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Genetic monitoring of a critically-endangered seal population based on field-collected placentas

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Genetic analyses of non-invasively collected samples are increasingly being used in the monitoring of wildlife populations and individuals. This study is the first describing the use of placentas as non-invasive genetic samples from a natural population. We collected 66 placentas from birth-lair sites of Saimaa ringed seals (*Phoca hispida saimensis*) after the breeding seasons, with the aim of obtaining DNA from both the pup and the mother. Umbilical cord samples proved to yield the pup genotypes, but mothers could not be genotyped with confidence. Comparisons with existing mtDNA and microsatellite reference data sets showed that placentas can be used for inferring population-level genetic parameters. Our microsatellite panel provided sufficient resolution for genetic identification of individuals but, due to the extremely low variability of the population, parentage and sibship could not be inferred reliably. Field-collected placentas could provide means for genetic monitoring of many other seal species as well.

Introduction

Genetic analyses of non-invasively collected samples, such as hair and faeces, are being increasingly utilized in wildlife research, management, and conservation. Applications of these methods include, for example, identification of species or individuals, and estimation of home range size, gene flow, local population size, and individual reproductive success (Waits & Paetkau 2005, Schwartz *et al*. 2007). Non-invasive approaches have become more or less established practice in studies on large terrestrial mammals, which are hard to find and capture (Arandjelovic *et al*. 2011, Kopatz *et al*. 2012, Davoli *et al*. 2013). In marine mammals, the collection of non-invasive samples is often more challenging, but shed skin (Swanson *et al*. 2006, Baker *et al*. 2013, Martinez-Bakker *et al*. 2013), faeces (Parsons *et al*. 2006, Valqui *et al*. 2010),

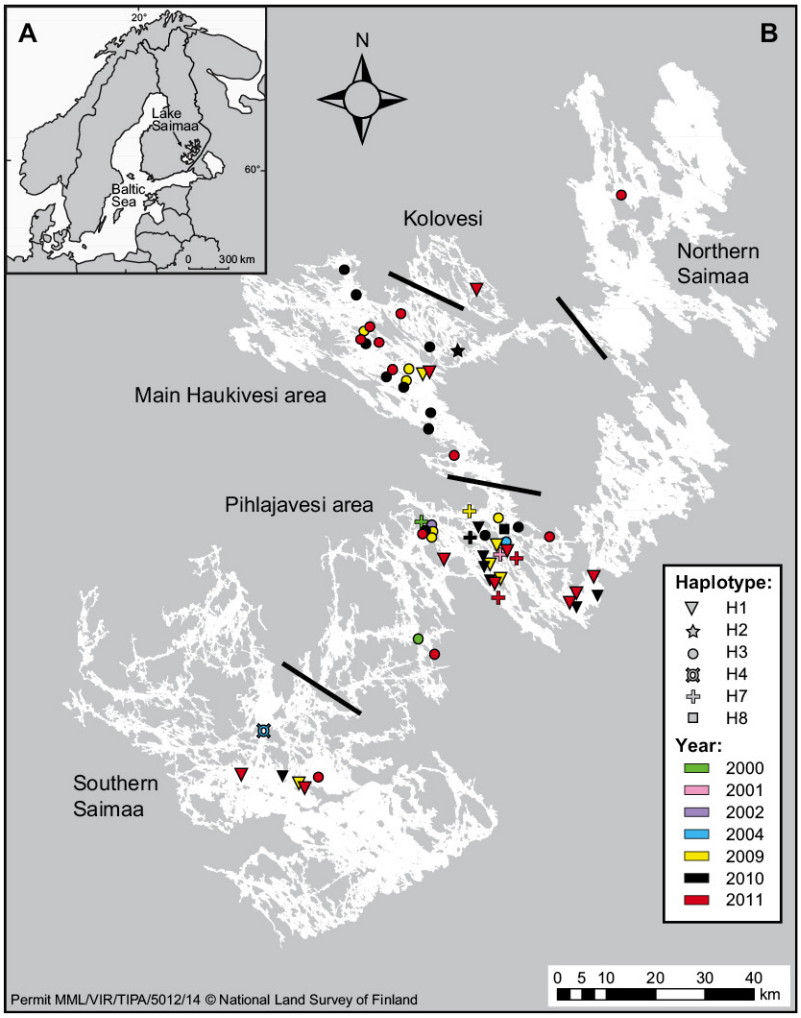

Fig. 1. (**A**) Location of Lake Saimaa in Finland, and (**B**) collection sites of Saimaa ringed seal placentas. Different symbols denote different mtDNA haplotypes and colours the year of collection.

and even environmental DNA from the water column (Foote *et al*. 2012) have been successfully used as a source of genetic information.

Here, we describe how field-collected placentas can be used for non-invasive genetic monitoring. The target population was the landlocked Saimaa ringed seal (*Phoca hispida saimensis*). This endemic subspecies inhabits Lake Saimaa in southeastern Finland (Fig. 1A). The population of currently circa 300 seals is threatened mainly by high mortality of juveniles due to entanglement in fishing gear and by climate change and, therefore, is classified as critically endangered (Rassi *et al*. 2010, Kovacs *et al*. 2012). Given the still-precarious situation of the subspecies, there is a clear need for continuous monitoring of the population, which could be substantially enhanced by remotely collected genetic samples. It has been estimated that around 70%–80% of the adult females give birth annually (Sipilä 2003), and that some 50–60 pups are born each spring. Placentas are relatively easy to collect post-partum: females give birth to a single pup in subnivean lairs dug into snowdrifts formed along shores of islands and islets (Sipilä 2003) and, after the breeding season, the placenta can often be found from the bottom of the lake within a few meters from the lair. Preservation of the tissue, and DNA, is extended by the low temperature of the water during and right after the ice-covered season.

Ideally, placentas could provide a unique opportunity for obtaining the DNA and genotypes of two different individuals from a single

sample, because mammalian placentas are composed of both foetal and maternal tissue. Like other carnivorans, pinnipeds have an endotheliochorial placenta (Stewart & Stewart 2009) with extensive intermingling of uterine and chorionic tissues, which causes a part of the uterine component to be torn away along with the placenta at birth. In the case of the Saimaa ringed seal, identification of females based on shed placentas could potentially be used to infer individual-level breeding-site fidelity and reproductive success, and could also lead to more accurate population-size estimates. Similarly, genotyping pups using placentas could yield information on long-term dispersal patterns and survival probabilities, if the individuals are recaptured and genotyped later.

The main aims of this study were to (1) investigate whether it is possible to identify seal mothers and/or offspring based on genetic profiling of field-collected placentas, and (2) find out the optimal spot for extracting DNA of each of these individuals from the placentas. We also wanted to (3) evaluate whether placentas could be used as a source of information in genotype-based mark–recapture and kinship studies, and (4) examine the utility of placentas in inferring population-genetic parameters of the Saimaa ringed seal using both nuclear (microsatellites) and mitochondrial (mtDNA control-region sequences) markers.

Material and methods

Sample collection and handling

Lake Saimaa is a large (ca. 4400 km^2) and shallow lake (mean depth 12 m, max. 85 m) with over 13 000 islands (Kuusisto 1999). Birth lairs are identified during annual lair censuses conducted throughout the lake during each April (Metsähallitus 2014). A few placentas used in this study were found from collapsed lairs during the censuses, but most placentas were collected by scuba diving from birth-lair sites after ice break-up in May, some 2–3 months after the birth of pups (*see* Auttila *et al*. 2014).

