Agriculture and Natural Resources 51 (2017) 212–217

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Cloning and comparative analysis of zinc-finger protein gene on Y-chromosome (*ZFY*) between Thai Bangkaew dog and other Thai canids

Ukadej Boonyaprakob,[a,*] Sommai Homsavart,[a] Jatuporn Noosud,[b] Rongdej Tungtrakanpoung[c]

[a] Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[b] Department of Companion Animal Clinical Science, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[c] Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand

ARTICLE INFO

Article history:
Received 11 July 2016
Accepted 25 December 2016
Available online 20 July 2017

Keywords:
Thai Bangkaew dogs
Thai canids
ZFX
ZFY

ABSTRACT

The Thai Bangkaew dog is a Spitz-type dog that originated in Thailand. Legend has it that the dog is descended from hybrids between a native female dog and a male wild canid. To examine the mysterious story about the ancestry of the Thai Bangkaew dog's paternal lineage, sequence variation was examined for the last intron of the Y-chromosome-specific zinc-finger gene, *ZFY*, and its X homolog for male Thai Bangkaew dogs and other male Thai canids, including the Thai ridgeback and mixed breed dogs, Asiatic jackals (*Canis aureus*) and a dhole (*Cuon alpinus*). A 1075-bp *ZFY* segment from DNA samples of Thai Bangkaew dogs was found to be 100% identical to the domestic dog *ZFY* and (if gaps are allowed) showed 81% and 92% identity to jackal *ZFY* and dhole *ZFY*, respectively. However, if gaps were treated as missing data, the 1045-bp *ZFY* sequence for the Thai Bangkaew dogs was 100% identical to domestic dog *ZFY* and 99.5% to jackal *ZFY* and dhole *ZFY*, respectively. In addition, the 959-bp Thai Bangkaew *ZFX* fragments were identical and showed 100% identity to domestic dog *ZFX*. These genetic data suggest that the Thai Bangkaew dogs still present today share a common male ancestor with modern dogs, rather than being the descendants of dhole or jackal/dog hybrids.

Introduction

The Thai Bangkaew dog has been described as an alert, loyal and intelligent dog and its reputation is not only as a good companion, but also as the best protector of territory and owners (Fédération Cynologique Internationale, 2013). The ancestry of the Thai Bangkaew dog remains virtually unknown, though there is an unsubstantiated claim that the Thai Bangkaew dog originated around the 1880s when the foundation stock was established by mating between a female Thai domestic dog living in the Bangkaew Temple (from which the breed's name is derived) and a wild male canid living in the surrounding forest (Fédération Cynologique Internationale, 2013). Furthermore, since there are only two wild canid species—Asiatic jackal (*Canis aureus*) and dhole (*Cuon alpinus*)—native to Thailand (Higham et al., 1980), the sire was thought to be either one of these wild dog species. Thus, the identity of the male ancestral line of the Thai Bangkaew dog remains a mystery and further work is needed to clarify the breed's identity.

DNA technologies are playing a significant role in genealogical research and genetic data inferred from DNA polymorphisms among individuals can determine connections between the host genes (Shriver and Kittles, 2004). With the benefit of polymerase chain reaction (PCR) and sequencing technology, it has become possible to determine hereditary links between two or more species by comparing the similarity of their DNA sequences (Kocher, 1992). The genetic data can also be used to disclose clues about ancestral relations. In mammals including *Canis* species, the Y-chromosome is present only in males and inherited from fathers (Tsubouchi et al., 2012), so comparisons of Y-chromosome variation are suitable for testing whether a set of males alive today shares a common male ancestor. In addition, the zinc-finger region of the Y-chromosome (*ZFY*), rarely recombines with its X-linked homolog (*ZFX*) in male meiosis and is species-specific (Tsubouchi et al., 2012). The *ZFY* sequence is thus used as a DNA marker for

* Corresponding author.
E-mail address: fvetudb@ku.ac.th (U. Boonyaprakob).

http://dx.doi.org/10.1016/j.anres.2016.12.007

discriminating between wild and domestic dog paternal ancestry (Galov et al., 2014).

