Female brown bears in Sør-Varanger, Norway: localities and mother-cub relationships analyzed by genetic methods

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Female brown bear with cubs in Sør-Varanger. Photo: Steinar Wikan.

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Abstract: Knowledge on the number of female brown bears, especially reproducing females, is important for the wildlife management. One of the largest and densest populations of brown bears in Norway is located in Sør-Varanger, Finnmark, Northern Norway. Observations of females with cubs are reported regularly in the region. Information on the relatedness among individuals is often unknown as well as specifics on the number of reproductions and relatedness among females within this population. We have utilized genetic data originating from feces and hair samples collected in Sør-Varanger in the years 2004-2014 to investigate female brown bear localities. In the same period, personnel from the Norwegian State Nature Inspectorate (SNO) have observed 9 female brown bears with potential female cubs (*a priori* probability of 0.5). Sampling areas of those female brown bears and their potential offspring showed substantial geographical vicinity suggesting overlapping home ranges. We then calculated the likelihood ratios for these relationships using the forensic software Familias for 18-mother-female cub relationships. For 10 of 18 such relationships, the genetic relationship between mother and female cub were confirmed as their observation in the field was suggestive of. Of the initially observed 9 female bears, 6 have produced 10 female cubs, which here could be confirmed by genetic methods. The remaining 3 females were not excluded to be mothers to their potential cubs, but these relationships cannot be confirmed without additional DNA analyses. Another family relationship could also be confirmed between two observed female bears, but the type of relationship could not be determined.

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

2

Sammendrag: Kunnskap om antallet hunnbjørner og særlig hunnbjørner som reproduserer, er viktig for naturforvaltningen. En av de største og tetteste bestandene av brunbjørn i Norge er lokalisert i Sør-Varanger i Finnmark. Observasjoner av hunnbjørner med unger blir rapportert regelmessig fra denne regionen. Informasjon om slektskap mellom individer er ofte ukjent så vel som spesifikke antall ynglinger og slektskap mellom hunner i bestanden. Vi har brukt genetiske data som stammer fra ekskrement- og hårprøver samlet inn i Sør-Varanger i årene 2004-2014 for å undersøke hunnbjørn lokaliteter. I samme periode har personell fra Statens naturoppsyn (SNO) observert 9 hunnbjørner med mulige unger (a priori sannsynlighet på 0,5). Innsamlingsområdene fra disse hunnbjørnene og deres mulige avkom viste betydelig geografisk nærhet, noe som tyder på overlappende hjemmeområder. Vi beregnet deretter sannsynlighetsforholdet for slektskapene ved å bruke programmet Familias for 18 mor-unge forhold. For 10 av 18 slike forhold kunne det bekreftes et genetisk slektskap mellom mor og hunnbjørn-unge slik som observasjonene i felten tydet på. Av de opprinnelig 9 hunnbjørnene som ble observert med unger har 6 hunnbjørner produsert 10 hunnbjørn-unger som her kunne bekreftes med genetiske metoder. De gjenstående 3 hunnbjørnene kunne ikke utelukkes å være mor til sine potensielle unger, men disse slektskapene kunne ikke bekreftes uten tilleggs analyser av DNA. Et annet familiært slektskap ble også bekreftet mellom to hunnbjørner uten at slektskapsforholdet kunne bestemmes.

Land/Country:	Norge/Norway
Fylke/County:	Finnmark (NO)
Kommune/Municipality:	Sør-Varanger (NO)
Sted/Lokalitet:	Øvre Pasvik (NO)

Godkjent / Approved

Prosjektleder / Project leader

_____
Snorre B. Hagen (Sign.)

_____
Alexander Kopatz (Sign.)

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

3

Table of Contents

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

4

Abstract

Knowledge on the number of female brown bears, especially reproducing females, is important for the wildlife management. One of the largest and densest populations of brown bears in Norway is located in Sør-Varanger, Finnmark, Northern Norway. Observations of females with cubs are reported regularly in the region. Information on the relatedness among individuals is often unknown as well as specifics on the number of reproductions and relatedness among females within this population. We have utilized genetic data originating from feces and hair samples collected in Sør-Varanger in the years 2004-2014 to investigate female brown bear localities. In the same period, personnel from the Norwegian State Nature Inspectorate (SNO) have observed 9 female brown bears with potential female cubs (a priori probability of 0.5). Sampling areas of those female brown bears and their potential offspring showed substantial geographical vicinity suggesting overlapping home ranges. We then calculated the likelihood ratios for these relationships using the forensic software Familias for 18-mother-female cub relationships. For 10 of 18 such relationships, the genetic relationship between mother and female cub were confirmed as their observation in the field was suggestive of. Of the initially observed 9 female bears, 6 have produced 10 female cubs, which here could be confirmed by genetic methods. The remaining 3 females were not excluded to be mothers to their potential cubs, but these relationships cannot be confirmed without additional DNA analyses. Another family relationship could also be confirmed between two observed female bears, but the type of relationship could not be determined.

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

5

1. Introduction

Although the brown bear (*Ursus arctos*) has been characterized as a solitary animal, kin-related spatial structure has been described in brown bears (Proctor et al. 2004; Støen et al. 2005): especially related female brown bears, which tend to be philopatric, often have overlapping home ranges. Male brown bears leave their natal area and disperse to further distances, probably to avoid inbreeding (McLellan and Hovey 2001). The number of females, in particular the ones reproducing, is important for the viability of a population, since a skewed sex ratio may have large influence on e.g. genetic drift and may lead to a loss in genetic variation. Also, the number of successful reproduction of bears in an area has become of high interest to the wildlife management nowadays, as a specific, maximum number of reproductions might be the goal to be achieved, e.g. to keep a population stable in size.

Sør-Varanger, including the Pasvik Valley, in Northern Norway houses one of the largest and densest brown bear populations in Norway (Schregel et al. 2012). Observations of females with cubs are reported regularly from the region (Wartiainen et al. 2009); however, little is known about the reproduction of this population in the far north of Europe. Since 2004, non-invasive genetic sampling (feces and hairs) has been conducted in Sør-Varanger (see e.g. Wartiainen et al. 2009; Kopatz et al. 2011 and 2012a). Methods of noninvasive genetic sampling to monitor and investigate brown bears or other rare and elusive mammals have become favored by wildlife researchers and managers during the last decade. Biological samples are collected and used for identification of animals with the help of the DNA contained in the sample (Taberlet et al. 1997; Waits and Paetkau 2005; Schwartz et al. 2006). Feces and hairs are the most common sources for DNA in brown bear monitoring and research and are widely in use (see e.g. Woods et al. 1999; Mowat and Strobeck 2000; Romain-Bondi et al. 2004; Kendall 1999; Bellemain et al. 2005; Thompson 2004; Waits & Paetkau 2005; Kendall et al. 2005, 2008a, 2008b, 2009; De Barba et al. 2010; Schregel et al. 2012).

A previous study investigated the sampling areas and pedigree of brown bears in Sør-Varanger from 2004 to 2008 (Wartiainen et al. 2009). At least 6 litters from 5 family groups were reported. At that time, three potential breeding females were known. Starting from 2004, biological samples from brown bears in Pasvik have been collected regularly. Since 2006 non-invasive genetic monitoring is implemented in the national monitoring scheme of brown bears in Norway (see e.g. Aarnes et al. 2013). Here, we use those samples and genotypes to investigate female brown bear localities and areas of abundance based on the locations the samples were collected as well as the kinship among individual female bears based on direct observations from the field by experienced personnel from the Norwegian State Nature Inspectorate (SNO).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

6

2. Materials and methods

Study area
The study area encompasses the municipality of Sør-Varanger in Finnmark, Northern Norway. The terrestrial area covers 3481.5 km^2. The area consists of arctic and boreal ecosystems in a mosaic of peat land and forest with Scots pine (*Pinus silvestris*) and downy birch (*Betula pubescens*).

