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Determining the subspecies composition of bean goose harvests in Finland using genetic methods

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Abstract:	Management of harvested species is of great importance in order to maintain a sustainable population. Genetics is, however, largely neglected in management plans. Here, we analysed the genetics of the bean goose (Anser fabalis) in order to aid conservation actions for the commonly hunted but declining subspecies, the taiga bean goose (A. f. fabalis). We used mitochondrial DNA (mtDNA) and microsatellites to determine the subspecies composition of the Finnish bean goose harvest, as the hunting bag is thought to comprise two subspecies, the taiga bean goose and the tundra bean goose (A. f. rossicus). The latter subspecies has a more stable or even increasing population size. Other eastern subspecies (A. f. serrirostris, A. f. middendorffii) could additionally be part of the Finnish hunting bag. We estimated genetic diversity, genetic structure and sex-biased gene flow of the different subspecies. Most of the harvested bean geese belonged to the taiga bean goose, whereas most of the tundra bean goose harvest was found to be geographically restricted to south-eastern Finland. The mtDNA data supported strong genetic structure, while microsatellites showed much weaker structuring. This is probably due to the extreme female philopatry of the species. The taiga bean goose had lowered genetic diversity compared to other subspecies, warranting management actions. We also detected A. f. serrirostris mtDNA haplotypes and evidence of interspecific hybridization with two other Anser species.

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Abstract

Management of harvested species is of great importance in order to maintain a sustainable population. Genetics is, however, largely neglected in management plans. Here, we analysed the genetics of the bean goose (*Anser fabalis*) in order to aid conservation actions for the commonly hunted but declining subspecies, the taiga bean goose *(A. f. fabalis*). We used mitochondrial DNA (mtDNA) and microsatellites to determine the subspecies composition of the Finnish bean goose harvest, as the hunting bag is thought to comprise two subspecies, the taiga bean goose and the tundra bean goose (*A. f. rossicus*). The latter subspecies has a more stable or even increasing population size. Other eastern subspecies (*A. f. serrirostris, A. f. middendorffii*) could additionally be part of the Finnish hunting bag. We estimated genetic diversity, genetic structure and sex-biased gene flow of the different subspecies. Most of the harvested bean geese belonged to the taiga bean goose, whereas most of the tundra bean goose harvest was found to be geographically restricted to south-eastern Finland. The mtDNA data supported strong genetic structure, while microsatellites showed much weaker structuring. This is probably due to the extreme female philopatry of the species. The taiga bean goose had lowered genetic diversity compared to other subspecies, warranting management actions. We also detected *A. f. serrirostris* mtDNA haplotypes and evidence of interspecific hybridization with two other *Anser* species.

Keywords: *Anser fabalis*, management, mtDNA, microsatellites, hybridization, sex-biased dispersal

Introduction

Management of harvested species is necessary to ensure that populations are maintained at a sustainable level. Sustainable hunting has been defined as 'the use of wild game species and their habitats in a way and at a rate that does not lead to the long-term decline of biodiversity or hinder its restoration' (Council of Europe 2007). Among the three conventionally recognised levels of biodiversity (ecosystem, species and genetic), genetics has largely been neglected in practical management, as well as in national and international policies (Laikre 2010), especially in relation to hunting. For example, the IUCN (International Union for Conservation of Nature) Red List at present lacks any genetic criteria (Rivers et al. 2014). Shortage of genetics in practical management is not due to the lack of research or scientific guidelines, but due to failure to consider genetic issues in management (Frankham 2010). Genetic factors, such as inbreeding and loss of genetic diversity compromise the viability of populations and may even lead to extinction (Frankham 2005). Harvesting itself may cause genetic changes, such as alteration of population subdivision and loss of genetic variation and local adaptations (Allendorf et al. 2008). Hence, genetic issues should be incorporated into management of harvested species in order to manage populations properly (Waples and Gaggiotti 2006; Palsbøll et al. 2007).

Only a few studies have focused on the incorporation of genetic aspects into management plans. Moyle et al. (2003) studied the species recovery plans (1977-1998) in the United States and they concluded that genetics had only a minor role and that the understanding of how genetics could be used to aid the species recovery was limited. However, in some cases genetics has been successfully incorporated into management actions. One famous example is the genetic restoration of the endangered Florida panther (*Puma concolor coryi*; U.S. Fish and Wildlife Service 2008; Johnson et al. 2010). The Florida panthers had low genetic variation, which was an indication of inbreeding that may have led to several defects, such as poor sperm quality, cryptorchidism (testicles not descending), kinked tail and cowlick on the back. Genetic rescue

with the translocation of eight Texas female pumas (*P. c. stanleyana*) led to increased population numbers and reduced the incidence of inbreeding defects (Johnson et al. 2010).

A process readily revealed by genetic analysis is hybridization, as defined as the interbreeding of individuals from genetically distinct populations (Short 1969). Hybridization is a serious conservation problem as demonstrated in many populations and species of plants and animals (Todesco et al. 2016). The risk of extinction by hybridization is increased by human activities such as translocations, husbandry and habitat disturbance, especially in the absence of reproductive barriers and when there is introgression (gene flow from one population to the other as a result from hybridization) to the rare species (Todesco et al. 2016). As an example, hybridization of the endangered red wolf (*Canis rufus*) with coyotes (*C. latrans*) has been considered as one of the greatest threats to the red wolf (Gese et al. 2015). The red wolf numbers declined due to land use changes, which allowed the coyotes to invade their range and hybridize with the red wolves, leading to a loss of almost all of the red wolf populations due to genetic mixing. The red wolf adaptive management plan (U.S. Fish and Wildlife Service 2013) has been successful at reducing the nuclear introgression of coyote genes into the red wolf (Gese et al. 2015).

Many European migrating waterfowl populations are of management concern and the EU Birds Directive and the African-Eurasian Waterbird Agreement (AEWA) provide the legal framework for sustainable management of migratory waterfowl populations. However, International Single Species Action Plans (ISSAPs) by AEWA fail to incorporate genetics into management actions and goals for the conservation of geese (Robinson and Colhoun 2006; Cranswick et al. 2012; Madsen and Williams 2012; Stroud et al. 2012; Marjakangas et al. 2015), except in the case of the lesser white-fronted goose (*Anser erythropus*; Jones et al. 2008). The ISSAP of the lesser white-fronted goose advocates development of genetic assessments and a strategy for genetic management and minimisation of interspecific introgression due to captive

breeding programs (Jones et al. 2008). Among goose species that are currently exploited by man, genetic methods have only been employed for the Canada goose (*Branta canadensis*), with the composition of the hunting bag genetically assessed (Inman et al. 2003; Scribner et al. 2003; Shorey et al. 2007; Mylecraine et al. 2008) and included in the Canada goose management plans (Canada Goose Committee 2008; Pacific Flyway Council 2015).

The bean goose (*Anser fabalis*) is currently divided into four subspecies: *A. f. fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii* (Fig. 1). However, taxonomy of the bean goose has been controversial for a long time with numerous changes (Delacour 1951; Sangster and Oreel 1996; Ruokonen and Aarvak 2011). Traditionally, five subspecies were recognised including also *A. f. johanseni* (Delacour 1951), whose validity as a subspecies has later been rejected by several authors (Burgers et al. 1991; Sangster and Oreel 1996; Ruokonen and Aarvak 2011). Historically, the pink-footed goose (*A. brachyrhynchus*) was also considered as a subspecies of the bean goose (Delacour 1951). More recently, Sangster and Oreel (1996) suggested that there are two species *A. fabalis* (including *A. f. fabalis* and *A. f. middendorffii*) and *A. serrirostris* (including *A. f. rossicus* and *A. f. serrirostris*). Two species was also proposed by Ruokonen et al. (2008) based on the mtDNA sequences, but with differing composition. *A. fabalis* was suggested to include three subspecies *A. f. fabalis, A. f. rossicus* and *A. f. serrirostris* whereas *A. middendorffii* forms another species, the Middendorf's goose, as it was clearly differentiated from the bean geese (Ruokonen et al. 2008). Further, Ruokonen et al. (2008) classified *A. brachyrhynchus* as a separate species based on the mtDNA, but a recent exon-based phylogenomics study identified a sister-species relationship of *A. brachyrhynchus* and *A. f. rossicus* (Ottenburghs et al. 2016a). This incongruence between different genetic markers could be due to very recent speciation that still can be seen as incomplete lineage sorting and/or hybridization in the bean goose-pink-footed goose complex (Ruokonen et al. 2000; Ottenburghs

et al. 2016a). However, the study of Ottenburghs et al. (2016a) did not include all the bean goose subspecies, leaving the evolutionary relationships of the complex still unresolved.

