RT: **GENETIC STRUCTURE OF TANA RIVER PRIMATES**

LINKS BETWEEN DISPERSAL, HABITAT FRAGMENTATION AND THE POPULATION GENETIC STRUCTURE OF TWO ENDEMIC, ENDANGERED FOREST PRIMATES: THE TANA RIVER RED COLOBUS (*PROCOLOBUS RUFOMITRATUS*) AND THE CRESTED MANGABEY (*CERCOCEBUS GALERITUS*).

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ABSTRACT:

We analyzed the population structures of the mtDNA variation of the Tana River red colobus and crested mangabey to determine how they are influenced by dispersal and habitat fragmentation. The colobus and mangabey are critically endangered primates endemic to gallery forests in eastern Kenya. The forests are a Pliocene-Pleistocene refugium that has recently undergone significant habitat loss and fragmentation due to human activities. Mangabey females are philopatric, so we expected their mtDNA variation to be homogeneous within forest patches but to be heterogeneous between patches. In contrast, colobus have a female-biased dispersal and so we expected their mtDNA variation to be homogeneous within and between forest patches. We expected both primates to exhibit low levels of genetic diversity due to genetic drift, and to show a strong correspondence between genetic and geographic distance due to disruption of gene flow between forest patches. We found high levels of haplotype and nucleotide diversity as well as high levels of sequence divergence between haplotype groups in both species. However, the red colobus had significantly higher genetic variation than the mangabey. Both species showed strong inter forest patch genetic structure, but a significant correspondence between genetic and geographic distances was found only for the mangabey.

INTRODUCTION:

The current genetic diversity of a species has been influenced by many factors in the past. Past fluctuations in climate are known to be a major influence on the distribution of populations and therefore their genetic structure (Hewitt 2000). In Africa, climate change caused major shifts in faunal assemblages during a time interval lasting 5.3 million years during the Pliocene-Pleistocene epochs (deMenocal 2004; Bobe & Behrensmeyer 2004). During this interval, lower temperatures and increased aridity in East Africa reduced and fragmented tropical forests and left them as isolated fragments along major rivers and on high elevation areas (Bobe & Behrensmeyer 2004). These forest refugia have provided important habitat for forest dependent non-human primates over evolutionary time in East Africa (Fleagle 1999). Thus, the current population genetic structure of primates endemic to these forests should reflect their histories in these refugia. Furthermore, primates endemic to these forests are now vulnerable to further loss of genetic diversity because of additional forest reduction and fragmentation caused by human activities (Mace & Balmford 2000).

There are two main consequences of habitat loss and fragmentation for genetic variation. First forest loss reduces the census and effective population sizes of primates and therefore increases the rates of genetic drift. Second, further habitat fragmentation would increase the distances between forest patches and thus disrupt gene flow among populations (Young & Clarke 2000; Frankham 1996). Although the nature of the habitat matrix and the vagility of a species both influence the degree of isolation, gene flow is usually greater among populations that are in close geographic proximity. Therefore, such populations should be more similar at neutral loci at equilibrium conditions (Wright 1978; Kimura & Weiss 1964).

Of course, gene flow between populations is usually achieved by the dispersal of individuals. Therefore, if we understand how the pattern of dispersal influences the genetic structure of populations, we can gain important insights into how forest loss and fragmentation affect species with different dispersal patterns, and therefore the extent of their vulnerability to current habitat change. Few studies have compared the population genetic structure of species with divergent patterns of dispersal to examine how they are influenced by habitat loss and fragmentation over evolutionary time (Johnson et al. 2003; Hutchison & Templeton 1999).

The Tana River forests of southeastern Kenya are an example of forest fragments whose origin dates back to the increasing aridity of the Pliocene-Pleistocene interval in East Africa (Bobe & Behrensmeyer 2004). The forests occupy the lower floodplain of the Tana River and are of great conservation importance. They are part of the east African coastal forests global biodiversity hotspot (Myers et al., 2000) and support a high diversity of rare plant and animal species (Andrews et al., 1975). In particular, they provide the only known habitat of two endemic primates: the Tana River red colobus (*Procolobus rufomitratus*) and the crested mangabey (*Cercocebus galeritus*). Both species are critically endangered (Hilton-Taylor 2000) and rank among the IUCN's top 25 most endangered primates (Grubb *et al.* 2003; Mittermeier *et al.* 2002). In addition to the natural forest fragmentation caused by the meandering of the river in its old stage, recent human

activities have further reduced and fragmented the forests causing precipitous declines in the primate populations, and extinctions in several of the fragments (Mbora & Meikle 2004). Thus, these forests offer a natural setting to study both short-term and long-term effects of forest loss and fragmentation on population genetic structure of endemic, endangered forest primates.

The population structure seen in mitochondrial DNA can be particularly useful in understanding the effects of forest loss and fragmentation on population genetic structure of forest primates. Mitochondrial DNA is maternally inherited (Gyllensten *et al.* 1985), lacks recombination (Hayashi *et al.* 1985) and exhibits rapid sequence evolution (Brown *et al.* 1979). Consequently, any mtDNA lineages that diverge in populations (e.g. in forest fragments) are independent clones that rapidly accumulate divergent sets of mutations through time. Thus, in species with male-biased dispersal, there should exist little or no variation within, and much variation between populations, *e.g.* in many macaque species (Melnick & Hoelzer 1992) and vervet monkeys (*Cercopithecus aethiops aethiops*; Shimada 2000). In contrast, female dispersal should lead to much differentiation within and less differentiation between populations; *e.g.* in the hamadryas baboons (*Papio hamadryas hamadryas*; Hapke *et al.* 2001).

We analyzed the population structures of mtDNA variation (ND4 region) of the Tana River red colobus and crested mangabey to determine how they are influenced by the pattern of dispersal and the changes in their forest habitat over time. The crested mangabey exhibits male-biased dispersal (Kinnaird 1992), while both sexes disperse in the red colobus (Marsh 1979). Thus, we expected the mtDNA variation in the mangabey to be relatively homogeneous within forest patches but to be heterogeneous between forest patches. Conversely, we expected the mtDNA variation of the red colobus to be relatively homogeneous within and between forest patches because females disperse in this species (Marsh 1979). We expected both primates to exhibit low levels of genetic diversity due to genetic drift in their relatively low populations, and for populations that were geographically close to one another to be more genetically similar because of greater gene flow (Wright 1978; Kimura & Weiss 1964).

MATERIALS AND METHODS:

Study area and Species

The study area comprises approximately 26 km^2 of gallery forest occurring in scattered patches of various sizes on both sides of the Tana River in eastern Kenya (Fig. 1; Mbora & Meikle 2004). These forests exist in an arid environment with an annual total rainfall of less than 400 mm. Forest is created and maintained by groundwater, and by periodic flooding of the river (Hughes 1990). The depth of the water table drops off rapidly from the edge of the river and limits the lateral extent of the forests to about 1 km on either side (Hughes 1990). The intervening matrix is mainly cultivated land, riparian grassland and dry shrubs.

We mapped the gallery forest using aerial photographs taken in 1994 and 1996, and selected 14 forest patches as study sites. We chose forest patches so that approximately equal forest area was sampled east and west of the Tana River to capture the range of

habitat conditions within the floodplain. We surveyed each study forest to determine the number of resident groups of colobus and mangabeys, and identified a subset of groups within each forest for detailed studies of group size, age and sex composition over time. We systematically selected social groups that were easy to locate and to identify using "marker" animals, as study groups. Since 2001, we have periodically surveyed the forests and monitored all the study groups (Mbora & Meikle 2004; Mbora, unpublished data).

The Tana colobus and mangabey are of similar body size but their behavioral ecology and life history strategies are quite different (Kinnaird1992; Marsh 1979). The red colobus is a habitat specialist with limited vagility, is almost exclusively arboreal and lives in relatively small social groups that exhibit high site fidelity (Marsh 1981). A canopy dweller, the colobus depends on a diet of mainly leaves obtained from a limited number of canopy tree species (Mbora & Meikle 2004; Marsh, 1981). Thus, it is relatively easy to locate and observe colobus groups, to maintain contact with them and to determine group composition. In contrast, the mangabey is a habitat generalist that is mostly terrestrial and highly vagile. It lives in much larger social groups and its diet comprises seeds and ripe fruit from a variety of tree species, and substantial amounts of animal prey (Kinnaird1992; Wieczkowski 2004). Mangabeys are quite skittish and it was necessary to get groups well habituated to human presence in order to determine their size and composition and to get fecal samples for mtDNA analysis. Consequently, detailed observation of mangabeys focus on fewer social groups than in colobus.