Placentas were searched for at birth-lair sites in the main breeding areas (Fig. 1B) in 2009 and 2010 (28 and 46 sites, respectively), and from all known sites in 2011 (50 sites). A total of 59 placentas were recovered (n_{2009} = 13, n_{2010} = 21, n_{2011} = 25), meaning that a placenta was found from 48% of the inspected lair sites. An unusual visual observation of nursed seal twins in 2009 was confirmed by our finding of two placentas only one meter apart on the lake bottom below a nearby birth-lair site (*see* below). In addition, seven placentas that had been collected in 2000–2006 and deposited in a tissue bank maintained by the University of Eastern Finland and Parks & Wildlife Finland were genotyped. All placentas were stored at –20 °C.

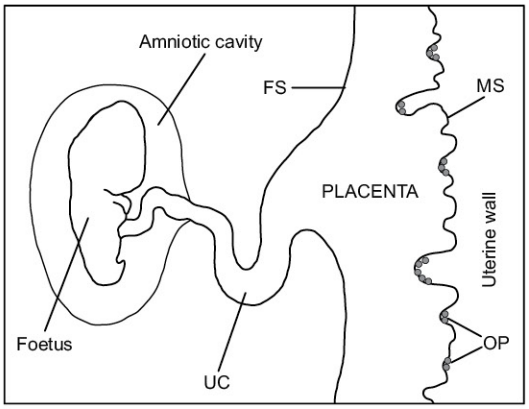

Fig. 2. Structure of the placenta, and different sampling spots used in this study: MS = maternal side, FS = foetal side, UC = umbilical cord, OP = orange particles.

The state of decomposition of each placenta was assessed visually using the following three-stage ordinal scale: (1) fresh, (2) partly decomposed and (3) decomposed. Of the 66 placentas sampled (Appendix), nearly half were classified as partly decomposed (48%), while 23% were categorized as fresh, and 29% as decomposed.

In order to identify the placental sampling spots from which the mother's and pup's DNA could be extracted separately, samples were taken from four different parts of each intact placenta (Fig. 2): maternal (i.e., uterine) side (MS); foetal (i.e., membrane) side (FS); umbilical cord, or in absence of it, a vein (UC); and orange particles (OP), which are bilirubine-containing particles found on the maternal side of the placenta (Van den Broeck 1904). We were able to take all four subsamples from 58 intact placentas (Appendix). For eight placentas with only shreds

of the membrane left, only an FS sample was taken. An additional blood sample (BS) was collected from four very fresh placentas that had been collected frozen on ice in April in 2000, 2001 and 2009. Altogether 244 tissue samples from 66 placentas were analysed, and for the laboratory analysis each sample was given a random number in order to prevent subjective interpretation of genotypes (i.e., samples were genotyped blind).

Samples from five pups (three stillborn and two by-caught pups with known natal sites) for which the corresponding placenta was available were used as reference samples. These samples were used to determine which of the sampling spots was optimal for obtaining the pup's genotype, and which for obtaining the mother's genotype.

Laboratory analyses

Total genomic DNA was extracted using the DNeasy Blood and Tissue Kits (Qiagen) according to the manufacturer's protocol. Each sample was genotyped at eleven microsatellite loci originally developed for other pinnipeds (annealing temperature (°C) and number of PCR cycles in parentheses): *Hg3.6* (58, 40), *Hg4.2* (61, 40), *Hg6.1* (58, 40), *Hg8.9* (58, 45), *Hg8.10* (53, 40), *SGPv9* (60, 40) (Allen *et al*. 1995), *Hgdii* (58, 40) (Allen *et al*. 1995, Twiss *et al*. 2006), *Hl15* (53, 43) (Davis *et al*. 2002), *SGPv10* (55, 40), *SGPv11* (55, 40) and *SGPv16* (51, 43) (Goodman 1997). These loci have previously been used in genetic analyses of the Saimaa ringed seal (*see* Valtonen *et al*. 2014), but the existing laboratory protocols were modified in order to optimise amplification success for placental DNA: PCR reactions contained 1 μl template DNA, 0.4 μM each primer, 0.6 U AmpliTaq Gold DNA polymerase (Applied Biosystems), 1X PCR buffer, 1.75 mM $MgCl_2$, 0.2 mM of each dNTP (Finnzymes), and 1 mg ml^{-1} BSA (Thermo Scientific) in a total reaction volume of 10 μl. Reactions were performed under the following conditions: 95 °C for 10 min followed by 40–45 cycles of 95 °C for 30 s, 51–61 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. PCR products were run on an ABI 3730 DNA Analyzer (Perkin Elmer Applied Biosystems), and genotypes were inferred using GENEMAPPER ver. 4.0 (Applied Biosystems).

MICRO-CHECKER ver. 2.2.3 (Van Oosterhout *et al*. 2004) was used for identifying possible genotyping errors (i.e., stuttering, allelic dropout, and null alleles) for UC samples, which were found to yield the pups' genotypes (*see* Results), with Bonferroni-adjusted 95% confidence intervals. FreeNA (Chapuis & Estoup 2007) was used to estimate the frequency of null alleles at each locus. In order to determine whether the data set was affected by allelic dropout due to poor sample quality, we used MICRO-DROP ver. 1.01 (Wang *et al*. 2012) to test for a correlation between the amount of missing data and homozygosity across individuals and loci. The mean error rate per locus and observed error rate per multilocus genotype were calculated by replicated genotyping of a subset of the samples: PCR was repeated three times for each locus for the UC sampling spots of the placentas of the twin individuals, and for the four UC samples for which a corresponding reference pup was available. In addition, a 704 bp-fragment from the 5′ domain of the mitochondrial control region was sequenced for all placentas as described by Valtonen *et al*. (2012).

Data analysis

A χ^2-test for homogeneity was used to test whether the four main placental sampling spots (MS, FS, UC, OP) differed with respect to overall genotyping success: a full 11-locus genotype *vs*. more than two alleles at any of the loci (suggesting mixture of pup's and mother's DNA), or an otherwise unclear genotype at any locus (e.g., locus did not amplify at all, or the signal of the shorter allele was weaker than that of the longer allele, which could indicate genotype mixture).

We compared the genotypes obtained from the different sampling spots (MS, FS, UC, OP, BS) within each placenta to investigate whether the mother's and/or pup's genotype could be inferred. Data from the five reference pups were used to determine the placental sampling spots that produced the best match with the corre-

sponding pup's genotype. PCR was repeated three times for all these placental samples, in order to acquire reliable genotyping results for comparison with the "correct" pup's genotype. As no reference samples of mothers were available, the optimal sampling spot for obtaining the mother's DNA was assessed indirectly, i.e., by comparing the multilocus genotypes of the reference pups with different placental sampling spots, in order to determine which ones produced clearly incompatible results.

The effect of the quality of the placenta (state of decomposition) on amplification success, measured as the number of loci successfully amplified from UC samples, which were found to yield the pups' genotypes (*see* Results), was tested using one-way ANOVA in SPSS Statistics ver. 19 (IBM).

Genetic diversity and isolation-by-distance

Population-level genetic parameters were estimated based on the UC samples, because these were found to yield the pups' genotypes (*see* Results). We estimated the number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, and Wright's inbreeding coefficients (F_{IS}) using ARLEQUIN ver. 3.5.1.2 (Excoffier & Lischer 2010). GENEPOP ver. 4.1.3 (Rousset 2008) was used to test for departures from Hardy-Weinberg equilibrium and for the presence of linkage disequilibrium between pairs of microsatellite loci. Haplotype (h) and nucleotide (π) diversities for the mtDNA control-region sequence data set were estimated using ARLEQUIN.