The bean goose subspecies are grouped into breeding forms (Delacour 1951) that inhabit different habitats in Fennoscandia and Russia (Fig. 1). The taiga breeding forms (*A. f. fabalis, A. f. middendorffii*) inhabit open or wooded mires, small lakes, ponds and streams (Nilsson et al. 1999) whereas the tundra breeding forms (*A. f. rossicus, A. f. serrirostris*) inhabit open tundra, usually near lakes or rivers (Van den Bergh 1999). The breeding forms differ slightly in body size and shape, bill morphology and coloration and plumage colour but due to large individual variation in morphology, the visual identification of each subspecies is challenging (Delacour 1951). Consequently, the breeding forms or the subspecies are not identified in goose counts or in hunting statistics.

Most of the European goose populations are currently expanding, while only the lesser-white fronted goose is showing a long-term decline and the red-breasted goose (*B. ruficollis*) and the taiga bean goose (*A. f. fabalis*) are showing short-term declines (Fox et al. 2010). The most recent population estimate for *A. f. fabalis* is 40,000-50,000 individuals at the end of the non-breeding season (Wetlands International 2016) which is less than half of the estimated 90,000-100,000 individuals in the 1990s (Nilsson et al. 1999). The taiga bean goose population was still expanding in 1970-1990 (Madsen 1991), but has been decreasing since then (Fox et al. 2010). The population trend of the western tundra bean goose *A. f. rossicus* has been stable (Fox et al. 2010) and estimated to be approximately 550,000 individuals (Wetlands International 2016). The decline of the taiga bean goose is of great management concern as the species is hunted throughout its range (Fig. 1) except in Great Britain, Norway, the Netherlands and Belgium, with thousands of taiga bean geese hunted in Finland, Sweden and Russia, and hundreds in Denmark (Hirschfeld and Heyd 2005).

Here, we aim to 1) determine the ratio between the declining *A. f. fabalis* and the stable *A. f. rossicus* in the Finnish hunting bag, 2) examine the possible presence of the eastern breeding taiga- and tundra bean geese in the hunting bag and search for possible hybridization between geese species and 3) provide estimates of genetic diversity, genetic structure and sex-biased gene flow for bean goose subspecies. We use mitochondrial DNA control region sequences and microsatellites to determine subspecies composition of the bean goose hunting bag in Finland. The mtDNA control region has been shown to separate well the different subspecies (Ruokonen et al. 2008) and we will also evaluate the usefulness of microsatellites in the subspecies identification.

Material and methods

Sampling and DNA extraction

The Finnish bean goose specimens (*n*=103) consisted of wings collected by hunters during the legal hunting seasons in years 2010-2013 (2010 *n*=9; 2011 *n*=26; 2012 *n*=64 and 2013 *n*=4). In 2010 the hunting season began on 10 September, in 2011 on 17 September, in 2012 on 26 September and in 2013 on 10 October, in southern and central parts of Finland. In northern and eastern parts (Lapland and Kainuu) the season began earlier. Each year the hunting season continued until the end of December. Since 2014, hunting of the bean goose has been completely forbidden in Finland. We also included Norwegian (*n*=8), Russian (*n*=39) and Finnish (*n*=8) samples of known breeding origin from years 1997-2006 (Fig. 1). Most of the latter samples (*n*=41) were included in a study by Ruokonen et al. (2008). In addition, we used mitochondrial control region sequences from GenBank published by Ruokonen et al. (2000, 2008): EU186805–EU186812 and AF159951 (*A. f. fabalis* haplotypes FAB1a, FAB1b and FAB3, *A. f. rossicus* haplotypes ROS2a and ROS2b, *A. f. serrirostris* haplotypes SER1a and SER1b and *A. middendorffii* haplotypes MID1 and MID5) as well as partial control region (219 bp) sequences

(Ruokonen et al. 2008) EU186813–EU186828 (*A. f. fabalis* FAB1, FAB3 and FAB6, *A. f. rossicus* ROS2, ROS3 and ROS4, *A. f. serrirostris* SER1, SER2 and SER3 and *A. middendorffii* MID1, MID2, MID4, MID5 and MID6). We extracted DNA using a DNeasy Blood and Tissue Sample Kit (Qiagen) according to manufacturer's instructions from 5 µl of blood (4 samples) or about 4 mg of muscle (all the rest). The samples with known breeding origin had their DNA extracted as in Ruokonen et al. (2008).

Mitochondrial DNA sequencing

We amplified the whole tRNAglu gene and almost the whole mitochondrial control region (11 bp from 3' end was excluded as in Ruokonen et al. 2008) that has been shown to distinguish the four bean goose subspecies (Ruokonen et al. 2008). The 1235 bp sequence was amplified in two fragments with primer pairs L16642/H411-AL and L334-AL/H1248 (Ruokonen et al. 2000), that were designed to contain mismatches to Numts (nuclear sequences of mitochondrial origin; Lopez et al. 1994). We performed PCR in 20 µl reaction volumes using Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific) and 50-100 ng of template-DNA. For PCRs with primers L16642/H411-AL, the thermal profile consisted of 98 °C for 30s, followed by 30 cycles of 98 °C for 10s, 52 °C for 20s and 72 °C 15s with a final extension of 72 °C for 10min. For PCRs with primers L334-AL/H1248, we used the same thermal profile except for primer annealing temperature of 59 °C for 30s and synthesis for 30s. Double-stranded sequencing of the PCR products with the PCR primers was performed using BigDye Terminator v.3.1 (Applied Biosystems) and the reactions were run on an ABI 3730 (Applied Biosystems). We aligned and manually edited sequences using BioEdit 7.2.5 (Hall 1999).

Microsatellite genotyping

For the microsatellite analysis, we chose 20 polymorphic loci originally designed for a closely related species, the pink-footed goose: Abra2, Abra3, Abra4, Abra5, Abra7, Abra9, Abra10, Abra12, Abra14, Abra15, Abra19, Abra23, Abra24, Abra29, Abra30, Abra35, Abra39, Abra43, Abra49 and Abra68 (Table 1; Noreikiene et al. 2012). The forward primers were fluorescently labelled with VIC, PET, FAM or NED. The microsatellite amplification was performed in two multiplexes using Type-it Microsatellite PCR Kit (Qiagen) in 10 µl volumes according to manufacturer's instructions. The annealing temperature was set to 60 °C (see Noreikiene et al. 2012). We performed the fragment analysis with an ABI 3730 and scored alleles with GeneMapper 5 (Applied Biosystems). We amplified all the samples twice to assess genotyping error between the two runs by calculating the number of mismatched genotypes divided by the number of reactions (Hoffmann and Amos 2005).

Mitochondrial DNA analysis

We estimated the genetic variation by calculating the number of haplotypes, haplotype diversity and nucleotide diversity with DnaSP v.5 (Librado and Rozas 2009). To estimate the differentiation between the subspecies, we calculated ϕ_{ST} values using the Tamura-Nei genetic distance (Tamura and Nei 1993) and alpha value 0.05 (significance tested with 10 000 permutations) with Arlequin 3.5.1.3 (Excoffier and Lischer 2010). We conducted AMOVA (analysis of molecular variance; Excoffier et al. 1992), as implemented in Arlequin 3.5.1.3, to partition the mtDNA diversity among subspecies derived from the phylogenetic analyses. Demographic and spatial population expansion of each subspecies was examined by calculating Tajima's *D* (Tajima 1989), Fu's *Fs* (Fu 1997) and R_2 (Ramos-Onsins and Rozas 2002) with coalescent simulations using the DnaSP v.5 and the mismatch distribution using Arlequin 3.5.1.3.