Collection of fecal samples and DNA extraction

In 2004 and 2005, from July to September, we collected fecal samples from study groups of colobus and mangabeys by following them from 0600hrs to 1130hrs, and then from 1500hrs until nightfall. Upon observing an animal defecate, we extracted a sample of the feces from the outermost part of the dung bolus using a sterile collecting stick while wearing latex gloves. The sample was placed into a tube containing 30 ml of 100% ethanol and labeled with a permanent marker to indicate the date, species identity, and coded to identify the troop and forest. The ethanol and sample were then mixed by inversion without shaking. The goal was to maintain the bolus form of the sample in order to avoid losing target cells along with the ethanol supernatant in the next step. After 36 hours, we carefully poured off the ethanol with the tube loosely capped, and transferred the remaining solid material into a new-labeled tube containing silica for further drying and storage (Nsubuga *et al.* 2004). The second tube was also labeled with a permanent marker as above. The samples were stored at a cool temperature in a tent in the field, and at -80° C after arrival in the laboratory. We made every effort to obtain samples from as many different individuals, within the study groups, as possible.

Approximately one fifth of the dried sample, (≅ 200 mg) was extracted using the QIAamp DNA Stool kit (Qiagen) according to the manufacturer's instructions with minor modifications (Nsubuga *et al.* 2004). The dried samples were vortexed in 1.6 mL of ASL buffer and left overnight (12–16 h) in an agitator at 25 °C. The intermediate steps followed the manufacturer's protocol, but we included an incubation step of 20 min followed by centrifugation for 2 min (Nsubuga *et al.* 2004) in the final step of the procedure where buffer AE elutes the DNA.

DNA for positive controls in PCR reactions (see below) was extracted from tissue samples following standard phenol extraction methods (Dowling *et al.* 1990). Colobus DNA was extracted from black and white colobus (*Colobus guereza*) muscle tissue donated by Dr. Cathi Lehn (American Zoo and Aquarium Association's Biomaterials Banking Advisory Group). Mangabey DNA was extracted from ethanol preserved muscle tissues acquired in Tana River following a fatal attack on a mangabey by an unidentified bird of prey in one of the forests in August 2005.

Genotyping and sequencing

We amplified and sequenced DNA from 53 colobus individuals from 10 forests, and 36 mangabey individuals from 6 forests; 40 colobus and 18 mangabey samples were drawn from study groups in the same forests. The following primer pair (obtained from Dr. C. Lehn) was used at a concentration of 5mM; STRETCHM (5'-RCT TGC GTT GAG GCG TTC TG, H11196) and ND4#1 (5'-CTT CTA ACA CTR ACC GCC TGA CT, L10952). The primers are located in the NADH 4 region corresponding to site 917-1119 of the mitochondrial genome (accession no. U92950). We used 1μL of the eluate from the extraction procedure as template in a 50μL polyemerase chain reaction (PCR) containing HotStarTaq DNA polymerase, PCR buffer with 3 mM $MgCl_2$ and 400μM each dNTP (Qiagen). We performed a hot start PCR cycle in an MJ Research PTC-200 Peltier thermal cycler under the following conditions: an activation step at 95^o C for 15 min; followed by 45 cycles at 95^o C for 30 s, 57.9^o C for 30 s and 72^o C for 1 min, and a final extension step at 72^o C for 10 min. Each reaction included positive and negative controls, and the success of the PCR was assessed by gel electrophoresis of 5 μL of the product.

The remainder of the PCR product was then purified using the QIAquick PCR purification kit (Qiagen) following the manufacture's protocol, and cloned using pGEM®-T Vector Systems (Promega Corporation). We deemed it necessary to clone the PCR products because initial sequencing yielded more than one unique sequence per sample. We prepared overnight cultures of cells containing pGEM® Vector by picking individual ampicillin-resistant colonies from fresh plates, inoculating 2ml of LB broth containing 100μg/ml ampicillin and shaking samples overnight at 37°C. We harvested the bacterial cells by centrifugation and purified single stranded DNA by extraction and precipitation using GenElute™ Plasmid Miniprep Kit (Sigma) following the manufacturer's protocol. Samples were then sequenced with an Abi Prism™ 3100 genetic analyzer (Applied Biosystems) using a BigDye Terminator CycleSequencing Kit (Applied Biosystems). Sequences were then aligned using SEQUENNCHER™ (version 4.5) and verified for accuracy; only unique sequences from the same stool sample were included in subsequent analyses. We sequenced 203 base pairs of the colobus, and 205 base pairs of the mangabey.

Data Analyses

We treated all samples from the same forest patch as comprising a population. To compare the mtDNA sequence variation of the two species, we calculated the haplotype diversity, nucleotide diversity (π), and the proportion of nucleotide polymorphisms (θ) for each species (Nei 1987) using DnaSP v. 4.10 (Rozas *et al.* 2003). To examine the relationships between haplotypes detected in the two species, we computed a minimum

spanning network between haplotypes using ARLEQUIN 2.0 (Schneider *et al.* 2000), and then used the connection lengths between samples (Operational Taxonomic Units) to draw a diagram of the minimum spanning network of haplotypes. We constructed a neighbor joining phylogenetic trees using MEGA version 3.1 (Kumar *et al.* 2004) using MODELTEST (Posada & Crandall 1998) to determine the appropriate nucleotide substitution model for the data set. To investigate the possibility of a past bottleneck in both species, we conducted an analysis of pairwise sequence mismatch distributions (Rogers 1995) using ARLEQUIN 2.0 (Schneider *et al.* 2000). The sequence mismatch distributions in a population that has experienced a population bottleneck should be smooth and have a peak whose position identifies the time of the bottleneck (Harpending 1994).

We conducted two analyses to elucidate the role of habitat fragmentation in shaping the population structure of the mtDNA variation among populations (forest patches) for each of the two species. First, we conducted an analysis of molecular variance (AMOVA) to determine how mtDNA variation was partitioned among and within populations (Excoffier *et al.* 1992). Second, we calculated the genetic distance between populations as pairwise F_{st} values (Weir & Cockerham 1984) and measured geographic distances between populations as linear centroid-to-centroid distances between forests using ArcMap GIS. We then tested for the correspondence between geographic and genetic distance using a mantel test (Mantel 1967) and linear regression analyses in the R-package (Casgrain *et al.* 2005).

RESULTS:

Overall, we found significantly greater levels of genetic variability in red colobus than in mangabeys. We identified 34 haplotypes among the 53 red colobus sequences, and 18 haplotypes among the 36 mangabey sequences (Table 1; Figure 2). In addition, when we compared metrics that account for differences in number of unique sequences for each species, we also found that red colobus had significantly greater haplotype and nucleotide diversity than mangabeys (Table 1). Comparison of the minimum spanning networks among haplotypes highlights the major difference that underlies these differences in genetic diversity. The red colobus haplotypes form seven distinct groups that are each separated from the next closest group in the network by 17-24 nucleotide substitutions (the average distance between adjacent groups was 20.5 mutational steps (Figures 2a & 3). The mangabey network contained only two such haplotype groups separated by 30 mutational steps from one another (Figures 2b & 3).

Although they differed in the overall levels of genetic diversity, the two species showed simpler patterns of spatial population structure. Five of the seven haplotype groups identified in the red colobus were represented in four or more populations; two groups were found in six populations (Figure 4a). Similarly, the mangabey network had one diverse haplotype group that was widely distributed among the various populations, and a second haplotype group, that was found in only three populations (Figure 4b).

The sequence mismatch distributions exhibited a distribution with a peak (Figure 5; Table 2). However, the smooth and peaked mismatch distribution pattern was much more clearly defined in the colobus than in mangabey (Figure 5).

The analysis of molecular variance (AMOVA) suggested that both species exhibit significant among population genetic differentiation (Colobus F_{st} = 0.095; Mangabey F_{st} = 0.236; Table 3a; Wright 1978). However, we found a significant association between genetic distance (population pair wise F_{st}) and geographic distance for the mangabey (Mantel's r = 0.482, P= 0.033), and not for the colobus (Mantel' r = 0.132, P= 0.18; Fig. 6). Despite the significant result for the mangabey, very little of the variance in the genetic distances of the mangabeys was actually explained by geographic distance (Linear regression, R^2 = 0.0.08; Fig. 6b).

DISCUSSION:

Our data revealed interesting and unexpected similarities in the population genetic structure of these species. First, both species had surprisingly high levels of haplotype and nucleotide diversity, as well as high levels of sequence divergence between haplotype groups (Figure 2; Table 1). Second, populations of both species showed strong among population genetic differentiation, but little or no correspondence between genetic and geographic distances. We believe that the high genetic diversity, high sequence divergence and strong genetic structure are a consequence of the effects of habitat fragmentation on the long-term effective population sizes of the species, their dispersal patterns, and the processes of social group formation over time. We discuss how these factors may combine to produce to the similarity in genetic structure below.