Sampling and data analyses
We used the data from samples collected noninvasively from 2004 to 2014 by opportunistic field sampling of feces and hairs (Wartiainen et al. 2008; Tobiassen et al. 2012) and systematic sampling with the help of hair traps in the area (Smith et al. 2007; Kopatz et al. 2011). Genetic analyses, PCR, fragment analysis and individual identification were not part of this study and have been described by Kopatz et al. (2012b) and Andreassen et al. (2012). Most samples were genotyped with 8 genetic markers (STRs). Being part of other research projects, a selection has also been previously genotyped with 12 microsatellite markers (see e.g. Schregel et al. 2012; Kopatz et al. 2014). Further, some individuals have been genotyped with 15 STRs.

Personnel of the Norwegian State Nature Inspectorate (SNO) records regularly brown bear females with cubs by observation. Such observations can be characterized as strong indication of relatedness of the bears observed, since unrelated brown bears tend to roam separately and solitary (Dahle and Swenson 2003; Dahle et al. 2006; Støen et al. 2006). Further, family members, especially related female brown bears are known to show overlapping home ranges (Støen et al. 2006; Zedrosser et al. 2007). In order to reveal geographical overlap and intersecting residency of female bears, we plotted all sampling locations of each individual female on a map.

Individual bear genotypes were analyzed based on ecological indications for family relationship: pedigree analyses were only performed for individuals, which have been observed and identified previously by the Norwegian State Nature Inspectorate (SNO) in the field together, suggesting family relationship. We used the latest version of the program Familias 3.1.6 (Egeland and Mostad 2000; Kling et al. 2014) to calculate likelihood ratios (LR) for genetic relationship. The LR shows which pedigree is more likely than others. Based on the field data, the *a priori* probability of being related/being not related is 0.5. This means a LR of 20 from the genetic analysis corresponds to a probability of 95% for relatedness. A value for LR above 20 would be considered as significant support (95% probability for relatedness) from DNA data for the pedigree in question while a LR value above 100 would be a very strong support (99% probability of relatedness). The Familias software is widely in use worldwide by human forensic laboratories and has been applied to numerous cases e.g. resolving family relations, individual identification after disasters etc. Calculating family relationships requires allele frequency data of the population in question and we deduced this information from the overall data we have recorded from the bear population in Sør-Varanger, genotyped with 12 STRs (Kopatz et al. 2012b). Based on our previous study we used a kinship correction (F$_{ST}$) of θ=0.09 (Andreassen et al. 2012).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

7

3. Results and discussion

A total of 47 individual genotypes from brown bears sampled in Sør-Varanger (2004-2014) have been extracted from our genetic database (data not shown). Field observations by the Norwegian State Nature Inspectorate (SNO) indicated motherhood for 9 female brown bears with 18 potential female cubs (Tab. 1). For those females and their potential female offspring, feces and hair has been collected throughout the past. Potential family groups have been plotted on a map with the following female bears as potential mothers: FI4 (Appendix 1), FI8, FI19 (Appendix 2), FI40 (Appendix 3), FI42 (Appendix 4), FI43/MO3 (Appendix 5), FI63/MO4 (Appendix 6), FI97 (Appendix 7) and FI98 (Appendix 8). Females without known reproduction were plotted together (Appendix 9 and 10). Overall, females and potential offspring showed close proximity of their sample locations and substantial overlap of their sampling area. Although we have not calculated home ranges, the data suggests overlapping territory of most of the females with their potential offspring, as it is expected from previous studies (Støen et al. 2005 and 2006; Zedrosser et al. 2007). The data also suggests that females, which may be unrelated or distantly related, share territories and probably have overlapping home ranges, especially in the southern part of the research area, namely the Pasvik Valley, as it has been reported earlier (see Appendices 1-10; Wartiainen et al. 2009).

We calculated the LRs for 18-mother-female cub relationships and 10 of those relationships confirmed the genetic relationships between mother and the potential female cub (with a LR>20; Tab. 2) as their observation in the field was suggestive of. FI42 and FI77 showed the highest LR=113.9 for their relationship and suggest 99% probability for relatedness. LR results for FI4-FI86, FI40-FI72, FI40-FI135, FI42-FI147, FI43/MO3-FI160, FI98/MO17-FI116 and FI98/MO17-FI117 showed 95% probability of relatedness. Mother-cub relations of FI19-FI40 and FI43/MO3-FI39/LL4 were just below the threshold of LR=20 (95% probability of relatedness). The bears FI149 and FI150 have been observed together in the field, suggesting family relationship. However, information from the field could not determine which individual is the mother and which one the cub. Genetic relatedness was supported by the results for of the pedigree calculation using 8 STRs: LR=50.8, suggesting significant probability of relatedness. The results suggests, that at least 6 female brown bears had female offspring during the last ten years in Sør-Varanger: mothers FI4, FI19, FI40, FI42, FI43/MO3 and FI98/MO17 and 10 female cubs showed significant likelihood with their potential mother using the genetic method (LR>20; Tab. 2). Reproduction for FI4, FI8, FI19 and FI40 was reported in the previous study, investigating the relatedness of bears from Sør-Varanger from 2004 to 2008 (Wartiainen et al. 2009). New individuals with female litter were FI42, FI43/MO3 and FI98/MO17. In addition, most probably there has been one more reproduction within family FI149/FI150.

Observations in the field are independent from the genetic analysis and therefore represent a strong likelihood of relatedness (*a priori* likelihood of 0.5). While for a part of such observations we found genetic indication, other relationships could not be confirmed with genetic methods, although field observations suggested such a relationship. One reason could be the number of genetic markers, STRs, used. While 8 STRs are sufficient for a feasible identification of individual brown bears, that number seems too low for revealing genetic relatedness among individuals in some cases. In human forensics 17 STRs are applied to scrutinize genetic relationships among humans, e.g. for parentage cases or after disasters. Here, we tested the use of data acquired during wildlife monitoring and including individuals genotyped with more than 8 STRs. Our results suggest that the panel of genetic markers used should be increased.

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

8

Tab. 1: Genetic registration of female brown bears in Sør-Varanger, Norway, based on collected feces and hair as well as their potential female offspring based on field observations by the Norwegian State Nature Inspectorate (SNO).

ID mother	Year of registrations (DNA)	ID of potential female offspring
FI4	2004, 2005, 2007, 2008, 2009	FI7, FI86
FI8	2004	FI10
FI19	2004, 2005, 2006, 2008, 2009, 2011, 2013, 2014	FI40, FI54
FI40	2005, 2006, 2007, 2008, 2009, 2011, 2012	FI72, FI135
FI42	2005, 2008, 2009, 2012, 2013	FI77, FI147
FI43/MO3	2005, 2007, 2008, 2009, 2010, 2011, 2012, 2013	FI39/LL4, FI109, FI110, FI111, FI160
FI63/MO4	2005 (Russia), 2007, 2008, 2009, 2010, 2011, 2012, 2013	FI129
FI97	2009, 2010, 2011, 2012, 2013	FI139
FI98/MO17	2010, 2011, 2012, 2013	FI116, FI117

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

9

Tab. 2: Analyses of the genetic relationship (mother-cub and family) with the program Familias 3.1.6. The table shows female brown bears in Sør-Varanger, Norway, and their potential female offspring based on field observations. Table includes the ID of the female bear and potential offspring, first year of DNA registration (NO=Norway, RU=Russia), LR=likelihood ratio, STRs=number of STRs as well as notes.