We used MEGA 6.06 (Tamura et al. 2013) to choose the appropriate DNA substitution model and selected the HKY+G model (Hasegawa et al. 1985) as both AIC (Akaike Information Criteria; 4486) and BIC (Bayesian Information Criterion; 5029) values supported this model with an alpha value of 0.05. We constructed a phylogenetic tree of the haplotypes inferred with DnaSP v.5 using MrBayes v.3.2.2. (Ronquist et al. 2012) with four incrementally heated MCMC chains for 1,000,000 generations, 100 as the sampling frequency, 0.05 as the Temp parameter and discarded 25% of the first trees as a burn-in, using otherwise the default parameters. The average standard deviation of split frequencies was 0.004. We also evaluated the convergence of the runs using Tracer v1.6 (Rambaut et al. 2014) by checking that the effective sample sizes (ESS) were >200 for all estimated parameters (> 3000 in our runs). The consensus tree was visualised using FigTree v1.4.2 (Rambaut 2006-2014). We also constructed a Maximum Likelihood tree with MEGA 6.06 using the HKY+G model with 1000 bootstrap replicates and using default parameters otherwise (the tree we present excluded gaps in the analysis; similar results were obtained when gaps were included). We constructed the trees using the 1235 bp control region sequences and using only the 219 bp hypervariable part of the control region in order to identify also haplotypes previously defined only by the shorter fragment (see Ruokonen et al. 2008). We constructed a Median-Joining network (Bandelt et al. 1999) using the program PopART (Leight and Bryant 2015) with ε set to zero. GenBank sequences of the mtDNA control region from the greylag goose (*A. anser*; GenBank Accession number AF159961), the greater white-fronted goose (*A. albifrons*; AF159958) and the pink-footed goose (AF159952 and AF159953) were used as outgroups.

Microsatellite analysis

We used Micro-Checker (Van Oosterhout et al. 2004) to search for null alleles and genotyping errors in the data and estimated the frequency of null alleles with the program FreeNA (Chapuis

and Estoup 2007). The samples were classified to subspecies based on their mitochondrial sequences and the following analyses were performed to these subspecies-groups. Deviations from Hardy-Weinberg Equilibrium (HWE) for each loci (Fisher exact test), Linkage Disequilibrium (LD) for each pair of loci in each population, R_{ST} (Slatkin 1995) values, observed (H_O) and expected (H_E) heterozygosities were estimated with Genepop 4.3 (Rousset 2008) and number of alleles, allele richness and pairwise F_{ST} (Wright 1951) values were estimated with FSTAT 2.9.3.2 (Goudet 1995). The effect of mutations on population differentiation was assessed with a permutation test (1000 permutations) implemented in the program SPAGeDi 1.5 (Hardy and Vekemans 2002) by testing if F_{ST}=R_{ST} (Hardy et al. 2003). Sequential Bonferroni correction was applied to Hardy-Weinberg tests, *F*-statistics and linkage equilibrium (Rice 1989). The unbiased probability of identity (P_{ID}) and the probability of identity of siblings (P_{ID} Sib) were estimated with the program Gimlet v.1.3.3 (Valière 2002).

In addition, population structure was studied using the program Genetix 4.05 (Belkhir et al. 2004) for a Factorial Correspondence Analysis (FCA) and the program Structure v.2.3.4 (Pritchard et al. 2000; Falush et al. 2003) for a clustering analysis. Structure was run first without prior information of populations, with a run length of 500,000 and burn-in 50,000 with the number of possible clusters (K) set from 1–7 and 8 iterations for each. The ancestry model was set to admixture and correlated allele frequencies were used. We inferred the most likely number of clusters on the basis of ΔK values estimated using the *ad hoc* approach of Evanno et al. (2005) implemented in Structure Harvester (Earl and vonHoldt 2012). We also ran Structure using prior population information (Usepopinfo) based on subspecies assignment from the mtDNA results. The run length was set to 1,000,000, burn-in to 100,000 and K=4 according to subspecies number (*A. f. fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii*) using population information in the ancestry model (Migprior=0.05) together with correlated allele frequencies.

Effects of year and wind direction

We tested for the difference in the numbers of *A. f. fabalis* and *A. f. rossicus* in the hunting bag between the years 2010–2012 with a χ^2-test, comparing the impact of easterly and northerly winds each year. We obtained wind direction data from the Finnish Meteorological Institute (2015) as determined in September and October each year for south-eastern Finland (Virolahti and Lappeenranta), where most of the *A. f. rossicus* were harvested. Year 2013 was excluded from the analyses due to a low sample size of geese (n=4).

Sex-biased dispersal

We estimated sex-biased gene flow for different subspecies by calculating the differentiation for males and females and the ratio of male to female gene flow using a F_{ST}-based method suggested by Hedrick et al. (2013; see also Hedrick et al. 2015) using their equations 7a and 7b. We also estimated the sex-biased dispersal between Canada goose populations using the same method with the data of F_{ST} values in Mylecraine et al. (2008) in order to conduct an interspecific comparison.

Results

Mitochondrial DNA

We obtained the tRNAglu and almost the whole control region sequence (1235 bp) which includes the hypervariable region (219 bp) from 121 bean geese of which 96 (out of 103) were the Finnish hunted geese of unknown breeding origin and 25 (out of 55) had a known breeding origin. We obtained only partial or no sequence from the rest of the samples and did not include these in further analyses. There were altogether 27 haplotypes of which 18 were from the hunted geese. We did not find any sequence length variation in the bean goose, however in

Anseriformes a C-stretch in the 5'-end can form a hairpin structure making the interpretation of the number of cytosines challenging.

The haplotype diversity was the highest in *A. f. middendorffii* and the second highest in *A. f. serrirostris* (Table 1) when the subspecies were compared. The lowest haplotype diversity was found in *A. f. fabalis*. Nucleotide diversity was also the highest in *A. f. middendorffii* and the lowest in *A. f. fabalis* (Table 1). 83% of the observed variation was explained by among subspecies and 17% by within subspecies variation in the AMOVA results. In all the subspecies comparisons the pairwise Φ_{ST} values were high (0.68-0.86; all $P< 0.001$; Table 2). *A. f. middendorffii* was the most differentiated from the rest of the subspecies and *A. f. rossicus* and *A. f. serrirostris* were the least differentiated from each other. Tajima's *D* and Fu's *Fs* were negative in all subspecies except in *A. f. serrirostris*, but significant only in *A. f. rossicus* (Table 1). Mismatch Distribution (MD) analysis and R_2 statistics with coalescent simulation indicated no population expansion for any of the populations (sum of squared deviation and R_2, all $P> 0.05$), but the raggedness-value for *A. f. rossicus* was significant (0.03, $P< 0.05$; Table 1).

Phylogenetic trees constructed by Bayesian and Maximum Likelihood methods produced similar tree topologies, though the Bayesian posterior probabilities for the different branches were higher than the Maximum Likelihood bootstrap support (Online resource 1). The *A. brachyrhynchus*-, *A. f. middendorffii*- and *A. f. fabalis/A. f. rossicus/A. f. serrirostris*-groups were clearly separated in the tree (posterior probabilities 1.00, 0.99 and 0.95, respectively), with the latter group further separated into *A. f. fabalis* and *A. f. rossicus/A. f. serrirostris*-groups. *A. f. middendorffii* was clearly separated as its own cluster, with high posterior probabilities (0.99) and bootstrap values (83%), as well as *A. f. fabalis* (1.00/82%), but the support for the *A. f. rossicus* and *A. f. serrirostris* groups was much lower (0.69/38%) (Online resource 1). The topology of the median-joining network was in accordance with the phylogenetic results with the haplotype Fa3 being the most common in *A. f. fabalis* and ROS2a in *A. f. rossicus* (Fig. 2).