The Tana River red colobus is one of a handful of primate species in which females transfer between social groups (Marsh 1979). However, this primate is also an arboreal habitat specialist in which little dispersal is assumed to occur between forest fragments. The high frequency of haplotypes shared among forests (Figure 4) suggests that dispersal does indeed occur between forest fragments (but see below for an alternative or complementary process that would lead to the same pattern of haplotype sharing). In contrast to the colobus, mangabeys exhibit male biased dispersal typical of cercopithecine monkeys (Kinnaird 1992). Thus, because female mangabeys are philopatric within forests, we expected the genetic variation in this species to be homogeneous within forests but to be heterogeneous among forests. However, our analyses showed that mangabey groups in many forests shared the same haplotypes. Since female philopatry in the Tana Mangabey is well established (Kinnaird 1992), the high level of haplotype sharing among forests is probably the result of shared common ancestry of the founding groups in those forests and the limited spread of new social groups in this primate (Melnick & Hoelzer, 1996). Among cercopithecine monkeys, new groups typically form by fissioning of existing groups along matrilines under conditions of environmental stress (*e.g.* food shortage in *Macaca sinica*: Dittus, 1988). Following group fissioning, daughter groups are usually characterized by a higher average level of within-group relatedness than the parent group (Melnick & Kidd 1983; Whitlock and McCauley, 1990). Because cercopithecine monkey social groups are generally characterized by low within group mtDNA diversity, group

fissioning followed by colonization of new areas should lead to homogeneity of mitochondrial haplotypes between forests (Melnick & Hoelzer 1996).
The high level of mtDNA haplotype diversity found in these two primates is atypical, most studies of primates have found low levels of mtDNA haplotype diversity among populations. For example, a study of bonobos (*Pan paniscus)*, found 4 haplotypes in 157 individuals drawn from 5 populations distributed across the Democratic Republic of Congo (Eriksson *et al.* 2004); a study of eastern gorillas (*Gorilla gorilla*) found 10 haplotypes in 107 individuals from 5 populations across central Africa (Jensen-Seaman & Kidd 2001); and just 26 haplotypes were found among 107 individuals from 4 populations of hamadryas baboons (*Papio hamadryas hamadryas*) distributed across Eritrea and Saudi Arabia (Winney *et al.* 2004). Similar low levels of mtDNA haplotype diversity are also commonly found in primate species that exhibit male-biased dispersal. For example, 24 haplotypes were found among 280 individuals sampled from 8 populations of Barbary macaques (*Macaca sylvanus*) distributed across Northern Africa and Gibraltar (Modolo *et al* 2005), and many species of macaques from Asia also show low levels of mtDNA haplotype diversity (*e.g.* Chu *et al.* 2005; Perwitasari-Farajallah *et al.* 2001). However, at least one other primate, the gray mouse lemur (*Microcebus murinus)* of Madagascar, also exhibits very high levels of mtDNA haplotype diversity within populations (Wimmer *et al.* 2002).

The mtDNA haplotype groups of both species in this study exhibited high levels of sequence divergences between haplotype groups, coupled with low levels of sequence divergence within groups (Figures. 2 and 3). Both the colobus and mangabey showed a mean sequence divergence between haplotype groups of 10% and 11% respectively. This too is atypical of non-human primates because primates usually exhibit low levels of sequence divergence. For example, mtDNA sequence divergence among eastern gorillas was found to be 0.8-1.8% (Jensen-Seaman & Kidd 2001), and that in long-tailed macaques was found to be 0-1.03% (Perwitasari-Farajallah *et al.* 2001). Nevertheless, high levels of mtDNA sequence divergence within the same populations are found in several other primate species: macaques, *Macaca cyclopis* (Chu *et al.* 2005); in the hamadryas baboon, *P. h. hamadryas* (Winney *et al.* 2004); and in the gray mouse lemur *(*Wimmer *et al.* 2002).
Why do the two Tana River primates exhibit high levels of haplotype diversity and high levels of sequence divergences among haplotype groups? High levels of sequence divergences between haplotype groups, coupled with low levels of sequence divergences within haplotype groups, are characteristic of populations that have experienced a population bottleneck in the past (Avise *et al.* 1987). In addition, studies show that an important source of different mtDNA lineages with large sequence divergences in the same populations is secondary contact between previously isolated populations (Avise *et al.* 1987; Taberlet *et al.* 1992). We believe that the history of habitat change in the study area could explain the high diversity of mtDNA haplotypes as well as the high levels of sequence divergences among haplotype groups.

Our analyses of pairwise sequence mismatch distributions suggest that the populations of the two primates experienced a major population bottleneck in the past (Rogers 1995; Figure 5; Table 2). Allowing for a large error associated with the estimation

of divergence time and based on the standard molecular clock rate for primate mtDNA of 2-4% per million years (Brown *et al.* 1979, 1982), the observed level of sequence divergence indicates that the haplotype groups observed have been diverging over the past 2-5.5 million years. Thus, these mtDNA polymorphisms seem to date back to the Pliocene-Pleistocene interval when major shifts occurred in the fauna of East African due to increased aridity (Bobe & Behrensmeyer 2004). Subsequently, the mtDNA polymorphisms may have been maintained by habitat heterogeneity in the landscape over time as follows. As far as we know, the Tana River forests have always been situated in the lower floodplain of the river. In this old stage, the river meanders widely and often changes course within the floodplain causing repeated fragmentation, isolation and reconnection of forest fragments over time (Mbora, personal observations). Consequently, primate populations in different forest patches must have experienced repeated extinctions and recolonizations over time, and therefore secondary contact between previously isolated mtDNA lineages can be assumed to have occured (Avise *et al.* 1987; Taberlet *et al.* 1992).

Our results have important implications for the conservation of these critically endangered primates. First, our finding that both species have very high levels of mtDNA diversity emphasizes the need to enhance protection and conservation measures for them. This is significant because some have argued, in the past, that because the populations of the two primates are very small, their genetic diversity may be already compromised and therefore may not be worth conserving (World Bank 1996 p. 23). Furthermore, our results show that the populations with the highest diversity haplotype groups (Figure 2) are located in the area of floodplain forest congruent with the location of the Tana River Primate National Reserve, which was established to protect these primates. Thus, we recommend that high priority be placed upon the protection and conservation of the primate populations found within the reserve. More generally, this study shows that endemic endangered primates can possess high levels of genetic diversity despite small fragmented populations. Thus, such species should not be discounted from conservation action because despite their small population size they may, evidently, possess remarkable resilience to genetic stochasticity.

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Figure 1: Study area indicating the distribution of the Tana River red colobus (a) and crested mangabey (b) study forests. Roman numeral codes identify the study populations.

Figure 2: Haplotype network of the Tana River red colobus (a) and crested mangabey (b). Colors represent different populations (forest patches) from which samples (OTUs) were obtained (Figure 1).

Figure 3: Neighbor joining tree of the red colobus (p) and the mangabey (cg) assuming Tamura-Nei Rates=gamma Shape=0.9513 Pinvar=0; calculated with Mega. Bootstrap values >50% (1000 reps) are shown along the branches.

Figure 4: Distribution of different haplotype groups in colobus (a) and mangabey (b) populations in the study area. Roman numeral codes identify the study populations, and haplotype groups as identified in Figure 2 above are indicated in brackets.

Figure 5: Sequence mismatch distributions for the Tana River red colobus (a) and crested mangabey (b).

Fig. 6: Analysis of isolation by distance; genetic distance (pairwise F_{st}) vs. geographic (m) distance between populations of Tana River red colobus (a) and crested mangabey (b).

LIST OF TABLES:

Table 1: Genetic variability of mtDNA in Tana River red colobus and crested mangabey

mtDNA attribute		**Colobus**	**Mangabey**
Number of Sites		203	196
Number of Sequences		53	36
Number of Segregating Sites (S)		83	52
Number of Mutations (Eta)		88	58
Number of Haplotypes		34	18
Haplotype Diversity (Hd)	Mean	0.96	0.80
	Variance	0.00	0.01
	Lower 95% CI	0.96	0.78
	Upper 95% CI	0.963	0.83
Nucleotide Diversity (Pi)	mean	0.12	0.05
	Variance	0.00	0.00
	Lower 95% CI	0.12	0.04
	Upper 95% CI	0.12	0.05
Theta (per site) from Eta		0.10	0.07
Theta (per site) from S	Mean	0.09	0.06
	Var (no recomb)	0.00	0.00
Theta (per site) from Pi		0.14	0.05
Theta (per site) from S		0.12	0.08
Theta (per site) from Eta		0.11	0.08
Average number of nucleotide differences	mean	24.33	9.27
	stochastic variance K (no recombination)	113.49	17.95
	Observed Variance	4.39	1.06
Raggedness		0.01	0.02
Fu's FS		-2.40	-1.48
P		0.04	0.08

Table 2: Results of the mismatch distribution analyses.