The presence of an isolation-by-distance pattern among placentas was tested by contrasting pairwise microsatellite- and mtDNA-based genetic relatedness with geographical distance on a logarithmic scale in SPAGEDI ver. 1.3 (Hardy & Vekemans 2009). We chose the kinship coefficient of Loiselle *et al.* (1995) as a pairwise estimator of genetic relatedness, as it is considered suitable for data sets containing rare alleles, and does not assume Hardy-Weinberg equilibrium (Vekemans & Hardy 2004). Ten spatial distance classes were created using the equal-frequency method, which produces distance intervals with uneven distances, but with roughly equal numbers of pairwise comparisons. The mean kinship coefficient of each distance class, as well as the overall regression slope, were tested for a significant departure from zero by 10 000 permutations, and standard errors were estimated by jackknifing over loci.

The utility of placentas for estimating population-level genetic parameters was evaluated by contrasting the aforementioned diversity and differentiation estimates with reference values obtained in previous genetic analyses of Saimaa ringed seals: Valtonen *et al.* (2012) sequenced the mtDNA control-region from 203 dead individuals (stillborns, weaned pups, and adults) and 12 placentas, and Valtonen *et al.* (2014) analyzed variation at 17 microsatellite loci in 172 seals. The placental samples used by Valtonen *et al.* (2012) were excluded from the mtDNA reference data (but included in our present data set), and the microsatellite data set of Valtonen *et al.* (2014) was in our main comparisons reduced to include the same 11 loci as our placental data. To correct for unequal sample sizes when comparing microsatellite allelic richness (A_R) and mtDNA haplotype richness (a), the reference microsatellite and mtDNA data sets were rarefied in HP-RARE (Kalinowski 2005) based on the numbers of placentas. Differences in microsatellite allele frequencies between placentas and the 11-locus reference data set were tested in GENEPOP using an exact G-test. Additionally, differences in allele frequencies were assessed between placentas and a subset of the reference data (n = 65) spanning the same time period as the placental samples (the 2000s). The correlation between mtDNA haplotype frequencies in the placental and reference data sets was tested using Spearman's rank-order correlation in SPSS.

Identification of individuals and kinship analyses

To evaluate whether our panel of 11 microsatellite loci allows reliable identification of Saimaa ringed seal individuals, we estimated the probability of identity (PI, i.e., the probability that two randomly chosen individuals have identical

multilocus genotypes) as well as the corresponding value for siblings (PI_{SIB}) using GENALEX ver. 6.41 (Peakall & Smouse 2006, 2012). For comparison, we calculated these probabilities also for the reference data set of Valtonen *et al.* (2014) using both 11 and 17 loci. For this and further analyses, missing data were not allowed at any loci; after exclusion of individuals with incomplete genotypes, the 11- and 17-locus reference data sets comprised 171 and 168 individuals, respectively.

GENALEX was also used to calculate the probability of exclusion (PE) in the placental and reference data sets, in order to estimate the power and utility of the 11- and 17-locus microsatellite panels in parentage analysis. PE was estimated for three alternative scenarios: PE_1 = probability for excluding a putative parent when the other parent is known; PE_2 = probability of exclusion of a putative parent when the other parent is unknown; PE_3 = probability of excluding two putative parents (Jamieson & Taylor 1997).

In order to determine the optimal number of loci for identification of Saimaa ringed seal individuals (*see* Waits & Paetkau 2005), we used MM-DIST (Kalinowski *et al.* 2006) to compute expected and observed mismatch distributions for placentas as well as for the 11- and 17-locus reference data sets. MM-DIST calculates probability distributions for genotypic differences, i.e., the numbers of loci that differ among individuals in a population. The program creates the observed distribution for the studied data set, as well as expected distributions for individuals with different degrees of relatedness: unrelated individuals, fullsibs, and parent–offspring pairs. The congruence between observed and expected MM-distributions, as well as between observed distributions estimated for the placental and 11-locus reference data sets, were tested using a χ^2-test for goodness of fit. In order to meet the test's assumption of all expected frequencies exceeding 1, the following mismatch categories were combined: for placentas, the two lowest and three highest categories; for the 11-locus reference data, the two lowest categories; for the 17-locus data, the three lowest and three highest categories; and for the comparison of observed MM-distributions in the placental and 11-locus reference data sets, the two lowest and two highest categories.

The adequacy of the 11- and 17-locus marker systems for inferring sibship and parentage was further studied using COLONY ver. 2.0.4.1 (Jones & Wang 2010). First, we inferred full- and half-sibs from the data on placentas alone; in this analysis, the aforementioned placentas of the confirmed twins were used as an additional reference to determine whether they could be recognised as siblings. Second, we introduced potential adult fathers ($n = 6$) and mothers ($n = 4$) from the reference data set into the analysis of placentas, in order to assign parentage and sibship simultaneously. Finally, we analysed the full microsatellite reference data to infer sibship and parentage based on 17 loci. Three independent replicate runs were carried out with each data set to ensure convergence. Allele frequencies were estimated for each data set by COLONY, polygamy was assumed for both sexes, and no sibship prior was used. For placentas alone, we used the full-likelihood method with high precision and 'long' run length. Otherwise similar settings were used for the analysis involving placentas and potential parents, but run length was set to 'medium'. For the large reference data with 17 loci, the pairwise-likelihood score method and 'very long' run length were chosen.

Results

Genotyping

Genotyping success varied among different placental sampling spots (Table 1). Full, unambiguous 11-locus genotypes were obtained for 51% of UC (umbilical cord/vein) samples, but for only 0%–12% of the other sampling spots. More than two alleles at one locus, indicating a mixture of the mother's and pup's DNA, were detected most often in MS (maternal side) samples and least frequently in UC samples (34.5% and 3.6%, respectively). A χ^2-test of homogeneity showed a highly significant difference in genotyping success among MS, FS, UC, and OP sampling spots ($\chi^2 = 56.45$, df = 6, $p < 0.001$).

When comparing the genotypes of the five reference pups with those of their corresponding

Table 1. Genotyping success at 11 microsatellite loci for each placental sampling spot. MS = maternal side, FS = foetal side, UC = umbilical cord/vein, OP = orange particles, BS = blood sample.

	MS		FS		UC		OP		BS		n_{total}
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Unambiguous genotype	5	8.6	8	12.1	28	50.9	7	12.1	0	0	48
More than 2 alleles	20	34.5	9	13.6	2	3.6	8	13.8	0	0	39
Unclear genotype	33	56.9	49	74.2	25	45.5	43	74.1	4	100	154
n_{total}	58		66		55		58		4		

placentas, UC samples were the only ones that produced completely matching multilocus consensus genotypes (Table 2). None of the remaining main sampling spots (MS, FS, OP) yielded a genotype that was both unambiguous and clearly different from the pup's genotype, indicating that the mothers' genotypes could not be reliably determined.

The MICRO-CHECKER analysis suggested the presence of null alleles for three loci (*Hg8.9*, *SGPv11* and *SGPv16*), but estimated null-allele frequencies were low ($r \leq 0.08$) at each locus, and the result is likely caused by the presence of population structure (Valtonen *et al.* 2014). Scoring errors suggested for *Hg8.9* were ruled out by rechecking the data independently by two researchers. Further, no significant correlation between the amount of missing data and homozygosity was found either across individuals ($r = 0.163$, $p = 0.123$) or loci ($r = –0.247$, $p = 0.738$). The estimated mean error rate per locus was 0.036, and the observed error rate per multilocus genotype 0.313. Degree of decomposition had no effect on amplification success in UC samples (one-way ANOVA: $F_{2,52} = 0.609$, $p = 0.548$).