One individual from the Finnish bean geese hunting bag carried the mtDNA sequence of the pink-footed goose (haplotype Br1, Fig. 2) and another individual carried the mtDNA of the white-fronted goose (haplotype Al1, Fig. 2). Three bean geese from Valdak Norway, sampled in 2003 represented the subspecies *A. f. rossicus* according to their mtDNA. In the Finnish bag, there was three of the four bean goose subspecies present on the basis of their mtDNA: *A. f. fabalis* 52% (*n*=53), *A. f. rossicus* 44% (*n*=45) and *A. f. serrirostris* 2% (*n*=2) (Online resource 2). The remaining 2% were the two individuals with the mtDNA of other geese species. The hunting locations of the subspecies varied geographically, with *A. f. fabalis* hunted throughout Finland but *A. f. rossicus* mostly in south-eastern Finland with only few individuals hunted outside that region (Fig. 3).

Microsatellites

Two loci (Abra3 and Abra4) failed to amplify, one locus (Abra49) showed ambiguous results and one locus (Abra35) contained null alleles in all subspecies studied and therefore these four loci were excluded, leaving 16 loci for further analyses. We succeeded in genotyping a total of 153 samples of which 103 (out of 103) were from the Finnish hunted geese and 50 (out of 55) were from the geese with a known breeding origin. Genotyping error between the two amplifications of the same samples averaged over loci was low for both the Finnish hunted geese (0.007) and for the geese of known breeding origin (0.026). The program Micro-Checker suggested null alleles (at a frequency of 0.00–0.24) at a few loci which deviated from the Hardy-Weinberg equilibrium ($P< 0.05$). These loci were however included in the further analysis because the deviation from Hardy-Weinberg was probably due to population structure; indications for null alleles were not constant across the loci or subspecies (except for the excluded Abra35) and the frequency of null alleles was low in most loci. Estimates of F_{ST} using the data corrected with FreeNA did not differ significantly from the uncorrected values, so the

existence of any null alleles did not bias our results. We did not find any Linkage Disequilibrium after Bonferroni correction. Numbers of alleles were highest in *A. f. fabalis* and *A. f. rossicus*, but these subspecies had the largest sample sizes (Table 1). The allelic richness, which takes into account the differences in the sample sizes did not vary much between the subspecies (3.3-3.8), and neither did the estimates for heterozygosity (H_O= 0.43-0.50; H_E= 0.51-0.57; Table 1). P_{ID} values varied from 0.07 to 0.91 and P_{ID} Sib values varied from 0.38 to 0.96 (Table 1).

The microsatellite markers did not show differentiation between the subspecies. Also the individuals with the mtDNA derived from another species clearly fell inside the intraspecific variation of the bean goose. The pairwise F_{ST} values were very low (≤0.03), especially when compared to the ϕ_{ST} values from the mtDNA (Table 2). The R_{ST} values were higher than the F_{ST} values except for the *A. f. fabalis – A. f. rossicus* pair (Table 2). A permutation test indicated that R_{ST} values were significantly higher ($P< 0.05$) than F_{ST} values only in two loci (Abra9 and Abra12), which indicates that only these two loci evolve under a strict stepwise mutations model. Thus, we used only F-statistics with the microsatellite loci. Factorial Correspondence Analysis (FCA) also showed high genetic similarity between the subspecies, although some slight differentiation was seen between *A. f. fabalis* and *A. f. rossicus* (Fig. 4). Structure analysis without prior population information gave K=3 as the most probable number of genetic clusters, but all individuals showed a high amount of admixture and no clear clustering (Online resource 3a). However, when we used the subspecies assignment based on the mtDNA results as prior population information (K=4), Structure clustered the individuals clearly according to their subspecies (Online resource 3b). All the individuals belonged to their subspecies with high likelihood (80-90%) except one *A. f. rossicus* individual that was admixed with *A. f. fabalis* (58% *rossicus* and 42% *fabalis*). The two geese with the mtDNA from a different species clearly belonged to bean goose according to nuclear genotypes (Online resource 3b).

Effects of year and wind direction

The frequency of the harvested subspecies varied between years (Table 3) with about 30% more *A. f. fabalis* in 2011 than in other years ($\chi^2 = 9.07$, $P < 0.05$). In 2010 and 2012 there were slightly more *A. f. rossicus* than *A. f. fabalis.* The prevailing wind direction varied between the years ($\chi^2 = 267.8$, $P < 0.01$) with year 2012 having more easterly winds than compared to other years (Online resource 4). The frequencies of *A. f. rossicus* as well as the easterly winds were higher in 2012 than in 2011. This indicates that the easterly winds shifted the migration of more *A. f. rossicus* individuals into Finland in autumn while prevailing northerly winds increased the proportion of *A. f. fabalis* in the hunting bag.

Sex-biased dispersal

Gene flow between the subspecies was much higher in the bean goose males than in the females and the ratio of gene flow between males and females (m_m/m_f) varied between (57.2-316; Table 4), with an average of 122. In the Canada goose, the m_m/m_f ratio varied from -0.35 to 17.40 between populations, with an average of 4.47. Compared to the bean goose, the Canada goose showed much lower m_m/m_f ratios, suggesting less sex-biased dispersal.

Discussion

Composition of the hunting bag

The Finnish bean goose harvests consisted mainly of the subspecies *A. f. fabalis* and *A. f. rossicus* as expected, since *A. f. fabalis* is the main subspecies breeding in Finland and *A. f. rossicus* is a regular passage migrant in Finland. Based on our results and the previous study by Ruokonen et al. (2008), we confirmed that the mtDNA-based classification of individuals to subspecies is powerful. The microsatellite data gave less clear results than mtDNA and the subspecies assignment could not be performed based on microsatellites alone. However, when

subspecies information obtained from mtDNA was used, the microsatellite data fit well with the subspecies assignments, supporting that the subspecies form coherent taxonomic entities.

The proportion of different subspecies fluctuated between years with *A. f. rossicus* harvested more than *A. f. fabalis* in most years but, on the whole, more *A. f. fabalis* were harvested. The fluctuation could be partly explained by the prevailing wind directions. When the easterly winds dominate, they shift the migration route of *A. f. rossicus* to the south-eastern Finland from Russia, whereas when northerly winds dominate, the migration route of *A. f. rossicus* stays mostly in Russia (Toivainen et al. 2014). This would result in more *A. f. rossicus* being hunted in Finland when easterly winds prevail during the migration time. It has been observed that winds shift the migration routes in other goose species as well (for example in the Brent goose *B. bernicla* and in the barnacle goose *B. leucopsis*; Green 2001). However, our results need further confirmation as sample sizes were rather low.

The subspecies composition in the hunting bag varied geographically, with *A. f. rossicus* hunted almost solely from south-eastern Finland along the Russian border, while *A. f. fabalis* was hunted evenly over the whole Finland (Fig. 3). This was predicted, as *A. f. fabalis* breeds in northern and central Finland and passes through the Åland archipelago located between Finland and Sweden to staging areas in southern Sweden (Nilsson 2011). In addition, Russian *A. f. fabalis* migrates also through Finland (Nilsson 2011). On the contrary, *A. f. rossicus* migrates along the eastern border of Finland via the Baltic countries to Central and Eastern Europe (Van den Bergh 1999). Interestingly, one *A. f. rossicus* individual was hunted in the Finnish Lapland and thus could originate from the quite recently reported *A. f. rossicus* population in the Norwegian Finnmark (Aarvak and Øien 2009) that migrates along the coast of Sweden (De Jong et al. 2013). Also a few *A. f. rossicus* individuals were harvested at or near the Finnish west coast. These birds could have used some alternative migration route or have wandered off the main migration routes.

In addition to these two main subspecies, two eastern tundra bean geese *A. f. serrirostris* mtDNA haplotypes were found among the hunted individuals. However, according to Ruokonen et al. (2008), several *A. f. serrirostris* haplotypes are found also in *A. f. rossicus*, thus the two individuals carrying *A. f. serrirostris* haplotypes might actually represent *A. f. rossicus* (see also Fig. 2), or they could as well be hybrids. It is possible that *A. f. serrirostris* is an occasional wanderer to Finland, providing an opportunity for hybridization, even though there are no previous reports of *A. f. serrirostris* in Finland. However, as the resolution in the microsatellites was not sufficient to separate these two subspecies, the identity of these birds remains unclear.