Species	τ	Observed mean	θ_0	θ_1	Raggedness
Colobus	27.41	24.57	3.62	81.99	0.013
	(21.14-39.14)	(18.69-31.38)	(0.0-9.07)	(55.38-450.12)	
Mangabey	1.20	9.94	1.59	5.12	0.021
	(0.0-7.854)	(0.74-5.61)	(0.0-4.97)	(1.47-5289.50)	

Values in brackets are 95% CI

Table 3a:AMOVA of the population structure of the Tana River red colobus

Source of Variation	Sum of squares (d)	Variance component	*P*	F_{st}	Percentage variation
Among populations	169.81 (10)	Va = 1.19	0.035	0.095	9.52
Within populations	485.54 (43)	Vb = 11.32			90.48

Table 3b:AMOVA of the population structure of the Tana River crested mangabey

Source of Variation	Sum of squares (d)	Variance component	*P*	F_{st}	Percentage variation
Among populations	59.87 (6)	Va = 1.218	0.024	0.24	24.64
Within populations	114 (29)	Vb = 3.93			90.48

Figure 1a

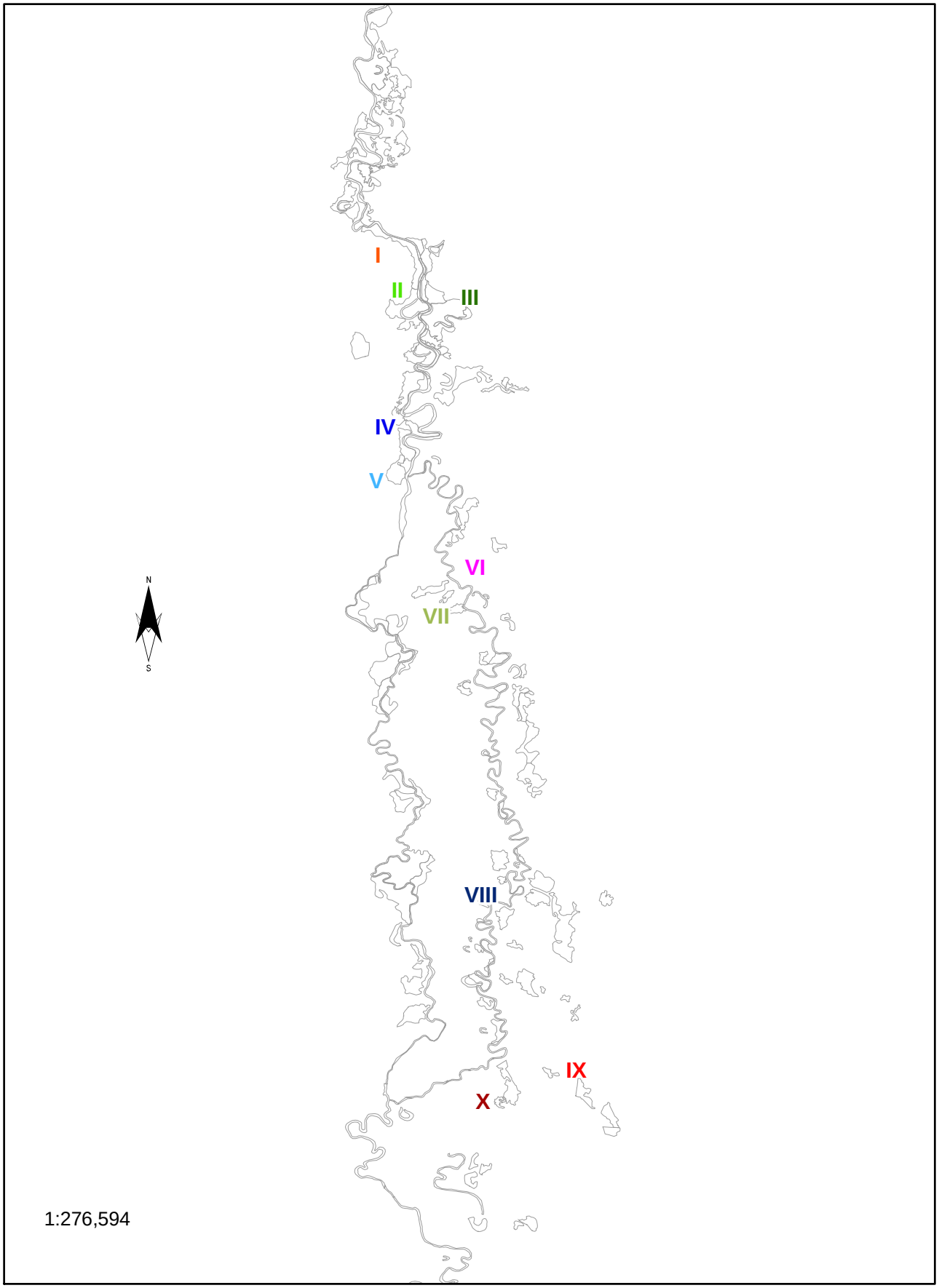


Figure 1b

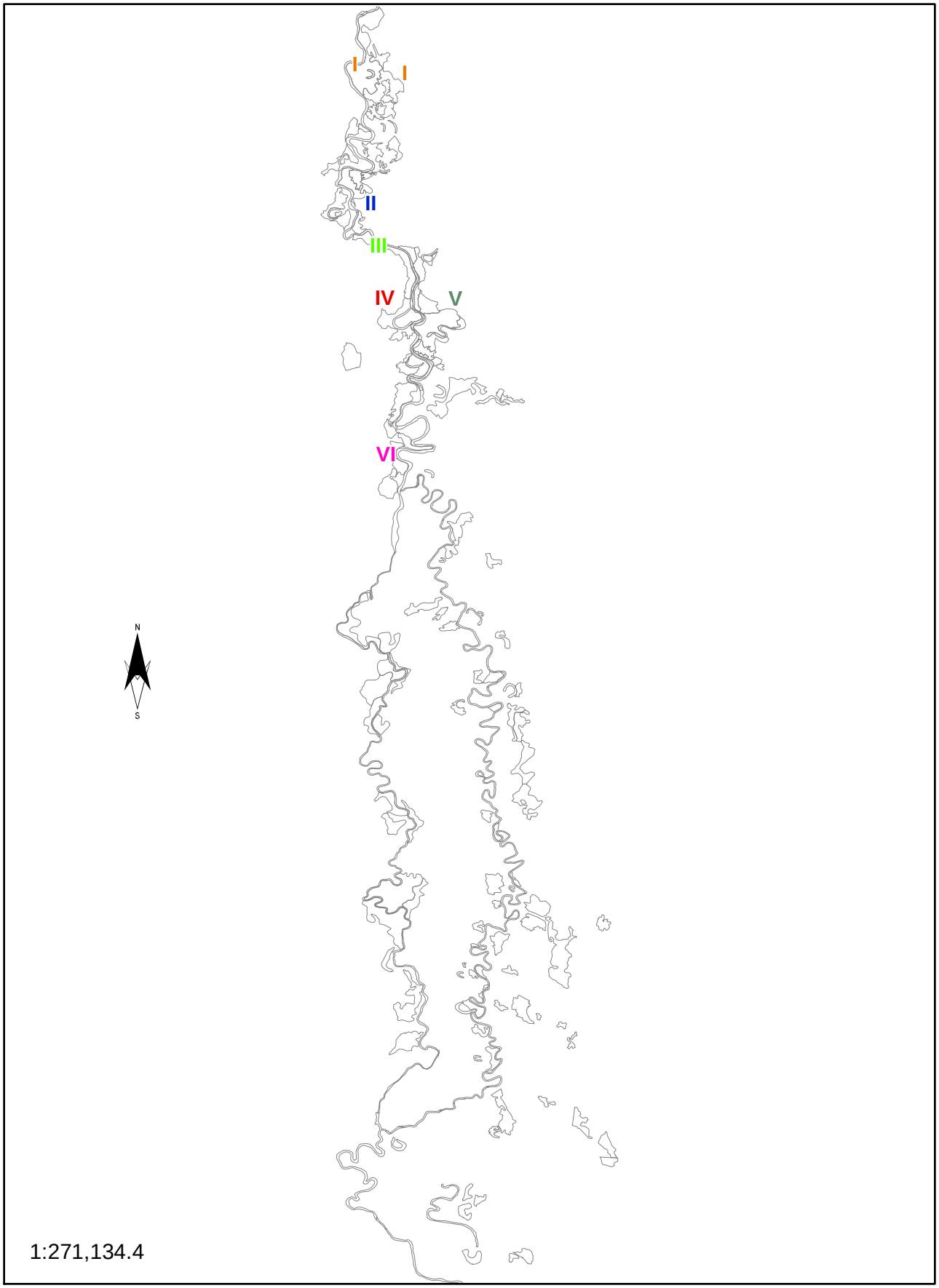
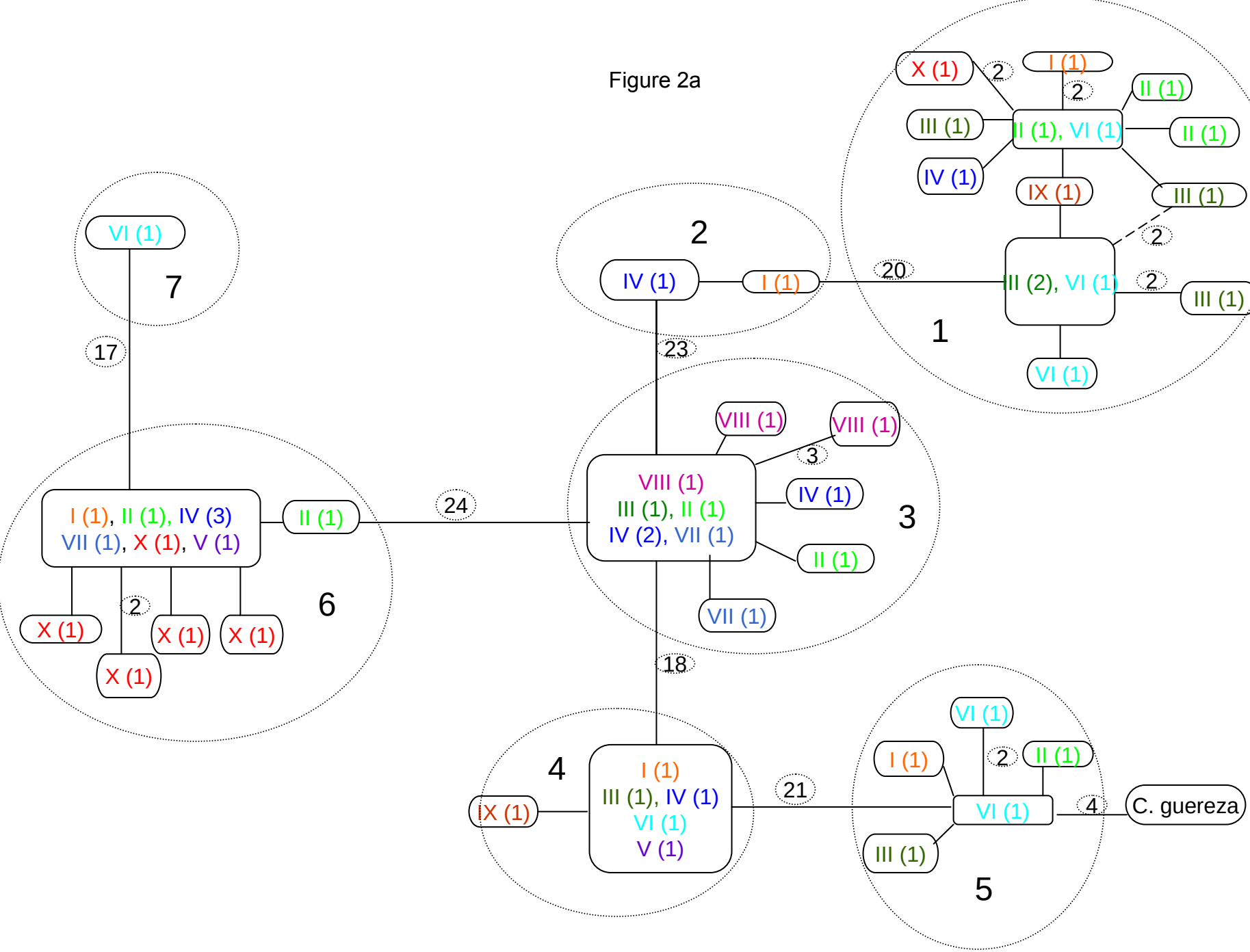