In order to investigate the evolutionary relationships between Thai Bangkaew dogs and other Thai canid species, this study sequenced about 1 kb of the zinc-finger region of the X and Y-chromosomes (*ZFX* and *ZFY*) from male Thai Bangkaew dogs, Thai domestic dogs (Thai ridgeback and mixed breeds), Asiatic jackals and dholes. A comparison of these sequences, allowed the question to be addressed of the genetic relationship between Thai Bangkaew dogs and Thai domestic or wild canids. The findings provide an opportunity to trace the paternal ancestor and understand the evolutionary history of the Thai Bangkaew dog.

Materials and methods

Samples and DNA extraction

The study was conducted in accordance with the Guidelines for the Use of Animals for Scientific Purposes, National Research Council of Thailand and was approved by the Animal Care and Use Committee of Kasetsart University, Bangkok, Thailand. For public-owned dogs, permission was obtained from each owner prior to sampling. Blood or plucked hair samples were obtained from three carnivore species (Table 1). Blood samples (about 1 mL) remaining after routine blood testing of healthy adult dogs ($n = 8$) were obtained from the Veterinary Teaching Hospital, Kasetsart University, Bangkhen campus, Thailand. The whole blood was centrifuged at $1500 \times g$ for 10 min at 4 °C. The plasma was removed and the remaining cells were washed at least twice with hypotonic lysis buffer to eliminate red blood cells (RBC). After RBC lysis, the remaining white blood cell pellet was used for extraction of DNA. Plucked hairs (40–60 hairs) from the top of the neck and the tip of the tail of the animals (male dogs, $n = 6$; female dogs, $n = 2$; male Asiatic jackal, $n = 2$; female Asiatic jackal, $n = 1$; female dhole, $n = 1$) were collected using a noninvasive hair sampling technique. Cells from a culture of ear skin fibroblast of a male dhole were kindly provided by Dr. Worawidh Wajjwalku, Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen campus, Thailand. Genomic DNA from all samples was isolated using the DNeasy® Blood & Tissue Kit (Qiagen; Hilden, Germany) according to the manufacturer's protocol.

Primer design and polymerase chain reaction amplification

A pair of primers for amplification of the canine *ZFX* and *ZFY* genes was designed based on the conserved regions between the *ZFY* cDNA of the domestic dog (JX964866), and human *ZFY* gene (AF114156). The sequence of the forward primer (dZFY1F) was 5′-CAA GTG CCC TCT TGC ACA TA-3′ and the reverse primer (dZFY2R) was 5′-TTC CAC AAA TCA TGC AAG GA-3′. An NCBI BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) also showed that the designed primers had sequence identity to the X-linked homolog (*ZFX*) of the dog gene. PCR amplification of canine *ZFX* and *ZFY* genes was carried out in a 50 μL reaction, with 50 ng of template DNA, 1 × concentrated PCR buffer without Mg^{2+}, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.2 mM each primer and 1.0 U Platinum Taq™ DNA Polymerase (Invitrogen, Life Technologies; Sao Paulo, Brazil). PCR reaction was performed in a GeneAmp® PCR system 9700 thermal cycler (Applied Biosystems; Foster City, CA, USA) with a thermal profile: 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min; followed by a final extension at 72 °C for 10 min.

Cloning and sequencing of the polymerase chain reaction products

PCR products were separated using electrophoresis in a 2.0% agarose gel and visualized using ethidium bromide staining. A small band (*ZFX*) and large band (*ZFY*) of each lane were excised separately, purified using a QIAquick Gel Extraction Kits (Qiagen; Valencia, CA, USA), and cloned by using the TOPO-TA Cloning Kit (Invitrogen; Carlsbad, CA, USA), following the manufacturer's instructions. Positive clones with expected insert sizes were selected using PCR screening, and incubated in Luria-Bertani broth with shaking at 37 °C overnight. Plasmids containing the insert DNA were isolated using the QIAprep Miniprep Kit (Qiagen; Valencia, CA, USA) and submitted to the First BASE laboratories (Malaysia) for automated sequencing. At least two clones for each band were selected for sequencing in both directions. The nucleotide sequences for final intron regions obtained in the present study were deposited in GenBank database under accession numbers KX065054 (dog *ZFY*), KX065055 (jackal *ZFY*), KX065056 (dhole *ZFY*), KX065057 (dog *ZFX*), KX065058 (jackal *ZFX*), and KX065059 (dhole *ZFX*).