We found one bird with mtDNA of the pink-footed goose and another with mtDNA of the greater white-fronted goose. The microsatellites indicated that these individuals are bean geese (Online resource 3b). Hence, this implies inter-specific hybridization and introgression of mtDNA to bean goose from other goose species. The bird with the pink-footed goose mtDNA looked morphologically like *A. f. rossicus*, except for yellower feet and bill than a normal bean goose (Tomas Aarvak, personal communication). The wing of the bird with the greater white-fronted goose mtDNA looked like a young greater white-fronted goose due to its grey colour and lack of clear white fringes of primary feathers typical of the bean goose (Petri Lampila, personal communication). These unusual morphological features suggest that these two birds were of hybrid origin. However, we did not have microsatellite data from pink-footed or white-fronted geese to confirm this result. In addition, the usage of microsatellite loci designed for another, although closely related, species could have limited the effectiveness of detecting hybrid individuals.

Genetic diversity, genetic structure and gene flow

The nuclear diversity in all subspecies, measured as observed heterozygosity, was low (0.43-0.50) compared for example to the greater white-fronted goose (0.67; Ruokonen et al. 2007).

However, it was at the same level as observed in the lesser white-fronted goose (0.51) that has been strongly declining in population size (Ruokonen et al. 2007). Mitochondrial haplotype diversities in *A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii* were higher (h = 0.68 – 0.86) and nucleotide diversities lower (π = 0.001 – 0.002) compared to several other geese (e.g. lesser white-fronted goose, h = 0.37 – 0.53, π = 0.003, pink-footed goose, h = 0.51, π = 0.003; Ruokonen et al. 2004, 2005). This could have resulted from a population growth after a past bottleneck (Grant and Bowen 1998). However, *A. f. rossicus* was the only subspecies showing signs of past population growth also by Tajima's *D*, Fu's *Fs* and the raggedness index and is the only population not in decline at present. *A. f. fabalis*, on the other hand, had lower haplotype and nucleotide diversities (h = 0.582, π = 0.00103) than the other subspecies, suggesting a possibility of a relatively recent bottleneck (Grant and Bowen 1998). The mitochondrial diversity of *A. f. fabalis* was close to the levels observed in other geese species that are declining or have had historically low population sizes (such as the above mentioned lesser white-fronted and the pink-footed goose).

The haplotype network clearly supported clustering of the bean goose into three separate groups: *middendorffii, fabalis* and a group including *rossicus* and *serrirostris* (Fig. 2). Divergence between the subspecies measured by the pairwise F_{ST} (0.01-0.03) or R_{ST} (0.01 – 0.07) values of microsatellite data (Table 2) was much lower than from the mtDNA (ϕ_{ST}: 0.68 – 0.86). The level of divergence in the bean goose microsatellites is comparable to values obtained from other goose species, for example the pairwise F_{ST} values between two wild populations of the lesser white-fronted goose was 0.01 (Ruokonen et al. 2007) and between populations of the Canada goose from 0.002 to 0.05 (Mylecraine et al. 2008).

This discrepancy in the amount of differentiation estimated from the two types of markers can partly be explained by differing effective population sizes of these markers, as mtDNA has four times smaller effective size than microsatellites. However, the extremely strong philopatry

in females can also have a great effect (Zink and Barrowclough 2008). When females return to nest at their natal sites, geographical structure is found in the maternally inherited mtDNA, but gene flow through males inhibits structuring in nuclear loci (Zink and Barrowclough 2008). We detected up to 300 times greater gene flow in males than in females and this seems to explain most of the difference between markers. This amount of sex-biased gene-flow is much larger than what we observed by performing the same calculations for the Canada goose (up to 17 times greater). Evidence of the sex-biased dispersal has been found also in the lesser-white fronted goose (Ruokonen et al. 2010) and the greylag goose (Nilsson and Persson 2001) but not in all goose species (e.g. in the lesser snow goose, *A. caerulescens*; Avise et al. 1992). The strong female philopatry could make the local taiga bean goose populations especially vulnerable to overharvesting, as local populations are not readily re-colonised after local extinction, due to the female site fidelity (Marjakangas et al. 2015).

Hybridization

Ducks and geese (Anseriformes) show the greatest propensity to hybridization in birds, with over 40% of the species doing so (Grant and Grant 1992; Ottenburghs et al. 2016b). For the declining taiga bean goose, hybridization can become a major threat as it brings genes from other species into the taiga bean goose. In geese, pair bonding takes place during the winter or early in the spring (Rohwer and Anderson 1988). In the winter, the geese are highly gregarious and may form mixed flocks with other goose species. Sometimes this may lead them to form inter-specific pairs with other goose species wintering in the same area, especially if there is a shortage of conspecific mates. The bean goose has previously been reported to hybridize at least with the pink-footed and the greater white fronted goose (McCarthy 2006; Kampe-Persson and Lerner 2007). These species use the same wintering areas as the bean goose, which may promote interspecific pairing and hybridization. The locations from where the putative hybrids were

hunted are in concordance with the possible hybrid origin. The putative pink-footed goose x bean goose hybrid was hunted at the Finnish coast of the Bothnian Bay that is along the migration route of the pink-footed goose (Hölttä 2013) and the putative greater white-fronted goose x bean goose hybrid was hunted in south-eastern Finland along the migration route of the greater white-fronted goose (Mooij et al. 1999; Fig. 3).

Taxonomy

The taxonomy of the bean goose-pink-footed complex is still not completely resolved. Our phylogeny corresponds to that of Ruokonen et al. (2008) as the same mtDNA region was used. However, Ottenburghs et al. (2016a) show incongruence in phylogeny when different genetic markers are used. This incongruence is probably caused by incomplete lineage sorting or speciation with hybridization (Ruokonen et al. 2000; Ottenburghs et al. 2016a). Our results show that the cross-species microsatellite panel did not help to resolve the bean goose phylogeny. However, a new microsatellite panel developed for the bean goose (Kleven et al. 2016) could resolve the shortcomings of our panel and should be tested in further studies. Further, a thorough genomic analysis with sampling across the entire range of the bean goose (Ottenburghs et al. 2016a) with all the subspecies and closely related species involved, should be carried out in order to resolve the taxonomic relationship of the bean goose-pink-footed goose complex. Also, elucidating the pattern of incomplete lineage sorting could be useful in order to explore the evolutionary forces that have acted during speciation within the genus *Anser* as was done by Scally et al. (2012) with human-great ape whole-genome sequences.

Management implications

In this study we found that over half of the Finnish bean goose bag consists of the declining taiga bean geese and that the tundra bean goose portion of the bag comes mainly from south-eastern

Finland. Our estimate is that, on average, 2200 taiga bean geese per year were hunted in Finland alone during our study period, which is far too many considering the fast decline of this subspecies. On the contrary, hunting of the tundra bean goose with a large and stable population could be permitted as long as it does not affect the taiga bean goose population. There is no knowledge of exact cause for the decline of the taiga bean goose, but potential reasons could be hunting (especially reproducing individuals), habitat destruction, increased predation, human disturbance and climate change. Interspecific competition with increasing numbers of whooper swans (*Cygnus cygnus*) at nesting sites (Kampe-Persson et al. 2005) or with other geese species in staging and wintering sites have also been suggested but not proven in any studies.

Conservation actions have already been made in Finland. Hunting of bean geese was seasonally restricted during 2010-2013, banned completely in 2014-2016 and a draft national management plan to protect the taiga bean goose was produced in 2014 (The Finnish Ministry of Agriculture and Forestry 2014). Also the International Taiga Bean Goose Management Plan was published in 2015 (Marjakangas et al. 2015). This is the first flyway conservation plan for a declining species that is still open for hunting. Unfortunately, genetic issues are not implemented in either of these management plans. Further conservation actions should be made, including a thorough study of the spatial population genetic structure of the breeding geese, continuation of restrictions for hunting at the sites where *A. f. fabalis* is the most common subspecies (at least in central and northern Finland) and management of breeding habitats.