Figure 2a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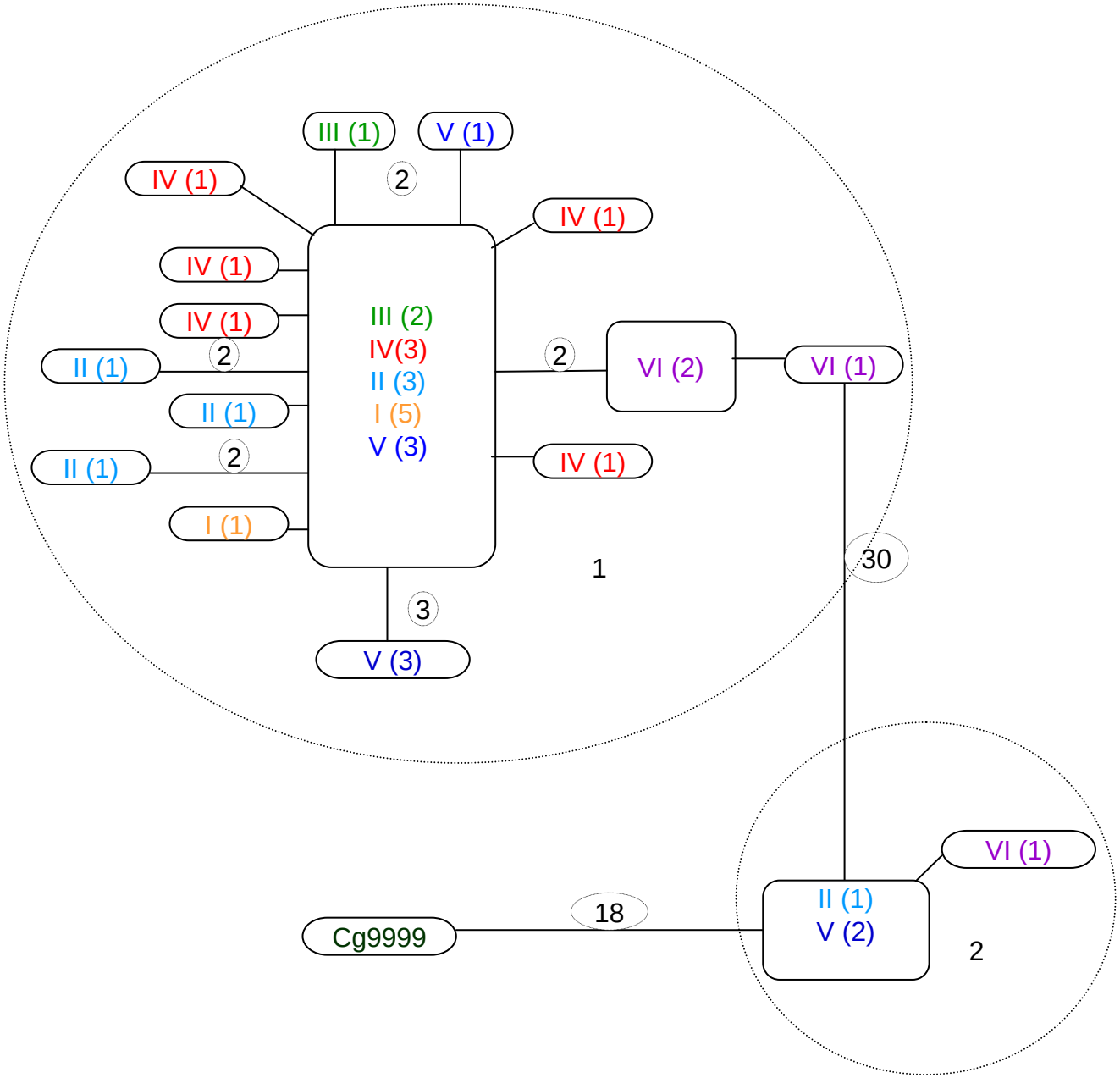
2
I (1)
2
II (1)
III (1)
II (1), VI (1)
II (1)
IV (1)
IX (1)
III (1)
2
20
II (2), VI (1)
2
III (1)
1
VI (1)
2
IV (1)
I (1)
23
VI (1)
7
17
VIII (1)
VIII (1)
3
VIII (1)
III (1), II (1)
IV (2), VII (1)
IV (1)
II (1)
3
VII (1)
I (1), II (1), IV (3)
VII (1), X (1), V (1)
II (1)
24
X (1)
2
X (1)
X (1)
X (1)
6
18
4
I (1)
III (1), IV (1)
VI (1)
V (1)
IX (1)
21
VI (1)
I (1)
2
II (1)
VI (1)
4
C. guereza
III (1)
5