Sequence analysis

Canine *ZFX* and *ZFY* sequences which were obtained following amplification with dZFY1F and dZFY2R were aligned with ClustalW (Thompson et al., 1994). Multiple base pairs behind the forward and reverse primers were manually removed from the 5′ end to the 3′ end of each amplified sequence in order to produce the length equal to the NCBI reference sequence for each species. Identical (redundant) sequences were removed from the alignment. All insertion and deletion sites were excluded from the analysis, and a phylogenetic tree was constructed using the neighbor-joining algorithm with 1000 bootstrap replications and a p-distance model in MEGA version 5.0 (Tamura et al., 2011). In addition, the wolf-like canids' *ZFY* and *ZFX* sequences obtained from GenBank (Table 2) were included in this analysis. The *ZFY* and *ZFX* sequences of Red fox (*Vulpes vulpes*) was used as an out-group.

Table 1
Species, breed and sex of Thai canids and the specific Thai localities used in the study.

Species	Breed	Sex	Samples (*n*)	Locality
Canis aureus (jackal)	–	Male	1	Bangkok (Dusit Zoo)
	–	Male	1	Kanchanaburi
	–	Female	1	Kanchanaburi
Cuon alpinus (dhole)	–	Male	1	Chachoengsao
	–	Female	1	Chachoengsao
Canis lupus familiaris (dog)	Thai Bangkaew	Male	3	Bangkok
	–	Male	6	Phitsanulok
	–	Female	2	Phitsanulok
	Thai ridgeback	Male	3	Bangkok
	Mixed breed	Male	2	Bangkok

Table 2
List of canine species used in phylogenetic analysis.

Scientific name	Common name	Accession numbers	
		ZFY	*ZFX*
Vulpes vulpes	Red fox	AB622140	AB622129
Cuon alpinus	Dhole	AB622143	AB622132
Canis mesomelas	Black-backed jackal	AB622144	AB622133
Canis aureus	Golden jackal	AB622145	AB622134
Canis latrans	Coyote	AB622146	AB622135
Canis lupus	Gray wolf	AB622147	AB622136
Canis familiaris	Dog	AB622147	AB622136

Table 3
Fragment lengths of *ZFX* and *ZFY* PCR products for each canine species.

Type of canid	Gene	Size (bp)	Gene	Size (bp)
Canis aureus (jackal)	*ZFX*	1000 ($n = 1$)	*ZFY*	1293 ($n = 1$) 1298 ($n = 1$)
Cuon alpinus (dhole)	*ZFX*	1000 ($n = 1$)	*ZFY*	1141 ($n = 1$)
Canis lupus familiaris (dog)	*ZFX*	999 ($n = 2$)	*ZFY*	1115 ($n = 11$) 1116 ($n = 3$)[a]

[a] Thai Bangkaew ($n = 1$); Thai ridgeback ($n = 2$).

Results

Polymerase chain reaction amplification and sequencing

DNA extracted from white blood cell pellets or plucked hairs of the canids was successfully amplified with the primer set designed in the present study. The amplified PCR product showed a single or two sex-specific bands from female or male genomic DNA corresponding to the *ZFX* or *ZFY* genes, respectively (Fig. 1). DNA samples from male jackals ($n = 2$) yielded double (XY) bands (1000 bp and 1293 bp or 1298 bp band, respectively), while female jackal ($n = 1$) had a single (XX) band (1000 bp). When this protocol was applied to dhole, two fragments of 1000 bp and 1141 bp were found for the male ($n = 1$) and a single product of 1000 bp was found for the female ($n = 1$). Similarly, DNA samples from male domestic dogs (Thai Bangkaew, Thai ridgeback, and mixed breed) yielded two fragments of 999 bp and 1115 bp ($n = 11$) or 1116 bp (Thai Bangkaew, $n = 1$ and Thai ridgeback, $n = 2$), while a single product of 999 bp was found in all females tested (Thai Bangkaew, $n = 2$). The size of the PCR products for each species is presented in Table 3.