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Figure captions

Fig. 1 Approximate breeding ranges of the bean goose subspecies (*Anser fabalis fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii*) marked with the subspecies name and their wintering ranges marked with dark shading (redrawn from BirdLife International and NatureServe 2014 and from Ruokonen et al. 2008). The western subspecies *A. f. fabalis* and *A. f. rossicus* overwinter in Europe and some eastern *A. f. fabalis* in central Asia. The eastern subspecies *A. f. serrirostris* and *A. f. middendorffii* overwinter in China, Korea and Japan. The sampling locations of the samples with known origin are marked with closed circles; for the locations of the hunted geese, see Fig. 3

Fig. 2 Median-joining network of the bean goose (*Anser fabalis*) haplotypes and outgroups (*A. brachyrhynchus, A. albifrons*) for the mitochondrial control region (1235 bp). The haplotypes named with three uppercase letters were previously described in Ruokonen et al. (2008). Letters a or b in the haplotype names denote haplotypes that were identical with the 219 bp hypervariable region but differ in the whole control region. The size of each circle is proportional to the frequency of each haplotype. Black slashes across branches indicate the number of mutational changes between the haplotypes

Fig. 3 Geographical distribution of the harvest locations of the Finnish bean goose subspecies (*Anser fabalis fabalis, A. f. rossicus* and *A. f. serrirostris*) based on mtDNA together with cases of mtDNA introgression, i.e. where the bean goose had the mtDNA of a different species, either *A. brachyrhynchus* or *A. albifrons*. The size of each circle is proportional to the frequency of each subspecies

Fig. 4 Microsatellite Factorial Correspondence Analysis (FCA) plot for the bean goose (*Anser fabalis*). The subspecies (*A. f. fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii*) were assigned based on mtDNA. The suggested hybrids with introgressed mtDNA (*A. brachyrhynchus* and *A. albifrons*) are also indicated

Dear Editor-in-Chief, Christian Gortázar

We are pleased to send you our revised manuscript (Ref.: Ms. No. EJWR-D-16-00127R2 Determining the subspecies composition of bean goose harvests in Finland using genetic methods European Journal of Wildlife Research). We have followed all the suggestions made by the reviewer and we have also shortened the Discussion of our manuscript. We have also made additional minor changes to the manuscript when, through our reading, we have noticed lack of clarity. We believe that out manuscript has further improved and hope that it is now suited for publication. Below you can find our responses to the reviewer's comments marked in blue italics.

Sincerely yours,
Johanna Honka

Dear Dr Honka,
one of the reviewers who had evaluated the original submission of your manuscript has commented on your revised version. As you can see the manuscript is now mostly satisfying, yet some minor changes are pending. As the Discussion is still a bit long, I invite you to undertake a text reduction, for instance summarizing results of other comparable studies.
Best regards
Massimo Scandura

We have shortened the Discussion by condensing the text and summarising results of other studies (please see below).

Reviewer #1: I have re-reviewed the manuscript by Honka et al. and find that it has been significantly improved compared to the original submission. The authors have done a very good job thoroughly addressing the reviewers' comments. In my opinion, Discussion is still long and a heavy to be read. It takes about 35% of the total length, more or less. I have a series of minor remarks that I hope can help further the understanding of the MS.

We have now shortened our Discussion, as suggested, from 2658 words to 2046 words, which equals two pages, hopefully making it also easier to read now.

Line xx:

28- delete comma after second parenthesis, and change "facilitate management" with "aid conservation";
34- delete "From...," and use "We estimated"; delete "were made ...diversity,"
38- move " We also ...species." to the end of the abstract
54- "Among the three conventionally recognized levels of biodiversity", I mean, there are many indeed ...
55- delete "overlooked and"
57- delete "the most ....species";
81- delete "and the harmful.... to" and replace with "as it has been proved in"

85- it could be nice spending just some words to explain differences between "hybridization" and "introgression", yet just in case authors agree to shorten a bit other parts in Discussion, otherwise Introduction too risks to be long as well;
91/92- delete "has a goal....and"
109- delete ", but the" and replace with ". However, taxonomy"
118/120: please, revise English
124- replace "as its own" with "separate"
128- Guess you meant "rapid speciation caused by hybridization", isn't it?
134- replace "environments" with "habitats"; delete "as shown" and use "(Figure 1)"
135- replace ", and" with ", whereas"
147- add "then" after "since"
154- ratio, in italic
158- delete "nuclear"
159- delete "as these ..challenging"
161-163: please, revise English and style
192- delete "to...amplification"
230/231- use capitals for spelling acronyms

We have now revised all the wording, English and style, except we did not change the word 'ratio', to italics because we actually couldn't see why it was suggested.

** 241- Why did you exclude gaps?

We performed maximum likelihood analyses both including gaps and excluding gaps and chose to present only the latter as the same tree topology was recovered, as now explained in the text.

255: Linkage Disequilibrium, LE; Hardy Weinberg Equilibrium, HWE
261-266: suggest removing paragraph
313-316: revise English and style
320- Mismatch .. add "Distribution (MD)"
322- R2, with 2 as subscript
413/415- revise English (syntax)
418/421- as above

We have revised all the wording, English and style.

** 471-476: so, I understand that, up to you, high haplotype diversity is 0.68-0.86 whereas low haplotype diversity is 0. 58: on which basis? Can you please test for these values to prove they are statistically different? "High" and "low" are qualitative descriptors only; as to me, 0.58 is not so low at all, depends on many things, you know.

We mean that 0.58 is low compared to other bean goose subspecies and species with high population numbers, we are sorry for being unclear. 0.58 is not as low as in the populations that are declining or have a historically low population size (h=0.37-0.53; lesser white fronted and pink-footed goose) but much less than species with a large population size such as the greater white fronted goose (h=0.89). We have now made efforts to make our comparisons clearer.

527-: "propensity to hybridization" (?)
525-529: see repetitions(hybrid/hybridization)
562: use "carried out" instead of "executed"
570: use "on average" between commas

575- may be a stop after "Finland" would be better

We have revised all the wording, English and style. We removed repetitions of hybridization by rewording.

Johanna Honka
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Christian Gortázar
Chief Editor
European Journal of Wildlife Research

April 22, 2016

Dear Dr. Gortázar

We are pleased to submit an original research article "Genetic methods for determining subspecies composition of bean goose harvests in Finland" for consideration for publication in the European Journal of Wildlife Research.

We believe that this manuscript is appropriate for publication by the European Journal of Wildlife Research because our manuscript focuses on wildlife and conservation genetics with clear management implications. Our aim was to produce genetic data to aid management decisions of a declining taiga bean goose subspecies (*Anser fabalis fabalis*) that is hunted throughout Europe. This research is the first study that genetically quantifies the subspecies composition of the bean goose harvests. Our study showed that most of the harvested geese belong to the declining taiga bean goose subspecies and that the harvesting of the abundant tundra bean goose (*A. f. rossicus*) subspecies was geographically restricted to southeastern Finland. We also detected eastern tundra bean goose (*A. f. serrirostris*) haplotypes and possible cases of interspecific hybridization between goose species.

This manuscript has not been published and is not under consideration for publication elsewhere. We declare no conflict of interest.

Thank you for your consideration of our manuscript.

