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En genetisk metod att identifiera mårdhund inom ett stort inhemskt samhälle av meso-köttätare

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Keywords: Raccoon dog, *Nyctereutes procyonoides*, PCR, carnivore, species specific primer

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ABSTRACTS

Introduced species often brings unforeseen and/or severe ecological impacts to ecosystem. These may be serving as vectors of disease, causing economic loss, ecosystem effects, reduction of biodiversity and interaction between species (e.g. predation, competition and introgression). So, it is important to do research about introduced species. Traditional research methods may be difficult when dealing with elusive introduced carnivores. Nowadays, non-invasive genetic sampling could be used to detect, identify and monitor invasive species. In this paper, I developed a protocol which uses 23 species specific primer pairs, based on mitochondrial DNA, to differentiate invasive raccoon dog and other native carnivores (European badger, red fox, pine marten, European otter, American mink, Eurasian lynx, domestic cat and domestic dog). PCR reactions were optimized by using muscle samples. Species identification requires amplification of one correct pair of primers and amplification failure of all the other primer pairs. My work offers an effective solution for the identification of invasive and native carnivores. In the future, this protocol can be used to monitor carnivore community in a large area by using field samples.

INTRODUCTION

Biological invasion consists of a species acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation or by the intentional introduction into a new area, in these cases, it may spread rapidly and conquer novel areas (Valery *et al.*, 2008). Introduced species often bring unforeseen and/or severe ecological impacts to ecosystems (Vitousek *et al.*, 1996). We can view these impacts from several aspects:

1) Vectors of disease For example, domestic chickens and some other species were introduced to Galápagos Islands. These species carry several diseases, such as, Newcastle disease, Marek's disease and avain pox. Now the native bird fauna on the islands experiences a long term and steady decline of population size (Wikelski *et al.*, 2004). In some locations, disease threatens native carnivores. During the 70s, Arctic fox (*Alopex lagopus semenovi*) on Mednyi Island suffered a dramatic decline, from about 600 individuals to less than 100, due to otodetic mange, probably introduced by domestic dogs *(Canis familiaris*) (Macdonald and Thom, 2001). In 1994, rabies virus and canine distemper virus (CDV) carried by domestic dogs outside the Serengeti National Park, triggered an epidemic which affected lions, hyenas and jackals in the Serengeti (Vanak and Gompper, 2009). Rabies can be a serious risk for endangered canids, such as Ethiopian wolf (*Canis simensis*). From 1988 to 1992, 42 individuals died and 35 individuals went missing in a population with 111 known wolves, most likely due to infection of rabies virus transmitted by domestic dogs. In the study site Web Valley, Ethiopia, 77% individuals were lost in 4 months (SilleroZubiri *et al.*, 1996). Raccoon dog (*Nyctereutes procyonoides*) is another example of a species shown to act as a vector for several diseases. In a study by Muller (2000) raccoon dog was shown to be the culprit in 16% of rabies cases in Estonia, 9% in Lithuania, 12% in Latvia, 7% in Poland and 0.7% in Russia (Muller, 2000). Invasive North American grey squirrels (*Sciurus carolinensis*) are vectors of squirrel poxvirus (SQPV), formerly called parapox, which is lethal to Eurasian red squirrels (*Sciurus vulgaris*) and is a significant component in their displacement (Sainsbury *et al.*, 2000; Tompkins *et al.*, 2002).

2) Economic loss In United States, invasive species cause environmental damages and losses worth almost $120 billion per year (Pimentel *et al.*, 2005). The invasion of star thistle (*Centaurea solstitialis*) causes water losses and an estimated US$ 16 – 56 million in

damages each year. The invasion of *Tamarix* is another example. It increases evapo-transpiration and narrows river channels result in fewer water supplies and more flood damages, which causes about $65 – 180 million and 50 million per year respectively. The introduction and spread of golden apple snail (*Pomacea canaliculata*) and European corn borer (*Ostrinia nubilalis*) have big impacts on food production and farm incomes (Chapin *et al.*, 2000).

3) Ecosystem effects According to Gordon (1998), introduced plant species can alter geomorphology, hydrology, biogeochemistry and disturbance. The introduction of the nitrogen-fixing tree (*Myrica faya*) and deep-rooted salt cedar (*Tamarix* sp.) are good examples. In Hawaii, the ecosystem is nitrogen-limited, but *Myrica faya* inputs five-fold more nitrogen to the ecosystem and changes most functional and structural properties of native forests. Deep-rooted salt cedar was introduced to Mojave and Sonoran Deserts of North America and increased the water and soil solutes accessed by vegetation, promoted productivity, and more surface litter and salts (Chapin *et al.*, 2000).