ID mother	ID of potential female offspring	Year of first registration (DNA)	LR	STRs	Notes
FI4		**2004 (NO)**		**15**	
	FI86	2008 (NO)	51.0	15	
	FI7	2004 (NO)	14.0	15	Dead 2009
	Family		17105.8		
FI8		**2004 (NO)**		**6**	
	FI10	2004 (NO)	7.3	12	Dead 2005
FI19		**2004 (NO)**		**15**	
	FI40	2005 (NO)	19.6*	15	
	FI54	2006 (NO)	7.0	15	Dead 2008
	Family		48.6*		
FI40		**2005 (NO)**		**15**	
	FI72	2007 (NO)	25.0	15	Dead 2010
	FI135	2012 (NO)	21.1	8	
	Family		1288.3		
FI42		**2005 (NO)**		**12**	
	FI77	2008 (NO)	113.9	12	
	FI147	2012 (NO)	30.4	12	
	Family		11435.3		
FI43/MO3		**2005 (RU)**		**12**	
	FI160	2013 (NO)	83.3	8	
	FI39/LL4	2005 (NO/FI)	19.9	12	Dead 2005
	FI109	2010 (NO)	10.3	8	
	FI110	2010 (NO)	3.1	8	
	FI111	2010 (NO)	1.4	8	
	Family		1891189.9		
FI63/MO4		**2005 (RU)**		**12**	
	FI129	2011 (NO)	15.7	8	
FI97		**2009 (RU)**		**8**	
	FI139	2012 (NO)	7.5	8	
FI98/MO17		**2010 (NO)**		**8**	
	FI116	2011 (NO)	23.5	8	
	FI117	2011 (NO)	22.5	8	
	Family		869.7		

Calculation based on 12 STRs

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

10

Besides increasing the number of genetic markers (STRs) for future studies we suggest including male bears to calculate full family pedigrees. The application of next generation sequencing techniques, i.e. SNPs, may be also be considered to analyze the genetic relationship among the brown bears in Sør-Varanger.

Individual identification of brown bears has become more and more important for wildlife management as well as in research. Nowadays, an effective management bases its decisions and actions on a feasible and correct identification of an animal and the genetic identification may determine the destiny of an individual bear. Therefore highest standards applied in human and animal forensic should be applied (see Linacre et al. 2011).
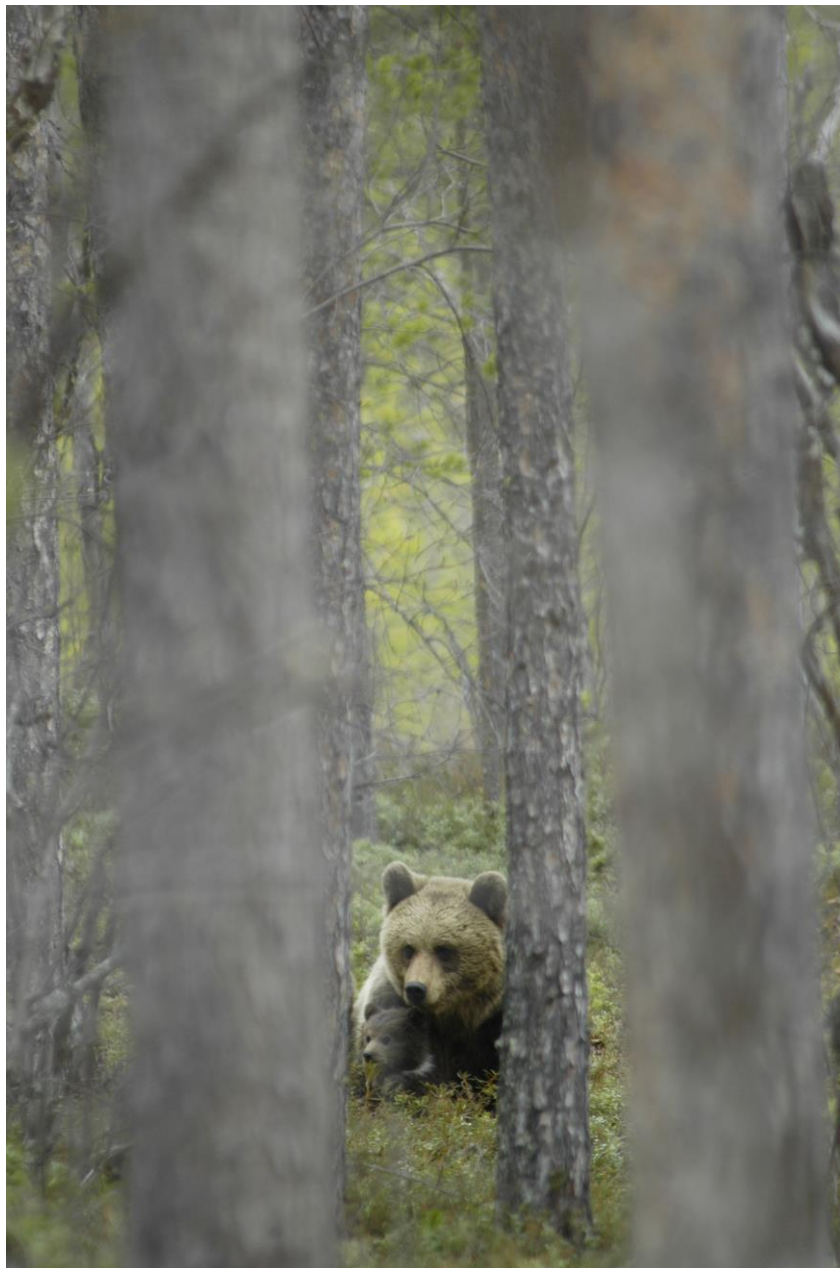
Fig. 1: Female brown bear with cub it Pasvik, Sør-Varanger, Norway. Photo: Steinar Wikan.

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

11

4. Conclusive remarks

We investigated the sampling locations of female brown bears in Sør-Varanger, Norway, registered by DNA from 2004 to 2014. In a second step, we used individual genotypes of bears, which had strong indication of being related based on observations in the field and calculated the pedigree. The results of this study can be summed up as follows:

- Sampling areas of female brown bears and their potential offspring showed substantial geographical vicinity suggesting overlapping home ranges.

- Observations in the field indicated relatedness of 9 female brown bears and 18 female cubs.

- Of those 9 female bears, 6 have reproduced 10 female cubs, which could be confirmed by genetic methods; including females FI4, FI19, FI40, FI42, FI43/MO3 and FI98/MO17.

- The remaining 3 of the 9 females were not excluded to be mothers to their potential cubs, but these relationships could not be confirmed. The results suggest that the panel of genetic markers used should be increased.

- Another family relationship could be confirmed between female bears FI149 and FI150. However, which bear is mother and which may be the cub remains unknown at this point.

- Low likelihood ratios for relatedness may have been caused by the number of genetic markers used for some individuals. Therefore we suggest increasing the number of STRs for a more feasible analysis of pedigrees.