Sincerely,
Johanna Honka

Table

Table 1 Population genetics statistics for the bean goose subspecies (*Anser fabalis fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii*). Above, data for mtDNA including sample size (*n*), number of haplotypes (*H*), haplotype diversities (*h*) and nucleotide diversities (π). Demographic population expansion tested with Tajima's *D* (*D*) and Fu's *F*s (*Fs*), sum of squared deviation (SSD), Raggedness-index and R_2 statistics. SD stands for standard deviation. Middle, data for microsatellites including sample size (*n*), number of alleles (*A*), allelic richness (A_R), observed (H_O) and expected heterozygosity (H_E), unique alleles and Hardy-Weinberg equilibrium test (χ^2, df=degrees of freedom). Below, data for microsatellite allele size ranges, unbiased probability of identity (P_{ID}) and probability of identity of siblings (P_{ID} Sib). Allele sequences and repeated motifs can be found in Noreikiene et al. (2012). Statistically significant values ($P< 0.05$) indicated with an asterisk and ($P< 0.001$) with two asterisks

Subspecies	*n*	*H*	*h* (SD)	π (SD)	*D*	*Fs*	SSD	Ragged-ness	R_2
fabalis	55	7	0.582 (0.062)	0.00103 (0.00011)	-0.182	-1.167	0.031	0.130	0.085
rossicus	49	9	0.676 (0.060)	0.00111 (0.00030)	-2.048*	-2.905	0.006	0.029*	0.068
serrirostris	4	3	0.733 (0.155)	0.00143 (0.00035)	1.386	0.688	0.108	0.293	0.278
middendorffii	9	6	0.855 (0.085)	0.00169 (0.00025)	-0.164	-1.607	0.383	0.044	0.140

	n	*A*	A_R	H_O	H_E	Unique alleles	χ^2	df
fabalis	63	96	3.834	0.502	0.571	8	∞**	32
rossicus	68	91	3.690	0.490	0.566	7	∞**	32
serrirostris	8	54	3.290	0.430	0.510	0	34.81	26
middendorffii	14	68	3.581	0.455	0.549	2	50.81	30

Locus	Allele size range (bp)	P_{ID} (locus)	P_{ID} Sib (locus)
Abra2	91-107	0.074	0.379
Abra5	119-131	0.129	0.430
Abra7	93-119	0.084	0.399

Abra9	180-196	0.172	0.483
Abra10	147-169	0.088	0.394
Abra12	95-109	0.166	0.475
Abra14	150-154	0.752	0.871
Abra15	188-202	0.101	0.408
Abra19	168-184	0.105	0.408
Abra23	272-288	0.116	0.415
Abra24	289-293	0.475	0.696
Abra29	205-207	0.912	0.956
Abra30	108-120	0.315	0.576
Abra39	118-139	0.156	0.470
Abra43	120-134	0.203	0.502
Abra68	120-130	0.303	0.575

Table

Table 2 Pairwise ϕ_{ST} values for bean goose subspecies (*Anser fabalis fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii*) for mtDNA above the diagonal and the pairwise F_{ST} values and the R_{ST} values (in parentheses) for microsatellites below diagonal. Statistically significant (after Bonferroni correction) values ($P< 0.05$) indicated with an asterisk and ($P< 0.001$) with two asterisks

Subspecies	*fabalis*	*rossicus*	*serrirostris*	*middendorffii*
fabalis		0.842**	0.791**	0.860**
rossicus	0.0256* (0.0058*)		0.679**	0.861**
serrirostris	0.0314* (0.0323*)	0.0120 (0.0255)		0.780**
middendorffii	0.0096 (0.0251)	0.0271* (0.0526*)	0.0282 (0.0664*)	

Table 3 The percentage of the harvested Finnish bean geese subspecies assigned to subspecies by mtDNA (*Anser fabalis fabalis*, *A. f. rossicus* and *A. f. serrirostris*). Year 2013 was excluded due to a low sample size.

	2010	2011	2012
Sample size	9	24	62
fabalis	44.4	76.0	42.2
rossicus	55.6	20.0	54.7
serrirostris		4.0	1.6
mtDNA of other species			1.6

Table

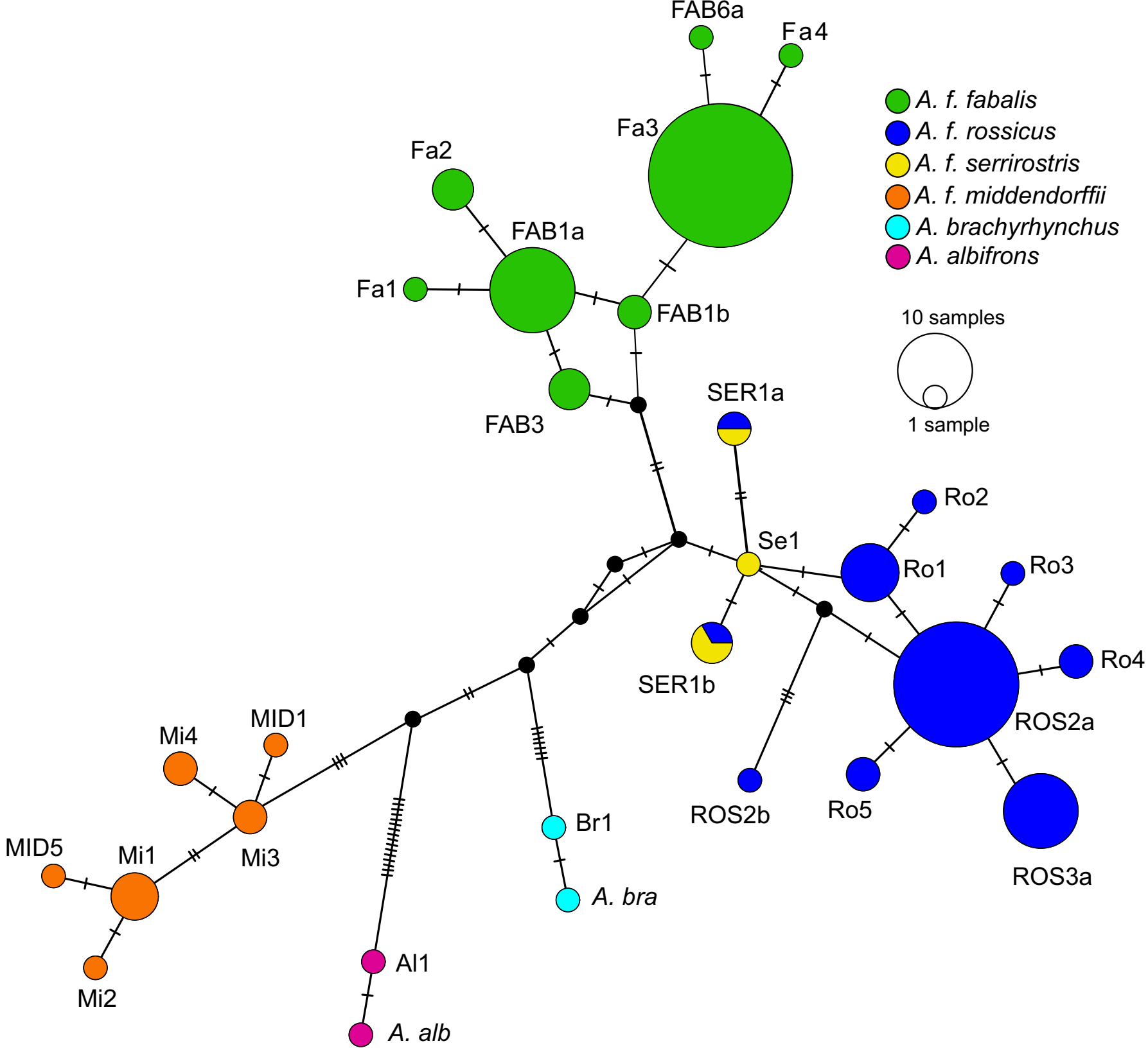
Table 4. F_{ST} values based on the microsatellites, differentiation in females based on the mtDNA ($F_{ST(f)}$) from ϕ_{ST}, calculated differentiation in males ($F_{ST(m)}$; Hedrick et al. 2013: equation 7a) and the ratio of the gene flow in males and females (m_m/m_f; Hedrick et al. 2013: equation 7b) in bean goose subspecies (*Anser fabalis fabalis, A. f. rossicus, A. f. serrirostris* and *A. f. middendorffii*)

		F_{ST}	$F_{ST(f)}$	$F_{ST(m)}$	m_m/m_f
fabalis	*rossicus*	0.026	0.842	0.050	100.3
fabalis	*serrirostris*	0.031	0.791	0.062	57.24
fabalis	*middendorffii*	0.010	0.860	0.019	316.1
rossicus	*serrirostris*	0.012	0.679	0.023	89.03
rossicus	*middendorffii*	0.027	0.861	0.053	109.9
serrirostris	*middendorffii*	0.028	0.780	0.056	60.03