4) Reduction of biodiversity In Clavero and García-Berthou (2005), invasive species are reported to be the leading cause of extinction of birds, and the second cause of the extinction of North American fish, world fish and mammals. Chapin *et al.* (2000) state that introduced species is projected to have the fourth largest global impact on biodiversity in the future. 18.4% of 941 endangered vertebrate species are attributable to introduced species in some extent. Introduced species may get advantage over native species. When Argentine ant (*Linepithema humilis*) invaded southern North America, all other native ants disappeared as it expanded (Macdonald and Thom, 2001).

5) Species interactions These inteactions can be partioned into three major categories;

a) Predation Ebenhard (1988) reviewed the impact of 118 introduced mammal species, of which 23 (19%) were carnivores. This is more than three times of its share in the mammalian fauna, suggesting that carnivores are more often introduced, presumable in efforts to control other species considered pests (i.e. rabbits, rats). These carnivores often become invasive and proliferate rapidly by using native prey that have not evolved appropriate defenses and thus fall easy prey to the newcomers. Introduced predators were responsible for extirpation or extinction of 61 bird species. 33 cases of them were caused by

cats (*Felis catus*) (Macdonald and Thom, 2001). Small Indian mongoose (*Herpestes auropunctatus*) have great impact on local amphibians and reptiles, also ground nesting birds in West Indies, Virgin Islands. Some species became extinct or local extirpation, others were "near extinction" (Macdonald and Thom, 2001). In Milicz Ponds reserve, Poland, when the population of hooded crow (*Corvus cornix*), main predator of coot (*Fulica atra*), declined, the number of coots and mean clutch size were still dropped due to the present of alien predators, such as, American mink (*Neovison vison*), raccoon dog and raccoon (*Procyon lotor*) (Rek, 2009). In the period of 1987/89 to 1998, the population of three gull species and two tern species in islands of Scotland decreased dramatically. The decline ranges from 37% to 49%. This phenomenon was caused by annual mink predation on eggs and chicks of seabirds.

b) Competition When Arctic fox (*Alopex lagopus*) was introduced to over 450 islands, the end result varied. If there were no red foxes (*Vulpes vulpes*) on the island, the Arctic fox flourished; but with red fox present on the island, the Arctic fox failed to establish itself and soon disappeared (Macdonald and Thom, 2001). Bailey (1992) provides evidence from both the literature and experimental data, that Arctic fox could not co-exist with red fox. After red fox was introduced to two islands in the Aleutians, native Arctic fox disappeared within four years. The reason behind this could be interference competition and/or direct predation (Bailey, 1992). In Holland, when red fox re-invaded this region in 1970s, the population of stoats (*Mustela erminea*) decreased and went extinct by 1985 due to aggressive competition (Macdonald and Thom, 2001). In Australian, since dingoes (*Canis lupus dingo*) were highly depended on wild-caught food, their dietary overlapped with that of two native carnivores, thylacine (*Thylacinus cynocephalus*) and Tasmanian devil (*Sarcophilus harrisii*). This resulted in the extinction of thylacine and extirpation of Tasmanian devil in mainland Australia (Vanak and Gompper, 2009). In general, when an introduced species compete with native species, three possible outcomes could occur: 1. the loser may lost part of its original distribution range, and the abundance may decline, or even go extinct; 2. The loser may change its spatial or temporal behavior to avoid competition, but the character of the involved species would not change; 3. A niche-shift may occur, and one or all species involved can coexist, in this scenario, character displacement could happen ultimately (Macdonald and Thom, 2001).

c) Introgression When two closely related species interbreed, offspring may be fertile, and if this phenomenon is frequent, one species may slowly be genetically wiped out, threatening species persistence. For example, recovery of the wisent (*Bison bonasus*) was threatened by interbreeding with plains bison (*Bison bison bison*) in Europe (Simberloff, 1996). The Florida panther (*Felis concolor coryi*), an endangered species in American, is also affected by introgression. This subspecies contains hybrids between the species and an illicitly released Latin American subspecies. This compromised an expensive, controversial endangered species rehabilitation project (Simberloff, 1996). Even without introgression, the existence of parental populations can be threatened. The male American mink start breeding earlier and larger, stronger than the European mink (*Mustela lutreola*), thus, the American mink will preempt the female European mink. In addition that the hybrid embryo will be resorbed before parturition, the reproduction of European mink is hindered. This may cause the population decline of the European mink (Maran and Henttonen, 1995).