- Brown bear management and research is based on individuals. Therefore, high quality standards have to be applied in the handling of the samples and laboratory analyses, but also further, in the post-processing and analysis of the genetic data. The pedigree analyses were performed with Familias, a program widely used in human forensics.

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

12

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Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

13

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Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

14

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Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

15

Appendix 1. Localities of samples found and identified for FI4 (red squares; 2004-2009) and potential female offspring FI7 (blue circles; 2005-2009) and FI86 (yellow circles; 2008).
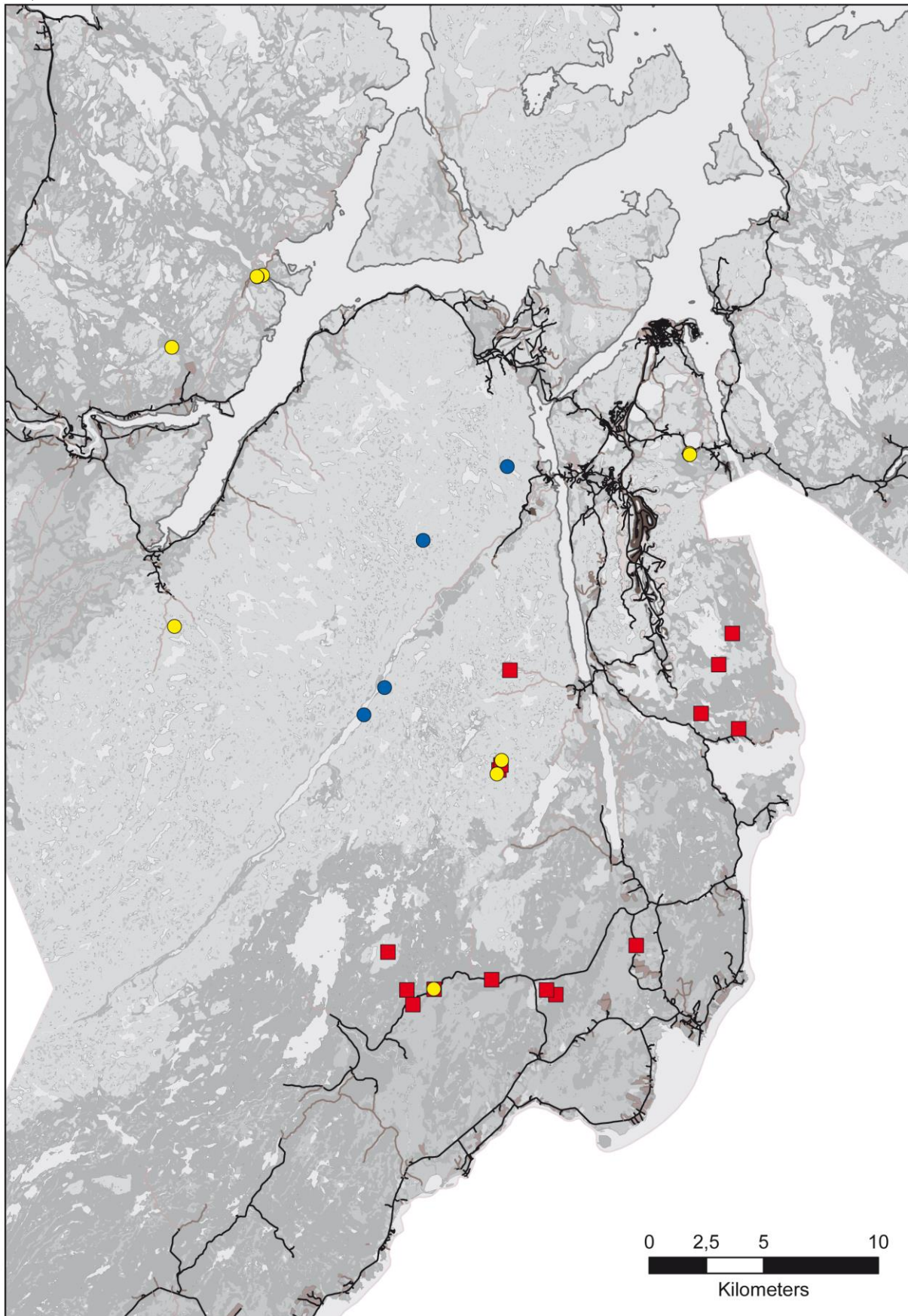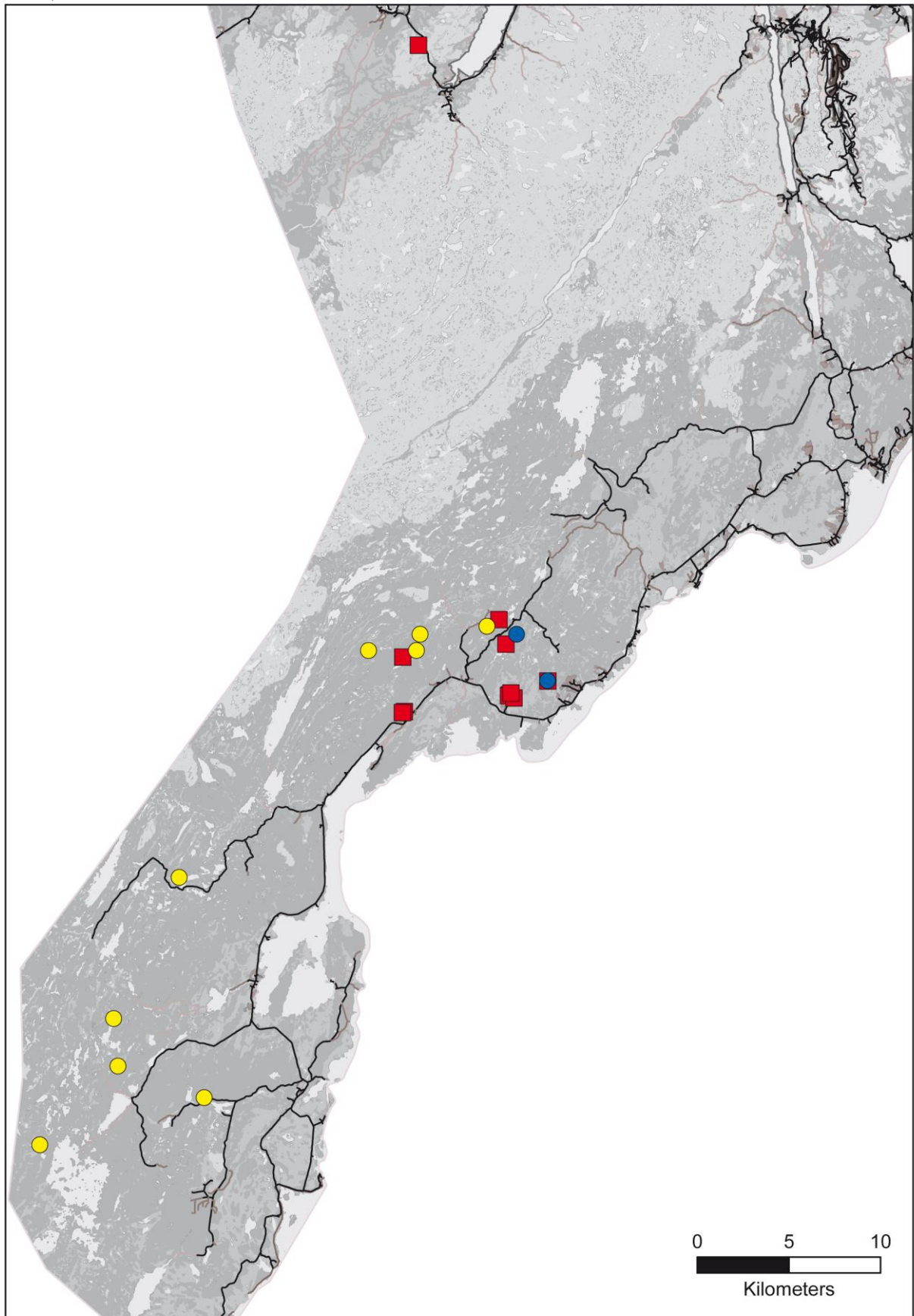
Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