Figure 2b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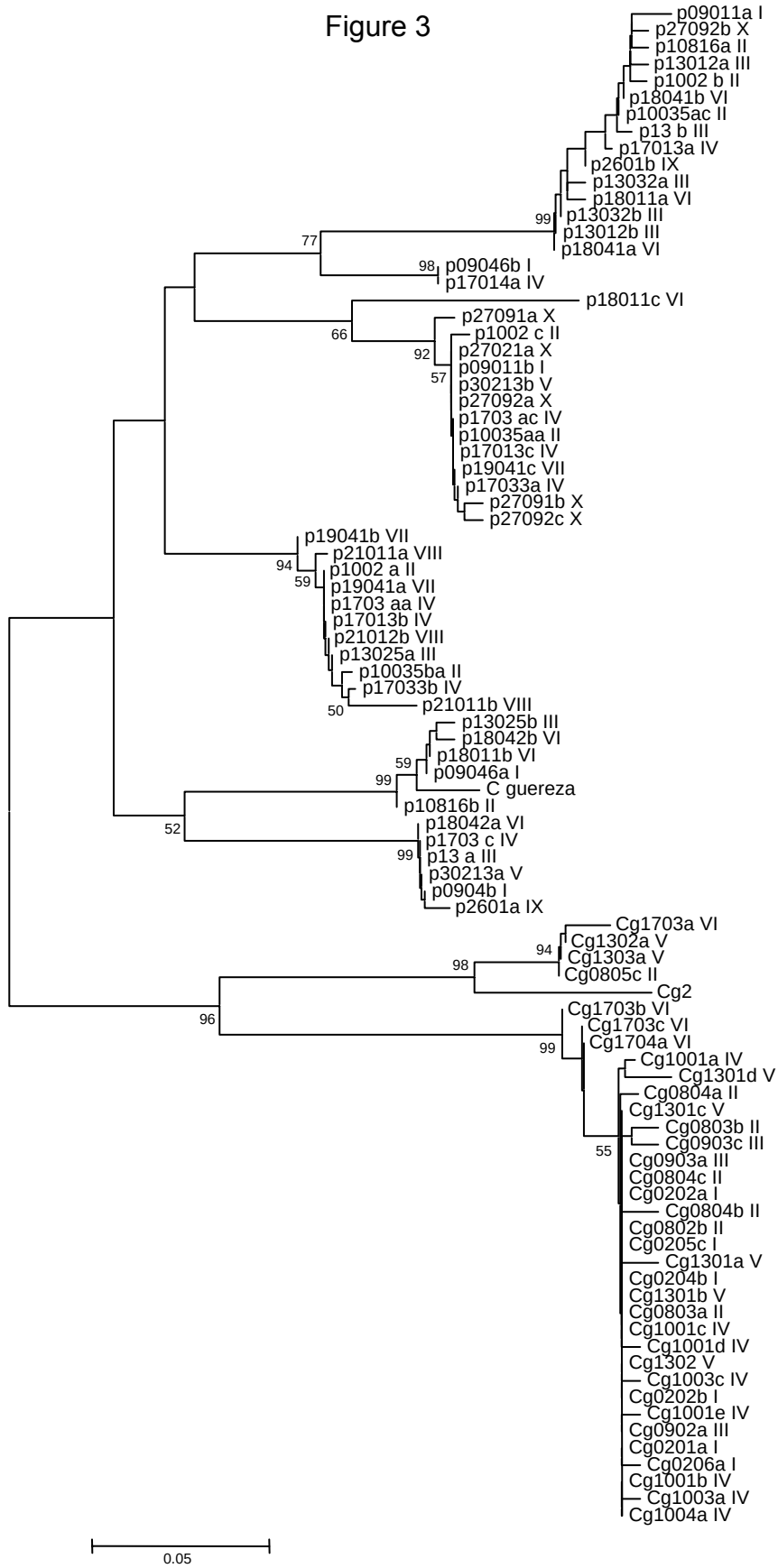
V (1)
2
IV (1)
IV (1)
IV (1)
IV (1)
III (2)
IV(3)
II (3)
I (5)
V (3)
II (1)
2
2
VI (2)
VI (1)
II (1)
II (1)
2
IV (1)
I (1)
1
30
3
V (3)
VI (1)
II (1)
V (2)
18
Cg9999
2

Figure 3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p27092b X
p10816a II
p13012a III
p1002 b II
p18041b VI
p10035ac II
p13 b III
p17013a IV
p2601b IX
p13032a III
p18011a VI
99
p13032b III
p13012b III
p18041a VI
77
98
p09046b I
p17014a IV
p18011c VI
66
p27091a X
p1002 c II
92
p27021a X
57
p09011b I
p30213b V
p27092a X
p1703 ac IV
p10035aa II
p17013c IV
p19041c VII
p17033a IV
p27091b X
p27092c X
p19041b VII
94
p21011a VIII
59
p1002 a II
p19041a VII
p1703 aa IV
p17013b IV
p21012b VIII
p13025a III
p10035ba II
p17033b IV
50
p21011b VIII
p13025b III
p18042b VI
59
p18011b VI
99
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C guereza
p10816b II
52
p18042a VI
p1703 c IV
99
p13 a III
p30213a V
p0904b I
p2601a IX
Cg1703a VI
94
Cg1302a V
Cg1303a V
98
Cg0805c II
Cg2
96
Cg1703b VI
Cg1703c VI
99
Cg1704a VI
Cg1001a IV
Cg1301d V
Cg0804a II
Cg1301c V
Cg0803b II
Cg0903c III
55
Cg0903a III
Cg0804c II
Cg0202a I
Cg0804b II
Cg0802b II
Cg0205c I
Cg1301a V
Cg0204b I
Cg1301b V
Cg0803a II
Cg1001c IV
Cg1001d IV
Cg1302 V
Cg1003c IV
Cg0202b I
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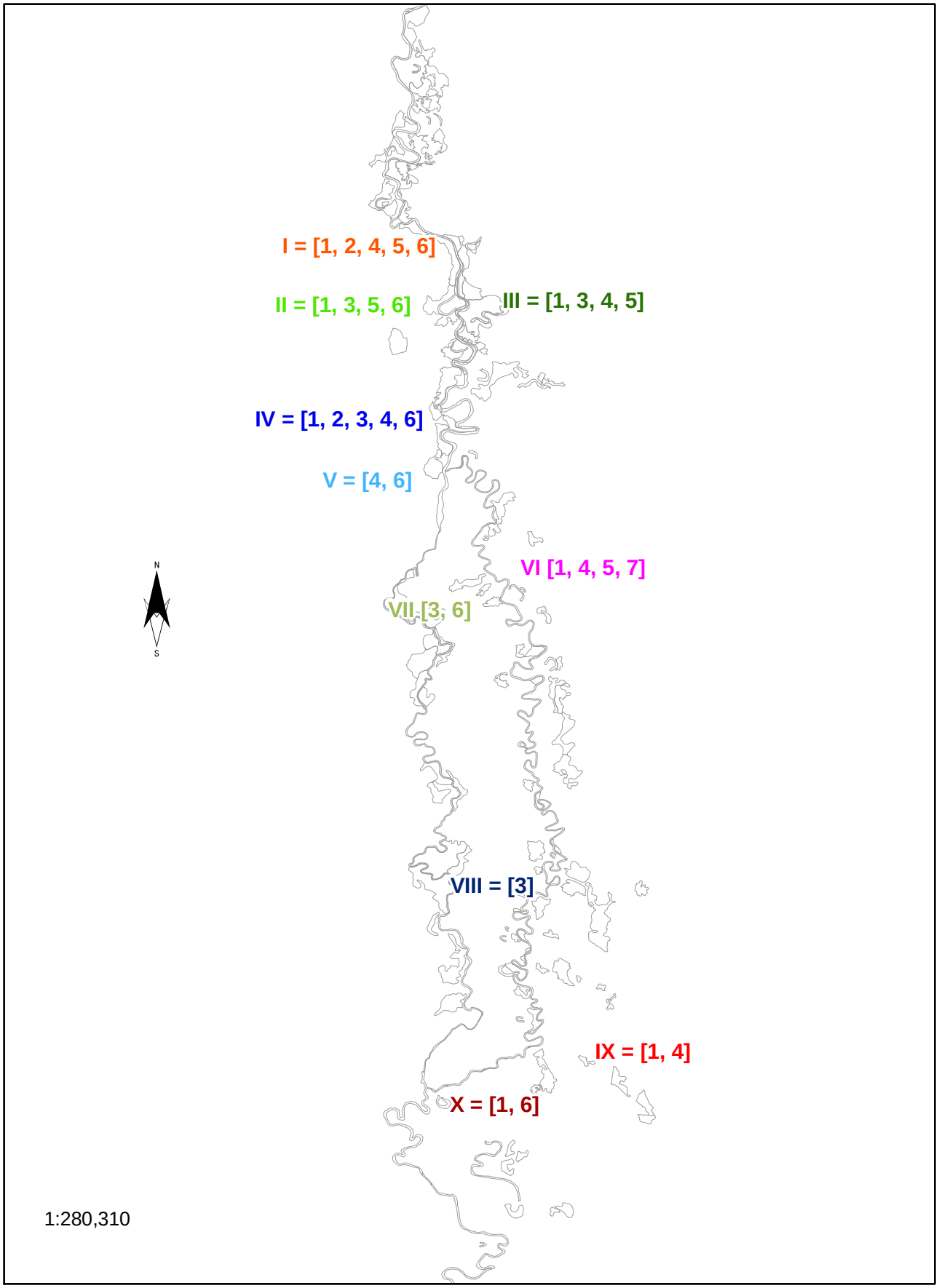
Cg1004a IV
0.05