Comparison of population-genetic parameters estimated from the placental and reference data sets

Based on the above, UC samples were taken to represent the genotypes of the pups. Unclear UC genotypes (i.e., longer allele producing a taller peak than the shorter allele, *see* above) were accepted as a true genotype, but samples with missing data or more than two alleles at any locus were discarded; hence, further analyses were conducted with the 47 placentas that had full UC genotypes at 11 microsatellite loci.

Population-level microsatellite diversity estimated on the basis of placentas was very low, but observed (H_O) and expected (H_E) heterozygosities corresponded closely with estimates obtained for the reference data sets of 11 and 17 loci (Table 3). Allelic richness (A_R) was slightly higher in the 11-locus reference data set than in the placental samples even after correcting for sample-size differences by rarefaction (Table 3), which was, however, expected given that the individuals in the reference data set represent a longer period and a wider geographic area, and that some placentas may represent siblings due to the fact that they had been collected from the same birth-lair sites during consecutive springs (*see* below).

F_{IS} values estimated for the pooled placental sample as well as for the two regional subsamples did not depart from zero at $p \leq 0.05$ (Table 3), but the pooled data set was not in Hardy-Weinberg equilibrium (Fisher's exact test over all loci, $p = 0.006$). However, when the Main Haukivesi ($n = 17$) and Pihlajavesi areas

Table 2. Congruence (i.e., percentage of full matches at 11 loci) between consensus genotypes of different placental sampling spots and those of corresponding reference pups (note that only an FS sample could be obtained from the placenta of Pup 3). MS = maternal side, FS = foetal side, UC = umbilical cord/vein, OP = orange particles.

	MS	FS	UC	OP
Pup 1	64	55	100	45
Pup 2	73	91	100	45
Pup 3	–	45	–	–
Pup 4	64	45	100	55
Pup 5	82	73	100	27

(n = 24; *see* Fig. 1B) were analysed separately, deviations from Hardy-Weinberg equilibrium were found neither over all loci nor at any individual locus after a sequential Bonferroni correction.

Significant linkage disequilibrium remained between loci *Hg8.9* and *Hl15* even after a sequential Bonferroni correction; this most likely reflects differentiation between the Pihlajavesi and Main Haukivesi areas. Allele frequencies in placentas and the 11-locus reference data set differed significantly (χ^2 = 41.03, df = 22, p = 0.008), but the difference disappeared after reducing the reference data to represent the same time span as the placentas (χ^2 = 23.17, df = 22, p = 0.392).

Six mtDNA haplotypes were detected in the 63 placentas successfully analysed for mtDNA variation (Fig. 1B; *see* Valtonen *et al*. 2012 for details of haplotypes). Haplotypes H1 and H3 dominated the sample with relative frequencies of 36.5% and 49.2%, respectively, while H7 comprised 9.5% of the haplotypes, and H2, H4, and H8 were found in a single placenta each. When considering all haplotypes (H1–H8) found by Valtonen *et al*. (2012), placental haplotype frequencies correlated strongly with estimates derived from the mtDNA reference data set (Spearman's ρ = 0.805, p = 0.016). Estimated haplotype (h ± SD) and nucleotide (π ± SD) diversities as well as haplotypic richness (a) were very low (0.625 ± 0.037, 0.005 ± 0.005, and 6.00, respectively), but similar to those observed for the reference data set (0.649 ± 0.021, 0.005 ± 0.005 and 6.69, respectively; the estimate of a in the reference data set was obtained by rarefaction to n = 63).

A weak but statistically significant negative relationship between relatedness and spatial distance between pairs of placentas was detected using both microsatellites (b = –0.025, p = 0.009; Fig. 3A) and mtDNA control-region sequences (b = –0.128, p < 0.001: Fig. 3B). These results corresponded well with the previous study of Valtonen *et al*. (2014).

Identification of individuals, and kinship analyses

The probabilities of identity (i.e., probability that two individuals share the same multilocus genotype; PI for randomly chosen individuals and PI_{SIB} for siblings), were very low, but slightly higher in placentas than in the 11-locus reference data set (Table 4). With six additional loci (17 loci in total), PI_{SIB} values were an order and PI values two orders of magnitude lower. The estimated probabilities of exclusion were sufficiently high ($p \geq 0.99$) for excluding two puta-

Table 3. Estimates of genetic diversity in the Saimaa ringed seal population based upon analyses of 11 microsatellite loci in UC samples of placentas, and 11 and 17 loci in the reference data set. Average total number of alleles (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) are given. Allelic richness (A_R) estimates in the reference data sets were obtained by rarefaction to the sample size (47, 17, or 24) in the corresponding sample in the placental data set. $P_{(HWE)}$ values indicating significant deviations from Hardy-Weinberg equilibrium are set in boldface.

Sample	n	N_A ± SD	A_R	H_O ± SD	H_E ± SD	F_{IS}	$P_{(HWE)}$
Placentas							
Total sample	47	2.91 ± 2.12	2.91	0.33 ± 0.24	0.35 ± 0.23	0.057^{ns}	**0.006**
Main Haukivesi area	17	2.64 ± 1.57	2.64	0.35 ± 0.27	0.39 ± 0.27	0.100^{ns}	0.396
Pihlajavesi area	24	2.60 ± 1.35	2.45	0.35 ± 0.23	0.34 ± 0.22	–0.020^{ns}	0.987
11-locus reference data							
Total sample	172	3.73 ± 4.10	3.38	0.35 ± 0.22	0.38 ± 0.24	0.073***	**< 0.001**
Main Haukivesi area	79	3.45 ± 3.56	2.90	0.38 ± 0.24	0.40 ± 0.25	0.038^{ns}	**< 0.001**
Pihlajavesi area	43	2.55 ± 1.63	2.35	0.32 ± 0.25	0.30 ± 0.23	–0.065^{ns}	0.280
17-locus reference data							
Total sample	172	3.47 ± 3.32	3.14	0.33 ± 0.21	0.36 ± 0.22	0.075***	**< 0.001**
Main Haukivesi area	79	3.18 ± 2.92	2.70	0.34 ± 0.23	0.35 ± 0.24	0.024^{ns}	**< 0.001**
Pihlajavesi area	43	2.59 ± 1.42	2.43	0.31 ± 0.25	0.30 ± 0.23	–0.034^{ns}	0.144

ns = not significant, *** p < 0.001.

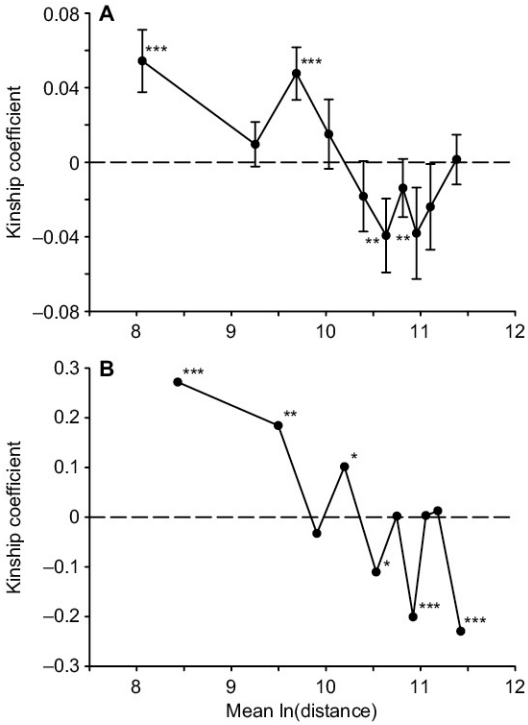

Fig. 3. Average Loiselle's kinship coefficient plotted against logarithmic distance between pairs of Saimaa ringed seal placentas. The plots are based on (**A**) 11 microsatellite loci, and (**B**) mtDNA haplotypes. Asterisks denote distance classes that differ significantly from mean kinship: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

tive parents (PE_3) for both reference data sets, but not for placentas. Moreover, none of the data sets provided enough resolution for excluding one parent (PE_1 and PE_2; Table 4).