Comparison of canine ZFX and ZFY amplicons

The PCR-amplified fragments for *ZFX* genes were very similar in size (Fig. 1 and Table 3) and displayed a high degree of sequence identities ranging from 99.1% to 99.6% for the three canine species. The BLAST search against nucleotide databases showed that the nucleotide sequences for the last intron of the Asiatic jackals, a dhole and domestic dogs obtained in this study had a 100% match for the *ZFX* gene in a Golden jackal (*Canis aureus*; AB622134), a dhole (*Cuon alpinus*; AB622132), and a dog (*Canis lupus*; AB622136), respectively.

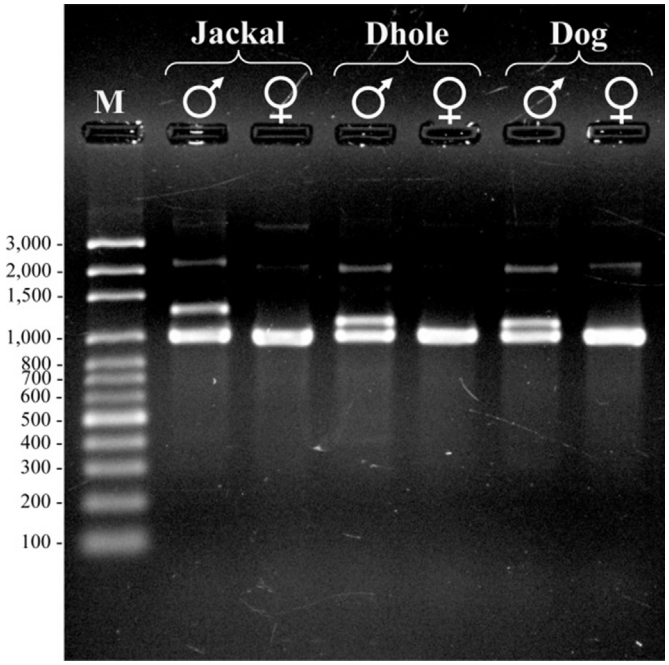

Fig. 1. Electrophoretic pattern of PCR amplified products from the final introns of *ZFX* and *ZFY* in 3 species of canids. Thai Bangkaew dogs were used as representatives of the domestic dogs. Male (♂) and female (♀) are shown for each species. M: 100 bp Ladder DNA Marker (Axygen BioScience; Union City, CA, USA).

Using the same set of PCR primers, the amplified products for the *ZFY* genes from male samples showed differences in size among species. The sequencing data showed that fragments at 1293 bp and 1298 bp were amplified from two male jackal individuals. Alignment of the two sequences showed that the difference in length (5 bp) was based on the number of T residues (11 T residues and 16 T residues) in the poly-T region. At the same location, 17 T residues were observed in the golden jackal *ZFY* reference sequence (AB622145), which also contained an additional 9-bp (GTAAATAGG) segment located at 35 bp upstream from this poly-T region (Table 4). The same region of the *ZFY* gene obtained from all male dogs (a Thai Bangkaew's sequence was used as a representative) is also demonstrated in Table 4. The DNA sample derived from the male dhole yielded a 1141-bp fragment. In the BLAST search results, the obtained 1141-bp sequence showed a 100% match for a dhole (*Cuon alpinus*) *ZFY* gene (AB622143).

