Others are Przewalski's Horse, the only true wild horse (San Diego Zoo Safari Park), California Condor (Los Angeles Zoo and San Diego Park), Corroboree Frog (Taronga Zoo, Sydney), Regent Honeyeater (Australian Zoos), Bellinger River Snapping Turtle (Taronga Zoo), Amur leopard (zoos worldwide) Golden Lion Tamarin (zoos worldwide) and Eastern Bongos (zoos worldwide) among others.

Although there is considerable work on the deer in the Asian region, information on many aspects of the *Rusa* in the IMA region is still lacking. Most literature have reported studies in South Asia, Thailand and Taiwan. Therefore, a lot more data needs to be gathered for successful conservation and management including identifying populations and delineating important deer habitat areas for protection in the IMA region. An effective deer conservation and management plan must be based on robust population data and current habitat assessments.

In general, there is a dearth of updated information in many aspects including sex/age ratio, survival rates, recruitment rates, mortality rates/causes that could facilitate the decision making process for population management. Comprehensive deer range maps reflecting density is also critical so that key habitats could be prioritized for conservation through habitat restoration or enhancement or even acquisition as an *in situ* facility. Habitats need to be monitored so that changes can be tracked over time and the data necessary for population modeling and recommending harvest strategies can be recommended. Furthermore, any restocking activities and other conservation interventions must

be evaluated. In the case of the two-endemic deer *Rusa* species in the Philippine islands, more surveys should be conducted on all major islands to determine their relative abundance, and the nature, threats and efficacy of existing protective measures (MacKinnon et al., 2015).

All data acquisition requires a lot of resources and efforts which is not possible for the government agencies to handle single handedly. Thus, coordinated efforts among universities, research institutes and NGOs with support from locals are essential. This will ensure the best available methods are used for optimal data collection and monitoring. In captive breeding, issues such as poor reproduction is still a major problem. Therefore, the relevant institutes must look into factors that are hindering the reproductive success. For instance, nutrition is considered to play a fundamental role in many aspects of the life history and biological processes of ungulates, from ovulation, conception, gestation, lactation, resistance to diseases and survival from diseases. In turn, many ecological factors influence nutrition of free-ranging ungulates, such as vegetation composition, soil type, phenological development among others. In ruminants such as deer, these nutrients require specific microflora for their digestion. Thus, any change in diet from the original to translocated habitats may be harmful as the rumen microflora are exposed to a new diet. Overcoming such issues are critical to ensure successful production of deer and can only be addressed through diligent research. Another area of research that is currently gaining much attention is the application of biotechnology. A conventional captive programme is challenging, which include limited physical space for animals, problems with animal husbandry, nutrition and general reproductive failure of the animal (Lasley et al., 1994). Techniques such as cloning, assisted reproductive techniques such as cryogenics of gametes/embryos, artificial insemination, and embryo transfer, somatic cloning could assist in the propagation of this endangered species (Lanza et al., 2000). This technique aspires to spread small fragmented populations of endangered species and also domestic breeds (Comizzoli et al., 2000).

An added incentive in *Rusa* conservation is the parallel protection of tigers which largely inhabit overlapping habitats (Corbett and Hill, 1992). The Sambar, when available is the dominant prey species in terms of biomass in South and Southeast Asia (e.g., Seidensticker and McDougal, 1993; Karanth and Sunquist, 1995; Biswas and Sankar, 2002). Given the significance of sambar in the diet of tigers and widespread documentation of prey depletion across the tiger's range (Ramakrishnan et al., 1999; Johnson et al., 2006; Datta et al., 2008), conservation of the Sambar will ensure the perpetuity of the tiger.

5. Conclusions

This study is the first comprehensive description of the genus *Rusa*, composed of the Sambar deer (*Rusa unicolor*), Javan rusa (*Rusa timorensis*), Visayan spotted deer (*Rusa alfredi*) and Philippine deer (*Rusa marianna*), inhabiting the Indo-Malaya Archipelago (IMA). An exhaustive search of the literature and information obtained from the wild life department and related institutes revealed that human intervention such as logging, hunting and habitat degradation or acquisition have adversely affected the populations of members of this genus which are under serious threat of extinction. Conservation efforts should be of high priority. While some measures are already in place, we recommend, better coordination among the various stakeholders for maintenance of their natural habitats, improved implementation of existing regulations and policies through effective monitoring and setting up of protected areas. Moving forward, we also recommend the application of conservation genetics approach for wild and captive population management.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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