Table 4. Probabilities of identity and exclusion estimated for Saimaa ringed seal placentas genotyped at 11 loci, as well as for the 11- and 17-locus reference data sets. PI = probability of identity for unrelated individuals, PI_{SIB} = probability of identity for siblings, PE_1 = probability for excluding a putative parent when the other parent is known, PE_2 = probability for excluding a putative parent when the other parent is unknown, PE_3 = probability of excluding two putative parents.

	Placentas	Reference data set	
		11 loci	17 loci
PI	2.098×10^{-4}	4.581×10^{-5}	4.817×10^{-7}
PI_{SIB}	1.695×10^{-2}	1.010×10^{-2}	1.193×10^{-3}
PE_1	0.878	0.930	0.978
PE_2	0.638	0.734	0.839
PE_3	0.972	0.990	0.999

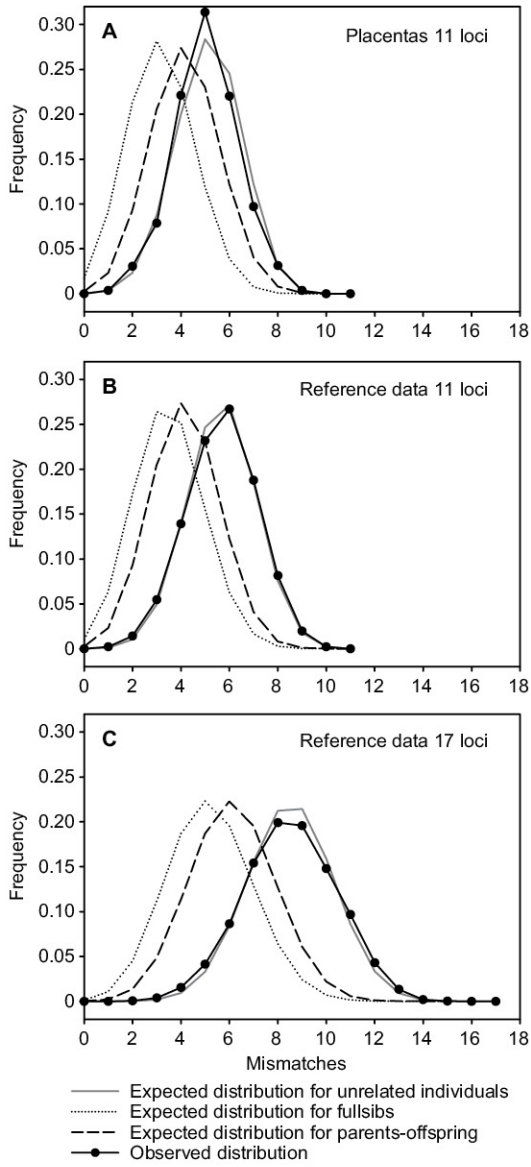

Fig. 4. Mismatch distributions among genotypes of Saimaa ringed seal placentas based on (**A**) 11 microsatellite loci, and within the reference data set based on (**B**) 11 loci and (**C**) 17 loci.

Mismatch distributions estimated for placentas and the 11- and 17-locus reference data sets were fairly consistent with the expected distributions of unrelated individuals (Fig. 4). Observed and expected distributions did not differ in the case of placentas ($\chi^2 = 9.85$, df = 8, $p = 0.276$), but a highly significant deviation was found in both reference data sets ($\chi^2 = 30.39$, df = 10,

$p < 0.001$ for the 11-locus reference data set, and $\chi^2 = 111.43$, df = 13, $p < 0.001$ for the 17-locus data set). Deviations from expectation are, however, not pronounced (Fig. 4B and C), and are probably explained by spatial genetic structure in the population (*see* above) and the relatively large sample sizes in the reference data sets. The observed mismatch distributions of placentas and the 11-locus reference data set differed significantly ($\chi^2 = 206.26$, df = 9, $p < 0.001$), because the distribution based on placental genotypes is slightly shifted towards smaller numbers (Fig. 4A and B).

No pairs of individuals with fully matching multilocus genotypes were found within any of the data sets. There were four pairs with a single mismatch (1MM-pairs) in the placental and 30 in the 11-locus reference data, whereas none were observed in the 17-locus data set. The number of 2MM-pairs was 33 for placentas, while 206 and 8 pairs were present in the 11- and 17-locus reference data sets, respectively. However, when the placental and 11-locus reference data sets were combined, we found five UC samples having full match with a genotype present in the reference data set; however, the matching reference individuals had died 3–30 years before collection of the placentas.

In COLONY analyses, replicate runs of each data set produced different results, and the analyses generally did not seem to have enough power to resolve relationships. The results suggested parentage of individuals that had died before the birth of suggested offspring, and also produced unrealistically numerous full- and half-sibling pairs. Moreover, the aforementioned placentas of seal twins were not recognised as siblings.

Discussion

The ecology of the critically endangered Saimaa ringed seal is well known as a result of three decades of intensive field research and telemetry studies (e.g., Hyvärinen *et al.* 1995, Rautio *et al.* 2009, Niemi *et al.* 2012). Nevertheless, as the population density is low, and the animal itself elusive and difficult to capture, all aspects of its ecology and behaviour cannot be studied using traditional monitoring approaches. Recent population-genetic analyses have demonstrated very low genetic variability in the population (Palo *et al.* 2003, Valtonen *et al.* 2012, Martinez-Bakker *et al.* 2013, Valtonen *et al.* 2014), and have revealed clear spatial structuring among the main breeding areas, indicating limited movement of Saimaa ringed seals and especially females (Valtonen *et al.* 2012, 2014). However, those analyses were mainly based on stillborn, by-caught, and stranded seals, of which only about 25 are recovered each year (Metsähallitus 2014). In addition, the samples were "retrospective" in the sense that the individuals were dead and, hence, removed from the population.

Under these circumstances, placentas collected from breeding sites could provide a highly useful source for non-invasive genetic sampling of live individuals: because of the thorough springtime breeding-site inspections conducted by Parks & Wildlife Finland (the authority responsible for monitoring and conservation of the Saimaa ringed seal), placentas of nearly half of the pups born each year can be recovered with a reasonable effort. Compared with other non-invasive samples such as hair and faeces, placentas provide a large amount of DNA, as well as the theoretical prospect of genotyping both the offspring and its mother from a single sample (cf. Stewart & Stewart 2009). As our analyses show, the pup's multilocus genotype can be reliably inferred from umbilical cord samples. Unfortunately, we did not succeed in genotyping the mothers, most likely due to mixture of maternal and offspring tissues and, therefore, genotypes, on the maternal side of the placenta.

Despite the limitation concerning the identification of breeding females, our results demonstrate that genetic tags obtained from umbilical cords could be used for studies on postnatal movements and long-term survival of Saimaa ringed seal individuals. Such studies could be conducted by comparing placental multilocus genotypes to tags that are later obtained from, for example, hair samples collected from haul-out sites, from live seals captured during telemetry studies, or from by-caught or stranded carcasses.