For the DNA samples from three domestic dog breeds (Thai Bangkaew, Thai ridgeback and mixed breeds), the PCR primer set amplified a product of 1115 bp (11/14; 78.6%) or 1116 bp (3/14; 21.4%) in length for the *ZFY* gene. Analysis against GenBank revealed that the 1115-bp fragment shared 100% sequence identity to nucleotide positions 654,623 to 655,737 of the *Canis lupus familiaris* chromosome Y genomic sequence (GenBank accession number KP081776). Sequence comparison showed that the 1116-bp fragment contained an additional A-residue at position 654,810 of the chromosome Y genomic sequence. When 40 bp primer sequences were excluded, a 1075-bp *ZFY* segment of the Thai Bangkaew dogs shared 100% sequence identity with the domestic dog *ZFY* (Thai ridgeback and mixed breed), and (if gaps are allowed) showed 81% and 92% identity to jackal *ZFY* and dhole *ZFY*, respectively. However, if alignment gaps were treated as missing data, the 1045-bp *ZFY* sequence for the Thai Bangkaew dogs was 100% identical to the domestic dog *ZFY* and, 99.5% to the both jackal *ZFY* and dhole *ZFY*. In addition, the 959-bp Thai Bangkaew *ZFX* fragments (excluding 40 bp primer sequences) were identical (100% sequence identity) to domestic dog *ZFX*, but shared 99.7% and 99.2% identity to jackal *ZFX* and dhole *ZFX*, respectively.

Phylogenetic analysis of Thai canid ZFY and ZFX

The last intron of the Y-chromosome-specific zinc-finger gene (*ZFY*) and its X homolog (*ZFX*) sequence data from three canid species endemic to Thailand and known canine reference sequence from GenBank were used to examine phylogenetic relationships between Thai Bangkaew dog and other wolf-liked canids. A 921-bp segment of the final intron of *ZFY* gene was compared and showed

Table 4
Comparison of nucleotide sequences from the *ZFY* gene final intron of the jackals and dogs. Dashes indicate gaps in one sequence compared with another.

Animals	Nucleotide Sequence	Reference
Golden jackal	CAAATAGGCTTTGAGTCAGAGTAAATAGGCATTGAGGACAGAGTAAAGTCTCAGGATATTTTAA $(T)_{17}$ AAAGA	AB622145
Asiatic jackal	CAAATAGGCTTTGAGTCAGA---------CATTGAGGACAGAGTAAAGTCTCAGGATATTTTAA $(T)_{16}$ AAAGA	This study
Asiatic jackal	CAAATAGGCTTTGAGTCAGA---------CATTGAGGACAGAGTAAAGTCTCAGGATATTTTAA $(T)_{11}$ AAAGA	This study
Dog[a]	CAAATAGGCTTTGAGTCAGA---------CATTGAGGACAGAGTAAAGTCT----------	This study

[a] a Thai Bangkaew's sequence was used as a representative.

that the *ZFY* sequences from all isolates obtained from three Thai domestic dog breeds (Thai Bangkaew, Thai ridgeback, and mixed breed) in this study, share 100% sequence identity to the *ZFY* final intron of the *Canis lupus* (*Canis lupus lupus*, gray wolf and *Canis lupus familiaris*, domestic dog; GenBank accession number AB622147). Then, a single *ZFY* sequence was used to represent them in the construction of a phylogenetic tree (Fig. 2A). Phylogenetic analysis of *ZFY* sequences showed that dog, gray wolf, coyote, golden jackal, Asiatic jackal, dhole and black-backed jackal were clustered together in the wolf-like canid clade similar to those reported by Tsubouchi et al. (2012).

For the analysis of the relationships between the canid's last intron segment of the *ZFX* gene, an 835-bp segment of the X homolog (*ZFX*) sequences from those animals were compared. The *ZFX* sequences from all three Thai domestic dog breeds showed 100% sequence identity to the *Canis lupus ZFX* gene (GenBank accession number AB622136). Then, a single sequence was selected as the representative of the group and used to build a phylogenetic tree. As expected, the neighbor joining tree shows that all the wolf-like canid species resided within the same clade, distinct from the clade of red fox, a representative of the outgroup species (Fig. 2B).