16
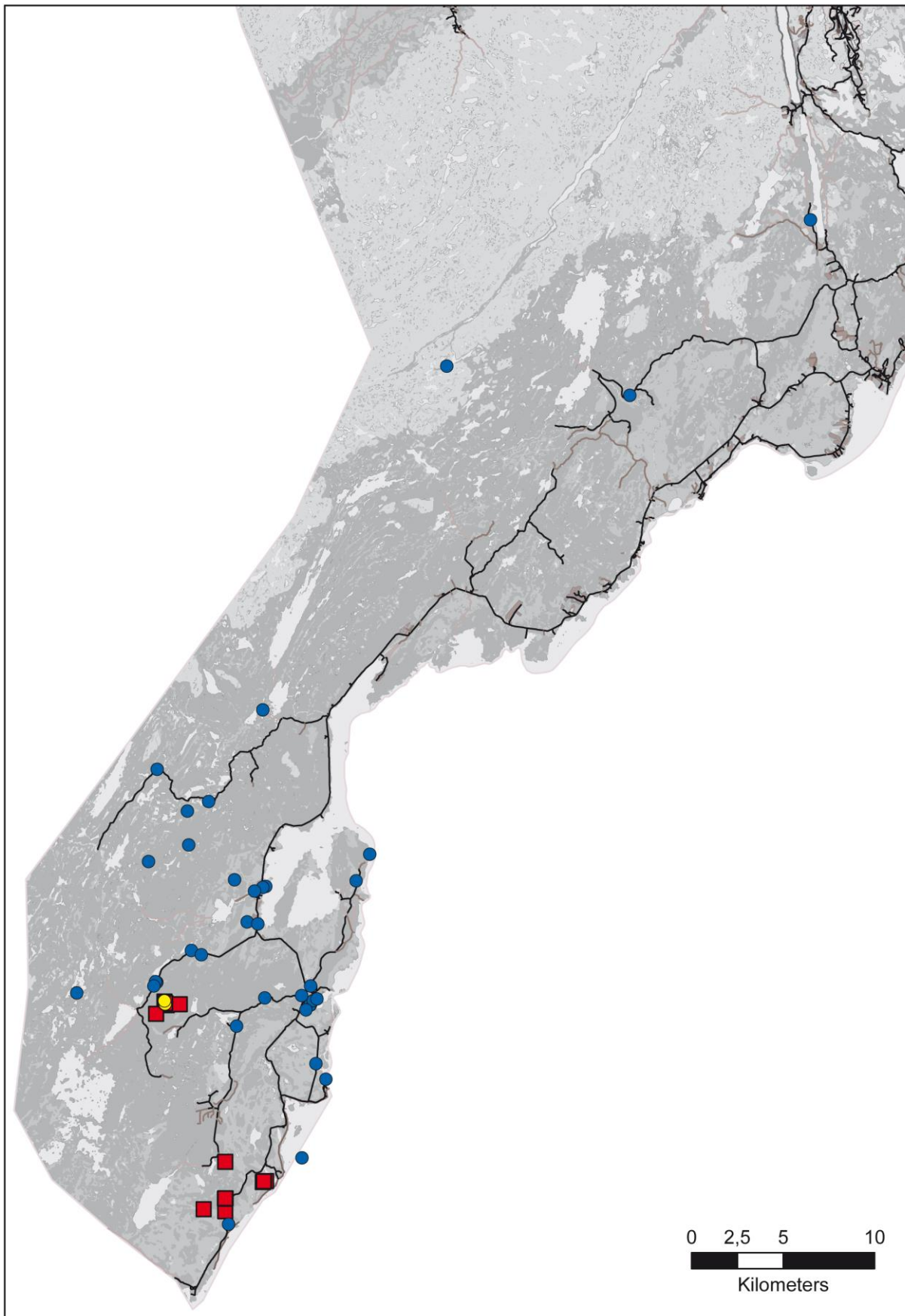
Appendix 2. Localities of samples found and identified for FI19 (red squares; 2005-2014) and potential female offspring FI40 (yellow circles; 2005-2012) and FI54 (blue circles; 2006-2008).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

17
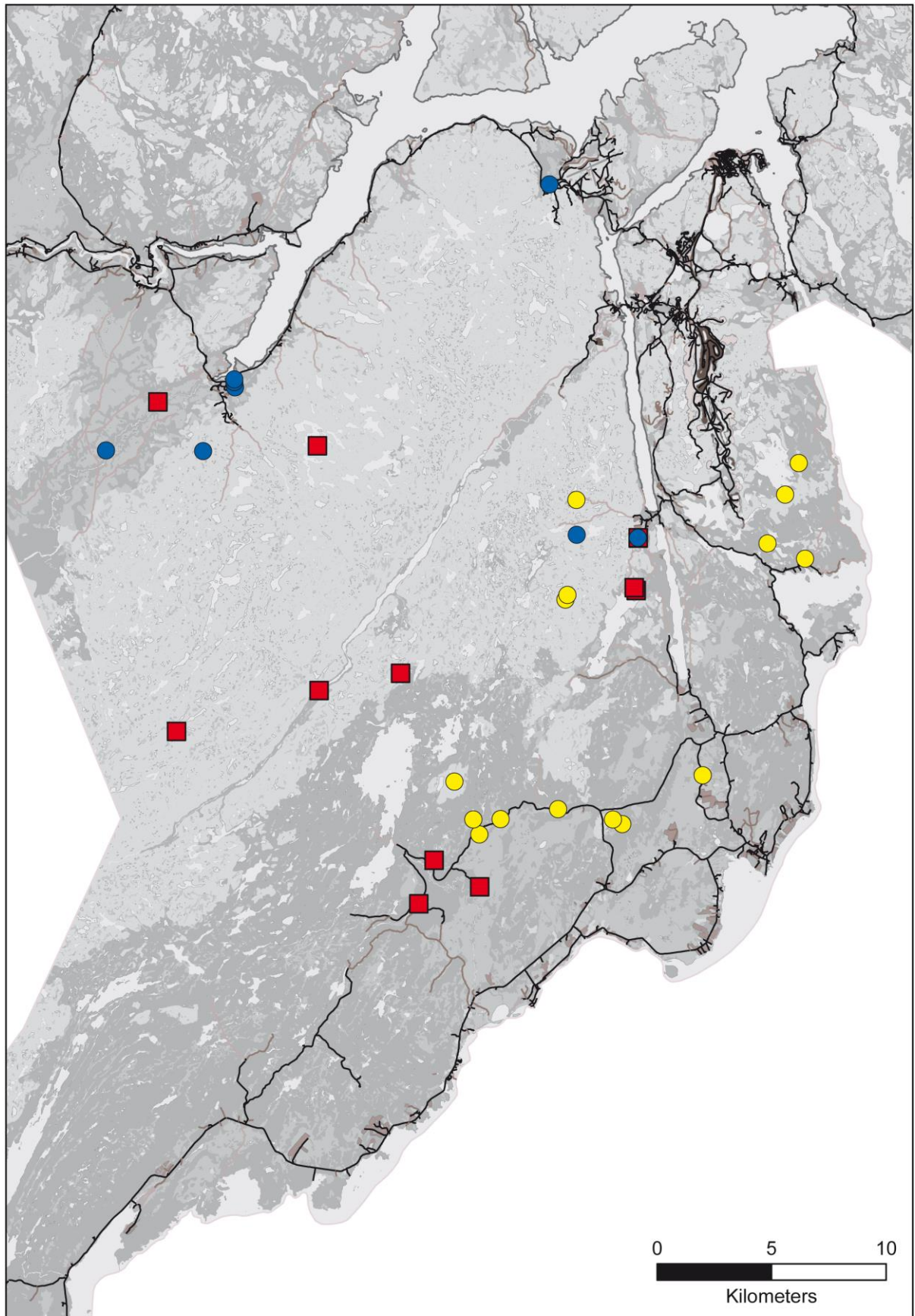
Appendix 3. Localities of samples found and identified for FI40 (red squares; 2005-2012) and potential female offspring FI72 (yellow circles; 2007-2010) and FI135 (blue circles; 2012-2014).