Figure 4a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V = [4, 6]
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VII [3, 6]
VIII = [3]
IX = [1, 4]
X = [1, 6]
N
S
1:280,310

Figure 4b

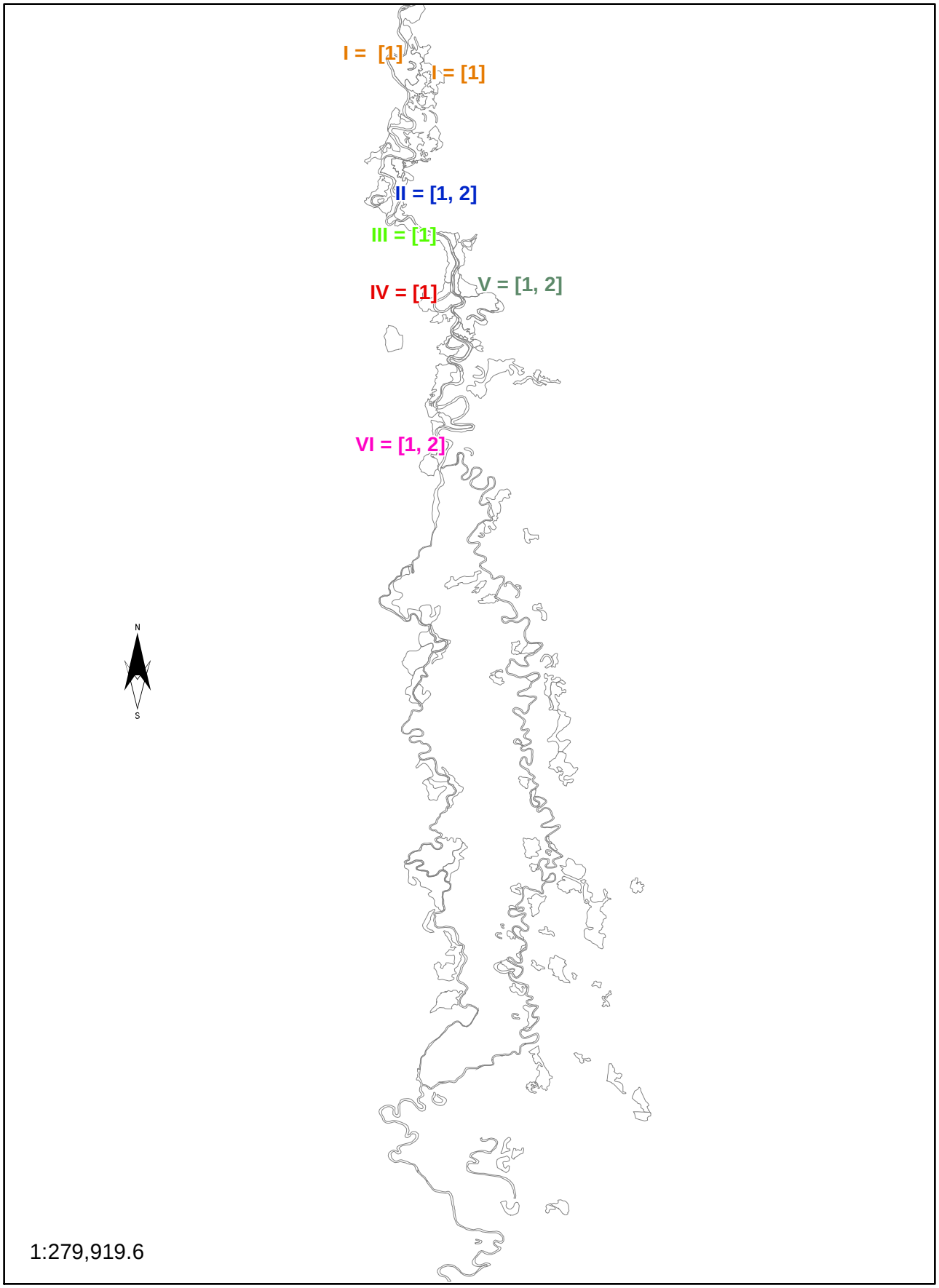


Figure 5a

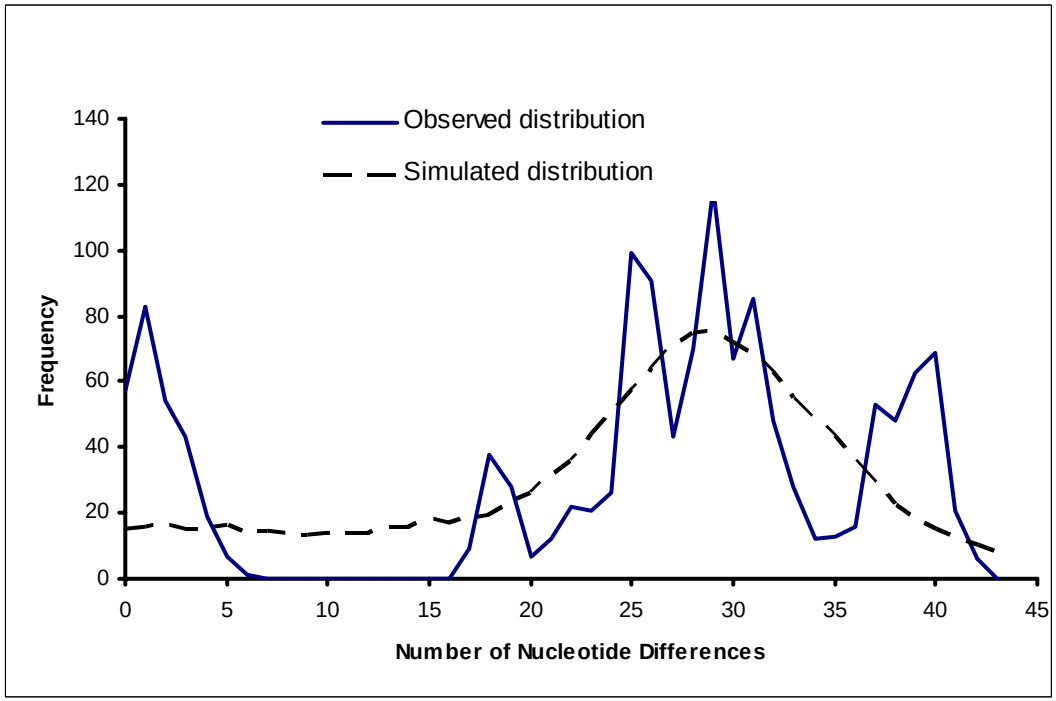

Observed distribution
Simulated distribution
Frequency
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Number of Nucleotide Differences