Since introduced species may have important and usually negative ecological and economical impacts, it is important to detect, identify, survey, monitor, and when feasible, manage the population size of introduced species. Traditionally, the research methods of introduced species could be mark-recapture, line transect or distance sampling etc. However, elusive species, true for most carnivores, are difficult to catch or observe. Nowadays, new remote and/or non-invasive methods are available, such as non-invasive genetic sampling. Non-invasive genetic sampling methods can be used to solve a broad range of problems, e.g. survey of population abundance (Kohn and Wayne, 1997; Marks *et al.*, 2009), identification of species, individual and sex, dietary, pathogens, reproduction and kinship etc (Kohn and Wayne, 1997; Taberlet *et al.*, 1999). There are many research have been done by using this method. Some of them use nuclear DNA, others use mitochondrial DNA. As source material for DNA it is possible to use shed hairs or feathers, feces, urine, saliva, buccal cells from food wodges, skins, eggshells and even skulls in owl pellets as DNA sources (Taberlet and Luikart, 1999; Taberlet *et al.*, 1999).

When this method is used in species and individual identification, PCR-RFLP (Restriction Fragment Length Polymorphism) or other techniques are typically used (Jiang *et al.*, 2011; Taberlet and Luikart, 1999). Species specific primers may also be used to identify a particular species. Spadaro *et al.* (2011) developed a primer pair to detect *Aspergillus carbonarius*, a fungus responsible for ochratoxin A (OTA) production in vineyards and

during wine production. The same approach was used to identify tomato DNA in guts of three insects, *Macrolophus pygmaeus*, *Helicoverpa armigera* and *Tuta absoluta* (Pumarino *et al.*, 2011). Boston *et al.* (2011) applied species specific primers to identify three *Myotis* species, namely, *Myotis mystacinus*, *Myotis brandtii* and *Myotis alcathoe*.

Kreisinger *et al.* (2010) use non-invasive genetic sampling method and microsatellite loci to determine the rates of extra-pair paternity and conspecific brood parasitism in mallards. This method was also used in the study of melanism in the jaguar by Haag *et al.* (2010), their study shows promising future of studying phenotypic polymorphisms by using non-invasive genetic sampling. The profiles of DNA extracted from teeth or other non-invasive samples can be used in the field of wildlife forensics (Caniglia *et al.*, 2010). This DNA-based species identification is helpful for the detection of illegal wildlife trade, poaching (Oliveira *et al.*, 2010). This approach can give accurate results compared with traditional morphological methods on identification of field fecal samples. Prugh and Ritland (2005) argued that even trained observers could not distinguish the scats of pine marten (*Martes martes*) and red fox reliably.

Raccoon dog, a small sized canid, is considered invasive in Europe. Its original range is from northern Indochina to the south-east corner of Russia, including China, Japan, Korea, Mongolia, Russia and Vietnam. It was introduced into Siberia and the European part of former Soviet Union from 1928 to 1955 as a fur-bearing animal, and then it spreads to northern and eastern Europe (Ansorge, 2009; Helle and Kauhala, 1995; Kauhala, 2004; Kauhala, 2008). Raccoon dog is a good disperser. In Finland, it has dispersed at a average rate of 20 km per year (Helle and Kauhala, 1991), rapidly colonizing new areas. A high reproduction rate is an important reason for the rapid colonization of this species. Among canids with similar size, raccoon dog has a very high reproductive capacity. The average litter size at birth of raccoon dog is 8.8 compared with 4 -6 for other canids' species. Raccoon dog has an average life-time production of 15 young, which is slightly higher than mammals in general. All these contribute to a high potential for raccoon dog to increase in numbers and disperse to large areas (Helle and Kauhala, 1995). In southern Finland, the density can reach 0.5 individuals / km^2 (Helle and Kauhala, 1995). Now the species can be seen in many European countries, including Sweden, the Netherlands, Switzerland, France and Norway (Helle and Kauhala, 1991). Climate is also an important factor affecting the distribution of the raccoon dog. The length of the growing season is the most important

single factor that causes the variation of the abundance of raccoon dog (Helle and Kauhala, 1991). In Finland, the population growth rate increased from northern Finland to southern and southeastern Finland as the annual mean temperature is also increased from northern to southern Finland. Climate can also affect the productivity of the female raccoon dog. The weight and fat reserves of juveniles in late autumn and the proportion of reproducing females decline when the snow depth increases (Kauhala and Helle, 1995).