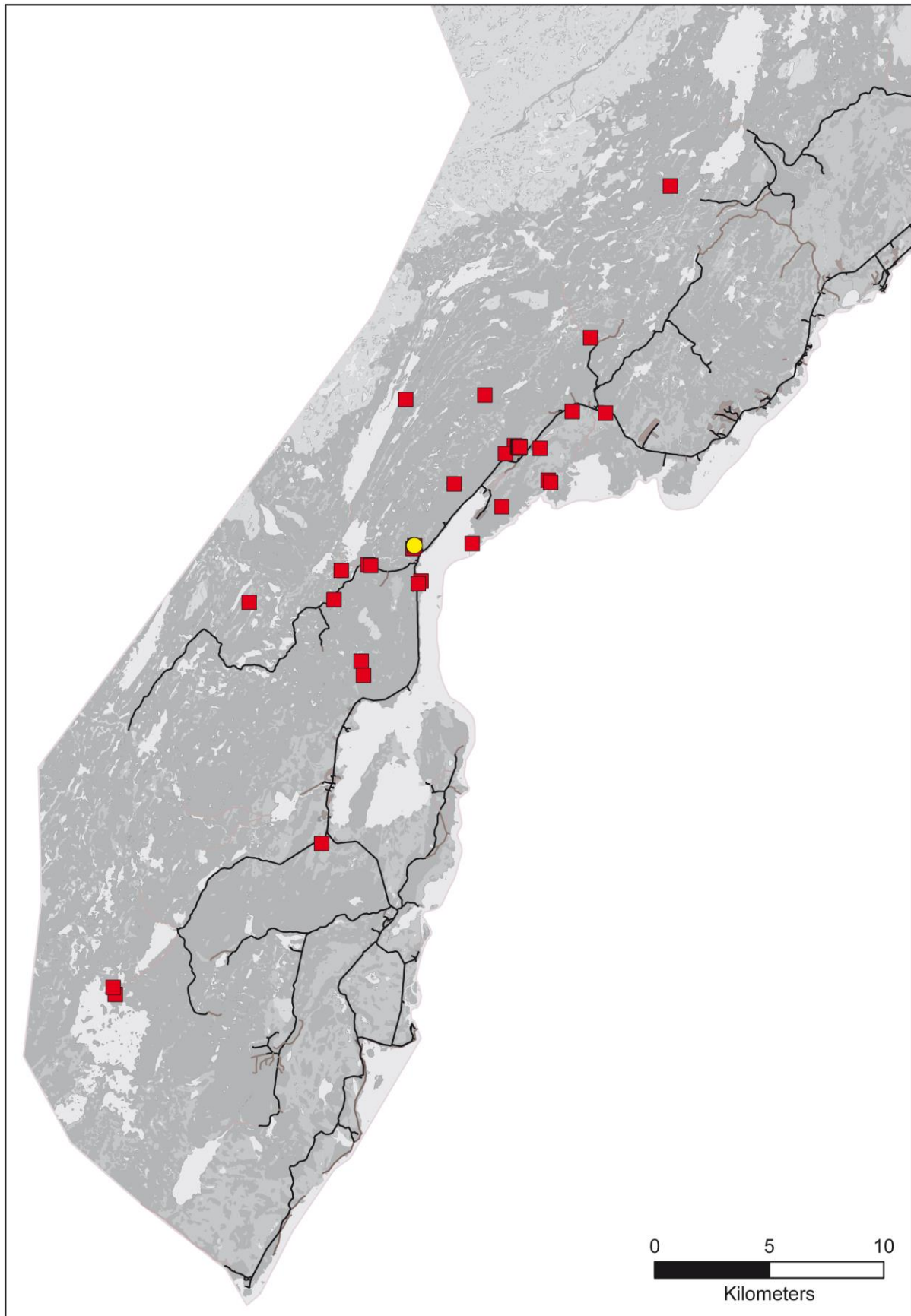Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

18

Appendix 4. Localities of samples found and identified for FI42 (red squares; 2009-2013) and potential female offspring FI77 (yellow circles; 2008-2013) and FI147 (blue circles; 2012-2013).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

19

Appendix 5. Localities of samples found and identified for FI43/MO3 (red squares; 2005-2012) and potential female offspring FI39/LL4 (blue circles; 2005), FI109 (green circles; 2010-2011), FI110 (magenta circles; 2010-2012), FI111 (light blue circles; 2010-2013) and FI160 (yellow circles; 2013).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