Discussion

Canids, like most placental mammals, have an XY/XX genetic sex determination system, in which the heterogametic sex is males (XY) and the homogametic sex is females (XX). As the Y-chromosome is present only in males and paternally inherited, genetic analysis of the Y chromosome, particularly the zinc-finger, Y-linked (*ZFY*) gene can then be used to trace the specific ancestral-descendant relationships (Galov et al., 2014). The *ZFY* gene and its homolog *ZFX* on the X chromosome are located outside the pseudoautosomal region of sex chromosomes and rarely recombine during meiosis (Page et al., 1987). In addition, both sex-linked genes are species-specific and so are used as a DNA marker for determining sex in canids (Lucchini et al., 2002). Tsubouchi et al. (2012) used PCR-based *ZFY*/*ZFX* assay with one pair of primers to simultaneously amplify the final introns of both genes in a single PCR, and examined the phylogenetic relationships in the family Canidae based on the *ZFY* and *ZFX* final intron sequences. This approach showed that the canid species were divided into three main clades—the red fox-like canids, the South American foxes, and the wolf-like canids—which agreed with previous phylogenetic studies using mitochondrial DNA (Wayne et al., 1997) and autosomal DNA (Bardeleben et al., 2005).

Thai Bangkaew dogs have some feral, canine characteristics, such as wild instincts, aggressive temperament, lion-like furry mane, or well plumed tails, and local legend holds that the Thai Bangkaew dog may have descended from a hybrid between a female domestic dog and a male wild canid (Fédération Cynologique Internationale, 2013). Since there is no fossil or living evidence of wolves existing in Thailand, two existing wild Thai canid species (Asiatic jackal and dhole) have become the suspected ancestor of

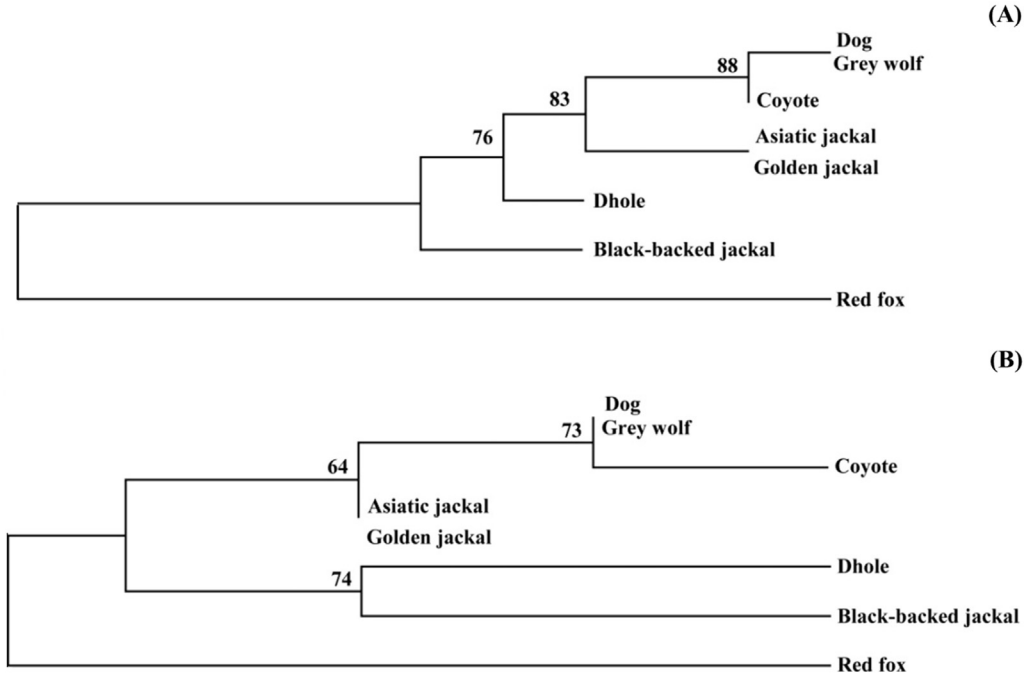

Fig. 2. Phylogenetic tree of final intron sequences from canid *ZFY* genes (A) and *ZFX* (B). The tree was constructed using the neighbor-joining method with the two-parameter distance model in MEGA version 5.0 (Tamura et al., 2011). Bootstrap support values (1000 replicates) are indicated above branches. A branch (Thai Asiatic jackal and Golden jackal) with less than 50% bootstrap support has been collapsed.

the Thai Bangkaew dog. As members of the wolf-like canids, jackals, dholes and domestic dogs are closely related species and have the same chromosome number ($2n = 78$), allowing them to interbreed and produce fertile offspring. Recently, genetic evidence of hybridization between golden jackal and domestic dog has been published (Galov et al., 2015). However, little is known about the existence of a hybrid between dogs and dholes.