The extremely low microsatellite diversity of the Saimaa ringed seal ($H_E = 0.36$; Valtonen *et al.* 2014) poses a clear challenge for genetically-based tagging (cf. Waits & Paetkau

2005). However, sufficient resolution for individual identification is provided by our panel of 17 microsatellite loci: the estimated probability of identity for unrelated individuals (PI = 4.8×10^{-7}) is lower, and that for siblings ($PI_{SIB} = 1.2 \times 10^{-3}$) very close, to the conservative threshold values (1×10^{-6} and 1×10^{-3}, respectively) recommended by McKelvey and Schwartz (2004). In the current population of approximately 300 seals (Metsähallitus 2014), our figures translate to 0.0001 expected full genotype matches for unrelated individuals and 0.36 matches for siblings, although the true numbers may be slightly higher due to the pronounced spatial genetic differentiation in the population found by Valtonen *et al*. (2012, 2014), i.e., because seals within each region tend to be more closely related and, thus, have more similar genotypes than individuals on average in the population. PI and PI_{SIB} values are higher for the 11-locus placental and reference data sets, and numbers of 1MM- and 2MM-pairs in their mismatch distributions (Fig. 4A and B) exceed the recommendations of Waits and Paetkau (2005) (< 1–2 1MM- and < 10 2MM-pairs in the data). Nevertheless, as pointed out by Waits and Paetkau (2005), the number of loci required also depends on the number of individuals to be compared, so even the 11-locus panel could be sufficient for spatially and/or temporally restricted surveys of the focal population.

Although our marker system allows genetic identification of individuals, the low population-level variability means that the limits of even the full 17-locus panel are reached when attempting to infer parentage or sibship among the sampled individuals and/or placentas. This is especially seen in the kinship analyses in COLONY, in which the output of all runs suggested implausible or impossible parentage and sibship. The unfortunate consequence of this is that one of our main goals could not be reached, because pups (placentas) collected from the same site during different springs could not be confidently determined to be siblings. At least some sibling pairs are likely to be present in our data set, because placentas having identical mtDNA haplotypes were collected from the same or closely located birth-lair sites in different years (Fig. 1B), but this could also be explained by the generally low level of mtDNA variation and the presence of an isolation-by-distance pattern within the lake (Fig. 3, and Valtonen *et al*. 2014). Importantly, however, the analytical impediments that follow from lack of marker resolution can be overcome by applying new, genome-scan based methods (e.g., Tokarska *et al*. 2009), so field-collected placentas undoubtedly can also be applied for studying breeding-site fidelity of females in the near future.

Interestingly, our results demonstrate that key population-level diversity and differentiation indices can be estimated based on placental samples. Both microsatellite and mtDNA diversities of placentas corresponded closely with previous results derived from large reference data sets comprising individuals found dead during a time span of 30 years (Valtonen *et al*. 2012, 2014), especially after applying rarefaction to correct for differences in sample size. No differences in microsatellite allele frequencies were detected between placentas and a subset of the reference data representing the same time span, the 2000s, and mtDNA haplotype frequencies in placentas and the reference data set were likewise highly correlated. Also the isolation-by-distance patterns of placentas were very similar to the findings of Valtonen *et al*. (2014).

Conclusions and further prospects

This study is the first describing the utility of placentas in non-invasive genetic monitoring of a natural population. Saimaa ringed seal placentas are relatively easily collected from birth-lair sites, and comparisons with existing reference data sets demonstrate that placentas can be used for estimating standard population-genetic parameters in separate breeding areas or within the whole lake. Genotyping pre-dispersal juveniles from umbilical cord samples provides a unique opportunity for tracking individuals from their natal site to later recapture(s) or death, yielding information on their dispersal patterns and long-term survival prospects. In addition, determining the pups' gender from umbilical cord samples using genetic markers (Curtis *et al*. 2007) would yield information on the sex ratio within the population and in different breeding

areas. Unfortunately, even our 17-locus microsatellite panel does not provide enough discriminatory power for pedigree construction and kinship analyses, due to the fact that this small population with extremely low genetic diversity and significant structuring (Valtonen *et al*. 2012, 2014) is inevitably inbred, but such analyses will undoubtedly be enabled by next-generation sequencing and genotyping technologies in the near future.

As our results show, high-quality DNA can be obtained from placentas, but wider application of our method is to some degree restricted by postnatal consumption of the placenta (placentophagia) by females in most mammalian species (Kristal *et al*. 2012). However, the order Pinnipedia is one of the few exceptions here, and genetic monitoring based on placental samples could be used, for example, for the Ladoga ringed seal (*P. h. ladogensis*), which inhabits Lake Ladoga in northwestern Russia, and which has breeding habits that closely resemble those of the Saimaa ringed seal (Kunnasranta *et al*. 2001). In addition, many other pinnipeds, such as grey seals (*Halichoerus grypus*), harbour seals (*Phoca vitulina*), and all otariids, typically give birth on land and regularly at the same locations (Riedman 1990), which offers a good opportunity for collection of placentas also from these species.

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References

Allen, P. J., Amos, W., Pomeroy, P. P. & Twiss, S. D. 1995: Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. — *Molecular Ecology* 4: 653–662.

Arandjelovic, M., Head, J., Rabanal, L. I., Schubert, G., Mettke, E., Boesch, C., Robbins, M. M. & Vigilant, L. 2011: Non-invasive genetic monitoring of wild central chimpanzees. — *PLoS One* 6: e14761, doi:10.1371/journal.pone.0014761.

Auttila, M., Niemi, M., Skrzypczak, T., Viljanen, M. & Kunnasranta, M. 2014: Estimating and mitigating perinatal mortality of the endangered Saimaa ringed seal (*Phoca hispida saimensis*) in a changing climate. — *Annales Zoologici Fennici* 51: 526–534.

Baker, C., Steel, D., Calambokidis, J., Falcone, E., González-Peral, U., Barlow, J., Burdin, A., Clapham, P. J., Ford, J. K. B. & Gabriele, C. M. 2013: Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. — *Marine Ecology Progress Series* 494: 291–306.

Chapuis, M.-P. & Estoup, A. 2007: Microsatellite null alleles and estimation of population differentiation. — *Molecular Biology and Evolution* 24: 621–631.

Curtis, C., Stewart, B. S. & Karl, S. A. 2007: Sexing pinnipeds with ZFX and ZFY loci. — *Journal of Heredity* 98: 280–285.

Davis, C., Gelatt, T., Siniff, D. & Strobeck, C. 2002: Dinucleotide microsatellite markers from the Antarctic seals and their use in other pinnipeds. — *Molecular Ecology Notes* 2: 203–208.

Davoli, F., Schmidt, K., Kowalczyk, R. & Randi, E. 2013: Hair snaring and molecular genetic identification for reconstructing the spatial structure of Eurasian lynx populations. — *Mammalian Biology* 78: 118–126.

Excoffier, L. & Lischer, H. E. L. 2010: Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. — *Molecular Ecology Resources* 10: 564–567.

Foote, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A, Salling, A. B., Galatius, A., Orlando, L. & Gilbert, M. T. 2012: Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. — *PLoS One* 7: e41781, doi:10.1371/journal.pone.0041781.

Goodman, S. J. 1997: Dinucleotide repeat polymorphisms at seven anonymous microsatellite loci cloned from the European harbour seal (*Phoca vitulina vitulina*). — *Animal Genetics* 28: 308–322.

Hardy, O. J. & Vekemans, X. 2009: *SPAGeDi 1.3: a program for spatial pattern analysis of genetic diversity*. — User's manual available at http://ebe.ulb.ac.be/ebe/SPAGeDi.html.