As other invasive species, raccoon dog can also cause some negative impacts to the ecosystems and humans, such as the spread of pathogens and parasites. Since raccoon dog is a good disperser and an important vector of rabies in Europe, the species can accelerate the spread of the disease. At the end of 1980s, raccoon dog served as a main vector (77%) of a rabies epidemic in Finland. Raccoon dog is also a potential vector of one fox tapeworm, *Echinococcus multilocularis*, a dangerous parasite that may infect humans, with lethal consequences if left untreated (Drygala *et al.*, 2010; Kauhala, 2004). The potential negative effects associated with an establishment of raccoon dogs in Sweden make it important to detect and monitor them at an early stage. However, direct observations are rare making it difficult to collect data based on traditional methods. Here non-invasive genetic sampling offers a solution.

In this paper, I have developed a protocol that can be used to detect and differentiate raccoon dog from other small-sized carnivores, such as red fox, raccoon and Eurasian badger etc., by using species-specific PCR.

METHODS

PRIMER DESIGN

In this study, 10 species are included. They are: raccoon dog, American mink, Eurasian badger, red fox, pine marten, European otter (*Lutra lutra*), raccoon, Eurasian lynx (*Lynx lynx*), domestic cat (*Felis domesticus*) and domestic dog (*Canis domesticus*). The designing of the primers are based on mitochondrial DNA, either cytochrome b gene or control region. The sequences used for the 10 concerned species were from GenBank, the accession numbers are: NC013700, GU256221, FJ888513 – 21, D83614 (raccoon dog), AF057129

(American mink), AB049790, AB049808, AB049809, EF689064, EF689065, X94922 (Eurasian badger), EF689062, X94929, AY928669 (red fox), AB051237, AF154975, AF448239, AF448240, AF448241, EF689072, EF987751 (pine marten), X94923, AF057124, EF689067 (European otter), AB291073, AB297804 (raccoon), EU818890, EU818893, L39269, D28902 (Eurasian lynx), AB194814, AB194815, AB194816 (domestic cat), EU789739 (domestic dog). FastPCR (PrimerDigital Ltd. Helsinki, Finland) was used to design primers, the primers with highest level of intraspecies homogeneity and interspecies heterogeneity and targeting on short fragments (less than 300 bp) were chosen for further test. In addition, the melting temperature of the chosen primers was between 50 and 60°C. All the primers were synthesized by Eurofins (Eurofins MWG Operon, Ebersberg, Germany). There are 102 primers and form 76 primer pairs in total (Table 1, not all data shown). Among them two primers are from Shimatani *et al.* (2008).

POLYMERASE CHAIN REACTION (PCR)

PCR optimization produced the following protocol for all primers: denaturation at 94˚C for 3 min; follows 20 cycles of denaturation at 94˚C for 20 sec, annealing at 60˚C for 30 sec in first cycle, and lower 0.5˚C each cycle, extension at 72˚C for 30 sec; then 15 cycles of denaturation at 94˚C for 20 sec, annealing at 50˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 5 min. To ensure that primers reliably amplified the correct fragments, while still ensuring that they did not produce false fragments in other species all primers were first tested with the correct species DNA, if it produces a good result, this primer pair goes to next step; secondly, the chosen primer pairs were tested against all the 10 species DNA. During the whole process, I have tested one to three samples for each species. If the primer pair only amplifies the target species DNA, it can be used for future field samples test. The PCR reaction system is 8 µl in volume, including 0.8 µl 10x PCR buffer, 0.48 µl 25 mM Mg^{2+}, 0.2 µl 2.5 mM dNTP, 0.07 µl AmpliTaq DNA polymerase, 0.25 µl 10 mM primer pairs, 1 µl DNA template and 5.2 µl ddH_2O. All the DNA templates were extracted from known species muscle samples by using commercial kits (Qiagen, Hilden, Germany). After extraction, the concentration of the DNA samples was tested. Before the PCR process, I diluted the DNA samples to the concentration of 20 to 50ng/µl by using ddH_2O.