Since there is no empirical evidence to support the speculation that the Thai Bangkaew dog is descended from jackal or dhole lineage, the present study designed a pair of gene-specific primers to separately amplify a portion of the last intron of the *ZFX* or *ZFY* genes in canids. In this process, the genotypic sex of each animal was determined and subsequent gene sequence analysis of the *ZFX* and *ZFY* segments was used to verify species identification and determine the genetic relationships between the three species of canids. The results did not support the claim that ancestors of the Thai Bangkaew dog originated from cross-breeding between a female dog and either a male jackal or dhole. Instead, the sequences found in all the Thai Bangkaew *ZFY* final intron segments were identical to sequences of the same region found in other Thai domestic dog breeds (Thai ridgeback and local mixed breeds) as well as sequences from the GenBank reference sequence from wolves and dogs. In addition, amplified *ZFX* sequences from the Thai Bangkaew and other domestic dogs were of equal size and shared 100% sequence identity with the *Canis lupus ZFX* gene.

In genetics, the last intron of *ZFY* is paternally inherited without recombination and so individuals that have a common paternal ancestor should have the same paternally derived *ZFY* (Tsubouchi et al., 2012). However, the present study showed that the nucleotide sequences of both the *ZFY* and *ZFX* gene final intron of the male Thai Bangkaew dogs are more closely related to that of domestic dog *ZFY* and *ZFX* than to that of male jackal or dhole *ZFY* and *ZFX*. Additionally, an examination of the fossil canid skull from Ban Chiang, Thailand found it was morphologically similar to that of the wolf and the domestic dog (Higham et al., 1980). It was also suggested that prehistoric domestic Thai dogs must be introduced since there is no evidence of native wolf in Thailand (Kijngam, 2010). The data taken together indicated that Thai Bangkaew dogs living today share a common ancestor with other domestic dogs; furthermore, the phylogenetic relationships within the Canidae based on *ZFY* and *ZFX* final intron sequences obtained in this study were in agreement with previous findings (Tsubouchi et al., 2012).

It is important to note that this study used a small sample size of wild male canids (one dhole and two jackals) due to the limited number of available wild dogs. However, for each wild canid species, the nucleotide sequence data obtained in the present study was supported by those sequences from the GenBank database. In addition, multiple Thai Bangkaew dog specimens were collected from the breeding population for which their genetic lines have been preserved by dog breeders in Phitsanulok province, Thailand. Thus, the current genetic data can be used to infer the likely evolutionary relationship between the Thai Bangkaew dogs and the other wild canid species. Based on the current findings, the 1075-bp *ZFY* sequences of the Thai Bangkaew dogs (where a gap was counted as one nucleotide substitution) shared only 81% and 92% identity to jackal *ZFY* and dhole *ZFY* sequences, while they showed a perfect 100% match to dogs (both Thai ridgeback and mixed breed) *ZFY*. Taken together, the results indicated that the Thai Bangkaew dogs and the other domestic breeds of dogs are the same species, namely *Canis lupus familiaris*. However, each breed of dogs through selective breeding over the years has changed physically and this has contributed to the differences among present day dog breeds.

Regardless of how the ancestral stock of the Thai Bangkaew dog became established, the breed was named after the place of the dog's origin—a Bangkaew village in Phitsanulok Province, Thailand. In the past, the Bangkaew village was surrounded by forest and the villagers lived on boats along river banks. During the rainy season, flooding caused a natural barrier that not only limited movement of animals, but also created habitat isolation. A consequence of continued inbreeding in the limited number of local dogs in the Bangkaew village and nearby areas might provide the foundation stock for today's Thai Bangkaew breed. Further selection and conservation projects as well as a campaign implemented by the Phitsanulok provincial livestock officers have made the breed well known and popular. Today, the Thai Bangkaew breed is accepted by the FCI, and regarded as part of the precious heritage of Thailand.