Figure 5b

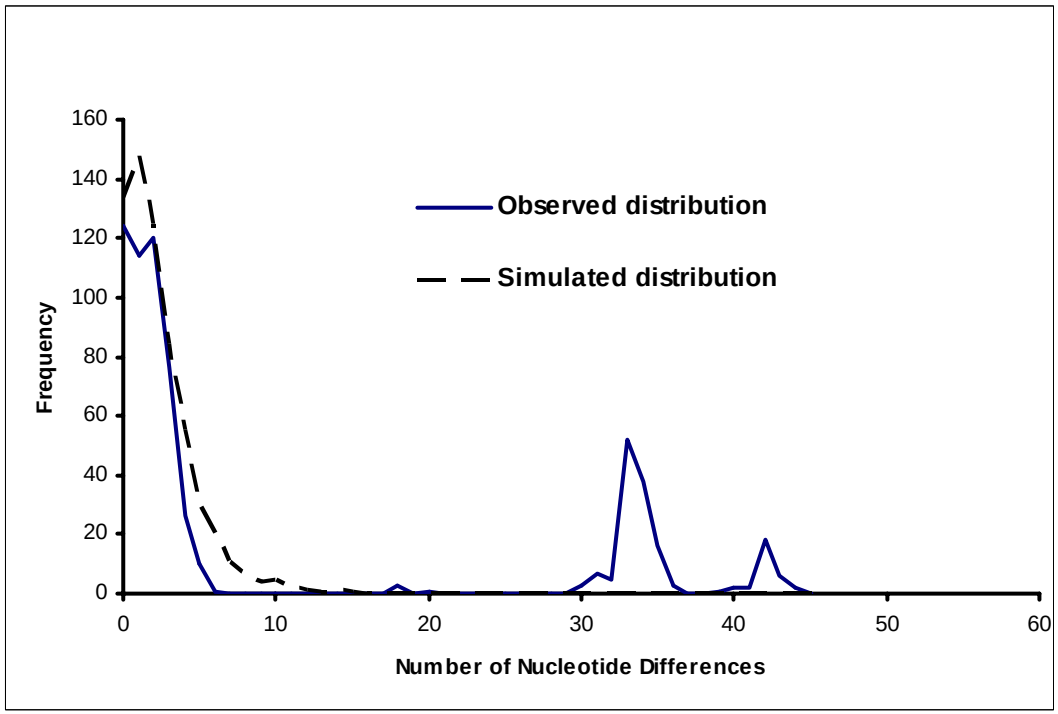
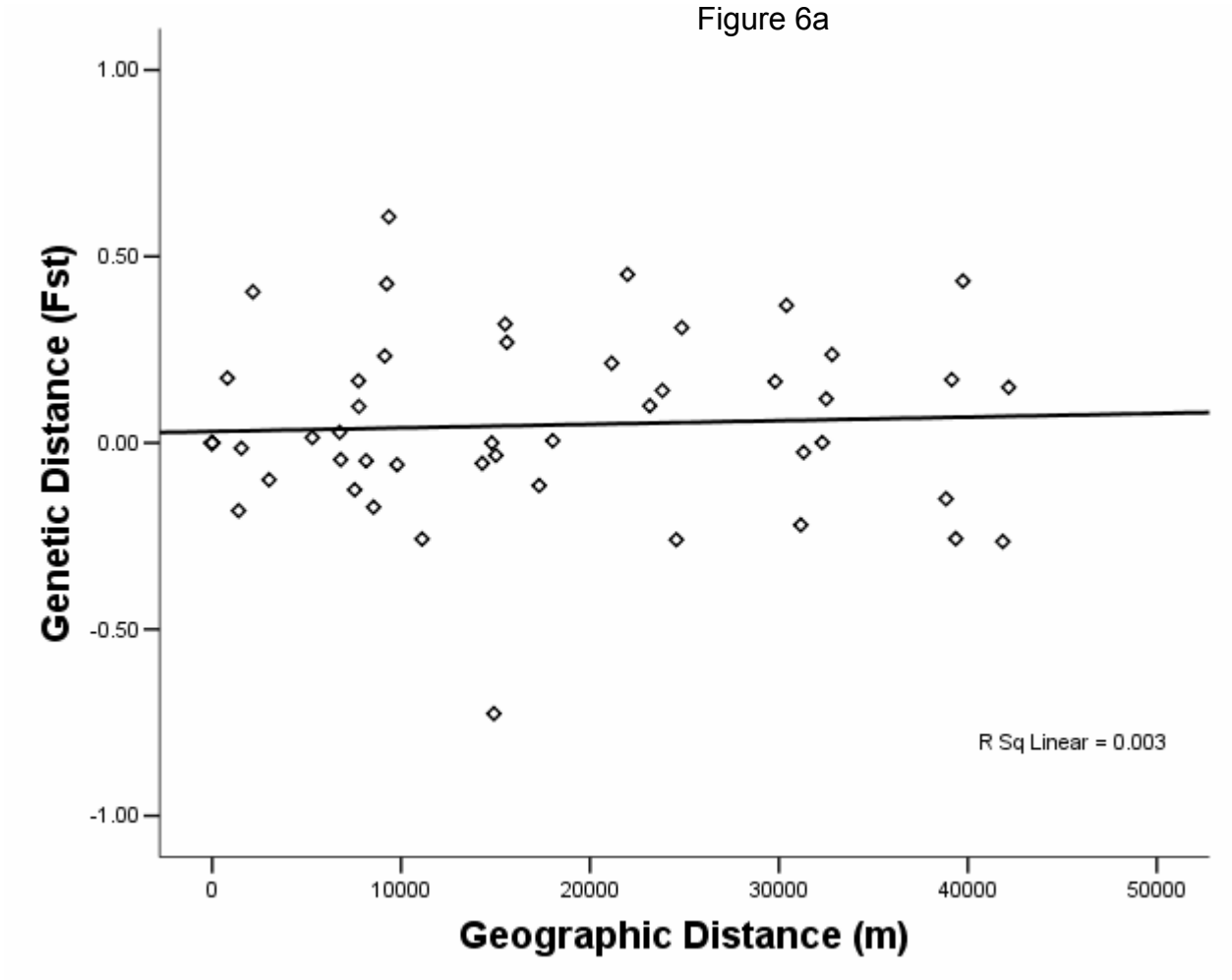

160
140
120
100
80
60
40
20
0
Frequency
0
10
20
30
40
50
60
Number of Nucleotide Differences
Observed distribution
Simulated distribution

Figure 6a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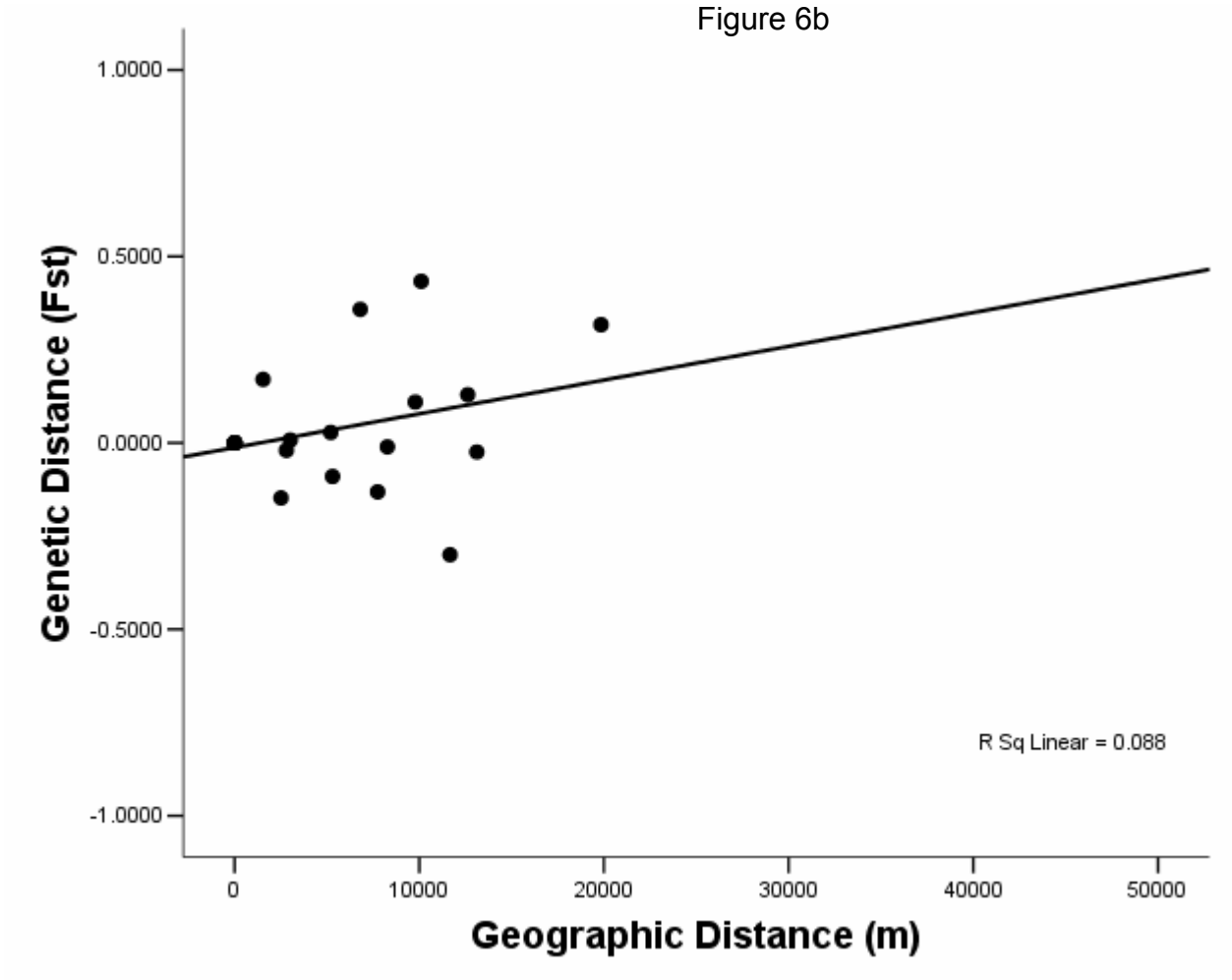
0.50
0.00
-0.50
-1.00
R Sq Linear = 0.003
0
10000
20000
30000
40000
50000
Geographic Distance (m)

Figure 6b


1.0000
0.5000
0.0000
-0.5000
-1.0000
Genetic Distance (Fst)
R Sq Linear = 0.088
0
10000
20000
30000
40000
50000
Geographic Distance (m)