Primer Pairs	*Forward Primer (start - end position)*	*Sequence (5' – 3')*	*Reverse Primer (start - end position)*	*Sequence (5' – 3')*	*Product's Length (bp)*
Npr5	823-842	ctattcgcctacgccatcct	969-990	gcattgactaagtggtcggaat	147
Ncr15*	466-485	gggaatctgctatcactcaa	589-608	ttgaaagcaagtccagctac	124
rac1*	110-130	ggtacatatccatgtattgtc	368-387	tagtaggattggatggagag	259
Mme1	55-73	attgacttacccgcaccat	110-128	aggattaggcagatcccga	74
Mme5	57-75	tgacttacccacaccatcc	110-128	aggattaggcagatcccga	72
Vvu4	166-185	acatctgacacagctactgc	290-308	tataagcctcgtcccacgt	143
Vvu5	177-198	agctactgctttctcatctgtc	290-308	tataagcctcgtcccacgt	132
Llu1	14-34	gcaaaactcacccattagcca	58-74	gatggcgcgggtagatc	66
Llu5	185-204	ccttctcatcagtcgcacac	293-309	gtacaggccgcgtccta	128
Mma3	281-297	tgttcctgcacgtcgga	394-412	tttgccctcatggcagaac	133
Mma4	95-112	atttcggctccctccttg	281-297	tccgacgtgcaggaaca	204
Mvi2	57-80	tgatctacctgctccatcaaacat	102-120	gcagattccgagtagggac	64
Mvi4	102-120	gtccctactcggaatctgc	248-265	tggaagctccatttgcgt	164
Mvc1*	308-328	ggccatgatagtcctcaatcc	467-487	tgactgctacgagccatacct	180
Mvc5*	64-82	tccctgatttcctcaccac	157-177	ctaatgcacgacgtacatagg	114
dog1	22-42	cacccactagccaaaattgtt	165-182	gtggctgtgtccgatgta	161
dog3	50-68	cattcattgacctcccagc	165-182	gtggctgtgtccgatgta	133
cat2	91-108	tgaaacttcggctccctt	248-263	gaagctccgttggcgt	173
cat3	104-123	cccttctaggagtctgccta	248-263	gaagctccgttggcgt	160
Plo1	122-142	tgcttctacagatcgcaacag	248-267	tatggaagctccgttagcgt	146
Plo4	289-305	cacgtaggacgaggctt	393-409	gtcctcatgggaggacg	121
Lly1	42-61	accactcattcatcgacctg	175-193	cgatgagaaggcggttgtt	152
Lly4	82-102	agcatgatgaaacttcggctc	175-193	cgatgagaaggcggttgtt	112

Table 1. The positions in the whole gene sequences, nucleotides sequences and PCR products' length of the 23 chosen primer pairs. The primer pairs with asterisk (*) are based on the control region of the mitochondrial DNA, others are based on cytochrome b gene.

The PCR products were electrophoresed on 1.5 % agarose gel stained with SYBR® Safe DNA gel stain (Invitrogen, Paisley, UK). The gel was visualized by using Kodak 1D Scientific Imaging Systems (Kodak, New Haven, US).

RESULTS

In total, 23 primer pairs were confirmed to be effectively amplifying corresponding species DNA, and not other species DNA (except two primer pairs). Among them, 3 primer pairs for raccoon dog and 4 primer pairs for mink, for other species, there are 2 primer pairs for each species. The length of the PCR product for each primer pairs is shown in table 1. For

these 23 primer pairs, 19 primer pairs are based on cytochrome b gene, and the other 4 are based on the control region of the mitochondrial DNA.

Figure 1 is the pictures of the PCR products visualized on a 1.5 % agarose gel.