Figure


rossicus and fabalis
serrirostris
rossicus
fabalis
middendorffii

Figure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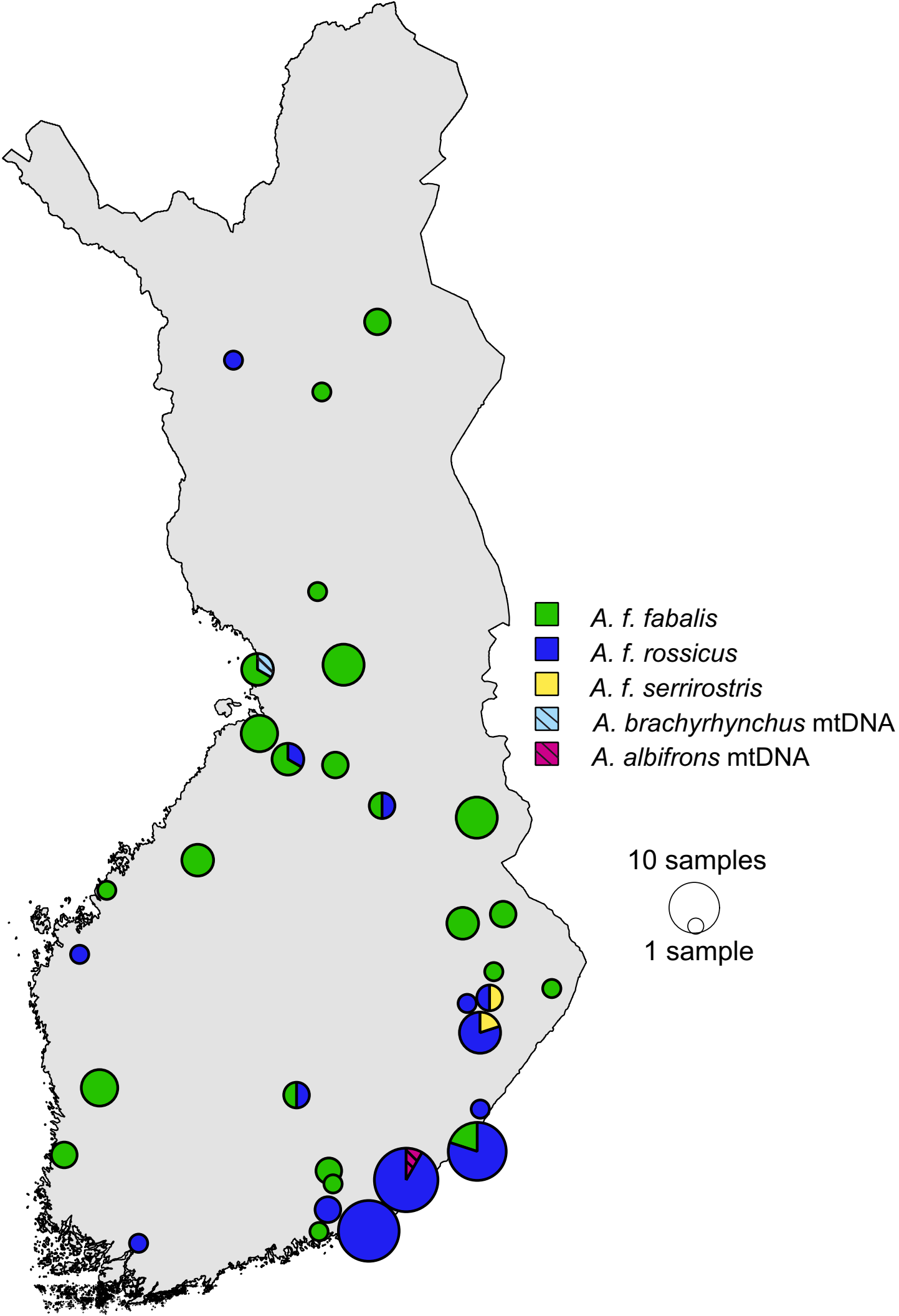
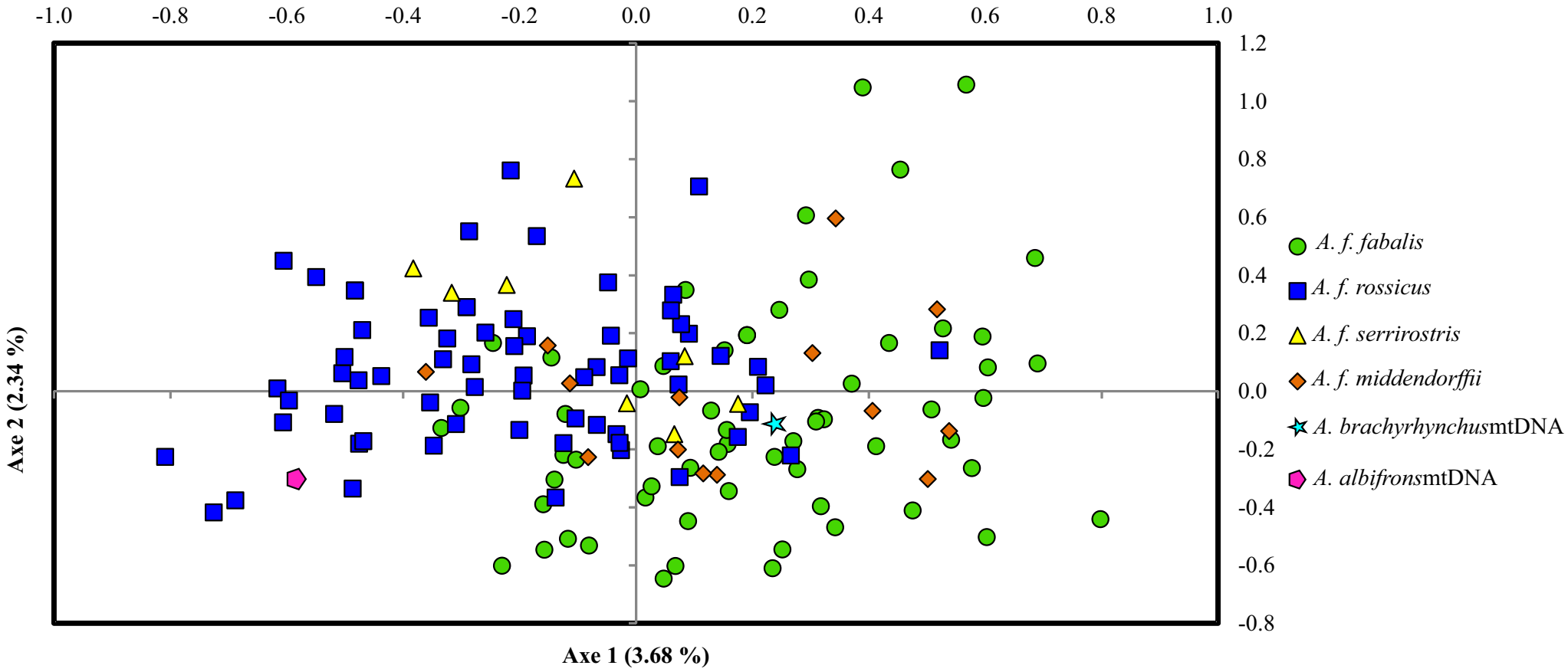
Fa4
Fa3
Fa2
FAB1a
Fa1
FAB1b
FAB3
SER1a
Se1
Ro2
Ro1
Ro3
Ro4
SER1b
ROS2a
ROS2b
Ro5
ROS3a
Br1
A. bra
MID1
Mi4
MID5
Mi1
Mi3
Mi2
Al1
A. alb
A. f. fabalis
A. f. rossicus
A. f. serrirostris
A. f. middendorffii
A. brachyrhynchus
A. albifrons
10 samples
1 sample

Figure


A. f. fabalis
A. f. rossicus
A. f. serrirostris
A. brachyrhynchus mtDNA
A. albifrons mtDNA
10 samples
1 sample

Figure


-1.0
-0.8
-0.6
-0.4
-0.2
0.0
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1.0
0.8
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0.0
-0.2
-0.4
-0.6
-0.8
Axe 2 (2.34 %)
Axe 1 (3.68 %)
A. f. fabalis
A. f. rossicus
A. f. serrirostris
A. f. middendorffii
A. brachyrhynchusmtDNA
A. albifronsmtDNA