Hyvärinen, H., Hämäläinen, E. & Kunnasranta, M. 1995: Diving behavior of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.). — *Marine Mammal Science* 11: 324–334.

Jamieson, A. & Taylor, S. C. 1997: Comparisons of three probability formulae for parentage exclusion. — *Animal Genetics* 28: 397–400.

Jones, O. R. & Wang, J. 2010: COLONY: a program for parentage and sibship inference from multilocus genotype

data. — *Molecular Ecology Resources* 10: 551–555.

Kalinowski, S T. 2005: HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. — *Molecular Ecology Notes* 5: 187–189.

Kalinowski, S. T., Sawaya, M. A. & Taper, M. L. 2006: Individual identification and distribution of genotypic differences between individuals. — *Journal of Wildlife Management* 70: 1148–1150.

Kopatz, A., Eiken, H., Hagen, S., Ruokonen, M., Esparza-Salas, R., Schregel, J., Kojola, I., Smith, M., Wartiainen, I., Aspholm, P., Wikan, S., Rykov, A., Makarova, O., Polikarpova, N., Tirronen, K., Danilov, P. & Aspi, J. 2012: Connectivity and population subdivision at the fringe of a large brown bear (*Ursus arctos*) population in North Western Europe. — *Conservation Genetics* 13: 681–692.

Kovacs, K. M., Aguilar, A., Aurioles, D., Burkanov, V., Campagna, C., Gales, N., Gelatt, T., Goldsworthy, S. D., Goodman, S. J., Hofmeyr, G. J. G., Härkönen, T., Lowry, L., Lydersen, C., Schipper, J., Sipilä, T., Southwell, C., Stuart, S., Thompson, D. & Trillmich, F. 2012: Global threats to pinnipeds. — *Marine Mammal Science* 28: 414–436.

Kristal, M. B., DiPirro, J. M. & Thompson, A. C. 2012: Placentophagia in humans and nonhuman mammals: causes and consequences. — *Ecology of Food and Nutrition* 51: 177–197.

Kunnasranta, M., Hyvärinen, H., Sipilä, T. & Medvedev, N. 2001: Breeding habitat and lair structure of the ringed seal (*Phoca hispida ladogensis*) in northern Lake Ladoga in Russia. — *Polar Biology* 24: 171–174.

Kuusisto, E. 1999: Basin and balances. — In: Kuusisto, E. (ed.), *Saimaa, a living lake*: 21–39. Tammi, Helsinki.

Loiselle, B. A., Sork, V. L., Nason, J. & Graham, C. 1995: Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). — *American Journal of Botany* 82: 1420–1425.

Martinez-Bakker, M. E., Sell, S. K., Swanson, B. J., Kelly, B. P. & Tallmon, D. A. 2013: Combined genetic and telemetry data reveal high rates of gene flow, migration, and long-distance dispersal potential in Arctic ringed seals (*Pusa hispida*). — *PLoS One* 8: e77125, doi:10.1371/journal.pone.0077125.

McKelvey, K. S. & Schwartz, M. K. 2004: Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. — *Journal of Wildlife Management* 63: 439–448.

Metsähallitus, Parks & Wildlife Finland 2014: *Saimaannorppa*. — Available at http://www.metsa.fi/saimaannorppa.

Niemi, M., Auttila, M., Viljanen, M. & Kunnasranta, M. 2012: Movement data and their application for assessing the current distribution and conservation needs of the endangered Saimaa ringed seal. — *Endangered Species Research* 19: 99–108.

Palo, J. U., Hyvärinen, H., Helle, E., Mäkinen, H. S. & Väinölä, R. 2003: Postglacial loss of microsatellite variation in the landlocked Lake Saimaa ringed seal. — *Conservation Genetics* 4: 117–128.

Parsons, K. M., Durban, J. W., Claridge, D. E., Herzing, D. L., Balcomb, K. C. & Noble, L. R. 2006: Population genetic structure of coastal bottlenose dolphins (*Tursiops truncatus*) in the northern Bahamas. — *Marine Mammal Science* 22: 276–298.

Peakall, R. & Smouse, P. E. 2006: GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. — *Molecular Ecology Notes* 6: 288–295.

Peakall, R. & Smouse, P. 2012: GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. — *Bioinformatics* 1: 6–8.

Rassi, P., Hyvärinen, E., Juslén, A. & Mannerkoski, I. (eds.), 2010: *The red list of Finnish species 2010*. — Ympäristöministeriö & Suomen ympäristökeskus, Helsinki.

Rautio, A., Niemi, M., Kunnasranta, M., Holopainen, I. J. & Hyvärinen, H. 2009: Vocal repertoire of the Saimaa ringed seal (*Phoca hispida saimensis*) during the breeding season. — *Marine Mammal Science* 25: 920–930.

Riedman, M. 1990: *The pinnipeds: seals, sea lions, and walruses*. — University of California Press, Berkeley, California, USA.

Rousset, F. 2008: GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. — *Molecular Ecology Resources* 8: 103–106.

Schwartz, M. K., Luikart, G. & Waples, R. S. 2007: Genetic monitoring as a promising tool for conservation and management. — *Trends in Ecology & Evolution* 22: 25–33.

Sipilä, T. 2003: Conservation biology of Saimaa ringed seal (*Phoca hispida saimensis*) with reference to other European seal populations. — Ph.D. thesis, University of Helsinki.

Stewart, R. E. A. & Stewart, B. S. 2009: Female reproductive systems. — In Perrin, W. F., Wursig, B. & Thewissen, J. G. M. (eds.), *Encyclopedia of marine mammals*, 2nd ed.: 423–428. Academic Press, San Diego.

Swanson, B. J., Kelly, B. P., Maddox, C. K. & Moran, J. R. 2006: Shed skin as a source of DNA for genotyping seals. — *Molecular Ecology Notes* 6: 1006–1009.

Tokarska, M., Marshall, T., Kowalczyk, R., Wójcik, J. M., Pertoldi, C., Kristensen, T. N., Loeschcke, V., Gregersen, V. R. & Bendixen, C. 2009: Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: the case of European bison. — *Heredity* 103*:* 326–332.

Twiss, S. D., Poland, V. F., Graves, J. A. & Pomeroy, P. P. 2006: Finding fathers: spatio-temporal analysis of paternity assignment in grey seals (*Halichoerus grypus*). — *Molecular Ecology* 15: 1939–1953.

Valqui, J., Hartl, G. B. & Zachos, F. E. 2010: Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (*Lontra felina*). — *Conservation Genetics* 11: 2067–2072.

Valtonen, M., Palo, J. U., Aspi, J., Ruokonen, M., Kunnasranta, M. & Nyman, T. 2014: Causes and consequences of fine-scale population structure in a critically endangered freshwater seal. — *BMC Ecology* 14: e22, doi:10.1186/1472-6785-14-22.

Valtonen, M., Palo, J. U., Ruokonen, M., Kunnasranta, M. & Nyman, T. 2012: Spatial and temporal variation in genetic diversity of an endangered freshwater seal. —

Conservation Genetics 13: 1231–1245.

Van den Broeck, A. J. P. 1904: The foetal membranes and the placenta of *Phoca vitulina*. — *Proceedings of the Royal Netherlands Academy of Arts and Sciences (KNAW)* 6: 610–619.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. 2004: MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. — *Molecular Ecology Notes* 4: 535–538.

Vekemans, X. & Hardy, O. J. 2004: New insights from fine-scale spatial genetic structure analyses in plant populations. — *Molecular Ecology* 13: 921–935.

Waits, L. P. & Paetkau, D. 2005: Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. — *Journal of Wildlife Management* 69: 1419–1433.