20
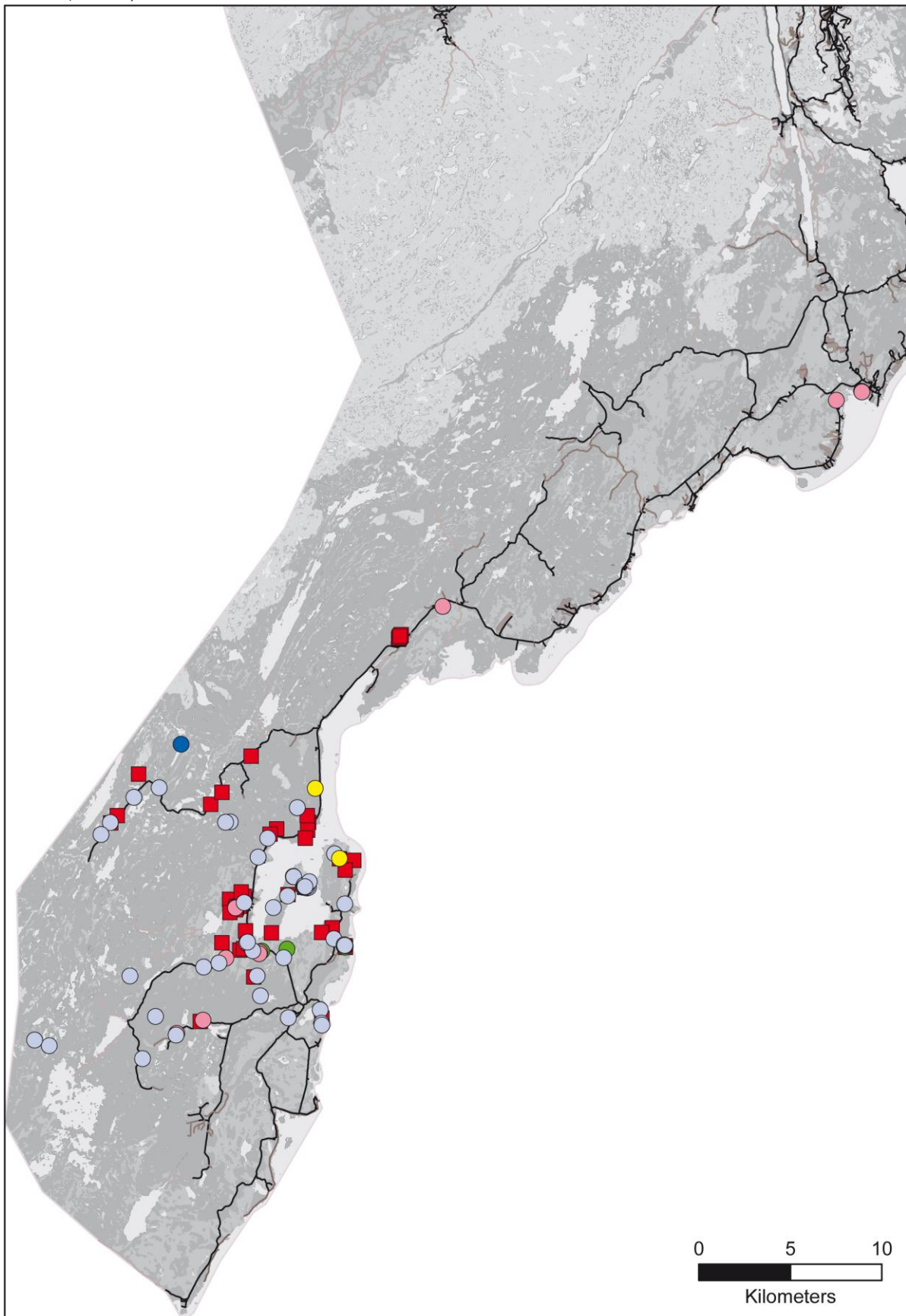
Appendix 6. Localities of samples found and identified for FI63/MO4 (red squares; 2007-2013) and potential female offspring FI129 (yellow circles; 2011).

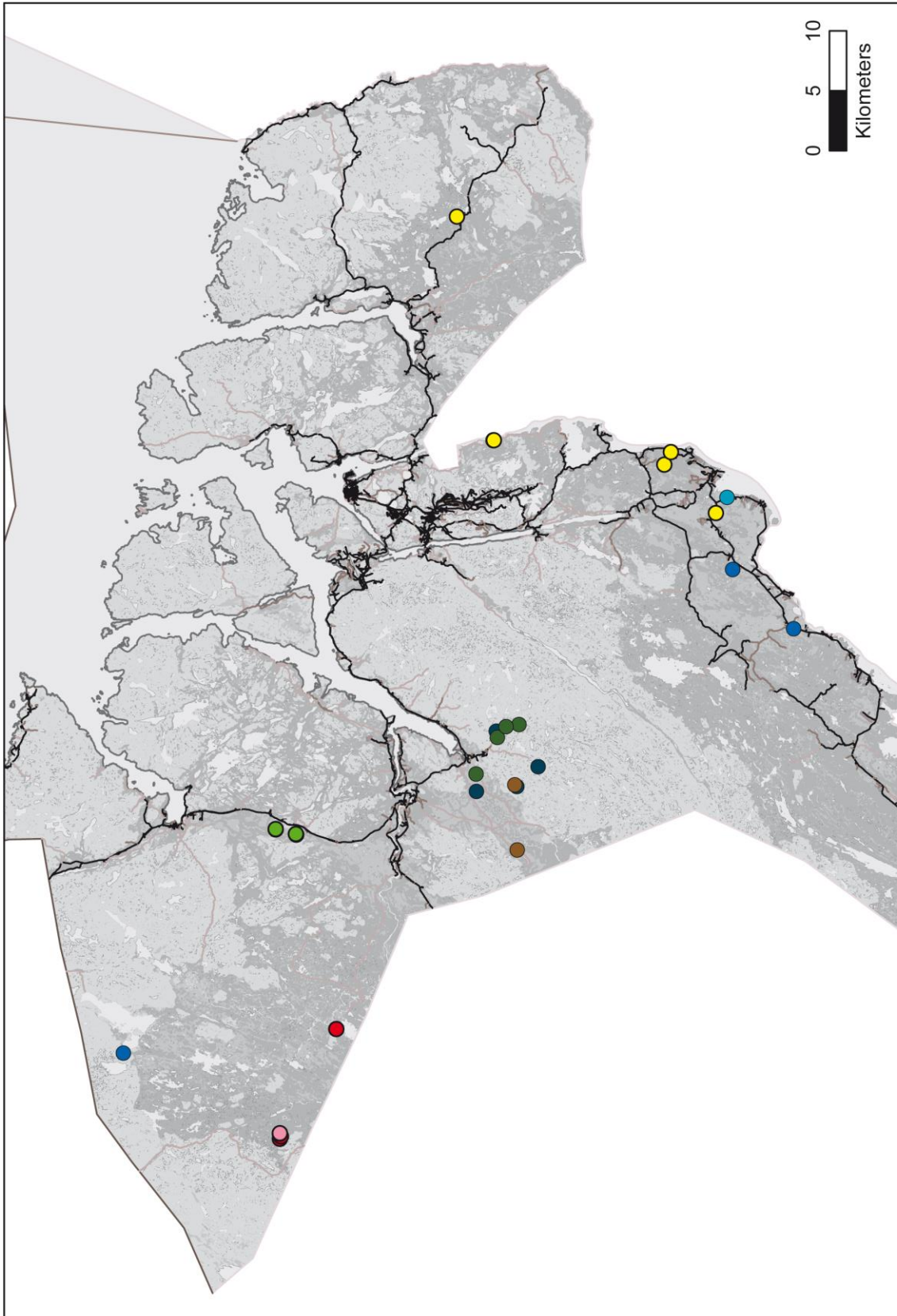Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

21

Appendix 7. Localities of samples found and identified for FI97 (red squares; 2009-2013) and potential female offspring FI139 (yellow circles; 2012-2013).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