In summary, this study was a first analysis of paternal inherited genes (*ZFY*/*ZFX* region) for the identification of genetic evidence to support the legend that Thai Bangkaew dogs have descended from wild/domestic dog hybrids. However, the results revealed that the Thai Bangkaew dog known today and other domestic dogs are genetically similar, suggesting that the Thai Bangkaew breed shares a common male ancestor with modern dog breeds, rather than being descendants of dhole or jackal/dog hybrids. The existence of the legendary ancestors of Thai Bangkaew dogs (hybrid offspring of domestic and now extinct wild dogs) still remains to be investigated.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors wish to thank Dr. Nisit Tungtrakanpoung for providing information about the history of the founding population for the Thai Bangkaew dog and Dr. Worawidh Wajjwalku for providing cultured fibroblast cells from a male dhole. The assistance of staff at the Dusit Zoo, Bangkok, Thailand and Dr. Nucharin Songsasen from the Smithsonian Conservation Biology Institute, is appreciated for kindly providing hair samples from jackals and dhole; also, pet owners allowed tissue samples to be taken from their dogs. The authors are grateful to the Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand for providing facilities and funding support.

References

Bardeleben, C., Moore, R.L., Wayne, R.K., 2005. A molecular phylogeny of the Canidae based on six nuclear loci. Mol. Phylogenetics Evol. 37, 815–831.

Fédération Cynologique Internationale, 2013. Thai Bangkaew Dog. FCI-Standard Number 358. Thuin, Belgium, 4 September 2014. www.fci.be/Nomenclature/Standards/358g05-en.pdf.

Galov, A., Sindičić, M., Gomerčić, T., Arbanasić, H., Baburić, M., Bošković, I., Florijančić, T., 2014. PCR-based Y chromosome marker for discriminating between golden jackal (*Canis aureus*) and domestic dog (*Canis lupus familiaris*) paternal ancestry. Conserv. Genet. Resour. 6, 275–277.

Galov, A., Fabbri, E., Caniglia, R., Arbanasić, H., Lapalombella, S., Florijančić, T., Bošković, I., Galaverni, M., Randi, E., 2015. First Evidence of Hybridization between Golden Jackal (*Canis aureus*) and Domestic Dog (*Canis familiaris*) as Revealed by Genetic Markers, 2 December 2015. http://rsos.royalsocietypublishing.org/content/2/12/150450.

Higham, C.F.W., Kijngam, A., Manly, B.J.F., 1980. An analysis of prehistoric canid remains from Thailand. J. Archaeol. Sci. 7, 149–165.

Kijngam, A., 2010. The mammalian fauna. In: Higham, C.F.W., Kijngam, A. (Eds.), The Origins of the Civilization of Angkor, Volume IV. The Excavation of Ban Non Wat. Part II: the Neolithic Occupation. The Thai Fine Arts Department, Bangkok, Thailand, pp. 189–197.

Kocher, T.D., 1992. PCR, direct sequencing, and the comparative approach. PCR Methods Appl. 1, 217–221.

Lucchini, V., Fabbri, E., Marucco, F., Ricci, S., Boitani, L., Randi, E., 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Mol. Ecol. 11, 857–868.

Page, D.C., Mosher, R., Simpson, E.M., Fisher, E.M., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., et al., 1987. The sex determining region of the human Y chromosome encodes a finger protein. Cell 51, 1091–1104.

Shriver, M.D., Kittles, R.A., 2004. Genetic ancestry and the search for personalized genetic histories. Nat. Rev. Genet. 5, 611–618.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Tsubouchi, A., Fukui, D., Ueda, M., Tada, K., Toyoshima, S., Takami, K., Tsujimoto, T., Uraguchi, K., et al., 2012. Comparative molecular phylogeny and evolution of sex chromosome DNA sequences in the family Canidae (Mammalia: Carnivore). Zool. Sci. 29, 151–161.
Wayne, R.K., Geffen, E., Girman, D.J., Koepfli, K.P., Lau, L.M., Marshall, C.R., 1997. Molecular systematics of the Canidae. Syst. Biol. 46, 622–653.