Wang, C., Schroeder, K. B. & Rosenberg, N. A. 2012: A maximum-likelihood method to correct for allelic dropout in microsatellite data with no replicate genotypes. — *Genetics* 192: 651–669.

Appendix. Collection information on the Saimaa ringed seal placentas included in this study. Collection date and location, samples taken (MS = maternal side; FS = foetal side; UC = umbilical cord/ vein; OP = orange particles; BS = blood) and quality (1 = fresh; 2 = partly decomposed; 3 = decomposed) of each placenta are given.

ID	Code	Collection date	Location	Samples	Quality
I-18	I-18_P_y2000	14 Apr. 2000	Pihlajavesi area	MS, FS, UC, OP, BS	1
I-19	I-19_P_y2000	17 Apr. 2000	Pihlajavesi area	MS, FS, UC, OP	2
I-20	I-20_P_y2001	12 Apr. 2001	Pihlajavesi area	MS, FS, UC, OP, BS	1
I-21	I-21_P_y2002	22 Apr. 2002	Pihlajavesi area	MS, FS, UC, OP	2
I-22	I-22_S_y2004	14 Apr. 2004	Southern Saimaa	FS	2
I-23	I-23_P_y2004	6 Apr. 2004	Pihlajavesi area	MS, FS, UC, OP	2
I-24	I-24_S_y2006	2006	Southern Saimaa	FS	2
I-26	I-26_S_y2009	18 Apr. 2009	Southern Saimaa	MS, FS, UC, OP, BS	1
I-27	I-27_H_y2009	19 Apr. 2009	Main Haukivesi area	MS, FS, UC, OP, BS	1
I-28	I-28_P_y2009	25 Apr. 2009	Pihlajavesi area	MS, FS, UC, OP	2
I-29	I-29_H_y2009	19 May 2009	Main Haukivesi area	MS, FS, UC, OP	3
I-30	I-30_H_y2009	19 May 2009	Main Haukivesi area	MS, FS, UC, OP	3
I-31	I-31_H_y2009	19 May 2009	Main Haukivesi area	MS, FS, UC, OP	3
I-32	I-32_P_y2009	28 May 2009	Pihlajavesi area	MS, FS, UC, OP	2
I-33A	I-33A_P_y2009	28 May 2009	Pihlajavesi area	MS, FS, UC, OP	3
I-33B	I-33B_P_y2009	28 May 2009	Pihlajavesi area	MS, FS, UC, OP	2
I-34	I-34_P_y2009	28 May 2009	Pihlajavesi area	MS, FS, UC, OP	2
I-35	I-35_P_y2009	28 May 2009	Pihlajavesi area	FS	3
I-36	I-36_P_y2009	29 May 2009	Pihlajavesi area	MS, FS, UC, OP	3
I-37	I-37_P_y2009	29 May 2009	Pihlajavesi area	MS, FS, UC, OP	3
I-38	I-38_P_y2010	14 May 2010	Pihlajavesi area	MS, FS, OP	2
I-39	I-39_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, UC, OP	1
I-40	I-40_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, UC, OP	3
I-41	I-41_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, UC, OP	2
I-42	I-42_P_y2010	11 May 2010	Pihlajavesi area	MS, FS, UC, OP	1
I-43	I-43_P_y2010	12 May 2010	Pihlajavesi area	MS, FS, UC, OP	1
I-44	I-44_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, UC, OP	2
I-45	I-45_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, OP	3
I-46	I-46_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, UC, OP	2
I-47	I-47_P_y2010	12 May 2010	Pihlajavesi area	MS, FS, UC, OP	3
I-48	I-48_P_y2010	13 May 2010	Pihlajavesi area	MS, FS, UC, OP	3
I-49	I-49_S_y2010	27 May 2010	Southern Saimaa	MS, FS, UC, OP	3
I-50	I-50_H_y2010	10 May 2010	Main Haukivesi area	MS, FS, UC, OP	3
I-51	I-51_H_y2010	3 May 2010	Main Haukivesi area	MS, FS, UC, OP	3
I-52	I-52_H_y2010	3 May 2010	Main Haukivesi area	MS, FS, UC, OP	2
I-53	I-53_H_y2010	12 May 2010	Main Haukivesi area	MS, FS, UC, OP	2
I-54	I-54_H_y2010	6 May 2010	Main Haukivesi area	MS, FS, UC, OP	1
I-55	I-55_H_y2010	12 May 2010	Main Haukivesi area	MS, FS, UC, OP	1
I-56	I-56_H_y2010	15 May 2010	Main Haukivesi area	MS, FS, UC, OP	2
I-57	I-57_H_y2010	12 May 2010	Main Haukivesi area	MS, FS, UC, OP	1
I-58	I-58_H_y2010	12 May 2010	Main Haukivesi area	MS, FS, UC, OP	2

continued

Appendix. Continued.

ID	Code	Collection date	Location	Samples	Quality
I-59	I-59_N_y2011	19 May 2011	Northern Saimaa	MS, FS, UC, OP	2
I-60	I-60_K_y2011	23 May 2011	Kolovesi	MS, FS, UC, OP	2
I-61	I-61_H_y2011	12 May 2011	Main Haukivesi area	FS	3
I-62	I-62_H_y2011	12 May 2011	Main Haukivesi area	MS, FS, UC, OP	3
I-63	I-63_H_y2011	16 May 2011	Main Haukivesi area	MS, FS, UC, OP	2
I-64	I-64_H_y2011	12 May 2011	Main Haukivesi area	MS, FS, UC, OP	2
I-65	I-65_H_y2011	13 May 2011	Main Haukivesi area	MS, FS, UC, OP	1
I-66	I-66_P_y2011	9 May 2011	Pihlajavesi area	MS, FS, UC, OP	1
I-67	I-67_P_y2011	9 May 2011	Pihlajavesi area	FS	2
I-68	I-68_P_y2011	10 May 2011	Pihlajavesi area	MS, FS, UC, OP	2
I-69	I-69_P_y2011	10 May 2011	Pihlajavesi area	MS, FS, UC, OP	2
I-70	I-70_P_y2011	10 May 2011	Pihlajavesi area	FS	2
I-71	I-71_P_y2011	11 May 2011	Pihlajavesi area	MS, FS, UC, OP	1
I-73	I-73_P_y2011	11 May 2011	Pihlajavesi area	MS, FS, UC, OP	1
I-74	I-74_P_y2011	11 May 2011	Pihlajavesi area	MS, FS, UC, OP	2
I-75	I-75_P_y2011	12 May 2011	Pihlajavesi area	MS, FS, UC, OP	2
I-76	I-76_P_y2011	12 May 2011	Pihlajavesi area	MS, FS, UC, OP	2
I-77	I-77_P_y2011	12 May 2011	Pihlajavesi area	MS, FS, OP	2
I-78	I-78_P_y2011	12 May 2011	Pihlajavesi area	MS, FS, UC, OP	3
I-79	I-79_S_y2011	7 May 2011	Southern Saimaa	MS, FS, UC, OP	3
I-82	I-82_S_y2011	8 May 2011	Southern Saimaa	FS	2
I-84	I-84_S_y2011	8 May 2011	Southern Saimaa	MS, FS, UC, OP	3
I-85	I-85_H_y2011	23 Apr. 2011	Main Haukivesi area	MS, FS, UC, OP	1
I-86	I-86_H_y2011	20 Apr. 2011	Main Haukivesi area	FS	2
I-87	I-87_H_y2011	2011	Main Haukivesi area	MS, FS, UC, OP	2