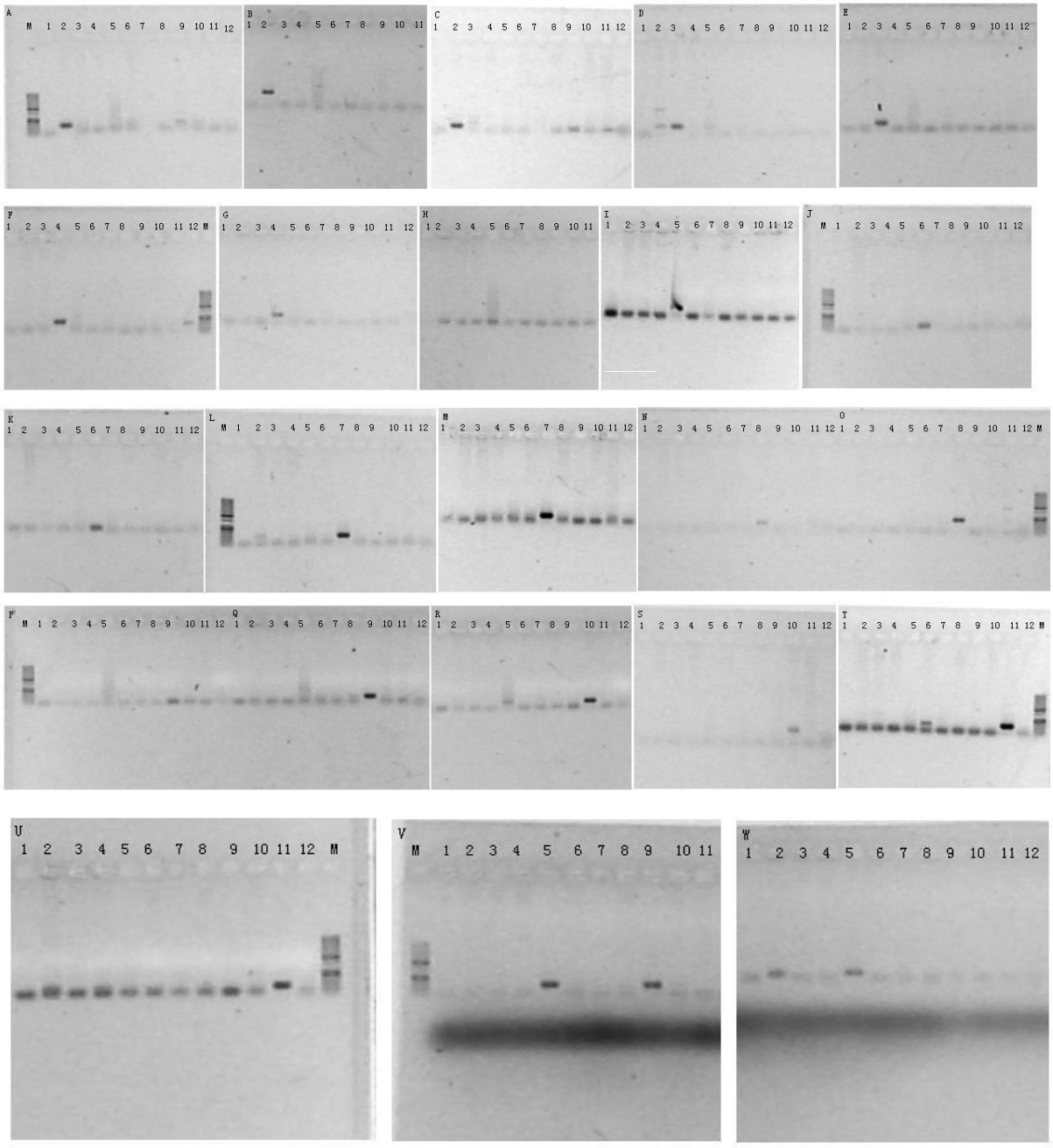

Figure 1. PCR amplification fragment of mtDNA in 1.5% agarose gel. M: 50 bp DNA ladder; Lane 1: negative control, 2: raccoon dog, 3: dog, 4: cat, 5: mink, 6: badger, 7: fox, 8: marten, 9: otter, 10: raccoon, 11: lynx, 12: field sample. A. primer pairs Npr 5; B. rac 1; C. Ncr15; D. dog1; E. dog3; F. cat2; G. cat3; H. Mvi2; I. Mvi4; J. Mme1; K. Mme5; L. Vvu4; M. Vvu5; N. Mma3; O. Mma4; P. Llu1; Q. Llu5; R. Plo1; S. Plo4; T. Lly1; U. Lly4; V. Mvc1; W. Mvc5.