22
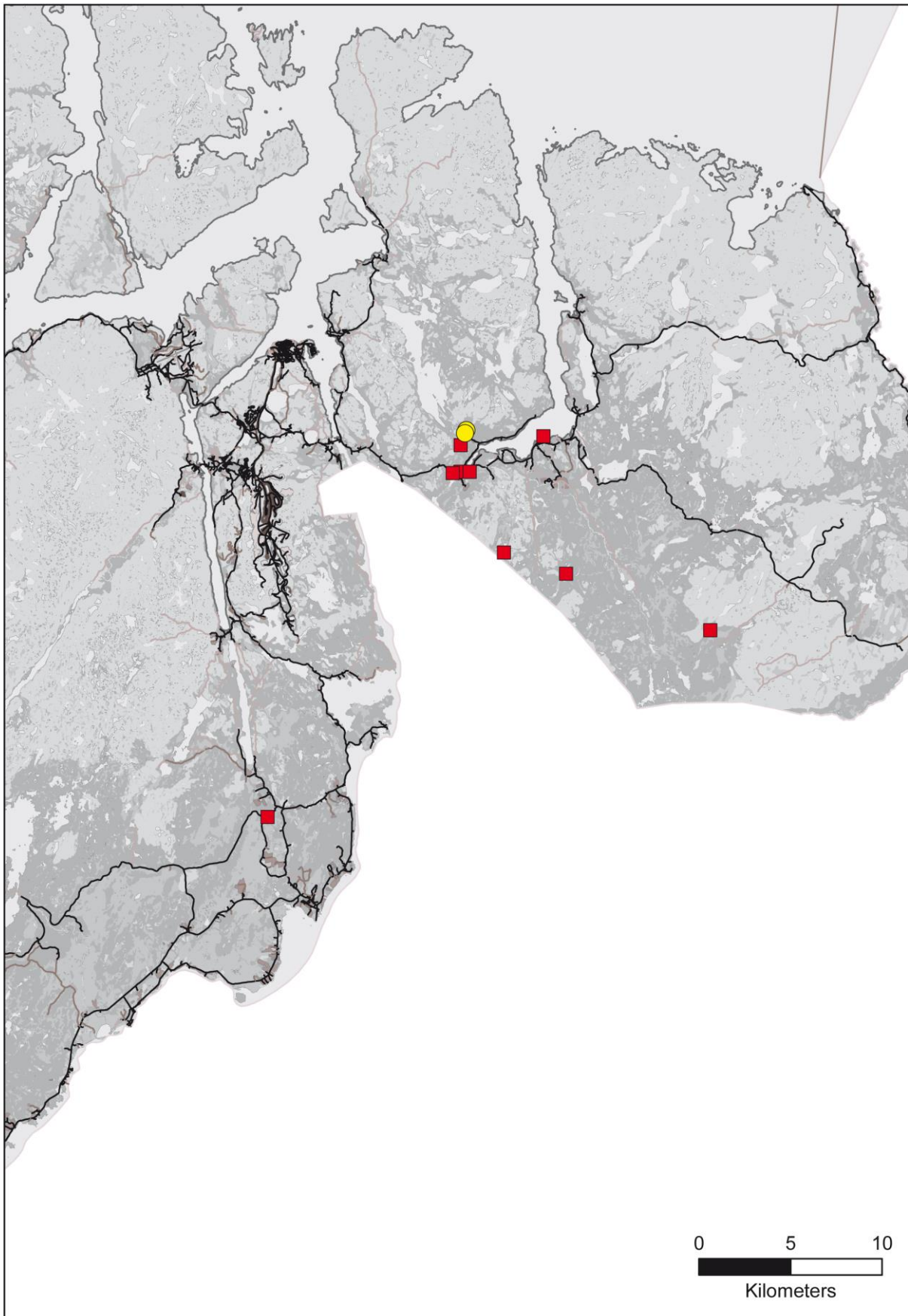
Appendix 8. Localities of samples found and identified for FI98/MO17 (red squares; 2010-2013) and potential female offspring FI116 (yellow circles; 2011-2012) and FI117 (blue circles; 2011).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

23
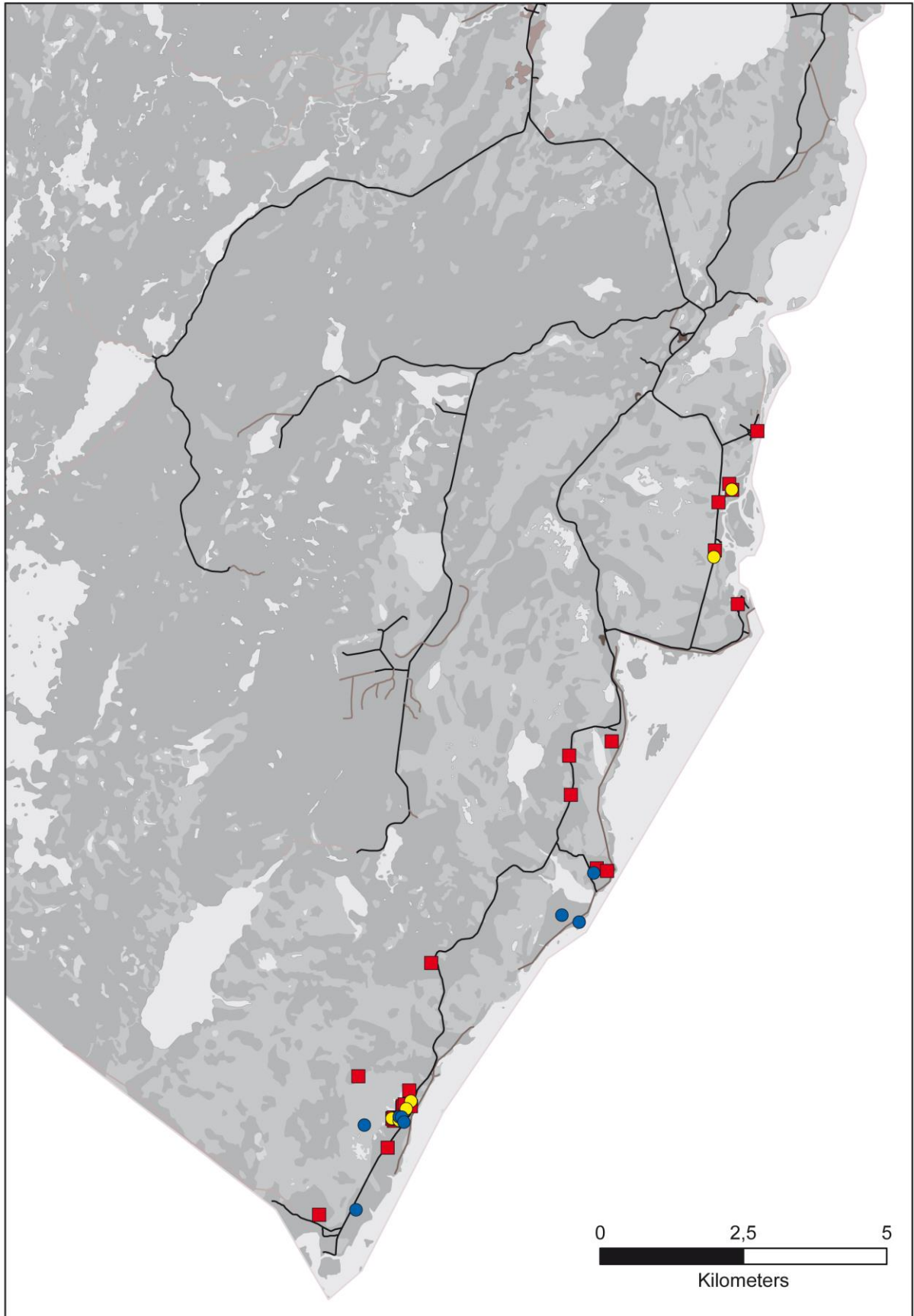
Appendix 9. Localities of samples found and identified for females FI14 (blue circles; 2008-2011), FI82 (cyan circle; 2008), FI89/LL29 (green circles; 2009), FI102 (yellow circles; 2010-2013), FI119 (dark blue; 2011-2012), FI121 (dark green; 2012), FI144 (brown; 2012), FI149 (magenta circles; 2013) and FI150 (red circles; 2013).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

24

Appendix 10. Localities of samples found and identified for females FI38/MO18 (light blue circles; 2005), FI74 (green circles; 2008-2013), FI95 (yellow circles; 2009-2010), FI148/LL8 (purple circle; 2012), FI157 (magenta circle; 2013) and FI167 (red circles; 2014).

Kopatz et al. Bioforsk Report Vol. 9 No. 167 2014

25