DISCUSSION

The use of molecular diagnostics, including specific primers to identify species or diets, is becoming widespread. But less research have been done on carnivores by using this method (Fernandes *et al.*, 2008; Mukherjee *et al.*, 2007; Shimatani *et al.*, 2008). My research finds a practical protocol to identify up to 10 carnivores in Sweden. For species identification, universal primers are often used and based on RFLP or sequencing some PCR fragment. But the application of these methods apply to large-scale survey meets several obstacles. For RFLP, it requires large fragments of PCR products, this is typically difficult from sources that often contain degraded DNA (for example feces). Sequencing, is expensive and hence unsuitable for large sample series (Mukherjee *et al.*, 2007). RAPD (Random Amplification of Polymorphic DNA) can also be used in species identification. But compared with species specific primers, the reproducibility of the results of RAPD is low (Rastogi *et al.*, 2007). So, species specific primers are an ideal alternative to RFLP, RAPD and sequencing for this study. It is more reliable, less expensive and save much time.

In my study, only when one particular band show up for one sample, a conclusion that the sample is belong to a particular species can be reached. But it is also possible that two bands can be seen for one sample (Shimatani *et al.*, 2008). In Shimatani *et al.* (2008), they tested with fecal samples and got seven samples (among 405 samples) amplified for two species. They argued that this may be caused by two reasons. First, one fecal sample might contain another species DNA due to predation; second, in case, two individuals of different species just dropped their feces in exactly the same place (Shimatani *et al.*, 2008). In the diet of American mink, some species of mustelids can be found. The remains of North American river otter (*Lontra canadensis*) were also found in the mink from Ontario, Canada (Shier and Boyce, 2009), possibly due to a scavenging event or by scent marking onto the feces of the other species.

Padial *et al.* (2002) did a study about the feeding habits of red fox and stone marten (*Martes foina*) in Mediterranean mountain habitats in Spain, they found six fox scats (among 856 scats) containing remains of marten. The authors argued that “asymmetric intraguild predation” could be the reason (Padial *et al.*, 2002). For raccoon dog, mammals compose a small percentage (less than 10 % throughout the year) of their diets (Hirasawa *et al.*, 2006;

Sasaki and Kawabata, 1994). In a mountainous area of Japan, the mammals in raccoon dog diets are mainly rodents and insectivores, although sika deer (*Cervus nippon*) and/or Japanese serow (*Capricornis crispus*) could also be identified (Sasaki and Kawabata, 1994), presumable from scavenging events. In my study, true cross amplification due to the consumption of a second species may occur, but the proportion should be low level since the probability of the 10 carnivores involved in this study takes other carnivores as prey is low. We could also reduce the occurrence of cross amplification by sampling the side surface of the feces. In Stenglein *et al.* (2010), they reached lower allelic dropout error rates by sampling the outside of the brown bear (*Ursus arctos*) feces and the side of the wolf (*Canis lupus*) feces. If a cross amplification do occur, we should check the origin of the samples and do some research about the ecology, especially the species interaction, of the carnivores in question. We need to keep in mind that even the same species can have different dietary in different habitats due to local variations (Hirasawa *et al.*, 2006).

In the future, this set of primer pairs will be used on the field fecal samples. In that case, the DNA quality would be low and degraded in different level (Kohn and Wayne, 1997). This can be influenced by storage method, season of collection, diet and age of feces (Stenglein *et al.*, 2010). In my work, I targeted on the PCR products' length between 64 – 259 bp, most of them are between 100 – 200 bp. Compared with long fragments, although the sample DNA was degraded, some short fragments will still be there, so the primer pairs that targeted on short fragments can produce good PCR products (Mukherjee *et al.*, 2007). On the contrary, if we target on long fragments, it is very likely that the primer pairs could not amplify any DNA due to that region is degraded or produce less consistent results (Kohn and Wayne, 1997). Shimatani *et al.* (2008) tested 405 fecal samples, 246 (60.74%) samples were successfully amplified and identified to species. And it could happen that the fecal samples result in slightly weaker bands compared with wing biopsies of three *Myotis* species (Boston *et al.*, 2011). In addition, there are 6 – 8 copies of mitochondrial genome in one mitochondrion and about 800 – 1000 mitochondria in each animal cell. Because of this, there are greater chances that we could get species DNA amplified when we use mitochondrial DNA (Haunshi *et al.*, 2009).

In conclusion, this paper describes the development of a genetic tool for the non-invasive identification of 10 carnivores in Sweden by using species-specific primers. This tool provide an effective and reliable method to screen field samples across large areas to

monitor carnivore community composition and dispersal patterns of the invasive raccoon dog. This method could also be used in tracking parasites, such as *Echinococus spp.*, among Swedish meso-carnivore community, contingent on the development of parasite specific markers.

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